

USING STABLE ISOTOPES TO CHARACTERIZE ORNAMENTAL AQUACULTURE
POND TROPHIC DYNAMICS AND MAXIMIZE FISH PRODUCTION

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2011

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To Shari, my sister Jaylynn, and my parents, without whom, this would not have been possible

ACKNOWLEDGMENTS

Thanks go out to committee chairs Drs. Charles E. Cichra and Roy P.E. Yanong, and committee members Drs. Thomas K. Frazer, Richard D. Miles and Joseph J. Delfino, for whose patience and understanding I will forever be in their gratitude. Special thanks go out to Craig A. Watson the director of the University of Florida's Tropical Aquaculture Laboratory in Ruskin, Florida, who generously provided funds and facilities that made this work possible. Additional thanks to the staff of the Fisheries Program at the University of Florida and staff and interns at the Tropical Aquaculture Laboratory who are too numerous to name in their entirety: Dr. Jeff Hill, Sonya Sampson, Ryan Schelb, Christopher Wilkinson, Tyler Pavlowich, Sherry Giardini, Mary Cichra, Carlos Martinez, Michael Krasilovsky, Scott Graves, Debra Poudner, Chris Daniels, and Beth Privett, and Julie-Anne and Ricardo Russo. Thanks go to my good friends Micah Alo, Richard Kline and Chris Tilghman who were always there to pitch in, bounce ideas off, or share a few beers.

I especially thank my late parents Ruth and Shih-Chien 'Dick' Kao whose love, affection and support will always be with me. Thanks go to my sister Jaylynn Milstein and her husband Michael for their love and generous financial assistance, and to my brother Jeff, whose eyes still glaze over at the mention of technical aspects of my research. Lastly, I thank Shari Hanson, whose love and support are beyond the measure of any scientific metric.

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LIST OF ABBREVIATIONS

$\delta^{13}\text{C}$	stable carbon isotope value
$\delta^{15}\text{N}$	stable nitrogen isotope value
$\Delta\delta^{13}\text{C}$	change (enrichment or depletion) in carbon isotope signature value
$\Delta\delta^{15}\text{N}$	change (enrichment or depletion) in nitrogen isotope signature value
C_H or MSI	Simplified Morisita's index of similarity
DIC	dissolved inorganic carbon
DIN	dissolved inorganic nitrogen
DOM	dissolved organic material
POM	particulate organic material
PSI	percent similarity index
%N	percent number
%V	percent volume
%FO	percent frequency of occurrence
PRO	processed commercial feed
UNP	unprocessed commercial feed (ground meal)
CSM	cottonseed meal (ground cottonseed)
INO	inorganic liquid nitrogen fertilizer
ANOVA	analysis of variance statistical test
IRI	index of relative importance
%IRI	index of relative importance standardized to percentage

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

USING STABLE ISOTOPES TO CHARACTERIZE ORNAMENTAL AQUACULTURE
POND TROPHIC DYNAMICS AND MAXIMIZE FISH PRODUCTION

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May 2011

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Fish production in pond aquaculture systems is highly variable among individual ponds; an obvious area of improvement would consist of maintaining uniformly high levels of production in ponds through the proper understanding and manipulation of pond trophic dynamics. One method used for understanding the flows of energy and matter within ponds is by tracing and quantifying the flow of energy and matter via the movement of stable isotopes, from allochthonous feed inputs, and live foods derived from allochthonous fertilizer inputs and autochthonous energy and matter sources within the pond's trophic system.

Using the common swordtail (*Xiphophorus hellerii*) live-bearer tropical fish as a model animal, indoor feeding trials were successfully used to validate carbon and nitrogen isotope tracing methods, and obtain mean carbon ($\text{‰ } \Delta\delta^{13}\text{C}$) and nitrogen ($\text{‰ } \Delta\delta^{15}\text{N}$) isotope enrichment magnitudes for use in outdoor pond trials.

Four nutrient types (commercial processed feed, commercial unprocessed feed, organic cottonseed meal fertilizer, and inorganic liquid fertilizer) that are widely used within the aquaculture industry, were applied to 24 ponds (6 ponds per treatment) evenly stocked with swordtail broodstock. At harvest, fish biomass production was greatest within the two feed

treatments after the 12-week trial. Inorganic fertilizer produced the lowest overall fish biomass, and least number of marketable swordtails (standard length > 31 mm).

Stable isotope analyses revealed that distinct nutrient movement patterns were present among pond treatments: (1) nutrients were moving in parallel through feed pond food webs, moving directly into fish and plankton (to varying degrees), (2) nutrients were moving in serial through inorganic fertilizer pond food webs: fertilizer → phytoplankton → small zooplankton → macrozooplankton → swordtails. The organic cottonseed fertilizer treatment was a special case, cottonseed meal performed as both a fertilizer and a directly ingestible high protein food source, trophic utilization and nutrient movement results from this treatment, were intermediate between parallel and serial nutrient movement observed within the other three treatments.

Large plankton (> 200 μm) assemblages were analyzed for taxa and abundance, and no qualitative assemblage differences were found that could be associated with high or low fish production performance. Temporal differences in plankton assemblage compositions and abundances were clearly present, likely due to plankton community succession resulting from competition and predation, but assemblages did not largely differ among pond nutrient treatment type. The generalist food habits of swordtails imply that swordtails are capable of efficiently preying upon large macrozooplankton, but due to the pharyngeal jaw arrangement within the swordtail buccal cavity, gut content analysis was not a viable method of determining swordtail diet or predation effects.

Twenty-four hour captive feeding trials demonstrated that swordtails were capable of reducing large plankton biomass and altering large plankton assemblage composition. Swordtails preferentially predated upon *Moina* daphnids relative to calanoid copepods in 24-hour incubation trials.

CHAPTER 1 INTRODUCTION

Background

Ornamental finfish aquaculture is the largest revenue component (65%) of Florida's aquaculture industry, and totaled over \$33 million in farm gate sales in 2005 (FASS/FDACS 2005), down from a near industry record of over \$42 million in 2001 (FASS/FDACS 2002). Over 5.45 million square meters of water surface were under tropical ornamental fish aquaculture cultivation in the state of Florida in 2001 (FASS/FDACS 2002). The ornamental fish trade in the U.S. was valued at \$660 million in foreign imports and \$182 million in exports (Adams et al. 2001, Larkin et al. 2001), not including domestic trade. By increasing domestic production in a more efficient and environmentally benign manner, fishing pressure on stocks of wild caught species and a half billion-dollar foreign trade deficit could be reduced, while increasing employment and economic revenue in local production areas and potentially diversifying income sources for local farmers (Chapman et al. 1997).

Tropical Fish Pond Aquaculture

Ornamental finfish pond production methods are conceptually simple and typically consist of either periodic stocking of aerated ponds with fry or eggs produced by brood stock held in tank systems, or from brood stock left to reproduce freely within a newly prepared pond (Tamaru et al. 2001). Ponds are broadcast-fed formulated commercial feeds daily, and often fertilizers are periodically applied to maintain phytoplankton bloom conditions to provide supplemental live feeds until fish are of marketable size. At harvest, fish are collected using nets or traps, graded for size and appearance, and sold. Typically, at the beginning of a production cycle, ponds are drained, de-mucked, and excavated if heavily eroded. Ponds are then sterilized via 'pH shock' by the application of hydrated lime [Ca(OH)₂] to remove or reduce potential predators,

competitors, pathogenic organisms, and pest species (including undesirable fish species) that can interfere with harvest and grading, lime application also has the added benefit of raising pond total alkalinity; fertilizer (e.g., cottonseed meal) is often added prior to filling and stocking to initiate and sustain a phytoplankton bloom (Boyd 1995, 1997, Kurten et al. 1999).

Florida Aquaculture Best Management Practices (FASS/FDACS 2001) are legally binding statutes which have been established in an attempt to improve aquaculture facilities design, management, and operations to better address growing public and government concerns with such issues as water usage, nutrient discharges to surface and ground waters, prevention of exotic species release, and general environmental degradation (FDACS 2000). Improved pond aquaculture management practices, such as the use of feeds with better growth efficiencies may reduce effluent nutrient levels (Boyd 1997, Cho and Bureau 1997), while simultaneously increasing production rates and production efficiencies, and therefore increase profitability given comparable feed prices.

Nutrient/feed quality evaluations are based upon the amount of growth in weight (protein and/or energy content, as opposed to gain in water weight) of an organism as a function of the amount of food consumed (usually as bulk weight). The higher the observed growth rate for a specific amount of feed or live food, the higher the quality of that feed or live food (Halver 1989). A major question in intensive pond aquaculture systems is the fate and efficacy of relatively expensive commercial feeds and relatively inexpensive fertilizers once they are introduced into the aquaculture pond ecosystem and food web; employing stable isotopes as tracers provides a promising approach in determining the ultimate fate of these nutrients.

Aquaculture Pond Trophic Ecology

Trophic ecology is the study of feeding relationships among organisms. These relationships are also impacted by biophysical, geophysical, and ecological processes. Crucial

processes such as when autotrophic producer organisms (primarily photosynthetic plants and bacteria) are able to utilize the energy in sunlight (photosynthetic organisms) or a reduced inorganic substrate (e.g., hydrogen sulfide), and fix inorganic nitrogen and carbon (in the soil, water, or air) into complex organic compounds (carbohydrates, lipids, and proteins) that contain chemical energy stored within their chemical bonds (NRC 1993, Jobling 2001). Heterotrophic consumer organisms may then consume these producer organisms and/or their byproducts, and they in turn may be consumed by predator organisms at higher trophic levels. Detritivores and heterotrophic decomposers (bacteria, fungi) ultimately return these organisms, their products, and wastes back into inorganic compounds (rem mineralization). These inorganic compounds can then again be utilized by autotrophic organisms under the proper conditions. The study of the flow of energy and the cycling of matter (nutrients) among trophic levels, and the factors that influence these trophic interactions are the major components of trophic ecology.

Trophic ecology makes up one of, if not the, fundamental basis of ecology. Obtaining sufficient energy and matter (nutrients) is the primary objective of every organism. Only by obtaining sufficient energy and nutrients are organisms able to maintain their physical and chemical structures, while performing necessary activities to grow, reproduce, and ultimately persist through ecological and evolutionary time.

The production and storage of biochemical energy and biomass from solar energy and inorganic nutrients by producer organisms drives and sustains almost all natural ecosystems. Notable exceptions include chemosynthetic sulfur bacteria in anoxic muds common in salt marshes and other estuarine environments, and chemosynthetic hydrogen sulfide bacteria in deep ocean cold 'black smoker' seeps and in deep ocean geothermal hot seeps and other aphotic environments (Corliss et al. 1979, Grassle 1985, Jannasch and Mottl 1985).

In contrast to natural aquatic ecosystems and extensive aquaculture systems (no intentional anthropogenic nutrient input), semi-intensive and intensive aquaculture systems rely upon human intervention in the form of supplemental nutrient inputs. These inputs may take the form of natural or formulated feeds, and organic or inorganic fertilizers, which are used singly or in combination to increase production of the desired species beyond that which could be supported naturally. Commercial formulated feeds are the most common form of supplemental nutrients used in the American aquaculture industry and come in two basic forms: nutritionally complete and incomplete feeds (Lovell 1998, Egna and Boyd 1997, Halver 2003). Complete feeds provide all of the nutritional needs of the target species, providing balanced, metabolically readily available, high quality and high-density nutrients (protein, lipids, carbohydrates, minerals, and vitamins) to produce high growth and survival rates, and promote good fish health (Royes 2003). Incomplete feeds are generally cheaper and supplement the nutritional needs of the target species, which must obtain a portion of their nutritional needs from live feeds or digestible organic matter within their environment. In addition to complete and incomplete feeds, two forms of feeds: cooked (properly heated) and extruded feeds, and ground meals (ingredients mechanically ground into small pieces or fine powders) are typically used in the American pond aquaculture industry. Heating (cooking) and extruding feed ingredients generally increases the digestibility and nutritional availability of some ingredients (starches, proteins), but may reduce or destroy other nutrients (non-heat stable proteins, amino acids, antioxidants, and vitamins), which may need to be reapplied (top dressed) following extrusion (Royes 2003, Barrows et al. 2008).

Aquaculture Pond Applied Nutrient Management Practices

Energy and matter, in the form of carbohydrates and lipids, must be present in sufficient quantities and forms nutritionally available to the culture animal (Lovell 1998). If energy in the

form of carbohydrates and lipids are insufficient for the needs of the fish, dietary proteins (amino acids) may be metabolized for energy. This is both expensive and wasteful, as protein is generally the greatest feed component expense and provides less energy on a per weight basis than lipids and carbohydrates. Additionally, high levels of amino acid catabolism are potentially lethal due to increased ammonia production as a direct by-product. Ammonia can quickly degrade water quality and become toxic under the proper conditions and in sufficient concentrations. Furthermore, energy to protein ratios must be properly balanced to promote good growth and prevent the development of nutritionally related pathological conditions and/or unmarketable physical characteristics (e.g., fatty fish fillets, fatty livers, dysfunctional liver diseases, etc.). Historically, fish meal has been the primary source of protein in fish feed formulations due to the high growth rates and high palatability observed with its use. Fish meal protein is a high quality protein for fish having an amino acid composition in good agreement with the amino acid requirement of most fish species (Halver 1989, Lim et al. 2004).

However, for the foreseeable future the use of plant based protein sources will continue to increase as limited availability, increasing cost, and potential non-sustainability of fish meal use makes plant based alternatives more attractive (Hardy 2003, Forster 2004, Barrows et al. 2008). Although plant based proteins are generally cheaper and more readily available than fish meal, their effectiveness in fish feed formulations is often inferior to fish meal based feeds due to lower palatability, suboptimal amino acid composition, and antinutritional factors (e.g., phytate, gossypol) when used at high concentrations; therefore, plant protein based feed formulations frequently need to be tested in research trials prior to their use in practical feeds (Day and Plascencia-Gonzalez. 2000, Lim et al. 2004, Yamamoto et al. 2007, Barrows et al. 2008).

Fertilizer alone is often applied to fish ponds, but more frequently it is applied in conjunction with feed for aquaculture pond production. Inorganic fertilizers in chemical forms available to phytoplankton, provide additional nitrogen, phosphorous, potassium (N-P-K), and trace elements necessary for enhanced plant growth depending upon fertilizer costs and local needs (regional geochemistry and water chemistry) required by the farmer. Organic fertilizers, in the form of plant and animal silage, manures, plant and animal meal by-products from oil processing and the food industry, also are often used to stimulate phytoplankton, protozoan, and bacterial/fungal detrital production (Schroeder 1987, FAO 1995, Halver 2002).

Both inorganic and organic fertilizers boost living food web component production, increasing 'natural' phytoplankton, zooplankton, and invertebrate biomass for the ultimate consumption and increased production of the pond's target species. These relatively low cost fertilizers utilize the pond's ecosystem to transfer nutrients to fish, and may prove to be a cost effective alternative to more expensive commercial feeds (Schroeder 1987, Barkoh 1996). Additionally, live foods may provide trace elements not present or in sufficient quantities in manufactured feeds, thereby enhancing growth, health, and appearance (marketability).

Periodic sampling of fish and examination of their gut contents for diet and trophic investigations is useful for describing short-time-scale nutritional processes, but is often temporally and spatially limited; due to the large amounts of time and labor involved in fish collection, processing, and analyzing and recording individual fish gut content items. Additionally, gut content analysis can bias diet composition data by overestimating the importance of prey components that resist digestion (e.g., chitinous crustaceans, shelled mollusks) and time of sampling in relation to a given fish species' feeding behavior (diurnal, nocturnal, crepuscular, seasonal differences, ontogenetic dietary changes, continuous feeding,

gorge and digest, etc.); furthermore, unidentified prey remains and empty stomachs, which are often a sampling artifact of regurgitation brought about by capture stress, are commonly observed during gut content analysis (Cailliet et al. 1986). Due to the logistical and budgetary constraints required to properly implement gut content analysis, it is usually limited temporally and spatially to a single season and/or geographical area. A major shortcoming of gut content analyses is that it does not address cumulative long-time-scale questions regarding the overall nutritional needs and predatory impact of fish upon their environment and local food web.

Trophic Dynamics Investigations Using Stable Isotopes

A long-time-scale analytical methodology is necessary to complement the short-time-scale analytical method of gut content analysis. Stable isotope tracer techniques have been successfully used to describe trophic processes in various ecosystems (Frazer 1996, Hansson et al. 1997, Velinsky and Fogel 1999, Gu et al. 2001, Lochmann et al. 2001, Dawson et al. 2002). Isotope tracer methods could potentially provide less biased trophic information than gut content analysis due to the cumulative sources of nutrition that are contained within the isotopic profile of the whole fish (DeNiro and Epstein 1978, 1981a, Saito et al. 2001, McCutchan et al. 2003, Olive et al. 2003). Due to the long-term relationship between dietary history and consumer organism tissue isotope signature, the advantages of using isotopic signature analyses in diet studies also can be problematic.

Short-term dietary changes such as intra-seasonal diet switching and heavy consumption of highly abundant, but ephemeral prey pulses may be important to the life history of the fish and the trophic ecology of the habitat, but remain undetected using stable isotope dietary analysis techniques. However, gut content analysis methods also can fail to properly evaluate prey that is only briefly abundant and heavily exploited by fish predators. Depending upon the questions being asked and the time scales involved, solely using either gut content analysis or stable

isotope techniques may make it difficult or impossible to answer the questions being asked by the researcher. Ideally, the complementary strengths of the two diet analysis methods would be employed simultaneously to investigate fish dietary habits. However, using both diet analysis techniques is not always possible due to logistical, budgetary or other constraints. For example, gut content analysis may not be possible when a high frequency of regurgitation from capture stress occurs or when fish employ pharyngeal teeth, detritivorous feeding habits, etc.

Alternately, isotope methods may provide little or no information regarding short-term dietary habits (seasonally ephemeral prey, daily prey or fish migrations, etc.) and feeding chronology patterns (diurnal, nocturnal, crepuscular).

By comparing and contrasting the trophic ecologies of aquaculture ponds that are undergoing different management techniques (feeds and/or fertilizers), it may be possible to identify management strategies (feeds or fertilizers, processed or unprocessed feed, complete or incomplete feeds) that are more cost effective for pond aquaculture practitioners, increasing the profitability and long-term economic viability of their operations.

Unfortunately, nutrients introduced into pond ecosystems from commercial feeds and fertilizers may ultimately end up as undesirable bacterial, fungal, algal, or animal biomass (i.e., predatory and pest insects, crayfish, frogs, turtles, etc), as opposed to increased fish biomass (desired nutrient sink). Feed and fertilizer inputs also may be lost to the sediments, and become unavailable for biological production (Boyd 1995, Boyd 1997, Tepe and Boyd 2003).

Employing stable isotopes as tracers in pond ecosystems may allow estimation of the relative efficiencies of fish production from commercial fish feeds and fertilizers, enabling producers to determine if higher commercial feed costs, versus relatively inexpensive fertilizer are justified.

CHAPTER 2 INDOOR SWORDTAIL FEEDING AND ISOTOPE METHODOLOGY VALIDATION TRIAL

The use of stable isotopes, within applied nutrients to investigate pond trophic dynamics, was first investigated using stable isotopes as tracers in an indoor study under controlled laboratory conditions. The purpose of the indoor trial was to determine the feasibility of using carbon and nitrogen isotopes as tracers of the transfer of manufactured feeds to their fish consumers in an indoor laboratory setting as an analog for outdoor pond food web processes. If juvenile fish from different feed (isotopically distinct) group treatments were isotopically distinct at the conclusion of the three-month trial, then these results could be taken as validation of the feed/nutrient isotope tracing methodology (DeNiro and Epstein 1978, 1981a, Fry 1991). Another important purpose of the indoor isotope tracing methodology validation trial was to determine the mean magnitude and sign (+, -) of isotopic enrichment for both carbon and nitrogen over a single trophic level in a simplified food web involving swordtails (*Xiphophorus hellerii*) in a controlled laboratory setting. Conducting indoor laboratory experimental trials, allowed for the complete control of what growing fry consumed.

Food web component carbon and nitrogen isotope signatures are used for contrasting and complimentary purposes (Hobson and Wassenaar 1999). Carbon signatures are primarily used to trace nutritional sources within a food web due to carbon's highly conserved isotopic signature, as carbon in a consumer is only minimally enriched relative to its food ($\sim 0.5 - 1 \text{ ‰}$ $\Delta\delta^{13}\text{C}$; DeNiro and Epstein 1978, 1981b, Fry 1991). In contrast, nitrogen exhibits a trophic level enrichment; consumers are enriched in ^{15}N relative to their food at a roughly constant level ($\sim 3.4 \text{ ‰}$ $\Delta\delta^{15}\text{N}$; DeNiro and Epstein 1981, Fry 1991), allowing trophic position within a food web to be predicted with a high degree of confidence (Kling and Fry 1992, Vander Zanden and

Rasmussen 1999, Vander Zanden and Vadeboncoeur 2002), given that basal nutrient nitrogen signature is known with a fair degree of certainty. Carbon and nitrogen isotope signatures tend to complement each other, in that nutrient/food sources believed to be primary sources of nutrition for a predator must have both plausible carbon and nitrogen signature magnitudes and mathematical signs (+, -). Trophic position of an organism can be estimated by comparing its isotopic signature relative to other organisms in the system. The expected magnitude of isotopic fractionation occurring between a consumer organism and lower trophic levels ($\sim 1.0\text{‰ } \Delta\delta^{13}\text{C}$, $\sim 3.4\text{‰ } \Delta\delta^{15}\text{N}$ per trophic level), believed to support the organism within the food web, will determine whether an organism or nutrient source is a plausible prey item or nutrient source for that organism, or at the very least, potentially within the trophic food web supporting the organism (Carpenter et al. 1985, Phillips and Gregg 2003.).

Materials and Methods

Experimental Feed Composition and Isotopic Signature Profiles

Five isonitrogenous and isocalorific feed formulations with varying proportions of animal (fish meal) and plant (wheat protein isolate) based proteins were fed to swordtail fry (*Xiphophorus hellerii*; Family Poeciliidae) in indoor tanks. Because feed formulations differed in their protein source inclusion rates, they also differed in their stable carbon and nitrogen isotope compositions. Stable isotopes of carbon (^{12}C , ^{13}C) and nitrogen (^{14}N , ^{15}N) were used as elemental tracers to characterize nutrient transfer from experimental flake feeds to ornamental fish fry. Additionally, carbon and nitrogen isotope fractionation magnitudes between feed and fry for a single trophic level were measured for each of the five experimental flake feed treatments.

The equation for determining carbon isotope signature ($\delta^{13}\text{C}$) is given as (Anderson et al. 1988, Knowles and Blackburn (eds.) 1993, Cook et al. 1998):

$$\delta^{13}\text{C} = \left(\frac{{}^{13}\text{R}_{\text{sample}} - {}^{13}\text{R}_{\text{std}}}{{}^{13}\text{R}_{\text{std}}} \right) \times 1000$$

where: $\delta^{13}\text{C}$ is the carbon delta signature; (‰),

${}^{13}\text{R}_{\text{sample}}$ is the ratio of $\left[\frac{{}^{13}\text{C}}{{}^{12}\text{C}} \right]$ in the sample material,

and ${}^{13}\text{R}_{\text{std}}$ is the ratio of $\left[\frac{{}^{13}\text{C}}{{}^{12}\text{C}} \right]$ in the standard material.

Similarly for nitrogen isotope signature determination ($\delta^{15}\text{N}$):

$$\delta^{15}\text{N} = \left(\frac{{}^{15}\text{R}_{\text{sample}} - {}^{15}\text{R}_{\text{std}}}{{}^{15}\text{R}_{\text{std}}} \right) \times 1000$$

where: $\delta^{15}\text{N}$ is the nitrogen delta signature; (‰),

${}^{15}\text{R}_{\text{sample}}$ is the ratio of $\left[\frac{{}^{15}\text{N}}{{}^{14}\text{N}} \right]$ in the sample material,

and ${}^{15}\text{R}_{\text{std}}$ is the ratio of $\left[\frac{{}^{15}\text{N}}{{}^{14}\text{N}} \right]$ in the standard material.

Broodstock and Fry Production

A total of 500 broodstock swordtails were used during the indoor portion of the study (400 female: 100 male) to produce fry for this trial. In a large greenhouse (Figure 2-1; 22 m length \times 9.1 m width), 10 epoxy-coated concrete burial vaults (Figure 2-2; 240 cm length \times 89.5 cm width \times 73.6 cm height; approximately 1,665 L volume) each held a group of broodstock (75 female: 25 male) in a cube-shaped ‘broodstock confinement’ basket (Figure 2-3; 66 cm length \times 66 cm width \times 49.5 cm height) lined with polyethylene mesh (0.635 cm \times 0.635 cm square plastic

mesh). As swordtails are primarily surface feeders, broodstock were fed TetraMin[®] commercial flake food as their staple diet. Floating flake feed was delivered to broodstock via automated belt feeders on 12-hour timers, feeders were refilled every morning [between 09:00-11:00 hrs (EST)]. Newly spawned fry dropped through the mesh basket where water currents within the vault moved fry away from confined broodstock, minimizing cannibalism. The vaults were supplied with filtered freshwater from a partial recirculation filter system that had a large drop bead filter, fluidized sand filter bed, UV sterilizer and degassing/bio-media trickle bed (Figures 2-1, 2-4); when temperatures were over 26° C, cooler (~ 22° C), degassed well water was automatically [nominal ¾ inch (1.9 cm) solenoid valve and submersible thermostat] shunted directly into the vats and excess water left the system through a screened drain.

Twenty randomly selected broodstock (10 female: 10 male) and 60 fry (trial day 0) were sacrificed for morphometric (standard length and weight), and stable isotope analyses (5 female, 5 male and 10 fry) immediately prior to the initiation of the indoor feeding trial; sacrificed broodstock and fry were not fed for 16 hrs prior to euthanasia to prevent biased weight measurement and isotopic contamination from ingested feed. Examination of excised gastrointestinal (GI) tracts from sacrificed broodstock (n = 8) not used for isotopic analysis, revealed that no undigested matter remained in the GI tract. Sacrificed broodstock were euthanized with an overdose of tricaine methanesulfonate (MS222) at a dose of 2.5 g/L, their length (SL mm) and wet weight (g) were recorded, and then frozen (-74 °C ± 2 °C) for several months prior to freeze drying. Samples were freeze dried at -57 °C under vacuum (1×10^{-4} Torr; Welch Chemstar™ vacuum pump model 1402N-60) for 24-48 hrs depending on sample wet weight) for further morphometric analysis (dry weight) and processing/preparation for isotopic analysis.

Remaining fry (0 – 14 days old) were transferred indoors and held within an elliptical polyethylene tank (103 cm length x 81 cm height x 61 cm width; approx. 265 L volume) on the same freshwater recirculating filter system as the indoor trial experimental test chambers (Figures 2-6 – 2-8; same room and water temperature). After a one-week acclimation period, fry were randomly allocated to one of five experimental flake-feed treatments beginning on 12 August 2005. Experimental feed regimes began the following day.

When necessary, animals used within this study were humanely sacrificed according to the current animal use guidelines of the University of Florida Institutional Animal Care and Use Committee (IACUC). The details of animal use for this study are on file at the University of Florida IACUC office (IACUC # D591).

Manufactured Feeds

Five flake feeds, containing wheat protein isolate (courtesy of Honeyville Grains, Inc.) and fishmeal, were manufactured at the Fish Technology Center (U.S. Fish and Wildlife Service) in Bozeman, Montana using a Buflovak[®] Double Drum Dryer (30.5 cm diameter × 45.7 cm length drum) running at 5.46 kg/cm² (78 psi) steam pressure and at temperatures between 68.3 – 81.1 °C. Each feed contained approximately 40% total protein by dry wt., but differed in their proportions of proteins derived from animal (FM - fish meal) and plant (WP - wheat protein isolate) protein sources: 0% FM and 100% WP%, 25% FM and 75% WP, 50% FM and 50% WP, 75% FM and 25% WP, and 100% FM and 0% WP (Figure 2-5). For the duration of the study, flake feeds were stored in air-tight polyethylene buckets at approximately 18 °C ± 3 °C (~65° F).

Experimental Design

Indoor trial experimental design consisted of five isocaloric and isonitrogenous experimental feeds fed to randomly selected individual fish, housed in 105 replicate aquaria

(Figures 2-6 – 2-8; 6.4-L rectangular polystyrene tanks; 21 aquaria per feed treatment) for a period of 90 days. Each tank (Figure 2-7) initially held 10 fry (1-14 days old) that were randomly allocated to each aquaria from a common tank in groups of five fry (1,050 total fry). Aquaria were held on a recirculation water system that utilized UV sterilization, a drop bead filter and a fluidized sand bed bioreactor to maintain water quality. Local well water (~ 11 mg/L CaCO₃ alkalinity, ~ 22 mg/L CaCO₃ hardness) was used to fill and maintain system volume; water lost from daily bead filter back flushing was automatically replaced from a gravity-fed head-tank reservoir and float valve contained in the system sump. A standard photoperiod (14 hrs light: 10 hrs dark) was maintained (Lovell 1998). Water temperature within the experimental system was maintained by setting the room thermostat to maintain a constant temperature of 24°C ± 2° C.

Fry were fed at an initial daily ration of 15% mean wet body weight based upon initial mean fry wet weight. Subsequent rations were adjusted based upon weight measurements occurring at 10-day intervals for the first thirty days, and weight measurements made every 15 days thereafter until termination of the trial. To measure fry weight change to calculate feed weight adjustments, one replicate (10 fry) from each of the five treatments was chosen at random at a predetermined time interval, and euthanized for weight and standard length measurement (and potential isotopic analysis). Sacrificed fry were not fed for 16 hrs prior to euthanasia to ensure ingested food would not bias weight measurements and future isotopic analysis. Following weight and standard length measurement, fry were stored frozen (-74 ± 2 °C) for a period from 12 – 18 months, then freeze dried and individually stored in polystyrene vials (32-ml volume) in 23-L air-tight polyethylene buckets that contained 250 g of desiccant (Dri-rite™: calcium sulfate/cobaltous chloride mixture). Daily ration level (% wet body weight) for all

replicates within a given time interval, was based upon the euthanized replicate with the highest mean fry weight. Ration for a given replicate was adjusted on a per-capita basis (initial 10 fry per replicate) following a daily morning mortality check prior to feeding. Daily ration was reduced to 10% mean body weight for days 31-90, with weighing adjustments occurring at days 45, 60 and 75.

Freeze Drying and Sample Preparation

Samples prepared for isotopic analysis were stored frozen, as mentioned above, at -74°C , and then freeze dried (Figures 2-9 – 2-11; Virtis[®] Sentry) at -57°C for 24 - 48 hrs (16 - 20 hrs for smaller specimens); a freeze drying pilot study was performed with swordtails of comparable weight and size to those in the main study to determine freeze drying durations after which no further sample weight loss occurred. Following freeze drying, whole fish samples were ball milled into a fine homogenous powder (Spex[®] 5100 ball mill). During the milling process, forceps were used to place fry samples into small stainless steel capsules each containing a stainless steel ball vibrated at a high speed from 20-80 seconds depending on fry weight (3500 rpm, 1/15 hp motor). Milling capsules, milling balls and forceps were sprayed with 70% isopropyl alcohol and swabbed with sterilized cotton swabs (Q-tips[™] Unilever Corp.) and laboratory wipes (KimWipes[™] Kimberly Clark Corp.) to remove trace contaminants and prevent carry-over between samples. As an added precaution, latex gloves were worn during the sample preparation process and were sprayed with 70% isopropyl alcohol and wiped with laboratory wipes between samples. A small amount of powdered fry material (150-1,300 μg), or entire fry if fry dry weight was less than 1,000 μg , were placed into preformed 7 mm x 4 mm tin sample capsules (Costech Analytical Industries, Inc.) and weighed to the nearest μg using a Cahn 25 Automatic Electrobalance[™] laboratory scale. Alcohol-swabbed forceps were used to fold capsules into a tight ball to prevent loss of sample material, folded capsules were then placed

into pre-cleaned and pre-sterilized polystyrene well plates and shipped to the University of Florida Center for Isotope Geoscience Laboratory (Department of Geology: UFCIGL) or to the Northern Arizona University Colorado Plateau Stable Isotope Laboratory (Department of Biological Sciences: NAUCPSIL) for stable isotope analyses. Samples were analyzed for carbon and nitrogen isotope composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) with an isotope ratio mass spectrometer [VG Prism (series II) mass spectrometer interfaced with a Carlo Erba CNS (carbon, nitrogen, sulfur) elemental analyzer (UFCIGL) or, a Thermo-Finnegan Delta Plus Advantage gas isotope-ratio mass spectrometer interfaced with a Costech Analytical ECS4010 elemental analyzer (NAUCPSIL)]. Triplicate weighed sub-sample isotopic analyses were made for ten randomly selected broodstock (five male, five female) to determine within-fish variation in isotopic signature values.

Isotopic Signature

Isotopic signatures of sampled material were reported in standard delta notation (δ) where:

$$\delta^{15}\text{N} = \left(\frac{{}^{15}\text{R}_{\text{sample}} - {}^{15}\text{R}_{\text{std}}}{{}^{15}\text{R}_{\text{std}}} \right) \times 1000 ,$$

${}^{15}\text{R}_{\text{sample}}$ is the ratio of $\left[\frac{{}^{15}\text{N}}{{}^{14}\text{N}} \right]$ in the sample material, and ${}^{15}\text{R}_{\text{std}}$ is the ratio of $\left[\frac{{}^{15}\text{N}}{{}^{14}\text{N}} \right]$ in the standard material.

$$\delta^{13}\text{C} = \left(\frac{{}^{13}\text{R}_{\text{sample}} - {}^{13}\text{R}_{\text{std}}}{{}^{13}\text{R}_{\text{std}}} \right) \times 1000 ,$$

${}^{13}\text{R}_{\text{sample}}$ is the ratio of $\left[\frac{{}^{13}\text{C}}{{}^{12}\text{C}} \right]$ in the sample material, and ${}^{13}\text{R}_{\text{std}}$ is the ratio of $\left[\frac{{}^{13}\text{C}}{{}^{12}\text{C}} \right]$ in the standard material.

The analytical standard for nitrogen is atmospheric nitrogen ($\delta^{15}\text{N}_{\text{air}} = 0 \text{ ‰}$) and the standard for carbon, historically is the Pee Dee Belemnite (PDB) formation carbon ($\delta^{13}\text{C}_{\text{PeeDee}} = 0 \text{ ‰}$); *Belemnitella americana* (Cephalopoda), an extinct Cretaceous limestone marine fossil from the Pee Dee formation in South Carolina, U.S.A. Unfortunately, the PDB formation has been exhausted and this carbon standard is no longer available. Carbon sources isotopically standardized to the PDB standard were used.

Elemental Carbon to Nitrogen Ratio

Elemental carbon to nitrogen ratio determination was performed on a limited basis during isotopic analysis at the NAU isotope analysis laboratory. Elemental carbon to nitrogen ratio was determined using a CNS (carbon, nitrogen, sulfur) elemental analyzer downstream from the isotope ratio mass spectrometer.

Statistical Analysis

Statistical analyses were performed using Microsoft[®] Excel and Prism[™] statistical software package version 4.03 (GraphPad Software Inc. 2005). Microsoft[®] Excel was used to calculate basic descriptive statistics (mean, standard deviation, standard error, 95% confidence intervals, etc.). Prism[™] was used to conduct parametric tests such as analysis of variance (ANOVA) and post-hoc comparison tests, unless otherwise noted.

Fry Growth and Survival

Final (24-36 hrs. postprandial) wet weights of fry were compared using a one-way analysis of variance (ANOVA) statistical test. 24-36 hours postprandial was deemed to be a sufficient time interval to ensure that most, if not all, swordtail gastrointestinal tracts were empty. Average fry survival rates among treatments also were compared using a one-way ANOVA test, after first performing an arcsine square root data transformation upon the survival

rate percentage data. Percentage data is typically binomial in distribution, requiring transformation to a normal distribution prior to ANOVA.

Isotopic Analyses

Carbon and nitrogen isotope signatures of randomly selected subsamples of broodstock females, males and initial fry used during the three-month trial were compared using a one-way ANOVA. Carbon and nitrogen isotope signatures of experimental feeds, and surviving harvest fry from the five treatment groups, also were compared using ANOVA (Zar 1984, Ott 2000).

Results

Fry Growth

Fry weight trajectories did not appear to qualitatively differ among treatments for randomly selected single replicates for the first three weighing periods (days 10, 20, 30). Fry growth at day 30 corresponded to an increase of 146 % from their initial mean weight $\bar{X} = 0.0089$ g (0.0082 to 0.0097 g 95 % CI, n = 60). Fry subsample mean weights began to visibly diverge among the five experimental treatments (Figure 2-12) on day 45; the mean weight of the 0% fishmeal: 100% wheat protein diet fry having a mean weight slightly less $\bar{X} = 0.021$ g (0.012 to 0.030 g 95 % CI, n = 10) than the mean treatment group with the most similar diet composition and nearest mean weight (25% fishmeal: 75% wheat protein: $\bar{X} = 0.046$ g, 0.045 to 0.050 g 95 % CI, n = 10). However, these differences were not statistical differences due to the lack of replication between the start and end of the experimental trial. These single tank replicates were periodically sacrificed (one per treatment) to determine fry weight changes over the twelve week course of the trial for daily feed ration adjustments, and to track isotopic changes in fry among treatments over time (Table 2-1).

Similar to the lower growth trajectory exhibited by the all plant diet pre-harvest fry (single replicates; Figure 2-12), all plant diet harvest fry average weight also was lower (Figure 2-13). At the conclusion of the 90-day trial, the 0% fishmeal: 100% wheat protein diet treatment fish ($\bar{X} = 0.136$ g, 0.1160 to 0.1566 g 95 % CI, n = 12) had a significantly lower average weight than those of the other diet treatments (Figure 2-13; $P < 0.01$, Tukey's multiple comparison test).

Fry Survival Rates

Indoor fry percent survival rates did not differ among experimental feed treatments at twelve week trial termination [Figure 2-14; one-way ANOVA (arcsin square root data transformation: $P = 0.1475$, $F = 1.765$, $\alpha_{(2,4)} = 0.05$]. Mean survival rates within replicates for the five experimental feed treatments were: 100%FM:0%WP $\bar{X} = 84.6\%$ (76.9 to 92.3 % 95 % CI, n= 14), 75%FM:25%WP $\bar{X} = 82.3\%$ (71.5 to 93.1 % 95 % CI, n =13), 50%FM:50%WP $\bar{X} = 82.3\%$ (74.8 to 89.8 % 95 % CI, n = 13), 25%FM:75%WP $\bar{X} = 88.0\%$ (80.4 to 95.6 % 95 % CI, n = 15), and 0%FM:100%WP $\bar{X} = 94.2\%$ (89.1 to 99.2 % 95 % CI, n =12).

Feed Isotopic Signatures

The experimental feeds differed in their carbon and nitrogen isotope signatures along a regular (monotonic) sequence (Figure 2-16), as would be expected by step-wise inclusion rates of plant and animal protein in their manufacture. The TetraMin[®] tropical fish staple flake control diet that was fed to female and male broodstock, isotopically differed from the five experimental feed diets for either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$, and frequently both.

Swordtail Isotopic Signatures

Initial male broodstock, female broodstock and (0 to 14-day-old) fry mean carbon isotope signatures $\delta^{13}\text{C}$ were: -21.63 ‰ (-21.80 to -21.45 ‰ 95 % CI, n=10), - 21.57 ‰ (-21.67 to - 21.47 ‰ 95 % CI, n=11), and -21.42 ‰ (-21.42 to -21.41 ‰ 95 % CI, n=21), respectively. $\delta^{13}\text{C}$

signatures did not differ significantly (Figure 2-15; one-way ANOVA: $P = 0.6053$, $F = 0.5085$, $\alpha_{(2,2)} = 0.05$).

Initial male broodstock, female broodstock, and (0 to 14-day-old) fry mean nitrogen isotope signatures ($\delta^{15}\text{N}$) were 10.81 ‰ (8.91 to 12.70 ‰ 95 % CI, $n=10$), 11.28 ‰ (9.54 to 13.01 ‰ 95 % CI, $n=11$), and 11.63 ‰ (8.72 to 14.54 ‰ 95 % CI, $n=21$), respectively. Only male broodstock and fry nitrogen signatures differed significantly (Figure 2-15; one-way ANOVA: $P = 0.004$, $F = 6.384$, $\alpha_{(2,2)} = 0.05$).

Similar to the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signature pattern of the five experimental feeds (Figure 2-16), pre-harvest fry $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures changed among treatment fry groups in a regular trajectory during the course of the trial (Figures 2-17 - 2-18). Following diet switching, isotopically similar fry within different treatments isotopically diverged and came to resemble their respective feeds over the twelve weeks of the trial. These pre-harvest differences were not statistical differences as only single replicates (one aquarium) from each treatment were analyzed for each of the periodic sampling periods prior to trial completion.

At harvest, carbon isotope ($\delta^{13}\text{C}$) signature differences among treatment groups were readily apparent (Figure 2-18). However, four pairwise comparisons of treatment fry $\delta^{13}\text{C}$ signatures did not significantly differ: (100FM:0WP and 75FM:25WP), (75FM:25WP and 50FM:50WP), (50FM:50WP and 25FM:75WP), and (75FM:25WP and 25FM:75WP), (Figure 2-18; one-way ANOVA: $P < 0.0001$, $F = 28.94$, $\alpha_{(2,4)} = 0.05$). Fry $\delta^{13}\text{C}$ signatures, reflected the carbon isotope signatures of their respective food sources. Nitrogen isotope signatures also differed among treatment groups (Figure 2-18; one-way ANOVA: $P < 0.0001$, $F = 39.11$, $\alpha_{(2,4)} = 0.05$).

At harvest, $\delta^{13}\text{C}$ signatures of fry differed from their respective feeds by an average of 0.64 ‰ (0.31 to 1.51 ‰ $\delta^{13}\text{C}$ 95 % CI) and nitrogen signatures differed by an average of 3.03 ‰ (1.82 to 8.09 ‰ $\delta^{15}\text{N}$ 95 % CI). The magnitudes of the differences in $\delta^{13}\text{C}$ values between fry and feed did not appear to change with plant protein inclusion level in the experimental feeds (Figure 2-19); however, differences in $\delta^{15}\text{N}$ signatures increased as plant protein inclusion increased, but these differences asymptote for the three experimental feeds with the highest plant content (Figure 2-20). Nitrogen isotope signature differences between harvest fry and their respective experimental feeds were the lowest for the two feeds that contained the most fish protein (100FM:0WP and 75FM:25WP), the fry and feed nitrogen isotope signature differences of these two groups significantly differed from those of the two treatments that contained the most wheat protein (Figure 2-20; $P < 0.05$ Tukey's multiple comparison test).

A dual (Cartesian) plot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for experimental feeds, control feed, broodstock, initial fry, and juvenile swordtails at harvest was created (Melville and Connolly 2003) to provide a visual representation of the contrasting carbon and nitrogen isotope signatures present among different groups (Figure 2-21). Monotonous changes in harvest fish isotopic signature, closely follow monotonous isotopic signature differences present within experimental feeds.

Elemental Carbon to Nitrogen Ratio Analysis

Elemental carbon to nitrogen ratios for indoor trial harvest fry did not significantly differ among treatments (Figure 2-22; one-way ANOVA: $P = 0.1320$, $F = 1.958$, $\alpha_{(2,4)} = 0.05$).

Elemental carbon to nitrogen ratio values did not differ greatly among fry treatments, actual mean elemental carbon to nitrogen ratio values for the indoor trial fry at harvest were:

100FM:0WP $\bar{X} = 4.31$ (0 to 8.83 95 % CI), 75FM:25WP $\bar{X} = 4.26$ (0 to 8.73 95 % CI),

50FM:50WP \bar{X} = 4.33 (0 to 8.88 95 % CI), 25FM:75WP \bar{X} = 4.74 (0 to 10.64 95 % CI), and 0FM:100WP \bar{X} = 4.90 (0 to 10.04 95 % CI).

Discussion

Fry Growth

Mean pre-harvest weights from different experimental feed treatments were almost identical for the first 30 days of the feeding trial. Fry growth followed an exponential growth trajectory, typical for juvenile fishes (Cailliet et al. 1986, Tamaru et al. 2001). After trial day 30, single replicate fry weights diverged and were often erratic among feed treatments (Figure 2-12). The 100% wheat-protein-diet fry growth rate lagged behind the other fry groups after day 30 and fish in this treatment group were smaller at the end of the 90-day trial. Although pre-harvest growth data were derived from single replicate sub-sample fry, it seems plausible that from day 30 onward, a missing dietary factor(s) limited the growth of the 100%-plant-protein diet fry and prevented them from attaining the size of fry from the other experimental feed treatments. This conclusion is supported by the finding that the 100% plant-protein-diet fry were significantly smaller than the other four experimental feed treatments at the end of the twelve-week trial (Figure 2-13). Wheat protein isolate was intentionally selected as a low-quality diet in that it is deficient in the essential amino acid lysine (1.6% by weight; NRC 1993). The two experimental diets (0% fishmeal: 100% wheat protein; 25% fishmeal: 75% wheat protein) with the lowest lysine content, had 0.57%, and 1.03% lysine, respectively by weight (certified analyses; ABC Laboratories Gainesville, Florida); lysine content of both diets were lower than the 1.43% lysine by weight minimum requirement established for tilapia (*Acanthopterygii*: closest related species listed *in* Fish Nutritional Requirements, NRC 1993) below which reduced growth occurs.

Final fry weights at the end of the 12-week indoor feeding trial did not differ among the four diets that contained fishmeal as a feed ingredient; only the 100% wheat-protein diet fry group exhibited an overall smaller size. Although reduced feed palatability may have been a factor in the reduced growth of the 100% wheat-protein-fed treatment group, a lack of differences in fry survival among treatment groups (Figure 2-14), tends to contradict lower palatability as a possible reason for the reduced growth observed for the 100% wheat-protein-fed treatment group.

Fry Survival Rates

The absence of fry survival rate differences among feed treatment groups at harvest, suggests that overall nutritional status of fry did not differ among treatments. Although lower feed palatability and/or amino acid deficiencies within the 100% plant-protein-diet may have produced sub-optimal growth of fry within this treatment, these deficiencies were sub-lethal.

Indoor Trial Fry Isotope Signature

Carbon isotope signatures of male broodstock, female broodstock, and 0 to 14-day old fry did not significantly differ (Figure 2-15). This was to be expected as all three groups were fed the same commercial feed (TetraMin[®]), and due to the typically low magnitude of carbon isotope fractionation, on the order of 0.25 to 1.0 ‰ per trophic level (DeNiro and Epstein 1978, 1981b, Gearing 1991, Fry 1991, Vander Zanden and Rasmussen 2001, Post 2002aa). Fry were slightly enriched in $\delta^{13}\text{C}$ relative to the control feed ($\Delta\delta^{13}\text{C}$ between feed and fry = 0.91 ‰) than both male ($\Delta\delta^{13}\text{C}$ feed and male broodstock: 0.70 ‰) and female ($\Delta\delta^{13}\text{C}$ feed and female broodstock: 0.75 ‰) broodstock. This was probably due to fry receiving a majority of their nutrition (lipids and carbohydrates) from maternally derived egg nutrients from their mothers (ovoviviparous

species); this may have placed fry into a 'hemi-trophic' level above their mothers, producing fry slightly enriched in their carbon isotope signature relative to both female and male broodstock.

Only male broodstock and 0 to 14-day-old fry nitrogen isotope ($\delta^{15}\text{N}$) signatures significantly differed, female broodstock did not differ from either male broodstock or 0 to 14-day-old fry (Figure 2-15). Of the three groups, fry were the most isotopically enriched in ^{15}N and were significantly enriched in ^{15}N relative to the male broodstock group. Again, this was possibly due to the fry being a 'hemi-trophic' level higher than their mothers as they gained maternal nutrition in the form of nitrogenous proteins and amino acids. Follicle development likely involves the same isotopic fractionation processes (e.g., enrichment due to molecular diffusion, enzyme kinetics, etc.) that occur during digestion, and may have caused fry to become isotopically enriched in the heavier nitrogen isotope relative to their mothers and the male broodstock (Vallowe 1957, Chong et al. 2004).

$\delta^{13}\text{C}$ signatures of pre-harvest fry from different treatments diverged (Figure 2-17) as of the first ration adjustment weighing; fry continued to isotopically diverge and more closely resemble their respective feeds (Figure 2-16) as the trial continued. At harvest, three fry pairwise comparisons did not differ in $\delta^{13}\text{C}$ signature (Figure 2-18). The latter finding demonstrated that fry groups that had the same initial carbon isotope signature became isotopically distinct over time when fed isotopically distinct feeds; and that $\delta^{13}\text{C}$ signatures can be used to identify the nutritional source of a consumer group for a single trophic level (swordtails). These results validated the isotope tracing methodology as a means of identifying the nutritional source of a particular fish group.

$\delta^{15}\text{N}$ signatures of fry also diverged (Figure 2-17) after the first ration adjustment period (day 10). Isotopically, fry increasingly resembled their respective feeds (Figure 2-16), while

becoming more distinct from each other. At harvest, all fry groups significantly differed in $\delta^{15}\text{N}$ signature (Figure 2-18) except for the same two treatments that did not differ for $\delta^{13}\text{C}$ signature, (75FM:25WP and 50FM:50WP), and (50FM:50WP and 25FM:75WP). This was further evidence that the indoor trial was successful in validating the stable isotope tracing method for identifying the trophic linkages between fish and their nutritional sources.

At harvest, fry carbon isotope signatures differed from their respective feeds by an average of 0.64 ‰ $\delta^{13}\text{C}$ (Figure 2-19; range: 0.26 to 0.86 ‰ $\delta^{13}\text{C}$). This difference was well within the range of carbon isotope enrichment values reported in the literature (typically ~ 0.4-1.0 ‰ $\delta^{13}\text{C}$) for a single upward step in trophic level (DeNiro and Epstein 1978, 1981b, Rodelli et al. 1984, Fry 1991).

Fry nitrogen isotope signatures differed from their respective feeds by an mean of 3.03 ‰ $\Delta\delta^{15}\text{N}$ (Figure 2-20; range: 1.84 to 3.95 ‰), which also was well within the range of nitrogen isotope enrichment values (3.0-4.0 ‰) reported in the literature for a single upward step in trophic level (DeNiro and Epstein 1978, 1981a, Minigawa and Wada 1984, Schoeninger and DeNiro 1984, Fry 1991). Both the observed carbon and nitrogen isotope enrichment results from the indoor twelve week swordtail fry feeding trial support the validity of using the stable isotope tracing method as a means of investigating trophic dynamics in an aquatic ecosystem (Glibert and Capone 1993).

Elemental Carbon to Nitrogen Ratios

Elemental Carbon to Nitrogen ratios have been used as a rough measure of an organism's health and nutritional state, with lower ratios tending to indicate that nitrogen (and protein) are not presently limiting to the organism (Valiela 1995, Williams et al. 2007). Elemental carbon to nitrogen ratios of indoor fry at harvest did not differ among the experimental feed treatment

groups (Figure 2-22), indicating that fry nutritional states probably did not biologically differ among treatment groups, and that all fish groups had approximately the same protein and energy [lipid and carbohydrate (glycogen)] content. Supporting the contention that all fry treatment groups were roughly consuming the same relative quantities of protein, carbohydrates and lipids (rations provided in excess of satiation – *ad libitum*), regardless of the potentially lower palatability of the 100% wheat protein isolate experimental feed (Arndt et al. 1999, Yamamoto et al. 2007, Barrows et al. 2008). Lower growth of the 100% plant protein fed fry treatment group was likely due to a lysine deficiency present within the all plant flake feed diet (NRC 1993).

Recirculated Well Water System

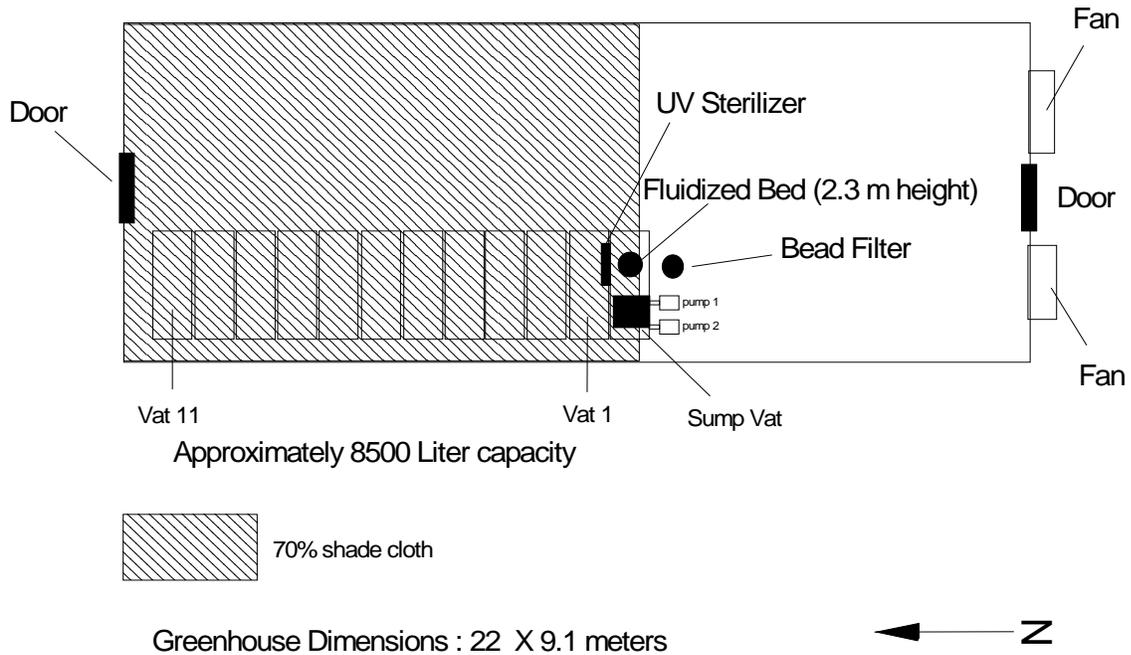


Figure 2-1. Greenhouse physical layout, location of concrete burial vaults and life support system for swordtail fry production and broodstock growout.



Figure 2-2. Epoxy coated concrete burial vault used to culture swordtail fry and broodstock in greenhouse (240 cm length \times 89.5 cm width \times 73.6 cm height: approx. 1,665 L volume).



Figure 2-3. Cage housing broodstock to minimize fry cannibalism, newly spawned fry fell through mesh. A single cage holding 25 male and 75 female broodstock fish was held within each epoxy coated concrete burial vault. Cage dimensions: 66 cm length \times 66 cm width \times 49.5 cm height.



Figure 2-4. Concrete vault freshwater recirculation/partial flow through swordtail fry production and growout system in greenhouse.



Figure 2-5. Five isonitrogenous and isocaloric experimental flake feeds used in the indoor feeding trial (from left to right: 100% fish meal protein: 0% wheat protein; 75% FM: 25% WP; 50% FM: 50% WP; 25% FP: 75% WP; 0% FP: 100% WP).

Indoor 3 month fry grow out trial: 5 treatments, 21 replicates, 105 tanks

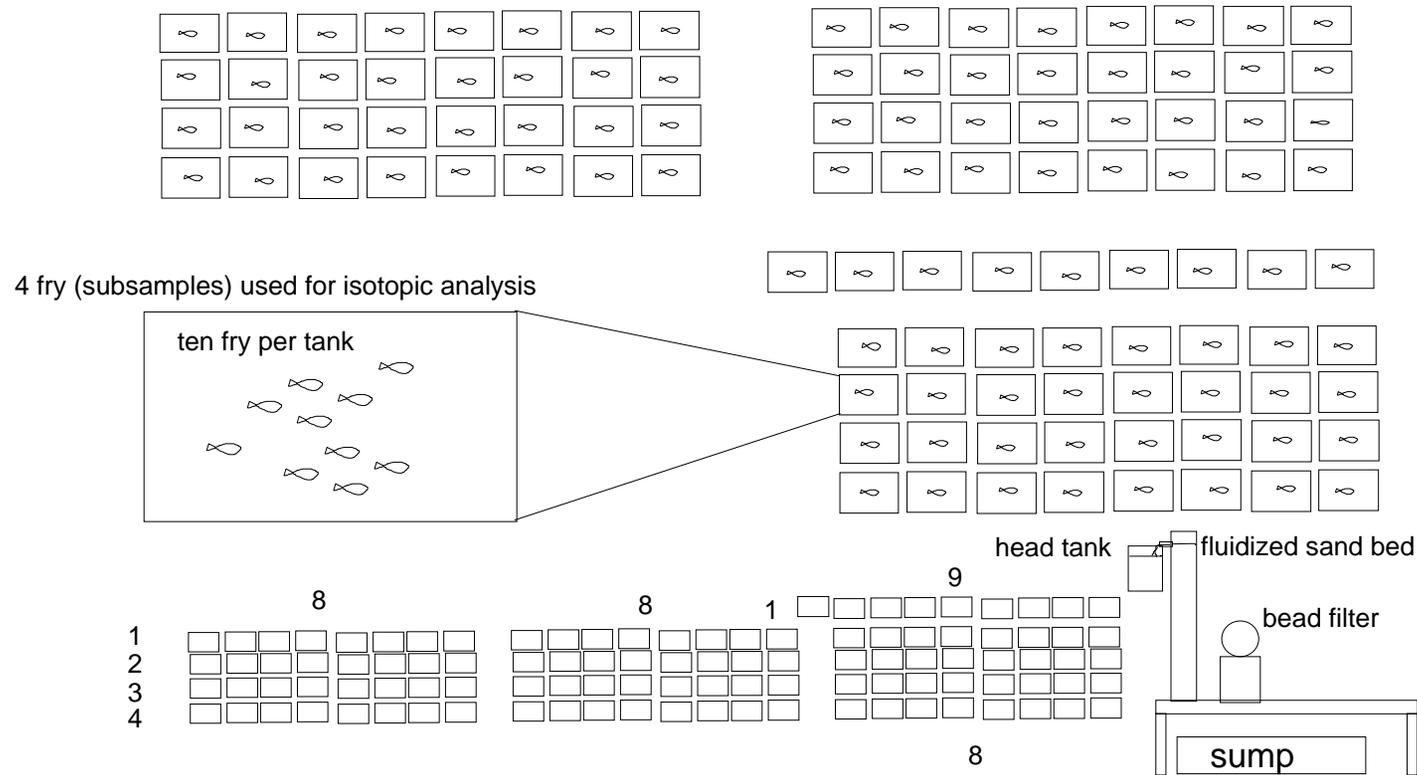


Figure 2-6. Physical layout and experimental design of the indoor feeding trial study.



Figure 2-7. Individual replicate aquarium (approx. 6.4 L) for indoor feeding and isotopic methodology validation trial.



Figure 2-8. Indoor fry feeding and isotope methodology validation trial aquaria batteries. Each aquaria was approximately 6.4 L in volume and held 10 fry at trial inception.



Figure 2-9. Virtis[®] Sentry freeze dryer.



Figure 2-10. Swordtails after 48 hrs in freeze dryer (-57°C , 1×10^{-4} Torr vacuum).



Figure 2-11. Desiccator used to store samples prior to weighing for dry weight and isotopic analysis sample preparation (attachment hardware not present in photo); approximately 0.5 kg Drierite™ (calcium sulfate) dessicant lining bottom.

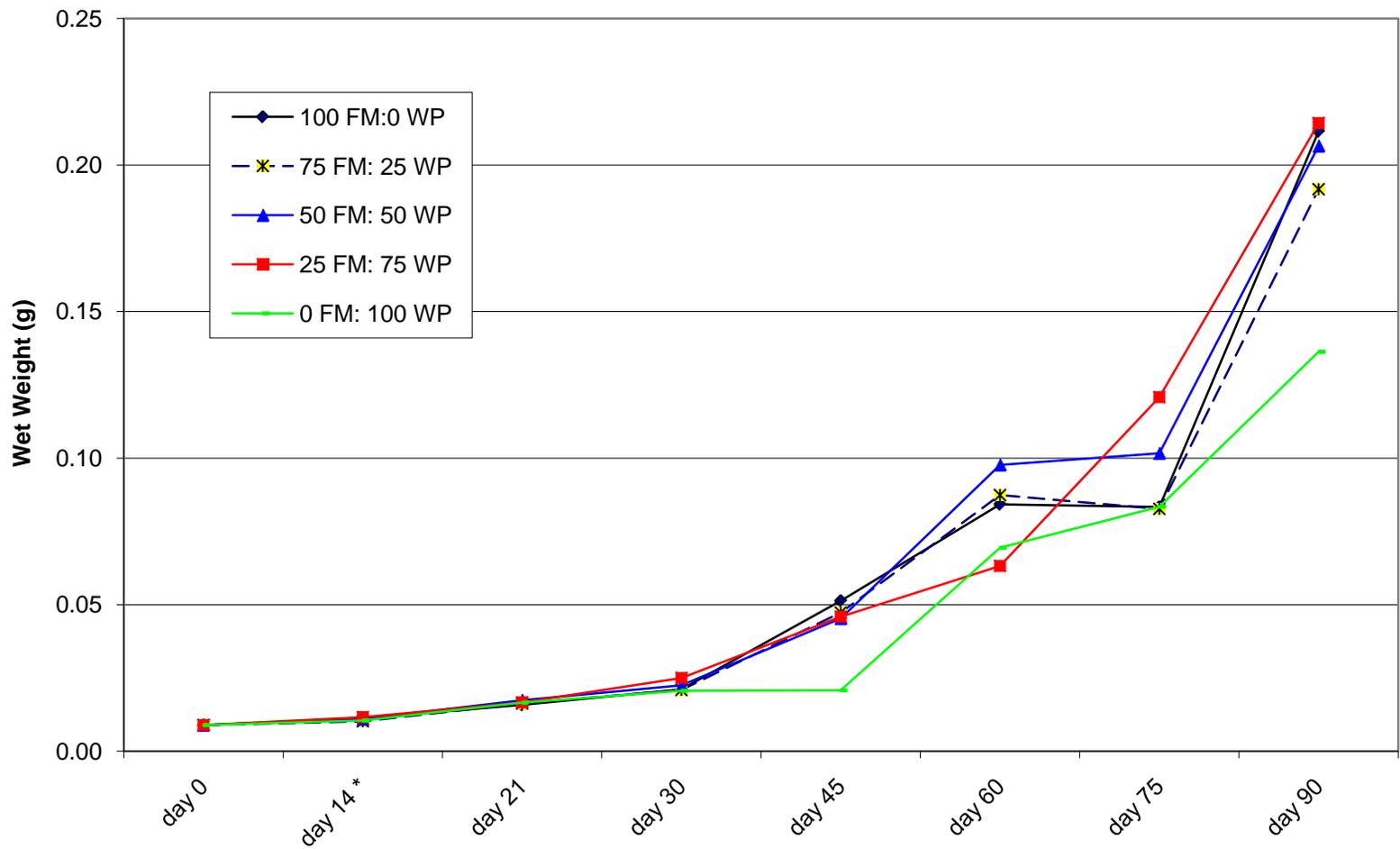


Figure 2-12. Mean swordtail fry (10 subsample fry / replicate aquaria) weight of randomly selected single replicate aquaria from each of five experimental feed treatments [% FM (fishmeal protein): % WP (wheat protein)], sacrificed at regular time intervals for daily ration feeding adjustment; * treatment mean of two replicates for 25 % FM: 75 % WP treatment.

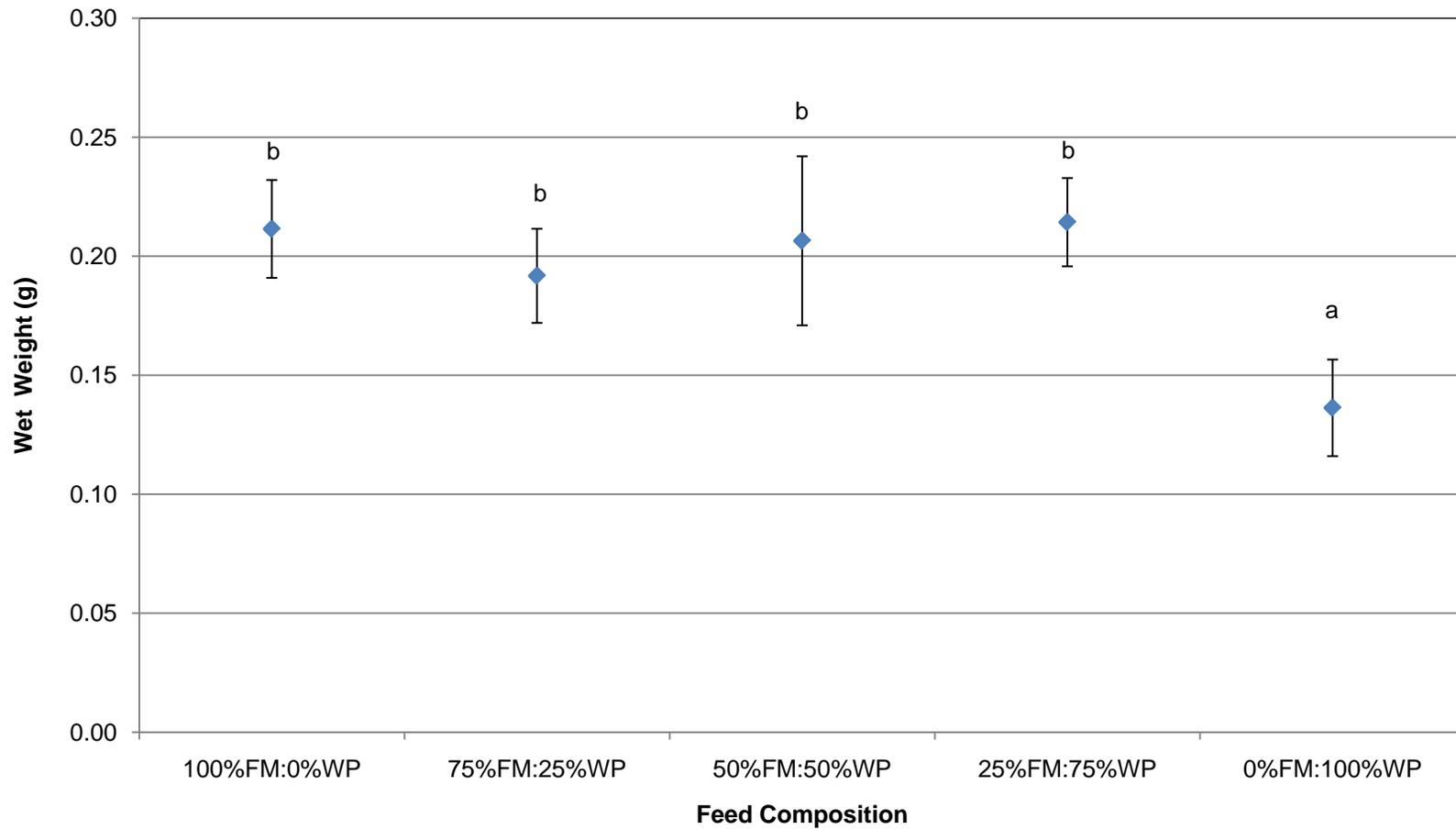


Figure 2-13. Mean swordtail fry weight (\pm 95 % CI) of five experimental feed treatments after 90 days (% fishmeal: % wheat protein isolate); differing letters denote significant differences between groups ($P < 0.05$, Tukey's multiple comparison test).

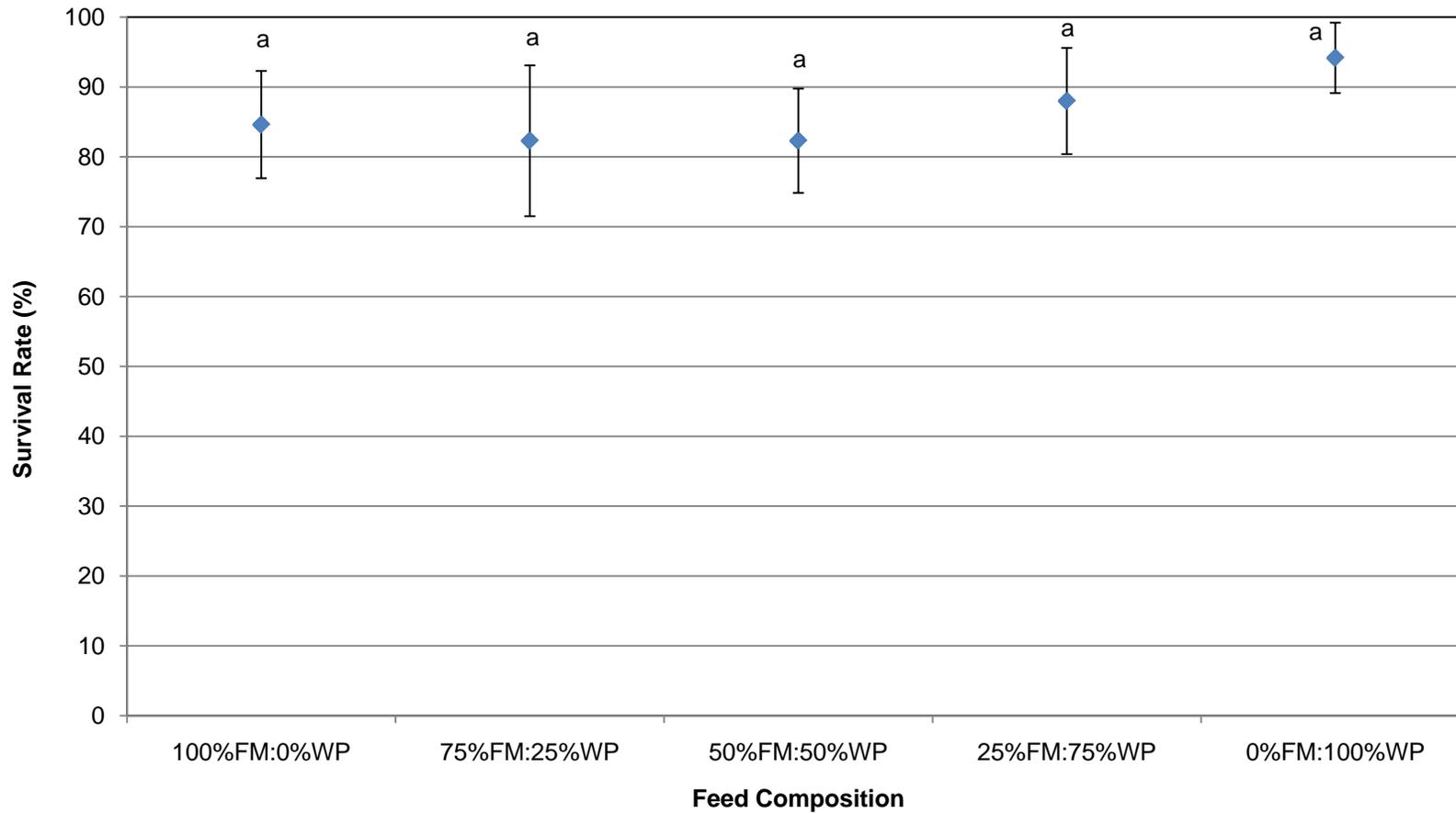


Figure 2-14. Swordtail fry survival rates for five feed treatments after 12 weeks (% survival \pm 95 % CI), differing letters denote significant differences between groups ($P < 0.05$, Tukey's multiple comparison test). ANOVA statistical test was performed upon arcsine transformed data. Treatment sample sizes (from left):14, 13, 13, 15, and12.

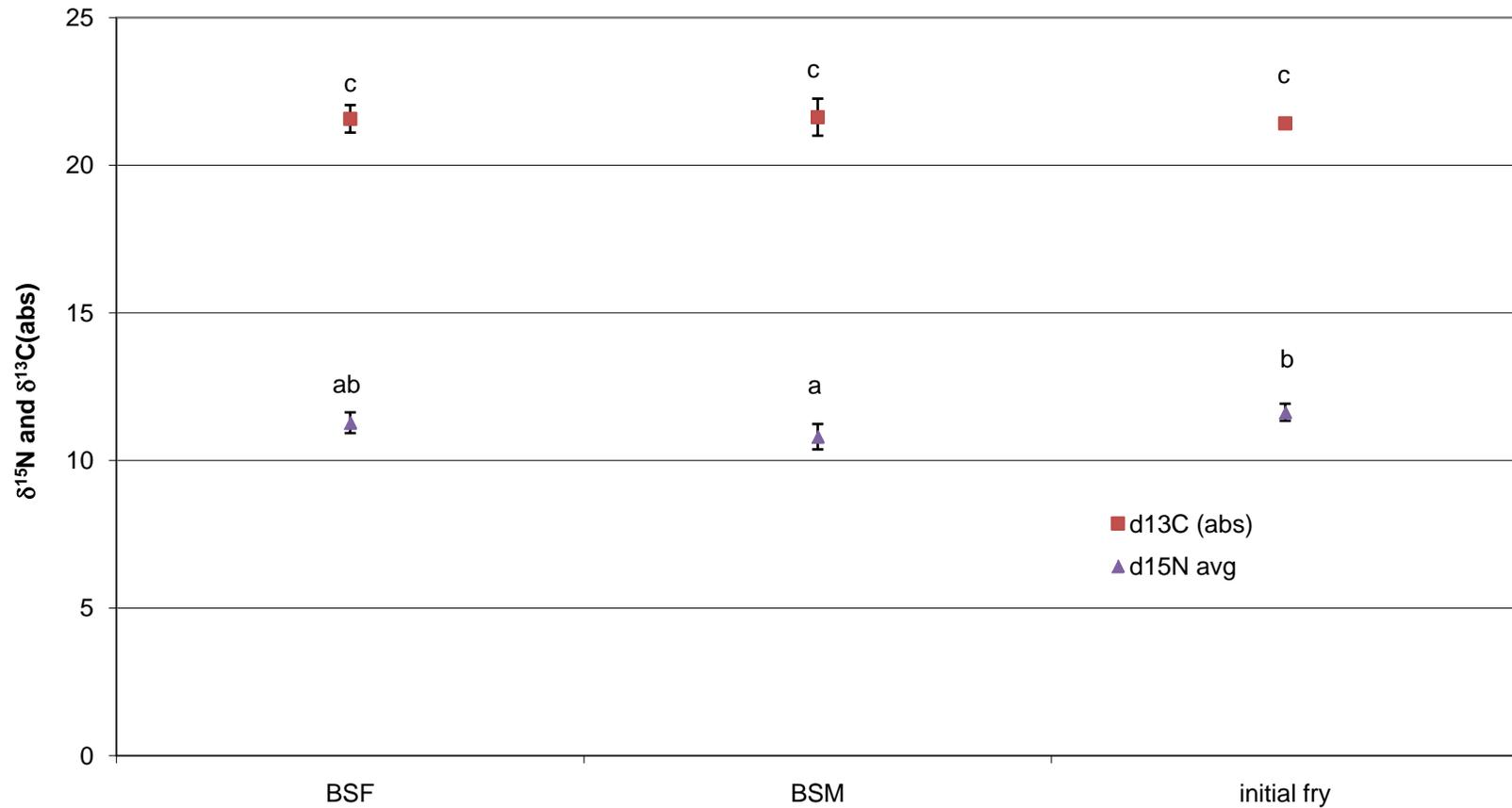


Figure 2-15. Isotopic signatures (\pm 95 % CI) of swordtail broodstock and initial fry for indoor feeding trial; BSF (female broodstock), BSM (male broodstock), $\delta^{13}\text{C}$ = absolute values; differing letters denote significant differences between groups ($P < 0.05$, Tukey's multiple comparison test). Treatment sample sizes (from left): 11, 10, and 21.

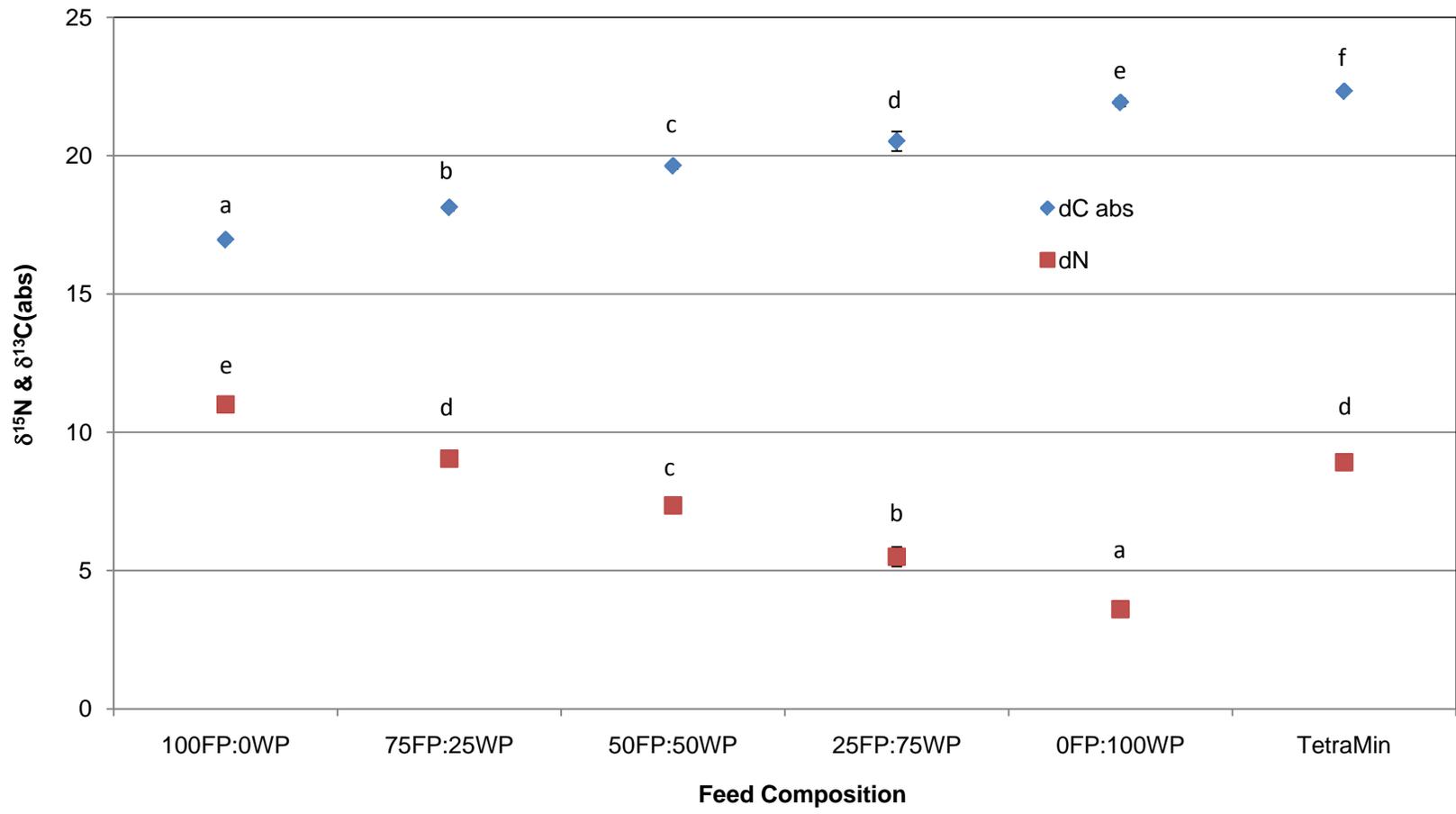


Figure 2-16. Six flake feed $\delta^{13}\text{C}$ (absolute value) and $\delta^{15}\text{N}$ signature values ($\pm 95\%$ CI); differing letters denote statistical differences between treatments ($P < 0.05$ Tukey's multiple comparison test) within elements, C or N. TetraMinTM registered trademark of the Tetra Werke Company. Treatment sample sizes (from left): 4, 4, 4, 4, 4, and 2.

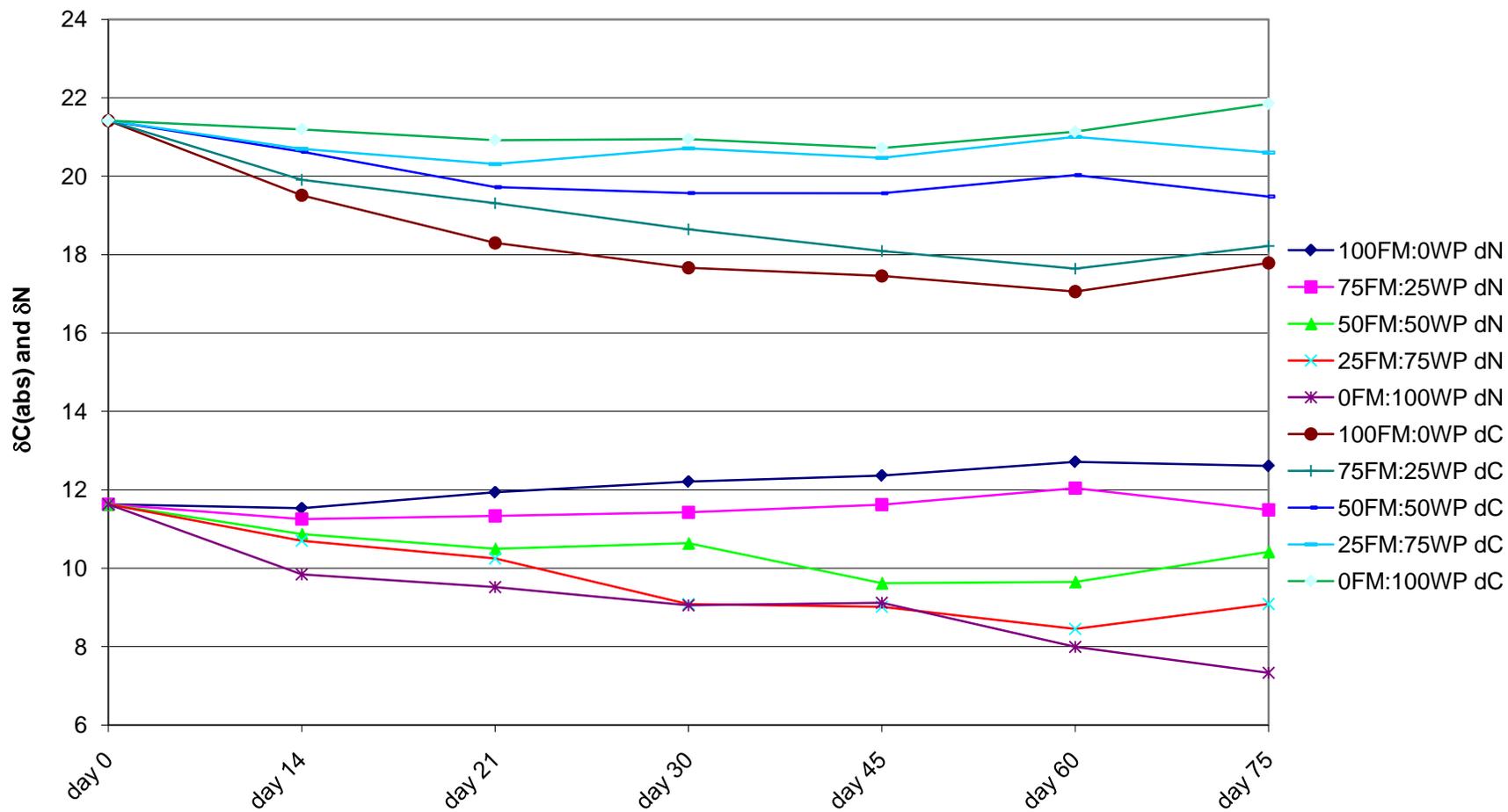


Figure 2-17. Mean pre-harvest swordtail fry isotope signature [$\delta^{13}\text{C}$ (abs), $\delta^{15}\text{N}$] trajectories over 90 day indoor trial, single replicates for each date and treatment combination (FM - % fishmeal: WP - % wheat protein isolate).

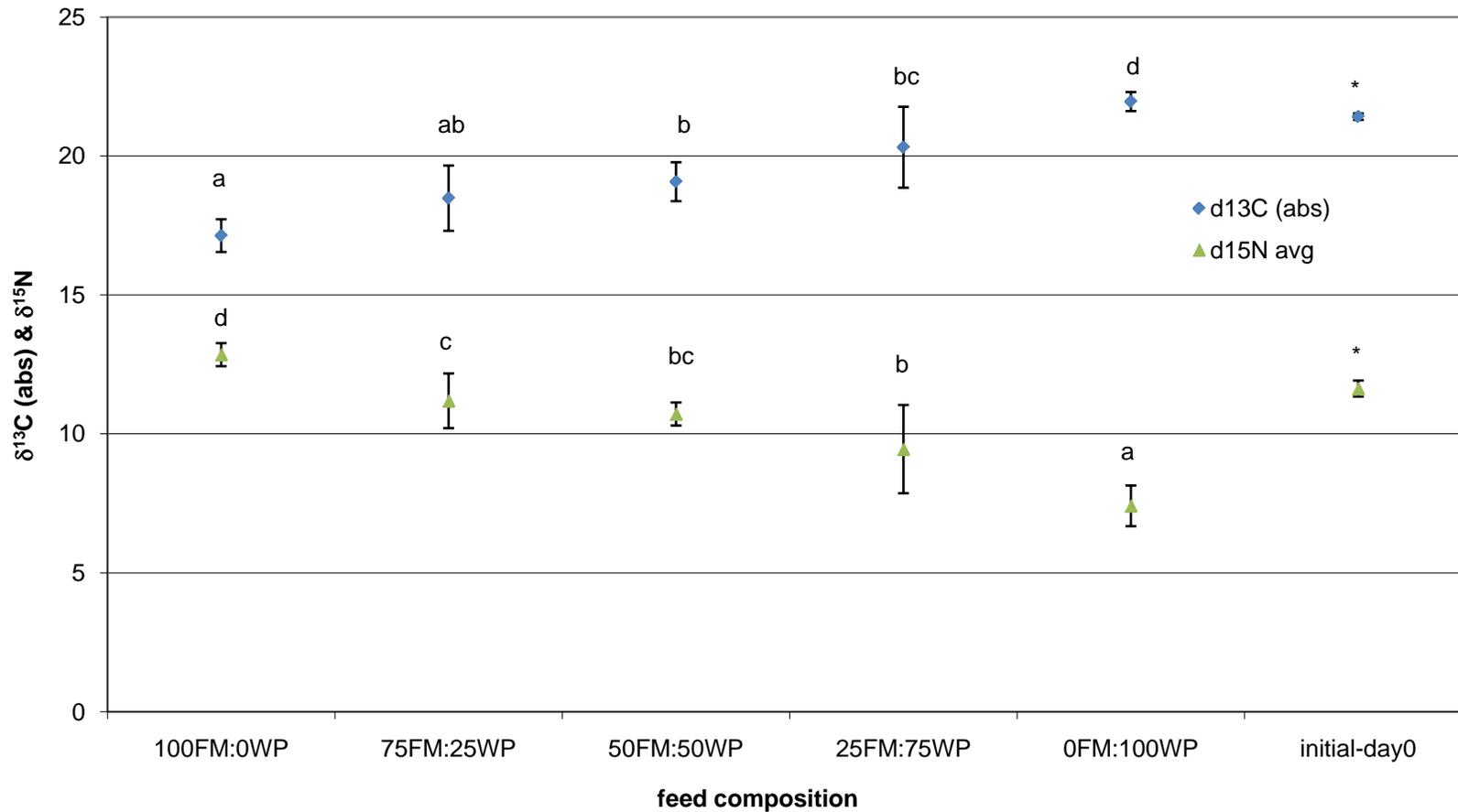


Figure 2-18. Indoor trial fry isotope signatures [$\delta^{13}\text{C(abs)}$, $\delta^{15}\text{N} \text{ ‰} \pm 95 \text{ ‰ CI}$] at harvest (90 days), * fry isotope signature values (day zero); differing letters denote significant differences between treatments ($P < 0.05$, Tukey's multiple comparison test) within elements, C or N. Treatment sample sizes (from left): 6, 6, 6, 5, and 6.

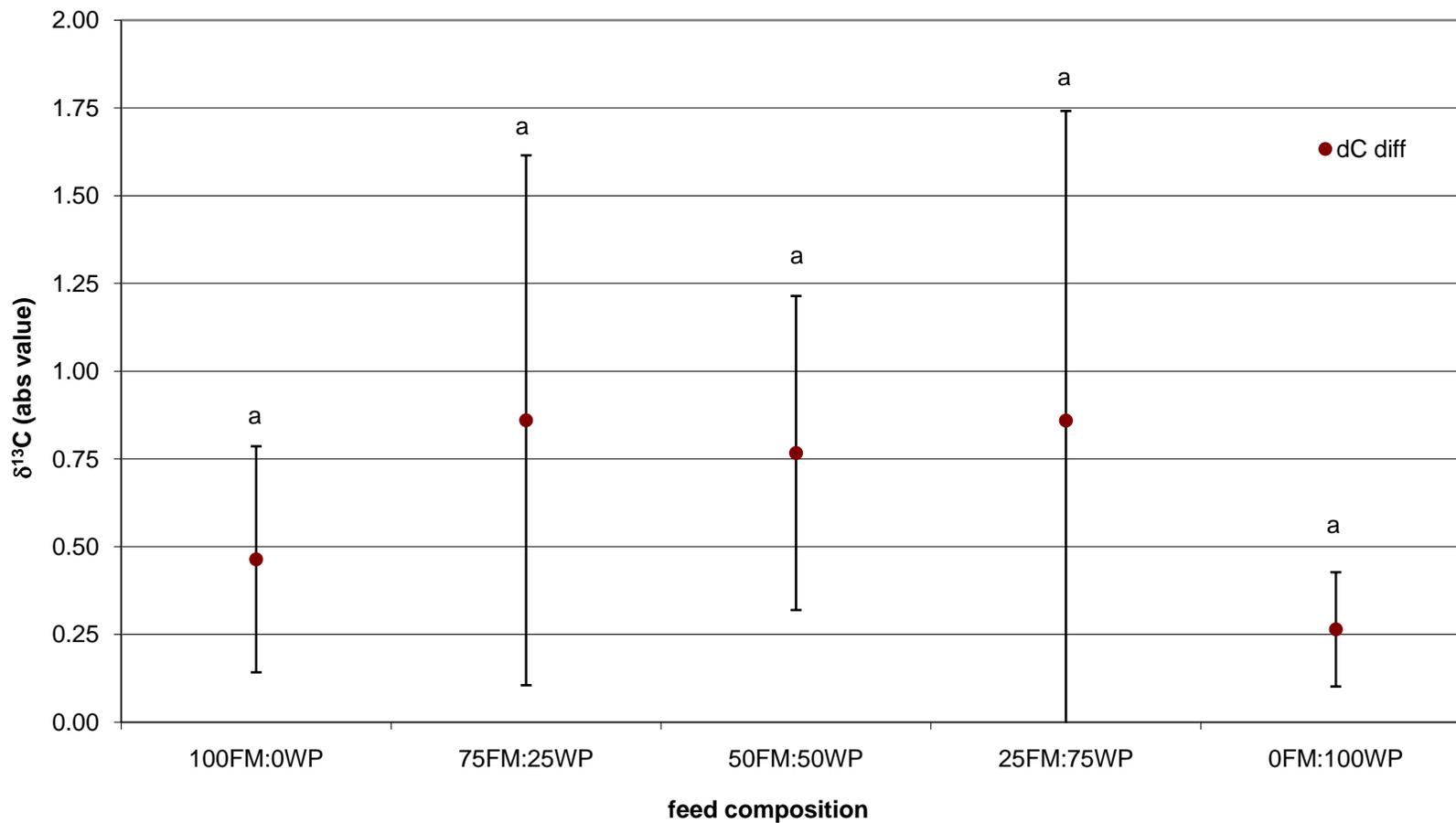


Figure 2-19. Carbon isotope signature differences ($\Delta\delta^{13}\text{C} \text{ ‰} \pm 95 \text{ ‰ CI}$) between harvest fry and respective experimental feed treatments after 90 days; differing letters denote significant differences between groups ($P < 0.05$, Tukey's multiple comparison test). Treatment sample sizes (from left): 6, 6, 6, 5, and 6.

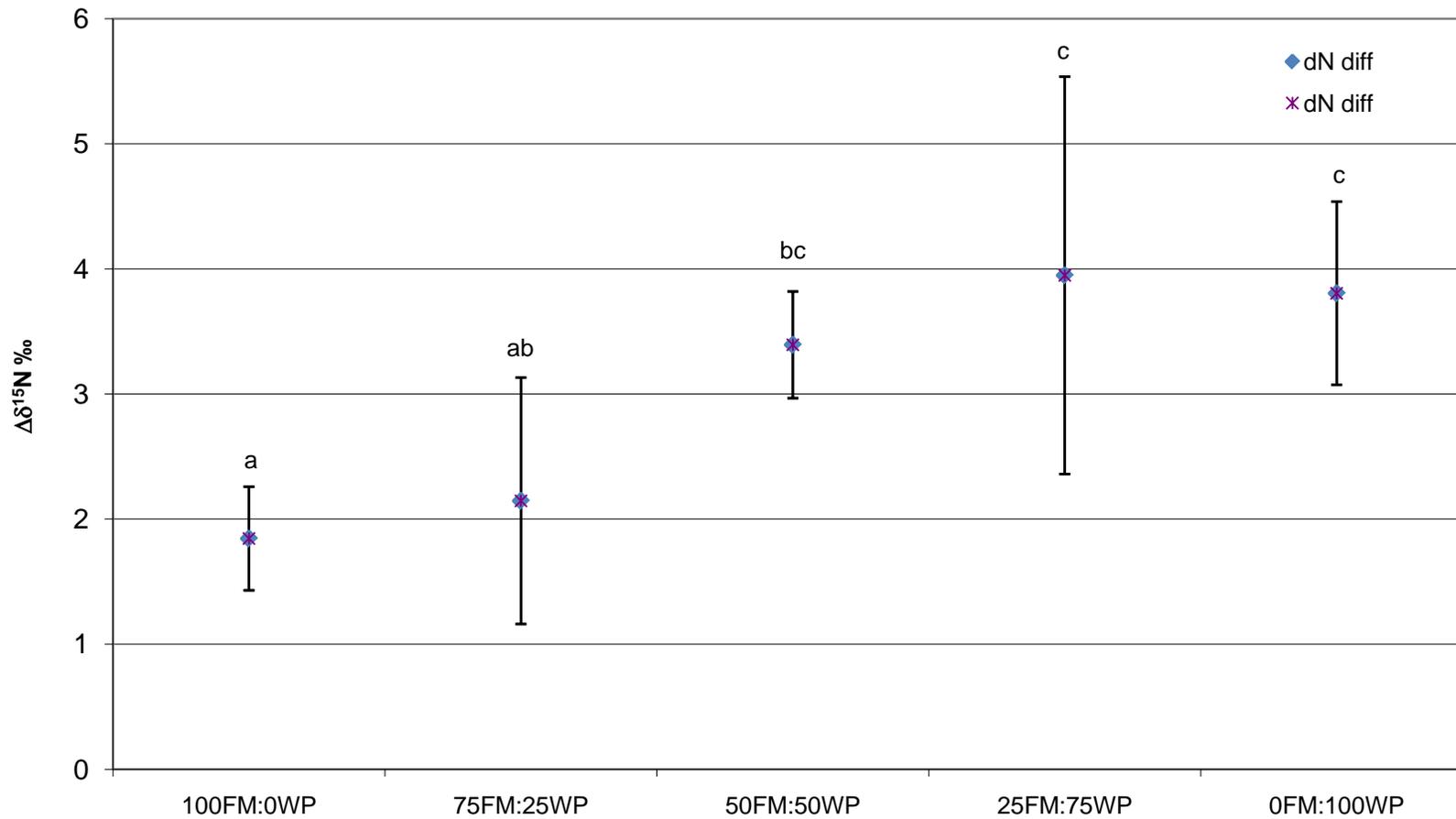


Figure 2-20. Nitrogen isotope signature differences ($\Delta\delta^{15}\text{N}$ ‰ \pm 95 % CI) between harvest fry and respective experimental feed treatments after 90 days, differing letters denote significant differences between groups ($P < 0.05$, Tukey's multiple comparison test). Treatment sample sizes (from left): 6, 6, 6, 5, and 6.

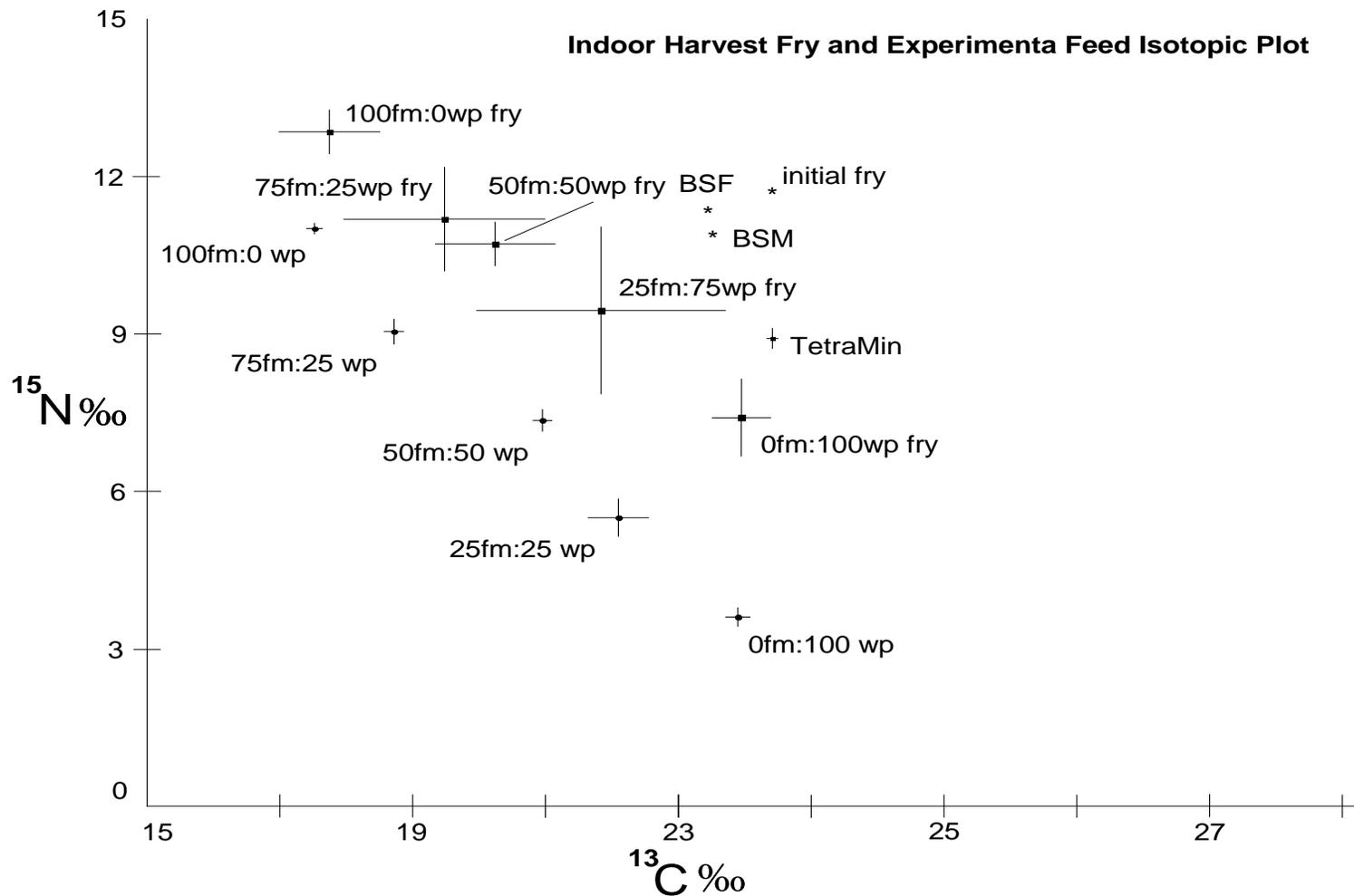


Figure 2-21. Cartesian plot of indoor feeding trial swordtail juveniles (3 months), initial fry, male (BSM) and female broodstock (BSF), and five experimental feeds (% fm: % wp, fm-fishmeal, wp –wheat protein) and control feed (Tetra Min) $\delta^{13}\text{C}$ (abs) and $\delta^{15}\text{N}$ signature values (‰ ± 95 % CI); confidence intervals omitted for swordtail initial fry, and broodstock for clarity.

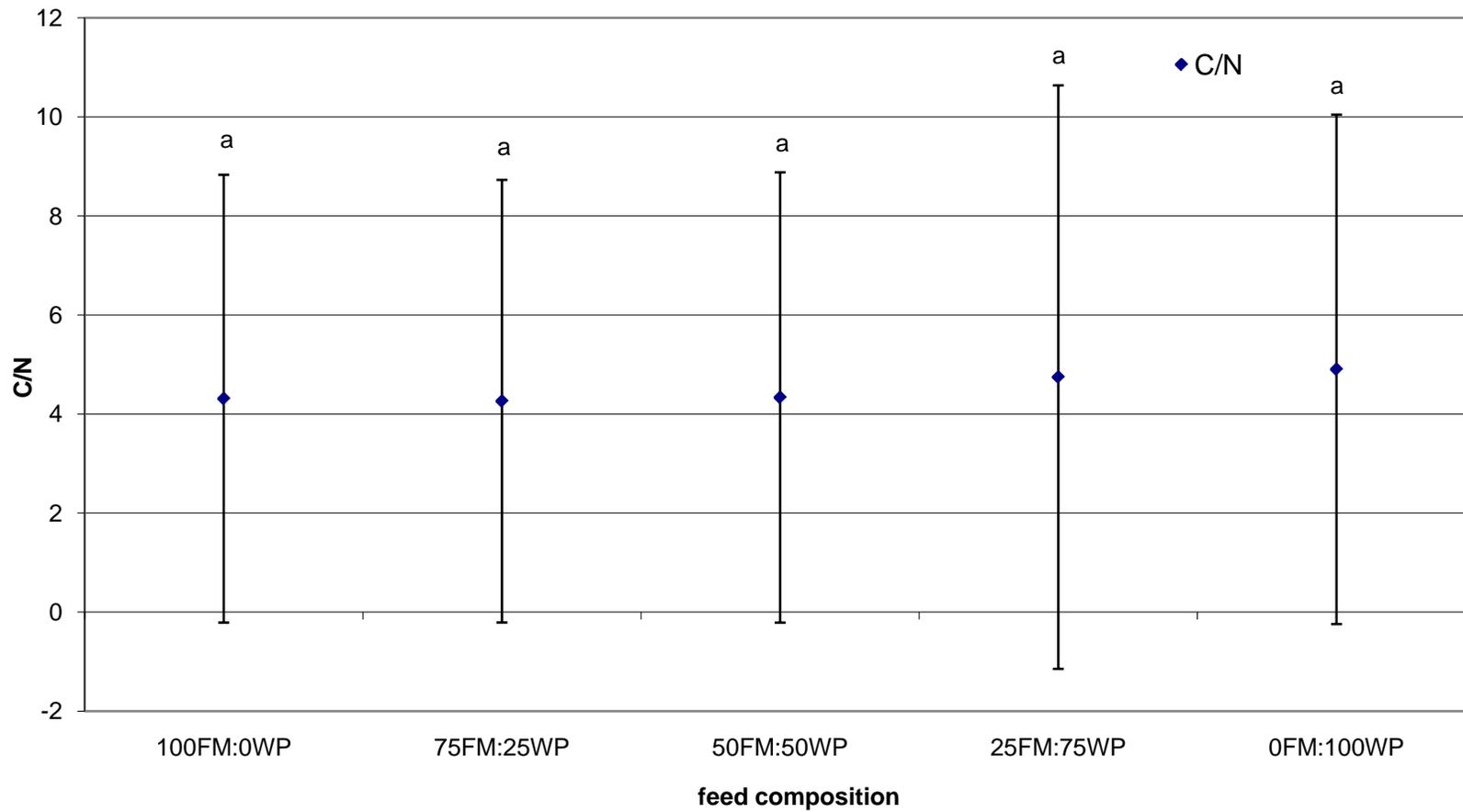


Figure 2-22. Indoor fry elemental [C]/[N] ratios (\pm 95 % CI) at harvest after 90 days on five experimental feeds (FM- % fishmeal, WP – % wheat protein) treatments; differing letters denote significant differences between treatments ($P < 0.05$, Tukey's multiple comparison test). Treatment sample sizes (from left): 6, 6, 6, 5, and 6.

Table 2-1. Twelve week indoor swordtail (*Xiphophorus hellerii*) fry feeding trial - daily per capita fry ration level as a function of time, day 0 to 10 ration based upon initial fry size at start of trial.

Days 0 – 10	Days 11-20	Days 21-30	Days 31-45	Days 46-60	Days 61-75	Days 75-90
15% ration (wet wt.)	15% ration (wet wt.)	15% ration (wet wt.)	10% ration (wet wt.)	10% ration (wet wt.)	10% ration (wet wt.)	10% ration (wet wt.)
0.00134 g feed/fish/day	0.0185 g feed/fish/day	0.0261 g feed/fish/day	0.0250 g * feed/fish/day	0.0513 g feed/fish/day	0.0977 g feed/fish/day	0.1208 g feed/fish/day
Treatment highest fry wt.	25%FM:75% WP	0%FM:100% WP	25%FM:75% WP	100%FM:0% WP	50%FM:50% WP	25%FM:75% WP

* calculation error resulted in lower feeding ration (still greater than 30 minute satiation level).

CHAPTER 3 OUTDOOR POND TRIAL SWORDTAIL BIOMASS PRODUCTION

Consistently high production rates are the goal of all aquaculture producers. Only by producing large numbers of high-quality livestock, can fish farmers sustain profitable operations when faced with low profit margins, potentially catastrophic livestock losses due to inclement weather (outdoor operations), and overseas competition. Increasing labor costs, operations overhead (electricity, feed, and transportation fuel costs), land prices, and consolidation of the retail pet store market have reduced the profit margin for ornamental fish producers.

The swordtail (*Xiphophorus hellerii*) is a popular ornamental fish in the freshwater tropical fish pet trade (Figures 3-2a-c). In the United States, swordtails and other ornamental livebearers are typically raised within earthen ponds in southern Florida, primarily centered within Polk, Hillsborough, Miami-Dade, and Palm Beach counties (FASS/FDACs 2005), where air temperatures rarely drop below 16°C (60°C) and groundwater from surficial aquifers is relatively close to the surface. Profit margins for producers are typically small and large numbers of fish of acceptable appearance and health are required to provide sufficient profits for producers.

A series of outdoor pond trials were performed to evaluate four common freshwater tropical fish species cultured using four different aquaculture pond management practices: processed commercial feed, unprocessed commercial feed, cottonseed meal (organic fertilizer), and an inorganic fertilizer (M. Krasilovsky unpublished data). In conjunction with one of these series of pond trials, involving the red wag swordtail (Figures 3-2a-c), isotopic analyses of planktonic assemblages within the ponds, as well as the applied feeds, fertilizers and fish, were conducted to determine the fate of applied nutrients (feeds and fertilizers), and if pond treatment trophic efficiencies and fish production were correlated.

Fish production rates were used to evaluate the efficacy of the four different pond (nutrient) management practices. Fish production differences among replicate ponds within management treatments, also were used to evaluate management techniques, as lower variation among replicate ponds is desirable. Production efficiencies of fish fed manufactured feeds, and live feeds derived from increased phytoplankton production through the addition of organic and inorganic fertilizers, were measured by harvesting ponds after a three-month grow-out period. Actual production efficiencies also were compared with production estimates predicted from trophic efficiencies determined from the isotopic signatures of the major food web components (Chapter 4).

Methods

Study Site

The study was conducted at the Tropical Aquaculture Laboratory (TAL), (University of Florida, Institute of Food and Agricultural Sciences [UF-IFAS], School of Forest Resources and Conservation), a satellite facility located in Ruskin, Florida, U.S.A. Twenty-four adjoining rectangular experimental ponds were used for this study. Each pond had similar physical dimensions (Figure 3-1a-d; 17.8 m length \times 8.6 m width \times 2 m avg. depth), surface area (0.015 ha) and water volumes [\sim 153,000 L (40,370 gallons) per pond]. Due to the high water table (typically 50 cm below grade) and permeable soil, ponds were filled primarily from a surficial aquifer with only sporadic inflow of supplemental well water necessary to maintain pond volume during the hotter summer months when higher rates of evaporation occurred.

The pond study began on 13 March, 2006, when ponds were initially drained and excess sediment and organic material was washed and removed from the substrate over a period of three days. Immediately following draining and washing, the pond substrate was sterilized with hydrated lime [$\text{Ca}(\text{OH})_2$] hand broadcast onto the pond bottom at approximately 800 grams per

pond (Figure 3-1a); lime application quickly raises the pH of any remaining pond water (Boyd 1974, 1995, 1997, Lin et al. 1997) and the pond substrate, killing many of the remaining organisms (potential predators, competitors and pathogens). Additionally, increased alkalinity provided by lime addition stimulates algal growth that supports the pond food web, supplementing the nutritional needs of broodstock and fry (Boyd 1995, 1997). Stocking occurred 14 days later on 27 March, 2006, when lower pond pH levels (< 8.0 pH) were deemed amenable to fish stocking and sufficient manpower was available.

Each pond was stocked with 250 (200 female: 50 male) redwag swordtail (*Xiphophorus hellerii*) broodstock (Figures 3-2a-b). Due to the large number of broodstock required for the study (6000), broodstock (> 4 cm S.L.) were obtained via seining and trapping from outside ponds that were initially stocked with red wag broodstock, and broodstock grown from fry kept in a greenhouse recirculation system. All broodstock were obtained from the same genetic stock, but ages and nutritional histories differed substantially. To account for these differences, prior to stocking, broodstock fish from the two sources were separated by sex and evenly distributed within ponds, to block for the effects of broodstock from different sources within the treatment ponds (Smart et al. 1998, 2001).

The 24 ponds were subjected to four different nutrient treatments with six replicate ponds per treatment: (1) processed feed (PRO), (2) unprocessed feed (UNP), (3) cottonseed meal organic fertilizer (CSM), and (4) nitrogen only (ammonium nitrate) inorganic fertilizer (INO). 'Unprocessed' feed was partially processed by milling, but differed from processed feed by not being heated during extrusion and further homogenized by regrinding. By heating the processed feed during extrusion, starches within the feeds become slightly gelatinized, increasing their digestibility and available caloric energy. In addition, anti-nutritional compounds (e.g., phytate,

lectins, and trypsin inhibitors) become inactivated when carefully heated (Halver 1989, Brown et al. 1997). The two commercial feeds were formulated using identical ingredients which were chosen and present in quantities deemed typical of high quality commercial tropical ornamental fish feeds utilized by local ornamental fish farmers in the area. These feeds were specially manufactured for TAL by Purina Animal Feeds Corporation (Table 3.1).

Ponds designated as feed treatment ponds were broadcast fed twice daily at dawn (approx. 300 g ~ 07:00 EST) and late afternoon (approx. 300 g ~ 17:00 EST), for a total of 600 g of feed per pond per day. Cottonseed meal fertilizer also was hand broadcast twice daily to their respective treatment ponds at the same times that processed and unprocessed feeds were applied. Inorganic liquid fertilizer (inorganic liquid fertilizer 11:0:37 – N:P:K) was administered according to weekly Secchi disk readings and additional fertilizer was applied if Secchi depths were less than 45 cm (Boyd 1979, 1995, Lin et al. 1997, Tepe and Boyd 2003) just after midday (13:00 EST). When dictated by Secchi depth values, inorganic fertilizer was applied at a rate of 4 kg/ha NH_4NO_3 : 8 kg/ha P_2O_5 . Fertilizers were applied to ponds to ensure the maintenance of a “green water” algal bloom to support pond food webs, as Secchi depths have been shown to correlate well with chlorophyll [a] concentrations in low turbidity/low color waters and were used to monitor phytoplankton abundance (Florida Lakewatch 2001).

Water Chemistry and Physical Environment

Temperature and dissolved oxygen concentrations were measured for each pond prior to the twice-daily feedings (dawn and late afternoon) throughout the twelve weeks of the outdoor-feeding trial. Standard water chemistry parameters: total alkalinity (mg/L CaCO_3 equivalent), total hardness (mg CaCO_3 equivalent), pH, total ammonia nitrogen, nitrite, nitrate, total phosphorous and chlorophyll [a] were measured weekly as part of another study (M. Krasilovsky unpublished data).

Pre-Harvest Fry Collection

Fry were collected weekly during the first three weeks (21 days) of the study, and were collected biweekly thereafter; fry were collected on days 7, 14, 21, 35, 49, 63, 77, and 84 of the 90-day experimental trial. Fry were collected from within the pond with a 74 cm (width) x 51 cm (length) 38 cm (bag depth) rectangular dip net with 1-mm square mesh. Fry were collected beginning at 10:00 hrs on the day of collection, fry were collected while walking through the shallow water and emergent weeds growing along the perimeter of each pond while periodically thrusting the net into the shallow weeds where fry were known to congregate. For each pond, a minimum of ten fry were placed into pre-sterilized (Whirlpak™ 2-ounce nominal size) polyethylene bags and placed into an insulated cooler lined within an ice water slurry. Upon return to the laboratory, fry were frozen and stored at (- 4 °C). Fry were stored frozen in an ice block (distilled water added) within their polyethylene sample bags to prevent dehydration from water ice sublimation ‘freezer burn’ for six to eight months prior to length and weight measurements. Fry were thawed prior to length measurement and gastrointestinal tracts and all internal organs were removed prior to being weighed (wet weight to the nearest ±0.001 gram) to reduce the bias of recently ingested prey organisms during isotopic analyses of fry. Fry length and weight measurements were made for a minimum of six fry from each pond for each of the eight sampling dates (~ 1,152 samples). After GI tract removal, and length and weight measurements, fry were individually placed upon preweighed aluminum foil “weighing boats” and placed into a - 70 °C (± 4 °C) ultra cold freezer (Revco™ Elite ultra-low temperature freezer) for 12 to 24 hrs prior to being freeze dried at - 57 °C (Virtis corporation model Sentry™ 16 freeze dryer) for 16 to 48 hrs depending upon specimen size. Fry dry weights were measured and fry were then stored in sterile, pre-cleaned, 0.7-ml polyethylene microcentrifuge tubes.

Harvest Fish Collection

The pond fry grow-out trial lasted for 90 days, and was terminated beginning the week of 18 June, 2006, with the initiation of pond harvesting. The application of all pond treatments ceased two days prior to the initiation of harvest. Swordtails were harvested over the course of four days using a 7.6 m length \times 1.8 m height 4 mm \times 4 mm square-mesh multifilament nylon beach seine with weighted lead line (Figure 3-3a). During harvest, two people on opposite banks dragged the seine along the long axis of the pond to the opposite bank where fish were concentrated and placed into 19-L plastic buckets approximately half filled with pond water. Swordtails were immediately transferred to large insulated coolers filled with aerated well water and transported to the TAL quarantine building, where fish were placed into large epoxy-coated concrete vaults (240 cm length \times 89.5 cm width \times 73.6 cm height; approx. 1,665 L volume) approximately $\frac{1}{4}$ filled with aerated well water; fish were then separated from debris and pest species (crayfish, tadpoles, insects, undesirable fish) and sorted into two size classes (small \leq 31 mm SL and large $>$ 31 mm SL) using a hand operated floating grate sorter (Figure 3-3b). Three subsamples (when possible) of 300 fish were taken from each of the two size classes for each pond, and subsample live wet weights, this allowed subsample and treatment mean fish live wet weight to be calculated (\bar{X} = subsample fish live wet weight grams/300 fish) for each fish size class. Additionally, smaller subsamples were taken from pond subsamples to determine individual morphometric mean swordtail standard lengths (SL) and live wet weights of the two size classes for each pond; small numbers (\sim 10-20) of fish were also frozen for eviscerated dry weight and isotopic determination.

Statistical Analyses

Comparisons among treatments and sampling dates were performed using two-way repeated measures ANOVA due to the non-independent nature of repeated samplings from

replicate ponds ($\alpha_{2\text{-tailed critical}} = 0.05$). Comparisons among treatments for time averaged data were performed using one-way ANOVA ($\alpha_{2\text{-tailed critical}} = 0.05$). Post hoc tests were performed using the Bonferroni post test (two-way repeated measures ANOVA; $\alpha_{2\text{-tailed critical}} = 0.05$) and Tukey's multiple comparison test (one-way ANOVA; $\alpha_{2\text{-tailed critical}} = 0.05$).

Results

Pre-harvest Fry Growth

Pre-harvest pond fry were sampled periodically during the 12-week outdoor grow-out trial: on days 7, 14, 21, 35, 50, 63, and 77 post stocking. Eviscerated fry dry weights generally increased over the 12-week trial (Figures 3-4 – 3-8). Nutrient treatment was not a significant factor in determining fry dry weight (2-way repeated measures ANOVA: $P_{(\text{nutrient})} = 0.2362$, $F_{(\text{nutrient})} = 1.535$, $\alpha_{(2,3)} = 0.05$), but time was significant (Figures 3-4 – 3-8; 2-way ANOVA: $P_{(\text{time})} < 0.0001$, $F_{(\text{time})} = 28.96$, $\alpha_{(2,6)} = 0.05$); to perform repeated measures analyses, two missing data points of 168, were replaced with their respective treatment and date mean values in order (PRO pond B10, 17 May 2006, and INO pond B9, 17 May 2006). Significant pairwise differences in dry fry weight and sampling date were found within individual pond nutrient treatments, (Figures 3-5 – 3-8; $P < 0.05$, Bonferroni post hoc test).

Fish Production at Harvest

Total fish biomass at harvest differed greatly among treatments (Figure 3-9; 1-way ANOVA, $P < 0.0001$, $F = 22.88$, $\alpha_{(2,3)} = 0.05$). PRO pond mean fish biomass was the highest $\bar{X} = 8.56$ kg (6.41 to 10.71 kg 95 % CI), followed in order from highest to lowest by UNP ponds $\bar{X} = 7.25$ kg (6.29 to 8.20 kg 95 % CI), CSM $\bar{X} = 5.25$ kg (4.79 to 5.70 kg 95 % CI), and INO $\bar{X} = 3.75$ kg (2.82 to 4.67 kg 95 % CI) ponds. PRO treatment ponds did not differ significantly

from UNP ponds, but both differed significantly from CSM and INO ponds, which did not significantly differ from each other.

Mean fish numbers at harvest significantly differed among treatments (1-way ANOVA, $P = 0.0115$, $F = 4.834$, $\alpha_{(2,3)} = 0.05$). PRO pond mean fish numbers were the highest $\bar{X} = 20,290$ (15,940 to 24,650 95 % CI), UNP $\bar{X} = 17,490$ (14,870 to 20,110 95 % CI) and INO $\bar{X} = 17,460$ (13,750 to 21,180 95 % CI) ponds had the next highest, and CSM treatment ponds had the lowest mean number $\bar{X} = 13,180$ (9,990 to 16,370 95 % CI). However, due to large within treatment variations in pond total fish numbers, post-hoc pairwise comparison tests found only one significant pairwise difference in total fish numbers among nutrient treatments, PRO v. CSM pond treatments (Figure 3-10).

Mean marketable ($SL > 31$ mm) fish numbers differed markedly and significantly among treatments (Figure 3-11; 1-way ANOVA, $P < 0.0001$, $F = 21.24$, $\alpha_{(2,3)} = 0.05$). PRO treatment ponds had the highest mean number of large fish $\bar{X} = 2,446$ (1790 to 3100 95 % CI), followed in order by UNP treatment ponds $\bar{X} = 1,919$ (1540 to 2300 95 % CI), CSM ponds $\bar{X} = 1,506$ (1360 to 1650 95 % CI), and INO ponds $\bar{X} = 424$ (277 to 570 95 % CI). Post hoc pairwise comparisons indicated that PRO treatment ponds had significantly more marketable fish than either of the fertilizer pond treatments, but did not differ from the UNP treatment, which had more marketable fish than the INO treatment, but did not differ from the CSM treatment; INO treatment numbers were significantly lower than those of the other treatments (Figure 3-11; $P < 0.05$).

Mean unmarketable ($SL \leq 31$ mm) fish numbers also differed greatly among treatments (Figure 3-12; 1-way ANOVA, $P = 0.0138$, $F = 4.610$, $\alpha_{(2,3)} = 0.05$). PRO ponds $\bar{X} = 17,850$ (14,190 to 21,510 95 % CI) and INO ponds $\bar{X} = 17,040$ (13,490 to 20,590 95 % CI) had the

highest numbers of small fish, followed by the UNP ponds $\bar{X} = 15,570$ (12,700 to 18,450 95 % CI) and lastly by the CSM ponds $\bar{X} = 11,670$ (8,410 to 14,940 95 % CI). Post hoc pairwise tests indicated that the PRO and INO ponds had significantly greater numbers of small fish than the CSM ponds, and the UNP pond numbers did not significantly differ from any other treatment (Figure 3-12; $P < 0.05$).

Large fish wet weight (SL > 31 mm) means at harvest did not significantly differ among treatments (Figure 3-13; 1-way ANOVA, $P = 0.8005$, $F = 0.3346$, $\alpha_{(2,3)} = 0.05$). PRO pond large fish mean wet weight $\bar{X} = 1.82$ g (1.62 to 2.02 g 95 % CI) was similar to UNP $\bar{X} = 1.71$ g (1.52 to 1.90 g 95 % CI), CSM $\bar{X} = 1.75$ g (1.52 to 1.90 g 95 % CI), and INO $\bar{X} = 1.87$ g (1.39 to 2.36 g 95 % CI) large fish mean weights. Weight variation (95 % CI) was greatest for the INO pond large harvest fish than for those of the other three treatments (Figure 3-13). The individual large harvest fish (SL > 31 mm) wet weight coefficient of variation values differed markedly among treatments (Figure 3-15).

Small fish wet weight (SL \leq 31 mm) means at harvest also did not significantly differ among treatments (Figure 3-14: 1-way ANOVA, $P = 0.0874$, $F = 2.536$, $\alpha_{(2,3)} = 0.05$). PRO pond small fish at harvest had a mean wet weight $\bar{X} = 0.23$ g (0.33 to 0.14 g 95 % CI) slightly lower than the UNP pond small fish $\bar{X} = 0.28$ g (0.36 to 0.20 g 95 % CI) and CSM pond small fish $\bar{X} = 0.28$ g (0.36 to 0.21 g 95 % CI), which had identical mean weights, and the INO pond small fish had the lowest mean wet weight $\bar{X} = 0.18$ g (0.25 to 0.12 g 95 % CI).

Discussion

Pre-harvest Fry Growth

Mean fry weights increased over the 12-week duration of the outdoor pond trial for all four pond nutrient treatments (Figure 3-4). However, fry mean weight trajectories were uneven

and mean fry weights dropped between the fourth and fifth weeks for the two fertilizer treatments and the fifth and seventh weeks for the two feed treatments (Figures 3-4 – 3-8). These drops in mean fry weight were likely due to an increase in overall reproductive output of adult females. Although fecundity increases as food supplies and temperatures increase (Milton and Arthington 1983, Dawes 1991, Hutchings 1993, Hopkins and Unwin 1997, Tamaru et al. 2001), the most likely factor impacting fry recruitment is that large numbers of older female fry had become sexually mature during these time periods.

In outdoor farm ponds in Hawaii, female fry produced at the beginning of the grow-out season, reached first age of reproduction at approximately 6-8 weeks of age (Tamaru et al. 2001); depending upon stocking density, food availability, photoperiod, temperature and other environmental factors. Age at first reproduction for swordtails has been reported by other investigators to occur between six to twelve weeks of age (Sohn 1977, Dawes 1991, Dussault and Kramer 1981, McKenzie et al 1983, Milton and Arthington 1983, Campton and Gall 1988, Tamaru et al. 2001). These reported time frames (6-8 weeks), coincided with the decrease in pre-harvest fry average weight observed within this study (Figure 3-4).

Pre-harvest fry samples were made of numerous cohorts as fry were continuously produced throughout the 12-week trial; based upon the observation of large variations in fry sizes (and presumably ages) within the periodic fry samples, and extensive knowledge of swordtail reproductive biology (Dawes 1991, Axelrod and Wischnath 1991, Tamaru et al. 2001). Mean fry weight increase was consistent with a logistic or Gompertz trajectory, but actual fry growth patterns (and instantaneous growth rates) could not be determined due to continuous spawning within the ponds that resulted in large numbers of fry of differing weights and ages (Cailliet et al. 1996).

Adult female reproductive output increases with increasing water temperatures (Dawes 1991, Tamaru et al. 2001). Increased water temperature decreases the age at first reproduction of female fry, and increases adult female fecundity with increasing water temperatures up to 29°C (Tamaru et al. 2001). Both air and pond surface water (17 - 31°C) temperatures rose dramatically as the trial progressed from mid March to mid June (Figure 3-16).

Total pond production also was likely enhanced by the effects of higher temperatures and longer photoperiods on phytoplankton production rates (Fogg and Thake 1987, Boyd 1979); increased primary production has been shown to lead directly to increased zooplankton production, both higher phytoplankton and zooplankton prey standing stocks are factors known to increase adult female swordtail fecundity (Kruger et al. 2001, Chong et al. 2004).

Harvest Fish

The two feed treatments had the highest mean harvest biomass yields, and had significantly higher fish yields than the two fertilizer pond treatments (Figure 3-9). Additionally, the two feed treatments individual large harvest fish weight coefficient of variation values were much lower than those of the two fertilizer treatments (Figure 3-13), indicating that individual fish weight variations were much higher within the two fertilizer treatments.

High total fish biomass at harvest is a desirable performance aspect for a conveniently applied pond nutrient. Another is large numbers of commercially marketable fish of sufficient and uniform size (usually total length), and appearance. High variation in size often indicates food limitation and/or overstocking (Figure 3-15), which may result in increased intraspecies aggression and social hierarchies that may create and amplify size disparities among or within cohorts (Hannes and Franck 1983, Hannes et al. 1984, Franck et al. 1985). Additionally, from a marketing perspective, size and color inconsistencies are extremely undesirable to potential

ornamental tropical fish buyers and retailers, and additional stress from handling and sorting is detrimental to appearance and increases fish mortality (Karplus et al. 2003).

Total fish numbers at harvest differed only between the processed feed and cottonseed meal treatments (Figure 3-10). Inorganic treatment total harvest did not significantly differ from the processed feed treatment, which had the highest number of fish among the four treatments. The INO treatment produced high fish numbers, but had the lowest mean fish biomass yield of the four treatments, resulting in a generally ‘stunted’ appearance of the fish within this treatment.

PRO ponds had the highest number of large marketable fish (SL > 31 mm), PRO treatment mean large harvest fish numbers were significantly greater than those of the two fertilizer treatments. UNP pond marketable fish numbers only differed significantly from the INO ponds, which had significantly fewer marketable fish than the other pond treatments (Figure 3-11).

PRO ponds also had the highest number of unmarketable fish (≤ 31 mm SL), followed closely by the UNP and INO treatments; the CSM ponds had significantly fewer smaller fish than both the PRO and INO ponds (Figure 3-12). The fact that INO ponds had the lowest total fish biomass, fewest large marketable fish, and high numbers of small unmarketable fish, again attests to the generally ‘stunted’ small fish that were harvested from these ponds.

Large fish (> 31 mm SL) mean weights did not differ among treatments at harvest (Figure 3-13). Small fish (≤ 31 mm SL) mean weights also did not differ among treatments at harvest (Figure 3-14). However, small fish mean weights varied among treatments more than their large fish counterparts (Figures 3-13 – 3-14). Greater weight variation among individual small harvest fish within the INO treatment ponds, relative to those of the other three treatments, may have been due to lower food availability within INO treatment ponds. Large numbers of small fry

were able to escape through the 4 mm × 4 mm square mesh of the seine, which may have slightly skewed the size frequency data results.

Water Chemistry and Physical Environment

Water temperature increased steadily throughout the 12 weeks of this outdoor pond feeding trial. Morning oxygen readings decreased slightly and late afternoon oxygen readings increased as pond heterotroph biomass, algal biomass, photoperiod, and water temperature increased from early spring to late spring/early summer (March-June). Oxygen levels were typically at their daily minimums during the early pre-dawn hours due to the respiration of pond organisms and lack of photosynthesis during nighttime hours, and increased to saturation ($\geq 100\%$ dissolved oxygen) or near saturation levels during the late afternoon (~ 16:00 hrs EST) depending on cloud cover. Feeding was postponed when oxygen concentration levels were below ~ 2.0 mg/L to prevent stress from potentially hypoxic conditions. Depending upon duration and severity of hypoxic and anoxic conditions, pond fish may become stressed and susceptible to disease or suffer mortality. Low oxygen conditions were only present on four occasions, and did not appear to cause any noticeable disease or mortality events. Water chemistry parameters such as pH, hardness and alkalinity were relatively stable during the trial. This was not unexpected as the surficial aquifer ground water source in this region flows through a karst geology that imparts high alkalinity to the water due to high concentrations of dissolved CaCO_3 and MgCO_3 . Total ammonia nitrogen (TAN: NH_3 , NH_4^+) and nitrite levels also were unremarkable (TAN typically < 2.5 mg/L, NO_2^- < 0.05 mg/L), but were sporadically higher within the INO treatment (Michael Krasilovsky unpublished data); most likely due to the weekly introduction of ammonium nitrate fertilizer, which immediately ionizes to ammonium (NH_4^+), and is subsequently oxidized to nitrate.

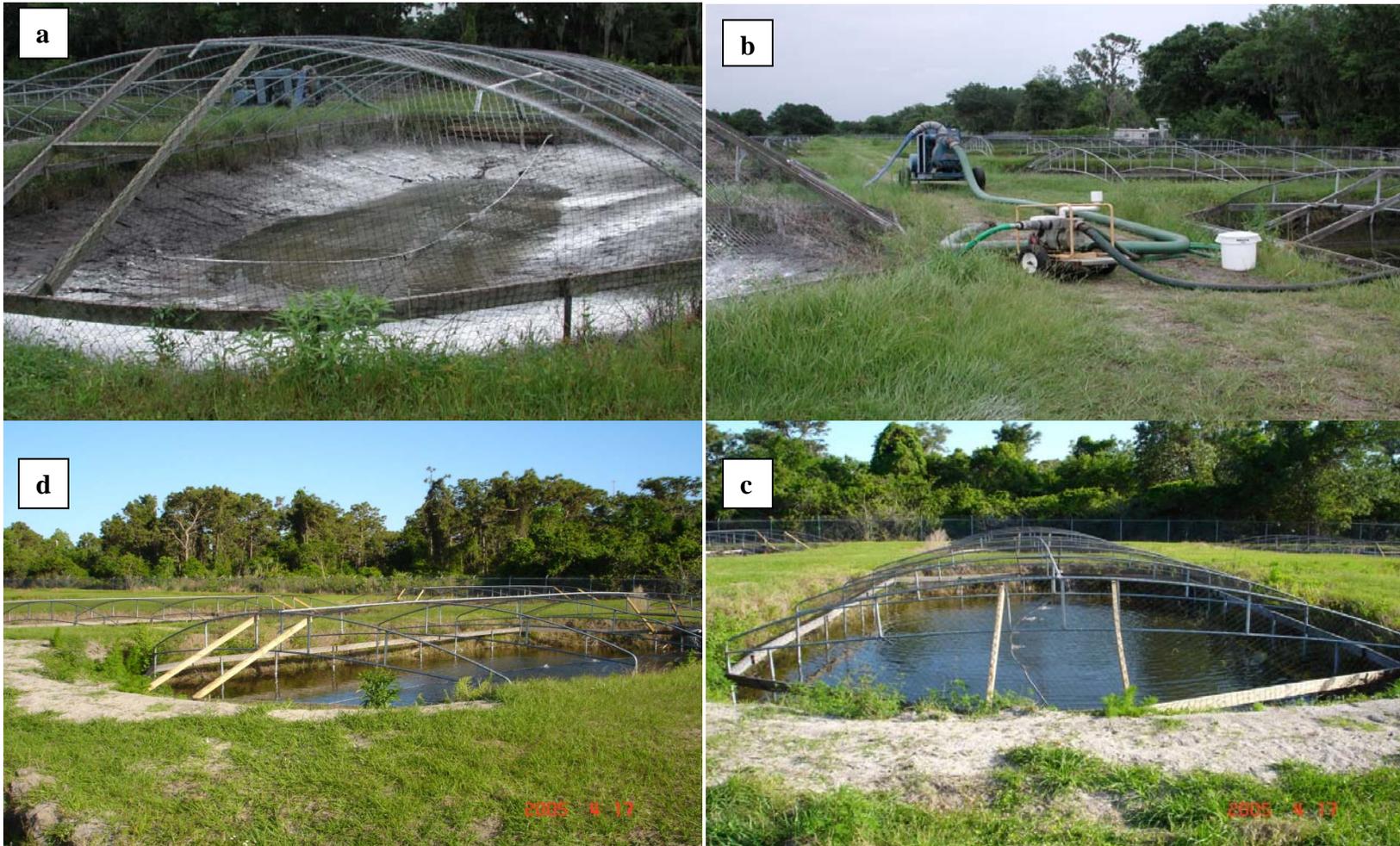


Figure 3-1a-d. Clockwise from top left: (a) drained and washed pond, hydrated lime applied to substrate visible as white powder, (b) diesel tractor engine pump used to drain ponds in background, smaller wash pump in foreground (c) pond filled with a combination of both groundwater and well water, (d) ponds have been refilled and are ready for stocking once pH levels have stabilized (~7-8 pH) and plankton blooms have become established, steel frame for bird netting/predator control.

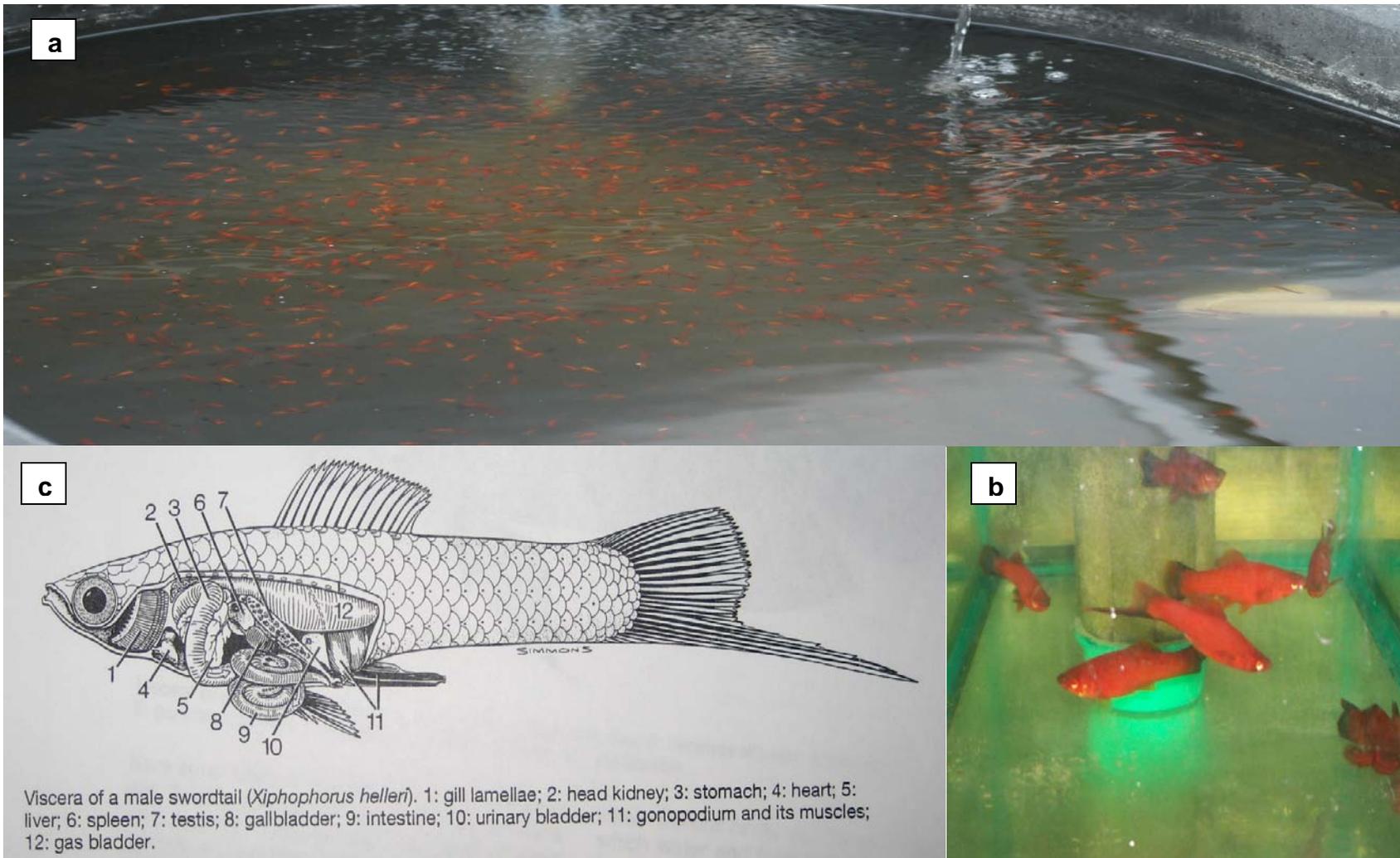


Figure 3-2a-c. Clockwise from top: (a) adult female and male redwag swordtails (*Xiphophorus hellerii*) held within large polyethylene tubs (~ 280 L) within a large greenhouse, (b) adult female and male red wag swordtails (note conspicuous 'sword' fin protrusion on ventral portion of caudal fin), (c) male swordtail (*Xiphophorus hellerii*) internal anatomy (Source: H.E. Evans *in* *Aquariology: Anatomy, Genetics, and Breeding*, Tetra Press 1992).

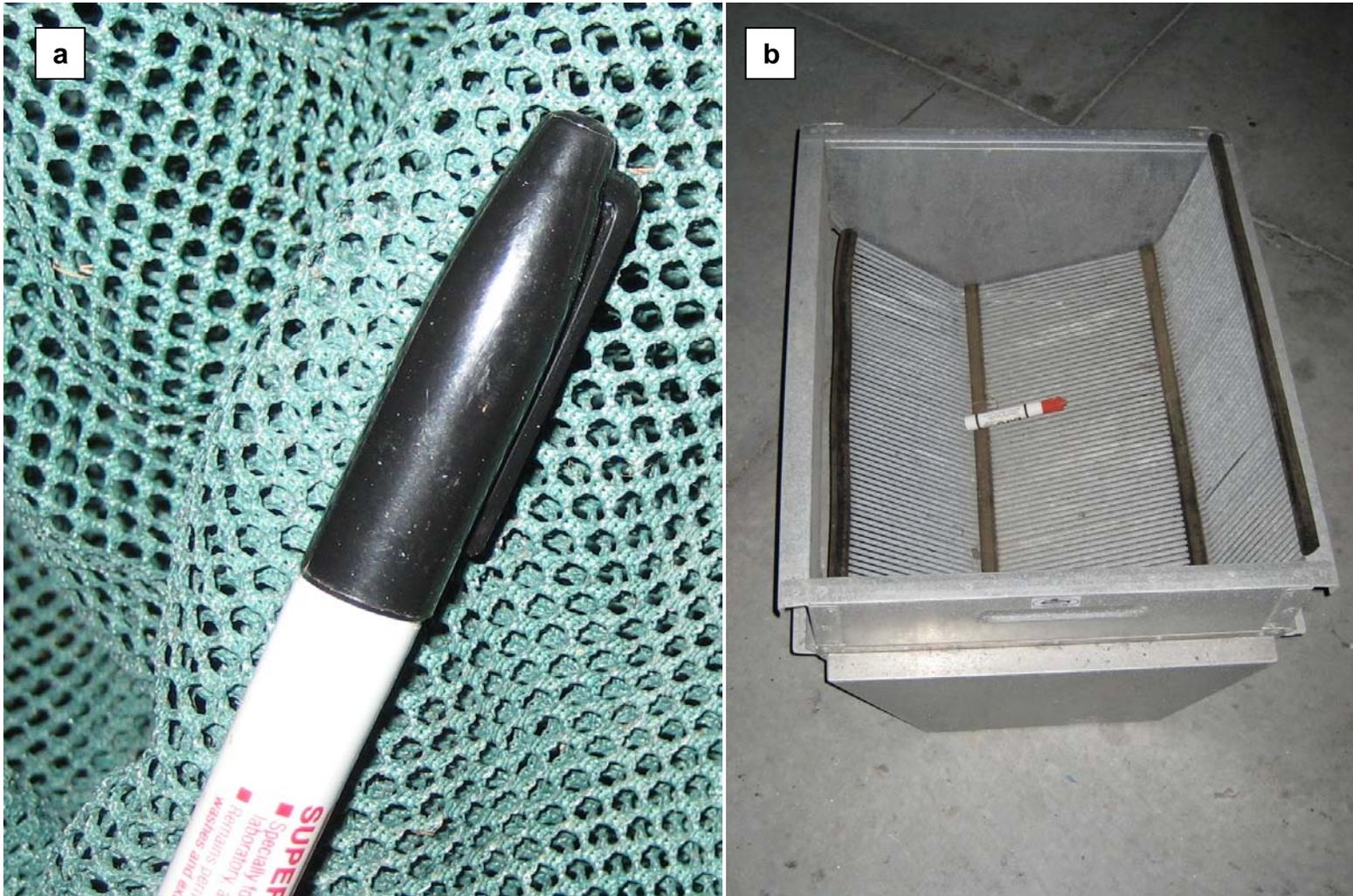


Figure 3-3a-b. Pond harvest equipment: (a) close-up of 7.6 m length \times 1.8 m height beach seine (4 mm \times 4 mm square mesh nylon multifilament), (b) floating fish grader, variety of removable grates insert into floating frame, inter-tine distance determines fish size/weight.

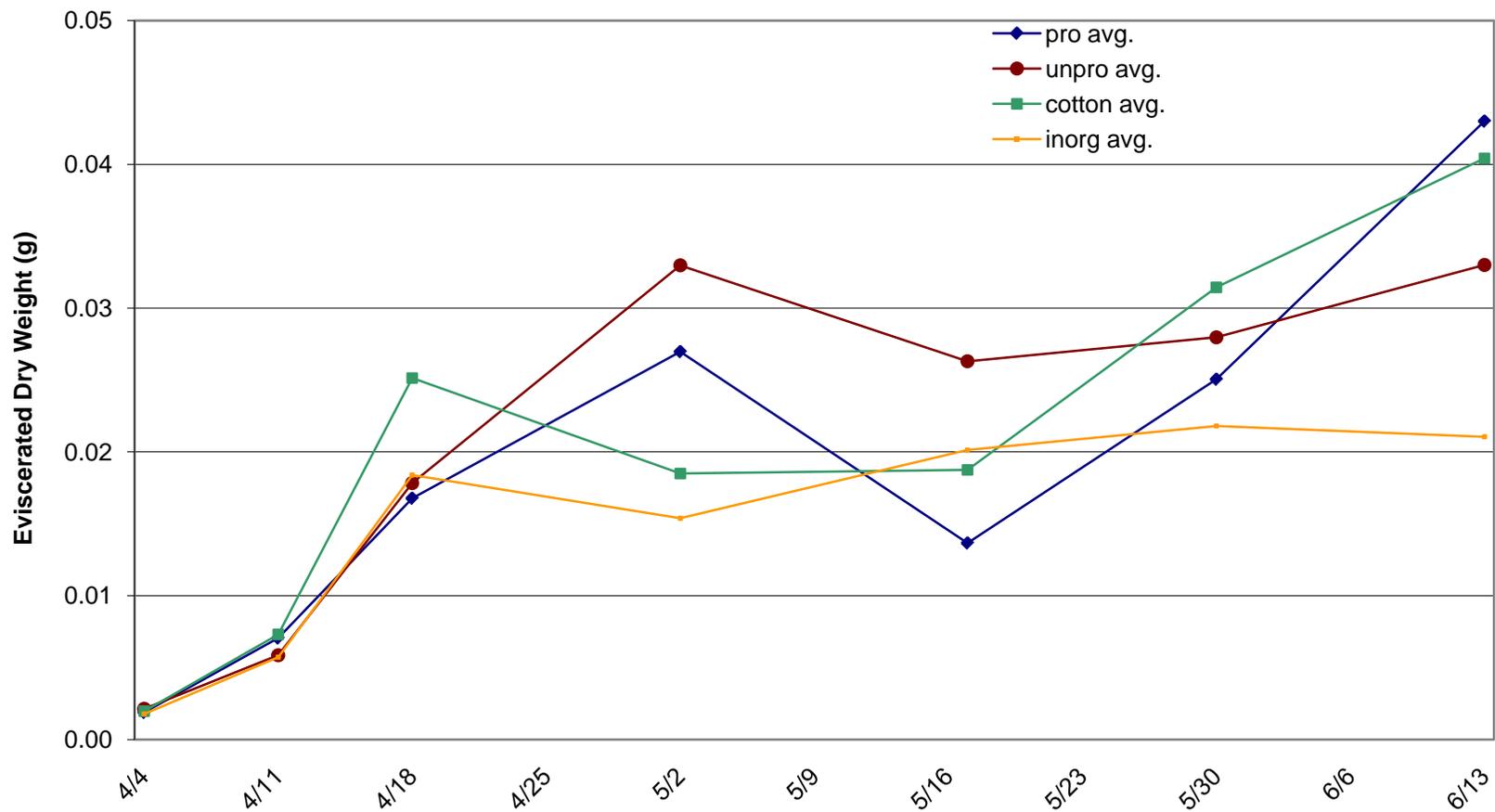


Figure 3-4. Pre-harvest fry mean dry weights, periodically sampled over 12 week pond trial (2006), n= 6 ponds per treatment.

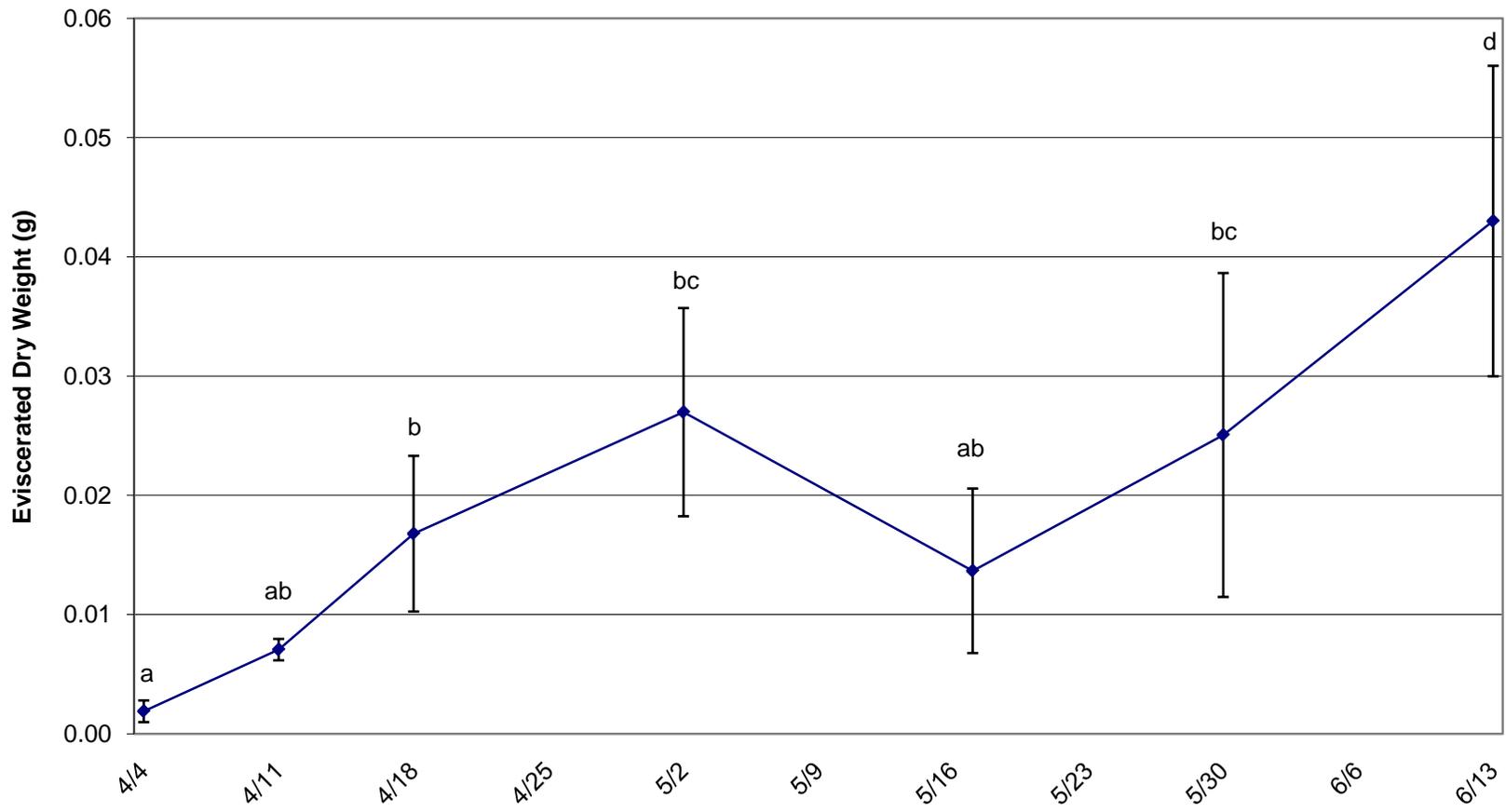


Figure 3-5. Processed feed (PRO) treatment mean fry dry weight (\pm 95% CI) sampled over 12 week trial (2006), six replicate ponds for each data point; differing letters denote statistical differences ($P < 0.05$).

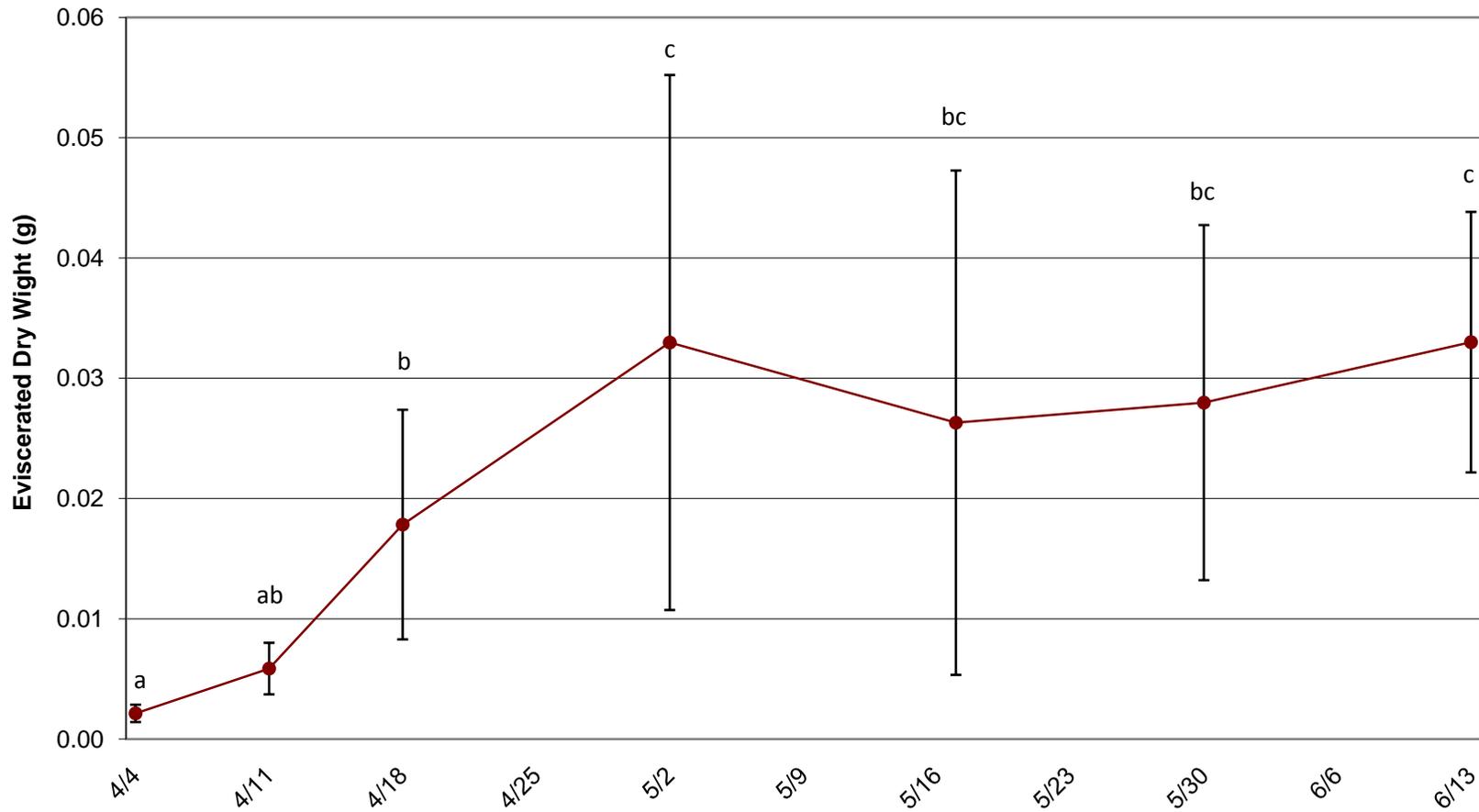


Figure 3-6. Unprocessed feed (UNP) treatment mean fry dry weights (\pm 95% CI) sampled over 12 week trial (2006), six replicate ponds for each data point; differing letters denote statistical differences ($P < 0.05$).

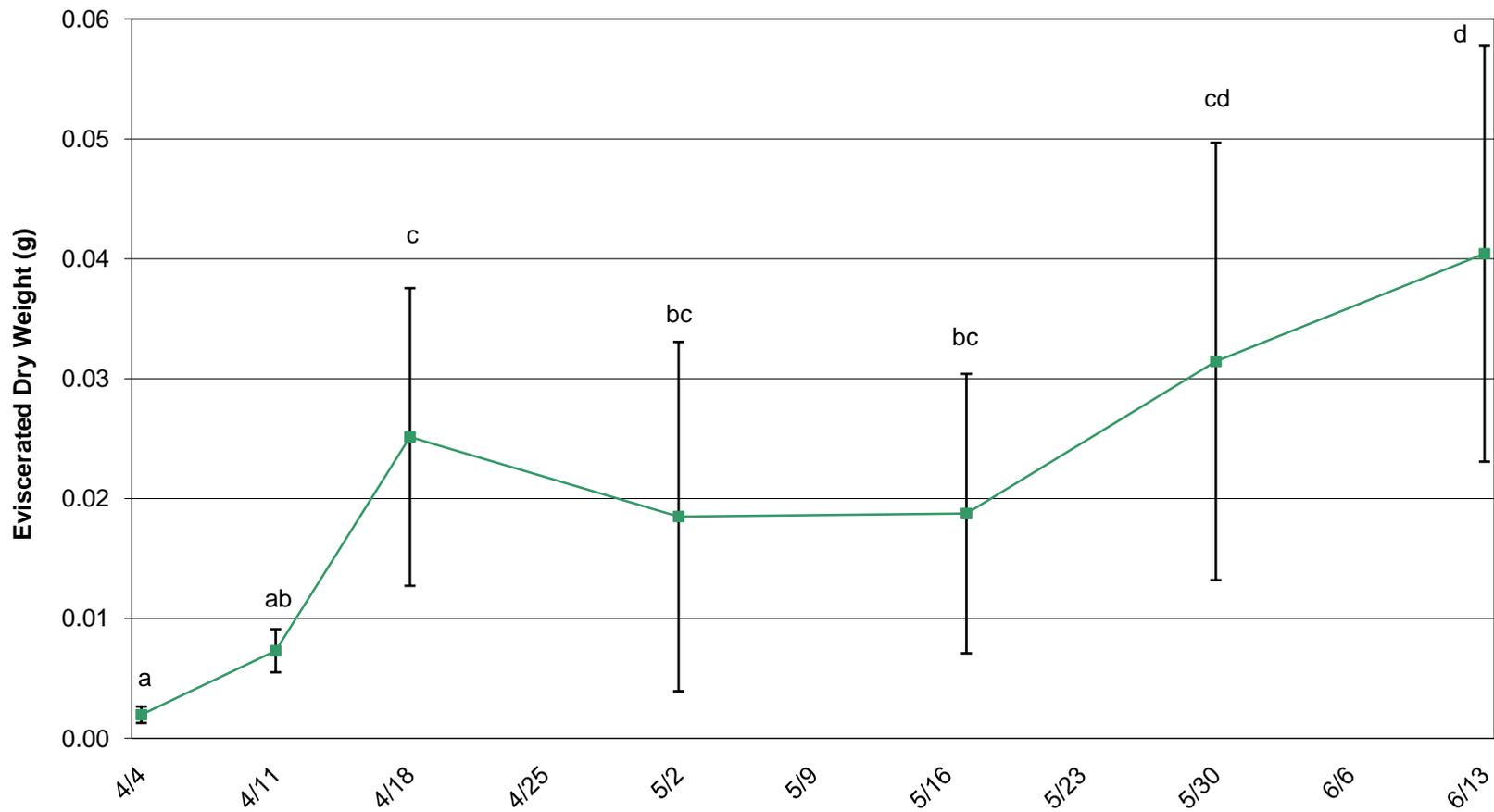


Figure 3-7. Cottonseed meal (CSM) treatment fry mean dry weights (\pm 95% CI) sampled over 12 week trial (2006), six replicate ponds for each data point; differing letters denote statistical differences ($P < 0.05$).

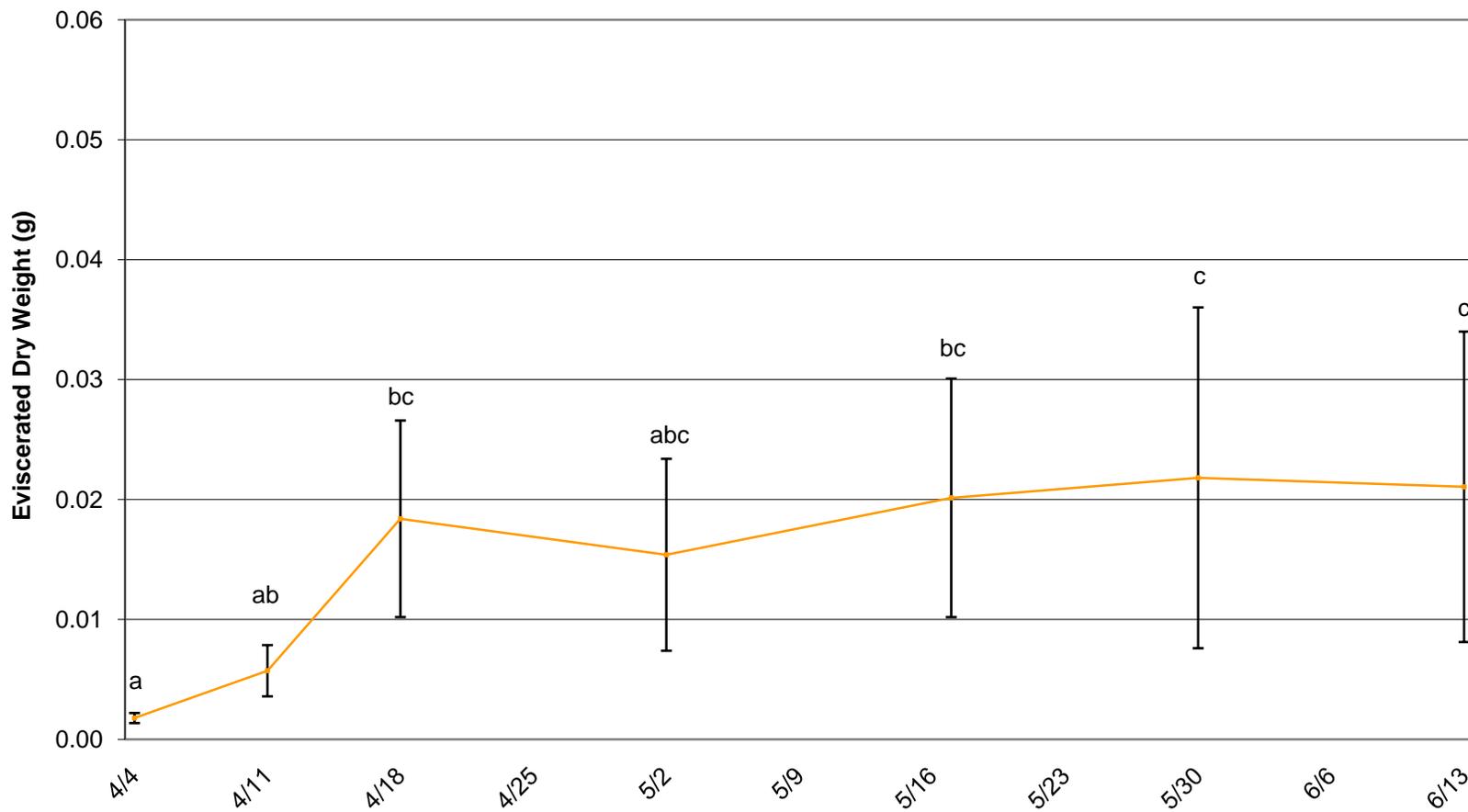


Figure 3-8. Inorganic fertilizer (INO) treatment mean fry dry weights (\pm 95% CI) sampled over 12 week trial (2006), six replicate ponds for each data point; differing letters denote statistical differences ($P < 0.05$).

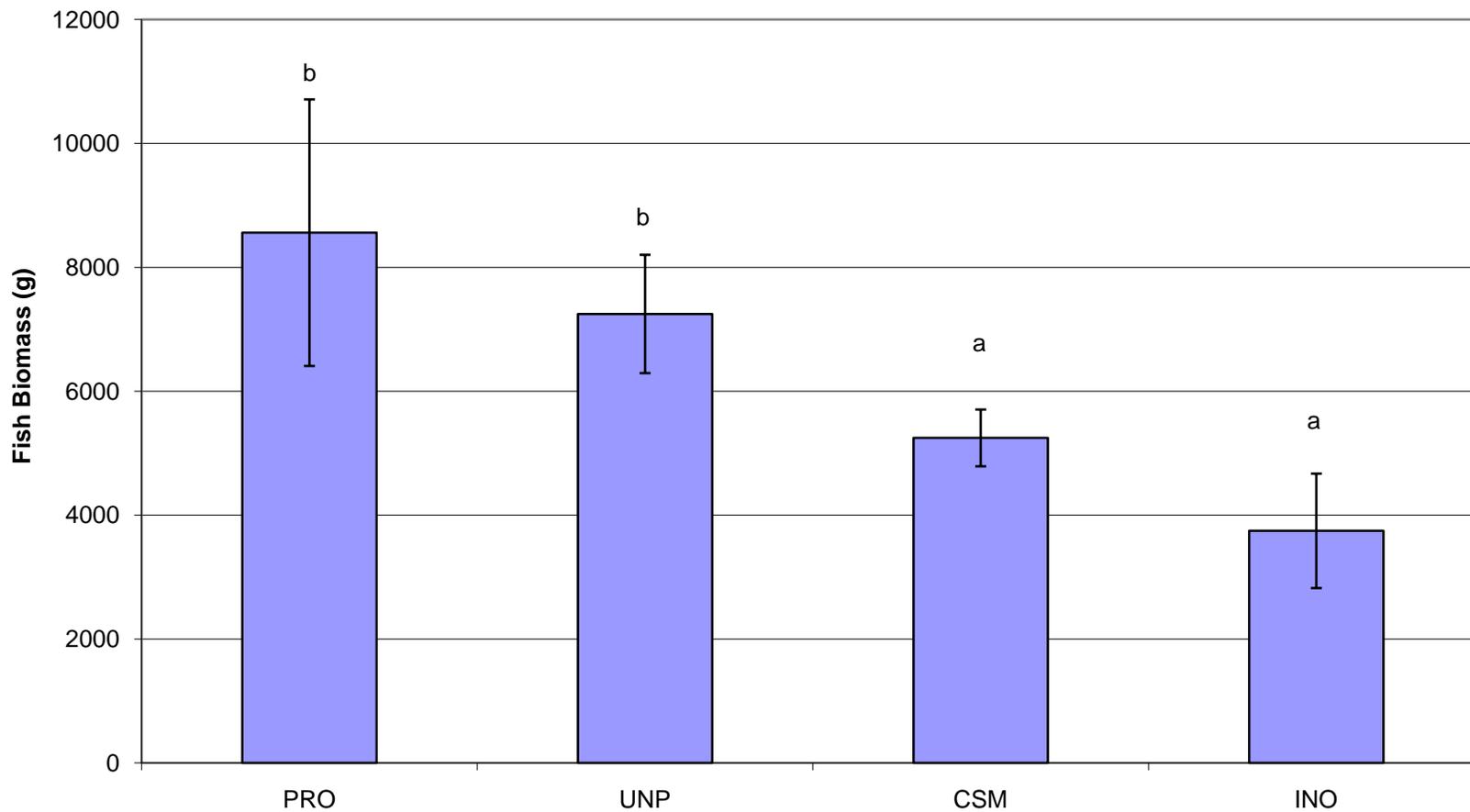


Figure 3-9. Mean fish biomass ($g \pm 95\%$ CI) at harvest, differing letters denote significant differences between treatments ($P < 0.05$, Tukey's multiple comparison test). Sample sizes: PRO (5), UNP (6), CSM (6), and INO (6).

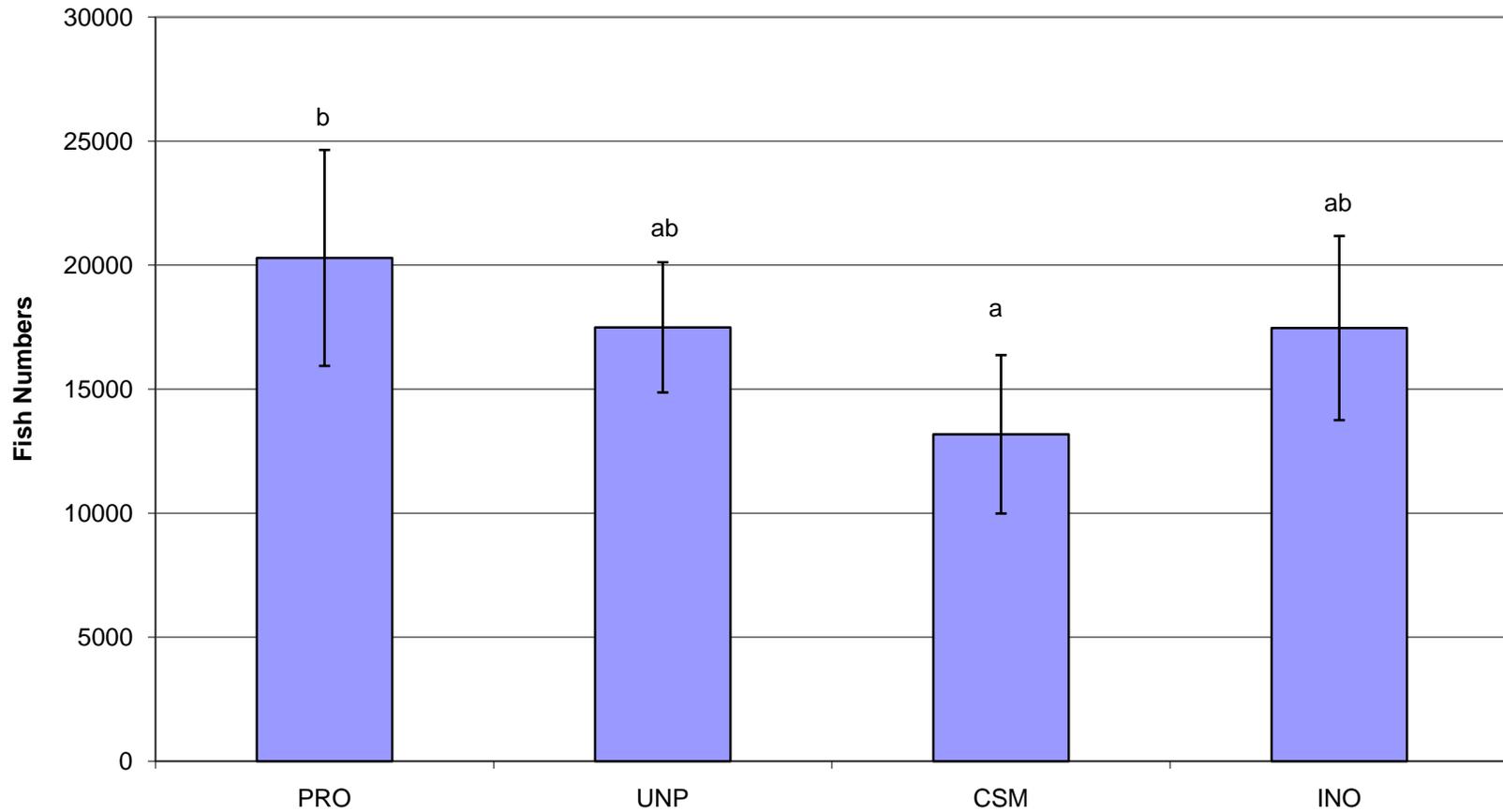


Figure 3-10. Mean fish numbers (#'s \pm 95% CI) at harvest, differing letters denote significant differences between treatments ($P < 0.05$, Tukey's multiple comparison test). Sample sizes: PRO (5), UNP (6), CSM (6), and INO (6).

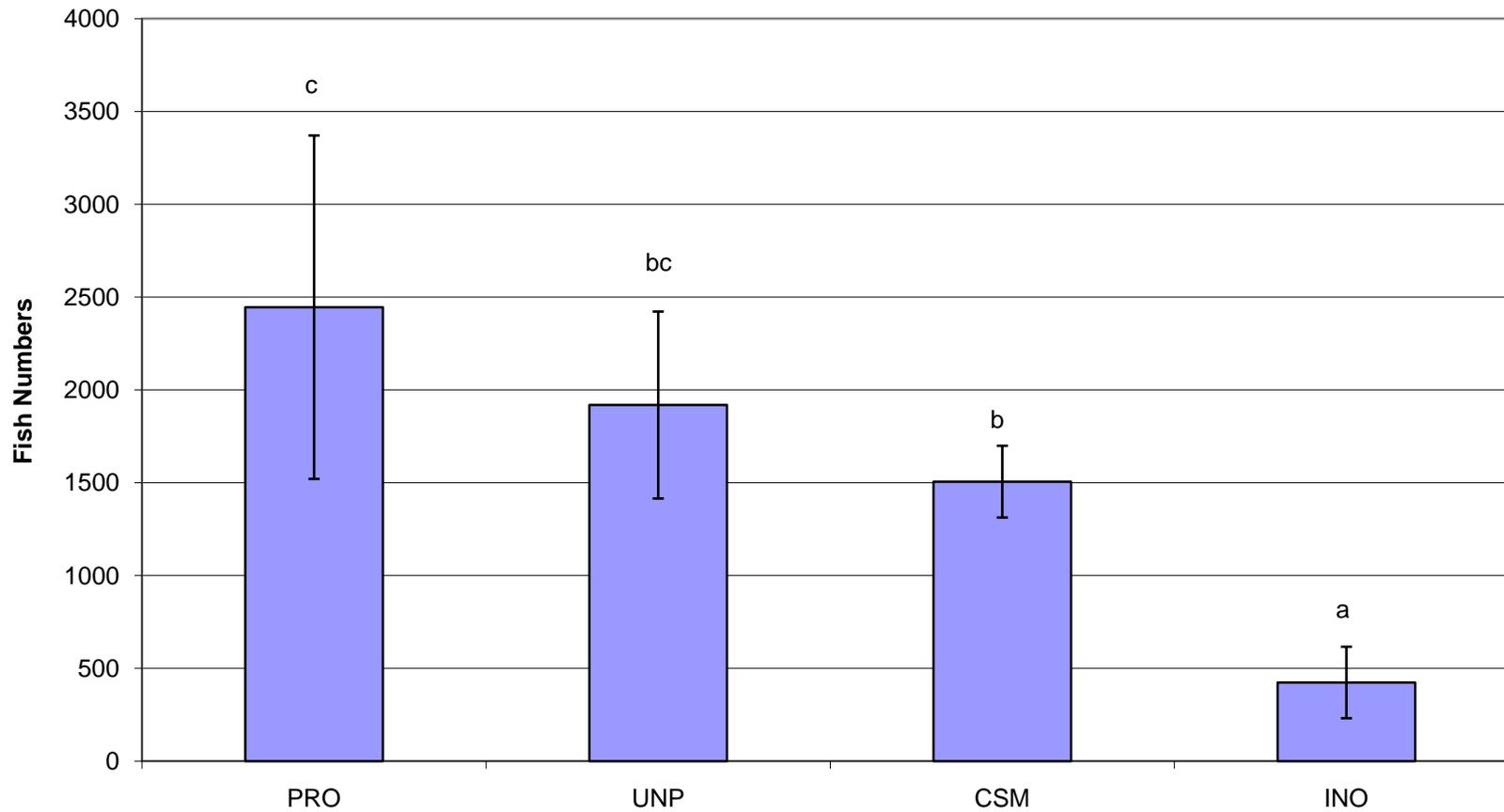


Figure 3-11. Mean marketable (> 31 mm SL) fish numbers (\pm 95% CI) at harvest, differing letters denote significant differences between treatments ($P < 0.05$, Tukey's multiple comparison test). Sample sizes: PRO (5), UNP (6), CSM (6), and INO (6).

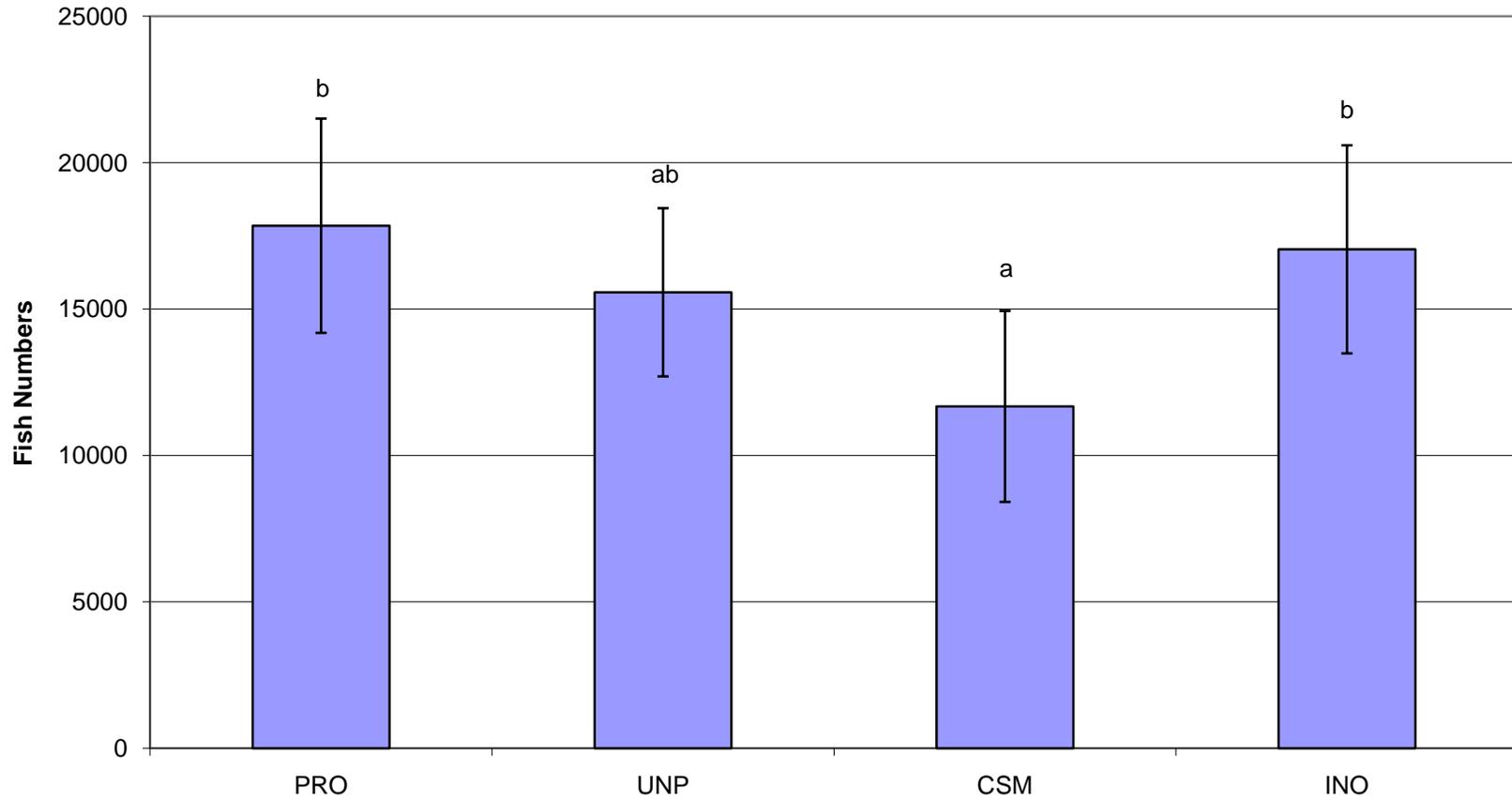


Figure 3-12. Mean unmarketable (≤ 31 mm SL) fish numbers ($\pm 95\%$ CI) at harvest, differing letters denote significant differences between treatments ($P < 0.05$, Tukey's multiple comparison test). Sample sizes: PRO (5), UNP (6), CSM (6), and INO (6).

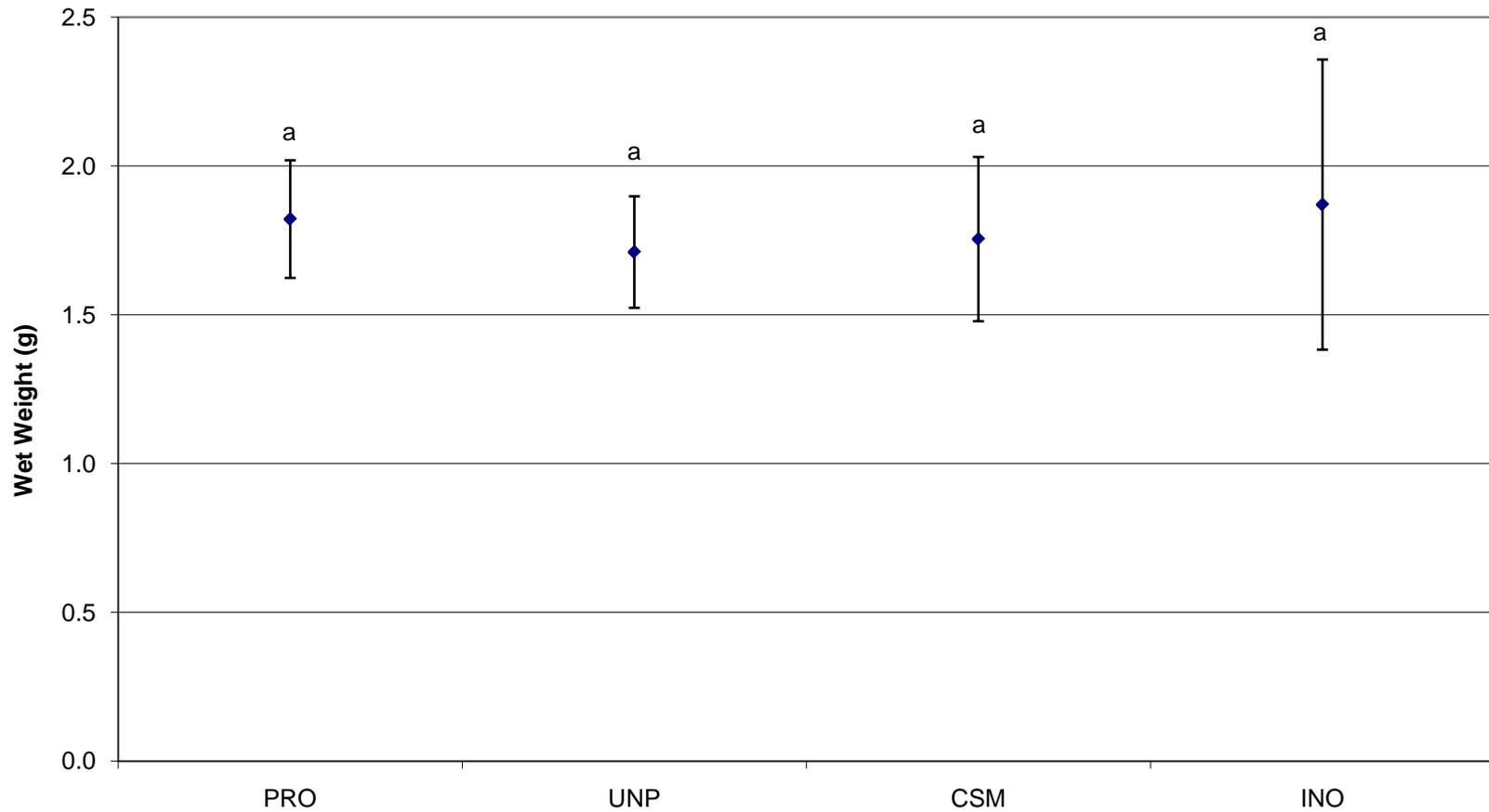


Figure 3-13. Mean individual marketable (> 31 mm SL) fish weights (\pm 95 % CI) at harvest, minimum 25 sub-sample fish from each replicate pond (data courtesy of M. Krasilovsky); differing letters denote significant differences between groups ($P < 0.05$, Tukey's multiple comparison test). Sample sizes: PRO (5), UNP (6), CSM (6), and INO (6).

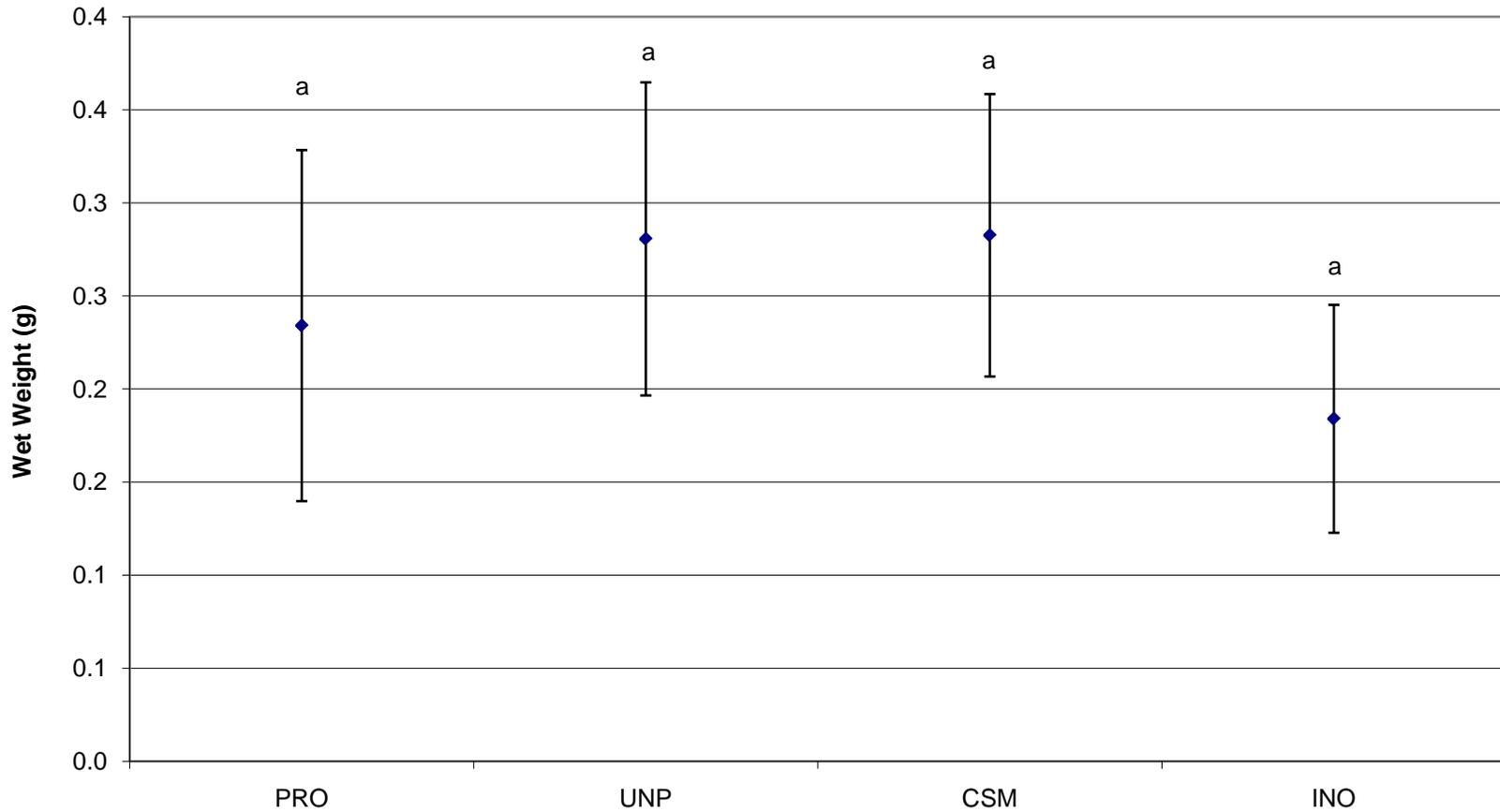


Figure 3-14. Mean individual unmarketable (≤ 31 mm SL) fish weights (± 95 % CI) at harvest, minimum 25 fish from each replicate pond (raw data courtesy of M. Krasilovsky); differing letters denote significant differences between groups ($P < 0.05$, Tukey's multiple comparison test). Sample sizes: PRO (5), UNP (6), CSM (6), and INO (6).

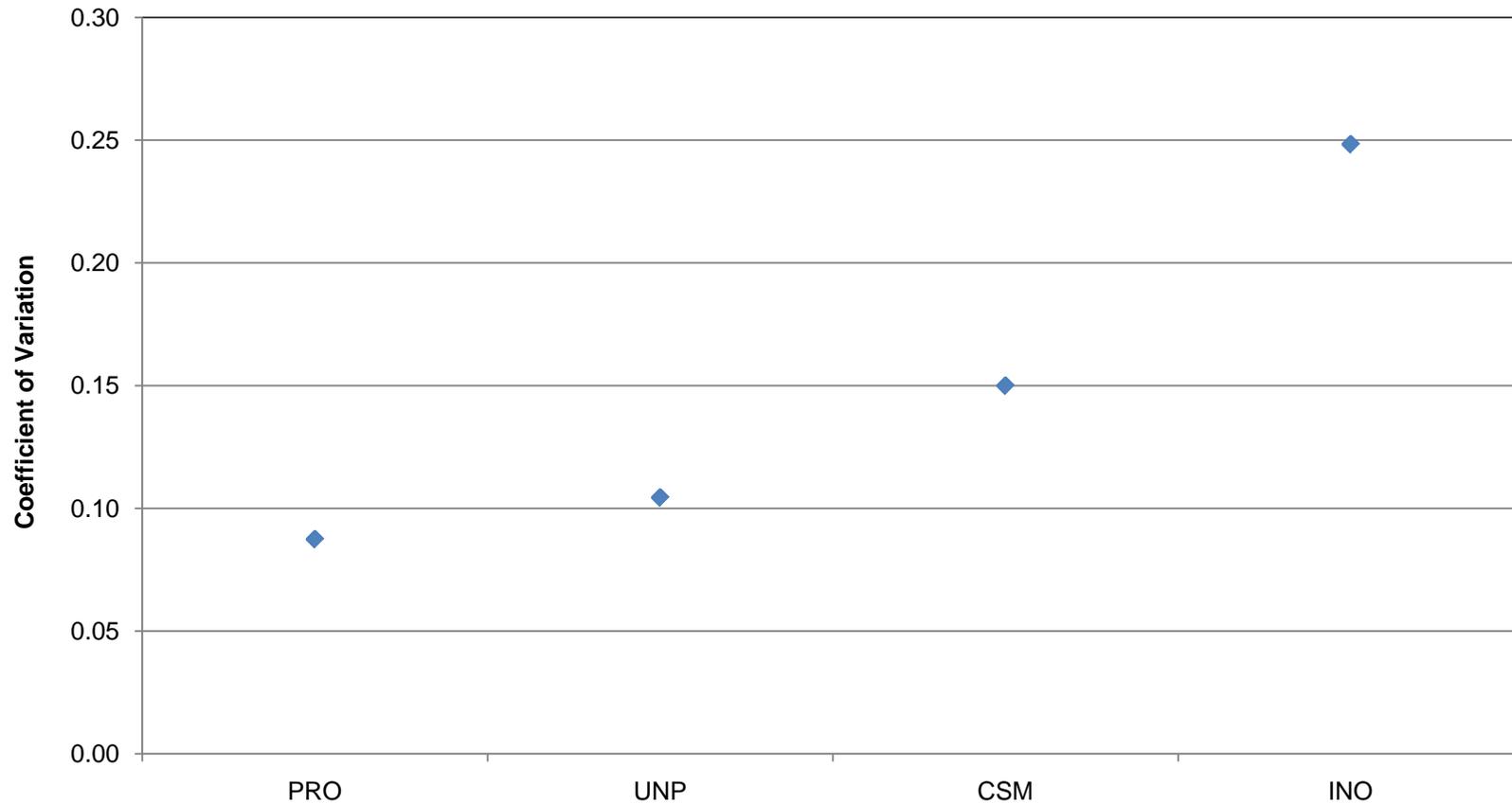


Figure 3-15. Large harvest fish (SL > 31 mm) individual fish wet weight coefficient of variation (sd/mean) values among applied pond nutrient treatments, minimum 25 sub-sample fish morphometric values from each replicate pond (data courtesy of M. Krasilovsky). Sample sizes: PRO (5), UNP (6), CSM (6), and INO (6).

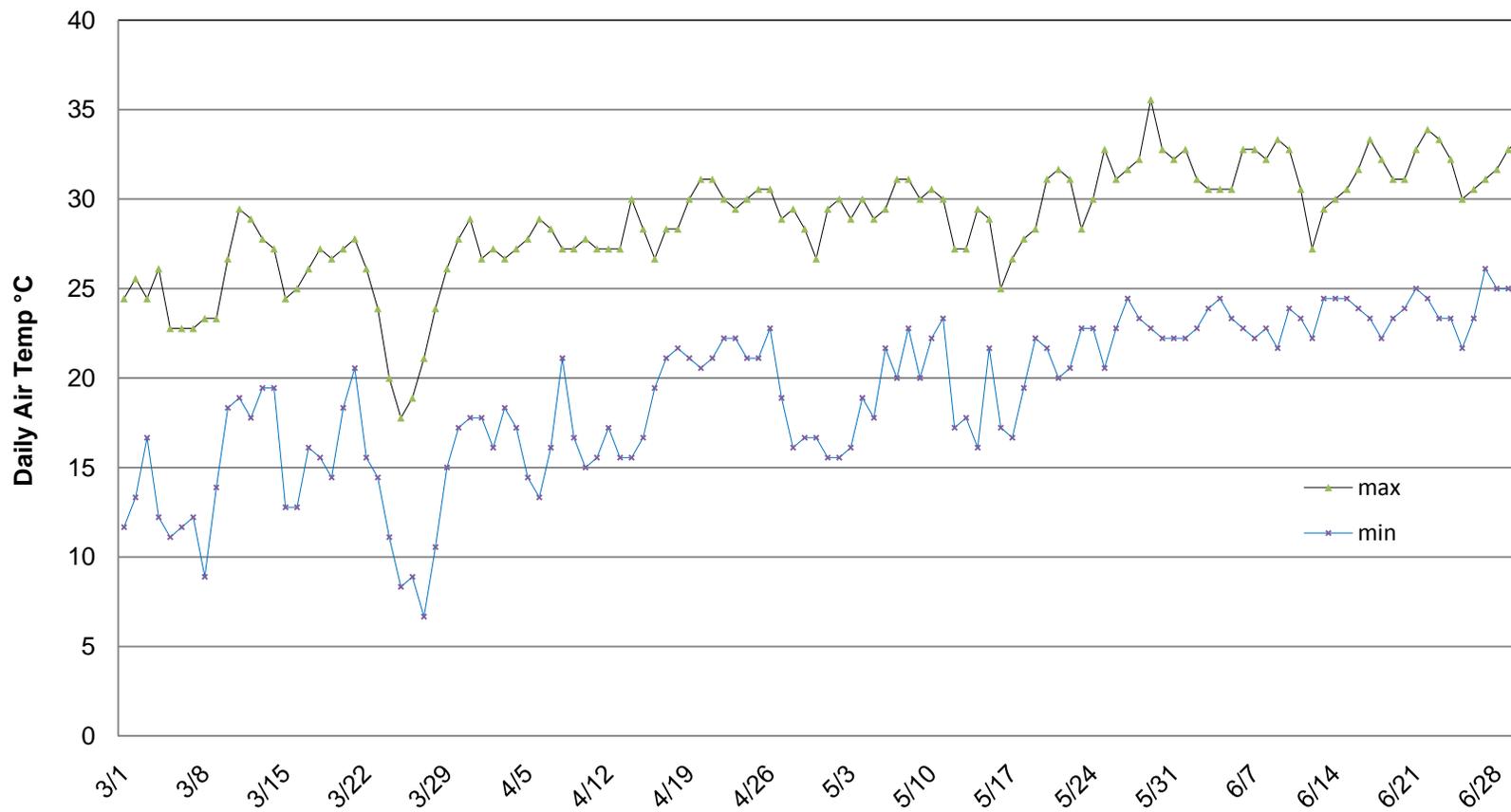


Figure 3-16. Air temperature daily maximum and minimum (°C) from 1 March to 30 June 2006 for Ruskin, Florida, USA (27° 42'57.72" N, 82° 15.70" W); data provided courtesy of the National Weather Service, Tampa Bay Area Station, Ruskin, Florida.

CHAPTER 4 OUTDOOR POND APPLIED NUTRIENT ISOTOPE TRACING TRIAL

In outdoor pond trials, commercial feed and fertilizer stable isotope signatures were used to follow nutrient flows within aquaculture pond trophic systems. Fish and plankton assemblage stable isotope signature determinations allowed the identification of potential nutritional sources utilized by fish and various planktonic taxa, evaluate the relative importance of potential nutrient sources, and describe trophic processes occurring within the different treatments (Harrigan et al. 1989, Fry 1991, Kling and Fry 1992, Gu et al. 1996, Lochmann and Phillips 1996, Hansson et al. 1997, Hoyer et al. 1998, Jones and Waldron 2003, Grey et al. 2004, Gregory-Eaves et al. 2007).

Commonly applied nutrients (feeds, organic and inorganic fertilizers) were applied to four pond treatments in a manner amenable to investigating research hypotheses rather than designed to maximize fish production and/or mimic typical commercial fish production methods. Fertilizers and feeds are typically applied in tandem to ornamental fish production ponds, but to reduce confounding factors in this study, fertilizers and feeds were used individually within designated treatment ponds after stocking brood fish.

Feeds and meals are commonly applied once/twice daily to commercial ponds near the study site (central and southern Florida); liquid fertilizers are typically applied either on a periodic basis (e.g., bi-weekly, weekly, semi-weekly), and/or on an as-needed basis, depending upon general pond appearance (absence, magnitude or even color of a conspicuous algal bloom). Cumulative labor expenses from daily applications of feeds and meals can become a major expense over the course of a single-production cycle (M. Krasilovsky unpublished data).

Cotton (*Gossypium hirsutum*) is a broadleaved, semi-tropical, C₃ dicotyledonous plant commonly grown within the southern and western United States (National Cotton Council of America website: cottonusa.org). Cottonseed meal is a high protein byproduct of cotton

processing, derived from cotton seeds and is a commonly applied organic fertilizer in aquaculture ponds within the southern United States (Teicher-Coddington et al. 1997).

In outdoor pond trials, isotopic compositions of fish, zooplankton, phytoplankton, and applied nutrients (feeds and fertilizers) were evaluated to determine nutrient fates (nutrient sinks) following nutrient application/inputs (two feeds, two fertilizers). In ponds that received commercial feeds, the lack of matching isotope signatures between fish and commercial fish feed inputs would indicate a lack of trophic coupling (0 % energy/nutrient transfer), and completely matching isotope signatures would indicate an ideal 100 % energy/nutrient transfer between the fish and applied feed (Lochmann and Phillips 1996, Lochmann et al. 2001). In ponds that only receive fertilizer, closer isotopic signatures between fish and fertilizer should indicate closer trophic coupling of fish to fertilizers. If fewer trophic levels separate fertilizers and fish, less undesirable organism production (e.g., crayfish, insects, tadpoles, and snails) and/or nutrient losses (e.g., mineralization, sediment losses, chemical precipitation due to redox conditions, and atmospheric outgassing) would be expected. This would likely indicate that applied nutrients assimilated by fishes were moving through fewer trophic intermediaries. With higher energy/nutrient transfer efficiencies, greater fish production should be observed relative to systems with lower energy/nutrient transfer efficiencies due to greater numbers of trophic intermediaries and/or sources of energy/nutrient loss (high pest species production, sediment losses, atmospheric volatilization, etc.), barring unforeseen losses from disease, predation, catastrophic weather events, etc.

In addition to pond fish and applied nutrient isotope signature measurements, periodic isotopic signature measurements of pond plankton fractions (small, medium and large plankton assemblages) also were made. Isotope signatures were used to determine trophic positions of

plankton assemblages (potential fish prey), fish fry, and small and large harvest fish within the different treatments. In addition to determining trophic positions, primary sources of nutrition for harvested fish and plankton size assemblages were identified, as well as the relative contributions (% basis) of plankton assemblages and applied nutrients to swordtail diets within the respective treatments.

The majority of aquatic, marine, and terrestrial food web studies that have utilized stable isotope tracers, have traced two biologically important elements, carbon and nitrogen within the ecosystem(s) of interest (Minigawa and Wada 1984, Fry et al. 1999, Frazer 1996, Gu et al. 2001). Natural abundances vary (DeNiro and Epstein 1978, 1981a, Fry 1991) for the stable isotopes of carbon [98.89 % [^{12}C] and 1.11 % [^{13}C]] and nitrogen (99.63 % [^{14}N] and 0.37 % [^{15}N]). The relative abundances of carbon and nitrogen isotopes vary within ecosystems according to primary producer origins (plant species/physiology), ecosystem type (terrestrial/aquatic/marine/estuarine), inorganic nutrient form [atmospheric carbon and nitrogen, or DIC and DIN in solution] and organism/taxa trophic position (1° producer, 1° consumer, 2° consumer, 3° consumer, herbivore, carnivore, omnivore) among others (Rounick and Winterbourn 1986, Peterson and Fry 1987, Takahashi et al. 1990, Fry 1991, Vander Zanden and Rasmussen 2001, Hall 2004, Gregory-Eaves et al. 2007).

Carbon isotope signatures are primarily used to identify food web base nutrient source(s) due to the high degree of carbon isotope signature conservation among primary producer(s) and their consumers within an ecosystem. Carbon isotope signatures typically undergo little isotopic change between trophic levels (enrichment $\sim 0.0 - 1.0$ ‰ $\Delta\delta^{13}\text{C}$), allowing basal nutrient sources within an ecosystem to be fairly easily identified (DeNiro and Epstein 1978, 1981b, Estep and Vigg 1985, Gregory-Eaves et al. 2007).

Nitrogen isotope signatures are primarily used to determine trophic relationships among organisms within an ecosystem (Peterson and Fry 1987, Wada 1980, Hideshige and Wada 1990, Fry et al. 1999, Vander Zanden and Rasmussen 1999, Yoshii et al. 1999, Frazer 1996, Gu et al. 2001, Fry 2006, De Brabandere et al. 2007). Within an ecosystem, nitrogen isotope enrichment magnitudes (typically 3.4 ‰ $\Delta\delta^{15}\text{N}$) between trophic levels are typically much greater than those occurring for carbon (DeNiro and Epstein 1978, 1981a). This allows nitrogen isotope signatures (measured via ^{14}N and ^{15}N content) to be used to determine an organism's relative trophic position (1° producer, 1°, 2°, and 3° consumer, etc.) as well as the number and identity of trophic-level intermediaries between an organism, or biological material of interest (e.g., excreted urine or feces) and the ecosystem's basal nutrient(s); if a thorough survey of ecosystem taxa (e.g., fish, zooplankton, phytoplankton, applied nutrients, etc.) nitrogen isotope signatures is undertaken (DeNiro and Epstein 1981, Minigawa and Wada 1984, Estep and Vigg 1985). Similar to carbon isotope tracers, nitrogen isotope tracers can also be used to identify presumed nutrient base(s) within a food web, but with a much lower degree of accuracy.

Within the outdoor pond trial, the primary working hypothesis, regarding the two commercial feed treatments, was that fish within these ponds were consuming and assimilating applied feeds largely to the exclusion of available live foods. Carbon and nitrogen contained within feeds would become sequestered within their respective treatment fish, and the movements of these nutrients could be traced and their assimilation verified using stable isotope analyses techniques. The primary working hypothesis, regarding the two fertilizer treatments, was that nutrients containing carbon and nitrogen would pass through a number of trophic guilds/intermediaries before becoming sequestered within the fish.

A primary goal of the study was to examine the relative magnitudes of enrichment occurring within ponds from applied nutrients to major planktonic guilds, and ultimately fish, not the actual isotope signature values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of the taxa within each pond treatment. Larger isotopic differences between fertilizer nutrients and fertilizer treatment fish relative to commercial feeds and feed treatment fish were expected, and these isotopic differences also were expected to be inversely related to target species (*Xiphophorus hellerii*) biomass production at harvest. Reasonable intermediate isotopic signature values for the postulated food chain guilds were viewed as validation of the primary working hypotheses concerning food web/chain structure, food chain length, and fish production efficiencies among the four pond nutrient treatments.

Methods

Plankton Sample Collection

Weekly water samples were taken from each of 24 aquaculture ponds (Chapter 3 methods) for 12 weeks and analyzed colorimetrically for basic water chemistry (pH, NO_2^- , TAN, hardness, alkalinity; Hach Laboratories: www.hach.com), and chlorophyll [a] concentrations. Duplicate weekly water samples were collected to determine the isotopic signatures (carbon and nitrogen) of three plankton size assemblages [large ($> 200 \mu\text{m}$); medium (32 - 200 μm); small (1 - 32 μm); DeVries and Stein 1991], and taxonomic composition and relative abundances of the large plankton ($> 200 \mu\text{m}$) assemblages within the ponds of the four (processed feed, unprocessed feed, cottonseed meal organic fertilizer, and liquid inorganic fertilizer) applied nutrient treatments (Chapter 5). An integrated water column sampler (Figure 4-2; 35 mm I.D. schedule 40 PVC pipe x 3.05 m length) with a gravity-operated one-way flap valve at the anterior to prevent sample loss was used to combine four pond subsamples (one from each bank) into one aggregate sample for each pond. The sampler was inserted (valve end first) into each

pond obliquely from the bank and care was taken to not touch the bottom in order to avoid entraining bottom sediment, detritus, and benthic organisms. Samplers and collection buckets were rinsed with an initial sample of pond water from the target pond which was then discarded to prevent cross-contamination of samples from previously sampled ponds.

Collected water was mixed vigorously and two one-liter samples were drawn from the aggregate sample for each pond for isotopic and taxonomic plankton analyses (Chapter 5), additionally, one 100-ml sample was taken for chlorophyll [a], and total nitrogen and phosphorous content analyses (M. Krasilovsky unpublished data). One set of one-liter pond water samples was immediately fixed with Lugol's solution preservative (APHA/AWWA 1999) and stored within light excluding containers for later filtering into plankton size assemblage fractions for microscopic analysis.

The remaining 'unfixed' one liter pond water samples were kept refrigerated (approx. 4 °C) after collection and serially filtered within 24 hrs through the following mesh sizes: 200 µm, 35 µm, and 1 µm. Retained particles from each sieve were vacuum filtered onto a Whatman GF/C pre-combusted glass fiber filter disk (47-mm diameter), placed into individual 58-ml (2-oz. nominal Whirlpak[®]) presterilized polyethylene bags and immediately frozen at -4 °C; sample disks were typically frozen for eight to twelve months prior to freeze drying. Prior to freeze drying, frozen samples were transferred to individual conical tubes and frozen in an ultra low temperature freezer (~ -74 ± 2 °C) for 16-24 hours.

Individual glass fiber filter disks were freeze dried for 24 hrs at approximately -56 to -60 °C. After freeze drying, individual filter disks were carefully folded with forceps and placed into sterile 1.7-ml microcentrifuge tubes; forceps were swabbed with alcohol (70% ethanol) and low-lint laboratory wipes (Kimwipes[®] Kimberly Clark Corp.) between samples to minimize cross

contamination. Due to the variable amount and small particle size of organic material on the two smallest size fraction disks (retained particles: 35 - 200 μ m and 1 - 35 μ m), these disks were sent directly to Northern Arizona University Colorado Plateau Stable Isotope Laboratory (NAUCPSIL). Once at NAUCPSIL, technicians with previous experience using the stable isotope mass spectrometer, estimated how much filter material and associated particulate matter to use in sample analysis (Richard Doucett pers. comm.). The large (> 200 μ m) plankton (primarily zooplankton; Chapter 5) fraction sample preparation procedure, consisted of: (1) removing the freeze dried sample disks from their presterilized microcentrifuge storage capsules, (2) sample disks were then carefully unfolded and attached to a small vibrating motor, (3) as the sample disk vibrated, its surface was lightly brushed using a small nylon bristle brush (Figure 4-3) to separate and concentrate retained particles into preweighed (± 0.001 mg) tin combustion capsules, and (4) sample and capsule were then weighed to the nearest (± 0.001 mg) using a Cahn[®] Electrobalance (model 25). Tin sample capsules were carefully folded and packed with forceps for shipment to NAUCPSIL for analysis. Instruments (forceps, aluminum funnel, acrylic capsule holder) were sprayed with 70% isopropyl alcohol and cleaned with low-lint laboratory wipes, or had particles removed (brushes) using filtered and dried ‘canned’ compressed air (Innovera[™] canned air duster) between samples to minimize possible cross contamination.

Isotopic Analyses

Carbon and nitrogen isotopic analyses ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) were performed upon pre-harvest fry samples and plankton collected at regular intervals during the outdoor pond trial (Chapter 3; methods). Weekly plankton samples were divided into three size fractions and presumed trophic assemblages consisting of a large (primarily) zooplankton fraction (> 200 μ m), a medium mixed plankton (rotifers and algae) fraction (32 - 200 μ m), and a small (primarily) phytoplankton

fraction (1 – 32 μm). Plankton size assemblage isotopic signatures were compared among treatments and sampling dates (a maximum of 12 weekly samplings for plankton) using two-way repeated measures ANOVA. Pairwise differences were determined using Bonferroni post comparison tests ($P < 0.05$), (Zar 1984, Ott 2000).

Time-Averaged Plankton Assemblage Isotopic Analyses

Plankton size assemblage isotopic data were time averaged ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) for each treatment. Time-averaged isotopic data were used for comparisons with other trophic groups (size assemblages) within the same treatment, and within the same trophic group (e.g., large zooplankton assemblage) among the other pond nutrient treatment groups. Comparisons were primarily made using one-way ANOVA, with pairwise differences made using Tukey's multiple comparison test ($P < 0.05$), (Zar 1994, Ott 2000).

Trophic Group Isotopic Signature Differences

Carbon and nitrogen isotopic signature differences among presumed trophic groups, and applied nutrient treatments were calculated as simple arithmetic differences, and analyzed. For example, isotopic differences between small harvest fish and large zooplankton were derived as:

$$\delta^{13}\text{C}_{(\text{sm fish-zoo})} = \delta^{13}\text{C}_{(\text{sm harvest fish})} - \delta^{13}\text{C}_{(\text{lg zooplankton})}; \text{ per pond treatment.}$$

$$\delta^{15}\text{N}_{(\text{sm fish-zoo})} = \delta^{15}\text{N}_{(\text{sm harvest fish})} - \delta^{15}\text{N}_{(\text{lg zooplankton})}; \text{ per pond treatment.}$$

Due to the small magnitude of carbon isotope differences between fish, nutrients and plankton groups, which often resulted in somewhat arbitrary positive and negative arithmetic signs for carbon isotope signature differences between groups, for statistical analyses involving carbon isotope signature differences, absolute values of carbon isotope signature differences were used (Motulsky 1995). For comparisons of trophic position differences between groups, simple arithmetic differences in their native values were used (non-absolute isotopic signature

values). For a given element, 95% confidence intervals for the differences between the isotopic signature means of different taxa were calculated using pooled standard error (pooled SE; Parker 1979).

Trophic Level Determination

Carbon isotope signature determinations are primarily used for identifying the basal nutrient source(s) for consumer organism(s) within a food web (DeNiro and Epstein 1978, Harrigan et al. 1989, Fry 1991, Zohary et al. 1994, Vander Zanden and Rasmussen 1999, Saito et al. 2000). Based upon the carbon isotope signature of a applied nutrients, primary producers, and consumer organism(s), the following equation was used to back calculate, and identify, the carbon isotope signature of the primary producer or anthropogenic nutrient source within a pond treatment's food web (modified from Saito et al. 2000):

$$\delta^{13}\text{C}'_{\text{prim}, i} = \delta^{13}\text{C}_i - (\text{TL}_i - 1) \cdot \Delta\delta^{13}\text{C}' \text{ (Equation 4.1)}$$

where:

$\delta^{13}\text{C}'_{\text{prim}, i}$ = estimated average carbon signature of primary producer/nutrient sources (‰) for consumer i .

$\delta^{13}\text{C}_i$ = measured carbon signature for consumer i (‰).

TL_i = estimated trophic level of consumer i ; ($\text{TL}_i \geq 1$).

$\Delta\delta^{13}\text{C}'$ = estimated trophic enrichment of carbon signature per trophic level.

For this study, the estimated carbon isotope signature trophic enrichment value per trophic level ($\Delta\delta^{13}\text{C}'$ or $\Delta\delta^{13}\text{C}$), was derived from the mean carbon isotope signature trophic enrichment rate observed in the indoor feeding trial (Chapter 2; $\Delta\delta^{13}\text{C} = 0.64$ ‰).

With slight modification, this equation also was used to determine the trophic difference between different consumer groups (e.g., primary, secondary, and tertiary consumers). Simple arithmetic differences in carbon and nitrogen isotope signatures were used to determine trophic distances between groups:

$$\delta\text{TDC}_{ij} = [(\delta^{13}\text{C}'_j - \delta^{13}\text{C}'_i) / \Delta\delta^{13}\text{C}] \cdot (-1) \quad (\text{Equation 4.2})$$

where:

δTDC_{ij} = carbon isotope signature derived trophic distance (levels)

estimate between taxon i, j .

$\delta^{13}\text{C}_i$ = (abs. value) carbon signature consumer i (‰).

$\delta^{13}\text{C}_j$ = (abs. value) carbon signature for consumer j (‰)

(assuming trophic level $j >$ trophic level i).

$\Delta\delta^{13}\text{C}$ = estimated trophic enrichment of carbon signature per trophic level.

Although carbon isotope signatures were used to calculate trophic distances, this was primarily performed as a redundant check upon trophic distances estimated using nitrogen isotope signatures. Carbon isotope signatures are primarily used to identify the basal nutrient source(s) within an ecosystem, and are considered superior to nitrogen for this purpose (Fry and Parker 1979, Haines and Montague 1979, Gu et al. 2001, Vander Zanden and Rasmussen 2001, McCutchan et al. 2003, Fry 2006). Nitrogen isotope signatures are primarily used to estimate the trophic position of consumer organisms within a putative food web, and are considered a better determinant of trophic position than carbon isotope signatures (Peterson and Fry 1987, Vander Zanden et al. 1997, Vander Zanden and Rasmussen 1999). An equation identical to that given for trophic distance calculation based upon carbon isotope signature, was used for trophic distance calculations using nitrogen isotope signatures:

$$\delta\text{TDN}_{ij} = (\delta^{15}\text{N}_j - \delta^{15}\text{N}_i) / \Delta\delta\text{N} \quad (\text{Equation 4.3})$$

where:

$\delta^{15}\text{N}_i$ = nitrogen signature consumer *i* (‰).

$\delta^{15}\text{N}_j$ = nitrogen signature for consumer *j* (‰).

(assuming trophic level *j* > trophic level *i*).

δTDN_{ij} = nitrogen isotope derived estimated trophic distance (levels)
between taxon *i*, *j*.

$\Delta\delta^{15}\text{N}$ = estimated trophic enrichment of nitrogen isotope signature per
trophic level.

The estimated nitrogen isotope signature trophic enrichment rate ($\Delta\delta^{15}\text{N}$) was derived from the indoor feeding trial (Chapter 2; $\Delta\delta^{15}\text{N} = 3.03$ ‰). The higher trophic enrichment rates observed for nitrogen isotope signatures (typically ~ 3.4 ‰ $\Delta\delta^{15}\text{N}$ per trophic level) relative to carbon signatures (typically ~ 0.0-1.0 ‰ $\Delta\delta^{13}\text{C}$ per trophic level) and associated high potential for overlap of carbon isotope signature error measures, is the primary reason that nitrogen isotope signatures rather than carbon isotope signatures are used to describe trophic positions within hypothetical food webs (DeNiro and Epstein 1979, 1981a, Minigawa and Wada 1984, Vander Zanden and Rasmussen 2001, McCutchan et al. 2003, Fry 2006).

Carbon and nitrogen isotope signature-based trophic source (primarily $\delta^{13}\text{C}$), and trophic position (primarily $\delta^{15}\text{N}$), estimates were made using the above equations to determine relative trophic distances from presumed basal nutrient sources, and other trophic groups (Levine 1980). The same equation (based upon Equation 4.3), was used to estimate a consumer organism's trophic position relative to primary producers on a continuous trophic scale (Vander Zanden et al. 1997):

$$TP'_i = (\delta^{15}N_i - \delta^{15}N_{pp}) \div \Delta\delta^{15}N_i \quad (\text{Equation 4.4})$$

where:

TP'_i = estimated trophic position of consumer i .

$\delta^{15}N_i$ = nitrogen isotope signature of consumer i .

$\delta^{15}N_{pp}$ = nitrogen isotope signature of presumed primary producer pp .

$\Delta\delta^{15}N$ = nitrogen isotope signature enrichment rate.

Due to the plankton sampling and plankton size fractionation (sieve choice) experimental design methodologies, plankton size assemblage trophic position (TP_i) and function (primary producer, primary consumer, secondary consumer, etc.) estimates were based upon trophic position assumptions based upon nominal plankton taxa size. Assuming that large zooplankton were more likely predate upon smaller zooplankton than *vice versa* (Carpenter et al. 1985, 1987, Briand and Cohen 1987, Cohen et al. 1993, Brett et al. 1994, Warren and Spencer 1996, Vander Zanden et al. 1999, Post 2002b, Hoeninghaus et al. 2008). The role of fish and applied nutrients within pond ecosystems were assumed to be the top and bottom of their food chains, respectively. Feeds (processed and unprocessed) and cottonseed meal, were considered to be equivalent to primary producers within living food webs. Cottonseed meal was a special case in that it is believed to promote algal growth as a plant or green ‘manure’, but is also a highly nutritious seed grain when consumed directly (Fowler 1980, Teichert-Coddington 1997). As opposed to producing discretely defined trophic level/position estimates (positive integer values), Equation 4.4 can produce a continuum of trophic position values (continuous variable), which are better suited for a highly omnivorous, and potentially cannibalistic species such as the swordtail (Dionne 1985, Jones et al. 1998a, 1998b, 2007, Kruger et al. 2001, Saito et al. 2000, Vander Zanden and Rasmussen 1997).

Isotopic Determination of Consumer Diet

Two diet estimation methods (Euclidean distance and linear mixing model) that utilize stable isotope signatures were employed to determine both large and small harvestable fish diets as percentages of introduced nutrients and potential plankton prey size assemblages. The Euclidean distance method is a deterministic method and therefore, error measures were not generated, the linear mixing method utilized a linear algebra software program (IsoSource) written for IBM™ PC compatible computers (IsoSource computer program, D. Phillips USEPA).

The Euclidean distance method utilized isotopic signature vector distances between presumed predator/consumer and prey/nutrient sources along the X · Y axes of an ordinate plane ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$). The adjusted Euclidean distance method is given as:

$$d_{ij} = \sqrt{(\delta^{13}\text{C}'_i - \delta^{13}\text{C}_j)^2 + (\delta^{15}\text{N}'_i - \delta^{15}\text{N}_j)^2} \quad (\text{Equation 4.5})$$

where:

d_{ij} = Euclidean isotopic signature distance between nutrient/organisms i, j .

$\delta^{13}\text{C}'_i$ = adjusted carbon isotope signature of predator/consumer i .

$\delta^{15}\text{N}'_i$ = adjusted nitrogen isotope signature of predator/consumer i .

$\delta^{13}\text{C}_j$ = carbon isotope signature of producer/prey/nutrient j .

$\delta^{15}\text{N}_j$ = nitrogen isotope signature of producer/prey/nutrient j .

Adjusted predator carbon and nitrogen isotope signatures ($\delta^{13}\text{C}'_i$, $\delta^{15}\text{N}'_i$), were obtained by subtracting the mean trophic enrichment values ($\delta\delta^{13}\text{C}$ and $\delta\delta^{15}\text{N}$; Equations 4.6-4.7) obtained in the indoor feeding trials (Chapter 2), from the unadjusted predator carbon and nitrogen isotope signatures ($\delta^{13}\text{C}_i$, $\delta^{15}\text{N}_i$):

$$\delta^{13}\text{C}'_i = \delta^{13}\text{C}_i - \Delta\delta^{13}\text{C} \quad \text{Equation 4.6}$$

$$\delta^{15}\text{N}'_i = \delta^{15}\text{N}_i - \Delta\delta^{15}\text{N} \quad \text{Equation 4.7}$$

Euclidean distance measures were then used to compute percent prey/nutrient contributions (percent C, N basis) to the predator (e.g., harvest fish) diet. Calculation of prey utilization using Euclidean distances used the reciprocal distance (% prey utilization inversely proportional to Euclidean trophic distance) equation given as (Kline et al. 1993, Ben-David and Schell 2001, Phillips 2001):

$$\%X = \left(\frac{1/DX'}{1/DA'+1/DB'+1/DC'+...1/Dn'} \right) \times 100 \quad (\text{Equation 4.8})$$

where:

$\%X$ = percent inclusion of prey/nutrient X in diet (% C, N basis)

DX' = adjusted Euclidean trophic distance predator/consumer to prey/nutrient X

(where X = prey/nutrient A, B, C... n; adjusted for trophic enrichment C, N)

DA' = adjusted Euclidean trophic distance prey/nutrient A to predator/consumer

DB' = adjusted Euclidean trophic distance prey/nutrient B to predator/consumer

DC' = adjusted Euclidean trophic distance prey/nutrient C to predator/consumer

Dn' = adjusted Euclidean trophic distance prey/nutrient n to predator/consumer

The linear mixing model utilizes a series of mass balance equations that are simultaneously solved for three unknown variables when two isotope tracing elements are used (Schwarcz 1991, Phillips 2001, Phillips and Koch 2002, Phillips and Gregg 2003). A Microsoft[®] Visual Basic[™] computer program IsoSource (ver. 1.3.1) was utilized to iteratively determine percent food source usage and error estimates based upon two isotope signature values and a three-food-source maximum (e.g., C, N; Phillips 2003 and Gregg, Phillips 2005, Benstead et al. 2006). The basic equations for the linear mixing model are given as (Harrigan et al. 1989):

$$\delta^{13}C'_{pred} = a \cdot \delta^{13}C_i + b \cdot \delta^{13}C_j + c \cdot \delta^{13}C_k \quad (\text{Equation 4.9})$$

$$\delta^{15}N'_{pred} = a \cdot \delta^{15}N_i + b \cdot \delta^{15}N_j + c \cdot \delta^{15}N_k \quad (\text{Equation 4.10})$$

$$1 = a + b + c \quad (\text{Equation 4.11})$$

where:

$\delta^{13}\text{C}'_{pred}$ = adjusted carbon isotope signature of the predator/consumer

$\delta^{15}\text{N}'_{pred}$ = adjusted nitrogen isotope signature of the predator/consumer

$\delta^{13}\text{C}_i$ = carbon isotope signature of prey i

$\delta^{13}\text{C}_j$ = carbon isotope signature of prey j

$\delta^{13}\text{C}_k$ = carbon isotope signature of prey k

$\delta^{15}\text{N}_i$ = nitrogen isotope signature of prey i

$\delta^{15}\text{N}_j$ = nitrogen isotope signature of prey j

$\delta^{15}\text{N}_k$ = nitrogen isotope signature of prey k

and a, b, c are proportions of diet consisting of prey $i, j,$ and $k,$ respectively.

Dual element (N, C) isotopic signature Cartesian plots ($\delta^{13}\text{C}, \delta^{15}\text{N}$) of male and female broodstock, initial fry, harvested fish, and experimental feeds were made for the indoor trial isotopic data (Chapter 2; Figure 2-21). Similarly, isotopic plots for each outdoor pond nutrient treatment that included small (≤ 31 mm SL) and large harvest fish (> 31 mm SL), applied pond nutrients (except inorganic fertilizer), and the three plankton size/trophic assemblages were also generated. Once target organisms (swordtails) and nutrient source candidates (feeds, plankton assemblages, etc.) were plotted on a carbon and nitrogen isotope signature Cartesian plane, potential nutrient source candidate's isotopic signature point coordinates were connected to describe a prey/food resource polygon. Polygons created by connecting the (X: $\delta^{13}\text{C},$ Y: $\delta^{15}\text{N}$) coordinates of each potential nutrient/prey group, defined the potential trophic space for each fish predator group (small and large harvest fish) within a given pond nutrient treatment. If a target species/consumer's isotopic coordinates are found to lie within the circumscribed resource

polygon, then a plausible dietary solution is possible (Kline et al. 1993, Ben-David et al. 1997, Ben-David and Schell 2001, Phillips 2001). Euclidean distance calculations and linear algebra techniques are then able to estimate relative use (% diet inclusion) among the isotopically analyzed potential food sources.

Results

Pond Nutrient Isotope Signatures

Pond nutrient carbon isotope signature differences were significant among the three pond nutrients analyzed [processed feed (PRO), unprocessed feed (UNP), and cottonseed meal (CSM) treatments, Figure 4-4; 1-way ANOVA, $P < 0.0001$, $F = 375.5$, $\alpha_{(2,2)} = 0.05$], the inorganic fertilizer (INO) pond nutrient did not contain carbon and was therefore excluded from the analysis. Processed feed $\delta^{13}\text{C}$ averaged -23.74‰ (-23.91 to -23.56‰ 95 % CI), unprocessed feed $\delta^{13}\text{C}$ averaged -23.95‰ (-24.36 to -23.54‰ 95 % CI), and cottonseed meal fertilizer $\delta^{13}\text{C}$ averaged -26.43‰ , (-26.54 to -26.32‰ 95 % CI). Both processed and unprocessed feeds differed from cottonseed meal $\delta^{13}\text{C}$ values, but did not differ from each other when analyzed with post-hoc pairwise testing ($P < 0.05$, Tukey's multiple comparison test).

Nitrogen isotope signatures also differed among pond treatments (Figure 4-5; 1-way ANOVA, $P < 0.0001$, $F = 235.7$, $\alpha_{(2,3)} = 0.05$). The processed feed $\delta^{15}\text{N}$ average of 4.69‰ (4.26 to 5.11‰ 95 % CI) and unprocessed feed $\delta^{15}\text{N}$ average of 4.80‰ (3.75 to 5.86‰ 95 % CI) were similar, the cottonseed meal $\delta^{15}\text{N}$ average of 3.79‰ (3.72 to 3.86‰ 95 % CI) was lower than both feeds, while the inorganic fertilizer $\delta^{15}\text{N}$ average of -0.08‰ (-0.55 to 0.39‰ 95 % CI) was the lowest of the four applied nutrients. Applied nutrient nitrogen signatures differed, except for the PRO and UNP treatments, which was expected given that both feeds were manufactured from identical ingredients. Significant pairwise differences were: PRO and CSM,

PRO and INO, UNP and CSM, UNP and INO, and CSM and INO, ($P < 0.05$, Tukey's multiple comparison test).

Pre-harvest Fry Isotopic Signatures

Limited carbon and nitrogen isotope signature analyses were performed for periodically sampled pre-harvest pond fry (Chapter 3; days 7, 14 and 21 post stocking). Pre-harvest fry carbon isotope signatures quickly diverged among treatments as the trial progressed (Figure 4-6). Carbon isotope signatures significantly differed for both nutrient treatment and sampling date (rep. meas. 2-way ANOVA: $P_{(\text{nutrient})} = 0.0004$, $F_{(\text{nutrient})} = 9.426$, $\alpha_{(2,3)} = 0.05$; $P_{(\text{time})} < 0.0001$, $F_{(\text{time})} = 31.89$, $\alpha_{(2,2)} = 0.05$, $P_{(\text{interaction})} = 0.0058$, $F_{(\text{interaction})} = 3.624$, $\alpha_{(2,6)} = 0.05$). Repeated measures ANOVA analyses requires a fully balanced experimental design with no missing data values (Zar 1984, Ott 2000, Motulsky 1995), so a single pseudo-value was generated for a missing PRO pond replicate $\delta^{13}\text{C}$ value (pond A11) by inserting the mean of the remaining PRO pond treatment replicates for the given sampling date (4 April 2006) into the data array. Fry were unavailable from this pond and sampling date due to the low standing stock of fry within this pond following the recent introduction of broodstock.

Several differences in pre-harvest fry carbon isotope signature were found among sampling dates within nutrient treatments (Figures 4-7 – 4-10), two treatments developed within-treatment differences between fry after only seven days (Figures 4-7, 4-9; PRO day 7 and day 14, CSM day 7 and day 14), whereas it took 14 days for fry $\delta^{13}\text{C}$ differences to develop for the remaining two treatments (Figures 4-8, 4-10; UNP day 7 and day 21, INO day 7 and day 21; $P < 0.05$, Bonferroni post hoc test). Within-nutrient-treatment pairwise differences were not significant between day 14 and day 21 fry for any of the four nutrient treatments. Pairwise differences in pre-harvest fry $\delta^{13}\text{C}$ between nutrient treatments for given sampling dates were:

PRO and CSM (day 14, 21), PRO and INO (day 21), UNP and CSM (day 14, 21), UNP and INO (day 21), CSM and INO (day 7, 14, 21); no pairwise differences between PRO and UNP treatments were present for any of the three sampling dates ($P < 0.05$, Bonferroni post hoc test).

Although, pond nutrient and sampling date were both significant factors determining pre-harvest pond fry $\delta^{13}\text{C}$ isotopic signature, interaction was also significant between the two factors, $P_{(\text{interaction})} = 0.0058$ (δC), $F_{(\text{interaction})} = 3.624$. Even though significant interaction occurred between factors, due to the high F values reported for the two factors in the analyses, both nutrient and sampling date factors likely had strong influences upon fry $\delta^{13}\text{C}$ signature, although nutrient and sampling date factor effects were potentially non-linear across levels (Motulsky 1995).

Pre-harvest fry nitrogen isotope signature values also diverged as the trial progressed (Figures 4-7 – 4-10). Nitrogen isotope signatures significantly differed for both nutrient treatment and sampling date (rep. meas. 2-way ANOVA: $P_{(\text{nutrient})} < 0.0001$, $F_{(\text{nutrient})} = 18.67$, $\alpha_{(2,3)} = 0.05$; $P_{(\text{time})} < 0.0001$, $F_{(\text{time})} = 21.59$, $\alpha_{(2,2)} = 0.05$; $P_{(\text{interaction})} < 0.0001$, $F_{(\text{interaction})} = 7.565$, $\alpha_{(2,6)} = 0.05$). To perform repeated measures ANOVA, a single pseudo-value replicate was generated for this data set ($\delta^{15}\text{N}$) due to the unavailability of fry for pond A11 for 4 April 2006; pseudo-value generation for nitrogen signature was identical to carbon signature pseudo-value generation described previously.

Within-nutrient treatment differences in pre-harvest fry nitrogen isotope signature among sampling dates were: CSM day 7 and day 14, INO day 7 and day 14, INO day 7 and day 21; no pairwise differences were found between day 14 and day 21 fry within any of the four nutrient treatments ($P < 0.05$, Bonferroni post hoc test). Pairwise fry $\delta^{15}\text{N}$ differences between nutrient treatments for given sampling dates were: PRO and CSM differed for all three dates (days 7, 14,

21), PRO and INO (days 14, 21), UNP and CSM (days 14, 21), UNP and INO (days 14, 21), and CSM and INO (day 7). PRO and UNP pre-harvest fish $\delta^{15}\text{N}$ signatures did not differ for any of the three sampling dates.

Although pond treatment and time were both significant factors determining $\delta^{15}\text{N}$ signature in pre-harvest pond fry, interaction was again significant between the two factors ($P_{(\text{interaction})} < 0.0001$); resulting in significant factor effects (nutrient treatment, sampling date), and potentially non-linear interactions between the levels of the two factors (Motulsky 1995).

Harvest Fish Isotope Signature Differences

Large marketable fish (> 31 mm SL) carbon isotope ($\delta^{13}\text{C}$) signatures differed among treatment groups at harvest (Figure 4-11; 1-way ANOVA, $P < 0.0001$, $F = 40.81$, $\alpha_{(2,3)} = 0.05$). PRO treatment large fish $\delta^{13}\text{C}$ mean -23.39 ‰ (-24.20 to -22.58 ‰ 95 % CI), UNP treatment $\delta^{13}\text{C}$ mean -23.26 ‰ (-23.93 to -22.58 ‰ 95 % CI), and the CSM treatment $\delta^{13}\text{C}$ mean -24.46 ‰ (-24.92 to -24.00 ‰ 95 % CI) were similar, but INO treatment large fish $\delta^{13}\text{C}$ mean -20.15 ‰ (-21.22 to -19.09 ‰ 95 % CI) was markedly higher than that of the other three nutrient groups. Pairwise post hoc testing determined that only the INO treatment large fish $\delta^{13}\text{C}$ significantly differed from those of the other three treatments (Figure 4-11; $P < 0.001$, Tukey's multiple comparison test).

Small unmarketable fish (≤ 31 mm SL) carbon isotope ($\delta^{13}\text{C}$) signatures also differed among treatments (Figure 4-12; 1-way ANOVA, $P < 0.0001$, $F = 50.72$, $\alpha_{(2,3)} = 0.05$). The PRO treatment small fish $\delta^{13}\text{C}$ signature mean -24.31 ‰ (-24.92 to -23.70 ‰ 95 % CI) was slightly less enriched than for larger (> 31 mm SL) fish -23.39 ‰ (-24.20 to -22.58 ‰ 95 % CI) from the same ponds, but was similar to both the UNP and CSM small harvest fish $\delta^{13}\text{C}$ means (UNP: -24.12 ‰, -24.50 to -23.74 ‰ 95 % CI; CSM -25.25 ‰, -26.22 to -24.28 ‰ 95 % CI), whereas

the INO small fish $\delta^{13}\text{C}$ signature mean -20.29‰ (-21.29 to -19.30‰ 95 % CI) was the highest among all treatments. Pairwise difference analyses found that INO small fish δC was significantly higher than the δC values of the other three treatments, which did not significantly differ from each other (Figure 4-12; $P < 0.05$, Tukey's multiple comparison test).

Large and small harvest fish carbon isotope signatures significantly differed within individual pond nutrient treatments (rep. meas. 2-way ANOVA: $P_{(\text{fish size})} = 0.0087$, $F_{(\text{fish size})} = 8.645$; $P_{(\text{nutrient})} < 0.0001$, $F_{(\text{nutrient})} = 101.0$; $P_{(\text{interaction})} = 0.5927$, $F_{(\text{interaction})} = 0.6507$). Mean carbon isotope signature difference between small and large fish within each nutrient treatment: PRO $\Delta\delta^{13}\text{C} = -0.917\text{‰}$ (-1.761 to -0.072‰ 95 % CI), UNP $\Delta\delta^{13}\text{C} = -0.137\text{‰}$ (-0.779 to 0.505‰ 95 % CI), CSM $\Delta\delta^{13}\text{C} = -0.503\text{‰}$ (-1.433 to 0.427‰ 95 % CI), and INO $\Delta\delta^{13}\text{C} = 1.194\text{‰}$ (-0.068 to 2.456‰ 95 % CI). Although significant differences in carbon signature were present between small and large harvest fish within individual pond treatments, post-hoc comparison tests were not able to identify significant individual pairwise treatment differences ($P > 0.05$, Bonferroni post hoc tests). Repeated measures analysis was performed due to the non-independent nature of small and large harvest fish sampled from the same replicate ponds.

Large fish (> 31 mm SL) nitrogen isotope ($\delta^{15}\text{N}$) signatures did not differ among treatment groups at harvest (Figure 4-11; 1-way ANOVA, $P = 0.0830$, $F = 2.612$, $\alpha_{(2,3)} = 0.05$). PRO treatment fish $\delta^{15}\text{N}$ signature mean 7.62‰ (6.78 to 8.46‰ 95%CI) was similar to the remaining three pond treatment large fish $\delta^{15}\text{N}$ means: UNP 7.82‰ (7.39 to 8.25‰ 95 % CI), CSM 8.26‰ (7.80 to 8.73‰ 95 % CI), and INO 7.44‰ (6.81 to 8.07‰ 95 % CI).

In contrast, small harvest fish (≤ 31 mm SL) nitrogen isotope signatures significantly differed among pond nutrient treatments (Figure 4-12; 1-way ANOVA, $P < 0.0001$, $F = 20.79$, $\alpha_{(2,3)} = 0.05$). PRO pond treatment small fish $\delta^{15}\text{N}$ signature mean 7.96‰ (7.59 to 8.33‰ 95 %

CI), UNP small fish $\delta^{15}\text{N}$ mean 8.18 ‰ (7.51 to 8.85 ‰ 95 % CI), and CSM small fish $\delta^{15}\text{N}$ mean 8.85 ‰ (7.98 to 9.71 ‰ 95 % CI) largely overlapped, while the INO small fish $\delta^{15}\text{N}$ mean 6.33 ‰ (5.87 to 6.79 ‰ 95 % CI) was considerably lower. Pairwise comparisons found that only the INO treatment small fish $\delta^{15}\text{N}$ signature mean differed from those of the other three groups (Figure 4-12; $P < 0.05$, Tukey's multiple comparison test).

Large and small harvest fish nitrogen isotope signature differences within individual pond treatments (e.g., PRO large fish $\delta^{15}\text{N}$ - PRO small fish $\delta^{15}\text{N}$) did not significantly differ among the four pond nutrient treatments (rep. meas. 2-way ANOVA: $P_{(\text{fish size})} = 0.7731$, $F_{(\text{fish size})} = 0.08564$; $P_{(\text{nutrient})} < 0.0001$, $F_{(\text{nutrient})} = 14.67$; $P_{(\text{interaction})} = 0.0012$, $F_{(\text{interaction})} = 8.165$). Small and large fish nitrogen isotope differences for each nutrient treatment averaged: PRO $\Delta\delta^{15}\text{N} = -0.339$ ‰ (-1.861 to 1.183 ‰ 95 % CI), UNP $\Delta\delta^{15}\text{N} = -0.137$ ‰ (-1.589 to 1.315 ‰ 95 % CI), CSM $\Delta\delta^{15}\text{N} = -0.503$ ‰, -2.105 to 1.099 ‰ 95 % CI), and INO $\Delta\delta^{15}\text{N} = 1.194$ ‰ (0.648 to 1.740 ‰ 95 % CI). Although significant differences in small and large harvest fish nitrogen isotope signature within individual pond treatments were not detected using repeated measures 2-way ANOVA, post-hoc comparison tests were able to identify a single significant small and large fish $\Delta\delta^{15}\text{N}$ signature difference within the INO pond treatment (H. Motulsky pers comm.), ($P < 0.05$, Bonferroni posttests). Repeated measures analysis was performed due to the non-independent nature of small and large harvest fish sampled from the same replicate ponds.

Large Zooplankton Isotope Signatures

Large zooplankton ($> 200 \mu\text{m}$) carbon isotope signatures ($\delta^{13}\text{C}$) significantly differed among nutrient treatments and sampling dates (Figure 4-13; rep. meas. 2-way ANOVA, $P_{(\text{nutrient})} < 0.0001$, $F_{(\text{nutrient})} = 23.09$, $\alpha_{(2,3)} = 0.05$; $P_{(\text{time})} < 0.0001$, $F_{(\text{time})} = 5.453$, $\alpha_{(2,10)} = 0.05$, $P_{(\text{interaction})} = 0.0189$, $F_{(\text{interaction})} = 1.688$, $\alpha_{(2,30)} = 0.05$). Large zooplankton carbon isotope

signatures ($\delta^{13}\text{C}$) only differed between the INO treatment and the other three pond nutrient treatments within some of the eleven weekly sampling periods. Furthermore, significant differences were only present for a small number of sampling periods, depending upon which treatments were compared ($P < 0.05$, Bonferroni post-hoc comparison test). The majority of pairwise differences in large zooplankton carbon signature between nutrients for a given sampling date occurred between the INO and CSM nutrient treatments (5 of 11 sampling periods), all of which occurred during the latter half of the trial.

Large zooplankton carbon isotope signatures differed among sampling dates within each of the four pond nutrient treatments (Figures 4-14 – 4-17). Pairwise differences in large zooplankton $\delta^{13}\text{C}$ signature between sampling dates for the PRO nutrient treatment (three differences) all involved a single sampling date (25 May 2006) that had a lower mean $\delta^{13}\text{C}$ signature value and relatively small variation among replicate ponds (Figure 4-14; lower $\delta^{13}\text{C}$ values higher on y-axis); UNP large zooplankton $\delta^{13}\text{C}$ signatures differed for a single pair of sampling dates (Figure 4-15; 4 May 2006 and 25 May 2006); CSM large zooplankton $\delta^{13}\text{C}$ differences (nine) among sampling dates all included a single sampling date (Figure 4-16; 25 May 2006), which again had a lower $\delta^{13}\text{C}$ value and relatively low variation among pond replicates. There were several INO large zooplankton $\delta^{13}\text{C}$ signature differences among sampling dates (Figure 4-17), the majority of these differences involved the first sampling date (6 April 2006); this date had a low mean $\delta^{13}\text{C}$ value, and greater variation among replicate ponds relative to samples collected near the end of the study ($P < 0.05$, Bonferroni post test). Due to insufficient large zooplankton biomass for isotopic analyses, primarily at the beginning of the trial, sixteen missing $\delta^{13}\text{C}$ values (single date and pond replicate) out of 264 date and replicate data points were replaced with ‘pseudovalues’ generated by replacing missing values with mean

values calculated from the remaining replicates for the given pond treatment and sampling date. To perform a two-way repeated measures ANOVA, a fully balanced experimental design is required that does not allow missing data. Although large F values for each factor indicated that both factors influenced large zooplankton $\delta^{13}\text{C}$ values, factor interactions (treatment \times time) also were significant; this indicated that factor effects may have been non-linear across levels within each factor (Motulsky 1995).

Large zooplankton nitrogen isotope signatures ($\delta^{15}\text{N}$) significantly differed among pond treatments and sampling dates for the last eight weeks of the study (Figure 4-13; rep. meas. 2-way ANOVA, $P_{(\text{nutrient})} < 0.0001$, $F_{(\text{nutrient})} = 91.41$, $\alpha_{(2,3)} = 0.05$; $P_{(\text{time})} < 0.0001$, $F_{(\text{time})} = 8.757$, $\alpha_{(2,7)} = 0.05$; $P_{(\text{interaction})} = 0.0002$, $F_{(\text{interaction})} = 2.764$, $\alpha_{(2,21)} = 0.05$). Samples from the first four weeks of the study were omitted as insufficient large zooplankton biomass had developed within the ponds to provide adequate sample material for nitrogen isotope analyses, given that only a single liter of water was filtered from each replicate pond for isotopic analysis. PRO and UNP large zooplankton $\delta^{15}\text{N}$ values did not differ for any of the eight sampling periods. Both PRO and UNP large zooplankton $\delta^{15}\text{N}$ values differed with CSM values, but only for three and two sampling dates, respectively; additionally, these dates occurred in the middle of the experimental trial and $\delta^{15}\text{N}$ isotopic differences were no longer detected during the last few weeks of the trial. INO large zooplankton $\delta^{15}\text{N}$ signature values differed more frequently with those of the other three treatments (PRO, UNP and CSM), than the three non-INO treatments differed from each other. PRO and UNP large zooplankton $\delta^{15}\text{N}$ signature values differed with INO large zooplankton $\delta^{15}\text{N}$ signature values (6 and 5 sampling dates, respectively) more frequently than did the CSM and INO large zooplankton $\delta^{15}\text{N}$ signature values (3 sampling dates). Interestingly, most of these differences occurred within the last four to six weeks of the three-month trial ($P <$

0.05, Bonferroni post-hoc comparison test). Thirty-five missing values of 264 data points (date and treatment replicate pond) were replaced by 'pseudovalues' generated by replacing the missing values with mean values calculated for the remaining replicates for the given nutrient treatment and sampling date (identical process for missing $\delta^{13}\text{C}$ values). The greater number of missing zooplankton $\delta^{15}\text{N}$ signature values was due to the higher detection threshold for nitrogen for the Thermo-Finnigan DELTAplus Advantage gas isotope-ratio mass spectrometer, used to determine carbon and nitrogen isotope ratios (R. Doucett, NAUCPSIL, pers. comm.). Higher detection thresholds require more biological material to generate reliable nitrogen isotope results. Additionally, biological materials typically have lower nitrogen content relative to carbon (% dry weight basis). Unfortunately, samples taken early in the study contained too little nitrogen to provide usable results, due to the low zooplankton standing stocks in these newly filled ponds.

Large zooplankton nitrogen isotope signature differences among sampling dates were present within the PRO, CSM and INO treatments (Figures 4-14, 4-16 – 4-17). No large zooplankton nitrogen isotope signature differences among sampling dates occurred within the UNP ponds (Figure 4-15). Pairwise differences between sampling dates for the PRO treatment (three differences) all involved a single sampling date (27 April 2006) that had a higher mean $\delta^{15}\text{N}$ signature value, and latter dates that had lower $\delta^{15}\text{N}$ signatures and lower variation among replicates (Figure 4-14). Within the CSM treatment, pairwise differences among sampling dates occurred due to two consecutive sampling dates (1, 8 June 2006) near the end of the study that had higher $\delta^{15}\text{N}$ signature values than earlier samples (Figure 4-16). Within the INO treatment, pairwise differences among sampling dates primarily occurred due to two consecutive samples (27 April 2006, 4 June 2006) collected earlier in the study that had higher $\delta^{15}\text{N}$ signatures and

greater variation in $\delta^{15}\text{N}$ signatures among pond replicates (Figure 4-17). Although a later date within the INO treatment also had a higher $\delta^{15}\text{N}$ signature value (1 June 2006).

Time-averaged large zooplankton $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures also were compared among pond nutrient treatments using 1-way ANOVA. Time-averaged large zooplankton carbon isotope signatures ($\delta^{13}\text{C}$) differed among treatments, agreeing with the 2-way repeated measures ANOVA results for nutrient treatments and sampling dates (Figure 4-18; 1-way ANOVA, $P < 0.0001$, $F = 23.57$, $\alpha_{(2,3)} = 0.05$). PRO pond large zooplankton $\delta^{13}\text{C}$ signature mean -23.10 ‰ (-23.61 to -22.59 ‰ 95 % CI) and UNP mean -23.40 ‰ (-24.21 to -22.58 ‰ 95 % CI) were roughly equal, and CSM -24.39 ‰ (-25.48 to -23.30 ‰ 95 % CI) and UNP values also overlapped, but INO $\delta^{13}\text{C}$ mean -20.82 ‰ (-21.49 to -20.16 ‰ 95 % CI) was higher than all other treatments. Pairwise testing found two treatment pairs that did not differ for large zooplankton $\delta^{13}\text{C}$ signature values: PRO and UNP, and UNP and CSM ($P > 0.05$, Tukey's multiple comparison test). The INO large zooplankton $\delta^{13}\text{C}$ signature value was significantly higher (^{13}C enriched) than the other three treatments. Although the CSM treatment large zooplankton assemblage was the most depleted in ^{13}C , it did not significantly differ from the PRO and INO treatments, but did differ from the UNP large zooplankton $\delta^{13}\text{C}$ values.

Time-averaged large zooplankton $\delta^{15}\text{N}$ signatures also significantly differed among pond treatments (Figure 4-18; 1-way ANOVA, $P < 0.0001$, $F = 37.30$, $\alpha_{(2,3)} = 0.05$). PRO treatment $\delta^{15}\text{N}$ mean 7.13 ‰ (6.89 to 7.37 ‰ 95 % CI), and UNP treatment $\delta^{15}\text{N}$ mean 7.35 ‰ (6.77 to 7.93 ‰ 95 % CI) confidence intervals largely overlapped. The CSM treatment $\delta^{15}\text{N}$ mean 6.29 ‰ (5.72 to 6.85 ‰ 95 % CI) was lower than both the PRO and UNP means, but higher than the INO mean 4.95 ‰ (4.60 to 5.31 ‰ 95 % CI), which was considerably lower than the other three treatments. Post hoc significance tests found that large zooplankton $\delta^{15}\text{N}$ signatures differed

from each other among all treatments, except for the PRO and UNP treatments (Figure 4-18; $P < 0.05$, Tukey's multiple comparison test).

Medium Plankton Isotope Signatures

Medium-size plankton assemblage (32 - 200 μm) carbon isotope signatures differed among pond nutrient treatments and sampling periods (Figures 4-19 – 4-22; 2-way rep. meas. ANOVA, $P_{(\text{nutrient})} < 0.0001$, $F_{(\text{nutrient})} = 23.70$, $\alpha_{(2,3)} = 0.05$; $P_{(\text{time})} < 0.0001$, $F_{(\text{time})} = 7.742$, $\alpha_{(2,11)} = 0.05$; $P_{(\text{interaction})} < 0.0001$, $F_{(\text{interaction})} = 2.773$, $\alpha_{(2,33)} = 0.05$). PRO and UNP, and PRO and CSM medium plankton carbon signatures did not differ within any of the twelve sampling periods ($P > 0.05$, Bonferroni posttests). PRO and INO signatures differed for the latter half (six) of the twelve-week study, UNP and CSM treatments differed for two consecutive sampling dates mid-trial (18 May 2006, 25 May 2006), UNP and INO treatments differed for four of the last six weeks of the study (11 May 2006, 1, 8 and 15 June 2006), and CSM and INO signatures differed for six of the last seven weeks of the study (4, 11, 18 and 25 May 2006, 1 and 8 June 2006; $P < 0.05$, Bonferroni post hoc tests).

Within-treatment differences in medium-size plankton carbon isotope signature occurred among sampling dates for all four pond nutrient treatments (Figures 4-19 – 4-22; $P < 0.05$, Bonferroni posttests). PRO treatment signatures did not vary greatly among sampling dates, most differences were between high carbon isotope signatures present during the first sampling dates and lower carbon isotope signatures later in the study; the lowest signature occurred during the last week of the study (15 June 2006; Figure 4-19). UNP treatment medium plankton carbon isotope signatures followed a time trajectory nearly identical to that of the PRO treatment, with the highest measured signature found during the first week of sampling, followed by a steady downward (lower $\delta^{13}\text{C}$ values progressing upward from abscissa) progression till trial

termination (Figures 4-19 – 4-20). CSM signatures also did not vary greatly over the twelve weeks, again the highest carbon isotope signature was during the first week of the study but remained fairly constant thereafter (Figure 4-21). INO signatures varied less than those of the other three treatments, fluctuating briefly during the first few weeks, and remaining fairly constant thereafter (Figure 4-22). A single pseudovalue out of 288 samples (4 treatments x 12 sampling dates x 6 replicate ponds) was generated for a missing signature (CSM pond A4 20 April 2006); sample was mistakenly discarded due to human error.

Medium-size plankton assemblage nitrogen isotope signatures differed among treatments, but sampling period was not a significant factor within treatments (Figures 4-19 – 4-22; 2-way rep. meas. ANOVA, $P_{(\text{nutrient})} = 0.0003$, $F_{(\text{nutrient})} = 10.36$, $\alpha_{(2,3)} = 0.05$; $P_{(\text{time})} = 0.3836$, $F_{(\text{time})} = 1.074$, $\alpha_{(2,11)} = 0.05$; $P_{(\text{interaction})} = 0.0450$, $F_{(\text{interaction})} = 2.773$, $\alpha_{(2,33)} = 0.05$). PRO and UNP signatures differed for only one of the twelve sampling periods (1 June 2006), PRO and CSM treatments did not differ for any of the twelve sampling dates, and PRO and INO differed for only two sampling periods during the first half of the twelve week trial (6 April 2006 and 27 April 2006). UNP and CSM signatures did not differ for any of the twelve sampling periods, UNP and INO signatures differed for a single sampling period (1 June 2006) and CSM and INO signatures differed most frequently with four pairwise differences (6 and 27 April 2006, 4 May 2006, and 1 June 2006; $P < 0.05$, Bonferroni posttests). Although it was determined that time was not a significant factor in explaining differences in medium-size plankton nitrogen isotope signature among sampling dates within applied nutrient treatments, post hoc pairwise difference analyses did find (rare) significant differences in signature between sampling dates ($P < 0.05$, Bonferroni posttests). In the PRO treatment, differences among sampling dates were infrequent and did not follow any discernable pattern (Figure 4-19), only a single date (1 June 2006) was

responsible for the two significant pairwise differences observed among sampling dates. Within the UNP treatment, pairwise differences in signature also were infrequent and due to a single pair of sampling dates (Figure 4-20; 4 May 2006 and 1 June 2006). CSM signatures did not differ among sampling dates (Figure 4-21). At the start of the trial (31 March 2006), a single sampling date with a high medium plankton nitrogen signature was responsible for the three pairwise differences (31 March 2006 and 27 April 2006; 31 March 2006 and 4 May 2006; 31 March 2006 and 1 June 2006) observed within the INO ponds (Figure 4-22). Out of 288 small plankton samples (4 treatments x 12 sampling dates x 6 replicate ponds), twelve pseudovalues were generated for missing (UNP ponds: A3 1 June 2006, A5 6 April 2006, B8 31 March, 18 May 2006; CSM ponds: A1 1 June 2006, B12 20 April 2006; INO ponds: B7 6 April, 1 June 2006) and outlier (UNP ponds: B8 20 April, 4 May 2006; CSM ponds: A6 13 April 2006, B12 27 April 2006) medium plankton nitrogen isotope signatures. Outlier values were defined as values that were more than two standard deviations away from the mean value of the remaining replicates (Zar 1984).

Time-averaged medium-plankton carbon and nitrogen isotope signatures also were compared using 1-way ANOVA. Time averaged medium size plankton carbon signature differences were found among pond nutrient treatments (Figure 4-23; 1-way ANOVA, $P_{(\text{nutrient})} < 0.0001$, $F_{(\text{nutrient})} = 23.72$, $\alpha_{(2,3)} = 0.05$). PRO $\delta^{13}\text{C}$ mean -24.15‰ (-24.15 to -23.86‰ 95 % CI) was similar to both UNP $\delta^{13}\text{C}$ mean -23.86‰ (-24.41 to -23.31‰ 95 % CI) and CSM $\delta^{13}\text{C}$ mean -24.76‰ (-25.59 to -23.93‰ 95 % CI), but the INO $\delta^{13}\text{C}$ mean -22.19‰ (-22.72 to -21.66‰ 95 % CI), had the highest $\delta^{13}\text{C}$ signature, differing from all three non-INO treatments. Significant differences were not found between the signatures of the PRO and UNP, and PRO and CSM treatments, but pairwise differences in carbon isotope signature were present between

the following pond nutrient treatment pairs: PRO and INO, UNP and CSM, UNP and INO, and CSM and INO treatments ($P < 0.05$, Tukey's multiple comparison test). Only one treatment pair (UNP and CSM) did not include the INO treatment medium plankton assemblage, which had a markedly higher carbon isotope signature (Figure 4-23; lower position on graph).

Time-averaged medium-size plankton assemblage nitrogen isotope signatures also differed among pond nutrient treatments (Figure 4-23; 1-way ANOVA, $P_{(\text{nutrient})} = 0.0003$, $F_{(\text{nutrient})} = 10.36$, $\alpha_{(2,3)} = 0.05$). PRO $\delta^{15}\text{N}$ mean 5.95 ‰ (5.04 to 6.85 ‰ 95 % CI) was similar to both UNP $\delta^{15}\text{N}$ mean 5.66 ‰ (5.28 to 6.15 ‰ 95 % CI) and CSM $\delta^{15}\text{N}$ mean 5.80 ‰ (4.71 to 6.90 ‰ 95 % CI), but all three were significantly higher than the INO $\delta^{15}\text{N}$ mean 3.51 ‰ (2.45 to 4.58 ‰ 95 % CI). Differences only occurred between the INO, and three non-INO treatments, no significant differences were detected between the remaining three nutrient treatments (Figure 4-23; $P > 0.05$, Tukey's multiple comparison test).

Small Plankton Isotope Signatures

Small-size plankton assemblage (1 - 32 μm) carbon isotope signatures differed among pond nutrient treatments and sampling periods within treatments (Figures 4-24 – 4-27; 2-way rep. meas. ANOVA, $P_{(\text{nutrient})} < 0.0001$, $F_{(\text{nutrient})} = 84.68$, $\alpha_{(2,3)} = 0.05$; $P_{(\text{time})} < 0.0001$, $F_{(\text{time})} = 14.62$, $\alpha_{(2,11)} = 0.05$; $P_{(\text{interaction})} = 0.0003$, $F_{(\text{interaction})} = 2.242$, $\alpha_{(2,33)} = 0.05$). PRO and UNP small plankton carbon isotope signatures did not differ for any of the twelve sampling periods, and PRO and CSM treatment signatures differed for only two sampling dates at the start of the outdoor pond trial (31 March 2006 and 6 April 2006). In contrast, PRO and INO signatures differed for the last nine sampling periods ($P < 0.05$, Bonferroni posttests). Similar to the PRO and CSM treatment comparison, UNP and CSM signature differences were present (to a slightly greater degree for the first five weeks) at the beginning of the study, but were absent in the latter

half of the study; UNP and INO signature differences followed a pattern similar to that of the PRO and INO treatments, initial similarities (four of first five sampling dates) were absent in the last seven weeks of the study. The two fertilizer (CSM and INO) treatment signatures differed for all twelve sampling dates ($P < 0.05$, Bonferroni posttests).

Within-pond nutrient treatment differences in small plankton carbon isotope signature among sampling dates occurred within all four pond nutrient treatments ($P < 0.05$, Bonferroni posttests). PRO treatment $\delta^{13}\text{C}$ signature was highest during the first week of the study (31 March 2006), and significantly differed from the signatures measured during the remaining eleven weeks of the study. PRO treatment $\delta^{13}\text{C}$ signatures slowly oscillated over the twelve weeks of the study, and did not appear to stabilize about a constant value (Figure 4-24; $P < 0.05$, Bonferroni posttests). The UNP signatures (Figure 4-25), followed a trajectory intermediate to that observed for the PRO small (a gradual oscillation) and the UNP medium plankton $\delta^{13}\text{C}$ signatures (high initial carbon signature then gradual decrease). CSM signatures oscillated from a high initial carbon signature (31 March 2006), gradually decreasing before finally increasing to $\delta^{13}\text{C}$ values (last three sampling periods) that did not statistically differ from the initial value (Figure 4-26). INO signatures differed among sampling dates due to three sampling dates (6, 13 April 2006 and 8 June 2006), but otherwise did not vary greatly (Figure 4-27). A single pseudo-value was generated for one missing sample (UNP pond B5 31 March 2006) out of 288 samples (4 treatments x 12 sampling dates x 6 replicate ponds), that was lost due to human error.

Small-size plankton assemblage nitrogen isotope signatures also differed among pond nutrient treatments and among sampling periods within pond treatments (Figures 4-24 – 4-27; 2-way rep. meas. ANOVA, $P_{(\text{nutrient})} < 0.0001$, $F_{(\text{nutrient})} = 46.69$, $\alpha_{(2,3)} = 0.05$; $P_{(\text{time})} < 0.0001$, $F_{(\text{time})} = 4.694$, $\alpha_{(2,11)} = 0.05$; $P_{(\text{interaction})} < 0.0001$, $F_{(\text{interaction})} = 3.118$, $\alpha_{(2,33)} = 0.05$). Among the three

non-INO treatments, $\delta^{15}\text{N}$ signatures did not differ among treatments for any of the twelve sampling periods. PRO and INO signatures differed for five of twelve sampling periods during the twelve-week trial, but differences did not appear to follow any temporal pattern with differences occurring at the beginning, middle and end of the study with equal likelihood. UNP and INO treatment signatures only differed twice among the twelve sampling dates and differences were non consecutive and therefore not part of a possible trend. The two fertilizer treatments had the highest frequency of significant differences in signature (five of twelve sampling dates), but again, differences were present at the beginning, middle and end of the study with no discernable temporal pattern.

Small-plankton nitrogen isotope signature differences among sampling dates within nutrient treatments were not present for either the PRO or UNP treatments (Figures 4-24 – 4-25; $P > 0.05$, Bonferroni posttests). CSM treatment small plankton nitrogen isotope signatures significantly differed among sampling dates, a gradual oscillation and overall increase occurred during the twelve weeks of the study (Figure 4-26). Within the INO treatment, $\delta^{15}\text{N}$ signatures were fairly constant (Figure 4-27), except for two notable sampling periods (6 April 2006 and 18 May 2006) with low signatures, which were not consecutive and were not part of any apparent trend in signature values. Two signature pseudovalues (PRO pond A8 31 March 2006 and UNP pond B5 31 March 2006) were generated for two missing samples, lost due to human error.

Time-averaged small plankton assemblage carbon isotope signature values significantly differed among pond nutrient treatments (Figure 4-28; 1-way ANOVA, $P_{(\text{nutrient})} < 0.0001$, $F_{(\text{nutrient})} = 84.59$, $\alpha_{(2,3)} = 0.05$). The PRO $\delta^{13}\text{C}$ mean -27.18‰ (-27.87 to -26.49‰ 95 % CI), and UNP mean -26.53‰ (-27.22 to -25.84‰ 95 % CI) were similar. The CSM $\delta^{13}\text{C}$ signature mean -28.64‰ (-29.26 to -28.01‰ 95 % CI) was the lowest, and the INO $\delta^{13}\text{C}$ mean -23.26‰

(-23.78 to -22.73 ‰ 95 % CI) was the highest of the four pond treatments. Pairwise comparisons among the four treatments found that the PRO and UNP treatments did not differ, and that the two fertilizer treatments significantly differed from both feed treatments, as well as from each other (Figure 4-28; $P < 0.05$, Tukey's multiple comparison test).

Time-averaged small plankton assemblage nitrogen isotope signatures also differed among pond nutrient treatments (Figure 4-28; 1-way ANOVA, $P_{(\text{nutrient})} < 0.0001$, $F_{(\text{nutrient})} = 46.70$, $\alpha_{(2,3)} = 0.05$). The PRO $\delta^{15}\text{N}$ mean 4.38 ‰ (3.59 to 5.18 ‰ 95 % CI), UNP $\delta^{15}\text{N}$ mean 4.47 ‰ (3.82 to 5.13 ‰ 95 % CI), and CSM $\delta^{15}\text{N}$ mean 4.29 ‰ (3.84 to 4.73 ‰ 95 % CI) were similar, but the INO $\delta^{15}\text{N}$ mean 1.19 ‰ (0.75 to 1.62 ‰ 95 % CI) was markedly lower. Pairwise difference testing, found that only the INO treatment differed from the other three treatments (Figure 4-28; $P < 0.05$, Tukey's multiple comparison test).

Pond Nutrient and Pond Fish Isotope Signature Differences

Time-averaged carbon isotope signature ($\delta^{13}\text{C}$) difference magnitudes (absolute value) between large harvest fish and their respective pond treatment nutrients were calculated and compared among treatments and significant differences were found (Figure 4-29; 1-way ANOVA, $P = 0.0005$, $F = 14.45$, $\alpha_{(2,2)} = 0.05$). PRO large harvest fish and processed feed carbon isotope differences [$\delta^{13}\text{C}_{(\text{fish})} - \delta^{13}\text{C}_{(\text{nutrient})}$] averaged $0.46 \Delta\delta^{13}\text{C} \text{ ‰}$ (-0.23 to $1.15 \Delta\delta^{13}\text{C} \text{ ‰}$ 95 % CI), UNP large fish and unprocessed feed $\delta^{13}\text{C}$ differences averaged $0.63 \Delta\delta^{13}\text{C} \text{ ‰}$ (1.32 to $-0.06 \Delta\delta^{13}\text{C} \text{ ‰}$ 95 % CI), and CSM large fish and cottonseed meal carbon isotope differences averaged $1.97 \Delta\delta^{13}\text{C} \text{ ‰}$ (1.51 to $2.43 \Delta\delta^{13}\text{C} \text{ ‰}$ 95 % CI), INO large harvest fish and inorganic fertilizer carbon isotope difference comparisons were not possible as the inorganic fertilizer did not contain carbon. Pairwise comparison tests found two significant differences in fish and nutrient carbon isotope difference magnitude; CSM large harvest fish and cottonseed

meal differed in carbon isotope signature values to a much greater degree than either the PRO or UNP large harvest fish, and their respective feed nutrients (Figure 4-29; $P < 0.05$, Tukey's multiple comparison test).

Time-averaged nitrogen isotope signature ($\Delta\delta^{15}\text{N}$) differences between large harvest fish and their respective pond treatment nutrients were calculated and compared among treatments and significant differences were found (Figure 4-29: 1-way ANOVA, $P < 0.0001$, $F = 91.50$, $\alpha_{(2,3)} = 0.05$). PRO treatment large harvest fish and processed feed $\delta^{15}\text{N}$ differences ($\delta\text{N}_{(\text{fish})} - \delta\text{N}_{(\text{nutrient})}$) averaged $2.94 \Delta\delta^{15}\text{N} \text{‰}$ (2.10 to $3.78 \Delta\delta^{15}\text{N} \text{‰}$ 95 % CI), UNP large harvest fish and unprocessed feed $\delta^{15}\text{N}$ differences averaged $3.05 \Delta\delta^{15}\text{N} \text{‰}$ (2.64 to $3.46 \Delta\delta^{15}\text{N} \text{‰}$ 95 % CI), CSM large harvest fish and cottonseed meal $\delta^{15}\text{N}$ differences averaged $4.48 \Delta\delta^{15}\text{N} \text{‰}$ (4.01 to $4.94 \Delta\delta^{15}\text{N} \text{‰}$ 95 % CI), and INO large harvest fish and inorganic fertilizer $\delta^{15}\text{N}$ differences averaged $7.52 \Delta\delta^{15}\text{N} \text{‰}$ (6.89 to $8.15 \Delta\delta^{15}\text{N} \text{‰}$ 95 % CI). Comparisons of nitrogen isotope difference magnitudes between large harvest fish and their respective pond nutrient among the different pond nutrient treatments were significant between all treatment pairs, except for the PRO and UNP treatments (Figure 4-29; $P < 0.05$, Tukey's multiple comparison test).

Time-averaged carbon isotope signature difference magnitudes between small harvest fish and their respective pond treatment nutrients were compared among treatments and no significant differences were found (Figure 4-30: 1-way ANOVA, $P = 0.0866$, $F = 2.971$, $\alpha_{(2,2)} = 0.05$). PRO treatment small fish and processed feed $\delta^{13}\text{C}$ difference averaged $0.64 \Delta\delta^{13}\text{C} \text{‰}$ (0.17 to $1.11 \Delta\delta^{13}\text{C} \text{‰}$ 95 % CI), UNP small harvest fish and unprocessed feed $\delta^{13}\text{C}$ difference averaged $0.29 \Delta\delta^{13}\text{C} \text{‰}$ (0.09 to $0.49 \Delta\delta^{13}\text{C} \text{‰}$ 95 % CI), and CSM small harvest fish and cottonseed meal $\delta^{13}\text{C}$ averaged $1.18 \Delta\delta^{13}\text{C} \text{‰}$ (0.22 to $2.15 \Delta\delta^{13}\text{C} \text{‰}$ 95 % CI). The magnitude of differences between feed nutrients and their respective small harvest fish carbon isotopes were

not significant among the three non-INO treatments (Figure 4-30; $P > 0.05$, Tukey's multiple comparison test). Again, the INO treatment small fish and applied inorganic fertilizer δC signature difference analysis was not possible due to the absence of carbon within the applied inorganic fertilizer.

Time-averaged nitrogen isotope signature differences between small harvest fish and their respective pond nutrients were compared among treatments and significant differences were found (Figure 4-30: 1-way ANOVA, $P < 0.0001$, $F = 37.98$, $\alpha_{(2,3)} = 0.05$). Nitrogen isotope signature ($\delta^{15}N$) differences between small fish and their respective nutrients averaged: PRO: $3.27 \Delta\delta^{15}N \text{ ‰}$ (2.91 to $3.64 \Delta\delta^{15}N \text{ ‰}$ 95 % CI), UNP: $3.37 \Delta\delta^{15}N \text{ ‰}$ (2.70 to $4.04 \Delta\delta^{15}N \text{ ‰}$ 95 % CI), CSM: $5.06 \Delta\delta^{15}N \text{ ‰}$ (4.19 to $5.92 \Delta\delta^{15}N \text{ ‰}$ 95 % CI), and INO: $6.41 \Delta\delta^{15}N \text{ ‰}$ (5.95 to $6.87 \Delta\delta^{15}N \text{ ‰}$ 95 % CI). The pattern of $\delta^{15}N$ signature differences between small harvest fish and their respective nutrients was identical to that found for large harvest fish and their respective feeds, differences were found among all treatment pair combinations except the PRO and UNP treatments (Figure 4-30; $P < 0.05$, Tukey's multiple comparison test).

Pond Fish and Large Zooplankton Isotope Signature Differences

Carbon isotope signature difference magnitudes between large (> 31 mm SL) harvest fish and their respective large zooplankton assemblages [$\delta^{13}C_{(fish)} - \delta^{13}C_{(lg.zooplankton)}$] were calculated and compared among treatments, but no significant differences in the magnitude (abs. value sign neutral) of $\delta^{13}C$ signature differences were found (Figure 4-31; 1-way ANOVA, $P = 0.0723$, $F = 2.758$, $\alpha_{(2,3)} = 0.05$). Large harvest fish and time averaged large zooplankton $\delta^{13}C$ signature differences averaged: PRO $0.65 \Delta\delta^{13}C \text{ ‰}$ (-0.94 to $2.24 \Delta\delta^{13}C \text{ ‰}$ 95 % CI), UNP $0.41 \Delta\delta^{13}C \text{ ‰}$ (-0.97 to $1.79 \Delta\delta^{13}C \text{ ‰}$ 95% CI), CSM $1.01 \Delta\delta^{13}C \text{ ‰}$ (-0.72 to $2.74 \Delta\delta^{13}C \text{ ‰}$ 95% CI), and INO $1.13 \Delta\delta^{13}C \text{ ‰}$ (-0.70 to $2.95 \Delta\delta^{13}C \text{ ‰}$ 95% CI) treatments.

Nitrogen isotope signature differences between large harvest fish and their respective large zooplankton assemblages [$\delta^{15}\text{N}_{(\text{fish})} - \delta^{15}\text{N}_{(\text{lg.zooplankton})}$] were calculated and compared among treatments and significant differences were found (Figure 4-32; 1-way ANOVA, $P = 0.0002$, $F = 11.42$, $\alpha_{(2,3)} = 0.05$). Nitrogen isotope signature differences between large harvest fish and time-averaged large zooplankton assemblages within the treatments were: PRO $0.70 \Delta\delta^{15}\text{N} \text{‰}$ (-1.04 to $2.44 \Delta\delta^{15}\text{N} \text{‰}$ 95 % CI), UNP $0.75 \Delta\delta^{15}\text{N} \text{‰}$ (-0.38 to $1.89 \Delta\delta^{15}\text{N} \text{‰}$ 95 % CI), CSM $1.98 \Delta\delta^{15}\text{N} \text{‰}$ (0.57 to $3.39 \Delta\delta^{15}\text{N} \text{‰}$ 95 % CI), and INO $2.49 \Delta\delta^{15}\text{N} \text{‰}$ (1.11 to $3.87 \Delta\delta^{15}\text{N} \text{‰}$ 95 % CI). Large harvest fish and large zooplankton nitrogen isotope signature difference magnitudes did not differ between the two feed treatments (PRO and UNP), nor between the two fertilizer treatments (CSM and INO), but the two feed treatments did significantly differ from those of the two fertilizer treatments (Figure 4-32; $P < 0.05$, Tukey's multiple comparison test).

Carbon isotope signature differences between small harvest fish and their respective treatment's time-averaged large zooplankton assemblage [absolute value ($\delta^{13}\text{C}_{(\text{fish})} - \delta^{13}\text{C}_{(\text{lg.zooplankton})}$)] were calculated and compared among treatments and were not significant (Figure 4-33; 1-way ANOVA, $P = 0.6421$, $F = 0.5697$, $\alpha_{(2,3)} = 0.05$). Time-averaged small harvest fish and large zooplankton $\delta^{13}\text{C}$ signature differences averaged: PRO $1.07 \Delta\delta^{13}\text{C} \text{‰}$ (-0.39 to $2.54 \Delta\delta^{13}\text{C} \text{‰}$ 95 % CI), UNP $1.03 \Delta\delta^{13}\text{C} \text{‰}$ (-0.12 to $2.18 \Delta\delta^{13}\text{C} \text{‰}$ 95 % CI), CSM $1.12 \Delta\delta^{13}\text{C} \text{‰}$ (-0.88 to $3.11 \Delta\delta^{13}\text{C} \text{‰}$ 95 % CI), and INO $0.73 \Delta\delta^{13}\text{C} \text{‰}$ (-1.06 to $2.52 \Delta\delta^{13}\text{C} \text{‰}$ 95 % CI).

Nitrogen isotope signature differences between small fish and their respective treatment large zooplankton [$\delta^{15}\text{N}_{(\text{fish})} - \delta^{15}\text{N}_{(\text{lg.zooplankton})}$] were calculated and significant differences were found among treatments (Figure 4-34; 1-way ANOVA, $P = 0.0025$, $F = 7.028$, $\alpha_{(2,3)} = 0.05$). Nitrogen isotope signature differences between small fish at harvest and time averaged large

zooplankton averaged: PRO 0.65 $\Delta\delta^{15}\text{N}$ ‰ (-0.80 to 2.11 $\Delta\delta^{15}\text{N}$ ‰ 95 % CI), UNP 1.11 $\Delta\delta^{15}\text{N}$ ‰ (-0.21 to 2.43 $\Delta\delta^{15}\text{N}$ ‰ 95 % CI), CSM 2.56 $\Delta\delta^{15}\text{N}$ ‰ (0.90 to 4.22 $\Delta\delta^{15}\text{N}$ ‰ 95 % CI), and INO 1.38 $\Delta\delta^{15}\text{N}$ ‰ (0.12 to 2.63 $\Delta\delta^{15}\text{N}$ ‰ 95 % CI). The magnitude of nitrogen isotope signature differences [$\delta^{15}\text{N}_{(\text{fish})} - \delta^{15}\text{N}_{(\text{lg.zooplankton})}$] between CSM small fish and large plankton were significantly greater than the differences between feed treatment small harvest fish and the respective large zooplankton assemblages, but INO treatment small harvest fish and large zooplankton nitrogen isotope signature differences magnitudes did not significantly differ from any other pond treatment (Figure 4-34; $P < 0.05$, Tukey's multiple comparison test).

Large Zooplankton and Pond Nutrient Isotope Signature Differences

Carbon isotope signature ($\delta^{13}\text{C}$) difference magnitudes between time-averaged large zooplankton and their respective pond nutrients were calculated and compared among treatments and differences were found (Figure 4-35: 1-way ANOVA, $P < 0.0001$, $F = 39.22$, $\alpha_{(2,2)} = 0.05$). Carbon isotope signature difference magnitudes between large zooplankton and their respective pond nutrient treatments [$\delta^{13}\text{C}_{(\text{large zooplankton})} - \delta^{13}\text{C}_{(\text{pond nutrient})}$] averaged: PRO 0.71 $\Delta\delta^{13}\text{C}$ ‰ (-0.71 to 2.13 $\Delta\delta^{13}\text{C}$ ‰ 95 % CI), UNP 0.88 $\Delta\delta^{13}\text{C}$ ‰ (-0.65 to 2.40 $\Delta\delta^{13}\text{C}$ ‰ 95 % CI), and CSM 3.03 $\Delta\delta^{13}\text{C}$ ‰ (0.79 to 5.28 $\Delta\delta^{13}\text{C}$ ‰ 95 % CI). The INO pond nutrient treatment (inorganic fertilizer) did not contain carbon and was omitted from analysis. Pairwise comparisons of carbon signature magnitude differences between large zooplankton and their respective pond nutrients found significant differences in isotope signature difference magnitudes between the PRO and CSM, and UNP and CSM treatments (Figure 4-35; $P < 0.05$, Tukey's multiple comparison test). Again, isotopic signature difference magnitudes between the isotopically examined taxa (i.e. large zooplankton $\delta^{13}\text{C}$) and their applied nutrients (feed $\delta^{13}\text{C}$), did not differ between the PRO and UNP treatments.

Nitrogen isotope signature ($\delta^{15}\text{N}$) differences between time-averaged large zooplankton and their respective pond nutrients were calculated and compared among treatments, and significant differences among treatments were found (Figure 4-36: 1-way ANOVA, $P < 0.0001$, $F = 51.68$, $\alpha_{(2,3)} = 0.05$). Nitrogen isotope signature differences between large zooplankton and their respective pond nutrient treatments [$\delta^{15}\text{N}_{(\text{large zooplankton})} - \delta^{15}\text{N}_{(\text{pond nutrient})}$] averaged: PRO 2.66 $\Delta\delta^{15}\text{N} \text{ ‰}$ (0.76 to 4.57 $\Delta\delta^{15}\text{N} \text{ ‰}$ 95 % CI), UNP 2.32 $\Delta\delta^{15}\text{N} \text{ ‰}$ (0.70 to 3.95 $\Delta\delta^{15}\text{N} \text{ ‰}$ 95 % CI), CSM 2.50 $\Delta\delta^{15}\text{N} \text{ ‰}$ (0.68 to 4.31 $\Delta\delta^{15}\text{N} \text{ ‰}$ 95 % CI), and INO 5.03 $\Delta\delta^{15}\text{N} \text{ ‰}$ (3.39 to 6.67 $\Delta\delta^{15}\text{N} \text{ ‰}$ 95 % CI). Pairwise treatment comparisons of large zooplankton and their respective pond nutrient nitrogen isotope signature differences only found significant differences between the INO and other three nutrient treatments, which did not differ from one another (Figure 4-36; $P < 0.05$, Tukey's multiple comparison test).

Small Plankton and Pond Nutrient Isotope Signature Differences

Time-averaged carbon isotope signature ($\delta^{13}\text{C}$) difference magnitudes between small plankton assemblages (1 – 32 μm) and applied nutrients differed among pond treatments (Figure 4-37; 1-way ANOVA, $P = 0.0124$, $F = 5.962$, $\alpha_{(2,2)} = 0.05$). Small plankton and feed nutrient carbon isotope difference averaged: PRO 3.44 $\Delta\delta^{13}\text{C} \text{ ‰}$ (2.75 to 4.13 $\Delta\delta^{13}\text{C} \text{ ‰}$ 95 % CI), UNP 2.58 $\Delta\delta^{13}\text{C} \text{ ‰}$ (1.89 to 3.27 $\Delta\delta^{13}\text{C} \text{ ‰}$ 95 % CI), and CSM 2.21 $\Delta\delta^{13}\text{C} \text{ ‰}$ (1.58 to 2.83 $\Delta\delta^{13}\text{C} \text{ ‰}$ 95 % CI). Again, due to the lack of carbon in the inorganic fertilizer, the INO treatment was omitted from this analysis. Pairwise difference analyses found that the small plankton and pond nutrient carbon isotope differences of the PRO and CSM treatments were significantly different, and again, the PRO and UNP treatments did not differ (Figure 4-37; $P < 0.05$, Tukey's multiple comparison test).

Time-averaged nitrogen isotope signature ($\delta^{15}\text{N}$) difference magnitudes between small plankton assemblages and pond nutrients, also differed among pond treatments (Figure 4-38; 1-way ANOVA, $P = 0.0178$, $F = 4.251$, $\alpha_{(2,3)} = 0.05$). Small plankton and pond nutrient nitrogen isotope differences averaged: PRO $0.58 \Delta\delta^{15}\text{N} \text{‰}$ (0.04 to $1.13 \Delta\delta^{15}\text{N} \text{‰}$ 95 % CI), UNP $0.58 \Delta\delta^{15}\text{N} \text{‰}$ (0.23 to $0.94 \Delta\delta^{15}\text{N} \text{‰}$ 95 % CI), CSM $0.55 \Delta\delta^{15}\text{N} \text{‰}$ (0.20 to $0.90 \Delta\delta^{15}\text{N} \text{‰}$ 95%CI), and INO $1.26 \Delta\delta^{15}\text{N} \text{‰}$ (0.83 to $1.70 \Delta\delta^{15}\text{N} \text{‰}$ 95 % CI). Non-INO treatment small plankton and applied nutrient $\delta^{15}\text{N}$ difference magnitudes were similar, and markedly lower than the INO treatment. Not surprisingly, pairwise treatment comparisons found that the INO treatment small plankton assemblage and inorganic fertilizer nitrogen isotope signature difference, was significantly greater than those of the other three treatments, which did not differ from each another (Figure 4-38; $P < 0.05$, Tukey's multiple comparison test).

Isotopically Derived Large Harvest Fish Diet

Euclidean distance estimates of PRO treatment large harvest fish diet contributions (Figure 4-39, Table 4-1) were: 70% processed feed, 7% large zooplankton, 16% medium plankton, and 7% small plankton. IsoSource model estimates of PRO large harvest fish diet (Table 4-1) were: 90.1% processed feed (87.74 to 92.46% 95 % CI), 0.2% large zooplankton (0.0 to 0.70% 95 % CI), 0.8% medium plankton (0.0 to 1.7% 95 % CI), and 8.9% small plankton (6.79 to 11.01% 95 % CI).

Euclidean distance estimates of UNP treatment large harvest fish diet (Figure 4-40, Table 4-1) were: 90% processed feed, 2% large zooplankton, 6% medium plankton, and 2% small plankton. IsoSource model derived estimates of UNP large harvest fish diet (Table 4-1) were: 91.1% processed feed (86.17 to 96.03% 95 % CI), 1.0% large zooplankton (0.0 to 2.05% 95 %

CI), 6.8% medium plankton (1.55 to 12.05 % 95 % CI), and 1.0% small plankton (0.16 to 1.84 % 95 % CI).

Euclidean distance estimates of CSM treatment large harvest fish diet (Figure 4-41; Table 4-1) were: 17% cottonseed meal, 23% large zooplankton, 51% medium plankton, and 9% small plankton. IsoSource model derived estimates of CSM large harvest fish diet (Table 4-1) were: 21.4% cottonseed meal (10.28 to 31.68 % 95 % CI), 22.0% large zooplankton (6.05 to 37.95 % 95 % CI), 53.6% medium plankton (29.25 to 77.95 % 95 % CI), and 1.1% small plankton (0.0 to 3.62 % 95 % CI).

Euclidean distance estimates of INO treatment large harvest fish diet (Figure 4-42, Table 4-1) were: 65 % large zooplankton, 25 % medium plankton, and 10 % small plankton. IsoSource model derived estimates of INO large harvest fish (Table 4-1) were: 86.5 % large zooplankton (82.72 to 90.28 % 95 % CI), 8.6 % medium plankton (2.41 to 14.79 % 95 % CI), and 4.9 % small plankton (1.75 to 8.05 % 95 % CI).

Isotopically Derived Small Harvest Fish Diet

Euclidean distance estimates of PRO treatment small harvest fish diet contributions (Figure 4-38, Table 4-1) were: 35 % processed feed, 14 % large zooplankton, 33 % medium plankton, and 19 % small plankton. IsoSource model derived estimates of PRO small harvest fish diet (Table 4-1) were: 43.5 % processed feed (37.79 to 49.21 % 95 % CI), 7.0 % large zooplankton (1.41 to 12.59 % 95 % CI), 14.8 % medium plankton (3.38 to 26.22 % 95 % CI), and 34.7 % small plankton (31.60 to 37.80 % 95 % CI).

Euclidean distance estimates of UNP treatment small harvest fish diet (Figure 4-39, Table 4-1) were: 37 % processed feed, 14 % large zooplankton, 32 % medium plankton, and 17 % small plankton. IsoSource model derived estimates of UNP small harvest fish diet (Table 4-1) were: 22.7 % processed feed (8.43 to 36.97 % 95 % CI), 13.4 % large zooplankton (8.36 to 18.44

% 95 % CI), 29.4 % medium plankton (10.93 to 47.87 % 95 % CI), and 34.5 % small plankton (31.98 to 37.02 % 95 % CI).

Euclidean distance estimates of CSM treatment small harvest fish diet (Figure 4-41, Table 4-1) were: 21% cottonseed meal, 25% large zooplankton, 40% medium plankton, and 14% small plankton. IsoSource model derived estimates of CSM small harvest fish diet (Table 4-1) were: 0.6 % cottonseed meal (0.0 to 1.44% 95 % CI), 63.3 % large zooplankton (61.73 to 64.87 % 95 % CI), 2.0% medium plankton (0.01 to 3.99 % 95 % CI), and 34.1% small plankton (33.16 to 35.04 % 95 % CI).

Euclidean distance estimates of INO treatment small harvest fish diet (Figure 4-42, Table 4-1) were: 34 % large zooplankton, 47 % medium plankton, and 19 % small plankton. IsoSource model derived estimates of INO treatment small harvest fish (Table 4-1) were: 67.3 % large zooplankton (64.05 to 70.55 % 95 % CI), 7.5 % medium plankton (1.52 to 13.48 % 95 % CI), and 25.3 % small plankton (22.36 to 28.24 % 95 % CI).

Isotopically Derived Pre-Harvest Fry Diet

Euclidean distance estimates of PRO treatment pre-harvest fry diet (Table 4-2) were: 30 % processed feed, 23 % large zooplankton, 38 % medium plankton, and 9 % small plankton. IsoSource model derived estimates of PRO treatment pre-harvest fish (Table 4-2) were: 49.8 % processed feed (45.71 to 53.89 % 95 % CI), 46.9 % large zooplankton (43.96 to 49.84 % 95 % CI), 3 % medium plankton (0.17 to 5.83 % 95 % CI), and 0.3 % small plankton (0.0 to 0.82 % 95 % CI).

Euclidean distance estimates of UNP treatment pre-harvest fry diet (Table 4-2) were: 24 % unprocessed feed, 19 % large zooplankton, 50 % medium plankton, and 8 % small plankton. IsoSource model derived estimates of UNP treatment pre-harvest fish (Table 4-2) were: 33.1 % unprocessed feed (19.35 to 46.85 % 95 % CI), 42.4 % large zooplankton (35.37 to 49.43 % 95 %

CI), 23.7 % medium plankton (4.81 to 42.59 % 95 % CI), and 0.7 % small plankton (0.0 to 1.64 % 95 % CI).

Euclidean distance estimates of CSM treatment pre-harvest fry diet (Table 4-2) were: 34 % cottonseed meal, 21% large zooplankton, 31% medium plankton, and 14% small plankton. IsoSource model derived estimates of CSM treatment pre-harvest fish (Table 4-2) were: 54.6 % cottonseed meal (50.30 to 58.90 % 95 % CI), 20.9 % large zooplankton (8.94 to 32.86 % 95 % CI), 23.2 % medium plankton (7.56 to 38.84 % 95 % CI), and 1.3% small plankton (0.0 to 2.66 % 95 % CI).

Euclidean distance estimates of INO treatment pre-harvest fry diet (Table 4-2) were: 32% large zooplankton, 55% medium plankton, and 13% small plankton. IsoSource model derived estimates of INO treatment pre-harvest fish (Table 4-2) were: 34.1 % large zooplankton (32.74 to 35.46 % 95 % CI), 65 % medium plankton (63.69 to 67.31 % 95 % CI), and 0.9 % small plankton (0.0 to 1.84 % 95 % CI).

Discussion

Pond Nutrient Carbon Isotope Signatures

Commercial feed (PRO and UNP) carbon isotope signatures differed from the applied cottonseed meal (CSM), but did not differ from each other due to their manufacture from identical ingredients (Figure 4-4). Commercial feeds were an indeterminate mixture of C₃ and C₄ crop plants and animal by-products, the primary protein sources within the two commercial feeds were soybean meal, wheat middlings, and fishmeal. Unfortunately, actual ingredient inclusion rates were not available from the manufacturer due to the proprietary nature of the commercial feeds [J. Herlyn (Purina Feed LLC) pers comm.].

Cottonseed meal is a high protein, commonly applied organic fertilizer derived from the harvesting and processing of cotton, a broadleaved dicotyledonous (C₃) crop plant (Fowler 1980,

Teichert-Coddington 1997). The $\delta^{13}\text{C}$ signature measured for cottonseed meal (C_3) in this study ($\bar{X} = -26.43 \text{‰ } \delta^{13}\text{C}$) agreed well with the mean $\delta^{13}\text{C}$ signature value reported in the literature for other terrestrial dicotyledonous plants (C_3 dicot $\sim -27.00 \text{‰ } \delta^{13}\text{C}$; O'Leary 1988, Boutton 1991). In contrast, terrestrial monocots typically have much higher carbon isotope signatures (C_4 monocot $\sim 13 \text{‰ } \delta^{13}\text{C}$; O'Leary 1988, Boutton 1991, Fry 2006).

The inorganic fertilizer (ammonium nitrate solution) used in the study, did not contain carbon, and therefore did not produce a carbon signature reading during carbon and nitrogen isotope analyses. For this reason, applied inorganic fertilizer nutrient and various INO treatment taxa carbon isotope signature difference calculations were not performed.

Pond Nutrient Nitrogen Isotope Signatures

Commercial feed (PRO and UNP) nitrogen isotope signatures differed from the two fertilizers (CSM and INO), which differed from each other (Figure 4-5). Again, the PRO and UNP commercial feeds did not differ due to their manufacture from identical ingredients.

Although cottonseed meal's nitrogen isotope signature significantly differed from that of the two feed treatments, it was only slightly lower than that of the two applied feeds (Figure 4-5). The INO fertilizer nutrient nitrogen isotope signature ($\bar{X} = -0.08 \text{‰ } \delta^{15}\text{N}$) was markedly lower than those of the other three nutrient treatments ($\delta^{15}\text{N}$ range: 3.79 to 4.80 ‰), due to its manufacture from atmospheric nitrogen ($\delta^{15}\text{N}_{(\text{atm. N})} = 0.00 \text{‰}$). The extremely low inorganic fertilizer $\delta^{15}\text{N}$, proved useful as a tracer within the inorganic fertilizer treatment ponds. Subsequent trophic levels/plankton size assemblages within the INO treatment ponds had markedly lower nitrogen isotope signature values than their commercial feed and organic fertilizer treatment counterparts (Figures 4-18, 4-23, 4-28).

Pre-harvest Fry Isotope Signatures

Due to budget and time constraints, pre-harvest fry from only three sampling intervals were analyzed for carbon and nitrogen isotope signatures to determine if, when, and at what weight, fry carbon and nitrogen isotopic signatures significantly diverged from presumably isotopically similar stock. Fry were not obtained for pond A11 (PRO) for one sampling date, 4 April 2006, due to low fry standing stock at the onset of the study; this may have been a result of the presence of invasive killifish, which ultimately necessitated the omission of this pond from data analyses at harvest. Many ponds had few fry at the beginning of the trial, and acquiring even a small number of fry took much more time than later in the study when standing stocks of fry were higher.

Broodstock for the outdoor pond trials were obtained from different sources (greenhouse vats and outdoor ponds) and had different nutritional histories (i.e., indoor fish: TetraMin™ Staple Flake; outdoor fish: Purina Aquamax®). Isotopically, offspring of mothers from different sources initially resembled their mothers and likely differed from fry of mothers from other sources. However, the initial **mean** isotopic signature of all broodstock and fry in the ponds should have been equal due to the random allocation of broodstock among the 24 replicate ponds of the four pond nutrient treatments (Zar 1984, Riley and Edwards 1998, Montgomery 2001).

Following diet switching by broodstock and as nascent fry consumed similar food items, it was expected that isotopic signatures among fry within ponds and treatments would converge (DeNiro and Epstein 1978, 1981a, Minagawa and Wada 1984, Fry 1991, 2006, Fry et al. 1996), and diverge among fry from differing nutrient treatment ponds over time. This was the observed pattern, as fry isotope signatures increasingly diverged over time (Figures 4-4 – 4-10).

Pre-Harvest fry Carbon Isotope Signatures

Initial fry carbon isotope signatures frequently differed with subsequent sampling dates within a given treatment: day 7 and day 14 fry (PRO and CSM treatments), and day 7 and 21 day fry (PRO, UNP and CSM treatments). However, no differences in carbon isotope signature were present between day 14 and day 21 fry within any of the four pond nutrient treatments, indicating that fry had reached carbon isotope equilibrium with their new nutrient sources by day 14.

Pre-harvest fry carbon isotope signature differences between treatments were also examined. Only the PRO and UNP fry did not differ at day 21, nor did they differ for the earlier day 7 and day 14 sampling periods. The lack of a $\delta^{13}\text{C}$ signature difference between the PRO and UNP pre-harvest fry was not unexpected as the PRO and UNP feeds were manufactured from the same ingredients. The finding that the two feed treatment fry groups did not differ from each other in carbon signature for any of the three sampling periods, and that both feed treatment fry groups differed from the two fertilizer fry groups by day 21 further supports the hypothesis that feeds applied to ponds were being consumed and assimilated by their intended target groups (swordtails). Another explanation for the observed feed treatment fry isotope signature similarities was that fry were utilizing live feeds (large zooplankton, medium and small plankton) that were obtaining their nutrition from the applied feeds, and that the exploited live food bases within the feed treatment ponds had similar isotopic signatures.

Cottonseed meal and inorganic fertilizer treatment pre-harvest fry carbon isotope signatures differed for all three sampling dates. Likely a result of differing carbon sources for primary producers within the CSM and INO treatment ponds (cottonseed meal decomposition, and abiotic carbon compounds, respectively).

Pre-harvest fry carbon isotope signature differences that developed over time (~ 14-21 days) from isotopically similar pre-harvest pond fry, indicate that nutrients derived from applied feeds and fertilizer nutrients resulted in isotopically distinct fry treatment groups over time. This finding was identical to that observed among indoor study fry fed five experimental feeds (Chapter 2; Figure 2-17).

Pre-Harvest Fry Nitrogen Isotope Signatures

Similar to pre-harvest fry carbon isotope signature results, pre-harvest fry nitrogen isotope signatures differed among sampling dates and pond nutrient treatments (Figures 4-4 – 4-7). The pattern of nitrogen isotope differences between fry collected on different sampling dates, but within a given pond nutrient treatment resembled that for carbon isotopes. If present, nitrogen signature differences were only between day 7 and day 14 (CSM and INO treatments) or day 7 and day 21 (INO treatment), no differences were present between day 14 and day 21. Within their respective treatments, PRO and UNP pond nutrient treatment pre-harvest fry did not isotopically differ for days 7, 14, or 21.

Nitrogen isotope signature differences, between fry from different pond nutrient treatments collected on the same sampling date, also followed a pattern similar to those found for carbon isotope signatures (Figure 4-6). Isotopic differences, that may not have been initially present (day 7), developed over time between fry of different pond nutrient treatments (day 14, day 21 and at harvest - day 90). PRO and UNP treatment pre-harvest fry, did not differ in nitrogen isotopic signature for any of the three time periods (day 7, 14, 21), which again was not surprising, as the two commercial feeds were manufactured from the same ingredients. Again, supporting the contention that fry within the PRO and UNP treatment ponds were either feeding upon and assimilating the applied commercial feeds, and/or were utilizing live prey that were

utilizing the applied feeds, or at the very least, utilizing live prey that were isotopically influenced by the application of the applied feeds.

Large Harvest Fish Carbon Isotope Signature Differences

PRO and UNP treatment large harvest fish carbon isotope signatures did not differ, which was not surprising as these two groups consumed feeds that were manufactured using identical ingredients. Although the mixture of C₃ and C₄ terrestrial plant crops (C_{3(dicots)} ~ -27.00 ‰ δ¹³C, C_{4(monocots)} ~ -13.00 ‰ δ¹³C; Boutton 1991, Fry 2006), fishmeal, and their respective inclusion rates in the commercial feeds produced a composite δ¹³C signature that differed from that of the CSM fertilizer (Figure 4-4), CSM treatment large harvest fish carbon isotope signatures, did not differ from those of the two feed treatments (Figure 4-12).

The finding and reason for why CSM treatment large harvest fish carbon isotope signatures did not differ from both the PRO and UNP fish is less clear. Cottonseed meal is a readily available and inexpensive terrestrial plant by-product from the cotton industry that is high in protein with an attractive amino acid profile for use as an animal feed (NRC 1993). It is likely that cottonseed meal acted as both an organic fertilizer that stimulated the production of living organisms (potential live feeds) within the ponds and as a high quality meal ‘feed’ that was directly consumed (pers. obs.) and partially assimilated by the fish (Fowler 1980, Teichert-Coddington et al. 1997).

Isotopic signature differences among the applied nutrients (Figure 4-3; PRO, UNP and CSM), and their respective plankton assemblage δ¹³C signatures (Figures 4-18, 4-23, 4-28; large, medium, and small plankton), were no longer present at the highest trophic levels – small and large harvest fish (Figures 4-11 – 4-12). Carbon isotope signature differences present between the two commercial feeds and cottonseed meal and their respective small plankton assemblages

(Figures 4-3, 4-28), were only 'partially' present within the medium plankton (Figure 4-23; PRO and CSM medium plankton – no $\delta^{13}\text{C}$ difference) and large zooplankton assemblages (Figure 4-18; UNP and CSM large zooplankton – no $\delta^{13}\text{C}$ difference); and as mentioned previously, were no longer present within the two harvest fish size classes (Figures 4-11 – 4-12).

The carbon isotope enrichment magnitude present between the applied cottonseed meal and CSM treatment large harvest fish indicates that interstitial trophic groups were present between the applied cottonseed meal and CSM harvest fish, which was the expected food chain/web pattern within the CSM treatment. A plausible explanation for why small plankton assemblage carbon isotope signature differences among the PRO, UNP and CSM treatments were no longer present among the small and large harvest fish groups of these treatments was due to the highly catholic and omnivorous feeding habits of the swordtail (Axelrod 1991, Tamaru et al. 2001). Swordtail diets (especially within the CSM treatment) likely consisted of numerous plant and animal sources from different trophic levels, which may have obscured carbon isotope signature differences present at the base and lower trophic levels (Figure 4-28) of the food web/chain, by the point that these nutrients had reached higher trophic levels.

INO large harvest fish carbon isotope signatures differed from those of the other three treatment groups (Figure 4-11), likely due to the fact that INO fish were only able to consume live plants and animals that ultimately obtained their carbon from autochthonous sources (Boyd 1979, 1995) within these ponds (remineralized inorganic carbon derived from bottom substrates, pond respiration, decaying organic matter), entering from the atmosphere (atmospheric CO_2 , dust particles, insects, organic debris, etc.) or carried in by ground water movements (aqueous carbonic acid, aqueous CO_2 , carbonates, etc.).

These largely inorganic forms (with the exception of insects and plant material entering the ponds) of carbon could not be directly utilized by INO pond treatment fish, requiring inorganic carbon to be fixed, assimilated or liberated by other pond organism (photosynthetic autotrophs, bacterial, and fungal decomposers) before entering the pond food web and becoming available to the target species (Boyd 1979, 1995, Schroeder 1987, Diana et al. 1991, Hall 2004, De Brabandere et al. 2007). The INO small plankton assemblage (primary phytoplankton) utilized this inorganic carbon pool(s), producing a composite carbon isotope signature differing from those of the other three treatments (Figure 4-28). Due to the $\delta^{13}\text{C}$ signature differences present between the INO and non-INO small plankton assemblages, it is also assumed that the carbon sources utilized by 1° producers also differed within the INO and non-INO treatments.

Assuming that non-anthropogenic or unintentional anthropogenic allochthonous and autochthonous carbon sources were equally available to all twenty-four treatment ponds, small plankton assemblage carbon isotope signature differences among treatments likely arose from autotroph assimilation of dissolved carbon compounds (DIC/DOC) leached, and/or remineralized from, the applied nutrients following consumption, assimilation, respiration, and excretion by (or death and decay of) pond heterotrophs (Boutton 1991, Diana et al. 1991, Fry 1991, Boyd 1997, Hoyer et al. 1998, Gregory-Eaves et al. 2007). Isotopically differing inorganic carbon pools should result in isotopically differing primary producer (small plankton assemblage) carbon isotope signatures within the feed and organic and inorganic fertilizer treatment ponds (Keeley et al. 1986, Kline et al. 1993, Fry 1999, Fry et al. 1999).

Unfortunately, inorganic carbon pool (DIC: aqueous CO_2 , HCO_3^- , and CO_3^{2-}) carbon isotope signatures were not measured within the different treatment ponds and their contribution to phytoplankton carbon isotope signatures relative to atmospheric carbon remains unknown.

Additionally, non-anthropogenic autochthonous and allochthonous inorganic carbon input magnitudes also were unknown.

With each additional trophic level occurring between an inorganic carbon source and the target species, an observable increase in the carbon isotope signature ($\delta^{13}\text{C}$ enrichment) of the target species occurs (DeNiro and Epstein 1978, Fry and Parker 1979). The carbon isotope signature difference between INO harvest fish and their presumptive basal carbon source(s) was likely greater in magnitude than that of the other three treatments, reflecting the greater number of trophic levels carbon was believed to have passed through before being assimilated by the fish. Unfortunately, the INO treatment DIC pool $\delta^{13}\text{C}$ signature was not isotopically measured, making it necessary to use the INO small plankton assemblage $\delta^{13}\text{C}$ signature in food web/chain length comparisons (Vander Zanden and Rasmussen 1999).

In addition to small plankton assemblage carbon isotope signature differences among the four treatments resulting from possible leaching and remineralization of the different applied nutrients, fundamentally different mechanisms by which carbon entered the two swordtail size classes likely occurred between the two feed treatments (via feed ingestion) and the two fertilizer treatments (via food web utilization, especially within the INO treatment). Primary producer carbon isotope signature differences among treatments (Figure 4-28), and/or direct ingestion of applied nutrients versus live food web utilization differences in swordtail nutrition were likely responsible for the large carbon isotope signature differences observed between INO large harvest fish and those within the other three treatments (Figure 4-11).

Large Harvest Fish Nitrogen Isotope Differences

Large harvest fish nitrogen isotope signatures did not differ among pond nutrient treatments (Figure 4-11). It was somewhat surprising that INO treatment large harvest fish

nitrogen isotope signatures did not differ from that of the other three treatments, given that the three INO plankton assemblages (large, medium, small) and INO small harvest fish nitrogen isotope signatures were significantly lower than their counterparts within the other three treatments (Figures 4-12, 4-18, 4-23, 4-28). INO treatment plankton assemblages were dependent upon applied inorganic fertilizer as their primary nitrogen source (Boyd 1974, 1979, Schroeder 1978, Yusoff and McNabb 1989, Knud-Hansen et al. 1993, Knud-Hansen 1998, Kastner and Boyd 1996, Ludwig et al. 1998), and had much lower $\delta^{15}\text{N}$ signatures than their non-INO treatment counterparts. Because atmospheric nitrogen was the feedstock used in the industrial manufacture of the inorganic fertilizer, this made the inorganic fertilizer's nitrogen signature [by definition $\text{N}_{2(\text{atm})}$ 0.00 ‰ $\delta^{15}\text{N}$] much lower than that of the next lowest applied nutrient (Figure 4-5; cottonseed meal = 3.79 ‰ $\delta^{15}\text{N}$).

Although the lack of large harvest fish $\delta^{15}\text{N}$ signature differences among treatments seems puzzling in light of the carbon isotope signature results, it was not surprising when it is known that nitrogen isotope signatures are less useful as nutrient source tracers than as a means of providing relative trophic position information (Hideshige and Wada 1990, Kling and Fry 1992, Minagawa and Wada 1984, Hansson et al. 1997, Fry et al. 1999, Fry 2006, Michener and Kaufman 2007). Nitrogen isotope signature enrichment rates can be highly variable between trophic levels, and enrichment rates of 1.5 to 4.0 ‰ for a single trophic level have been reported (DeNiro and Epstein 1981, Fry 2006).

Application of the four different pond nutrient regimes was expected to have resulted in large harvest fish nitrogen isotope signature differences among the four pond treatments. Omnivory by swordtails may explain the lack of nitrogen isotope signature differences among large harvest fish within pond treatments (Figure 4-11). If swordtails were consuming plant and

animal prey, as well as animal prey from different trophic levels, this more trophically varied 'food web' diet would result in a greater loss of the basal nutrient's isotopic and trophic identity among their higher trophic level consumers, than would have resulted from a less trophically varied (e.g., strict zooplanktivore) and more simplistic dietary food chain (1° producer → 1° consumer → 2° consumer → 3° consumer → etc.) originally postulated for swordtails within the fertilizer treatment ponds. Food chains originally postulated for feed treatment ponds were even more simplistic (applied feed → swordtails). These simple food chains were somewhat unrealistic given what is known of swordtail dietary habits and managed pond food web structure.

A more intriguing explanation for the lack of differences in large harvest fish $\delta^{15}\text{N}$ signature among the four pond treatments, and the INO treatment large harvest fish in particular, is the possibility of high rates of cannibalism of small fry and juveniles by larger fish within the ponds (Dione 1985, Jones et al. 1998a, 1998b, 2007, Beaudoin et al. 1999, Saito et al. 2000, Kruger et al. 2001). Cannibalism seems plausible in light of the lower nitrogen isotope signature of the INO taxa (small, medium, and large plankton assemblages, and small harvest fish) relative to their counterparts in the other three treatments, and the lack of large harvest fish $\delta^{15}\text{N}$ signature differences among the four pond treatments (Figures 4-11 – 4-12, 4-18, 4-23, 4-28). Significant cannibalism by large harvest fish within the INO treatment, would place INO large harvest fish at a higher trophic level and increase their nitrogen isotope signatures to a greater degree than if they were strictly consuming non-fish live foods. Additionally, small fish and fry (≤ 31 mm SL) made up a large percentage of the fish within the INO treatment ponds (Figures 3-10 – 3-12), large numbers of size 'stunted' fish have been cited as being an indicator of food limitation (Yusoff and McNabb 1989, Osenberg et al. 1992, Hopkins and Unwin 1997, Ingram

2009). Evidence of food limitation for INO treatment harvest fish was provided by the large variation (Chapter 3; Figures 3-13, 3-15) in INO treatment individual large fish weights and coefficient of variation (weight) values, relative to those of the other three treatments, also which are often indicators of food limitation (Ali 1990, Diana et al. 1991).

Small Harvest Fish Carbon Isotope Signatures

Small harvest fish carbon isotope signatures followed a trajectory similar to that of large harvest fish; INO treatment small harvest fish were more enriched in ^{13}C relative to those of the other three treatments (Figure 4-11 - 4-12; note: higher $\delta^{13}\text{C}$ values, closer to abscissa). Again, the higher INO small fish carbon isotope signature was likely due to a combination of the greater number of trophic levels believed to be separating INO small harvest fish from their basal nutrient source, and the higher carbon isotope signature of the INO treatment small plankton assemblage and its possible contribution to swordtail diet (Figures 4-18, 4-23, 4-28).

Another possible factor contributing to higher INO small and large harvest fish $\delta^{13}\text{C}$ signatures is that the INO treatment basal inorganic carbon pool $\delta^{13}\text{C}$ signature was considerably higher than those of the other treatments. This may have been the case if carbon from the non-INO treatment nutrients were present at high enough concentrations as remineralized DIC, to influence small phytoplankton $\delta^{13}\text{C}$ within their respective ponds during carbon fixation; resulting in primary producers that isotopically resembled ($\delta^{13}\text{C}$) their respective applied nutrients. Unfortunately, due to budget, time and logistical constraints, $\delta^{13}\text{C}$ signatures of basal inorganic carbon pools within the various treatment ponds were not measured.

Small Harvest Fish Nitrogen Isotope Signatures

In contrast to INO large harvest fish nitrogen isotope signatures, small harvest fish nitrogen isotope signatures differed from those of the other three treatments (Figure 4-12). Only

INO pond treatment fish were entirely dependent upon live food generated within the pond ecosystem, as no directly ingestible nutrients were applied to the INO treatment ponds. Fish within the PRO, UNP, and to a lesser degree CSM pond treatments may have been partly or even largely independent of any reliance upon live foods. Small harvest fish within the PRO, UNP, and CSM treatments were consuming applied nutrients, and/or pond organisms that were consuming, or at a minimum isotopically influenced by the applied nutrients (Figure 4-5; INO treatment lowest $\delta^{15}\text{N}$ signature), in quantities sufficient to raise their nitrogen isotope signatures to values greater than those within the INO treatment. The pattern of higher $\delta^{15}\text{N}$ signatures of the non-INO treatments' large, medium and small plankton assemblages compared to their INO treatment counterparts (Figures 4-18, 4-23, 4-28), was identical to that found for plankton assemblage $\delta^{13}\text{C}$ signatures (i.e., INO δC and δN differing from those of the non-INO treatments).

As previously suggested, non-piscine heterotrophs (e.g., zooplankton) may have been consuming applied nutrients, and autotrophic organisms (e.g., phytoplankton) may have been absorbing organic compounds leached from the applied nutrients or utilizing remineralized inorganic carbon and nitrogen derived from other sources. Dissolved inorganic nitrogen (DIN) may have been derived from remineralized applied nutrients, primarily in the form of excreted ammonia and urea, or as free amino acids, or remineralized nitrite and nitrate. This was likely the reason that different plankton size assemblages (including phytoplankton) had similar nitrogen isotope signatures within treatments (PRO, UNP and CSM), and why patterns of nitrogen isotope signature differences for different taxa groups (e.g., small plankton or small harvest fish) were similar among treatments (Figure 4-12, 4.28; i.e., INO treatment taxa consistently had lowest $\delta^{15}\text{N}$).

Although INO small fish mean weight did not statistically differ from those of the three other treatments, INO small fish mean weight (0.18 g) was lower (22 % less; Figure 3-14) than that of the next lowest mean (PRO: 0.23 g). Because fish size is generally proportional to fish mouth gape, which is directly correlated with potential prey size, it is likely that INO treatment small harvest fish fed on smaller prey, from lower trophic levels than their counterparts within the non-INO treatments (Werner and Hall 1979, Dussault and Kramer 1981, Lemly and Dimmick 1982, Schmitt and Holbrook 1984a, b, Post and McQueen 1987, Arthington 1989, Holbrook and Schmitt 1992, 1994, Osenberg et al. 1992, Mittelbach and Osenberg 1993, Wainwright and Richard 1995, DeVries et al. 1998, Cailliet et al. 1996, Gu et al. 1996a, b, Mittelbach et al. 1999). The combination of lower INO small harvest fish weight and higher INO treatment small fish numbers (Chapter 3; Figures 3-12, 3-14), may have caused INO small fish diets to shift in favor of smaller prey due to both mouth gape and/or prey abundance limitations, due to increased intraspecific competition.

Smaller, lower trophic level prey also was a possible explanation for the lower $\delta^{15}\text{N}$ signature of the INO treatment small harvest fish relative to their counterparts in the other three treatments. As previously mentioned, the complete dependence of INO treatment small harvest fish upon live foods produced within the ponds, and the lower $\delta^{15}\text{N}$ signatures observed for the INO treatment plankton assemblages (Figures 4-18, 4-23, 4-28), may have manifested itself as the lower $\delta^{15}\text{N}$ signature of INO small harvest fish. The lower nitrogen isotope signature (tracer signal) of the INO treatment's inorganic fertilizer basal nutrient relative to the non-INO treatment basal nutrients appears to have propagated throughout the INO treatment food web.

Harvest Fish Size Class Isotope Signature Differences

As previously mentioned, the small carbon isotope signature difference observed between small and large harvest fish within a given treatment, may have been due to ontogenetic differences between small and large fish; with smaller fish eating smaller food items from lower trophic levels relative to larger fish due to gape size limitations, and behavioral differences in microhabitat preference (Schmitt and Holbrook 1984a, Holbrook and Schmitt 1992, Cailliet et al. 1996, Mittlebach and Osenberg 1993). Ontogenetic dietary differences are consistent with the observation that smaller fish and fry were closely associated with the submerged and emergent vegetation present in the shallow water areas adjacent to pond perimeters. Aggregations of presumably higher trophic level large macroscopic zooplankton were frequently observed at the surface in deeper portions of the pond, where larger fish were known to congregate, and smaller fish were absent.

Large Zooplankton Carbon Isotope Differences

Large zooplankton carbon isotope signatures did not differ among the PRO, UNP, and CSM treatments for any of the 10 weekly sampling dates, only the INO treatment large zooplankton carbon isotope signatures differed from those of the other treatments for any of the 10 sampling dates. During the last three weeks of the study, INO treatment large zooplankton $\delta^{13}\text{C}$ (range: -19.76 to -19.88‰) signatures were considerably higher than those of the other three treatments (mean values: -24.25 ‰ CSM, -23.16 ‰ UNP, -22.59 ‰ PRO). The frequency with which the INO treatment large zooplankton $\delta^{13}\text{C}$ signatures differed from those of the other three treatments varied: PRO and INO treatments (one sampling period), UNP and INO (two periods), and CSM and INO (five periods). The majority of between-treatment differences in large zooplankton $\delta^{13}\text{C}$ occurred during the last five weekly sampling periods. All five CSM and

INO large zooplankton $\delta^{13}\text{C}$ isotopic signature differences occurred during the last five sampling dates. A decrease in variation and stabilization about a mean $\delta^{13}\text{C}$ value (range: -19.76 to -19.88 ‰) was observed for the INO treatment large zooplankton, and was most likely responsible for the observed differences between the INO and CSM treatments near the end of the trial (Figure 4-17). The frequency and temporal pattern of large zooplankton carbon isotope signature differences between the two fertilizer treatments, suggests that isotopic equilibrium between the CSM and INO large zooplankton assemblages and their respective applied nutrients was not reached until approximately half way into the pond trial. This supports the contention that carbon within the cottonseed meal and inorganic fertilizer ponds were derived from different sources, which was fortunate, in that this likely produced the isotopically contrasting CSM and INO small phytoplankton assemblage, and subsequent trophic level taxa $\delta^{13}\text{C}$ differences (Figures 4-18, 4-24, 4-28).

The absence of large zooplankton carbon isotope signature differences between the PRO and UNP treatments for the 10 sampling dates was not surprising as both presumably received a large portion of their nutrition from applied feeds that had identical chemical compositions. The fact that the PRO and UNP large zooplankton carbon isotope signatures did not differ from each other, but differed from those of the two fertilizer treatments, supports the hypothesis that pond organisms (not limited to fish) in the feed treatment ponds were either assimilating, or at a minimum, isotopically influenced by the applied feeds. Additionally, both feeds were ground to a fine powder, so the possibility of consumption and assimilation by heterotrophic macrozooplankton seems plausible.

Large zooplankton carbon isotope signatures differed among sampling dates within each of the four pond nutrient treatments. However, within treatments, large zooplankton carbon

isotope signature differences among sampling dates did not appear to follow any discernable pattern (Figures 4-14 – 4-17). Within-treatment carbon isotope signature differences among sampling dates were more frequent within the two fertilizer treatments. The CSM treatment had the greatest number of pairwise differences (10), followed by the INO treatment (7). Only three differences were present within the PRO treatment and only one within the UNP treatment. Although the CSM treatment had the greatest number of pairwise differences in large zooplankton $\delta^{13}\text{C}$ signature among sampling dates, all significant pairwise differences involved a single date that had an unusually low carbon signature (25 May 2006, $-28.92\text{‰ } \delta^{13}\text{C}$). INO treatment large zooplankton δC signature differences among sampling dates, also only involved a single date with a high carbon signature (4 April 2006, $-18.95\text{‰ } \delta^{13}\text{C}$) and the three earliest sampling dates, which had relatively low carbon signatures (Figure 4-17). These differences were likely due to decreasing $\delta^{13}\text{C}$ signature variation among sampling date means as the trial progressed; large zooplankton $\delta^{13}\text{C}$ signature 95 % CI near the end of the trial were noticeably narrower than those at the start of the trial (Figures 4-16 – 4-17). Although variation decreased with time for both large zooplankton carbon and nitrogen signatures for the PRO, UNP, and CSM treatments, this decrease was greatest within the INO pond nutrient treatment. The higher frequency of large zooplankton carbon isotope signature differences between treatments during latter sampling periods suggests that large zooplankton (and other pond taxa) had come to isotopic equilibrium with their prey organisms and applied nutrients (Figure 4-13-4.17).

The smaller number of isotopic signature differences among sampling dates within the INO treatment, may have been due to higher levels of chemical and isotopic homogeneity of carbon and nitrogen pools within the INO treatment. The atmospheric nitrogen used in the manufacture of the liquid inorganic fertilizer (Haber-Bosch process) was chemically and

isotopically homogenous (Haber 1920). Presumably, the carbon pools utilized by primary producers within the INO pond treatment also were relatively large, well mixed, and isotopically homogenous and stable over the three-month study (atmospheric carbon dioxide, dissolved ground water carbon dioxide and carbonates; Boutton 1991). Although atmospheric carbon isotope signatures undergo seasonal and latitudinal variations, primarily due to vegetative growing seasons and carbon fixation due to photosynthesis (Estep and Vigg 1985, Takahashi et al. 1990, Zohary et al. 1994, Hall 2004), these changes should have been relatively minor during the short duration of this study.

In contrast to the isotopic homogeneity of the carbon pool utilized by the INO pond treatment primary producers, the potential for carbon isotope variation among the commercial feed (e.g., animal and plant material), and cottonseed meal particles was relatively high. Organisms consuming these nutrients may not be assimilating particles of uniform chemical and isotopic composition (more so within the UNP and CSM treatments), and therefore, individual consumer organisms may vary slightly in their chemical and isotopic makeup (C. Watson pers comm.).

Isotopic changes also may have been occurring as these perishable materials (feeds and cottonseed meal) aged and oxidized, were subjected to changes in humidity and temperature, and underwent bacterial and fungal decomposition. Feeds, cottonseed meal and liquid fertilizers were stored in a climate controlled room at a temperature of approximately 21°C (+/- 4°C). However, humidity and temperature levels noticeably fluctuated throughout the day, depending on ambient temperature and weather conditions. The inorganic fertilizer treatment is believed to have been relatively stable due to its simple chemical composition, storage conditions (cool, dark

room), and highly concentrated form that would tend to inhibit microbial growth, in comparison to the applied organic nutrients.

Unfortunately, to perform repeated measures ANOVA, it was necessary to replace 16 missing large zooplankton carbon isotope signature values (of 264 possible single date and pond replicate combinations) with ‘pseudovalues’. Missing carbon isotope signature values were the result of insufficient quantities of biological material required for isotope ratio mass spectrometry analysis. This usually occurred at the beginning of the trial, when recently drained and sterilized ponds were still in the initial stages of colonization by aquatic organisms; cooler weather and shorter photoperiods also contributed to lower standing stocks of phytoplankton and zooplankton (Fogg and Thake 1987, Malin and Paerl 1994, Hall 2004, Hoeninghaus et al. 2008)

Lower plankton standing stocks early in the study relative to later sampling periods, were almost certainly responsible for the significant ‘time’ factor, in the two-factor repeated-measures ANOVA. It was not clear how the significant time and nutrient treatment factor interactions were auto-correlated to produce any obvious trends (Zar1984, Sokal and Rohlf 1981).

Time-Averaged Large Zooplankton Carbon Isotope Signatures

Time-averaged large zooplankton $\delta^{13}\text{C}$ signature differences among the four treatments, found that only two of the six possible treatment pair combinations did not significantly differ: PRO and UNP, and UNP and CSM (Figure 4-18). PRO and UNP large zooplankton $\delta^{13}\text{C}$ isotope signatures did not differ, possibly due to direct consumption of the isotopically identical PRO and UNP feeds by these relatively large macrozooplankton (> 200 μm), which may have been large enough to directly consume applied feeds (Chapter 5). The reason why time averaged UNP and CSM large zooplankton carbon isotope signatures did not differ is less clear (Figure 4-18), and somewhat puzzling given the presence of $\delta^{13}\text{C}$ signature differences among

the applied feed and cottonseed meal (Figure 4-4), and their respective small plankton assemblages (Figure 4-28). Within the feed and cottonseed meal treatments, carbon isotope signature differences present at the base of the food chain/web, may have become attenuated as carbon progressed to higher trophic levels before becoming statistically insignificant (PRO, UNP, and CSM treatments) within the two top trophic levels examined (Figures 4-11 – 4-12, 4-18; harvest fish, large zooplankton). As previously mentioned, this may have occurred because plankton size assemblages only approximately consisted of functional trophic groups (i.e. 1° producer, 1° consumer, 2° consumer), and each plankton assemblage likely contained a mixture of autotrophs and heterotrophs (Chapter 5), as well as heterotrophic organisms (bacteria, fungi, protists, zooplankton) from different trophic levels (e.g., the medium and large plankton assemblages). Potential large zooplankton omnivory and carnivory from different trophic levels may have further dampened large zooplankton assemblage carbon isotope signature differences among treatments, and among trophic guilds/plankton assemblages within treatments (Kling and Fry 1992, Vander Zanden and Rasmussen 2001). The low magnitude of carbon signature enrichment typically observed ($\sim 0.4-1.0 \text{ ‰ } \Delta\delta^{13}\text{C}$) with each successive trophic level, also likely contributed to the lack of carbon isotope signature differences at higher trophic levels that were present at lower trophic levels (Fry 2006).

The INO large zooplankton assemblage carbon isotope signature was significantly higher than those of the non-INO treatments. Additionally, INO treatment small (primarily phytoplankton), and medium (phytoplankton and rotifers) plankton assemblages, also had carbon isotope signatures higher than their counterparts in the three other treatments (Figures 4-23, 4-28). This was the carbon isotope signature pattern expected for the INO plankton assemblages relative to those within the non-INO treatments; given the primary alternate hypothesis of

parallel nutrient movement within the two feed treatments, and serial nutrient movement within the two fertilizer treatments (more so within the INO than CSM treatment).

The prediction that the INO large plankton assemblage carbon isotope signature would be higher than those of the non-INO treatments, was based upon the assumption that PRO, UNP, and CSM treatment plankton assemblage organisms (small, medium and large) either directly consumed applied nutrients (heterotrophs), indirectly utilized applied nutrients via prey that assimilated applied nutrients (heterotrophs), and/or that primary producers within the non-INO treatments utilized dissolved inorganic carbon (DIC) partially derived from remineralized applied nutrient carbon. Applied nutrients were likely remineralized into DIC following consumption and assimilation by heterotrophic organisms, which could subsequently be utilized by photosynthetic autotrophs (Rounick and Winterbourn 1986, Cabana and Rasmussen 1994, Valiela 1995, Grey et al. 2005, Hall 2004, Gregory-Eaves 2007). If non-INO treatment taxa (plankton and fish) were isotopically influenced by their respective applied nutrients (including via remineralization) to a greater degree than by nutrients derived from food chain/production resulting from autochthonous and non-anthropogenic allochthonous inorganic nutrient sources, these taxa (fish and plankton) should have had carbon isotope signature values more similar to one another (Figure 4-43) compared to taxa within the INO treatment (e.g., greater processed feed treatment $\delta^{13}\text{C}$ similarity between large fish and large zooplankton, compared to their inorganic fertilizer treatment counterparts).

In contrast, INO pond treatment organisms were predicted to have a longer food web/chain between them and their basal autochthonous carbon source(s), becoming more enriched in ^{13}C with each trophic level, and having higher $\delta^{13}\text{C}$ signatures relative to their counterparts in the other three treatments. This carbon isotope signature difference pattern among the four

treatments was not limited to the large zooplankton assemblages. Among the four treatments, carbon isotope signature differences remained the same within all of the postulated ‘trophic’ groups (Figures 4-11 - 4-12, 4-18, 4-23, 4-28; small and large harvest fish, large, medium and small plankton size assemblages): PRO and UNP groups did not differ in $\delta^{13}\text{C}$ signatures, CSM group $\delta^{13}\text{C}$ signatures were slightly lower than other groups, and INO group $\delta^{13}\text{C}$ signatures were significantly higher. PRO and UNP taxa carbon isotope signature similarities were likely due to the manufacture of both feeds from identical ingredients (identical batch/supplier), and the direct (fish, large zooplankton) and indirect (smaller zooplankton and phytoplankton) utilization of the applied feeds by the PRO and UNP treatment pond taxa.

As previously mentioned, an alternate explanation for higher INO taxa $\delta^{13}\text{C}$ signatures relative to their non-INO counterparts is that the DIC pool utilized by INO primary producers had a higher $\delta^{13}\text{C}$ signature than those of the three non-INO treatments; and this higher carbon isotope signature signal was propagated throughout the INO treatment food web taxa, resulting in their higher carbon isotope signatures relative to their non-INO taxa counterparts (Figures 4-11 – 4-12, 4-18, 4-23, 4-28). Additionally, both processes (greater carbon enrichment due to trophic processes – due to longer food chain, and higher INO DIC $\delta^{13}\text{C}$ signature) may have been occurring simultaneously within the INO pond treatment.

However, a higher INO DIC pool $\delta^{13}\text{C}$ signature relative to the non-INO DIC pool $\delta^{13}\text{C}$ signatures scenario seems unlikely, due to the large and relatively uniform (isotopically) inorganic carbon pool(s) typically utilized by freshwater autotrophs (Zohary et al. 1994, Yoshii et al. 1999, Fry 2006). The same non-anthropogenic inorganic carbon pool was likely present within all experimental ponds to some degree, and its influence upon pond taxa carbon isotope signatures likely did not differ among treatments.

Baseline DIC pool carbon isotope signatures for the four pond treatments would have been extremely useful in determining which of the above explanations (if any) was correct, or at least more likely. Unfortunately, DIC carbon isotope signatures were not measured within the four pond treatments due to budget, labor, logistical and time constraints.

Large Zooplankton Nitrogen Isotope Signature Differences

Large zooplankton nitrogen isotope signature values also differed among sampling dates and treatments. Similar to large zooplankton carbon isotope signature results, large zooplankton nitrogen isotope signatures did not differ between PRO and UNP treatments for any of the eight sampling dates. The higher $\delta^{15}\text{N}$ signatures of the two feed treatment large zooplankton assemblages differed less frequently with the CSM fertilizer treatment, than they did with the INO fertilizer treatment: PRO and CSM (3 differences), UNP and CSM (2 differences), PRO and INO (6 differences), and UNP and INO (5 differences). The two fertilizer treatment large zooplankton $\delta^{15}\text{N}$ signatures (CSM and INO), only differed three times during the study. Differences in large zooplankton $\delta^{15}\text{N}$ signature between treatments did not follow any discernable temporal pattern, as differences between treatments were present at the beginning, middle and end of the study with equal likelihood, and were frequently not consecutive.

Large zooplankton $\delta^{15}\text{N}$ signature differences among sampling dates within individual treatments were found for the PRO, CSM, and INO treatments, with no differences among sampling dates present for the UNP treatment. The majority of these pairwise differences involved latter dates and their smaller confidence intervals, and earlier dates when more variation among replicates occurred, especially within the INO treatment (Figures 4-14 - 4-17). Large zooplankton $\delta^{15}\text{N}$ signature variations were markedly lower than $\delta^{13}\text{C}$ signature variations for the same sampling dates and treatments. Although $\delta^{15}\text{N}$ isotope signature variations about the

means were lower (narrower error margins), significant differences in large zooplankton $\delta^{15}\text{N}$ signature among dates were less frequent, due to the flatter (more uniform) time series trajectories of $\delta^{15}\text{N}$ signatures relative to $\delta^{13}\text{C}$ signatures (Figures 4-13 – 4-17). The lower frequency of large zooplankton $\delta^{15}\text{N}$ signature differences relative to $\delta^{13}\text{C}$ signature differences, between and within treatments among the eight sampling dates, was likely a function of both the fewer sampling dates analyzed, and the smaller differences among sampling date means (Figures 4-13 – 4-17).

Unfortunately, it was necessary to omit large zooplankton $\delta^{15}\text{N}$ signature results from the first three weeks of the study due to insufficient large zooplankton biomass in the ponds. Additionally, it was necessary to replace 34 missing sampling date and replicate pond $\delta^{15}\text{N}$ values (of 192 combinations) with ‘pseudovalues’ generated in the same manner as missing $\delta^{13}\text{C}$ signature values, before conducting repeated measures ANOVA. A greater number of missing $\delta^{15}\text{N}$ data occurred than for $\delta^{13}\text{C}$ data. Due to the lower molar content of nitrogen relative to carbon within biological materials, nitrogen has a higher detection threshold than carbon for the isotope ratio mass spectrometer used in this study; hence a greater quantity of biological material is required to adequately perform nitrogen isotope signature analysis (R. Doucett pers. comm.).

Time-Averaged Large Zooplankton Nitrogen Isotope Differences

Time-averaged large zooplankton $\delta^{15}\text{N}$ signature differences were significant among treatments (Figure 4-18). Similar to time-averaged large zooplankton $\delta^{13}\text{C}$ signature results, time-averaged large zooplankton $\delta^{15}\text{N}$ signatures for the PRO and UNP treatments did not differ. Time-averaged CSM large zooplankton $\delta^{15}\text{N}$ signature differed from all other groups, as did the INO large zooplankton $\delta^{15}\text{N}$ signature, which had the lowest $\delta^{15}\text{N}$ isotope signature of all four treatments (Figure 4-18).

The lack of large zooplankton nitrogen isotope signature differences between the PRO and UNP treatments, was likely due to many heterotrophs (including, but not limited to large zooplankton, i.e., fish, etc.) in these ponds either receiving direct nutrition from the isotopically identical applied feeds (Figures 4-11 - 4.12, 4-18, 4-23, 4-28), indirectly by the consumption of a lower trophic group(s) that depended heavily upon the applied feeds, or by consuming primary producers which had utilized and become isotopically influenced by remineralized carbon and nitrogen originally derived from the applied feeds. Direct consumption of the two commercial feeds and to a lesser extent cottonseed meal in the non-INO treatment ponds by pond zooplankton should have produced shorter food chains/webs than those occurring within the INO fertilizer treatment (less trophic distance to the applied and presumed basal nutrients within the INO treatment). Shorter food chains/webs were the observed pattern for time-averaged nitrogen isotope differences between large zooplankton and their applied pond nutrients (Figure 4-36), within the non-INO treatments.

Time-averaged CSM large zooplankton nitrogen isotope signatures were intermediate between those of the two feed and inorganic fertilizer treatments (Figure 4-18), indicating that CSM treatment pond trophic processes may have been intermediate to those occurring within the feed and inorganic treatment ponds. In that cottonseed meal was being utilized by direct consumption as a high protein seed 'meal', and as a green manure that releases nutrients to stimulate primary production during bacterial and fungal decomposition. CSM food chain/web trophic distances may have been longer than those occurring within the two feed treatments, but short in comparison to trophic distances present between the inorganic fertilizer basal nutrient and INO treatment heterotrophs. However, food chain/web lengths did not appear to be significantly greater within the CSM treatment in comparison to the two feed treatments, as

nitrogen isotope signature differences between CSM large zooplankton and the applied cottonseed meal did not significantly differ from the nitrogen isotope signature differences between feed treatment large zooplankton and applied feeds within the two feed treatments (Figure 4-36).

INO treatment pond organisms were wholly dependent upon the applied inorganic fertilizer and likely limited autochthonous nutrients as their basal nitrogen sources. The longer INO pond food web/chain produced greater nitrogen isotope enrichment between the INO treatment nitrogen pool (fertilizer plus autochthonous and non-anthropogenic allochthonous nitrogen) and its dependent organisms. Due to the extremely low $\delta^{15}\text{N}$ signature of the basal INO fertilizer (inorganic fertilizer $\sim 0.00\text{‰}$ $\delta^{15}\text{N}$), isotopic enrichment did not increase the $\delta^{15}\text{N}$ signature of the various INO pond treatment trophic guilds/size assemblages to values equal to or greater than their counterparts in the other three treatments (Figures 4-11-4-12, 4-18, 4-23, 4-28). Although INO large zooplankton $\delta^{15}\text{N}$ signatures were lower than their counterparts within the non-INO treatments, the $\delta^{15}\text{N}$ signature difference between the applied basal nutrient and large zooplankton was much greater within the INO treatment than within the non-INO treatments. This indicated that applied inorganic fertilizer nitrogen had passed through a greater number of trophic intermediaries, and had become isotopically enriched before contributing to somatic growth within the INO treatment taxa (Figures 4-18, 4-36, 4-38).

Medium Plankton Assemblage Isotopic Signatures

Medium plankton assemblage carbon isotope signatures differed among pond nutrient treatments and among sampling dates within pond treatments. Again, PRO and UNP treatment medium plankton carbon signatures did not differ during the 10 weekly sampling periods, supporting the contention that commercial feed treatment plankton assemblages (large

zooplankton, medium and small plankton) either directly, or indirectly derived the majority of their nutrients from the applied feeds as opposed to autochthonous or non-anthropogenic allochthonous nutrient sources.

Although the PRO and CSM treatment applied nutrient compositions (Chapter 3; Table 3-1) and carbon isotope signatures differed (Figure 4-4), PRO and CSM medium plankton assemblage carbon signatures did not differ for any of the 10 sampling periods analyzed. Cottonseed meal was believed to function as a combination of a directly consumable nutrient and organic fertilizer for stimulating phytoplankton production. Heterotroph consumption of both cottonseed meal and live foods within the CSM ponds may have obliterated any isotopic differences (within a given taxa) with the other three treatments that would have been imparted by consumption of the applied cottonseed meal nutrient alone. Additionally, the cottonseed meal carbon isotope signature only marginally differed from that of the two commercial feeds (Figure 4-4; note truncated y-axis scale).

Only two sampling period differences in medium plankton assemblage carbon isotope signature occurred between the UNP and CSM treatments near the second half of the 12-week trial (consecutive sampling dates: 18 and 25 June 2006). Infrequent UNP and CSM medium plankton carbon isotope signature differences may have occurred for the same reason the PRO and CSM treatments lacked differences, namely that the two feed treatments had identical isotopic compositions. Only the INO treatment medium plankton carbon isotope signatures consistently differed from those of the other three pond treatments, the majority of differences occurred during the latter sampling periods of the trial. This indicated that isotopic equilibrium had become established within the different treatment ponds during this time.

Medium plankton assemblage carbon isotope signatures differed among sampling periods within each treatment, but significant differences in medium plankton carbon isotope signatures measured at the start and end of the pond trial, only occurred within the non-INO treatments. Additionally, medium plankton assemblage carbon isotope signatures followed a similar pattern within all three non-INO treatments, initially high $\delta^{13}\text{C}$ values declined to lower values at the end of the trial (Figures 4-19 – 4-22). This decrease was less pronounced within the CSM treatment, and as mentioned above, nonexistent within the INO treatment (Figure 4-22). The lack of INO medium plankton $\delta^{13}\text{C}$ differences at the start and end of the trial may have been due to the isotopic homogeneity of the carbon pool utilized by INO treatment autotrophs (primarily small plankton assemblage). Potential isotopic homogeneity may have lead to a more rapid establishment of isotopic equilibrium ($\delta^{13}\text{C}$) of pond autotrophs (small plankton assemblage) and heterotrophs (medium and large plankton assemblages) within the INO treatment.

The conclusion that carbon isotope signatures reached isotopic equilibrium over time, also was supported by the observation that when significant pairwise differences among sampling periods were present within a treatment, differences were generally between isotopically higher (lower on graph) means at the beginning of the trial and isotopically lower means later in the trial (Figures 4-19 – 4-22). The lack of medium plankton assemblage carbon isotope signature differences among consecutive sampling periods in the latter half of the study, indicates that medium plankton assemblage carbon isotope signatures had stabilized about an mean equilibrium value.

Time-Averaged Medium Plankton Carbon Isotope Differences

Time-averaged medium plankton assemblage carbon isotope signatures differed among treatments (Figure 4-23). Again, PRO and UNP medium plankton assemblage carbon isotope

signatures did not differ, nor did PRO and CSM treatment medium plankton assemblage carbon isotope signatures differ. The INO medium plankton assemblage carbon isotope signature was significantly higher than those of the other three treatments. This indicated that the carbon incorporated by the INO treatment medium plankton assemblage may have moved through more trophic intermediaries, becoming more enriched in the heavier carbon isotope (^{13}C) than the medium plankton assemblages of the other treatments. As stated previously, an alternate or simultaneous process occurring within the INO treatment ponds was that carbon fixed by primary producers within the INO ponds was from an inorganic carbon pool that had a much higher carbon isotope signature.

Unfortunately, due to the lack of baseline carbon isotope signatures for the DIC pools being utilized by primary producer organisms within the different treatment ponds, a starting point for measuring carbon isotope fractionation occurring within the different treatment pond ecosystems was unavailable. This makes any inferences about the actual length of the food chain in these systems partially supposition.

Medium Plankton Assemblage Nitrogen Isotope Signatures

Medium size plankton assemblage nitrogen isotope signatures differed among pond nutrient treatments, but did not differ among sampling periods within nutrient treatments. Medium plankton assemblage nitrogen isotope signature differences between pond treatments did not appear to follow any temporal pattern among the 12 sampling dates, and the number of nitrogen isotope signature differences was small compared to that found for carbon signature differences. The reason for the lower frequency of medium plankton assemblage $\delta^{15}\text{N}$ signature differences between treatments among sampling dates was likely due to the higher variation of nitrogen isotope signatures relative to those for carbon isotope signatures (Figures 4-19 – 4-22).

This was somewhat unexpected, as large plankton assemblage $\delta^{15}\text{N}$ signatures generally had lower variation among replicates than their $\delta^{13}\text{C}$ signature counterparts (Figures 4-14 – 4-17). Greater $\delta^{15}\text{N}$ signature variation among replicates within the medium plankton assemblages, relative to replicates within the large plankton assemblages may have been due to greater trophic diversity of organisms within the medium plankton assemblage relative to those within the large plankton assemblages. Large plankton assemblage taxa primarily consisted of large zooplankton (Chapter 5), whereas the medium plankton assemblage was a trophically diverse mixture of phytoplankton and zooplankton (pers. obs.), consisting of a diverse mixture of herbivores, carnivores, and omnivores. This artificial and unrealistic characterization of the medium plankton size assemblage as a discrete trophic group/unit was due to it consisting of trophically diverse organisms, primarily mixed phytoplankton and zooplankton (e.g. rotifers, eggs, copepod nauplii, protists). The more trophically diverse medium plankton assemblage may have produced more nitrogen isotope signature variation among its replicates, than that produced by a more trophically discrete and trophically functional group such as the large plankton assemblage (Figure 4-14-4.17; Chapter 5, primarily zooplankton).

PRO and UNP medium plankton assemblage nitrogen isotope signatures differed infrequently, differing for only one of the twelve weekly sampling dates. As previously mentioned, this was likely due to the two feeds being manufactured from identical ingredients and the large influence of the applied feeds upon the isotopic signatures of the taxa within their respective treatment ponds. The medium plankton assemblage was a mixture of smaller zooplankton (pers. obs.; rotifers, nauplii, protists, ciliates) and phytoplankton, and was likely isotopically influenced by heterotroph consumption (feeds, small zooplankton and phytoplankton) and phytoplankton that utilized DIN derived from remineralized applied feeds.

Cottonseed meal treatment medium plankton nitrogen isotope signatures did not differ from those within either feed treatment for any of the 12 weekly samples. This was likely due to the greater variation in medium plankton nitrogen isotope signature enrichment magnitudes frequently observed among replicate samples, relative to carbon isotope enrichment values (Rounick and Winterbourn 1986, Post 2002aa). Additionally, commercial feed nitrogen isotope signatures (Figure 4-5) only marginally differed from that of the applied cottonseed meal (~ 1.0 ‰ $\delta^{15}\text{N}$). The two fertilizer treatment (CSM and INO) medium plankton assemblage $\delta^{15}\text{N}$ signatures differed most frequently (4 of 12 sampling periods) among the six possible pairwise treatment comparisons; however, CSM and INO medium plankton $\delta^{15}\text{N}$ signature differences did not appear to follow any discernable temporal pattern.

Although repeated measures ANOVA did not find significant differences in medium plankton nitrogen isotope signatures among sampling dates within treatments, pairwise post hoc tests (Bonferroni, $P < 0.05$) did find a small number of differences among sampling periods within pond treatments (Motulsky 1995; H. Motulsky pers com.) that did not appear to follow any chronological pattern (Figures 4-19 – 4-20, 4-22). The INO treatment had the highest frequency of significant differences in medium plankton nitrogen isotope signature among sampling periods, due to a single sampling date (31 March 2006) at the start of the trial that had a higher nitrogen isotope signature than later sampling periods.

Again, more pseudovalues were necessary for the medium plankton nitrogen isotope analyses (8 of 288) than were required for the carbon isotope analyses (1 of 288), due to the higher nitrogen detection threshold limit of the isotope ratio mass spectrometer. Notably, less pseudovalues were required for the medium plankton assemblage isotopic analysis than for the large plankton assemblage isotopic analysis, presumably due to the greater biomass standing

stocks (~10X biomass increase per trophic level decrease) generally present at lower trophic levels within an ecosystem (Paine 1980, Briand and Cohen 1987, Pauly and Christensen 1995, Valiela 1995, Vander Zanden et al. 1999, Saito et al. 2000, Post 2002b, Hoeninghaus et al. 2008).

Time-Averaged Medium Plankton Nitrogen Isotope Differences

Time-averaged medium plankton assemblage nitrogen isotope signatures also differed among treatments, with only the INO treatment mean differing from those of the other three treatments (Figure 4-23). INO medium plankton assemblage nitrogen isotope signature was markedly lower than the other three treatments, mirroring the pond nutrient (Figure 4-5) and time-averaged large zooplankton (Figure 4-18) nitrogen isotope results. Again, this was likely due to the low nitrogen isotope signature ($\delta^{15}\text{N} = -0.08\text{‰}$) of the applied inorganic nitrogen fertilizer nutrient and presumed basal nitrogen source for the INO treatment ponds.

Small Plankton Carbon Isotope Differences

Small plankton size assemblage carbon isotope signatures differed among pond nutrient treatments and among sampling periods within pond treatments. Again, PRO and UNP small plankton carbon isotope signatures did not differ for any of the 12 weekly sampling periods. The two feed and cottonseed meal treatments differed for only a small number of early sampling intervals: PRO and CSM (2), and UNP and CSM (5). In contrast, pairwise treatment comparisons involving the INO treatment, differed for the majority of the latter sampling periods: PRO and INO (last 9 periods), UNP and INO (8 of last 9 periods), and the CSM and INO treatments differed for all 12 weekly sampling periods. The two differing trends in small plankton carbon isotope signatures, diverging (PRO and INO, UNP and INO) and converging (PRO and CSM, UNP and CSM) values among treatments, took from two to five weeks before small plankton isotopic equilibriums were established with their presumed primary carbon

source(s) – applied nutrients or autochthonous carbon source in the case of the INO treatment ponds.

Small plankton assemblage carbon isotope signature differences among sampling dates occurred within all treatments (Figures 4-24 – 4-27). Small plankton carbon isotope signatures followed similar trajectories within all pond treatments. Initially high small plankton $\delta^{13}\text{C}$ signature values gradually oscillated before ending at a slightly lower carbon isotope signature value. Final small plankton carbon isotope signature values differed from initial values in the two commercial feeds, but not in the case of the two fertilizer treatments. The two fertilizer treatment small plankton carbon isotope signatures were more constant, and had flatter trajectories compared to the two feed treatments (Figures 4-24 – 4-27), resulting in no carbon isotope signature differences between the initial and final sampling periods. This may have been due to the slow and even release of DIC from the applied organic cottonseed meal (bacterial and fungal mediated remineralization/decay) within the CSM treatment ponds, and a large, isotopically homogenous and unmeasured DIC source(s) within the INO treatment ponds (e.g., atmospheric CO_2 , and groundwater carbonates).

Time-Averaged Small Plankton Carbon Isotope Differences

Time-averaged small plankton assemblage carbon isotope signatures differed among the four treatments (Figure 4-28). Only the PRO and UNP treatment time-averaged small plankton assemblage carbon isotope signatures did not differ.

However, it remains unclear how the small plankton assemblage (primarily small phytoplankton) could obtain carbon (and nitrogen) from applied feeds designed for consumption by relatively large heterotrophs (Figure 4-4). It is possible that carbon (and nitrogen) compounds from fine feed particles applied to ponds could have leached directly into the water where they

were assimilated by pond phytoplankton (Schroeder 1983, Lochmann and Phillip 1996, Lovell 1998, Lochmann et al. 2001). Although it is more likely that dissolved inorganic carbon and nitrogen compounds (DIC and DIN) originally derived from applied feeds (remineralized) entered aqueous solution and became available to primary producers following consumption, assimilation, decomposition, respiration and excretion of feed nutrients by the various heterotrophic pond taxa (Boyd 1979, 1997, Coman et al. 2003, Grey et al. 2004). If sufficient applied feed nutrient leaching and remineralization occurred to isotopically influence the dissolved inorganic carbon (and nitrogen) nutrient pools available to primary producers within the feed ponds, taxa that utilized primary production within these ponds also would have isotopic compositions influenced by the applied feeds, even if these taxa were unable to utilize the feeds via direct consumption. The similarity of the PRO and UNP plankton (large, medium and small) assemblage carbon isotope signatures, and the finding that the INO plankton assemblage carbon signatures were significantly lower than those of the other three treatments, supports the hypothesis that each of the four pond treatments received the majority of its carbon from the applied nutrient, or a uniform non-anthropogenic (autochthonous and/or allochthonous) carbon pool in the case of the INO treatment. Even at the lowest trophic levels (small plankton/phytoplankton), feed nutrients appear to have isotopically influenced feed treatment pond taxa.

Small Plankton Assemblage Nitrogen Isotope Signature Differences

Small plankton assemblage nitrogen isotope signatures differed among pond nutrient treatments and among sampling dates within treatments (Figures 4-24 – 4-27). Small plankton assemblage nitrogen isotope signature differences between treatments only occurred among the pairwise treatment combination comparisons that involved the INO treatment (PRO and INO, UNP and INO, and CSM and INO).

Small plankton assemblage nitrogen isotope differences between the three non-INO and INO treatments did not follow any temporal trends with differences occurring at the beginning, middle and end of the study with equal likelihood. Additionally, the number of differences occurring between the non-INO and INO treatments also did not follow any apparent trend: PRO vs. INO (5 differences), UNP vs. INO (2 differences) and CSM vs. INO (5 differences).

Again, the reason why INO small plankton nitrogen isotope signatures differed from those of the three non-INO treatments was likely due to the low nitrogen isotope signature of the applied inorganic fertilizer ($\sim 0.00\text{‰ } \delta^{15}\text{N}$). Additionally, the applied liquid inorganic fertilizer was formulated to be in a chemical (aqueous ammonium nitrate) form easily assimilated by pond autotrophs. The INO small plankton assemblage consisted primarily of phytoplankton, and should have been the plankton size fraction most capable of utilizing the applied inorganic fertilizer, and therefore should be the plankton assemblage most isotopically influenced by the applied inorganic fertilizer.

Within pond nutrient treatments, small plankton nitrogen isotope signature differences among sampling dates only occurred for the two fertilizer treatments (Figures 4-26 – 4-27). No within treatment differences among sampling dates occurred for the two feed treatments (Figures 4-24 – 4-25).

Small plankton nitrogen isotope trajectories differed slightly within the two fertilizers treatments (Figures 4-26 – 4-27). CSM small plankton nitrogen isotope signatures followed a gradual sinusoidal oscillation similar to that observed for CSM small plankton carbon signature, whereas INO small plankton nitrogen isotope signature values followed a generally flat trajectory. Within the INO ponds, significant differences in small plankton assemblage nitrogen isotope signatures among sampling dates were due to two markedly low nitrogen isotope

signature means on two non-consecutive sampling dates (Figure 4-27; 6 April 2006 and 18 May 2006).

Time-Averaged Small Plankton Nitrogen Isotope Differences

Time-averaged small plankton assemblage nitrogen isotope signatures also differed among pond nutrient treatments (Figure 4-28). Only the INO small plankton nitrogen isotope signature differed from those of the other three treatments, partially mirroring the nitrogen isotope signature pattern of the four applied nutrient treatments (Figure 4-5). Again, this supports the contention that the nitrogen pools being utilized by the food webs within the different pond treatments were primarily derived from the applied nutrients, and not by autochthonous or non-anthropogenic allochthonous nitrogen sources.

Fewer pseudovalues were necessary to produce a fully balanced data set for the 2-way repeated measures ANOVA for small plankton assemblage isotope signature difference analyses than for the medium plankton isotopic analyses, which in turn required fewer pseudovalues than the large plankton isotopic analyses. Again, this was likely due to the greater quantities of biological material commonly observed at lower trophic levels ~ 10X biomass increase with trophic level decrease (Paine 1980, Valiela 1995); given that the same volume of pond water was filtered to obtain each of the three plankton size fractions (small, medium and large plankton assemblages/trophic guilds).

Nutrient Movement within Fish and Plankton Size Assemblages

Parallel movement of isotopically homogenous nutrients within an ecosystem's taxa, should produce taxa that have similar isotopic signatures. As all taxa are consuming the same or similar nutrients, and all taxa are located at similar trophic positions within the ecosystem's food web.

In contrast, serial movement of nutrients within an ecosystem should produce large isotopic differences between the lowest and highest trophic levels, due to serial enrichment as nutrients move through the food chain/web. As nutrients move from applied basal nutrients to primary producer to primary consumer to secondary consumer and to tertiary consumer, and so forth, the carbon and nitrogen of these nutrients become increasingly enriched in the heavier isotopes of carbon and nitrogen ($\sim 0.64 \Delta\delta^{13}\text{C}$, $\sim 3.03 \Delta\delta^{15}\text{N}$). This not only results in increasing isotopic signature with increasing trophic level, but also greater isotopic signature difference (e.g., carbon, nitrogen, sulfur, etc.) between the presumed basal nutrient and top predator with increasing ecosystem trophic complexity/food chain length (with the exception of high rates of omnivory, cannibalism, or generalist feeding habits).

Nutrient movement within pond treatments appeared to have been primarily in parallel within the two feed treatments, serially within the inorganic fertilizer treatment, and intermediate to parallel and serial within the cottonseed meal treatment. This conclusion is based primarily upon the small range of mean nitrogen isotope signatures among the five taxa/size assemblages (small, medium and large plankton, small and large harvest fish) within the two feed treatments relative to the larger range of values (Figure 4-43; greater SD among taxa) observed in mean nitrogen isotope signatures within the two fertilizer treatments, and the INO treatment in particular. Taxa carbon isotope signature ranges were largely equal among treatments, demonstrating the highly conserved basal nutrient carbon isotope signature phenomena which occurs due to the low isotopic enrichment rate of carbon (Figure 4-43).

Pond Nutrient and Harvest Fish Carbon Isotope Signature Differences

Due to the small magnitudes of carbon isotope signature differences between taxa/trophic guilds, statistical comparisons of carbon isotope signature differences among treatments were

‘sign neutral’, and used absolute values of arithmetic differences in taxa $\delta^{13}\text{C}$ signatures. Actual small harvest fish and applied nutrient carbon isotope signature difference mean value signs were preserved and frequently differed (Figure 4-30; i.e., PRO and UNP $\Delta\delta^{13}\text{C}_{(\text{small fish} - \text{nutrient})} > 0$, INO $\Delta\delta^{13}\text{C}_{(\text{small fish} - \text{nutrient})} < 0$). Carbon isotope signature differences between processed and unprocessed feed and their respective large harvest fish were well within published values (0.35 ‰ $\Delta\delta^{13}\text{C}$ PRO, 0.69 ‰ $\Delta\delta^{13}\text{C}$ UNP) for a single trophic level (DeNiro and Epstein 1978, Peterson and Fry 1987, Fry 2006). The carbon isotope signature difference magnitude between the applied cottonseed meal and CSM large harvest fish was significantly greater than those of the two commercial feeds and their respective large harvest fish (Figure 4-29). Utilizing the carbon isotope enrichment value measured for an increase of one trophic level obtained from the indoor feeding trial ($\bar{X} = 0.64$ ‰ $\Delta\delta^{13}\text{C}$ per trophic level), and the carbon isotope differences found between the two commercial feeds and their respective large harvest fish, the trophic distance estimate derived from carbon isotope signature differences corresponded to roughly one trophic level between large harvest fish and the applied nutrients within the two feed treatments (Equation 4.2; 0.54 PRO, 0.99 UNP). These trophic difference magnitudes (only using carbon isotope data) were roughly those expected, assuming that large harvest fish in the feed ponds were receiving the majority of their nutrition from the applied feeds.

Latent carbon isotope signatures from fry and juvenile diets of small and medium plankton may have resulted in the observed feed treatments’ large harvest fish carbon isotope signatures being slightly less than the expected value (1.0 trophic level difference) based solely upon a feed based diet (eq. 4.2; Vander Zanden and Rasmussen 1999, Vander Zanden et al. 1997, 1999, Vander Zanden and Vadeboncoeur 2002, Saito et al. 2000). Overall however, these results indicate that feed treatment large harvest fish were deriving most of their nutrition from applied

feeds rather than live foods within the ponds, but with the possibility that fry and juveniles were deriving large portions of their nutrition from non-feed (live) food sources before undergoing an ontogenetic shift in diet to applied feeds.

The two diet composition estimation methods that utilized both carbon and nitrogen isotope signatures, Euclidean distance (ED) and IsoSource (IS), agreed well with the trophic distance estimates derived solely from carbon isotope signature differences for the two feed treatment large harvest fish groups. Estimated applied feed/nutrient usage: ~ 90% Euclidean distance (ED), and 70% IsoSource (IS) for PRO large harvest fish, ~ 91% ED and 87% IS for UNP large harvest fish.

Carbon isotope signature differences between cottonseed meal and their large harvest fish ($1.97\text{ ‰ } \Delta\delta^{13}\text{C CSM}$) corresponded to approximately three (3.08) trophic levels, based upon a trophic distance estimate derived solely from carbon isotope signature results, and indoor carbon isotope enrichment rates ($0.64\text{ ‰ } \Delta\delta^{13}\text{C}$; Chapter 2). Indicating that cottonseed meal served primarily as a nutrient source for the CSM treatment pond food web, and may have made only a minor contribution to the direct nutrition of CSM treatment fish (based solely upon $\delta^{13}\text{C}$ results), even though fish were regularly observed feeding upon cottonseed meal broadcast twice daily upon the pond surface (pers. obs.). Three trophic levels (3.08 CSM carbon signatures only) separating the CSM treatment large harvest fish and their applied cottonseed meal nutrient seems a reasonable trophic distance estimate given what is known of tropical freshwater lentic ecosystems (Estep and Vigg 1985, Cabana and Rasmussen 1994, Yoshii et al. 1999); if finely ground cottonseed meal was being directly utilized by rotifers and other small heterotrophs, which were in turn consumed by larger zooplankton, prior to being consumed by CSM treatment fish. However, this simple ($\delta^{13}\text{C}$ only) interpretation of CSM large harvest fish diet was only

marginally supported by the two diet estimation methods that utilized both carbon and nitrogen isotope signature data (Table 4-1; CSM large harvest fish diet: cottonseed meal ~ 12% ED and 21.4% IS, large zooplankton ~ 16% ED and 22% IS, medium plankton ~ 65% ED and 53.6 IS, small plankton ~ 6% ED, 1.1 IS).

The simple food chain initially postulated CSM treatment was: cottonseed meal → small plankton → medium plankton → large zooplankton → large harvest fish. A slightly more complex and realistic, but still simplistic CSM treatment food web diagram (Figure 4-44) was created that was roughly supported by the two (ED and IS) dual element (C, N) diet estimation methods, and observation of CSM treatment fish regularly grazing upon cottonseed meal at the pond surface. This alternative scenario to the simple linear food chain described above postulated that CSM harvest fish primarily received their nutrition from the applied cottonseed meal and large zooplankton assemblage, and that the large zooplankton assemblage primarily derived their nutrients as described in the simple linear food web/chain scenario: cottonseed meal → small plankton → medium plankton → large zooplankton. Nutrients utilized by CSM treatment primary producers were likely remineralized DIC and DIN from respiration and excretion (following CSM consumption), decay of cottonseed meal, or volatile nutrients (free amino acids, sugars) leached directly from the cottonseed meal into aqueous solution. The CSM large harvest fish carbon and nitrogen isotopic signature plot position was on a line, roughly midway between the isotopic signature plot positions of the applied cottonseed meal and the CSM large zooplankton assemblage, which would be expected if CSM large harvest fish were primarily deriving their nutrition from these two sources (Figure 4-41). Unfortunately, the isotopic signature plot collinearities of the cottonseed meal, CSM large zooplankton assemblage, and CSM medium plankton assemblages, may have erroneously biased the ED and IS CSM large

harvest fish diet estimates in favor of the CSM medium plankton assemblage (Figures 4-41, 4-53).

The inorganic fertilizer used for the INO treatment did not contain carbon, preventing a trophic distance estimate based solely upon carbon isotope signatures between the INO fertilizer and INO large harvest fish. Back calculations were made to determine the trophic distance and carbon isotope signature value (and likely identity) of the basal primary producer within the INO pond food web. It was expected that the INO small plankton assemblage ($-23.26\text{‰ } \delta^{13}\text{C}$) would be the primary candidate for the basal trophic group (1°) within the INO treatment food web. However, the carbon isotope signature distance ($3 \text{ trophic levels} \times 0.64\text{‰ } \delta^{13}\text{C}$ per trophic level or $1.92\text{‰ } \delta^{13}\text{C}$) between the INO treatment large harvest fish (Figure 4-11; INO lg fish - $20.15\text{‰ } \delta^{13}\text{C}$) and the primarily mixed rotifer and phytoplankton INO medium plankton assemblage (estimated as 1° consumer; INO med plank $-22.19\text{‰ } \delta^{13}\text{C}$) had the closest isotopic fit to the back calculated primary producer (Table 4-3; estimated 1° producer ~ mean $-22.50\text{‰ } \delta^{13}\text{C}$) group postulated to be the base of the food web within the INO treatment ponds (Figure 4-28). The INO treatment medium plankton assemblage was only slightly less enriched in ^{13}C than the back calculated INO treatment 1° producer (INO med plank $-22.19\text{‰ } \delta^{13}\text{C}$, INO sm plank $-23.26\text{‰ } \delta^{13}\text{C}$). However, the $\delta^{13}\text{C}$ signature sign of the INO medium plankton assemblage relative to the back calculated INO pond primary producer [$\delta^{13}\text{C}_{(\text{post } 1^\circ \text{ prod})} < \delta^{13}\text{C}_{(\text{med plank})}$] indicated that it was unlikely that the INO medium plankton assemblage was the base of the food chain within the INO treatment ponds. Whereas the INO small plankton assemblage was of the correct $\delta^{13}\text{C}$ signature sign and magnitude [$\delta^{13}\text{C}_{(\text{post } 1^\circ \text{ prod})} > \delta^{13}\text{C}_{(\text{sm plank})}$] to indicate that it was a possible candidate for the base of the INO treatment pond food chain.

Large Harvest Fish and Applied Nutrient Nitrogen Isotope Differences

Nitrogen isotope signature differences between large harvest fish and their respective pond treatment nutrients differed among all groups, except the PRO and UNP treatments. The finding that nitrogen isotope enrichment magnitudes between large harvest fish and their respective applied nutrients did not differ for the two feed treatments provides support for the contention that fish within the applied feed ponds were consuming and assimilating feed nutrients into their bodies for somatic growth, largely to the exclusion of pond live prey. If feed treatment large harvest fish $\delta^{15}\text{N}$ enrichment levels had differed, it could have been argued that one or both of the feed treatment fish groups were obtaining a significant portion, or even a majority of their nutrition from a non-feed source (Figure 4-29).

There is a remote possibility that both PRO and UNP large harvest fish were obtaining most of their nutrition from an isotopically similar, non-anthropogenic basal nutrient source. However, I feel this is unlikely as the most conspicuous potential live food sources within the two feed treatments (small, medium, and large plankton) were surveyed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signature profiles, and their profiles did not indicate that live foods were major prey items for large harvest fish within these treatments. Additionally, harvest fish nitrogen isotope signatures differed among treatments (INO fish lower $\delta^{15}\text{N}$; Figures 4-11-4-12), indicating that some, if not all of the applied nutrients were responsible for the detectable isotopic signature differences of harvest fish within their respective pond treatments. Namely, that isotopic signature differences in harvest fish among treatments were an indicator of dietary source differences among treatments (DeNiro and Epstein 1978, 1981a, Fry et al. 1999, Fry 2006).

Nitrogen signature differences between feed treatment large harvest fish and their respective feeds were extremely close ($2.94\text{‰ } \Delta\delta^{15}\text{N}$ PRO, $3.05\text{‰ } \Delta\delta^{15}\text{N}$ UNP) to the mean

nitrogen enrichment magnitude observed for a single trophic level change in the indoor feeding trials (Chapter 2; 3.03 ‰ $\Delta\delta^{15}\text{N}$ per trophic level). Which produced estimated trophic distances (Equation 4.4) of approximately one trophic level separating commercial feeds and large harvest fish within both feed treatments (Table 4-4; $\delta^{15}\text{N}$ 0.97 trophic level PRO, $\delta^{15}\text{N}$ 1.00 trophic level UNP). Nitrogen isotope signature based trophic distance estimates between commercial feeds and their respective large harvest fish agreed well with those estimated solely using carbon isotope data ($\delta^{13}\text{C}$ 0.54 trophic level PRO, $\delta^{13}\text{C}$ 0.99 trophic level UNP), especially for the UNP treatment.

Trophic distance estimates derived from nitrogen isotope signature differences between large harvest fish and their applied fertilizer nutrients were: 1.5 trophic levels separating cottonseed meal and the CSM large harvest fish ($\delta^{15}\text{N}$ 1.48 trophic level CSM), and approximately 2.5 trophic levels separating the inorganic fertilizer and INO large harvest fish ($\delta^{15}\text{N}$ 2.48 trophic level INO).

Based on nitrogen isotope signature differences (Equation 4.4), the greatest trophic distance between swordtail top predators and applied basal nutrients within the four nutrient treatments was estimated to be the inorganic fertilizer treatment, followed by the cottonseed meal treatment, and lastly by the two commercial feed treatments (Figures 4-29-4-30, Tables 4-4, 4-10). Nitrogen isotope signature based trophic distance estimates, supported the main alternate hypothesis, that fish primarily utilized feeds, and that fertilizer-treatment fish primarily obtained their nutrients from the applied fertilizers via the pond food webs.

Carbon and nitrogen isotope signature based trophic distance estimates (Equations 4.2, 4.4) between CSM large harvest fish and their cottonseed meal basal nutrient ($\delta^{13}\text{C}$ 3.08 trophic levels, $\delta^{15}\text{N}$ 1.48 trophic levels) did not agree as closely as their feed treatment counterparts, but

did not dramatically differ (difference $\sim 2X$). The initially postulated CSM treatment food chain: cottonseed meal \rightarrow small plankton \rightarrow medium plankton \rightarrow large zooplankton \rightarrow CSM large harvest fish, translated to an expected overall nitrogen isotope signature difference of 12.12 ‰ $\delta^{15}\text{N}$ between CSM large harvest fish and the applied cottonseed meal nutrient, which was much greater than the actual measured difference (4.48 ‰ $\delta^{15}\text{N}$). This indicated that CSM large harvest fish and/or their zooplankton prey were likely consuming organisms from different trophic levels than those strictly postulated by the simple linear food chain model (e.g., omnivory absent).

Alternately, or in conjunction with this explanation, is that actual trophic assemblages/guilds did not concur with the three plankton size assemblages (experimental artifacts of mesh size selection), which is almost certainly the case. If plankton size assemblages and trophic positions do not directly correspond, plankton size assemblage isotope signatures will not be an accurate representation of distinct trophic levels/guilds within treatment ponds, and trophic distance estimates using Equations 4.2 and 4.4 will deviate from actual trophic distances occurring between trophic groups/guilds. Both carbon and nitrogen diet estimation methods (ED and IS) concluded that CSM large harvest fish diet was a composite of the four isotopically analyzed components (Table 4-1). This indicated that CSM treatment swordtails were omnivorous generalists and part of a complex food web (Figure 4-44), as was indicated within the examined literature (Axelrod 1991, Tamaru et al. 2001).

Inorganic fertilizer nitrogen (~ 0.00 ‰ $\delta^{15}\text{N}$) was incorporated into primary producers (small plankton assemblage) with a small degree of isotopic nitrogen enrichment (Table 4-4; INO small plankton assemblage and fertilizer: 1.19 ‰ $\Delta\delta^{15}\text{N}$), which commonly occurs within nitrogen-rich (eutrophic) ecosystems (Wada and Hattori 1978, Wada 1980, Evans et al. 1996,

Pennock et al. 1996, Waser et al. 1998, Waser et al. 1999, Grey et al. 2004, Hall 2004, De Brabandere et al. 2007, Gregory-Eaves 2007). Actual INO large harvest fish and inorganic fertilizer nitrogen isotope differences translated to a trophic distance of ~ 2.5 trophic levels. This total food chain trophic distance was close to the expected number of trophic levels (3) believed to exist within the INO treatment ponds based upon the simple food chain: inorganic fertilizer → small plankton → medium plankton → large zooplankton → INO large harvest fish. The trophic distance between INO large harvest fish and the INO small plankton assemblage (1° producers), calculated solely from nitrogen isotope signature differences (Equation 4.4), and the predicted number of trophic levels present within the simple food web (three trophic levels), differed by only ~ 0.5 trophic levels, assuming small plankton assemblage and fertilizer $\delta^{15}\text{N}$ signature values were roughly equal. The calculated trophic distance of 2.5 is even more plausible than the estimated 3.0, given the omnivorous and possible juvenile ontogenetic dietary habits of swordtails (Tables 4-4, 4-10).

Small Harvest Fish and Pond Nutrient Isotopic Differences

Unlike large harvest fish, carbon isotope signature difference magnitudes between small harvest fish and pond nutrients did not differ among pond treatments (Figure 4-30). As was previously mentioned, statistical comparisons among treatments were ‘sign neutral’ and used absolute value differences. Actual carbon isotope signature difference value signs between groups (e.g., PRO small fish $\delta^{13}\text{C}$ and processed feed $\delta^{13}\text{C}$) frequently differed (Figure 4-30).

Carbon isotope signature differences between the two commercial feeds and their respective small harvest fish translated to roughly one negative trophic level shift (-0.89 PRO, -0.27 UNP). These nonsensical results (negative enrichment signs) indicated that commercial feeds were not as predominant a dietary component for feed-treatment small harvest fish as feeds

were for large harvest fish (Table 4-1). This conclusion also was supported by the PRO and UNP small harvest fish ED and diet estimates. When comparing the diets of PRO large harvest fish (Table 4-1; PRO_(lg fish): processed feed ~ 90% ED and 70% IS), and small harvest fish, feed only marginally remained the primary dietary component of small harvest fish (Table 4-1; PRO_(sm fish): processed feed 35% ED and 43.5% IS). The lesser importance of applied feed for small harvest fish, relative to their large fish counterparts, also occurred within the UNP treatment ED and IS diet estimates (Table 4-1; UNP_(sm fish): unprocessed feed 37% ED and 22.7% IS). Dietary (trophic) estimates based solely upon carbon isotope signature differences (i.e., a single element; Figure 4-52), are inferior to estimates based solely upon nitrogen isotopes, or estimates based upon both carbon and nitrogen signatures (DeNiro and Epstein 1978, 1981a, Fry 2006). It is likely that large proportions of other dietary items (live feeds), were obscuring the carbon isotope signatures of ingested feeds within PRO and UNP small harvest fish (Table 4-1), possibly due to a greater juvenile dependence upon live foods relative to older fishes.

Roughly two trophic levels (1.85; Table 4-1, based on $\Delta\delta^{13}\text{C}$) separated the CSM small harvest fish and the cottonseed meal pond nutrient. This was roughly one trophic level less than the calculated trophic distance between the CSM treatment large harvest fish and the cottonseed meal pond nutrient (Table 4-1, based on $\Delta\delta^{13}\text{C}$), and approximately 2 trophic levels less than that predicted by the simple, initially postulated linear food web (cottonseed meal → small plankton → medium plankton → large zooplankton → small fish). It is much more likely that actual CSM treatment pond trophic interactions resembled a complex food web (Figure 4-44), as supported by the isotopic signature data and the ED and IS CSM harvest fish diet estimates (Table 4-1), rather than the initially postulated simple, but longer food chain.

Unlike small harvest fish from the two commercial feed treatments, CSM small harvest fish may actually have been utilizing the applied nutrient to a greater degree than large harvest fish, lessening the isotopic distance separating CSM small fish and their applied CSM nutrient. Alternately or in addition to this possibility, CSM small harvest fish may have been utilizing the small and medium plankton assemblages to a greater degree than their large harvest fish counterparts. Large and small CSM harvest fish were probably exploiting the different plankton trophic guilds to differing degrees (ontogenetic dietary habit shift, trophic specialization, microhabitat utilization), including the three plankton size assemblages and through direct consumption of cottonseed meal (Lemly and Dimmick 1982, Holbrook and Schmitt 1992, Jones and Waldron 2003, Melville and Connolly 2003). This seems entirely possible for an omnivorous, generalist diet species such as the swordtail, especially if mouth gape and microhabitat usage differences between small fry/juveniles and adult fish size classes were present. Omnivory and carnivorous predation from multiple trophic levels for fish and larger invertebrates were likely responsible for the shorter trophic distance actually measured (1.85 trophic levels; Table 4-1 $\Delta\delta^{13}\text{C}$), versus the four trophic levels/distance that were predicted to exist between the CSM small harvest fish and the applied cottonseed meal (Briand and Cohen 1987, Post 2002a, 2002b, Cohen et al. 2002).

Cottonseed meal may have been the best basal nutrient source candidate for CSM treatment small harvest fish, based solely on arithmetic $\delta^{13}\text{C}$ signature differences, and the indoor trial carbon isotope enrichment rate (Chapter 2; $-0.64\text{‰ } \Delta\delta^{13}\text{C}$ per trophic level). However, the two diet estimation methods (ED and IS) that utilized both carbon and nitrogen isotope data to determine fish diet, came to highly divergent estimates, both from each other and the simple carbon isotope signature difference based trophic distance estimation method (Table

4-1; CSM_(sm fish): cottonseed meal 22% ED and 0.6% IS, large zooplankton 25% ED and 63.3% IS, medium plankton 39% ED and 2.0% IS, small plankton 14% ED and 34.1% IS).

Conspicuous differences between the CSM small harvest fish ED and IS diet estimations, and the high diet inclusion (percentage) estimate for medium plankton (ED), may have been due to the CSM medium plankton assemblage's isotopic composition being intermediate (isotopically collinear) to CSM large zooplankton and the applied cottonseed meal nutrient (Figures 4-41, 4-53; Schwarcz 1991, Phillips 2001). This may have produced an overestimation of the importance of the CSM medium plankton assemblage within the CSM small harvest fish diet.

Additionally, fry and juvenile ontogenetic dietary habits, and the lower body weights of small harvest fish relative to large harvest fish, may have contributed to greater isotopic variation of small fish (relative to large fish) for both carbon and nitrogen. Due to the possible effects of greater juvenile herbivory and omnivory (ontogenetic dietary habit differences), a more trophically diverse and lower trophic level diet (planktonic and epiphytic plant and animal prey) along the pond perimeter, changes in diet due to seasonal plankton community changes/succession, and the greater effects any dietary changes would produce within a smaller fish's isotopic profile because of lower fish to prey weight ratios. Greater small harvest fish isotopic variation, due to greater dietary niche breadth and lower fish mass relative to potentially shifting food source availabilities, would likely contribute to greater errors in small harvest fish diet estimates derived from methods that utilize organism isotope signatures. A roughly 4X initial individual organism weight gain, is generally considered the minimum weight increase necessary to properly describe and differentiate organism dietary shifts in diet studies which utilize stable isotope techniques (Fry 2006).

INO small harvest fish carbon isotope signature (INO sm fish $-20.29\text{‰ } \delta^{13}\text{C}$) was used to trophically back calculate the carbon isotope signature of the basal plankton assemblage within the INO treatment food web (small fish estimated 1° producer $-22.21\text{‰ } \delta^{13}\text{C}$), which differed only slightly from the INO large harvest fish estimated basal trophic group carbon isotope signature (large fish estimated 1° producer $-22.07\text{‰ } \delta^{13}\text{C}$). The INO treatment plankton assemblage with the $\delta^{13}\text{C}$ signature closest in value to the estimated basal plankton group was the small plankton assemblage ($-23.26\text{‰ } \delta^{13}\text{C}$). This relatively large isotopic difference ($1.05\text{‰ } \delta^{13}\text{C}$) between the actual and estimated small plankton carbon isotope signatures was likely due to plankton size assemblages being somewhat erroneously attributed to different functional trophic assemblages due to plankton sieve selections, rather than reflecting actual trophic divisions within the pond ecosystem. Unfortunately, the large variations observed in $\delta^{13}\text{C}$ signature data, relative to the typically low ^{13}C enrichment rates (Chapter 2; $\Delta\delta^{13}\text{C} = 0.64\text{‰}$) that occur between trophic levels, made predictions of prey carbon isotope signature values via back calculation of $\delta^{13}\text{C}$ signatures of limited value within the INO treatment, relative to $\delta^{15}\text{N}$ signature back calculation analysis (Smith and Walker 1980, Peterson and Fry 1987, Post 2002a, Fry 2006).

Similar to what may have occurred within the CSM treatment, the lower average body weight of INO small harvest fish (Chapter 3; Figure 3-14) relative to INO large harvest fish, may have been a factor contributing to the large ED and IS diet estimate differences between INO small and large harvest fish. In that, low average body weights of INO small harvest fish, and dietary differences among individual fish, may have inflated variation in dietary estimates due to the greater isotopic influence of prey and potential diet differences upon smaller fish. Additionally, higher INO swordtail cannibalism rates due to food limitation, which may be

indicated by the relatively greater population percentage of small fish (Chapter 3; Figure 3-12) within the INO treatment, and size variation among individual INO large harvest swordtails (Chapter 3; Figure 3-15), may have further increased INO large harvest fish isotopic signatures ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) relative to their small harvest fish counterparts.

Small Harvest Fish and Applied Nutrient Nitrogen Isotope Differences

Nitrogen isotope signature differences between small harvest fish and their respective nutrients differed among all pairwise treatment combinations, except for the PRO and UNP treatments. Trophic distance estimates based upon nitrogen isotope signature differences between small harvest fish and their respective applied nutrients, and the mean rate of nitrogen enrichment (Chapter 2; $3.03 \text{ ‰ } \Delta\delta^{15}\text{N}$) per trophic level derived from the indoor trials, were approximately: a single trophic level within the two feed treatments (1.08 PRO, 1.11 UNP), ~ 1.7 trophic level (1.67 CSM) within the CSM treatment, and roughly two trophic levels (2.12 INO) within the INO treatment.

Trophic position/distance estimates ($\delta^{15}\text{N}$ signature only) for groups believed to be trophic intermediaries between small harvest fish and pond nutrient treatments, or directly driving fish production in the case of applied feeds, were well within expected values. One trophic level between the two commercial feeds and their respective small harvest fish indicated that these fish were likely obtaining the majority of their nutrition from the applied feeds. In contrast to the $\delta^{13}\text{C}$ signature trophic distance estimates, the estimated number of trophic levels for feed treatment small harvest fish was fractionally higher than an integer value of one, unexpectedly making small harvest fish within the feed treatment ponds, appear to be trophically higher than their large harvest fish counterparts (Table 4-4).

A possible explanation for why feed treatment small harvest fish had higher estimated trophic positions than their large harvest fish counterparts was that smaller fish were obtaining a larger portion of their nutrition from live foods than larger fish. This is a reasonable assumption if smaller fish were feeding in the shallow, weedier areas of the pond. Also, their smaller mouth gape, may have forced them to utilize planktonic and epiphytic fauna and flora to a higher degree than larger fish. Zooplankton consuming applied feeds would tend to have nitrogen isotope signatures on par with fish that directly consumed applied feeds (Figures 4-11 – 4-12, 4-18), and phytoplankton that were utilizing remineralized DIN derived from applied feeds, also would have nitrogen isotope signatures similar or closer to that of the applied feeds (Figure 4-28). If feed treatment zooplankton and phytoplankton were being heavily grazed upon by small juveniles and fry to a greater degree than their large harvest fish counterparts, these small fish would become further enriched in ^{15}N , and tend to appear to be at a higher trophic level than their large fish counterparts (Figures 4-11 – 4-12; small feed fish $\delta^{15}\text{N} >$ large feed fish $\delta^{15}\text{N}$). Larger fish (pers. obs.) were more often in the deeper areas of the pond, where heavy grazing and lack of cover may have reduced zooplankton standing stocks, and reduced their importance as a food source for larger fish within the two feed treatments. These processes may have been occurring to a lesser extent within the CSM treatment (Figures 4-11 - 4-12; small CSM fish $\delta^{15}\text{N} >$ large CSM fish $\delta^{15}\text{N}$), but did not appear to be occurring within the INO pond treatment (small INO fish $\delta^{15}\text{N} <$ large INO fish $\delta^{15}\text{N}$).

The limitations of only using simple arithmetic differences in the isotopic signatures of a single element to evaluate dietary candidates was apparent, as isotopic distance information of a single element only evaluates potential food sources along a single dimension. Different food source candidates may have similar isotopic signatures for one element (e.g., carbon), but differ

for another (e.g., nitrogen). If only a single element (carbon) is examined, two (or more) potential dietary candidates may appear to be isotopically similar, or even identical (Figure 4-52). If this is the case, no conclusions can be drawn as to which of the food sources is being utilized to a greater degree if differential prey/nutrient utilization is present (Schwarcz 1991, Ben-David and Schell 2001, Phillips 2001, Fry 2006).

When carbon and nitrogen isotope enrichment differences between feed treatment small harvest fish and their applied feeds were examined together, it was clear that carbon isotope signature data alone could not adequately determine trophic position (Table 4-4; -0.89 PRO, -0.27 UNP – resulting in negative trophic levels between small feed fish and applied feeds). When carbon and nitrogen isotope signatures were used in conjunction, more differentiation was possible among potential prey candidates, making determinations of which prey candidates were isotopically plausible and which were not, easier, and in some cases, possible (Figures 4-39 – 4-42).

Diet Estimates Using Both Carbon and Nitrogen Isotope Signatures

Although the Euclidean distance (ED) and IsoSource linear algebra program (IS) fish diet estimates were previously mentioned, a more thorough examination and interpretation of those results are given here. Both the ED and IS PRO and UNP large harvest fish diet estimates (Table 4-1) agreed well with simple arithmetic differences that used either carbon or nitrogen signatures (~ 1 trophic level) in concluding that applied feeds were the primary source of nutrition for large harvest fish within the two feed treatments. This supported the primary alternate hypothesis, that large feed treatment fish primarily utilized feed for their nutritional needs. PRO feed treatment large fish diet was estimated to be primarily composed of the applied feed (70% ED, 90.1% IS), as was the UNP treatment large fish diet (90% ED, 91.1% IS). PRO and UNP small harvest fish

diet estimates were much more catholic, consisting of roughly equal proportions of applied feeds and the three plankton size assemblages (Table 4-1).

CSM large harvest fish ED and IS diet estimates were roughly similar, and both concluded that the medium plankton assemblage was the primary nutrient source (51% ED, 53.6% IS). CSM small harvest fish ED and IS diet estimates differed greatly, with the ED method estimating medium plankton (40%) assemblage as the primary dietary component, with nearly equal percentages of the applied cottonseed meal and two remaining plankton assemblages, whereas the IS method estimated CSM small harvest fish diet as being roughly two-thirds large zooplankton to one-third small plankton (Table 4-1). As previously mentioned the CSM medium plankton assemblage's isotopic coordinates lie roughly mid-way and almost directly upon a line connecting the isotopic coordinates of the CSM large zooplankton assemblage and the applied cottonseed meal nutrient (Figure 4-41). This has been cited as a major confounding factor in delineating diets based upon isotopic signatures, as a diet consisting of equal proportions of CSM large zooplankton and cottonseed meal will produce an isotopic signature similar to a diet consisting primarily of CSM medium plankton (Figure 4-52) within a consumer organism (such as the swordtail), given equal assimilation efficiencies (Schwarcz 1991, Ben-David and Schell 2001, Phillips 2001, Fry 2006).

For INO treatment large harvest fish, both the ED and IS methods estimated that large zooplankton was the primary dietary component (65% ED, 86.5% IS) with smaller contributions by medium (25% ED, 8.6% IS) and small (10% ED, 4.9% IS) plankton assemblages. INO treatment small harvest fish dietary estimates somewhat differed for the two methods, medium plankton (47% ED) followed by large zooplankton (34% ED) were the primary food sources according to the ED method, whereas large zooplankton was the primary food source (67.3% IS)

followed by small phytoplankton (25.3% IS) for the IS method. Again, isotopically collinear $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signature plots for INO large, medium, and small plankton may have biased the ED INO small harvest fish diet estimates in favor of INO medium plankton.

Large and small harvest fish lined up vertically (similar $\delta^{13}\text{C}$, dissimilar $\delta^{15}\text{N}$: large fish enriched ~ 1.1 ‰ $\Delta\delta^{15}\text{N}$ relative to small fish) within the INO treatment isotopic plot (Figure 4-42), but lined up horizontally within the isotopic plots of the other three treatments (Figures 4-39 – 4-42). This indicated there was a greater trophic difference between the INO small and large harvest fish groups relative to their non-INO treatment counterparts, whereas there was greater trophic similarity between small and large harvest fish within the non-INO treatment groups relative to those within the INO treatment. These findings suggest that limited, direct consumption of smaller fish by larger fish (cannibalism) may have occurred within the INO treatment ponds, although INO treatment fry and small harvest fish were not included as a potential prey for INO large harvest fish using the ED and IS diet estimation methods.

Although ED and IS large harvest fish diet estimates differed slightly in their predicted inclusion rates (% of diet), dietary importance rankings were similar between the two methods. Both methods determined that feeds were the primary nutrients for feed treatment large harvest fish, and that medium plankton and large zooplankton were the primary food sources for CSM and INO large harvest fish, respectively.

Simple arithmetic differences in the isotopic signatures of nutrients/taxa for a single element ($\Delta\delta^{13}\text{C}$ or $\Delta\delta^{15}\text{N}$), also suggested (via trophic distance estimates) that large harvest fish derived most of their nutrition from applied feeds within the two feed treatments and from the pond food web in the two fertilizer treatments. Although it was an expected outcome of the trial

that fish ate applied fish feeds and that fish fertilized ponds utilized live foods, actual confirmation or rejection of the primary alternate hypothesis was desirable.

Dietary importance rank agreement between the Euclidean distance and IsoSource computer program was much lower for small harvest fish compared to large harvest fish within all four treatments (Tables 4.5-4.8). When small harvest fish dietary component estimates were ranked in order of importance from highest to lowest, no rank orders completely concurred between the two diet estimation methods for any of the four pond nutrient treatments. Again, this may have been due to the more generalized diet believed to occur for smaller fish, as well as their lower predator to prey weight ratios. This would tend to increase small harvest fish isotopic variation within treatments due to dietary differences of individual fish (Estep and Vigg 1985, Beaudoin et al. 1999).

Large Zooplankton and Harvest Fish Isotope Differences

Carbon isotope signature differences (signed values) between large harvest fish and their respective large zooplankton assemblages (Chapter 2; $0.64\text{‰ } \Delta\delta^{13}\text{C}$ per trophic level) translated to roughly -0.41 PRO, 0.21 UNP, -0.44 CSM, and 1.18 INO trophic levels. As previously mentioned, negative trophic distance estimates denote isotopic depletion of a presumed predator group relative to a presumed prey group (i.e., presumed predator has a lighter isotope signature than presumed prey), which indicates a lack of trophic coupling (resource utilization) between groups (e.g., large zooplankton and large harvest fish).

These results indicate that large harvest fish in the PRO and CSM ponds were not heavily utilizing the large zooplankton standing stocks within their ponds, according to carbon isotopic signature differences. PRO and CSM large zooplankton assemblages likely utilized processed feed and cottonseed meal directly, accounting for the lower carbon isotope signature difference

magnitudes and negative trophic distance (unutilized resource) estimates between large harvest fish and large zooplankton within these treatments (Figures 4-11, 4-18, 4-31). Within their respective treatments, processed feed and cottonseed meal nutrients appeared to be moving directly into large harvest swordtails and the large zooplankton assemblages (parallel), indicating a lack of a strong and direct trophic coupling between these two taxa.

CSM large harvest fish and large zooplankton carbon isotope signature difference results were more difficult to interpret. Although cottonseed meal was applied as an organic fertilizer, larger fish were regularly observed ingesting and may have been partially assimilating finely ground cottonseed meal (pers. obs.), which contains high amounts of crude protein. Contradicting this observation, both the ED and IS diet estimations indicated that CSM large harvest fish primarily consumed medium plankton assemblage prey (Table 4-7), which may have been an erroneous estimate caused by the isotopic composition collinearities of the applied cottonseed meal, CSM large zooplankton, and medium plankton assemblages (Figures 4-41, 4-53). Being visual predators (Axelrod 1991, Jones et al. 1998a, 1998b, 2007), it is more likely that CSM large harvest fish were consuming both cottonseed meal and large zooplankton (Figure 4-44), which could produce an isotopic signature profile similar to that of a diet consisting primarily of CSM medium plankton within a fish consumer (Figure 4-41).

UNP large harvest fish and large plankton carbon isotope signature difference results were less clear, and appeared to indicate that UNP large harvest fish were primarily consuming applied feeds and limited amounts of large zooplankton. Alternately, large UNP fish may have been more dependent upon live feeds when smaller relative to their PRO large fish counterparts, but no obvious cause for such a scenario makes this seem unlikely. Regardless, the positive trophic distance estimate (Table 4-4) and low carbon isotope signature difference between UNP

large harvest fish and large zooplankton indicated that (based on $\delta^{13}\text{C}$) the large zooplankton assemblage was probably a minor UNP large harvest fish nutrient source.

As expected, INO large harvest fish and large zooplankton $\delta^{13}\text{C}$ signature differences indicated that fish were likely utilizing large zooplankton as a major food source within the INO ponds. INO large harvest fish carbon isotope signatures were higher (positive trophic distance/proper magnitude ~ 1.0 trophic level) than their large zooplankton assemblages (Figures 4-11, 4-18; Table 4-4), making large zooplankton a plausible prey item candidate for INO large harvest fish.

Within their respective pond treatments, small harvest fish and large zooplankton carbon isotope signature difference magnitudes corresponded to trophic distance estimates of -1.84 PRO, -1.14 UNP, -1.67 CSM, and 0.95 INO (Chapter 2; $0.64\text{‰ } \Delta\delta^{13}\text{C}$ per trophic level). These trophic distance estimates (Table 4-4), indicated that feed and CSM treatment small harvest fish groups were not (wrong sign, $\delta^{13}\text{C} < 0$) obtaining the majority of their nutrition from the large plankton assemblage.

In contrast, the IS and ED diet estimation methods predicted that feed treatment small harvest fish were obtaining most of their nutrition from the applied feeds, except for the UNP small harvest fish IS diet estimate, which indicated that small plankton were their primary nutrient source $\sim 34.5\%$ (Table 4-1). IS and ED diet estimates also determined that feed treatment small harvest fish were receiving large portions of their nutrition from the medium and small plankton assemblages: (PRO) $\sim 15\text{-}33\%$ medium and $\sim 20\text{-}35\%$ small plankton, and (UNP) $\sim 29\text{-}32\%$ medium and $\sim 17\text{-}35\%$ small plankton (Table 4-1). Interestingly, feed treatment small harvest fish were estimated to be consuming relatively minor amounts of large zooplankton (Table 4-1; PRO 0.2-14%, UNP 1-14%; IS and ED). This may have been due to the tendency

for large zooplankton to congregate at the surface in deeper areas of the pond, and small fish to inhabit the shallower, weedier areas along the pond perimeters (pers. obs). Unfortunately, a thorough survey of large zooplankton densities in relation to distance from shore and water depth was not conducted due to time constraints. Based on carbon isotope signature differences (Table 4-4), CSM small harvest fish did not appear to be heavily utilizing large zooplankton, but may have been consuming prey from other trophic levels, or directly ingesting cottonseed meal as was postulated for CSM large harvest fish.

In contrast, the IsoSource diet estimate determined large zooplankton to be the primary nutrient source for CSM small harvest fish, and the ED method ranked large zooplankton as their second most important food item (Table 4-7). Interestingly, although the IS method ranked large zooplankton as the primary food source for CSM small harvest fish, the IS method ranked medium plankton as the primary prey of CSM large harvest fish (Table 4-7). Given what is known of predator feeding behavior (mouth gape and maximum prey particle size) from optimal foraging theory (Schmitt and Holbrook 1984a, b, Osenberg et al. 1992, Mittlebach et al. 1999) and microhabitat differences between small and large CSM treatment fish and large zooplankton assemblages (deeper water), it seems unlikely that CSM large harvest fish would be utilizing more medium than large zooplankton, while CSM small harvest fish would be consuming more large plankton than medium plankton. Again, this may be due to the collinear isotopic signature plot positions ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of medium and large plankton, and cottonseed meal within the CSM treatment (Figures 4-41, 4-44, 4-53).

The ED diet estimation method ranked plankton assemblages and applied cottonseed meal in the same increasing order of dietary importance for CSM small and large harvest fish (Table 4-7; medium plankton, large zooplankton, cottonseed meal, and small plankton, respectively).

Medium plankton were ranked higher in dietary importance than large plankton for CSM large harvest fish, which again may have been due to the collinear isotopic plot positions of the potential food items (Figures 4-41, 4-53) and associated potential diet estimate bias. Regardless of these potential issues, because the IS and ED methods use both carbon and nitrogen isotope data, more confidence can be attributed to their estimates due to the simultaneous use of isotopic signatures two element, rather than that of only a single element.

INO small harvest fish appeared to be primarily utilizing large zooplankton, based on trophic distances derived from carbon isotope signature differences (Table 4-4; ~ 0.95 trophic levels) between INO small harvest fish and large zooplankton. This was not surprising given the lack of an ingestible applied organic nutrient and the visually mediated feeding behavior of swordtails.

However, the two methods used to estimate INO small harvest fish diets (IS and ED), both utilize carbon and nitrogen isotope signatures, and produced different dietary importance rankings for the three plankton assemblages that were isotopically analyzed (Table 4-8). The IS diet estimate predicted that large zooplankton were the major nutrient source for INO small harvest fish, whereas the ED method predicted medium plankton to be their major food source (Table 4-8). Similar to the CSM treatment results, Euclidean distance INO small harvest fish diet estimation may be biased in favor of the INO medium plankton assemblage, due to the carbon and nitrogen isotope signature plot position of the medium plankton assemblage being roughly midway, and nearly collinear to the INO small and large plankton plot points (Figures 4-42, 4-53).

If small phytoplankton and epiphytic algae are important food sources for INO small fry and juveniles living within the physical cover (predator avoidance) of emergent and submerged

plants in the shallows along the pond perimeter, and large zooplankton become important adult prey items as juvenile fish grow and move to deeper water, a dietary history consisting primarily of mixed small phytoplankton (juvenile diet) and large zooplankton (adult diet), could isotopically resemble a diet consisting primarily of medium plankton using the Euclidean distance diet estimation method and INO plankton isotopic signature values (Figures 4-42, 4-45).

INO pre-harvest fry ED and IS diet estimates (Table 4-2), closely agreed with INO small harvest fish ED diet estimates (Table 4-1), with the exception of a small increase in the diet inclusion rate of the small plankton assemblage (19%) in the INO small harvest fish ED diet estimate. The INO pre-harvest fry (ED) and small harvest fish (ED and IS) diet estimations indicated that medium plankton were a more important food item than small plankton (largely autotrophs) for this group of fish (Table 4-2). The INO pre-harvest fry IS estimate, had much higher dietary inclusion rates for large and small plankton, at the expense of a lower inclusion rate for the medium plankton assemblage [Table 4-1: INO small harvest fish (medium plankton 55% ED, 65% IS); Table 4-2: INO pre-harvest fish (7.5% IS medium plankton)].

Fish size series (fry, juvenile and adult) isotopic profiles proved useful within the different treatment ponds as a means of determining if natal fry and small juvenile fish were obtaining most, or at least a detectable portion of their nutrition from small phytoplankton and epiphytic algae. INO pre-harvest fish (fry and juvenile fish) isotope signatures, indicated that it was more likely that INO small harvest fish were heavily preying upon the INO medium plankton assemblage, than an isotopically similar composite diet of INO small and large zooplankton (Figure 4-42). This dietary scenario is suggested by the direct observation of large macrozooplankton assemblages congregating near the water surface in the deeper, central portions of the ponds where smaller swordtails were largely absent. A more thorough survey of

fry isotopic signatures would have been useful in determining the role, if any, of small phytoplankton in the direct nutrition (cannibalism) of larger swordtails within the INO treatment ponds.

Large Harvest Fish and Large Zooplankton Assemblage Nitrogen Isotope Differences

The INO large harvest fish and large zooplankton nitrogen isotope signature difference was significantly greater than those of the other three groups. As a general visual trend (not statistical), harvest fish and plankton assemblage nitrogen isotope signature differences were much lower within the two feed treatments, than those within the two fertilizer treatments (Figure 4-32; Table 4-4).

Nitrogen isotope signature differences between large harvest fish and their respective large zooplankton assemblages corresponded to trophic distances of 0.08 PRO, 0.22 UNP, 0.65 CSM and 0.82 INO (Chapter 2; $3.03 \text{ ‰ } \Delta\delta^{15}\text{N}$ per trophic level). Nitrogen isotope signature-derived trophic distance estimates between large harvest fish and large zooplankton assemblages for the PRO, UNP and INO treatments agreed well with those derived from carbon isotope signatures (Table 4-4). Specifically, it seems unlikely that large zooplankton were a major food item for feed treatment large harvest fish, as much higher trophic distances (~ 1.0) would have been expected between predators and prey. It seems likely that INO large harvest fish were receiving a large portion of their nutrition from the large zooplankton assemblage, according to nitrogen isotope signature differences between the two taxa. In addition to nitrogen isotope signature difference based trophic distance estimates, and as stated earlier, PRO and UNP large harvest fish IS and ED diet estimates indicate that feeds were their primary nutrients sources (Tables 4-1, 4-5 - 4-6, 4-10).

Nitrogen isotope signature based trophic distance estimates, indicated that large harvest fish from the two fertilizer treatments may have utilized large zooplankton standing stocks to a greater degree than their counterparts within the two feed treatments, and more so within the INO treatment ponds than within the CSM treatment ponds (Tables 4-4, 4-10). Nitrogen isotope signature difference derived trophic distance estimates did not eliminate CSM large zooplankton as a potential major dietary component for CSM large harvest fish, unlike the trophic distance derived from carbon isotope signature differences (Table 4-4; $\delta^{13}\text{C}$ -0.44 trophic levels). Again, this suggests that nitrogen isotope signature based trophic distance estimates are superior to those provided by carbon isotope signatures.

When CSM taxa carbon and nitrogen isotope signatures were considered simultaneously for diet estimation (via the ED and IS methods), several plausible CSM large harvest fish dietary scenarios occurred. One possibility was that CSM large harvest fish were subsisting primarily upon the medium plankton assemblage, which was predicted by the ED and IS diet estimation methods (Table 4-1). Another possible scenario was that CSM large harvest fish were primarily consuming cottonseed meal and large zooplankton in roughly equal amounts (Figure 4-44). As previously mentioned, both of these dietary scenarios were isotopically valid, due to the CSM medium plankton assemblage's carbon and nitrogen isotope signature plot point being roughly midway and collinear upon the line connecting the carbon and nitrogen isotope signature plot points of the cottonseed meal and the CSM large zooplankton assemblage (Figure 4-41). CSM large harvest fish IS and ED diet estimates may be inaccurately biased in favor of the CSM medium plankton assemblage (Schwarcz 1991, Ben-David and Schell 2001, Phillips 2001, Fry 2006), again, due to the isotopic signature collinearity of the applied cottonseed meal, and CSM medium and large plankton assemblages (Figures 4-41, 4-45, 4-53).

However, the CSM large harvest fish ED and IS dietary estimates indicate that the large zooplankton assemblage was not their primary dietary component (Table 4-1). Both CSM pre-harvest fish ED and IS diet estimates ranked the medium plankton assemblage second in prey importance only to cottonseed meal (Table 4-2). The CSM small harvest fish ED diet estimate ranked medium plankton as the primary dietary source, and only the small harvest fish IS diet estimate indicated that large zooplankton were their primary prey item (Table 4-1).

Even though the ED and IS CSM fish diet estimates (except IS small harvest fish) did not indicate that the CSM large zooplankton assemblage was the primary prey group for CSM fish size classes (fry, small and large), in the opinion of this researcher, large zooplankton did play a major role in the CSM fish diets based upon direct observation of cottonseed meal consumption and the general observation of large zooplankton's microhabitat preference for deeper water. Of the two isotopically plausible CSM fish diets [(a) cottonseed meal and large zooplankton or (b) medium plankton], a diet of cottonseed meal and large zooplankton appeared to be a more plausible diet for CSM treatment fish (especially large harvest fish) than one composed primarily of prey derived from the medium plankton assemblage.

The INO large harvest fish and large zooplankton nitrogen isotope signature difference derived trophic distance estimate (0.80 trophic levels), was close to the theoretical value expected for a difference of one trophic level (Tables 4-4, 4-10). Combined with the INO large harvest fish and large zooplankton carbon isotope signature difference derived trophic distance estimate [1.18 trophic levels ($\delta^{13}\text{C}$)], nitrogen and carbon derived trophic distance estimates between INO large harvest fish and large zooplankton closely corroborated each other (correct sign and magnitudes), and were both close to the values expected for a direct predator and prey relationship.

INO large harvest fish IS and ED diet estimates (Table 4-1) agreed well with the simple trophic distance estimates derived from carbon and nitrogen isotope signature differences between INO large harvest fish and large zooplankton (Tables 4-4, 4-10). Both INO large harvest fish IS and ED diet estimates ranked large zooplankton as the primary food source (Table 4-8) of INO large harvest fish by wide margins (Table 4-1; 86.5% IS, 65% ED).

Small Harvest Fish and Large Zooplankton Isotope Differences

Nitrogen isotope signature difference magnitudes between small harvest fish and large zooplankton differed among treatments (Figure 4-34). Again, nitrogen isotope signature differences between small harvest fish and their respective large zooplankton assemblages did not differ within the two feed treatments. The CSM small harvest fish and large zooplankton nitrogen isotope signature difference did not significantly differ from those of the other three treatment groups. Only the INO treatment small harvest fish and large zooplankton isotope signature difference was greater than those of the two feed treatments. Nitrogen isotope signature differences between small harvest fry and their respective large zooplankton, corresponded to trophic distances of 0.18 PRO, 0.34 UNP, 0.81 CSM, and 0.43 INO (Chapter 2; $\Delta\delta^{15}\text{N}$ 3.03 ‰ per trophic level).

The nitrogen isotope signature based trophic distance estimates for the two feed treatments were small, making it unlikely that feed treatment small harvest fish were heavily utilizing their respective large zooplankton assemblages. This conclusion was supported by the feed treatment small harvest fish ED and IS diet estimates, which were trophically broader than their large fish counterparts. The feed treatment small harvest fish ED and IS diet estimates, indicated lower consumption of feed and higher consumption of the three plankton assemblages, compared to feed treatment large harvest fish diet estimates (Table 4-1). Although feed marginally remained

the most important small harvest fish dietary component, according to the PRO small harvest fish ED and IS, and UNP small harvest fish ED diet estimates. The UNP small harvest fish IS diet estimate ranked medium and small plankton as their primary nutrient sources, but only slightly higher than feed and large zooplankton (Table 4-1).

Nitrogen isotope signature based trophic distance estimates between small harvest fish and large zooplankton calculated for the two fertilizer treatments, indicate that CSM and INO small harvest fish likely obtained a major portion of their nutrition from large zooplankton, INO small fish to a greater degree than CSM small fish. Latent isotope signals resulting from natal diets that consisted largely of lower trophic level phytoplankton and zooplankton (e.g., rotifers, protists, bacterial flocs) may have been present within all pond treatments; resulting in somewhat indeterminate trophic level/position estimations (including omnivory) for small size class fish among treatments, individual fish within treatments, and fractional (non-integer) trophic level/position determinations for large size class fish from the two fertilizer treatments.

The CSM small harvest fish and large zooplankton assemblage nitrogen isotope signature difference derived trophic distance estimate, concurred with the IS CSM small fish diet estimate, which indicated that large zooplankton were the primary food source of CSM small harvest fish (Table 4-1). However, the carbon-based CSM small harvest fish and large zooplankton trophic distance estimate was of the incorrect sign to indicate large zooplankton were the major food source for CSM small harvest fish (Table 4-4; $\delta^{13}\text{C}$ -0.44 trophic levels).

The carbon isotope signature difference derived CSM small harvest fish and large zooplankton assemblage trophic distance ($\Delta\delta^{13}\text{C}$ incorrect sign) and ED diet estimation, both indicated that large zooplankton were not the primary prey of CSM small harvest fish. According to the ED diet estimate, CSM small harvest fish were consuming almost equal

proportions of medium plankton, large zooplankton, cottonseed meal, and small plankton (Table 4-1; in increasing order of importance). This seems to be the more likely scenario, given that the CSM small harvest fish ED diet estimate ranked potential prey/food sources in the same order of importance as the CSM large harvest fish ED and IS diet estimates (Table 4-7). Additionally, when carbon and nitrogen isotope signature difference based trophic distance estimates for the CSM small harvest fish were considered simultaneously, large zooplankton did not appear to be the primary dietary component (Table 4-4; incorrect $\Delta\delta^{13}\text{C}$ sign). Again, CSM small harvest fish diet estimates (ED and IS) may have been incorrectly biased in favor of the medium plankton assemblage due to its intermediate carbon and nitrogen isotopic signatures relative to cottonseed meal and the CSM large zooplankton assemblage (Figures 4-41, 4-53).

Only the INO small harvest fish and large zooplankton assemblage carbon and nitrogen isotope signature difference derived trophic distance estimates were of the correct signs and magnitudes, to indicate that INO large zooplankton were a plausible primary prey item for INO small harvest fish (Table 4-1).

INO small harvest fish ED and IS diet estimates gave conflicting results. The ED estimate indicated that medium plankton were the primary dietary item, followed by large zooplankton and small plankton, whereas the IS estimate, indicated that large zooplankton were the primary prey by a fairly large margin (~ 67%), followed by small plankton (~ 25%), and little medium plankton (Tables 4-1, 4-8).

Unfortunately, and similar to what may have been occurring within the CSM treatment, isotopic collinearities of the INO small, medium and large plankton assemblages (Figures 4-42, 4-53), may have biased the INO small harvest fish ED diet estimate in favor of the INO medium plankton assemblage. However, from the INO large harvest fish ED and IS diet estimates (Table

4-1; large zooplankton primary prey), and what is known from optimal foraging theory that visual predators tend to preferentially select large common prey (Brooks 1968), it would seem much more likely that large and medium plankton would be more important nutritional sources for INO small harvest fish. However, small plankton may be an important natal food for INO treatment swordtails, and may still influence their overall isotopic composition, even after swordtails switch diets as they mature; although this did not appear to be indicated by the INO pre-harvest fry isotope ED and IS diet estimate results (Table 4-2).

An additional and major issue that may have contributed to increasing INO swordtail diet estimate errors is that the INO treatment taxa isotopic signature plot (Figure 4-42) may have omitted a major food source(s) from the diet of the INO swordtails. The food resource polygon drawn within the INO treatment $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signature plot, does not include the INO small and large harvest fish isotopic signature plots within its boundaries (Figure 4-42), which indicates that a major food source may have been omitted from isotopic analyses. Small epiphytic plants and animals may have been important prey for INO fry and young juveniles, but due to time and budgetary constraints, samples of this potential prey component were not collected for isotopic analysis.

Large Zooplankton and Pond Nutrient Isotopic Differences

Isotopic signature differences between large zooplankton and their respective pond nutrients differed among treatments for both carbon and nitrogen (Figures 4-35 - 4-36). Carbon isotope signature differences between large zooplankton and pond nutrients translated to approximately one trophic level (Table 4-4; 0.99 PRO, 0.87 UNP) for the two feed treatments, and over three trophic levels (3.19 CSM) for the CSM treatment. It was likely that feed nutrients were being directly utilized by the large zooplankton assemblage within the two feed treatments,

and that cottonseed meal nutrients were moving through a number of trophic levels before becoming sequestered within the CSM treatment large zooplankton assemblage. Due to the absence of carbon within the applied inorganic fertilizer, the trophic distance between the INO treatment large zooplankton and small plankton assemblages (primarily phytoplankton) was estimated to be slightly over one trophic level (1.24 INO), which was somewhat less than expected (~ 2.0). The expected trophic distance was based upon a simple and somewhat unrealistic, postulated INO treatment food chain model: small plankton assemblage (phytoplankton) ← medium plankton assemblage (rotifers and other heterotrophs) ← large zooplankton. The shorter than estimated trophic distance between the INO small plankton and large zooplankton assemblages, was likely due to a combination of experimental artifacts resulting from sieve size selections that resulted in plankton size assemblages that did not coincide with functional trophic groups (Chapter 5; e.g., large phytoplankton within the large ‘zooplankton’ assemblage), and omnivory and predation from multiple trophic levels (Figure 4-45).

Large zooplankton and applied nutrient trophic distance estimates derived from nitrogen isotope signature differences were approximately one trophic level for the two feed (0.88 PRO, 0.77 UNP) and CSM fertilizer (0.82 CSM) treatments. Nitrogen isotope signature differences indicated that almost two trophic levels (1.66 INO) separated the INO treatment large zooplankton and applied inorganic fertilizer, and slightly more than one trophic level (1.24 INO) separated the INO treatment large and small plankton assemblages. This indicates that the applied INO fertilizer was being assimilated by phytoplankton with a detectable degree of nitrogen isotope signature enrichment (0.44 ‰ $\delta^{15}\text{N}$), implying that nitrogen was not limiting within the INO treatment ponds (Wada and Hattori 1978, Wada 1980, Peterson and Fry 1987,

Pennock et al. 1996, Waser et al. 1998, 1999). The similar nitrogen isotope signatures of the applied inorganic fertilizer and the INO small plankton assemblage indicates that fertilizer nitrogen was being assimilated by phytoplankton before being consumed and assimilated by the large zooplankton assemblage, and possible smaller (< 200 µm) zooplankton intermediaries (Table 4-4). This seems a reasonable assumption as the applied inorganic fertilizer had to be assimilated by the ponds autotrophic community before entering the food web in the INO treatment ponds.

Both the ED and IS diet estimations indicated that commercial feeds were the primary nutrient sources for large zooplankton assemblages within both feed treatments (Table 4-9). Within the CSM treatments, the IS method ranked cottonseed meal as the primary nutrient, whereas the ED method ranked medium plankton, followed closely by cottonseed meal, as the primary nutrients of the large zooplankton assemblage. INO treatment large zooplankton diets were estimated to consist primarily of medium plankton followed closely by small plankton using the ED method. Small plankton was identified to be the primary dietary component of INO large zooplankton using the binomial linear algebra (Table 4-9; non-IsoSource) dietary estimation method (Ben-David and Schell 2001, Fry 2006).

The role of autochthonous and non-human mediated allochthonous carbon in phytoplankton nutrition was unknown due to the lack of baseline (no applied nutrient control treatment) inorganic carbon pool isotope signature information for the different pond treatments. Due to the absence of carbon within the INO treatment's basal nutrient, the INO small plankton assemblage (phytoplankton) was analyzed as the nutrient base of the INO treatment food web (Zohary et al. 1994, Yoshii et al. 1999, Hall 2004, Gregory-Eaves 2007).

Unfortunately, plankton assemblages were artificially separated on the basis of size rather than as discrete functional trophic groups (1° producer, 1° and 2° consumers, etc.). These artificial and somewhat arbitrary plankton size divisions and trophic interactions (trophic positions/feeding habits, e.g., omnivory) likely contributed to trophic positions that were not as clearly defined as proposed within the highly oversimplified INO treatment food chain: small plankton (primary producers) → medium plankton (rotifers other heterotrophs) → large zooplankton → fish. This also may have contributed to the non-integer carbon isotope enrichment magnitudes observed among the postulated trophic guilds (Tables 4-4, 4-10).

Small Plankton and Nutrient Isotope Differences

From a limited, qualitative microscopic examination of the preserved small plankton assemblage (1- 32 µm) samples, small plankton assemblages were found to consist primarily of small green algae. This conclusion also was supported by the vivid green color of the small plankton filtrate (refrigerated/unpreserved) when present upon the white Whatman® GF/C filter disks in comparison to the brown to bright red color of the medium and large plankton filtrates.

Unlike the medium plankton assemblage, which was largely a mixture of phytoplankton (e.g., green algae, diatoms) and zooplankton (e.g., rotifers, nauplii), the small plankton assemblage appeared to largely coincide with an actual functional trophic group (1° producers). Because the small plankton assemblage coincided with the trophic group that converts solar energy and inorganic carbon (DIC) and nitrogen (DIN) into energy containing organic compounds, a more thorough examination of this group is warranted.

Carbon isotope signature differences between small plankton and pond nutrients differed only between the PRO and CSM treatments (Figure 4-37). The large differences were likely due to phytoplankton within these assemblages utilizing autochthonous and remineralized applied nutrient DIC rather than direct utilization of applied feeds, as would have occurred for

heterotrophic organisms. The large carbon isotope signature differences, between the two feed and CSM small plankton assemblages and their respective applied nutrients, were equivalent to 0.86 (PRO), 0.64 (UNP), and 0.55 (CSM) photosynthetic inorganic carbon fixation isotopic signature enrichment levels (Table 4-4), assuming a primary producer isotopic enrichment rate of $-19\text{ ‰ } \Delta\delta^{13}\text{C}$ typical for C_3 aquatic plants when $[\text{CO}_2]$ is high (Peterson and Fry 1987, O'Leary 1988). These values seem plausible given what is currently known of aquatic photosynthetic carbon fixation dynamics (Finlay and Kendall 2007).

A lack of direct trophic coupling between the small plankton assemblages and applied feed nutrients was expected within the PRO and UNP pond treatments, as these plankton assemblages consisted primarily of phytoplankton that are likely unable to directly utilize nutrients (organic compounds: starches, lipids, proteins) designed for animal consumption (Lochmann and Phillips 1996, Lochmann et al. 2001, Grey et al. 2004).

Within the CSM treatment, the limited ability of phytoplankton to directly utilize applied cottonseed meal also was expected, as the nutrients are primarily in the form of starches and proteins that are largely unavailable to autotrophs. Cottonseed meal nutrients were believed to be unavailable to primary producers, prior to their remineralization to DIC and DIN via bacterial and fungal decomposition, and following ingestion and digestion by heterotrophic organisms (Landau 1992, Teicher-Coddington et al. 1997).

Although non-INO treatment primary producers were unable to directly utilize feed and cottonseed meal nutrients directly, based on the carbon isotope signature differences between non-INO treatment small plankton assemblages and applied nutrients, it seems that primary producers were able to utilize remineralized DIC derived from recycled applied nutrients in addition to isotopically unmeasured autochthonous and non-human mediated allochthonous DIC

and atmospheric carbon sources. Because small plankton assemblages were composed primarily of phytoplankton autotrophs, they appear to have been isotopically influenced by applied nutrients, even when these nutrients were intended for large heterotrophs. By altering the carbon isotope signatures of the inorganic carbon pools utilized by autotrophs, applied nutrients within the non-INO treatments may have influenced small plankton assemblage carbon isotope signatures. This may explain why feed treatment small plankton carbon isotope signatures did not differ from each other, but differed from those of the two fertilizer treatments, and why each of the two fertilizer treatments small plankton carbon isotope signatures differed from those of the other three treatments (Figure 4-28). If non-anthropogenic DIC was the predominant form of DIC available to pond primary producers, then no small plankton assemblage carbon isotope signature differences would be expected among treatments; additionally, all small plankton assemblage carbon isotope signature values would be expected to have been near that measured for the INO treatment (Figure 4-28), due to the absence of applied carbon within the INO treatment ponds.

After several weeks of twice daily feed, and once daily cottonseed meal additions, applied nutrient carbon had apparently been recycled by repeated mineralization and photosynthetic carbon fixation of applied nutrients within the PRO, UNP, and CSM treatments. Inorganic carbon originally derived from applied nutrients, was utilized by pond primary producers, which was reflected in the small plankton assemblage carbon isotope signature differences observed among treatments (Figure 4-28).

The range of carbon isotope signature values observed among the three non-INO treatment small plankton assemblages (-28.64 to -26.53 ‰ $\delta^{13}\text{C}$), were in the upper ranges reported for phytoplankton assemblages in the literature (-42 to -26 ‰ $\delta^{13}\text{C}$ Deines 1980; -28 to -27 ‰ $\delta^{13}\text{C}$

Yoshioka et al. 1994), but less enriched than other studies (-23.2 to -17.9 ‰ $\delta^{13}\text{C}$ Zohary et al. 1994).

The INO small plankton assemblage carbon isotope signature was higher than that of the other three treatments (Figure 4-28). This was probably due to autochthonous (mineralization of pond sediments, decaying organic matter, organism respiration, etc.) and allochthonous inorganic carbon from non-human mediated sources (groundwater, atmospheric, etc.), which resulted in a labile inorganic carbon pool with a composite isotopic signature higher than those of the non-INO treatment ponds.

Isotopic enrichment processes for inorganic carbon uptake by aquatic autotrophs are strongly biased against the heavier ^{13}C , due to diffusion rate differences and carbon fixation enzyme kinetics (RuBisCo; ~ - 20 ‰ $\Delta\delta^{13}\text{C}$, Peterson and Fry 1987), unless DIC availability is extremely low relative to photosynthetic demand. Typically, algal fractionation values are heavily influenced by the isotopic signature of the available DIC (primarily aqueous CO_2 and HCO_3^-), and the relative availability of these two chemical species (Rounick and Winterbourn 1986, Peterson and Fry 1987, Fogel et al. 1992). Generally, when aqueous $[\text{CO}_2]$ is high and/or phytoplankton growth rates are low, isotopic carbon fractionation between the DIC pool and the algae is highest (Finlay and Kendall 2007). Isotopic discrimination during the fixation of carbon dioxide and carbonate during photosynthesis (i.e., RuBisCo, C_3 , PEP carboxylase, C_4 metabolic differences), tends to mask or obliterate the isotopic signature identity of the carbon pool being utilized, if the carbon pool is sufficiently large relative to carbon demand (Smith and Walker 1980, Yoshii et al. 1999, Finlay and Kendall 2007). Additionally, seasonal changes as well as latitudinal gradients in the atmospheric carbon dioxide pool carbon isotope signature can occur, due to terrestrial photosynthetic carbon demands (Estep and Vigg 1985, Fogel et al. 1992,

Cifuentes et al. 1998, Takahashi et al. 1990, Zohary 1994, Grey et al. 2001, Fry 2006), which can add to carbon isotope signature variability and carbon pool identification uncertainty.

Unfortunately, dissolved inorganic carbon pool isotope signatures of the four treatments were not measured during the study due to logistical, time and budgetary constraints. Additionally, due to the lack of baseline carbon isotope signatures for the DIC pool and pond taxa (phytoplankton, zooplankton, fish) in the absence of anthropogenically applied nutrients (no applied nutrient control treatment), the causes of carbon isotope differences among the taxa in the various treatments are partially conjecture, especially in comparisons involving primary producers (small plankton assemblage).

A remote possibility remains that the carbon isotope signatures of the DIC pools naturally differed among ponds, and that these differences resulted in observable isotopic differences among the respective small phytoplankton assemblages, and taxa that trophically depended upon them. However, due to the random assignment of experimental ponds among treatments, it would be unlikely that these differences would occur to a greater degree within a given treatment.

Small Plankton Assemblage Applied Nutrient Nitrogen Isotope Differences

The INO small plankton and inorganic fertilizer nitrogen isotope signature difference (INO 1.26 ‰ $\Delta\delta^{15}\text{N}$) was higher than that of the other three treatments, but was still much lower than the 3.03‰ $\Delta\delta^{15}\text{N}$ nitrogen enrichment magnitude observed in the indoor trial (Chapter 2), and the mean enrichment magnitude levels reported in the literature for heterotroph predation for one trophic level (~ 3.4 ‰ $\delta^{15}\text{N}$; Peterson and Fry 1987, Kling and Fry 1992, Gu et al. 1996, Post 2002a).

The INO small plankton assemblage nitrogen isotope signature was lower than those of the other three treatments, which did not differ from one another (Figure 4-28). Again, this was likely due to the lower nitrogen isotope signature of the INO treatment food web/chain's presumed basal nutrient (Figure 4-5; inorganic fertilizer - 0.08 ‰ $\delta^{15}\text{N}$) and the small initial $\delta^{15}\text{N}$ difference among the three non-INO applied nutrients (Figure 4-5 cottonseed meal and feeds $\Delta\delta^{15}\text{N} < 1.0$ ‰); assuming that non-INO treatment small plankton assemblages heavily utilized remineralized feed and cottonseed meal nitrogen (Figure 4-38).

Small plankton assemblage $\delta^{15}\text{N}$ signature differences among treatments indicated that small plankton assemblages were significantly influenced by the addition of applied nutrients, as were their respective food webs (Figures 4-11 – 4-12, 4-18, 4-23). However, whether (1) only the non-INO treatment phytoplankton assemblage nitrogen signatures were influenced by applied feed and cottonseed meal nutrient additions, or (2) only the INO phytoplankton assemblage nitrogen signature was altered by the application of the applied nutrient (i.e., inorganic fertilizer), or (3) all four treatments' small plankton assemblages were isotopically influenced by their respective applied nutrients, was unknown due to the lack of baseline small plankton assemblage isotopic signature data in the absence of applied nutrients ('no applied nutrient' control treatment), and the lack of aqueous inorganic (DIN: NH_4^+ , NO_2^- , NO_3^-) and organic nitrogen chemical species (urea, labile free amino acids) isotope data within the INO and non-INO treatment ponds. Regardless, the presence of nitrogen isotope signature differences among the four small plankton assemblages, strongly suggests that small plankton assemblages were utilizing inorganic nitrogen pools (DIN) with differing isotopic signatures (Figure 4-28).

Lower nitrogen isotope enrichment magnitudes between small plankton assemblages and their applied nutrients were not surprising given that small plankton assemblages consisted

primarily of autotrophs, which typically exhibit low nitrogen isotope enrichment rates (0-1 ‰ $\Delta\delta^{15}\text{N}$) relative to their inorganic nitrogen sources (Finlay and Kendall 2007). Because nitrogen is often a limiting nutrient for plants within an ecosystem, plant nitrogen uptake typically results in little to no nitrogen isotope enrichment. Large nitrogen isotope enrichment magnitudes between photosynthetic autotrophs and their DIN pools tend to occur when available nitrogen pools are extremely large and nitrogen uptake is low, usually resulting from low algal growth and reproductive rates (Cifuentes et al. 1988, Needoba 2004, Finlay and Kendall 2007).

Because phytoplankton nitrogen uptake mechanisms differ from nitrogen uptake processes occurring during prey/particle ingestion by animals (Montoya and McCarthy 1995, Cowey and Walton 1989), phytoplankton are not able to directly utilize applied feed nutrients (possible exception of leaching) formulated for carnivorous or omnivorous fish. Due to feed nitrogen being primarily in the form of proteins, applied feed nitrogen was largely unavailable to primary producers within the feed treatment ponds (Tyler et al. 2003). Phytoplankton and other primary producers obtain labile nitrogen (urea, NH_4^+ , NO_3^- , NO_2^- , N_2 [cyanobacteria, legumes via mutualistic bacteria]) via diffusion across cell membranes (Fogg and Thake 1987, O'Leary 1988, Valiela 1995). Uptake kinetics of these different nitrogenous chemical species, and their typically low (limiting nutrient) concentrations, often result in fairly low nitrogen isotope signature enrichment magnitudes relative to those observed between heterotrophs and their plant or animal prey (Wada 1980, Wada and Hattori 1978, DeNiro and Epstein 1981, Vander Zanden et al. 1997, Waser et al. 1998). Additional factors known to influence phytoplankton nitrogen isotope enrichment magnitudes relative to their primary nitrogen sources are: inorganic nitrogen pool characteristics (molecular size/chemical species, ionic charge, isotopic signature, concentration/availability – as mentioned previously), phytoplankton characteristics (species, cell

size, somatic growth rate, senescence, nutritional history, population growth rate, etc), temperature (via effects on photosynthesis and respiration), photoperiod, light levels (via effects on photosynthesis – e.g., photoinhibition, algal self shading), water chemistry (turbidity, pH, redox), ecosystem trophic state, among others (Fogg and Thake 1987, Vander Zanden and Rasmussen 1997, Vander Zanden et al. 1997, 1999).

Although feed treatment small phytoplankton assemblages and applied feeds were not trophically coupled via direct consumption, they seemed to be isotopically coupled, resulting in small isotopic signature differences ($\sim 0.60\text{‰ } \Delta\delta^{15}\text{N}$) between feed treatment small plankton assemblages and the heavily recycled and remineralized feed nitrogen they were presumably utilizing (Figure 4-38). Phytoplankton within the small plankton assemblages of the two feed treatments were utilizing DIC and DIN derived from applied feeds, possibly due to decomposition of uneaten feed and from respiration, egestion and excretion of pond taxa that had consumed applied feeds.

The nitrogen isotope signature difference between the CSM small plankton assemblage and applied cottonseed meal also was small ($\sim 0.55\text{‰ } \Delta\delta^{15}\text{N}$), and much lower than that observed within the INO treatment ($1.26\text{‰ } \Delta\delta^{15}\text{N}$). These small nitrogen isotope signature differences indicated probable trophic and isotopic coupling. The most plausible explanation for these small differences (Figure 4-38), is that processes believed to be occurring within ponds for applied nutrient carbon, were also occurring for nitrogen. Feed and cottonseed meal nitrogen were being remineralized and recycled within the non-INO pond food webs into DIN that was then available for assimilation by phytoplankton. DIN may have been a limiting nutrient for phytoplankton within these ponds, resulting in low nitrogen isotope signature enrichment between the non-INO treatment small plankton assemblages and their respective inorganic

nitrogen pools. Proteins within applied feeds and cottonseed meal were eventually metabolized to ammonium (NH_4^+) by pond organisms and transformed by bacteria into nitrite and nitrate. Subsequently, primary producers within the small plankton assemblage may have assimilated these nutrients for amino acid synthesis, protein synthesis, growth, and reproduction (Bilby et al. 1996, Pennock et al. 1996, Needoba et al. 2004). Even largely unpalatable blue-green algae has been shown to influence the nitrogen isotope signature of higher trophic levels by fixing atmospheric nitrogen before eventually undergoing remineralization and recycling into more palatable green algae, which then entered the food web (Estep and Vigg 1985, Valiela 1995, Piola et al. 2008).

The larger nitrogen isotope signature difference between the INO small phytoplankton and inorganic fertilizer treatment, relative to those of the two feed treatments (Figure 4-38), may have been due to higher DIN concentrations within the INO treatment ponds (Figure 4-46). Higher algal nitrogen turnover rates, due to greater nitrogen availability ('luxury uptake') within the INO ponds, may have produced greater $\delta^{15}\text{N}$ signature enrichment magnitudes within INO pond algal cells relative to their non-INO primary producer counterparts (Fogel and Cifuentes 1993, Pennock et al. 1996, Needoba et al. 2004).

However, the much lower nitrogen isotope signature difference (and $\delta^{15}\text{N}$ based trophic distance estimate) between the CSM small phytoplankton and applied cottonseed meal fertilizer ($\sim 0.55 \text{‰ } \delta^{15}\text{N}$), compared to that occurring within the INO treatment (Figure 4-38; Table 4-4), was somewhat puzzling given that nitrogen availability within the two fertilizer treatments did not differ (Figure 4-46). A possible explanation for the lower nitrogen isotope signature difference between the CSM small plankton and applied cottonseed meal in the presence of high DIN, was that higher algal standing stocks within the CSM treatment ponds (Figure 4-49;

chlorophyll [a]), may have reduced nitrogen availability due to resource competition among phytoplankton and other aquatic and semi-aquatic primary producers. Per capita phytoplankton nitrogen availability (DIN/algal biomass) was lower within the CSM ponds relative to the INO ponds, which may have reduced the nitrogen isotope signature enrichment differences between phytoplankton and their DIN pool, due to greater competition among CSM pond phytoplankton for available nitrogen and lower overall nitrogen turnover rates within the algal cells (Fogg and Thake 1987, Fogel and Cifuentes 1993, Pennock et al. 1996, Needoba et al. 2004).

Interestingly, the nitrogen isotope signature difference derived trophic distance estimate (0.55 trophic levels) between CSM small plankton and the applied cottonseed meal was identical to that calculated from carbon isotope signature differences (Tables 4-4, 4-10; Equations 4.2-4.3). This close agreement between two trophic distance estimates derived from the isotopic differences of two elements (C, N), implies that the theoretically predicted carbon and nitrogen isotopic enrichment rates ($-19\text{‰ } \Delta\delta^{13}\text{C}$, $\sim 0\text{‰ } \Delta\delta^{15}\text{N}$) for primary producers and their inorganic carbon and nitrogen pools were very close to the actual isotopic enrichment rates occurring within the cottonseed meal treatment ponds (O'Leary 1988).

As previously stated, isotopic enrichment magnitudes for inorganic nitrogen uptake by aquatic primary producers, are typically low, on the order of $0\text{-}1\text{‰ } \Delta\delta^{15}\text{N}$ depending primarily upon inorganic nitrogen availability and phytoplankton nitrogen demands (Pennock et al. 1996, , Needoba et al. 2004, Finlay and Kendall 2007). This was supported by the low nitrogen isotope signature difference between the INO small plankton assemblage and applied inorganic nitrogen fertilizer (Figure 4-38; $1.26\text{‰ } \Delta\delta^{15}\text{N}$), which was the treatment in which the greatest confidence was present that the applied nutrient was a major, if not primary, phytoplankton nitrogen source. Recycled nitrogen, from applied nutrients within the non-INO treatments, was the likely primary

nitrogen source for autotrophs as indicated by the small nitrogen isotope differences measured between the non-INO treatment small plankton assemblages and their respective applied nutrients (Figure 4-38; 0.55-0.58 ‰ $\Delta\delta^{15}\text{N}$). However, non-anthropogenic allochthonous nitrogen input magnitudes were not measured, nor 1° producer $\delta^{15}\text{N}$ signatures in the absence of anthropogenic nitrogen input, therefore, it is not certain that applied nutrients were the primary sources of nitrogen within the ponds.

However unlikely, the presence of a major non-anthropogenic nitrogen source(s) could not be eliminated as baseline fish production, DIN pool size, and taxa isotopic signatures in the absence of applied nutrients could not be measured due to the lack of a ‘no applied nutrient’ control treatment. Because baseline fish production in the absence of nutrient application could not be compared to fish production resulting from anthropogenic nutrient addition, it is not entirely certain that applied nutrients were responsible for observed fish production or observed differences in fish production among the four treatments. In the absence of anthropogenic nutrient input, it was assumed that nitrogen availability is the primary limiting factor in phytoplankton production, and ultimately fish production within aquaculture ponds in the area. This is a reasonable assumption, given that phosphorous, which is the usual limiting nutrient for freshwater primary production, is generally not limiting within freshwater ponds in the study area; due to large phosphate deposits within the limestone Karst geology of central and southern Florida (Lakewatch #103).

Due to the lack of a ‘no applied nutrient’ control treatment, and difficulty of measuring DIN fluxes and DIN pool isotopic signatures occurring within the different treatment ponds, their potential contribution to pond taxa $\delta^{15}\text{N}$ signatures and taxa production are unknown. Because atmospheric nitrogen and the applied inorganic fertilizer (INO) nitrogen isotope signatures were

nearly identical ($\sim 0.00 \text{ ‰ } \delta^{15}\text{N}$), it was not possible to eliminate atmospheric nitrogen as the primary nitrogen source responsible for biological production within the INO treatment ponds. However, the lack of a large cyanobacteria/blue-green algae (which can fix atmospheric nitrogen into plant biomass) blooms within any of the 24 treatment ponds (pers. obs.), made this scenario unlikely.

Algal standing stocks were estimated using chlorophyll [a] concentration data measured weekly within each pond (Figures 4-47 – 4-50). Time-averaged chlorophyll [a] concentrations only differed for the CSM treatment, which was significantly higher than those within the other three treatments (Figure 4-51). This indicated that cottonseed meal ponds had higher phytoplankton standing stocks (Florida Lakewatch 2001), but due to the lack of primary consumer grazing rate information or direct measurements of primary production rates, it remains unknown whether primary production rates were greater within the CSM treatment ponds relative to the other treatments. Primary production rate information would have been extremely useful as evidence of whether higher algal production was responsible for the higher, but not significantly greater total fish production rate mean observed for the CSM treatment relative to the INO treatment (Figure 3-9).

Time-averaged total ammonia nitrogen concentrations were significantly higher in the two fertilizer treatments than within the two feed treatments (Figure 4-46). This was expected and may have been responsible for the higher phytoplankton standing stock observed within the CSM ponds, although a higher algal standing stock was not observed within the INO ponds. This may have been due to higher, unmeasured primary consumer grazing rates within the INO ponds and/or due to the more labile and possibly volatile nature of inorganic fertilizer nutrients being unable to maintain high phytoplankton growth rates and standing stocks relative to the

slow nutrient release nature of the cottonseed meal fertilizer (Kastner and Boyd 1996). However, higher zooplankton grazing rates within the INO ponds did not appear to be occurring, as large zooplankton standing stocks did not differ among ponds for the four treatments (Chapter 5; Figures 5-6, 5.12). Although higher predation upon zooplankton by swordtails within the two fertilizer treatments, may have kept zooplankton standing stocks low (trophic cascades), masking the presence of greater zooplankton grazing pressures upon algae within the CSM and INO ponds (Mallin and Paerl 1994, Hansson and Tranvik 1997, Tranvik and Hansson 1997, Cohen et al. 2002).

Again, although there was weekly algal standing stock (Figure 4-47 -4.51) and treatment ammonia nitrogen levels (Figure 4-46) information, phytoplankton production rate and zooplankton grazing rate information was lacking. Due to this lack of information, the quantitative contribution primary producers may have made to food webs of the two fertilizer treatments, as well as any supplemental contributions that phytoplankton may have made to the food webs or to fish production within the ponds of the two commercial feed treatments, remains unknown (Diana et al. 1991, Knud-Hansen et al. 1993).

In conjunction with an absence of DIN pool $\delta^{15}\text{N}$ signature information, a lack of primary production rate information, means that the effects of primary production rates upon the magnitude of primary producer nitrogen isotope signature fractionation within the treatments is also unknown. As higher phytoplankton growth rates typically result in lower $\delta^{15}\text{N}$ signature fractionation magnitudes between 1° producers and the exploited DIN pool (Evans et al. 1997, Finlay and Kendall 2007).

The INO small plankton assemblage (primarily phytoplankton) was slightly more enriched (1.26 ‰ $\Delta\delta^{15}\text{N}$) than the applied inorganic fertilizer, at an enrichment rate similar to that found

for marine phytoplankton ($1.015 \text{ ‰ } \Delta\delta^{15}\text{N}$; Wada 1980). Due to the isotopic similarity of the applied inorganic nitrogen fertilizer and atmospheric nitrogen ($\sim 0.00 \text{ ‰ } \delta^{15}\text{N}$), it was not unexpected that nitrogen isotope signature enrichment between the INO small plankton assemblage and applied inorganic nitrogen fertilizer, was similar to that measured between atmospheric nitrogen and nitrogen fixing cyanobacteria mats ($1.5\text{-}1.7 \text{ ‰ } \Delta\delta^{15}\text{N}$) in a coastal lake in New South Wales, Australia (Piola et al. 2008).

If similar nitrogen isotope enrichment rates between phytoplankton and their respective inorganic nitrogen pools occurred within the non-INO treatments ($\sim 1.26 \text{ ‰ } \Delta\delta^{15}\text{N}$ enrichment rate), this might have allowed back calculation of the nitrogen isotope signatures of the inorganic nitrogen pools (DIN) utilized by the non-INO treatment small plankton assemblages. However, lower inorganic nitrogen concentrations within the two feed treatment ponds (Figure 4-46) may have reduced nitrogen isotope fractionation rates [$\Delta\delta^{15}\text{N} \neq 1.26 \text{ ‰ } \delta^{15}\text{N}$ (INO enrichment rate)] between inorganic nitrogen pools, and primary producers within the feed treatment ponds (Figure 4-38). Making back calculation of the $\delta^{15}\text{N}$ signature of the non-INO inorganic nitrogen pools (DIN), unreliable (Michener and Lajtha 2077).

Again, the presence of a ‘no applied nutrient’ control pond treatment would have been useful in that it would have provided baseline isotopic profile information for algal, zooplankton and fish taxa, as well as baseline production rate information for these taxa in the absence of applied nutrients. Baseline ‘no applied nutrient’ small plankton/phytoplankton isotope signatures would have been useful in back calculating the isotopic signature profiles of non-anthropogenic DIC and DIN pools being utilized by pond autotrophs at the base of the pond food webs/chain. With an additional expenditure of time, money and labor, actual pond DIC and DIN pool isotopic signature information could have been measured within ‘no applied nutrient’

control ponds. This information could have been used to verify the DIC and DIN pool isotopic signature estimates derived from back calculation of 'no applied nutrient' pond taxa isotope signatures. This would have allowed calculation of the contribution, if any, made by autochthonous and non-human mediated allochthonous (groundwater DIN, wind blow dust, leaf litter, insects, etc.) nutrients, as well as that of applied nutrients to primary production within the respective treatment ponds, allowing rudimentary quantitative, and improved qualitative, estimates of primary production originating from applied nutrient contributions, and therefore, rough estimates of potential live food contributions to fish production among the four treatments. Making it easier to evaluate any increase in fish biomass and economic benefits (if any), provided by the different applied nutrients, and the general efficacy of their use.

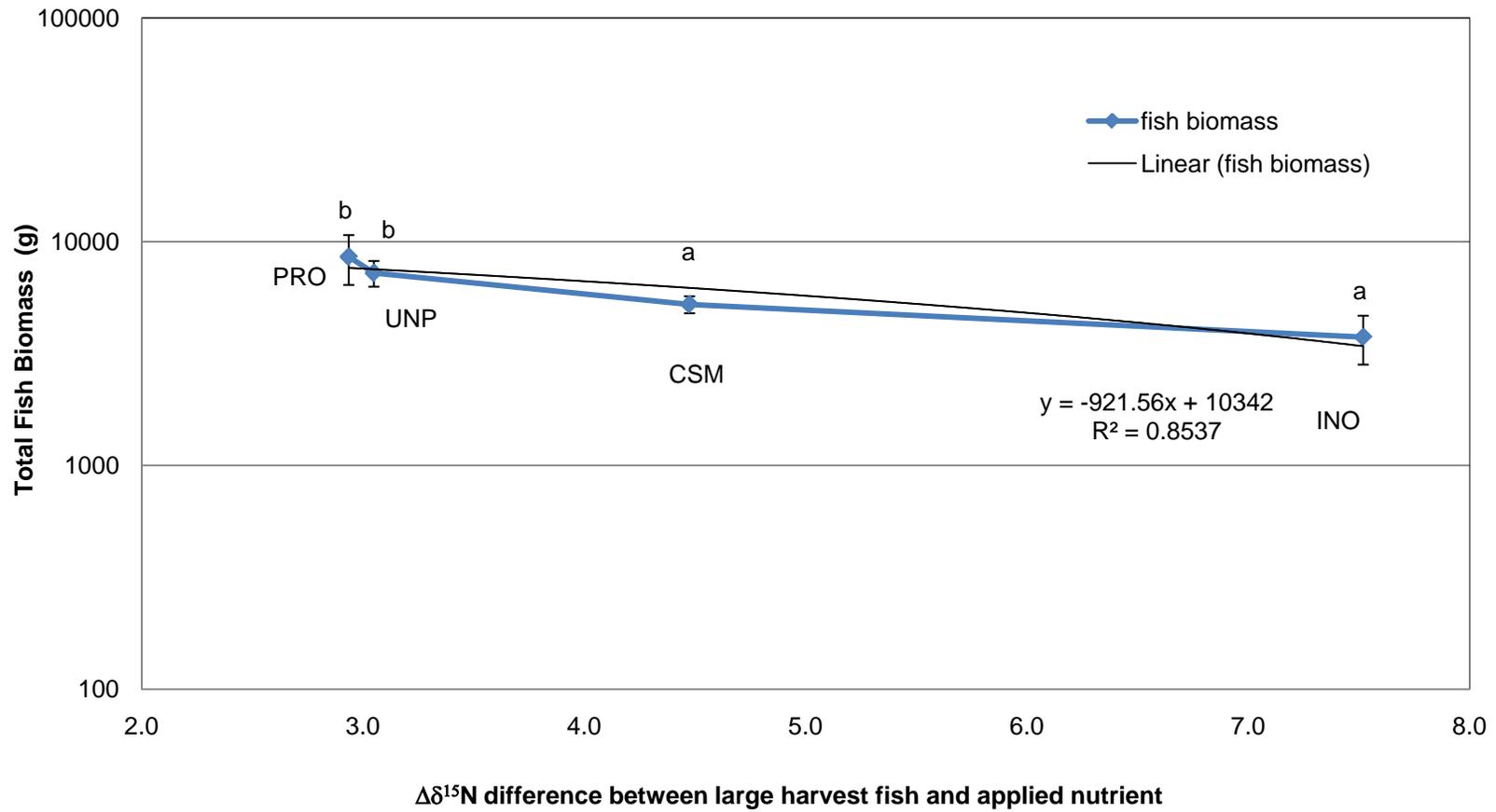


Figure 4-1. Average large harvest fish (swordtail) biomass (\pm 95 % CI) as a function of nitrogen isotope signature differences (semi-log plot) between large harvest fish and applied nutrient treatments; differing letters denote significant differences between groups ($P < 0.05$, Tukey's multiple comparison test). Sample sizes: PRO (5), UNP (6), CSM (6), and INO (6).

Figure 4.2: Integrated pond water sampler

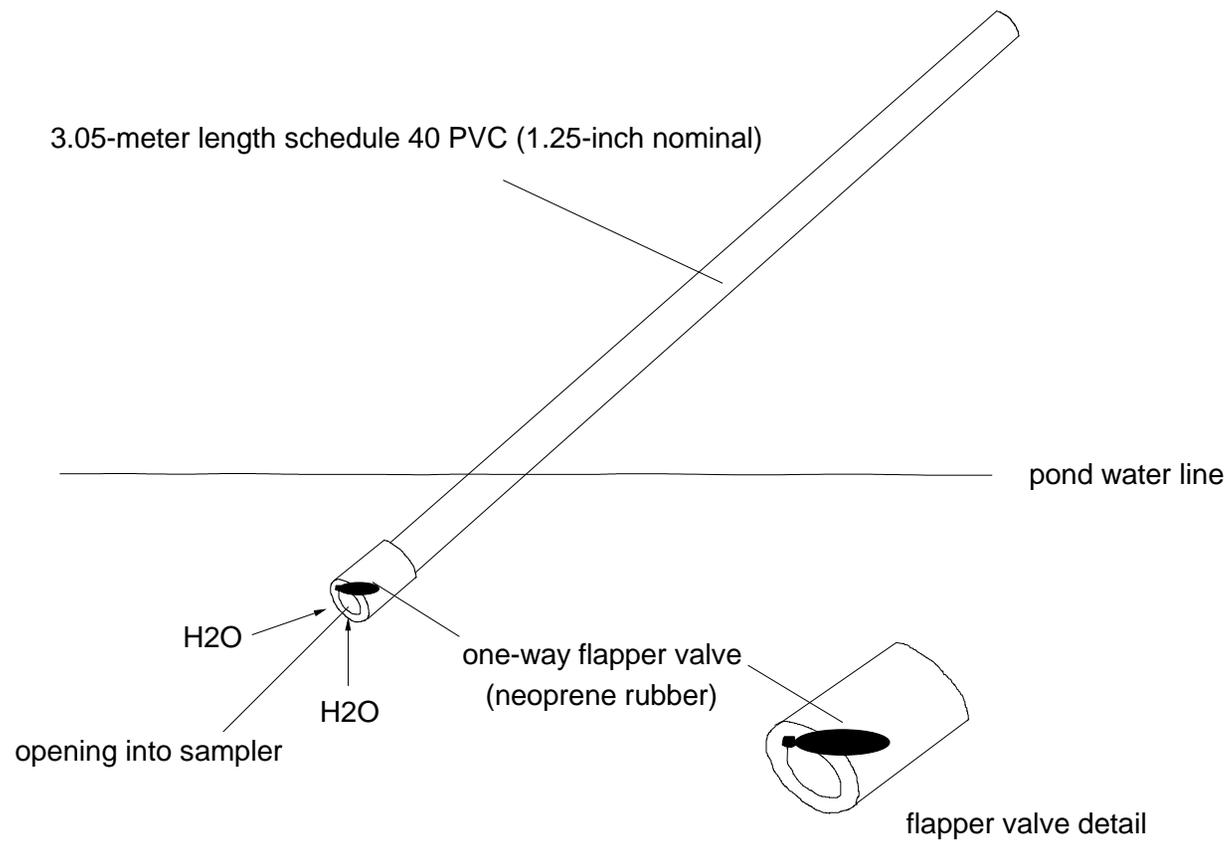


Figure 4-2. Integrated-depth pond water column sampler.

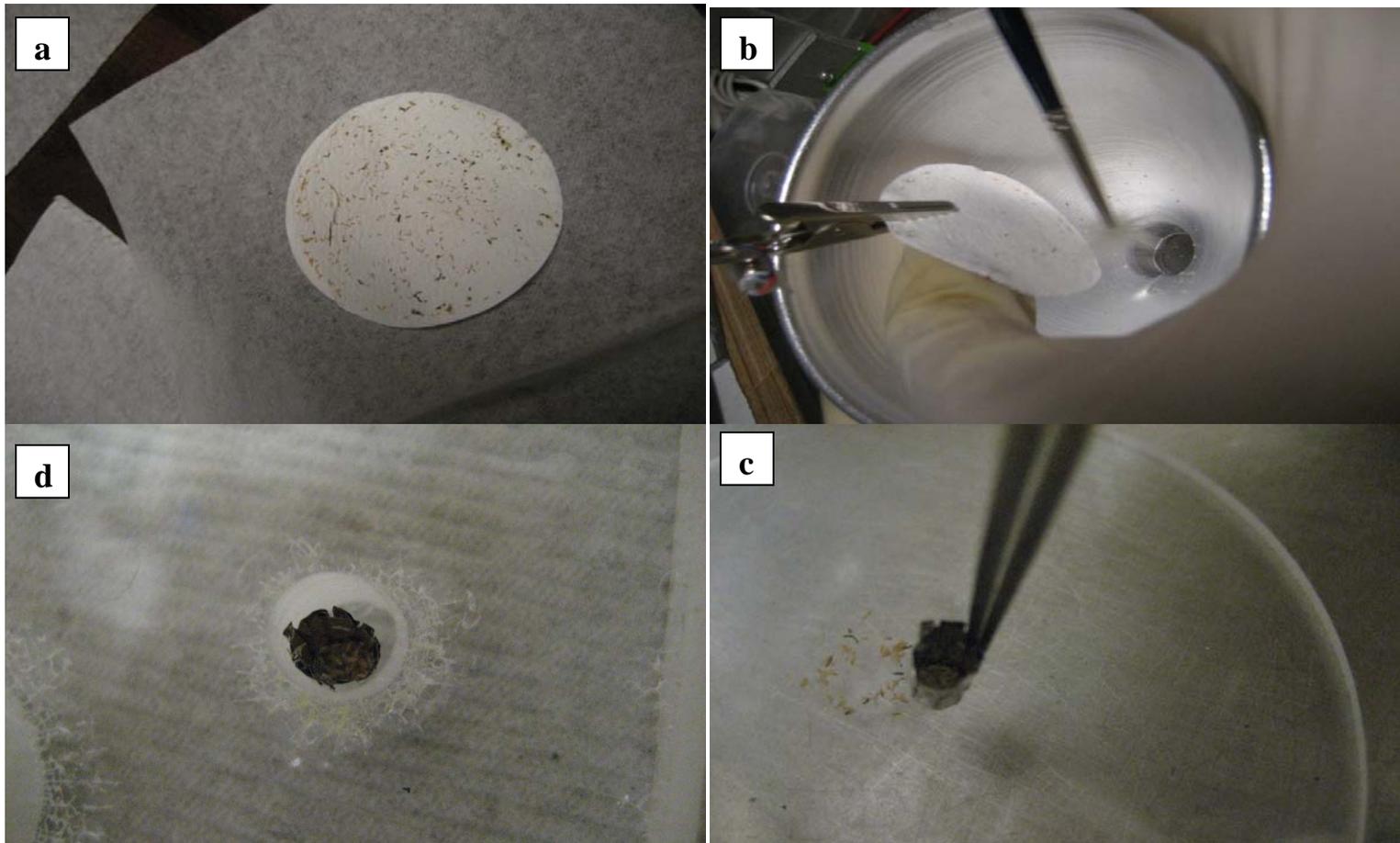


Figure 4-3a-d. Clockwise from top left: (a) freeze dried large zooplankton size assemblage on Whatman™ GF/C pre-combusted glass filter disc (47 mm dia.), (b) filter disc attached to small vibrating motor while particles are brushed into funnel, (c) open tin foil sample capsule containing plankton sample, (d) tin capsule with sample prior to being closed, folded and placed into analysis tray.

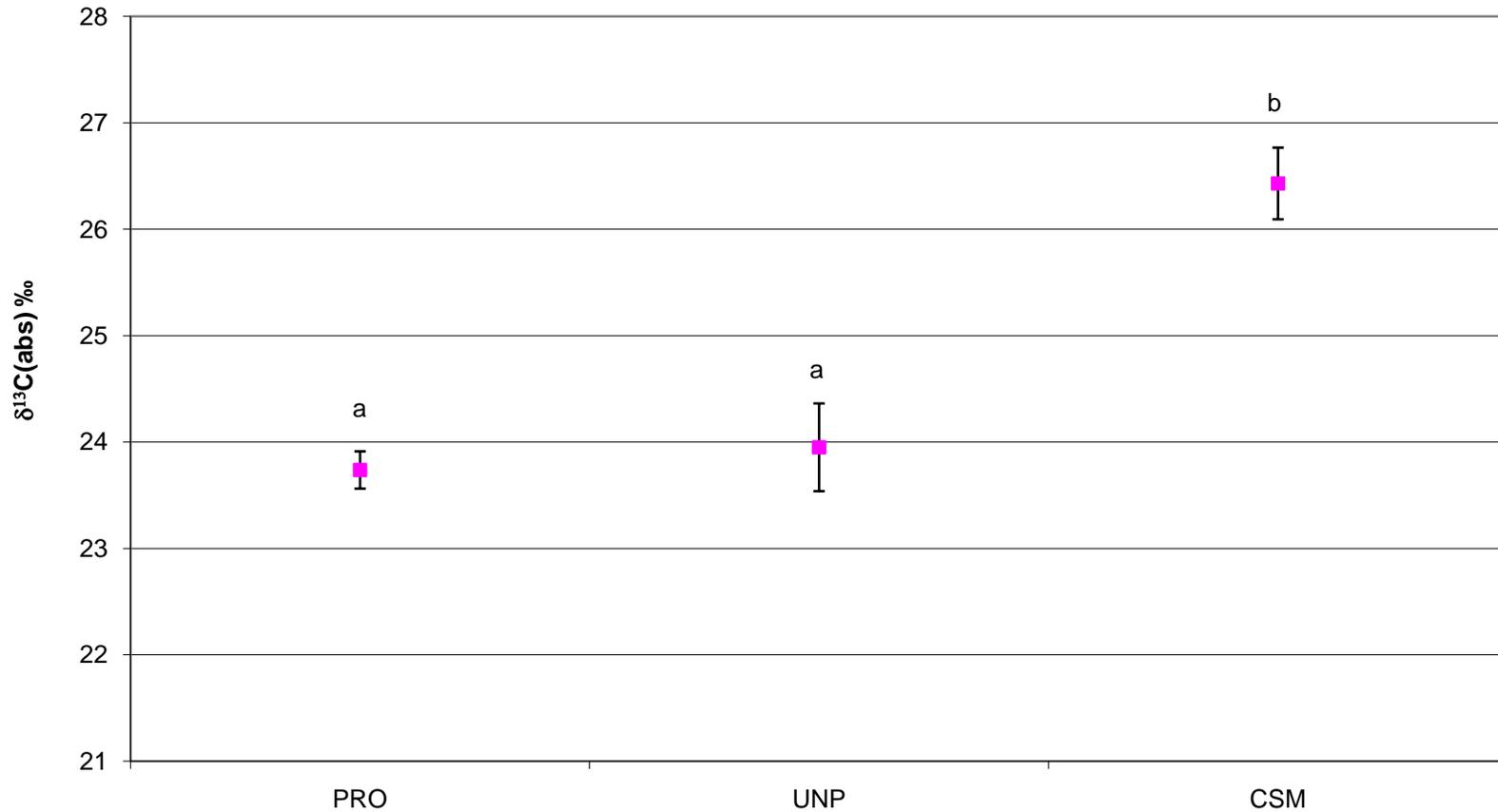


Figure 4-4. Mean carbon isotope signatures ($\delta^{13}\text{C } \text{‰} \pm 95 \text{ \% CI}$) of three pond nutrient treatments [processed feed (PRO), unprocessed feed (UNP), and cottonseed meal fertilizer (CSM)], $n = 3$ (PRO), 3 (UNP), and 2 (CSM); unshared letters denote statistical differences between treatments ($P < 0.05$, Tukey's multiple comparison test).

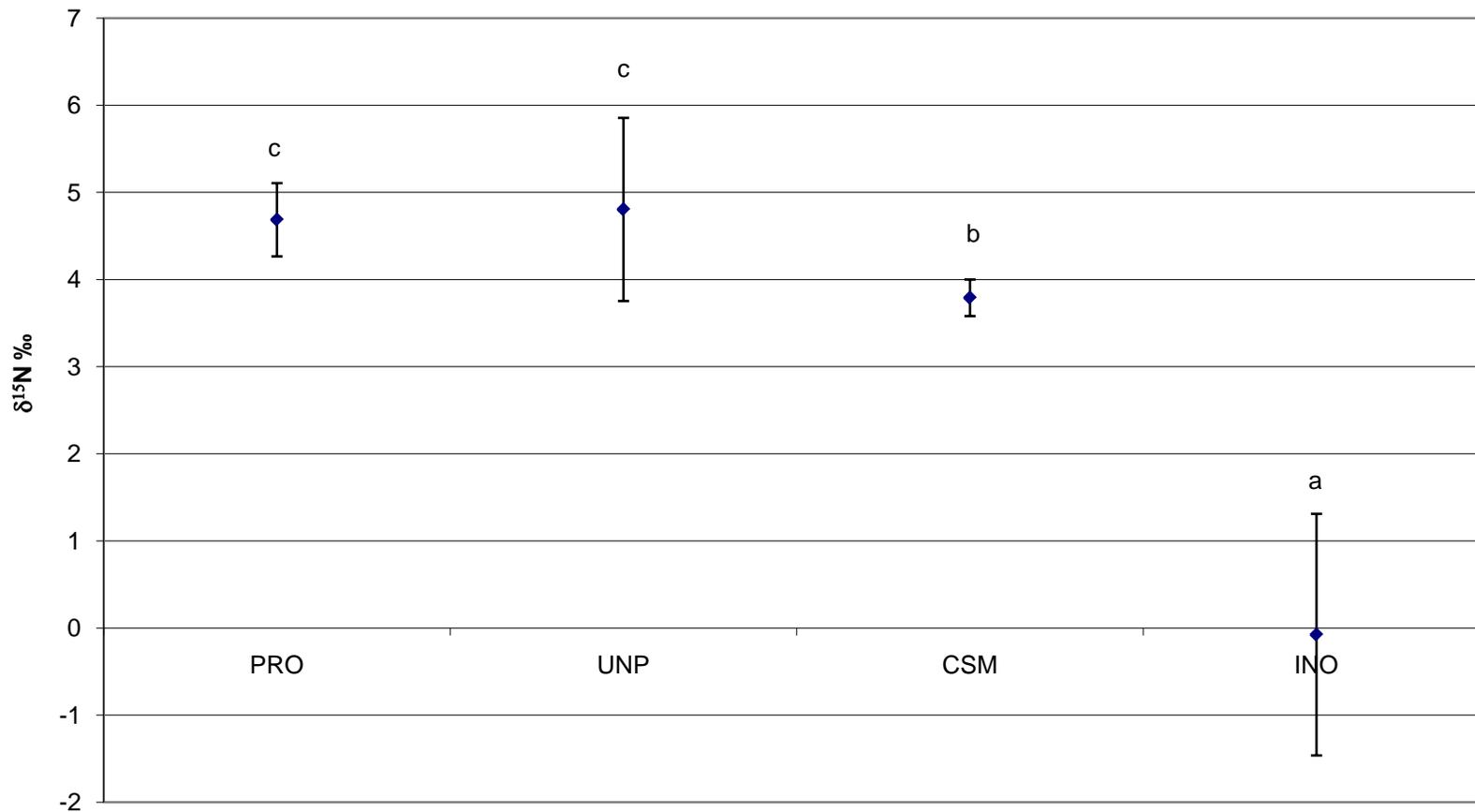


Figure 4-5. Mean nitrogen isotope signatures ($\delta^{15}\text{N}$ ‰ \pm 95 % CI) of applied pond nutrient treatments [processed feed (PRO), unprocessed feed, cottonseed meal (CSM), and inorganic fertilizer (INO)], n = 3 (PRO), 3 (UNP), 2 (CSM), and 2 (INO); unshared letters denote statistical differences between treatments ($P < 0.05$, Tukey's multiple comparison test).

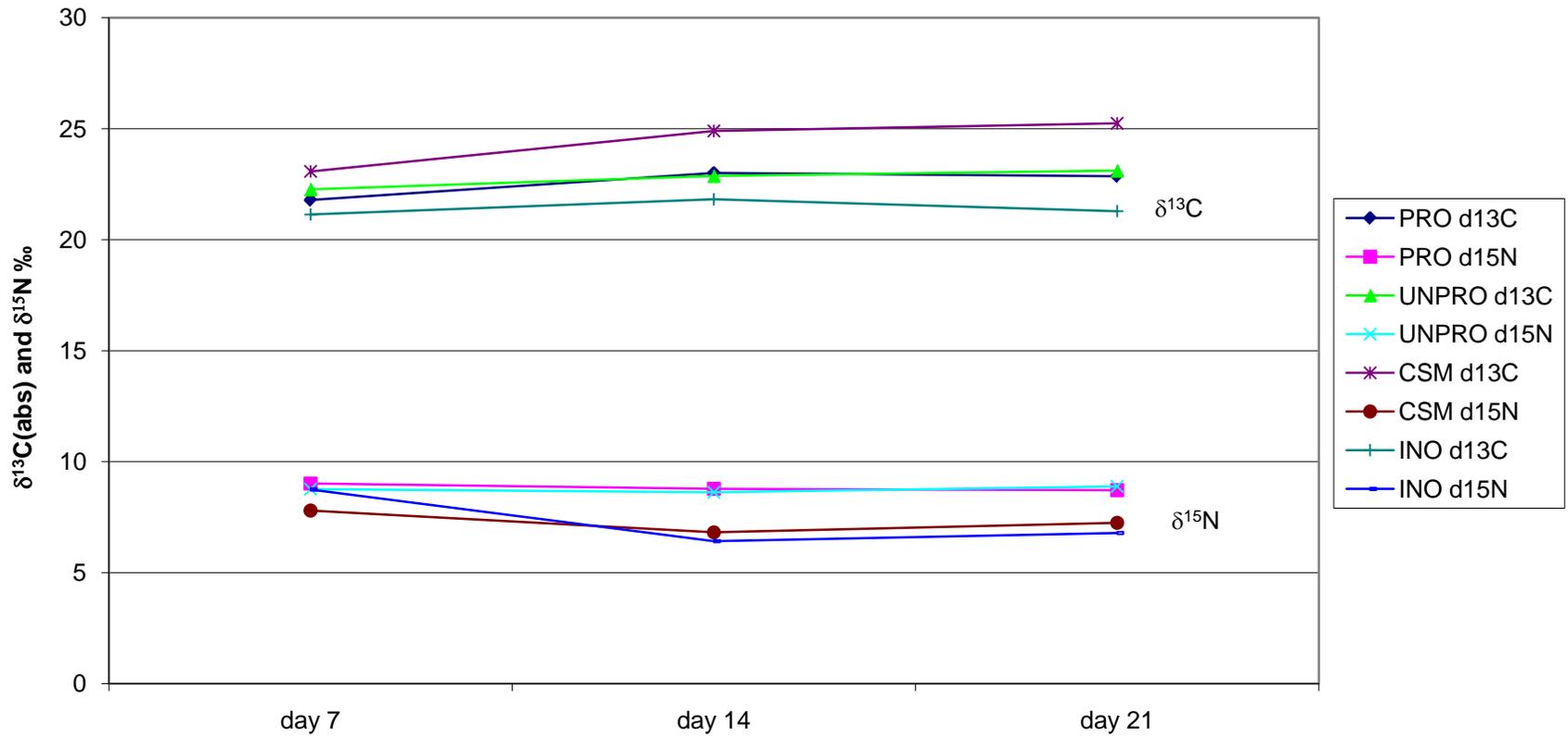


Figure 4-6. Average pre-harvest pond swordtail fry carbon (absolute value) and nitrogen isotope signatures ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) for three weekly sampling dates among four nutrient treatments [processed (PRO) and unprocessed (UNP) feed, cottonseed meal (CSM) and inorganic (INO) fertilizer]; n = 6 replicate ponds per treatment.

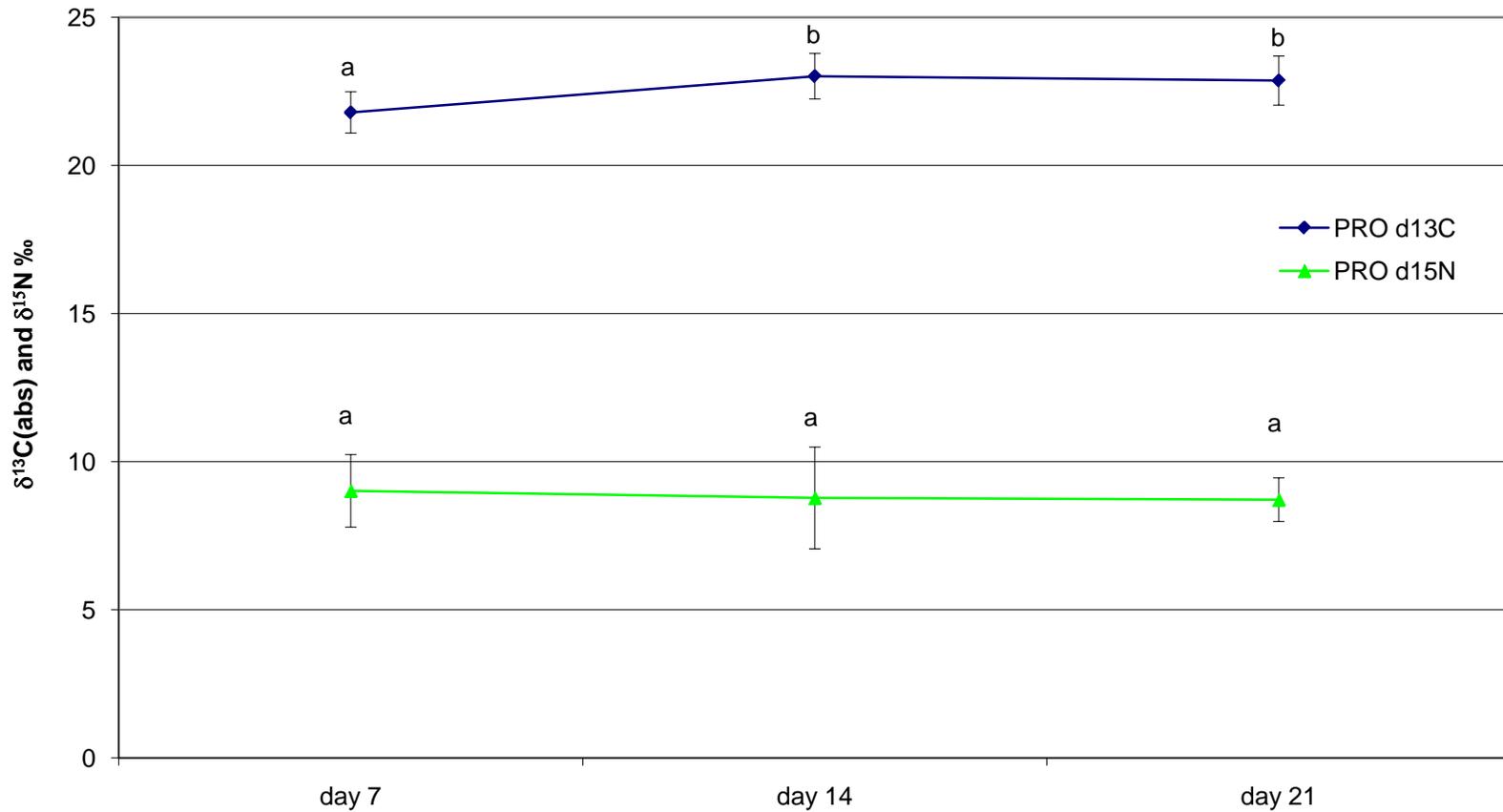


Figure 4-7. Average processed feed (PRO) treatment pre-harvest swordtail fry carbon and nitrogen isotope signatures [$\delta^{13}\text{C}$ (abs. value), $\delta^{15}\text{N} \text{‰} \pm 95 \text{ \% CI}$] for three weekly sampling dates; $n = 6$ replicate ponds per treatment, unshared letters denote statistical differences within element, C or N ($P < 0.05$, Tukey's multiple comparison test).

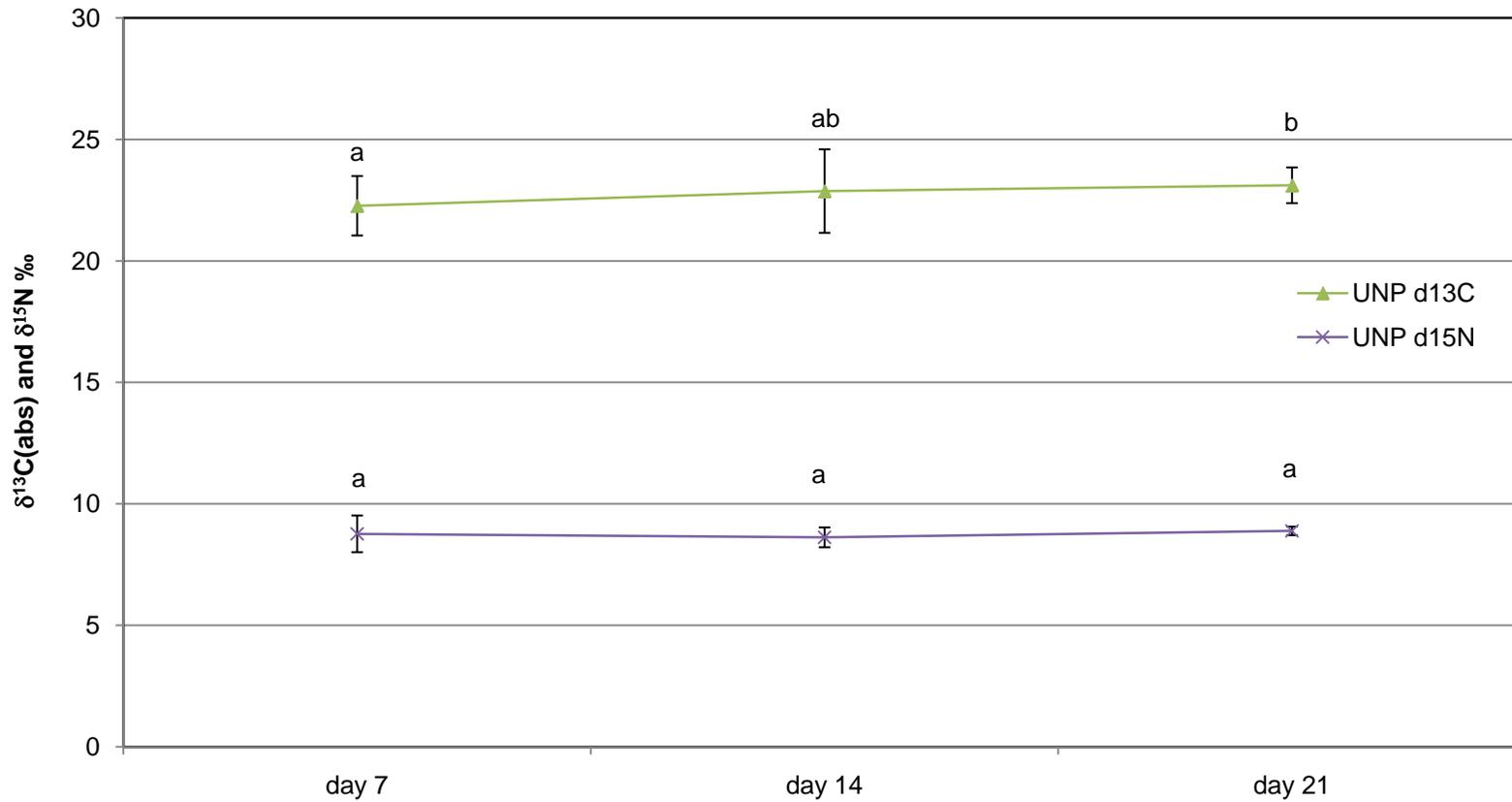


Figure 4-8. Average unprocessed feed (UNP) pond treatment pre-harvest swordtail fry carbon and nitrogen isotope signatures [$\delta^{13}\text{C}$ (abs. value), $\delta^{15}\text{N}$ ‰ \pm 95 % CI] for three weekly sampling dates; $n = 6$ replicate ponds per treatment, unshared letters denote statistical differences within elements, C or N, ($P < 0.05$, Tukey's multiple comparison test).

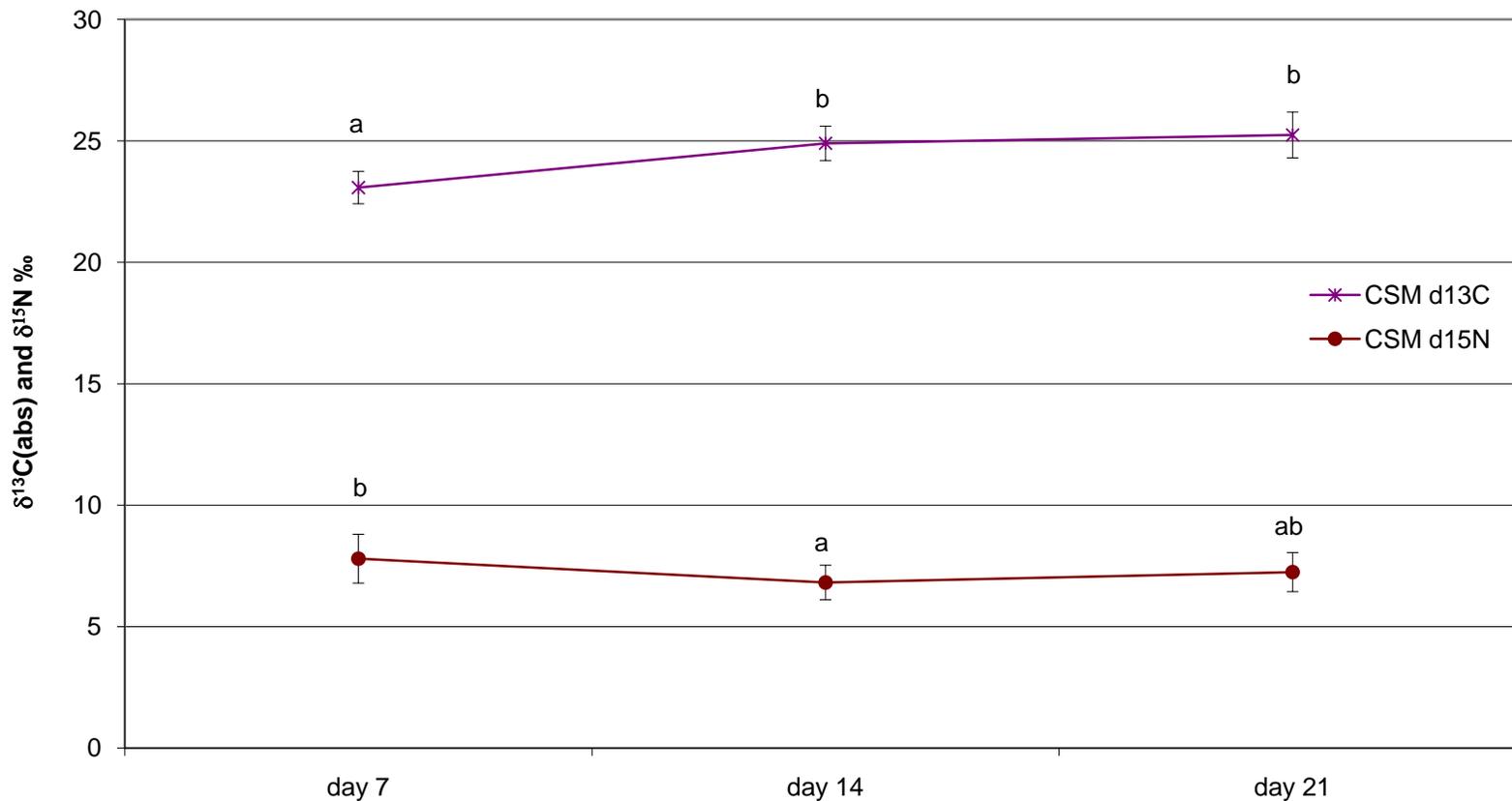


Figure 4-9. Average cottonseed meal (CSM) pond treatment pre-harvest swordtail fry carbon and nitrogen isotope signatures [$\delta^{13}\text{C}$ (abs. value), $\delta^{15}\text{N} \text{‰} \pm 95 \text{ \% CI}$] for three weekly sampling dates; $n = 6$ replicate ponds per treatment, unshared letters denote statistical differences within elements, C or N ($P < 0.05$, Tukey's multiple comparison test).

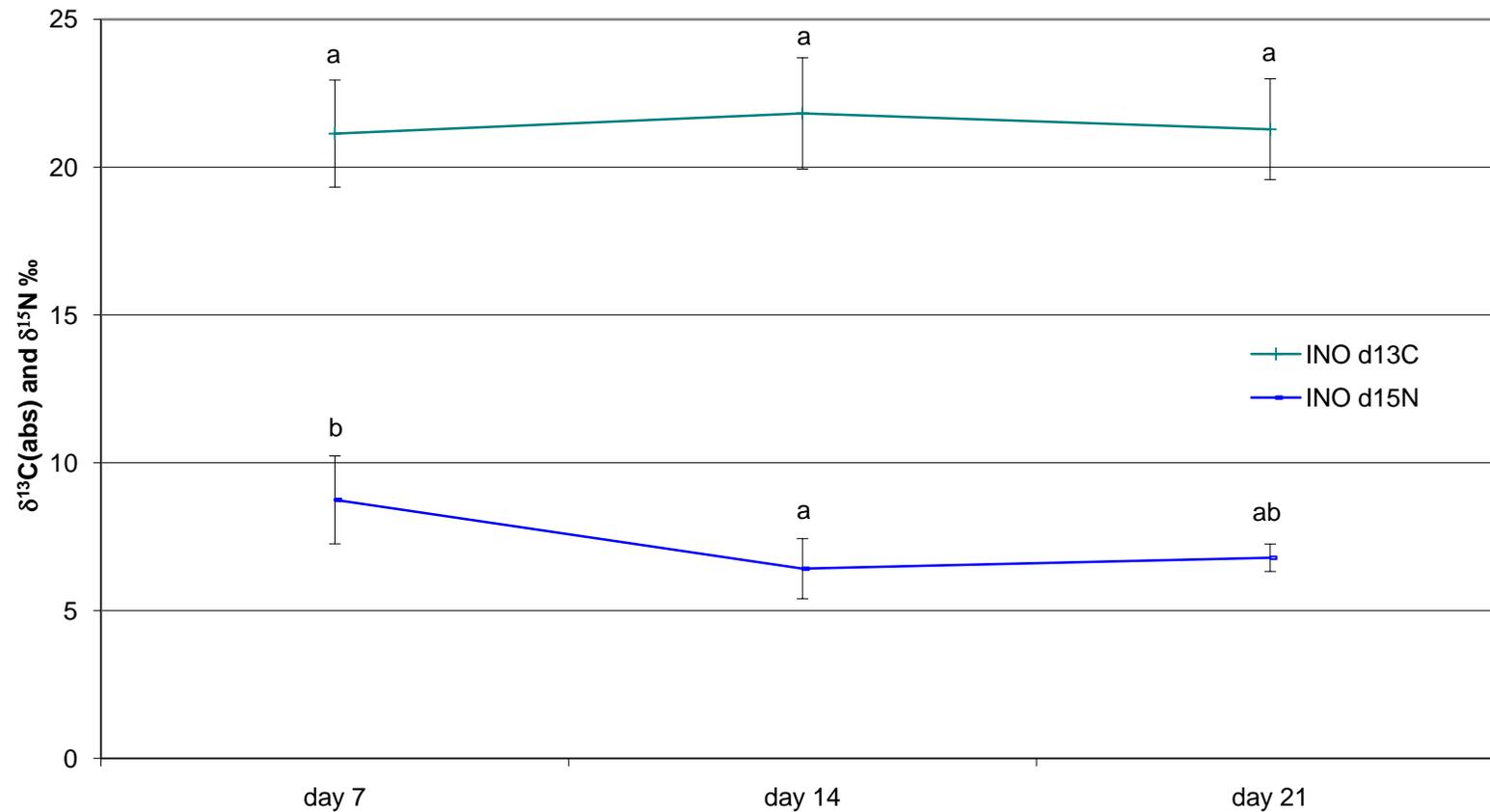


Figure 4-10. Average inorganic fertilizer (INO) treatment pre-harvest swordtail fry carbon and nitrogen isotope signatures [$\delta^{13}\text{C}$ (abs. value), $\delta^{15}\text{N}$ ‰ \pm 95 % CI] for three weekly sampling dates; n = 6 replicate ponds per treatment, unshared letters denote statistical differences within elements, C or N ($P < 0.05$, Tukey's multiple comparison test).

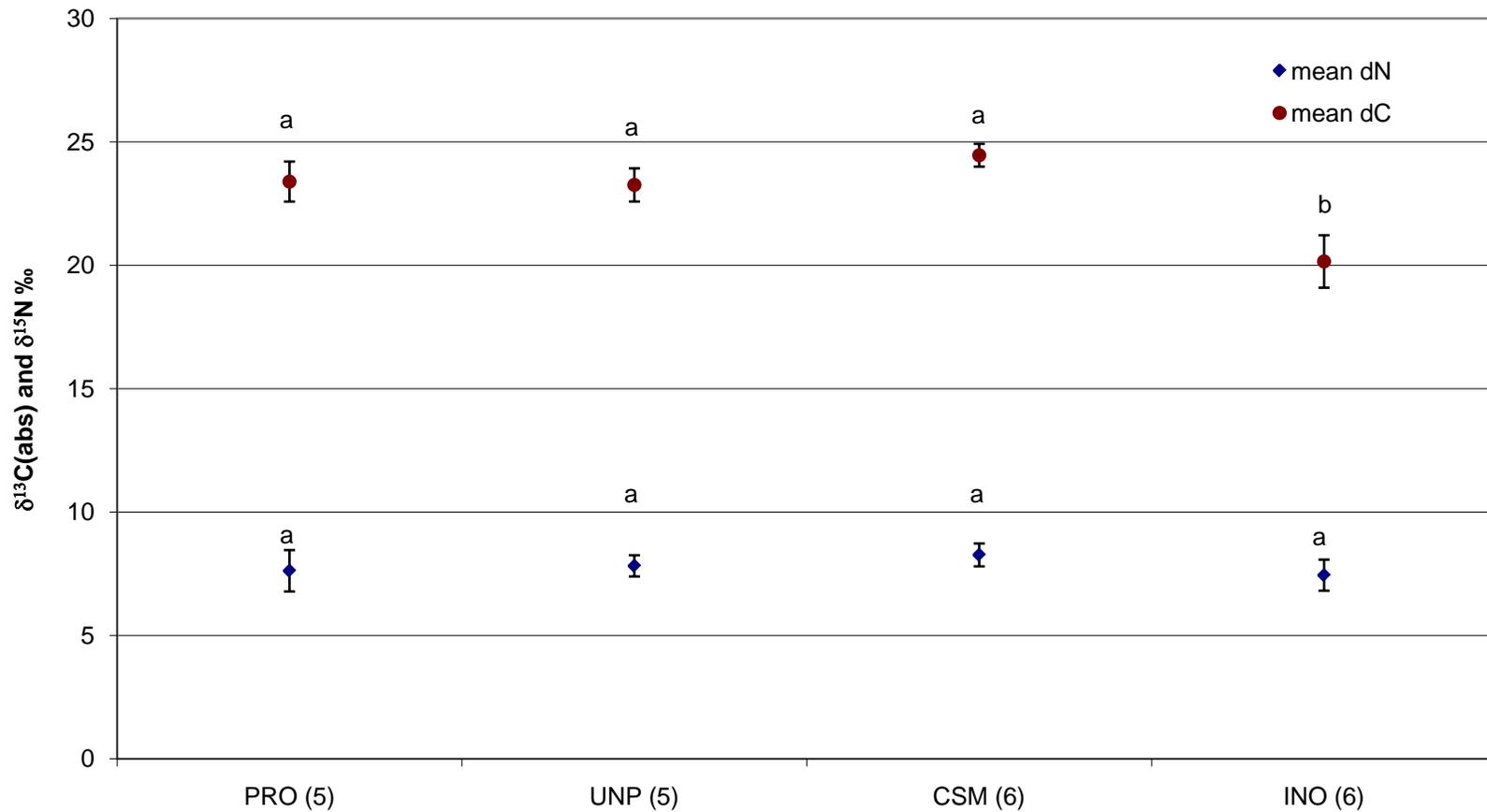


Figure 4-11. Time-averaged pond harvest large swordtail (> 31 mm SL) carbon and nitrogen isotope signatures [$\delta^{13}\text{C}$ (abs. value), $\delta^{15}\text{N} \text{‰} \pm 95 \text{ \% CI}$] among four nutrient treatments, numbers in parentheses denote sample size; unshared letters denote statistical differences within elements, C or N, ($P < 0.05$, Tukey's multiple comparison test).

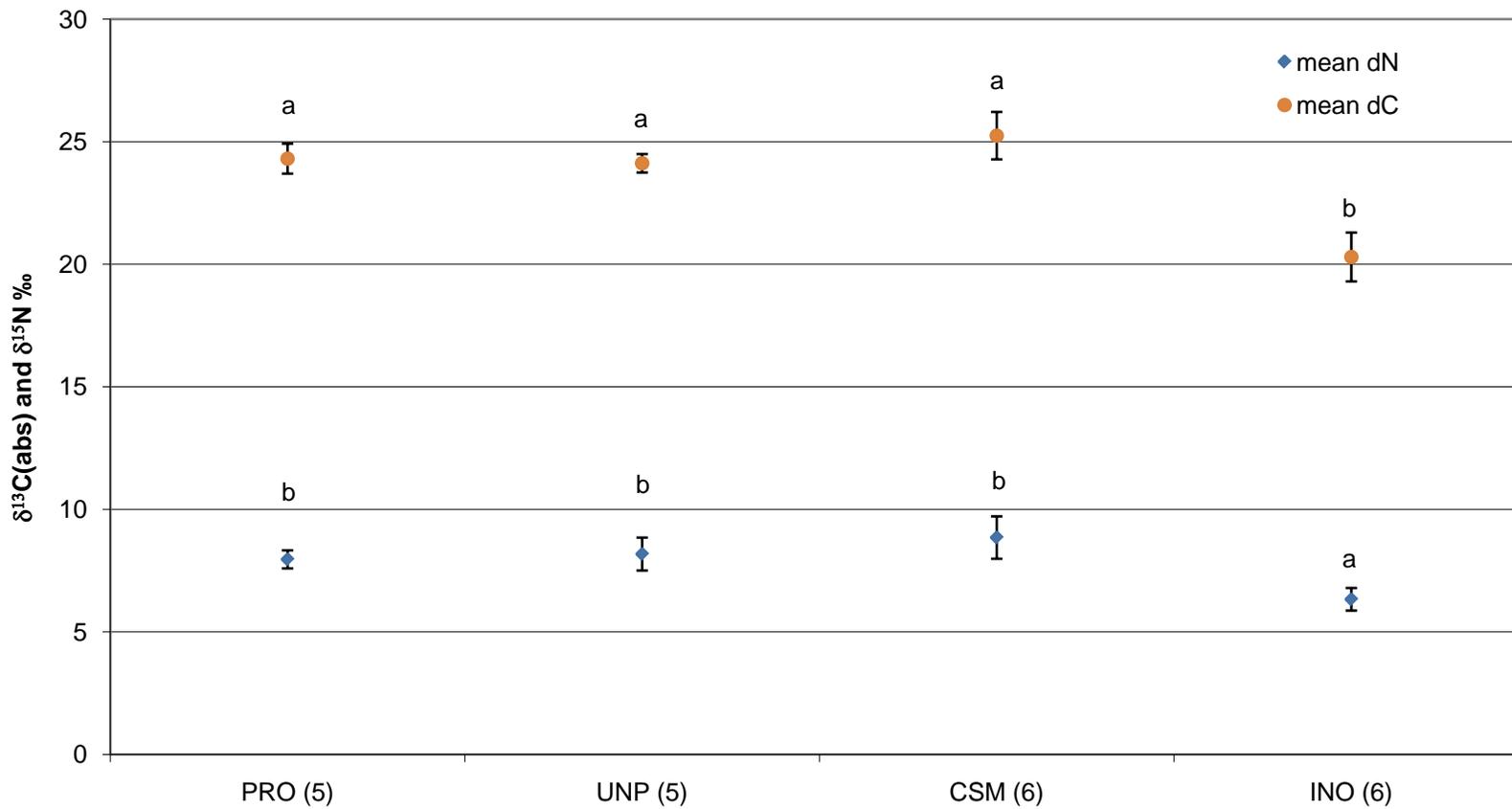


Figure 4-12. Average pond harvest small fish ($SL \leq 31$ mm) carbon and nitrogen isotope signatures [$\delta^{13}\text{C}$ (abs. valve), $\delta^{15}\text{N}$ ‰ \pm 95 % CI] among four pond nutrient treatments, numbers in parentheses denote sample size; unshared letters denote statistical differences ($P < 0.05$, Tukey's multiple comparison test).

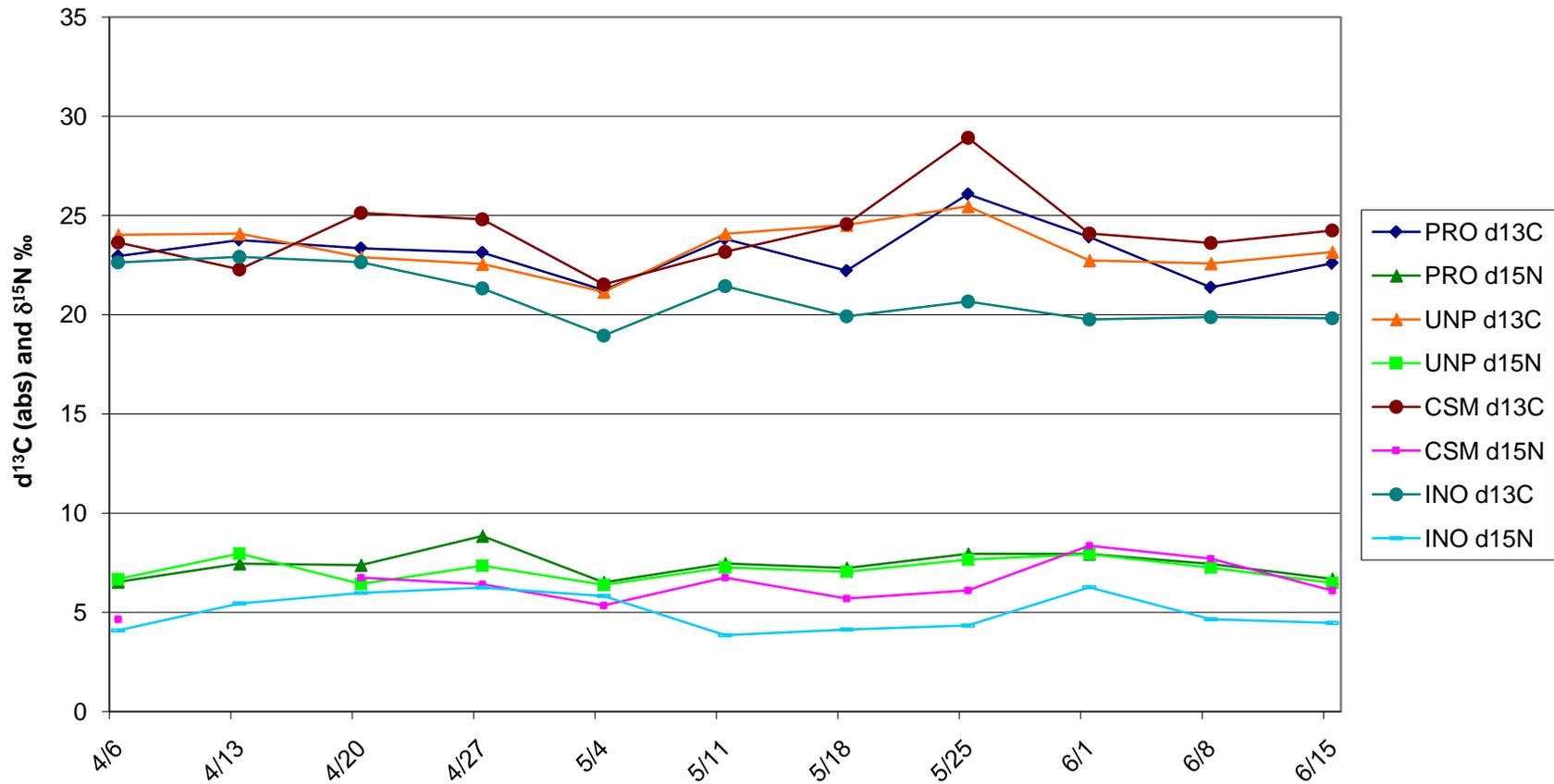


Figure 4-13. Pond trial large zooplankton (> 200 μm) carbon [$\delta^{13}\text{C}$ (abs. value)] and nitrogen ($\delta^{15}\text{N}$) isotope signatures for 11 weekly sampling periods among four pond nutrient treatments, n = 6 ponds per treatment.

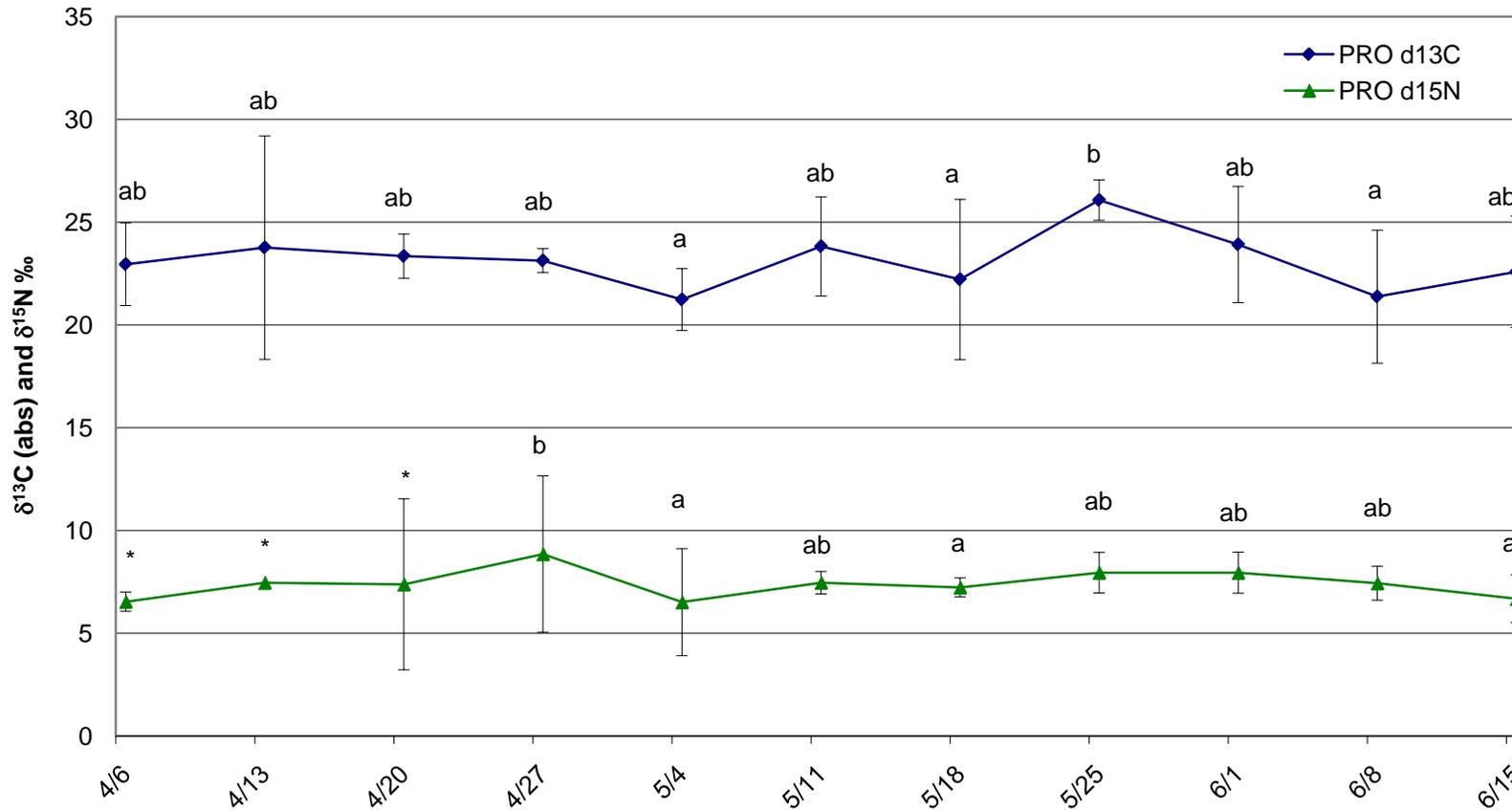


Figure 4-14. Processed feed (PRO) treatment large zooplankton assemblage carbon and nitrogen isotope signatures [$\delta^{13}\text{C}$ (abs. value), $\delta^{15}\text{N}$ ‰ \pm 95 % CI] among 11 weekly sampling periods, n = 6 ponds per treatment; unshared letters denote statistical differences within elements, C or N, ($P < 0.05$, Bonferroni post test), “*” too few replicates for statistical analysis.

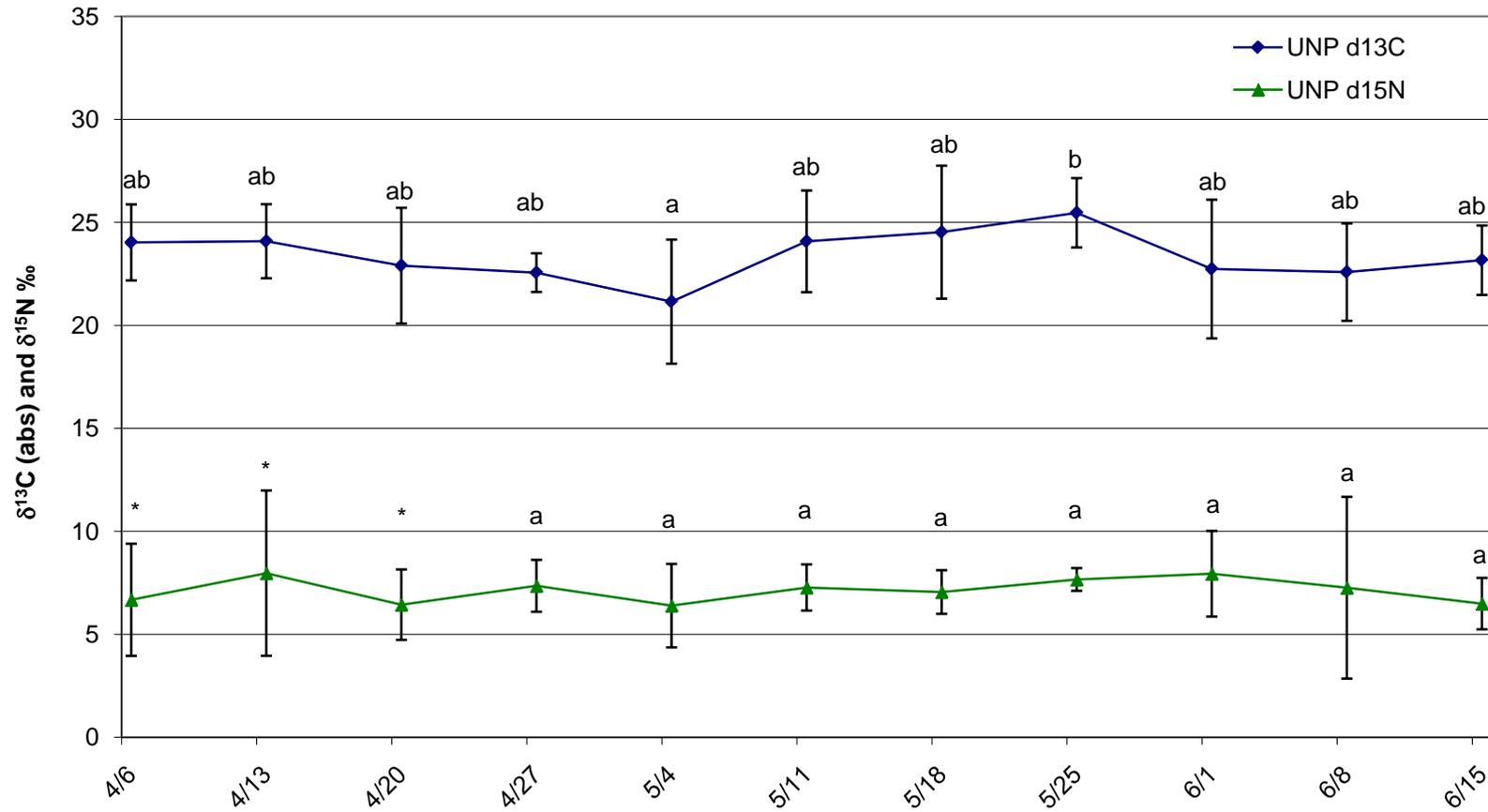


Figure 4-15. Unprocessed feed (UNP) treatment large zooplankton assemblage carbon and nitrogen isotope signatures [$\delta^{13}\text{C}$ (abs. value), $\delta^{15}\text{N}$ ‰ \pm 95 % CI] among 11 weekly sampling periods, n = 6 ponds per treatment; unshared letters denote statistical differences within elements, C or N, ($P < 0.05$, Bonferroni post test), ‘*’ too few replicates for statistical analysis.

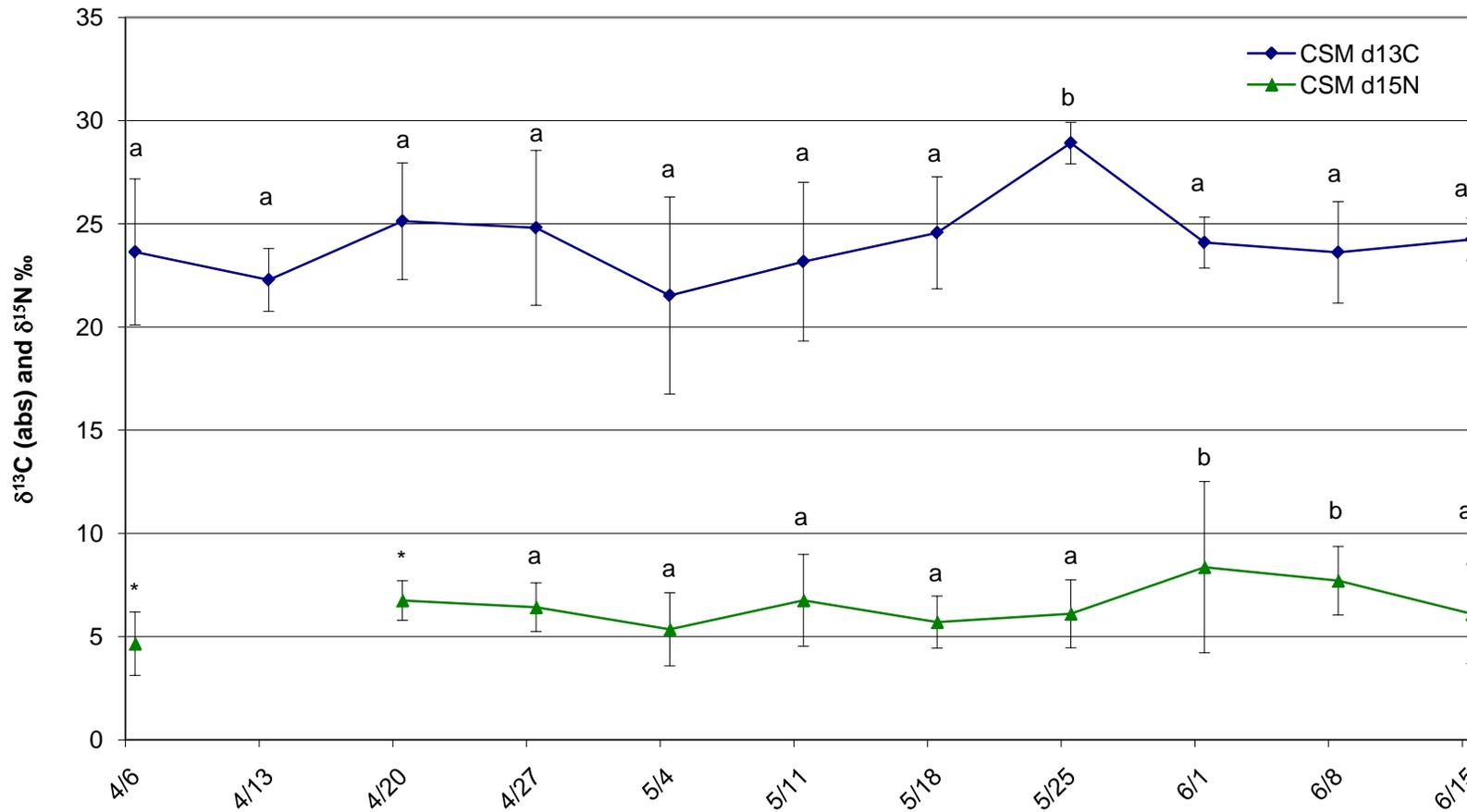


Figure 4-16. Cottonseed meal (CSM) treatment large zooplankton (> 200 μm) assemblage carbon and nitrogen isotope signatures [$\delta^{13}\text{C}$ (abs. value), $\delta^{15}\text{N}$ ‰ \pm 95 % CI] among 11 weekly sampling periods, n = 6 ponds per treatment; unshared letters denote statistical differences within elements, C or N, ($P < 0.05$, Bonferroni post test), “*” too few replicates for analysis.

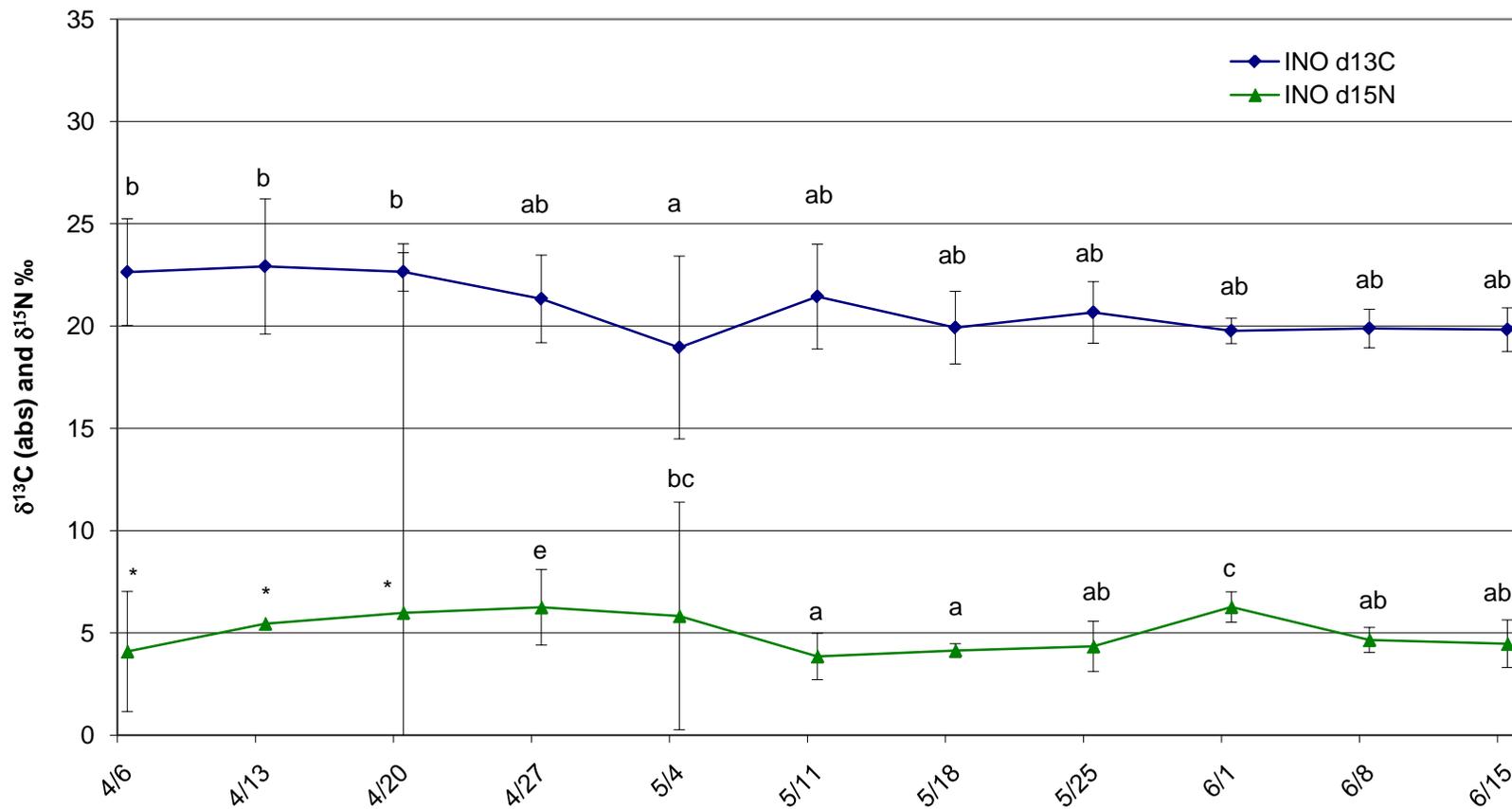


Figure 4-17. Inorganic fertilizer treatment large zooplankton (> 200 μm) assemblage carbon and nitrogen isotope signatures [$\delta^{13}\text{C}$ (abs. value), $\delta^{15}\text{N}$ ‰ \pm 95 % CI] among 11 weekly sampling periods, n = 6 ponds per treatment; unshared letters denote statistical differences within elements, C or N, ($P < 0.05$, Bonferroni post test), ‘*’ too few replicates for statistical analysis.

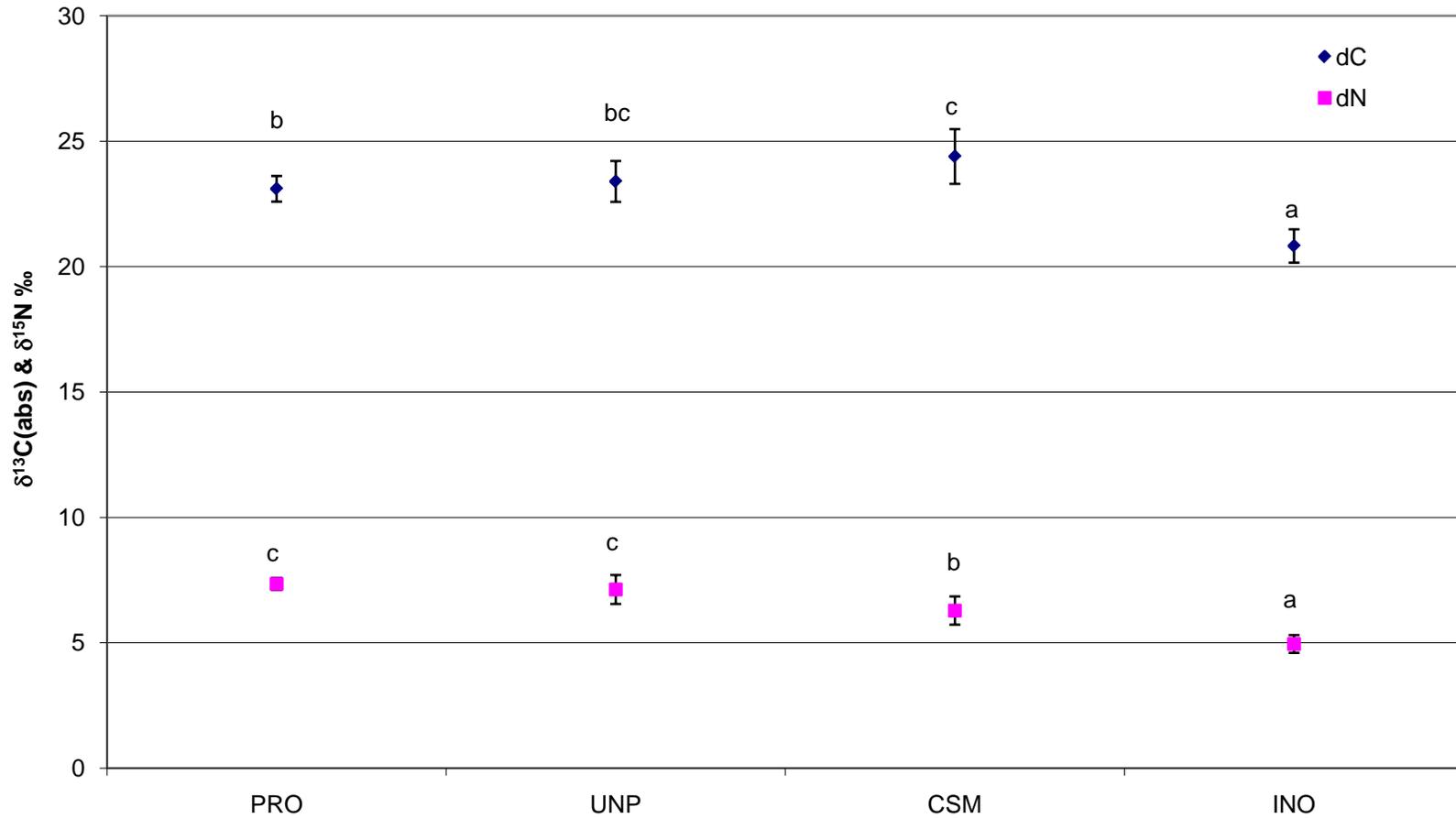


Figure 4-18. Time-averaged large zooplankton (> 200 μm) carbon and nitrogen isotope signatures [$\delta^{13}\text{C}$ (absolute value), $\delta^{15}\text{N}$ ‰ \pm 95 % CI] among four pond nutrient treatments, n = 6 ponds per treatment; unshared letters denote statistical differences within elements, C or N, ($P < 0.05$, Tukey's multiple comparison test).

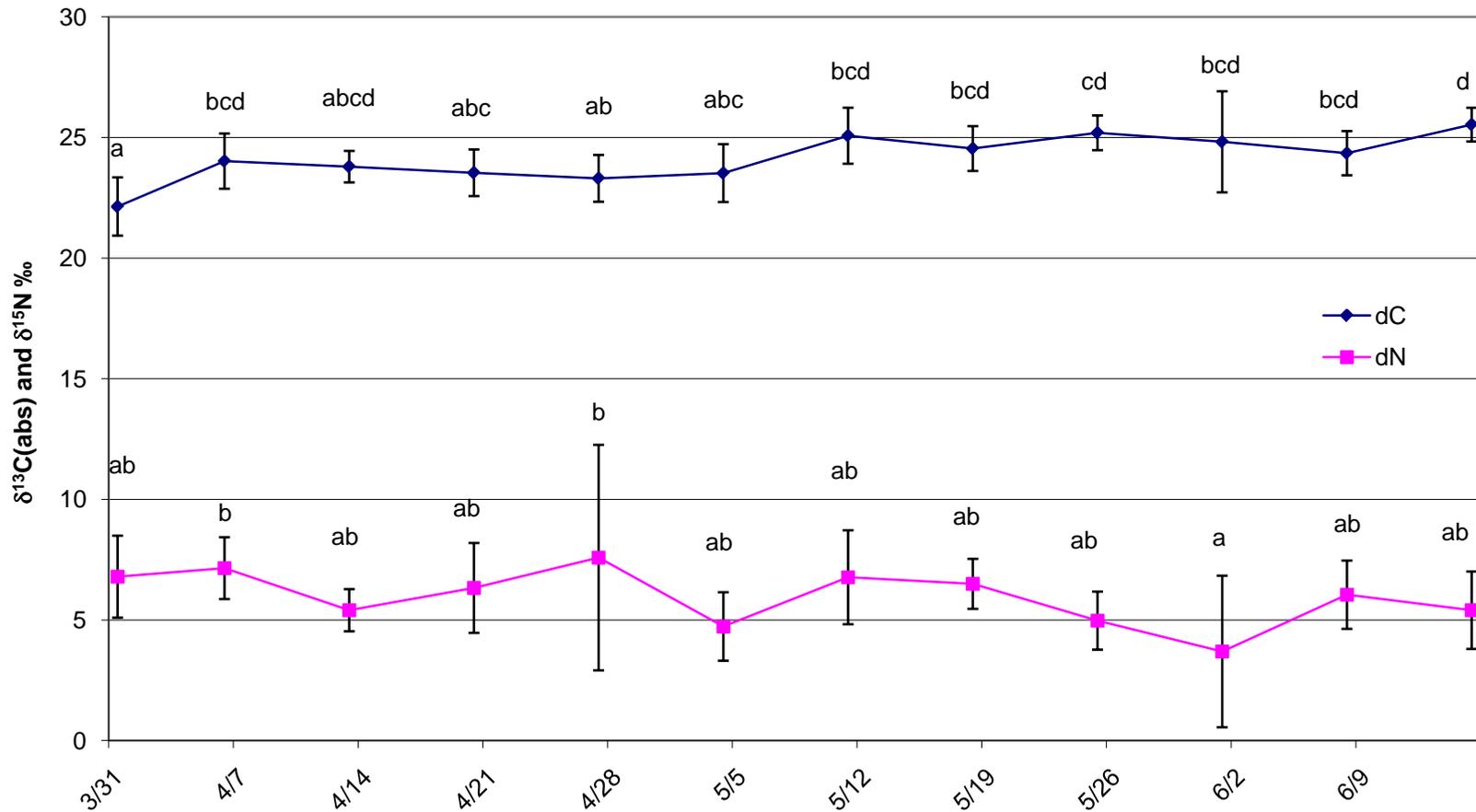


Figure 4-19. Processed feed (PRO) medium zooplankton (32 – 200 μm) assemblage carbon and nitrogen isotope signatures [$\delta^{13}\text{C}$ (absolute value), $\delta^{15}\text{N} \text{‰} \pm 95 \%$ CI] among 12 weekly sampling periods, $n = 6$ ponds per treatment; unshared letters denote statistical differences within elements, C or N, ($P < 0.05$, Bonferroni post test).

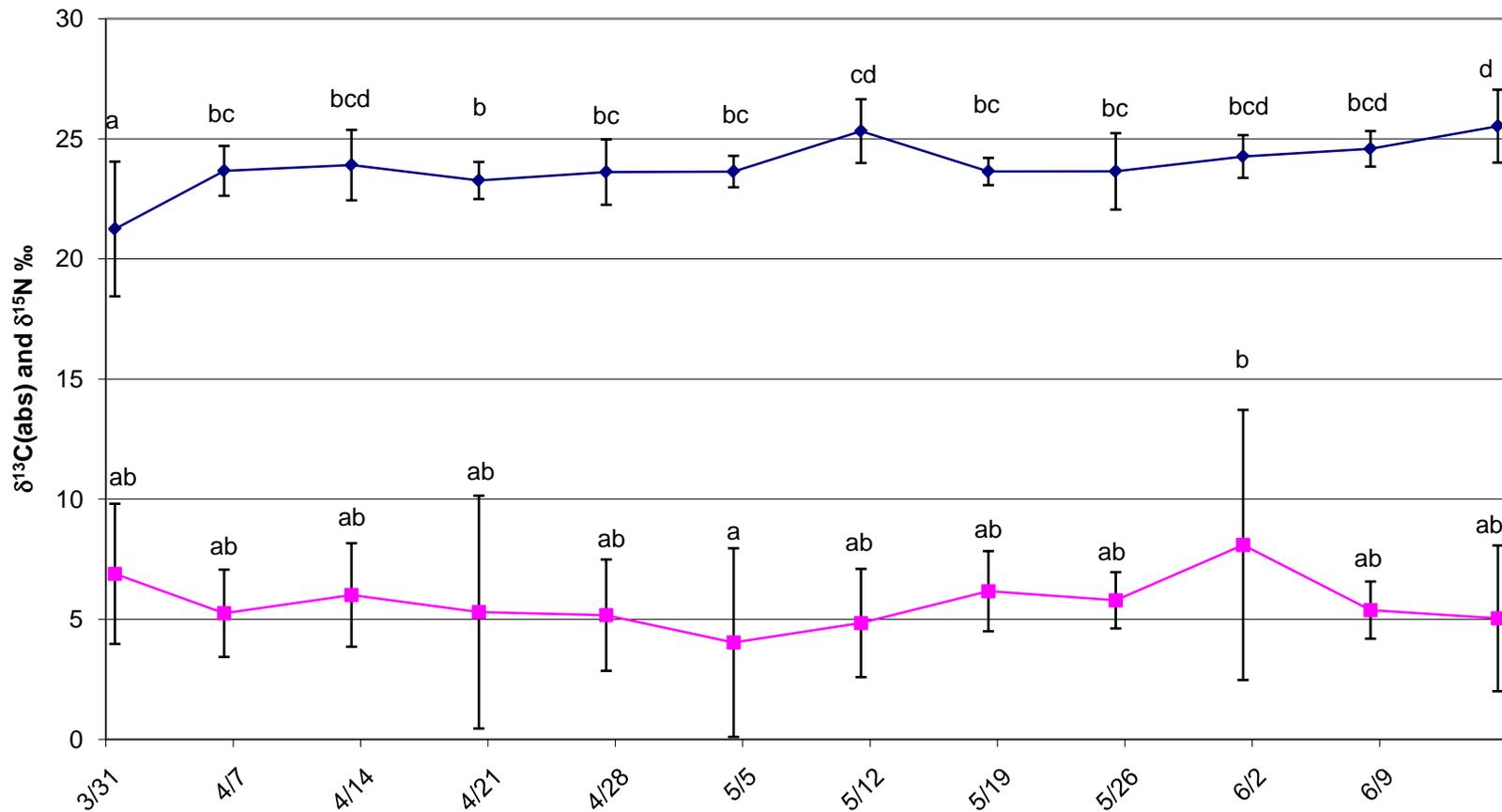


Figure 4-20. Unprocessed (UNP) feed medium plankton assemblage carbon and nitrogen isotope signatures [$\delta^{13}\text{C}$ (absolute value), $\delta^{15}\text{N} \text{‰} \pm 95 \text{ \% CI}$] among 12 weekly sampling periods, $n = 6$ ponds per treatment; unshared letters denote statistical differences within elements, C or N, ($P < 0.05$, Bonferroni post test).

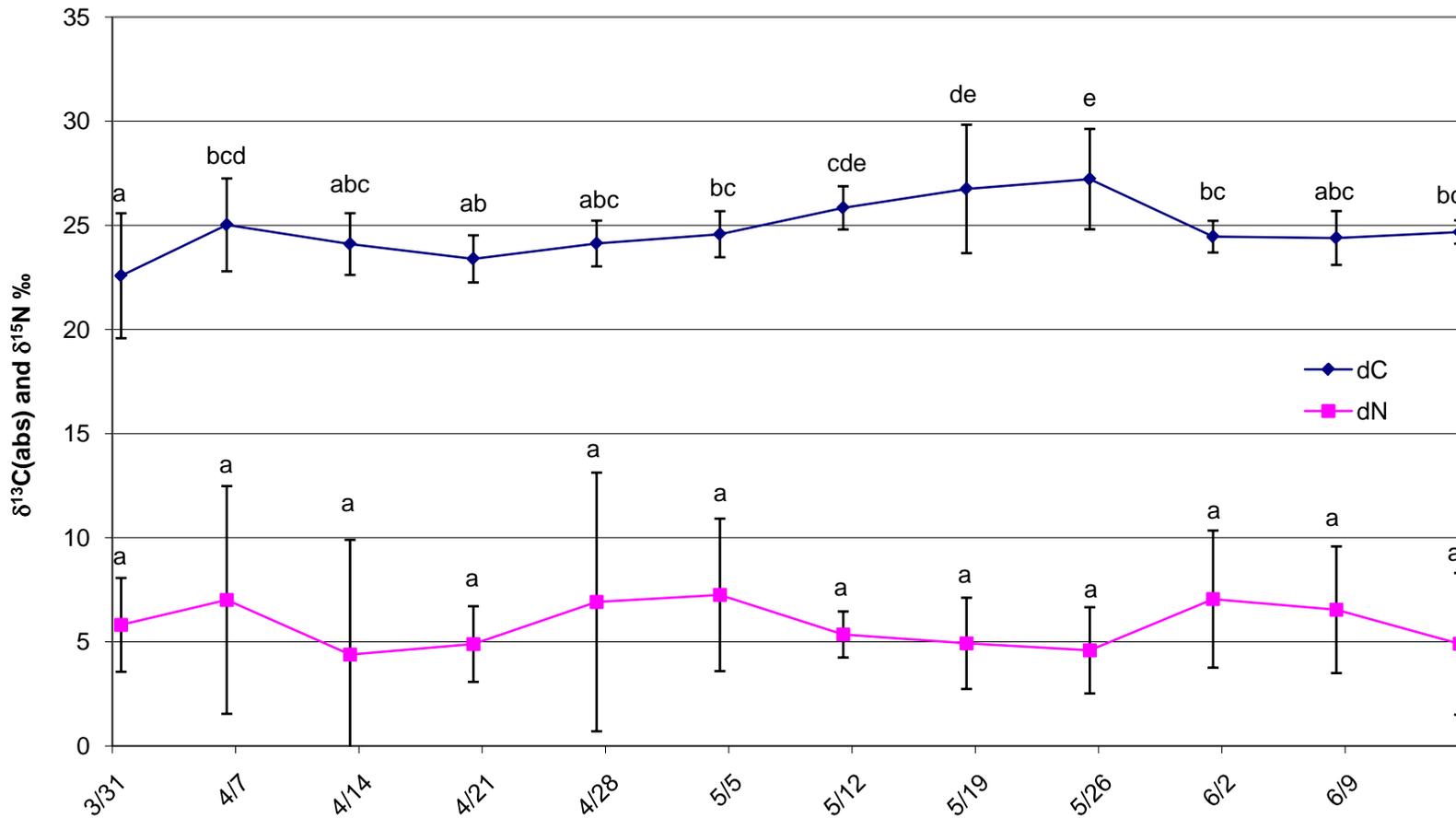


Figure 4-21. Cottonseed meal (CSM) medium plankton assemblage carbon and nitrogen isotope signatures [$\delta^{13}\text{C}$ (absolute value), $\delta^{15}\text{N} \text{‰} \pm 95\% \text{ CI}$] among 12 weekly sampling periods, $n = 6$ ponds per treatment; unshared letters denote statistical differences within elements, C or N, ($P < 0.05$, Bonferroni post test).

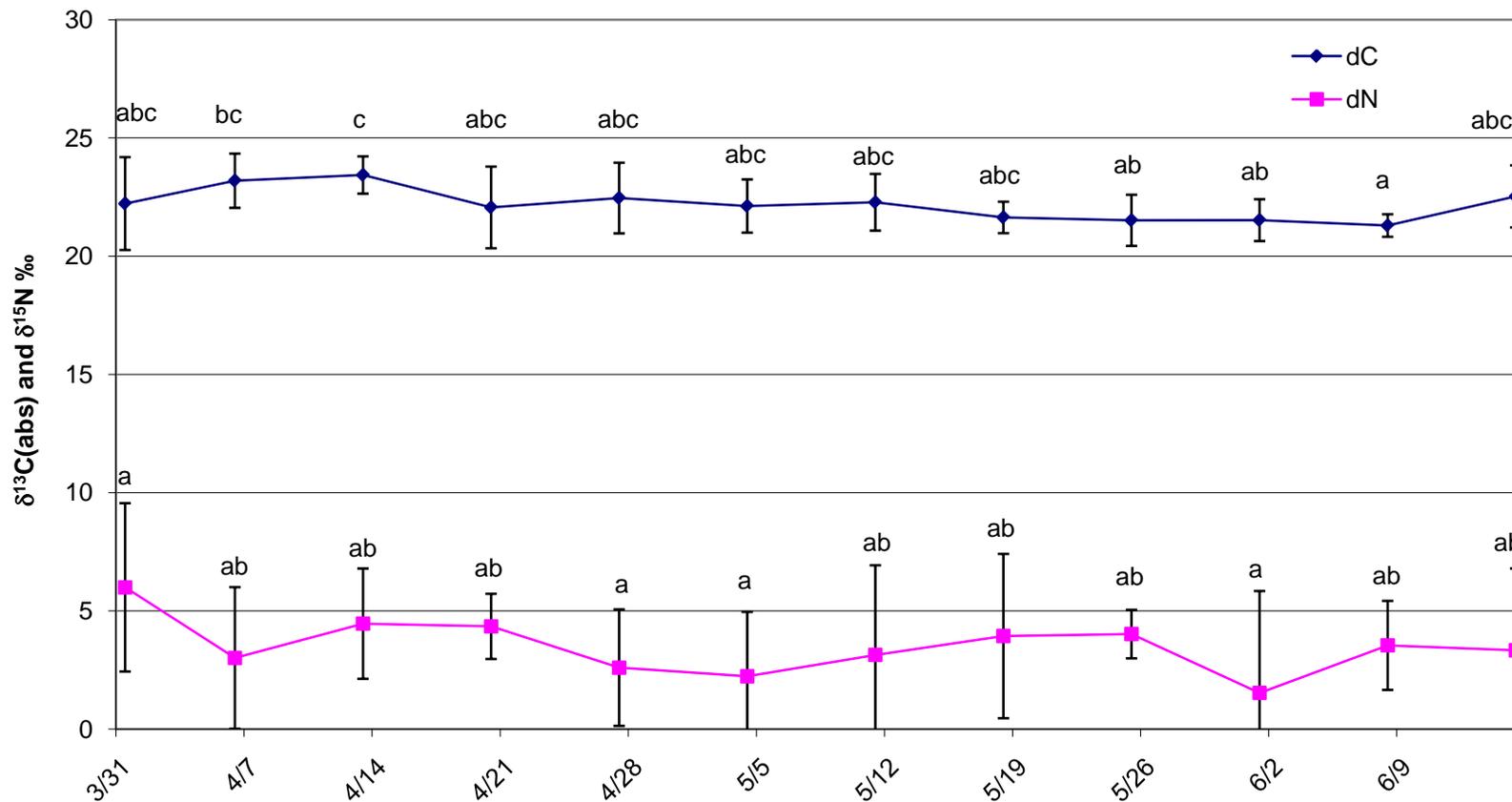


Figure 4-22. Inorganic fertilizer (INO) medium plankton (32 – 200 μm) assemblage carbon and nitrogen isotope signatures [$\delta^{13}\text{C}$ (absolute value), $\delta^{15}\text{N}$ ‰ \pm 95 % CI] among 12 weekly sampling periods, n = 6 ponds per treatment; unshared letters denote statistical differences within elements, C or N, ($P < 0.05$, Bonferroni post test).

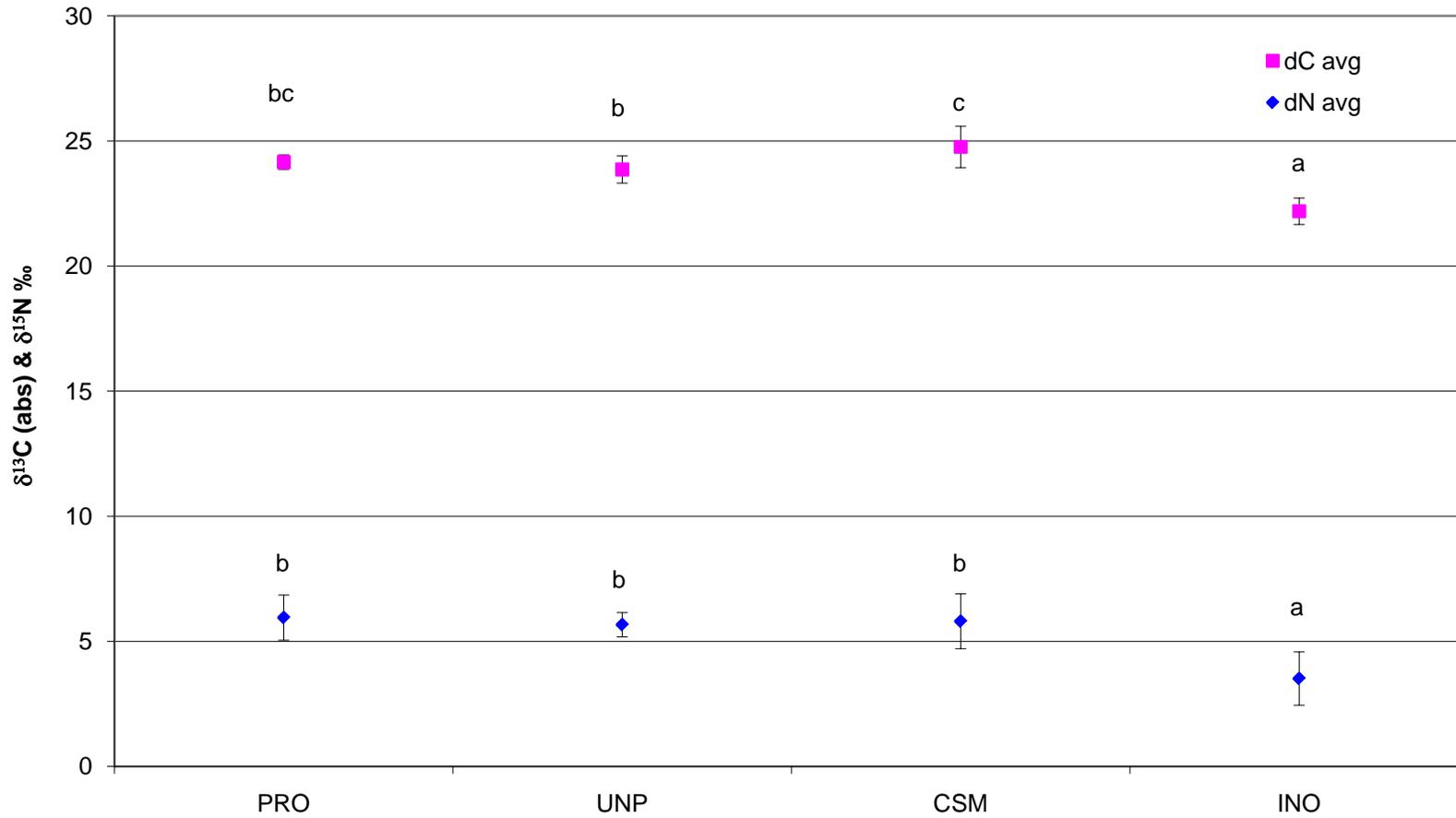


Figure 4-23. Time-averaged medium plankton (32 – 200 μm) carbon and nitrogen isotope signatures [$\delta^{13}\text{C}$ (absolute value), $\delta^{15}\text{N}$ ‰ \pm 95 % CI] among 12 weekly sampling periods, $n = 6$ ponds per treatment; unshared letters denote statistical differences within elements, C or N, ($P < 0.05$, Tukey's multiple comparison test).

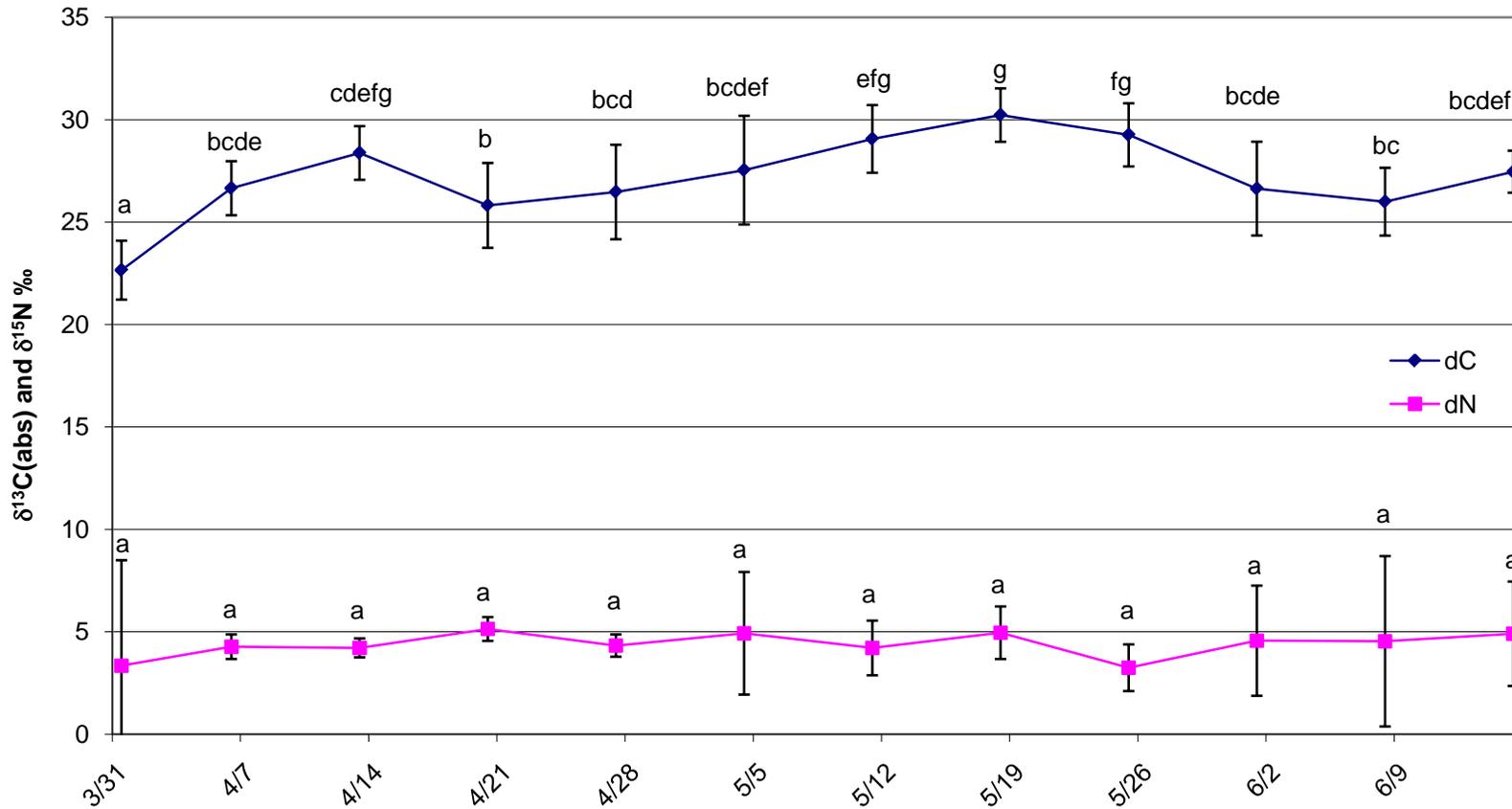


Figure 4-24. Processed feed (PRO) small plankton (1 – 32 μm) assemblage carbon and nitrogen isotope signatures [$\delta^{13}\text{C}$ (absolute value), $\delta^{15}\text{N}$ ‰ \pm 95 % CI] among 12 weekly sampling periods, n = 6 ponds per treatment; unshared letters denote statistical differences within elements, C or N, ($P < 0.05$, Bonferroni post test).

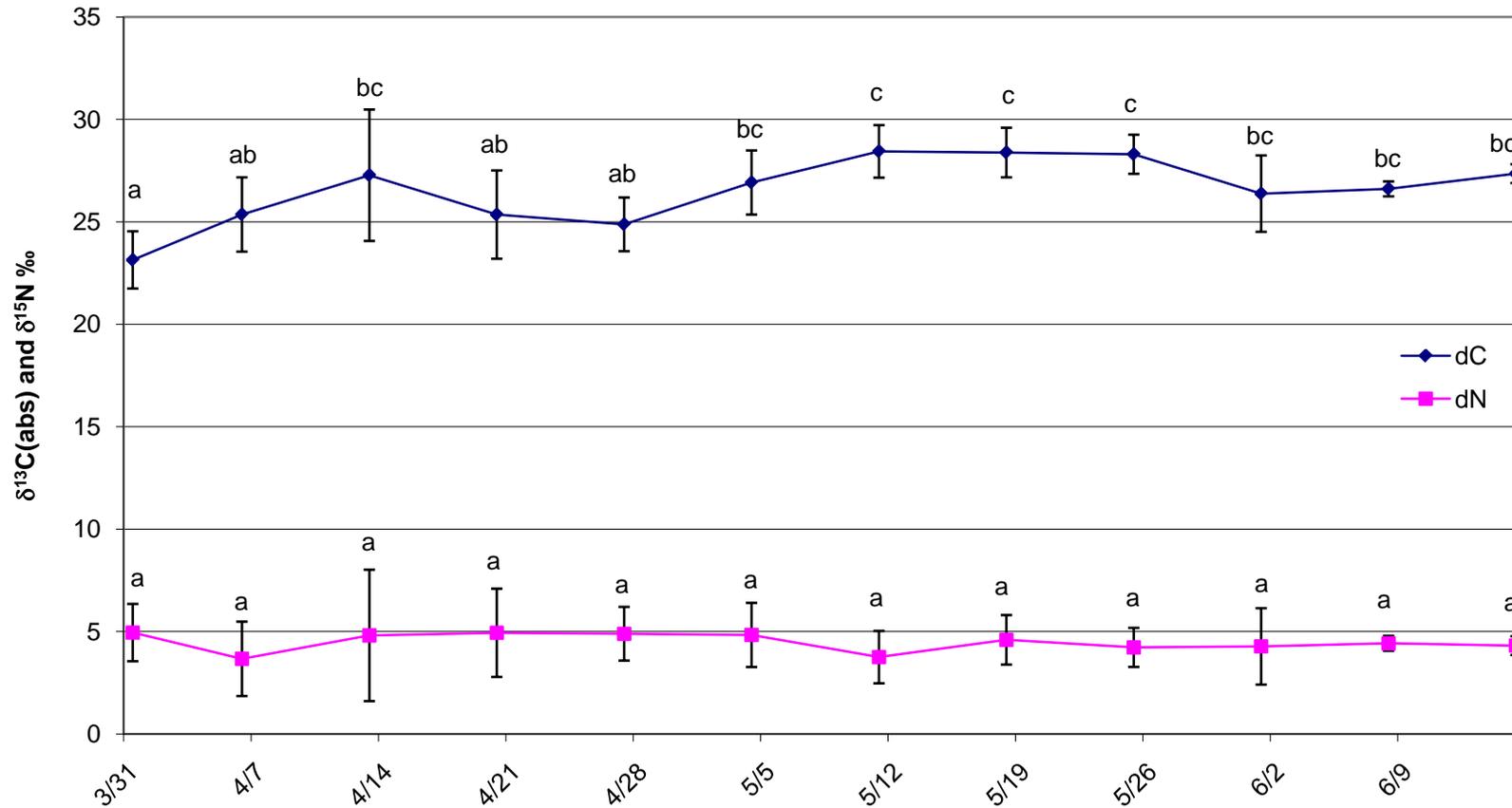


Figure 4-25. Unprocessed feed (UNP) small plankton (1 – 32 μm) assemblage carbon and nitrogen isotope signatures [$\delta^{13}\text{C}$ (absolute value), $\delta^{15}\text{N} \text{‰} \pm 95 \%$ CI] among 12 weekly sampling periods, $n = 6$ pond per treatment; unshared letters denote statistical differences within elements, C or N, ($P < 0.05$, Bonferroni post test).

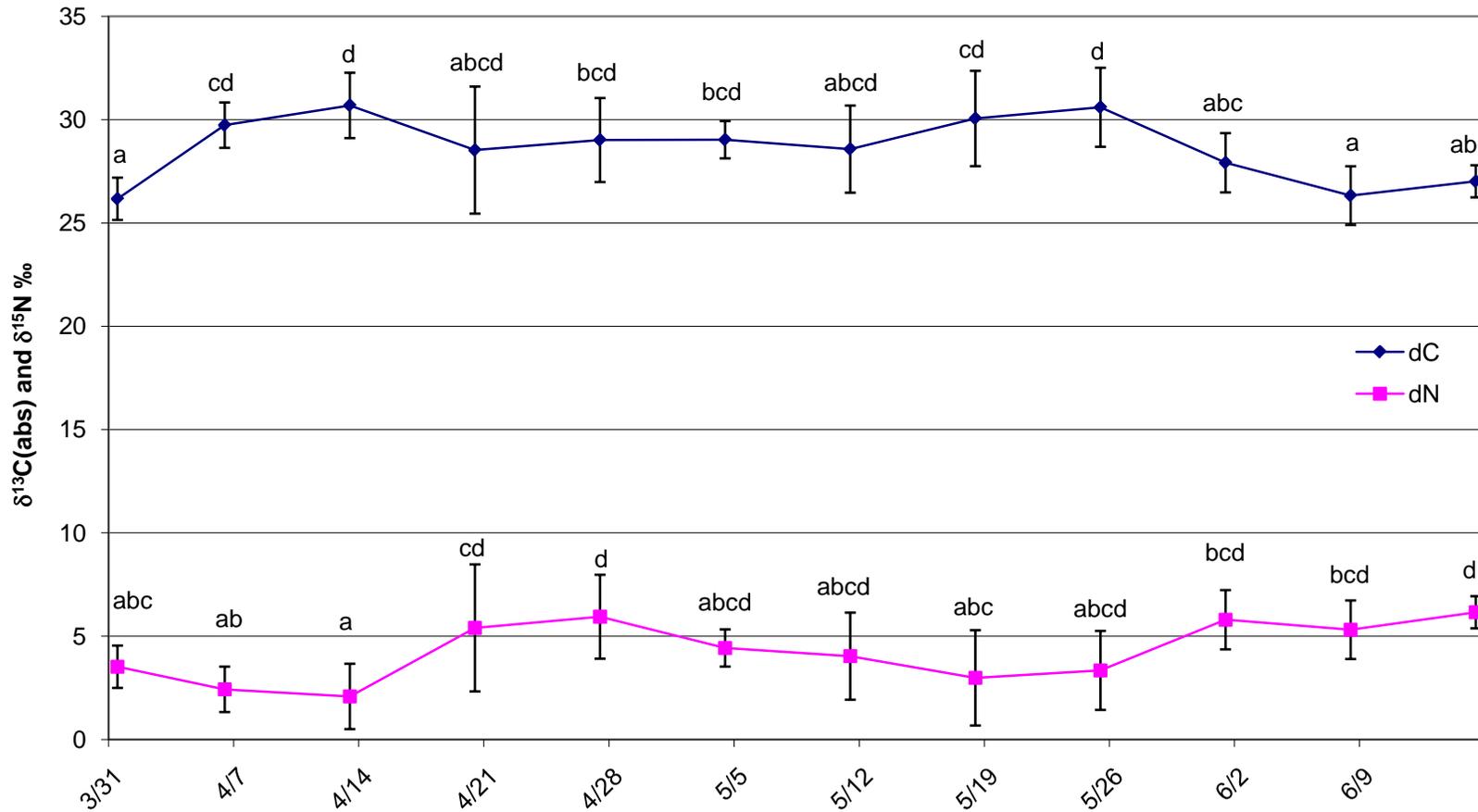


Figure 4-26. Cottonseed meal (CSM) small plankton (1 – 32 μm) assemblage carbon and nitrogen isotope signatures [$\delta^{13}\text{C}$ (absolute value), $\delta^{15}\text{N} \text{‰} \pm 95 \%$ CI] among 12 weekly sampling periods, $n = 6$ ponds per treatment; unshared letters denote statistical differences within elements, C or N, ($P < 0.05$, Bonferroni post test).

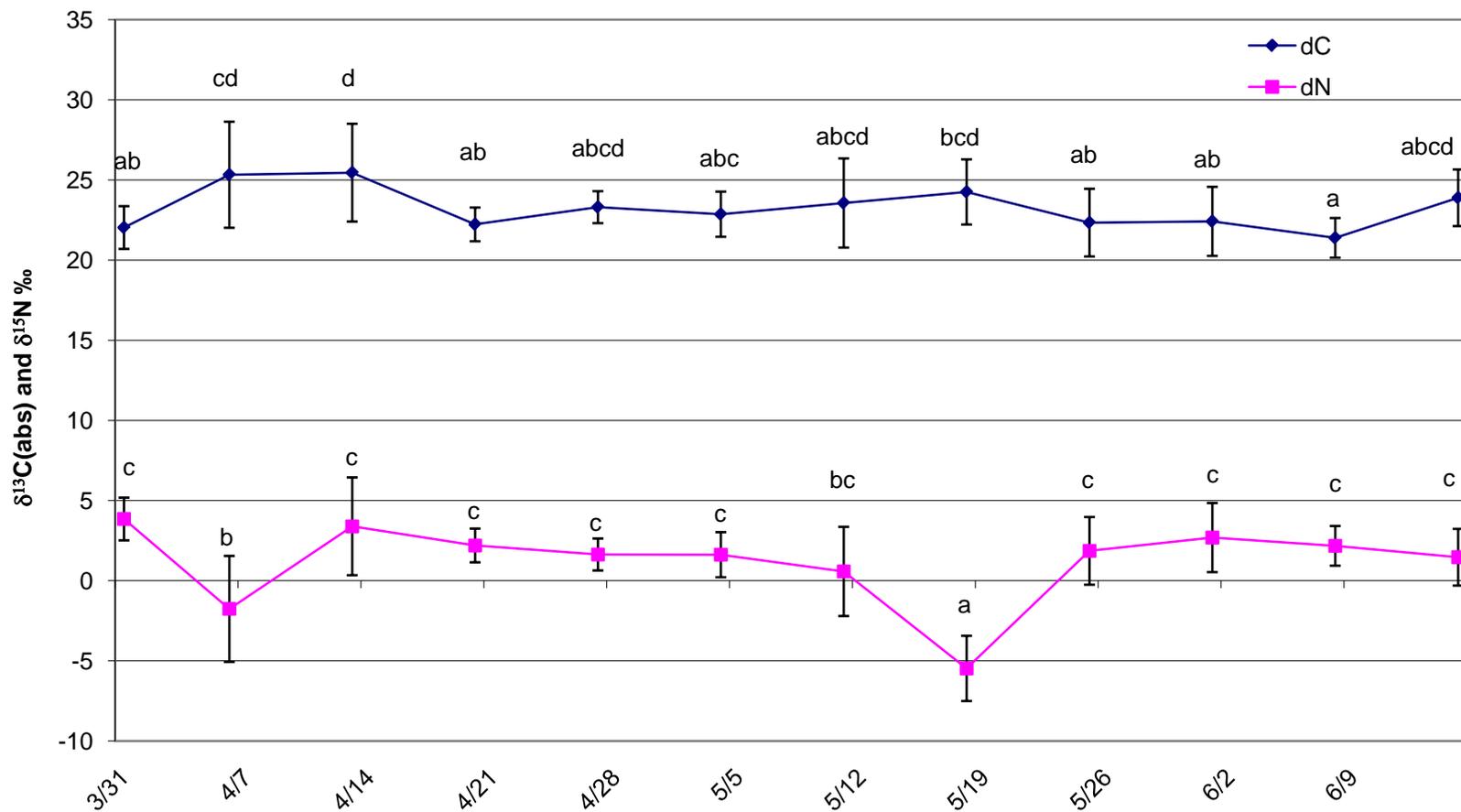


Figure 4-27. Inorganic fertilizer (INO) small plankton (1 – 32 μm) assemblage carbon and nitrogen isotope signatures [$\delta^{13}\text{C}$ (absolute value), $\delta^{15}\text{N}$ ‰ \pm 95 % CI] among 12 weekly sampling periods, n = 6 ponds per treatment; unshared letters denote statistical differences within elements, C or N, ($P < 0.05$, Bonferroni post test).

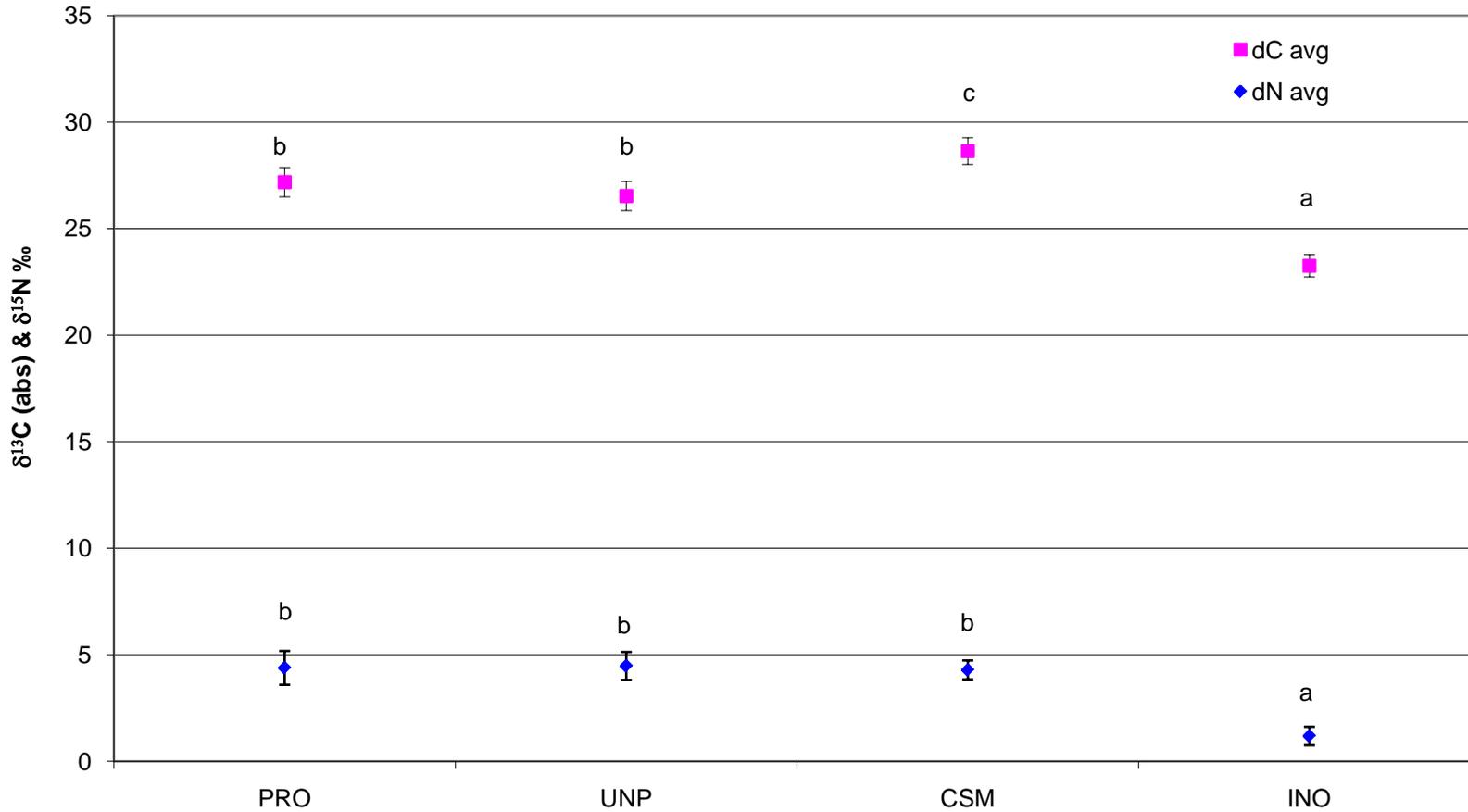


Figure 4-28. Time-averaged small plankton (1 – 32 μm) carbon and nitrogen isotope signatures [$\delta^{13}\text{C}$ (absolute value), $\delta^{15}\text{N}$ ‰ \pm 95 % CI] among 12 weekly sampling periods, $n = 6$ ponds per treatment; unshared letters denote statistical differences within elements, C or N, ($P < 0.05$, Tukey's multiple comparison test).

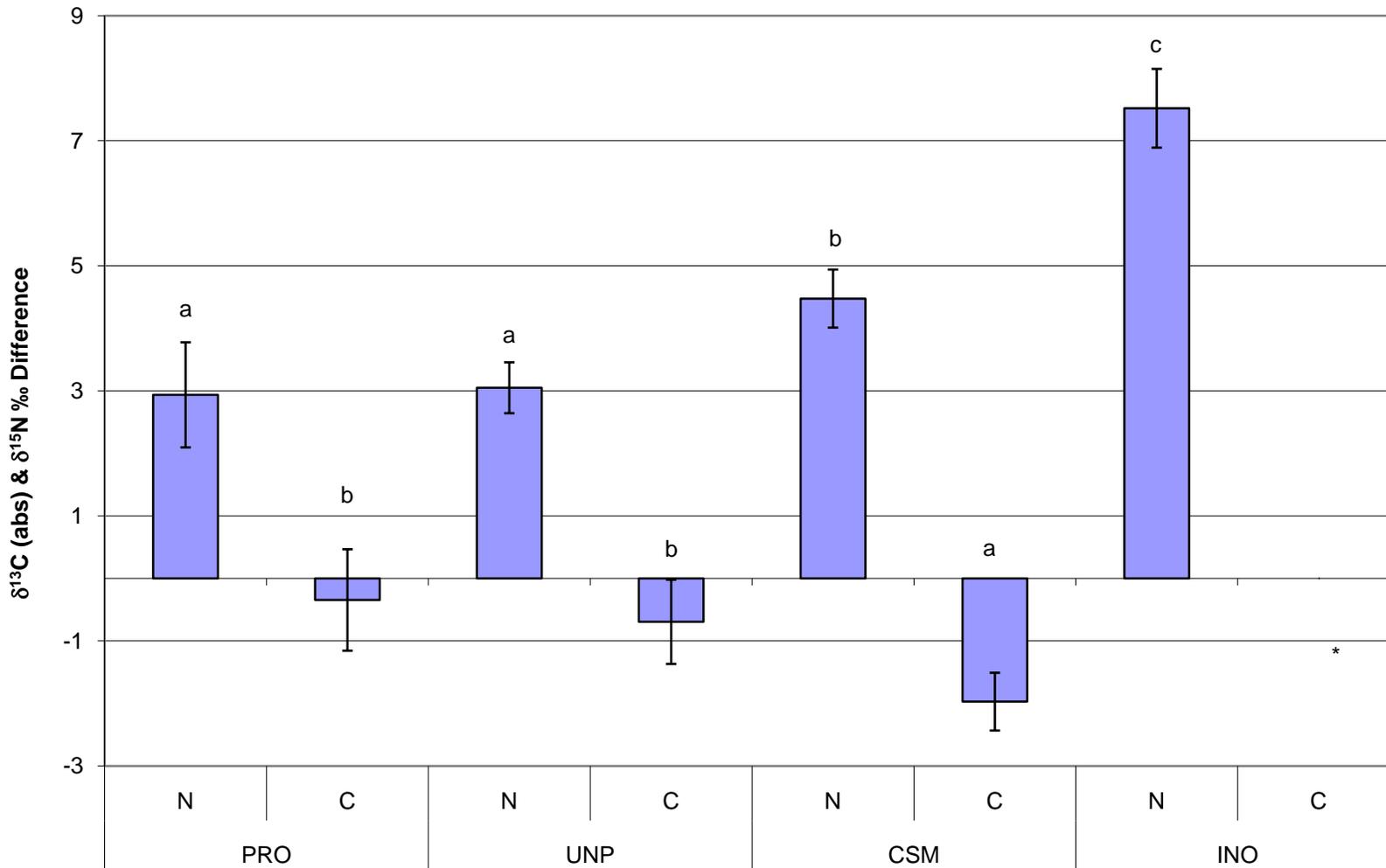


Figure 4-29. Average pond trial large (> 31 mm SL) swordtail and nutrient source isotope signature difference magnitudes ($\Delta\delta^{13}\text{C}$, $\Delta\delta^{15}\text{N} \text{ ‰} \pm 95\% \text{ CI}$) at harvest, $n = 6$ ponds per treatment; unshared letters denote statistical differences within elements, C or N, ($P < 0.05$, Tukey's multiple comparison test).

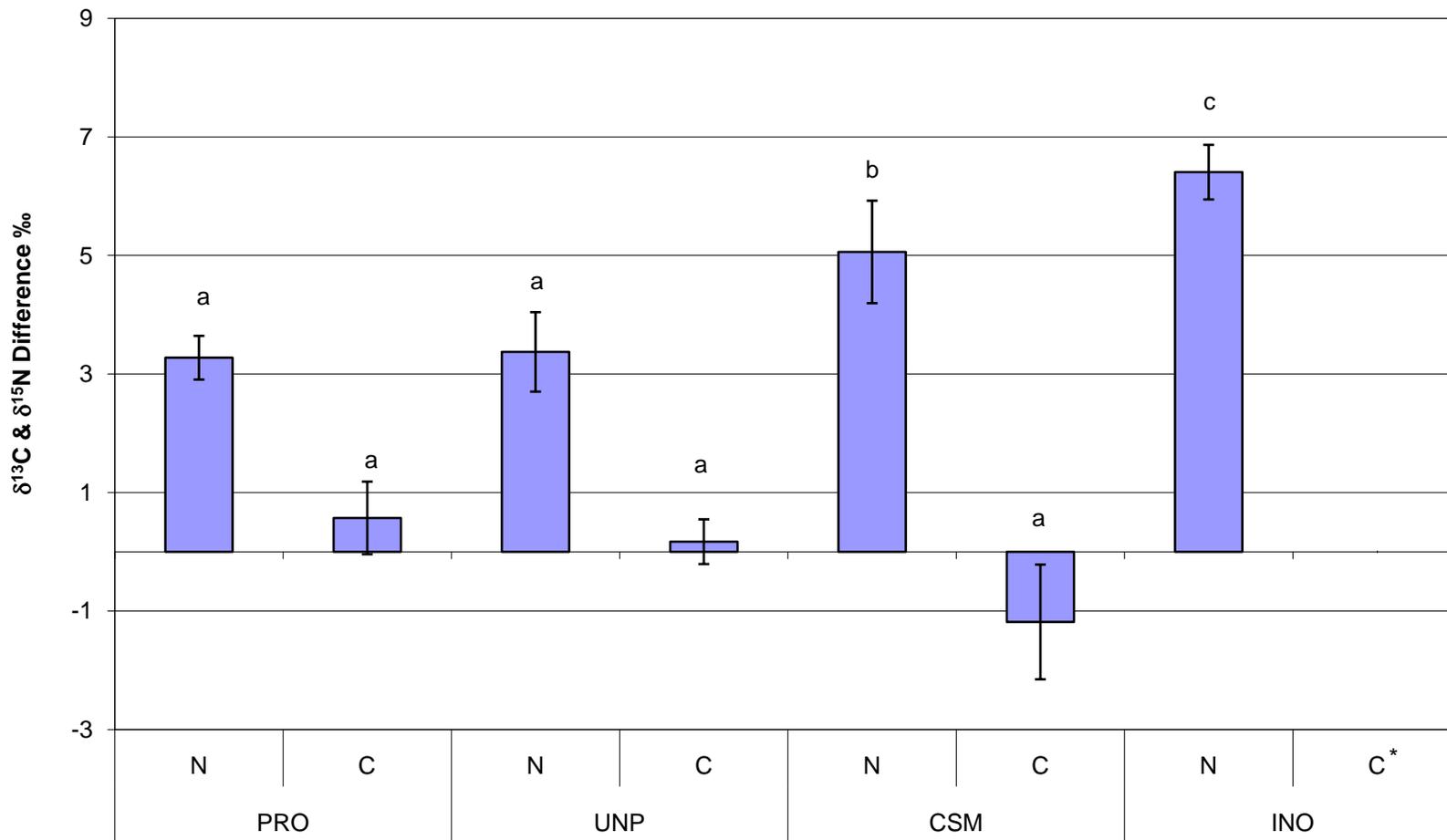


Figure 4-30. Average pond trial small (≤ 31 mm SL) swordtail and nutrient source isotope signature difference magnitudes ($\Delta\delta^{13}\text{C}$, $\Delta\delta^{15}\text{N}$ ‰ \pm 95 % CI) at harvest, $n = 6$ ponds per treatment; unshared letters denote statistical differences within elements, C or N, ($P < 0.05$, Tukey's multiple comparison test).

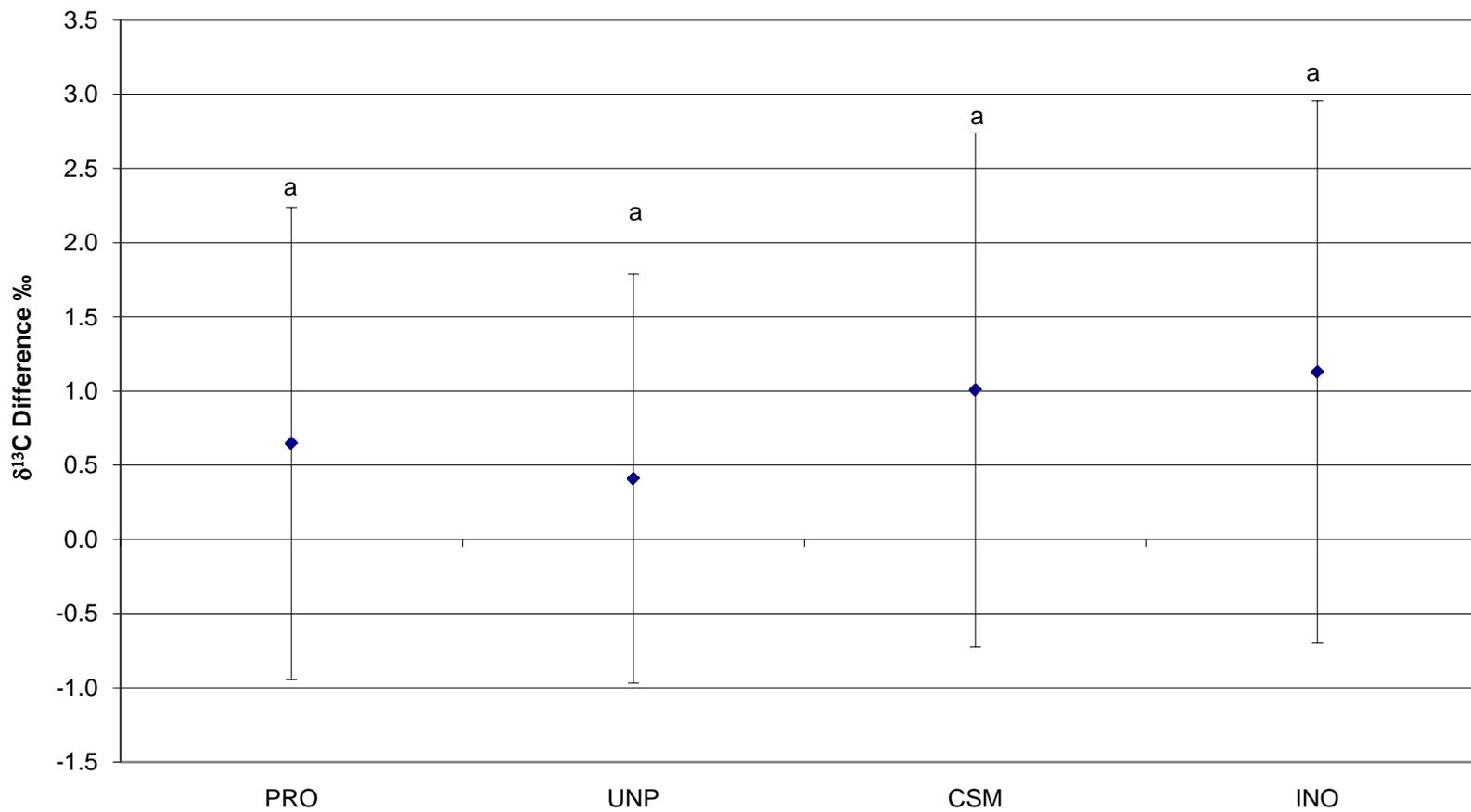


Figure 4-31. Average pond trial large (> 31 mm SL) swordtail and large zooplankton carbon isotope signature difference magnitudes (sign neutral) at harvest ($\Delta\delta^{13}\text{C}$ ‰ \pm 95 % CI), n = 6 ponds per treatment; unshared letters denote statistical differences (P < 0.05, Tukey's multiple comparison test).

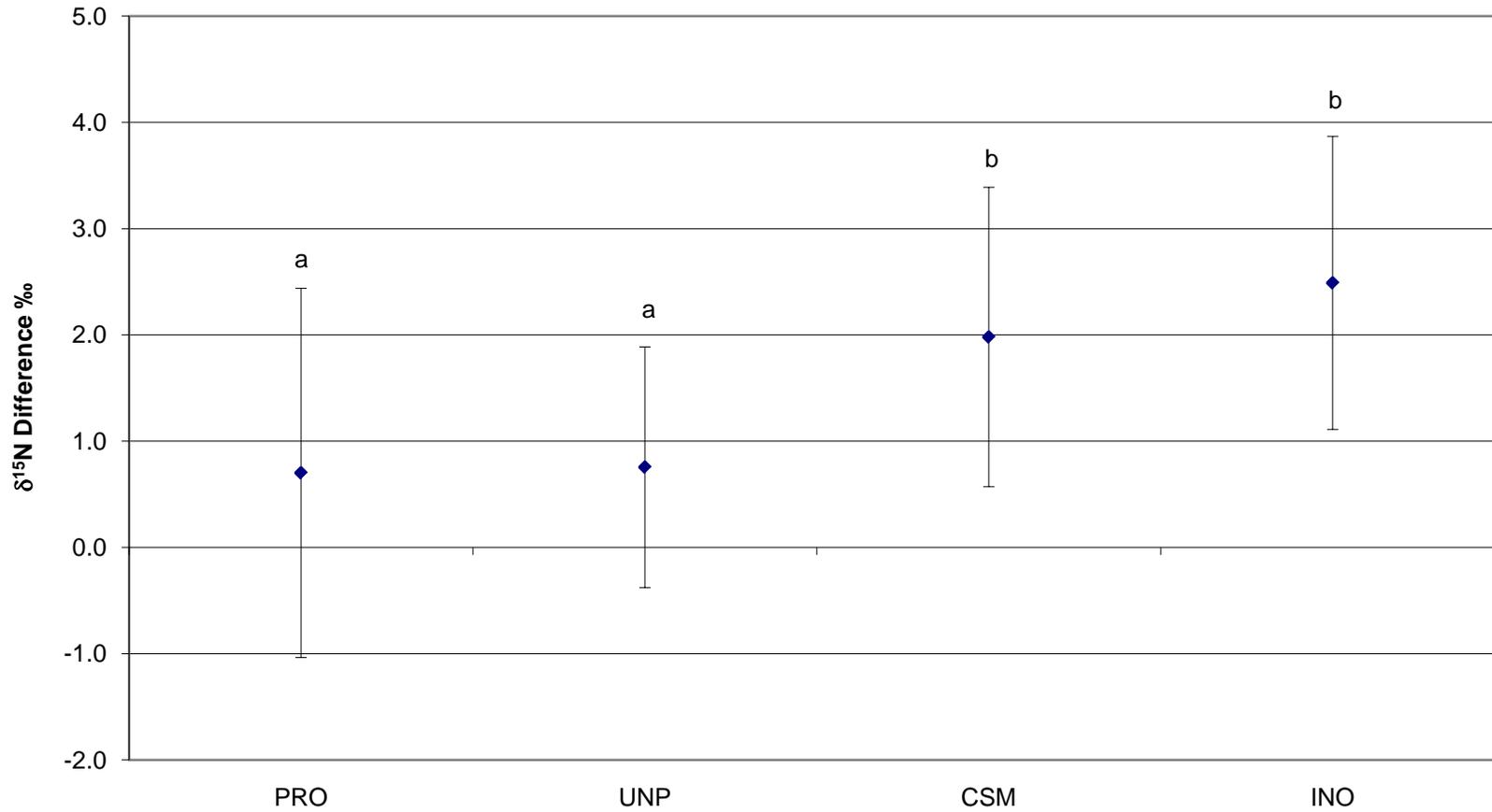


Figure 4-32. Average pond trial large (> 31 mm SL) swordtail and large zooplankton nitrogen isotope difference magnitudes (sign neutral) at harvest ($\Delta\delta^{15}\text{N}$ ‰ \pm 95 % CI), n = 6 ponds per treatment; unshared letters denote statistical differences ($P < 0.05$, Tukey's multiple comparison test).

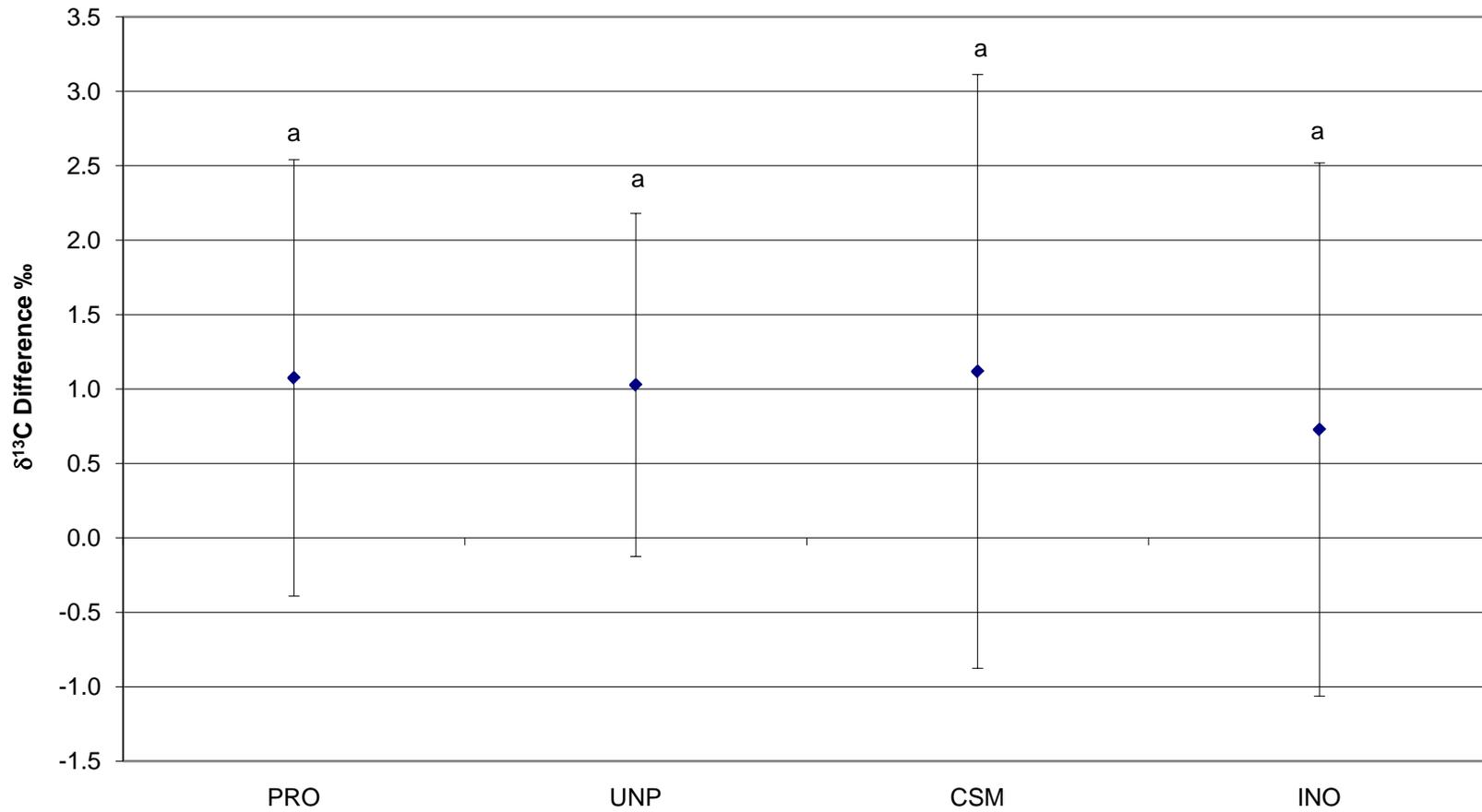


Figure 4-33. Average pond trial small (≤ 31 mm SL) swordtail and large zooplankton carbon isotope difference magnitudes (sign neutral) at harvest ($\Delta\delta^{13}\text{C}$ ‰ \pm 95 % CI), $n = 6$ ponds per treatment; unshared letters denote statistical differences ($P < 0.05$, Tukey's multiple comparison test).

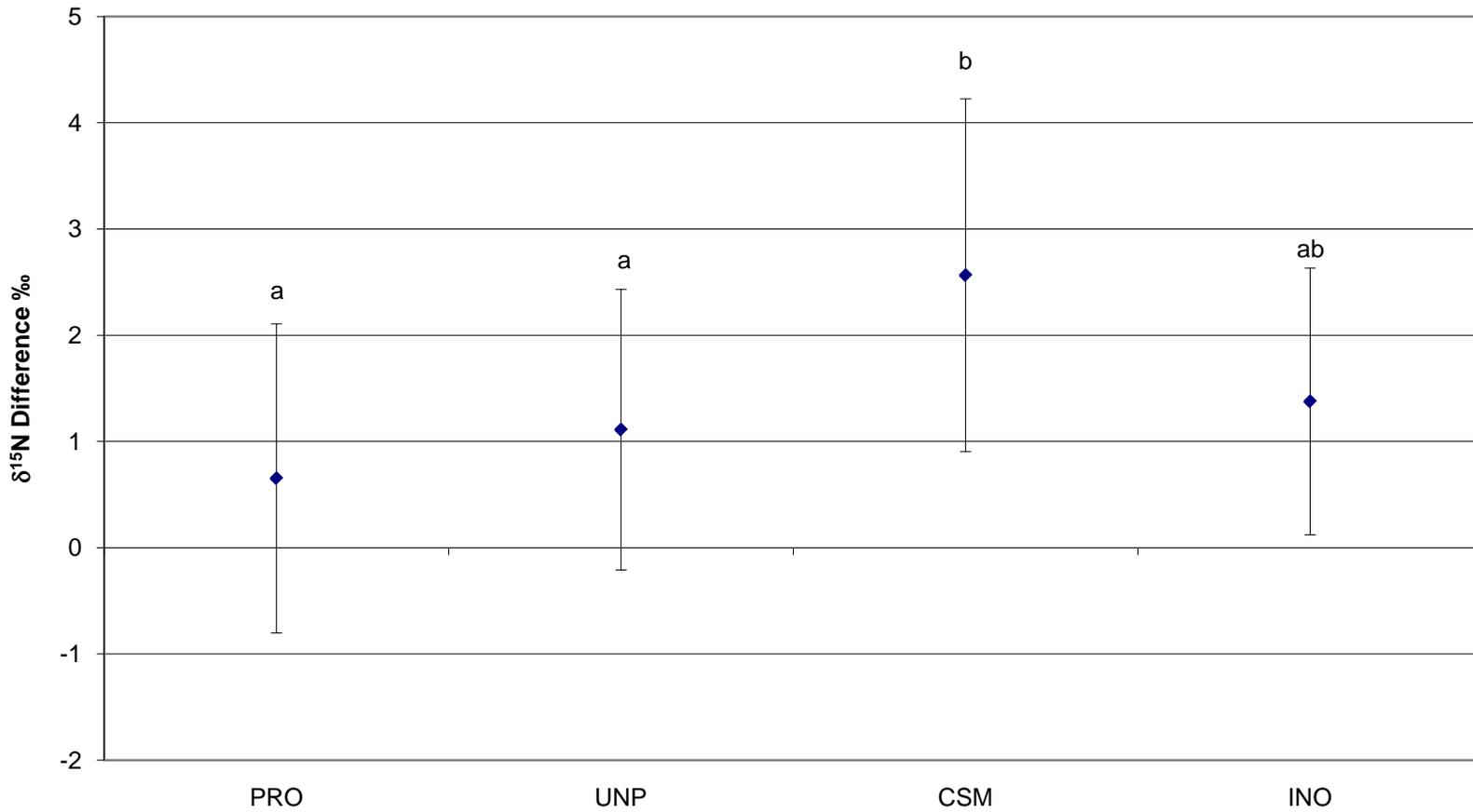


Figure 4-34. Average pond trial small (≤ 31 mm SL) swordtail and large zooplankton nitrogen isotope difference magnitudes (sign neutral) at harvest ($\Delta\delta^{15}\text{N}$ ‰ \pm 95 % CI), $n = 6$ ponds per treatment; unshared letters denote statistical differences ($P < 0.05$, Tukey's multiple comparison test).

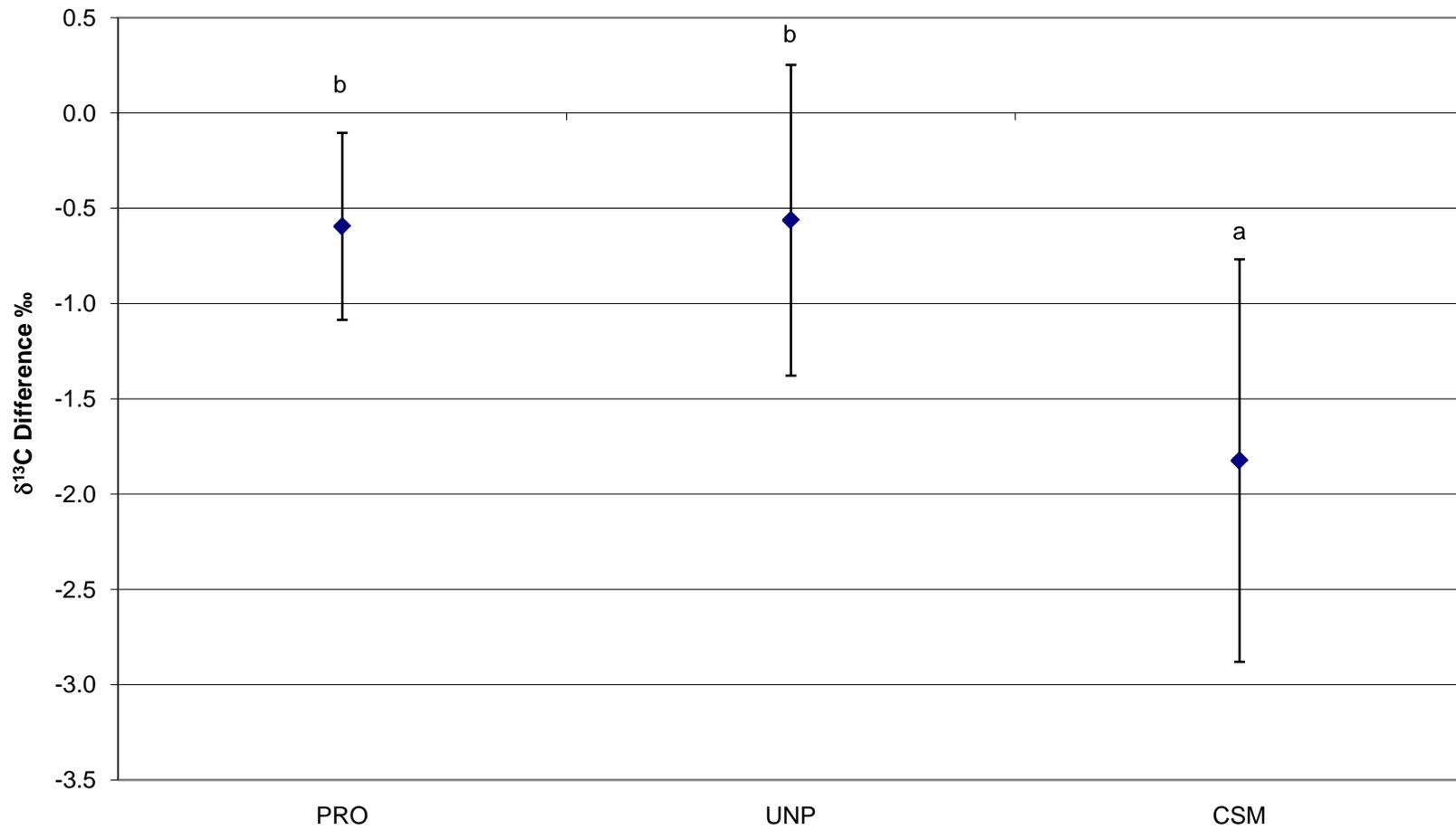


Figure 4-35. Average pond trial large zooplankton and applied nutrient carbon isotope signature (sign neutral) difference magnitudes ($\Delta\delta^{13}\text{C}$ ‰ \pm 95 % CI), n = 6 ponds per treatment; unshared letters denote statistical differences ($P < 0.05$, Tukey's multiple comparison test).

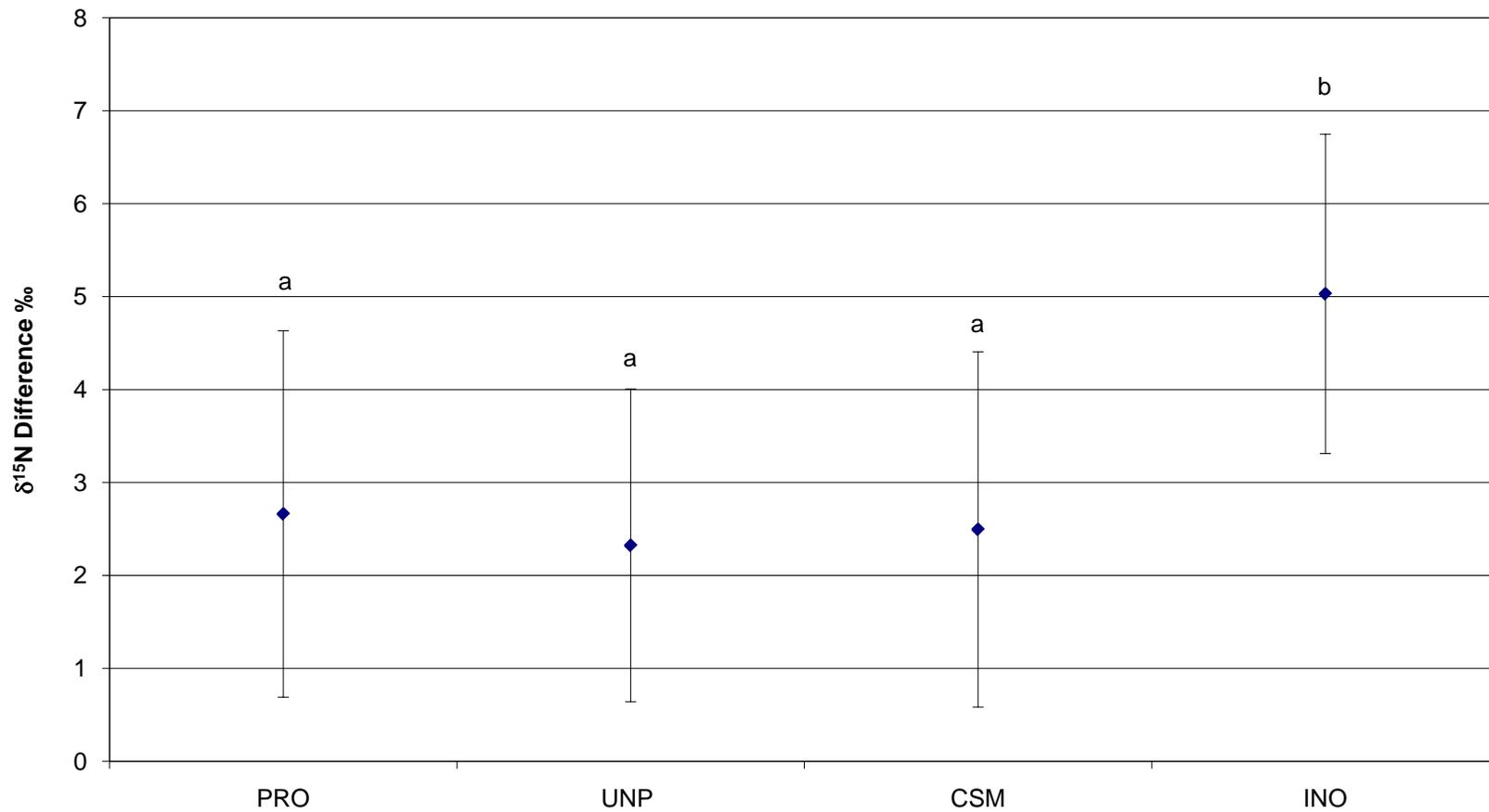


Figure 4-36. Average pond trial large zooplankton and applied nutrient nitrogen isotope signature (sign neutral) difference magnitudes ($\Delta\delta^{15}\text{N}$ ‰ \pm 95 % CI), n = 6 ponds per treatment; unshared letters denote statistical differences ($P < 0.05$, Tukey's multiple comparison test).

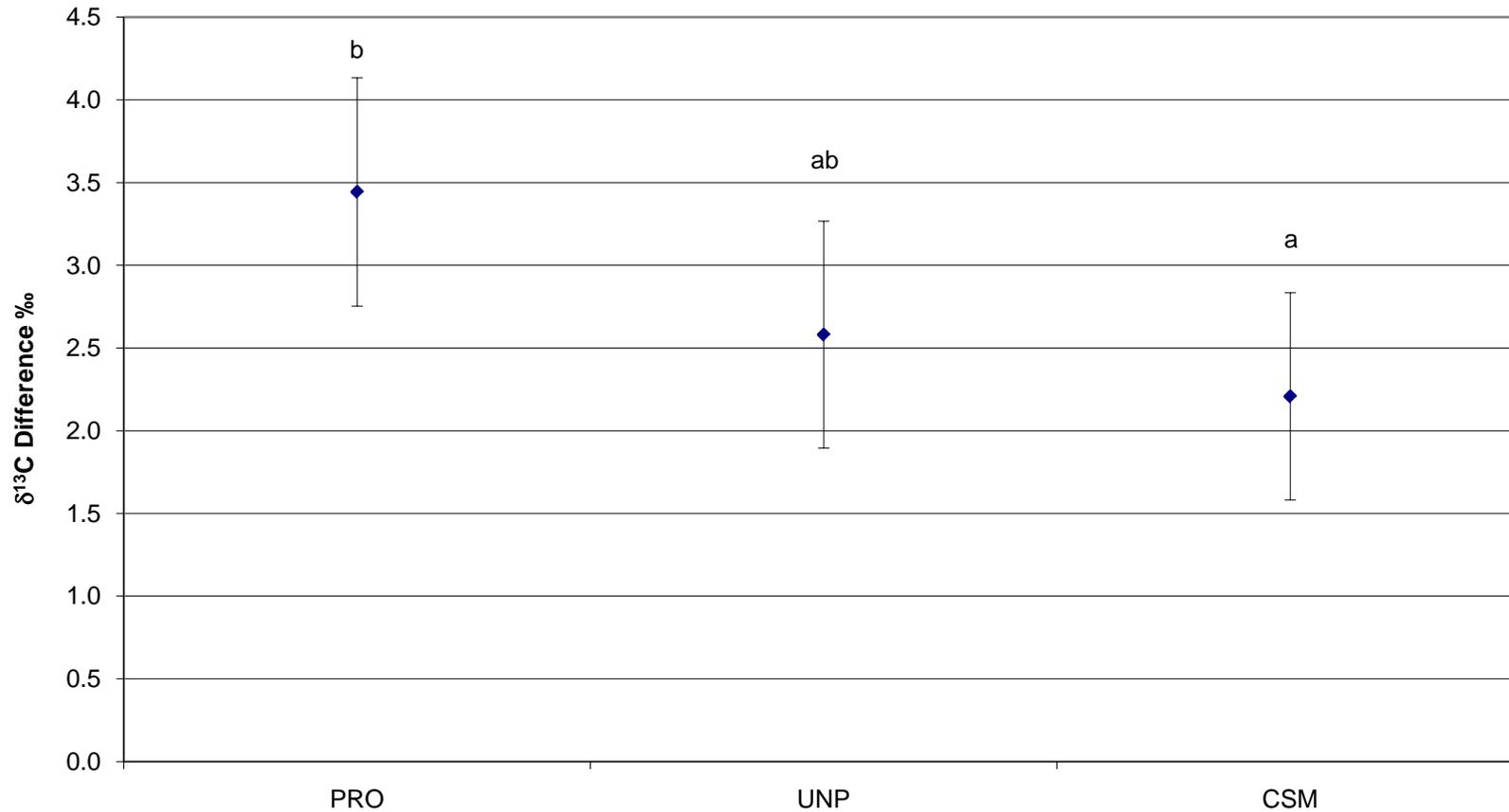


Figure 4-37. Average pond trial small plankton and applied nutrient carbon isotope signature (sign neutral) difference magnitudes ($\Delta\delta^{13}\text{C}$ ‰ \pm 95 % CI), n = 6 ponds per treatment; unshared letters denote statistical differences ($P < 0.05$, Tukey's multiple comparison test).

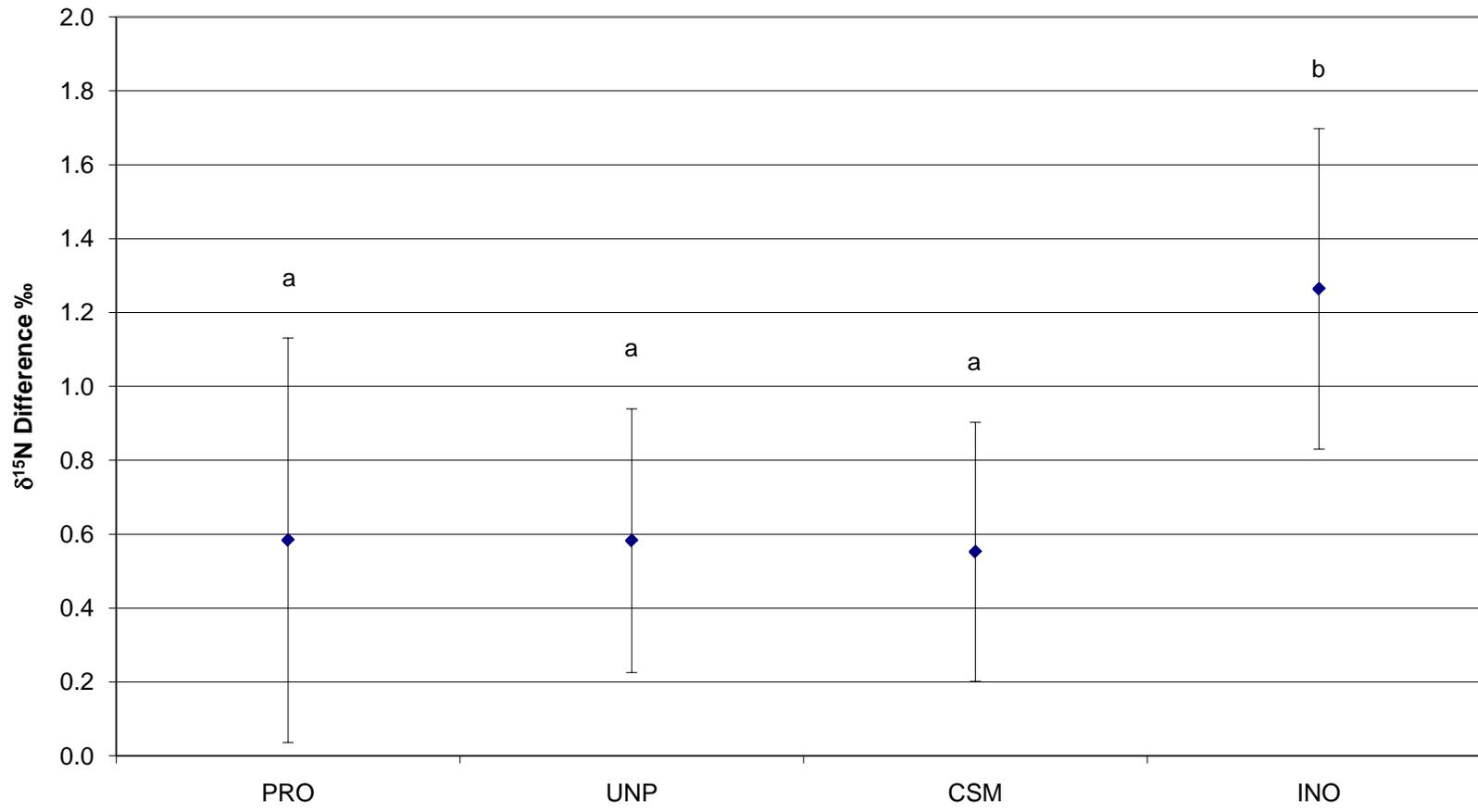


Figure 4-38. Average pond trial small plankton and applied nutrient nitrogen isotope signature (sign neutral) difference magnitudes ($\Delta\delta^{15}\text{N}$ ‰ \pm 95 % CI), n = 6 ponds per treatment; unshared letters denote statistical differences ($P < 0.05$, Tukey's multiple comparison test).

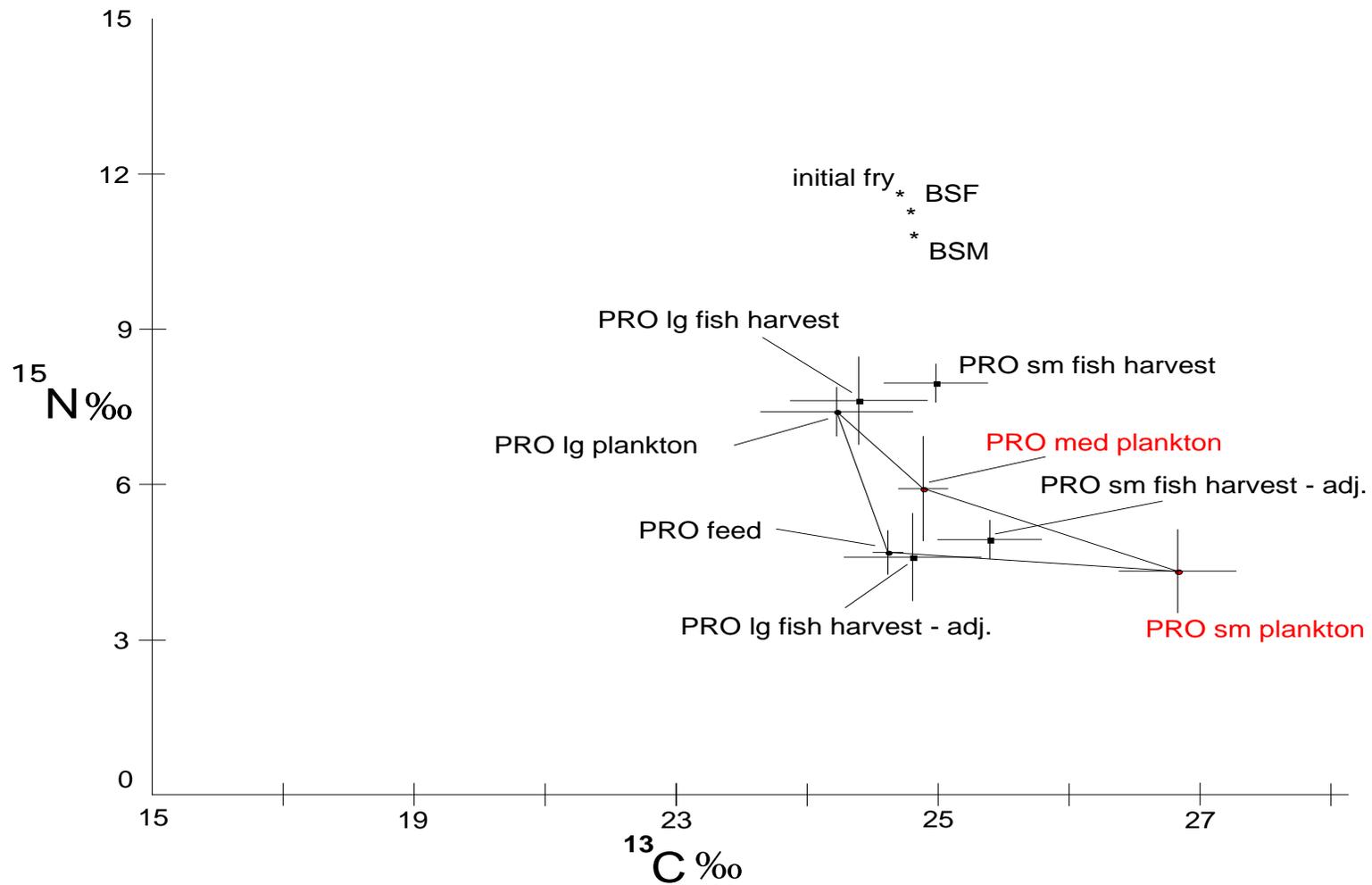


Figure 4-39. Cartesian plot of processed feed (PRO) treatment harvest swordtail, processed feed, and three plankton size assemblage $\delta^{13}\text{C}$ (absolute value) and $\delta^{15}\text{N}$ values ($\text{‰} \pm 95\%$ CI); $n = 6$. Broodstock (BSF - Female, BSM - Male), and initial fry $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ value error measures omitted for clarity; $n = 11, 10,$ and $21,$ respectively.

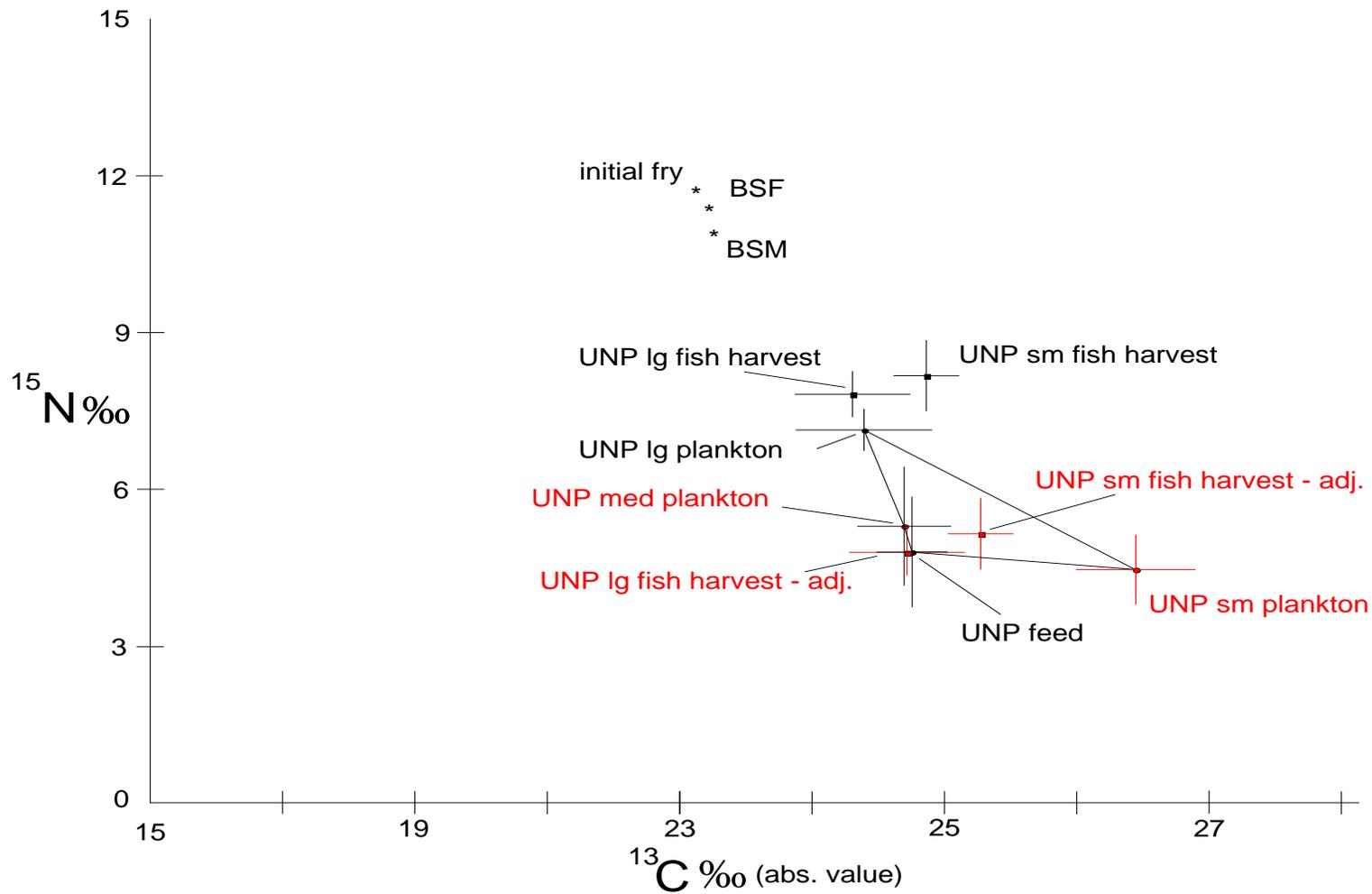


Figure 4-40. Cartesian plot of unprocessed feed (UNP) treatment harvest swordtail, processed feed, and three plankton size assemblage $\delta^{13}\text{C}$ (absolute value) and $\delta^{15}\text{N}$ values ($\text{‰} \pm 95\%$ CI); $n = 6$. Broodstock (BSF - Female, BSM - Male), and initial fry $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ value error measures omitted for clarity; $n = 11, 10,$ and $21,$ respectively.

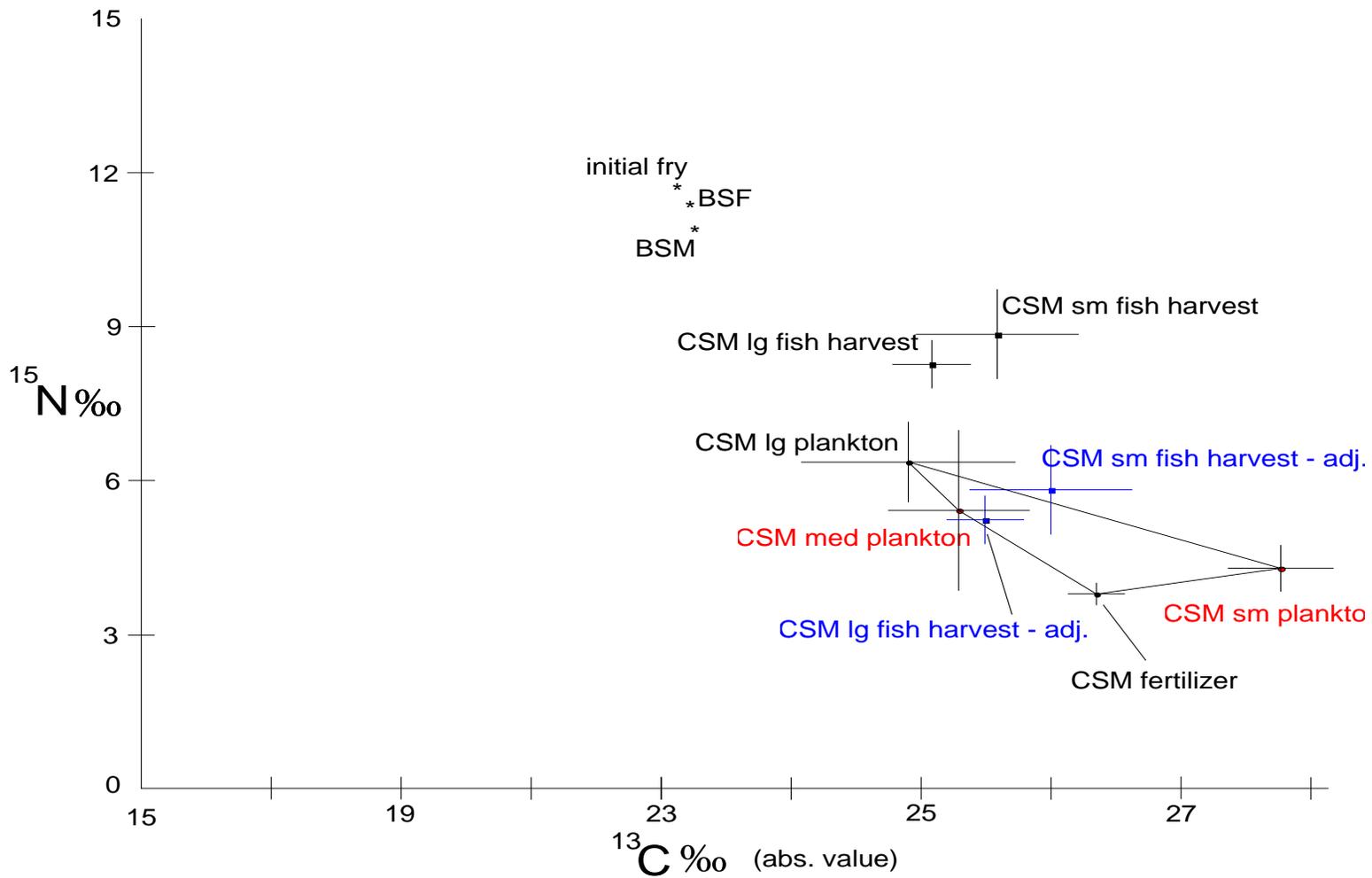


Figure 4-41. Cartesian plot of cottonseed meal (CSM) treatment harvest swordtail, cottonseed meal, and three plankton size assemblage $\delta^{13}\text{C}$ (absolute value) and $\delta^{15}\text{N}$ values ($\text{‰} \pm 95\%$ CI); $n = 6$. Broodstock (BSF - Female, BSM - Male), and initial fry $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ value error measures omitted for clarity; $n = 11, 10,$ and $21,$ respectively.

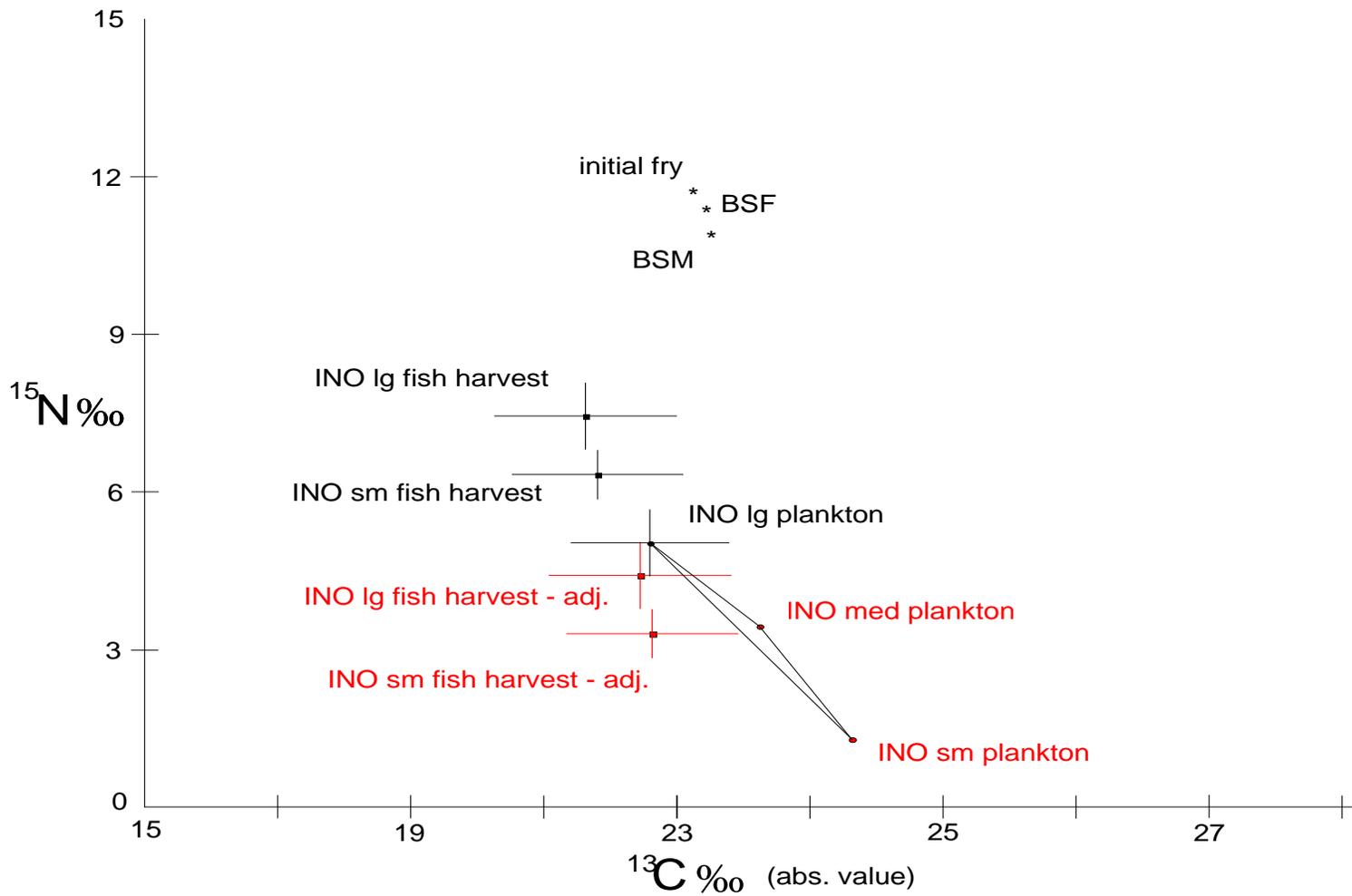


Figure 4-42. Cartesian plot of inorganic fertilizer (INO) treatment harvest swordtail, and three plankton size assemblage $\delta^{13}\text{C}$ (absolute value) and $\delta^{15}\text{N}$ values ($\text{‰} \pm 95\%$ CI); $n = 6$. Broodstock (BSF - Female, BSM - Male), and initial fry $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ value error measures omitted for clarity; $n = 11, 10,$ and $21,$ respectively.

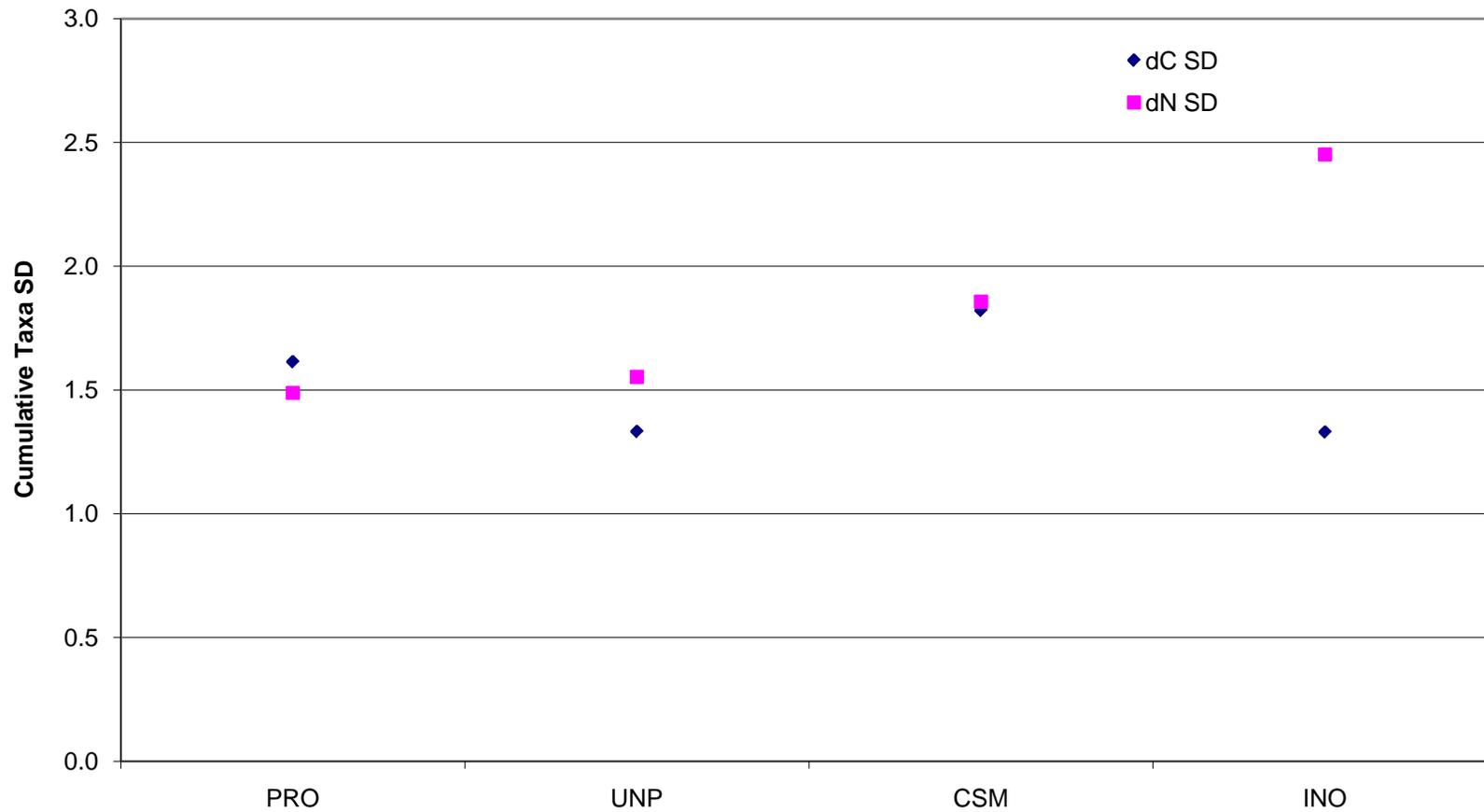


Figure 4-43. Isotopic variation among five pond groups (small, medium, and large plankton assemblages, small and large harvest swordtails) for four applied pond nutrients, n = 6 ponds per treatment.

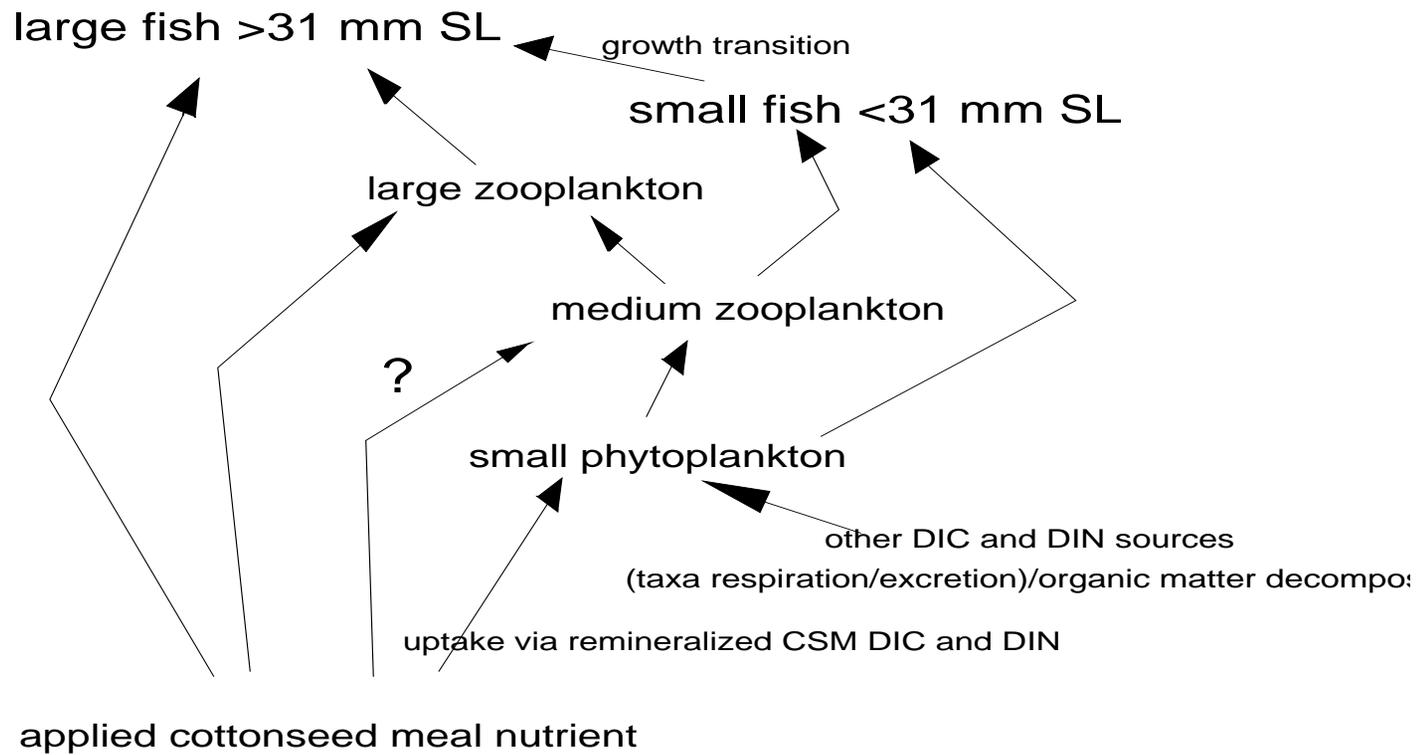


Figure 4-44. Postulated cottonseed meal (CSM) food web based upon isotopically derived Euclidean distance and IsoSource program swordtail diet estimates (Table 4-1); small phytoplankton (small plankton assemblage: 1-32 μm), medium zooplankton (medium plankton assemblage: 32-200 μm), and large zooplankton (large plankton assemblage: 200+ μm).

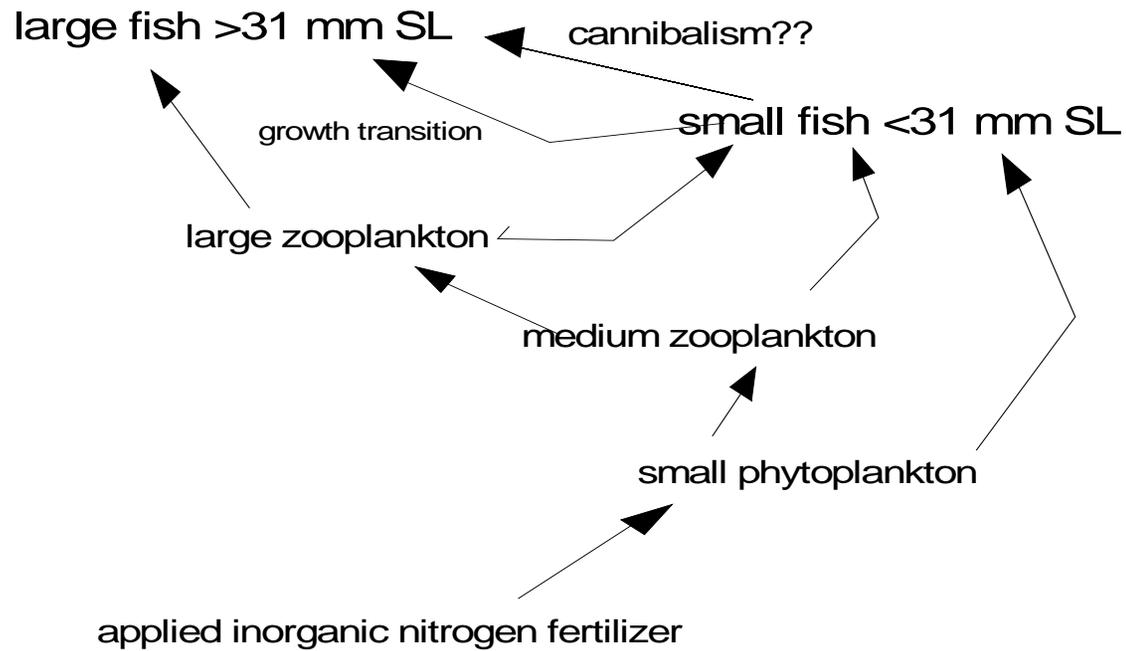


Figure 4-45. Postulated inorganic fertilizer (INO) food web based upon isotopically derived Euclidean distance and IsoSource program swordtail diet estimates (Table 4-1); small phytoplankton (small plankton assemblage: 1-32 μm), medium zooplankton (medium plankton assemblage: 32-200 μm), and large zooplankton (large plankton assemblage: 200+ μm).

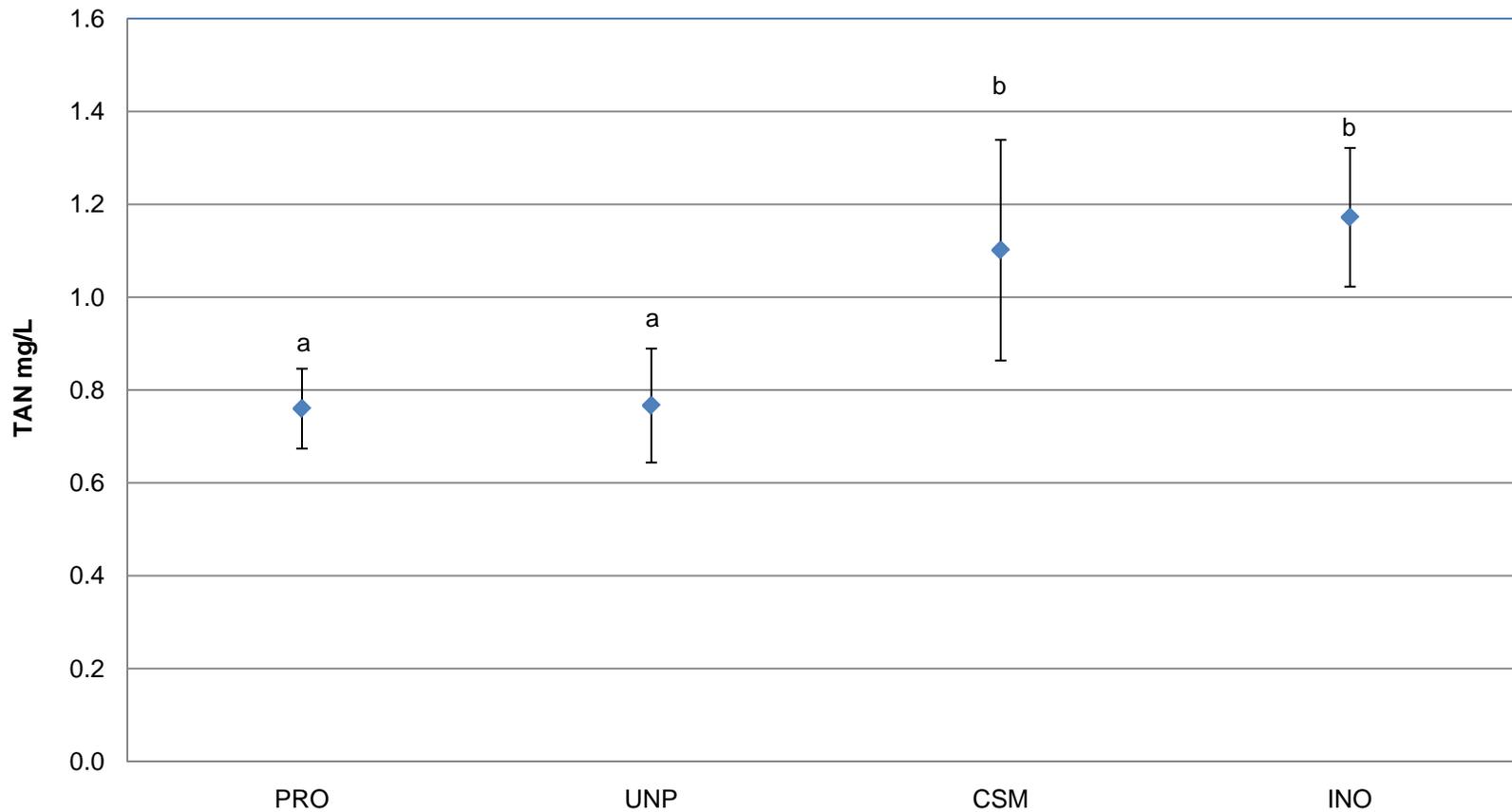


Figure 4-46. Average total ammonia nitrogen (TAN) concentrations measured for 12 weekly sampling periods (mg/L \pm 95 % CI), n = 6 ponds per treatment; unshared letters denote statistical differences ($P < 0.05$, Tukey's multiple comparison test).

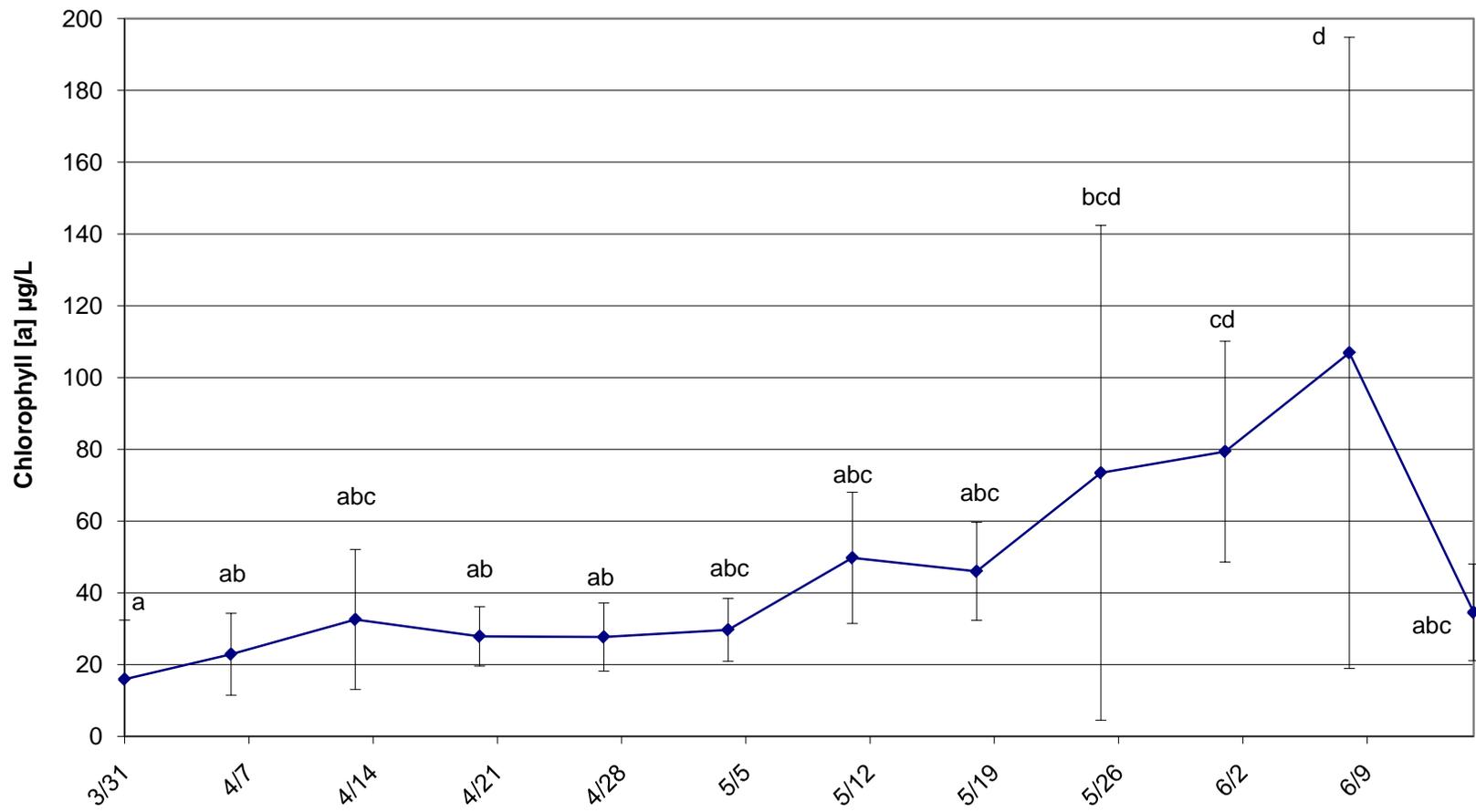


Figure 4-47. Processed feed (PRO) treatment average pond chlorophyll [a] among 12 weekly sampling periods ($\mu\text{g/L} \pm 95\% \text{ CI}$), $n = 6$ ponds per treatment; unshared letters denote statistical differences ($P < 0.05$, Bonferroni post test).

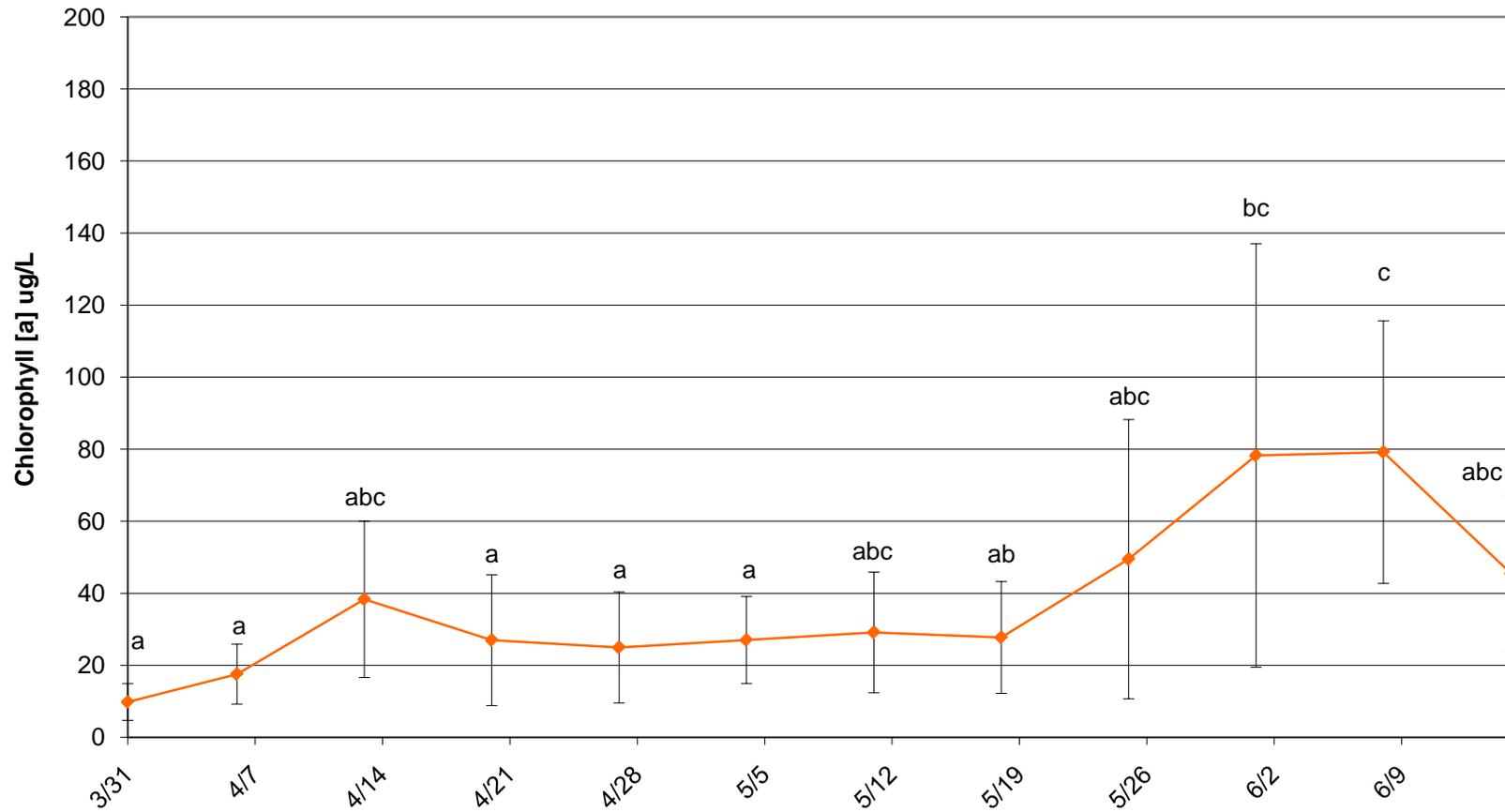


Figure 4-48. Unprocessed feed (UNP) treatment average pond chlorophyll [a] among 12 weekly sampling periods ($\mu\text{g/L} \pm 95\% \text{ CI}$), $n = 6$ ponds per treatment; unshared letters denote statistical differences ($P < 0.05$, Bonferroni post test).

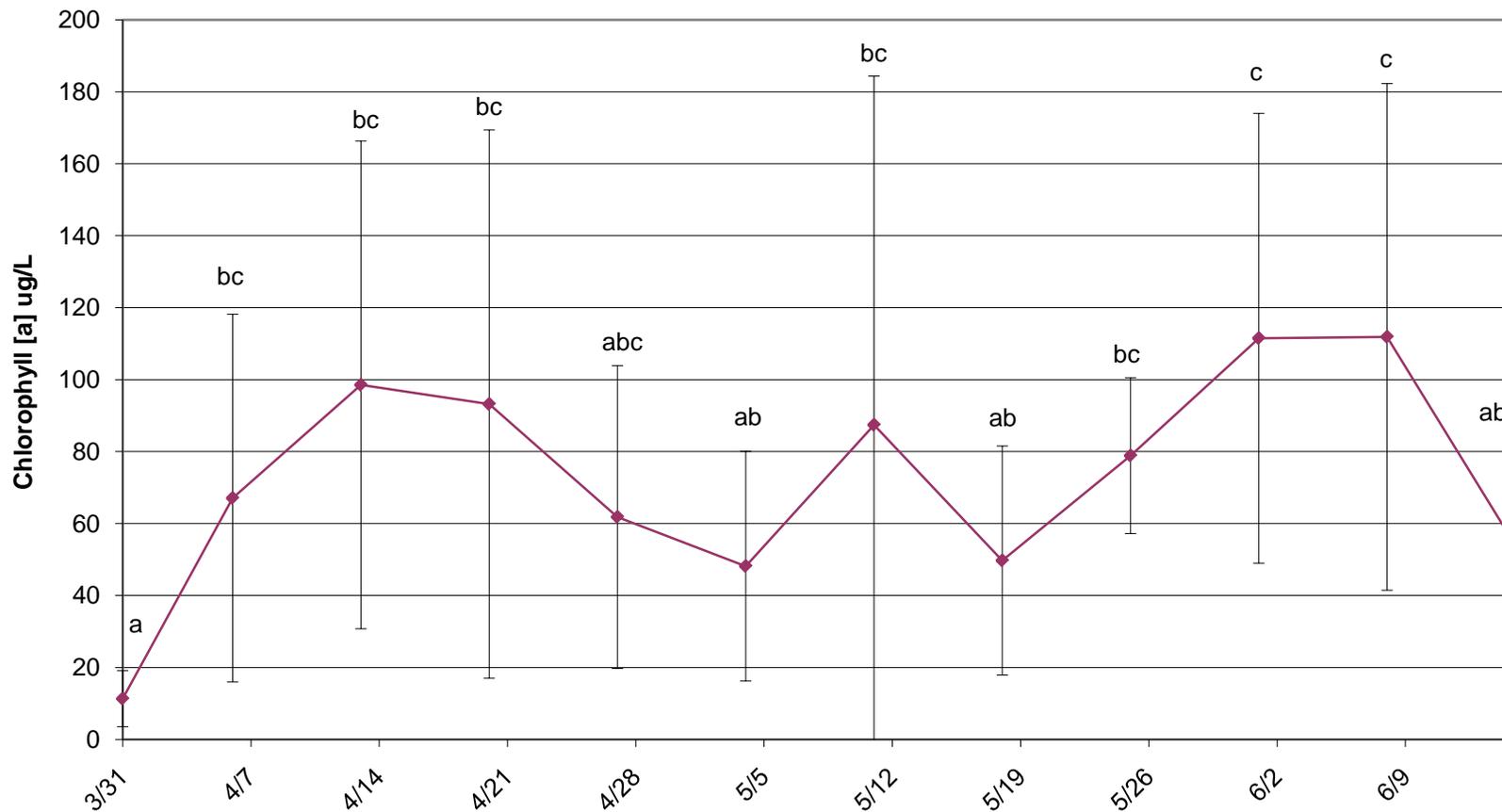


Figure 4-49. Cottonseed meal fertilizer (CSM) treatment average pond chlorophyll [a] among 12 weekly sampling periods ($\mu\text{g/L} \pm 95\%$ CI), $n = 6$ ponds per treatment; unshared letters denote statistical differences ($P < 0.05$, Bonferroni post test).

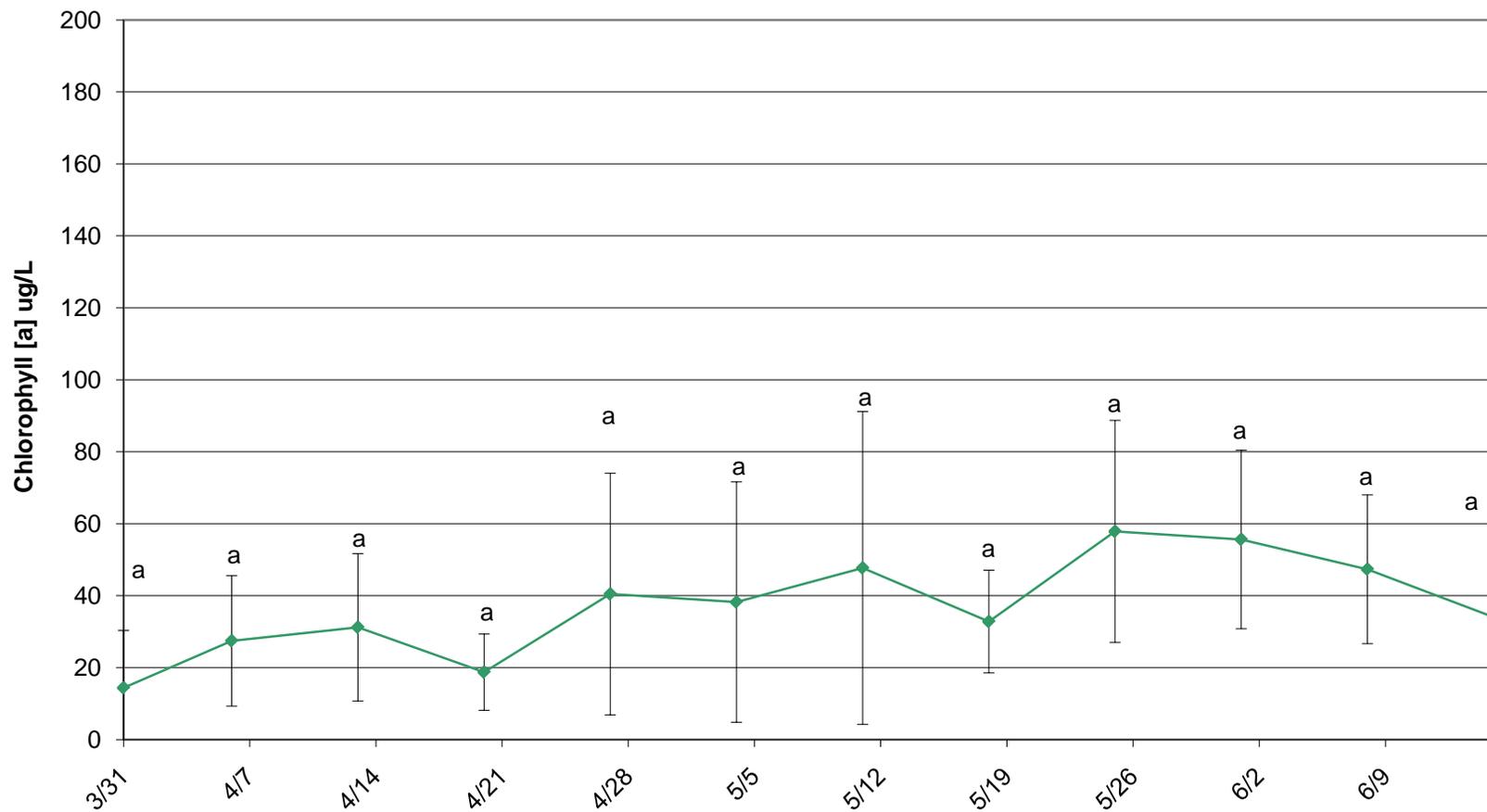


Figure 4-50. Inorganic fertilizer (INO) treatment average pond chlorophyll [a] among 12 weekly sampling periods ($\mu\text{g/L} \pm 95\% \text{ CI}$), $n = 6$ ponds per treatment; unshared letters denote statistical differences ($P < 0.05$, Bonferroni post test).

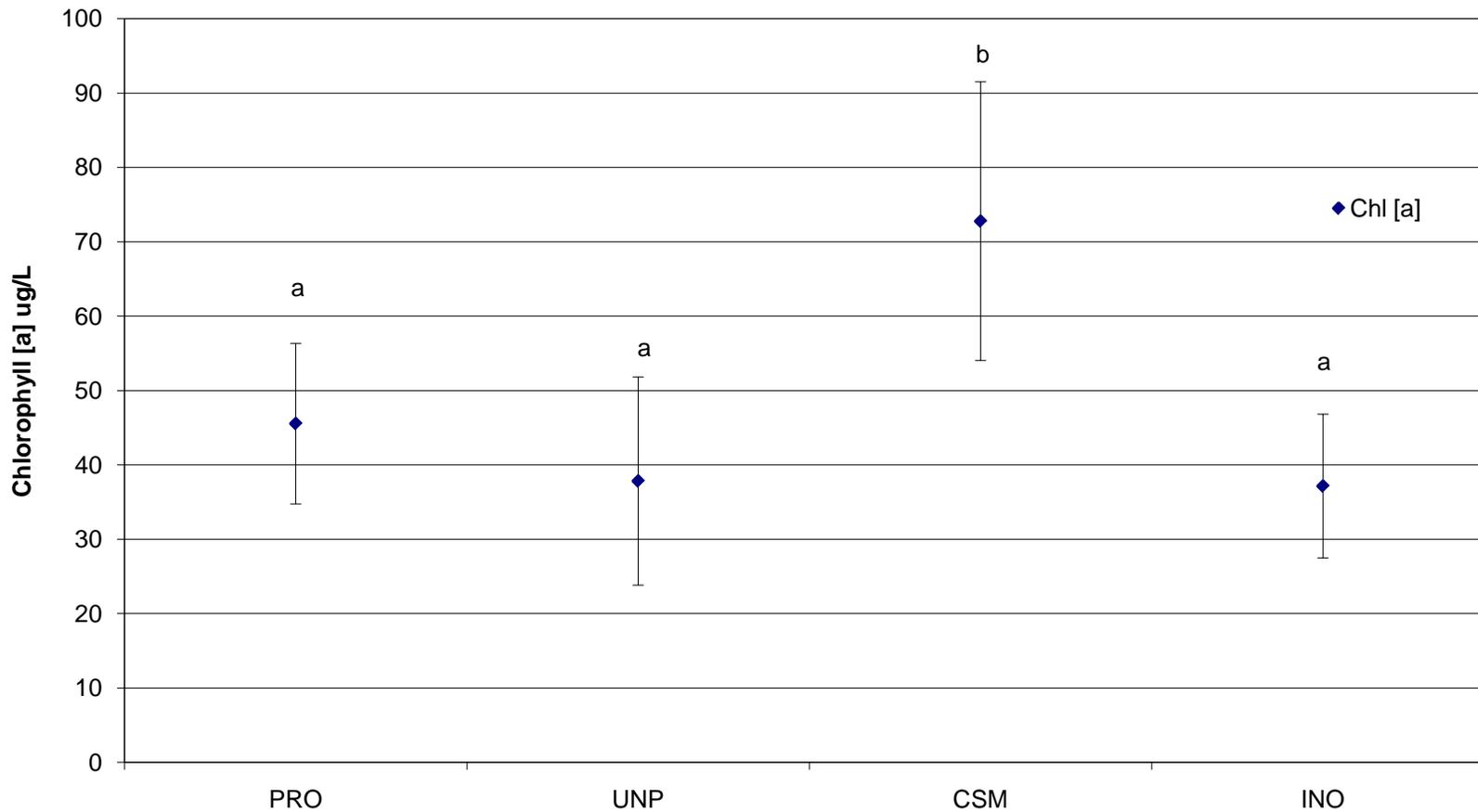


Figure 4-51. Time-averaged mean chlorophyll [a] concentrations ($\mu\text{g/L} \pm 95\% \text{ CI}$) measured over 12 weekly sampling periods among four pond treatments, $n = 6$ ponds per treatment; unshared letters denote statistical differences ($P < 0.05$, Tukey's multiple comparison test).

composite diets isotopic similarities

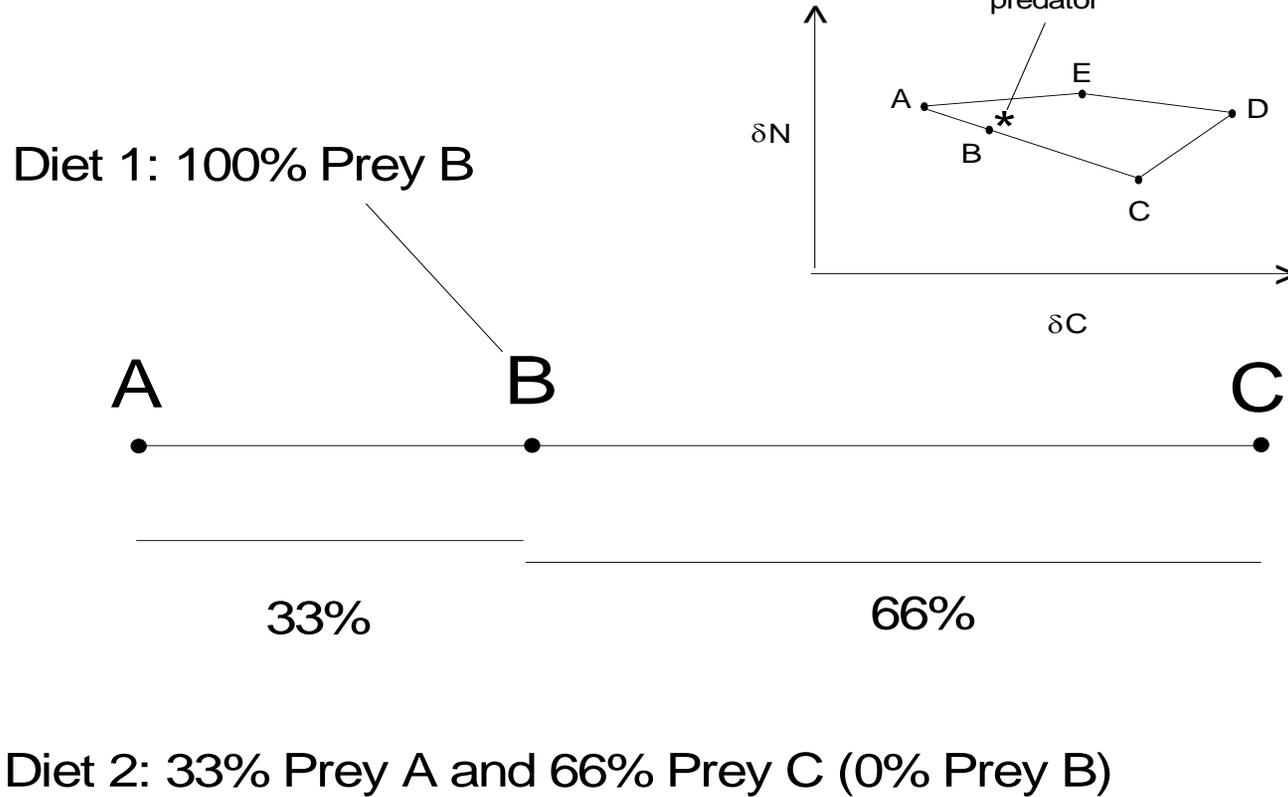


Figure 4-52. Diagram illustrating potential isotopic collinearity ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) of two hypothetical composite diets (diet 1 and 2), and associated dietary prey inclusion rate bias; given isotopic signatures of predator and three potential prey (A, B, C), diet 1 and diet 2 could not be isotopically discernable as separate diets. Therefore the probabilities of diet 1 or diet 2 being the more likely 'true' diet cannot be determined.

Table 4-1. Dietary estimates of harvest fish derived from isotopic results from IsoSource linear mixing model computer program (\pm 95% CI) and Euclidean distance measurements; raw Euclidean distance measure values also included.

Target Species	Presumptive Nutrient Source			
	Feed/Fertilizer	Large Plankton	Medium Plankton	Small Plankton
PRO Large Fish	90.1 % IS (87.7-92.5%) 70 % EDIM 0.31 EDM	0.2 % IS (0.0-0.7%) 7 % EDIM 2.96 EDM	0.8 % IS (0.0-1.8%) 16 % EDIM 1.36 EDM	8.9 % IS (6.8-11.0%) 7 % EDIM 3.16 EDM
PRO Small Fish	43.5 % IS (37.8-49.2%) 34 % EDIM 1.24 EDM	7.0 % IS (1.4-12.6%) 14 % EDIM 3.07 EDM	14.8 % IS (3.4-26.2%) 33 % EDIM 1.29 EDM	34.7 % IS (31.6-37.8%) 19 % EDIM 2.31 EDM
UNP Large Fish	91.1 % IS (86.2-96.0%) 90 % EDIM 0.06 EDM	1.0 % IS (0.0-2.1%) 2 % EDIM 2.40 EDM	6.8 % IS (1.6-12.1%) 6 % EDIM 0.51 EDM	1.0 % IS (0.2-1.8%) 2 % EDIM 2.71 EDM
UNP Small Fish	22.7 % IS (8.4-37.0%) 37 % EDIM 0.88 EDM	13.4 % IS (8.4-18.4%) 14 % EDIM 2.42 EDM	29.4 % IS (10.9-47.9%) 32 % EDIM 1.04 EDM	34.5 % IS (32.0-37.0%) 17 % EDIM 1.89 EDM
CSM Large Fish	21.4 % IS (11.1-31.7%) 17 % EDIM 1.96 EDM	22.0 % IS (6.1-38.0%) 23 % EDIM 1.48 EDM	53.6 % IS (29.3-78.0%) 51 % EDIM 0.66 EDM	1.1 % IS (0.0-3.6%) 9 % EDIM 3.66 EDM
CSM Small Fish	0.6 % IS (0.0-1.44%) 21 % EDIM 2.10 EDM	63.3 % IS (64.9-61.7%) 25 % EDIM 1.80 EDM	2.0 % IS (0.0-4.0%) 40 % EDIM 1.13 EDM	34.1 % IS (33.2-35.0%) 14 % EDIM 3.15 EDM
INO Large Fish	----- ----- ----- 36.07* EDM	86.5 % IS (82.7-90.3%) 65 % EDIM 0.63 EDM	8.6 % IS (2.4-14.8%) 25 % EDIM 1.66 EDM	4.9 % IS (1.8-8.1%) 10 % EDIM 4.06 EDM
INO Small Fish	----- ----- ----- 6.83* EDM	67.3 % IS (64.1-70.6%) 34 % EDIM 1.73 EDM	7.5 % IS (1.5-13.5%) 47 % EDIM 1.26 EDM	25.3 % IS (22.4-28.2%) 19 % EDIM 3.14 EDM

IS: IsoSource Program; ($\bar{X} \pm 95\%$ CI)

(<http://www.epa.gov/wed/pages/models/stableIsotopes/isotopes.htm>)

EDIM: Euclidean Distance Inclusion Measurement

EDM: Euclidean Distance Measure

* inorganic fertilizer treatment $\delta^{13}\text{C} \sim -15\%$ (aqueous HCO_3 ; Boutton 1991)

Table 4-2. Dietary estimates of pre-harvest fish derived from isotopic results from IsoSource linear mixing model computer program ($\pm 95\%$ CI) and Euclidean distance measurements; raw Euclidean distance measure values also included.

Target Species	Feed/Fertilizer	Presumptive Nutrient Source		
		Large Zooplankton	Medium Plankton	Small Plankton
PRO	49.8 % IS	46.9 % IS	3 % IS	0.3 % IS
Pre-Harvest Fry	(45.7-53.9%)	(44.0-49.8%)	(0.2-5.8%)	(0.0-0.8%)
	30 % EDIM	23 % EDIM	38 % EDIM	9 % EDIM
	1.24 EDM	1.60 EDM	0.97 EDM	4.23 EDM
UNP	33.1 % IS	42.4 % IS	23.7 % IS	0.7 % IS
Pre-Harvest Fry	(19.4-46.9%)	(35.4-49.4%)	(4.8-42.6%)	(0.0-1.6%)
	24 % EDIM	19 % EDIM	50 % EDIM	8 % EDIM
	1.10 EDM	1.42 EDM	0.52 EDM	3.43 EDM
CSM	54.6 % IS	20.9 % IS	23.2 % IS	1.3 % IS
Pre-Harvest Fry	(50.3-58.9%)	(8.9-32.9%)	(7.6-38.8%)	(0.0-2.7%)
	34 % EDIM	21 % EDIM	31 % EDIM	14 % EDIM
	1.46 EDM	2.30 EDM	1.57 EDM	3.59 EDM
INO	-----	34.1 % IS	65 % IS	0.9 % IS
Pre-Harvest Fry	-----	(32.7-35.5%)	(62.7-67.3%)	(0.0-1.8%)
	-----	32 % EDIM	55% EDIM	13 % EDIM
	-----	1.37 EDM	0.79 EDM	3.33 EDM

IS: IsoSource Program; ($\bar{X} \pm 95\%$ CI)

(<http://www.epa.gov/wed/pages/models/stableIsotopes/isotopes.htm>)

EDIM: Euclidean Distance Inclusion Measurement

EDM: Euclidean Distance Measure

* inorganic fertilizer treatment $\delta^{13}\text{C} \sim -15\%$ (aqueous HCO_3^- ; Boutton 1991)

Table 4-3. Back calculated primary producer carbon isotope signature ($\delta^{13}\text{C}$) estimates based upon actual (*) and hypothetical pond guild carbon signature results and trophic level estimations (assuming 0.64 ‰ $\Delta\delta^{13}\text{C}$ enrichment per trophic level).

Taxon	Estimated trophic level				
	level	PRO	UNP	CSM	INO
Small Plankton 1 -32 μm *	1.0	-27.18	-26.53	-28.64	-23.26
Medium Plankton 32-200 μm	2.0	-24.79	-24.50	-25.40	-22.83
Large Zooplankton $\geq 200 \mu\text{m}$	3.0	-24.34	-24.34	-25.88	-22.47
Small Fish	3.5	-25.91	-25.72	-26.85	-21.89
Large Fish	4.0	-25.31	-25.18	-22.07	-22.07
Mean estimated 1° producer $\delta^{13}\text{C}$		-25.51	-25.25	-26.63	-22.50
Applied nutrient $\delta^{13}\text{C}$ signature for reference		-23.74	-23.95	-26.43	n/a

* actual small plankton assemblage carbon isotope signature results.

Table 4-4. Isotopically derived trophic differences among harvest fish predators, applied nutrients and plankton size assemblages (via $\delta^{15}\text{N}$); based upon $\delta^{13}\text{C}$ provided as supplemental information. Assuming 0.64 ‰ $\Delta\delta^{13}\text{C}$, 3.03 ‰ $\Delta\delta^{15}\text{N}$, enrichment rates from indoor feeding trial (Chapter 2).

Trophic distance estimate		PRO	UNP	CSM	INO
Lg Fish / Nutrient	$\delta^{15}\text{N}$	0.97	1.01	1.48	2.48
	$\delta^{13}\text{C}$	0.54	1.09	3.08	n/a
Lg Fish / Lg Zooplankton	$\delta^{15}\text{N}$	0.07	0.23	0.62	0.80
	$\delta^{13}\text{C}$	-0.41	0.21	-0.44	1.18
Lg Fish / Med Plankton	$\delta^{15}\text{N}$	0.60	0.66	0.81	1.30
	$\delta^{13}\text{C}$	1.28	0.80	0.47	3.18
Lg Fish / Sm Plankton	$\delta^{15}\text{N}$	1.04	1.10	1.31	2.06
	$\delta^{13}\text{C}$	5.92	5.20	6.53	4.85
Sm Fish / Nutrient	$\delta^{15}\text{N}$	1.08	1.11	1.67	2.11
	$\delta^{13}\text{C}$	-0.89	-0.27	1.85	n/a
Sm Fish / Lg Zooplankton	$\delta^{15}\text{N}$	0.18	0.34	0.81	0.43
	$\delta^{13}\text{C}$	-1.84	-1.14	-1.67	0.96
Sm Fish / Med Plankton	$\delta^{15}\text{N}$	0.71	0.78	1.01	0.93
	$\delta^{13}\text{C}$	-0.15	-0.56	-0.76	2.96
Sm Fish / Sm Plankton	$\delta^{15}\text{N}$	1.15	1.22	1.51	1.70
	$\delta^{13}\text{C}$	4.48	3.77	5.30	4.63
Lg Zoo / Nutrient	$\delta^{15}\text{N}$	0.88	0.77	0.82	1.66
	$\delta^{13}\text{C}$	0.99	0.87	3.19	n/a
Lg Zoo / Med Plank	$\delta^{15}\text{N}$	0.46	0.48	0.16	0.48
	$\delta^{13}\text{C}$	1.64	0.72	0.58	2.14
Lg Zoo / Sm Plank	$\delta^{15}\text{N}$	0.98	0.88	0.66	1.24
	$\delta^{13}\text{C}$	6.37	4.90	6.63	3.80
Med Plank / Nutrient	$\delta^{15}\text{N}$	0.42	0.28	0.66	1.18
	$\delta^{13}\text{C}$	-0.65	0.14	2.61	n/a
Med Plank / Sm Plankton	$\delta^{15}\text{N}$	0.52	0.39	0.50	0.77
	$\delta^{13}\text{C}$	4.73	4.17	6.06	1.67
Sm Plank / Nutrient *	$\delta^{15}\text{N}$	0.58	0.58	0.55	1.26
	$\delta^{13}\text{C}$	0.86	0.64	0.55	n/a

Negative values denote probable lack of trophic linkage, values near unity denote roughly one trophic level difference and high probability of direct trophic linkage, values greater than one denote probable distant linkage or lack of trophic linkage.

* assuming -19 ‰ $\Delta\delta^{13}\text{C}$ enrichment rate between primary producers (C_3) and carbon pool $\delta^{13}\text{C}$ (Fry 2006), and ~ 0.0 ‰ $\Delta\delta^{15}\text{N}$ enrichment rate between primary producers and exploited nitrogen pool $\delta^{15}\text{N}$ (Fogel and Cifuentes 1993, Fry 2006).

Table 4-5. Processed feed (PRO) pond treatment dietary components ranked in order of percentage importance (1 highest, 4 lowest) for the Euclidean distance and IsoSource linear algebra computer program.

	Processed Feed	Lg. Plankton	Med. Plankton	Sm. Plankton
Large Fish IS	1	4	3	2
Large Fish ED	1	3.5	2	3.5
Small Fish IS	1	4	3	2
Small Fish ED	1	4	2	3

Table 4-6. Unprocessed feed (UNP) pond treatment dietary components ranked in order of percentage importance (1 highest, 4 lowest) for the Euclidean distance and IsoSource linear algebra computer program.

	Unprocessed Feed	Lg. Plankton	Med. Plankton	Sm. Plankton
Large Fish IS	1	3.5	2	3.5
Large Fish ED	1	3.5	2	3.5
Small Fish IS	3	4	2	1
Small Fish ED	1	4	2	3

Table 4-7. Cottonseed meal (CSM) pond treatment dietary components ranked in order of percentage importance (1 highest, 4 lowest) for the Euclidean distance and IsoSource linear algebra computer program.

	Cottonseed Meal	Lg. Plankton	Med. Plankton	Sm. Plankton
Large Fish IS	3	2	1	4
Large Fish ED	3	2	1	4
Small Fish IS	4	1	3	2
Small Fish ED	3	2	1	4

Table 4-8. INO pond treatment dietary components ranked in order of percentage importance (1 highest, 4 lowest) for the Euclidean distance and IsoSource linear algebra computer program.

	Large. Plankton	Medium Plankton	Small Plankton
Large Fish IS	1	2	3
Large Fish ED	1	2	3
Small Fish IS	1	3	2
Small Fish ED	2	1	3

Table 4-9. Food source inclusion rates for large zooplankton derived from isotopic signature results, IsoSource program, linear mixing models, and Euclidean distance measurements.

Target Species	Feed/Fertilizer	Presumptive Nutrient Source	
		Medium Plankton	Small Plankton
PRO Large Zooplankton	93.1 % IS (90.7-95.5%) 54 % EDIM 1.29 EDM	0.3 % IS (0.0-0.8%) 31 % EDIM 2.27 EDM	6.6 % IS (4.4-8.8%) 15 % EDIM 4.69 EDM
UNP Large Zooplankton	73.8 % IS (62.6-85.0%) 45 % EDIM 1.39 EDM	6.4 % IS (1.3-11.5%) 39 % EDIM 1.55 EDM	19.8 % IS (12.1-27.5%) 16 % EDIM 3.85 EDM
CSM Large Zooplankton	59.2 % IS (55.1-63.3%) 38 % EDIM 2.92 EDM	38.5 % IS (35.6-41.4%) 41 % EDIM 2.57 EDM	2.2 % IS (0.2-4.2%) 21 % EDIM 5.18 EDM
INO Large Zooplankton	----- ----- ----- 36.61* EDM	33 % Linear Model ($\delta^{15}\text{N}$ only) 53 % EDIM 1.69 EDM	67% Linear Model ($\delta^{15}\text{N}$ only) 47 % EDIM 1.89 EDM

IS: IsoSource Program; \bar{X} , (95% CI lower limit – 95% CI upper limit)
<http://www.epa.gov/wed/pages/models/stableisotopes/isotopes.htm>

Linear Model Using Binomial Solution (INO treatment only)

EDIM: Euclidean Distance Inclusion Measurement

EDM: Euclidean Distance Measure

* inorganic fertilizer ~ -15 ‰ $\delta^{13}\text{C}$ (aqueous HCO_3^- ; Boutton 1991)

Table 4-10. Trophic level estimates of plankton assemblages and harvest fish within treatments, trophic levels relative to treatment small plankton assemblage $\delta^{15}\text{N}$ values only.

Taxon	$\delta^{15}\text{N}$ difference ($\Delta\delta^{15}\text{N}$)	PRO	UNP	CSM	INO
Hypothetical trophic level					
Small plankton 1 -32 μm	$\Delta\delta^{15}\text{N}$	4.38	4.47	4.29	1.19
	1.0	1.0	1.0	1.0	1.0
Medium plankton 32-200 μm	$\Delta\delta^{15}\text{N}$	5.95	5.66	5.80	3.51
	2.0	1.5	1.4	1.5	1.8
Large plankton $\geq 200 \mu\text{m}$	$\Delta\delta^{15}\text{N}$	7.35	7.13	6.29	4.95
	3.0	2.0	1.9	1.7	2.2
Small fish SL ≤ 31 mm	$\Delta\delta^{15}\text{N}$	7.96	8.18	8.85	6.33
	3.5	2.2	2.2	2.5	2.7
Large fish SL > 31 mm	$\Delta\delta^{15}\text{N}$	7.62	7.82	8.26	7.44
	4.0	2.1	2.1	2.3	3.1

Taxa trophic position relative to primary producer (Vander Zanden and Rasmussen 1999); e.g., PRO Small Fish trophic position: $(7.96-4.38)/3.03 + 1.0$ (autotroph) ≈ 2.2 trophic levels. Nitrogen isotope enrichment magnitude value from Chapter 2 ($\Delta\delta^{15}\text{N} = 3.03 \text{‰}$ per trophic level).

CHAPTER 5 LARGE ZOOPLANKTON ASSEMBLAGE TAXA IDENTIFICATION AND ENUMERATION

Large (> 200 μm) plankton assemblage taxa were examined and compared among pond nutrient treatments (primarily zooplankton), in an attempt to identify and enumerate major taxa associated with differing pond management methods (feeds versus fertilizers), and fish production rates. Zooplankton taxa and numbers also were compared among weekly sampling dates to characterize large size assemblage zooplankton community change over time.

Large plankton assemblages within the different ponds treatments were compared using the simplified Morisita's similarity index (MSI) and the percent similarity index (PSI), to determine if plankton assemblage commonalities occurred among the different treatments (Krebs 1998). Also, if common plankton assemblage characteristics occurred within treatments: (1) were assemblage differences present among treatments? (2) were these differences along a predictable nutritional/nutrient type grade?; i.e. were feed treatment large plankton community taxa more similar to each other than either feed treatment large plankton community was to those of the two fertilizers and vice versa?

Because direct microscopic analyses of swordtail gut contents were not possible due to the finely ground and largely unidentifiable prey items that resulted from the action of the swordtails' pharyngeal teeth, more indirect dietary habit investigation methods were employed. One indirect method was the use of large plankton assemblage community analyses [index of relative importance (%IRI)], which were performed as proxies for potential swordtail prey/diets. Potential large plankton prey taxa abundances (% number and % volume) and indices of relative importance were assumed to be correlated with their potential importance as prey items for swordtails. Although this indirect method of characterizing possible swordtail diets was used, additional lines of evidence and more direct dietary habit analysis methods, other than gut

content analysis, also were utilized (Chapter 4: fish and pond taxa isotopic signature analyses; Chapter 6: 24-hour captive feeding trials).

Isotopic signature data for applied pond nutrients (inorganic fertilizer – nitrogen only), plankton assemblages, and swordtails were used to generate isotopically derived swordtail diet estimates (Chapter 4), which were used to roughly quantify potential predation pressure occurring for the different plankton size assemblages and dietary inclusion rates for non-INO applied nutrients. Additionally, 24-hour controlled feeding trials were performed with individual swordtails incubated with pond water to determine potential swordtail predation effects upon the large zooplankton communities within the various treatment ponds (Chapter 6).

Deterministic large plankton assemblage similarity estimates (MSI and PSI), were calculated for pairwise treatment comparisons among the four applied pond nutrient treatments using mean %IRI values calculated for each treatment, for each of the 10 analyzed weekly sampling dates (Krebs 1998, Cortes 1997). Due to the deterministic nature of the similarity (MSI and PSI) indices, generated using large plankton assemblage taxa %IRI values, error measures were lacking for these similarity value estimates and conclusions regarding large plankton community similarities were based upon arbitrarily chosen threshold ‘critical’ values (Zaret and Rand 1971, Cailliet and Barry 1978, Lindquist 1998). A Bayesian resampling bootstrap computer program was developed to generate similarity index values (MSI and PSI) and error measures from major large plankton assemblage taxa %N within the large zooplankton assemblages occurring within the various treatment ponds. Resampled indices and error measures were produced using replicate pond large plankton assemblage datasets (Appendix B: MSI/PSI/%N bootstrap computer program) that were repeatedly sampled over numerous iterations. Error measure calculations allowed statistical comparisons of large zooplankton

assemblage MSI and PSI to be made among sampling dates within pond nutrient treatment pair comparisons.

Methods

Plankton Sample Collection

A more detailed description of methods used for weekly pond plankton sample collections for stable isotope analyses and microscopic examination is given in Chapter 4 (Methods). A brief summary of the methods used to collect plankton for microscopic examination is given below.

Weekly pond water samples (1 L/pond) were taken from twenty-four replicate ponds (13:00-15:00 EST), that were randomly and evenly assigned into four applied pond nutrient treatments (6 replicate ponds per treatment) for a period of twelve weeks. Samples were fixed within one hour of collection using Lugol's preservative (APHA 1989) and stored in light-proof containers. At a later date, preserved samples were serially filtered through three sieves (200- μm , 35- μm , and 1- μm square mesh); resulting in three size fractions (identical size fractions as for isotope analyses; Chapter 4) that were stored in light proof storage boxes in 9-ml brown flint glass vials containing ~ 6-10 drops of Lugol's solution. Sample water, filtered through the final sieve ($< 1 \mu\text{m}$) was discarded.

Large Plankton Assemblage Examination and Processing

Preserved large plankton assemblages were examined for zooplankton, phytoplankton, and incidental organisms by identifying and enumerating all taxa via light microscopy (Olympus BH-2 binocular microscope with 4X, 10X, 20X, 40X objectives and 10X ocular for 40X, 100X, 200X and 400X total magnification). Large plankton assemblage samples were serially examined using a Sedgewick-Rafter plankton counting cell in 1-ml aliquots until the entire sample was surveyed. The Sedgewick-Rafter counting cell slide had a 50 mm x 20 mm

graduated grid base and depth of 1 mm for a total volume of exactly one milliliter, the graduated volume and grid base allows for quantitative assessment of organism densities and measurement of organism linear dimensions, respectively. Microscopically examined (processed) samples were then returned to their original flint glass sample vials for archival purposes.

Large plankton samples from the first two weeks of the 12-week trial were discarded due to a general lack of organisms present within pond water samples during the first two weeks of the 12-week study (31 March 2006 and 6 April 2006). Absence of large plankton were likely due to pond sterilization with hydrated lime prior to stocking, cooler water temperatures, and shorter photoperiods associated with late winter/early spring at the start of the outdoor pond trial. Microscopically examined large plankton assemblage samples thus consisted of weekly samples from four pond nutrient treatments with six replicate ponds per treatment over a course of ten weeks ($4 \times 6 \times 10 = 240$) samples. Unfortunately, one sample was lost due to human error (from pond B1 13 April 2006, UNP feed treatment).

Limited samples for the two smaller size fractions: medium (35-200 μm), and small (1-35 μm) were examined for randomly selected ponds and dates. Unfortunately, due to the large number of individual organisms per sample, their morphological similarity, and large amounts of flocculent material in the sample, only a cursory examination was feasible, making organism identification and enumeration extremely impractical and time consuming for the medium (mixed phytoplankton and small zooplankton) and small plankton (primarily phytoplankton) size fractions. The two smaller plankton size assemblages were ultimately excluded from quantitative microscopic analyses.

Large Plankton Assemblage Taxa Identification

Large plankton (primarily zooplankton) assemblage fractions were analyzed to determine assemblage organisms present by taxon, number and estimated volume. All large plankton were

identified to order and lower when possible and notable plankton organisms were photographed depending on its commonality/novelty, possession of distinguishing characteristics, and determination of linear dimensions for volumetric estimation.

Twenty randomly selected digital photomicrographs of each major planktonic item were measured using the 1.0 mm × 1.0 mm grid of the Sedgwick-Rafter cell as a size reference under known magnification. The determination of linear dimensions allowed rough volumetric approximations (length x width x height) of items based upon simple geometric solids (sphere, cube, cone, etc.) or combination of solids that roughly corresponded to the geometry of the item.

With minor differences depending upon taxon shape, the general taxon volume estimate equation is given as (as an example, cumulative volume of cube hexahedron taxon):

$$l \times w \times h \times n \quad (\text{Equation 5.1})$$

where: l = length in μm ,

w = width in μm ,

h = height in μm ,

n = number of a given taxon.

Taxon percent volume (%V) and number (%N) were calculated as the cumulative taxon volume or number as a percentage of the total volume or number of large zooplankton items (all taxa) within a sample. Taxon percent frequency of occurrence (%FO) was calculated as the percentage of treatment replicates where a given taxon was present. For the present study, the maximum number of replicates was six ponds for each of the four pond nutrient treatments.

Large Plankton Community Analyses

Large zooplankton (> 200 μm ; primarily zooplankton) assemblage community analyses were performed using the index of relative importance (IRI), which is a compound index that combines metrics of taxon number, weight or volume (physical size), and frequency of

occurrence within the sample population, and by inference, the community population under investigation (Pinkas et al. 1971, Cortes 1997). Specifically, percent index of relative importance (%IRI) analyses were used to evaluate taxa importance within the plankton community to facilitate comparisons among replicates, treatments and potential comparisons with past and future studies, and differing ecosystems (Cortes 1997). In addition to taxa %IRI values, zooplankton taxa densities (taxa number/L water sample) and zooplankton taxa volume (ml zooplankton taxa/L water sample) estimates also were calculated.

Advantages of using %IRI are that a single value is used to describe multiple community metrics, greatly simplifying calculations, interpretation of data, and comparisons among studies. For example, if a numerically dominant taxon has a small individual volume or biomass and is found in just a few samples within the community, this numerical dominance (and associated bias) is offset by its small size and limited distribution within the community, thereby reducing its community IRI value and giving a more realistic, less biased measure of the taxon's importance within the community. This is an advantage when evaluating a community component as a potential prey item for a predator species when direct stomach content data are unavailable. The index of relative importance for a plankton community (IRI) is given by the formula (Pinkas et al. 1971):

$$IRI_i = (\% N_i + \% V_i) \times \% FO_i \quad (\text{Equation 5.2})$$

where: %N_i = the numerical % fraction of a single plankton community component/taxa (i) among the total number of plankton community items within a sample,
 %V_i = the percentage volume fraction of a single plankton community component/taxa (i) among the total community volume within a sample,

%FO_{*i*} = the frequency that a single plankton community component/taxa (*i*) occurs within replicate samples from a given population.

IRI values range from a maximal value of 200,000, when a single community component is contained within all samples, to a value of zero, when a component is absent from the community. Due to the extremely large range of IRI values that a given community component can have, Cortes has advocated %IRI as a transformation of raw IRI values in order to put the 'relative importance index' concept into a more intuitively meaningful context (Cortes 1997).

Percent IRI is given as:

$$\% \text{ IRI}_i = \left(\frac{\text{IRI}_i}{\sum_{i=1}^n \text{IRI}_i} \right) \times 100 \quad (\text{Equation 5.3})$$

where: IRI_{*i*} = the raw IRI value for the *i*th community component,

%IRI_{*i*} = the percentage of the total IRI contributed by the *i*th community component.

Percent IRI (%IRI) is a data transformation of raw IRI values standardized to the total IRI values calculated for a given sample. Again, advantages of using %IRI over raw IRI values is that transformed %IRI values allow for easier interpretation of the community components' relative importance to the community and potential predator species. It also facilitates comparisons among different samples within and among different studies occurring in different geographical areas and time periods. An arbitrary %IRI cutoff value of 0.5% was used to limit plankton analyses to those assemblage components with the greatest potential nutritional impact on pond fry and broodstock. Thirty large plankton assemblage components regularly had %IRI values equal to or greater than 0.5%, thereby meeting the cutoff criterion (Table 5.1).

Two different methods of calculating %IRI were employed, the first consisted of using large zooplankton assemblage taxa data from pooled replicates (pooled %IRI), and the second consisted of using individual replicate data (unpooled %IRI).

Large zooplankton assemblage taxa and potential swordtail prey pooled %IRI values (Equations 5.2-5.3) are useful in describing which taxa were present within the different pond nutrient treatments as a whole. Unpooled large zooplankton taxa %IRI values (Equations 5.4) are useful in that they describe the taxa present within the different pond nutrient treatments on an average basis among replicate ponds within the different pond nutrient treatments. Unpooled average relative importance values, allow variation of taxa importance among ponds within treatments to be examined, and how these variations might affect potential predators within different ponds and among the different pond treatments. Large plankton assemblage taxa unpooled %IRI values were calculated as:

$$IRI_{i (unpooled)} = (\bar{X}_{\%N_i} + \bar{X}_{\%V_i}) \times \%FO_i \quad (\text{Equation 5.4})$$

where: $\bar{X}_{\%N_i}$ is the mean %N for taxon i among replicate ponds,

$\bar{X}_{\%V_i}$ is the mean %V for taxon i among replicate ponds,

$\%FO_i$ is the %FO for taxon i among the replicate pond population,

$IRI_{i (unpooled)}$ is the pooled IRI for taxon i .

Percent Number, Volume, and Frequency of Occurrence Graphs

Using a compound index, such as the IRI, had the advantage of reducing the numerical bias of a small and highly numerous community or prey item, by incorporating the item's relative size into the index, or conversely, a large, but numerically rare item. Unfortunately, IRI and %IRI also have the disadvantage of eliminating information present in individual metrics such as the relative number, relative volume, and the prevalence of an item (frequency of

occurrence) in the community or predator's diet (rare or common). Variations in potential prey availability (as %N and %V) among individual prey assemblage populations or among individual predator's diets cannot be examined when metrics are combined into a single compound metric such as IRI and %IRI.

To address the loss of information that occurs when individual large plankton assemblage metrics (%N, %V, %FO) are combined into a single compound index, three vector graphs of these metrics were produced for each sampling date analyzed within each of the four pond nutrient treatments (Cailliet et al. 1996). Large plankton assemblage three vector graphs consisted of the percent number of an assemblage component as a percentage of all components on the positive Y-axis, percent volume of an assemblage component as a percentage of the total assemblage volume displayed on the negative Y-axis, and the frequency of occurrence (%FO) of the assemblage component among the six pond replicates along the positive X-axis. The relative size of the parallelogram created on the graph utilizing percent number (%N), percent volume (%V), and percent frequency of occurrence (%FO) data for an assemblage component, denoted the taxon's importance (positively correlated) in relation to the sum total area prescribed by all large plankton assemblage components within replicate samples for a given treatment (Table 5.1, 30 large plankton assemblage taxa, each %IRI > 0.5%).

Graphical plots of %N, %V, and %FO also were calculated in two different ways using pooled and unpooled values. Pooled values were created by summing the counts of a given assemblage component (taxon) among all replicates within a given pond nutrient treatment and calculating its percentage of all organisms counted within all replicates for a given treatment (pooled %N), or as the total volume of a given assemblage component (taxon) summed among all replicates as a percentage of the volume of all large zooplankton assemblage components

summed among all replicates within a given treatment (pooled %V); %FO calculation was identical in the case of pooled and unpooled values. Unpooled values were calculated as the mean %N or %V of a given assemblage component among the six replicates of each pond nutrient treatment; the advantage of unpooled values was that it allowed the calculation of error measures for either %N or %V for a given assemblage component and allowed evaluation of individual variation among replicates for given plankton assemblage components.

Major Large Plankton Assemblage Taxa Analyses

Detailed analyses [weekly large plankton density values (plankton number/L)] of the three large plankton assemblage components that regularly displayed the highest %IRI values ('major prey') among pond nutrient treatments and weekly sampling dates also were performed. Major large plankton assemblage taxa analyses were performed to determine if different patterns in availability of these potential 'major' prey occurred among the pond treatments and/or weekly sampling dates.

Large Plankton Assemblage Similarity Indices

In an attempt to determine if the magnitude of large zooplankton community similarities/dissimilarities between different treatment ponds were correlated with fish production rates, large plankton assemblage taxa similarities among pond nutrient treatments were examined using large plankton assemblage taxa %IRI, %N, and two qualitative similarity indices. The similarity indices used to compare large plankton assemblages between treatments and among sampling dates within treatments were the simplified Morisita's similarity index and percent similarity index (Horn 1966, Krebs 1998). The simplified Morisita's index (MSI) is given as (Krebs 1998):

$$\text{Simplified Morisita's Index } C_H = \frac{2 \cdot \sum_{i=1}^n p_{ij} \cdot p_{ik}}{\sum_{i=1}^n p_{ij}^2 + \sum_{i=1}^n p_{ik}^2} \quad (\text{Equation 5.5})$$

where: p_{ij} = the proportion of taxon i of treatment assemblage j ,

p_{ik} = the proportion of taxon i of treatment assemblage k ,

n = the number of taxa categories for assemblages j and k .

A widely used but arbitrary significance level of 0.65 was defined as the critical value for the simplified Morisita's index (Equation 5.5) denoting assemblage taxa similarity (Zaret and Rand 1971, Cailliet and Barry 1978).

The percent similarity index (PSI) is given as (Krebs 1998):

$$PSI = \left(\sum \min . p_{ij} \cdot p_{ik} \right) \cdot 100 \quad (\text{Equation 5.6})$$

where: p_{ij} = the proportion of taxon i of treatment assemblage j ,

p_{ik} = the proportion of taxon i of treatment assemblage k ,

n = the total number of taxa categories for assemblages j and k ,

$\min . p_{ij} \cdot p_{ik}$ = either p_{ij} or p_{ik} , which ever is smaller.

Again, an arbitrary critical value of 60% was chosen to denote similarity between large plankton assemblage between treatments using the percent similarity index (PSI; Equation 5.6). This value was based upon PSI critical values chosen by previous researchers (Horn 1966, Cailliet and Barry 1978, Lindquist 1998). Higher PSI critical values have been chosen by others in describing plankton assemblage communities (e.g., 80%; Silver 1971, 1975), but these values was deemed too high to be useful within the current study.

Each of the two similarity indices (MSI and PSI) was calculated using two different methods: deterministic and Bayesian bootstrap similarity indices values. Large plankton assemblage MSI and PSI were estimated for each pond nutrient treatment pair and weekly sampling date. Deterministic MSI and PSI values were calculated using %IRI values (%IRI \geq 0.5%) of the major large plankton assemblage taxa. Bootstrap resampling similarity index values were estimated using taxon counts within each replicate pond, which were then transformed to %N values (%N \geq 0.5%) and repeatedly sampled (resampled) using a computer program (Appendix B) written in BASIC to estimate resampled MSI and PSI means and their error measures for the ten sampling periods, during which large plankton assemblage taxa were examined. Bootstrap MSI and PSI values were estimated by resampling from dataset pools composed of the actual replicate pond large zooplankton assemblage taxa (%N) data, error measures were generated based upon numerous draws (iterations) using t-scores, draw pool degrees of freedom, and standard error measurements of the individual iterations to produce 95% confidence intervals for the mean bootstrap MSI and PSI values estimated for each treatment pair and sampling date (Zar 1984, Ott 2000).

Additionally, bootstrap percent similarity index (PSI) mean values were generated with varying numbers of replicate data sets randomly pooled (draw size) for analyses (4, 6, 8, 15, and 20), and the number of iterations performed (10, 50, 100, 500, 1000, and 5000) to observe the behavior of the various index estimates and error measures under differing conditions.

Statistical Analyses

Total large zooplankton assemblage densities (numbers/L) and volumes (mm^3/L) were compared among treatments and 10 weekly sampling dates using repeated measures two-way ANOVA statistical analyses. Time-averaged differences in large zooplankton assemblage total densities and volumes were compared among treatments using one-way ANOVA. Densities of

the three major large zooplankton groups (consistently highest %IRI values) were compared among treatments and among 10 weekly sampling dates using ANOVA.

Treatment pair large zooplankton assemblages were determined to be similar when deterministic large zooplankton assemblage taxa simplified Morisita's and percent similarity indices were above an arbitrarily chosen critical threshold value (0.65 MSI, 60% PSI).

Bootstrap MSI and PSI values, generated using large zooplankton assemblage taxa %N randomly selected from replicate dataset pairs (6 pairs per iteration) were compared using 95 % CI overlaps among sampling dates within treatment pair comparisons (Liberty™ BASIC 4.0 computer program; Appendix B). This allowed statistical comparisons and conclusions to be drawn regarding differences in indices similarity values among sampling dates (e.g., were large zooplankton assemblages of the PRO and CSM treatments more similar for latter sampling dates than they were for earlier sampling dates?).

Results

Large Zooplankton Production Analyses

Differences in large plankton volumes (mm^3/L pond water sample) among the four pond nutrient treatments and 10 weekly sampling periods were compared (Figures 5-1-5-5; rep. meas. 2-way ANOVA: $P_{(\text{nutrient})} = 0.1305$, $F_{(\text{nutrient})} = 2.114$, $\alpha_{(2,3)} = 0.05$; $P_{(\text{time})} < 0.0001$, $F_{(\text{time})} = 8.327$, $\alpha_{(2,9)} = 0.05$; $P_{(\text{interaction})} = 0.2118$, $F_{(\text{interaction})} = 1.231$, $\alpha_{(2,27)} = 0.05$). Although the nutrient treatment was not significant, post-hoc pairwise testing found a single pairwise difference among the four nutrient treatments and ten sampling dates for total large plankton volume, CSM 8 June 2006 and INO 8 June 2006 ($P < 0.05$, Bonferroni post-hoc multiple comparison test). Time was highly significant and numerous pairwise differences in large plankton volume were found among sampling dates within individual pond nutrient treatments (Figures 5-2 – 5-3, 5-5); these differences usually occurred between samples collected early in the trial, when large plankton

volumes were low, and mid and late samples when plankton standing stocks were considerably higher (Figure 5-1). Only the CSM pond nutrient treatment did not have any differences among sampling dates (Figure 5-4).

Time-Averaged Large Plankton Volumes

Time-averaged large (> 200 μm) plankton volumes did not differ among the four pond nutrient treatments (Figure 5-6; rep. meas. 1-way ANOVA, $P = 0.1812$, $F = 1.746$, $\alpha_{(2,3)} = 0.05$). PRO pond treatment large plankton volume mean $\bar{X} = 3.64 \text{ mm}^3/\text{L}$ (2.33 to 4.94 mm^3/L 95 % CI), UNP mean $\bar{X} = 4.08 \text{ mm}^3/\text{L}$ (2.47 to 5.70 mm^3/L 95 % CI), CSM mean $\bar{X} = 2.55 \text{ mm}^3/\text{L}$ (2.09 to 3.01 mm^3/L 95 % CI), and INO mean $\bar{X} = 3.08 \text{ mm}^3/\text{L}$ (2.05 to 4.10 mm^3/L 95 % CI) were all of similar magnitudes. Interestingly, the CSM treatment exhibited much lower variation among its six replicate ponds than the other three treatments (Figure 5-6).

Mean large zooplankton density (number/L) differences among the four pond nutrient treatments and 10 weekly sampling periods also were compared (Figures 5-7 – 5-11; rep. meas. 2-way ANOVA, $P_{(\text{nutrient})} = 0.4978$, $F_{(\text{nutrient})} = 0.8204$, $\alpha_{(2,3)} = 0.05$; $P_{(\text{time})} < 0.0001$, $F_{(\text{time})} = 6.574$, $\alpha_{(2,9)} = 0.05$; $P_{(\text{interaction})} = 0.5084$, $F_{(\text{interaction})} = 0.9730$, $\alpha_{(2,27)} = 0.05$). Again, although the nutrient factor was found to be non-significant, a single significant difference in large zooplankton numbers were found during pairwise comparisons of pond treatments, CSM 8 June 2006 and INO 8 June 2006 ($P < 0.05$, Bonferroni post-hoc multiple comparison test). This was the same post hoc pairwise difference observed for zooplankton volume. Several differences in mean large zooplankton density were found among sampling periods within each pond nutrient treatments, except for the PRO treatment (Figure 5-8). The UNP treatment had several pairwise differences that resulted from low densities at the start of the trial and much higher densities mid and late trial (Figure 5-9). CSM pairwise differences all involved a single sampling date, due to

a single pond that had *Filinia* rotifer densities two orders of magnitude higher than all other replicates (Figure 5-10; 1,644 rotifers in pond A6 on 11 May 2006; mean of 23.4 rotifers in five remaining ponds). Two non-consecutive sampling dates (11 May 2006, 8 June 2006) were involved in all pairwise differences for the INO treatment (Figure 5-11).

Time-Averaged Large Plankton Densities

Time-averaged large plankton assemblage density differences among treatments were not present (Figure 5-12; rep. meas. 1-way ANOVA, $P = 0.4424$, $F = 0.9244$, $\alpha_{(2,3)} = 0.05$). PRO pond treatment large zooplankton density mean $\bar{X} = 143.6$ plankton/L (202.1 to 85.1 plankton/L 95 % CI), UNP mean $\bar{X} = 183.0$ plankton/L (256.0 to 110.1 plankton/L 95 % CI), CSM mean $\bar{X} = 132.8$ plankton/L (211.1 to 54.5 plankton/L 95 % CI), and INO mean $\bar{X} = 153.3$ plankton/L (255.0 to 51.7 mm³/L 95 % CI) were all of similar magnitudes.

Outdoor Pond Trial Major Large Plankton Groups

Major large plankton groups were defined as the three assemblage components/taxa which consistently had the highest %IRI values among the thirty commonly found assemblage taxa (%IRI > 0.5 %) among the four nutrient treatments (Figures 5-13 – 5-20). The three highest IRI values among the thirty most common large plankton assemblage components/taxa (Figures 5-21-5.36, Table 5.1), consistently included: large Calanoid copepods (*Diaptomous* spp.; Figure 5-37a), daphnids (*Moina macrocopa*; Figure 5-37b), and rotifers (*Filinia* spp.; Figure 5-37c). Other rotifers and ostracods made sizable contributions to the large plankton assemblage (Figure 5-38e-h)

Copepod Densities for 10 Weekly Sampling Periods

Weekly *Diaptomous* copepod (LHC) densities (number/L) differed significantly among sampling dates within nutrient treatments, but not among nutrient treatments (Figures 5-22, 5-26,

5-30, 5-34: rep. meas. 2-way ANOVA, $P(\text{nutrient}) = 0.1516$, $F(\text{nutrient}) = 1.966$, $\alpha(2,3) = 0.05$, $P(\text{time}) < 0.0001$, $F(\text{time}) = 8.007$, $\alpha(2,9) = 0.05$, $P(\text{interaction}) = 0.4750$, $F(\text{interaction}) = 0.9973$, $\alpha(2,27) = 0.05$). No pairwise treatment differences in copepod density were found for any of the sampling dates ($P > 0.05$, Bonferroni post hoc test). Within individual pond treatments, copepod density differences among dates were found for the PRO, UNP, and INO nutrient treatments, but were not found within the CSM nutrient treatment (Figures 5-22, 5-26, 5-30, 5-34).

Within the two feed treatments, differences in copepod density among the weekly periods were between samples collected early in the study and samples gathered mid and late trial, when copepod standing stocks were considerably higher (Figures 5-22, 5-26). Extremely large densities of copepods for pond A5 (UNP) on 4 May 2006 (A5: 297 copepods/L; remaining replicates mean: $\bar{X} = 33$ copepods/L) and pond A12 (UNP) on 18 May 2006 (A12: 685 copepods/L; remaining replicates mean: $\bar{X} = 118$ copepods/L) contributed to the extremely large variation for these dates (Figure 5-26). Copepod densities among weekly sampling periods for the INO treatment roughly followed the same trajectories as the PRO and UNP treatments; low copepod densities occurred at the start of the trial, steadily increasing before reaching a maximum mid-trial, which then slowly tapered off declined toward the end of the trial (Figure 5-34). Of the four treatments, only the CSM weekly copepod densities did not differ among sampling periods (Figure 5-30).

A single UNP sampling date and pond large zooplankton assemblage sample [13 April 2006/pond (B1)] was lost due to human error. To perform repeated a measures 2-way ANOVA, a 'pseudovalue' was generated by replacing the missing value with the mean copepod count of the five remaining replicates. Due to the early date of the missing sample (first week of 12-week

trial), low plankton standing stocks had developed, potentially minimizing the impact of the missing value on overall plankton density means.

Mean rotifer (*Filinia* spp.) densities did not differ among pond nutrient treatments, but did differ among sampling intervals within treatments (rep. meas. 2-way ANOVA, $P(\text{nutrient}) = 0.6776$, $F(\text{nutrient}) = 0.5135$, $\alpha(2,3) = 0.05$; $P(\text{time}) = 0.0001$, $F(\text{time}) = 4.006$, $\alpha(2,9) = 0.05$; $P(\text{interaction}) = 0.9656$, $F(\text{interaction}) = 0.5508$, $\alpha(2,27) = 0.05$). *Filinia* spp. densities did not significantly differ among weekly sampling dates within either feed treatment (Figures 5-23, 5-27). In contrast to the PRO and UNP pond treatments, *Filinia* spp. densities differed among sampling dates for CSM and INO nutrient treatments (Figures 5-31, 5-35). The extremely large variation about the mean observed on 11 May 2006 for the CSM treatment was due to a single pond having a rotifer density two orders of magnitude higher than the overall mean of the remaining ponds for this sampling date (Figure 5-31; pond A6: 1644 rotifers/L; remaining replicate mean $\bar{X} = 23.4$ rotifers/L). The INO treatment also had large variation about a single sampling date due to the presence of high densities of *Filinia* spp. for a single pond (Figure 5-35; 11 May 2006 pond B7: 1128 rotifers/L; remaining replicate mean $\bar{X} = 42$ rotifers/L). As stated previously, a single replicate for the UNP nutrient treatment large zooplankton assemblage was lost and a pseudo-value was generated for the missing *Filinia* spp. density (13 April 2006 UNP).

Daphnids did not appear in substantial numbers until well into the 12-week trial, typically mid-trial (~ week six) or later (Figures 5-24, 5-28, 5-32, 5-36). Daphnid frequencies were compared among nutrient treatments and sampling dates within individual treatments (rep. meas. 2-way ANOVA, $P(\text{nutrient}) = 0.0767$, $F(\text{nutrient}) = 2.561$, $\alpha(2,3) = 0.05$; $P(\text{time}) < 0.0001$, $F(\text{time}) = 9.293$, $\alpha(2,9) = 0.05$, $P(\text{interaction}) = 0.0220$, $F(\text{interaction}) = 1.705$, $\alpha(2,27) = 0.05$). Although pond nutrient treatment was not found to be a significant factor, significant

differences between pond treatments were detected using post-hoc pairwise comparisons (Bonferroni posttests; Motulsky pers comm.). Only three pairwise differences in daphnid density were found between pond nutrient treatments, all involving the INO treatment for a single sampling date (8 June 2006, PRO and INO, UNP and INO, and CSM and INO); during the sampling date of 8 June 2006, INO treatment daphnid density was considerably lower than mean daphnid densities of the three other treatments (Figure 5-36). Daphnid density differences among sampling dates occurred within each of the four treatments, with among sampling date differences typically occurring between early sampling periods, when daphnids were absent and mid and late sampling periods, when daphnids were present in substantial numbers (Figures 5-24, 5-28, 5-32, 5-36).

Daphnid frequencies in the PRO treatment varied significantly among sampling dates, with daphnids largely absent from ponds until mid-trial, with significantly higher numbers in the latter half of the trial; interestingly, daphnids were present within the PRO treatment ponds sooner and in larger numbers than in the other three treatments, first appearing in the 18 May 2006 samples (Figure 5-24), approximately two weeks prior to the remaining three treatments (Figures 5-28, 5-32, 5-36). Daphnid densities in the UNP treatment differed significantly among sampling dates and followed a pattern similar to the PRO treatment (Figure 5-28). Daphnid densities in the CSM treatment did not significantly differ among sampling dates, probably due to the low daphnid density means measured during the trial and high variations observed during the last weeks of the trial (Figure 5-32). INO daphnid frequencies also did not differ among weekly sampling intervals, although means increased during the latter weeks of the study. INO daphnid densities followed a trajectory similar to the other treatments, with daphnids absent during the first half to two-thirds of the trial and a steady increase thereafter until trial termination. Within

the INO treatment, all pairwise differences in daphnid density involved a single sampling date (INO 8 June 2006) that was significantly higher than all other sampling dates (Figure 5-36).

Large Plankton Assemblage Simplified Morisita's and Percent Similarity Indices

Similarity indices were calculated as deterministic values and using bootstrap Monte Carlo resampling estimates. Deterministic estimates were calculated using weekly large plankton assemblage %IRI values (Figures 5-38 - 5-51), and bootstrap estimates were calculated using weekly large plankton assemblage taxa %N values (Figures 5-52 – 5-73).

Deterministic Morisita's Similarity and Percent Similarity Indices

Deterministic simplified Morisita's index value (MSI) comparisons between treatments did not appear to dramatically differ between treatment pairs (Figure 5-38). MSI values followed similar temporal patterns with high similarity among treatments at the start of the trial, likely due to the low large plankton abundances and limited taxonomic range present following pond sterilization and cooler, shorter-photoperiod days early in the study; MSI values dipped sharply and oscillated before steadily increasing to high similarity levels between treatments before trial termination. Of the six possible pairwise treatment comparisons of large plankton assemblage MSI similarities, the PRO and INO (Figure 5-42; exception 18 May 2006), and UNP and CSM (Figure 5-42) treatments appeared to have the most similar large plankton assemblages over the 12-week course of the trial. Large plankton assemblages were examined for only ten of the twelve weeks of the trial due to the lack of organisms present during the first two weeks of the trial following pond sterilization and the colder, shorter-photoperiod days associated with late winter/early spring. These factors likely reduced phytoplankton production to levels that only allowed negligible zooplankton production, and/or insufficient time had passed for phytoplankton and zooplankton colonization and reproduction to occur within the ponds. Unfortunately, only five UNP large plankton assemblages were present within the dataset for

MSI and PSI estimation on 13 April 2006 due to the loss of a replicate due to human error (pond B1).

PRO and UNP large plankton assemblages had similar MSI values ($MSI \geq 0.65$) for seven of the ten weeks that were examined (Figure 5-39). Additionally, of the three weekly sampling periods that were dissimilar ($MSI < 0.65$), all occurred during the first two thirds of the 12-week trial, and only two were for consecutive sampling periods (18 May and 25 May 2006).

PRO and CSM large plankton assemblages had similar MSI values for eight of the ten weeks that were examined (Figure 5-40). The two sampling periods, when PRO and CSM treatment large plankton assemblages were not similar, also were nonconsecutive (27 April 2006 and 18 May 2006).

PRO and INO large plankton assemblage MSI values were similar for nine of the ten weekly sampling dates examined (Figure 5-41). The single mid-trial sampling date that the PRO and INO treatments' large plankton assemblages were dissimilar, again was 18 May 2006.

UNP and CSM large plankton assemblage MSI values were similar for all ten of the weekly sampling dates examined (Figure 5-42). Additionally, the UNP and CSM MSI trajectories only oscillated between 0.67 and 1.0 compared to the erratic MSI value oscillations occurring between the other pairwise treatment combinations (e.g., Figure 5-43, UNP and INO treatments).

UNP and INO large plankton assemblage MSI values were similar for seven of the ten weekly sampling dates examined, although two large declines in MSI values occurred (Figure 5-43). Of the three dissimilar sampling periods (27 April, 18 May, and 25 May 2006), only two were consecutive and all three roughly occurred during the middle of the 12-week trial.

CSM and INO large plankton assemblage MSI values were similar for seven of the ten weeks examined (Figure 5-44), and none of the three dissimilar sampling dates (27 April, 25 May, and 8 June 2006) were consecutive. A qualitative examination of the CSM and INO MSI value time series data indicates that these two treatments had the most dissimilar large plankton assemblages over the 12-week outdoor trial, as indicated by the large oscillations and three low MSI minima observed during the trial (Figure 5-44, 27 April 2006 MSI = 0.24).

Large MSI value oscillations occurred among five of the six pairwise treatment combinations, with the single exception of the UNP and CSM treatment comparison. However, MSI values were uniformly high (Figures 5-38 – 5-43) during the last few weeks (1, 8, 15 June 2006) of the trial, with the exception of the CSM and INO treatment pair (Figure 5-44).

Percent similarity index (PSI) values exhibited less variation among treatment pair comparisons than their MSI counterparts (Figures 5-38, 5-45). Of the six possible pairwise treatment comparisons, the PRO and INO, UNP and CSM, and UNP and INO appeared to display the greatest large plankton assemblage similarities (Figures 5-48 - 5-50). Surprisingly, only one sampling date and treatment pair (PRO and CSM treatments) comparison displayed large plankton assemblage taxa PSI ‘dissimilarity’ (PSI < 0.6 or 60%) among the four pond nutrient treatments and ten weekly samples that were examined and compared for the 12-week trial (Figures 5-45, 5-47).

PRO and UNP large plankton assemblage PSI values were significant (PSI ≥ 60%) for all ten of the sampling dates that were examined (Figure 5-46), all PSI values oscillated between 61 and 91 percent similarity. No PSI value trends appeared to be occurring during the twelve weeks other than minor oscillations resulting from slight differences in PSI values among consecutive weekly sampling periods.

The PRO and two fertilizer treatment comparisons (PRO and CSM, PRO and INO) PSI value trajectories were unremarkable (Figures 5-47 – 5-48). PRO and CSM large plankton assemblage PSI values were similar for all but one sampling period (27 April 2006, PSI = 55%), the remaining sampling periods did not differ dramatically (Figure 5-47). PRO and INO large plankton assemblage PSI values were similar for all ten weekly sampling periods examined (Figure 5-48). PRO and INO PSI values displayed gradual oscillations that were centered between 70-80%, with values rising slightly toward the end of the trial.

UNP and CSM large plankton assemblage PSI values also were similar for all ten of the weekly sampling periods (Figure 5-49). Aside from a low initial PSI value at the start of the trial (13 April 2006, PSI = 62%), UNP and CSM PSI values were high (90-95%), before declining slightly and stabilizing (~ 80%) during the last few weeks of the trial. A smooth PSI value oscillation occurred between the UNP and CSM treatment pair during the ten weeks that the large plankton assemblage taxa were examined (Figure 5-49).

UNP and INO large plankton assemblage PSI values were similar for all ten weekly sampling periods (Figure 5-50). PSI values slowly increased to a maximum value mid-trial (18 May 2006) before declining only slightly during the last half of the trial. Little variation occurred in UNP and INO treatment large plankton PSI values during the ten weekly sampling periods, producing a smooth PSI time series trajectory (Figure 5-50).

CSM and INO large plankton taxa PSI values were similar among all ten weekly sampling periods (Figure 5-51). Two PSI minima occurred during the trial (27 April and 8 June 2006) when PSI values dropped to the low 60s; however six of the ten sampling periods had PSI values well over 80%.

Bootstrap Morisita's Similarity and Percent Similarity Indices

Bootstrap resampling estimates of MSI and PSI were performed for taxon counts (%N) between different treatments for different draw pool sizes (3, 4, 5, 6, 8, 15 and 20 replicates), and iteration rates (10, 50, 100, 500 and 1000 iterations). Draw pool sizes and iteration rates were varied to observe how these indices reacted to different resampling parameters (Figures 5-52A – 5-55A; Appendix A). MSI and PSI values generally exhibited lower variation at higher draw and iteration rates (Figures 5-53 – 5-55, 5-68 – 5-73). Unfortunately, only five UNP treatment large plankton assemblages were present within the dataset for resampling estimation on 13 April 2006 because a replicate was lost due to human error (pond B1). Bootstrap generated large plankton community MSI and PSI means and 95% confidence interval estimates among pond nutrient treatment pairs and sampling dates are summarized in Table 5.2.

Within pairwise treatment comparisons, differences in weekly large plankton assemblage MSI values (%N) occurred for all comparisons except between the CSM and INO treatments (Figures 5-56 – 5-61). MSI treatment pair values followed a similar trajectory pattern: a gradual increase from initial values, followed by a steep decline, followed by a gradual increase before declining to near constant values during the last few weeks of the 12-week trial. The CSM and INO MSI values displayed a more gradual oscillation than those of their five treatment pair counterparts (Figure 5-61).

PRO and UNP treatment pair bootstrap large plankton assemblage MSI values differed for nine of the pairwise sampling date combinations (45 total comparisons) among the ten weekly sampling periods examined (Figure 5-56; $P < 0.05$, Bonferroni post test). The majority of these differences were due to a single period early in the study (27 April 2006), which had a low MSI value (MSI = 0.09) between the two feed treatments. MSI values leveled off during the latter half of the 12-week trial, possibly as a result of a more stable plankton community composition

with greater abundance within feed treatment ponds. Taxa were similar (%N MSI > 0.65) between the PRO and UNP treatments for six of the ten sampling dates (Figure 5-56), and the majority of these ‘similar assemblage’ sampling periods occurred during the latter two thirds of the trial.

PRO and CSM large plankton assemblage bootstrap MSI values followed a time series trajectory similar to those of the PRO and UNP MSI values (Figures 5-56 – 5-57). Of the nine PRO and CSM MSI pairwise sampling date differences that occurred among the ten sampling dates examined, seven were due to a single date (27 April 2006, MSI = 0.06) with a noticeably lower MSI value ($P < 0.05$, Bonferroni post test). Again, MSI slowly increased from a minimal value early in the trial (27 April 2006), reached a maximum at mid-trial, before declining slightly to a fairly constant value for the last few weeks. During only two of the ten weekly sampling periods examined were PRO and CSM treatment large plankton taxa significantly similar, although three additional sampling dates were extremely close (0.63-0.64) to the arbitrarily chosen MSI critical value ($C_{H(\text{critical})} = 0.65$).

PRO and INO large plankton assemblage taxa time series bootstrap MSI values (Figure 5-58) also followed a pattern similar to those of the three other PRO treatment pairwise comparisons (Figures 5-55 – 5-57). Of the eight pairwise differences in PRO and INO MSI values that occurred among the ten sampling dates ($P < 0.05$, Bonferroni post test), five occurred due to a single sampling date (27 April 2006). MSI values gradually increased from the 27 April 2006 minimum before reaching a maximum four weeks later, and then slightly decreased to a plateau for the last few weeks (Figure 5-58). Large plankton taxa of the PRO and INO treatments were found to be similar ($MSI \geq 0.65$) for only four of the ten sampling periods examined, all of which were in the last five weeks of the trial (Figure 5-58).

UNP and CSM large plankton assemblage time series bootstrap MSI values (Figure 5-59) followed a trajectory slightly different from the pairwise PRO treatment similarity comparisons (Figures 5-56 - 5-58; PRO and UNP, PRO and CSM, PRO and INO). In contrast to the PRO treatment's pairwise MSI comparisons, the UNP and CSM MSI minimum value occurred at 4 May 2006 rather than 27 April 2006, slowly rising to a maximum on 25 May 2006 before decreasing to a plateau for the last few weeks of the trial. Large plankton taxa of the UNP and CSM treatments were found to be similar for only three (two consecutive) of the ten sampling periods examined ($P < 0.05$, Bonferroni post test), although two sampling dates (1 June and 15 June 2006, $MSI = 0.64$) were near the critical value (Figure 5-59).

UNP and INO large plankton assemblage time series bootstrap MSI value time series trajectory also resembled that of the UNP and CSM trajectory (Figure 5-60). The UNP and INO MSI minima occurred on 4 May 2006, before slowly rising to a plateau for the last four weeks. Of the seven pairwise differences that occurred among the ten sampling dates ($P < 0.05$, Bonferroni post test), all were due to a single sampling date (4 May 2006) that occurred fairly early in the 12-week trial. UNP and INO assemblages were found to be similar for seven of the ten sampling periods ($MSI \geq 0.65$), five of which occurred in the last five weeks of the trial (Figure 5-60).

Only the CSM and INO large plankton assemblage time series bootstrap MSI values did not differ (overlapping 95 % CI) among the ten weekly sampling periods (Figure 5-61; $P > 0.05$, Bonferroni post test). CSM and INO MSI values smoothly oscillated over the ten weeks for which large plankton assemblage taxa were examined. Six of the ten weekly samples had similar large plankton assemblages ($MSI \geq 0.65$) between the CSM and INO treatments. One additional date had an MSI close to the critical value (8 June 2006, $MSI = 0.64$).

Differences in weekly large plankton assemblage bootstrap PSI values occurred for all treatment pair combinations except the CSM and INO combination (Figures 5-62 – 5-67). All other treatment combinations displayed similar bootstrap PSI temporal patterns: initial values were followed by a gradual increase, then by a sharp decrease, that slowly increased to a maximal level before decreasing to a nearly constant level for the last few weeks. The CSM and INO treatment pair displayed more gradual oscillations in PSI values than those of their five treatment pair counterparts.

These sharp decreases in large plankton assemblage MSI and PSI values for 27 April and/or 4 May 2006, were most likely due to the application of diuron herbicide (Karmax®; DuPont Chemical Corp.) on 19 April 2006 to control a filamentous algae outbreak in pond A7 (processed feed). All ponds were treated with diuron to control any potential filamentous algae that had not yet been visually detected and to eliminate potential experimental bias associated with differing replicate pond management practices.

PRO and UNP large plankton assemblage bootstrap PSI values, among the ten weekly sampling periods, followed a trajectory similar to that estimated for their bootstrap MSI values (Figures 5-56, 5-62). PSI minima early in the study gradually increased to a maximum four weeks later, before declining to a stable plateau for the last three weeks. Of the nine pairwise differences in PSI observed during the 12-week trial ($P < 0.05$, Bonferroni post test), six were due to a single sampling date with a low PSI value (27 April 2006). On only one of the ten sampling dates (25 May 2006), did the PSI value denote significant similarity ($PSI \geq 60\%$) between the PRO and UNP large plankton assemblage taxa (Figure 5-62). This was in stark contrast to the deterministic PRO and UNP treatment-pair large plankton assemblage PSI time series values (Figure 5-46)

The PRO and CSM large plankton taxa bootstrap PSI value trajectory was similar to that observed for the MSI and PSI value trajectories of the PRO and UNP treatments (Figures 5-56, 5-62 – 5-63). A PSI minimum early in the trial (27 April 2006), gradually increased before falling to a stable value for the last few weeks. Of the eleven pairwise sampling period differences in large plankton taxa PSI values for the PRO and CSM treatments ($P < 0.05$, Bonferroni post test), seven were due to a single date (27 April 2006). PRO and CSM large plankton assemblages were found to be similar ($PSI > 0.60$) for only a single date (25 May 2006) among the ten dates examined.

The PRO and INO treatment-pair large plankton assemblage bootstrap PSI value trajectory differed somewhat from that of the other pairwise PRO treatment comparisons (Figures 5-62 – 5-64; PRO and UNP, PRO and CSM). The PRO and INO treatment-pair PSI minimum observed early in the study (27 April 2006), gradually increased to a plateau earlier and without the maximum peak and slight decline observed within the other PRO treatment PSI comparisons (Figures 5-62 – 5-63). Of the 12 pairwise differences in PRO and INO PSI values observed among the ten sampling dates (Figure 5-64; $P < 0.05$, Bonferroni post test), seven were due to a single low PSI value (27 April 2006), and the remaining five were due to a consecutive sample (4 May 2006), with a relatively low PSI value. Within the PRO and INO time series comparisons, only two nonconsecutive dates (25 May 2006 and 15 June 2006) were found to be similar ($PSI \geq 60\%$) among the ten dates (Figure 5-64).

UNP and CSM large plankton assemblage bootstrap PSI values followed a trajectory similar to those produced by the PRO pairwise treatment comparisons (Figures 5-62 – 5-65; PRO and UNP, PRO and CSM, PRO and INO). A PSI minimum slightly later than that encountered within the PRO treatment comparisons (5 May versus 27 April 2006), gradually increased to a

maximum that sharply decreased before settling to a plateau for the last few weeks. UNP and CSM assemblages were dissimilar for most of the ten weeks ($P < 0.05$, Bonferroni post test). Bootstrap PSI values were similar ($PSI \geq 60\%$) for only two consecutive weekly samples (18 May and 25 May 2006).

UNP and INO large plankton taxa bootstrap PSI values also had their minimum value on 4 April 2006 (Figure 5-66), but followed a PSI trajectory more similar to that of the PRO and INO treatment PSI values (Figure 5-64). PSI smoothly increased from a minimum value, before reaching a plateau earlier than within the other treatment pair comparisons (Figures 5-62 – 5-63, 5-65). Within the UNP and INO comparisons, eight pairwise PSI value differences that occurred among the ten dates ($P < 0.05$, Bonferroni post test), were due to a single date (4 May 2006), which had a low PSI value estimate. UNP and INO assemblages were similar ($PSI \geq 60\%$) for only two nonconsecutive samples (25 May and 15 June 2006) of the ten sampling dates, although two dates (18 May and 1 June 2006) had PSI values near the critical value (Figure 5-66).

The CSM and INO large plankton taxa bootstrap PSI values displayed a smoothly oscillating trajectory similar to their MSI counterpart (Figures 5-61, 5-67). Again, similar to the CSM and INO MSI time series values, no significant differences in MSI occurred among the ten dates ($P > 0.05$, Bonferroni post test). On only two nonconsecutive dates were the CSM and INO assemblages similar (Figure 5-67; 25 May and 15 June 2006). Additionally, the PSI values measured for these two samples were only marginally higher than the arbitrarily chosen 60% critical value (63% PSI on 25 May 2006, and 60% PSI on 15 June 2006).

Bootstrap PSI estimates also were performed with 20 resampled replicates drawn randomly with replacement (from the actual six replicate pond sample pool) to determine the effects of increased draws per iteration (with 1000 iterations per weekly PSI value estimate) would have

upon the similarity index (Figures 5-68 – 5-73). Overall large plankton assemblage PSI trajectory patterns remained the same among pairwise treatment combinations (Figures 5-62 - 5-73), but the overall variation in mean values was much lower, and greater “curve smoothing” occurred as draw numbers were increased (e.g., Figures 5-67, 5-73). Again, only five UNP treatment large plankton assemblages were present within the dataset for resampling estimation on 13 April 2006 because a replicate was lost due to human error (pond B1).

Discussion

Large Zooplankton Assemblage Production Volumes

Only one large zooplankton volume (mm^3/L of pond water) difference occurred among the four pond nutrient treatments and ten weekly pond water sampling dates (CSM and INO 8 June 2006; Figure 5-1). This indicated that large zooplankton standing stocks did not differ among treatments, and that presumably, prey availabilities also did not differ for potential large zooplankton predators (e.g., swordtails) within the different treatment ponds. Although large zooplankton standing stocks did not differ among treatments (except CSM and INO treatments on 8 June 2006), large zooplankton production rates may have differed among treatments if grazing rates differed sufficiently and occurred at high enough magnitudes among treatments to mask differential zooplankton production rates (trophic cascades) that may have occurred among treatment ponds (Carpenter et al. 1985, 1987, Post and McQueen 1987, Cohen et al. 2002).

When volume comparisons were performed among sampling dates within treatments, differences in large zooplankton volume were found within all nutrient treatments except the CSM treatment (Figure 5-2 - 5-5). Differences followed similar patterns in the PRO, UNP and INO treatments (Figures 5-2 – 5-3, 5-5). Low volumes/standing stocks were associated with the cooler, shorter photoperiod of late winter/early spring samples, which significantly differed from higher large zooplankton volumes/standing stocks that occurred during the warmer, longer

photoperiod late spring samples. Large zooplankton assemblage volume time series trajectories followed pond phytoplankton standing stocks as measured via chlorophyll [a] concentrations (Chapter 4; Figures 4-48 – 4-52). Although large zooplankton volumes increased from early to late spring, volumes decreased prior to the onset of summer. This may have been due to a number of factors, such as temperatures above those optimal for phytoplankton and zooplankton production, algal photoinhibition from increased solar insolation, decreased rainfall, lower oxygen tension due to increased pond metabolism from increasing temperature and increased biomass/organic loading, or other environmental factors, acting either singly or in concert. Alternately, or in addition to the above factors, biological factors such as limited large zooplankton food availability due to increased competition, species succession (competitive exclusion) or increased zooplankton grazing by predators, may also have been factors in reducing large zooplankton volume/biomass (Boyd 1974, Pearl and Slobodkin 1976, Yusoff and McNabb 1989, Diana et al. 1991, Knud-Hansen et al. 1993, Kastner and Boyd 1996, Ludwig et al. 1998, Brown et al. 2000, Saito et al. 2001, Hall 2004).

Time-averaged large zooplankton volumes (mm^3/L pond water) did not differ among nutrient treatments (Figure 5-6), which indicates that large zooplankton standing stocks, and their potential as a food source for fish did not differ among treatments. However, equal zooplankton standing stocks among treatments does not necessarily indicate equal zooplankton production rates among treatments - i.e., differential zooplanktivore grazing and/or zooplankton reproduction rates may have occurred among treatments. Interestingly, the pattern of small fish (≤ 31 mm SL) production numbers at harvest (Chapter 3; Figure 3-10), somewhat resembled the time-averaged large zooplankton standing stock volume trends among the four applied nutrient treatments (Figure 5-6). Although large zooplankton standing stocks did not significantly differ

among treatments, further research with more replicate ponds and/or larger water sample volumes may find a significant correlation between mean fish numbers/biomass at harvest and large zooplankton biomass.

Large Zooplankton Assemblage Production Numbers

Only one difference in large zooplankton density (plankton/L) was found among the four nutrient treatments and ten sampling periods (CSM on 8 June and INO on 8 June 2006). This was the same pairwise treatment and sampling date difference found for large zooplankton volumes (Figure 5-7). Within-treatment large zooplankton density comparisons among sampling dates found significant pairwise differences for the UNP, CSM, and INO treatments, but no differences among dates were found for the PRO treatment (Figures 5-8 – 5-11). Similar to large zooplankton volume results, pairwise differences in large zooplankton densities among sampling dates within the UNP, CSM, and INO pond treatments all involved low zooplankton densities that occurred early in the study, when pond biota were colonizing newly sterilized [hydrated lime application: $\text{Ca}(\text{OH})_2$] ponds during the colder, shorter photoperiod days (e.g., Figure 5-10; 13 April 2006) and for mid-trial dates, when large zooplankton standing stocks were significantly larger (e.g., Figures 5-9 – 5-11; 11 May 2006). Pairwise differences, among dates for the UNP, CSM, and INO treatments, all involved a single date with a high zooplankton density (11 May 2006), and earlier dates, when large zooplankton densities were low due to low water temperature, short photoperiod, etc.

As previously mentioned, diuron herbicide (Karmax®; DuPont Chemical Corp.) was applied to all ponds on 19 April 2006 to control filamentous algae, but did not appear to drastically reduce large zooplankton densities, as the herbicide was applied early in the trial, when standing stocks of planktonic algae and large zooplankton were relatively low (Figures 5-1 – 5-5, 5-7 – 5-11). A slight decrease in phytoplankton standing stocks may have occurred

immediately following diuron application, but the relatively rapid increase in chlorophyll [a] and large zooplankton volumes and densities following herbicide application, and the short half life of diuron in aqueous solution (~ 20 hours; EPA 2003), suggests that this decrease was short lived (Havens 1994, Perschbacher et al. 2002, Perschbacher and Ludwig 2004).

Large zooplankton assemblage densities followed similar time trajectories in all four treatments (PRO less pronounced variation): low numbers occurred for the first few weeks, numbers then steadily rose to a maximum value before declining slightly near the end of the trial (Figures 5-7 – 5-11). Significant differences in large plankton densities between sampling dates within treatments, usually resulted from comparisons between earlier sampling dates when large plankton numbers were extremely low, and higher plankton numbers during the middle and latter portions of the trial. Only the PRO treatment large zooplankton densities did not differ among sampling dates (Figure 5-8). The PRO and UNP large zooplankton assemblage density trajectories were somewhat flatter and more constant than the inorganic fertilizer treatment, and may have been due to the regular (twice daily) and even application of feed nutrients in contrast to the large periodic increases of nutrients from weekly applications of the inorganic liquid fertilizer (Boyd 1979, 1995, Kastner and Boyd 1996). CSM treatment large plankton assemblage density was fairly constant among sample dates, with differences due to a single sample interval (1 May 2006) when high numbers of large plankton were observed (Figure 5-10). Cottonseed meal application may have resulted in a slower, controlled release of nutrients within the CSM treatment ponds (Kastner and Boyd 1996, Tepe and Boyd 2003). Slower nutrient release and a potential as a directly ingestible food source for fish, may make organic plant materials such as cottonseed meal, a more attractive fertilizer option than inorganic liquid fertilizers. Slowly released nutrients likely allowed pond food webs to be sustained with a constant supply of

nutrients, and potentially under less ecosystem stress, by avoiding large oscillations in plankton numbers that could lead to plankton crashes and potential starvation. Sufficient food availability results in uninterrupted growth, higher reproduction rates, and enhanced immune system function for the target species (Dussault and Kramer 1981, Diana et al. 1991, Hopkins and Unwin 1997, James and Sampath 1998, Knud-Hansen 1998, Ludwig et al. 1998, Kruger et al. 2001, Lochmann et al. 2001, Royes and Chapman 2003, Ingram 2009). When zooplankton are heavily preyed upon by fish, high zooplankton densities have been shown to accelerate swordtail growth and recruitment rates (Kruger et al. 2001, Tamaru et al. 2001).

Additionally, slow-release fertilizers may improve water quality by preventing large spikes in pond ammonia and nitrite levels that can occur with the application of more volatile organic (e.g., animal manures) and inorganic fertilizers, high levels of which are stressors of fish, potentially resulting in lower growth and reproduction rates, increased disease susceptibility, and increased mortality. High pond biomass conditions have a greater potential for anoxic or hypoxic conditions due to a large biomass of respiring organisms that can consume available dissolved oxygen in excess of oxygen production during prolonged overcast conditions and/or anoxic bottom water displacement following heavy rains, or oxygen depletion from a high biomass of decaying organisms following a large algal die off, all of which can create the potential for catastrophic production losses (Tepe and Boyd 2003).

The use of control ponds with broodstock, but without intensive nutrient addition, would have been useful in determining zooplankton volumes (standing stocks) and community composition in the presence of a presumed increase in predation pressure (no allochthonous nutrient inputs) from an omnivorous, non-demersal, visual predator fish species (Dussault and Kramer 1981, Axelrod 1991, Jones et al. 1998, Tamaru et al. 2001). Additionally, control ponds

without broodstock fish could have provided useful baseline information regarding large zooplankton volumes and community composition in the absence of fish predation.

Unfortunately, neither ‘no nutrient’ nor ‘no broodstock’ control treatments were incorporated into this study’s experimental design.

Outdoor Pond Trial Major Large Zooplankton Groups

The three major large zooplankton taxa which had the highest %IRI values over the 12-week trial were copepods, rotifers and daphnids (Figures 5-13 – 5-20, 5-37). Of the three major large zooplankton taxa, two (*Diaptomous* copepods and *Filinia* rotifers) followed similar population trajectories within the four pond nutrient treatments (Figures 5-21, 5-25, 5-29, 5-33). Typically, initial zooplankton numbers were low as ponds were slowly colonized following pond draining and sterilization, gradually rising to a maximum near mid-trial, followed by a slight decrease during the latter half of the trial; this pattern was observed for both *Diaptomous* copepods and *Filinia* rotifers (Figures 5-21 - 5-23, 5-25 – 5-27, 5-29 – 5-31, 5-33 – 5-35). Daphnid (*Moina macrocopa*) population numbers followed a different pattern, in which daphnids were completely absent from samples until near mid-trial, when numbers would slowly increase until trial termination (Figures 5-24, 5-28, 5-32, 5-36).

Among the major large zooplankton taxa, copepods and rotifers appeared to be early colonizers, and displayed high reproductive potentials typical of an early succession or ‘r’ selected colonizer species, whereas daphnids did not typically appear until mid-trial, and once observed, their numbers slowly and steadily increased, appearing to display lower reproductive potential, possibly through superior competition, typical of a ‘K’ selected climax species (Pianka 1970, Paine 1980, Post and McQueen 1987).

Copepod Density for 10 Weekly Sampling Periods

Copepod densities did not differ among nutrient treatments, but did differ between sampling dates within individual pond nutrient treatments (Figures 5-22, 5-26, 5-30, 5-34). Significant differences in copepod densities between sampling dates within treatments were usually due to low copepod densities and low variation among replicate ponds at the beginning of the 12-week trial and much higher densities and greater variation among replicates at mid and late trial. Copepods appeared to follow an early colonizer or an 'r' species reproductive strategy within the experimental ponds, and followed the same general population growth trajectory regardless of experimental treatment over the twelve week trial.

Rotifer Density for 10 Weekly Sampling Periods

Rotifer density trajectories were similar to that of copepods in the four pond nutrient treatments and density differences were not significant between treatments for any given sampling period. However, rotifer density differences between sampling periods were significant within nutrient treatments. The general pattern observed for *Filinia* rotifer densities was similar to that found for *Diaptomous* copepods: low numbers at the onset, were followed by a gradual increase to a maximum value approximately midway through the study, with a slight decline toward the end (Figures 5-23, 5-27, 5-31, 5-35). Extremely high rotifer counts for a single pond within the CSM treatment and a single pond within the INO treatment on the same sampling date (11 May 2006), produced high mean values and large 95% confidence intervals for the CSM and INO treatments for this date (Figures 5-31, 5-35). These high counts also were responsible for the significant pairwise differences in *Filinia* rotifer densities among sampling dates within the CSM and INO treatments. All pairwise differences in rotifer density between dates involved the 11 May 2006 sample within the CSM and INO treatments.

Daphnid Density for 10 Weekly Sampling Periods

Daphnid densities differed among pond nutrient treatments, but only for a single date (8 June 2006), which had relatively high densities for the PRO and INO treatments, and relatively low densities for the CSM treatment (Figures 5-24, 5-32, 5-36). This single date also generated pairwise differences in daphnid density among sampling dates within treatments, all PRO and INO daphnid density differences among sampling periods involved this date (Figures 5-24, 5-36). A different date (15 June 2006), with a high daphnid density, generated all of the pairwise differences among sampling dates detected within the UNP treatment (Figure 5-28).

Interestingly, CSM daphnid densities did not differ among dates, which may have been due to the 'slow release' nature of the cottonseed meal as opposed to the nutrient spikes of the inorganic fertilizer. This possibly resulted in gradual *Moina macrocopa* population changes within the CSM ponds (Figure 5-36), due to a constant phytoplankton standing stock prey pool for daphnids.

Pond Large Zooplankton Assemblage %IRI Trends

As previously mentioned, the three primary large zooplankton groups according to %IRI rankings were copepods, rotifers and daphnids; twenty-seven other taxa were routinely found in the samples, but in lower numbers and/or in fewer samples. Copepods were the most important item within all four pond nutrient treatments (Figures 5-13 – 5-20). Daphnids increased in importance late in the trial and until trial termination a few weeks later in mid-June 2006.

Interestingly, %IRI trajectories of the three major groups were basically similar within all four treatments, copepod numbers underwent gradual fluctuations but maintained their dominance, rotifer numbers underwent large increases and declines, and daphnids underwent a steady increase in abundance after being absent during the first half of the trial. Most interesting was an inverse %IRI trajectory relationship between the copepod and rotifer groups, evident in their

%IRI graphs (Figures 5-17 – 5-20); plots of the two trajectories produced roughly inverse images of each other. Also, when rotifers were at a low %IRI, typically near the end of the trial, the daphnid group would appear and mirror the copepod group's %IRI trajectory. The inverse relationship between copepod and rotifer abundances was most likely due to copepod predation on rotifer standing stocks or possibly competition between these groups for a limited phytoplanktonic food source. In the case of the inverse %IRI trajectories for copepods and daphnids, the dominance of daphnids at the end of the trial was most likely due to daphnids being superior competitors, typical of a 'K' strategy life history species and not due to predation of daphnids upon copepods, based on their similar sizes and feeding habits (Pianka 1970, Brett et al. 1994, Mallin and Paerl 1994, Burns and Schallenberg 2001a, 2001b).

Densities of the three major large zooplankton groups (copepods, rotifers, daphnids) also were individually graphed for each pond nutrient treatment over the 12 weeks of the trial, but the inverse %IRI relationships observed between copepods relative to rotifers and daphnids were not as readily apparent for taxa densities (Figures 5-21, 5-25, 5-29, 5-33). Arguably, copepod numbers and rotifer numbers were weakly inversely proportional to each other for the PRO and UNP pond nutrient treatments. Daphnids, which are considered superior competitors, have been known to frequently out compete copepods, which are considered early colonizers (Paine 1980, Gilbert 1988, 1989, Larson et al. 2007).

Similar to large plankton assemblage taxa %N and %V estimates (Appendix 5A), %IRI were calculated using both pooled and unpooled data. Pooled %IRI data displayed large plankton assemblage taxa trends within treatments as a whole (Figures 5-13 – 5-16), whereas unpooled %IRI data stressed mean trends occurring among the different large plankton assemblage taxa over time (Figures 5-17 – 5-20) and individual variation among replicate ponds

within treatments. Unpooled %IRI large plankton assemblage time series plots appear to be slightly less erratic than those of pooled %IRI plots (e.g., Figures 5-15, 5-19; CSM treatment), which was expected as these %IRI values were derived from averaged large plankton assemblage taxa %N and %V data.

Deterministic Similarity Indices

Deterministic simplified Morisita's similarity indices (utilizing pooled %IRI) between large plankton assemblage taxa of the four nutrient treatments oscillated erratically between treatment pairs, but generally followed similar trends among the six possible pairwise treatment comparisons (Figure 5-38). Similarity between the large plankton assemblages of different treatments was high at the start of the trial, probably due to the general low diversity and lack of large zooplankton following recent pond sterilization, cold water temperatures, and short photoperiods associated with late winter/early spring. High water clarity (Secchi disk readings, pers. obs.) and low chlorophyll [a] concentrations (Chapter 4; Figures 4-48 – 4-51) at the beginning of the trial, indicated that insufficient time had elapsed following pond sterilization, and/or the previously cited factors, may have prevented large 'algal blooms' within ponds. Low phytoplankton concentrations would provide insufficient food for many herbivorous zooplankton, which also would retard herbivorous, carnivorous, and omnivorous zooplankton numbers/biomass production. Additionally, colonization of these ponds from neighboring ponds via eggs/spores/cysts/juvenile/adults, attached to insects or water fowl, wind borne, or from resting egg/cysts within the pond sediments, may not have hatched/emerged due to unsuitable environmental conditions (cold temperatures, short photoperiod) and/or lack of sufficient time following pond sterilization (pH shock) and subsequent refilling with ground and well water.

Pond large plankton assemblages became increasingly diverse over time (Appendix A; Figures 5A-1 - 5A-80). Among the majority of pairwise treatment combinations, after a slight

increase in MSI values, a sharp decrease occurred on one of two sampling dates (27 April or 4 May 2006), this marked decrease occurred between most treatments pairs (Figures 5-56 – 5-60), but only a slight decrease occurred between the CSM and INO treatments (Figure 5-61). This sharp decrease in MSI likely occurred due to diuron herbicide application on 19 April 2006, although similarity indices did not decrease uniformly among all pond treatment pairs compared. MSI quickly rebounded, then dropped slightly before reaching a plateau of uniformly high values ($MSI \geq 0.65$) among all six treatment pair combinations (Figure 5-38 – 5-44). Of the six possible pairwise treatment combinations, the UNP and CSM treatments were consistently the most similar, with their MSI ranging from 0.67 to 1.0, and with much lower fluctuations among sampling periods (Figure 5-42). Interestingly, aside from a single sampling date with a low MSI value, the PRO and INO treatments had consistently similar large plankton assemblages ($MSI > 0.65$) among the ten sampling dates (Figure 5-41).

The MSI significance level of 0.65 was arbitrary chosen by previous researchers, and does not signify actual functional similarities between assemblages, especially when comparing studies and different ecosystems (Zaret and Rand 1971, Cailliet and Barry 1978, Lindquist 1998). Additionally, the MSI was designed to compare resource utilization differences among predator groups, rather than plankton assemblage community differences among treatments (Krebs 1998).

Deterministic MSI values between treatment pairs (Figure 5-38) indicate that large plankton assemblage differences between treatments were more a function of time rather than applied nutrient type. The trajectory that large plankton assemblage similarities followed did not appear to be a function of nutrient type, i.e., the large plankton assemblages of the two commercial feeds did not display a greater similarity to each other than either did to the two

fertilizer treatment, and vice versa. All large zooplankton assemblages appear to have converged over time, becoming more similar over the course of the 12-week pond trial (Figures 5-38 – 5-44). All treatment pair combinations had significantly similar large plankton assemblage MSI values ($MSI > 0.65$) for the last two weeks of the trial (Figures 5-39 – 5-44), while for the second to last week of the trial, the CSM and INO treatment MSI were just significant at 0.65 (Figure 5-44).

Large zooplankton assemblage taxa deterministic percent similarity index values (using pooled %IRI) between treatments did not follow any general trends (Figure 5-45). PSI values were uniformly high throughout the 12-week trial, and did not appear to be noticeably higher between any two treatment pairs (Figures 5-46 – 5-51). Within all six possible treatment pair combinations, only a single large plankton assemblage treatment pair/sampling date dissimilarity occurred, which was between the PRO and CSM treatment pairs (27 April 2006, $PSI = 55$). The sharp decline observed in MSI observed for 27 April or 4 May 2006 (Figures 5-38 – 5-44), did not translate into sharp declines in PSI for these dates, as declines were either much lower in magnitude (Figures 5-46 – 5-48, 5-51) or largely absent (Figures 5-49 – 5-50). Unexpectedly, PRO and UNP PSI value trajectories oscillated among sampling dates to the greatest degree among the six pairwise treatment combinations (Figure 5-46). Due to the similarity of the two applied feed nutrients, the PRO and UNP time series PSI values were expected to be the most similar (highest PSI values) and least variable among sampling dates within the different treatment combinations. The UNP and CSM, and UNP and INO PSI trajectories appeared to display the smoothest transitions among sampling dates (Figures 5-49 – 5-50). This may indicate that unprocessed ‘uncooked meal’ feed may have acted in a manner similar to the two fertilizer nutrients within the UNP pond ecosystems, rather than the processed (cooked via heat

extrusion) and reground PRO feed, which was designed to be directly ingested by the fish as a highly metabolizable, high-quality nutrient source.

PSI value trajectories did not appear to indicate increasing large plankton assemblage similarity over time as indicated by some MSI trajectories (Figures 5-39 – 5-44). The paucity of dissimilar large plankton assemblage PSI sampling date treatment pair combinations, relative to the numerous dissimilar Morisita's index time series large plankton assemblage differences within treatments tends to indicate that MSI is superior to the PSI in detecting large plankton assemblage taxa differences (higher index sensitivity). This also was supported by the working hypothesis that large plankton assemblage community differences between treatments would tend to be present earlier in the study. At the start of the trial, large zooplankton community taxa successions had not yet occurred and low phytoplankton standing stocks/productivity were well below carrying capacity. At the onset of the study, even small differences in large zooplankton assemblage composition would be amplified by the similarity indices, due to the low number of zooplankton taxa and their relative abundances (Krebs 1998). Large zooplankton taxa numbers and abundances increased later in the study. MSI values indicated that large plankton assemblages were more dissimilar earlier in the study, while PSI indicated that large plankton assemblages were similar throughout the study. Additionally, MSI may have a greater ability than PSI to detect major disturbances to a pond's large plankton assemblage, such as when diuron was applied on 19 April 2006, which likely affected large plankton assemblages sampled on 20 and 27 April 2006 (Figures 5-38 – 5-44). In contrast to MSI, PSI did not noticeably change during this time (20 and 27 April 2006), with the possible exception of the PRO and CSM PSI values (Figure 5-47).

The number of similar ($MSI \geq 0.65$) large plankton assemblages that occurred between pairwise treatment comparisons among the ten weekly sampling periods differed depending upon whether MSI values were deterministically derived using pooled %IRI values (Figures 5-39 – 5-44), or obtained from bootstrap generated MSI values using taxon %N (Figures 5-56 – 5-61). Likewise, the number of similar ($PSI \geq 60\%$) large plankton assemblages that occurred between treatment pairs, also differed greatly depending upon whether PSI was deterministically estimated based upon pooled %IRI (Figures 5-46 – 5-51) or via bootstrapping using taxon %N (Figures 5-62 – 5-67).

The difference in large plankton assemblage similarity frequencies between treatment pairs ranged between 0 and 7 for MSI, and between 8 and 9 for PSI calculated deterministically using %IRI values or via bootstrap resampling methods using %N (Table 5.2). Closer examination of large plankton assemblage similarity frequencies among pairwise treatment combinations, indicate that MSI frequency differences appeared to be much more consistent and robust than PSI frequency differences ($\bar{X} = 3.33, MSI$; $\bar{X} = 8.33, PSI$) for both the deterministic and bootstrap methods, even when considering that some of the discrepancies in the number of similar large plankton assemblages observed between the deterministic and bootstrap similarity estimates were due to the different plankton community metrics (%IRI versus %N) used to calculate each of the indices (MSI, PSI).

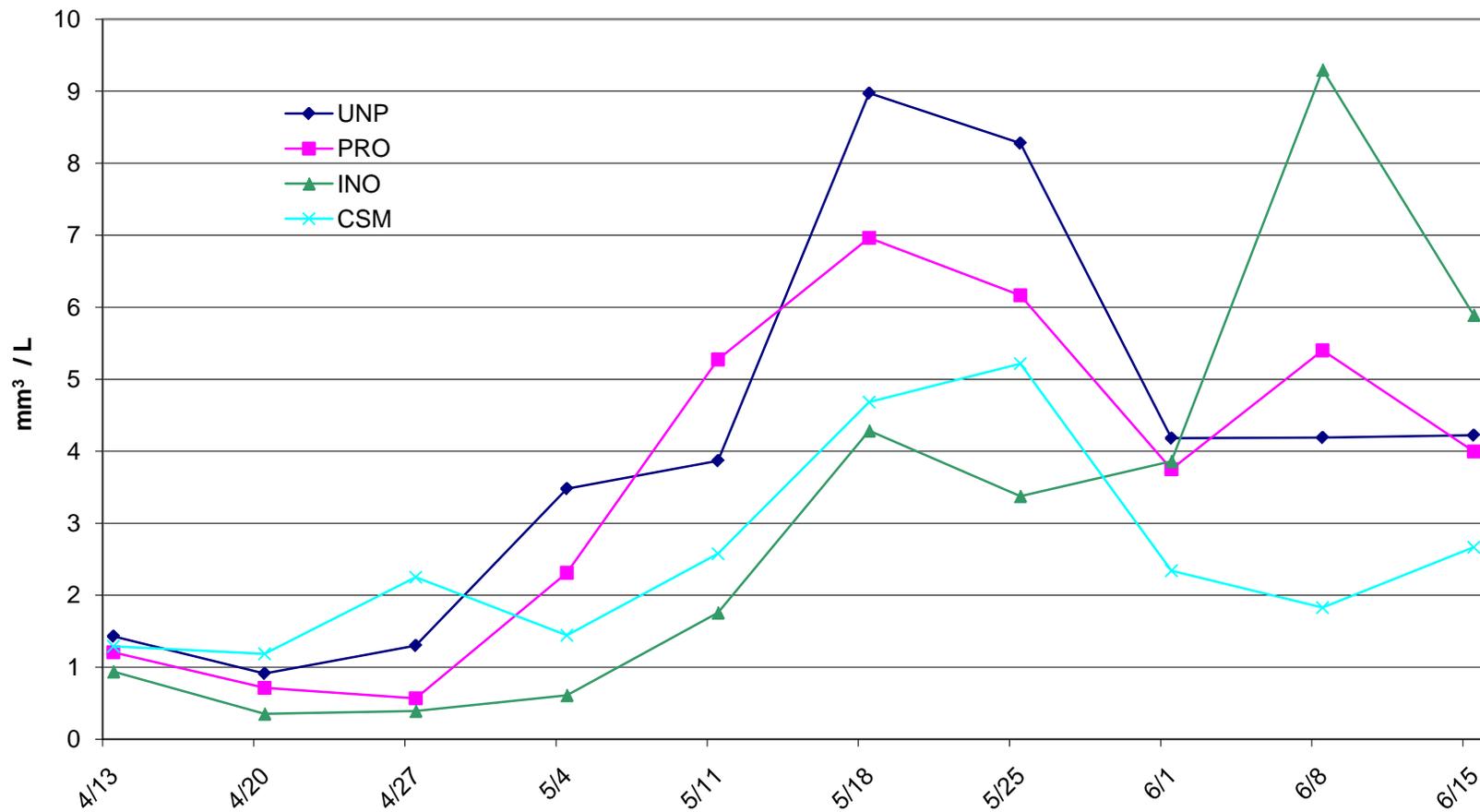


Figure 5-1. Large plankton (> 200 μm) assemblage mean volumes for 10 weekly sampling periods for four pond nutrient treatments: processed feed (PRO), unprocessed feed (UNP), cotton seed meal (CSM), and inorganic liquid fertilizer (INO); $n = 6$ ponds per treatment.

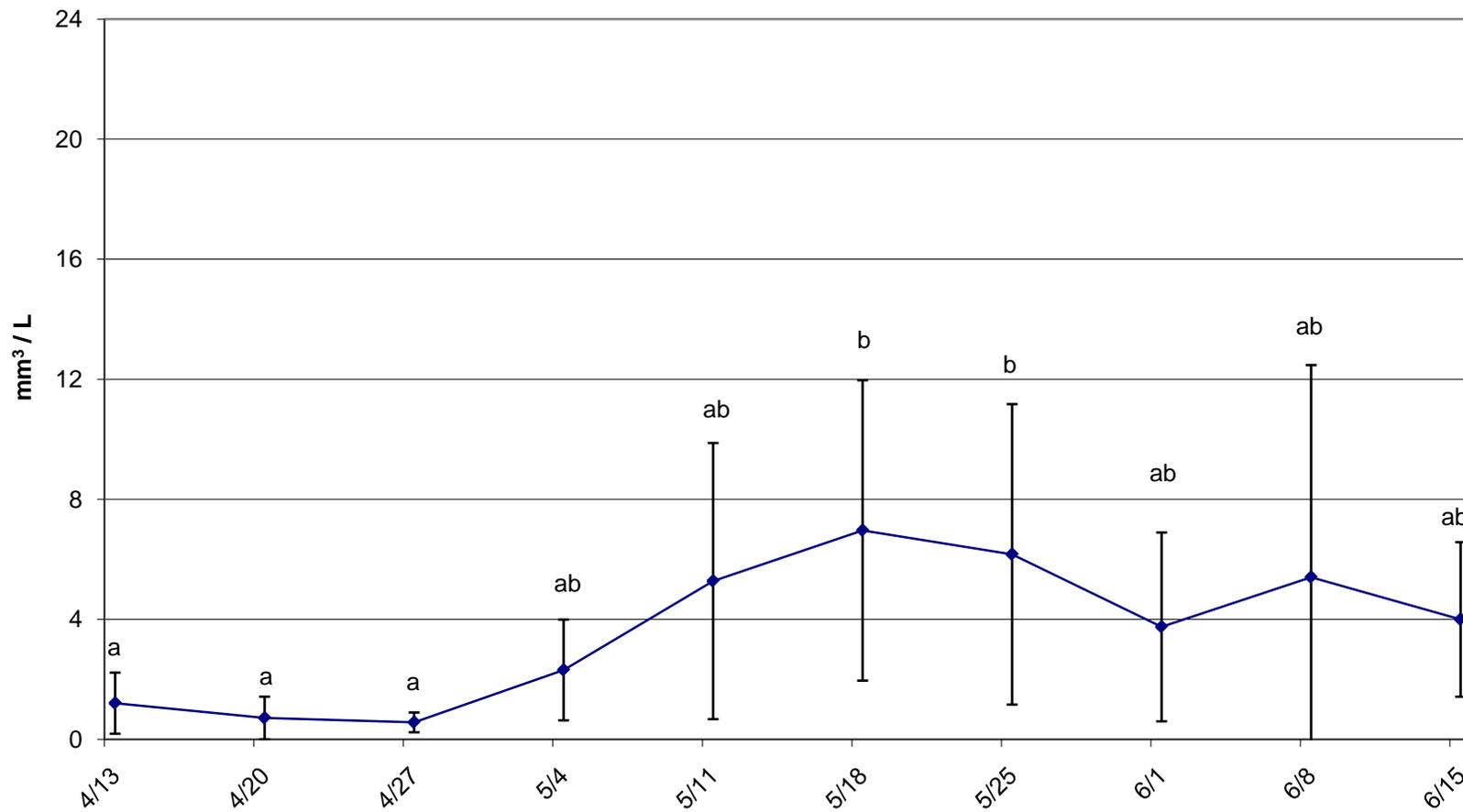


Figure 5-2. Processed feed (PRO) treatment mean large plankton (> 200 μm) assemblage volumes ($\text{mm}^3/\text{L} \pm 95\% \text{ CI}$) for 10 weekly sampling periods, unshared letters denote significant differences between sampling periods ($P < 0.05$ Bonferroni post test); $n = 6$ replicate ponds.

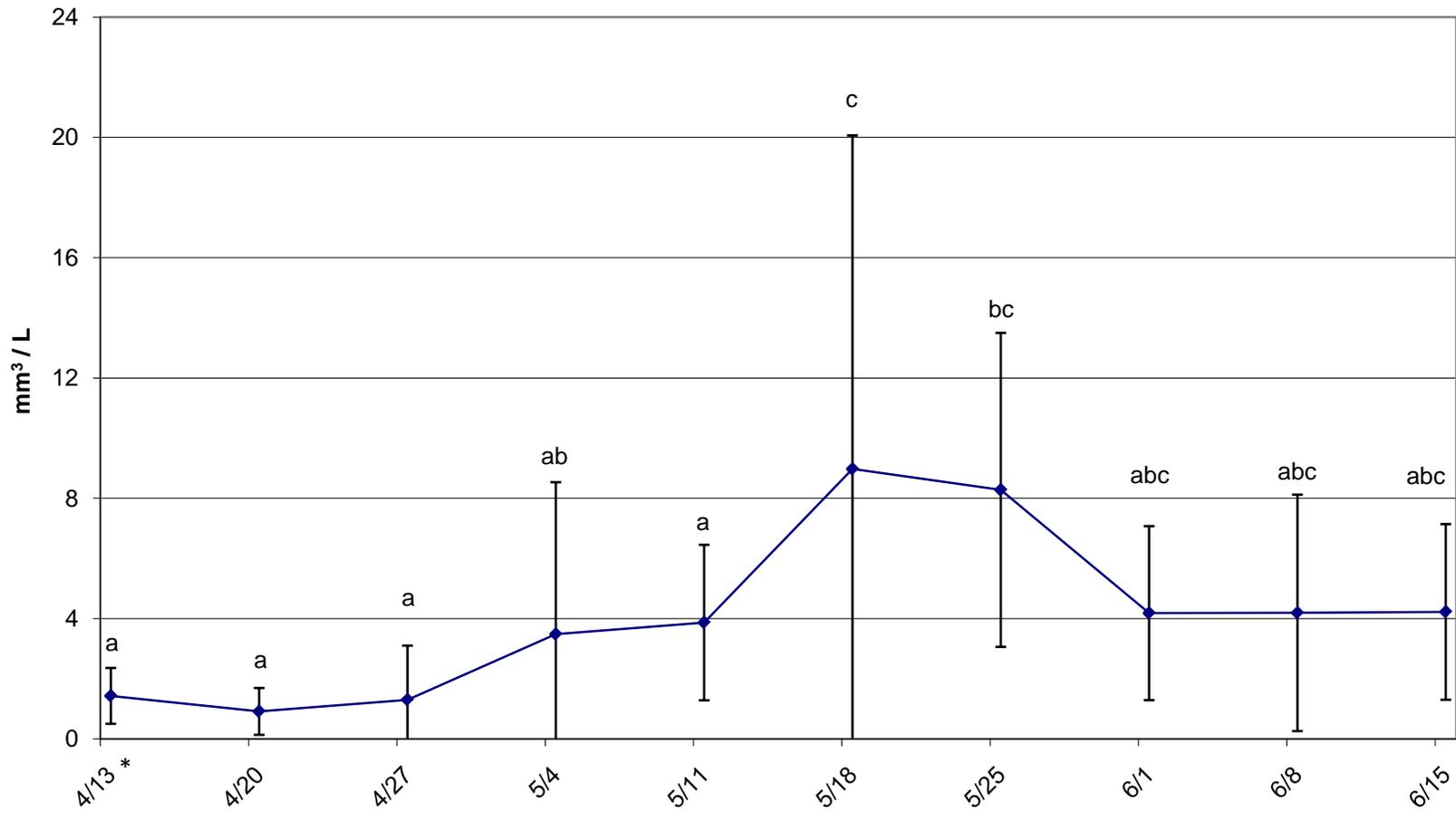


Figure 5-3. Unprocessed feed (UNP) treatment large plankton (> 200 μm) assemblage mean volumes ($\text{mm}^3/\text{L} \pm 95\% \text{ CI}$) for 10 weekly sampling periods, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ replicate ponds (* only five replicate samples available for this date).

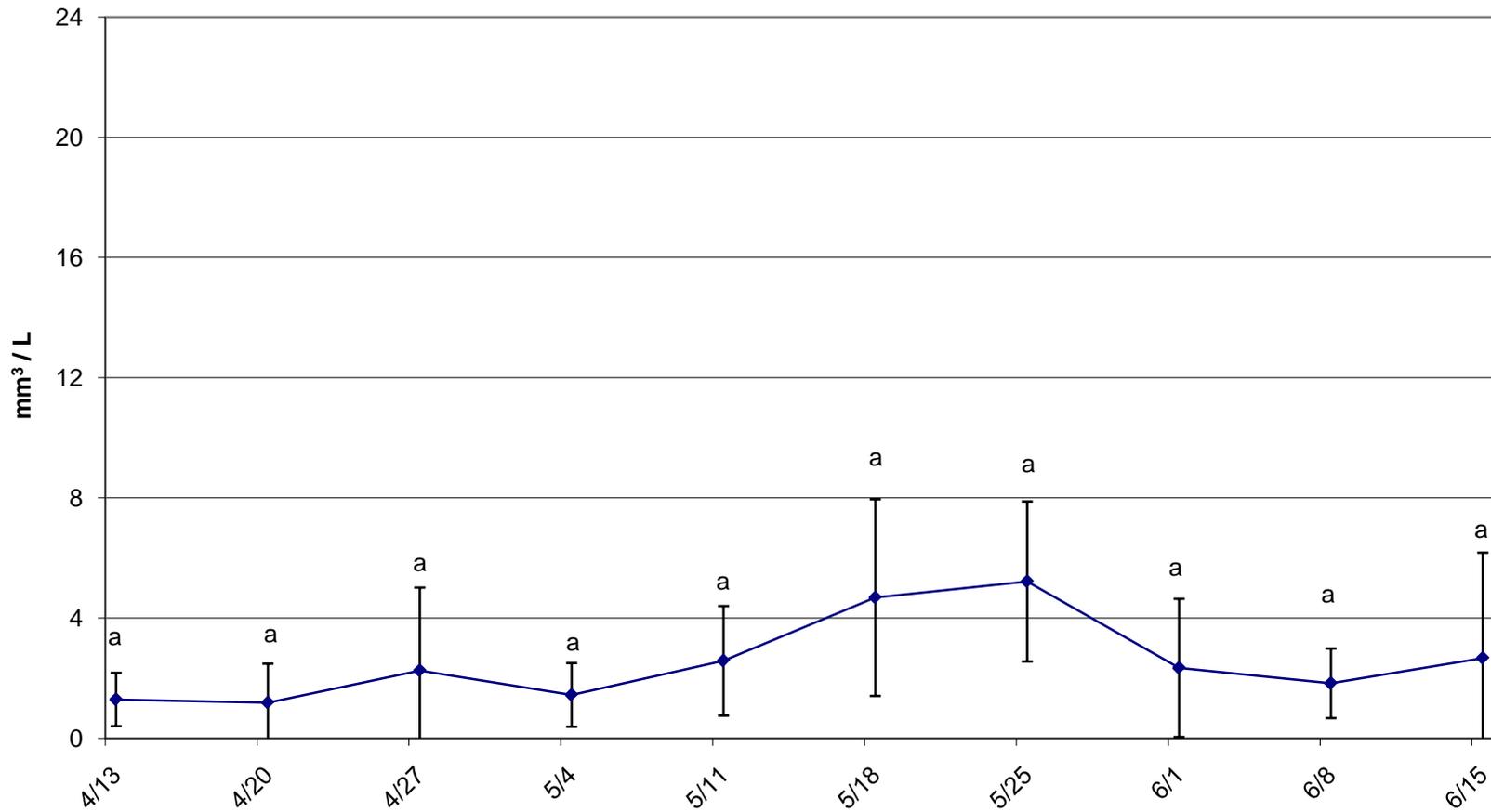


Figure 5-4. Cottonseed meal (CSM) treatment large plankton (> 200 μm) assemblage mean volumes ($\text{mm}^3/\text{L} \pm 95\% \text{ CI}$) for 10 weekly sampling periods, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ replicate ponds.

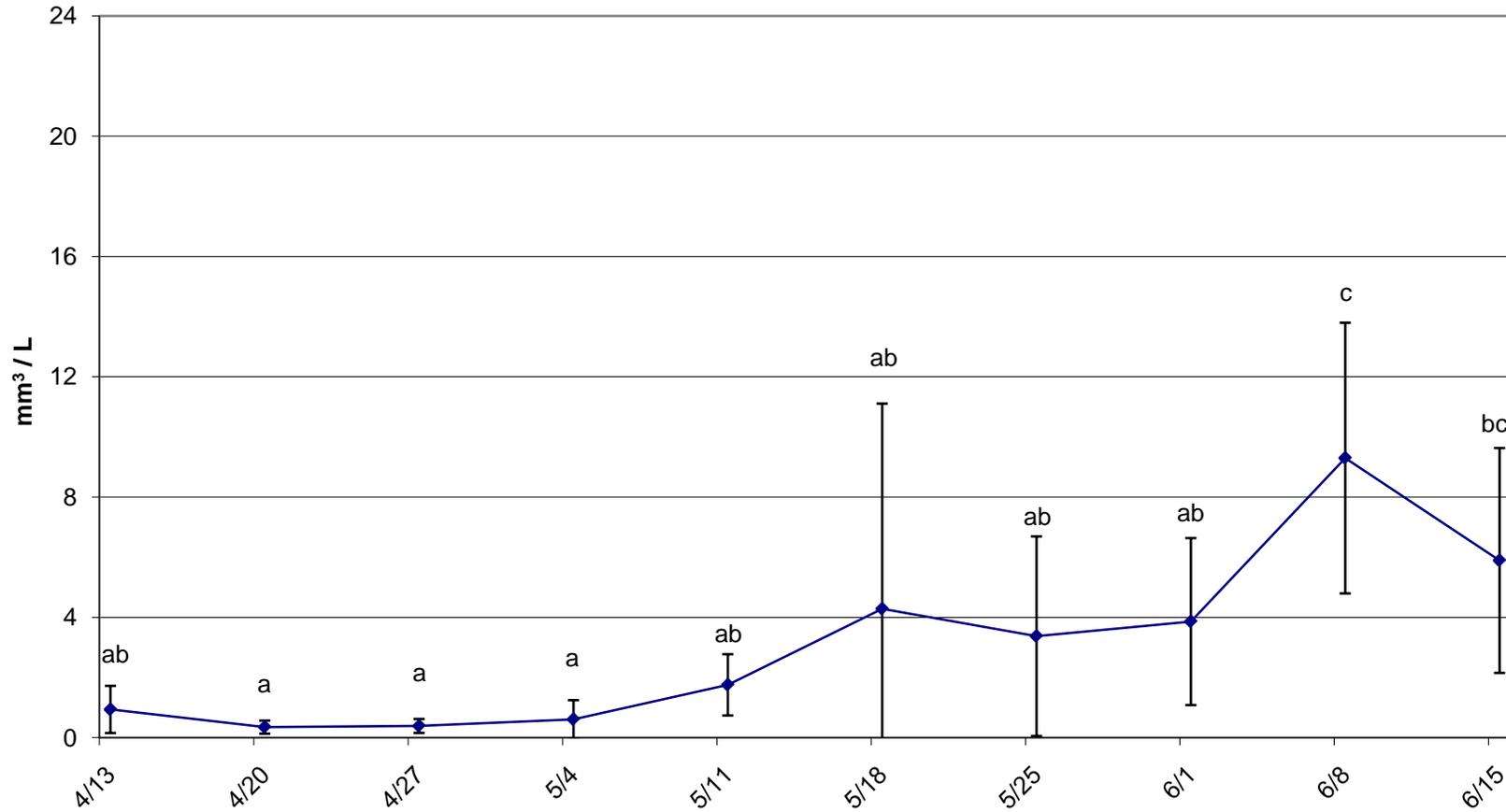


Figure 5-5. Inorganic fertilizer (INO) treatment large plankton (> 200 μm) assemblage mean volumes ($\text{mm}^3/\text{L} \pm 95\% \text{ CI}$) for 10 weekly sampling periods, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ replicate ponds.

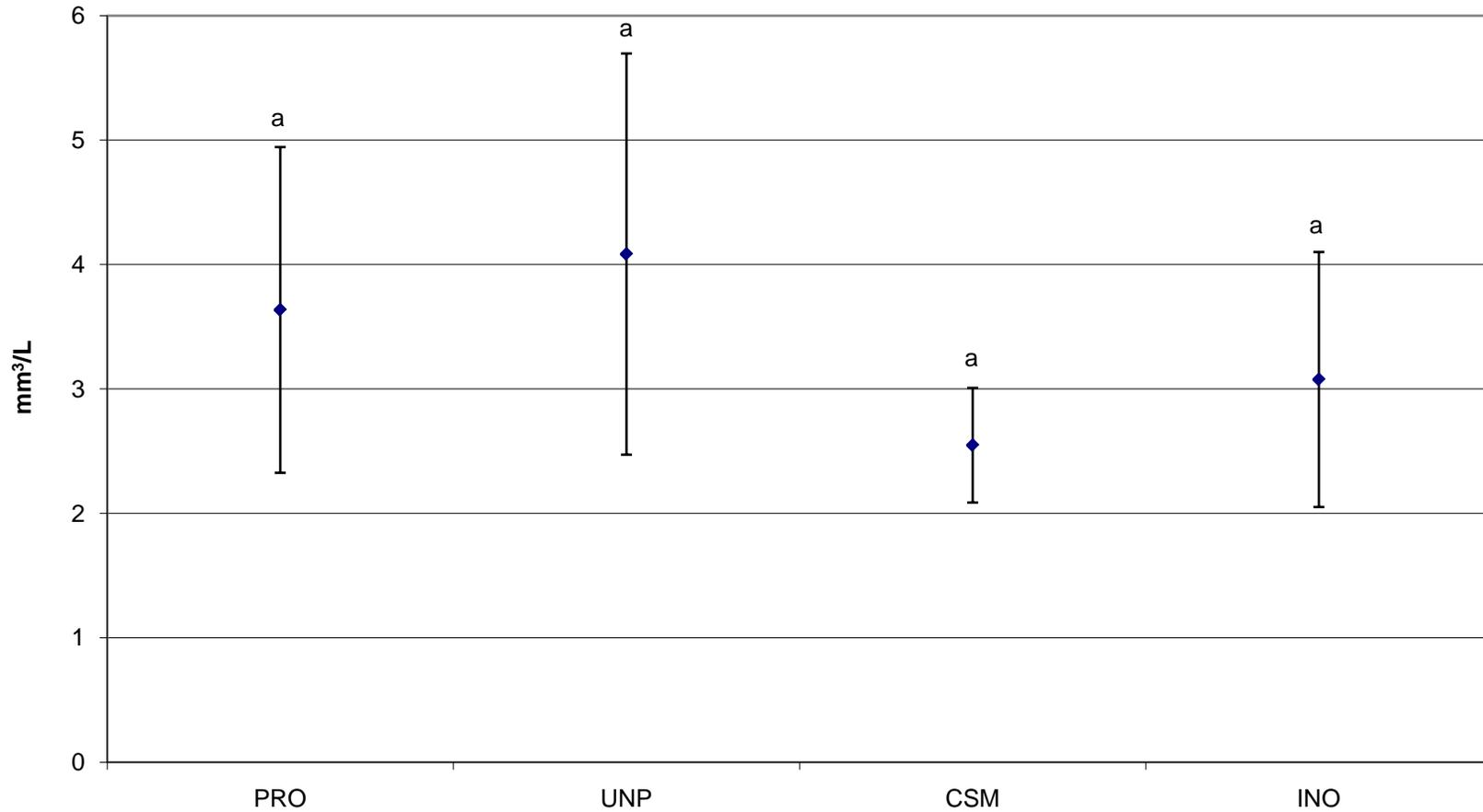


Figure 5-6. Time-averaged large plankton (> 200 μm) assemblage mean volumes ($\text{mm}^3/\text{L} \pm 95\% \text{ CI}$) for four nutrient treatments; unshared letters denote statistical differences between treatments ($P < 0.05$, Tukey's multiple comparison test); $n = 6$ ponds per treatment.

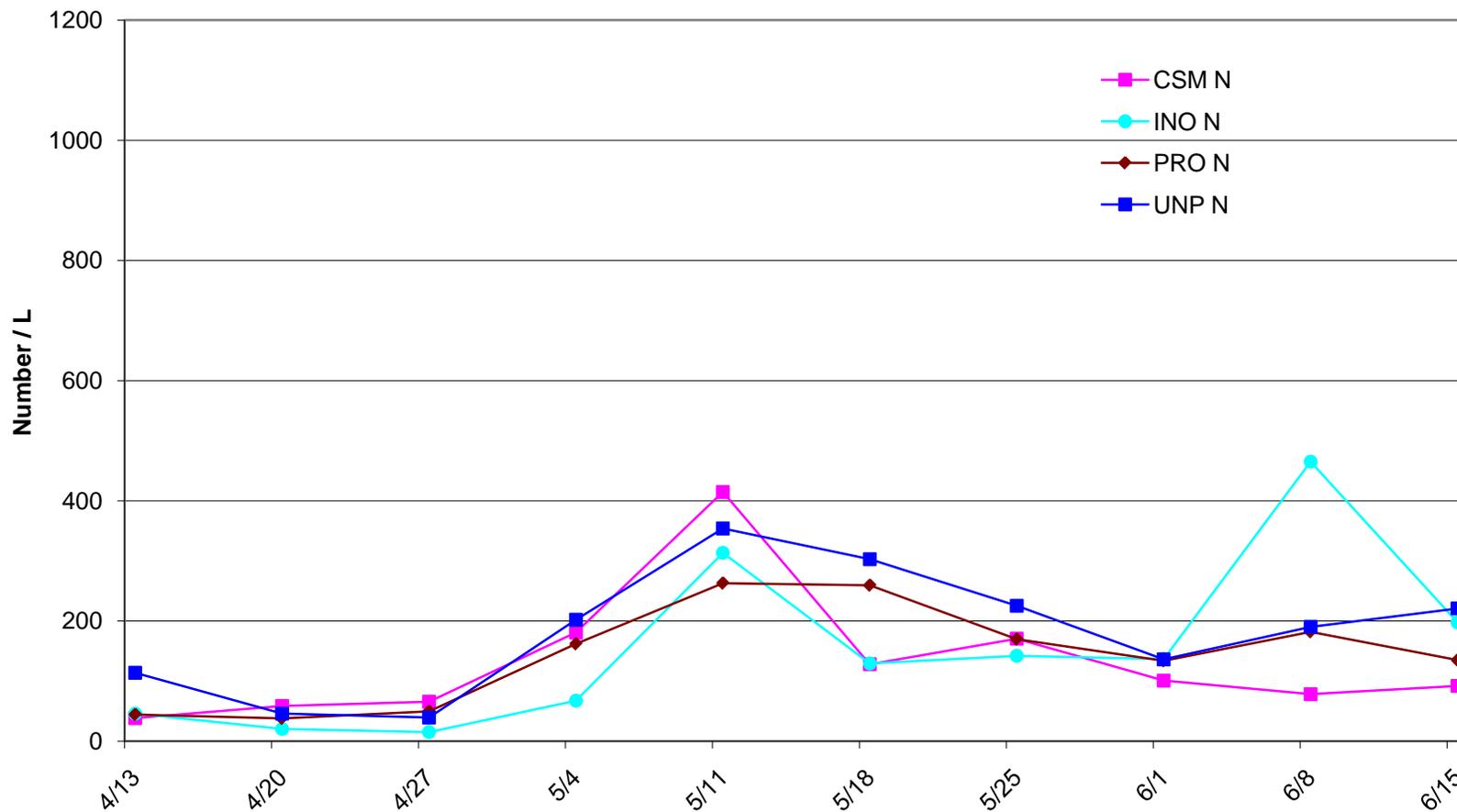


Figure 5-7. Large plankton (> 200 μm) assemblage mean densities (number/L) among 10 weekly sampling periods for four nutrient treatments: processed feed (PRO), unprocessed feed (UNP), cottonseed meal (CSM), and inorganic fertilizer (INO); n = 6 ponds per treatment.

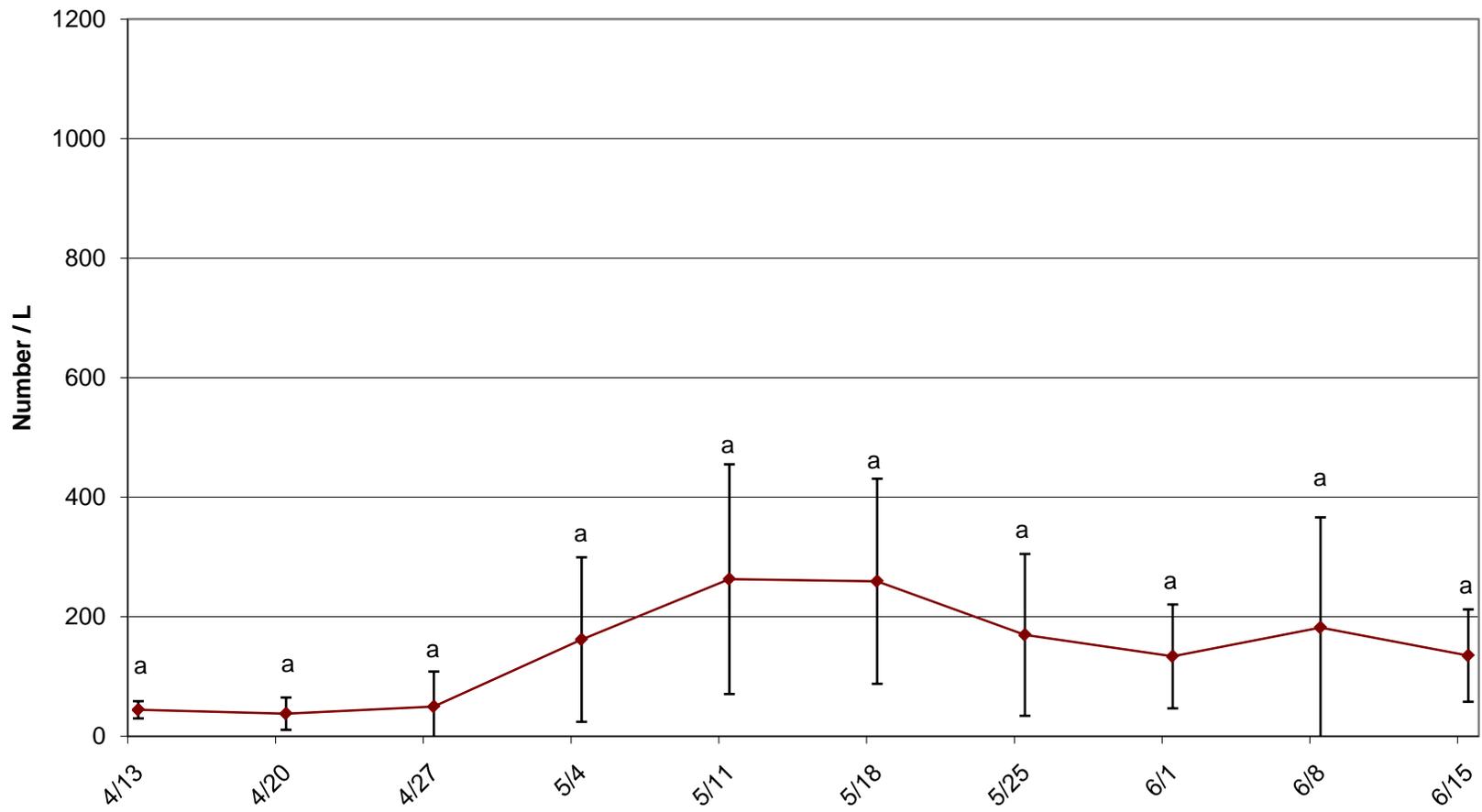


Figure 5-8. Processed feed (PRO) treatment large plankton (> 200 μm) assemblage mean densities (number/L ± 95% CI) among 10 weekly sampling periods, unshared letters denote statistical differences between sampling periods (P < 0.05, Bonferroni post test); n = 6 replicate ponds.

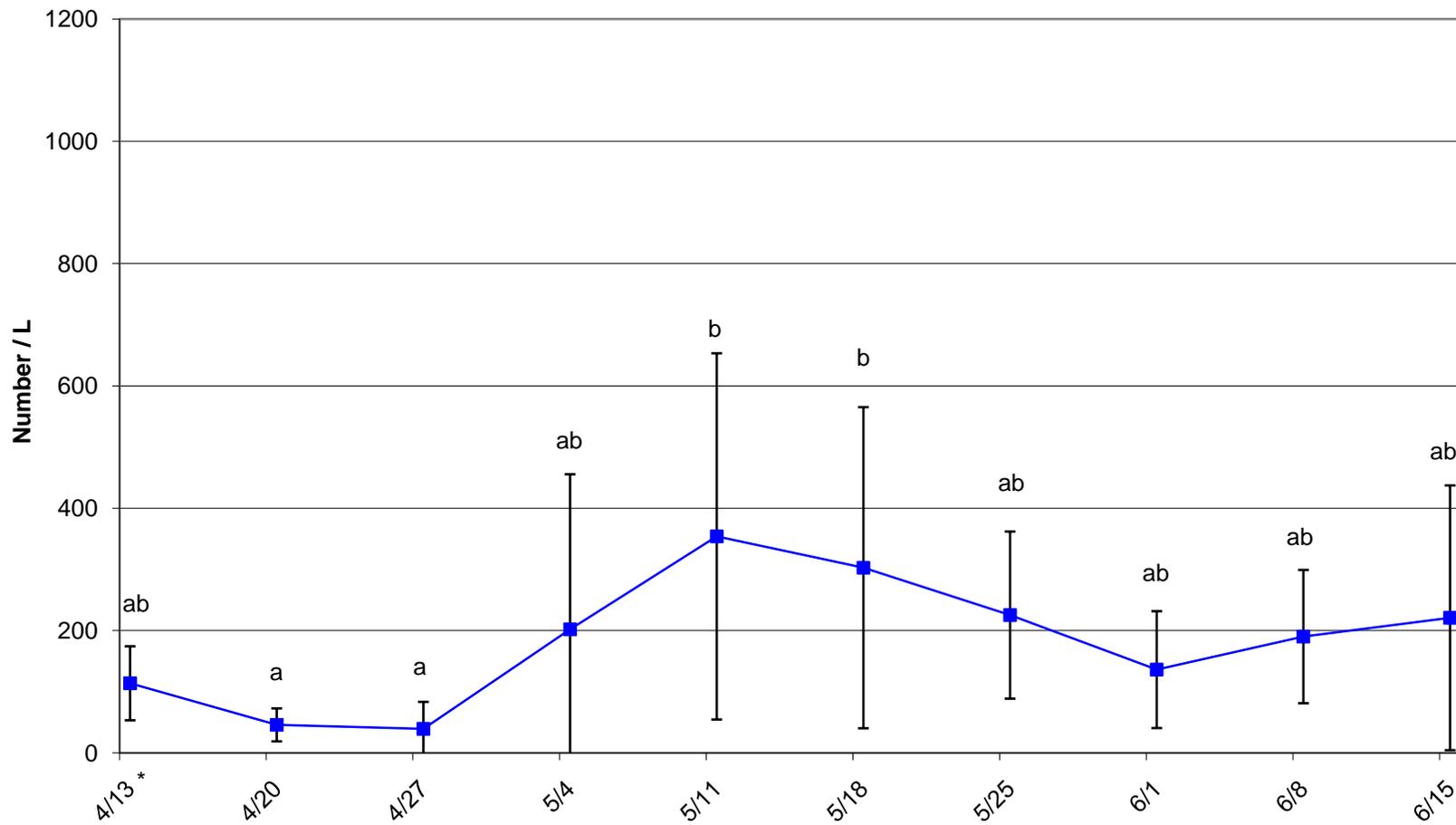


Figure 5-9. Unprocessed feed (UNP) treatment large plankton (> 200 μm) assemblage mean densities (number/L \pm 95 % CI) among 10 weekly sampling periods, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ replicate ponds, * only 5 replicate samples available for this sampling date.

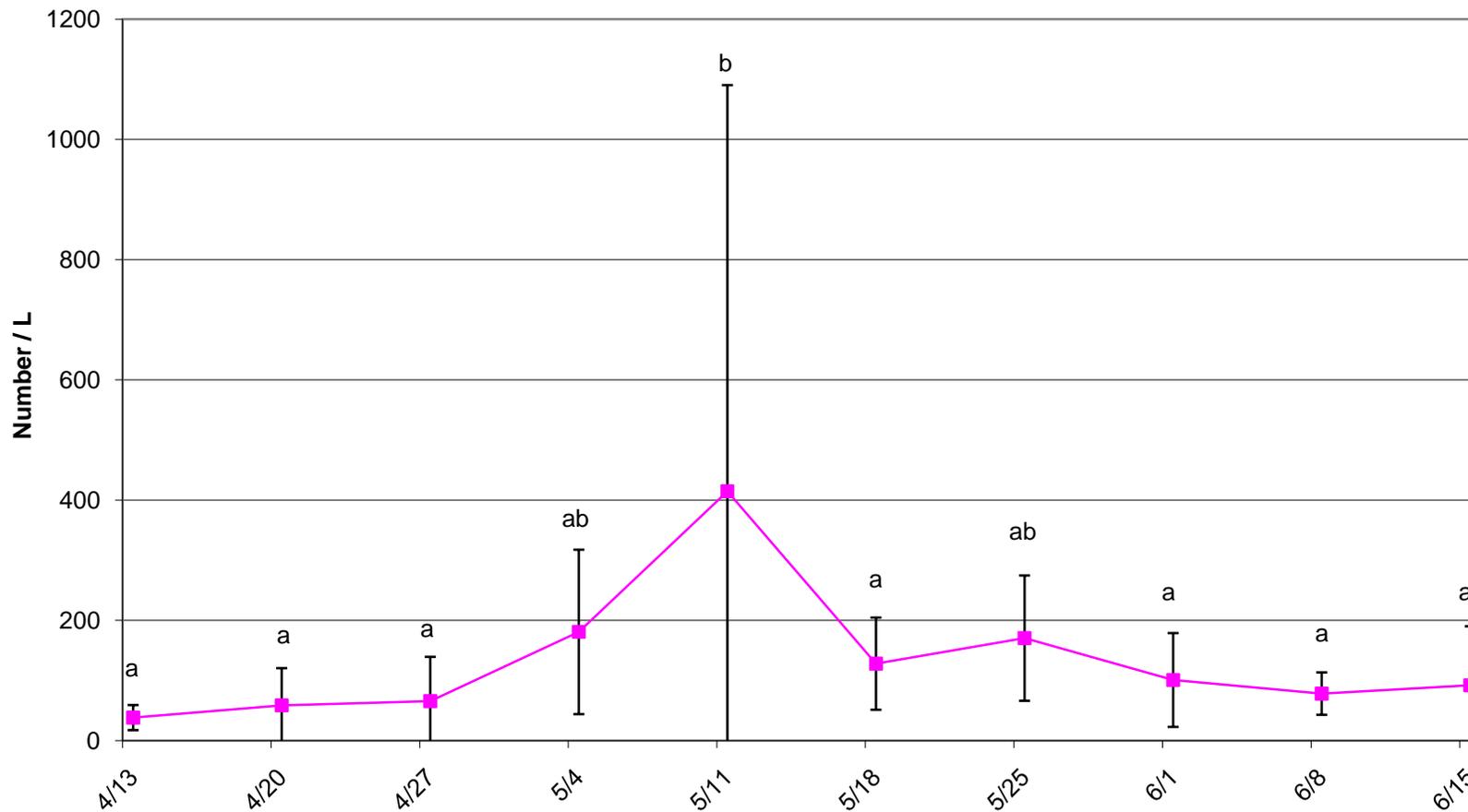


Figure 5-10. Cottonseed meal (CSM) treatment large plankton (> 200 μm) assemblage mean densities (number/L \pm 95 % CI) among 10 weekly sampling periods, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ replicate ponds.

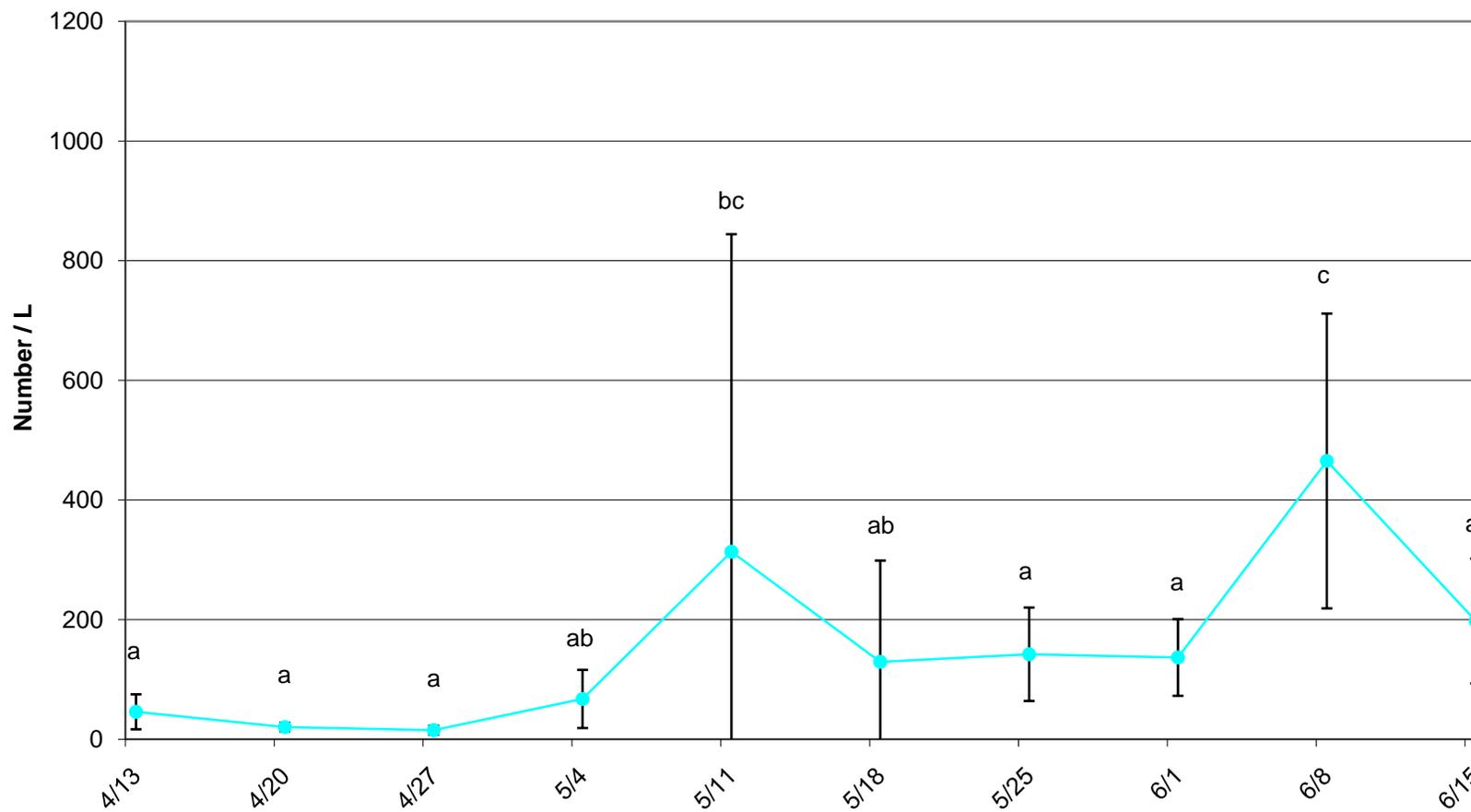


Figure 5-11. Inorganic fertilizer (INO) treatment large plankton (> 200 μm) assemblage mean densities (number/L \pm 95 % CI) among 10 weekly sampling periods, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ replicate ponds.

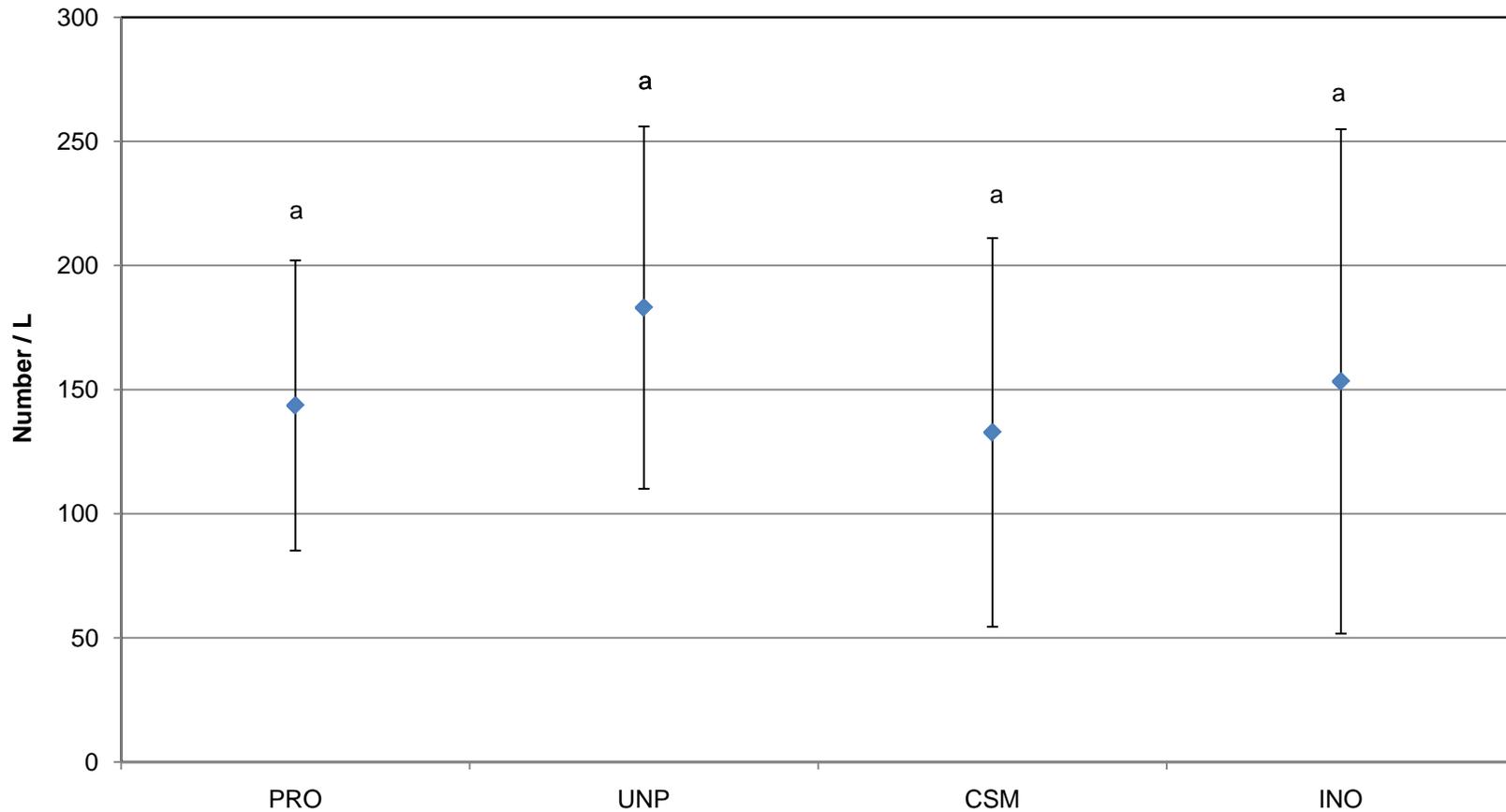


Figure 5-12. Time-averaged large plankton (> 200 μm) assemblage mean densities (number/L \pm 95% CI) for four nutrient treatments: processed feed (PRO), unprocessed feed (UNP), cottonseed meal, and inorganic fertilizer (INO), unshared letters denote statistical differences between treatments ($P < 0.05$, Tukey's multiple comparison test); $n = 6$ ponds per treatment.

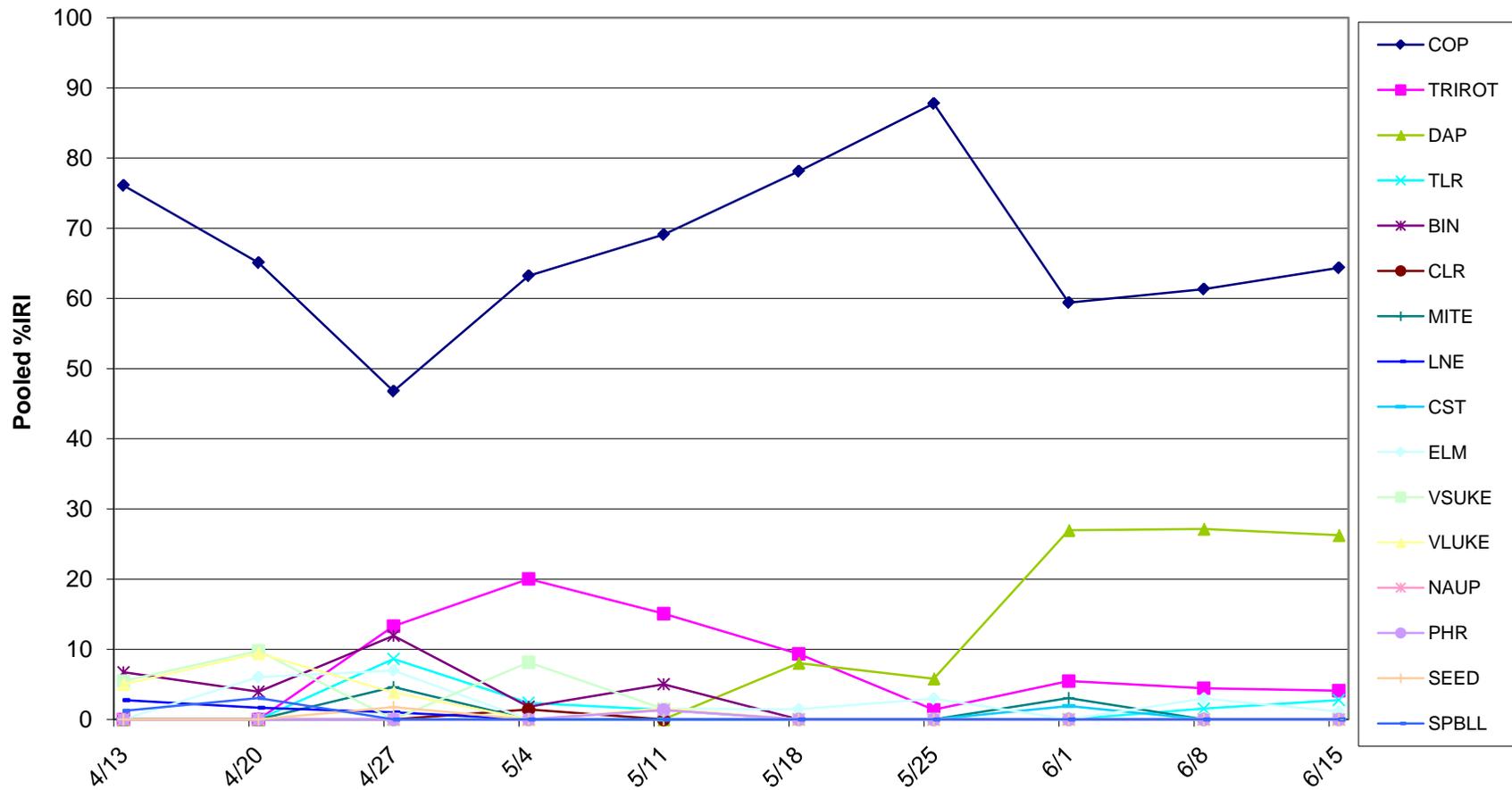


Figure 5-13. Processed feed (PRO) treatment large (> 200 μm) plankton assemblage pooled %IRI (index of relative importance) for 10 weekly sampling periods, n = 6 replicate ponds.

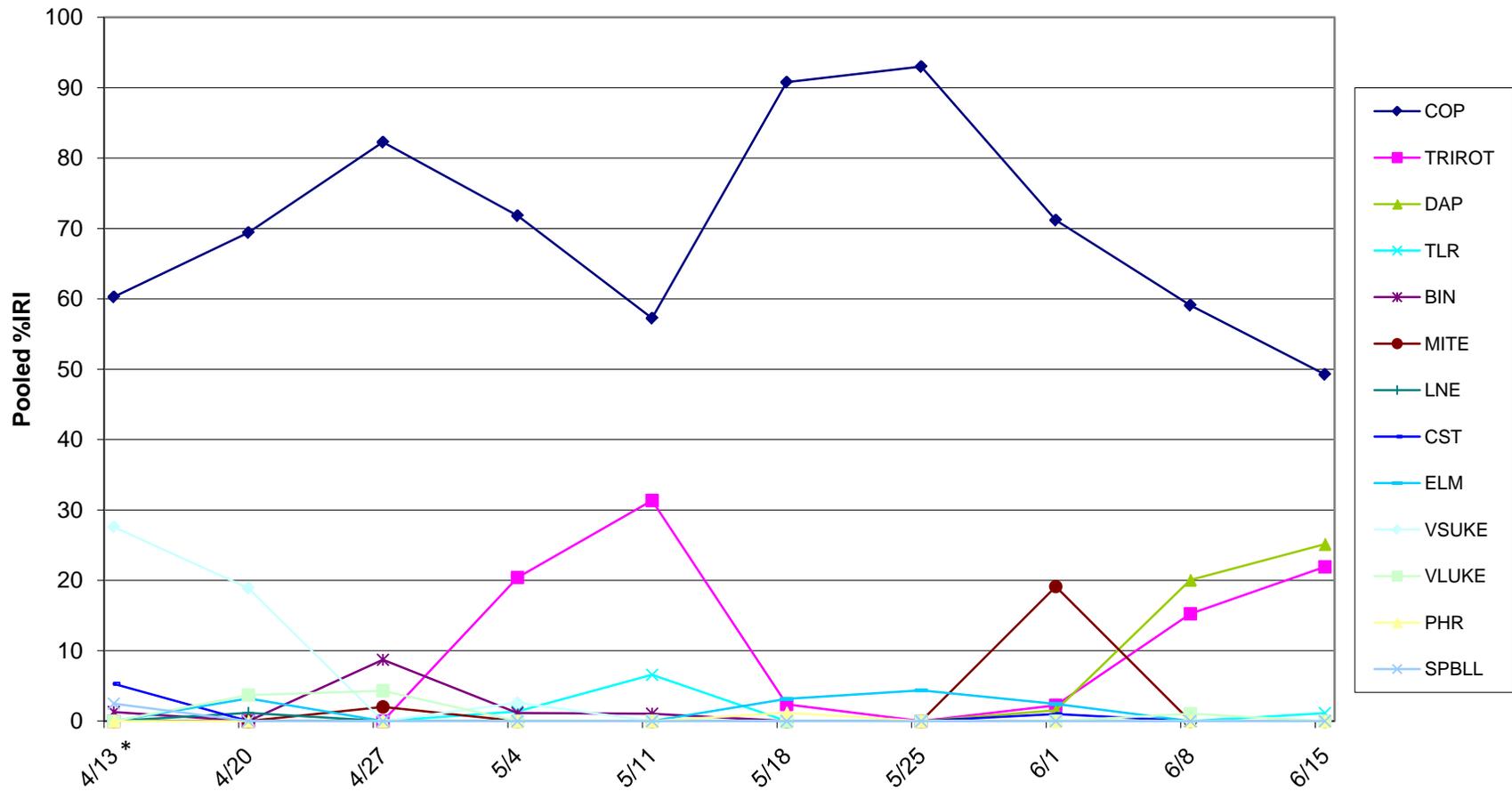


Figure 5-14. Unprocessed feed (UNP) treatment large (> 200 μm) plankton assemblage %IRI (index of relative importance) for 10 weekly sampling periods; data pooled from six replicate aquaculture ponds, * only five replicate samples available for this date.

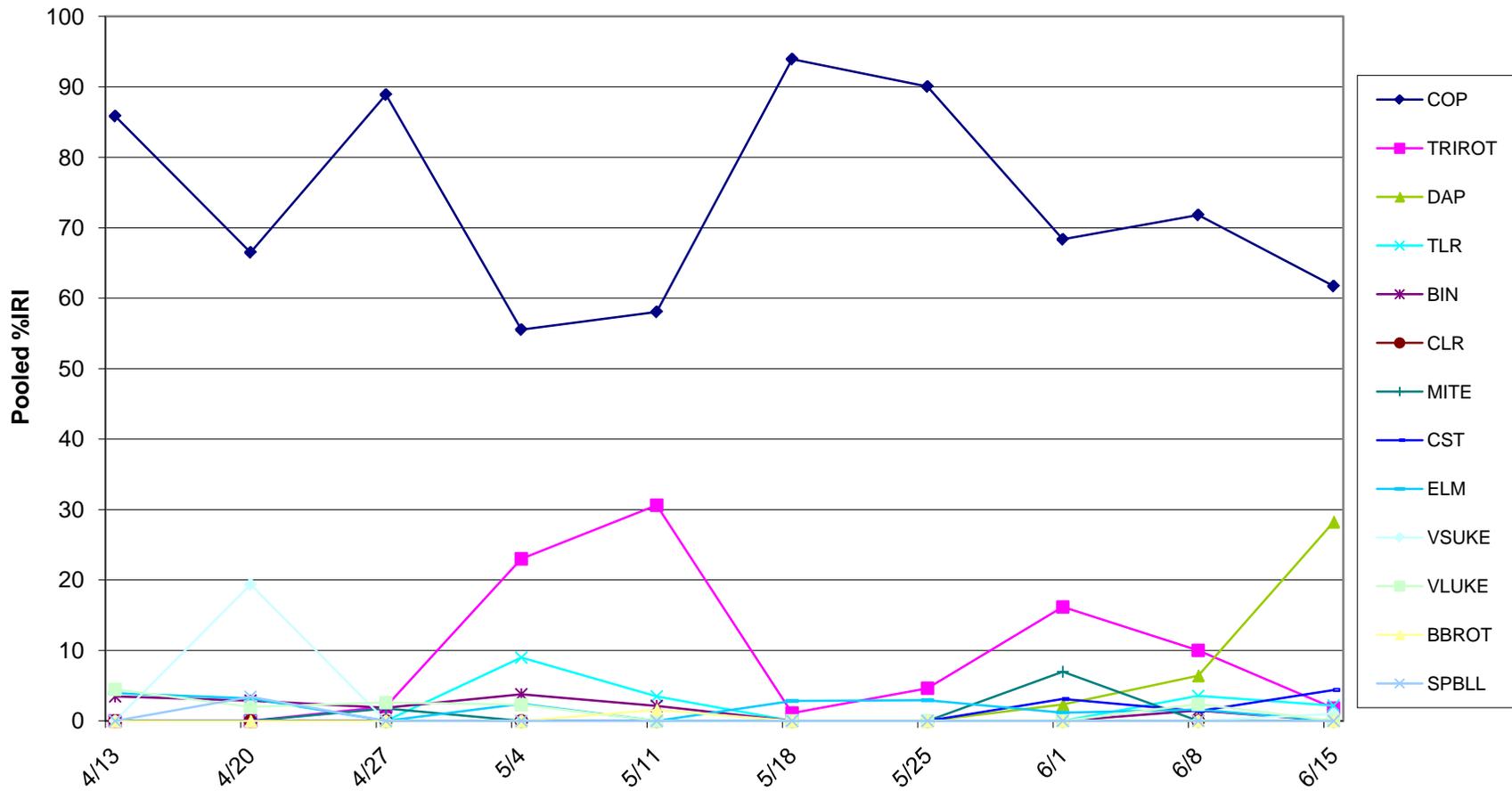


Figure 5-15. Cottonseed meal (CSM) treatment large (> 200 μm) plankton assemblage %IRI (index of relative importance) for 10 weekly sampling periods, data pooled from six replicate ponds.

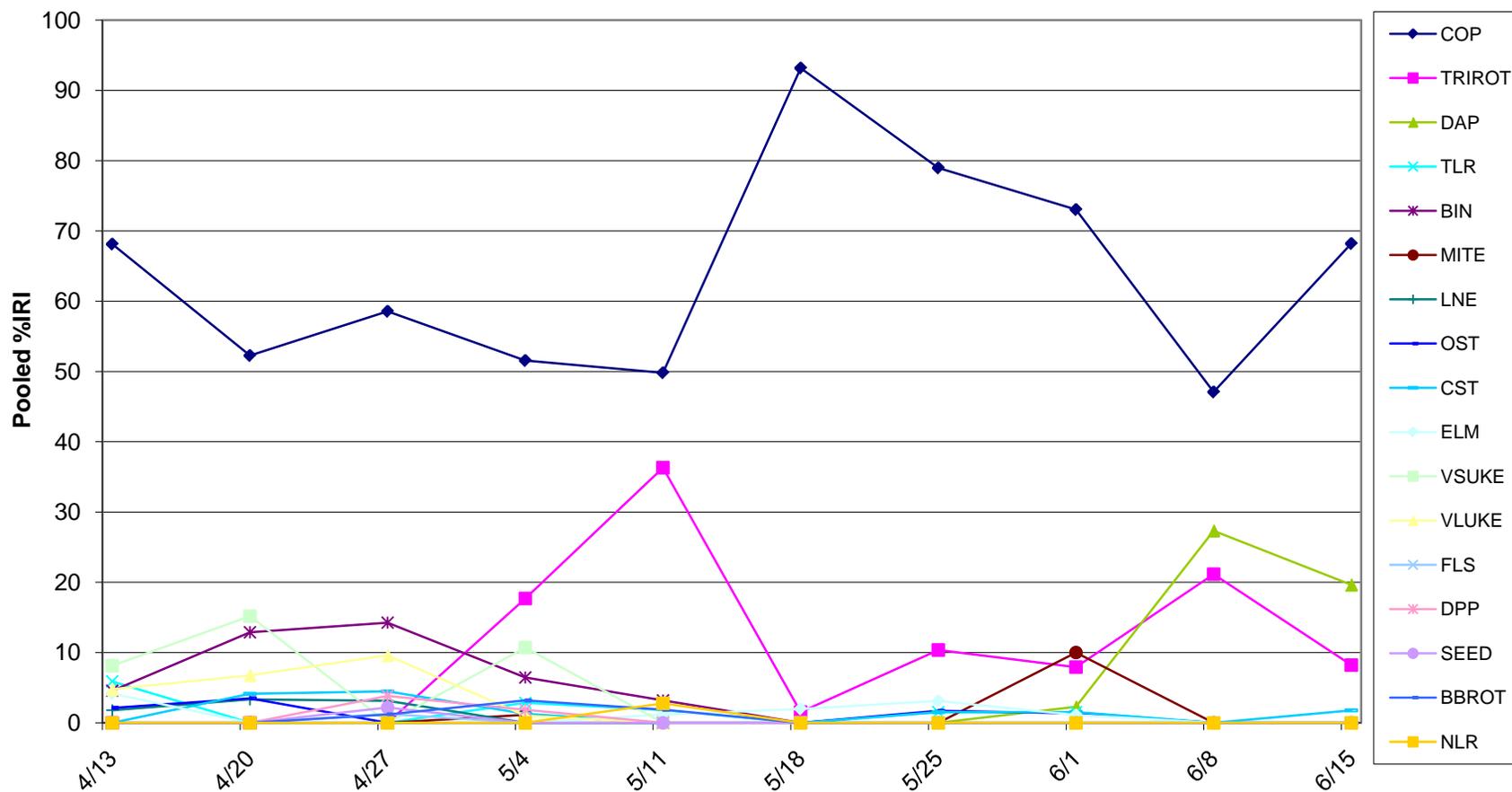


Figure 5-16. Inorganic fertilizer (INO) treatment large (> 200 μm) plankton assemblage %IRI (index of relative importance) for 10 weekly sampling periods, data pooled from six replicate ponds.

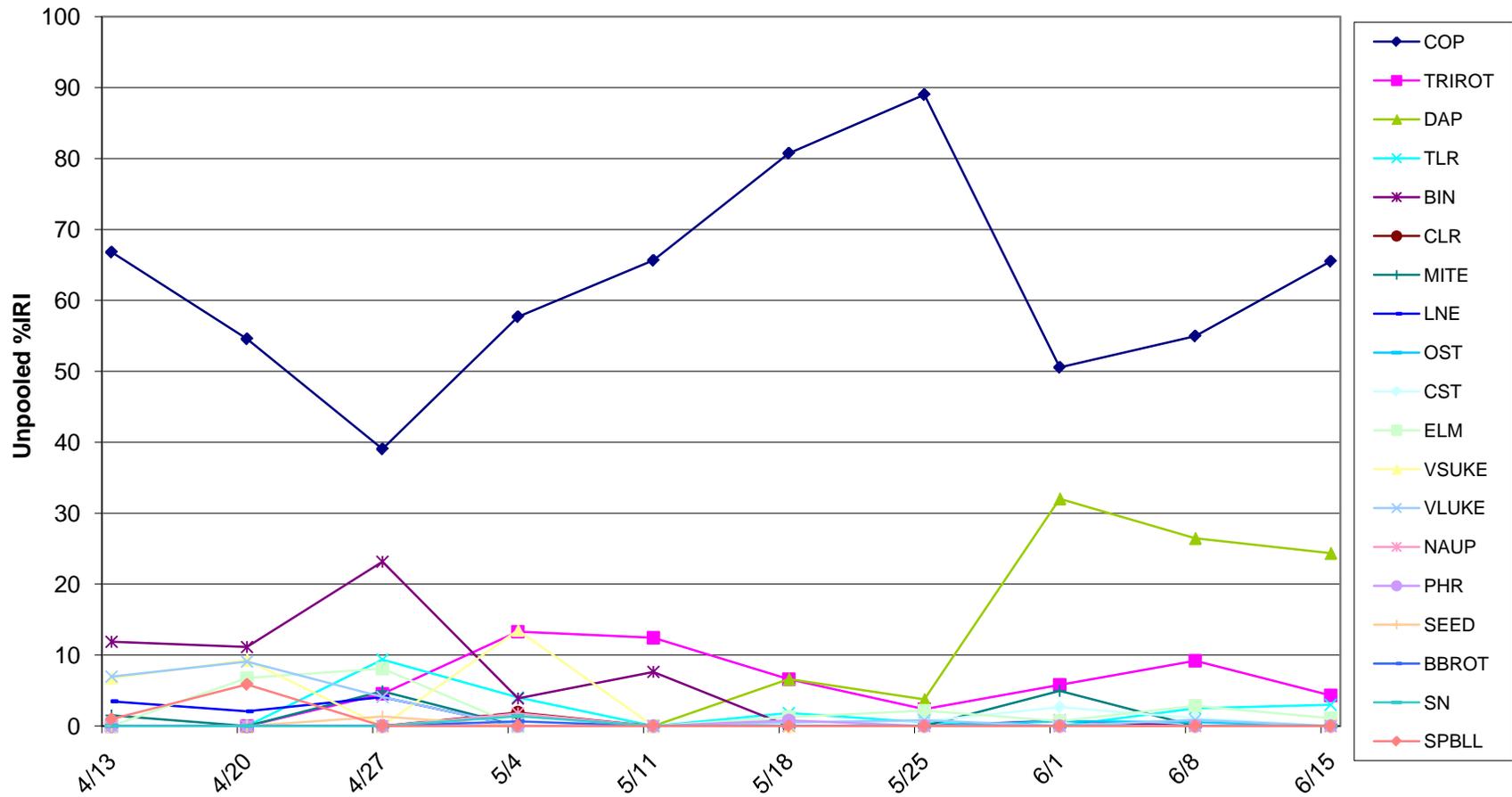


Figure 5-17. Processed feed (PRO) treatment large (> 200 μm) plankton assemblage unpooled %IRI (index of relative importance) for 10 weekly sampling periods, n = 6 replicate ponds.

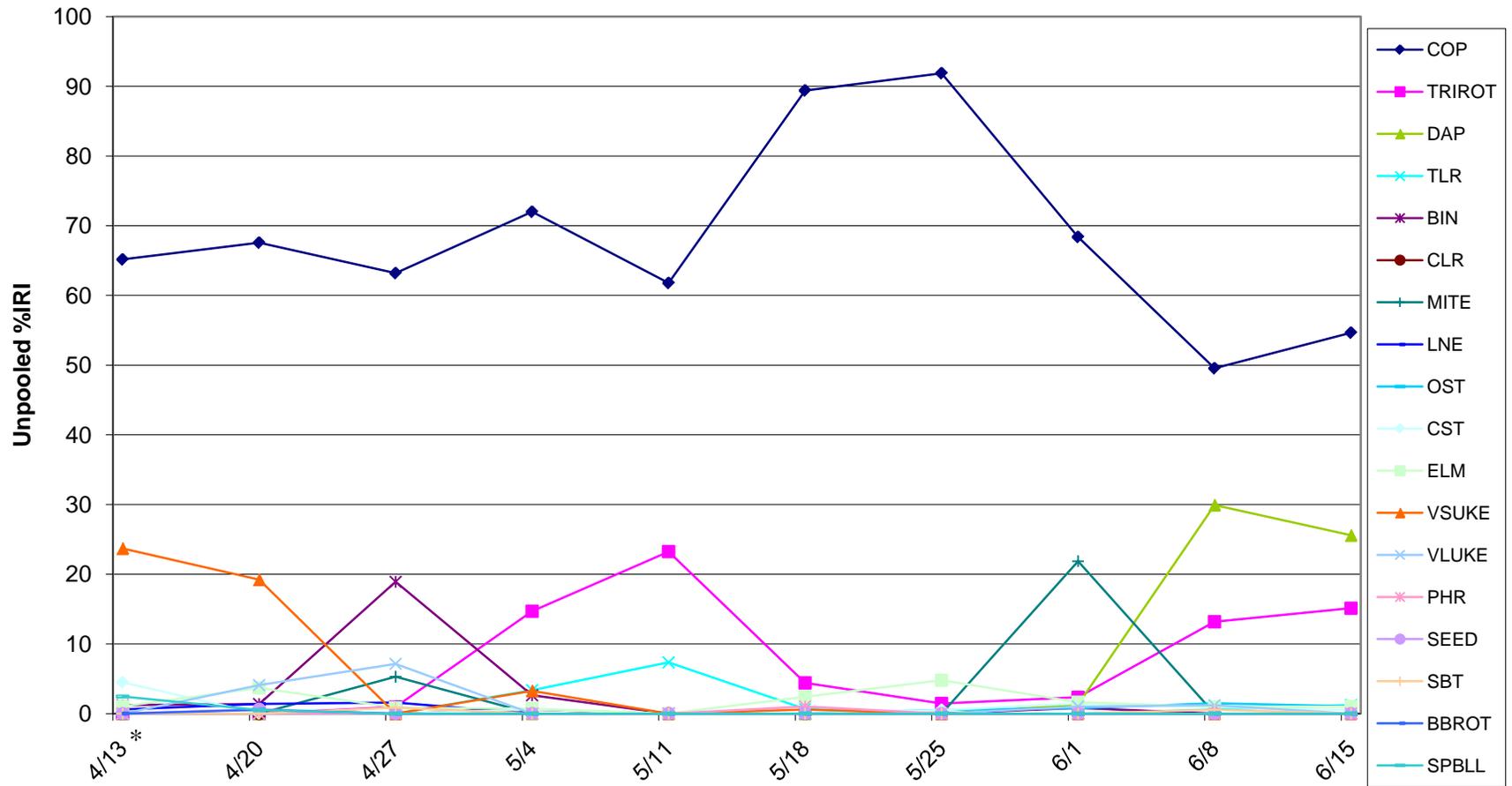


Figure 5-18. Unprocessed feed (UNP) treatment large (> 200 μm) plankton assemblage unpooled %IRI (index of relative importance) for 10 weekly sampling periods; n = 6 replicate ponds, * only five replicate samples for this sampling period.

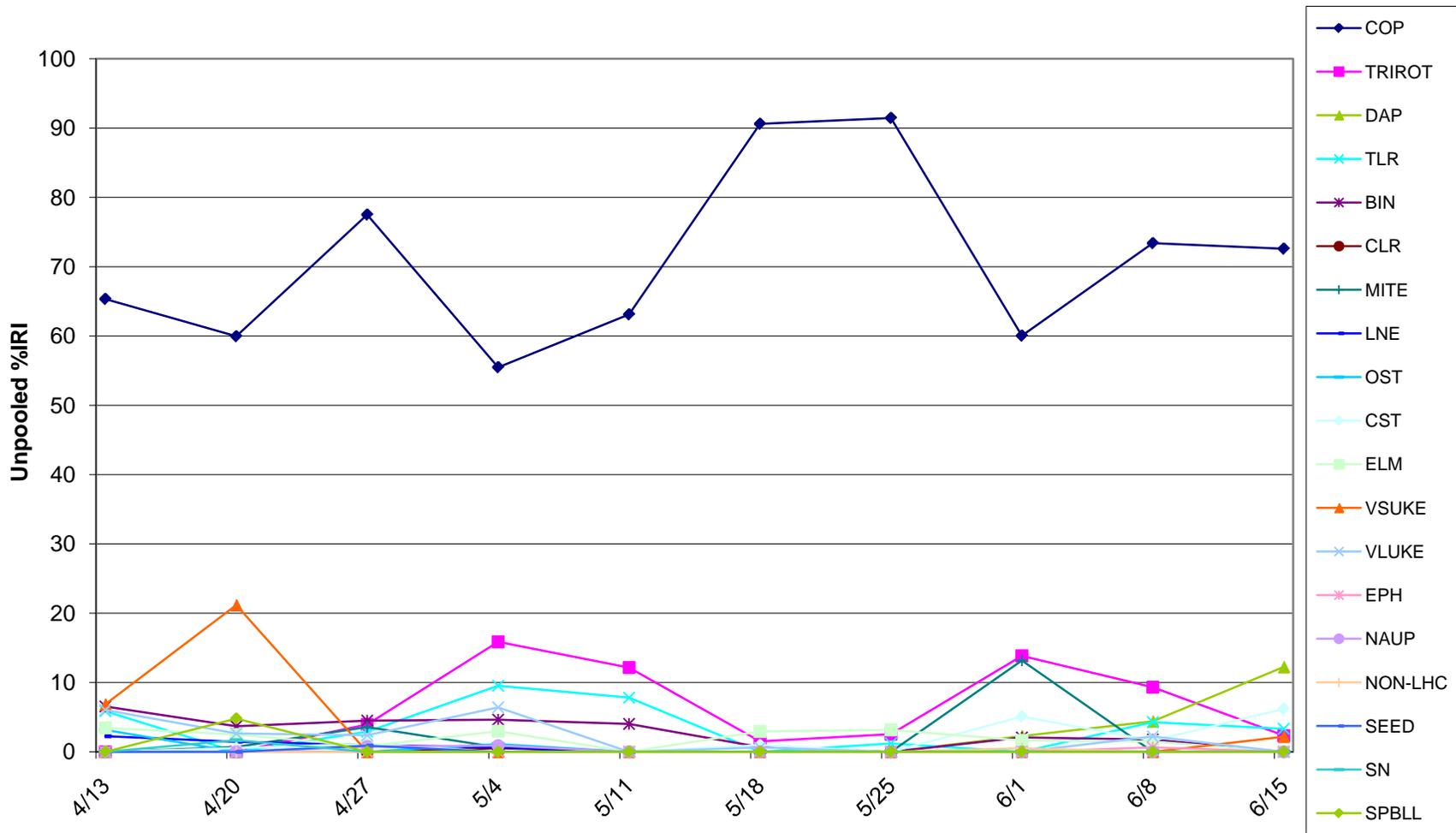


Figure 5-19. Cottonseed meal (CSM) treatment large (> 200 μm) plankton assemblage unpooled %IRI (index of relative importance) for 10 weekly sampling periods, n = 6 replicate ponds.

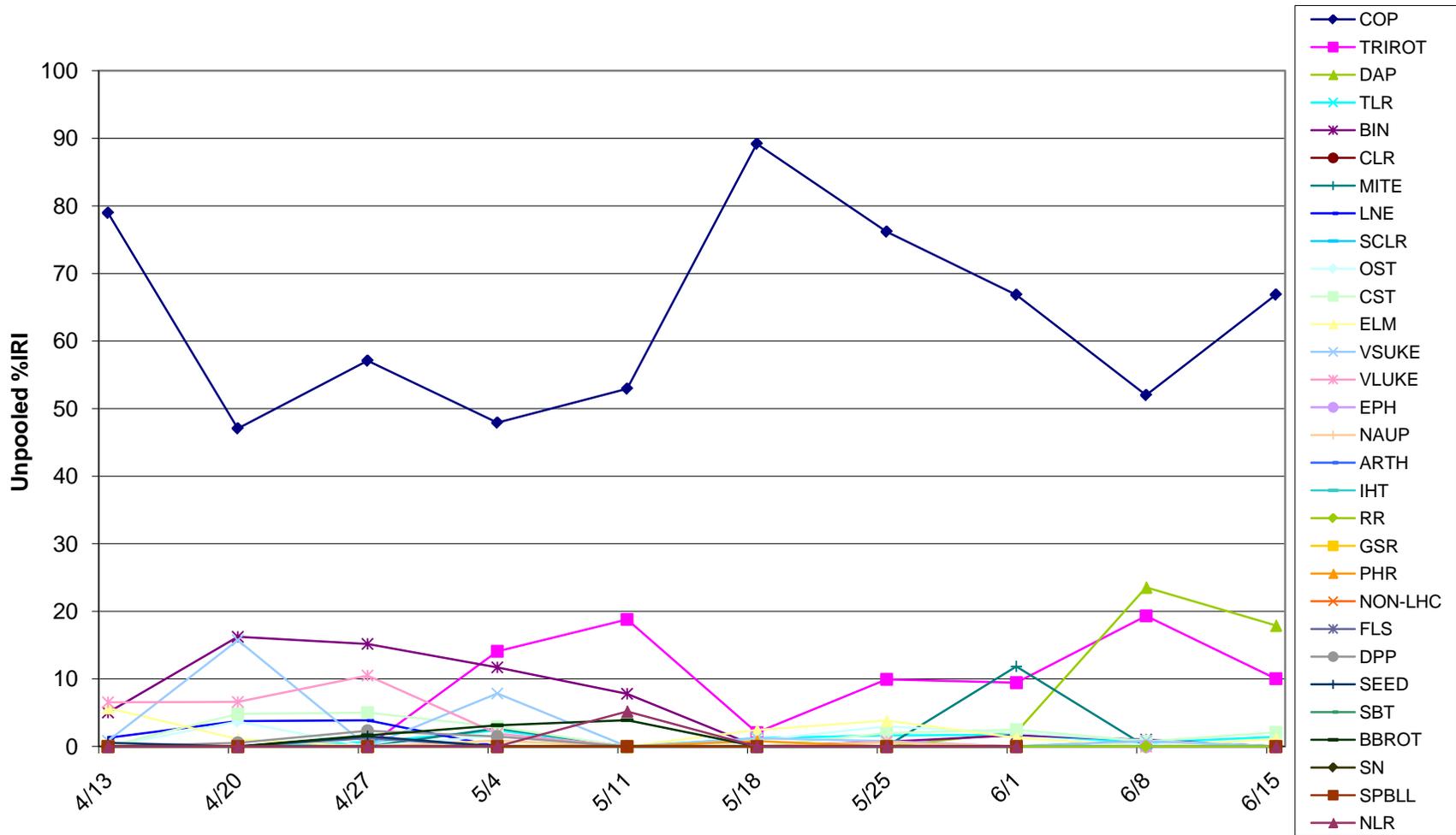


Figure 5-20. Inorganic fertilizer (INO) treatment large (> 200 μm) plankton assemblage unpooled %IRI (index of relative importance) for 10 weekly sampling periods, n = 6 replicate ponds.

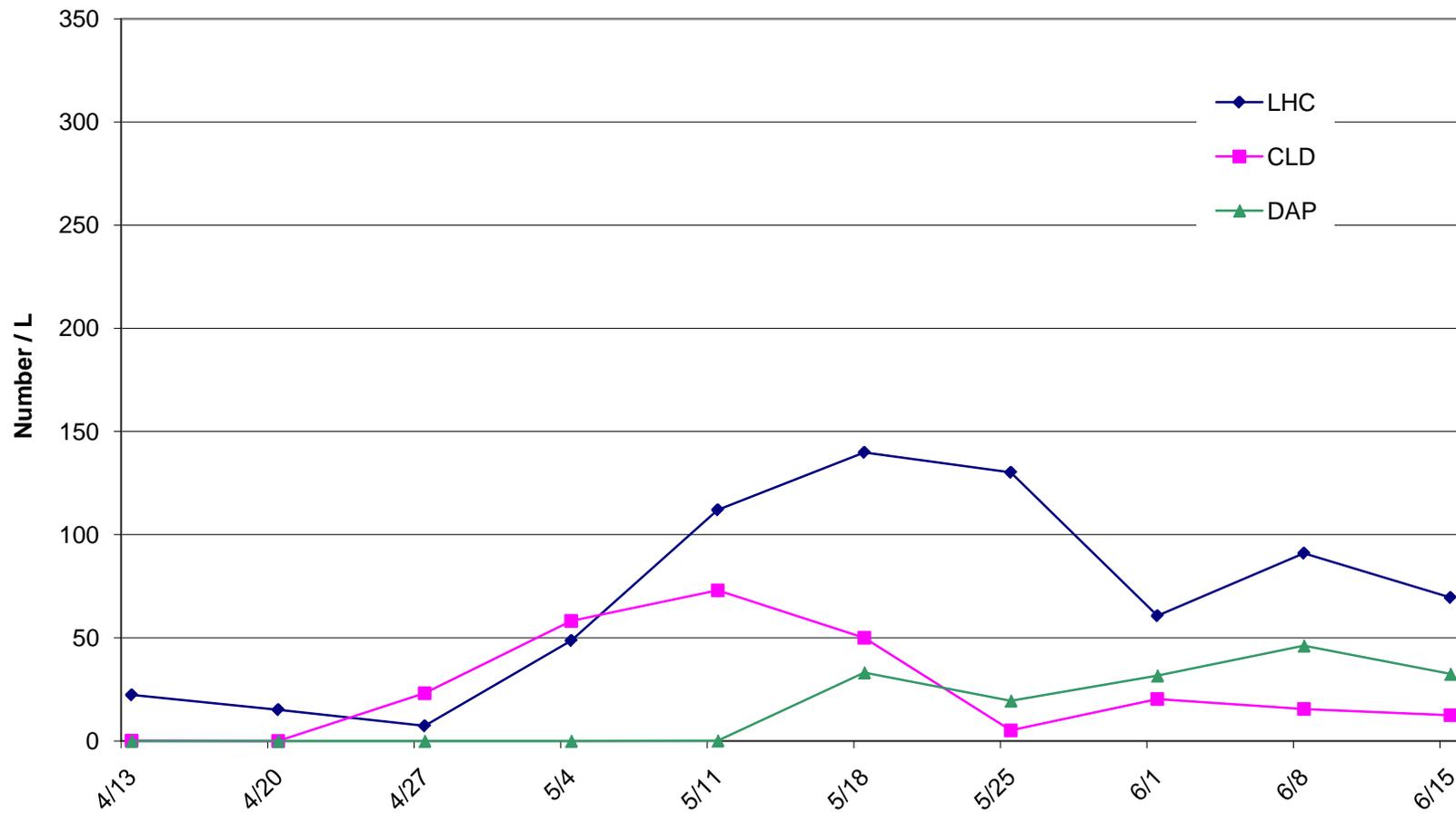


Figure 5-21. Processed feed (PRO) treatment major (highest %IRI) large (> 200 μm) plankton assemblage taxa [*Diaptomous* copepods (LHC), *Filinia* rotifers (CLD), *Moina macrocopa* (DAP; Daphnid)] densities for 10 weekly sampling periods; n = 6 replicate ponds.

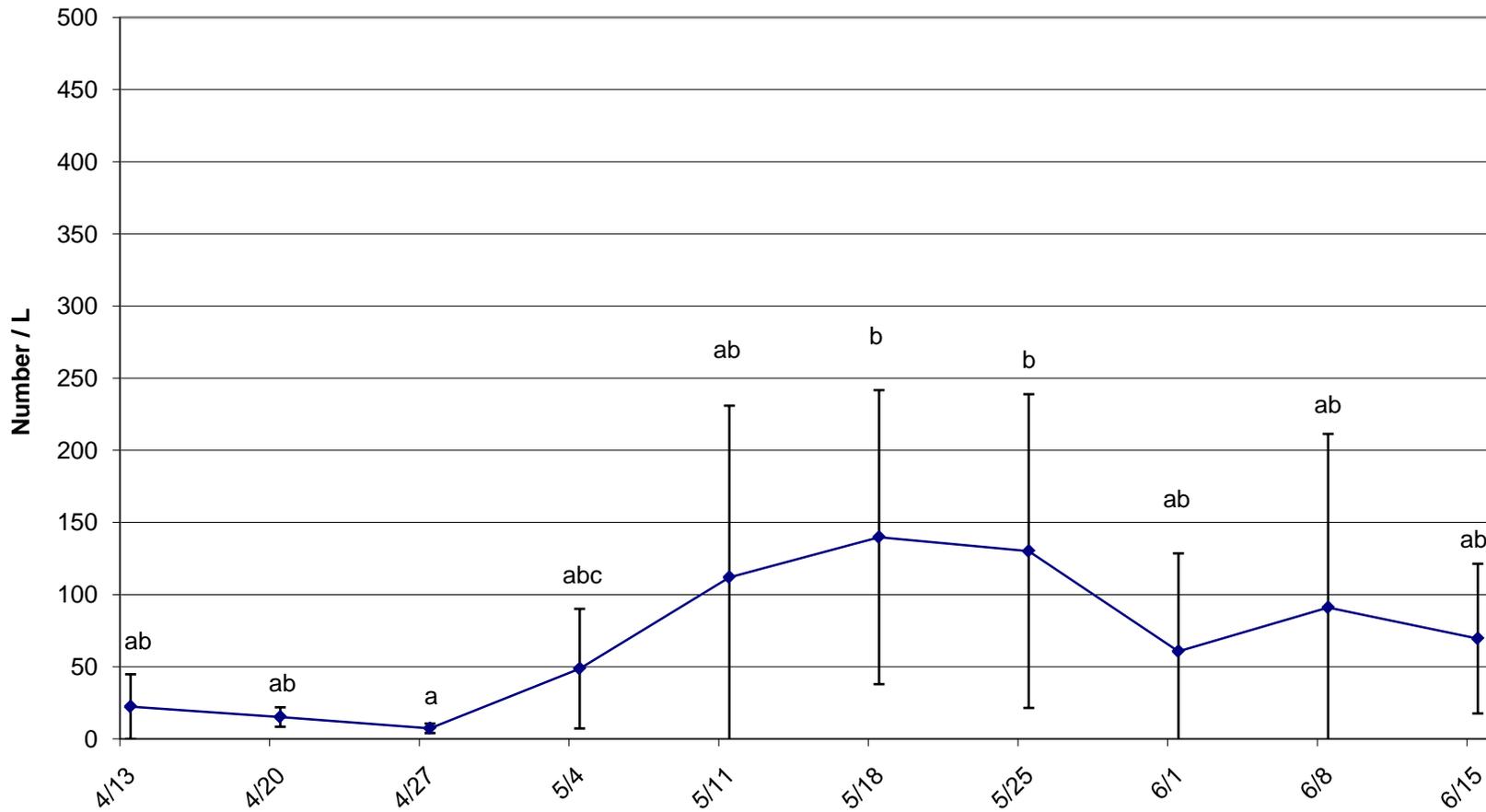


Figure 5-22. Processed feed (PRO) treatment *Diaptomous* copepod (LHC) densities (number/L \pm 95 % CI) for 10 weekly sampling periods, differing letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ replicate ponds.

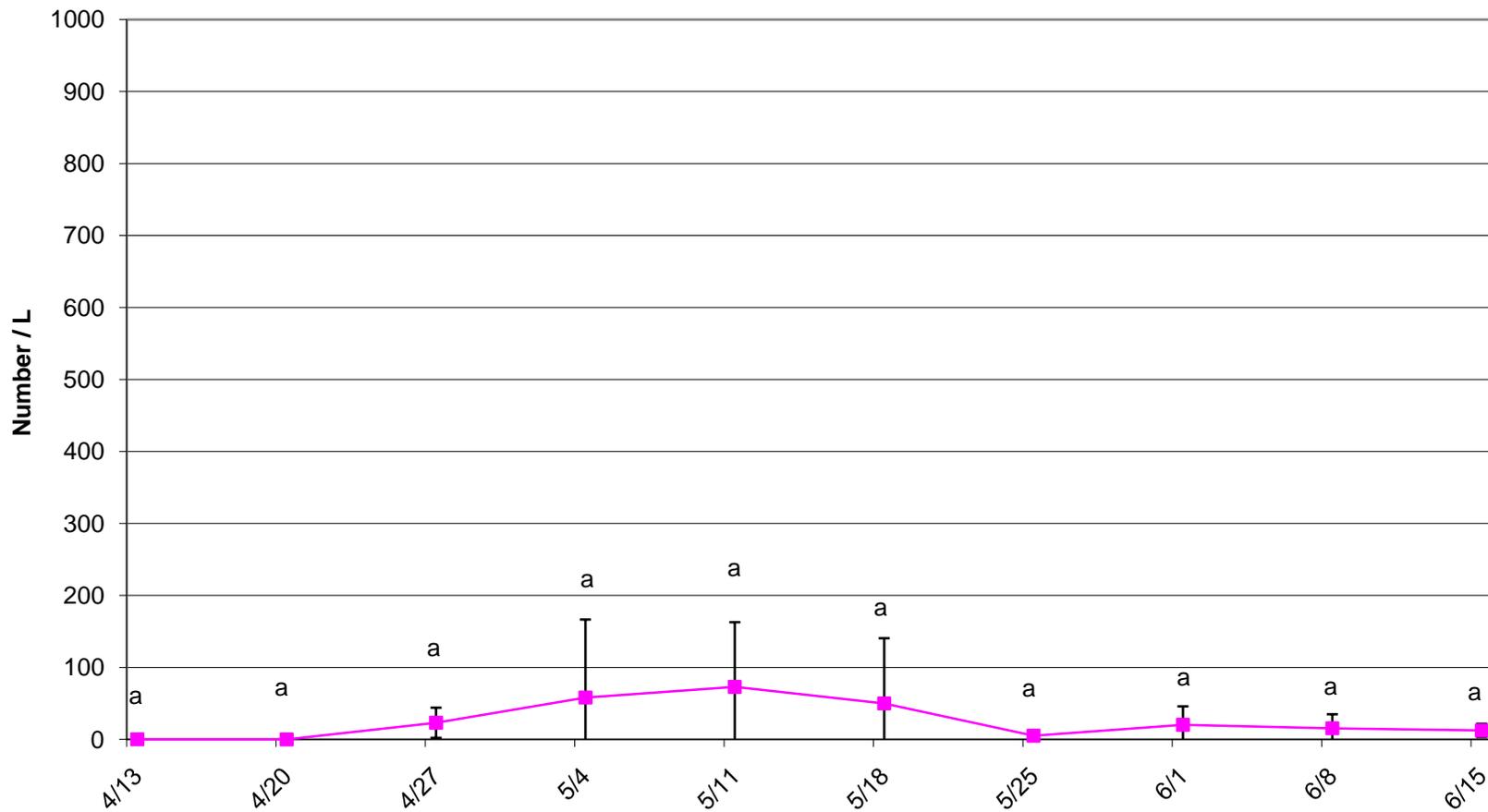


Figure 5-23. Processed feed (PRO) treatment *Filinia* rotifer (CLD) densities (number/L \pm 95 % CI) for 10 weekly sampling periods, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ replicate ponds.

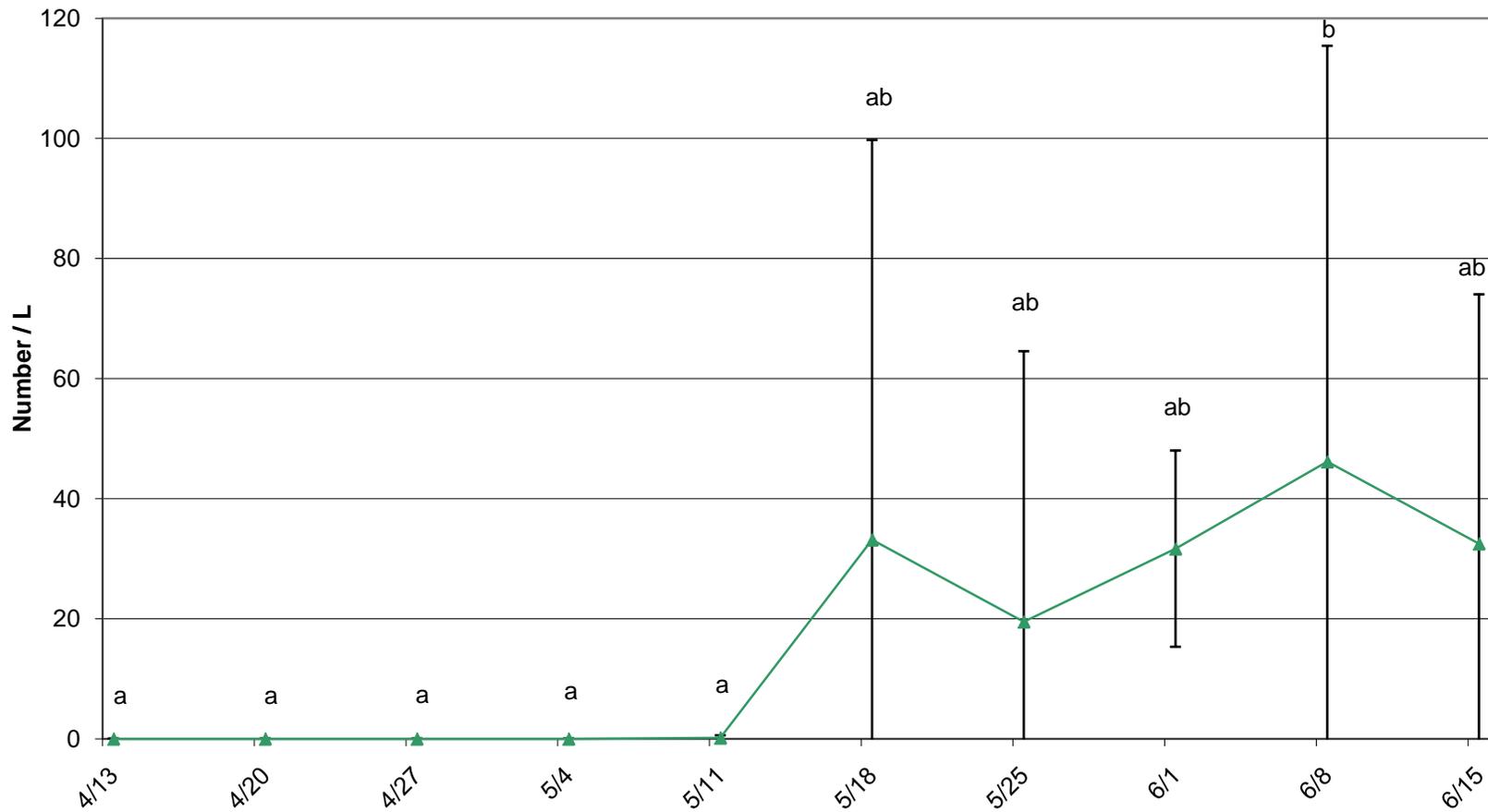


Figure 5-24. Processed feed (PRO) treatment *Moina macrocopa* (DAP; Daphnid) densities (number/L \pm 95 % CI) for 10 weekly sampling periods, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ replicate ponds.

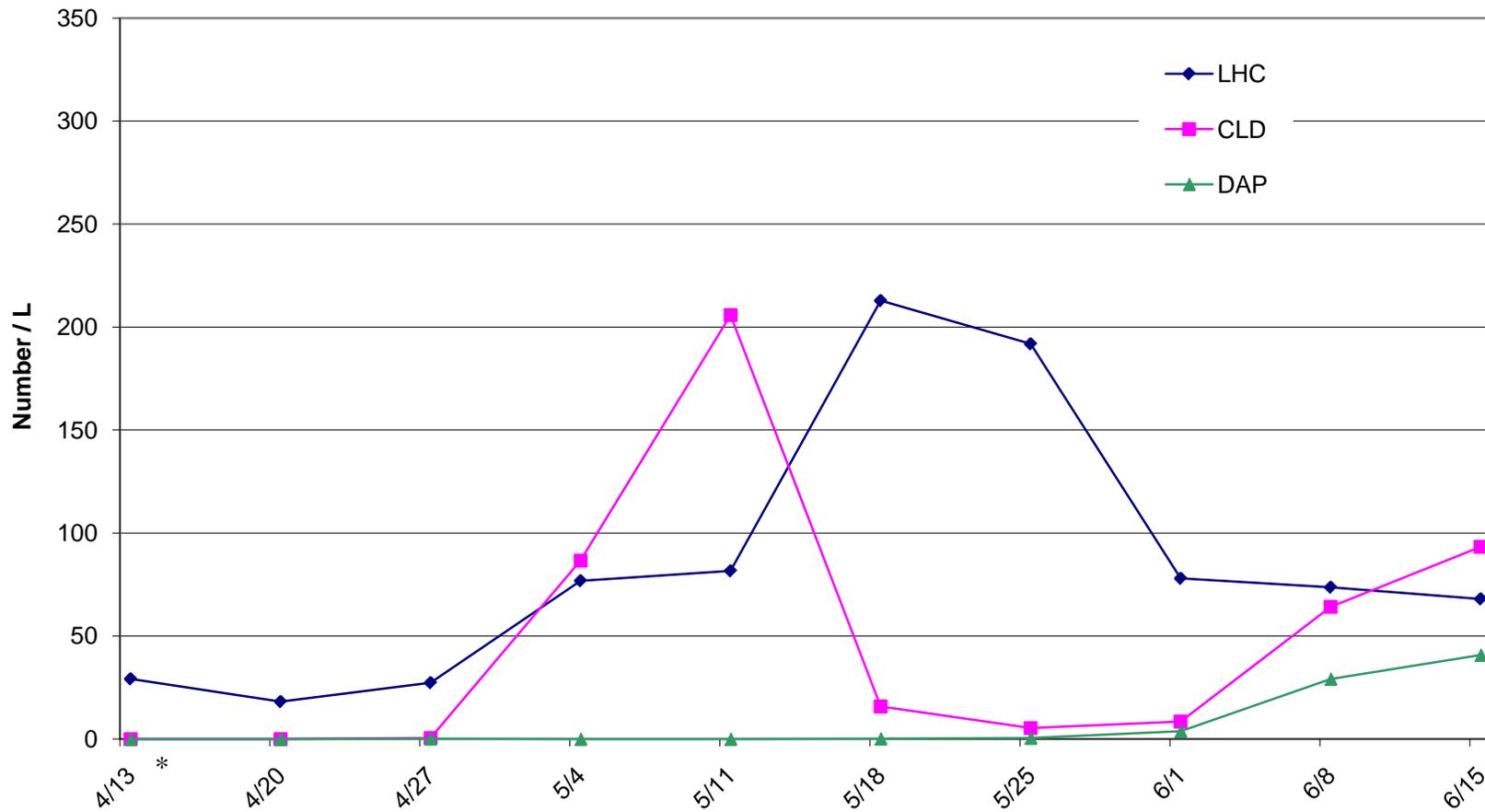


Figure 5-25. Unprocessed feed (UNP) treatment major (highest %IRI) large plankton assemblage taxa [*Diaptomous* copepods (LHC), *Filinia* rotifers (CLD), *Moina macrocopa* (DAP; Daphnid)] densities for 10 weekly sampling periods; n = 6 replicate ponds.

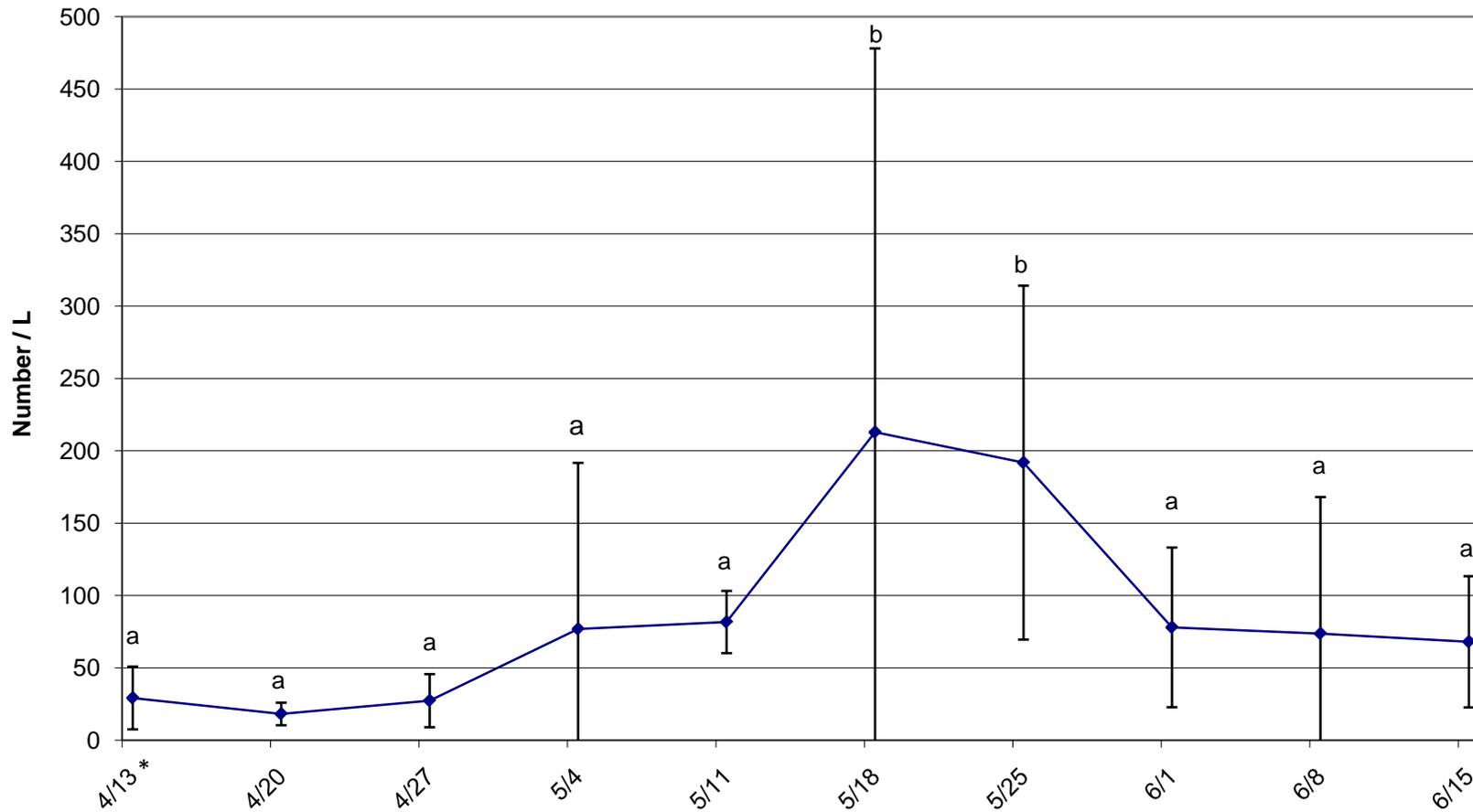


Figure 5-26. Unprocessed feed (UNP) treatment *Diaptomous* copepod (LHC) densities (number/L \pm 95% CI) for 10 weekly sampling periods, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ replicate ponds, * only five replicate samples available for this date.

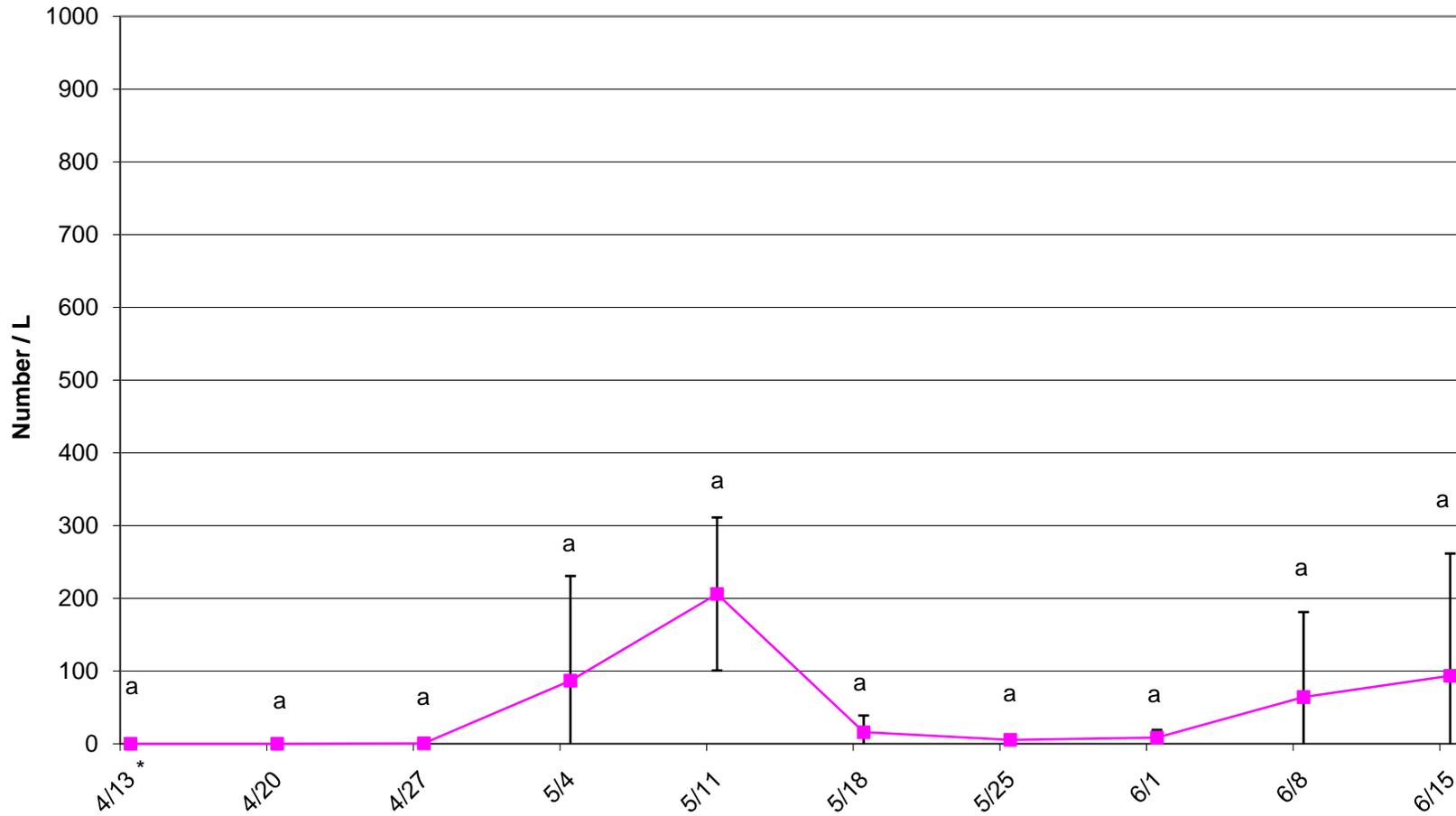


Figure 5-27. Unprocessed feed (UNP) treatment *Filinia* rotifer (CLD) taxa densities (number/L \pm 95% CI) for 10 weekly sampling periods, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ replicate ponds, * only five replicate samples available for this sampling date.

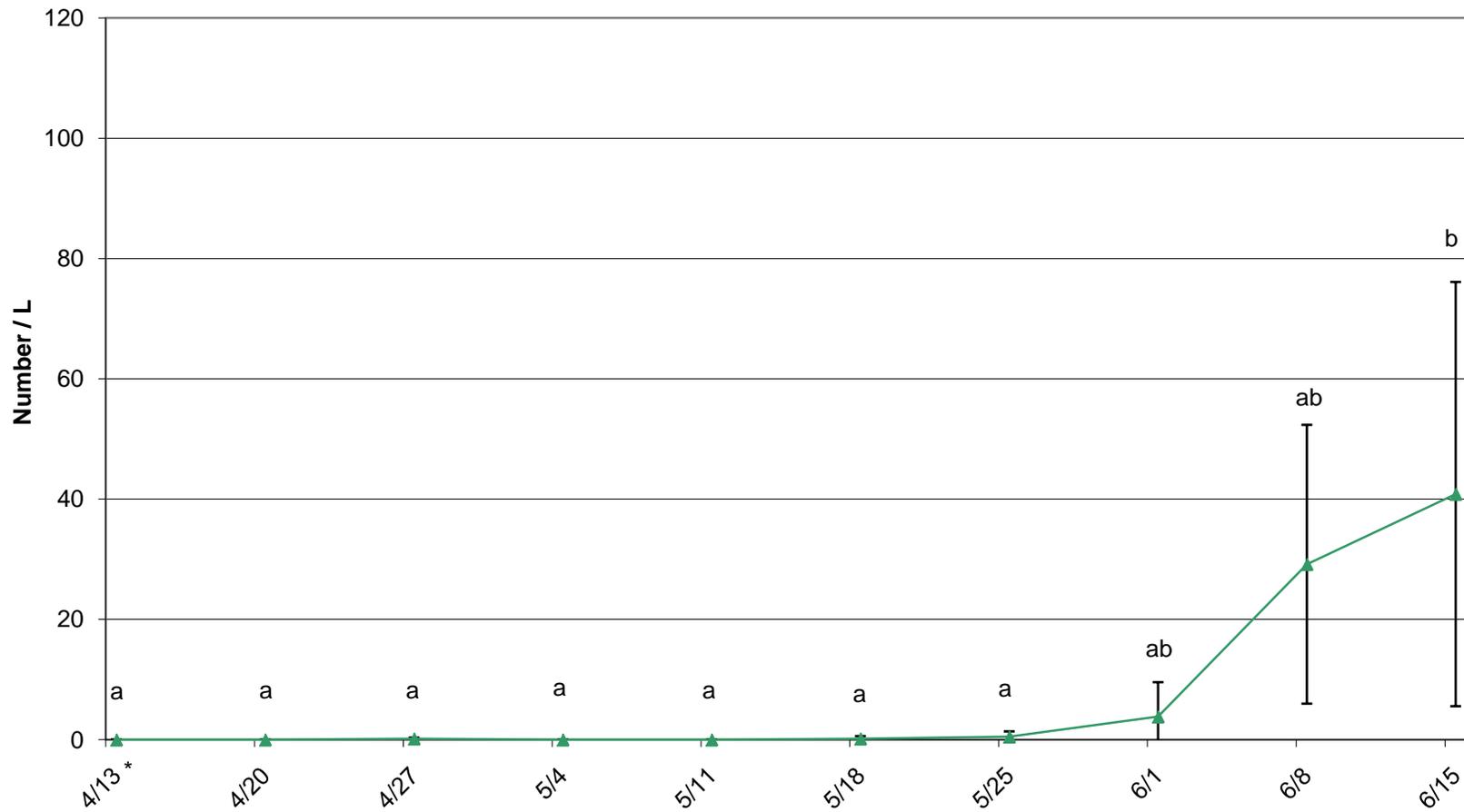


Figure 5-28. Unprocessed feed (UNP) treatment *Moina macrocopa* (DAP; Daphnid) taxa densities (number/L \pm 95% CI) for 10 weekly sampling periods, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ ponds per replicate, * only 5 replicate samples available for this sampling date.

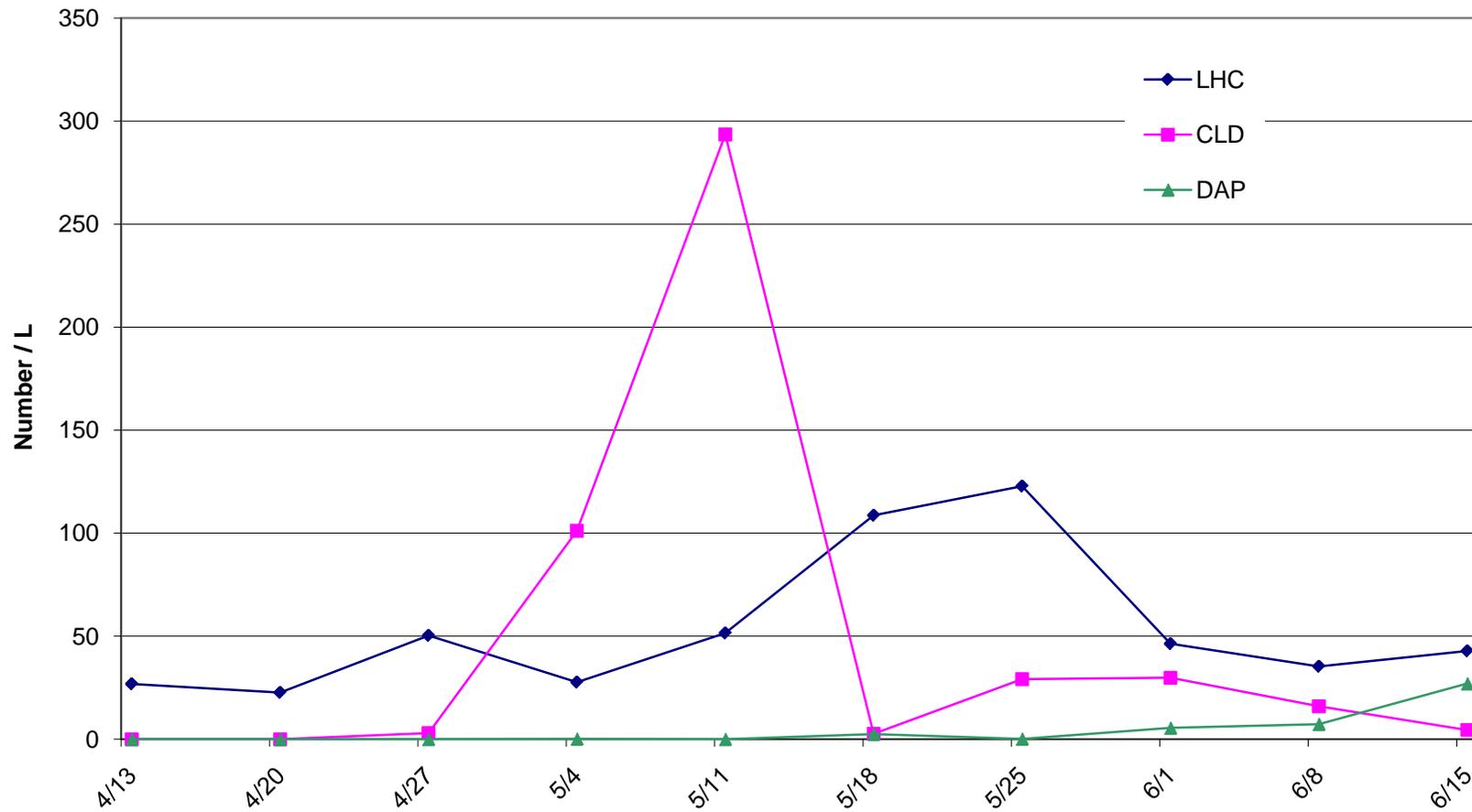


Figure 5-29. Cottonseed meal fertilizer (CSM) treatment major (highest %IRI) large (> 200 μm) plankton assemblage taxa [*Diaptomous* copepods (LHC), *Filinia* rotifers (CLD), *Moina macrocopa* (DAP; Daphnid)] densities for 10 weekly sampling periods; n = 6 ponds per treatment.

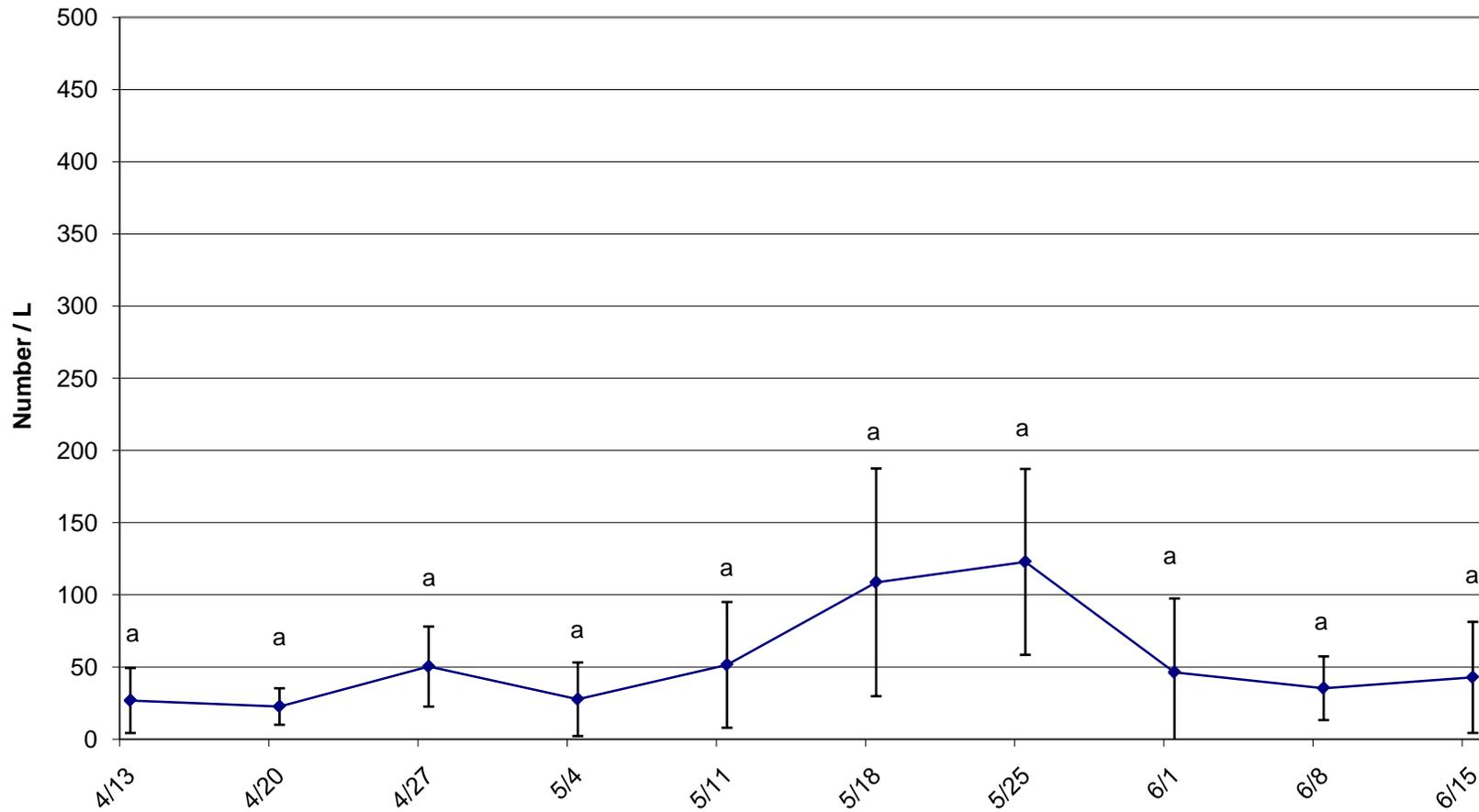


Figure 5-30. Cottonseed meal fertilizer (CSM) treatment *Diaptomous* copepod taxa densities (number/L \pm 95 % CI) for 10 weekly sampling periods, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ replicate ponds.

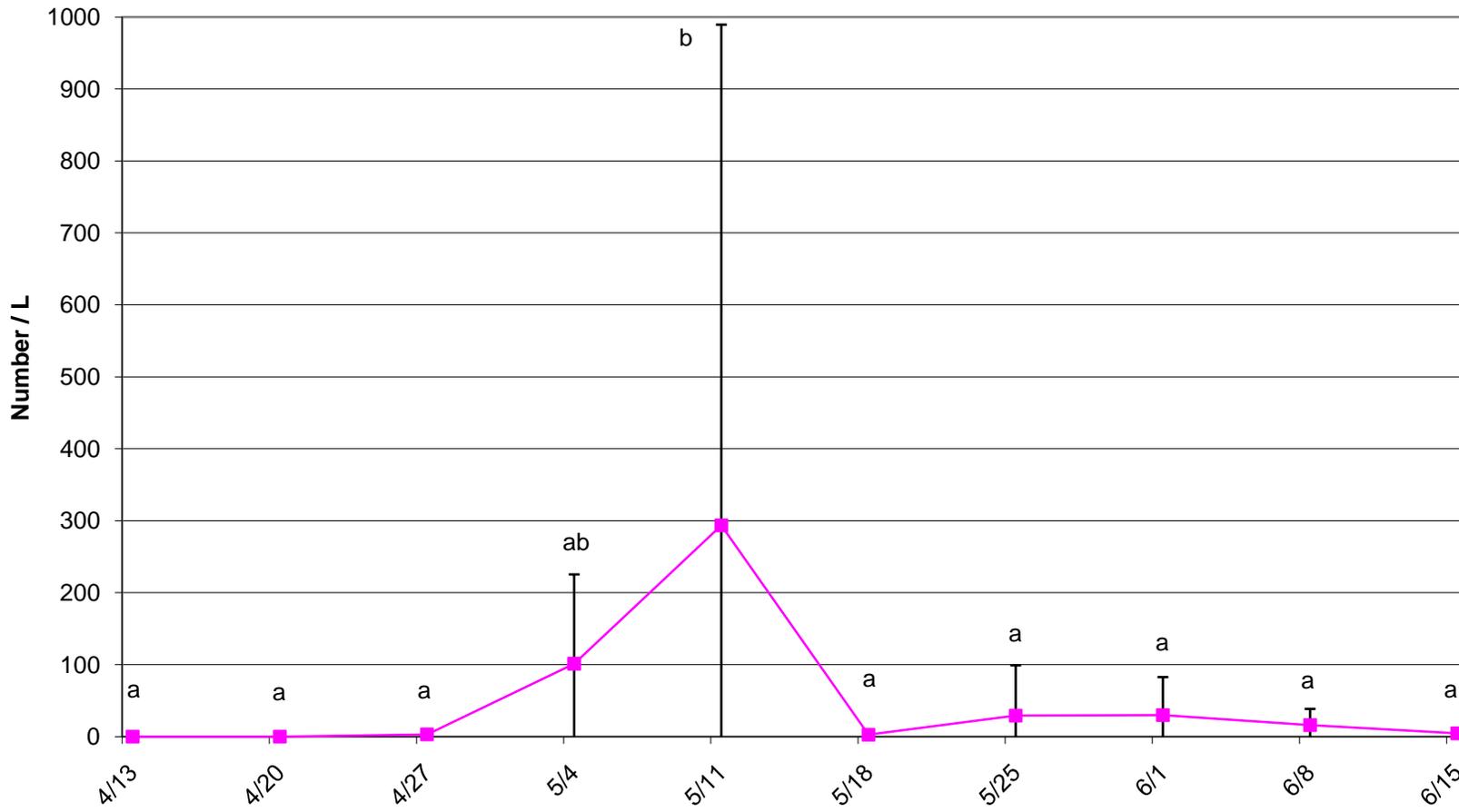


Figure 5-31. Cottonseed meal fertilizer (CSM) treatment *Filinia* rotifer taxa densities (number/L \pm 95 % CI) for 10 weekly sampling periods, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ replicate ponds.

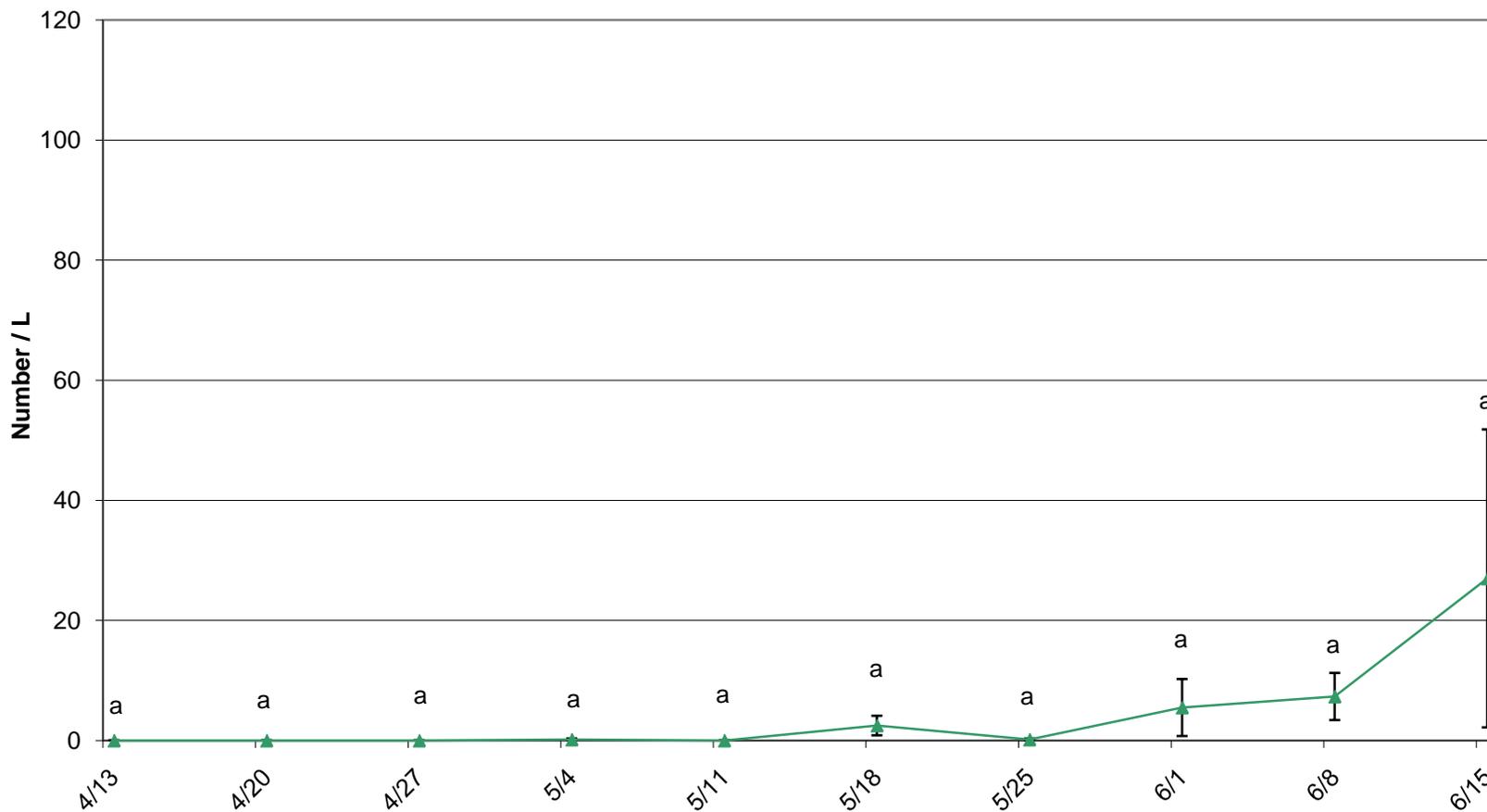


Figure 5-32. Cottonseed meal fertilizer (CSM) treatment *Moina macrocopa* (DAP; Daphnid) taxa densities (number/L \pm 95 % CI) for 10 weekly sampling periods, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ replicate ponds.

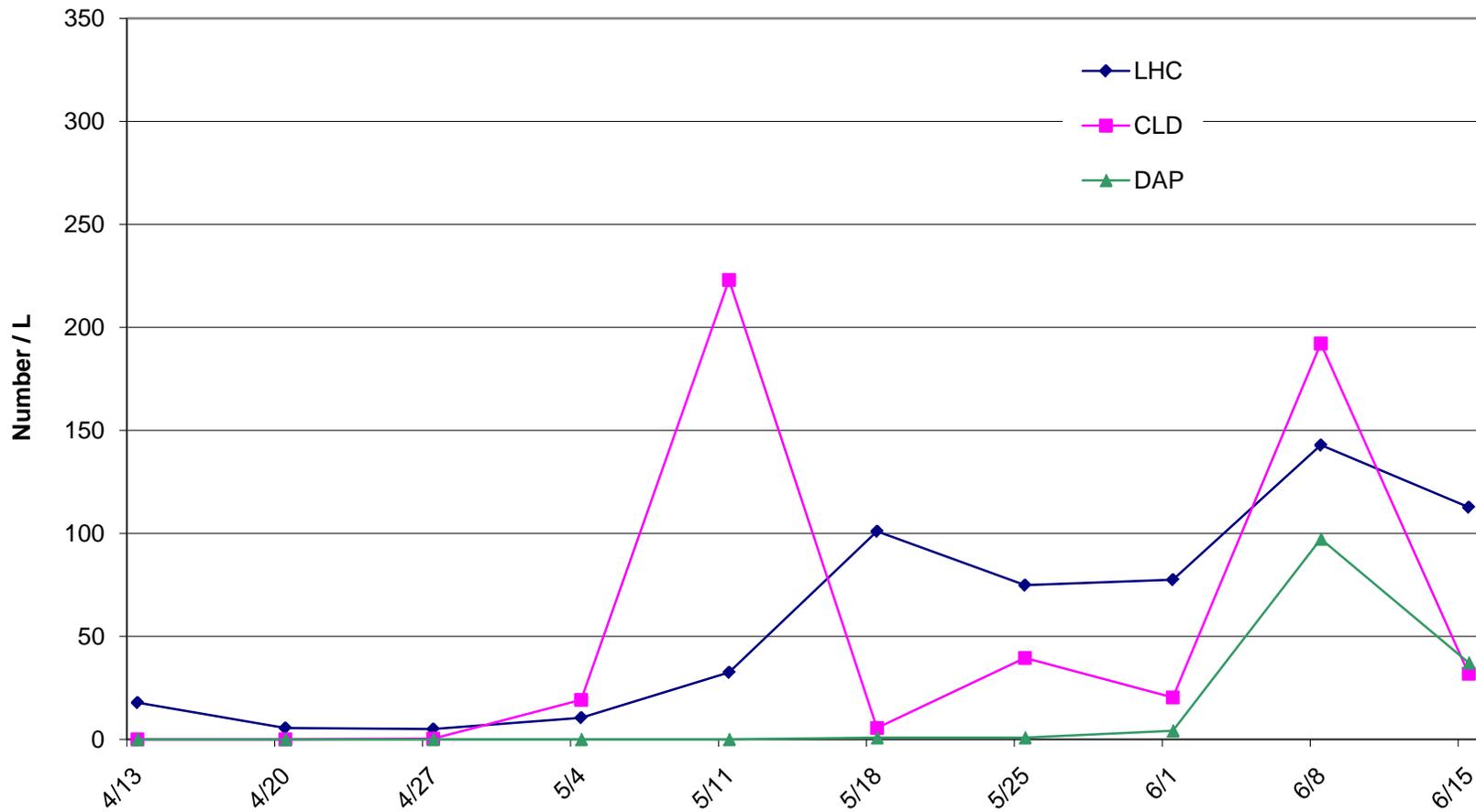


Figure 5-33. Inorganic fertilizer (INO) treatment major (highest %IRI large ($> 200 \mu\text{m}$) plankton assemblage taxa [*Diaptomous* copepods (LHC), *Filinia* rotifers (CLD), *Moina macrocopa* (DAP; Daphnid)] densities for 10 weekly sampling periods; $n = 6$ replicate ponds.

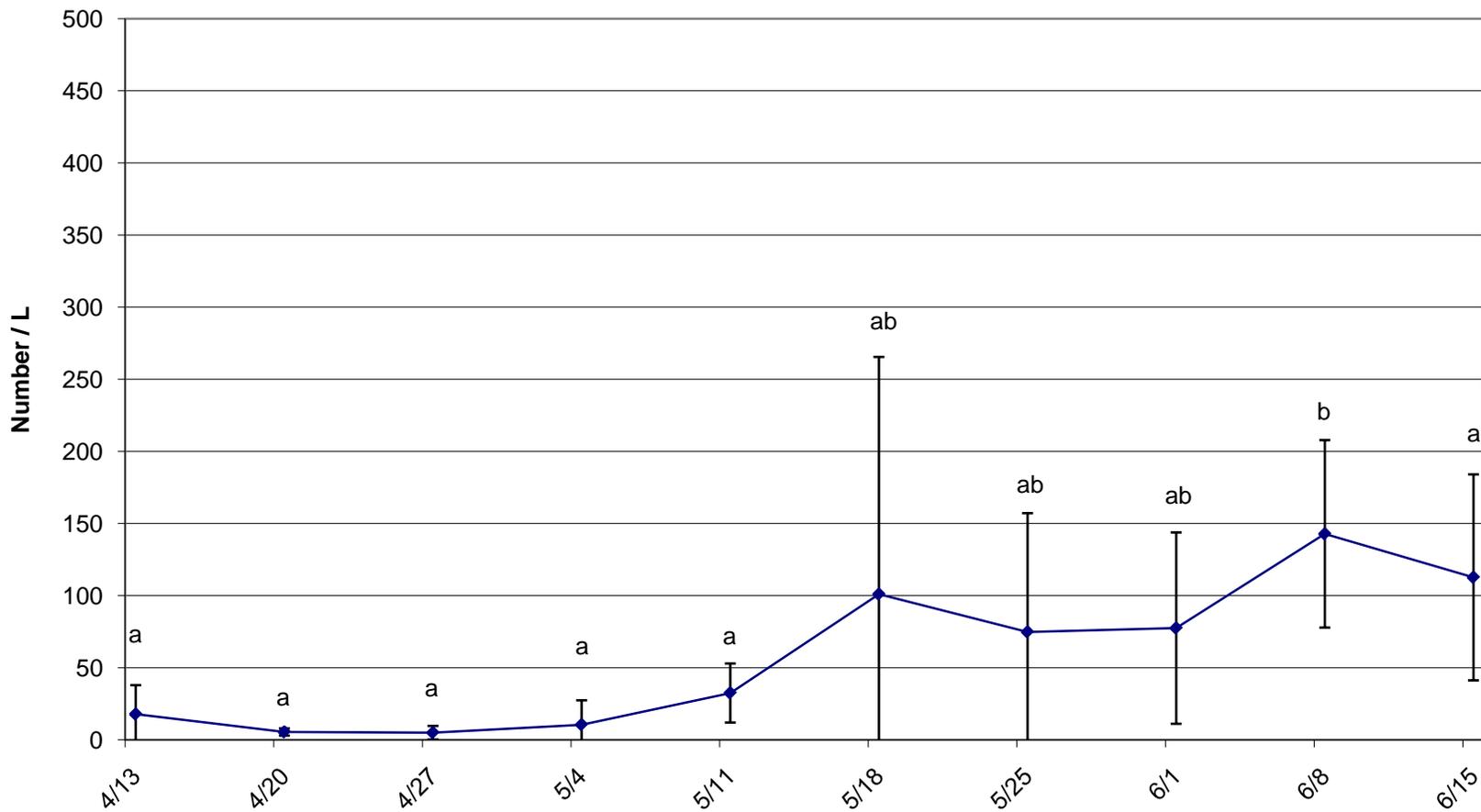


Figure 5-34. Inorganic fertilizer (INO) treatment *Diaptomous* copepod (LHC) taxa densities (number/L \pm 95% CI) for 10 weekly sampling periods, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ replicate ponds.

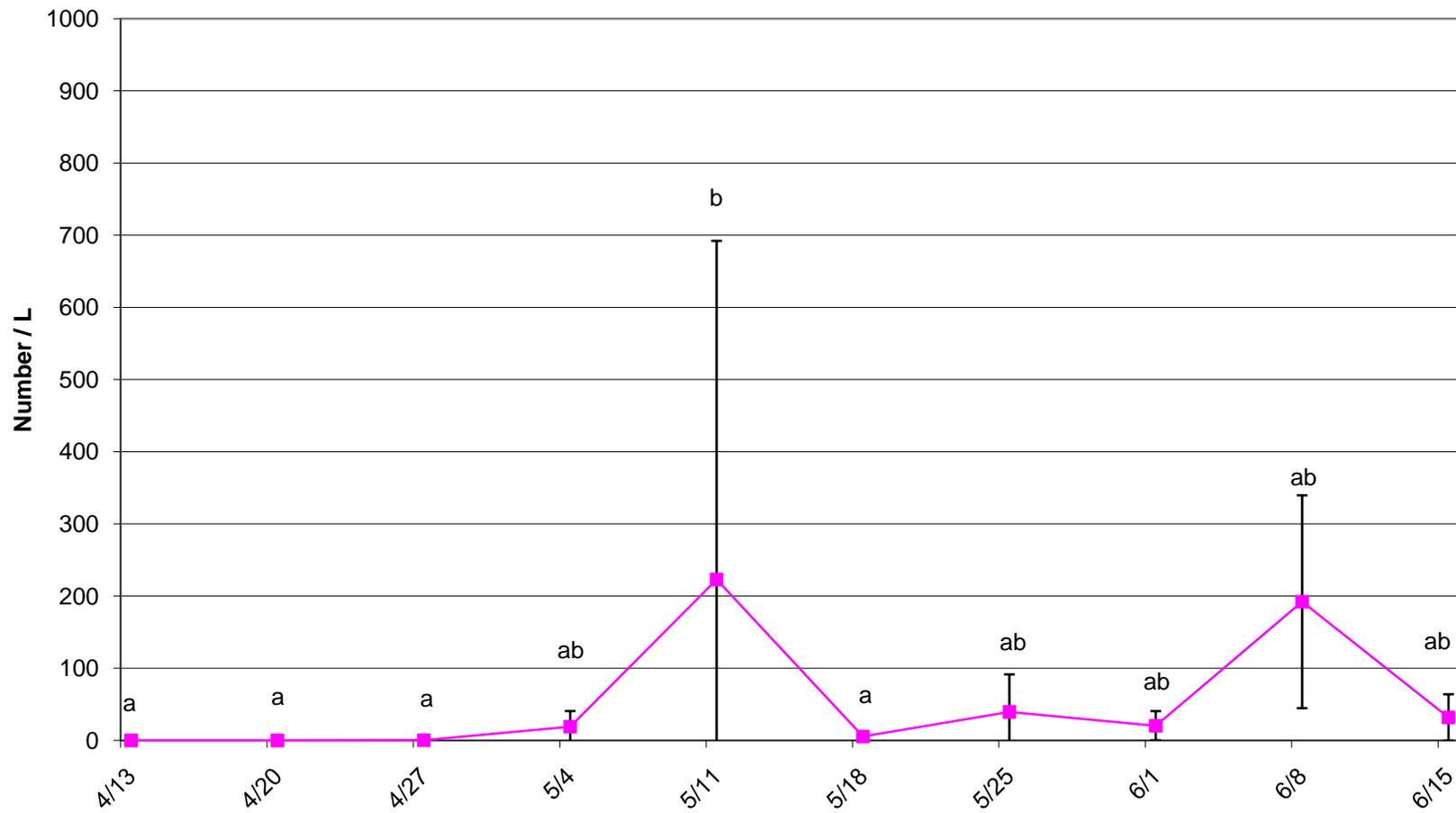


Figure 5-35. Inorganic fertilizer (INO) treatment *Filinia rotifer* (CLD) taxa densities (number/L \pm 95% CI) for 10 weekly sampling periods, unshared letters denote statistical differences between treatments; n = 6 replicate ponds.

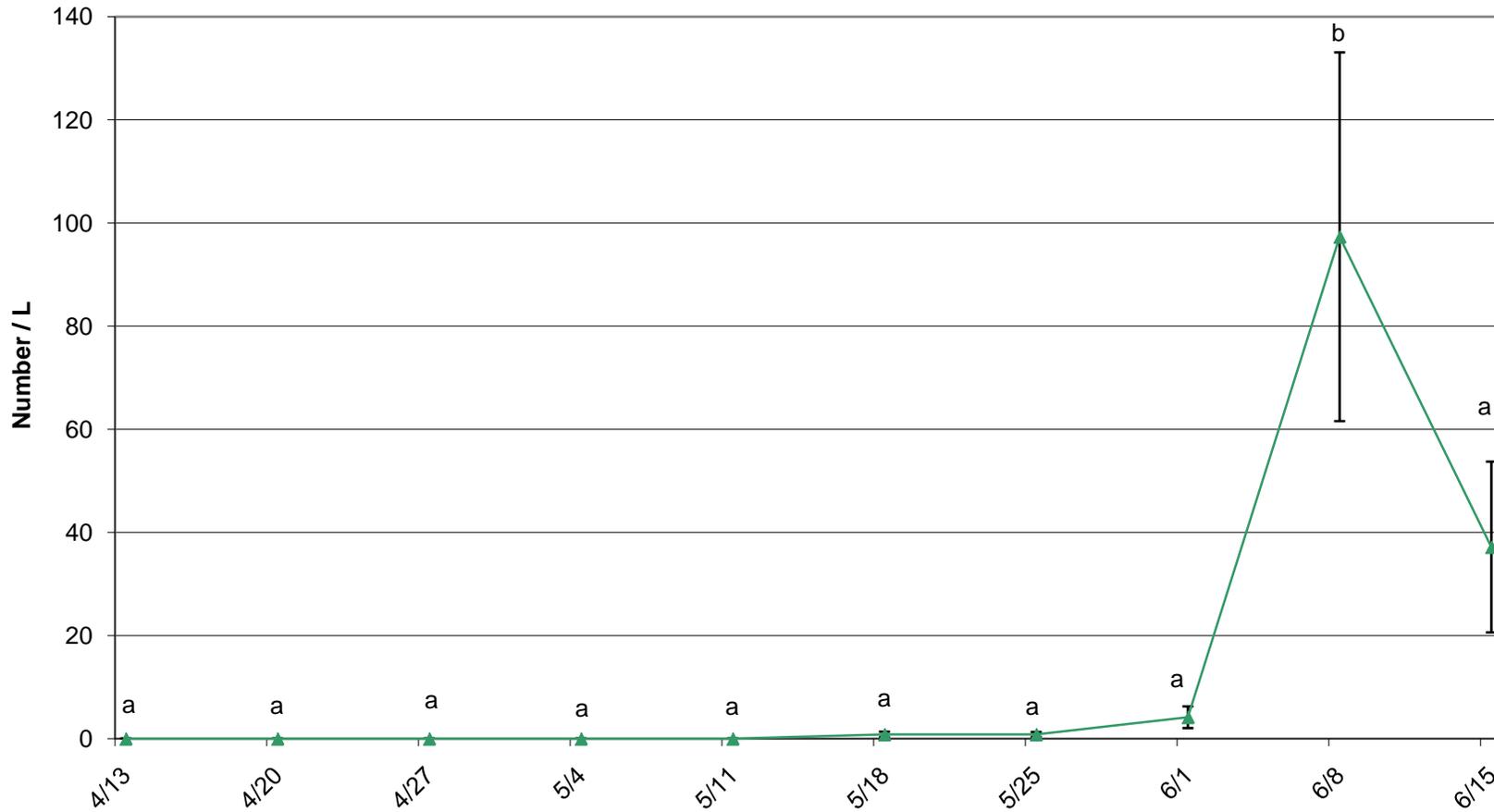


Figure 5-36. Inorganic fertilizer (INO) treatment *Moina macrocopa* (DAP; Daphnid) taxa densities (number/L \pm 95% CI) for 10 weekly sampling periods, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ replicate ponds.

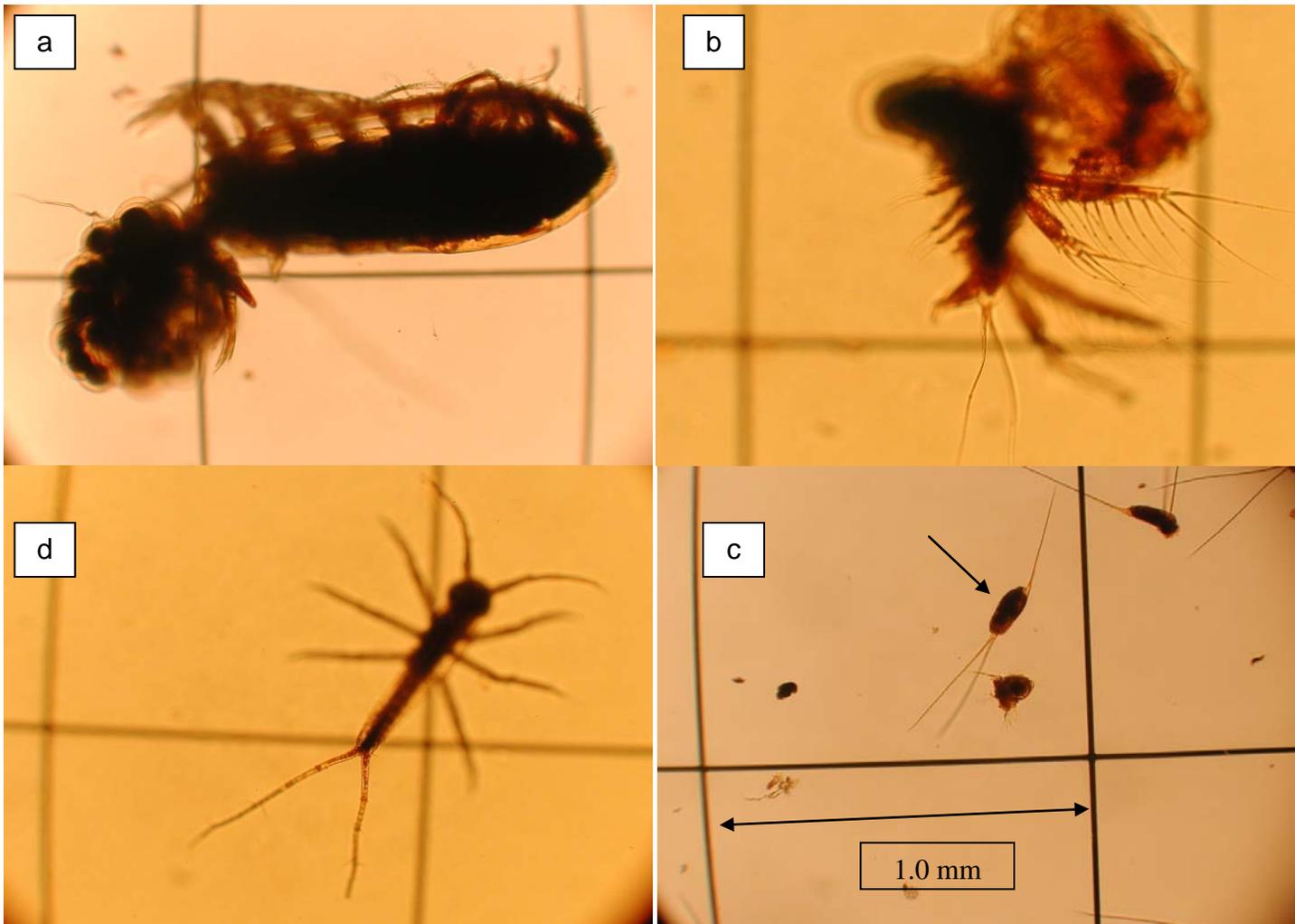


Figure 5-37a-d. Large ($> 200 \mu\text{m}$) plankton assemblage taxa, clockwise from upper right: (a) Calanoid copepod – *Diaptomous spp.*, (b) daphnid – *Moina macrocopa*, (c) rotifers – mainly *Filinia spp.*, (d) aquatic insect – (Ephemeroptera or Odonata); grid background (1.0 mm \times 1.0 mm).

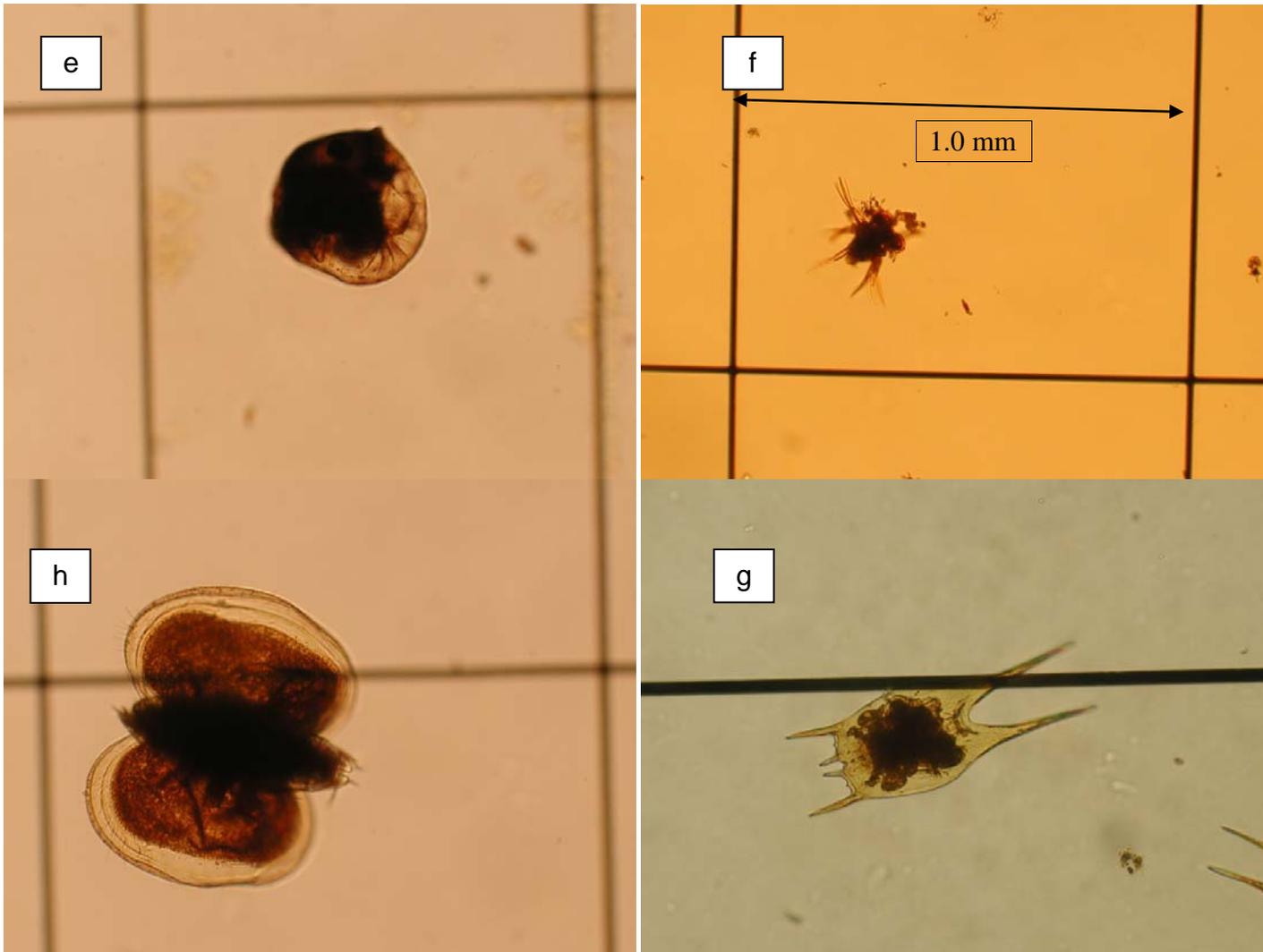


Figure 5-37e-h. Large plankton assemblage taxa, clockwise from upper right: (e) spherical rotifer, (f) rotifer – *Hexarthra spp.*, (g) rotifer – *Brachionus spp.*, (h) unknown ostracod; grid background (1.0 mm × 1.0 mm).

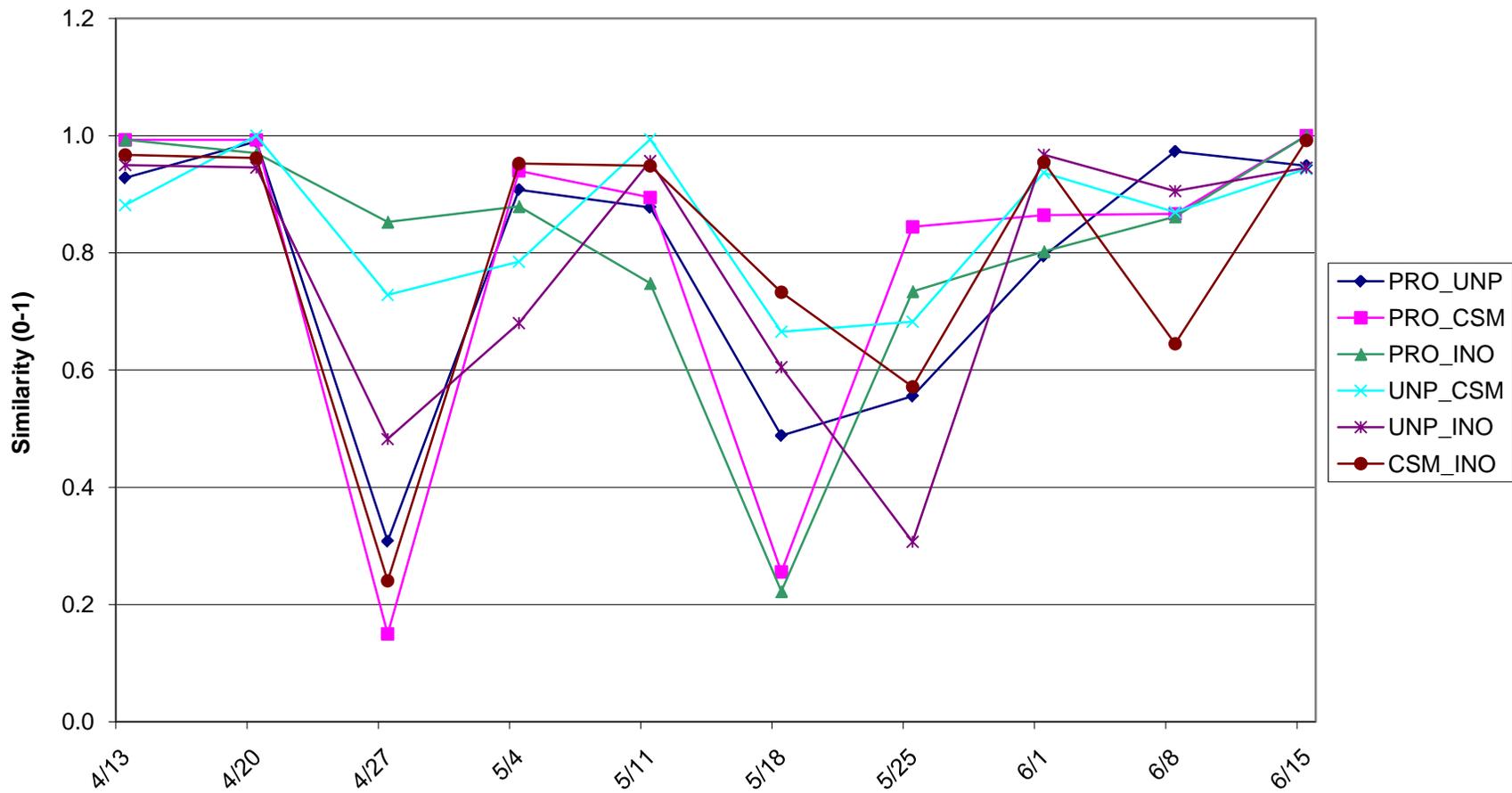


Figure 5-38. Large (> 200 μm) plankton assemblage treatment pair deterministic MSI (simplified Morisita's similarity index) values, generated from taxa pooled %IRI values for 10 weekly sampling periods. Four nutrient treatments: processed feed (PRO), unprocessed feed (UNP), cottonseed meal fertilizer (CSM), and inorganic fertilizer (INO).

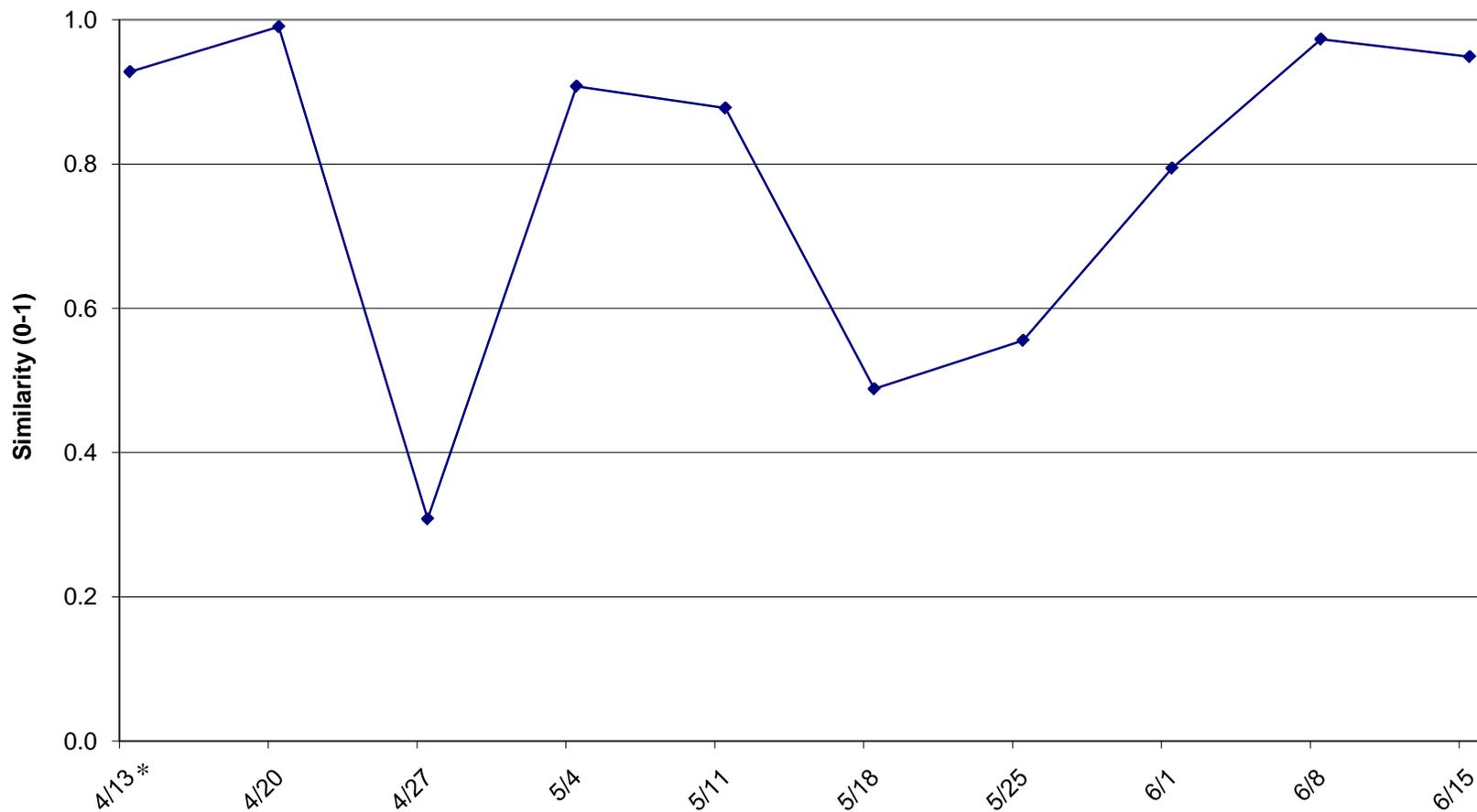


Figure 5-39. Processed feed (PRO) and unprocessed feed (PRO) treatment deterministic MSI values for 10 weekly sampling periods generated from large (> 200 μm) plankton assemblage taxa %IRI values (critical value $C_H \geq 0.65$); $n = 6$ replicate ponds per treatment, * only five UNP treatment replicate samples available for this date.

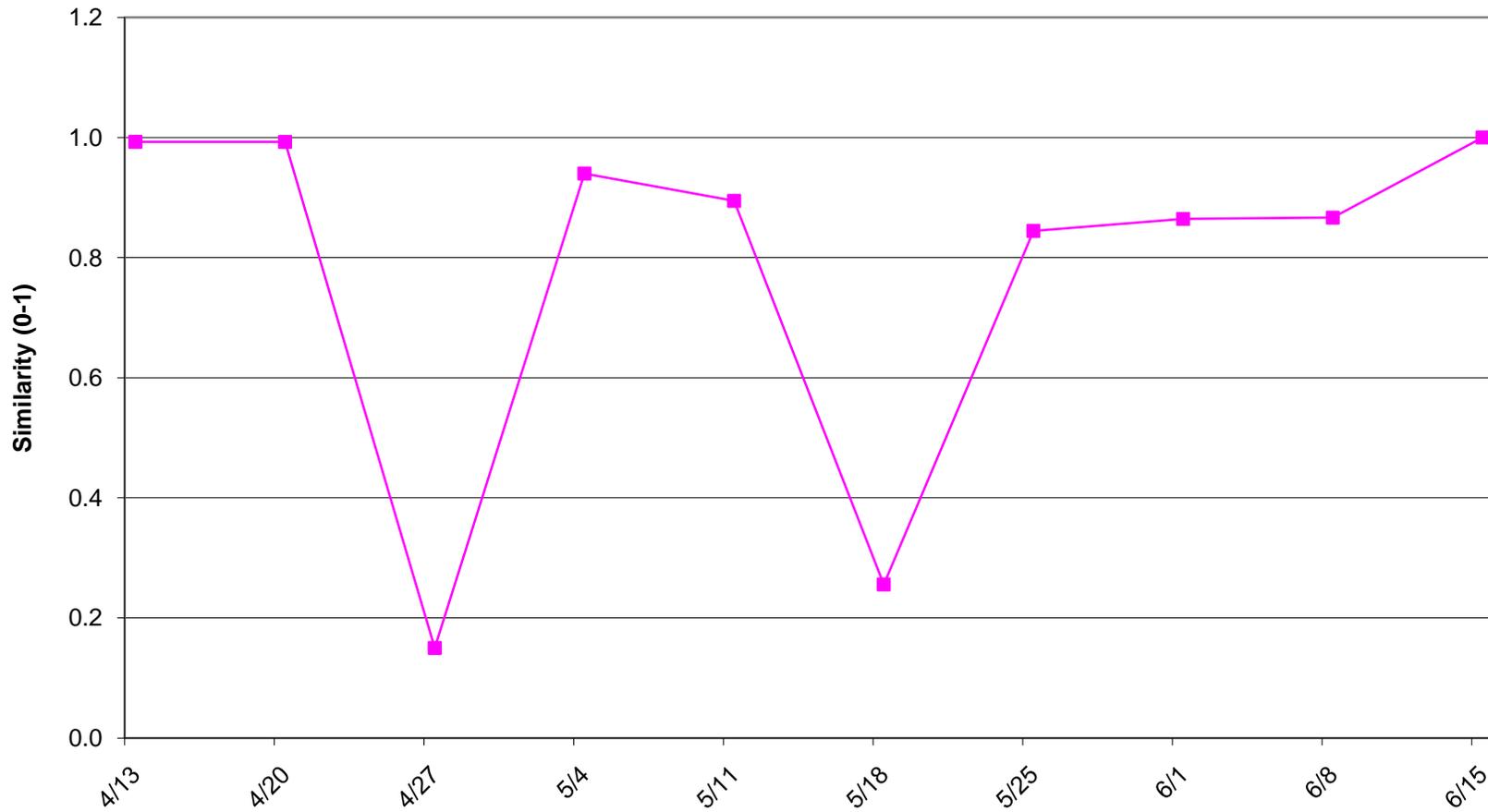


Figure 5-40. Processed feed (PRO) and cottonseed meal (CSM) fertilizer treatment deterministic MSI values for 10 weekly sampling periods generated from large (> 200 μm) plankton assemblage taxa %IRI values (critical value $C_H \geq 0.65$); n = 6 replicate ponds per treatment.

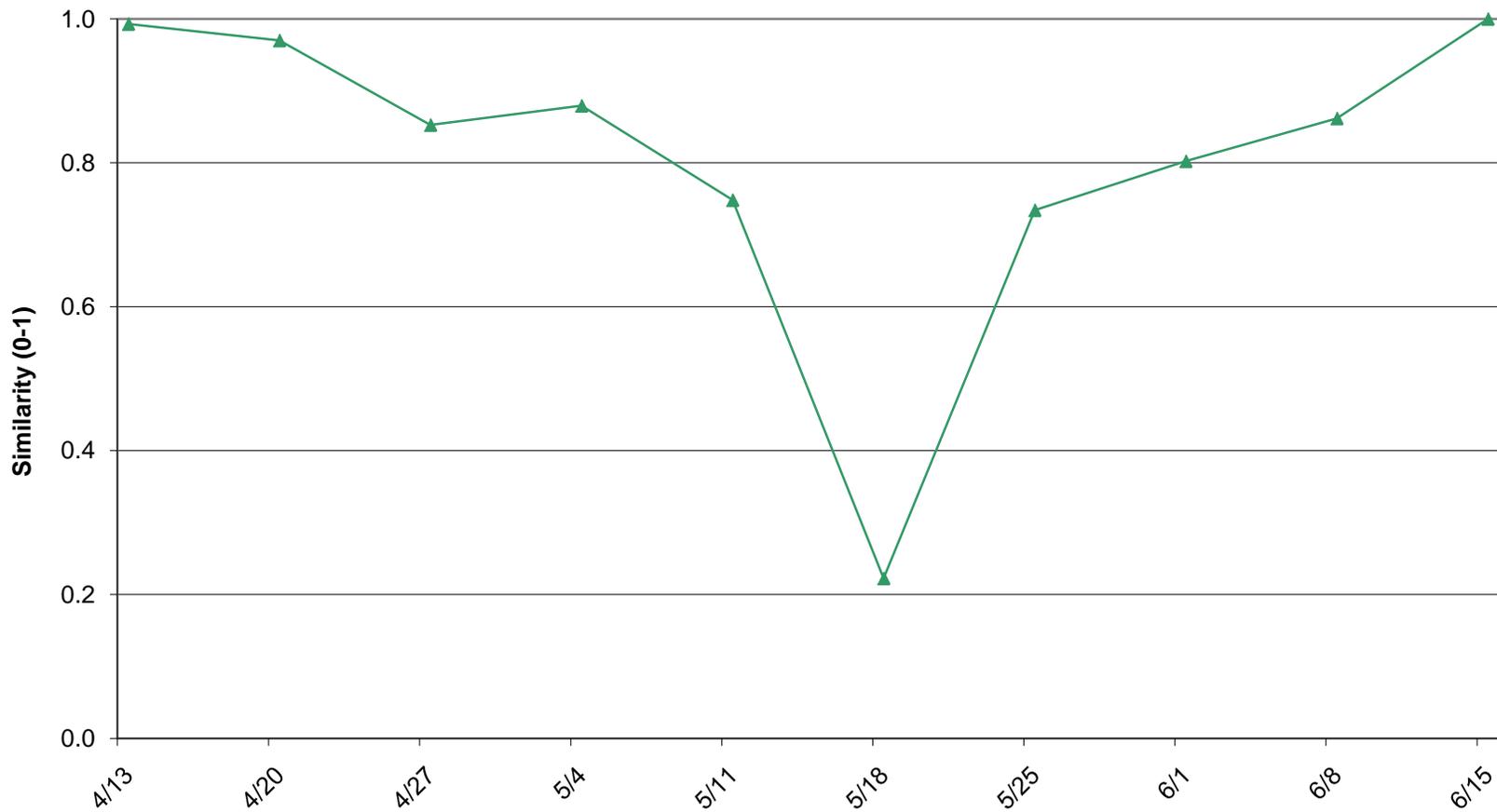


Figure 5-41. Processed feed (PRO) and inorganic (INO) fertilizer treatment deterministic MSI values for 10 weekly sampling periods generated from large (> 200 μm) plankton assemblage taxa %IRI values (critical value $C_H \geq 0.65$); $n = 6$ replicate ponds per treatment.

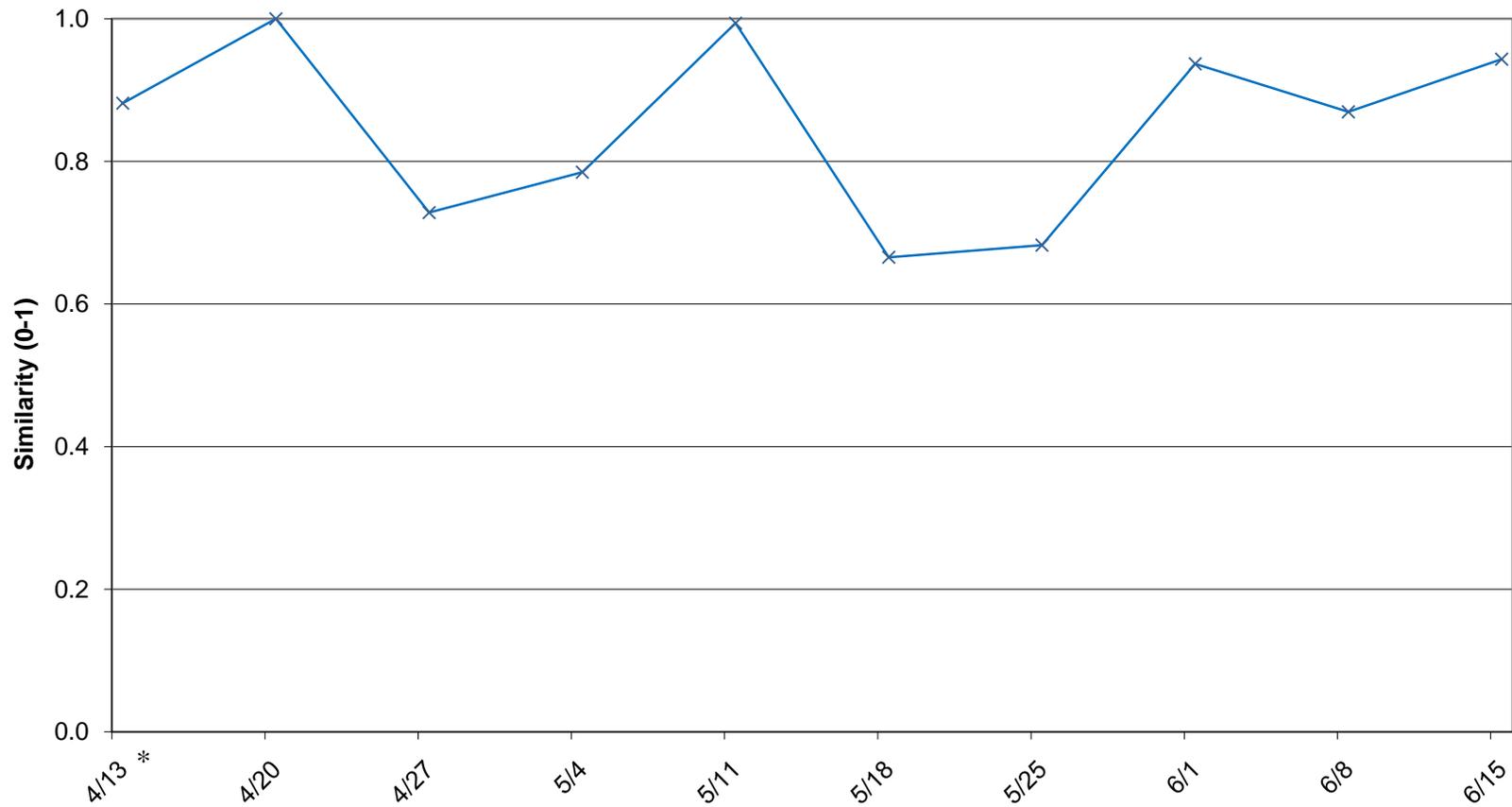


Figure 5-42. Unprocessed feed (UNP) and cottonseed meal (CSM) fertilizer treatment deterministic MSI values for 10 weekly sampling periods generated from large (> 200 μm) plankton assemblage taxa %IRI values (critical value $C_H \geq 0.65$); $n = 6$ replicate ponds per treatment, * only five replicate samples available for this sampling date.

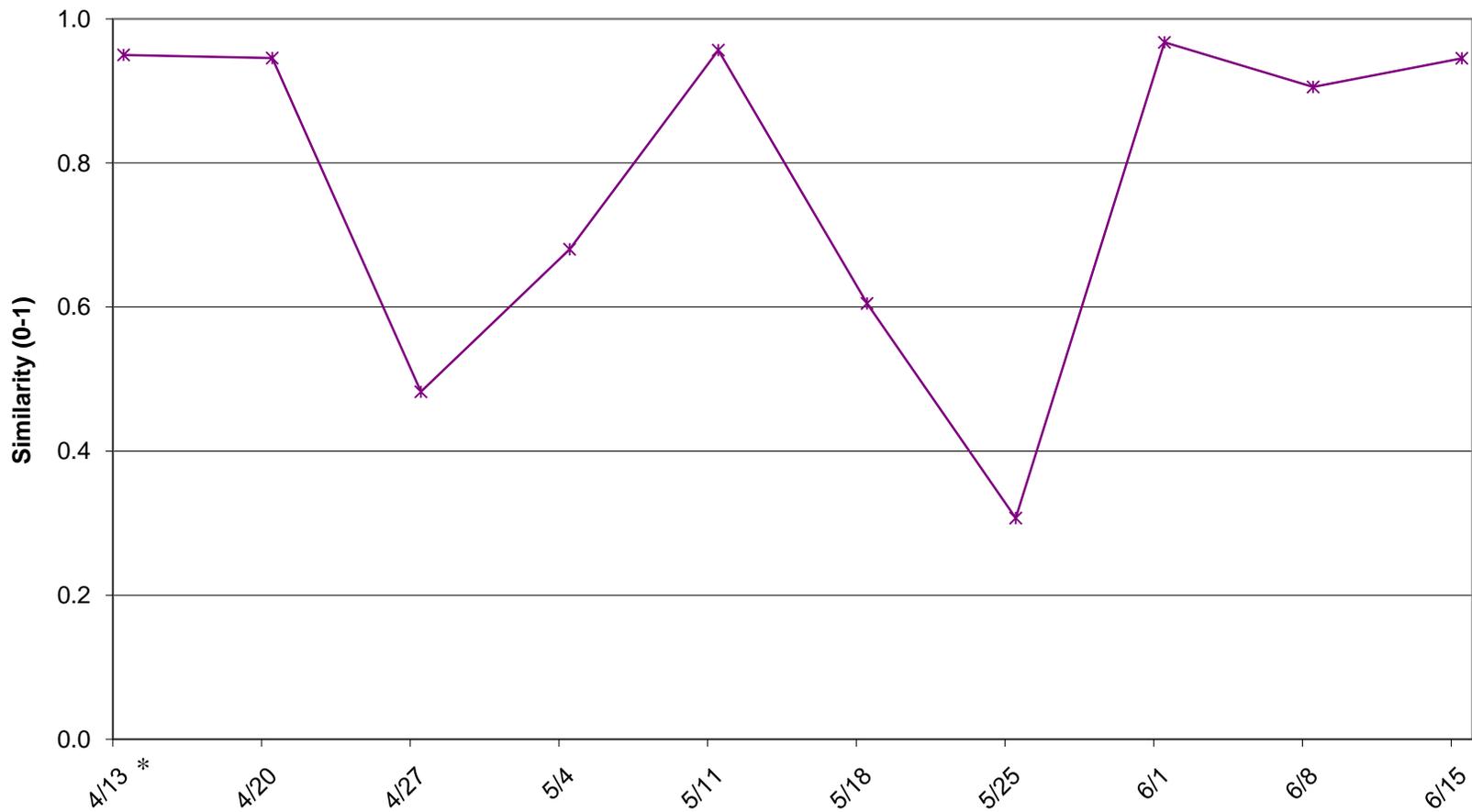


Figure 5-43. Unprocessed feed (UNP) and inorganic fertilizer (INO) treatment deterministic MSI values for 10 weekly sampling periods generated from large (> 200 μm) plankton assemblage taxa %IRI values (critical value $C_H \geq 0.65$); n = 6 replicate ponds per treatment, * only five replicate samples available for this sampling date.

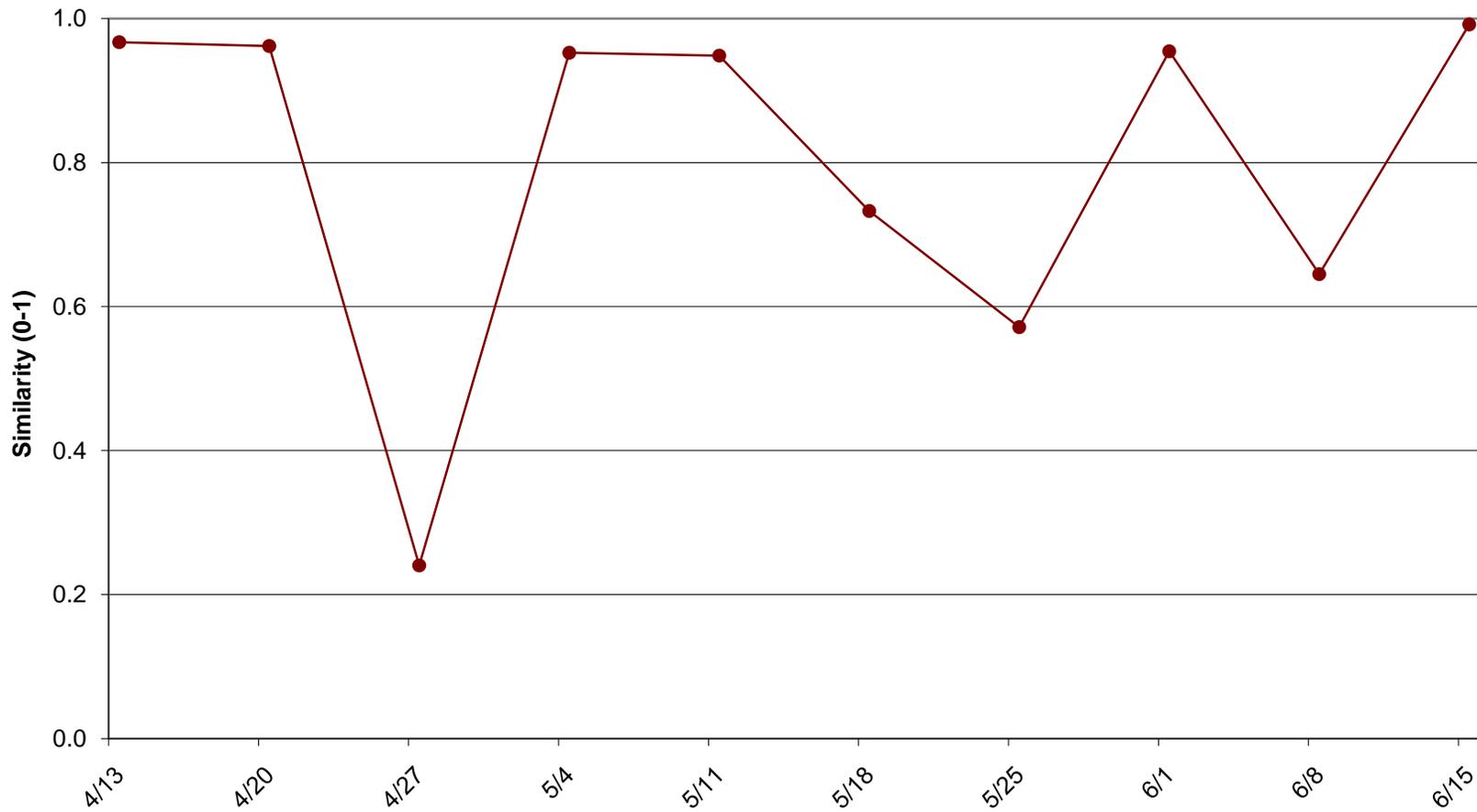


Figure 5-44. Cottonseed meal (CSM) and inorganic (INO) fertilizer treatment deterministic MSI values for 10 weekly sampling periods generated from large (> 200 μ m) plankton assemblage taxa %IRI values (critical value $C_H \geq 0.65$); n = 6 replicate ponds per treatment.

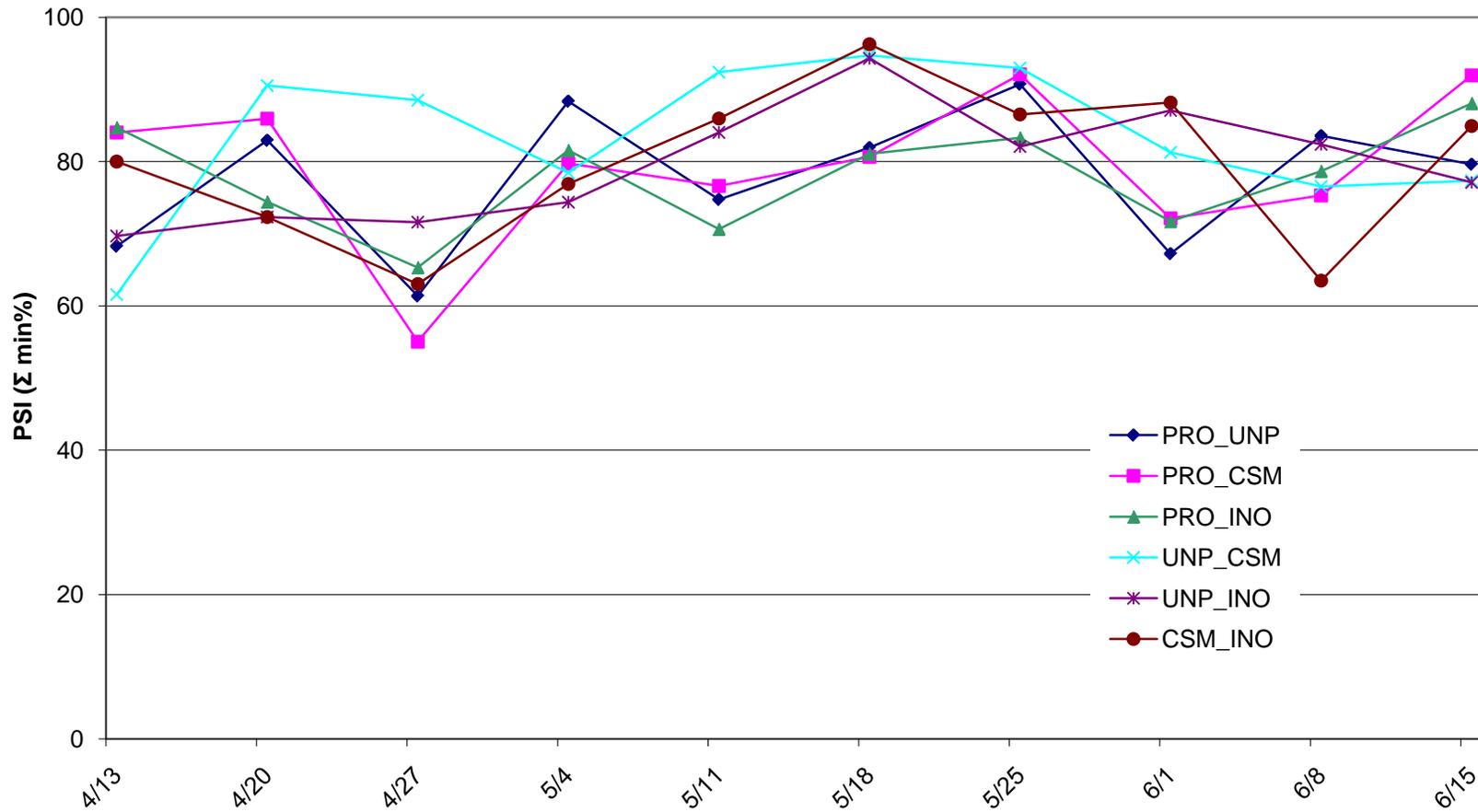


Figure 5-45. Large (> 200 μm) plankton assemblage treatment pair deterministic PSI (percent similarity index) values, generated from taxa pooled % IRI values for 10 weekly sampling periods. Four nutrient treatments: processed feed (PRO), unprocessed feed (UNP), cottonseed meal fertilizer (CSM), and inorganic fertilizer (INO).

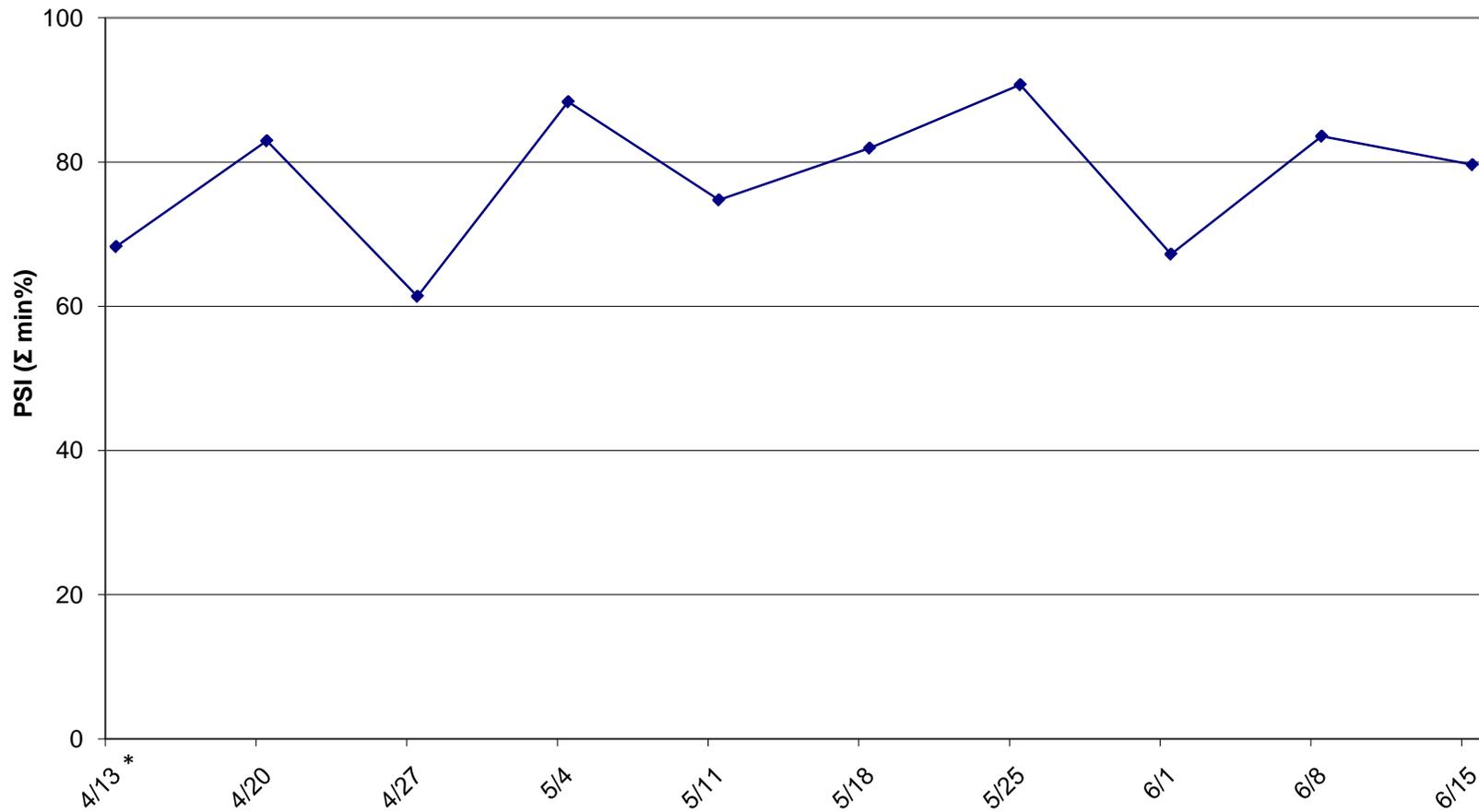


Figure 5-46. Processed (PRO) and unprocessed (UNP) feed treatment deterministic PSI (percent similarity index) values for 10 weekly sampling periods, generated from large (> 200 μm) plankton assemblage taxa %IRI values, six replicate ponds per treatment ($\text{PSI}_{(\text{critical value})} \geq 0.60$) ; * five UNP treatment replicate samples, see text.

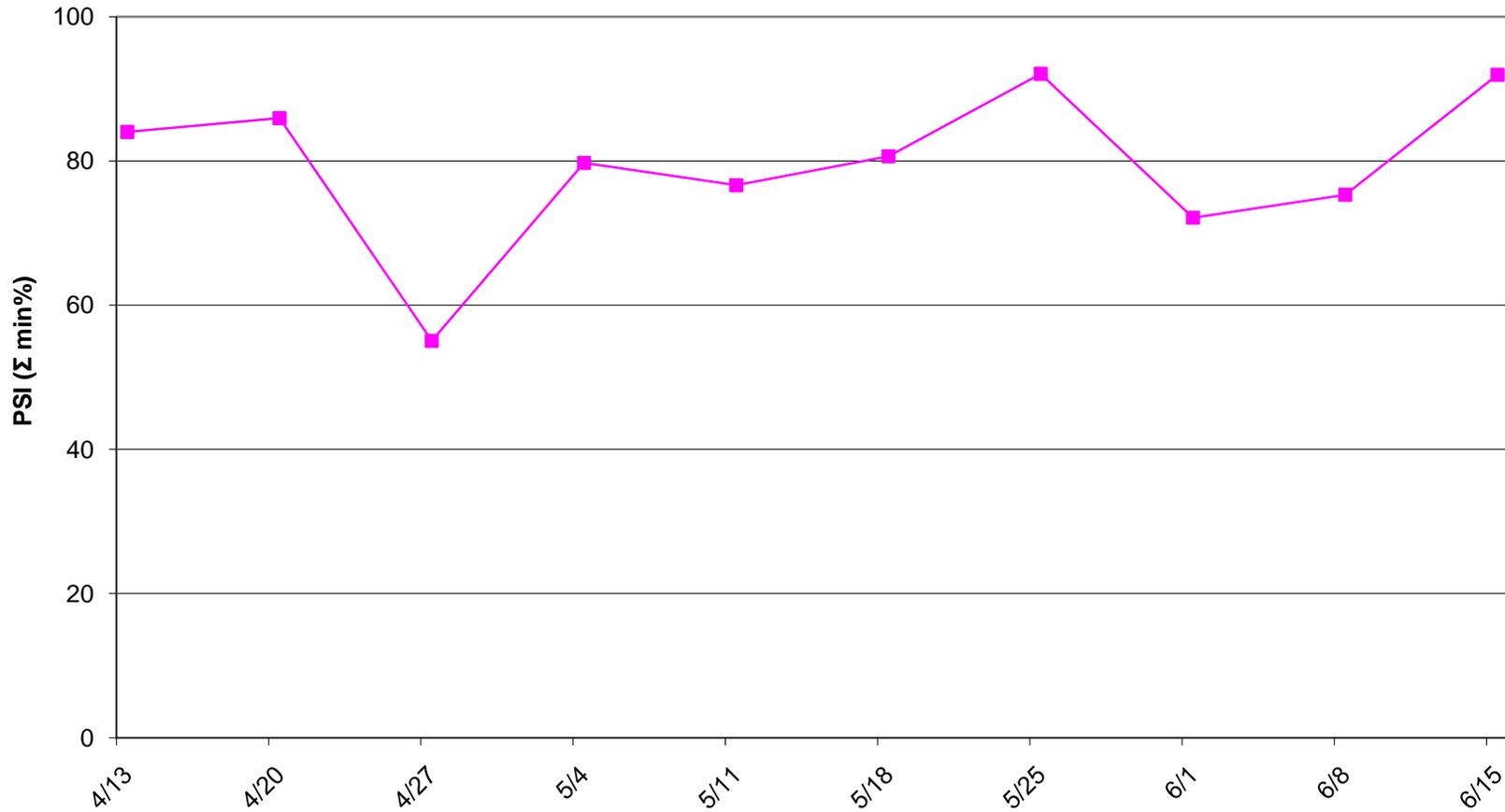


Figure 5-47. Processed feed (PRO) and cottonseed meal fertilizer (CSM) treatment deterministic PSI (percent similarity index) values for 10 weekly sampling periods, generated from large ($> 200 \mu\text{m}$) plankton assemblage taxa %IRI values, six replicate ponds per treatment ($\text{PSI}_{(\text{critical value})} \geq 0.60$).

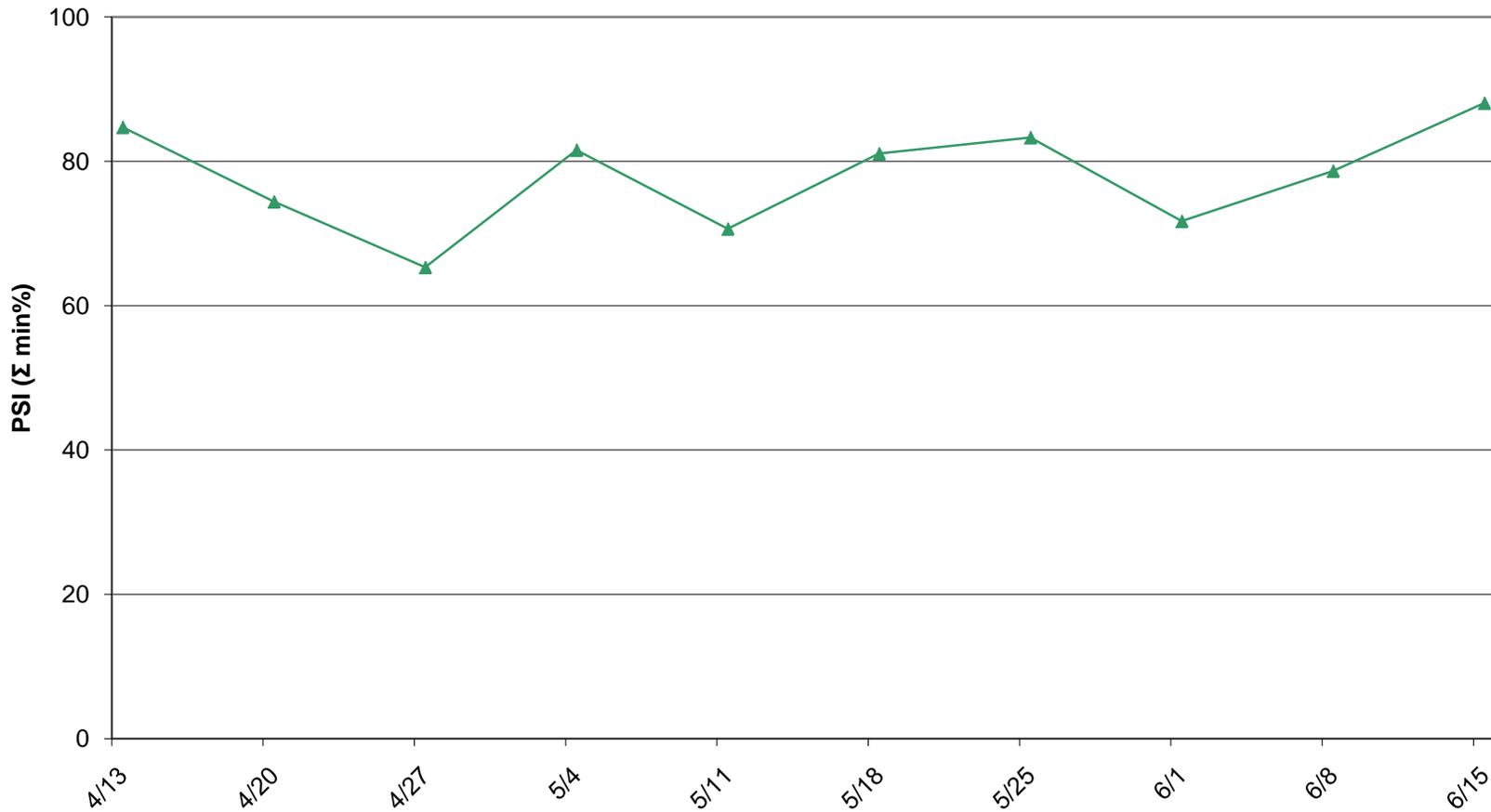


Figure 5-48. Processed feed (PRO) and inorganic fertilizer (INO) treatment deterministic PSI (percent similarity index) values for 10 weekly sampling periods, generated from large (> 200 μm) plankton assemblage taxa %IRI values ($PSI_{(critical\ value)} \geq 0.60$), n = 6 ponds per treatment.

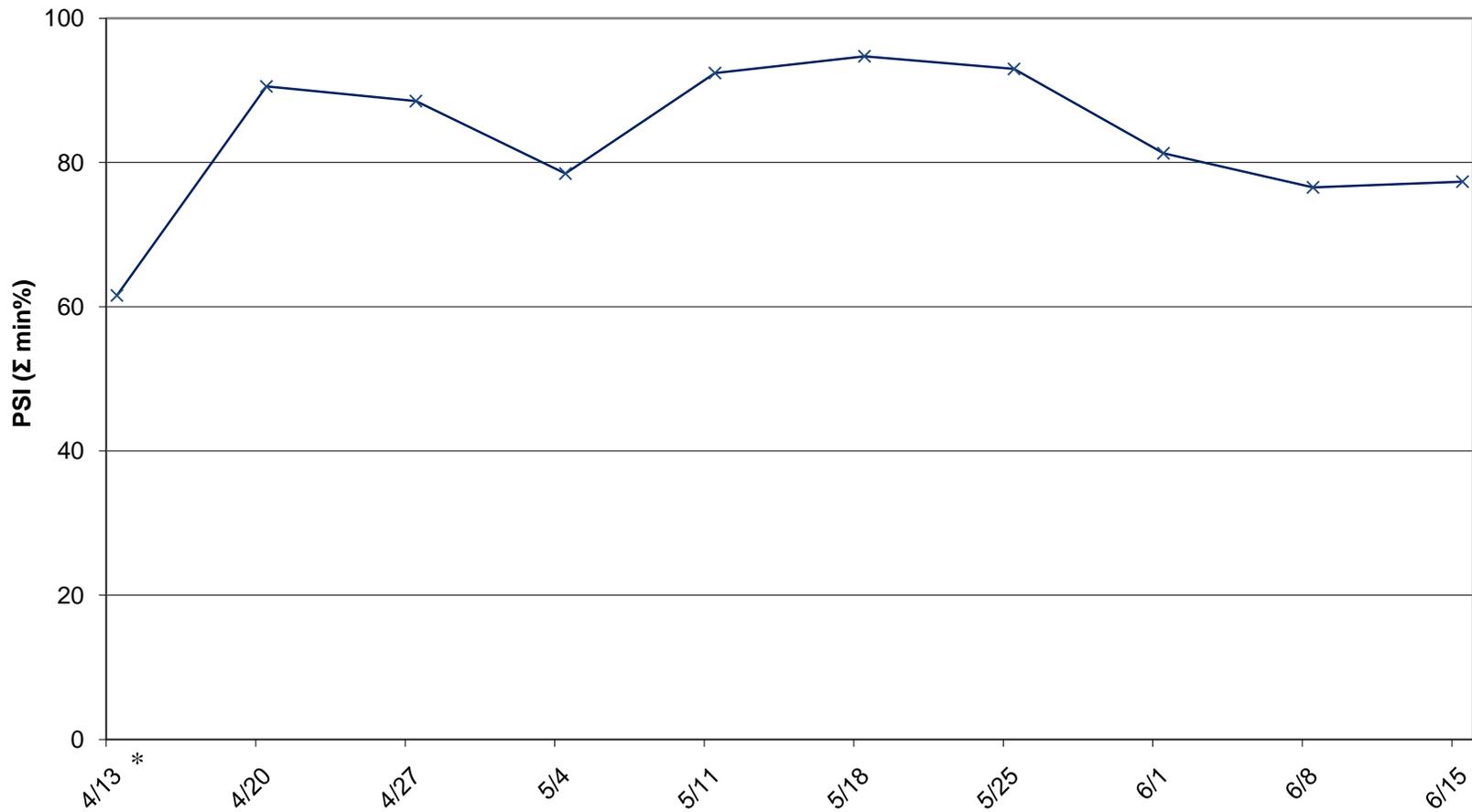


Figure 5-49. Unprocessed feed (UNP) and cottonseed meal (CSM) fertilizer treatment deterministic PSI (percent similarity index) values for 10 weekly sampling periods, generated from large (> 200 μm) plankton assemblage taxa %IRI values (critical value $PSI \geq 0.60$); n = 6 ponds per treatment.



Figure 5-50. Unprocessed feed (UNP) and inorganic fertilizer (INO) treatment deterministic PSI (Percent Similarity Index) values for 10 weekly sampling periods, generated from large (> 200 μm) plankton assemblage taxa %IRI values (critical value $\text{PSI} \geq 0.60$); $n = 6$ ponds per treatment, * only 5 UNP treatment replicate samples available for this date.

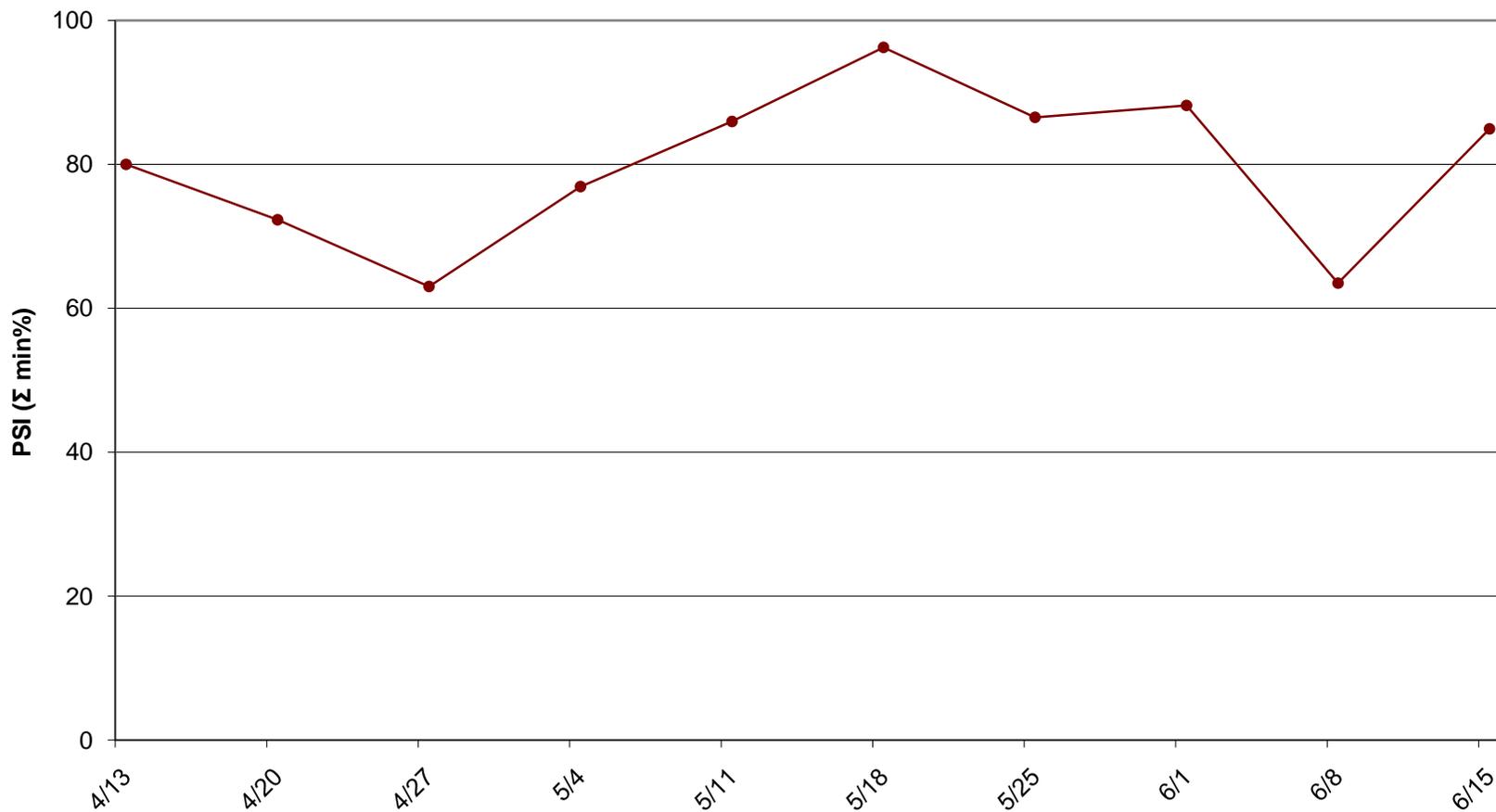


Figure 5-51. Cottonseed meal (CSM) and inorganic fertilizer (INO) treatment deterministic PSI (Percent Similarity Index) values for 10 weekly sampling periods, generated from large (> 200 μm) plankton assemblage taxa %IRI values (critical value $\text{PSI} \geq 0.60$); $n = 6$ ponds per treatment).

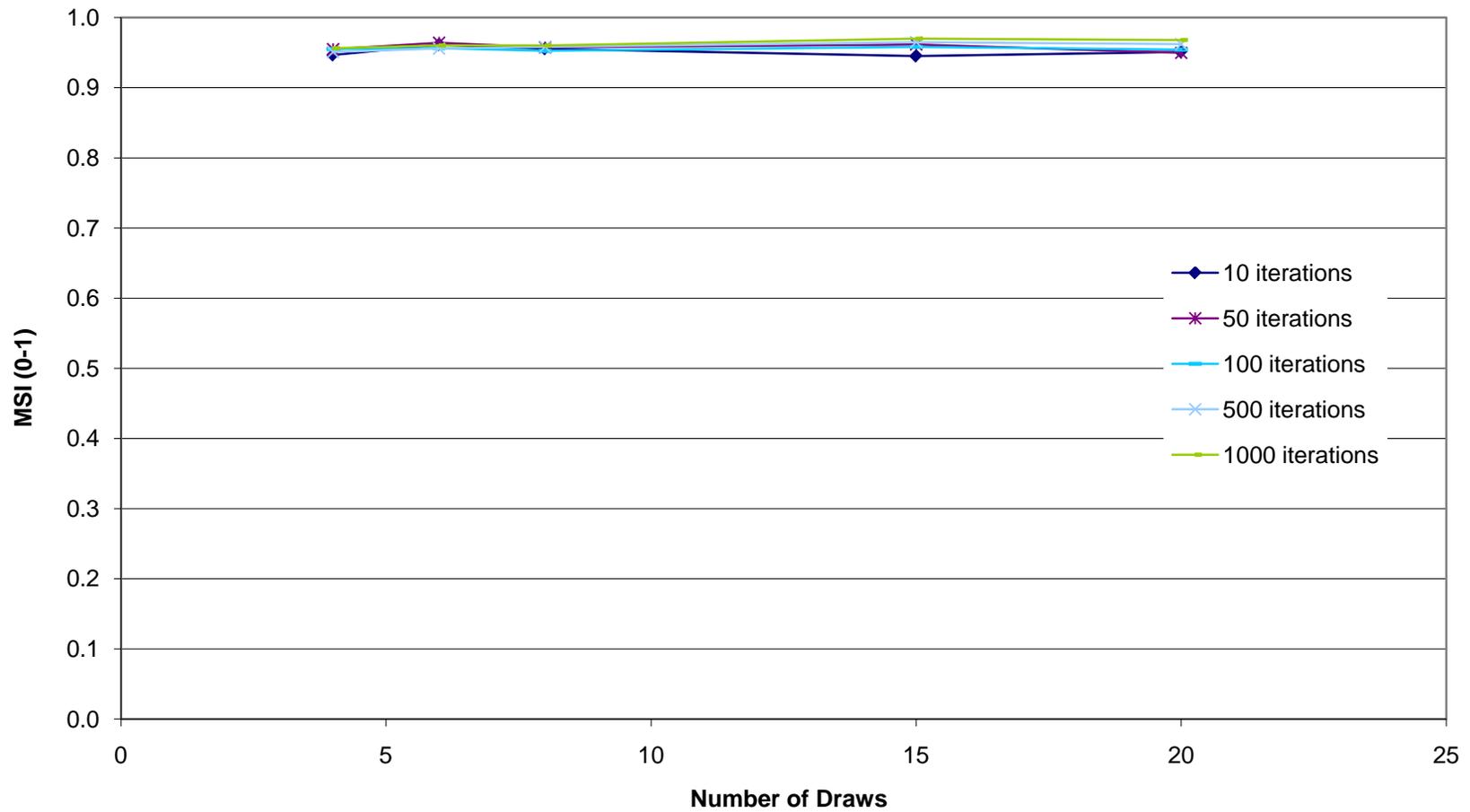


Figure 5-52. Processed (PRO) and unprocessed (UNP) feed treatment bootstrap MSI (Simplified Morisita's Index) values (critical value $C_H \geq 0.65$) generated from different numbers of randomly drawn replicates and iterations using large ($> 200 \mu\text{m}$) plankton taxa %N values from a single sampling date (25 May 2006), $n = 6$ replicate ponds.

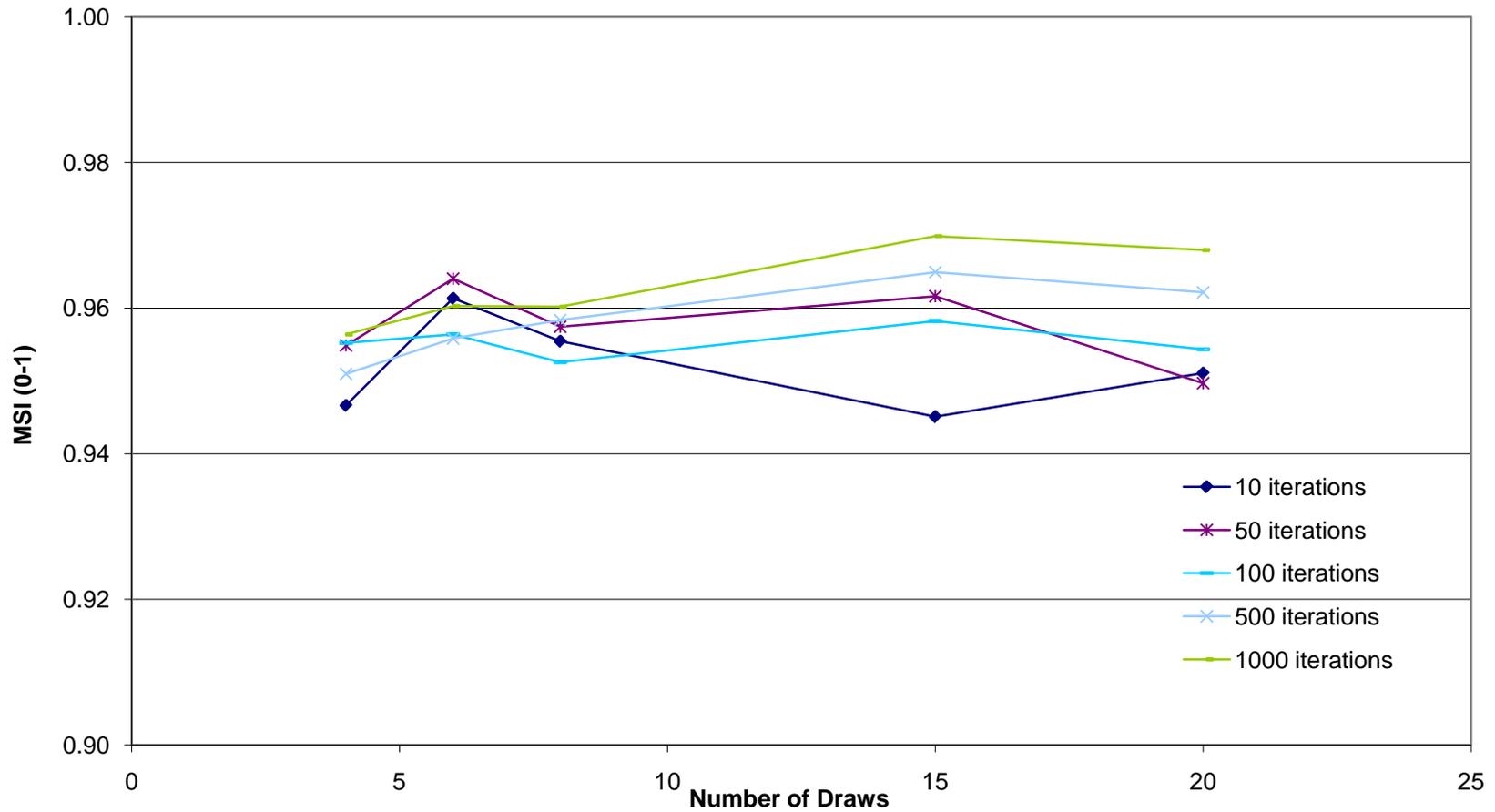


Figure 5-53. Scale adjusted processed (PRO) and unprocessed (UNP) feed treatment bootstrap MSI (simplified Morisita's Index) values (critical value $C_H \geq 0.65$) generated from different numbers of randomly drawn replicates and iterations using large ($> 200 \mu\text{m}$) plankton assemblage taxa %N values from a single sampling date (25 May 2006), $n = 6$ replicate ponds.

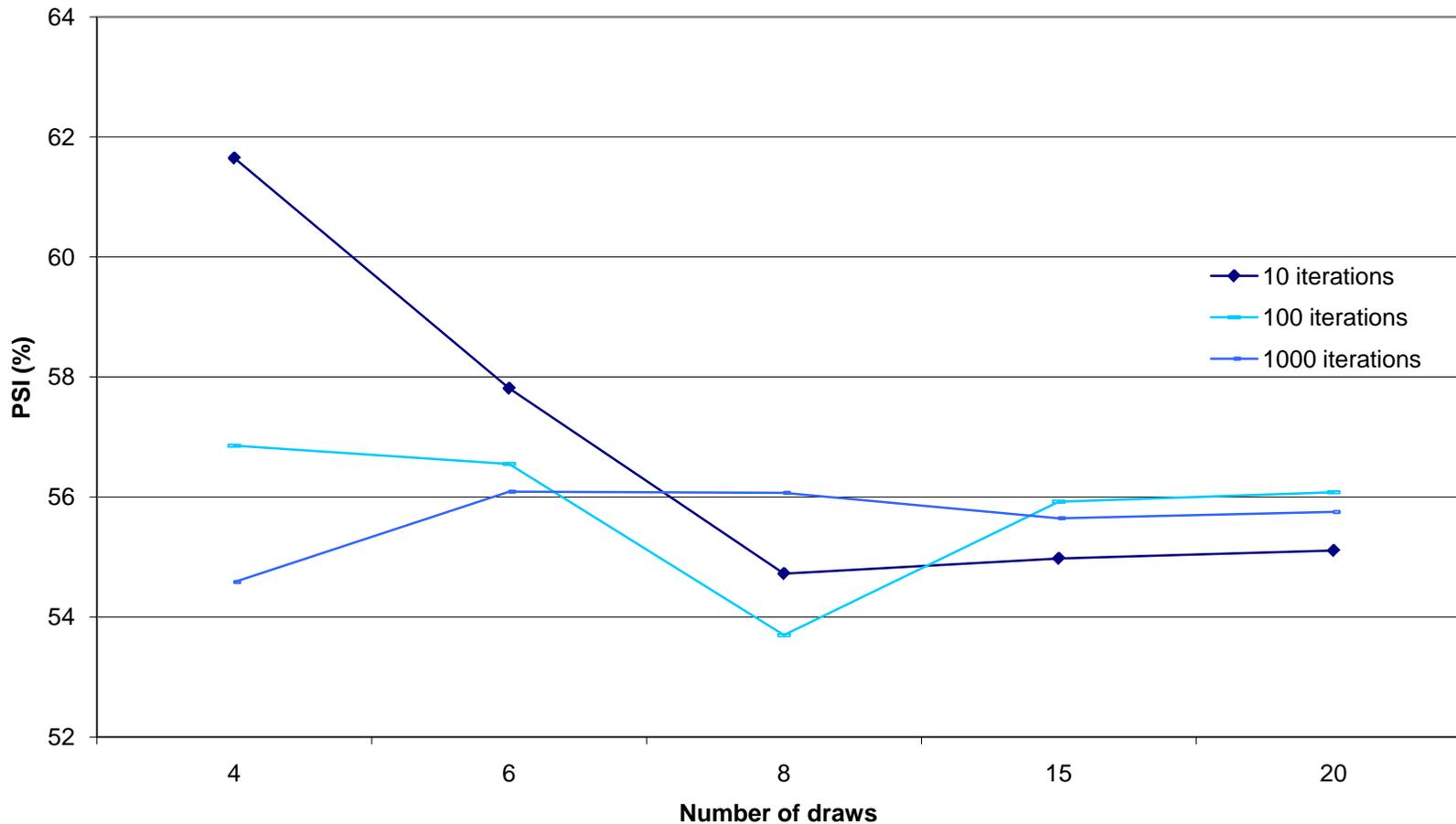


Figure 5-54. Processed feed (PRO) and inorganic fertilizer (INO) treatment bootstrap PSI (Percent Similarity Index) values (critical value $PSI \geq 0.60$) generated from differing numbers of randomly drawn replicates and iterations using large ($> 200 \mu\text{m}$) plankton assemblage taxa %N values from a single sampling date (25 May 2006); $n = 6$ replicate ponds.

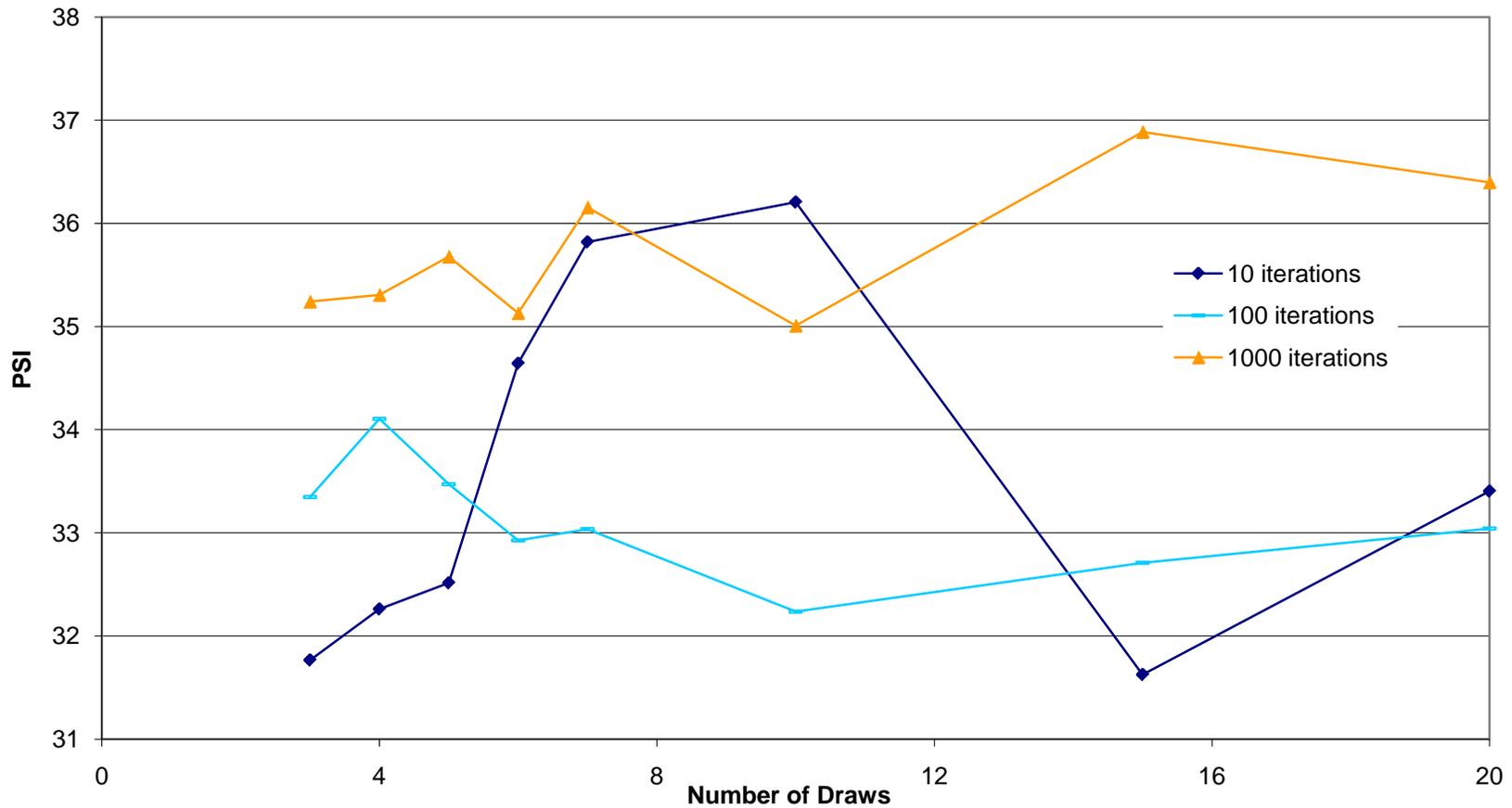


Figure 5-55. Processed (PRO) and unprocessed (UNP) feed treatment bootstrap PSI (Percent Similarity Index) values (critical value $PSI \geq 0.60$) generated from differing numbers of randomly drawn replicates and iterations using large ($> 200 \mu\text{m}$) plankton assemblage taxa %N values from a single sampling date (13 April 2006), $n = 6$ replicate ponds.

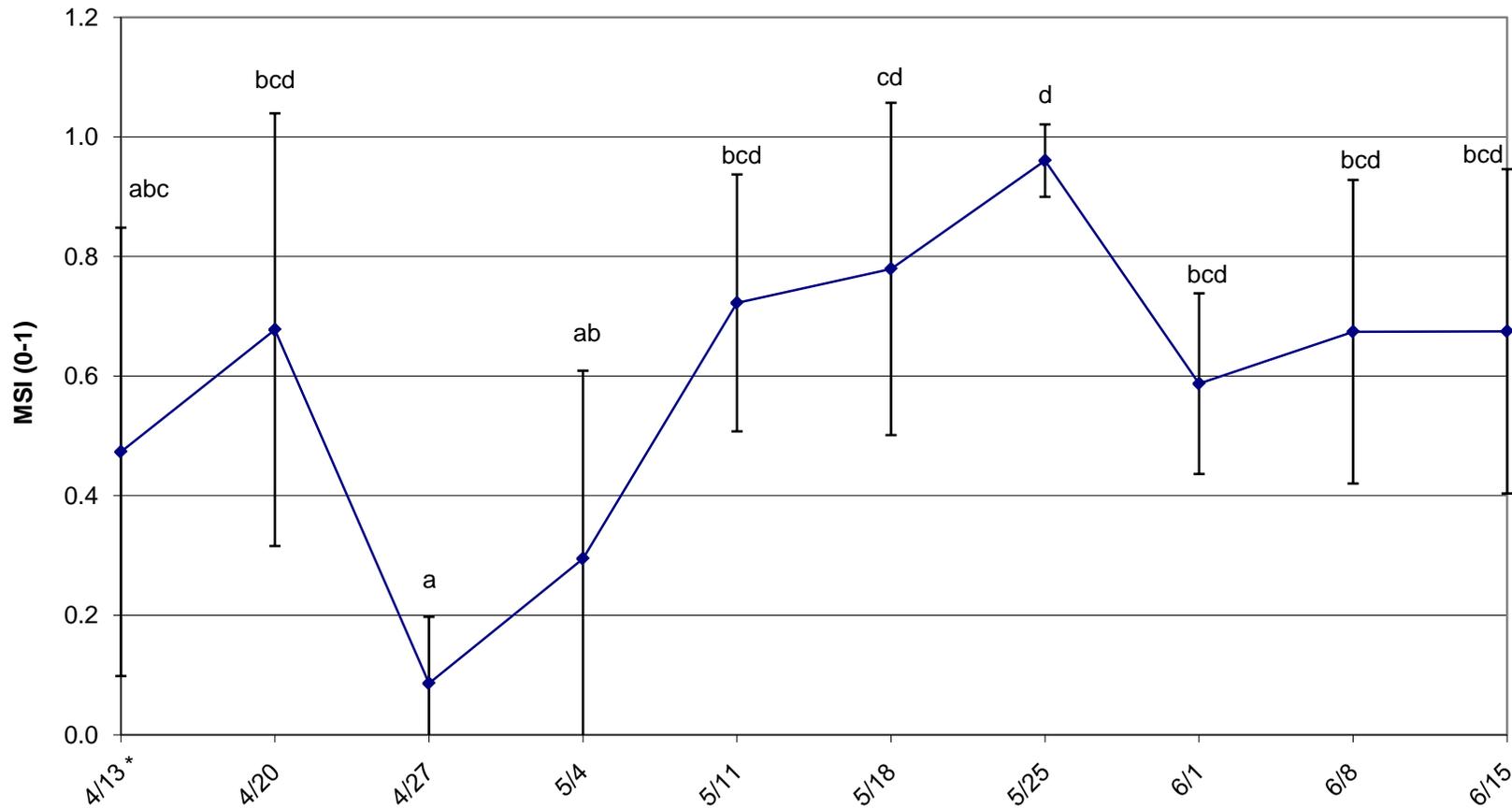


Figure 5-56. Processed (PRO) and unprocessed (UNP) feed treatment bootstrap MSI (simplified Morisita's similarity index; critical value $C_H \geq 0.65$) values (\pm 95% CI) generated from 1000 iterations per sampling period using large ($> 200 \mu\text{m}$) plankton assemblage taxa %N values, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ ponds per treatment, * only 5 UNP replicate samples available for this date.

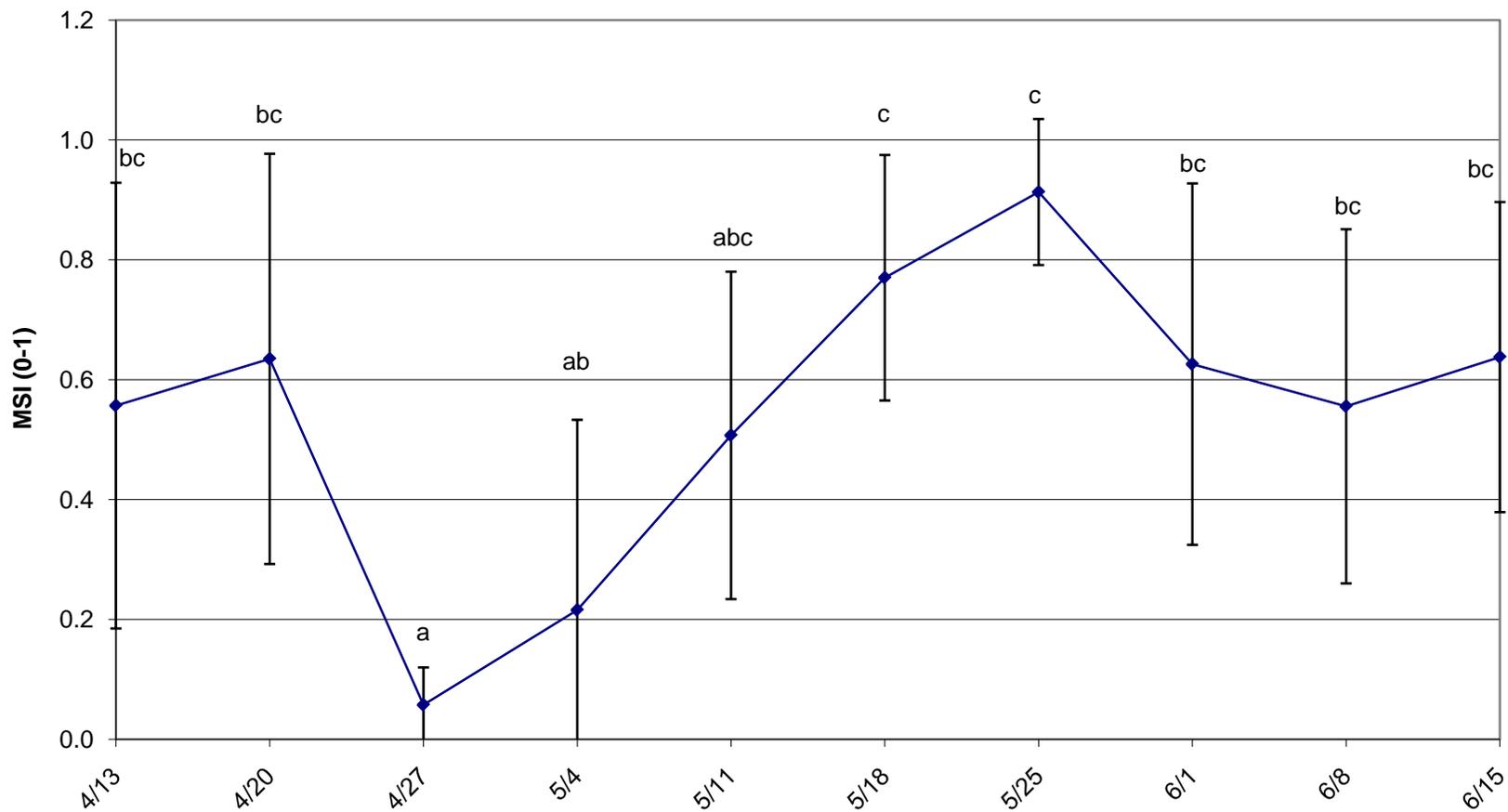


Figure 5-57. Processed feed (PRO) and cottonseed meal fertilizer (CSM) treatment bootstrap MSI (simplified Morisita's similarity index; critical value $C_H \geq 0.65$) values (\pm 95% CI) generated from 1000 iterations per sampling period using large ($> 200 \mu\text{m}$) plankton assemblage taxa %N values, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ ponds per treatment.

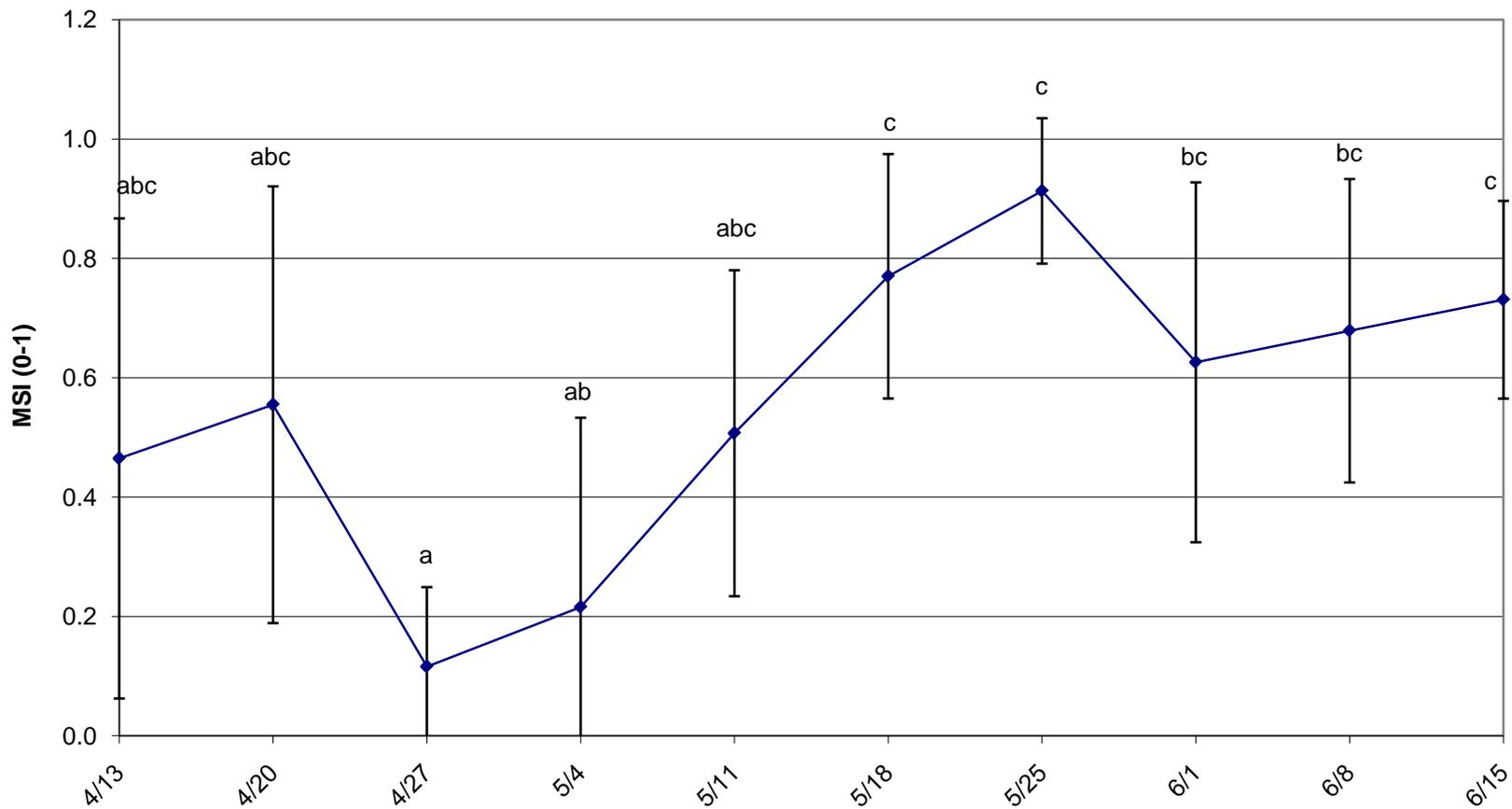


Figure 5-58. Processed feed (PRO) and inorganic fertilizer (INO) treatment bootstrap MSI (simplified Morisita's similarity index; critical value $C_H \geq 0.65$) values ($\pm 95\%$ CI) generated from randomly drawn replicates at 1000 iterations per sampling period using large ($> 200 \mu\text{m}$) plankton assemblage taxa %N values, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ ponds per treatment.

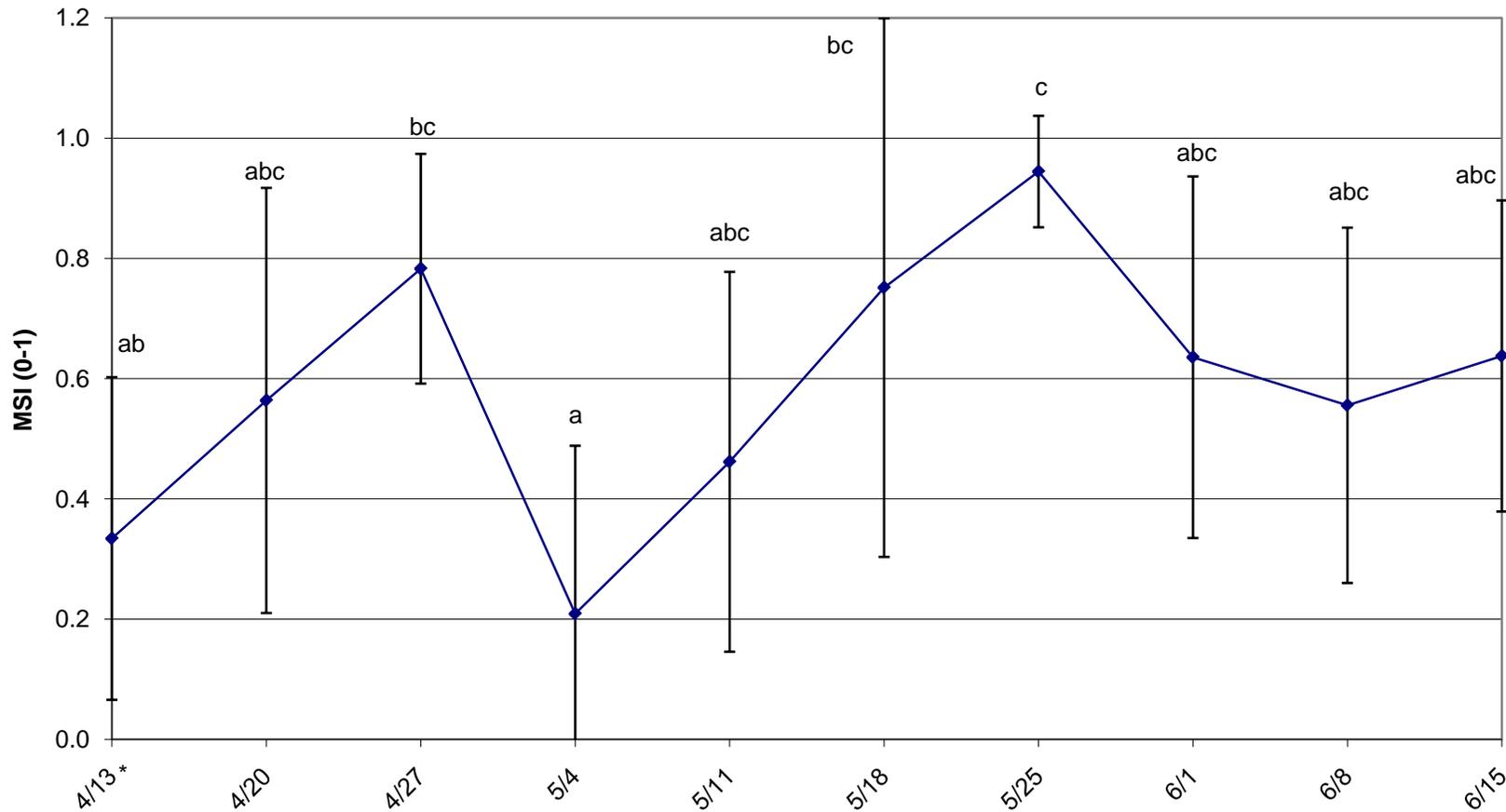


Figure 5-59. Unprocessed feed (UNP) and cottonseed meal fertilizer (CSM) treatment bootstrap MSI (simplified Morisita's similarity index; critical value $C_H \geq 0.65$) values ($\pm 95\%$ CI) generated from 1000 iterations per sampling period using large ($> 200 \mu\text{m}$) plankton assemblage taxa %N values, unshared letters denote statistical differences between sampling dates ($P < 0.05$, Bonferroni post test); $n = 6$ ponds per treatment, * only 5 UNP replicate samples available for this date.

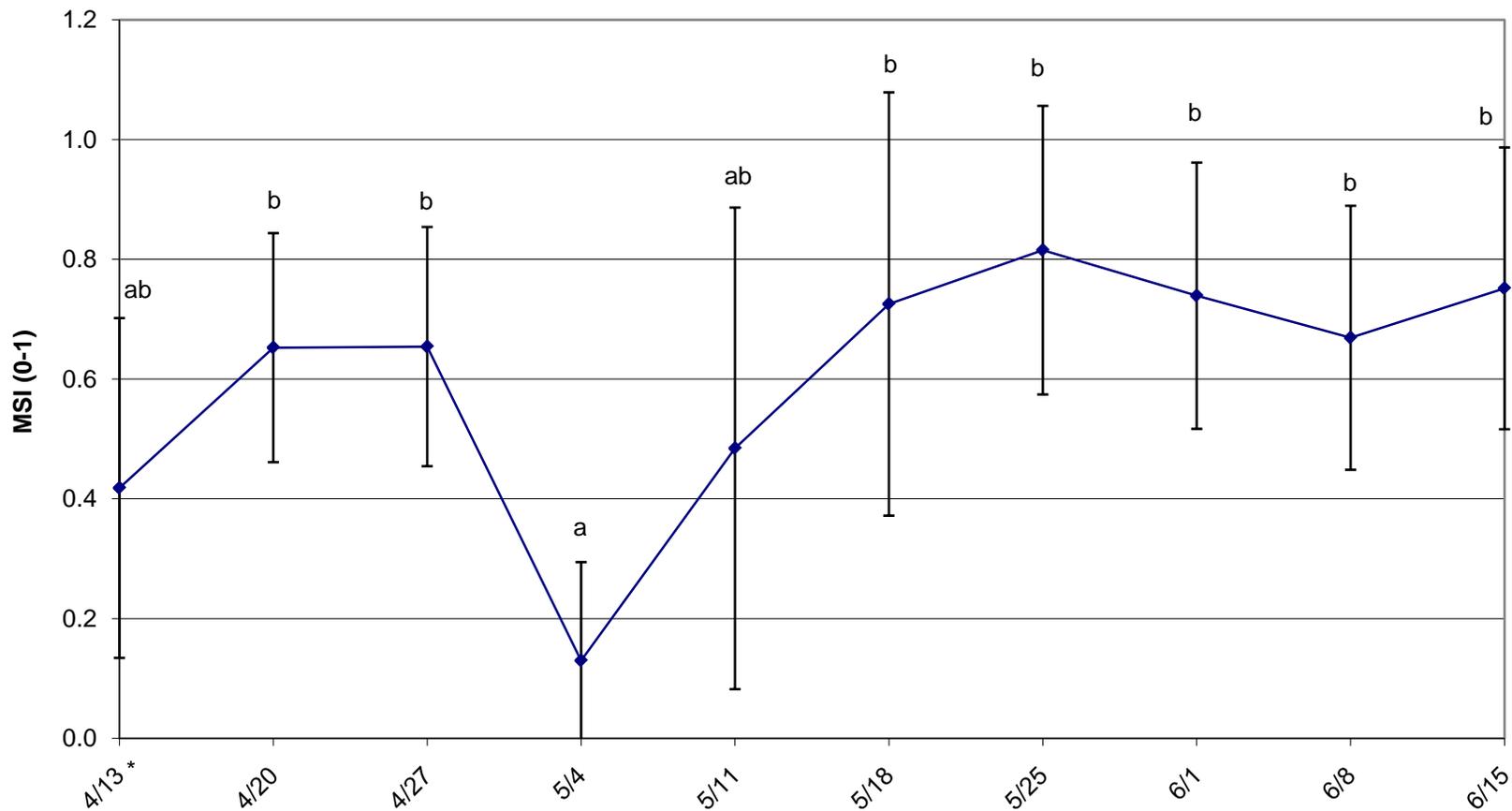


Figure 5-60. Unprocessed feed (UNP) and inorganic fertilizer (INO) treatment bootstrap MSI (simplified Morisita's similarity index; critical value $C_H \geq 0.65$) values (\pm 95% CI) generated from 1000 iterations per sampling period using large ($> 200 \mu\text{m}$) plankton assemblage taxa %N values, unshared letters denote statistical differences between sampling periods; n = 6 ponds per treatment; * only 5 UNP replicate samples available for this sampling date.

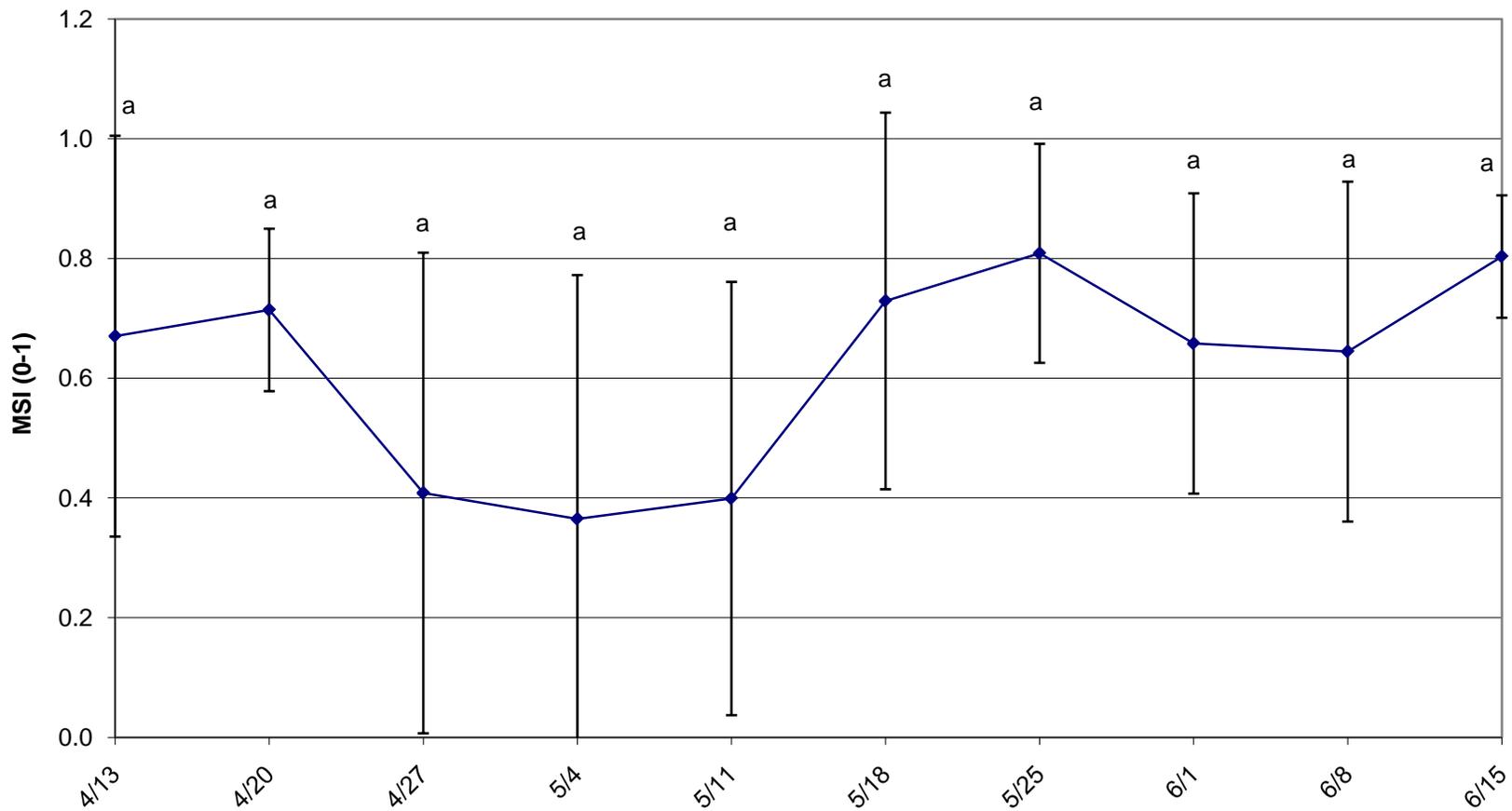


Figure 5-61. Cottonseed meal (CSM) and inorganic fertilizer (INO) treatment bootstrap MSI (simplified Morisita's similarity index; critical value $C_H \geq 0.65$) values ($\pm 95\%$ CI) generated from randomly drawn replicates at 1000 iterations per sampling period using large ($> 200 \mu\text{m}$) plankton assemblage taxa %N values, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ ponds per treatment.

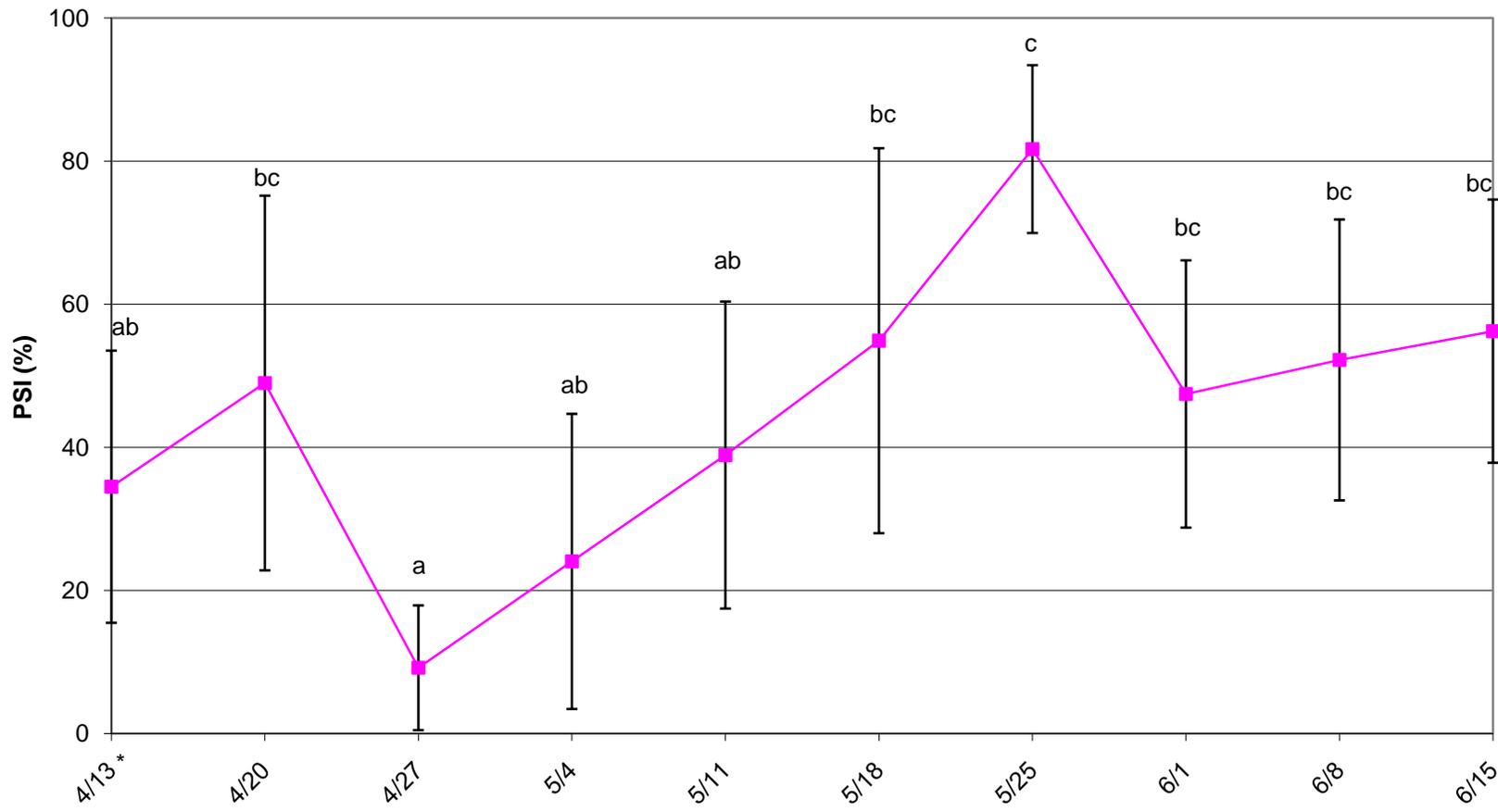


Figure 5-62. Processed (PRO) and unprocessed (UNP) feed treatment bootstrap PSI (percent similarity index; critical value $PSI \geq 0.60$) values ($\pm 95\%$ CI) generated from 1000 iterations per sampling period using large ($> 200 \mu m$) plankton assemblage taxa %N values, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ ponds per treatment, * only 5 UNP replicate samples available for this sampling date.

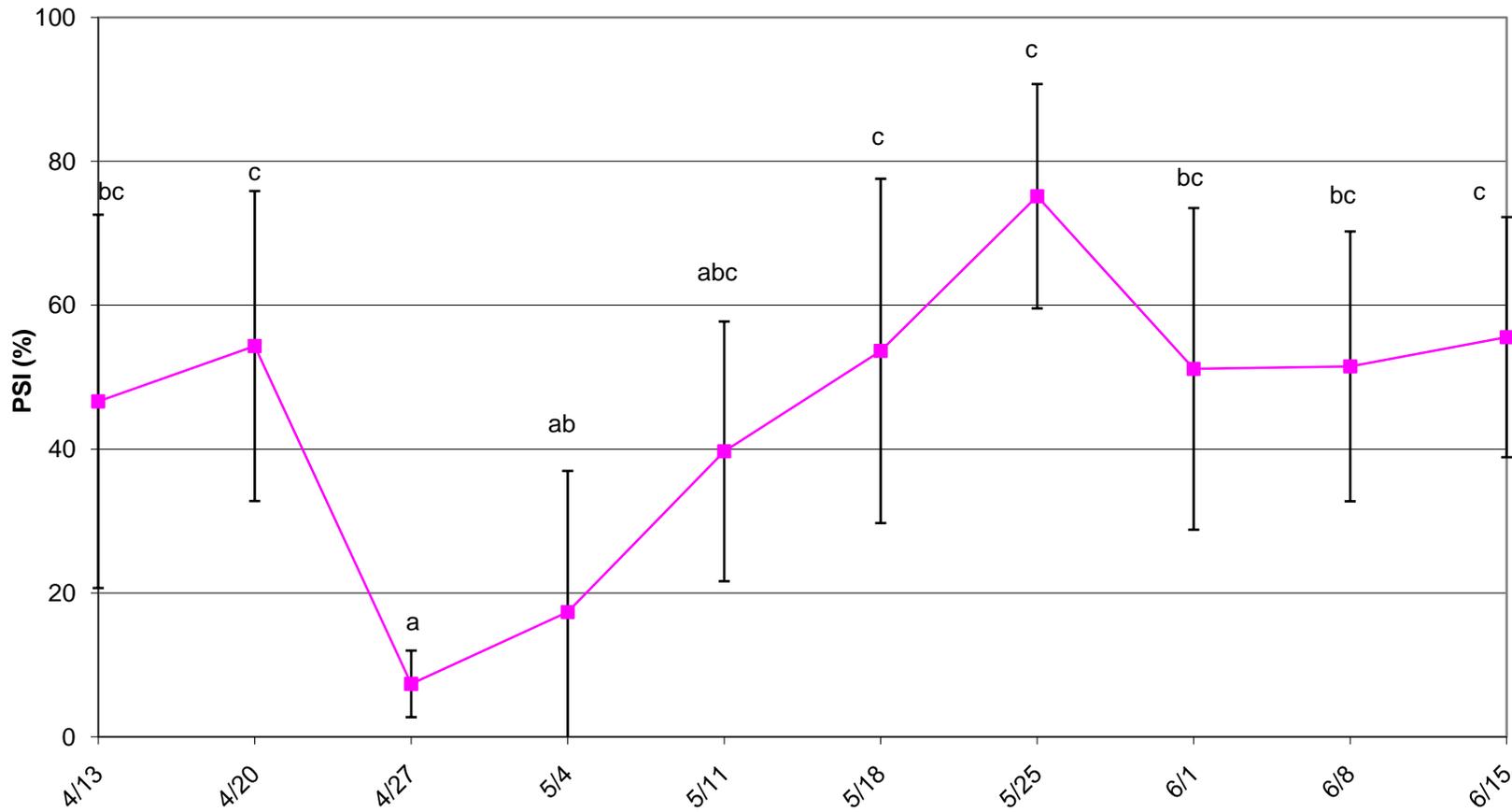


Figure 5-63. Processed feed (PRO) and cottonseed meal fertilizer (CSM) treatment bootstrap PSI (percent similarity index; critical value $PSI \geq 0.60$) values ($\pm 95\%$ CI) generated from randomly drawn replicates at 1000 iterations per sampling period using large ($> 200 \mu\text{m}$) plankton assemblage taxa %N values, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ ponds per treatment.

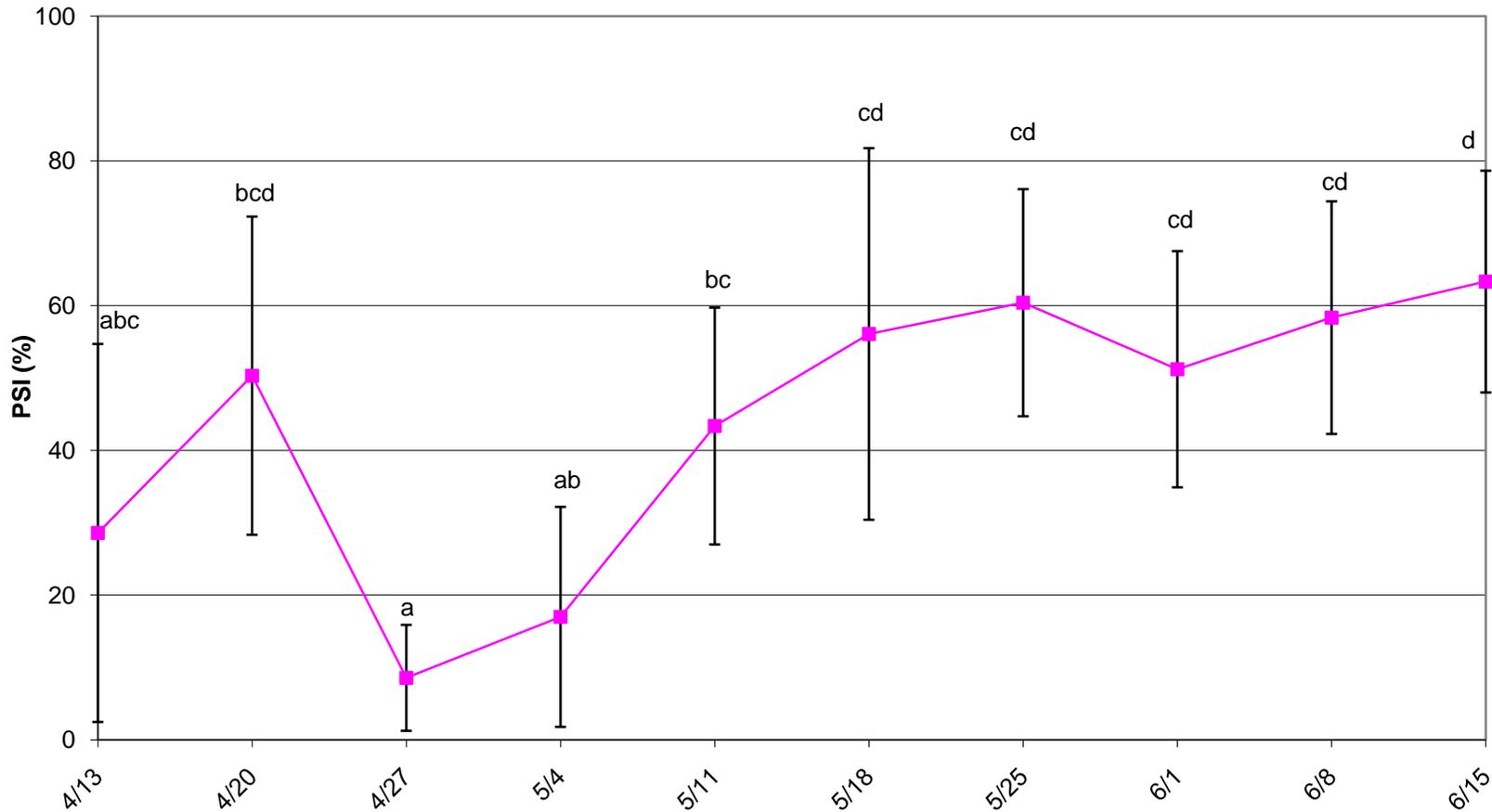


Figure 5-64. Processed feed (PRO) and inorganic fertilizer (INO) treatment bootstrap PSI (percent similarity index; critical value $PSI \geq 0.60$) values ($\pm 95\%$ CI) generated from randomly drawn replicates at 1000 iterations per sampling period using large ($> 200 \mu\text{m}$) plankton assemblage taxa %N values, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ ponds per treatment.

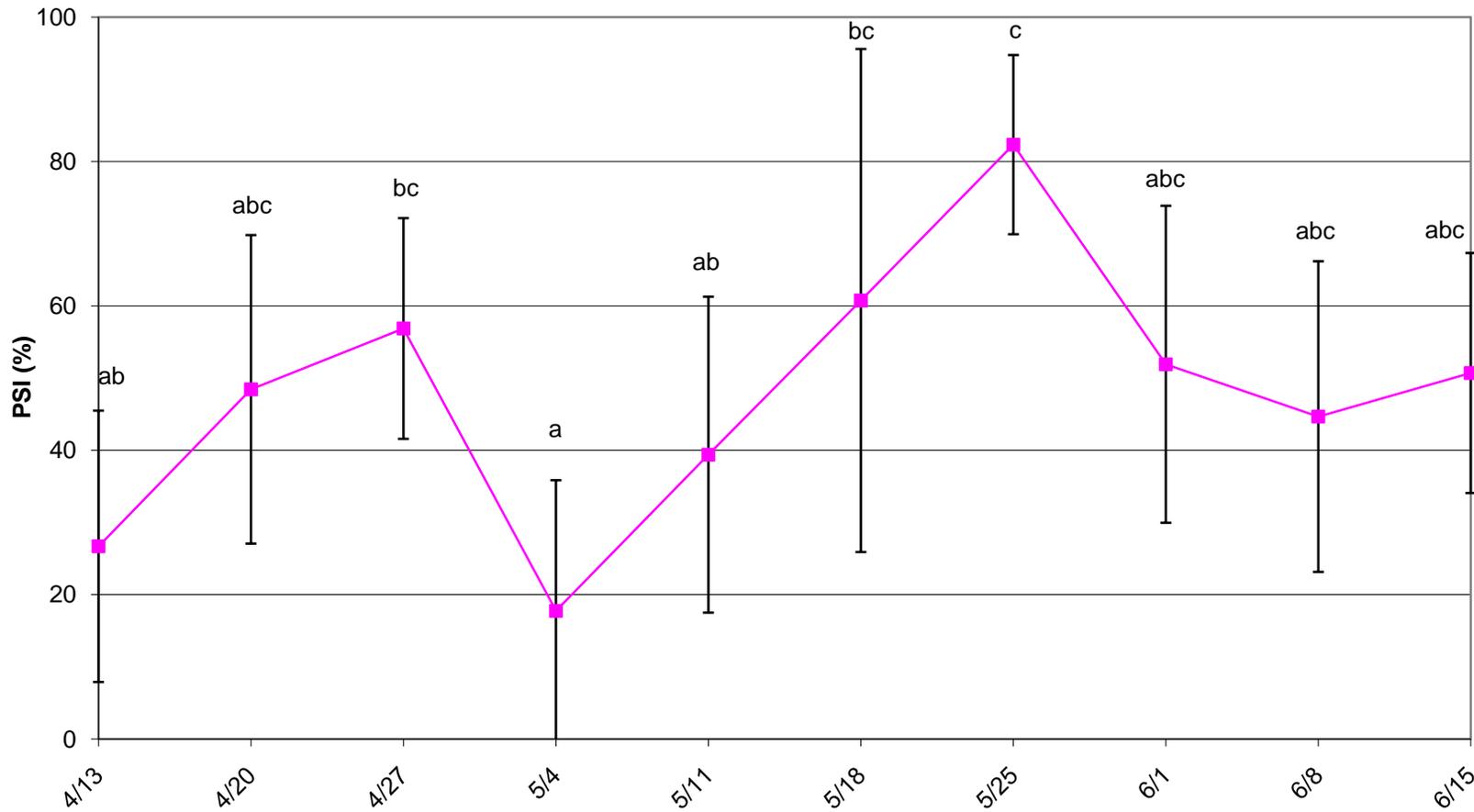


Figure 5-65. Unprocessed feed (UNP) and cottonseed meal fertilizer (CSM) treatment bootstrap PSI (percent similarity index; critical value $PSI \geq 0.60$) values ($\pm 95\%$ CI) generated from 1000 iterations per sampling period using large ($> 200 \mu\text{m}$) plankton assemblage taxa %N values, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ ponds per treatment, * only 5 UNP replicate samples available for this sampling date.

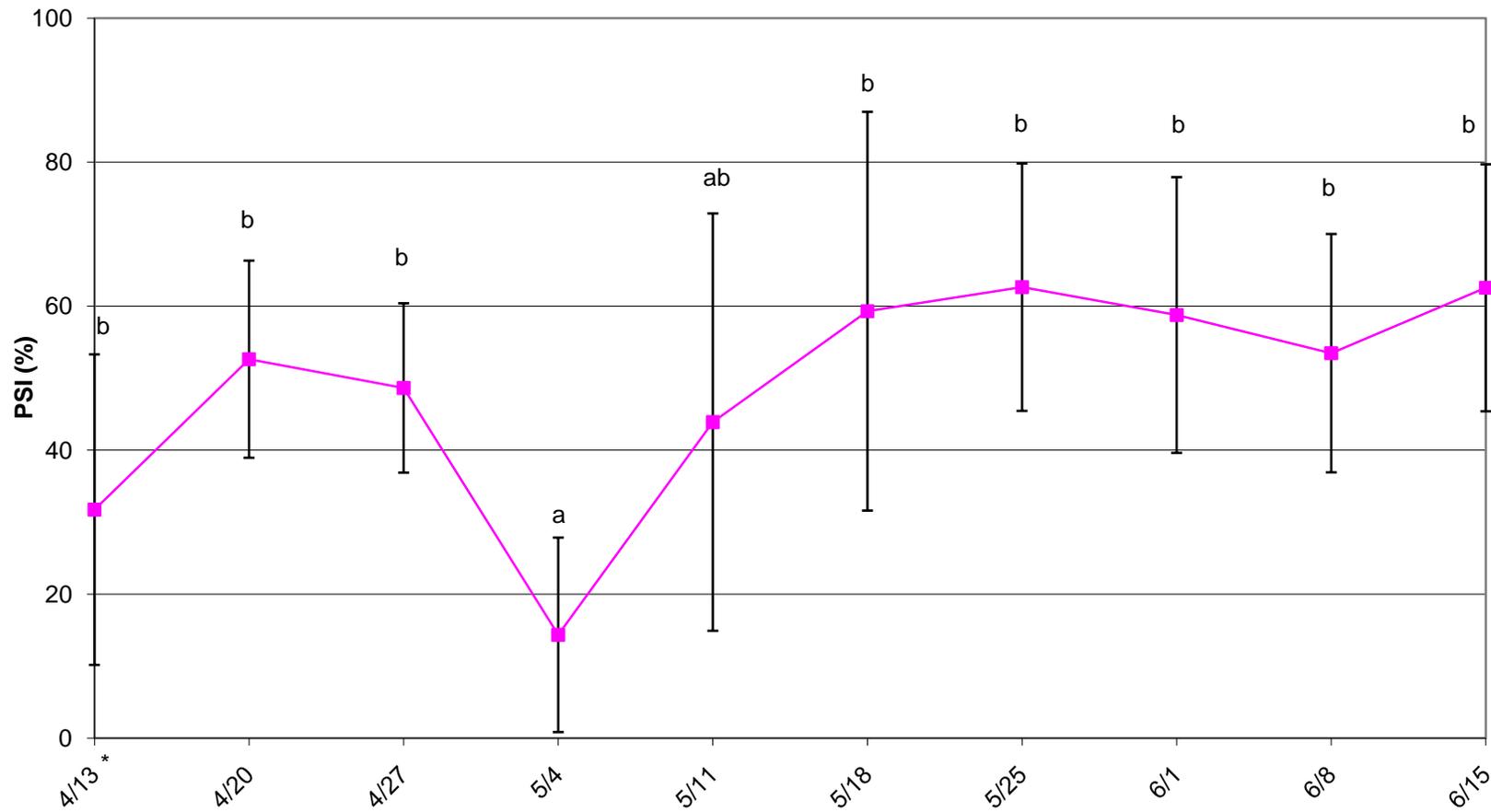


Figure 5-66. Unprocessed feed (UNP) and inorganic fertilizer (INO) treatment bootstrap PSI (percent similarity index; critical value $PSI \geq 0.60$) values ($\pm 95\%$ CI) generated from 1000 iterations per sampling period using large ($> 200 \mu\text{m}$) plankton assemblage taxa %N values, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ ponds per treatment, * only 5 UNP replicate samples available for this sampling period.

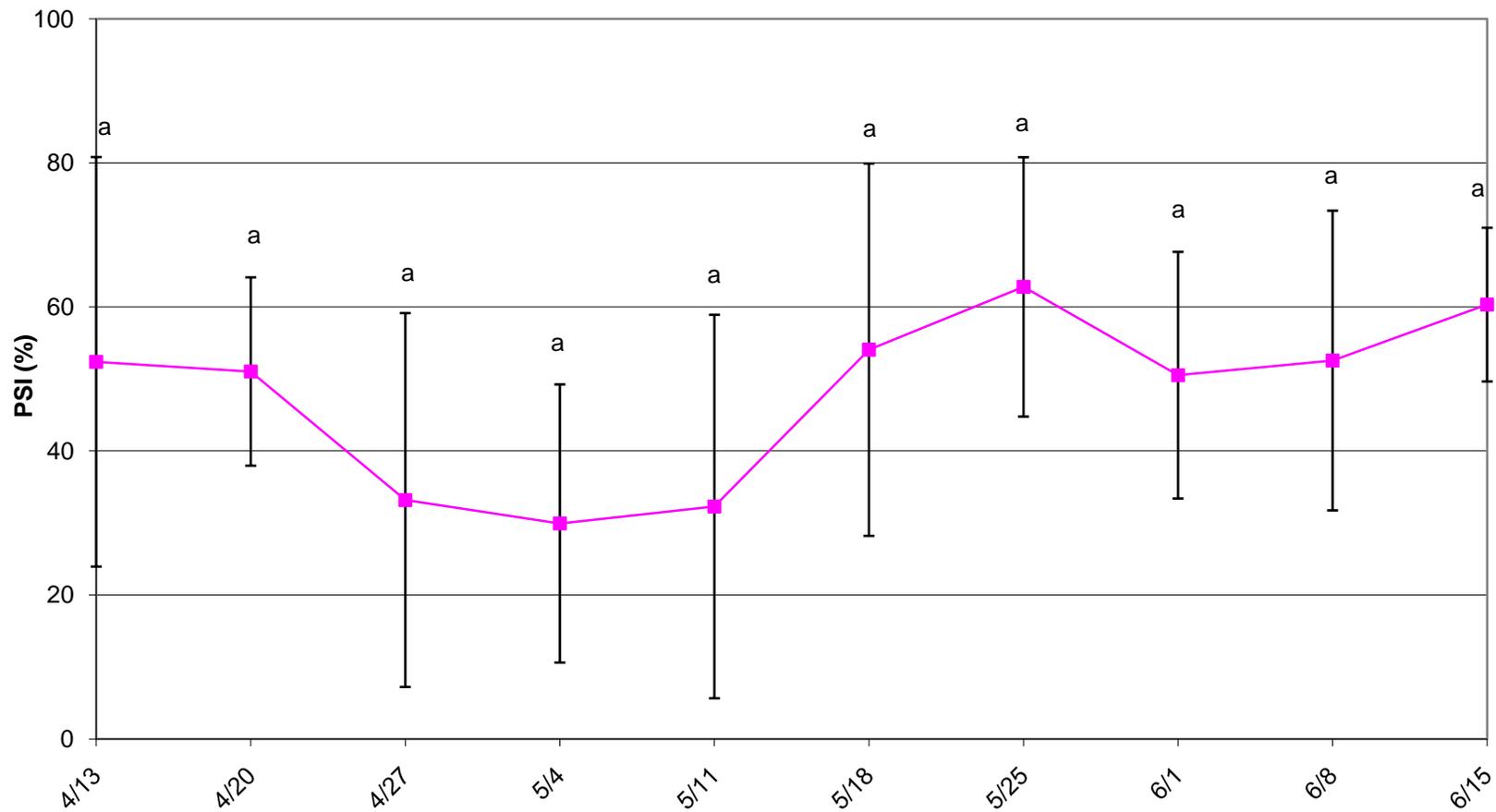


Figure 5-67. Cottonseed meal (CSM) and inorganic fertilizer (INO) treatment bootstrap PSI (percent similarity index; critical value $PSI \geq 0.60$) values ($\pm 95\%$ CI) generated from randomly drawn replicates at 1000 iterations per sampling period using large ($> 200 \mu\text{m}$) plankton assemblage taxa %N values, unshared letters denote statistical differences between sampling periods ($P < 0.05$, Bonferroni post test); $n = 6$ ponds per treatment.

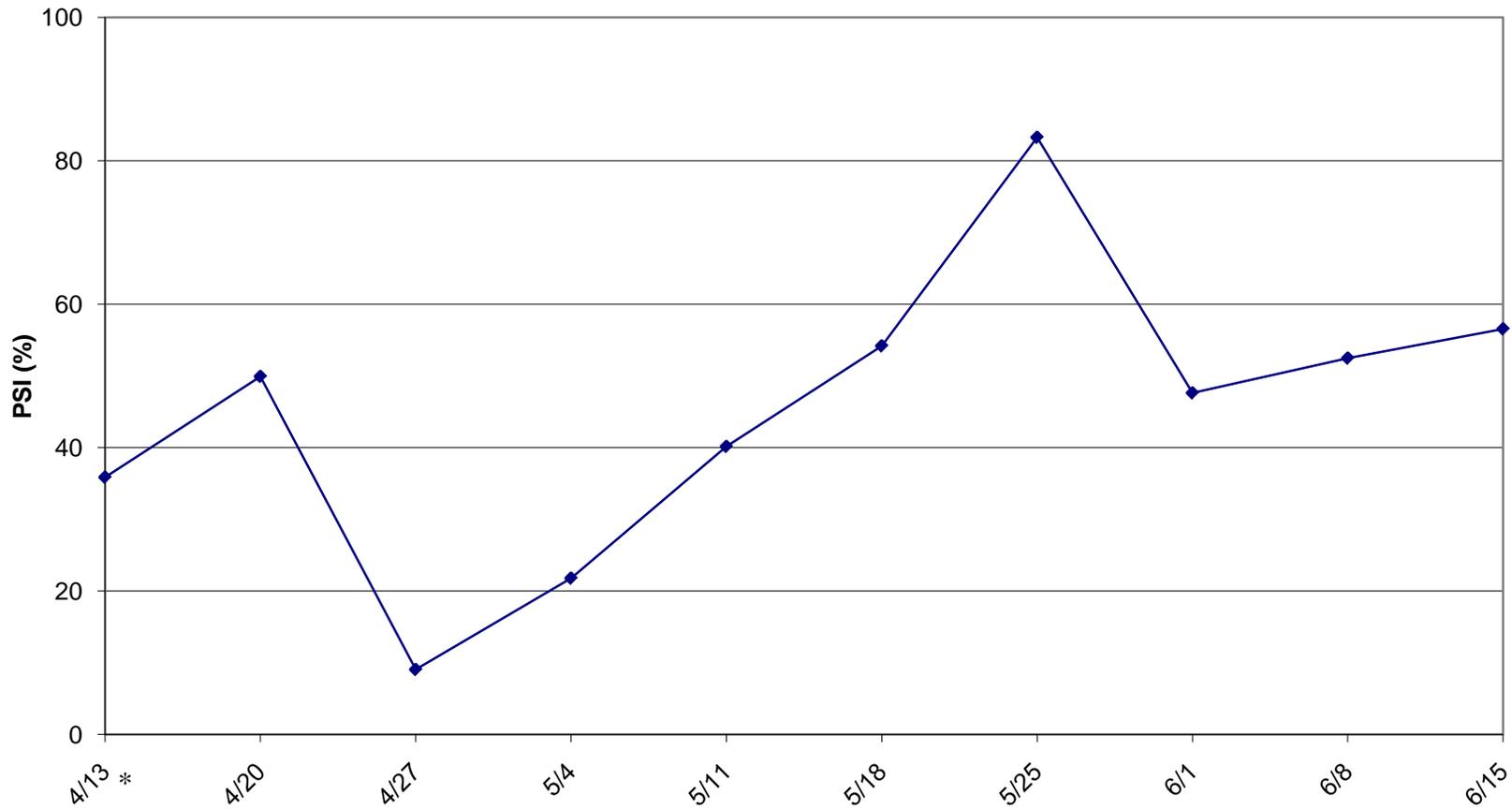


Figure 5-68. Processed (PRO) and unprocessed (UNP) feed treatment bootstrap PSI (percent similarity index; critical value $PSI \geq 0.60$) values generated from 20 randomly drawn replicates at 1000 iterations per sampling period using large plankton assemblage taxa %N values; n = 6 ponds per treatment, * only 5 UNP replicate samples available for this sampling date..

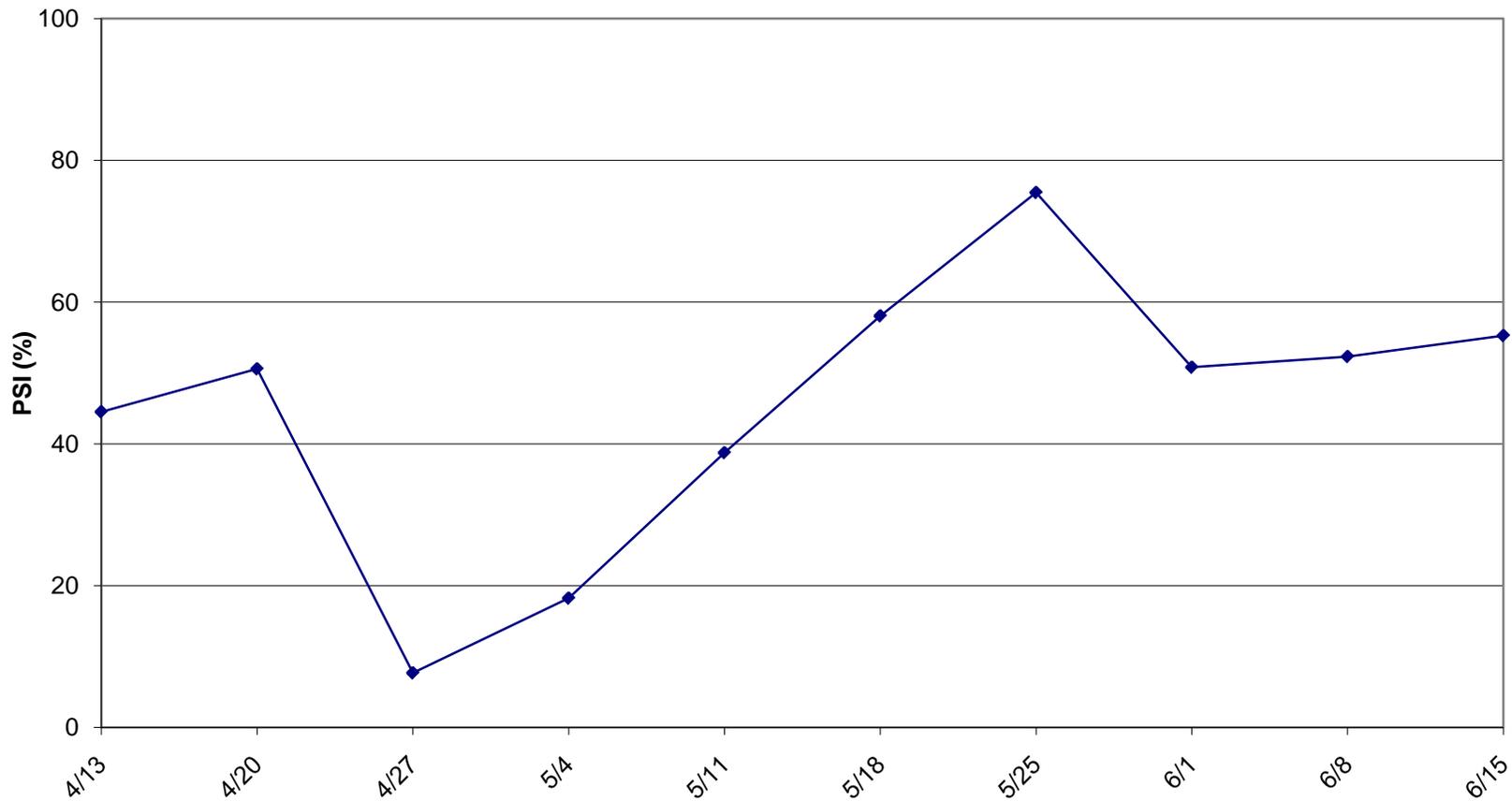


Figure 5-69. Processed feed (PRO) and cottonseed meal (CSM) fertilizer treatment bootstrap PSI (percent similarity index; critical value $PSI \geq 0.60$) values generated from 20 randomly drawn replicates at 1000 iterations per sampling period using large ($> 200 \mu m$) plankton assemblage taxa %N values; $n = 6$ ponds per treatment.

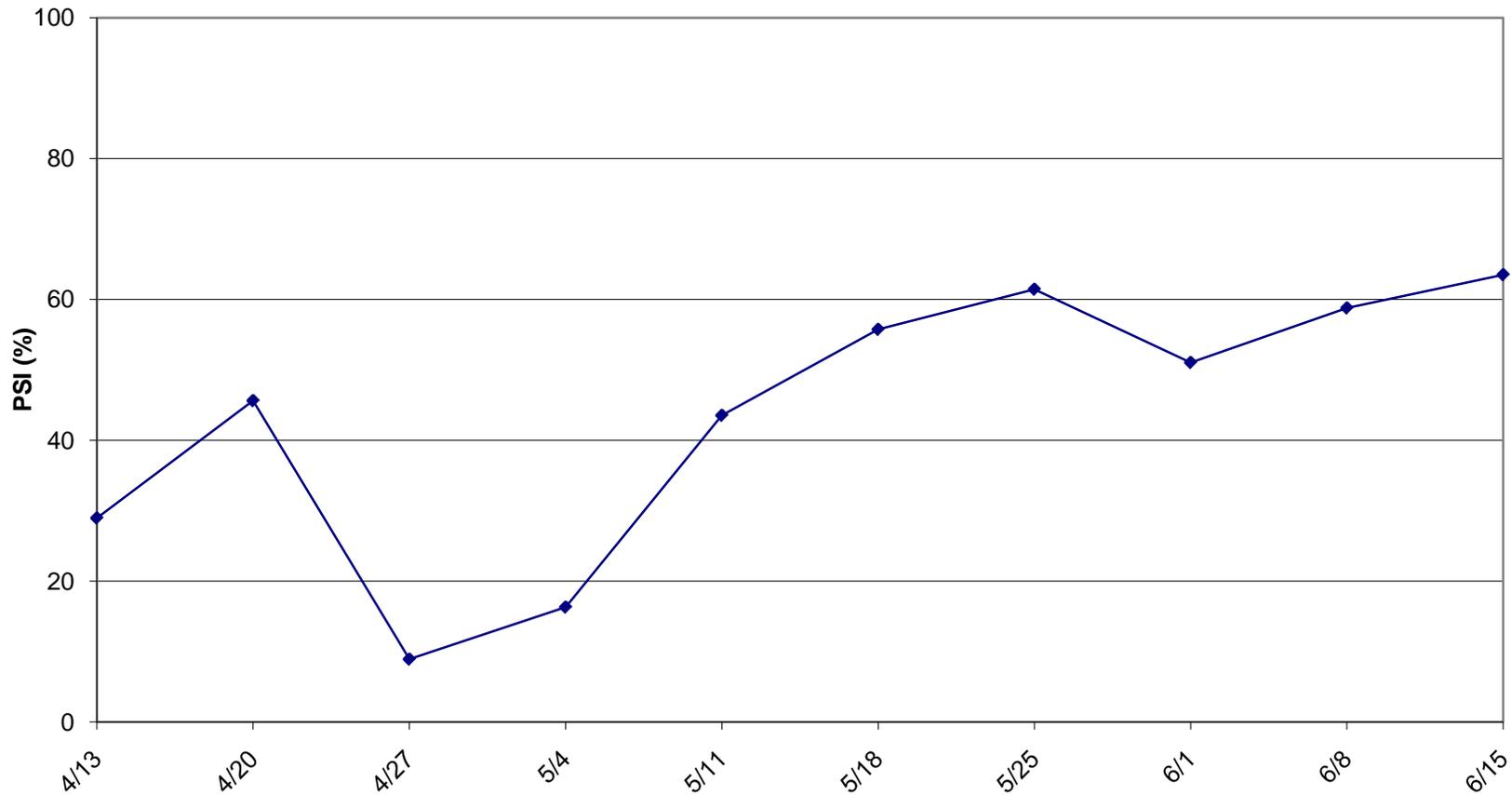


Figure 5-70. Processed feed (PRO) and inorganic fertilizer (INO) treatment bootstrap PSI (percent similarity index; critical value $\text{PSI} \geq 0.60$) values generated from 20 randomly drawn replicates at 1000 iterations per sampling period using large ($> 200 \mu\text{m}$) plankton assemblage taxa %N values; $n = 6$ ponds per treatment.

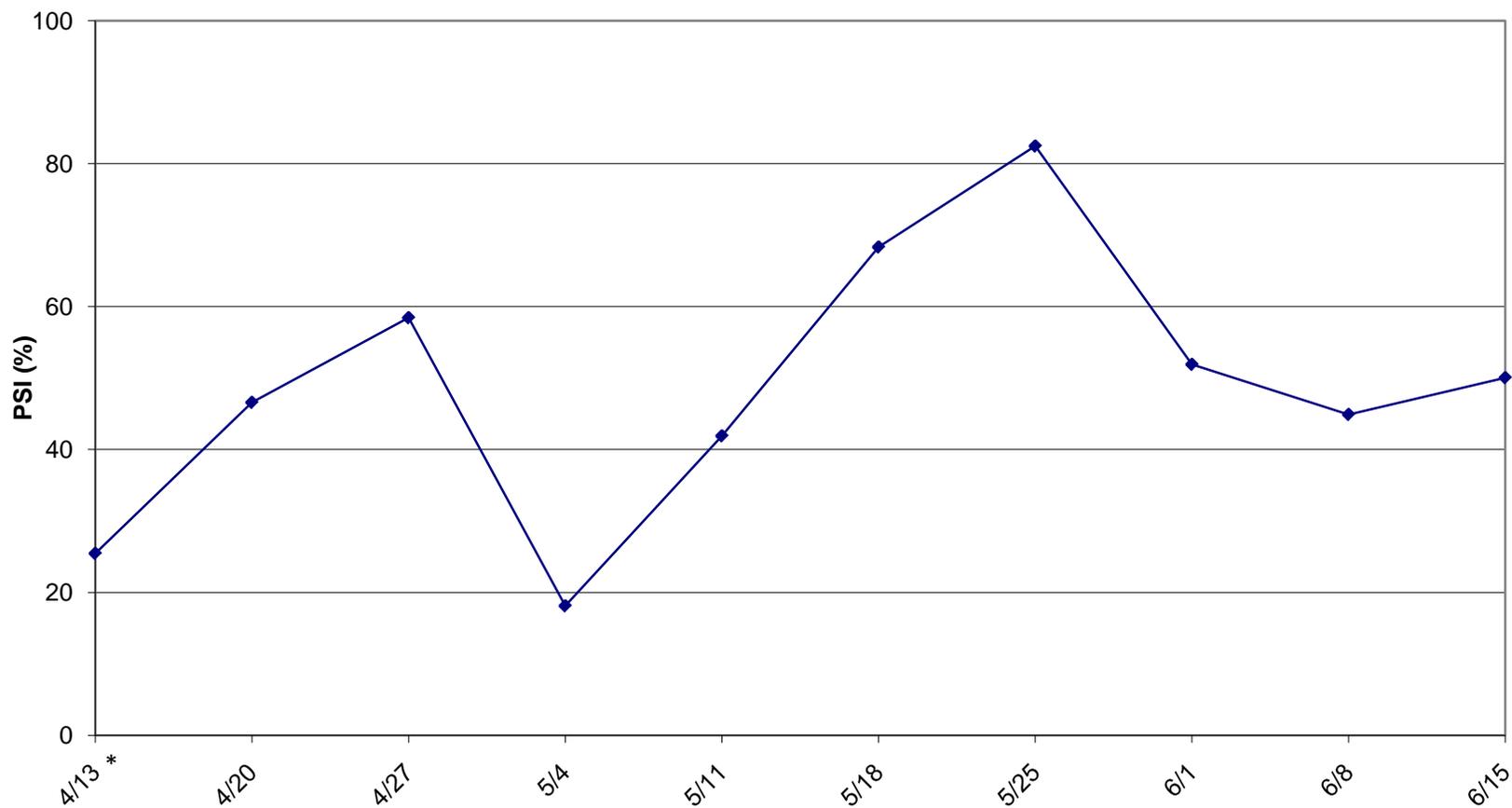


Figure 5-71. Unprocessed feed (UNP) and cottonseed meal fertilizer (CSM) treatment bootstrap PSI (percent similarity index; critical value $PSI \geq 0.60$) generated from 20 randomly drawn replicates at 1000 iterations per sampling period using large ($> 200 \mu\text{m}$) plankton assemblage taxa %N values; $n = 6$ ponds per treatment, * only 5 UNP replicate samples available for this date.

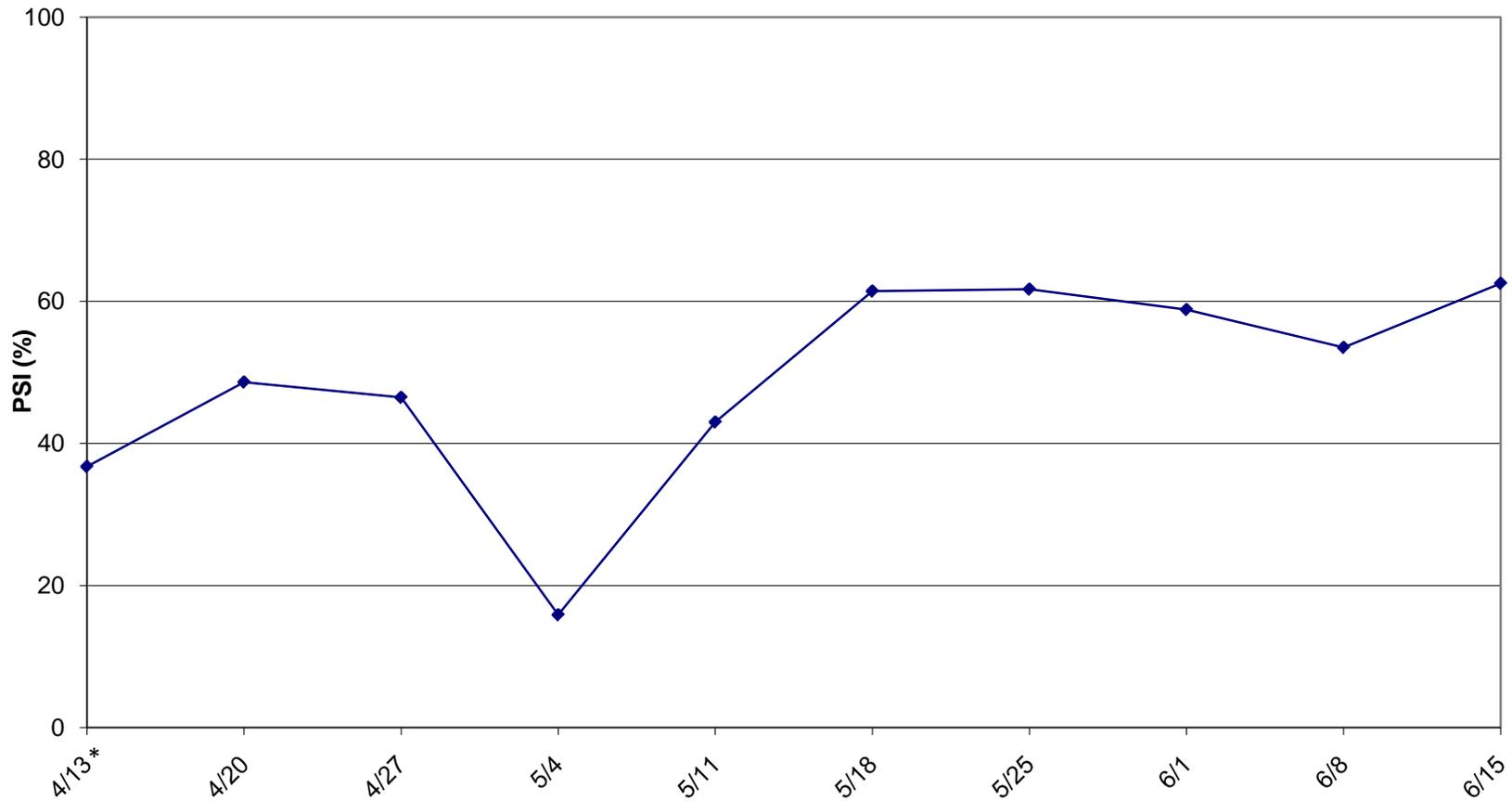


Figure 5-72. Unprocessed feed (UNP) and inorganic fertilizer (INO) treatment bootstrap PSI (percent similarity index; critical value $PSI \geq 0.60$) values generated from 20 randomly drawn replicates at 1000 iterations per sampling period using large ($> 200 \mu\text{m}$) plankton assemblage taxa %N values; $n = 6$ ponds per treatment, * only 5 UNP replicate samples available for this date.

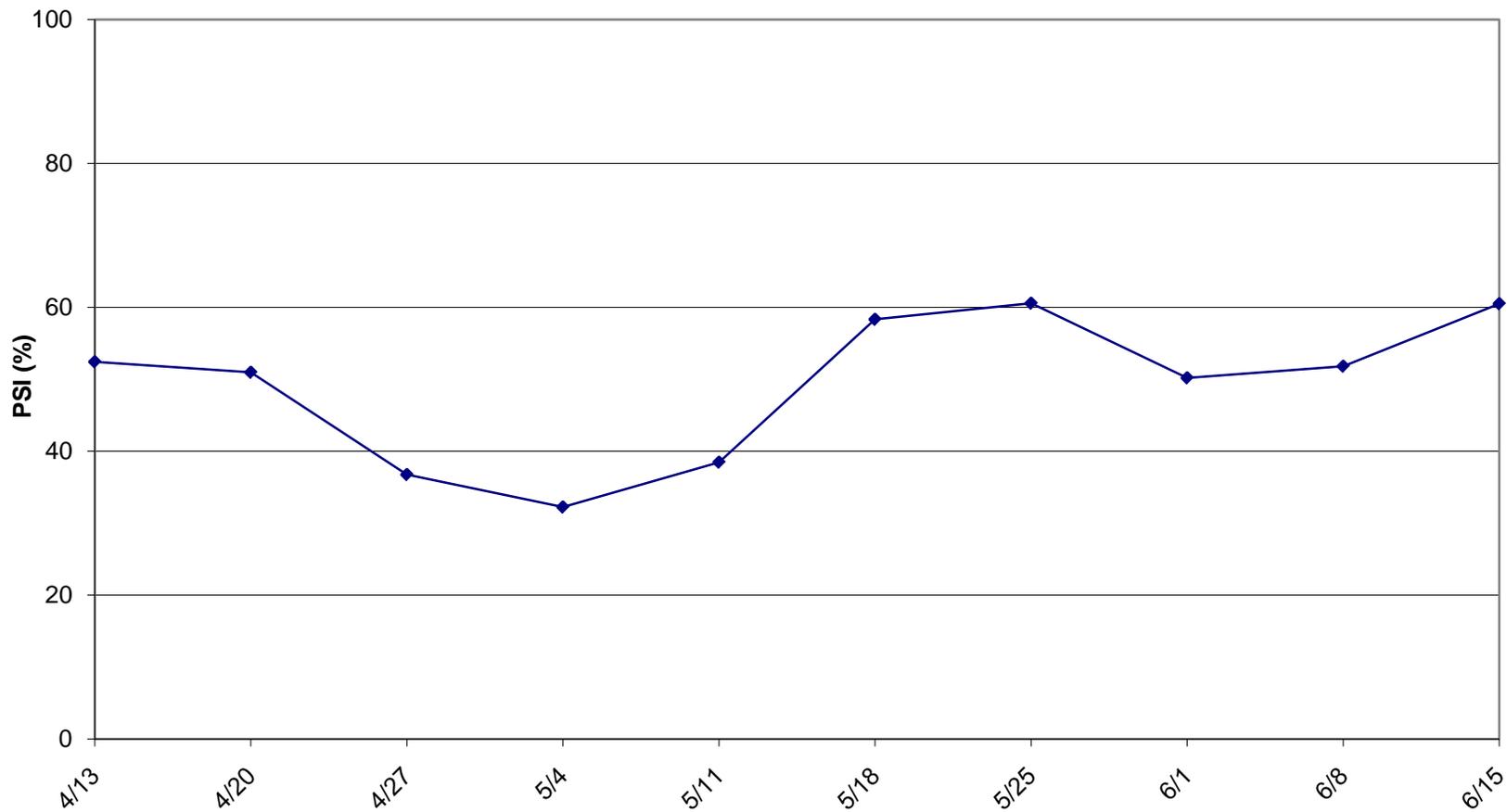


Figure 5-73. Cottonseed meal (CSM) and inorganic fertilizer (INO) treatment bootstrap PSI (percent similarity index; critical value $PSI \geq 0.60$) values generated from 20 randomly drawn replicates at 1000 iterations per sampling period using large ($> 200 \mu\text{m}$) plankton assemblage taxa %N values; $n = 6$ ponds per treatment.

Table 5-1. 30 most common large zooplankton assemblage components and their approximate volumes calculated via photomicrographs.

Taxa	Length mm	Width mm	Height mm	Diameter mm	Volume mm ³	Sample Size
Non-Diaptomous Copepods	0.4078	0.1429	0.1429		1.5147	5
Diaptomous Copepods	0.7526			0.2486	0.0408	41
Tri-Rotifers <i>Filinia</i> spp.	0.1276			0.0521	0.0003	12
Ball Rotifers					0.0351	13
Statoblasts					0.0029	6
Daphnids <i>Moina macrocopa</i>					0.0318	10
Insect Larvae	0.7893	0.1595			0.0152	4
Ostracods	0.5020	0.2969	0.3475		0.0307	9
Tooth Rotifers	0.2312	0.1902	0.0996		0.0025	4
Nematodes	1.5729	0.0290			0.0010	4
Water Mites	0.4102	0.2415	0.2293		0.0201	9
'Nauplii-like' Rotifers	0.0970	0.0656			0.0001	12
True Nauplii	0.1224	0.0659			0.0005	4
Gastropods	1.4457	0.8920	0.8920		0.6695	3
Egg Mass					0.0171	5
Flower Stamens	0.9953	0.3734	0.2073		0.0392	3
'Grape' Rotifers	0.1513	0.0758	0.0758		0.0005	4
'Spike Cup' Rotifers	0.0872	0.0513	0.0513		0.0001	1
Ball Fungi					0.0004	2
Chironomids	1.7473			0.1370	0.0471	6
Ephemera	0.5251			0.1443	0.0029	6
Hymenoptera	1.1285	0.2161	0.2161		0.0305	4
Coleoptera	0.6764	0.1878	0.1646		0.0155	9
Springtail	1.4444	0.3827	0.3827		0.1108	1
Dipteran	1.0590	0.4927	0.4789		0.1299	3
Insect heads	0.3119	0.1889	0.1487		0.0055	3
<i>Pediastrum</i>	0.1407	0.0230	0.0003		0.0003	2
Pope Rotifers	0.0699	0.0568	0.0437		0.0001	1
Grass Seeds	1.9562	0.6737	0.6890		0.4201	4
Misc Rotifers	0.1304	0.0978	0.0978		0.0007	1

Table 5-2. Frequency of similar large plankton assemblages between treatment pairs according to the simplified Morisita's similarity index (MSI) and percent similarity index (PSI) using deterministic (%IRI) and resampling (%N) methods.

Determination Method	PRO/UNP	PRO/CSM	PRO/INO	UNP/CSM	UNP/INO	CSM/INO
MSI Deterministic	7	8	9	10	7	7
PSI Deterministic	10	9	10	10	10	10
MSI Bootstrap	6	2	4	3	7	6
PSI Bootstrap	1	1	1	2	2	2

CHAPTER 6 24-HOUR SWORDTAIL CAPTIVE FEEDING EXPERIMENTS

Swordtail (*Xiphophorus hellerii*; Family Poeciliidae) dietary habits in semi-tropical aquaculture ponds were investigated to determine their dependence upon applied anthropogenic nutrients and live foods derived from the plankton community. Swordtail dietary habits were investigated using a powerful, but indirect method - stable isotope signature analysis (Chapter 4). Carbon and nitrogen stable isotope signatures of swordtails, applied nutrients [two commercial feeds, cottonseed meal fertilizer, liquid inorganic fertilizer (nitrogen only)], and three pond plankton size fractions (small, medium, large), were measured to determine the contribution of applied nutrients and pond plankton to swordtail diets within four nutrient treatments. Unfortunately, swordtail internal anatomy made conventional gut content analysis impractical. Pharyngeal jaws within the swordtail buccal cavity, grind prey items into a fine, homogenous food bolus.

An alternative to gut content analysis is the use of captive feeding trials, consisting of timed incubation trials in which one or more fish are allowed to feed at will (*ad libitum*) upon quantified prey assemblages or known nutrient sources. At trial termination, control treatments, incubated without fish, are compared to those incubated with fish and differences in recovered plankton assemblage taxa composition and abundances are compared. Any differences are attributed to fish predation.

To compliment the findings from more indirect methods such as stable isotope dietary analyses (Chapter 4), and potential swordtail prey large plankton assemblage community analyses (Chapter 5), in the absence of direct gut content analysis, 24-hour captive feeding trials were employed to investigate swordtail dietary habits/preferences (Axelrod 1991, Tamaru et al. 2001, Kruger 2001).

Although captive feeding trials were performed under artificial conditions at fish predator densities that were likely higher than those occurring within the ponds, this was a direct method of evaluating the swordtails ability to reduce zooplankton biomass and/or alter the large zooplankton community occurring within the controlled feeding trials, and by proxy, within actual aquaculture ponds.

Methods

24-Hour Captive Feeding/Plankton Grazing Experiment

Twenty-four hour captive feeding/plankton grazing experiments were performed (17 – 18 September 2007) to evaluate the effects of juvenile swordtails (naïve to potential live plankton prey) from randomly selected ponds (orthogonally blocked within nutrient treatment/pond water source) on large zooplankton assemblages (size > 200 µm). Three liters of pond water were collected from each of 16 ponds [four replicate ponds from each of four pond nutrient treatments: processed feed (PRO), unprocessed feed (UNP), cottonseed meal fertilizer (CSM), and inorganic liquid fertilizer (INO)]. Each three-liter integrated-depth water sample (Chapter 4 Methods) was gently agitated to ensure sample homogeneity and split into three one-liter samples, each assigned to one of three treatments: (1) a single one-liter pond water sample that was immediately fixed using 10 ml of Lugol's solution (APHA/AWWA 1989) following collection (Initial), (2) a flint glass jar containing one liter of pond water and no fry for 24 hrs (NF), and (3) a flint glass jar containing one liter of pond water and a single randomly selected juvenile swordtail for 24 hrs (Fish). Aeration was not provided, but all jars (16 NF: 16 Fish) were exposed to the atmosphere for gas exchange. Experimental jars with fish and control jars without fish were placed onto wooden beams (8.9 cm × 8.9 cm × 1.8 m) in random order and incubated for 24 hours within a 70% shade cloth covered greenhouse (Chapter 2, Figure 2-1) that was temperature controlled at the upper end (~ 30 °C) by thermostatically controlled ventilation

fans. Natural fluctuations in temperature occurred due to ambient weather and solar insolation levels (15-16 September 2007: 33.3 °C daily high both dates; (NOAA) National Weather Service, Ruskin, Florida).

The purpose of the 'Initial' control treatment was to provide a baseline for the large zooplankton community prior to the 24-hour duration of the two experimental treatments (NF and Fish). It was assumed that the zooplankton assemblage of the Initial treatment would have the greatest plankton community diversity and individual plankton taxa abundances, as this plankton assemblage was not subject to potential swordtail predation and captivity stressors within a confined space (i.e., potential sub-optimal temperature regime, dissolved oxygen, light levels, etc.). The zooplankton within the Initial treatment were immediately 'fixed' following collection, as Lugol's preservative is also a non-vital stain.

The purpose of the NF treatment was to provide an *in situ* baseline control for the Fish treatment. It was assumed that the NF would have a zooplankton community intermediate in complexity and abundance to that of the Initial and Fish treatments due to intra-plankton assemblage predation and possible large zooplankton mortality due to stress from experimental conditions.

The 'Fish' group simulates potential swordtail predation pressure upon large plankton assemblage communities within the ponds. It was assumed that the Fish treatment plankton community complexity and taxa abundances would be the lowest due to direct predation from the omnivorous, visually mediated swordtail predators.

Randomly chosen juvenile swordtails ($\bar{X} = 16.8 \text{ mm SL} \pm 6.3 \text{ 95 \% CI}$) taken from the greenhouse spawning and grow-out systems (Chapter 2, Figures 2-1 – 2-2) were used for the captive feeding experiment. Swordtails had only received commercial flake fish food (Tetra

Min™ tropical staple flake food) and were naïve to the presence and consumption of live planktonic prey, as the water source within these culture systems was from a well.

Jars were covered with nylon mesh (3 mm square) held in place by rubber bands to permit air exchange with the water surface (~ 2 cm headspace), but not allow juvenile fish to escape. Control jars, without fish, also were covered. Jars were kept in a greenhouse under natural photoperiod and slightly higher than ambient temperatures (26-30° C), away from human activity during the trial. No fry mortality occurred during the trial. All fish were euthanized for later weight and length measurement. No identifiable prey items were found during gastrointestinal tract examination, but food bolus material was regularly encountered. At trial termination, jars were gently agitated prior to Lugol's solution fixation (10 mls), to collect as much particulate matter as possible. Initial, NF, and Fish large plankton assemblages were filtered, concentrated, and sealed into small brown flint glass screw-top vials containing 8-9 drops (~ 0.4 ml) of Lugol's preservative (APHA/AWWA 1989; Chapter 5 Methods). Large zooplankton assemblages were microscopically examined using a Sedgewick-Rafter counting slide and individual plankton were enumerated by taxa (Chapter 5 Methods).

Descriptive Parameters, Indices and Statistical Analyses

Captive feeding trial large zooplankton assemblage volumes (plankton mm³/liter) and densities (total plankton #/liter; individual taxa #/liter) were calculated and compared among experimental treatments (Initial, NF, Fish) and pond nutrient treatments using 2-way repeated measures ANOVA. Methods used for taxa volume estimation (Equation 5.1) and total large zooplankton assemblage volume and density estimates were identical to those used for the (March – June 2006) pond trial large zooplankton assemblages (Chapter 5 Methods).

Captive feeding trial large zooplankton assemblages were compared using the simplified Morisita's similarity index (MSI) and percent similarity index (PSI) calculated both

deterministically using taxa %IRI and stochastically using taxa %N and bootstrap resampling methods using equations 5.2-5.6 (Chapter 5 Methods, Appendix 5B). Only large plankton assemblage taxa that had %IRI values > 0.5%, were reported or used in the similarity indices analyses. Large zooplankton assemblage taxa communities were deemed similar if indices were greater than arbitrarily chosen ‘threshold’ values ($MSI \geq 0.65$; $PSI \geq 60\%$; Chapter 5 Methods).

All descriptive parameter and indice calculations (mean, error measures, %IRI, MSI, PSI) were made using either Microsoft[®] Excel[™] 2003 or the bootstrap Morisita’s and percent similarity indices program written in BASIC (Appendix 5B). All statistical comparisons were made using the GraphPad Prism 4.0 statistical package (GraphPad Software, Inc. 2005).

Results

Large Plankton Taxa % Number, % Volume, % Frequency of Occurrence

The 30 most important (Chapter 5; highest % IRI) large plankton assemblage taxa, were identified and enumerated using the same criteria as within Chapter 5 (Methods, Table 5-1).

Large plankton assemblage taxa percent number (%N), volume (%V), and frequency of occurrence (%FO) were measured for pooled and unpooled data (Figures 6-1 – 6 -24).

Qualitative differences among the Initial, NF, and Fish treatments within the four nutrient treatments, were the most conspicuous for daphnids (DAP – *Moina macrocopa*), which were greatly reduced in the presence of swordtails.

24-Hour Feeding Trial Large Plankton Volume Differences

Pond nutrient treatment (PRO, UNP, CSM and INO) and experimental treatment (Initial, NF and Fish) large zooplankton volumes (mm^3/L) did not significantly differ for the nutrient treatment factor, but significantly differed for the experimental treatment factor (Figure 6-25; rep. meas. 2-way ANOVA, $P_{(\text{nutrient})} = 0.375$, $F_{(\text{nutrient})} = 1.132$, $\alpha_{(2,3)} = 0.05$; $P_{(\text{experimental treatment})} < 0.0001$, $F_{(\text{experimental treatment})} = 15.68$, $\alpha_{(2,2)} = 0.05$; $P_{(\text{interaction})} = 0.480$, $F_{(\text{interaction})} = 0.949$, $\alpha_{(2,6)} =$

0.05). Three pairwise differences within the experimental treatment factor (Initial, NF, and Fish) were found, PRO Initial and PRO Fish, UNP Initial and UNP Fish, and UNP NF and UNP Fish, ($P < 0.05$, Bonferroni posttests). No pairwise differences were found among the nutrient treatments within levels of the experimental treatment (Initial, NF and Fish; $P > 0.05$, Bonferroni post test).

Fish and NF treatments had numerically lower large zooplankton volumes than Initial treatments, and Fish treatments had lower volumes than either the Initial or NF treatments (Figure 6-25). However, statistical differences were only present within the PRO and UNP treatments due to high levels of variation among replicates.

PRO large zooplankton experimental treatment volume averages (Figure 6-26) were: Initial $\bar{X} = 11.7 \text{ mm}^3/\text{L}$ (0 to 27.3 mm^3/L 95 % CI), NF $\bar{X} = 8.4 \text{ mm}^3/\text{L}$ (0 to 17.8 mm^3/L 95 % CI), and Fish $\bar{X} = 2.9 \text{ mm}^3/\text{L}$ (1.6 to 4.3 mm^3/L 95 % CI). PRO large zooplankton volumes differed significantly among the experimental treatment groups (Figure 6-26): Initial, NF and Fish. Post hoc comparison tests found a single pairwise difference between the PRO Initial and Fish experimental treatment groups ($P < 0.05$, Bonferroni).

UNP large zooplankton volume averages (Figure 6-27) were: Initial $\bar{X} = 12.3 \text{ mm}^3/\text{L}$ (0 to 25.3 mm^3/L 95 % CI), NF $\bar{X} = 10.8 \text{ mm}^3/\text{L}$ (0 to 22.2 mm^3/L 95 % CI), and Fish $\bar{X} = 2.5 \text{ mm}^3/\text{L}$ (1.7 to 3.3 mm^3/L 95 % CI). UNP large zooplankton volumes significantly differed among the three treatment groups (Figure 6-27). Post hoc comparison tests found two significant pairwise treatment differences: Initial and Fish, and NF and Fish ($P < 0.05$, Bonferroni post test).

CSM large zooplankton volume averages (Figure 6-28) were: Initial $\bar{X} = 5.3 \text{ mm}^3/\text{L}$ (0 to 11.2 mm^3/L 95 % CI), NF $\bar{X} = 4.2 \text{ mm}^3/\text{L}$ (0 to 8.6 mm^3/L 95 % CI), and Fish $\bar{X} = 2.1 \text{ mm}^3/\text{L}$

(0.5 to 3.6 mm³/L 95 % CI). CSM large zooplankton volumes did not differ significantly among the three experimental treatment groups (Figure 6-28; $P > 0.05$ Bonferroni posttests).

INO large zooplankton volume averages were: Initial $\bar{X} = 10.2$ mm³/L (5.3 to 15.0 mm³/L 95 % CI), NF $\bar{X} = 8.4$ mm³/L (6.4 to 10.5 mm³/L 95 % CI), and Fish $\bar{X} = 4.9$ mm³/L (2.2 to 7.6 mm³/L 95 % CI). INO pond treatment large zooplankton volumes did not differ among the three experimental treatment groups (Figure 6-29; $P > 0.05$ Bonferroni posttests).

Due to pond nutrient treatment not being a significant factor in the repeated measures 2-way ANOVA ($P = 0.3753$), the four pond nutrient treatments (PRO, UNP, CSM and INO) were pooled among the three experimental treatments (Initial, NF, and Fish) to increase the number of replicates from four to sixteen, increasing the statistical power available to analyze differences among the three levels (Initial, NF, and Fish) of the captive feeding trial.

Following pooling, significant differences in total large zooplankton numbers were found among the three experimental treatment groups (rep. meas. 1-way ANOVA, $P < 0.0001$, $F = 15.85$, $\alpha_{(2,2)} = 0.05$). Two pairwise differences were found: Initial and Fish, and NF and Fish. No significant difference was found between the NF and Fish groups (Figure 6-30; $P < 0.05$, Tukey's Bonferroni post test).

24-Hour Feeding Trial Large Plankton Densities

Large zooplankton densities (total numbers/L) did not significantly differ among pond nutrient treatments, but significantly differed for experimental treatments (Initial, NF, and Fish; rep. meas. 2-way ANOVA, $P_{(\text{nutrient})} = 0.423$, $F_{(\text{nutrient})} = 1.007$, $\alpha_{(2,3)} = 0.05$; $P_{(\text{experimental treatment})} < 0.0001$, $F_{(\text{experimental treatment})} = 16.28$, $\alpha_{(2,2)} = 0.05$; $P_{(\text{interaction})} = 0.423$, $F_{(\text{interaction})} = 1.315$, $\alpha_{(2,6)} = 0.05$). Numerically, Initial treatment large zooplankton density averages were highest among the three experimental treatments, followed by NF treatment averages. Fish treatment averages had

the lowest averages (Figure 6-31). Two pairwise differences were found between the different experimental treatment groups within both the UNP and INO treatments: Initial and Fish, and NF and Fish ($P < 0.05$, Bonferroni post test).

PRO large zooplankton density averages differed among experimental treatment groups (Figure 6-32). PRO density averages were: Initial $\bar{X} = 354$ zooplankton/L (0 to 833 zooplankton/L 95 % CI), NF $\bar{X} = 260$ zooplankton/L (49.0 to 470 zooplankton/L 95 % CI), and Fish $\bar{X} = 85.0$ zooplankton/L (46.0 to 124 zooplankton/L 95 % CI). Within the PRO pond treatment, the Initial zooplankton density was significantly greater than both the NF and Fish treatments, which did not significantly differ from each other (Figure 6-32; $P < 0.05$ Bonferroni post test).

UNP large zooplankton densities differed significantly among the three treatments (Figure 6-33). UNP density averages were: Initial $\bar{X} = 365$ zooplankton/L (0 to 760 zooplankton/L 95 % CI), NF $\bar{X} = 208$ zooplankton/L (0 to 646 zooplankton/L 95 % CI), and Fish $\bar{X} = 142$ zooplankton/L (0 to 357 zooplankton/L 95 % CI). Two pairwise differences in density were found among the three experimental treatments: Initial and Fish, and NF and Fish. No significant difference was found between the Initial and NF treatments (Figure 6-33; $P < 0.05$, Bonferroni posttests).

CSM large zooplankton densities did not significantly differ among the three treatments (Figure 6-34, $P > 0.05$, Bonferroni post test). CSM density averages were: Initial $\bar{X} = 149$ zooplankton/L (0 to 317 zooplankton/L 95 % CI), NF $\bar{X} = 116$ zooplankton/L (0 to 233 zooplankton/L 95 % CI), and Fish $\bar{X} = 100$ zooplankton/L (1.0 to 200 zooplankton/L 95 % CI).

INO pond treatment large zooplankton densities did not significantly differ among the three captive feeding trial experimental treatment groups (Figure 6-35; $P > 0.05$, Bonferroni post

test). INO pond treatment large zooplankton density averages among the experimental treatments were: Initial $\bar{X} = 289$ zooplankton/L (179 to 399 zooplankton/L 95 % CI), NF $\bar{X} = 241$ zooplankton/L (165 to 316 zooplankton/L 95 % CI), and Fish $\bar{X} = 114$ zooplankton/L (74.9 to 153 zooplankton/L 95 % CI).

Similar to large zooplankton volume comparisons, the lack of differences in large zooplankton densities among pond nutrient treatments was used as justification to pool nutrient treatment groups within their respective experimental treatment groups (Initial, NF and Fish), increasing sample size from four to sixteen and increasing the statistical power for experimental treatment comparisons (Figure 6-36; rep. meas. 1-way ANOVA, $P_{(\text{exp. Treat.})} < 0.0001$, $F_{(\text{exp. Treat.})} = 15.31$, $\alpha_{(2,2)} = 0.05$). Among the three experimental treatment groups, significant differences were found between the Initial and Fish, and NF and Fish treatment groups, no differences were found between the Initial and NF groups ($P < 0.05$, Bonferroni post test).

24-Hour Feeding Trial Major Zooplankton Taxa Differences

The three major zooplankton taxa [Chapter 5; *Diaptomous* copepods, *Filinia* rotifers, and daphnids (*Moina macrocopa*)] examined in outdoor pond trials conducted during spring 2006, were individually examined within the large zooplankton assemblages obtained during the 24-hour captive feeding trials. Average densities (plankton/L) of the three major taxa generally decreased in a predictable and monotonous manner among the three experimental treatments (in the following order: Initial, NF, and Fish) within each of the four pond nutrient treatments (Figures 6-37 – 6-55).

Copepod densities did not significantly differ among nutrient treatments, but did significantly differ among 24-hour experimental (Initial, NF, and Fish) treatments (Figures 6-37 - 6-40; rep. meas. 2-way ANOVA, $P_{(\text{nutrient})} = 0.555$, $F_{(\text{nutrient})} = 0.730$, $\alpha_{(2,3)} = 0.05$; $P_{(\text{exp. Treat.})} =$

0.0005, $F_{(\text{exp. Treat.})} = 10.80$, $\alpha_{(2,2)} = 0.05$; $P_{(\text{interaction})} = 0.939$, $F_{(\text{interaction})} = 0.285$, $\alpha_{(2,6)} = 0.05$).

Post hoc pairwise comparisons of experimental treatment groups, within individual pond nutrient treatments, found only one significant difference in copepod density, PRO Initial and Fish experimental treatment groups (Figure 6-37; $P < 0.05$, Bonferroni post test).

PRO copepod densities among the three experimental treatments (Initial, NF, and Fish) averaged: Initial $\bar{X} = 160$ copepods/L (0 to 403 copepods/L 95 % CI), NF $\bar{X} = 119$ copepods/L (0 to 316 copepods/L 95 % CI), and Fish $\bar{X} = 63.0$ copepods/L (29.4 to 96.6 copepods/L 95 % CI). As previously mentioned, the PRO Initial treatment copepod density was significantly higher than that of the Fish treatment.

UNP copepod densities (number/L) did not significantly differ among the feeding trial treatments (Figure 6-38; $P > 0.05$ Bonferroni posttests). UNP copepod densities averaged: Initial $\bar{X} = 117$ copepods/L (35.5 to 199 copepods/L 95 % CI), NF $\bar{X} = 118$ copepods/L (16.7 to 220 copepods/L 95 % CI), and Fish $\bar{X} = 49.3$ copepods/L (25.9 to 72.6 copepods/L 95 % CI).

CSM copepod densities did not significantly differ among the 24-hour feeding trial experimental treatments (Figure 6-39; $P > 0.05$ Bonferroni posttests). CSM copepod densities averaged: Initial $\bar{X} = 104$ copepods/L (1.9 to 206 copepods/L 95 % CI), NF $\bar{X} = 84$ copepods/L (0 to 182 copepods/L 95 % CI), and Fish $\bar{X} = 47$ copepods/L (12.0 to 82.5 copepods/L 95 % CI).

INO copepod densities did not significantly differ among the feeding trial treatments (Figure 6-40; $P > 0.05$ Bonferroni posttests). INO copepod densities averaged: Initial $\bar{X} = 168$ copepods/L (112 to 2236 copepods/L 95 % CI), NF $\bar{X} = 139$ copepods/L (122 to 156 copepods/L 95 % CI), and Fish $\bar{X} = 102$ copepods/L (54 to 150 copepods/L 95 % CI).

Due to the non-significance of the pond nutrient factor during the 2-way repeated measures ANOVA comparison of copepod density among pond nutrient treatments and experimental

treatments (Initial, NF, and Fish), experimental treatment groups were pooled across pond nutrient treatments to increase statistical power (unpooled $n = 4$, pooled $n = 16$) and a 1-way repeated measures ANOVA was performed on the pooled data (rep. meas. 1-way ANOVA, $P = 0.0001$, $F = 12.60$, $\alpha_{(2,2)} = 0.05$). Average copepod density of the pooled experimental treatment groups were: Initial $\bar{X}_{(pooled)} = 137$ copepods/L (92.3 to 182 copepods/L 95 % CI), NF $\bar{X}_{(pooled)} = 115$ copepods/L (77.1 to 153 copepods/L 95 % CI), and Fish $\bar{X}_{(pooled)} = 65.4$ copepods/L (49.2 to 81.6 copepods/L 95 % CI). Two significant pairwise differences were found among the three experimental treatments (Initial, NF, and Fish): Initial and Fish, and NF and Fish (Figure 6-41; $P < 0.05$, Bonferroni post test).

Filinia rotifer did not significantly differ among pond nutrients, but did significantly differ among experimental treatments (Figure 6-42-6.45; rep. meas. 2-way ANOVA, $P_{(nutrient)} = 0.6958$, $F_{(nutrient)} = 0.4898$, $\alpha_{(2,3)} = 0.05$; $P_{(exp. Treat.)} = 0.0408$, $F_{(exp. Treat.)} = 3.665$, $\alpha_{(2,2)} = 0.05$; $P_{(interaction)} = 0.2212$, $F_{(interaction)} = 1.498$, $\alpha_{(2,6)} = 0.05$). Although the nutrient treatment factor F value was not significant, post hoc pairwise comparisons found a single pairwise difference among nutrient treatments (H. Motulsky pers. comm.), Initial PRO and Initial CSM ($P < 0.05$, Bonferroni posttests). Two pairwise differences were found between experimental groups within the PRO treatment: PRO initial and PRO NF, and PRO initial and PRO Fish ($P < 0.05$, Bonferroni post test).

PRO *Filinia* densities averaged (Figure 6-42): Initial $\bar{X} = 18.3$ rotifers/L (0.0 to 57.4 rotifers/L 95 % CI), NF $\bar{X} = 2.3$ rotifers/L (0.0 to 7.3 rotifers/L 95 % CI), and Fish $\bar{X} = 0.8$ rotifers/L (0.0 to 1.5 rotifers/L 95 % CI). *Filinia* density within the Initial treatment was significantly higher than for the NF and Fish treatments ($P < 0.05$, Bonferroni post tests).

UNP *Filinia* densities did not significantly differ among the three 24-hour feeding trial treatments (Figure 6-43; $P > 0.05$, Bonferroni post test). UNP *Filinia* densities averaged: Initial $\bar{X} = 6.5$ rotifers/L (0.0 to 21.1 rotifers/L 95 % CI), NF $\bar{X} = 2.0$ rotifers/L (0.0 to 6.5 rotifers/L 95 % CI), and Fish $\bar{X} = 0.8$ rotifers/L (0.0 to 2.3 rotifers/L 95 % CI).

CSM *Filinia* densities did not differ significantly among the 24-hour feeding trial treatments (rep. meas. 1-way ANOVA, $P = 0.4640$, $F = 0.8750$, $\alpha_{(2,2)} = 0.05$). CSM *Filinia* densities (Figure 6-44) averaged: Initial $\bar{X} = 1.5$ rotifers/L (0.0 to 3.6 rotifers/L 95 % CI), NF (no fish 24 hours) $\bar{X} = 3.0$ rotifers/L (0.0 to 8.0 rotifers/L 95 % CI), and fish (single fish 24 hours) $\bar{X} = 2.5$ rotifers/L (0.0 to 8.4 rotifers/L 95 % CI).

INO *Filinia* densities among the 24-hour feeding trial treatments, did not differ significantly (rep. meas. 1-way ANOVA, $P = 0.3343$, $F = 1.322$, $\alpha_{(2,2)} = 0.05$). INO *Filinia* densities (Figure 6-45) averaged: Initial $\bar{X} = 5.5$ rotifers/L (0.0 to 21.0 rotifers/L 95 % CI), NF $\bar{X} = 3.0$ rotifers/L (0.0 to 10.5 rotifers/L 95 % CI), and Fish $\bar{X} = 1.8$ rotifers/L (0.0 to 7.3 rotifers/L 95 % CI).

Due to the non-significance of the nutrient factor in the 2-way repeated measures ANOVA comparison of *Filinia* density among pond nutrient and 24-hour trial experimental treatments (Initial, NF, and Fish), experimental treatments were pooled across nutrient treatments. As a result of this increased experimental treatment group (Initial, NF, and Fish) sample size [$n = 4_{(\text{unpooled})}$ to $n = 16_{(\text{pooled})}$] and associated statistical power, significant differences in experimental treatment *Filinia* densities were detected (rep. meas. 1-way ANOVA, $P = 0.0493$, $F = 3.333$, $\alpha_{(2,2)} = 0.05$). Average *Filinia* densities of the pooled treatments were: Initial $\bar{X}_{(\text{pooled})} = 7.9$ rotifers/L (0.4 to 15.4 rotifers/L 95 % CI), NF $\bar{X}_{(\text{pooled})} = 2.6$ rotifers/L (0.9 to 4.3 rotifers/L 95 % CI), and Fish $\bar{X}_{(\text{pooled})} = 1.4$ rotifers/L (0.1 to 2.7 rotifers/L 95 % CI). Although the pooled F

statistic was significant, no pairwise differences among experimental treatments (Initial, NF, and Fish) were detected (Figure 6-46; $P < 0.05$, Bonferroni post test).

Daphnid densities did not significantly differ among nutrients, but did differ among feeding trial treatments (Figures 6-47 – 6-50; rep. meas. 2-way ANOVA, $P_{(\text{nutrient})} = 0.3380$, $F_{(\text{nutrient})} = 1.241$, $\alpha_{(2,3)} = 0.05$; $P_{(\text{exp. Treat.})} = 0.0024$, $F_{(\text{exp. Treat.})} = 7.847$, $\alpha_{(2,2)} = 0.05$; $P_{(\text{interaction})} = 0.4674$, $F_{(\text{interaction})} = 0.9683$, $\alpha_{(2,6)} = 0.05$). Post hoc comparison tests did not find any differences in daphnid density between nutrient treatments within individual experimental treatments (Initial, NF, Fish), but a single difference in daphnid density between experimental treatments within nutrient treatment was found, UNP Initial and UNP Fish ($P < 0.05$, Bonferroni posttests).

PRO daphnid densities among the 24-hour feeding trial treatments did not significantly differ (Figure 6-47; Bonferroni posttests). PRO daphnid densities averaged: Initial $\bar{X} = 151$ daphnids/L (0.0 to 480 daphnids/L 95 % CI), NF $\bar{X} = 92.8$ daphnids/L (0.0 to 211 daphnids/L 95 % CI), and Fish $\bar{X} = 5.5$ daphnids/L (0.1 to 10.9 daphnids/L 95 % CI).

UNP daphnid densities averaged: Initial $\bar{X} = 218.5$ daphnids/L (0.0 to 601 daphnids/L 95 % CI), NF $\bar{X} = 153$ daphnids/L (0.0 to 414 daphnids/L 95 % CI), and Fish $\bar{X} = 7.0$ daphnids/L (0.0 to 22.4 daphnids/L 95 % CI). Within the UNP treatment, Initial average daphnid density was significantly higher than that within the Fish treatment ($P < 0.05$, Bonferroni post test).

CSM daphnid densities differences did not significantly differ among the 24 hour feeding trial treatments (Figure 6-49; $P > 0.05$, Bonferroni posttests). CSM daphnid densities averaged: Initial $\bar{X} = 29.5$ daphnids/L (0.0 to 118 daphnids/L 95 % CI), NF $\bar{X} = 14.0$ daphnids/L (0.0 to 55.4 daphnids/L 95 % CI), and Fish $\bar{X} = 2.0$ daphnids/L (0.0 to 7.4 daphnids/L 95 % CI).

INO daphnid densities did not significantly differ among the 24 hour feeding trial treatments (Figure 6-50; $P > 0.05$, Bonferroni post test). INO daphnid densities averaged: Initial

$\bar{X} = 85.8$ daphnids/L (0.0 to 175 daphnids/L 95 % CI), NF $\bar{X} = 73.0$ daphnids/L (33.2 to 113 daphnids/L 95 % CI), and Fish $\bar{X} = 8.0$ daphnids/L (0.0 to 18.7 daphnids/L 95 % CI).

Due to the non-significance of the pond nutrient factor, experimental group treatments were pooled across pond nutrient treatments (unpooled $n = 4$, pooled $n = 16$) to increase statistical power. This resulted in significant differences in daphnid density among experimental treatments (Figure 6-51; rep. meas. 1-way ANOVA, $P = 0.0027$, $F = 7.276$, $\alpha_{(2,2)} = 0.05$).

Average pooled daphnid densities were: Initial $\bar{X}_{(\text{pooled})} = 117$ daphnids/L (29.2 to 206 daphnids/L 95 % CI), NF $\bar{X}_{(\text{pooled})} = 80.7$ daphnids/L (28.3 to 133 daphnids/L 95 % CI), and Fish $\bar{X}_{(\text{pooled})} = 5.3$ daphnids/L (2.0 to 8.7 daphnids/L 95 % CI). Two pairwise differences in daphnid density were found, Initial and Fish, and NF and Fish (Figure 6-51; $P < 0.05$, Bonferroni post test).

24-Hour Feeding Trial Major Taxa %IRI

Out of the thirty taxa commonly found (%IRI > 0.5%) in the large zooplankton assemblages from the (spring 2006) 12-week outdoor pond trials (Chapter 5), only two (Copepod - *Diaptomous spp.* and Daphnid - *Moina macrocopa*; Figures 5-37a, b) were commonly found within the one-liter samples recovered from the 24-hour captive feeding trials. Large plankton assemblage taxa percent number, percent volume, and percent frequency of occurrence graph taxa abbreviations (Figures 6-1 – 6-24) are identical to those listed in Appendix A.

Among three of the four pond nutrient treatments, *Diaptomous* copepods were the most important large plankton community component and potential swordtail prey item group within the Initial 24-hour captive feeding experimental treatment. The single exception was the UNP treatment, which had *Moina* as the primary taxa (Figure 6-56). *Moina* was the second most important large plankton assemblage component and potential swordtail prey item within the

non-UNP treatments (Figure 6-56). Non-UNP Initial experimental treatment unpooled %IRI values were similar to pooled %IRI values (Figures 6-56, 6-59) except for the UNP pond treatment, which had nearly equal *Diaptomous* copepod and *Moina* pooled %IRI values (Figure 6-59).

The NF treatment large plankton community taxa pattern was identical to that observed within the Initial experimental treatment among the four pond nutrient treatments (Figure 6-57). Within the large zooplankton assemblage of the NF treatment, *Diaptomous* copepods were the most important group, followed by *Moina* daphnids in the non-UNP pond nutrient treatment. Within the UNP NF experimental treatment group, *Moina* was again the most important taxon, followed by *Diaptomous* copepods. Unpooled NF large zooplankton assemblage %IRI values were similar to pooled %IRI values (Figures 6-57, 6-60).

The Fish experimental treatment large plankton assemblage community %IRI values were markedly different from either the Initial or NF control treatments (Figure 6-58). Copepod relative %IRI, %N and %V values were much greater within the Fish treatment compared to within the Initial and NF treatments (Figures 6-1-6-24), although actual copepod numbers were dramatically lower (Figures 6-37 – 6-41). Conversely, daphnid relative %IRI, %N and %V values were markedly lower within the Fish treatment compared to within the Initial and NF treatments (Figures 6-1 – 6-24). Fish treatment unpooled %IRI values did not differ greatly from those of pooled %IRI values, although unpooled *Pediastrum* algae %IRI values were slightly greater than their pooled %IRI value counterparts (Figures 6-54, 6-57).

24-Hour Feeding Trial Similarity Indices

Large plankton assemblage community similarities among the three 24-hour captive feeding trial treatments were estimated using deterministically derived simplified Morisita's and percent similarity indices (MSI) values using large plankton assemblage taxa %IRI values

(Chapter 5 Methods). Among the four pond nutrient treatments, the 24-hour captive feeding trial large plankton community MSI values were significantly similar ($MSI \geq 0.65$) for all pairwise treatment comparisons (Figure 6-62; Initial and NF, Initial and Fish, NF and Fish), although the Initial and NF treatment large plankton communities consistently displayed greater similarity to each other ($MSI \sim 1.0$), than either treatment displayed to the Fish treatment (MSI range: 0.78-0.98), which had lower MSI values, as well as greater variation among pond nutrient treatments. UNP Initial and Fish, and NF and Fish pairwise large plankton community similarities were numerically lower than those of the other three pond nutrient treatments (Figure 6-62).

Among the 24-hour feeding trial treatments, large plankton assemblage PSI values displayed a pattern similar to those of the simplified MSI, except for the PRO pond nutrient treatment (Figure 6-63). The PRO large plankton community Initial and NF PSI value was markedly lower than those of the other three treatments, although all four pond nutrient treatment Initial and NF PSI values were considered similar ($PSI \geq 60\%$). Initial and Fish PSI values were generally lower than their Initial and NF counterparts for all pond nutrient treatments, except for the PRO treatment. Similarly, NF and Fish PSI values were generally lower than their Initial and Fish counterparts, except for the UNP treatment.

Bootstrap Large Plankton Assemblage Similarity Indices

Two-way repeated measures ANOVA large plankton assemblage community comparisons, using bootstrap-generated MSI estimates (using %N), did not find significant differences among the three experimental treatments (Initial, NF, Fish) within given pond nutrient treatments (Figures 6-64 - 6-67), but did find significant differences among the four pond nutrient treatments within experimental treatments (rep. meas. 2-way ANOVA, $P_{(\text{nutrient})} = 0.0041$, $F_{(\text{nutrient})} = 5.255$, $\alpha_{(2,3)} = 0.05$, $P_{(\text{exp. treat.})} = 0.1786$, $F_{(\text{exp. treat.})} = 1.808$, $\alpha_{(2,2)} = 0.05$, $P_{(\text{interaction})} = 0.3124$, $F_{(\text{interaction})} = 1.234$, $\alpha_{(2,6)} = 0.05$).

Numerically, INO Initial and NF large plankton community similarities (MSI values) were significantly greater than those of the PRO and UNP treatments ($P < 0.05$, Bonferroni post test). Although large plankton assemblage MSI values did not significantly differ among the three possible pairwise experimental treatment combinations (Initial and NF, Initial and Fish, NF and Fish) within any of the four pond nutrient treatments, Initial and NF MSI values were numerically greater than the Initial and Fish, and NF and Fish MSI values for the UNP, CSM and INO treatments (Figures 6-64 – 6-67). Suggesting that the large plankton assemblages of the two control treatments that had not been subjected to fish predation (Initial, NF), had greater large plankton community similarities, at least on a taxa % number basis. However, these MSI differences were not statistically significant among the three experimental treatments within each of the four pond nutrient treatments. Bootstrap large plankton assemblage MSI values between the Initial and NF experimental treatments were: PRO $\bar{X} = 0.55$ (0.16 to 0.95 95 % CI), UNP $\bar{X} = 0.73$ (0.36 to 1.11 95 % CI), CSM $\bar{X} = 0.90$ (0.76 to 1.04 95 % CI), INO $\bar{X} = 0.92$ (0.81 to 1.03 95 % CI). Bootstrap large plankton assemblage MSI values between the Initial and Fish experimental treatments were: PRO $\bar{X} = 0.78$ (0.42 to 1.14 95 % CI), UNP $\bar{X} = 0.52$ (0.05 to 0.99 95 % CI), CSM $\bar{X} = 0.69$ (0.34 to 1.03 95 % CI), INO $\bar{X} = 0.88$ (0.74 to 1.03 95 % CI). Bootstrap large plankton assemblage MSI values between the NF and Fish experimental treatments were: PRO $\bar{X} = 0.48$ (0.33 to 0.63 95 % CI), UNP $\bar{X} = 0.58$ (0.12 to 1.03 95 % CI), CSM $\bar{X} = 0.68$ (0.35 to 1.02 95 % CI), INO $\bar{X} = 0.83$ (0.70 to 0.97 95 % CI). According to the arbitrarily chosen similarity threshold value ($MSI \geq 0.65$), PRO Initial and NF, UNP Initial and Fish, and UNP NF and Fish large plankton communities were no longer similar (Cailliet and Barry 1978, Lindquist 1998).

Bootstrap-generated large plankton assemblage PSI values did not differ among the three experimental treatments within any given pond nutrient treatment, or among the four pond nutrient treatments within any given experimental treatment (Figures 6-68 – 6-71; rep. meas. 2-way ANOVA, $P_{(\text{nutrient})} = 0.2348$, $F_{(\text{nutrient})} = 1.486$, $\alpha_{(2,3)} = 0.05$, $P_{(\text{exp. treat.})} = 0.3540$, $F_{(\text{exp. treat.})} = 1.069$, $\alpha_{(2,2)} = 0.05$, $P_{(\text{interaction})} = 0.3540$, $F_{(\text{interaction})} = 0.3415$, $\alpha_{(2,6)} = 0.05$). The Initial and NF similarity index (PSI) values were higher than those of the Initial and Fish, and NF and Fish PSI values for the same three pond nutrient treatments as the MSI value comparisons (UNP, CSM and INO). This indicates that fish presence may have reduced large plankton taxa abundance and changed the large plankton community structure to the extent that initially similar large plankton communities were less similar (Initial, NF, Fish). Initial and NF treatment large plankton assemblage bootstrap PSI values were: PRO $\bar{X} = 45.0$ (13.6 to 76.4 95 % CI), UNP $\bar{X} = 60.5$ (24.8 to 96.2 95 % CI), CSM $\bar{X} = 71.4$ (51.0 to 91.8 95 % CI), INO $\bar{X} = 75.7$ (61.2 to 90.2 95 % CI). Initial and Fish treatment large plankton assemblage bootstrap PSI values were: PRO $\bar{X} = 60.0$ (32.1 to 88.0 95 % CI), UNP $\bar{X} = 43.7$ (12.3 to 75.1 95 % CI), CSM $\bar{X} = 53.4$ (22.6 to 84.3 95 % CI), INO $\bar{X} = 68.8$ (52.4 to 85.2 95 % CI). NF and Fish treatment large plankton assemblage bootstrap PSI values were: PRO $\bar{X} = 35.2$ (25.9 to 44.6 95 % CI), UNP $\bar{X} = 47.0$ (14.7 to 79.4 95 % CI), CSM $\bar{X} = 52.8$ (23.8 to 81.9 95 % CI), INO $\bar{X} = 60.9$ (46.2 to 75.6 95 % CI).

According to the arbitrarily chosen PSI threshold critical value ($\text{PSI} \geq 60\%$), following 24-hour trial duration without fish predation (NF), and 24-hour trial duration with fish predation (Fish), large zooplankton assemblages were no longer similar to the Initial treatment within the UNP and CSM (Initial and NF), and PRO, UNP and CSM (Initial and Fish) treatments.

To determine if increasing the number of pond replicates would result in greater sensitivity to differences (increased statistical power) among the 24-hour treatments (Initial, NF, Fish), replicate large plankton assemblages from the four pond nutrient treatments were pooled among experimental treatments, increasing sample size from four to sixteen replicates for each treatment. Pooled bootstrap PSI values, among the feeding trial treatments, did not significantly differ (Figure 6-73). The same treatment-pair comparison PSI value rankings (Initial and NF – highest, NF and Fish – lowest) occurred for both the pooled and unpooled treatment (non-PRO) data. Pooled average bootstrap PSI values between feeding trial treatment pair values: Initial vs. NF PSI $\bar{X} = 59.4$ (48.8 to 70.0 95 % CI), Initial vs. Fish PSI $\bar{X} = 56.2$ (44.6 to 67.8 95 % CI), NF vs. Fish PSI $\bar{X} = 50.2$ (39.5 to 60.8 95 % CI).

All three pooled bootstrap large zooplankton community PSI similarity comparisons (Initial and NF, Initial and Fish, NF and Fish) were ‘not similar’ according to the arbitrarily chosen PSI critical value (PSI \geq 60%). Due to the nature of resampling statistical techniques, it was not deemed necessary to perform arcsine square root transformations upon raw percentage data for the PSI comparisons due to the normal distributions that typically result from large numbers of sample iterations (1000 iterations; Simon 1997).

Discussion

Large Zooplankton Assemblages

The majority of taxa, and those with the greatest importance (highest %IRI) within the large plankton community, were zooplankton, with minor exceptions (e.g., *Pediastrum spp.* phytoplankton, Division Chlorophyta - green algae). Because the majority of large plankton assemblage taxa were zooplankton, large plankton assemblages are frequently referred to as ‘large zooplankton’ assemblages within this document.

24-Hour Captive Feeding Trials

Two-way repeated measures ANOVA found that large zooplankton assemblage volumes did not differ among pond nutrient treatments within the captive feeding trial experimental treatments, but that large zooplankton volumes did differ among captive feeding trial treatments within given pond nutrient treatments (PRO Initial and Fish, UNP Initial and Fish, and UNP NF and Fish). These results indicate that feeding trial treatment was a factor in determining large zooplankton volume within the PRO and UNP treatments, and that both time and/or fish presence effected large zooplankton volumes after 24 hours (Figures 6-26 – 6-27). Fish are likely consuming large zooplankton, at least within the UNP treatment (Figure 6-27). Fish also may be consuming large zooplankton within the three remaining non-UNP treatments, as average volumes were numerically lower within the Fish treatment relative to their Initial and NF treatment counterparts within all pond nutrient treatments (Figures 6-26 – 6-30). However, Fish treatment plankton volumes were not statistically lower than the Initial and NF feeding trial treatments within the CSM and INO pond nutrient treatments. However, the supposition that fish were grazing upon large zooplankton is reasonable given the limitations of the experimental design utilized for the 24-hour captive feeding trial, and the fact that direct feeding was not observed due to the desire to avoid observer effects, and that gut content analyses on fish of this size and species had previously proven fruitless.

Because pond nutrient treatment was not found to be a significant factor, nutrient treatments were pooled within the three experimental treatments (Initial, NF, and Fish). This increased the sample size from four to sixteen, and increased the statistical power of the analysis. Pooling seemed reasonable in this instance, as fish were not obtained from within the ponds that were used for plankton sample collection (feeding history), and the general qualitative similarity of large plankton communities among pond treatments (Motulsky 1995). Large plankton

communities from each of the four pond nutrient treatments, appeared to be qualitatively and quantitatively similar, except for the UNP large plankton community which had higher %IRI, % number and % volume for daphnids than for copepods (Figures 6-1, 6-4, 6-7, 6-10, 6-52).

Pooling nutrient treatments resulted in two pairwise experimental treatment differences (Initial v. Fish, and NF v. Fish). Trial duration no longer had an effect upon large zooplankton volume as the Initial and NF experimental treatment volumes no longer differed (Figure 6-30). These results indicate that fish presence in the experimental treatments was the only major factor in reducing large zooplankton volume among pooled samples, and that fish predation was likely the cause of the observed decrease (Figure 6-30). Although time also was a factor in reducing large plankton community volumes, time alone was not a significant factor in reducing large zooplankton volumes among pooled samples (Figure 6-30). Trial duration likely resulted in large plankton volume reduction from numerous causes such as intra-plankton predation, food limitation, and environmental stressors (temperature, DO, etc.). However, time was not as strong a factor in reducing large plankton biomass as fish presence, and large plankton volume reductions resulting from trial duration alone were not present among the pooled nutrient treatment data set.

Similar to total large plankton volume differences among pond treatments and 24-hour feeding trial treatments, pond nutrient treatment was not a factor in determining large zooplankton densities. However, within a given pond nutrient treatment, total large zooplankton density averages uniformly decreased among feeding trial treatments in the following order: Initial, NF, and Fish (Figures 6-31 – 6-35). Within pond treatments, total plankton density averages noticeably dropped among the three experimental treatments (Figures 6-32 – 6-35), although statistical differences were not always present due to large variation among replicates.

Again, this suggests that both fish presence and time (24-hour trial duration) decrease large plankton abundance due to swordtail planktivory and co-occurring factors unrelated to fish predation (e.g., intra-plankton predation, starvation, DO stress, temperature extremes, etc.). Large plankton density differences among the 24-hour feeding trial experimental treatments were present only within the UNP and INO pond nutrient treatments, where the presence of a single swordtail (Fish) had lowered large plankton densities relative to either the Initial or NF (no-fish) treatments over a 24-hour period (Figures 6-33, 6-35). As with large plankton volume differences among experimental treatments, large plankton density differences among experimental treatments, also indicated that fish presence, and likely fish planktivory, was responsible for the lower large plankton biomasses recovered from these treatments after 24 hours.

The finding that pond nutrient was not a significant factor in determining large plankton densities, was used as justification for pooling replicates within experimental treatment groups. Pooled one-way repeated measures ANOVA found large plankton density differences between the Fish experimental group and the two remaining treatment groups (Initial and NF), which did not differ from each other (Figure 6-36). Again, these results indicate that fish presence and likely planktivory, but not trial duration (24 hours) had a detectable effect upon large plankton assemblage biomass. Although these results indicate that trial duration had a small, but statistically insignificant effect upon biomass within the experimental design of the 24-hour captive feeding trial.

Major Taxa Densities among 24-Hour Trial Treatments

Within individual pond nutrient treatments, the three major large zooplankton taxa (*Diaptomous* copepods, *Filinia* rotifers, and daphnids – *Moina macrocopa*) densities decreased within feeding trial treatments in the following order: Initial, NF, and Fish (Figure 6-37-6.40).

Filinia rotifer density differences among the three feeding trial treatments were not significant, due to the low densities present within samples, regardless of feeding trial treatment (Initial, NF, and Fish).

Diaptomous copepod densities were numerically lower within the Fish treatment relative to the two remaining treatments (Initial, NF) in all four pond nutrient treatments (Figure 6-37-6.40), but was only significantly lower within the INO treatment (Figure 6-40). Similar to large plankton volume and density, when nutrient treatments were pooled, the Fish treatment copepod density was significantly less than that of the Initial and NF treatments (Figure 6-41).

Within given feeding trial treatments (Initial, NF, or Fish), *Filinia* densities did not differ among pond nutrient treatments, but within nutrient treatments, densities differed among feeding trial treatments. However, a single pairwise difference in *Filinia* densities was found between the PRO and CSM treatments for the Initial treatment according to post hoc tests. Post hoc differences can occur among treatments, even when ANOVA does not detect a significant difference among treatments (Bonferroni post test, GraphPad Prism 4.0; Motulsky 1995, Zar 1984). Within pond nutrient treatments, significant differences in *Filinia* density among the feeding trial treatments were only found within the PRO pond treatment (PRO Initial and NF, PRO Initial and Fish). Lack of differences among the *Filinia* experimental treatments within the non-PRO treatments was puzzling at first, but upon closer examination, it can be seen that Initial, NF and Fish treatment rotifer numbers were extremely low (range 2-18 rotifers/L; Figures 6-42-6-46, 6-52, 6-54). Logically, the Initial experimental treatment should have had the highest number of rotifers of the three experimental treatments, which was not the case for the CSM treatment (Figure 6-44). Initial experimental treatment samples were immediately fixed with Lugol's solution upon collection from the pond and had not been subjected to zooplankton or

fish predation, or possible stress and mortality from 24 hours of captivity (Figures 6-42 – 6-46). A major problem with small sample sizes is that significant differences among treatments can easily occur due to random sampling differences (Krebs 1998, Zar 1984). Rotifer densities for the 24-hour captive feeding trials (mid-September 2007) were extremely low relative to the majority of samples acquired during the 12-week swordtail pond trials performed the previous year (March – June 2006), possibly due to seasonal effects.

Even when pond nutrient treatments were pooled, no significant differences in *Filinia* densities among 24-hour feeding trial treatments were found (Figure 6-46). Again, this may have been due to the low average *Filinia* densities among the pooled Initial, NF, and Fish treatments, and large variation among replicates within the Initial treatment.

Daphnid densities did not differ among pond nutrient treatments, but did differ among the three feeding trial treatments (Initial, NF, Fish). Within each of the four pond nutrient treatments, *Moina macrocopa* density averages decreased in the following order: Initial, NF and Fish (Figures 6-47 – 6-50). However, only a single significant difference (UNP Initial and UNP Fish) in *Moina* density was found among the three captive feeding trial experimental treatments (Figure 6-48). In an attempt to determine if daphnid density differences among the three experimental treatments could be better discerned with increased sample size, samples were pooled across the four pond nutrient treatments. Among the pooled data, two of the three possible pairwise treatment comparisons were significant; the Fish treatment had less daphnids than either the Initial or NF treatments, which did not differ from each other (Figure 6-51). This further supports the contention that fish were grazing on large zooplankton, and daphnids in particular, to the extent that the Fish treatment had significantly less daphnids than either of the control treatments (Initial, NF).

Although pooling treatment groups is not ideal, due to the general similarities in the Initial plankton assemblage communities among the four pond nutrient treatments, a strong argument against pooling did not appear to be present (Figures 6-1, 6-4, 6-7, 6-10, 6-56, 6-59). Additionally, average densities, that did not statistically differ prior to data pooling, differed after pooling, which may indicate the presence of strong experimental treatment effects that were not detected due to inadequate replication (four samples), or experimental treatment limitations (e.g., possible insufficient trial duration – 24 hrs, and/or insufficient pond water sample volumes – 1 L, or predator number). Insufficient replication and trial duration likely occurred during the 24 hour captive feeding trial, due to time constraints, only four pond replicates were obtained from each of the four pond nutrient treatments (PRO, UNP, CSM and INO) when six replicate ponds were available.

Pooling nutrient treatments also was partially justified as no significant differences in densities of the three major large zooplankton taxa groups (copepods, *Filinia* rotifers, daphnids), occurred among the Initial treatments of the four pond nutrient treatments (Figure 6-52-6.55). Additionally, the main objective of the 24-hour feeding trial was to determine the relative effects of swordtail feeding on plankton assemblages from four different pond nutrient regimes, and not to evaluate differences in plankton assemblages among pond nutrient treatments *per se*.

Additionally, live food naïve fish from a single source were used for the feeding trials. Fish were not obtained from ponds that had received different applied nutrients. This ruled out previous nutritional history from outdoor pond applied nutrients (e.g., feeds, cottonseed meal), or live prey exposure as potential experimental artifacts or biases among swordtails randomly assigned to large plankton assemblages obtained from different pond nutrient treatments.

24-Hour Trial Community %IRI Values

Within each of the 24-hour feeding trial treatments (Initial, NF, Fish), large plankton assemblage community %IRI values were fairly similar among pond nutrient treatments (Figures 6-56 – 6-61). Interestingly, copepod and daphnid %IRI values appeared to be inversely proportional within the Initial and NF control treatments (treatments lacking potential fish predator), as copepod and daphnid %IRI values were never simultaneously low or high (Figures 6-52 – 6-53, 6-55 – 6-56). This was similar to the inversely proportional copepod and daphnid %IRI relationship observed during the 2006 12-week outdoor pond trial (Chapter 5; Figures 5-13 – 5-20).

The Fish treatment showed the most dramatic differences in large plankton taxa %IRI values, compared to their Initial and NF control treatment counterparts (Figures 6-54, 6-57). Relative decreases in daphnid %IRI, percent volume (%V), and percent number (%N), coupled with simultaneously increases in copepod %IRI, %V, and %N, indicate that swordtails were preferentially selecting daphnid prey within the Fish treatment during the 24-hour feeding trial. Swordtails also were utilizing copepods and other prey, as overall densities of copepods, daphnids, and total large plankton volumes and densities decreased within the Fish treatment relative to the Initial and NF control treatments (Figures 6-1 – 6-24, 6-38 – 6-41, 6-47 – 6-57).

Although copepod density was reduced within the Fish treatment, copepod relative importance (%IRI) increased in the presence of swordtail predation. The large antennae of Calanoid copepods may have made them a less attractive food source relative to daphnids, as the large antennae of copepods may have increased copepod prey handling time, or reduced fish feeding efficiency by clogging gill rakers, or by producing physical irritation (Chapter 5; Figure 5-37a,b).

24-Hour Trial Simplified Morisita's and Percent Similarity Indices

Within pond nutrient treatments, large plankton community Morisita's similarity (MSI), and to a lesser degree percent similarity (PSI), indices were higher between the Initial and NF feeding trial control treatments, than they were between the Initial and Fish, and NF and Fish comparisons (Figures 6-62 – 6-63). Notable exceptions were the PRO Initial and NF PSI values, which were markedly lower than those of the other three pond nutrient treatments (Figure 6-63). Within nutrient treatments, Initial and Fish, and NF and Fish, large plankton community pairwise MSI and PSI values did not appear to greatly differ from each other, but both values were dramatically lower than those of the Initial and NF pairwise MSI and PSI values (Figures 6-62 – 6-63). These results indicate that the presence of a single fish predator for a 24-hour period fundamentally changed previously similar large plankton communities (NF and Fish).

Bootstrap Initial, NF, and Fish MSI and PSI Estimates

Captive feeding trial large plankton community bootstrap MSI values did not differ among the feeding trial treatment comparisons (Initial and NF, Initial and Fish, NF and Fish) within any of the four pond nutrient treatments, but MSI values did differ among pond nutrient treatments within given feeding trial treatment-pair comparisons: Initial and NF MSI PRO and INO, Initial and Fish MSI UNP and INO. The reason that these particular experimental treatment-pair MSI values differed among pond nutrient treatments was due to the greater taxa % number similarities of the INO Initial and NF large plankton assemblages (Figures 6-22 – 6-23; positive X-axis) relative to those of the PRO treatment (Figures 6-13 – 6-14), which may have been due to the large number of small 'spball' taxa (believed to be fungal spores or unidentified cysts), within the PRO NF large plankton assemblage, which were largely absent within the PRO Initial treatment. The reason the Initial and Fish experimental treatment MSI values differed between the UNP and INO treatments was due to the large numbers of *Pediastrum spp.* green algae

present within the Fish treatment assemblage of the UNP treatment (Figure 6-18), that were absent within the UNP Initial plankton assemblage (Figure 6-16).

Generally, bootstrap-generated MSI values of experimental treatment combinations containing swordtails (Initial and Fish, NF and Fish) were lower than those of the Initial and NF treatment combination, except for those of the PRO pond treatment. However, variation among replicate large plankton assemblages was fairly high, resulting in the absence of significant bootstrap MSI value differences among the experimental treatment pair combinations.

Bootstrap large plankton community PSI values did not differ among experimental treatment group pair combinations within nutrient treatments, or within experimental treatment group pair combinations among the four pond nutrient treatments. Large plankton community bootstrap PSI difference patterns within pond nutrient treatments, and among the three experimental treatment group pair combinations, followed the same pattern as that of the bootstrap MSI values. With the exception of the PRO nutrient treatment, experimental treatment pairwise combination MSI value rankings were the highest between the Initial and NF treatments, which were then followed by the Initial and Fish, or NF and Fish treatment pairs (Figures 6-69 – 6-71). Initial and Fish, and NF and Fish MSI values were nearly identical.

The reason the Initial and NF large plankton assemblages were less similar (lower MSI and PSI values) within the PRO nutrient treatment were likely due to the relatively large numbers of ‘spball’ spores or cysts within the NF (no-fish) experimental treatment, rather than a fundamental difference in the PRO Initial and NF large plankton community assemblages (Figures 6-1 – 6-2, 6-13 – 6-14, 6-62 – 6-63).

Because no significant differences in large plankton community PSI values occurred among feeding treatment-pair combinations within given pond nutrient treatments, pond nutrient

treatments were pooled to increase the experimental treatment sample size from four to sixteen. Again, no significant differences occurred among the three pooled experimental treatment combinations (Figure 6-72), which may have been due to the large variation in taxa % numbers within replicate plankton assemblages.

Interestingly, only within the UNP and CSM treatments were the Initial and NF MSI values considered similar, and the remaining two pairwise-treatment combinations (Initial and Fish, NF and Fish) not considered similar, according to the arbitrarily chosen critical threshold value ($MSI_{(critical)} \geq 0.65$). The three feeding trial treatment plankton assemblage MSI comparisons (Initial and NF, Initial and Fish, NF and Fish), were all similar within the INO nutrient treatment ($MSI_{(critical)} \geq 0.65$).

Similarly, UNP and CSM Initial and NF PSI values were considered similar ($PSI_{(critical)} \geq 60\%$), and the Initial and Fish, and NF and Fish PSI values were not similar (Figure 6-69-6.70). Again, the INO experimental treatment PSI values were all similar (Figure 6-71).

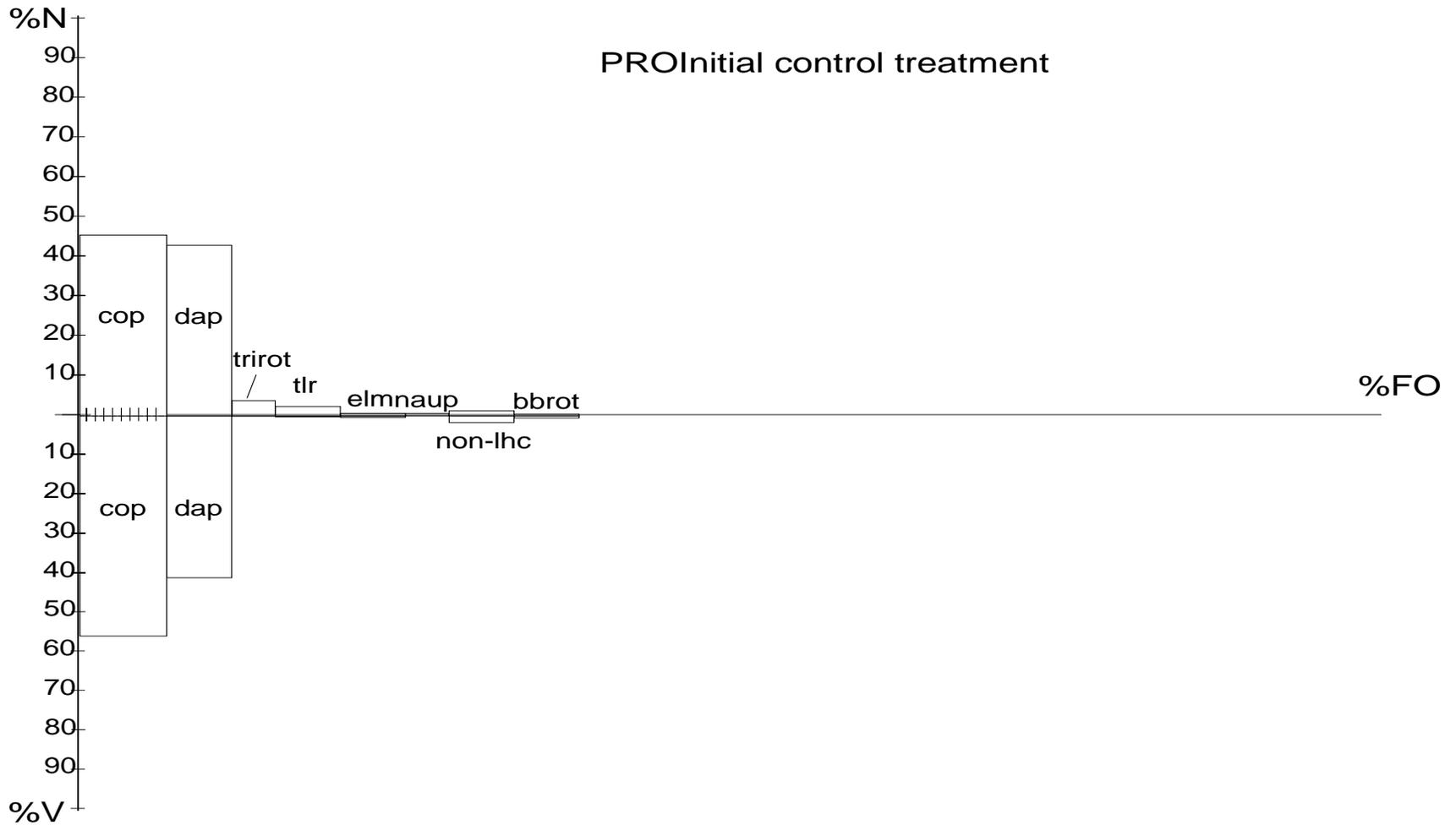


Figure 6-1. Initial (0 hrs) processed feed (PRO) treatment large zooplankton community pooled taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for 24-hour feeding trial, taxa %N, %V, or %FO \geq 0.5%; n = 4.

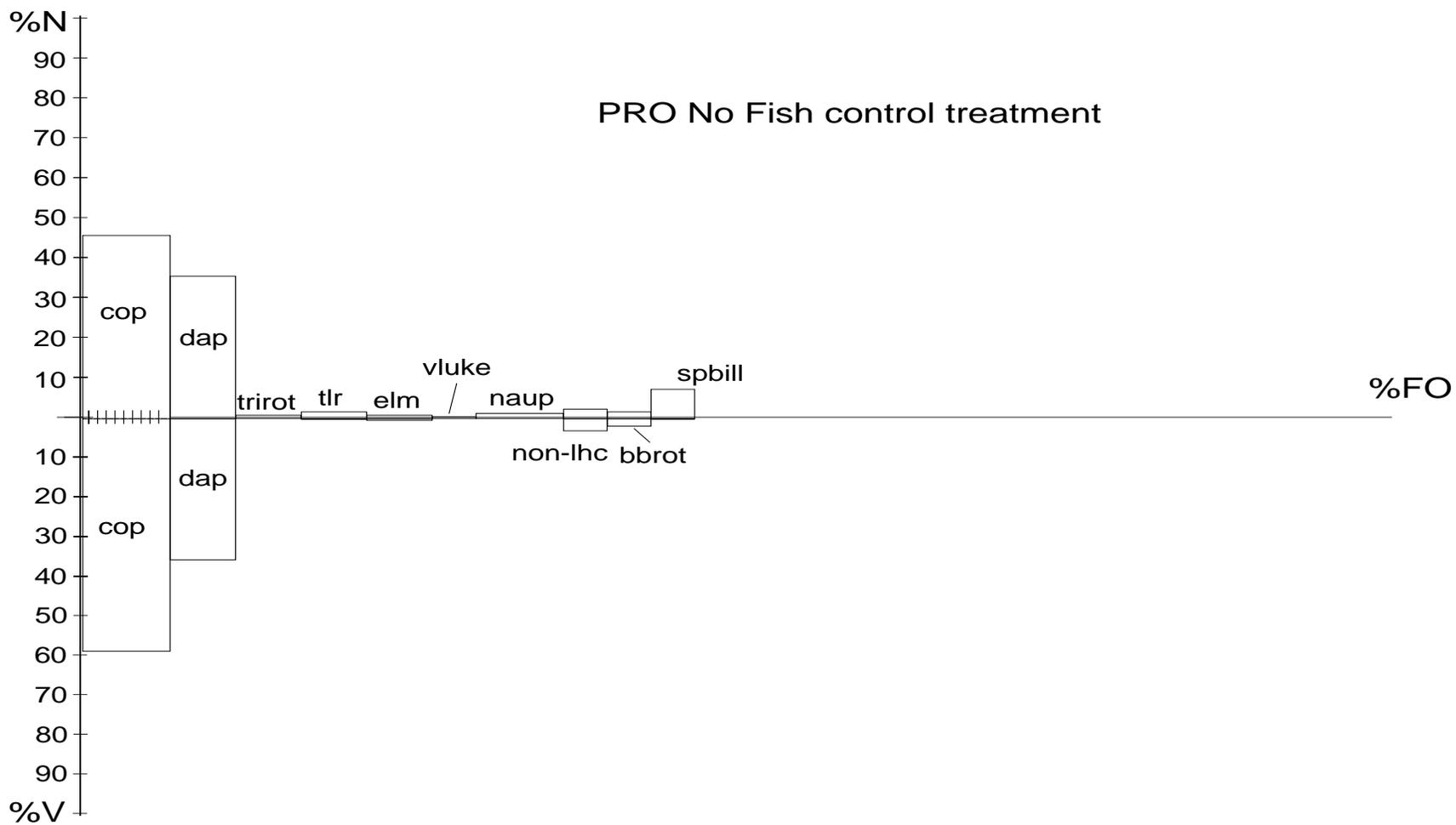


Figure 6-2. NF (no fish 24 hr incubation) processed feed treatment (PRO) large zooplankton pooled community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for 24-hour feeding trial, taxa %N, %V, or %FO \geq 0.5%; n = 4.

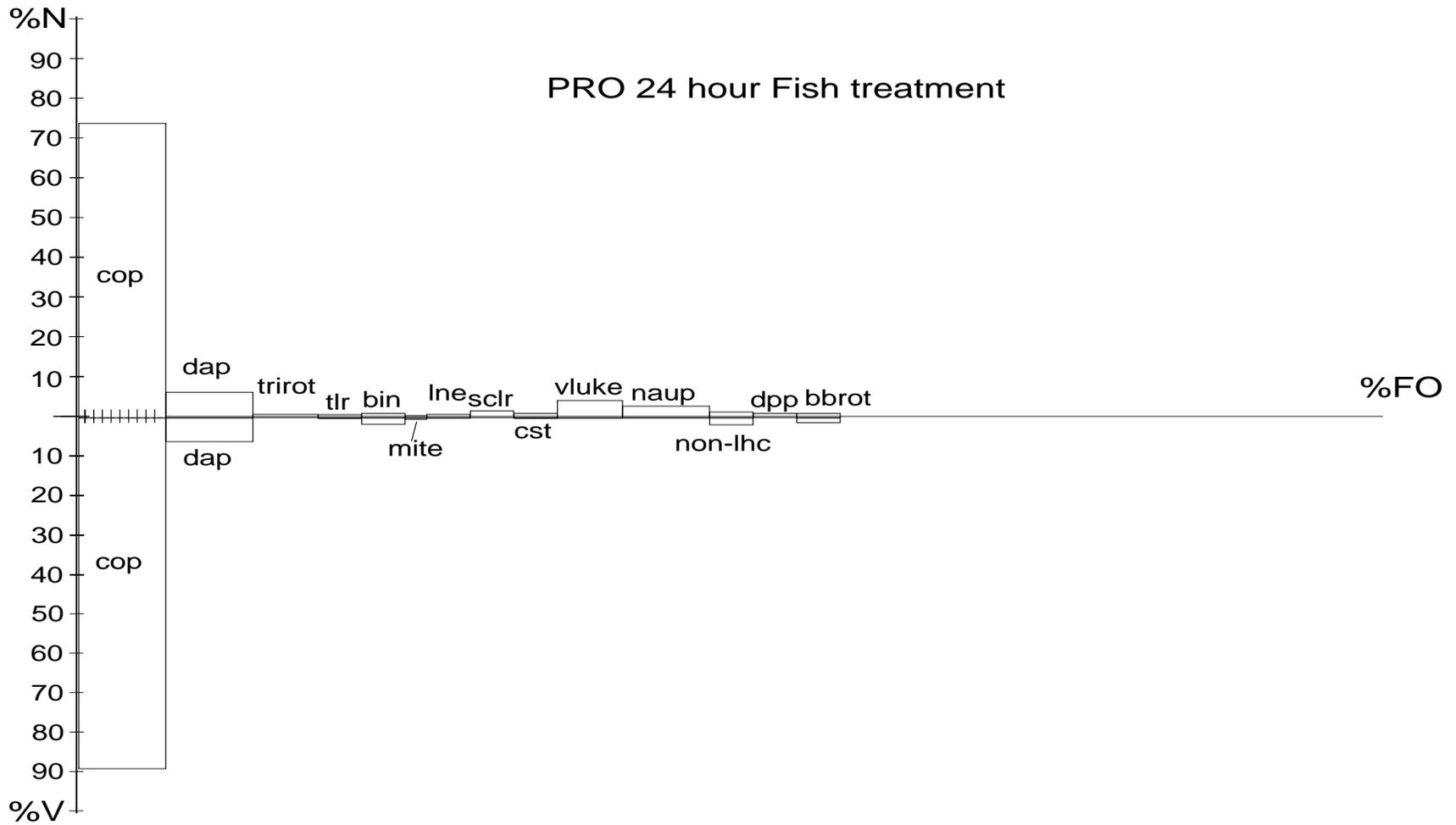


Figure 6-3. Fish (single fish 24 hr incubation) processed feed (PRO) treatment large zooplankton pooled community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for 24-hour feeding trial, taxa %N, %V, or %FO \geq 0.5%; 1-L water sample per pond, n = 4 ponds.

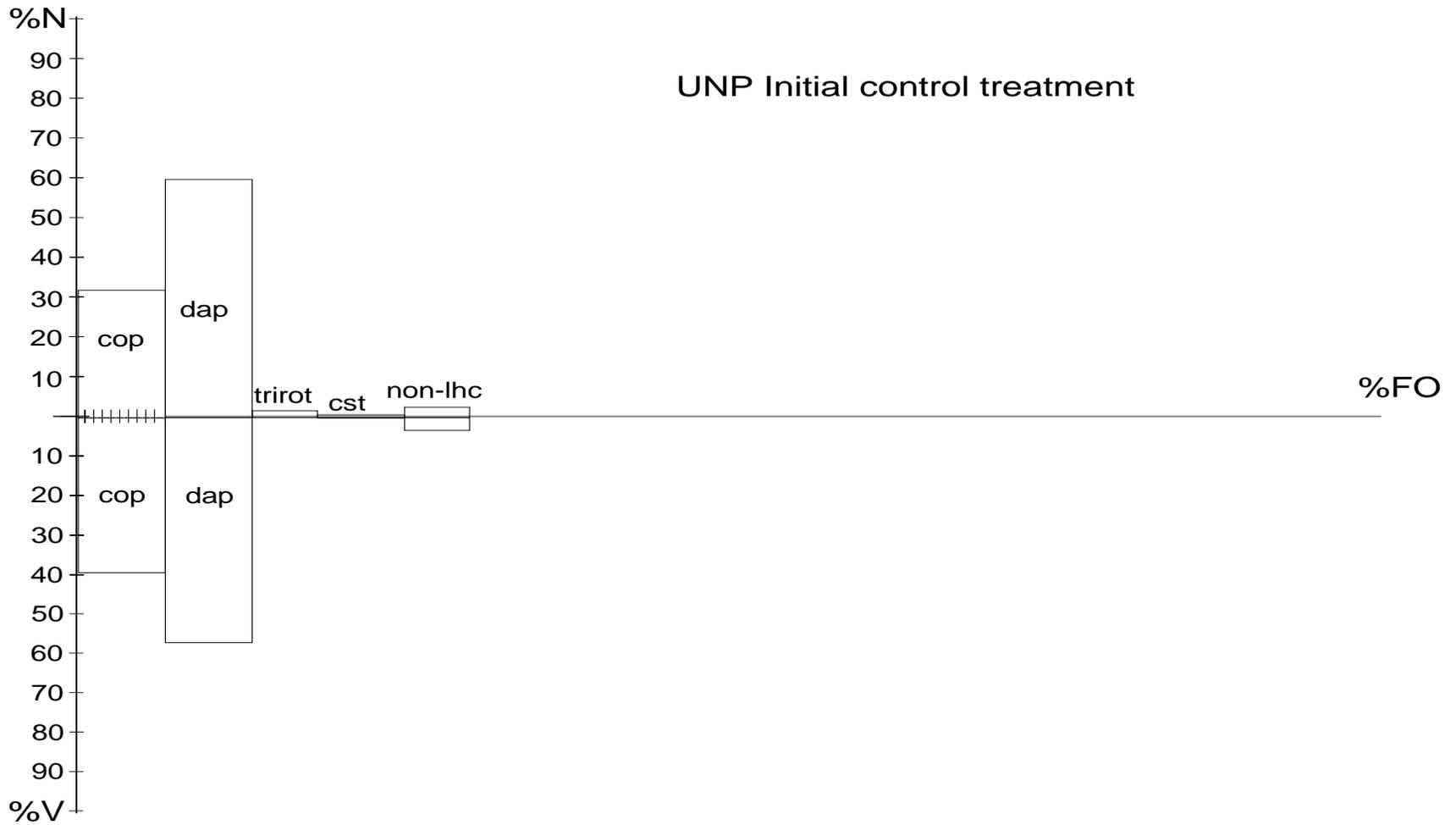


Figure 6-4. Initial (0 hrs) unprocessed feed (UNP) treatment large zooplankton pooled community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for 24-hour feeding trial, taxa %N, %V, or %FO \geq 0.5%; 1-L water sample per pond, n = 4 ponds.

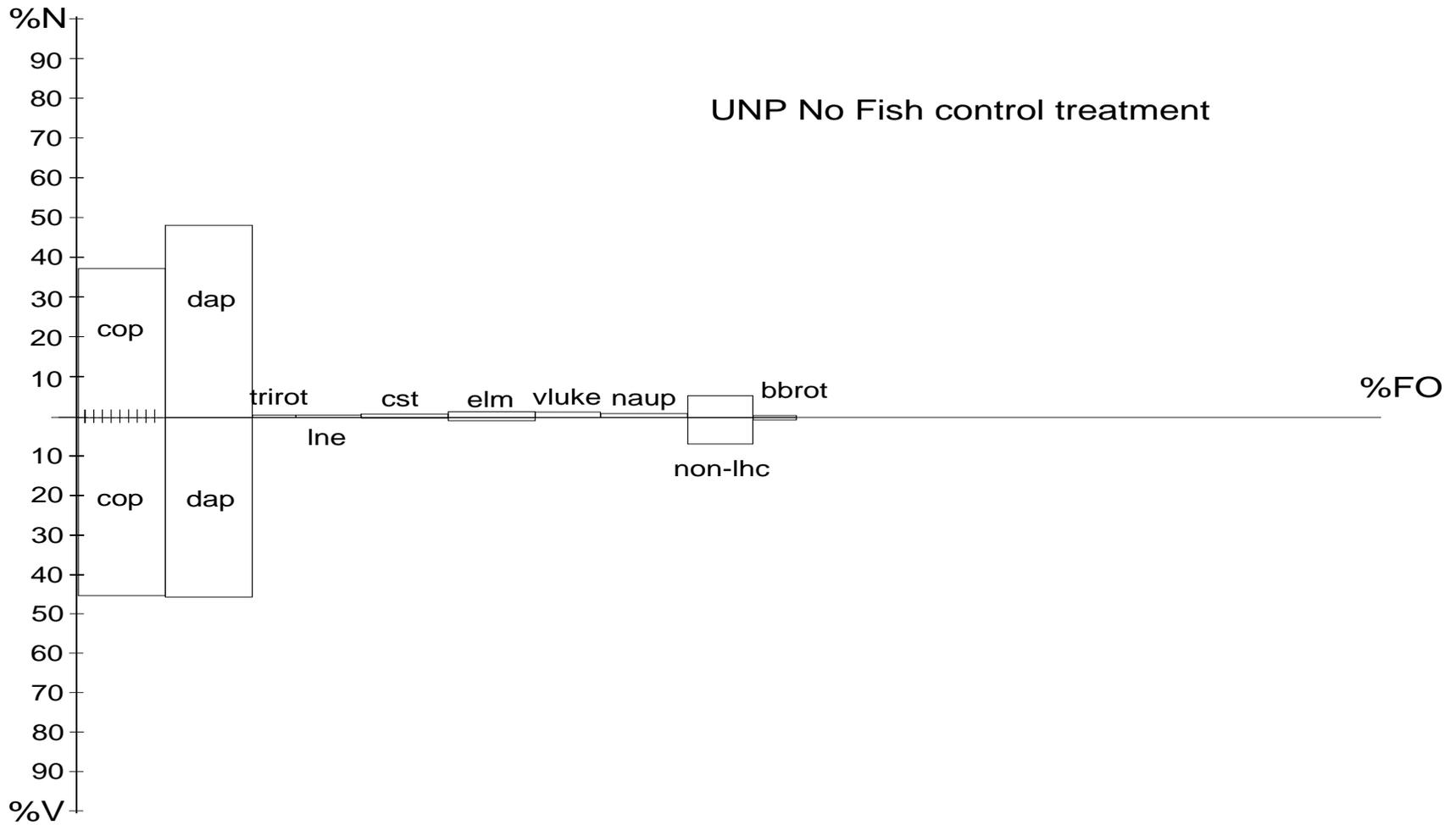


Figure 6-5. NF (no fish 24 hr incubation) unprocessed feed (UNP) treatment large zooplankton pooled community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for 24-hour feeding trial, taxa %N, %V, or %FO \geq 0.5%; 1-L water sample per pond, n = 4 ponds.

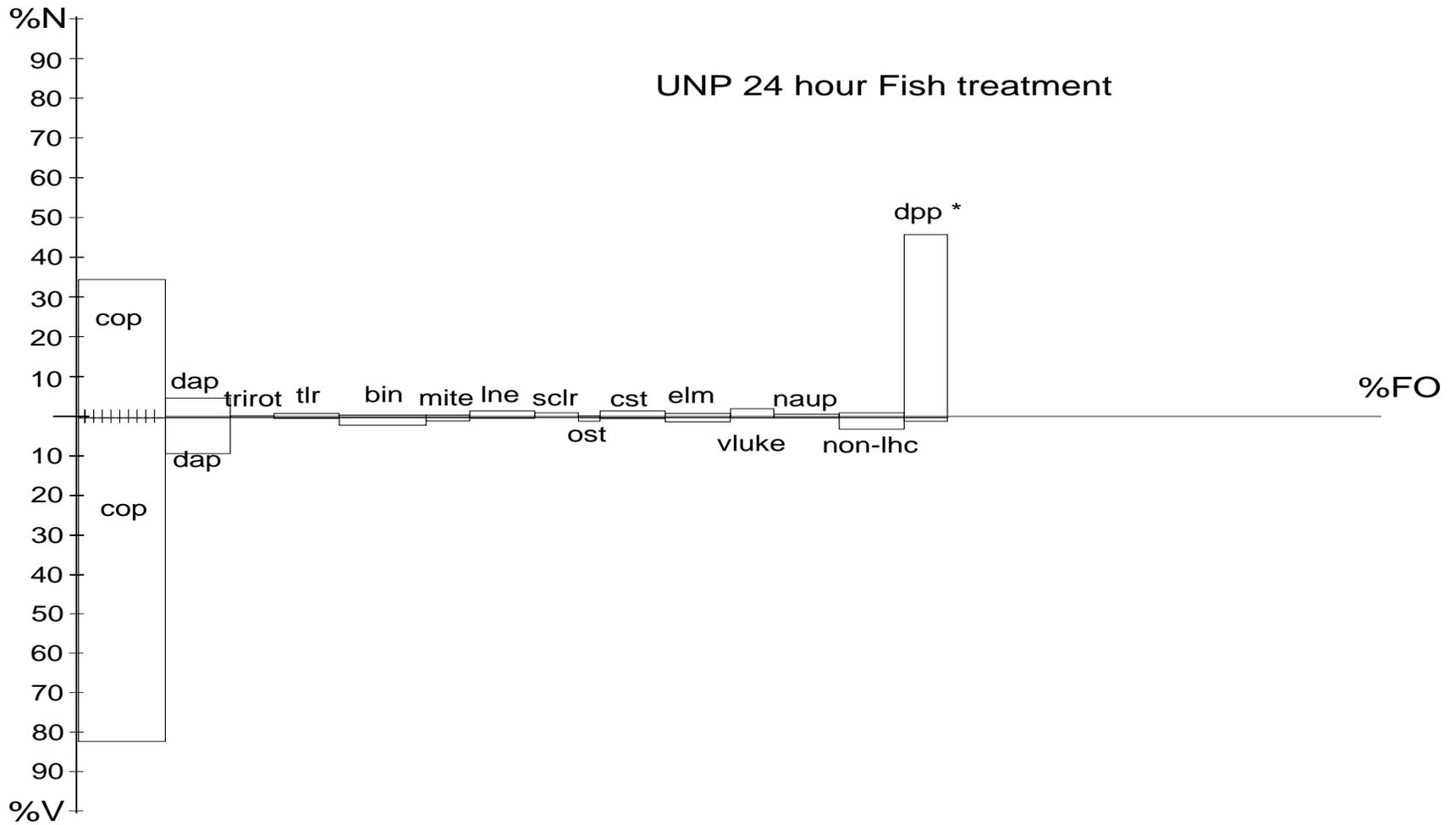


Figure 6-6. Fish (single fish 24 hr incubation) processed feed (PRO) treatment large zooplankton pooled community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for 24-hour feeding trial, taxa %N, %V, or %FO \geq 0.5%; 1-L water sample per pond, n = 4 ponds.

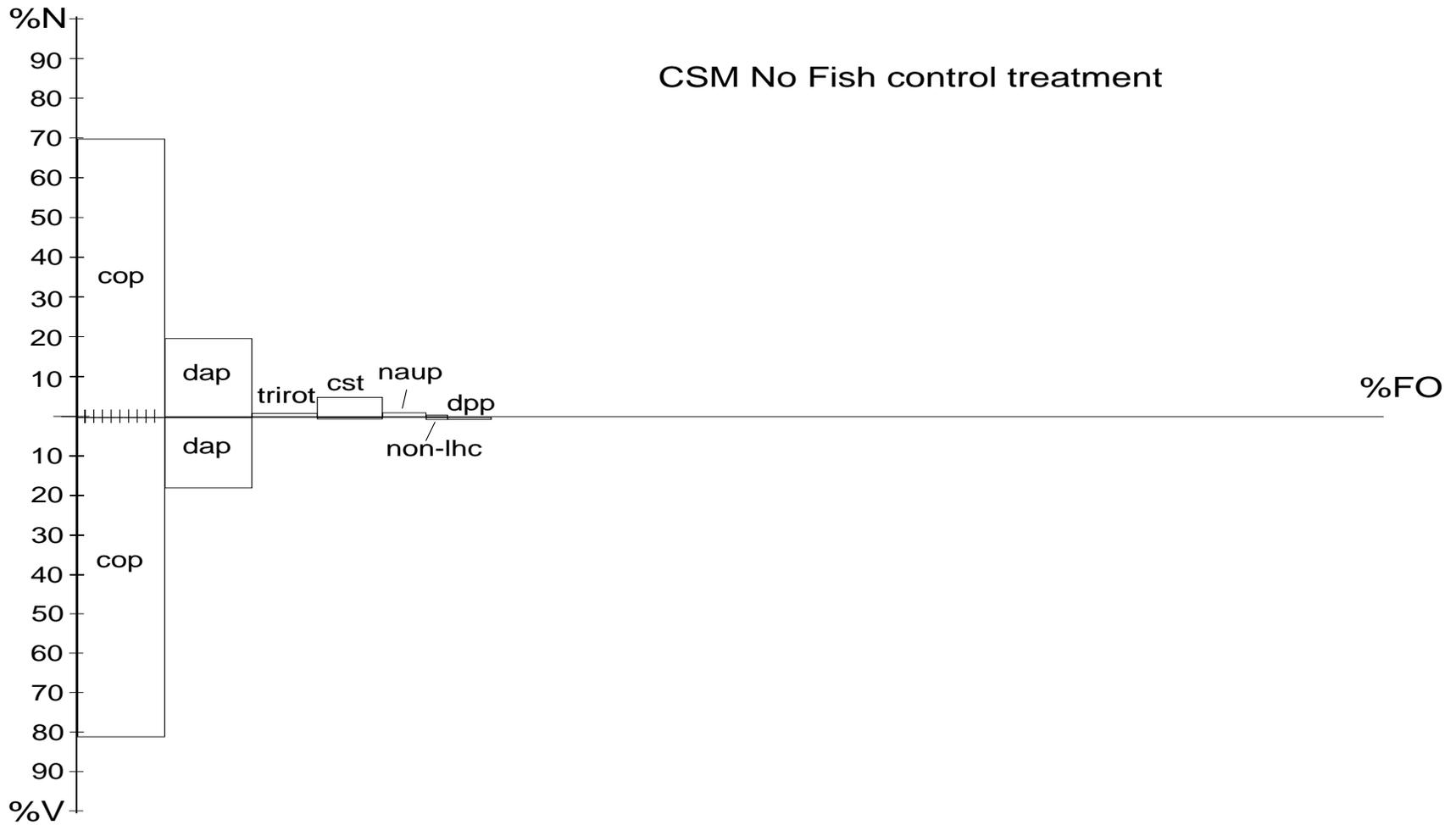


Figure 6-7. Initial (0 hrs) cottonseed meal (CSM) treatment large zooplankton pooled community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for 24-hour feeding trial, taxa %N, %V, or %FO \geq 0.5%; 1-L water sample per pond, n = 4 ponds.

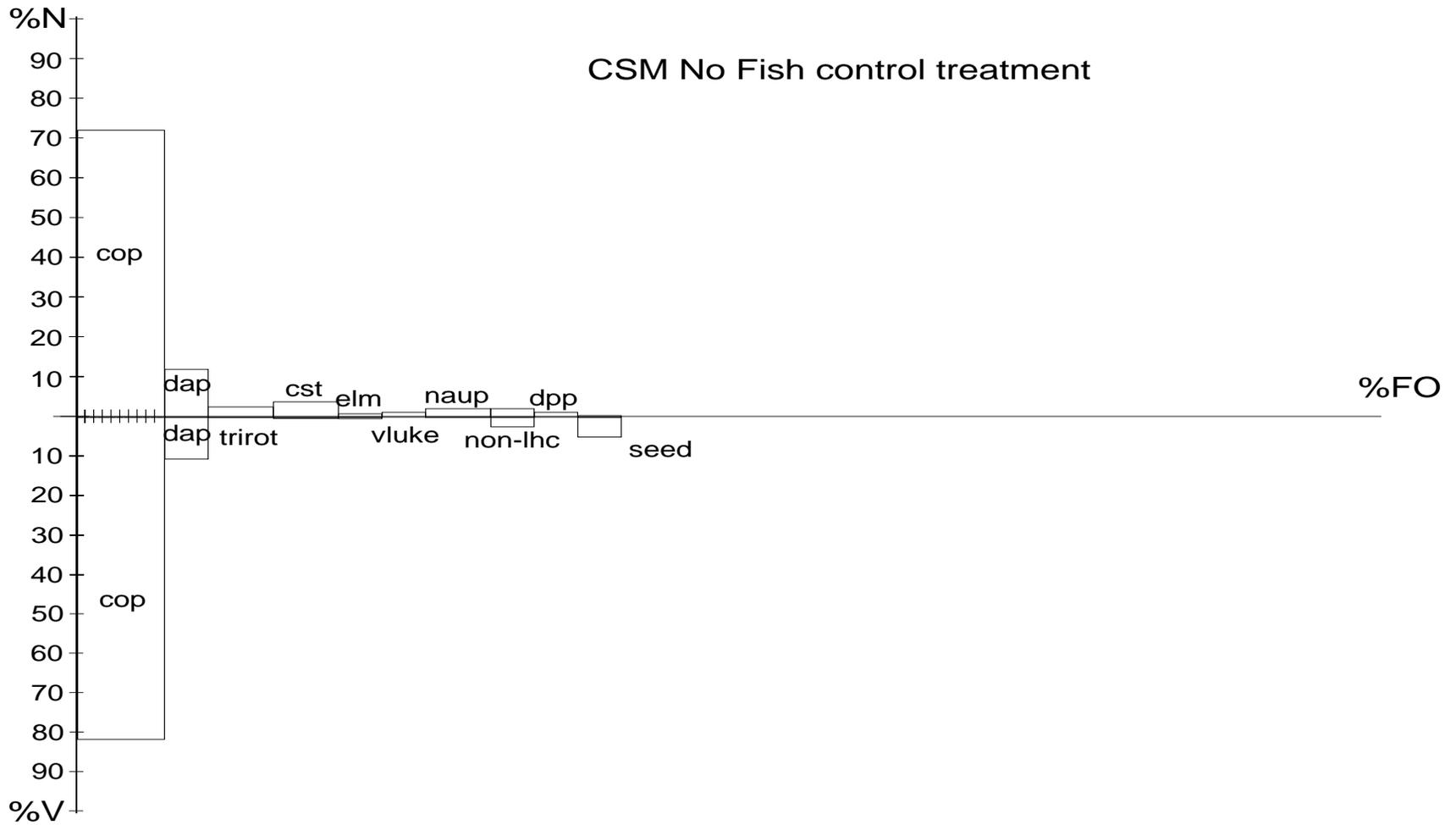


Figure 6-8. NF (no fish 24 hr incubation) cottonseed meal (CSM) treatment large zooplankton pooled community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for 24-hour feeding trial, taxa %N, %V, or %FO \geq 0.5%; 1-L water sample per pond, n = 4 ponds.

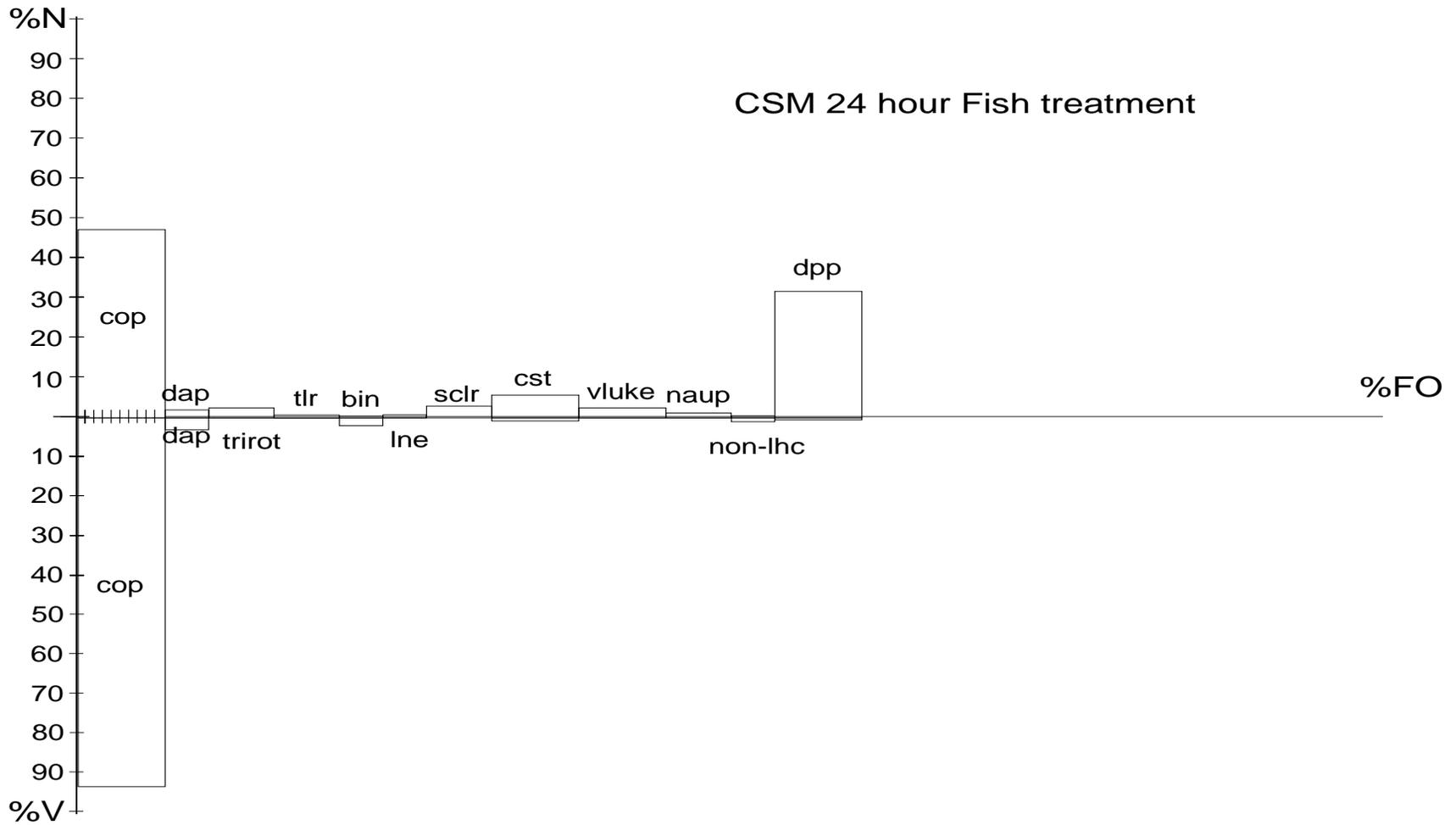


Figure 6-9. Fish (single fish 24 hr incubation) cottonseed meal (CSM) treatment large zooplankton pooled community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for 24-hour feeding trial, taxa %N, %V, or %FO \geq 0.5%; 1-L water sample per pond, n = 4 ponds.

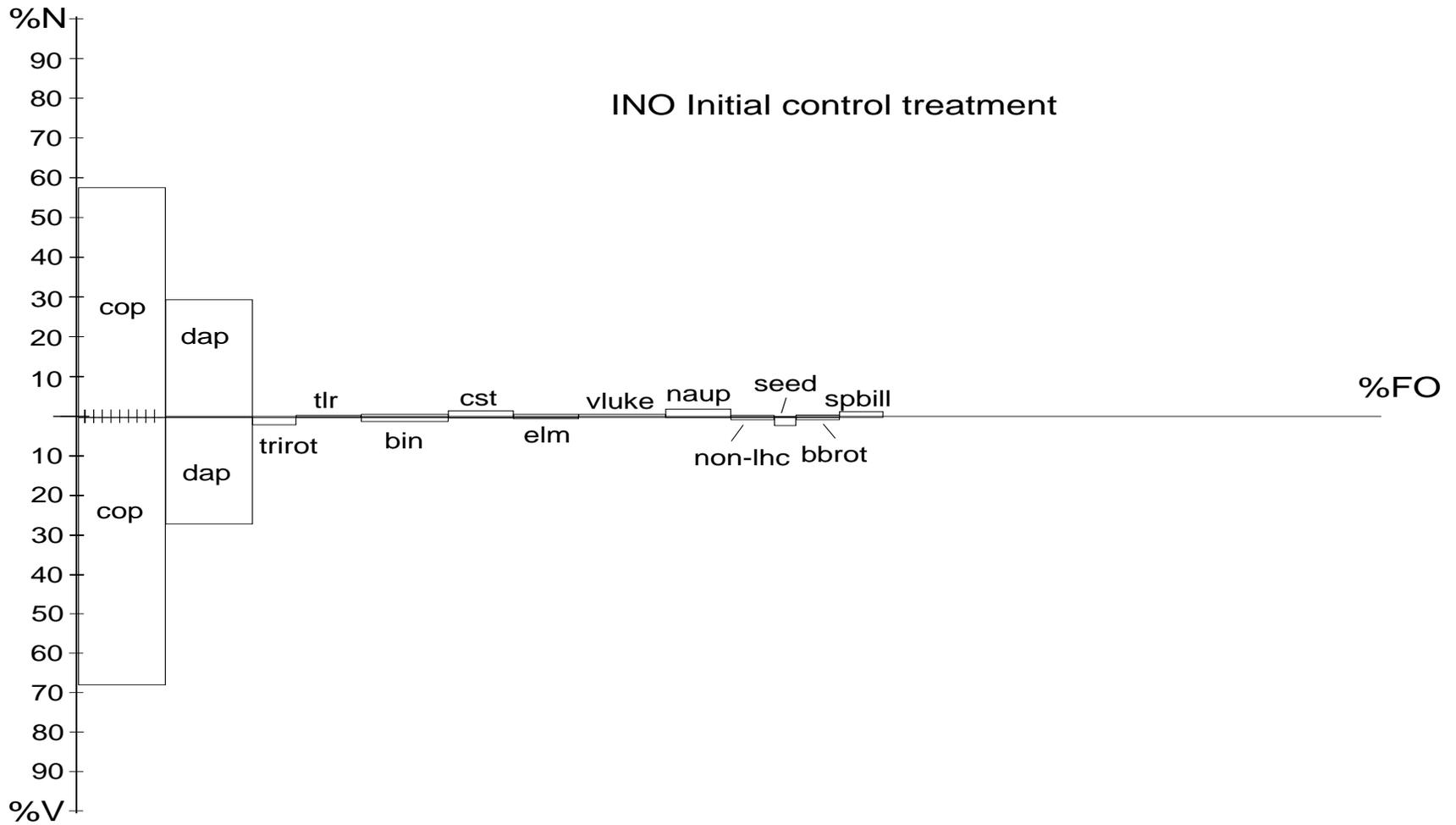


Figure 6-10. Initial (0 hrs) inorganic fertilizer (INO) treatment large zooplankton pooled community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for 24-hour feeding trial, taxa %N, %V, or %FO \geq 0.5%; 1-L water sample per pond, n = 4 ponds.

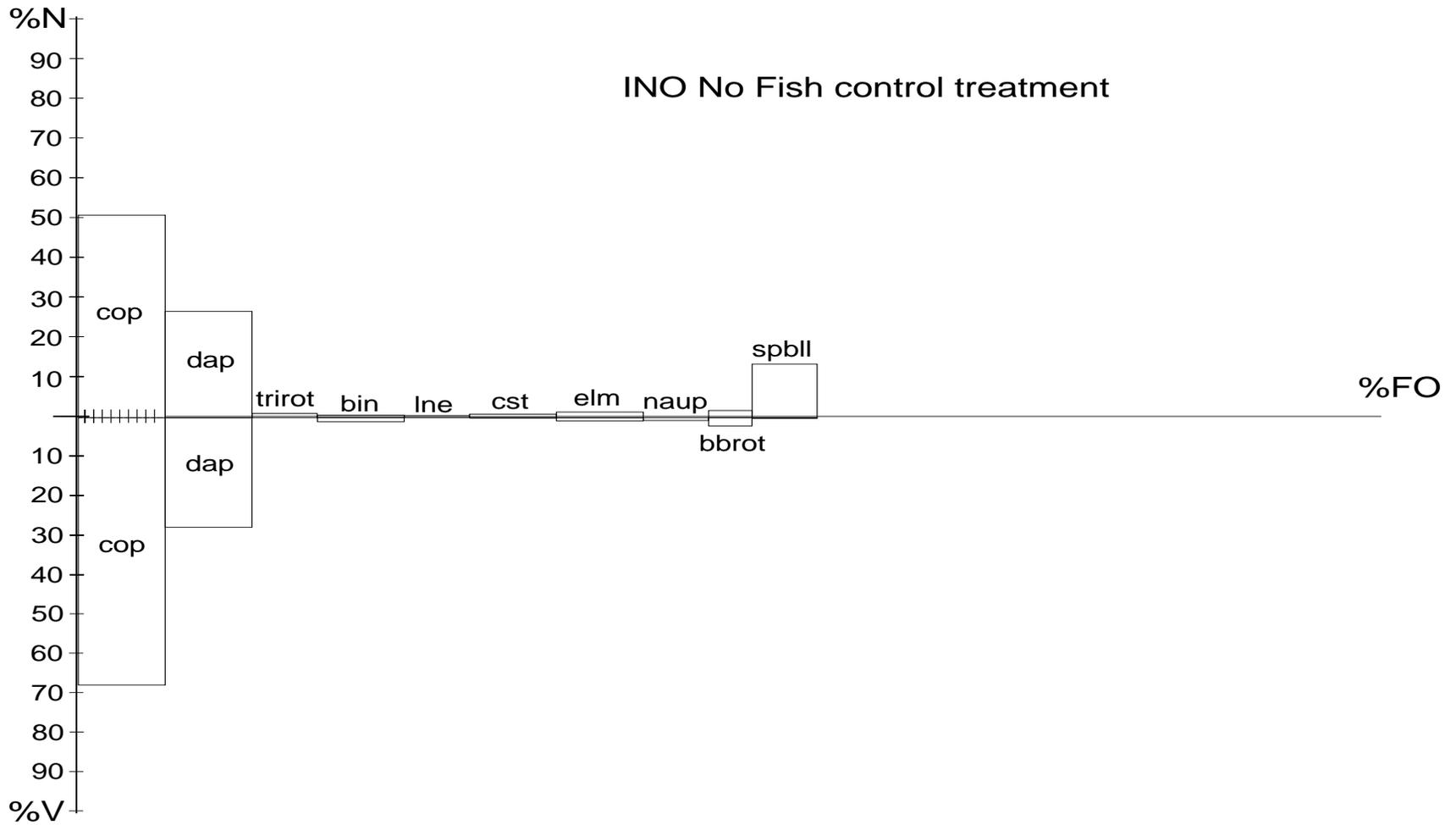


Figure 6-11. NF (no fish 24 hr incubation) inorganic fertilizer (INO) treatment large zooplankton pooled community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for 24-hour feeding trial, taxa %N, %V, or %FO \geq 0.5%; 1-L water sample per pond, n = 4 ponds.

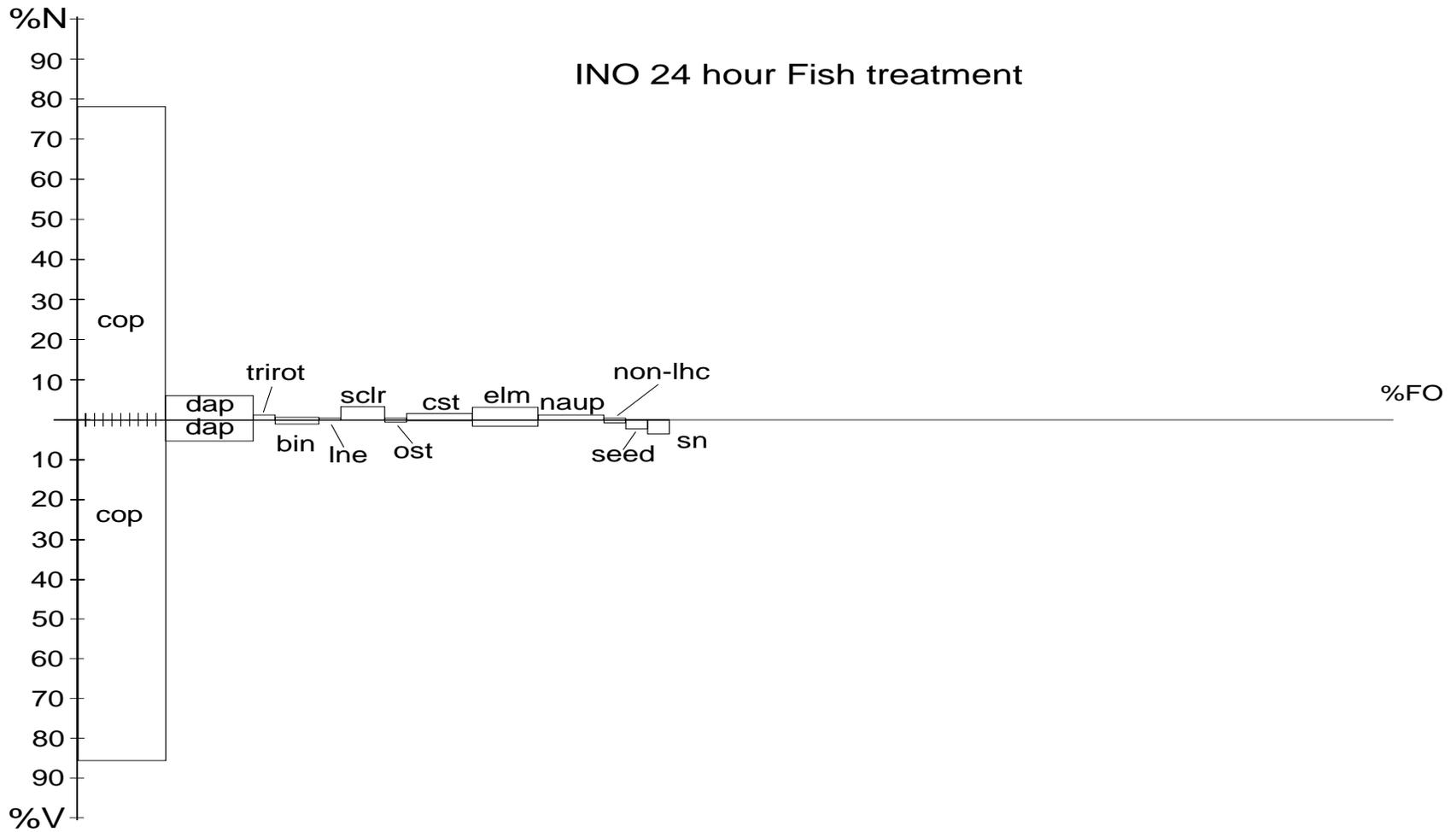


Figure 6-12. Fish (no fish 24 hr incubation) inorganic fertilizer treatment large zooplankton pooled community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for 24-hour feeding trial, taxa %N, %V, or %FO \geq 0.5%; 1-L water sample per pond, n = 4 ponds.

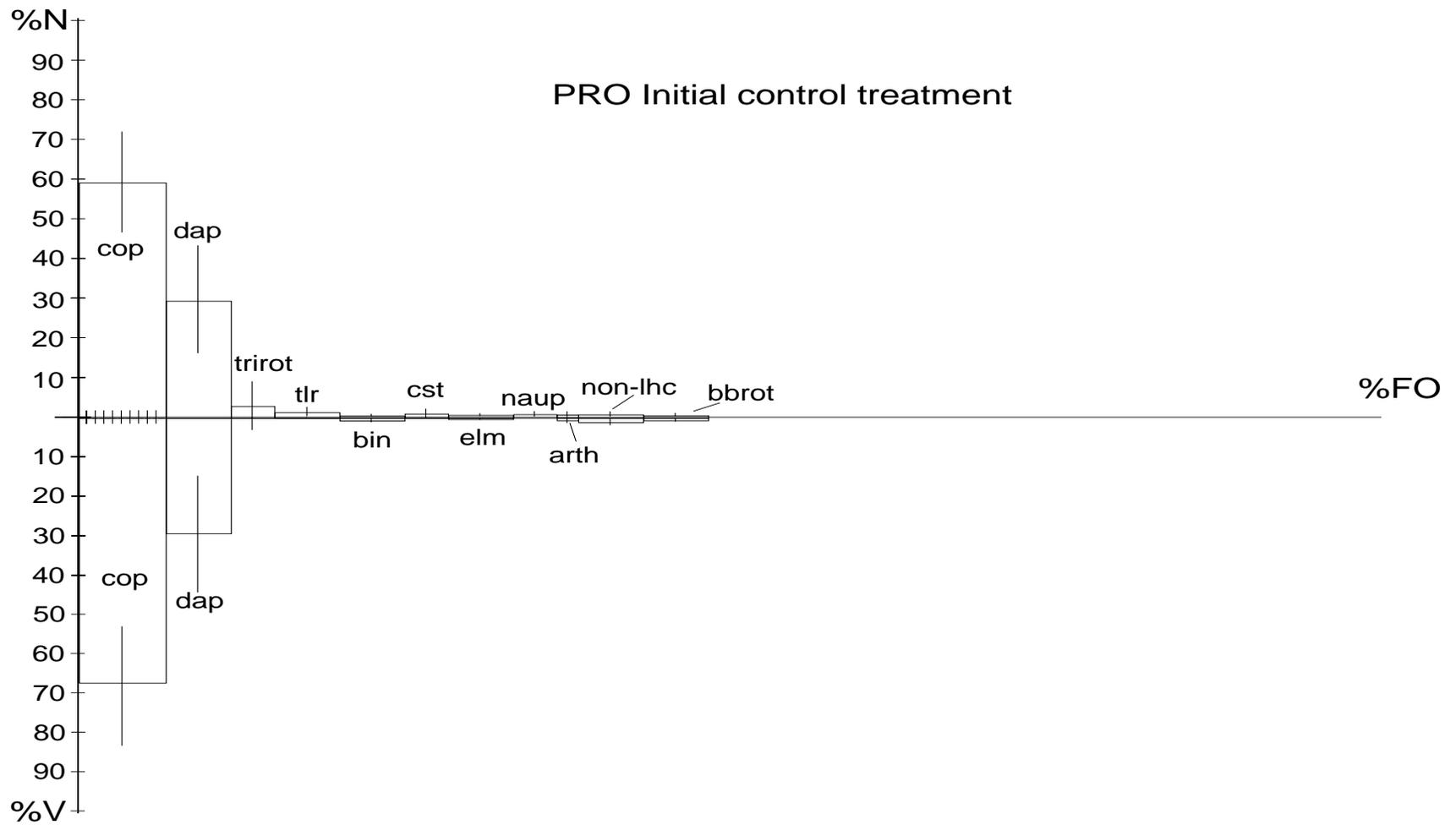


Figure 6-13. Initial (0 hrs) processed feed (PRO) treatment large zooplankton unpooled community taxa parameters: % number (% N \pm 1 SE), % volume (% V \pm 1 SE), and frequency of occurrence (% FO) for 24-hour feeding trial, taxa %N, % V, or %FO \geq 0.5%; 1-L water sample per pond, n = 4 ponds.

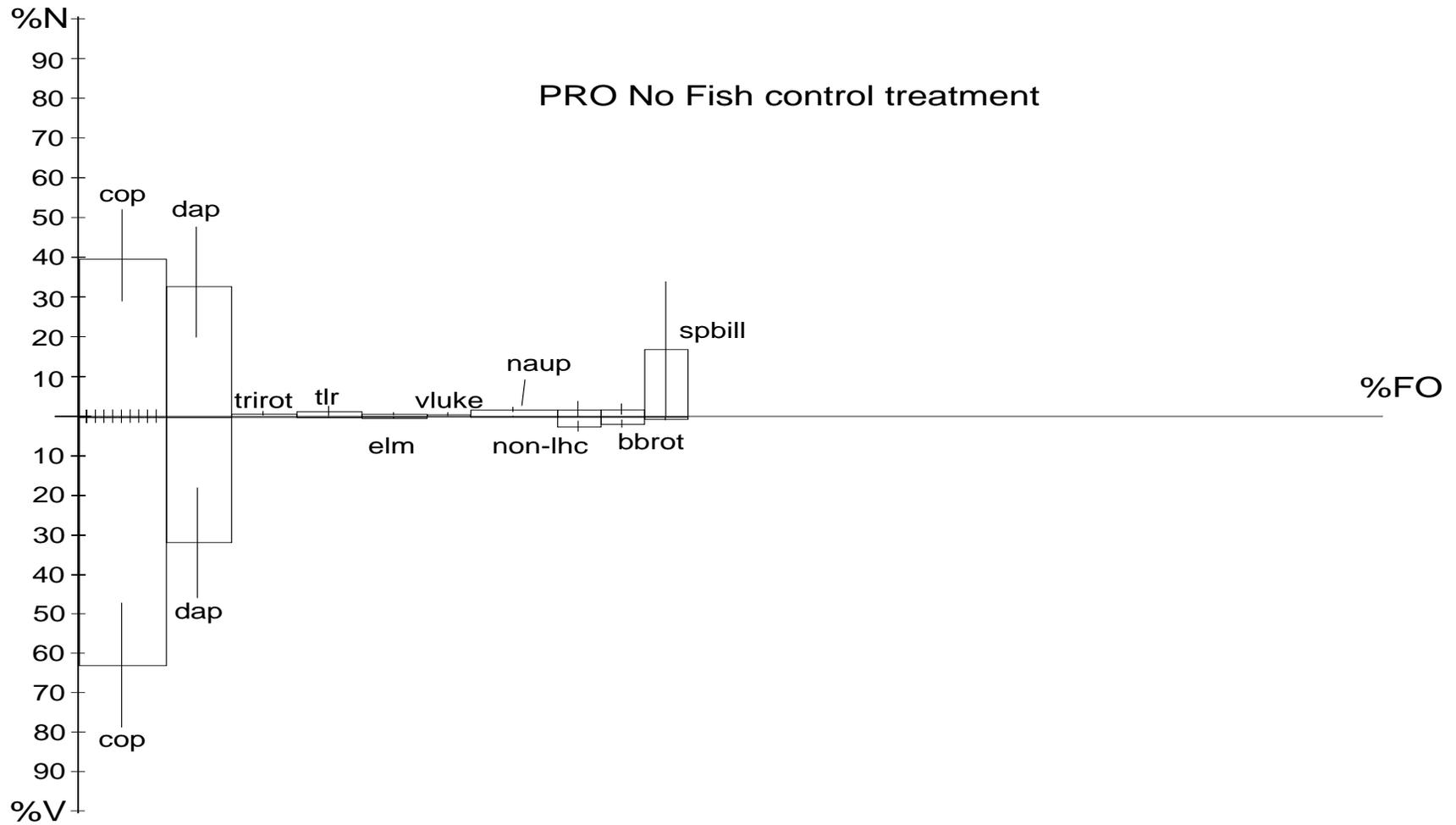


Figure 6-14. NF (no fish 24 hr incubation) processed feed (PRO) treatment large zooplankton unpooled community taxa parameters: % number (%N \pm 1 SE), % volume (%V \pm 1 SE), and % frequency of occurrence (%FO)] for 24-hour feeding trial, taxa %N, % V, or %FO \geq 0.5%; 1-L water sample per pond, n = 4 ponds.

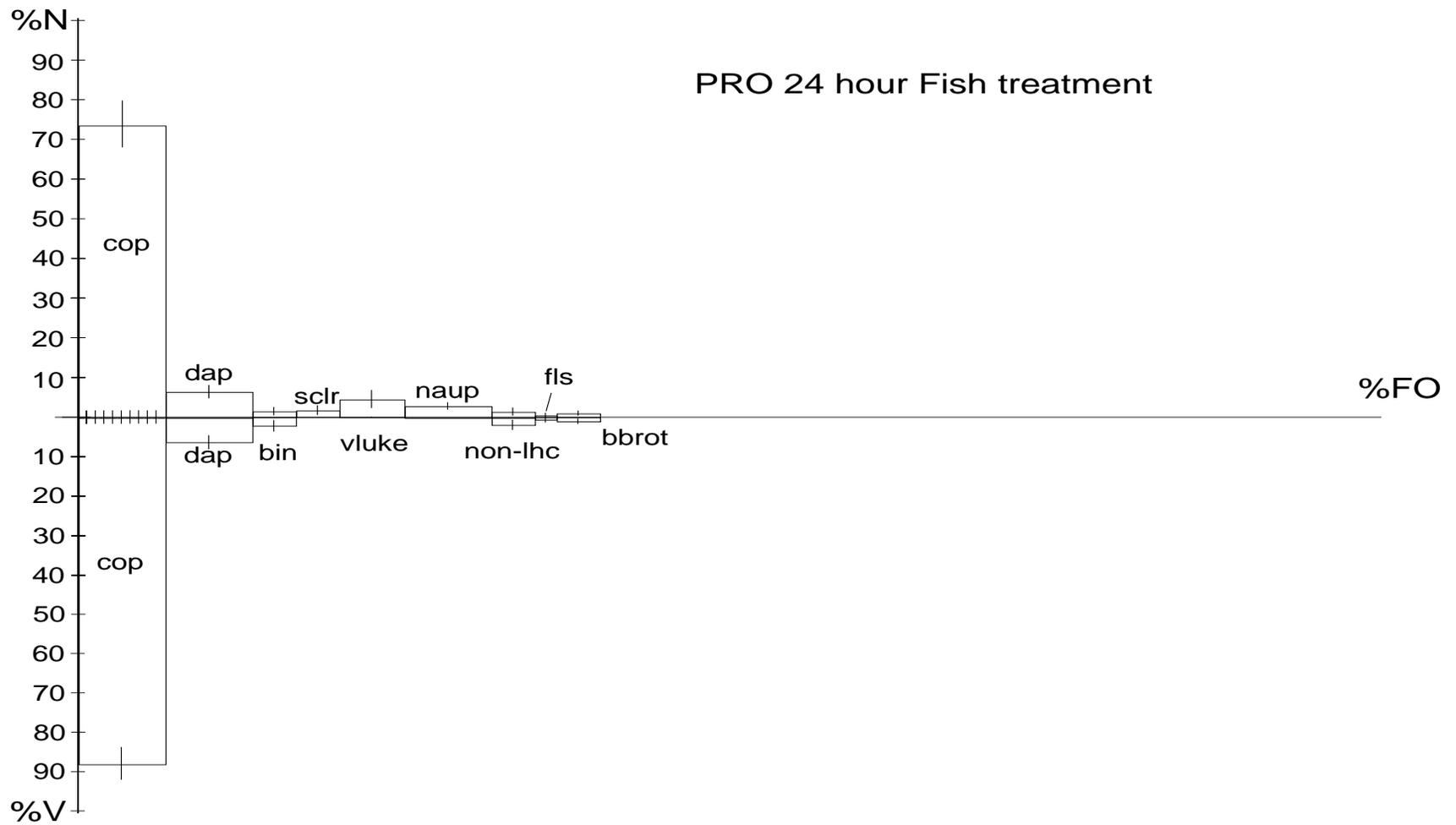


Figure 6-15. Fish (single fish 24 hr incubation) processed feed (PRO) treatment large zooplankton unpooled community taxa parameters: % number (%N \pm 1 SE), % volume (%V \pm 1 SE), and % frequency of occurrence (%FO) for 24-hour feeding trial, taxa %N, % V, or %FO \geq 0.5%; 1-L water sample per pond, n = 4 ponds.

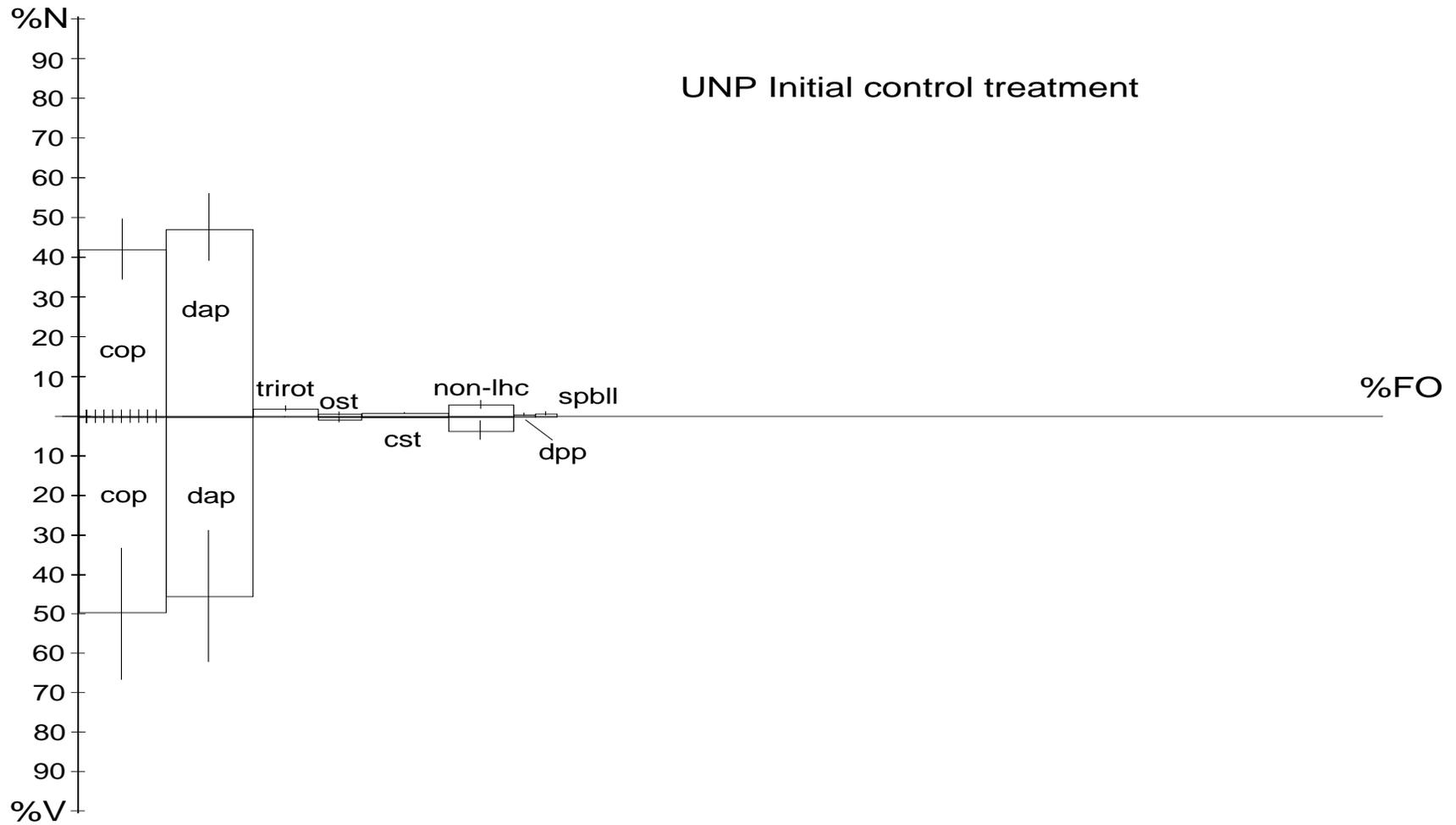


Figure 6-16. Initial (0 hrs) unprocessed feed (UNP) treatment large zooplankton unpooled community taxa parameters: % number (N \pm 1 SE), % volume (%V \pm 1 SE), and frequency of occurrence (%FO) for 24-hour feeding trial, taxa %N, %V, or %FO \geq 0.5%; 1-L water sample per pond, n = 4 ponds.

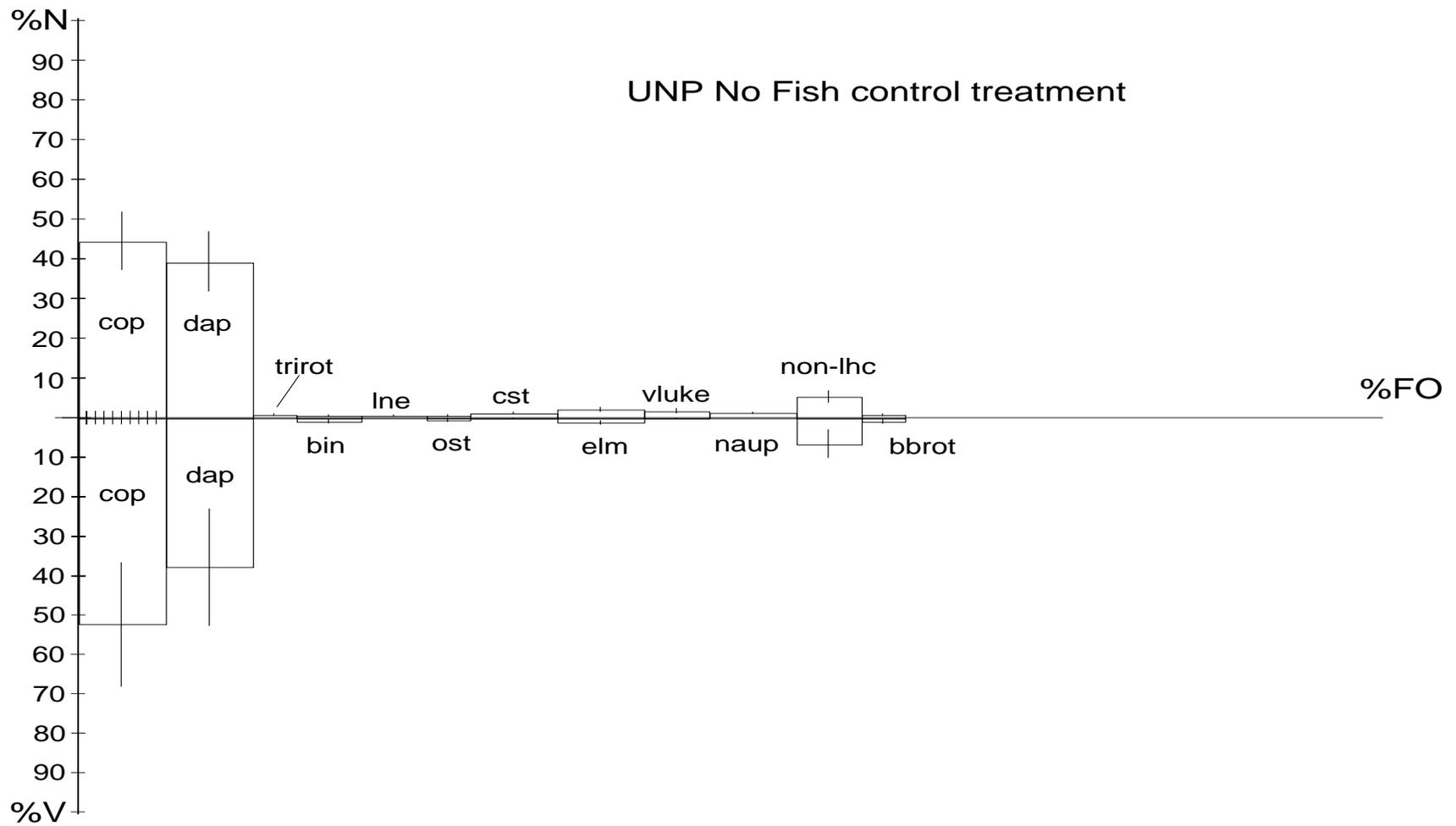


Figure 6-17. NF (no fish 24 hr incubation) unprocessed feed (UNP) treatment large zooplankton unpooled community taxa parameters: % number (%N \pm 1 SE), % volume (%V \pm 1 SE), and % frequency of occurrence (%FO) for 24-hour feeding trial, taxa %N, % V, or %FO \geq 0.5%; 1-L water sample per pond, n = 4 ponds.

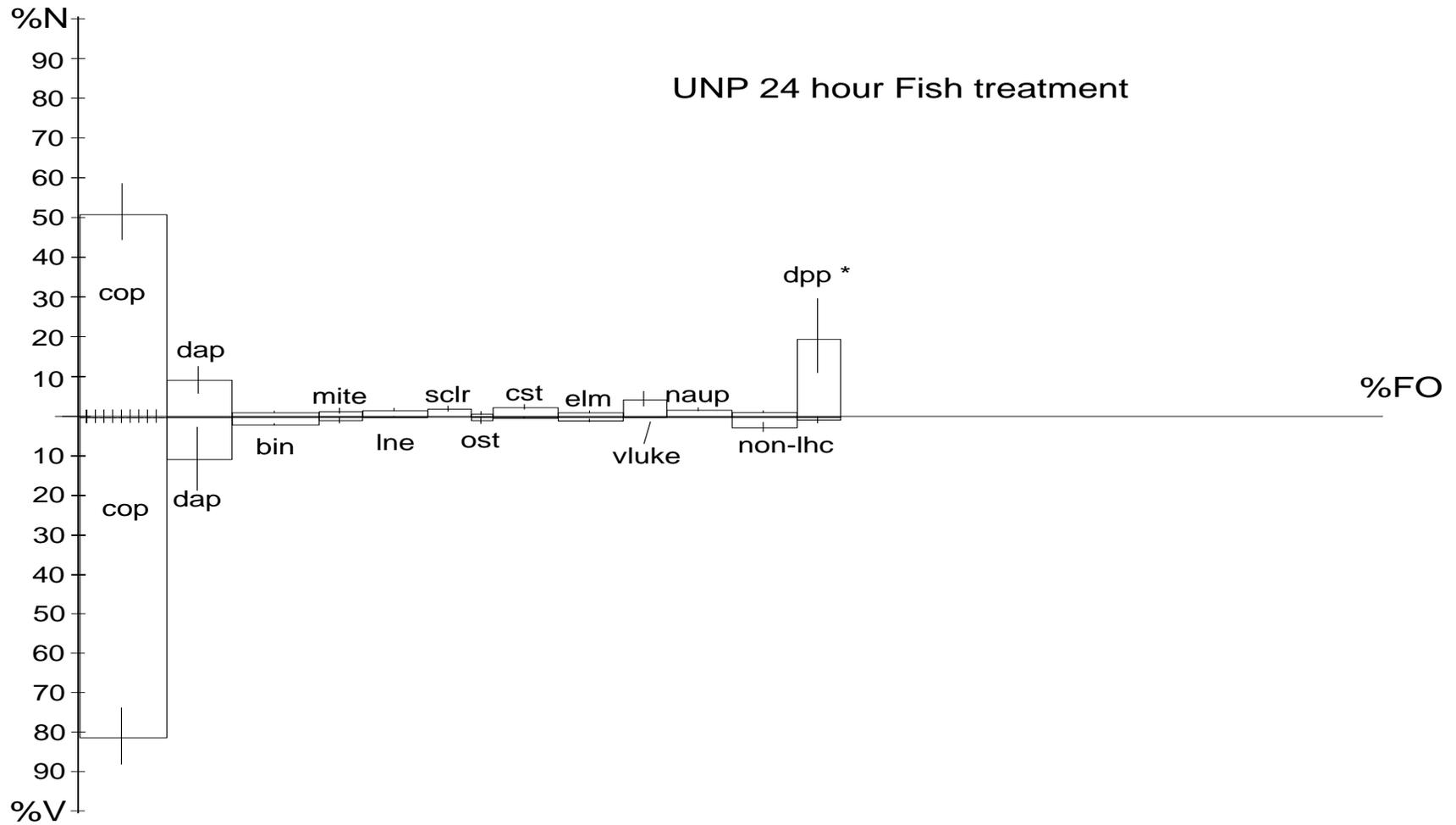


Figure 6-18. Fish (single fish 24 hr incubation) unprocessed feed (UNP) treatment large zooplankton unpooled community taxa parameters: % number (%N \pm 1 SE), % volume (%V \pm 1 SE), and % frequency of occurrence (%FO) for 24-hour feeding trial, taxa %N, %V, or %FO \geq 0.5%; 1-L water sample per pond, n = 4 ponds.

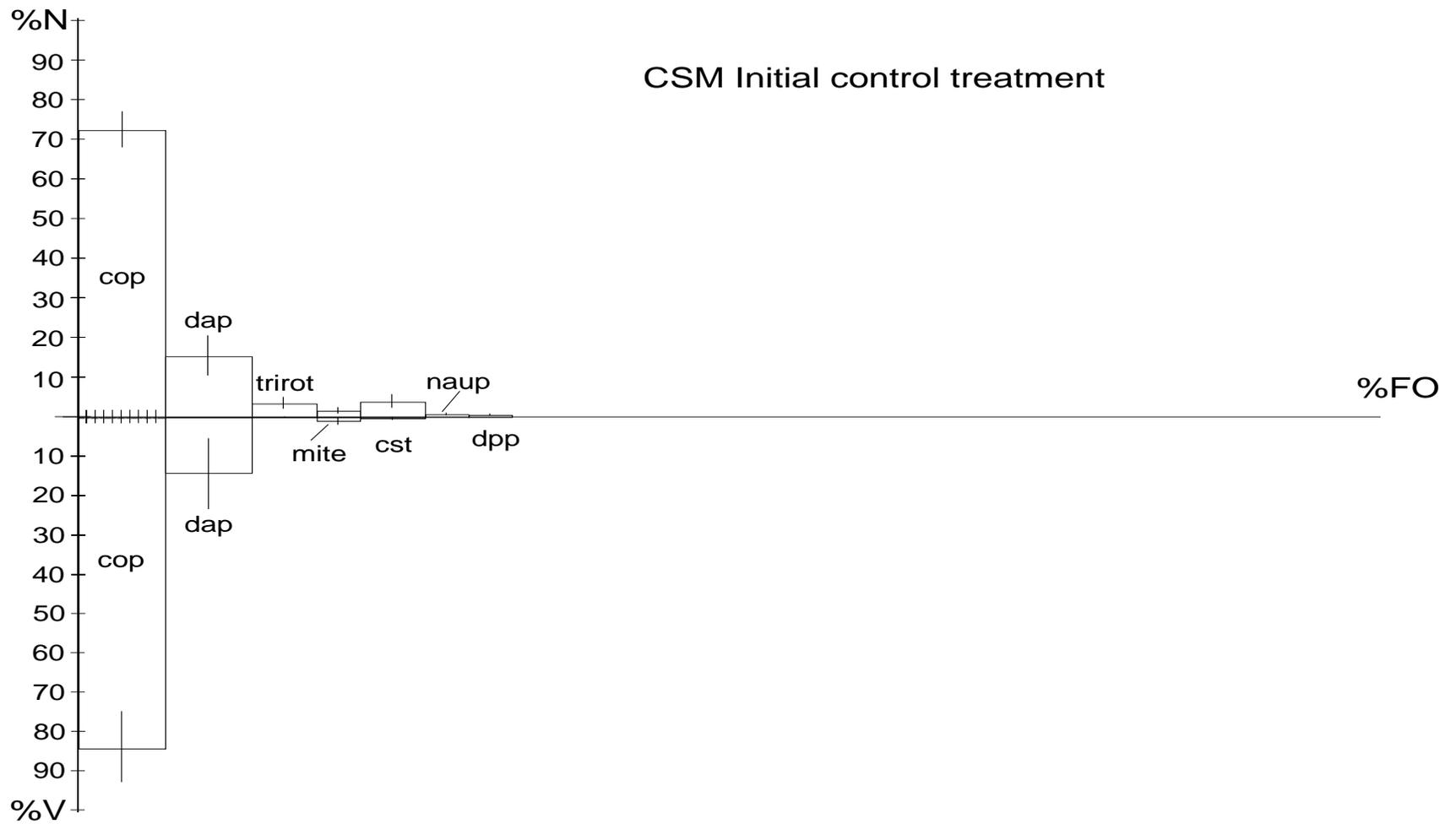


Figure 6-19. Initial (0 hrs) cottonseed meal fertilizer (CSM) treatment large zooplankton unpooled community taxa parameters: % number ($N \pm 1$ SE), % volume ($\%V \pm 1$ SE), and frequency of occurrence ($\%FO$) for 24-hour feeding trial, taxa $\%N$, $\%V$, or $\%FO \geq 0.5\%$; 1-L water sample per pond, $n = 4$ ponds.

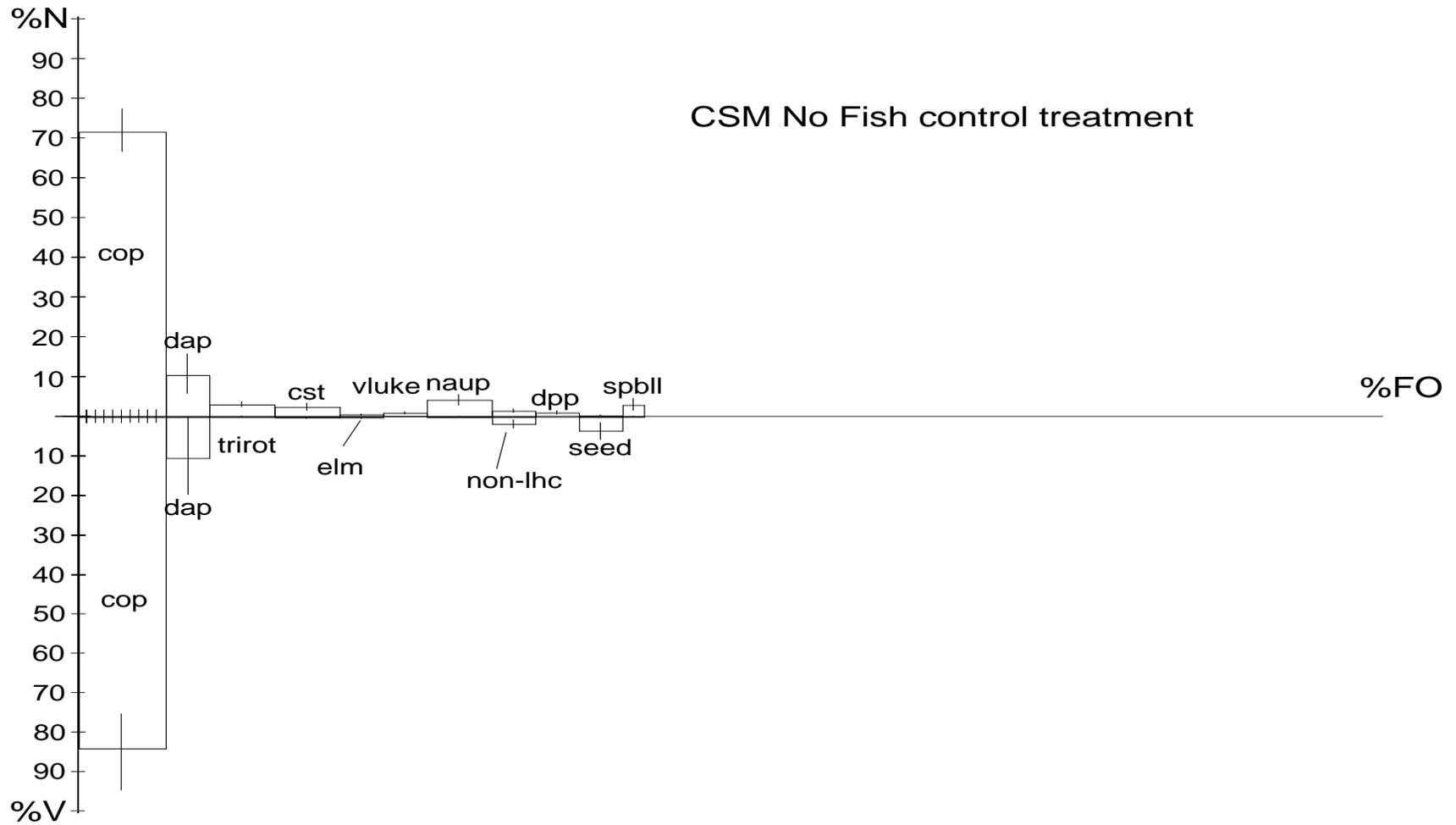


Figure 6-20. NF (no fish 24 hr incubation) cottonseed meal (CSM) treatment large zooplankton unpooled community taxonomic parameters: % number (%N \pm 1 SE), % volume (%V \pm 1 SE), and % frequency of occurrence (%FO) for 24-hour feeding trial, taxa %N, %V, or %FO \geq 0.5%; 1-L water sample per pond, n = 4 ponds.

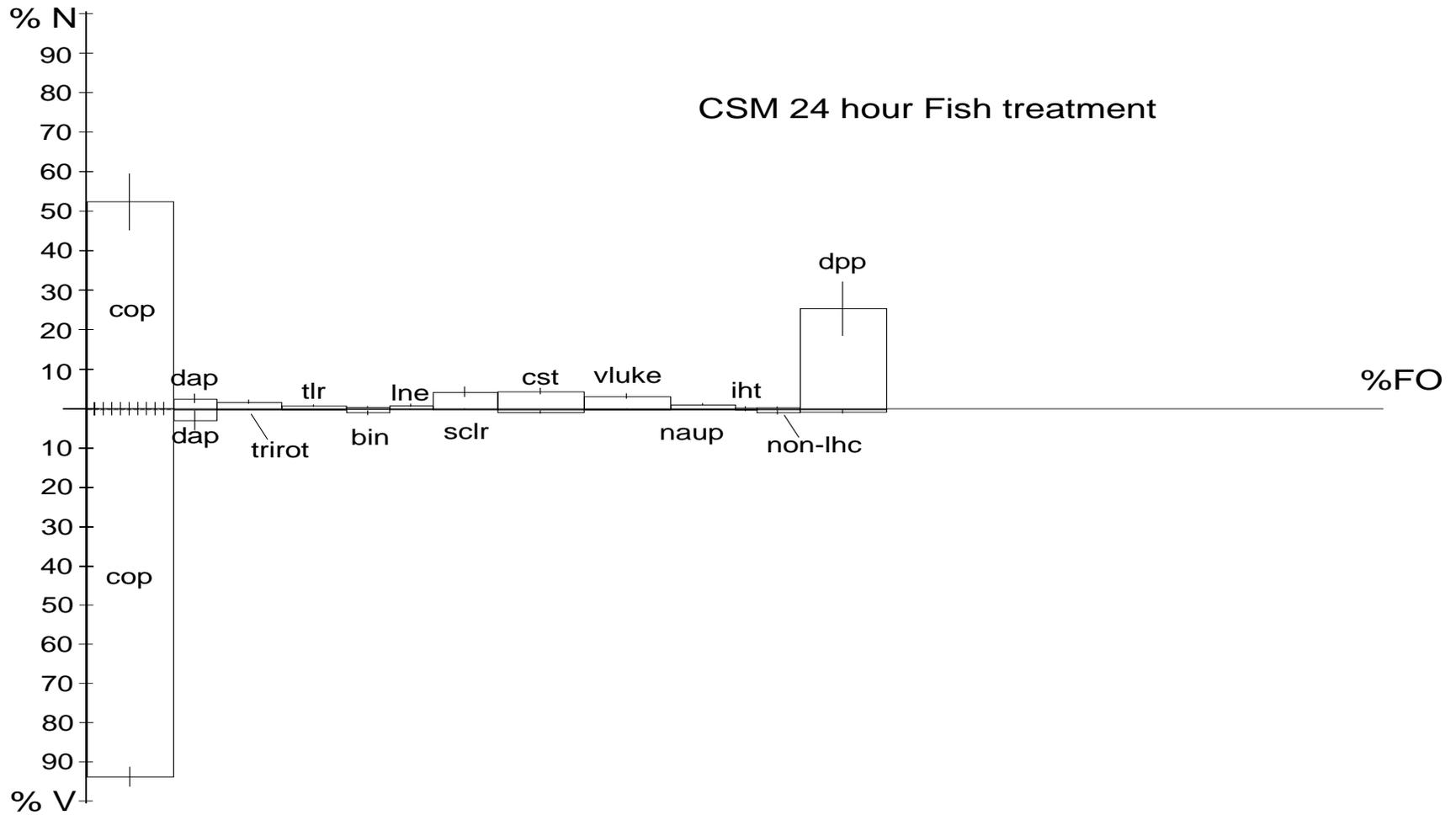


Figure 6-21. Fish (single fish 24 hr incubation) cottonseed meal (CSM) treatment large zooplankton unpooled community taxa parameters: % number (%N \pm 1 SE), % volume (%V \pm 1 SE), and % frequency of occurrence (%FO) for 24-hour feeding trial, taxa %N, %V, or %FO \geq 0.5%; 1-L water sample per pond, n = 4 ponds.

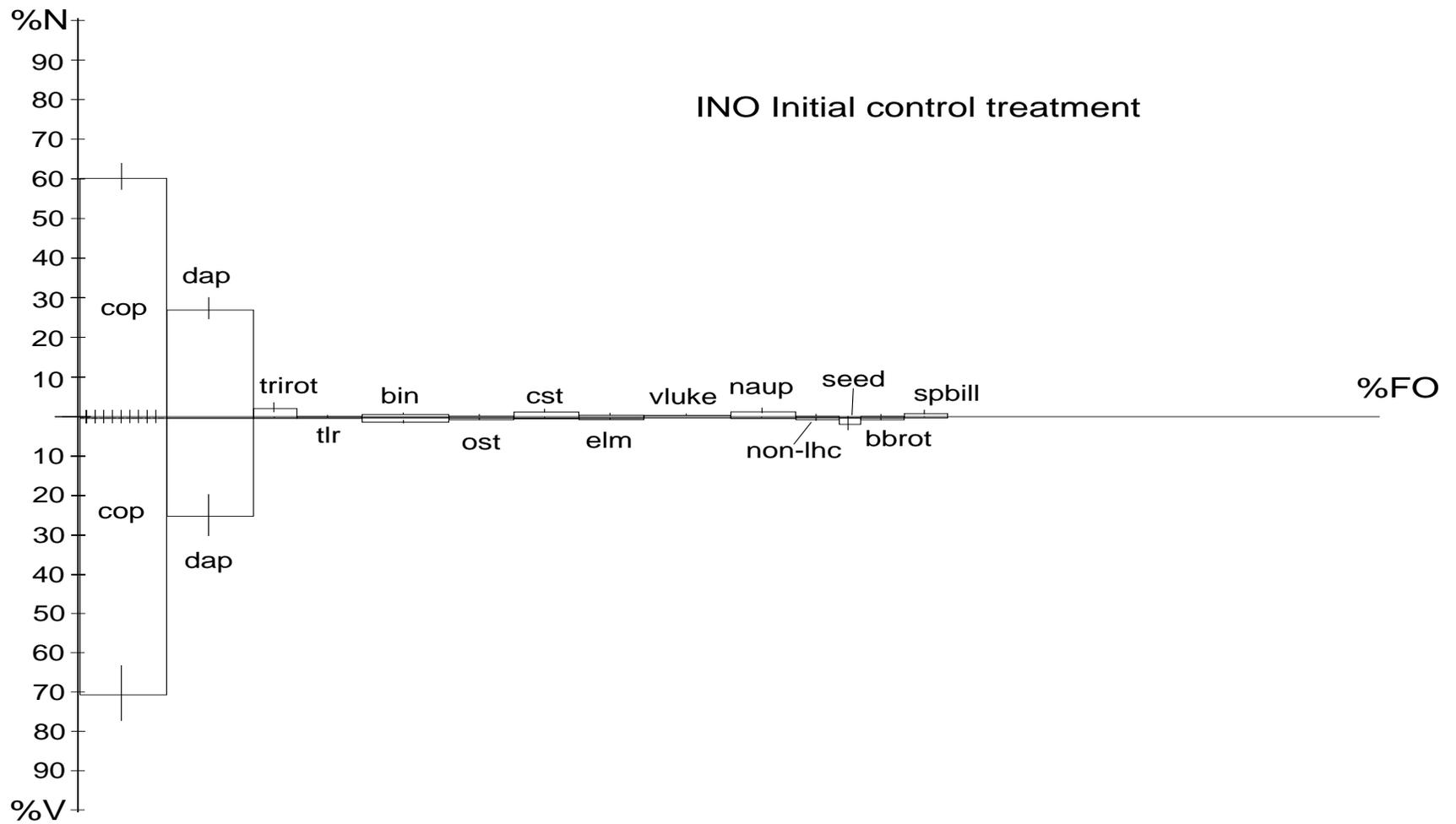


Figure 6-22. Initial (0 hrs) inorganic fertilizer (INO) treatment large zooplankton unpooled community taxa parameters: % number (N \pm 1 SE), % volume (%V \pm 1 SE), and frequency of occurrence (%FO) for 24-hour feeding trial, taxa %N, %V, or %FO \geq 0.5%; 1-L water sample per pond, n = 4 ponds.

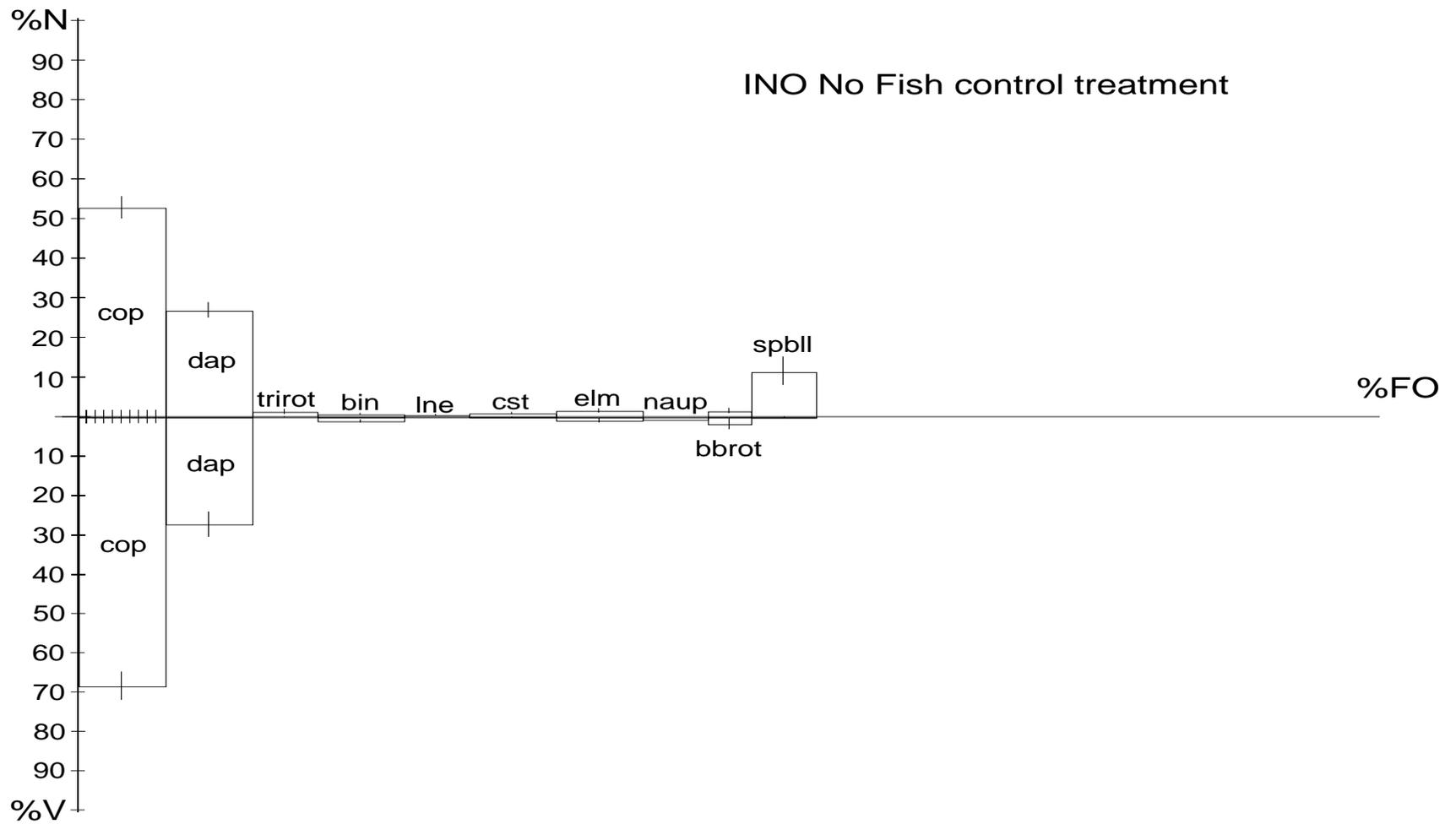


Figure 6-23. NF (no fish 24 hr incubation) inorganic fertilizer (INO) treatment large zooplankton unpooled community taxa parameters: % number (%N \pm 1 SE), % volume (%V \pm 1 SE), and frequency of occurrence (%FO) for 24-hour feeding trial, taxa %N, %V, or %FO \geq 0.5%; 1-L water sample per pond, n = 4 ponds.

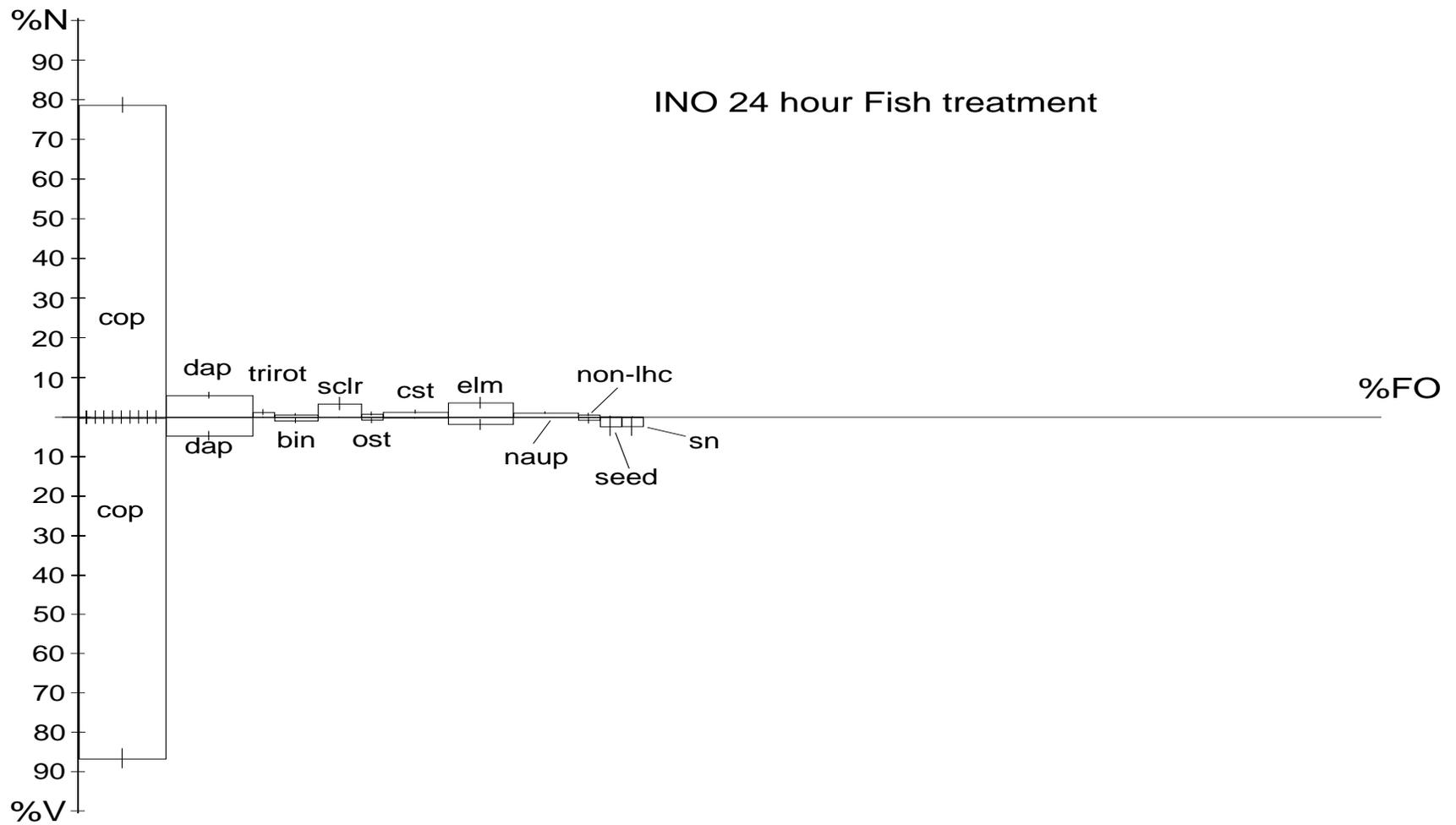


Figure 6-24. Fish (single fish 24 hr incubation) inorganic fertilizer (INO) treatment large zooplankton unpooled community taxa parameters: % number (%N \pm 1 SE), % volume (%V \pm 1 SE), and % frequency of occurrence (%FO) for 24-hour feeding trial, taxa %N, %V, or %FO \geq 0.5%; 1-L water sample per pond, n = 4 ponds.

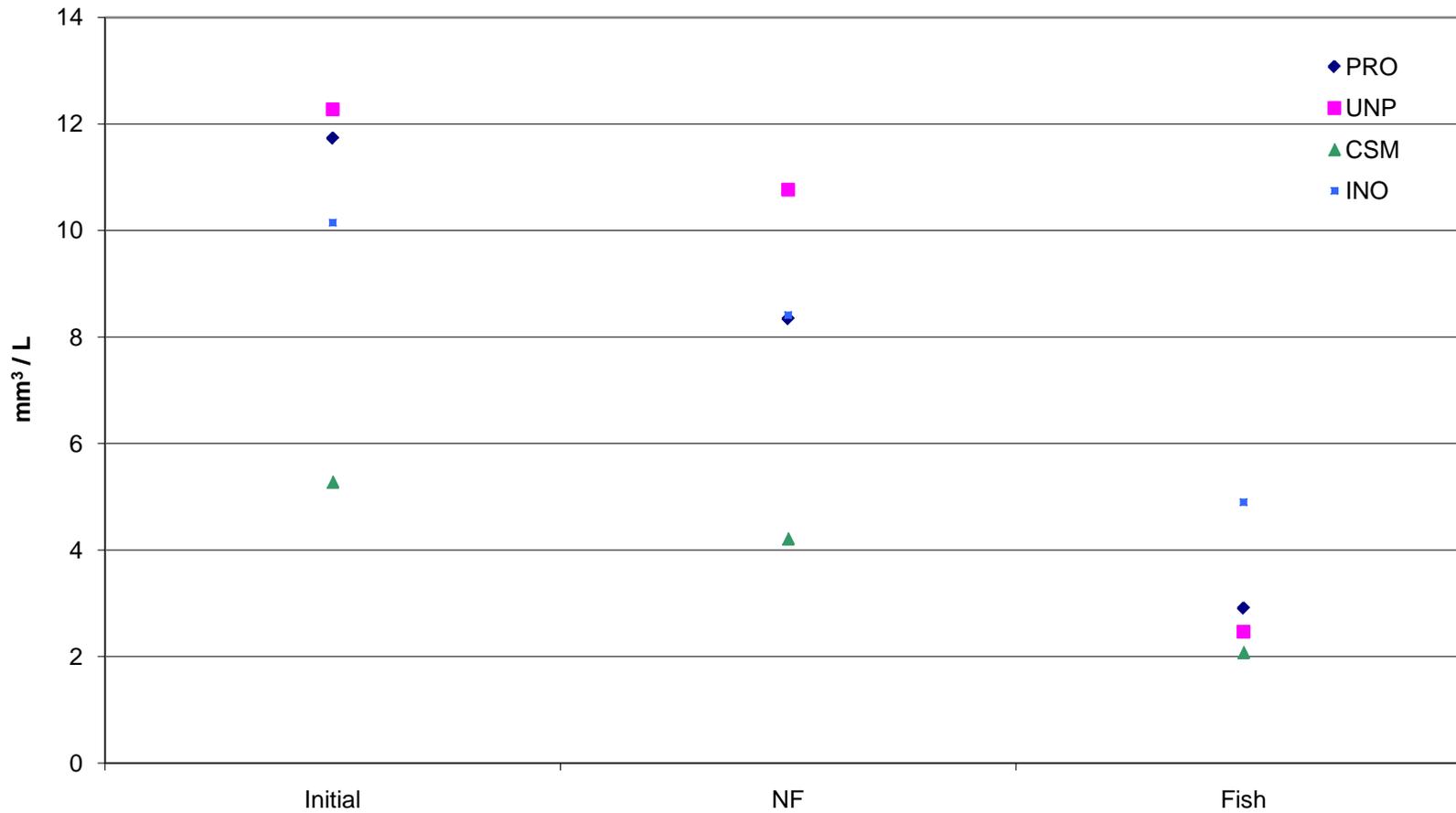


Figure 6-25. 24-hour feeding trial large (> 200 μm) plankton assemblage taxa total volumes (mm^3/L) among four pond nutrient treatments [processed feed (PRO), unprocessed feed (UNP), cottonseed meal fertilizer (CSM), inorganic fertilizer (INO)] and three treatments [Initial, NF (no fish), Fish]; 1-L water sample per pond, n = 4 ponds.

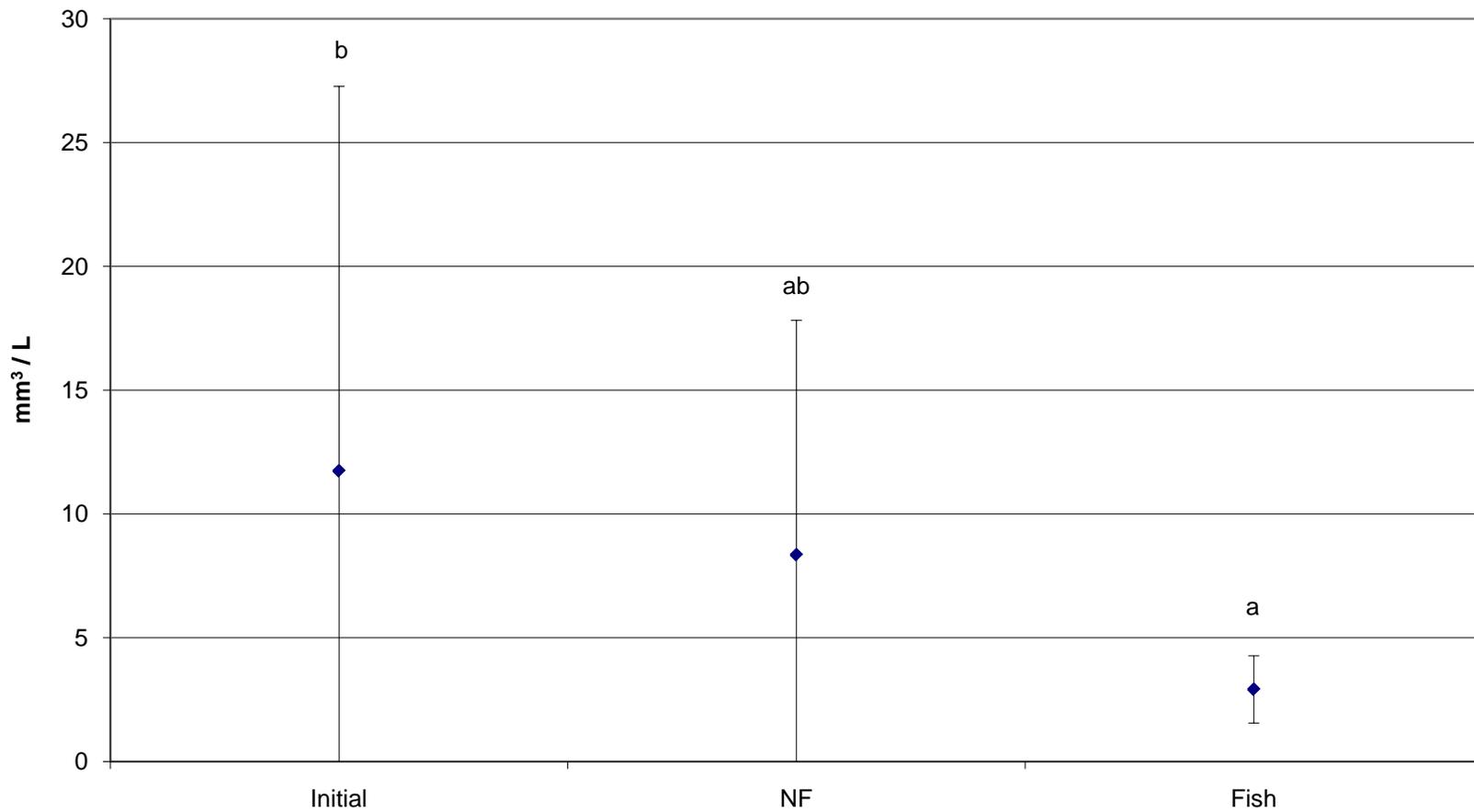


Figure 6-26. Processed feed (PRO) treatment 24-hour feeding trial large (> 200 μm) plankton assemblage volumes ($\text{mm}^3/\text{L} \pm 95\% \text{ CI}$) within three treatments [Initial, NF (no fish), Fish], four replicates per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

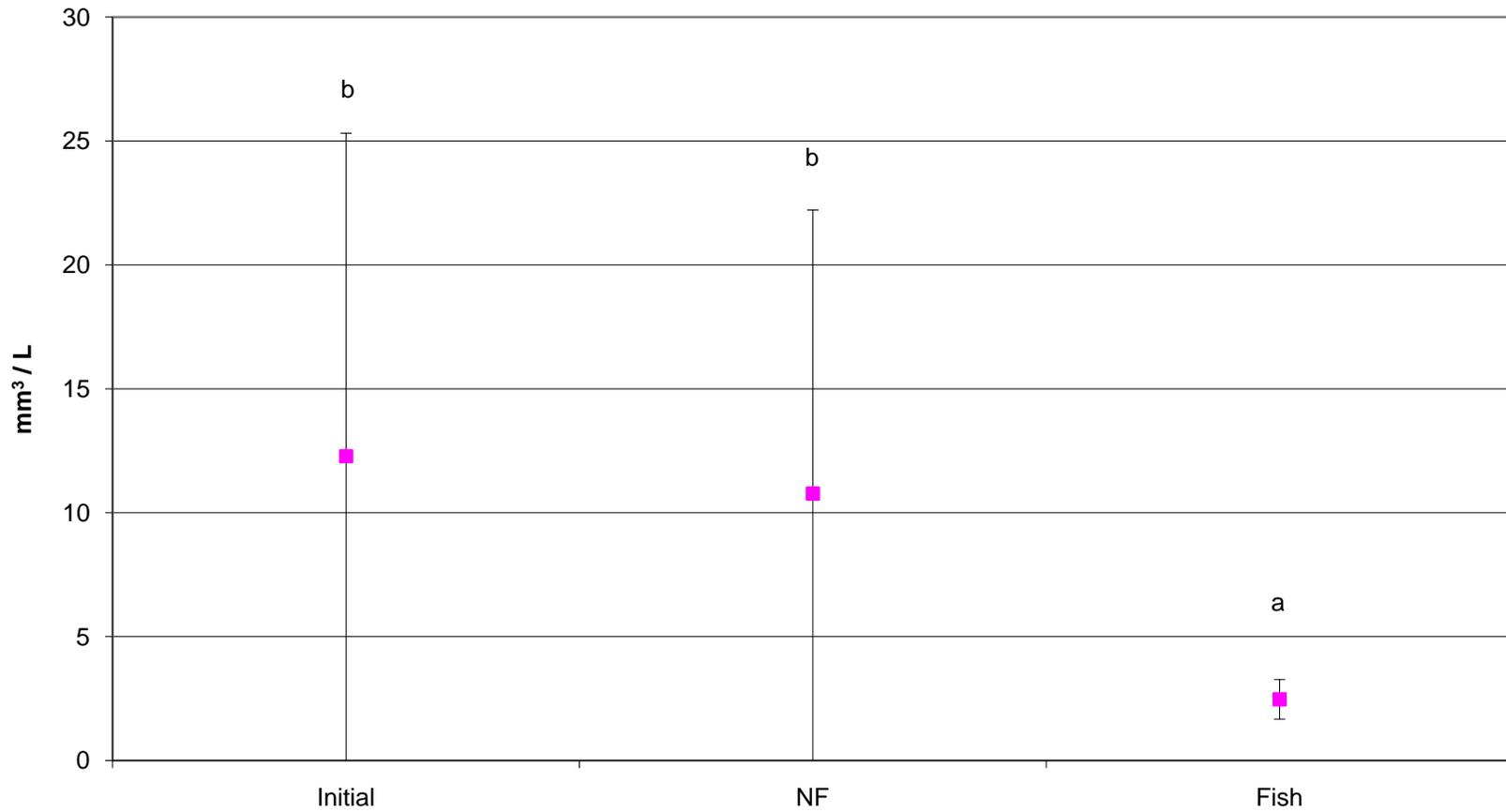


Figure 6-27. Unprocessed feed (UNP) treatment 24-hour feeding trial large (> 200 μm) plankton assemblage volumes ($\text{mm}^3/\text{L} \pm 95\%$ CI) within three treatments [Initial, NF (no fish), Fish], four replicate pond water samples per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

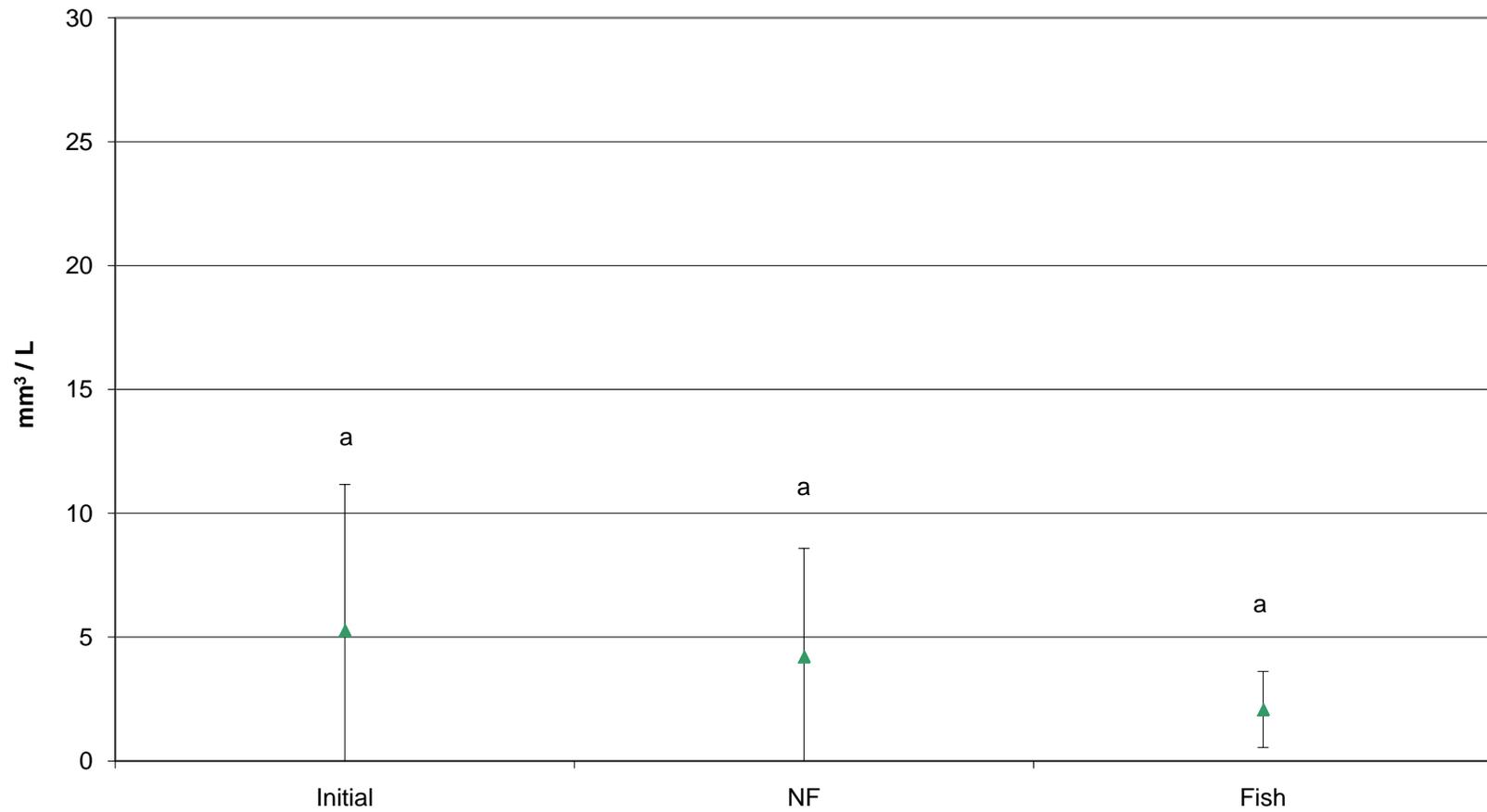


Figure 6-28. Cottonseed meal fertilizer (CSM) treatment 24-hour feeding trial large (> 200 μm) plankton assemblage volumes ($\text{mm}^3/\text{L} \pm 95\% \text{ CI}$) within three treatments [Initial, NF (no fish), Fish], four replicate pond water samples per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

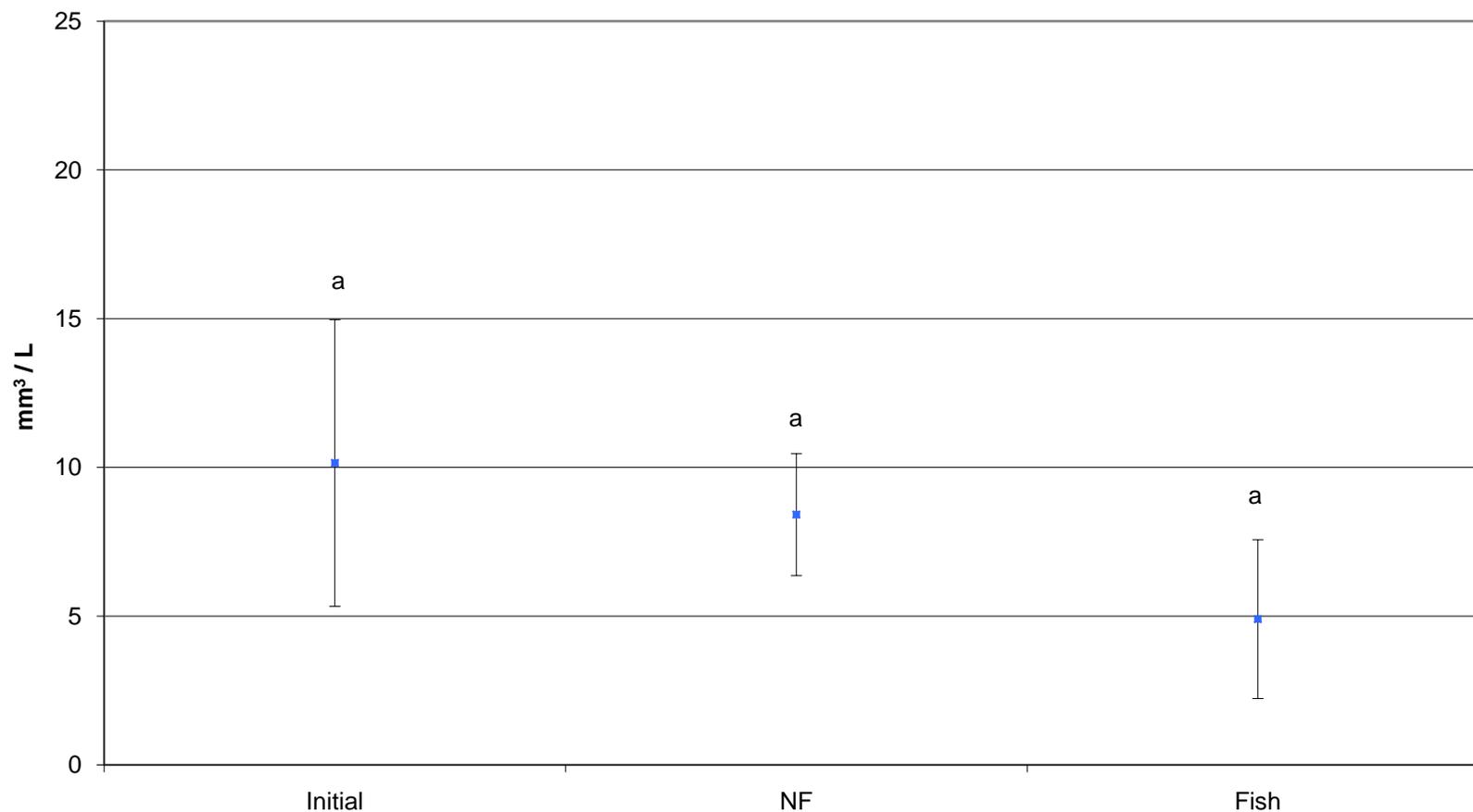


Figure 6-29. Inorganic fertilizer (INO) treatment 24-hour feeding trial large (> 200 μm) plankton assemblage volumes ($\text{mm}^3/\text{L} \pm 95\%$ CI) within three treatments [Initial, NF (no fish), Fish], four replicate pond water samples per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

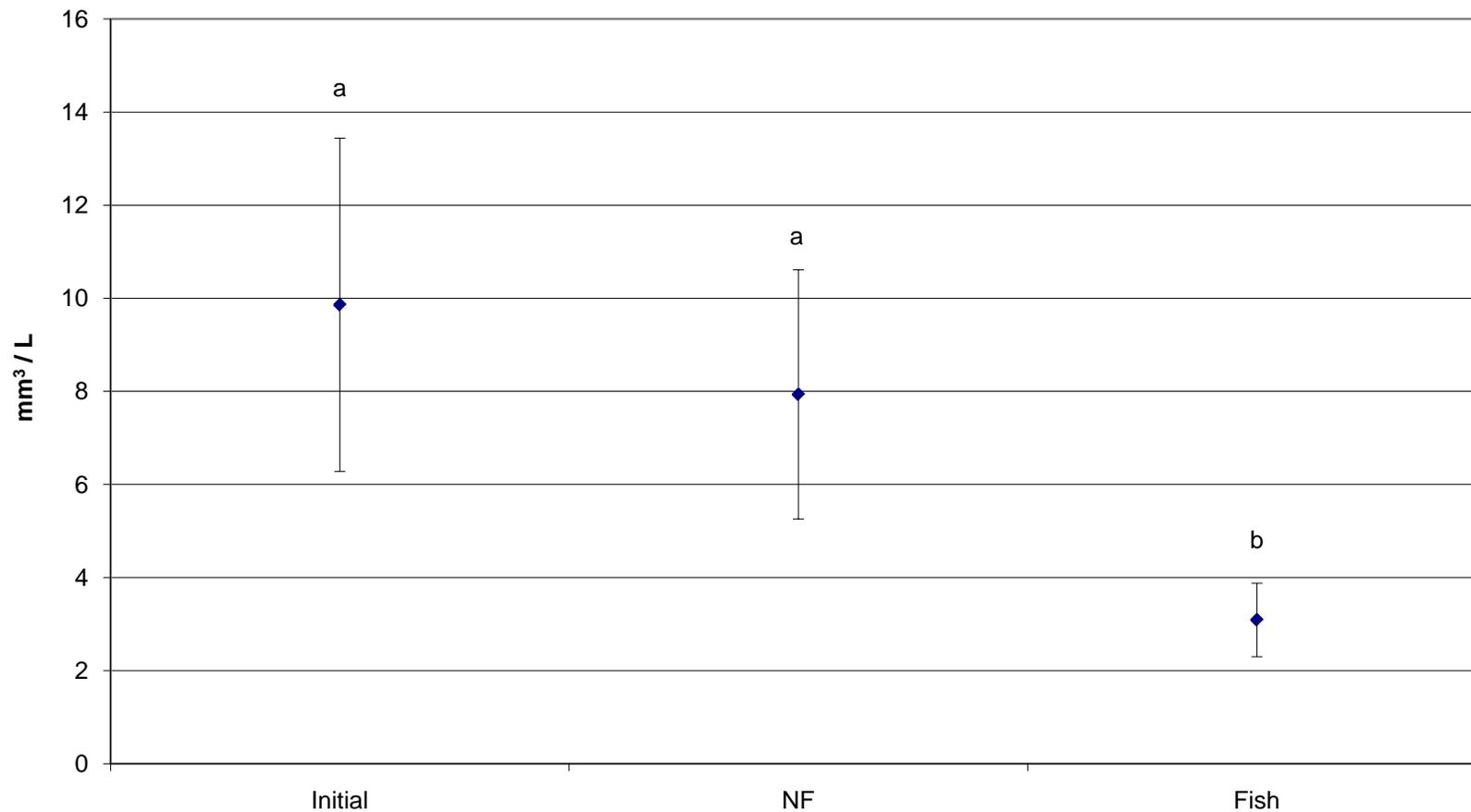


Figure 6-30. Pooled (among nutrient treatment) 24-hour feeding trial large (> 200 μm) plankton assemblage volumes ($\text{mm}^3/\text{L} \pm 95\%$ CI) within three treatments [Initial, NF (no fish), Fish], 16 pooled 1 L replicate pond water samples per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

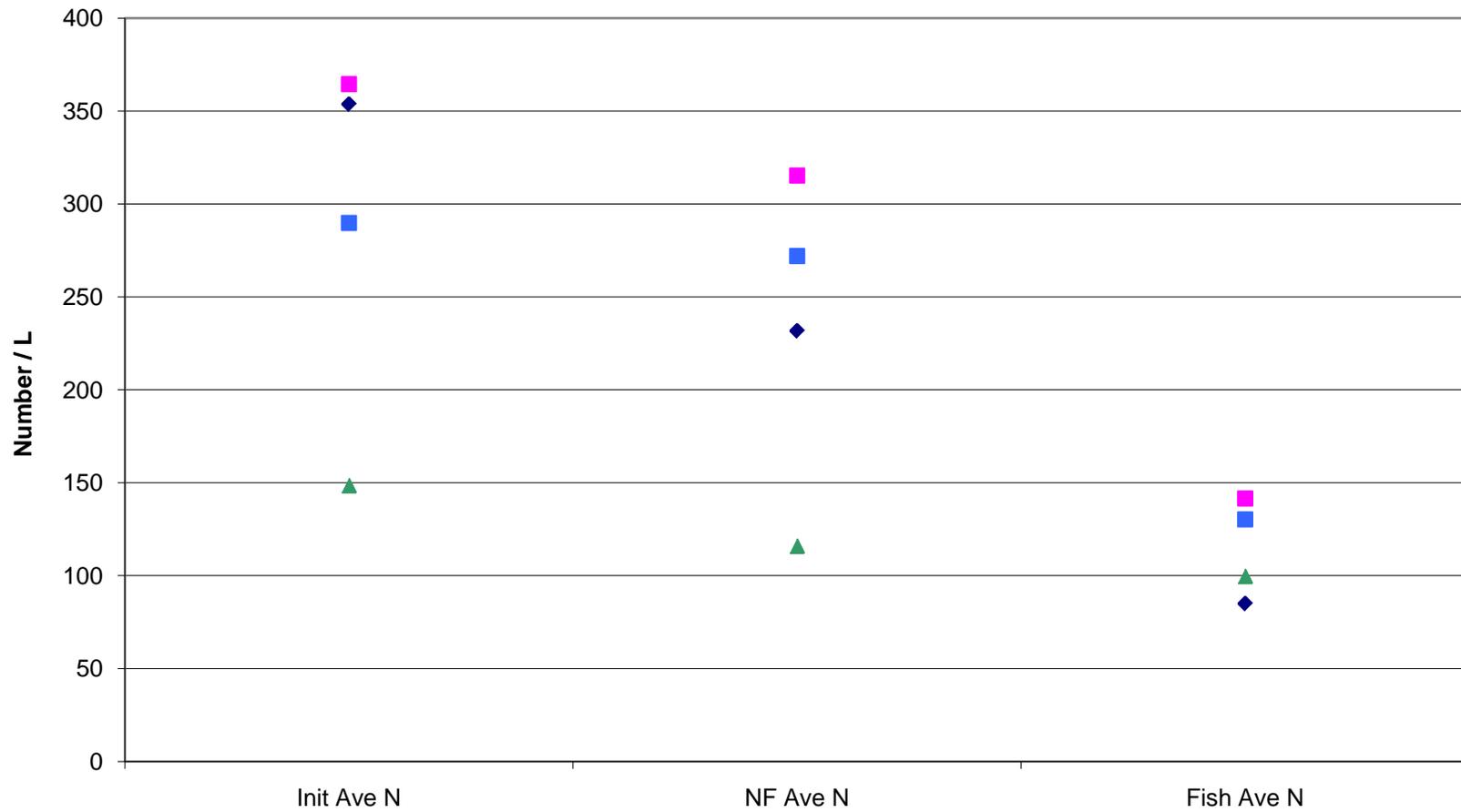


Figure 6-31. 24-hour feeding trial large (> 200 μm) plankton assemblage densities (plankton number/L) among four pond nutrient treatments [processed feed (PRO), unprocessed feed (UNP), cottonseed meal fertilizer (CSM), inorganic fertilizer (INO)] and three treatments [Initial, NF (no fish), Fish].

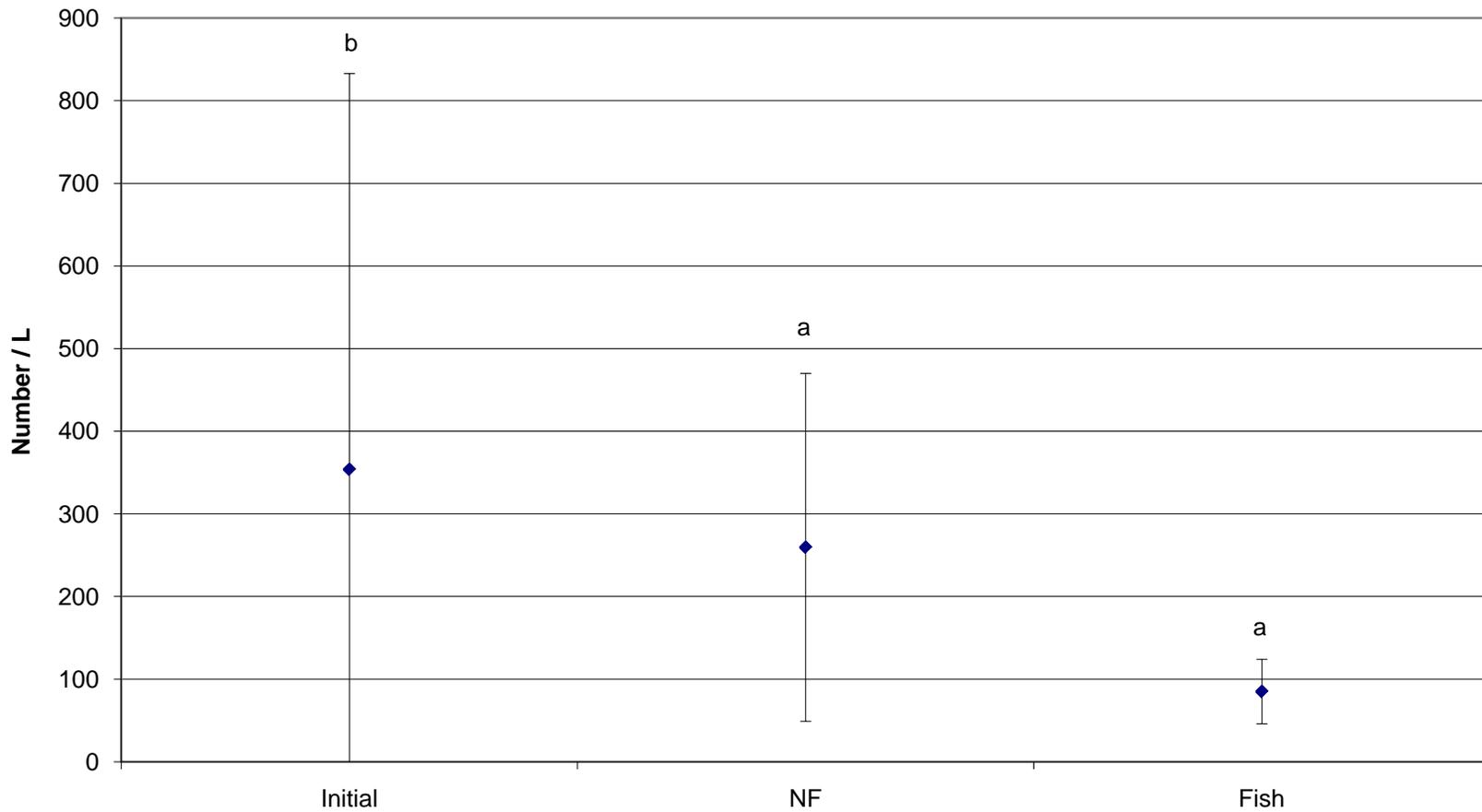


Figure 6-32. Processed feed (PRO) treatment 24-hour feeding trial large (> 200 μm) plankton assemblage densities (plankter number/L \pm 95% CI) within three treatments [Initial, NF (no fish), Fish], four replicate samples per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

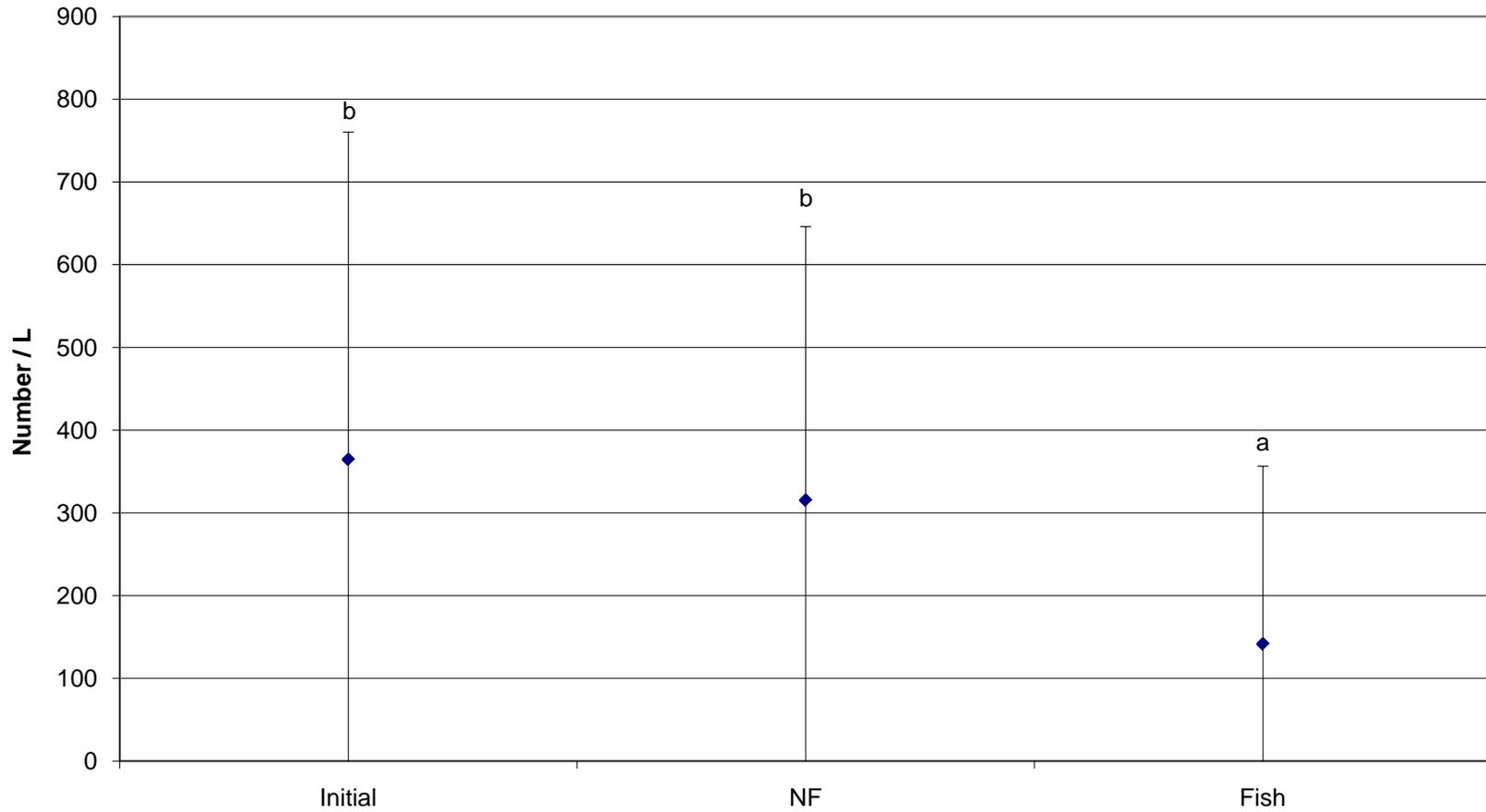


Figure 6-33. Unprocessed feed (UNP) treatment 24-hour feeding trial large (> 200 μm) plankton assemblage densities (plankton number/L \pm 95% CI) within three treatments [Initial, NF (no fish), Fish], four replicate samples per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

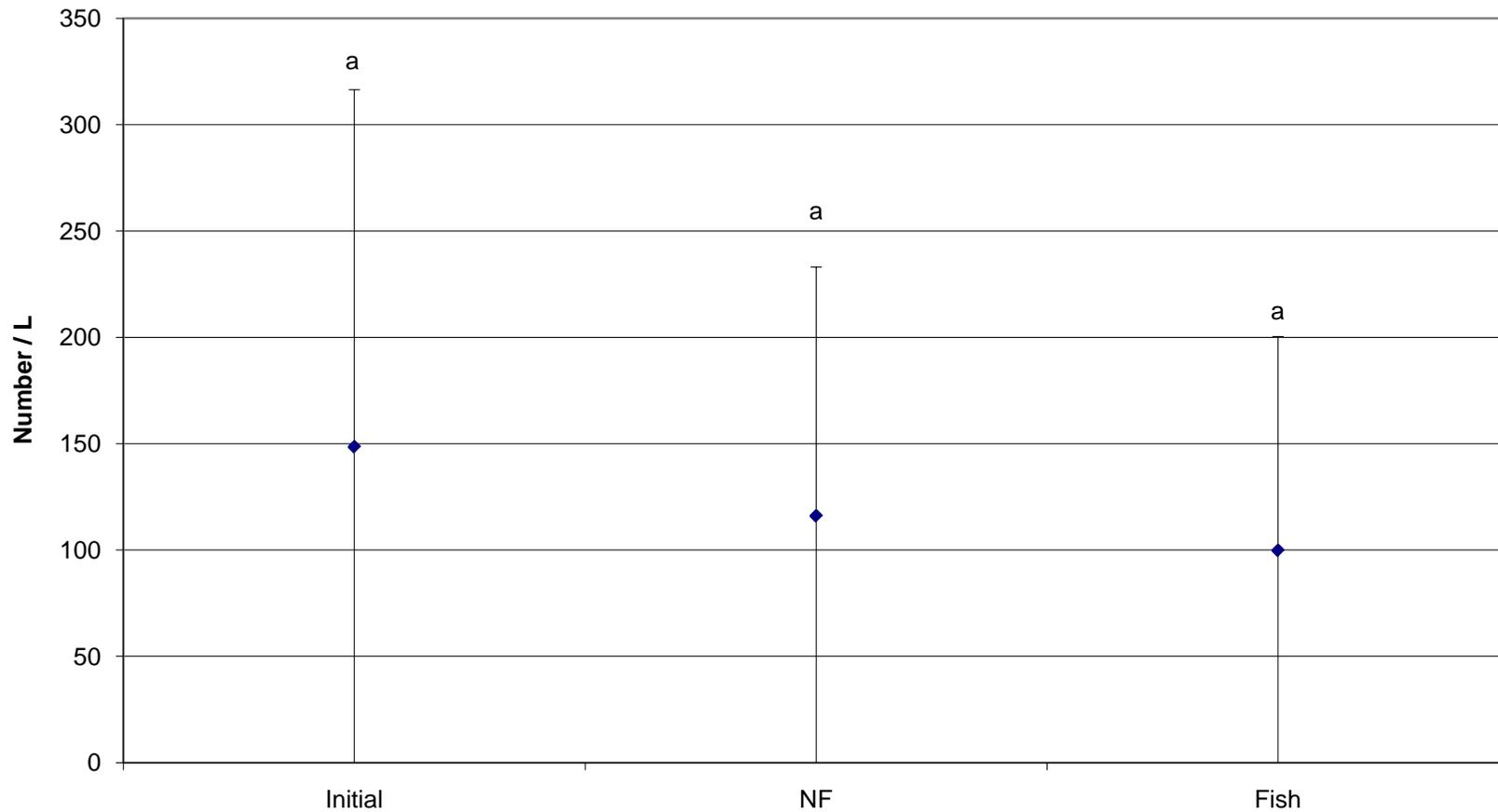


Figure 6-34. Cottonseed meal fertilizer (CSM) treatment 24-hour feeding trial large (> 200 μm) plankton assemblage densities (plankter number/L \pm 95% CI) within three treatments [Initial, NF (no fish), Fish], four replicate samples per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

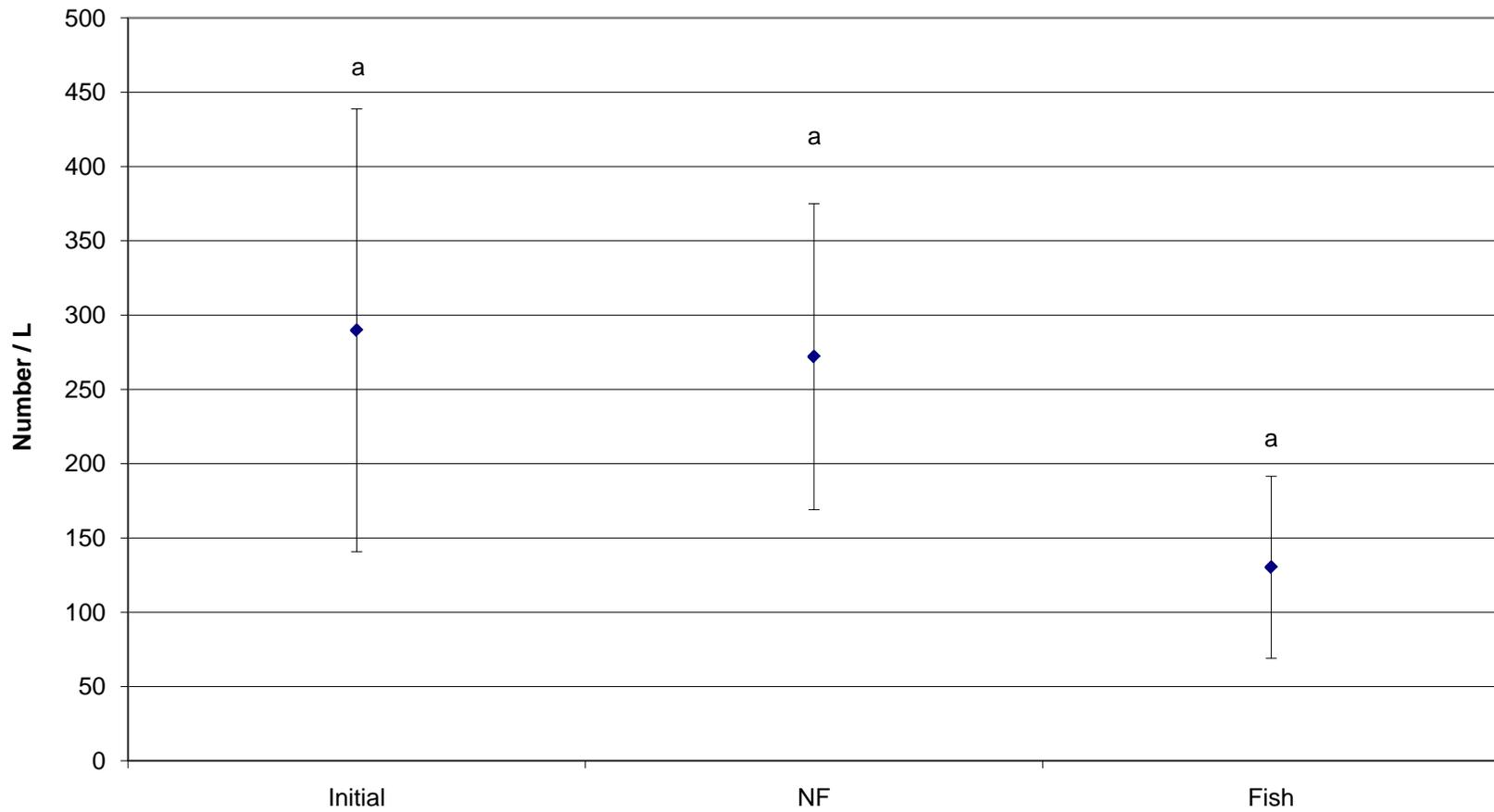


Figure 6-35. Inorganic fertilizer (INO) treatment 24-hour feeding trial large (> 200 μm) plankton assemblage densities (plankton number/L \pm 95% CI) within three treatments [Initial, NF (no fish), Fish], four replicate samples per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

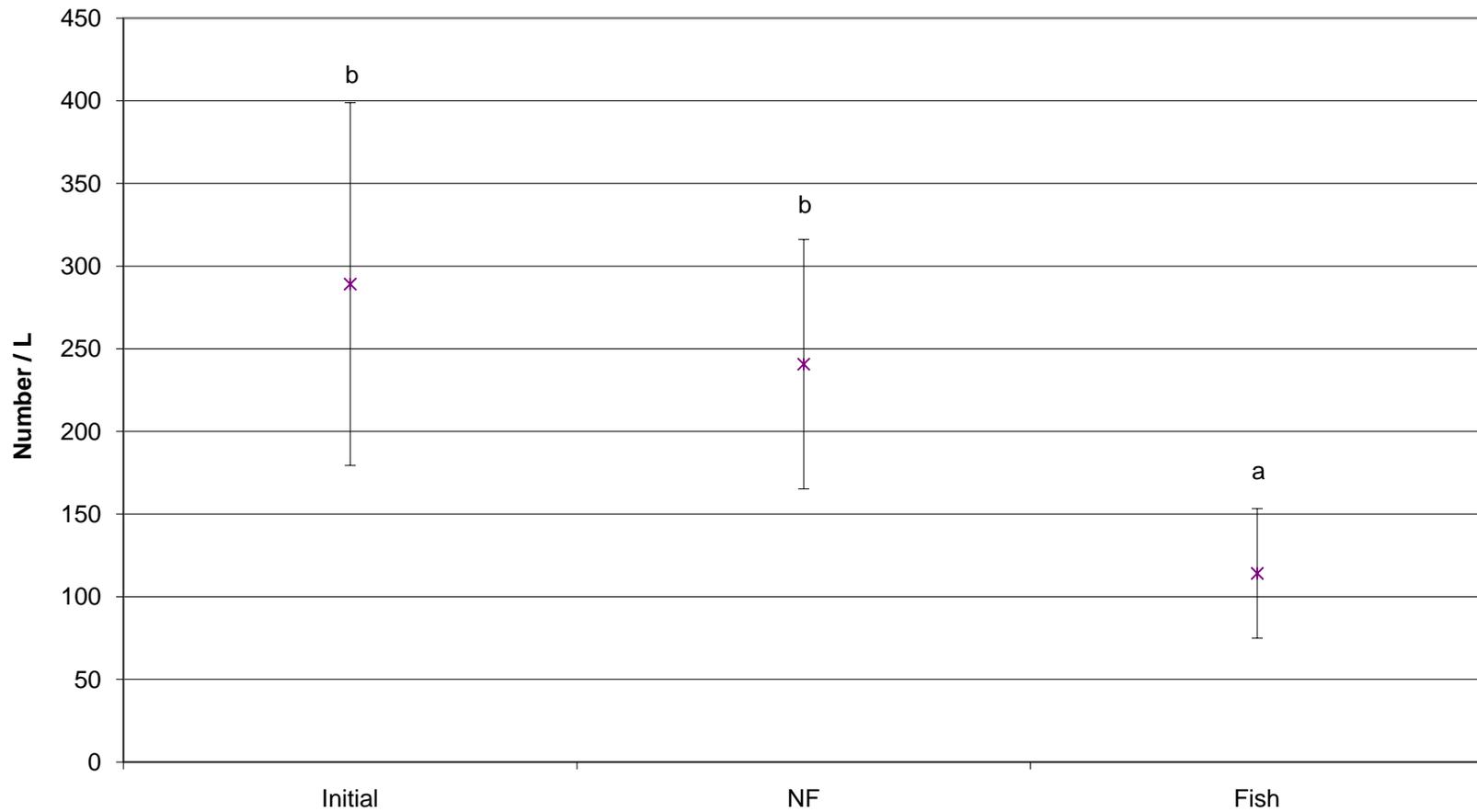


Figure 6-36. Pooled (among nutrient treatment) 24-hour feeding trial large (> 200 μm) plankton assemblage densities (plankton number/L \pm 95% CI) within three treatments [Initial, NF (no fish), Fish], 16 pooled replicates per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

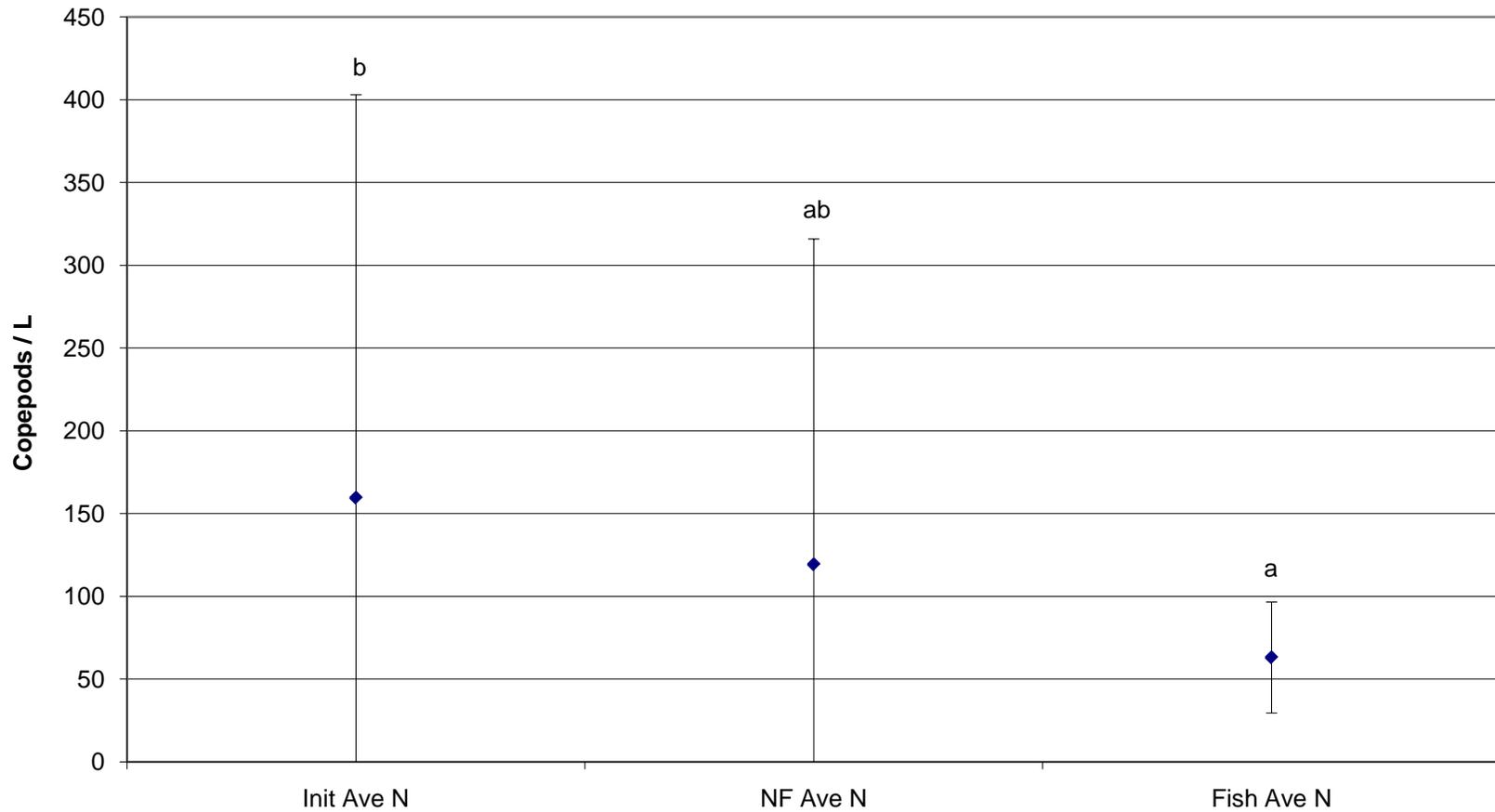


Figure 6-37. Processed feed (PRO) treatment 24-hour feeding trial large (> 200 μm) plankton assemblage *Diaptomous* copepod densities (copepods/L \pm 95% CI) within three treatments [Initial, NF (no fish), Fish], four replicates per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

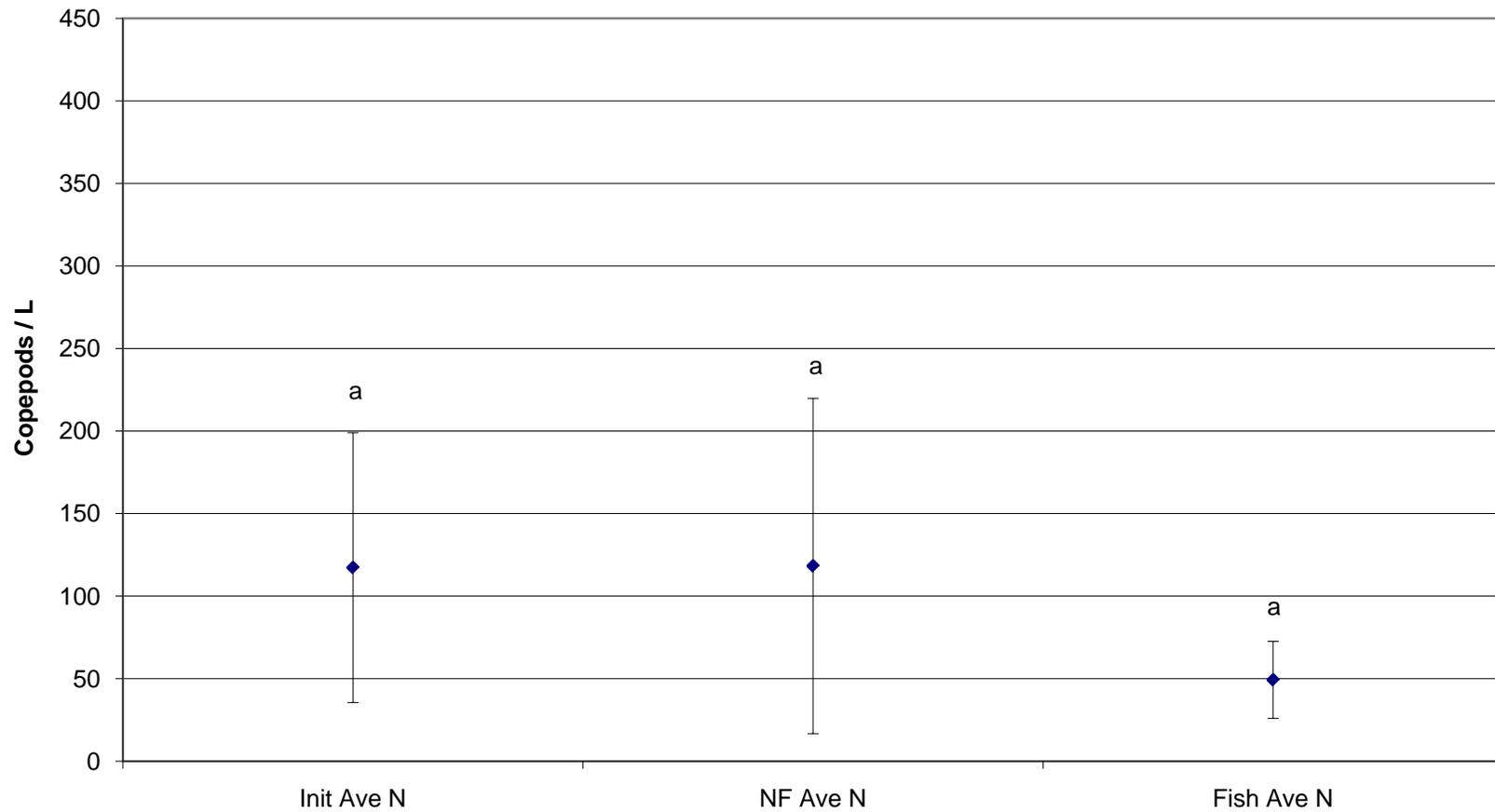


Figure 6-38. Unprocessed feed (UNP) treatment 24-hour feeding trial large (> 200 μm) plankton assemblage *Diaptomous* copepod densities (copepods/L \pm 95% CI) within three treatments [Initial, NF (no fish), Fish], four replicates per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

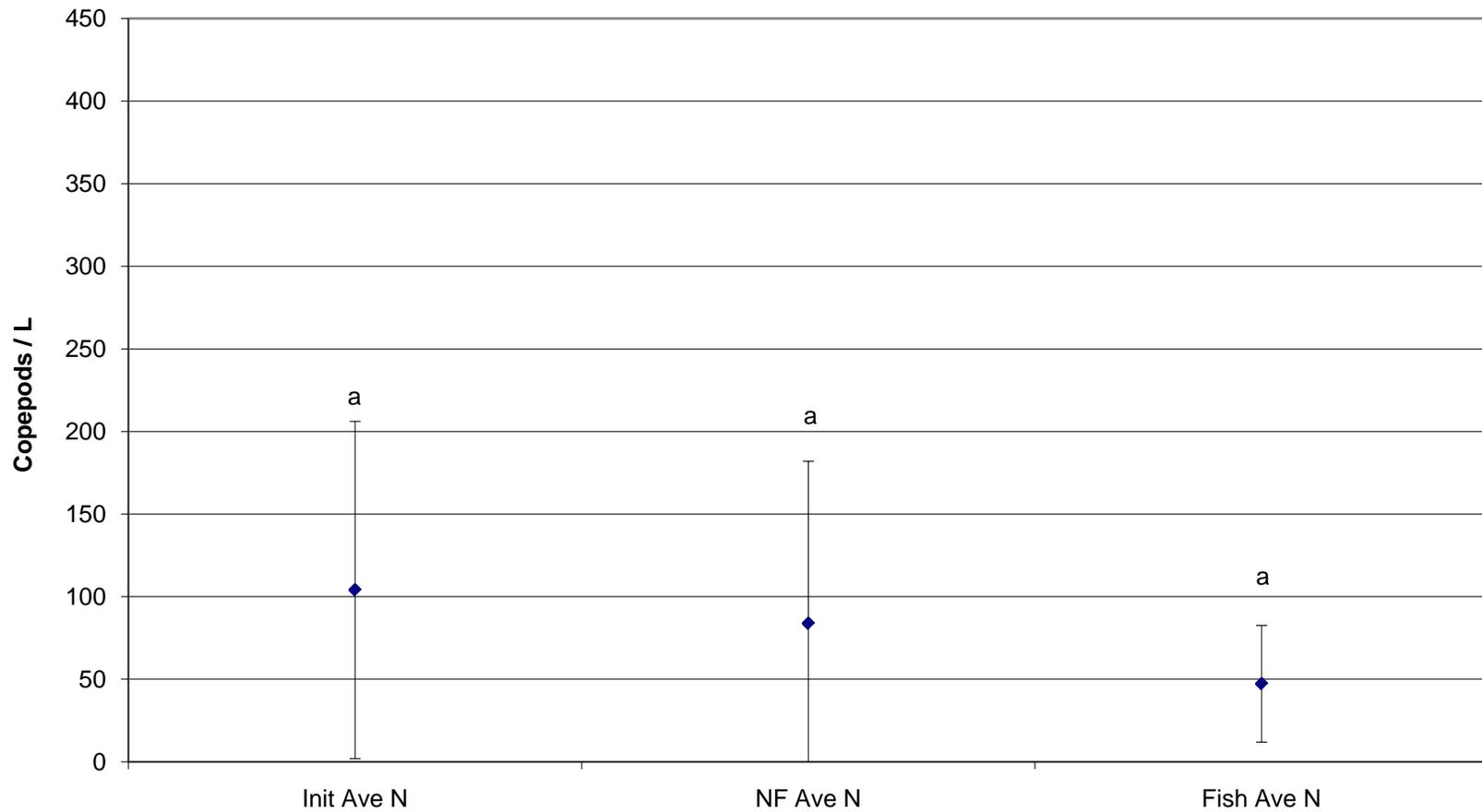


Figure 6-39. Processed feed (PRO) treatment 24-hour feeding trial large (> 200 μm) plankton assemblage *Diaptomous* copepod densities (copepods/L \pm 95% CI) within three treatments [Initial, NF (no fish), Fish], four replicates per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

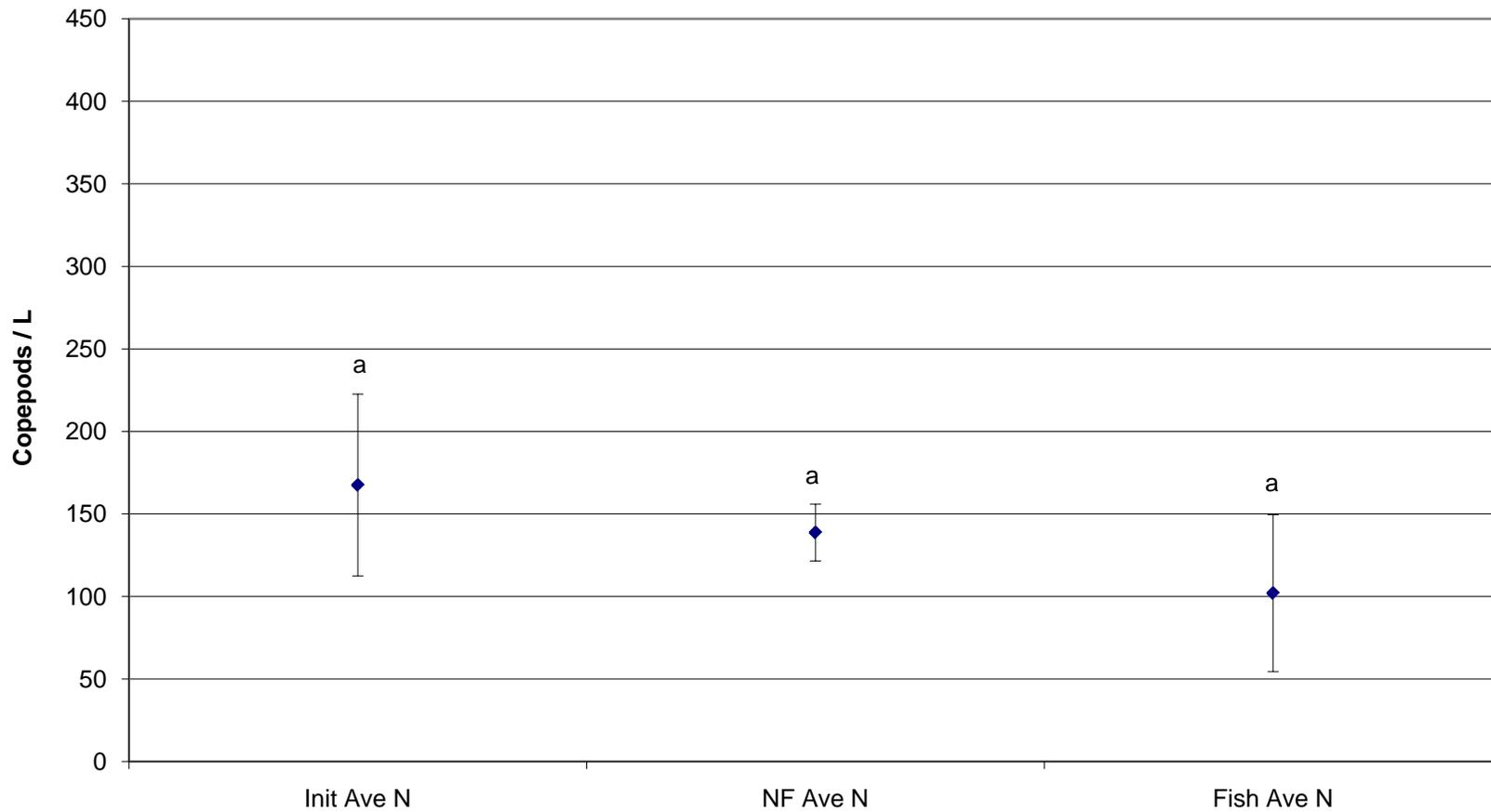


Figure 6-40. Inorganic fertilizer (INO) treatment 24-hour feeding trial large (> 200 μm) plankton assemblage *Diaptomous* copepod densities (copepods/L \pm 95% CI) within three treatments [Initial, NF (no fish), Fish], four replicates per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

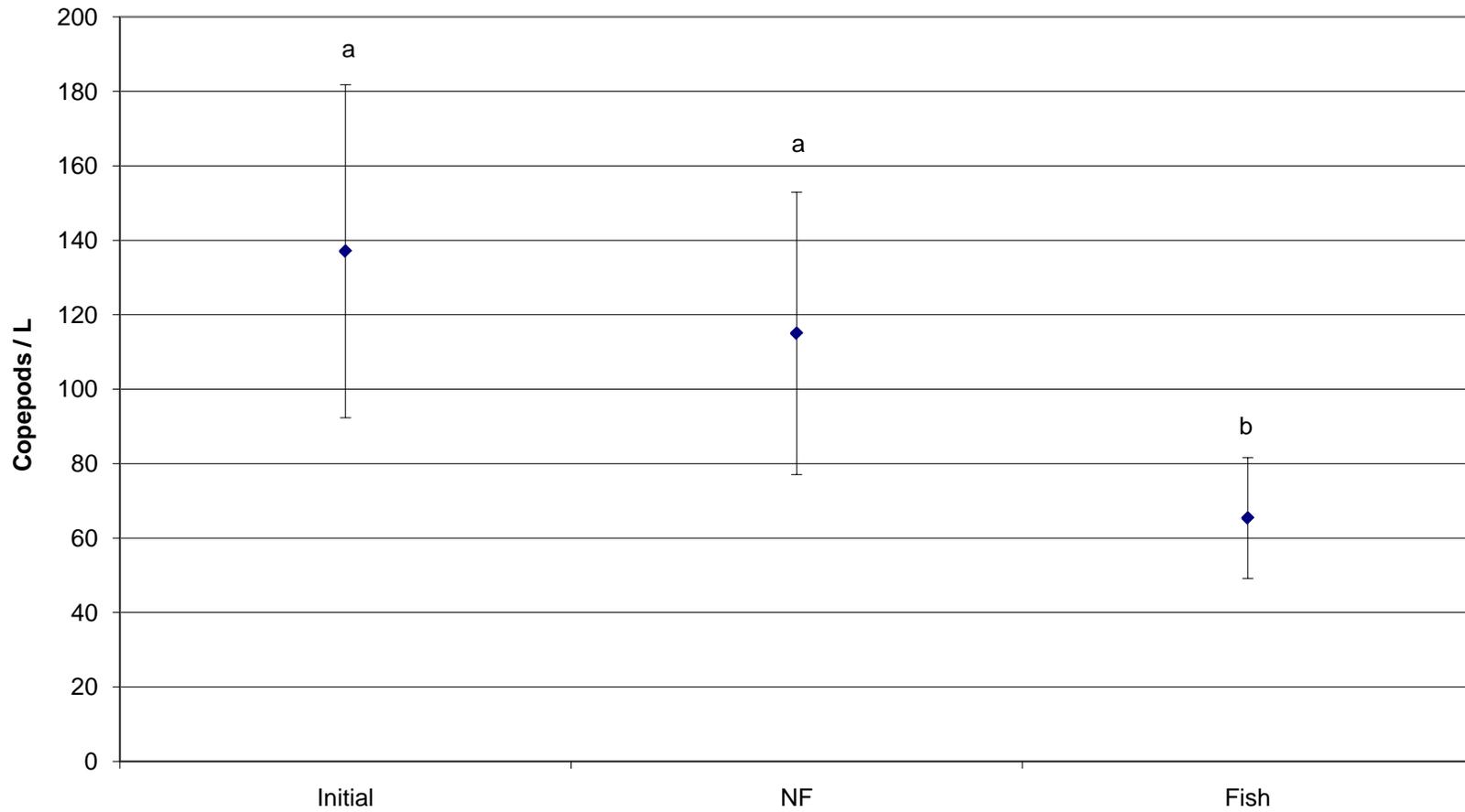


Figure 6-41. Pooled (among nutrient treatment) 24-hour feeding trial large ($> 200 \mu\text{m}$) plankton assemblage *Diaptomous* copepod densities (copepods/L \pm 95% CI) within three treatments [Initial, NF (no fish), Fish], 16 replicates per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

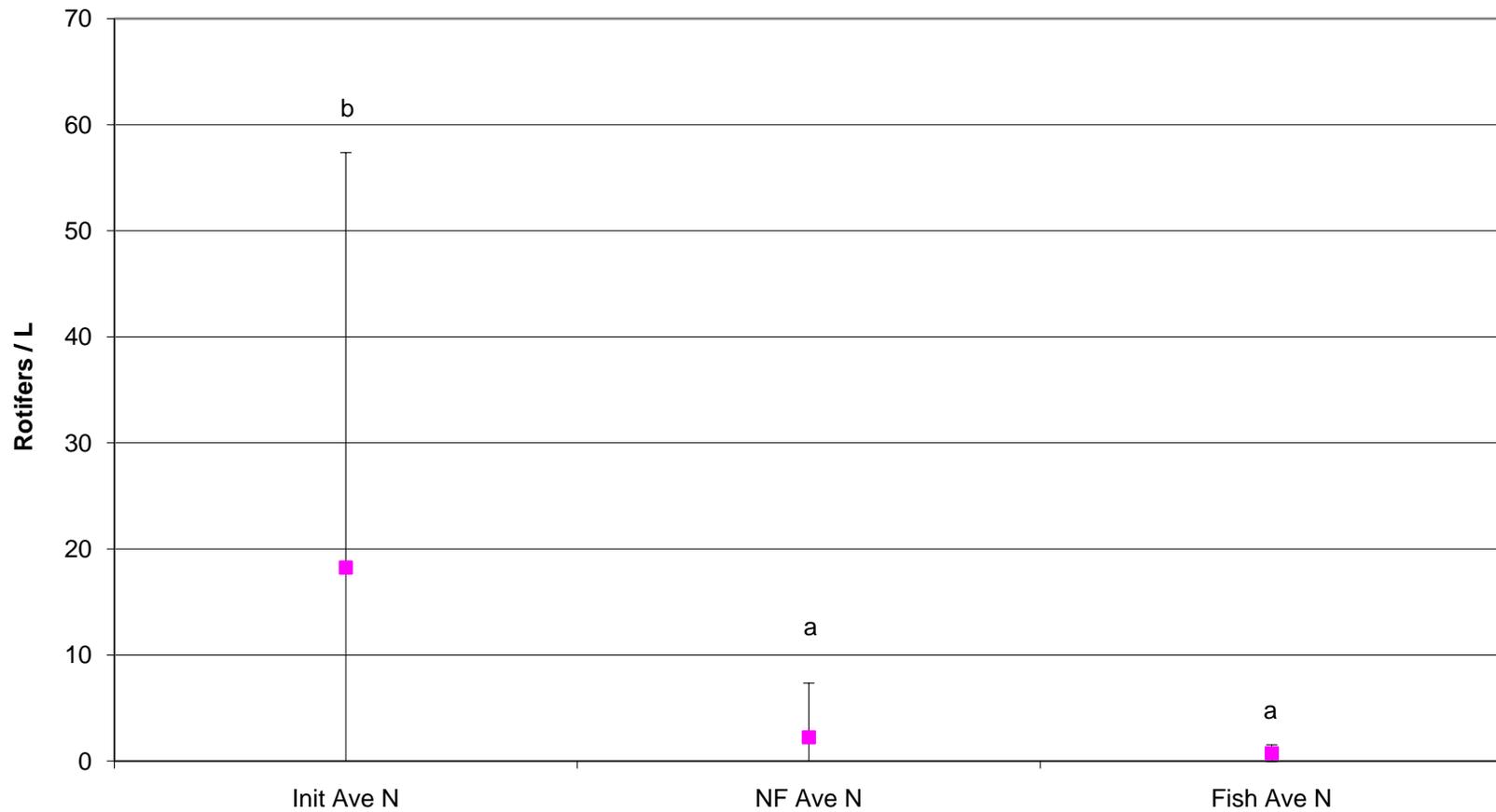


Figure 6-42. Processed feed (PRO) treatment 24-hour feeding trial large (> 200 μm) plankton assemblage *Filinia* rotifer densities (rotifers/L \pm 95% CI) within three treatments [Initial, NF (no fish), Fish], four replicate water samples per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

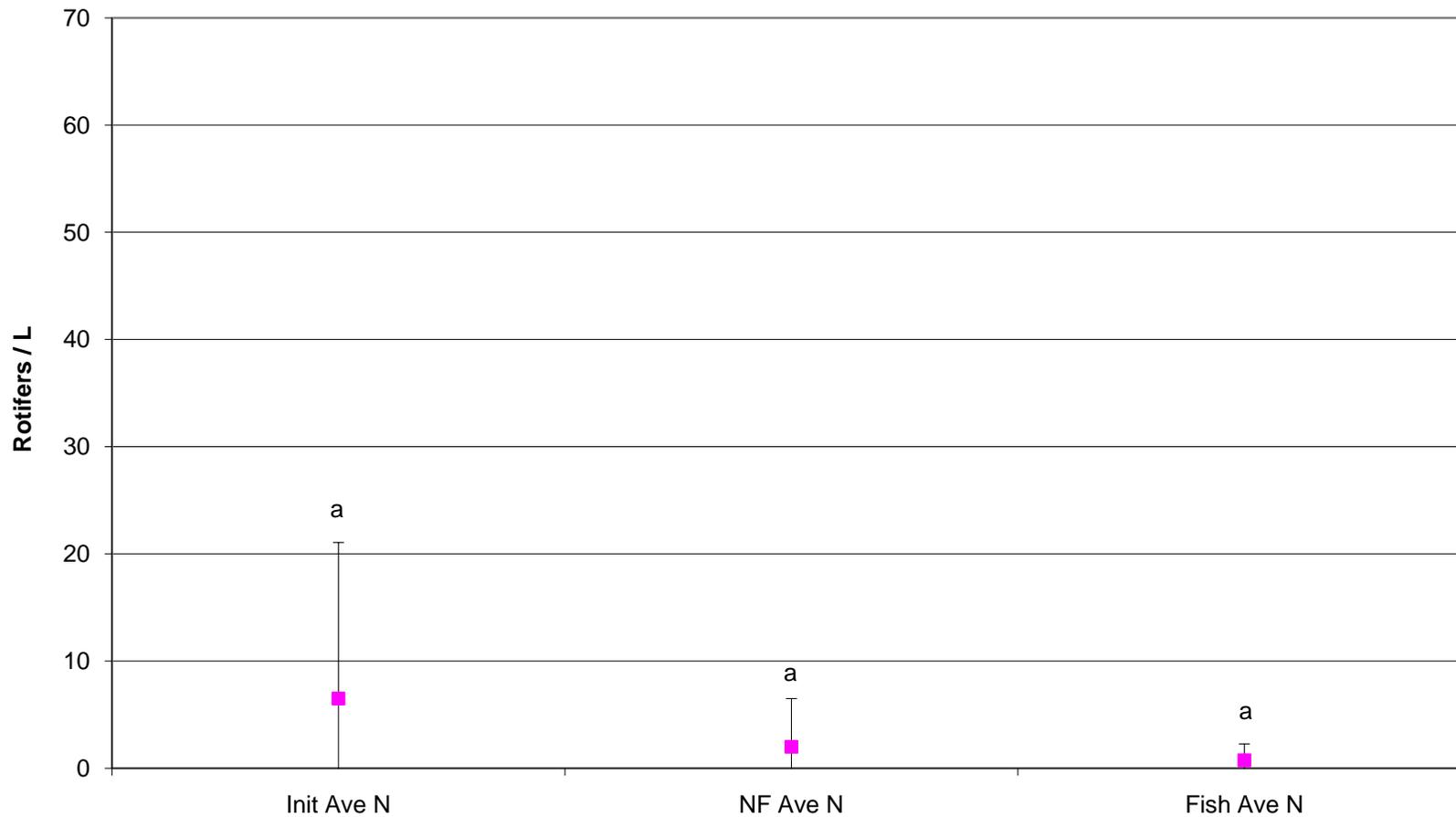


Figure 6-43. Unprocessed feed (UNP) treatment 24-hour feeding trial large (> 200 μm) plankton assemblage *Filinia* rotifer densities (rotifers/L \pm 95% CI) within three treatments [Initial, NF (no fish), Fish], four replicate water samples per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

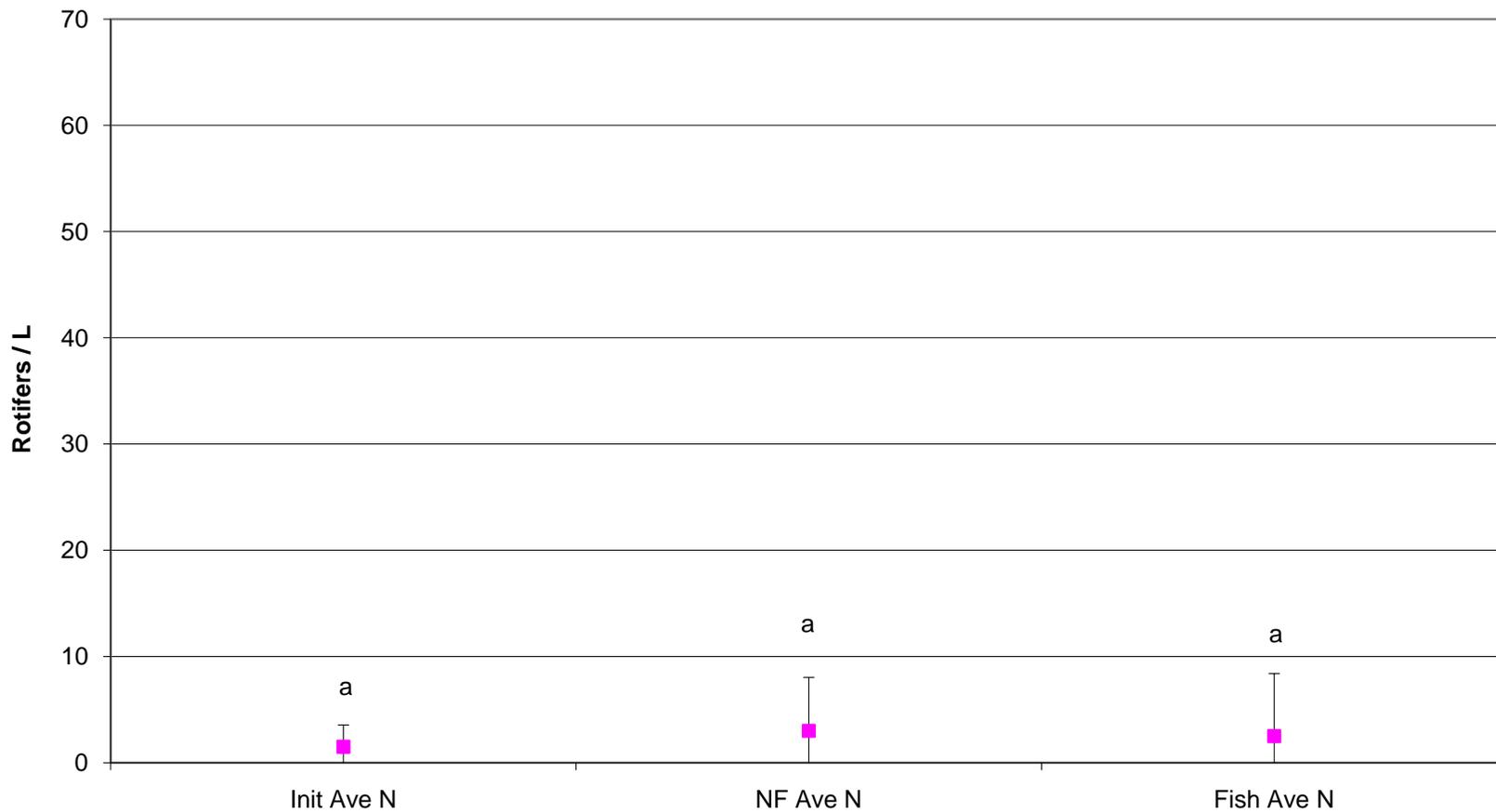


Figure 6-44. Cottonseed meal fertilizer (CSM) treatment 24-hour feeding trial large (> 200 μm) plankton assemblage *Filinia* rotifer densities (rotifers/L \pm 95% CI) within three treatments [Initial, NF (no fish), Fish], unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test); four replicates per treatment.

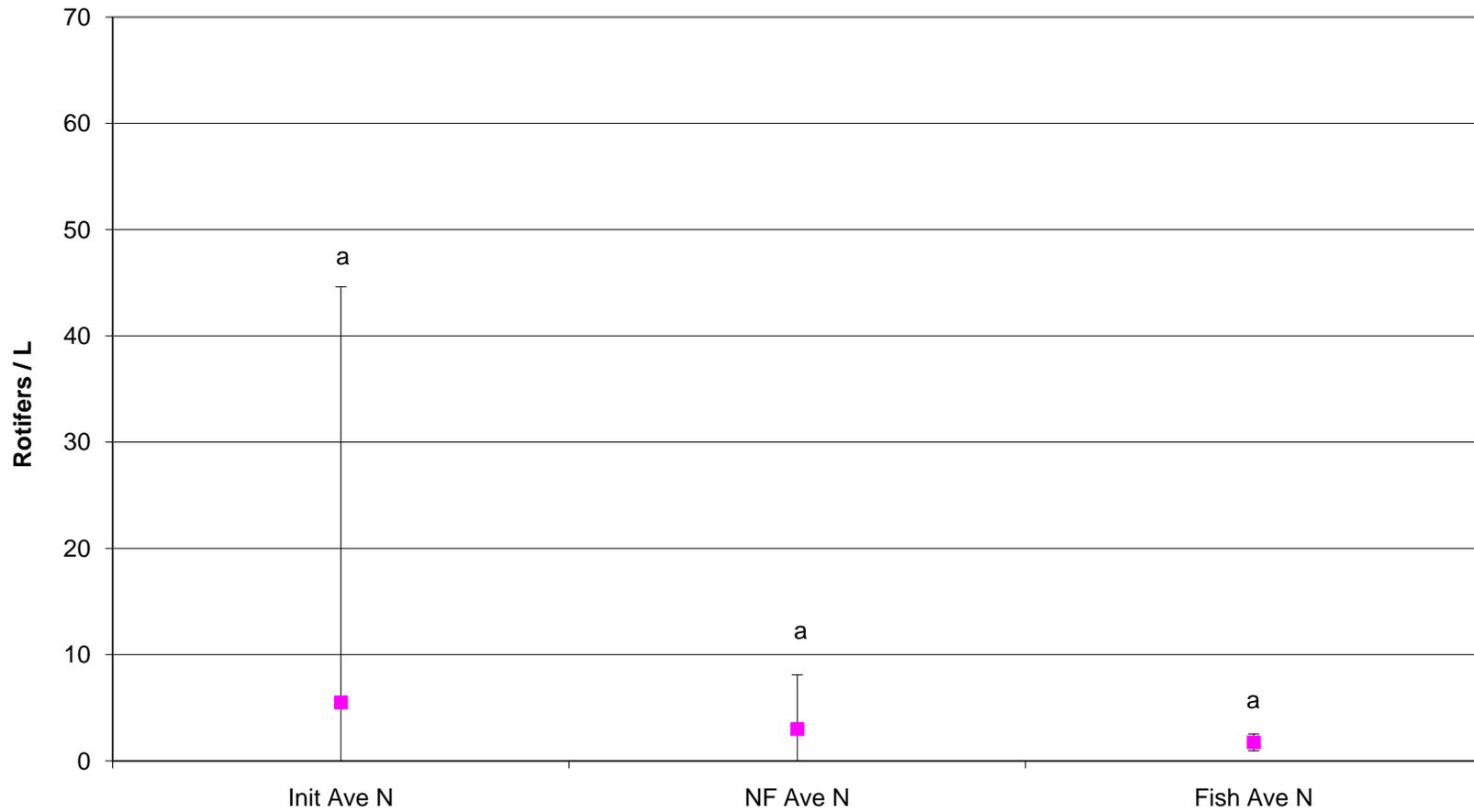


Figure 6-45. Inorganic fertilizer (INO) treatment 24-hour feeding trial large (> 200 μm) plankton assemblage *Filinia* rotifer densities (rotifers/L \pm 95% CI) within three treatments [Initial, NF (no fish), Fish], four replicate water samples per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

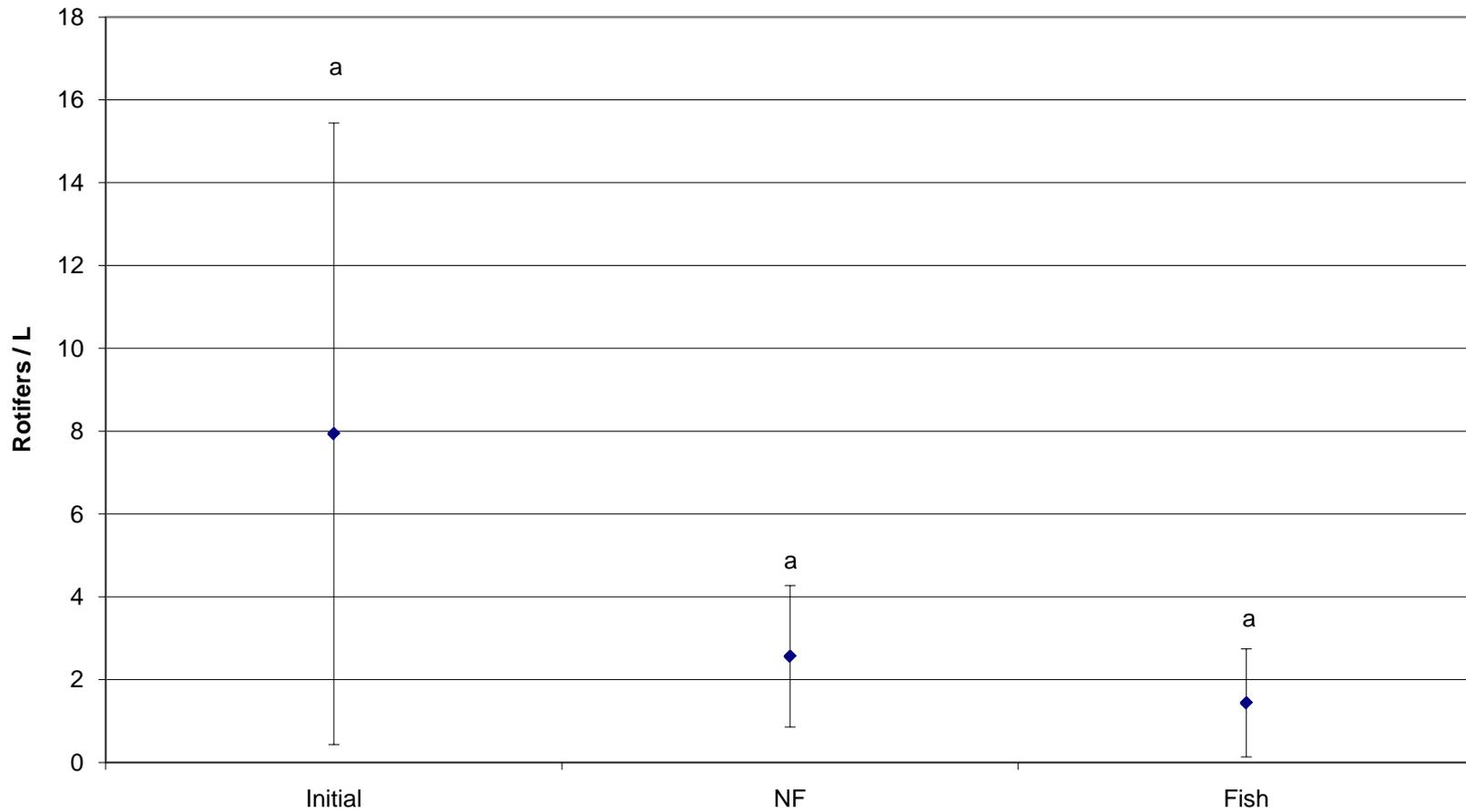


Figure 6-46. Pooled (among nutrient treatment) 24-hour feeding trial large (> 200 μm) plankton assemblage *Filinia* rotifer densities (rotifers/L \pm 95% CI) within three treatments [Initial, NF (no fish), Fish], 16 replicate water samples per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

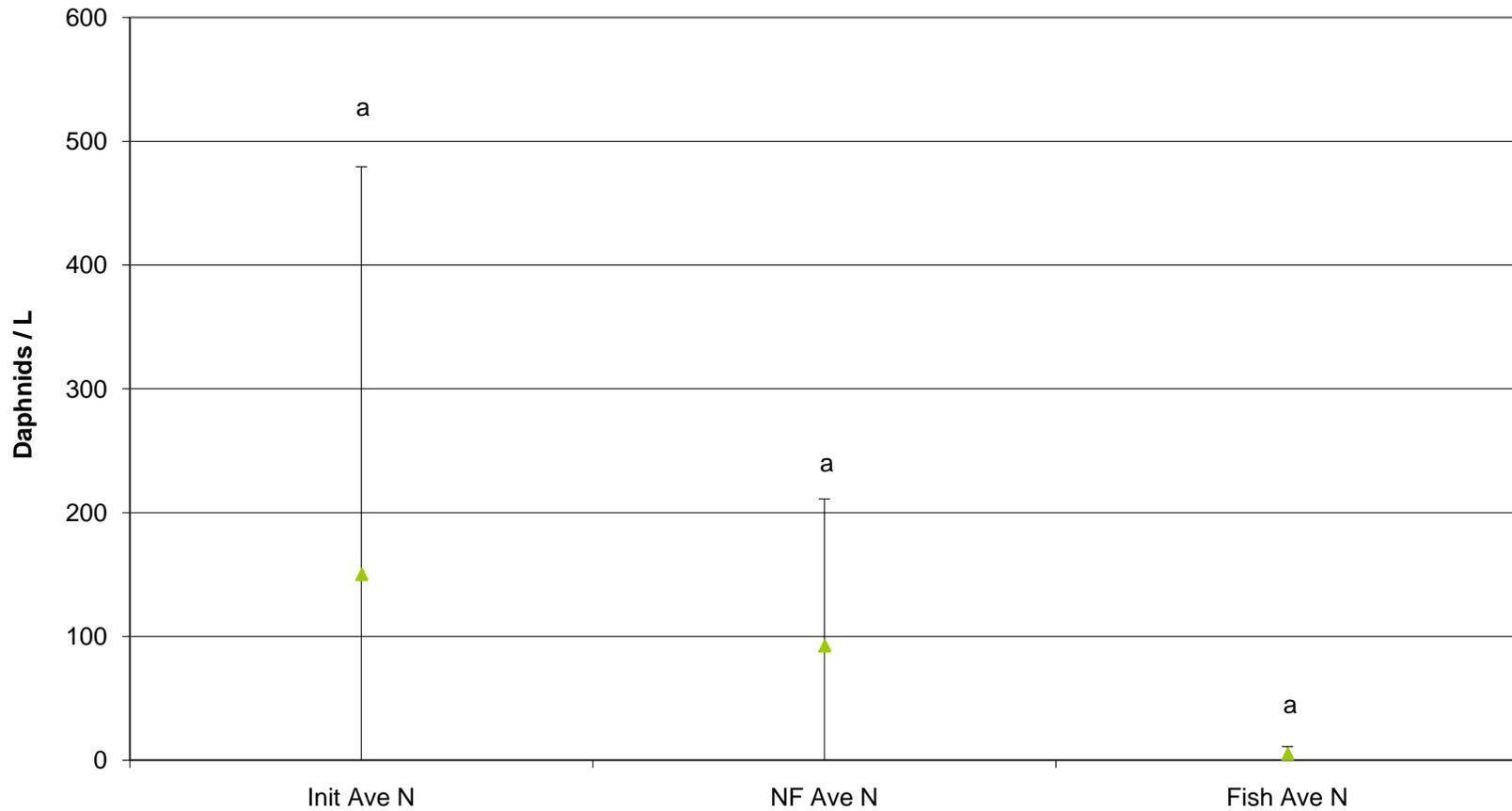


Figure 6-47. Processed feed (PRO) treatment 24-hour feeding trial large (> 200 μm) plankton assemblage *Moina macrocopa* (Daphnid) densities (daphnids/L \pm 95% CI) within three treatments [Initial, NF (no fish), Fish], four replicates per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

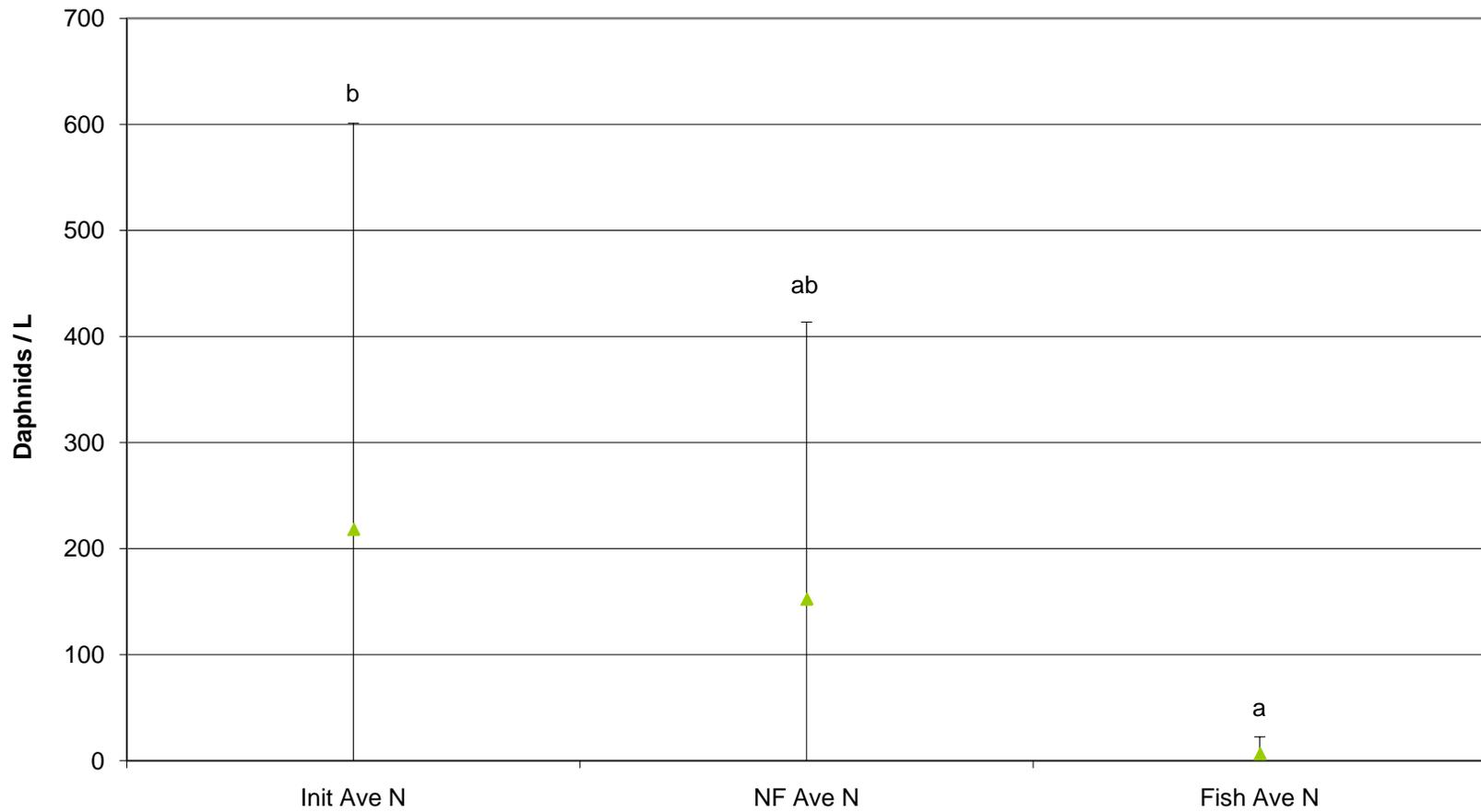


Figure 6-48. Unprocessed feed (UNP) treatment 24-hour feeding trial large (> 200 μm) plankton assemblage *Moina macrocopa* (Daphnid) densities (daphnids/L \pm 95% CI) within three treatments [Initial, NF (no fish), Fish], four replicates per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

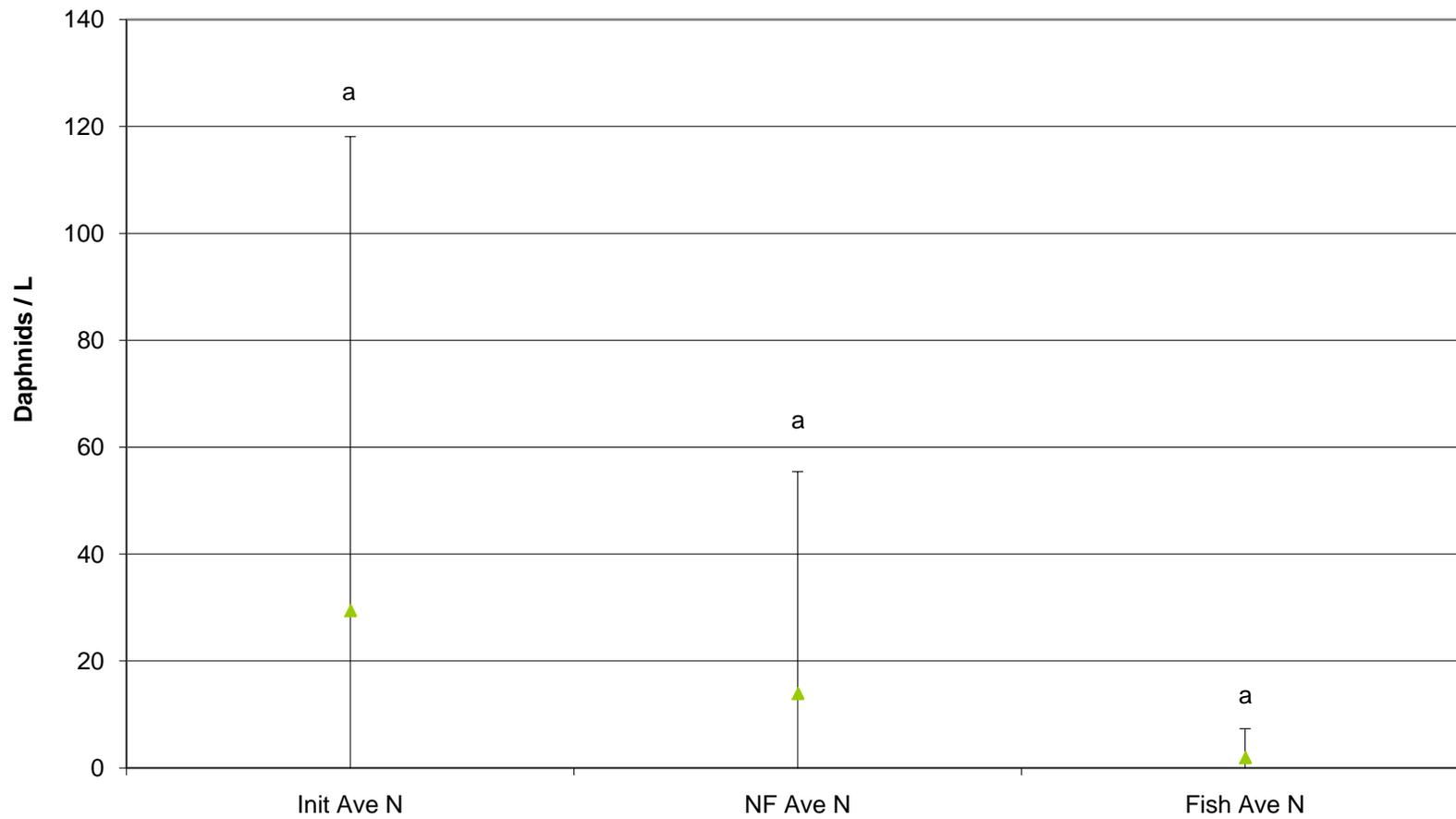


Figure 6-49. Cottonseed meal fertilizer (CSM) treatment 24-hour feeding trial large (> 200 μm) plankton assemblage *Moina macrocopa* (Daphnid) densities (daphnids/L \pm 95% CI) within three treatments [Initial, NF (no fish), Fish], four replicates per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

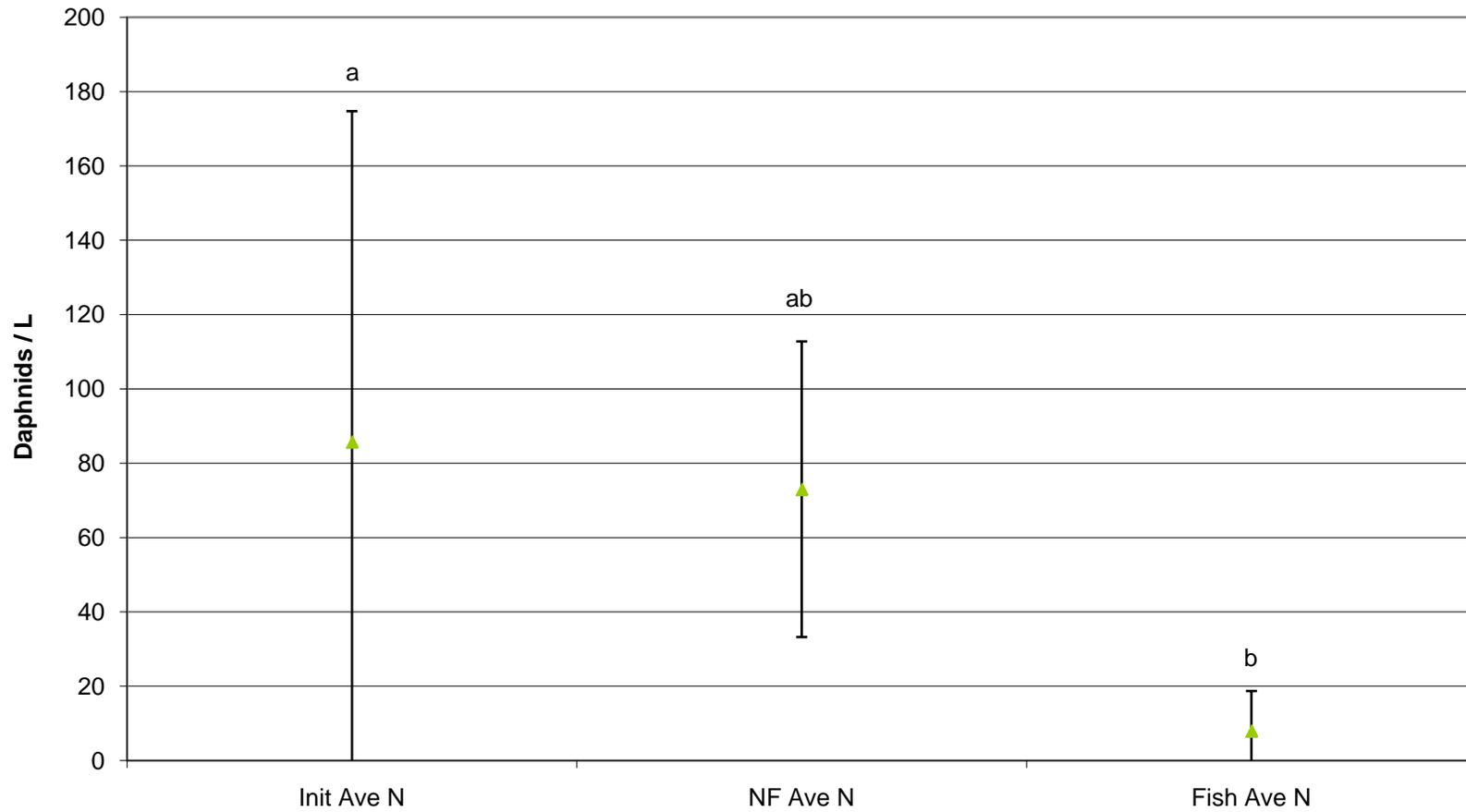


Figure 6-50. Inorganic fertilizer (INO) treatment 24-hour feeding trial large (> 200 μm) plankton assemblage *Moina macrocopa* (Daphnid) densities (daphnids/L \pm 95% CI) within three treatments [Initial, NF (no fish), Fish], four replicates per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

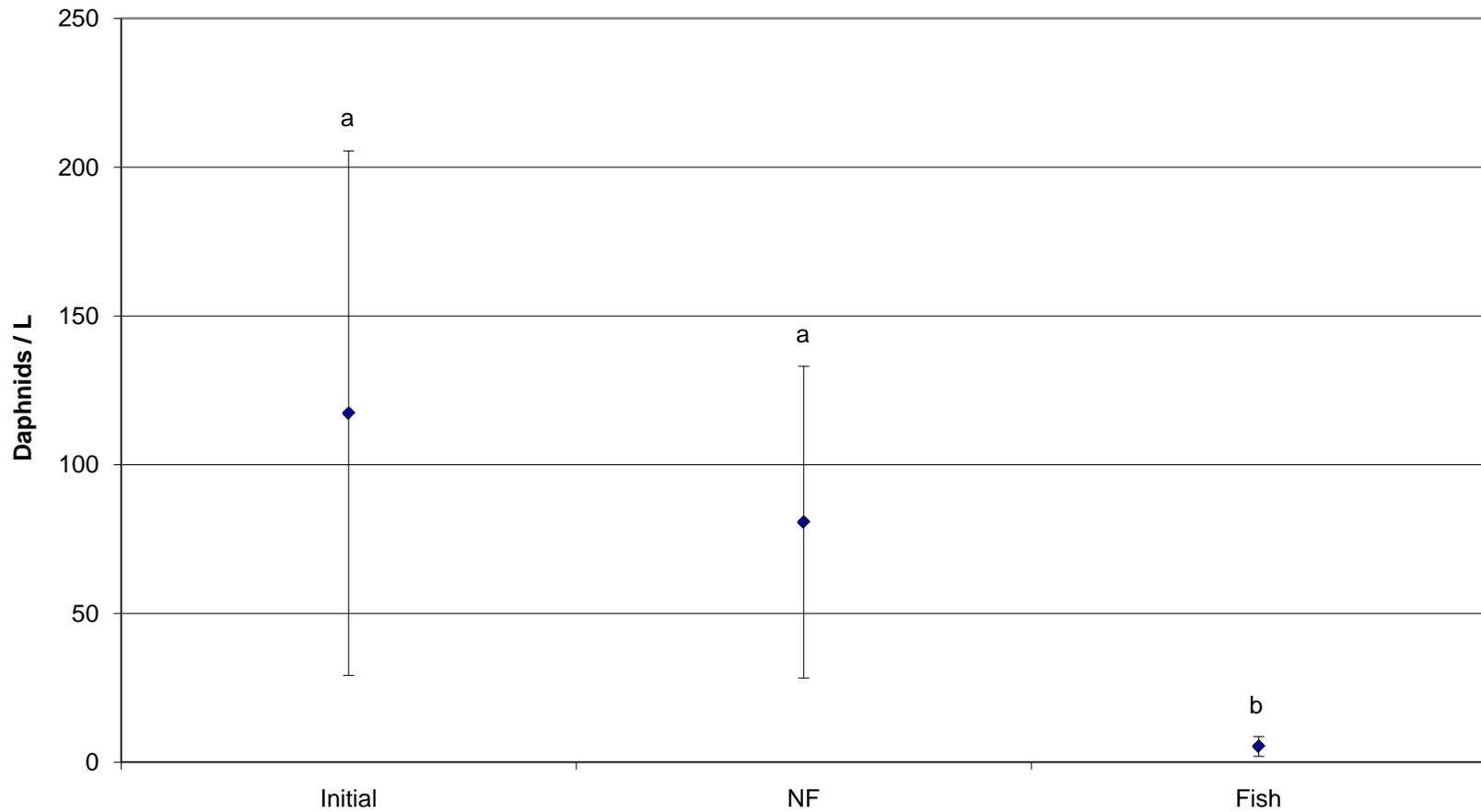


Figure 6-51. Pooled (among nutrient treatment) 24-hour feeding trial large (> 200 μm) plankton assemblage *Moina macrocopa* (Daphnid) densities (daphnids/L \pm 95% CI) within three experimental treatments [Initial, NF (no fish), Fish], 16 samples per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

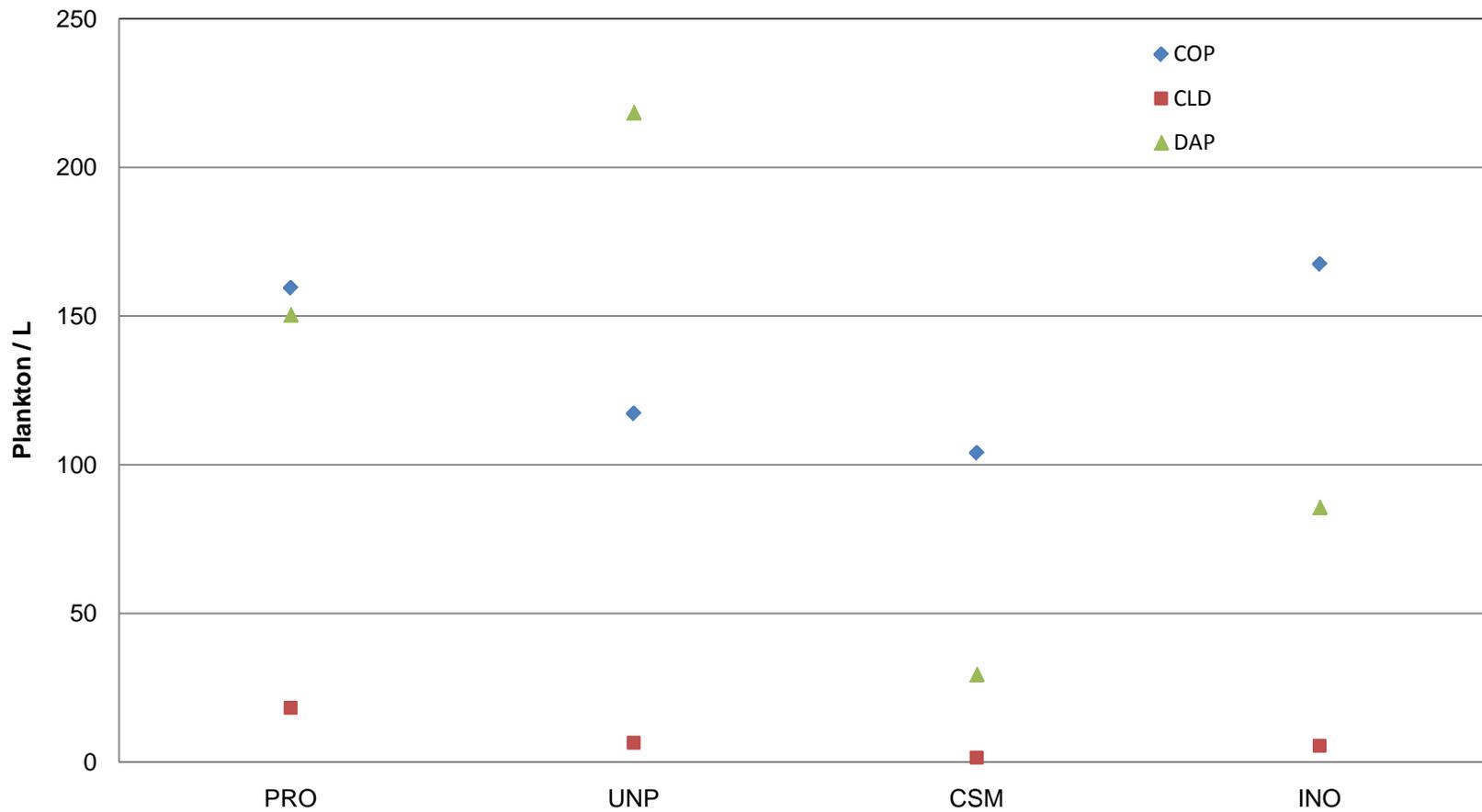


Figure 6-52. 24-hour feeding trial Initial control treatment major taxa densities (plankton number/L) within four pond nutrient treatments [processed feed (PRO), unprocessed feed (UNP), cottonseed meal fertilizer (CSM), inorganic fertilizer (INO)]; four 1 L replicate water samples per treatment.

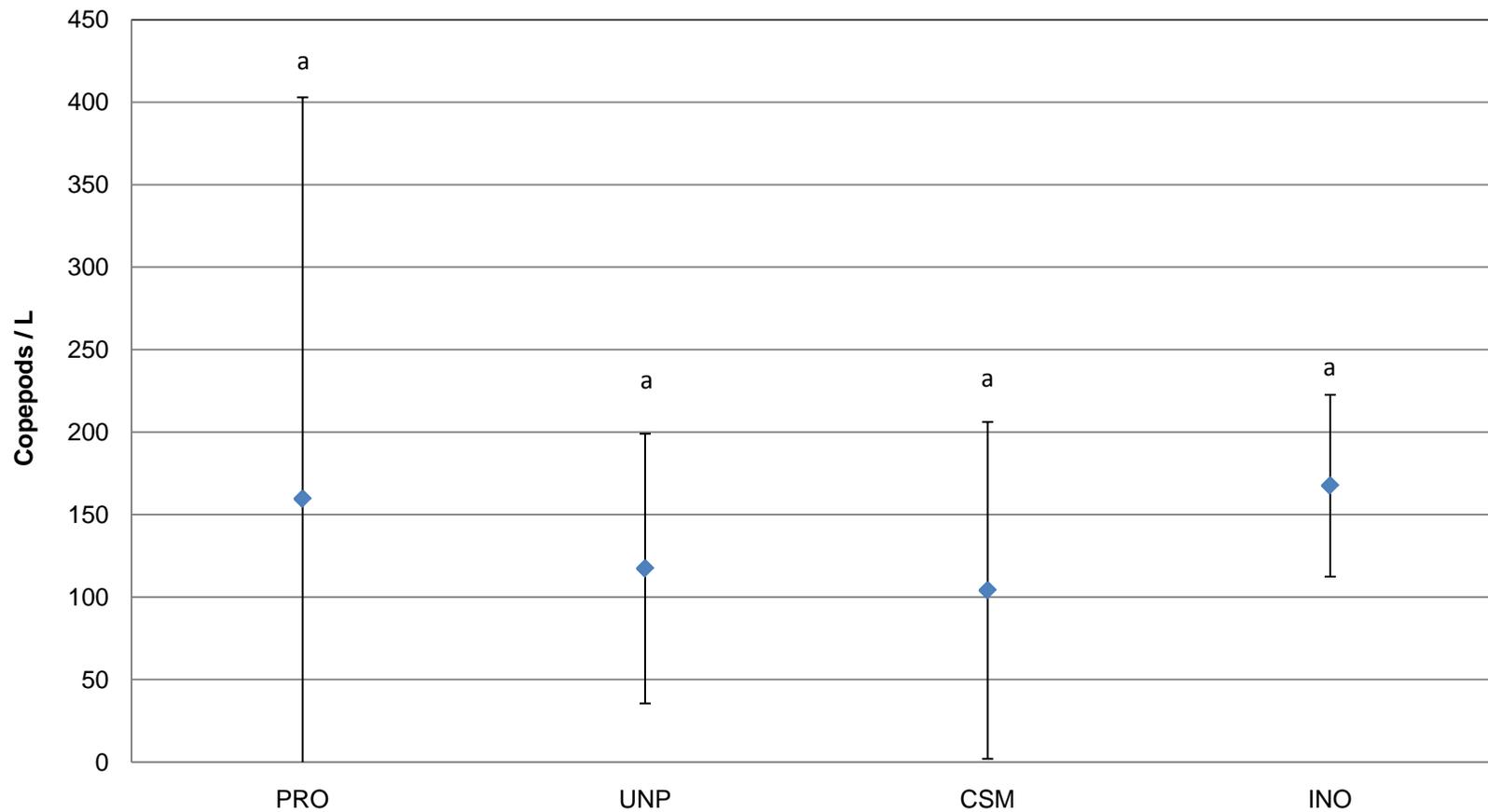


Figure 6-53. 24 hr feeding trial Initial treatment Diaptomous copepod densities (copepods/L \pm 95% CI) among four treatments [processed feed (PRO), unprocessed feed (UNP), cottonseed meal fertilizer (CSM), inorganic fertilizer (INO)], four replicates per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

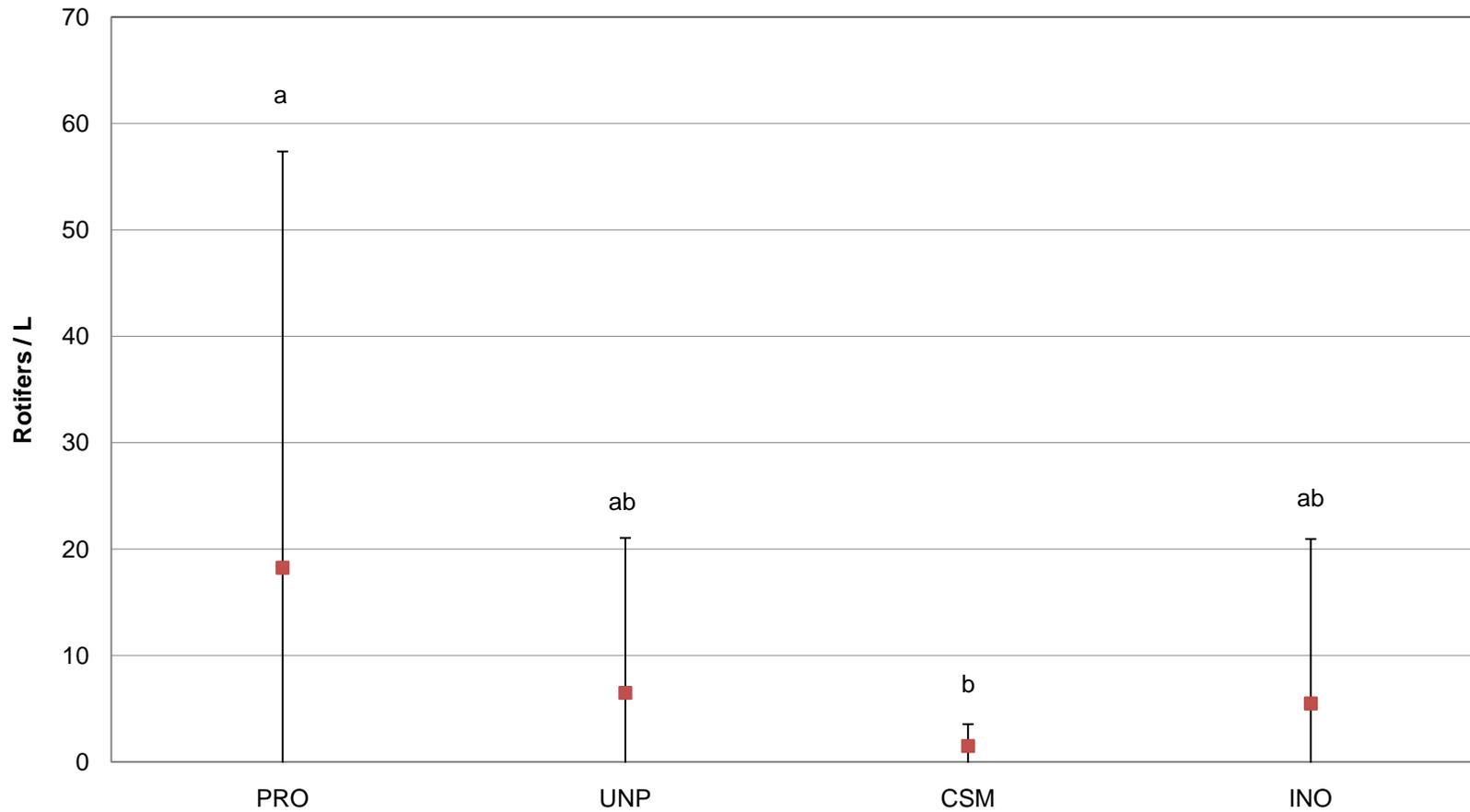


Figure 6-54. 24 hr feeding trial Initial treatment *Filinia spp.* rotifer densities (rotifers/L \pm 95% CI) within large (> 200 μ m) plankton assemblages [processed (PRO) and unprocessed (UNP) feed, cottonseed meal (CSM), and inorganic fertilizer (INO)], unshared letters denote statistical differences ($P < 0.05$ Bonferroni posttests); four replicates per treatment.

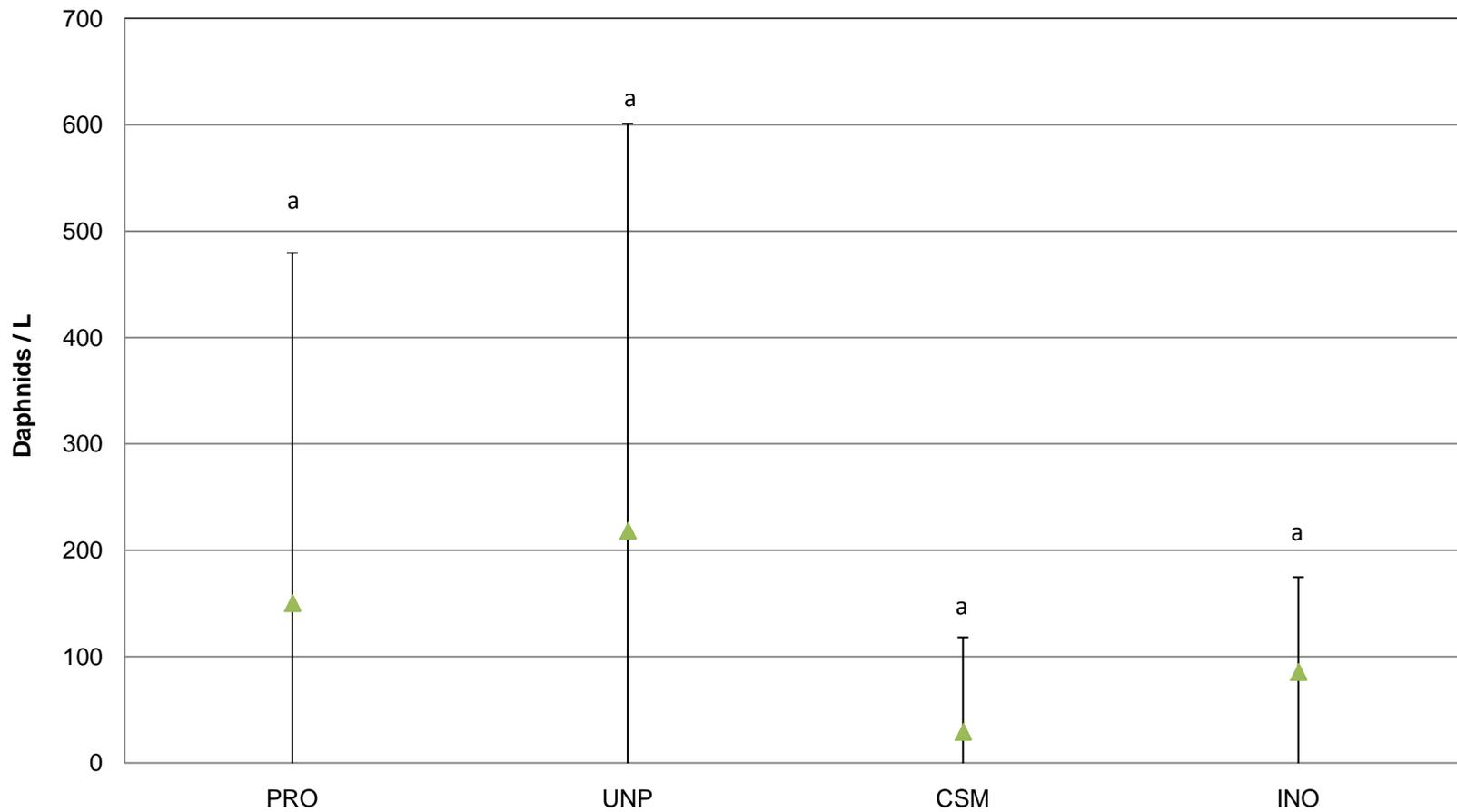


Figure 6-55. 24 hr feeding trial Initial treatment *Moina macrocopa* (Daphnid) densities (daphnids/L \pm 95% CI) within four nutrient treatments [processed (PRO) and unprocessed (UNP) feed, cottonseed meal (CSM) and inorganic (INO) fertilizer], four replicates per treatment; unshared letters denote statistical differences between treatments ($P < 0.05$, Bonferroni post test).

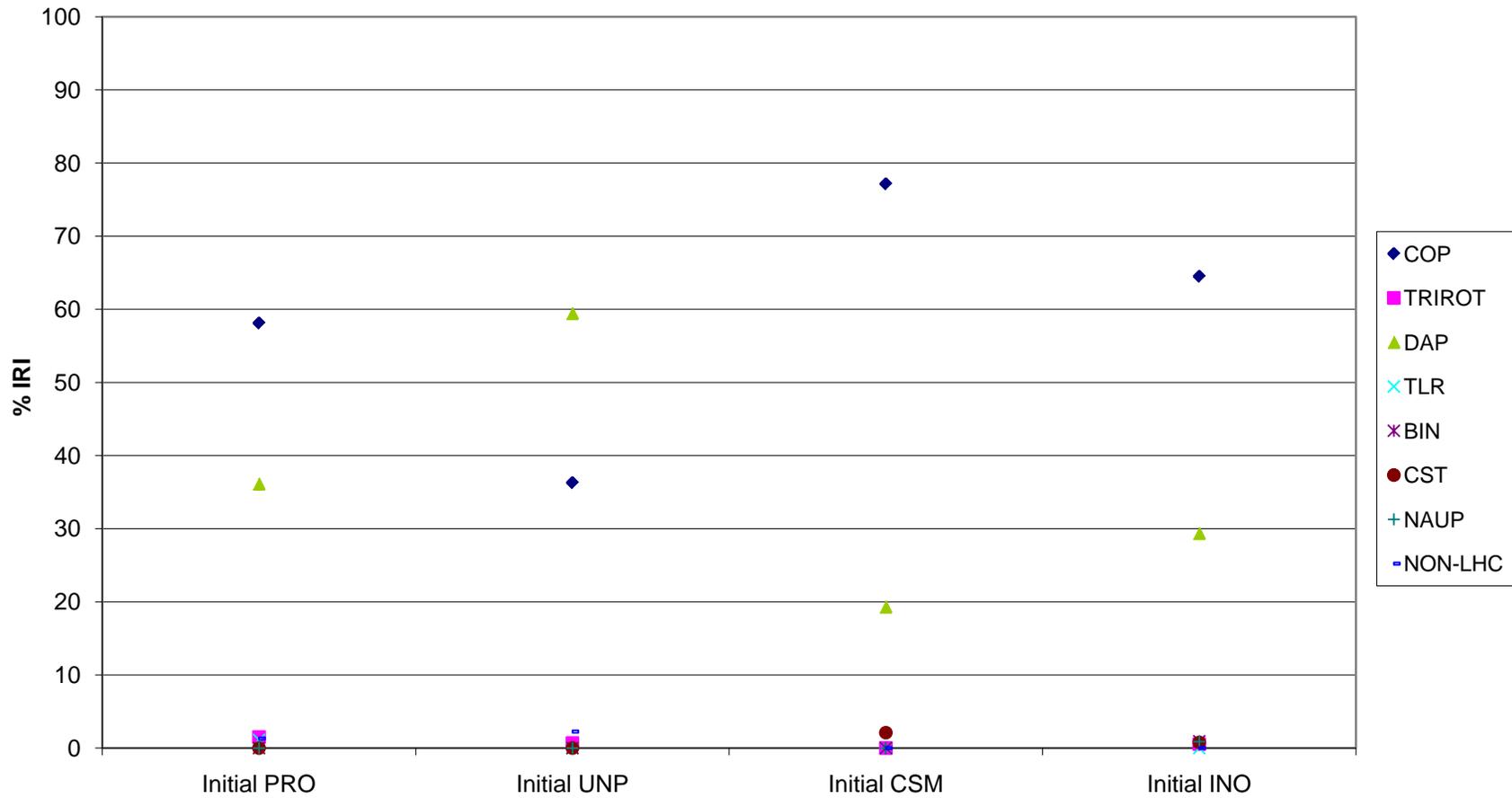


Figure 6-56. 24-hour feeding trial Initial (0 hrs) treatment major (taxa %IRI > 0.5%; IRI: index of relative importance) large (> 200 μm) plankton assemblage taxa %IRI values pooled among four nutrient treatments [processed (PRO) and unprocessed (UNP) feed, cottonseed meal (CSM) and inorganic (INO) fertilizer].

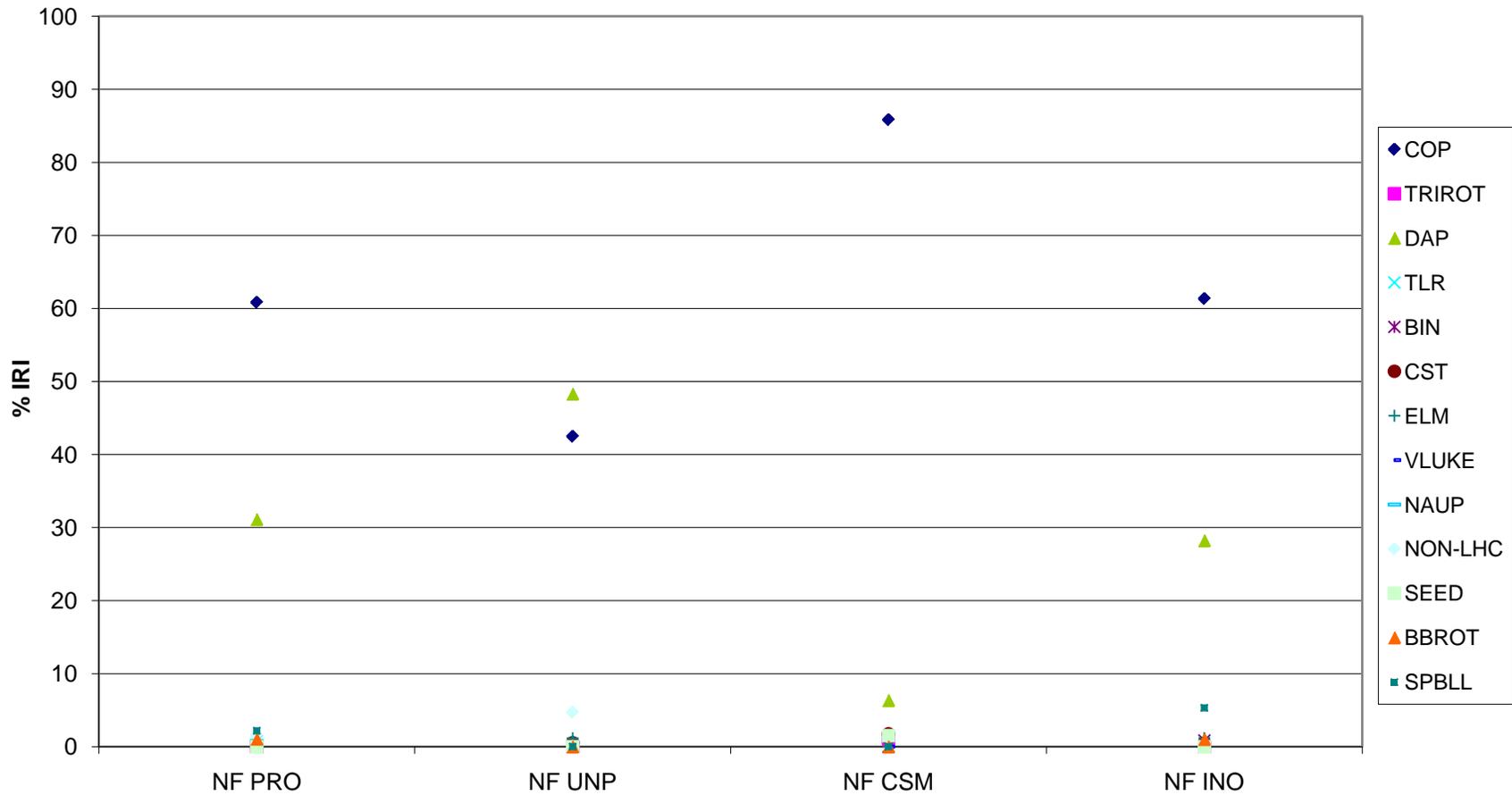


Figure 6-57. 24-hour feeding trial NF (no fish + 24 hrs) treatment major (taxa %IRI > 0.5%; IRI: index of relative importance) large (> 200 μm) plankton assemblage taxa %IRI values pooled among four pond nutrient treatments [processed (PRO) and unprocessed (UNP) feed, cottonseed meal (CSM) and inorganic (INO) fertilizer].

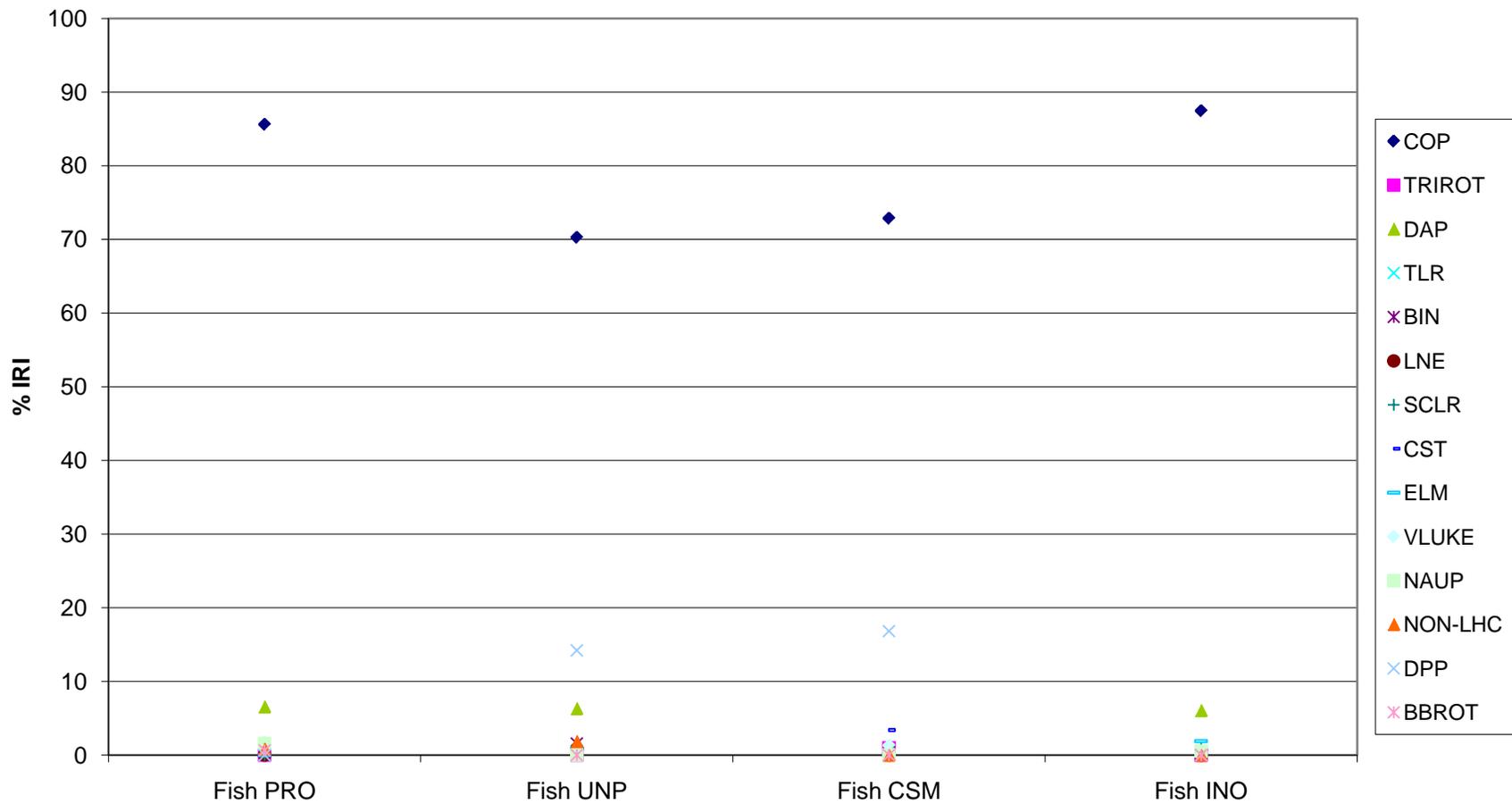


Figure 6-58. 24-hour feeding trial Fish (one fish + 24 hrs) treatment major (taxa %IRI > 0.5%; IRI: index of relative importance) large (> 200 μm) plankton assemblage taxa %IRI values pooled among four pond nutrient treatments [processed (PRO) and unprocessed (UNP) feed, cottonseed meal (CSM) and inorganic (INO) fertilizer].

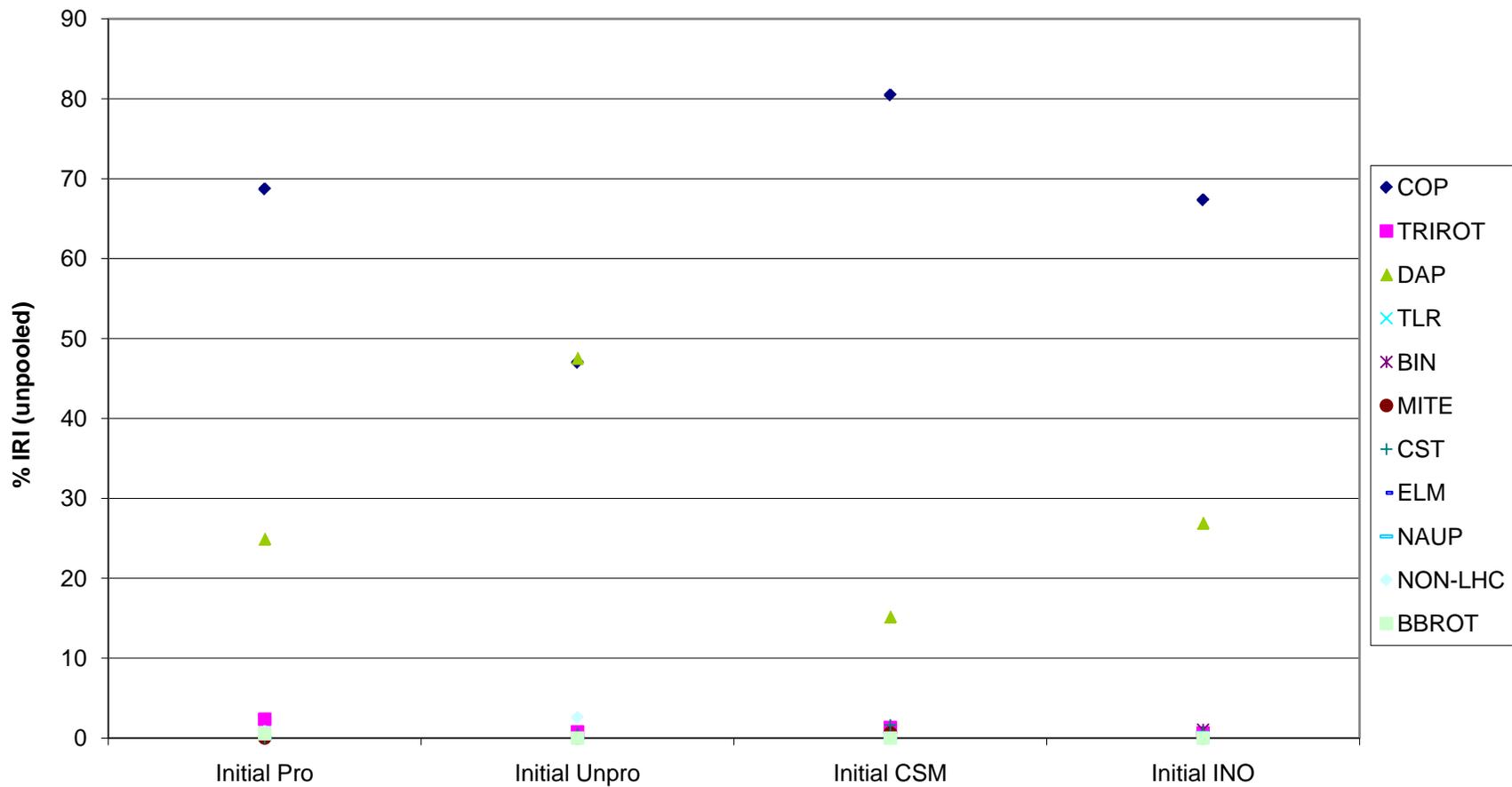


Figure 6-59. 24-hour feeding trial Initial (no fish + 0 hrs) treatment major (taxa %IRI > 0.5%; IRI: index of relative importance) large (> 200 μm) plankton assemblage taxa unpooled %IRI values among four pond nutrient treatments [processed (PRO) and unprocessed (UNP) feed, and cottonseed meal (CSM) and inorganic (INO) fertilizer].

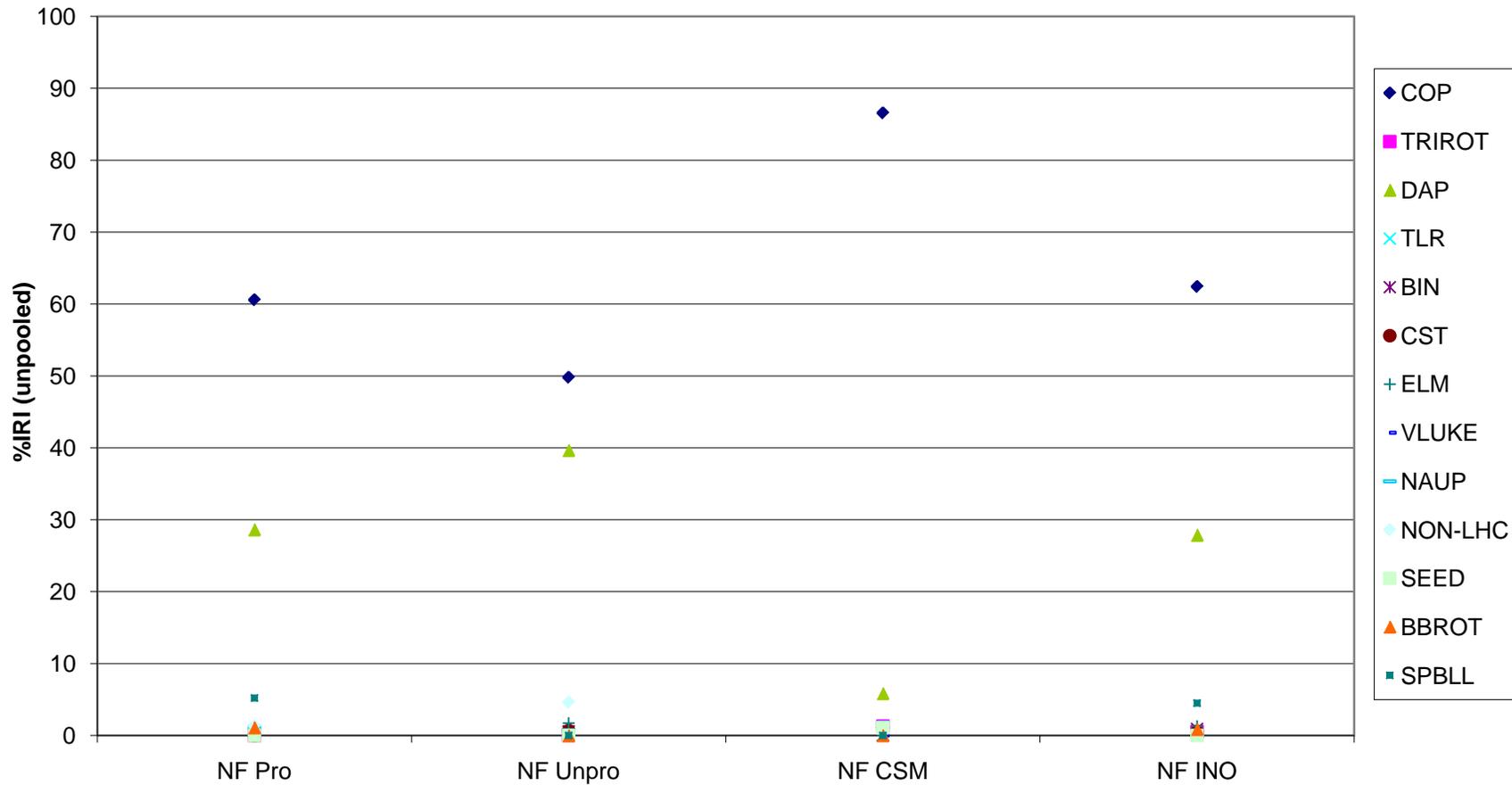


Figure 6-60. 24-hour feeding trial NF (no fish + 24 hrs) treatment major (taxa %IRI > 0.5%; IRI: index of relative importance) large (> 200 μm) plankton assemblage taxa unpooled %IRI values among four pond nutrient treatments [processed (PRO) and unprocessed (UNP) feed, and cottonseed meal (CSM) and inorganic (INO) fertilizer].

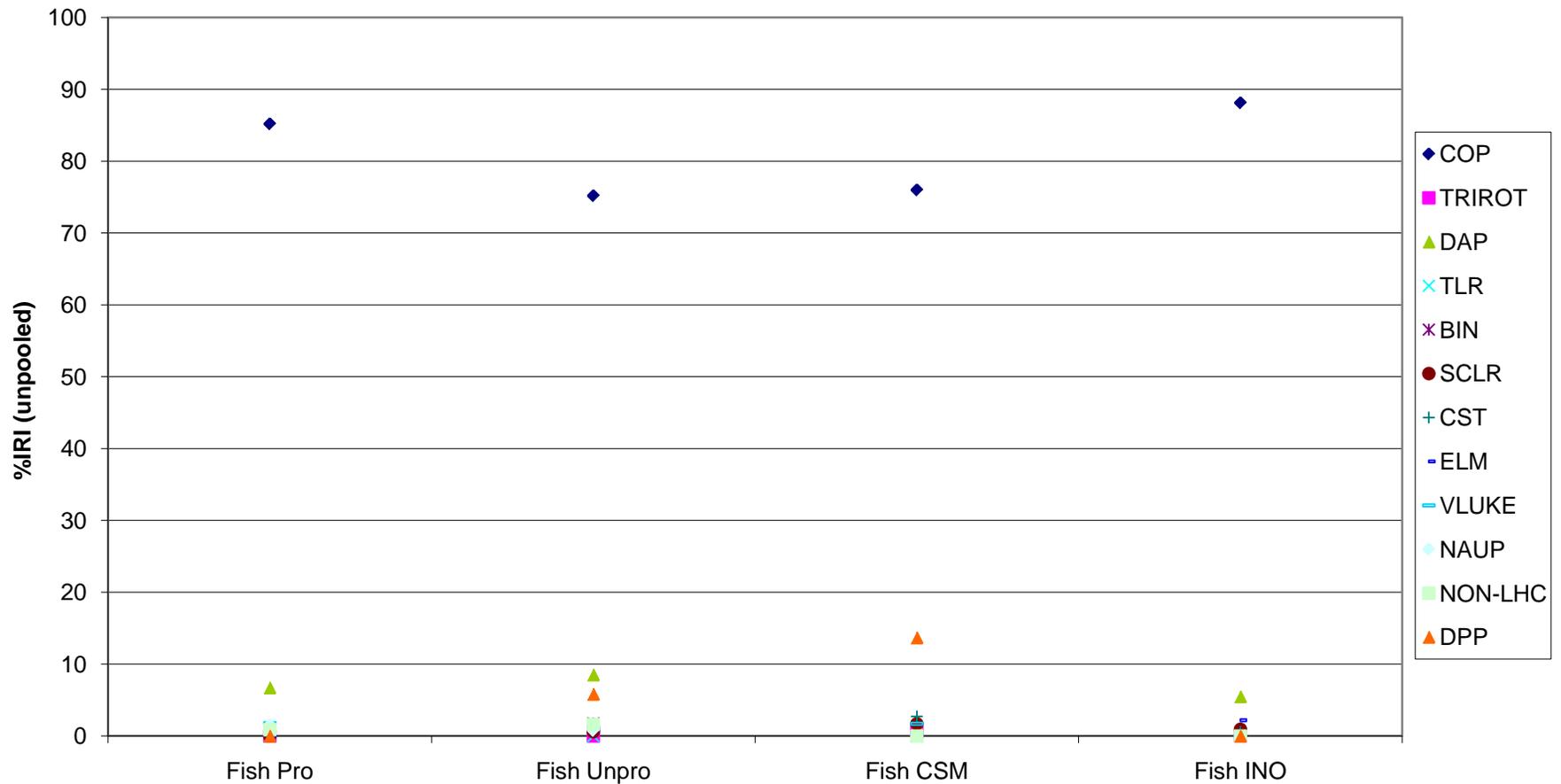


Figure 6-61. 24-hour feeding trial Fish (one fish + 24 hrs) treatment major (taxa %IRI > 0.5%; IRI: index of relative importance) large (> 200 μm) plankton assemblage taxa unpooled %IRI values among four pond nutrient treatments [processed (PRO) and unprocessed (UNP) feed, and cottonseed meal (CSM) and inorganic (INO) fertilizer].

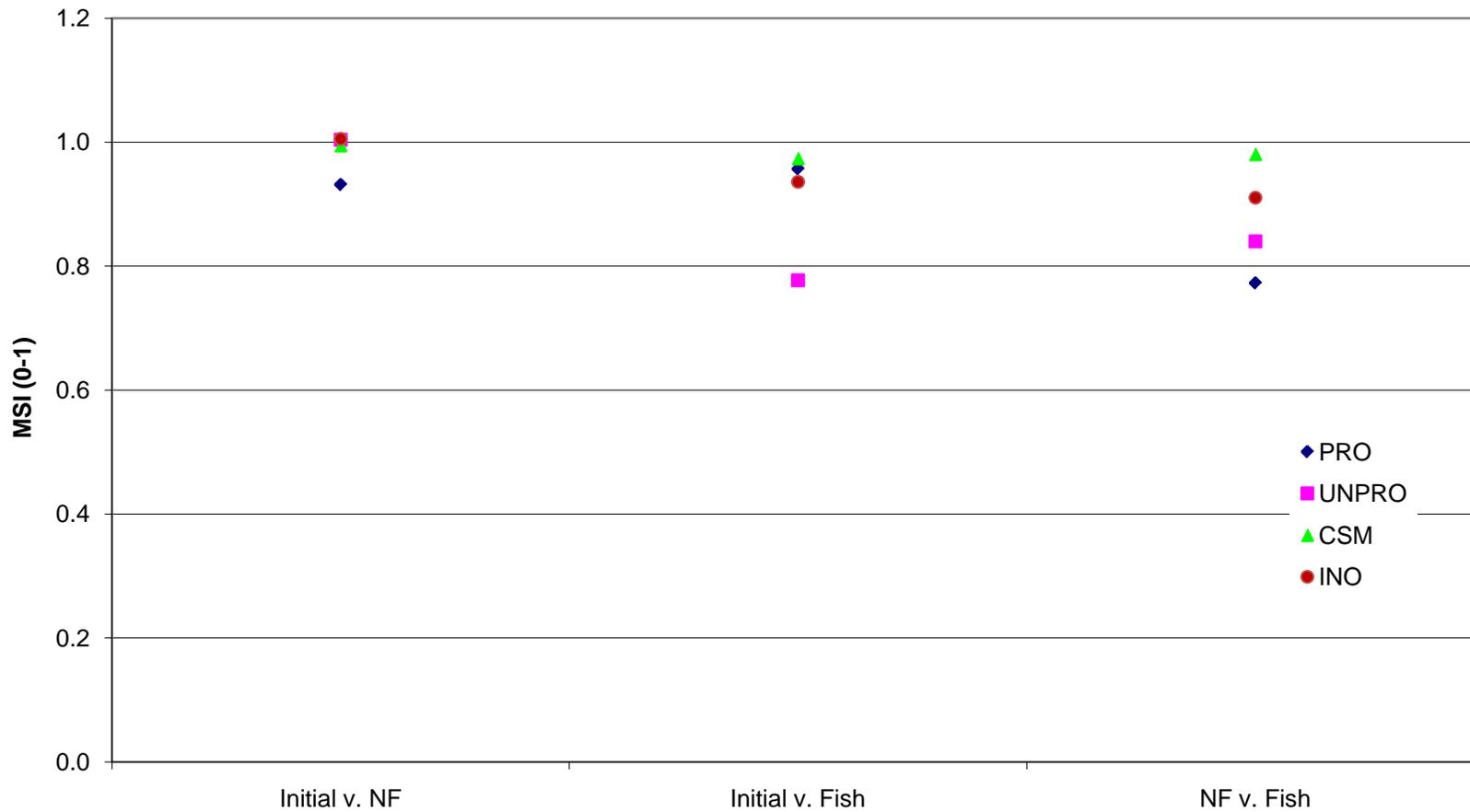


Figure 6-62. Deterministic large plankton assemblage simplified Morisita's similarity index values (using taxa %IRI) between 24-hour feeding trial treatments within four pond nutrient treatments [processed (PRO) and unprocessed (UNP) feed, and cottonseed meal (CSM) and inorganic (INO) fertilizer].

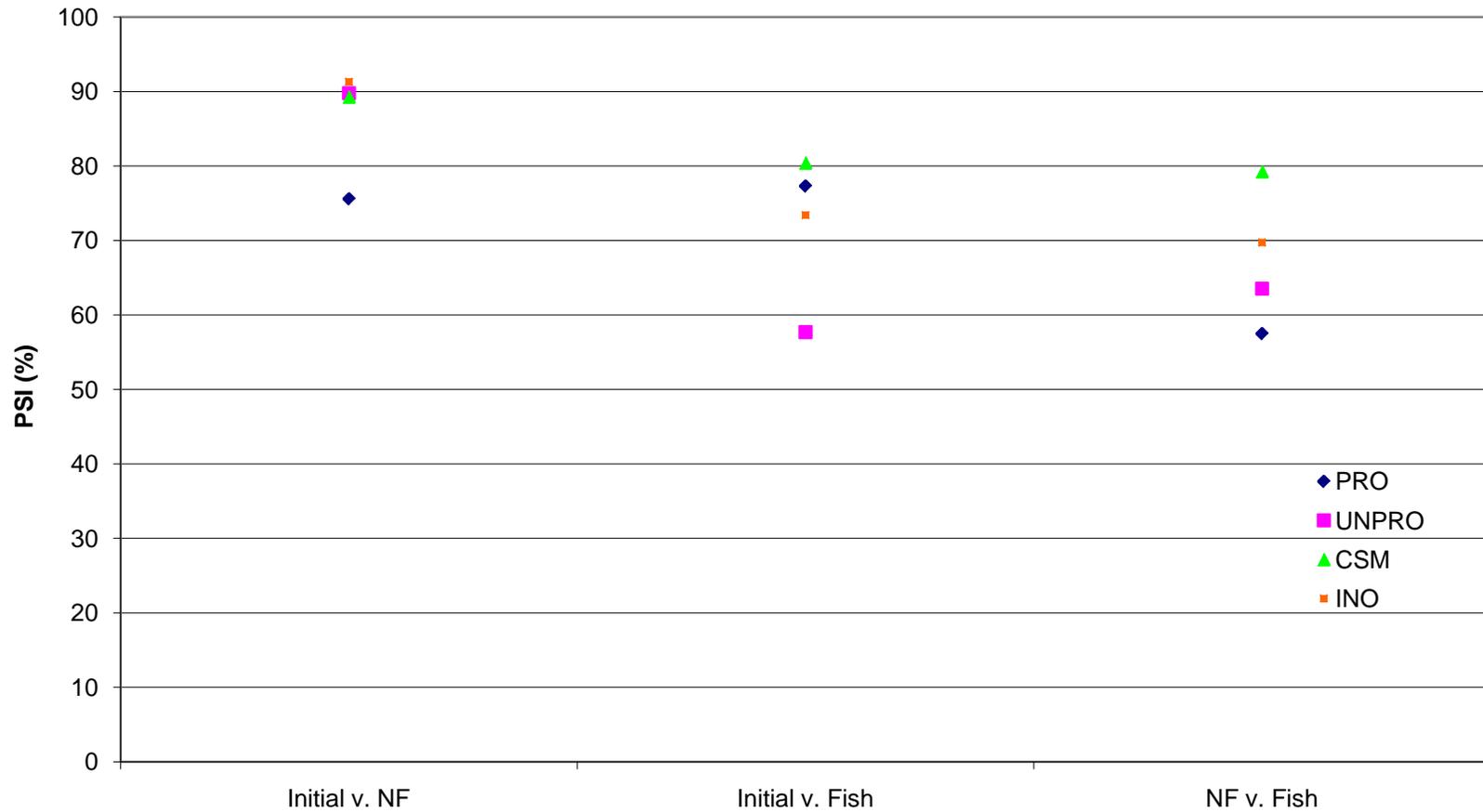


Figure 6-63. Deterministic large plankton assemblage percent similarity index values [using taxa %IRI (index of relative importance)] between 24-hour feeding trial treatments within four pond nutrient treatments [processed (PRO) and unprocessed (UNP) feed, and cottonseed meal (CSM) and inorganic (INO) fertilizer]; four replicates per treatment.

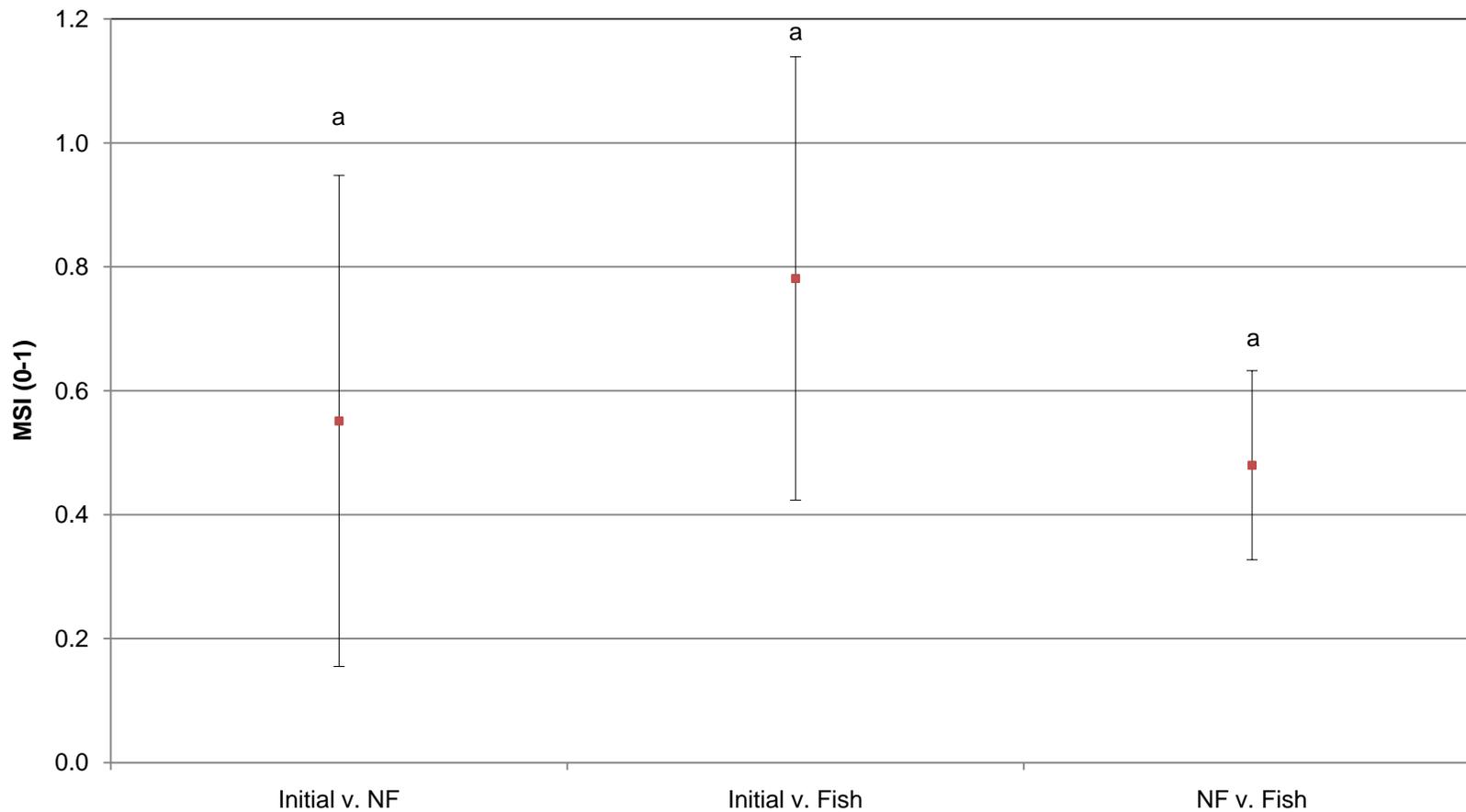


Figure 6-64. 24-hour feeding trial large (> 200 μm) plankton assemblage bootstrap (taxa % number; 1000 iterations) simplified Morisita's similarity index ($\text{MSI} \pm 95\% \text{ CI}$) between treatments within the processed feed (PRO) treatment, four replicate water samples; unshared letters denote statistical differences ($P < 0.05$, Tukey's multiple comparison test).

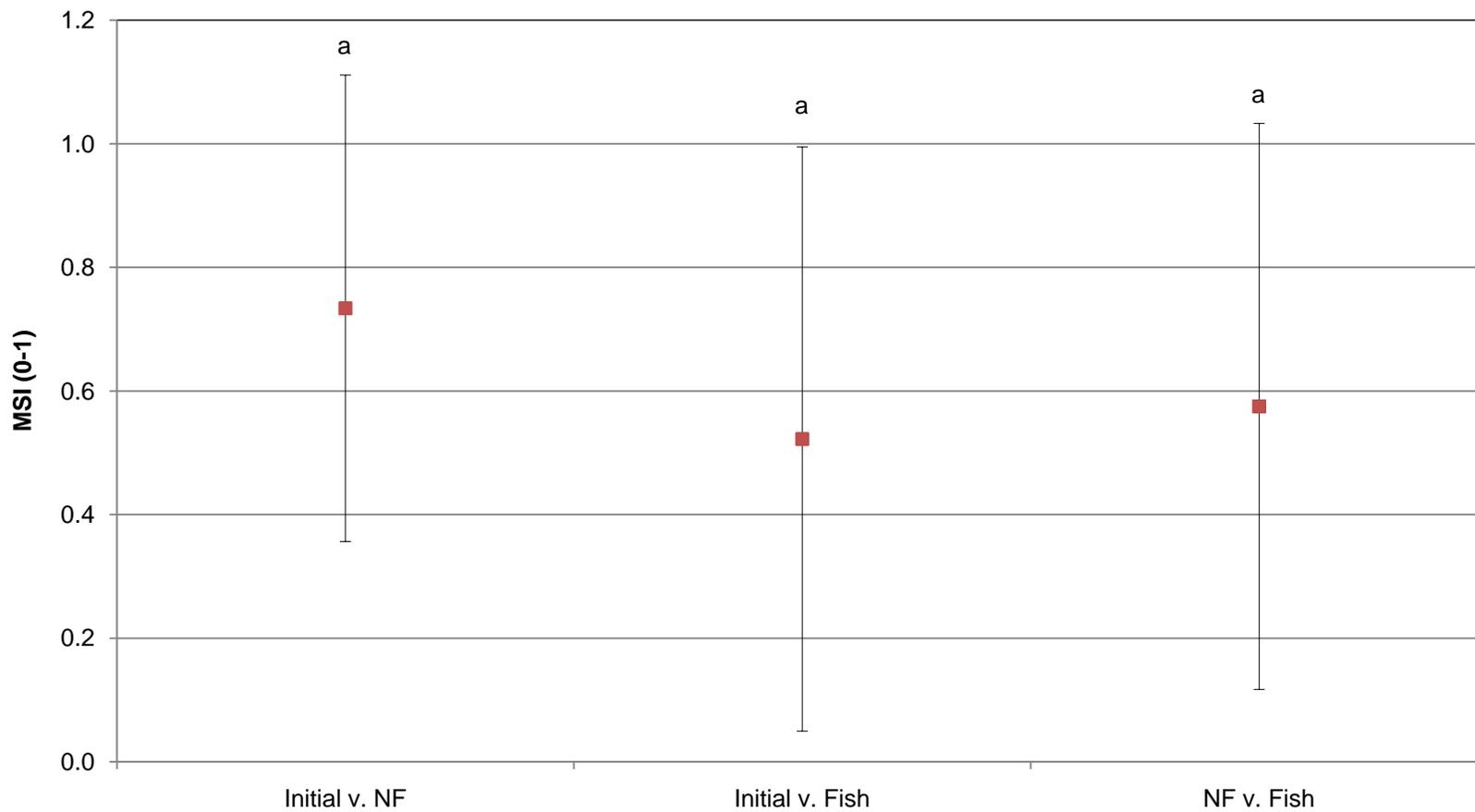


Figure 6-65. 24-hour feeding trial large (> 200 μm) plankton assemblage bootstrap (taxa % number; 1000 iterations) simplified Morisita's similarity index ($\text{MSI} \pm 95\% \text{ CI}$) between treatments within the unprocessed feed (UNP) treatment, four replicate water samples; unshared letters denote statistical differences ($P < 0.05$, Tukey's multiple comparison test).

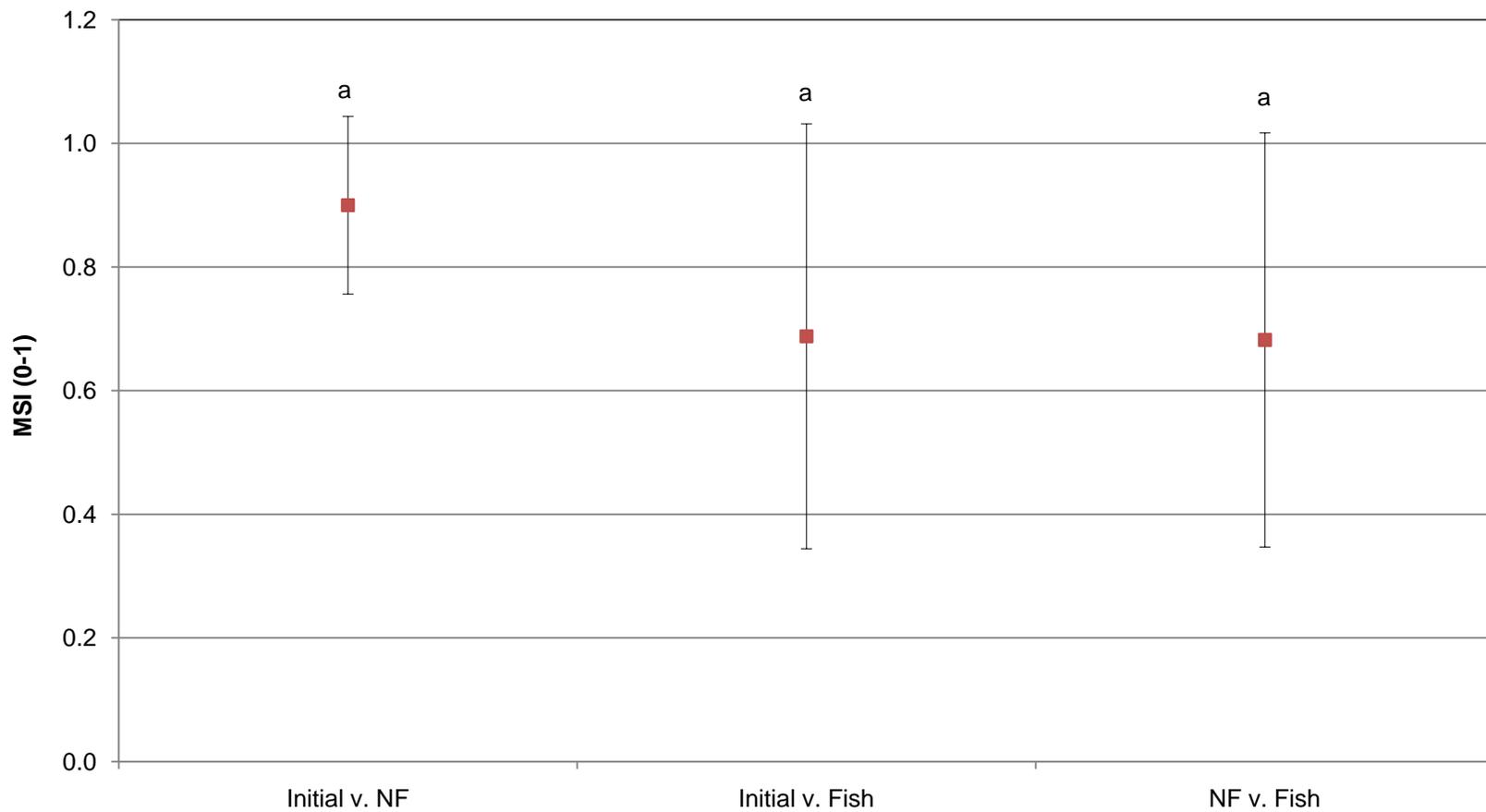


Figure 6-66. 24-hour feeding trial large (> 200 μm) plankton assemblage bootstrap (taxa % number; 1000 iterations) simplified Morisita's similarity index ($\text{MSI} \pm 95\% \text{ CI}$) between treatments within the cottonseed meal (CSM) treatment, four replicates per treatment; unshared letters denote statistical differences ($P < 0.05$, Tukey's multiple comparison test).

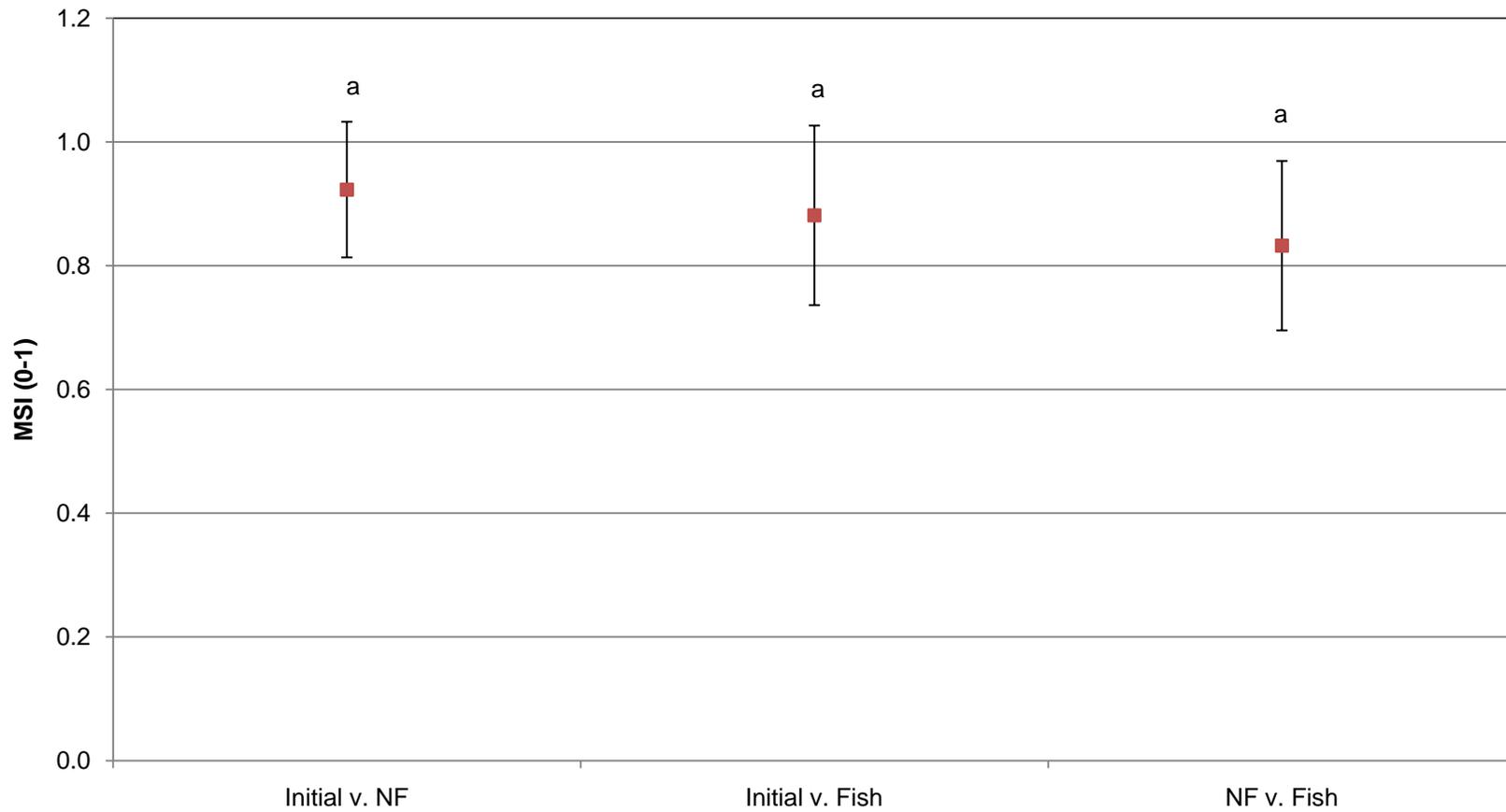


Figure 6-67. 24-hour feeding trial large (> 200 μm) plankton assemblage bootstrap (taxa % number: 1000 iterations) simplified Morisita's similarity index ($\text{MSI} \pm 95\% \text{ CI}$) between treatments within the inorganic fertilizer (INO) treatment, four replicates per treatment; unshared letters denote statistical differences ($P < 0.05$, Tukey's multiple comparison test).

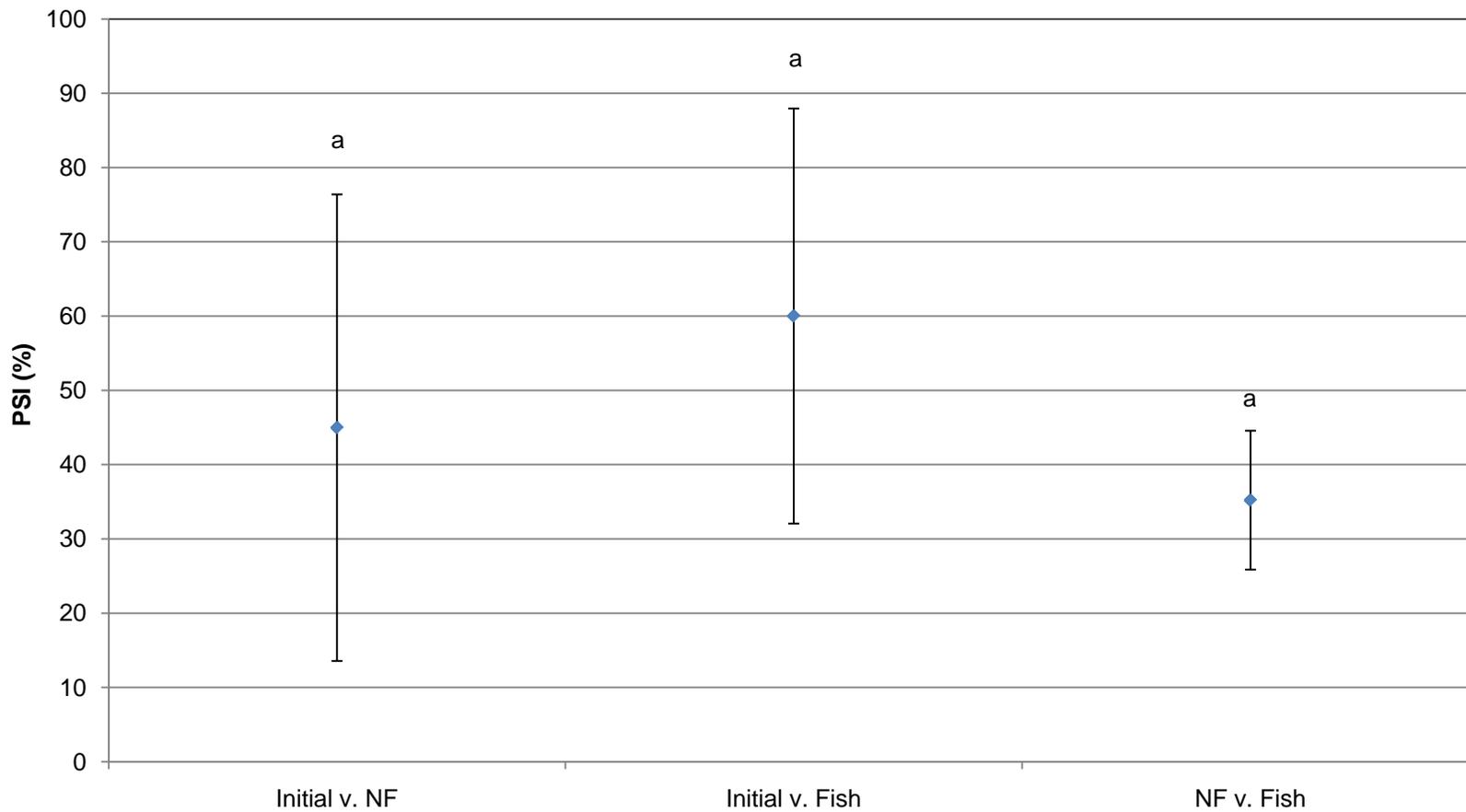


Figure 6-68. 24-hour feeding trial large (> 200 μm) plankton assemblage bootstrap (taxa % number; 1000 iterations) percent similarity index (PSI \pm 95% CI) between treatments within the processed feed (PRO) pond nutrient treatment, four replicates per treatment; unshared letters denote statistical differences ($P < 0.05$, Tukey's multiple comparison test).

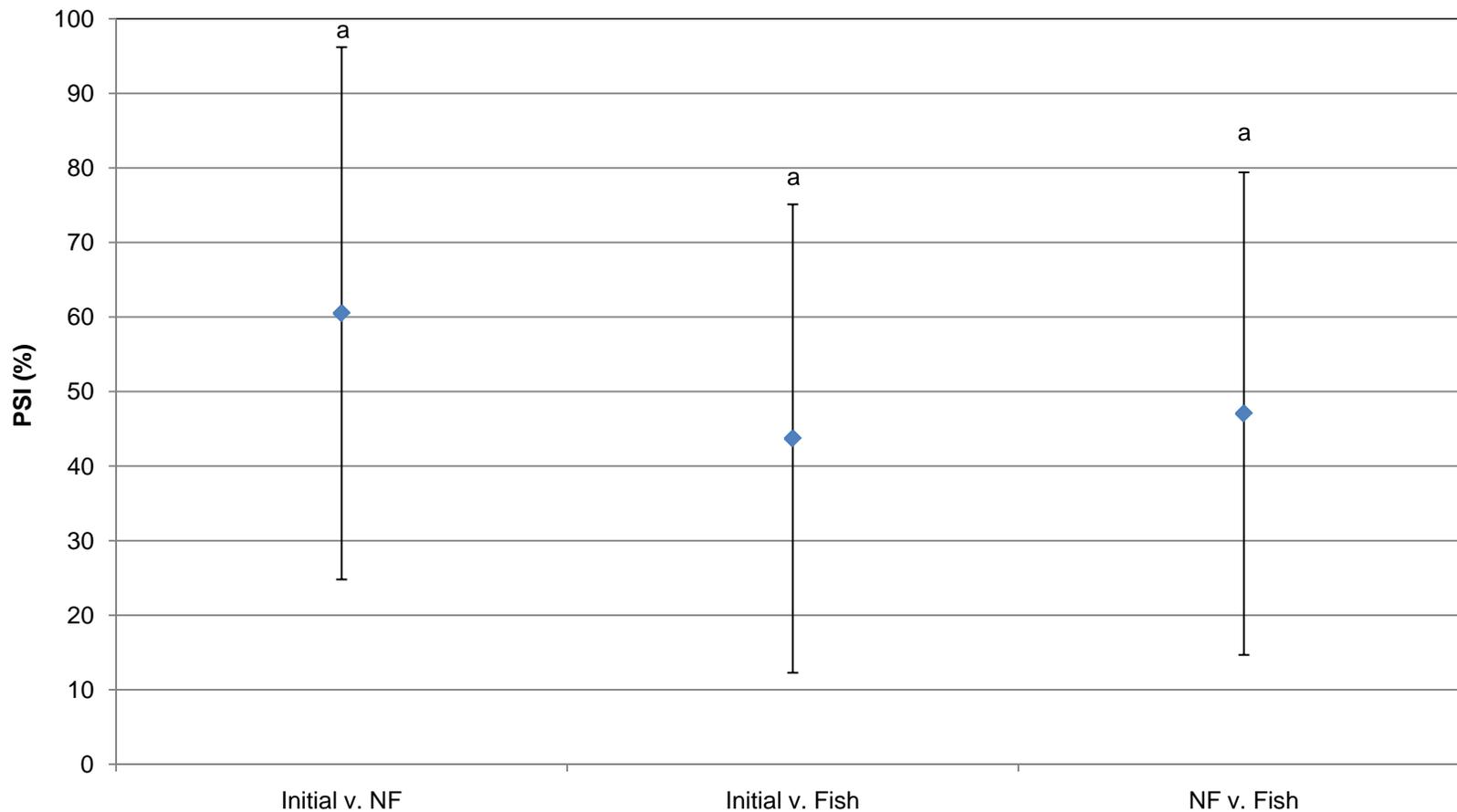


Figure 6-69. 24-hour feeding trial large (> 200 μm) plankton assemblage bootstrap (taxa % number; 1000 iterations) percent similarity index ($\text{PSI} \pm 95\% \text{ CI}$) between treatments within the unprocessed feed (UNP) treatment, four replicates per treatment; unshared letters denote statistical differences ($P < 0.05$, Tukey's multiple comparison test).

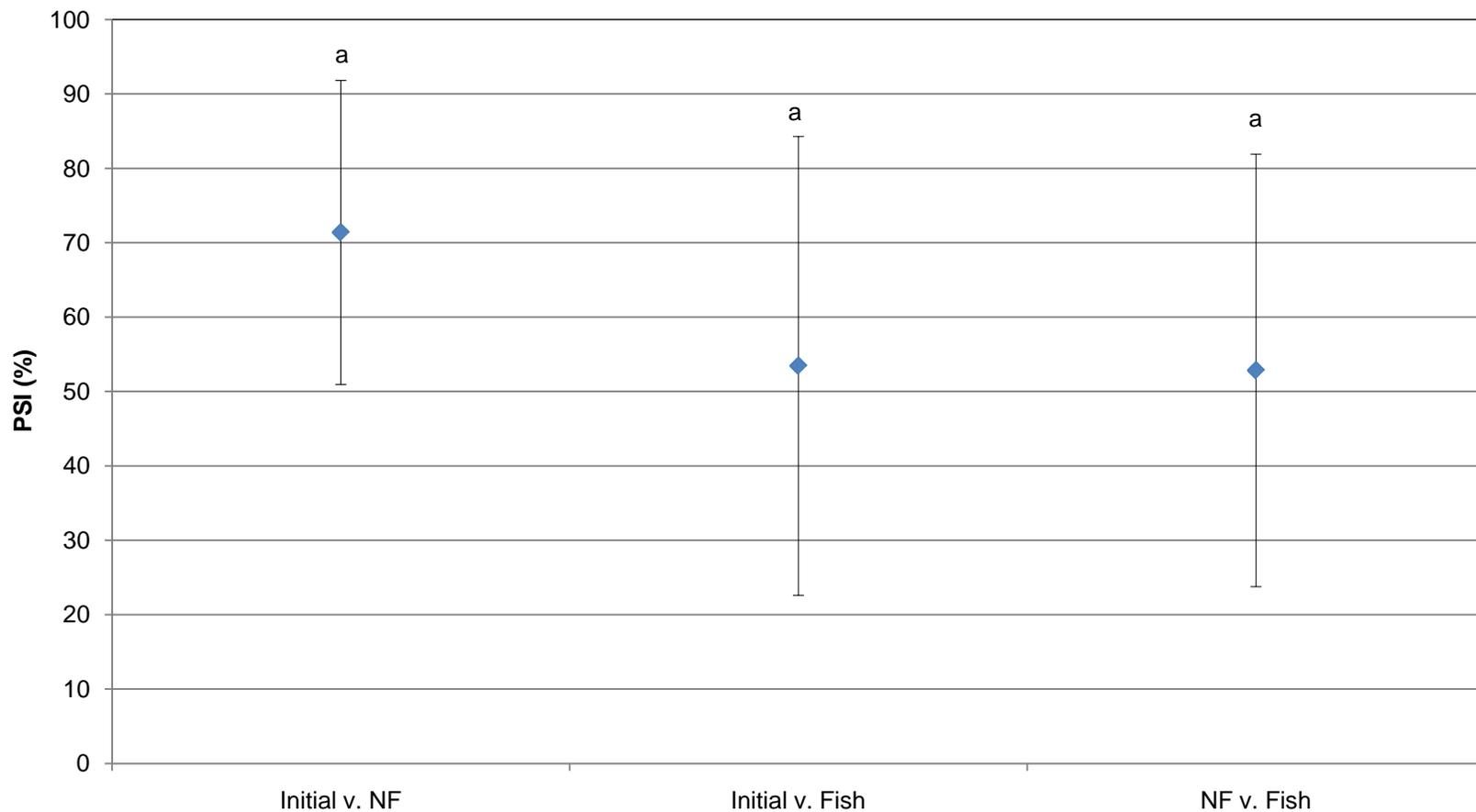


Figure 6-70. 24-hour feeding trial large (> 200 μm) plankton assemblage bootstrap (taxa % number; 1000 iterations) percent similarity index ($\text{PSI} \pm 95\% \text{ CI}$) between treatments within the cottonseed meal fertilizer (CSM) treatment, four replicates per treatment; unshared letters denote statistical differences ($P < 0.05$, Tukey's multiple comparison test).

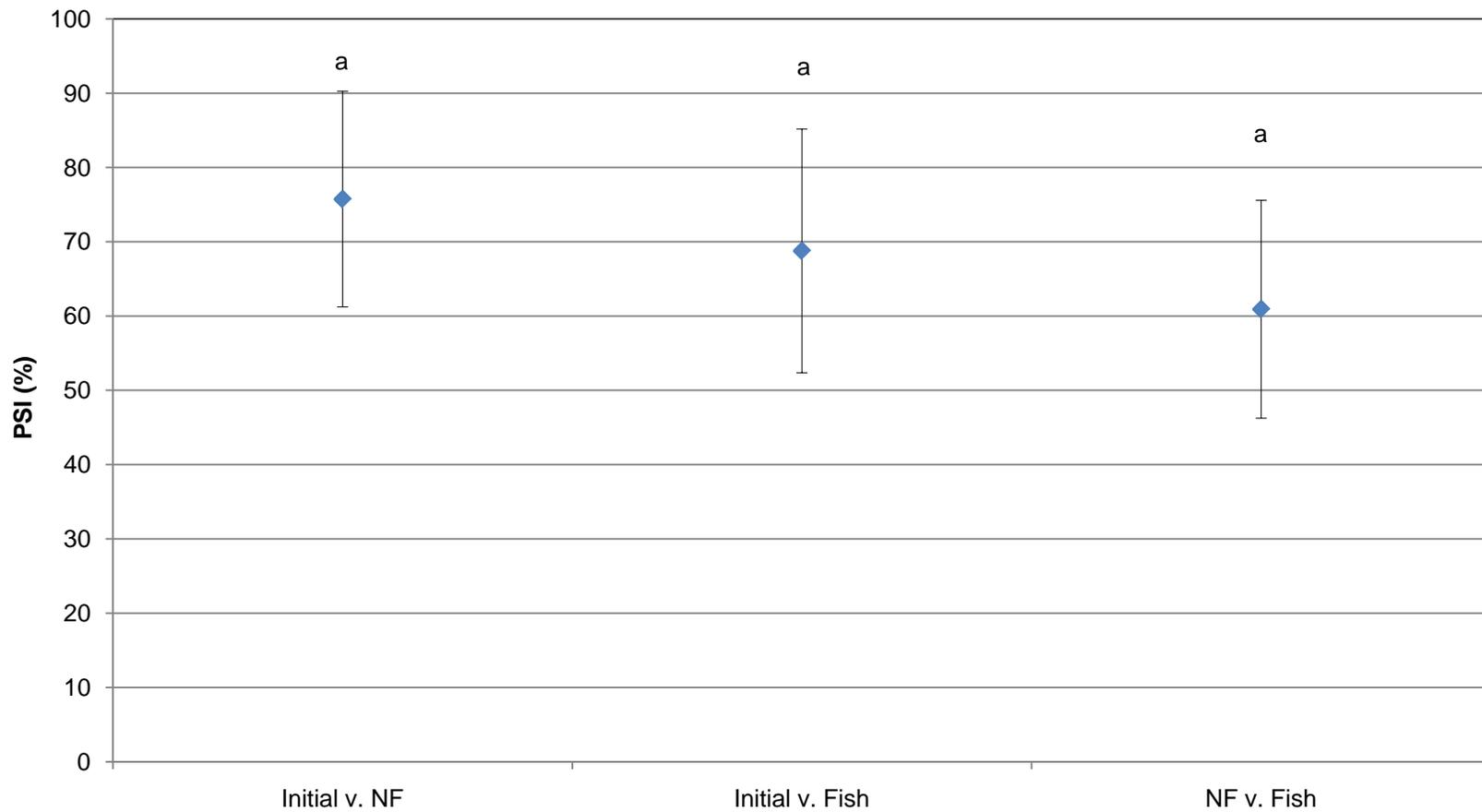


Figure 6-71. 24-hour feeding trial large (> 200 μm) plankton assemblage bootstrap (taxa % number; 1000 iterations) percent similarity index ($\text{PSI} \pm 95\% \text{ CI}$) between treatments within the inorganic fertilizer (INO) treatment, four replicates per treatment; unshared letters denote statistical differences ($P < 0.05$, Tukey's multiple comparison test).

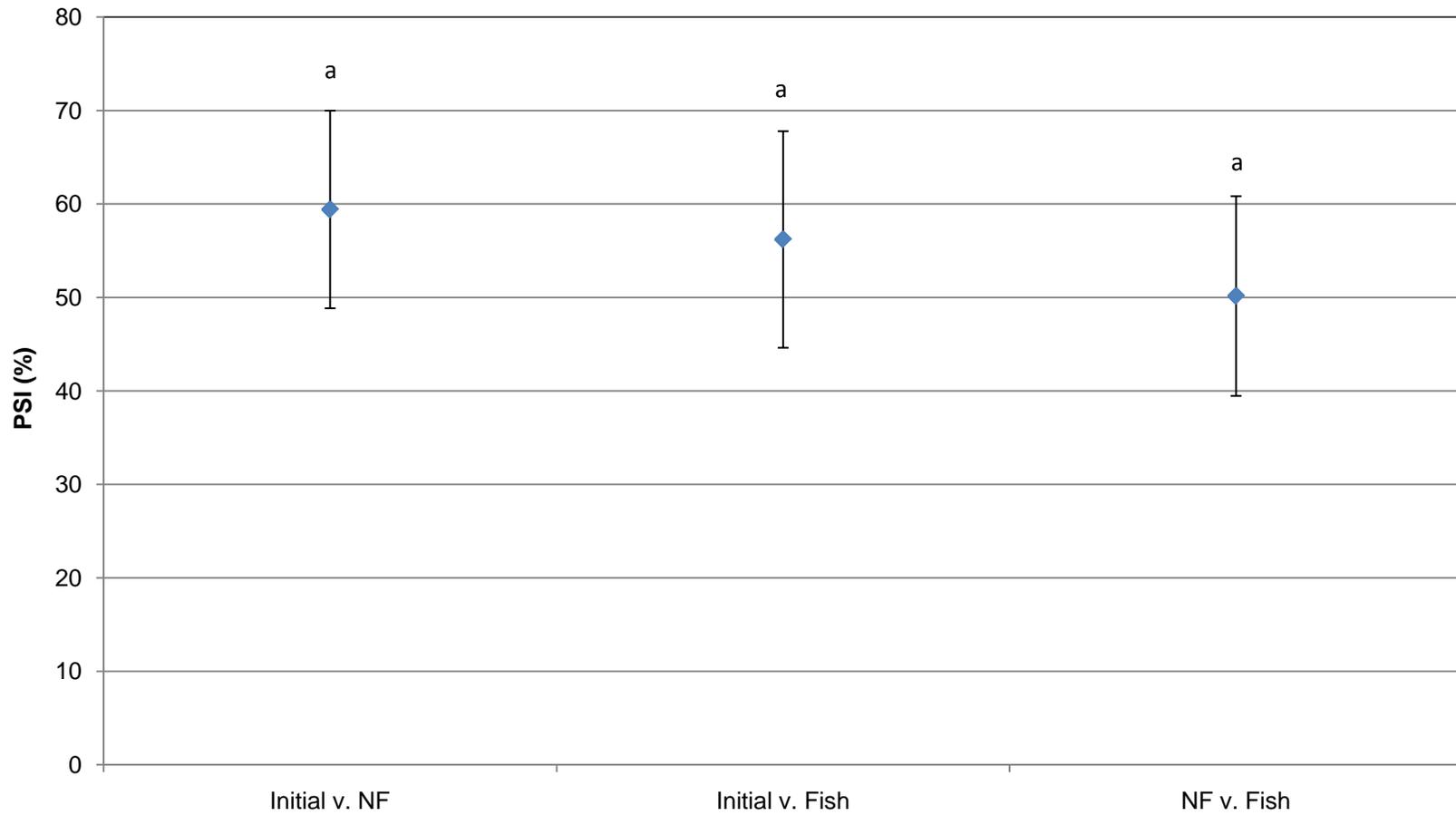


Figure 6-72. 24-hour feeding trial large (> 200 μm) plankton assemblage bootstrap (taxa % number; 1000 iterations) percent similarity index ($\text{PSI} \pm 95\% \text{ CI}$) between treatments within pooled pond nutrient treatments, 16 replicates per treatment; unshared letters denote statistical differences ($P < 0.05$, Tukey's multiple comparison test).

CHAPTER 7 SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

The overall goal of this research was to provide insight into the trophic dynamics occurring within commercial tropical fish ponds. The use of robust stable isotope measurement techniques, allowed description of the trophic interactions occurring in tropical fish ponds that had been previously based upon a vast, but incomplete knowledge base. Hopefully, this study has added scientific and practical aquaculture management information to this knowledge base.

Indoor Fry Growth and Isotopic Tracing Validation Trial

The primary objective of the indoor feeding trial was to validate the isotope tracing methodology by feeding five experimental feeds with differing isotopic profiles to swordtail fry, and determine if different fry groups subsequently developed unique isotopic profiles. An additional objective of the indoor trial was to derive carbon and nitrogen enrichment magnitudes for a single trophic level, and apply these values to outdoor pond trials to estimate trophic positions of pond taxa based upon their carbon and nitrogen isotope signatures.

Indoor Fry Growth

The 100% wheat-protein-diet fry exhibited less growth than those of the other four feed treatments from day 30 onward, and were significantly smaller than the other groups at the end of the trial (Figure 2-12). Although it seems likely that lysine deficiency was responsible for reduced fry growth within this treatment, lower feed palatability of the 100% wheat-protein-diet may have reduced feed consumption, resulting in lower growth. Unfortunately, due to *ad libitum* feeding (instead of restricted rations), and the impracticality of measuring uneaten food, it is unknown whether feed palatability or suboptimal amino acid profile was responsible for the reduced growth in the 100% wheat-protein-diet fed fry.

Again, the finding that elemental carbon to nitrogen ratios of fry did not differ among treatment groups at harvest, tends to contradict, but not rule out reduced feed palatability as a possible reason for the lower growth observed for the 100% wheat-protein-diet fry. Lack of carbon to nitrogen ratio differences suggests that the overall nutritional status of fry did not differ among treatments as nitrogen and carbon ratios are considered direct indicators of protein (nitrogen), and carbohydrate and lipid (carbon) content (Valiela 1995). Elemental carbon to nitrogen ratios suggest that the sub-optimal amino acid composition of the 100% wheat-protein-diet may have been the primary reason for reduced fry growth observed within this treatment.

The lack of differences in both fry survival rates and fry elemental carbon to nitrogen ratios among experimental feed treatments suggest that the nutritional states of fry did not differ among treatments. An overall protein, lipid and carbohydrate deficiency from malnutrition and feed aversion would likely manifest itself in survival rate differences due to a chronic protein and energy deficiency. If the 100% wheat-protein-diet fry were eating less feed due to palatability issues, it would be expected that both elemental carbon to nitrogen ratios (and therefore nitrogen content) and treatment fry survival rates would differ from fry of the other treatments due to lower total nitrogen (protein) intake (Dabrowki et al. 2007).

However, reduced palatability of the 100% wheat-protein-diet cannot be ruled out. Cold-water salmonids have been shown to differentiate lysine deficient diets from complete diets and have exhibited less growth and lower consumption on lysine-deficient diets (Yamamoto et al. 2001). In this study, lysine was present in the 100% wheat-protein-diet, but at levels considered insufficient for maximum growth (NRC 1993). Conversely, increased food consumption to compensate for low lysine feed, may have led to increased SDA (specific dynamic activity) and

greater energy expenditure in the 100% wheat-protein-diet, possibly to the point growth was reduced (Tandler and Beamish 1981, Jobling 1983).

Ultimately, the lower growth rate of the 100% wheat-protein-diet fry was of minor importance as the primary objective of the study was to validate the ability of the isotope tracing method to differentiate consumer groups that had consumed isotopically different food sources. This was demonstrated by the isotopic signature profiles of the five experimental feeds, which were isotopically ‘mirrored’ by the isotopic signatures exhibited by the pre-harvest and harvest swordtail fry (Figures 2-16 – 2-18).

Initial Fry Isotope Signature

Female broodstock, male broodstock, and initial fry carbon isotope signatures did not differ at the start of the trial, whereas significant nitrogen isotope signature differences were present between the male broodstock and initial fry (Figure 2-15). These findings clearly contrast the differing strengths and purposes of carbon and nitrogen isotope signature determinations, which helped to validate the isotope tracing methodology. Carbon signatures were highly conserved between fry offspring and broodstock females; all three groups had similar carbon isotope signatures due to their direct nutrition from the control feed (TetraMin[®] tropical flake feed) in the case of the female and male broodstock, and more indirect maternal nutrition (lecitrophic) in the case of fry. In contrast to carbon signature results, fry nitrogen signatures were significantly higher than male broodstock, and numerically higher, but not significantly higher than female broodstock. This indicates that fry (via maternal egg nutrition) were a quasi-trophic level enriched relative to male broodstock, and that fry were significantly enriched in the heavier nitrogen isotope (¹⁵N), illustrating the trophic position identifying capability of nitrogen isotope ratio determinations.

Pre-harvest fry carbon and nitrogen isotope signatures diverged quickly and in a predictable manner (Figure 2-15). Changes in isotopic signatures appeared to follow fairly linear trajectories even though pre-harvest fry isotopic profile data points were all from single replicates, demonstrating that isotopically identical fry undergo systematic changes in isotope signature following diet switching to a food, with a different isotopic profile. These results support the validity of the carbon and nitrogen isotope tracing methods.

At harvest, carbon and nitrogen isotope signatures significantly differed among the majority of treatments, except for four pairwise treatment comparisons (10 possible) for carbon and two pairwise treatment comparisons (10 possible) for nitrogen (Figure 2-18). Relative to carbon, greater nitrogen signature differences occurred among harvest fry, which likely contributed to the greater number of harvest fry nitrogen signature differences. Isotopically similar consumers largely differed after 12 weeks of consuming isotopically contrasting diets; additionally, isotopic differences of fry reflected the isotopic differences of the experimental feeds they consumed in a regular and predictable manner (Figures 2-18, 2-21).

These findings support the use of the isotopic tracing method as a valid means of determining the primary food sources of a consumer. Additionally, the observed mean carbon and nitrogen isotope signature enrichment magnitudes were well within published values for a simple, single trophic level food chain. This allowed aquaculture pond food sources to be identified with a high degree of confidence, and also allowed aquaculture pond food web organism trophic position to be identified with a high degree of confidence.

Fry Growth and Survival Rates

Paradoxically, the feed treatment that displayed the lowest overall growth (100% plant protein diet), also displayed the highest overall fry survival rate, although overall survival rates did not significantly differ among treatment groups. Increasing aggressive behavior among fry

(pers. obs.) within replicate aquaria (10 sub-sample fry at trial inception) may have been responsible for some observed mortality. As fry grew and matured, aggression among tank mates may have increased due to the onset of sexual maturity and/or physical crowding due to fry somatic growth. Lower overall growth of fry within the 100% plant protein diet treatment also may have resulted in lower rates of intra-specific aggression due to the delayed onset of sexual maturity and/or less physical crowding due to nutritional differences among the applied experimental feeds. Resulting in a higher fry survival rate at harvest for the 100% plant protein diet treatment group, again, this difference in survival rate was not statistically significant.

Indoor-Trial Experimental Design Improvements

Ten subsample fry were housed within each aquarium replicate at trial inception. Unfortunately, noticeable aggression among fry was observed in the last 2 to 3 weeks of the trial, resulting in nearly a dozen suspected mortalities (pers. obs.). Whether these mortalities resulted from overcrowding due to increased somatic growth and/or increased ontogenetic aggression is not known (Sohn 1977, Hannes and Franck 1983, Hannes et al. 1984, Dionne 1985, Franck et al. 1985, Campton and Gall 1988, Ribowski and Franck 1993, Jones et al. 1998). Ideally, and in possible future studies, it may be advantageous to have single fry housed within aquaria. Survival rates were relatively high (~80-90%) with ten fry per aquarium, and would likely be higher with a single fish per tank. Additionally, aggressive behavior and social hierarchies may have led to reduced feed intake/stunting and mortality in smaller and less dominant individuals. Using a single fish per replicate aquarium may eliminate these confounding factors, and increase the experimental rigor (experimental design orthogonality and statistical power) and statistical strength of any conclusions produced (Sokal and Rohlf 1981, Zar 1984).

Ideally, the single daily feeding would have been equally divided into two or three meals spaced into morning and evening, or morning, mid-day and evening; multiple small meals

typically result in better feed and protein utilization efficiency, and higher growth rates than a single daily feeding (Boujard and Corraze 1995, Boujard et al. 1996, Boilliet et al. 2004, G lineau et al. 1996). Unfortunately, due to the large number of man-hours required to provide weighed rations (± 0.001 gram accuracy) for daily feedings (~ 120 minutes), and alternate-day tank cleaning (~ 4-5 hrs), fry were fed only once daily between 11:00-13:00 hrs (EST).

Fry were fed at a high initial daily ration level of 15% body weight per day (dry feed wt. /wet fry wt. x 100). This level of ration was greater than the 30-minute satiation level, which is the ability of fish within a tank to completely consume a meal within 30 minutes (Lovell 1998, Halver 2003). Lower initial ration levels (below 30-minute satiation level) would have been desirable as maximal fish growth was not the primary objective of the indoor trial. The primary research objectives of the indoor trial were to produce maximum feed protein utilization in fry from five different feed treatments, and measure the resulting carbon and nitrogen isotope fractionation magnitudes between fry and their respective feed treatments, to validate feed source identification methods and calculate mean isotopic enrichment magnitudes. These objectives would have been more easily achieved and the resulting conclusions less limited without the confounding factor of *ad libitum* feeding, as equivalent nitrogen budgets may not have been established for individual replicates for all five experimental feed treatments; feed consumption rates may have differed among replicates and/or treatments, due to factors such as feed palatability, appearance, visual contrast with tank background, or tactile and olfactory cues. Unfortunately, the five experimental indoor feeds varied greatly in appearance (Figure 2-5).

The isotopic nutrient tracing and enrichment magnitude measurements for a single trophic level objectives were met with moderate success (Figure 2-21). Additional objectives, such as feed quality evaluation of the experimental feeds, would have been more easily achieved and the

resulting conclusions less limited without the confounding factor of *ad libitum* feeding, as equivalent nitrogen budgets may not have been established for fry groups among all five experimental feed treatments. Feed consumption rates may have differed among replicates and/or treatments, due to factors such as feed appearance (visual contrast with white tank background), feed tactile and olfactory cues, or swordtail behavioral issues (aggression, feeding hierarchies), etc.; for example, the five experimental indoor feeds varied greatly in appearance (Figure 2-5). Feed palatability also may have been a factor that reduced relative feed consumption rates. Reduced consumption of feeds with high plant content has been observed in many salmonid studies (Fowler 1980, Refstie 1998, Arndt et al. 1999, Regost et al. 1999, Yamamoto et al. 2007, Barrows et al. 2008). With sub-satiation ration levels, the probability that treatment fish were consuming differing amounts of feed would likely have been reduced.

Outdoor Pond Production Trial

Prior to harvest, mean fry growth was periodically monitored during the 12-week outdoor pond trial. At harvest, swordtail biomass was significantly greater within the two commercial feed treatment ponds than within the two fertilizer treatment ponds (Figure 3-9). Additionally, marketable fish (> 31 mm SL) numbers were highest within the two feed treatments, and the inorganic fertilizer ponds had significantly fewer marketable swordtails than those of the three other treatments (Figure 3-11).

Pre-harvest Fry Growth

Five to seven weeks into the 12-week outdoor pond trial, increased female broodstock recruitment from precocious female fry and higher fecundity due to increased water temperature, led to an increase in the numbers of young/small fry within study ponds, reducing mean pre-harvest fry weights. Pre-harvest fry mean weights differed among sampling dates within given pond nutrient treatments (Figures 3-5 – 3-8), but pre-harvest fry mean weights did not differ

among nutrient treatments for given sampling dates. Unfortunately, the presence of any true differences in fry growth rates among nutrient treatments were obscured by the continuous production of multiple age cohorts; also, the mathematical function describing fry growth could not be determined due to the presence of multiple cohorts and continuous spawning.

A major problem that occurred during pre-harvest fry sample collection was the tendency for larger, presumably older swordtails to congregate near the surface at the center of the pond, which prevented their capture with the sampling method employed to capture pre-harvest fry (wading along the pond perimeter with a dip net). Aggregations of larger fish were routinely observed at the surface, associating with the center aeration line that bisected ponds along the longest axis in the deepest section (Figure 3-1a,c,d).

Fish at Harvest

Lack of weight uniformity among large harvest fish within the INO ponds (Figures 3-13, 3-15), may have been a consequence of food limitation resulting in large numbers of stunted fish. Again, this would be an undesirable performance trait for an applied nutrient as uniform livestock size is desired by both wholesalers and retail buyers (Karplus et al. 2003).

Under certain situations, large numbers of small unmarketable fish may actually be desirable as future marketable stock during times when fish livestock numbers are low and wholesale prices are high. If making a choice strictly between the two fertilizers evaluated in the outdoor pond trial, cottonseed meal application alone would produce higher marketable fish yields than the inorganic fertilizer, due to the inorganic fertilizer's relatively poor fish production performance (Figures 3-9, 3-11).

Among the different metrics used to analyze pond treatments at harvest (total biomass, total fish numbers, marketable fish numbers, unmarketable fish numbers), mean marketable fish numbers at harvest was the best measure of a pond's financial value (Tamaru et al. 2001).

Among the four treatments, the two feed treatments (PRO and UNP) significantly produced more marketable fish than the two fertilizer (CSM and INO) treatments (Figure 3-11).

If commercial feed and cottonseed fertilizer application costs (costs of feed, fertilizer, and labor) are negligibly greater than inorganic fertilizer application, the harvest data tends to support the concerted use of commercial feed and periodic cottonseed meal fertilizer application.

Cottonseed meal may enhance fish production by increasing the production of supplemental live feeds, which may be important as primary feeds for fry and young juveniles (Chapters 4-6); supplemental live feeds also may provide necessary growth factors (omega 3 and 6 fatty acids) that increase somatic growth rates and broodstock fecundity, immune system boosters (vitamins, minerals, antioxidants), and color enhancing pigments that may increase ornamental fish appearance and marketability (Royes and Chapman 2003, Ezhil et al. 2008).

Although the PRO and UNP marketable fish numbers at harvest did not statistically differ, the PRO treatment mean was approximately 500 (~ 20%) marketable fish per pond higher than the UNP treatment (Figure 3-11). As marketable fish numbers are the best measure of livestock value, this makes commercial feeds an obvious choice over fertilizer application alone to maximize production. Additionally, not only did PRO ponds produce the highest numbers of large marketable fish, PRO ponds also produced the highest numbers of small unmarketable fish; large numbers of small unmarketable fish also may be desirable, as livestock that can be harvested later when market conditions are better (supplies low and wholesale prices high) or empty ponds are available for further fry grow-out ('mover' ponds). PRO and UNP treatments also displayed the lowest variations in individual large harvest (SL > 31 mm) fish weight (Figures 3-13, 3-15), which also is desirable, as tropical fish wholesalers prefer fish of uniform size, as well as appearance.

Water Chemistry and Pond Physical Environment

Water temperature increased steadily during the 12-week trial (Figure 3-16) from late March to mid-June. Increased water temperatures likely increased swordtail fecundity, growth rates and zooplankton and phytoplankton biomass, as well as pond respiration rates and reduced oxygen solubility in water (Hall 1964, Boyd 1979, 1995, Milton and Arthington 1983, Kruger et al. 2001).

Experimental Design Considerations and Further Research

Swordtails (Family Poeciliidae) were not an ideal fish species choice for this study. The reproductive life history of this species made individual fish somatic growth and fry survival rate calculations impossible using standard life table generation methods (Pianka 1970, Pearl and Slobodkin 1976, Cailliet et al. 1996, Hutchings 1993). Reproductive and survival rate calculations were not possible, due to the continuous reproduction of swordtail broodstock, and reproductive recruitment of older, precocious fry at approximately five to seven weeks of age, (Figure 3-4).

Stocking ponds with known numbers of eggs, or fry from a highly fecund egg laying fish species with an age of first reproduction greater than the 12-week study duration, would have allowed the growth of a single fry cohort to be followed via periodic sampling prior to harvest (single age cohort), as well as allowed the calculation of fry survival rates at harvest. Additionally, genetic diversity could have been reduced by using highly inbred broodstock to produce the fry/eggs used in the outdoor pond trials, increasing treatment effects on fish size and production, while reducing size variation due to genetic and age differences among fry/eggs. By using sexually immature fish of known age, and prior knowledge that excess bioenergetic resources (beyond maintenance, activity, and specific dynamic activity needs) are largely

channeled into somatic growth, stronger conclusions could be made regarding preferred pond nutrient treatment choices.

An important advantage of having a ‘no applied nutrient/extensive aquaculture’ control treatment would have been the possession of actual baseline fish production information. Baseline ‘extensive management’ fish production information could have provided a means by which to evaluate absolute fish production performances of the various applied nutrient treatments (Chapter 3, relative fish production). Because the study’s experimental design lacked a ‘no applied nutrient’ control treatment, only relative fish production rate comparisons could be made among the different experimental treatments (Chapter 3). Fish production resulting solely from applied nutrient application (absolute fish production) could have been easily calculated for each treatment by subtracting mean baseline ‘no applied nutrient’ fish production values from the mean gross fish production values measured for each of the four pond treatments (Chapter 3). Because baseline extensive aquaculture management scenario fish production values were not available, the actual increase in fish production realized from anthropogenic nutrient addition is somewhat conjecture.

Regardless of fish production increases resulting from human mediated nutrient addition, the relative contributions of autochthonous (recycled nutrients from previous production cycles, regeneration of nutrients from sediments) and non-anthropogenic allochthonous (groundwater nutrients, windblown dust, leaves, terrestrial insects, etc.) nutrient sources to total pond fish production could still not be measured and evaluated solely by the addition of a ‘no applied nutrient’ control treatment. Further information regarding non-human mediated allochthonous nutrient inputs [i.e., magnitudes (mass balance) and isotopic signatures of nitrogen within windblown dust and groundwater DIN inputs] would still need to occur before the relative

impacts of autochthonous and non-anthropogenic allochthonous nutrients sources upon pond fish production could be properly evaluated.

Isotopic Investigation of Pond Trophic Dynamics

Carbon and nitrogen isotope signatures of four applied nutrient treatments were traced to three plankton size assemblages and swordtails within 24 aquaculture ponds. Applied nutrients primarily moved in parallel to swordtails and plankton assemblages within the two feed treatments, serially within the inorganic fertilizer treatment (fertilizer → phytoplankton → zooplankton → fish), and intermediate between parallel and serial within the organic cottonseed fertilizer ponds. Additionally, swordtail yields at harvest reflected these nutrient movement patterns, with more direct movement of nutrients into swordtails (applied commercial feed treatments), producing greater overall swordtail yields at harvest (Figure 4-1). Poor fish yields, observed within the inorganic fertilizer treatment (Chapter 3), were reflected in the greater number of trophic levels believed to separate swordtails and the applied basal nutrient indicated by isotopic tracing.

Pond Nutrient Isotope Signatures

As expected, processed and unprocessed feed carbon and nitrogen isotope signatures did not differ due to their manufacture from identical ingredients. Applied cottonseed meal isotope signatures ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) differed from both feeds and from the inorganic fertilizer's nitrogen signature. The inorganic fertilizer nitrogen signature differed from the other applied nutrients, and although the inorganic fertilizer did not contain carbon, INO treatment taxa carbon isotope signatures differed noticeably from those of the other three treatments. Fortunately, these differences in nutrient carbon (or INO pond DIC) and nitrogen isotope signatures produced contrasting isotopic signatures among the respective pond taxa, of the different pond nutrient treatments. Isotopic signature differences among pond nutrient treatments for given taxa was

necessary to determine whether applied nutrients had altered pond taxa isotope signatures in the absence of baseline taxa isotopic signatures from a ‘no applied nutrient’ control treatment.

Although isotopic signature differences among treatments within a given taxa were not necessary to investigate trophic dynamics occurring within nutrient treatment ponds; within nutrient treatment trophic dynamic investigations required that taxa from different trophic levels exhibit relative isotopic signature differences. Allowing an applied nutrient to be traced as it progressed through its respective food webs/chains.

Applied nutrient isotope signature differences, and differences among treatments for given taxa, helped reduce the magnitude of the problem created by the lack of baseline taxa isotope signatures that would have been provided by a ‘no applied nutrient’ control treatment.

Unfortunately, applied nutrient isotopic signature differences did not address a major problem created by this lack of baseline taxa isotopic signature information, which was whether anthropogenic nutrient applications were of sufficient magnitude and/or nutrients were in chemical forms suitable to produce isotopic differences within given taxa, among the four different treatments (via primary producer assimilation). Whether applied nutrient applications were capable of isotopically altering the various pond taxa within the different pond treatments, was uncertain at the onset of the outdoor pond trial portion of the study.

A lack of isotopic signature differences within individual food web guilds among the different pond nutrient treatments (Figure 4-18; e.g., PRO and INO large zooplankton $\delta^{15}\text{N}$ values), would not have made it possible to reject the null hypothesis that ‘applied nutrients had no effect upon the isotopic composition of the food web components of their respective treatment ponds’. This would indicate that isotopically contrasting applied nutrients had not been assimilated by pond taxa to an appreciable degree, either because applied nutrients were not in a

suitable chemical form and/or applied nutrients had not been present in sufficient quantities to produce any noticeable effects, and allowing for the remote possibility that autochthonous or non-anthropogenic allochthonous (e.g., groundwater NO_3^-) nutrient sources were primarily responsible for biomass production within the treatment ponds, rather than the applied nutrients. As a result, only relative trophic interaction conclusions regarding taxa from different trophic levels within individual treatments can be made using taxa stable isotope signatures. If ‘no applied nutrient’ control treatment baseline taxa isotope signature values had been available, experimental treatment taxa isotope signature differences with those of the ‘no applied nutrient’ control taxa could have been used to determine trophic processes likely occurring within the various experimental treatment ponds, even if given taxa isotopic signatures had not differed among the applied nutrient treatments (e.g., PRO and INO large zooplankton $\delta^{13}\text{C}$ and/or $\delta^{15}\text{N}$).

If taxa did not isotopically differ within a given treatment, than we could not have rejected the null hypothesis ‘applied nutrients do not alter pond trophic dynamics’. It would remain uncertain whether applied nutrients were in the proper chemical form and/or amounts necessary to alter pond trophic dynamics, or responsible for any differences in fish production among pond nutrient treatments.

If pond taxa isotope signatures had not differed within given treatments, then identification of functional trophic processes occurring within the different pond treatments could not have been easily made using the stable isotope signature tracing methodology. This would have prevented answering two questions central to this study: (1) what trophic processes are occurring within the different treatment pond? (e.g., are feeds driving fish production within the feed treatment ponds?), and (2) are nutrients moving directly and efficiently to the target species or are nutrients moving inefficiently through several trophic intermediaries before (or even if)

becoming sequestered within the target species? Only empirical measurements (e.g., fish production rates) could have been used to evaluate treatment efficacies, and then only if significant differences in harvest fish production were present among treatments. This would still have left unanswered these two questions central to this study.

As previously mentioned, the problems created by the lack of external (absolute standard) baseline taxa isotope signatures, were largely offset by the presence of contrasting isotopic signatures of individual taxa among treatments. The nitrogen and carbon isotope signature contrasts provided by the INO treatment food web components relative to those of the other three nutrient treatments, acted as an 'internal standard' control (relative standard) for the outdoor trial isotope tracing methodology (Sawyer et al. 2003). The often contrasting isotopic signatures of given taxa from different pond treatments (e.g., PRO and INO large plankton assemblage $\delta^{15}\text{N}$), implies that some (if not all) of the applied nutrients altered the isotopic signatures of taxa, and by proxy, the trophic dynamics of the ponds in which they were applied.

It remains a possibility that liquid inorganic fertilizer application did not result in increased fish production. It is possible that the applied inorganic liquid fertilizer nitrogen was not in the proper chemical form and/or in sufficient quantities to alter INO treatment pond trophic dynamics. Liquid inorganic fertilizer may have immediately become bound within the sediments following application and therefore unavailable to pond primary producers.

Having greater isotopic contrasts among the applied nutrient sources would have been desirable, in that it would have allowed greater confidence to be placed in the food web interpretations and conclusions. However, high isotopic contrasts were not necessary to successfully trace pond treatment nutrients, as naturally contrasting isotopic signatures within the selected applied nutrient sources appeared to have been adequate. Additionally, although

artificially enriched or depleted nutrient sources were available, their high cost and the large quantities required for the present study made their use prohibitive.

Production biomass and isotopic measurement of incidental taxa (crayfish, tadpoles, large insects, and snails) and pond sediments also would have been useful in identifying some of the more conspicuous unintended fates of applied nutrients. This also may have allowed nutrient losses resulting from pest species production to be measured, and the identification of which species were the largest nutritional drains/sinks upon pond food webs, other than the intended swordtail target species. If given incidental fauna were isotopically identified and present in sufficiently large numbers (e.g., over a selected biomass threshold), then pond management countermeasures might be implemented to reduce the impact of these more problematic species, and hopefully increase swordtail fish production. Unfortunately, due to time and budgetary issues, incidental pond fauna were not isotopically examined nor were their production rates.

In addition to the desire for baseline fish production (Chapter 3), fish and plankton assemblage isotopic signature information, having large zooplankton community composition and relative abundance information (Chapter 5) in the absence of fish predators ('no fish control treatment'), also might have been useful in providing important insights into pond food web dynamics. Adding these two control treatments ('no applied nutrient' and 'no fish'), would have allowed a better evaluation of the effects of typical aquaculture industry pond management practices on pond food web dynamics and fish production rates. A 'no fish' control treatment would have provided large plankton assemblage biomass and community information in the absence of swordtail production. Differences in large plankton assemblage communities in the presence and absence of swordtail predation may be used to identify preferred macro-zooplankton taxa prey. Pond management strategies might then be altered to enhance production

of preferred macro-zooplankton prey, potentially increasing swordtail production. Regardless, strong inferences regarding pond food web dynamics in the absence of this information were possible in the absence of ‘no nutrient’ and ‘no fish’ control treatments.

Due to anatomical (e.g., pharyngeal teeth), life history (e.g., continuous reproduction – mixed size/age cohorts), and ecological (e.g., omnivorous generalist versus zooplanktivore specialist) aspects of the model animal, the determination of functional trophic processes that occurred within the various treatment ponds, was addressed using pond taxa isotopic signatures. Understanding functional trophic processes occurring within aquaculture ponds allows the prediction of relative production efficiencies of different pond management strategies (feeds, fertilizers, or both). Functional process models also can tell why certain strategies are better at producing fish than others, which from a scientific viewpoint, is much more interesting and satisfying than what can be learned from a simple empirical model.

Dynamical ecosystem trophic functional models were the primary objectives of this study. However, empirical models and measurements are still vital in determining what has actually occurred (input and output rates) within an aquaculture pond system, and in evaluating the validity (‘ground truthing’) of different functional ecosystem models.

Pre-Harvest Fish Isotopic Signatures

Limited isotopic analyses of consecutively sampled (weekly) pre-harvest fish followed expected trajectories, initially similar fry isotope signatures diverged among treatments over time, and fry signatures stabilized about mean values for latter sampling dates within treatments. Fry from the two feed treatments did not differ isotopically ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) for any of the three sampling dates analyzed, most likely because the two applied feeds were made from the same ingredients. Mean fry weight gains of 650 to 1,150 % initial weight (Chapter 2; indoor trial

initial fry weight value) were necessary before $\delta^{13}\text{C}$ signature differences between fry from different treatments were detectable between the PRO and INO, and UNP and INO treatments, which occurred at approximately three weeks post stocking. At two weeks post stocking, pre-harvest fry weight gains were much lower (Chapter 2; 200 to 250 % initial fry wt) and fewer pairwise carbon isotope signature differences between treatment fry were present (i.e., PRO and INO, and UNP and INO $\delta^{13}\text{C}$ did not differ at two weeks post stocking). This was roughly half of the minimum biomass gain necessary (~ 4X initial weight) to be isotopically detectable following diet switching (Fry 2006, T. Frazer pers. comm.).

During the initial weeks of the study, feed treatment pre-harvest fry may have been able to consume greater quantities of the finely ground applied feeds, due to low predation pressure from the lower density of adult fish (250 broodstock/pond) present at the onset of the study, and lower overall swordtail biomass, which may have increased feed availability for small fry and young juvenile fish due to lower competition for applied feed from within and among-fry and juvenile cohorts. The previously hypothesized greater fry and juvenile swordtail dependence upon live foods, may have taken several weeks to develop as large zooplankton standing stocks increased over time (Figure 5-1), along with the increasing fry, juvenile, and adult swordtail numbers and biomass observed at harvest (Figures 3-9 – 3-10). Increasing competition over time for feeds from other fry, juveniles, and adults, and increasing potential predation pressure (cannibalism) from adults, may have caused microhabitat shifts for both fry and juvenile swordtails to the more vegetated areas along pond perimeters, and a trophic shift to epiphytic and planktonic live foods due to reduced availability of applied feed in these areas.

Harvest Fish Isotopic Signatures

As expected, large harvest fish isotope signatures of the two feed treatments did not differ for either carbon or nitrogen. This was likely due to their consumption of the chemically and isotopically identical ingredients used to manufacture the processed and unprocessed feeds. Larger fish were regularly observed feeding (pers. obs.) upon the applied feeds and finely ground cottonseed meal at the surface in the deeper, central portions of the non-INO treatment ponds. Within the non-INO treatment ponds, smaller fish were rarely seen at the surface and may not have been able to exploit the applied feeds and cottonseed meal as readily as larger fish. Casual observations of differences in feeding behavior and micro-habitat usage between large and small fish, such as feeding at the surface, water depth, and association with plant cover, may have combined with morphological limitations such as maximum mouth gape and food particle size differences to produce the nitrogen isotope signature differences observed between co-occurring small and large fish in the non-INO treatment ponds. Additionally, during periodic pre-harvest fry sampling using a large, fine mesh 'vat' net while wading within the shallows along pond perimeters, few adult fish were captured (pers. obs.).

Only the INO large harvest fish carbon isotope signature differed from those of the other three treatments. This was interesting in that only the INO treatment food web's carbon sources were derived from autochthonous and non-anthropogenic allochthonous sources that apparently had a composite carbon isotope signature differing greatly from those of the non-INO treatment nutrients. Carbon dioxide in the atmosphere, aqueous solution, and ground water carbonates were the most likely sources of inorganic carbon for photosynthetic organisms within the INO treatment ponds (Hall 2004). Additionally, carbon released through the decomposition of organic material (leaves, cellulose, dead plants and animals), and carbon dioxide released during the respiration of pond organisms, also contributed to the dissolved inorganic carbon pool

utilized by INO treatment pond primary producers. Primary production resulting from dissolved inorganic carbon (DIC) fixed by pond phytoplankton and submergent and emergent vascular plants, formed the base of the food web within the INO treatment ponds (Smith and Walker 1980, Rounick and Winterbourn 1986, Peterson and Fry 1987, Zohary et al. 1994, Takahashi et al. 1999, Vander Zanden and Rasmussen 2001).

Within the non-INO treatments, applied nutrient carbon isotope signature differences were the result of the differing origins of their constituent ingredients: plant/animal, terrestrial/aquatic. Terrestrial cereal crops such as corn and wheat are C₄ monocotyledonous plants ($\delta^{13}\text{C}_4 = -13\text{‰}$; Boutton 1991), while cotton (*Gossypium hirsutum*) and soybean (*Glycine max*) are C₃ dicotyledonous plant (C₃ $\sim -27\text{‰}$ $\delta^{13}\text{C}$; Boutton 1991). The commercial feeds were manufactured using a combination of both C₃ (soybean meal) and C₄ plants [rice bran (*Oryza sativa*) and wheat middlings (*Triticum spp.*)] that resulted in composite carbon isotope signature values of $\sim -24\text{‰}$ $\delta^{13}\text{C}$. The cottonseed meal carbon isotope signature value was -26.43‰ ($\delta^{13}\text{C}$), which is close to the value reported for C₃ (-27‰ $\delta^{13}\text{C}$) plants in the literature (Minagawa and Wada 1984, O'Leary 1988, Finlay and Kendall 2007). Organic carbon within the INO treatment trophic guilds was most likely derived from INO pond DIC fixed via the C₃ pathway utilized by freshwater green algae (INO small plankton $\delta^{13}\text{C} = -23.26\text{‰}$).

Large harvest fish nitrogen isotope signatures did not differ among treatments, which was somewhat surprising given the conspicuous nitrogen isotope signature differences among the four applied nutrient treatments. However, given that nitrogen isotope signature enrichment rates are much larger and typically more variable than carbon isotope enrichment rates between trophic levels, the lack of nitrogen isotope signature differences at the highest trophic levels are understandable (Kling and Fry 1992, Fry et al. 1999, Vander Zanden and Rasmussen 1999, Fry

2006.). Additionally, the presence of more diffuse trophic food webs, as opposed to simple linear food chains, within the two fertilizer treatments, likely contributed to the lack of nitrogen isotope signature differences within large harvest swordtails. Nutrients within the ponds of the two fertilizer treatments, moved through more trophic levels, than within those of the two feed treatments, prior to becoming assimilated by swordtails within their respective treatment ponds. The low inorganic fertilizer nitrogen isotope signature ($\sim 0.00 \text{‰ } \delta^{15}\text{N}$) was enriched via the inorganic fertilizer pond food web to such a degree that INO large swordtail $\delta^{15}\text{N}$ signature values did not statistically differ from those of the two feed treatments. An initial nitrogen isotope signature difference of $\sim 4.7 \text{‰ } \Delta\delta^{15}\text{N}$ ($4.61 \text{‰ } \Delta\delta^{15}\text{N}$ PRO/INO, $4.88 \text{‰ } \Delta\delta^{15}\text{N}$ UNP/INO) existed between the two feed treatments and the inorganic nitrogen fertilizer.

The fewer isotopic differences present among higher trophic level taxa than among lower taxa within the four treatments, were likely due to the attenuation of isotopic differences (tracer signals) present among the applied nutrients. Tracer signal attenuation, likely resulted from zooplankton omnivory, zooplanktivore predation upon multiple trophic levels, predation within (e.g., medium size rotifers consuming medium size phytoplankton) and between plankton assemblages, including cannibalism (e.g., adult copepod predation upon copepod nauplii), as well as omnivory and cannibalism among swordtails (Dionne 1985, Jones et al. 1998, Tamaru et al. 2001).

Cannibalism among swordtails may have been particularly common within the INO treatment, as indicated by the large nitrogen isotope signature difference (trophic difference) between the two INO harvest fish size classes compared to those of their non-INO treatment counterparts. INO ponds had higher percentages of smaller, possibly stunted fish (Chapter 3; Figure 3-12). The greater coefficient of variation (Chapter 3; Figure 3-15) among individual

INO large harvest fish mean weights relative to those within the other three treatments, also may suggest low prey availability within the INO ponds compared to the other treatments (Holbrook and Schmitt 1992, 1994, Holbrook and Unwin 1997, Mittlebach et al. 1999). Greater predation on limited planktonic live foods may have increased swordtail cannibalism rates within the INO ponds.

Alternately, higher nitrogen isotope signature differences between INO small and large harvest fish (Figure 4-42) may have occurred due to the complete reliance of INO swordtails upon live foods, and fish size stunting and high size variation resulting from food limitation. Lower trophic level estimates for INO small harvest fish relative to INO large harvest fish, may have been due to ontogenetic dietary differences arising from ontogenetic microhabitat and morphological differences, such as mouth gape limitations. Food limitation may have reduced the trophic niche breadth of INO swordtail size classes, as well as producing a trophic shift among smaller fry and juveniles toward smaller, trophically lower prey items, resulting in greater swordtail size class nitrogen isotope signature (and $\Delta\delta^{15}\text{N}$ derived trophic distance estimates) differences within the INO relative to the CSM treatment. The high population percentage of small fish, within the INO ponds relative to the CSM ponds, may have resulted in greater swordtail food limitation, within the INO treatment ponds due to lower small plankton availability [Figures 3-11 – 3-12 (Chapter 3), 4-52].

Departures from a strictly linear trophic food chain (omnivory, cannibalism, etc.) may have weakened or even obliterated isotopic tracer signal differences present at the base and lower trophic levels as they progressed to the higher trophic levels within the ponds. This was evident among the harvest fish size classes, as small harvest fish isotopic signature differences among the four treatments, were no longer present among their large harvest fish counterparts.

Small Harvest Fish Isotope Differences

Unlike INO large harvest fish, which only differed from their non-INO treatment counterparts for carbon isotope signatures, INO small harvest fish differed from their non-INO treatment counterparts for both carbon and nitrogen isotope signatures. Similarly, large, medium, and small plankton assemblages displayed nearly identical carbon and nitrogen isotope signature difference patterns among treatments - feed treatment taxa and harvest fish did not isotopically differ, but feed treatment taxa frequently differed from those of the two fertilizer treatments, which frequently differed from each other. Again, this was likely due to the manufacture of the two feeds from identical materials. This suggests that, in addition to feed treatment swordtails, feed treatment plankton assemblages also were isotopically influenced by applied feed nutrients; possibly due to direct feed consumption by larger macrozooplankton, and remineralization and subsequent assimilation of feed nutrients by feed treatment pond primary producers and any heterotrophs that nutritionally utilized them within the medium and small plankton assemblages.

Swordtail Ontogenetic Dietary Differences

As previously mentioned, fry and juvenile swordtail microhabitat associations with dense plant cover along pond perimeters, may have been due to factors such as lower adult swordtail predation pressure and greater food availability. Appropriately-sized prey associated with plants, may have been limited due to mouth gape limitations of fry and juvenile swordtails. As a result, small harvest fish may have obtained a greater proportion of their nutrition from smaller, lower trophic-level live foods (e.g., greater omnivory) within the vegetation than large harvest fish that congregated at the surface in the non-vegetated deeper areas of the ponds.

Small and large harvest fish carbon isotope signature differences followed similar patterns among treatments: non-INO harvest fish carbon isotope signatures were lower than their INO

counterparts. This indicated that INO harvest fish, and the taxa they either directly or indirectly (e.g., phytoplankton) consumed, utilized a DIC-basal nutrient pool distinct from those utilized within the non-INO treatments. Additionally, the INO DIC pool acted as a ‘quasi’ no applied nutrient control for carbon isotope signature values from the non-INO treatments, as this carbon was not anthropogenic in origin. This also provided circumstantial evidence that non-INO nutrient applications were of sufficient magnitudes, and in forms (feeds, seed meal) sufficient to alter carbon isotope signatures among the various taxa within the non-INO treatment ponds.

Paradoxically, non-INO large harvest fish nitrogen isotope signatures were lower than their small fish conspecifics, suggesting that non-INO large harvest fish were trophically lower than non-INO small harvest fish, which was a non sequitur conclusion based upon what is known of fish biology and aquatic ecosystems. This could have occurred if larger fish within these treatments were primarily utilizing isotopically lower ($\delta^{15}\text{N}$) nutritional sources (applied feeds and cottonseed meal), while fry and juvenile swordtails were heavily utilizing live pond prey (plankton assemblages) that also were utilizing these same applied nutritional sources. Potential live prey may have been directly consuming applied nutrients or have been isotopically influenced by applied nutrients via primary producer assimilation of DIC and DIN derived from remineralized applied nutrients. This was supported by the finding that plankton assemblage isotope signatures (medium and large plankton) were frequently enriched (^{15}N) relative to the applied nutrient treatment (Figures 4-39 – 4-42). If ontogenetic differences in resource utilization/partitioning were occurring for non-INO swordtails for applied pond nutrients (feeds and cottonseed meal) and live pond foods. This could have resulted in small (≤ 31 mm SL) swordtails, within the non-INO ponds, being more isotopically enriched than larger swordtails ($>$

31 mm SL), resulting in smaller fish being perceived as being at a higher trophic level than larger individuals.

In contrast, due to the chemical form of the inorganic nitrogen fertilizer used within the INO ponds, INO small harvest fish were obtaining their nutrition from live foods. Because primary producers were assimilating the inorganic fertilizer nitrogen within the INO ponds, heterotrophs that utilized this primary production and those occurring further up the food chain (including swordtails), became further enriched with each increasing trophic level. As a result of the INO pond food web structure, the INO large harvest fish (apex predator) displayed the greatest nitrogen isotope signature difference (trophic distance) relative to the applied inorganic nitrogen fertilizer, followed by INO small harvest fish. This was the expected pattern of nitrogen isotope signature increase from lower to higher trophic levels within the INO ponds (monotonous increase in $\delta^{15}\text{N}$ signature).

In summary, higher small harvest fish isotope signatures, relative to large harvest fish within the non-INO treatments, were primarily due to the parallel movements of applied nutrients into the various pond taxa. Greater dependence of small fish upon live foods within the non-INO treatments may explain their higher isotope signatures relative to their large harvest fish counterparts. This gave the false impression that non-INO small harvest fish were at higher trophic levels than non-INO large harvest fish, which was not possible given what is known of fish feeding behavior and ecosystem dynamics. In contrast, the serial movement of applied nutrients through the various pond plankton taxa and possible swordtail cannibalism likely explain INO small harvest fish having lower isotope signatures than INO large harvest fish. This was an interesting finding, in that it clearly illustrated a major flaw in relying solely upon

isotopic signature results when interpreting trophic dynamics within an aquatic ecosystem and the need for additional, corroborating trophic analyses methods (Chapters 5-6).

Plankton Isotope Signatures

Non-INO plankton assemblages had lower $\delta^{13}\text{C}$ signatures than their INO counterparts. This again indicated that INO organisms were utilizing a different carbon source (DIC) than those within the non-INO treatments - applied nutrients and/or DIC derived primarily from applied nutrients.

The finding that the INO plankton assemblages (small, medium, large) had much lower nitrogen isotope signatures than their counterparts in the other three pond nutrient treatments was reasonable if the applied inorganic nitrogen was first assimilated by pond primary producers, which were then utilized by heterotrophic organisms. Inorganic fertilizer was the presumed nitrogen source for the INO food webs, and had a much lower nitrogen isotope signature than the applied nutrients of the other three treatment groups (INO - 0.08 ‰ $\delta^{15}\text{N}$), due to its manufacture from atmospheric nitrogen feedstock (Haber 1920). The lower nitrogen isotope signatures observed in the INO pond trophic guilds (plankton and harvest fish) were likely a reflection of the lower nitrogen isotope signature of the INO food web basal nutrient (inorganic fertilizer). This provided indirect evidence that INO pond organisms utilized the applied inorganic fertilizer.

There is a remote possibility that the primary nitrogen source driving production within the INO treatment ponds was actually derived from the fixation of atmospheric nitrogen via cyanobacteria (blue-green algae). If the primary nitrogen source within the INO food webs was derived from blue-green algae production, the nitrogen isotope signature of the resulting taxa would have values similar to those observed using the inorganic liquid fertilizer; again, due to the manufacture of the applied inorganic liquid fertilizer from atmospheric nitrogen. However,

this does not appear likely as conspicuous mats of blue green algae were not observed during the 12-week pond trial. A heavy infestation of filamentous green algae occurred in one pond (pers. obs.), but was quickly controlled (~2 days) by mechanical removal. Herbicide was then applied to all ponds as a preventative measure (prevent reinfestation) early in the study (diuron[®] during week 4).

It was still possible that the applied inorganic nitrogen fertilizer did not alter pond primary production rates and was not utilized by pond taxa, due to the chemical form and/or amount of fertilizer applied to ponds. Applied nutrients may have become bound to bottom sediments and become unavailable to pond heterotrophs. In the absence of a 'no applied nutrient' control, it remains uncertain whether INO treatment pond swordtail production rates are higher than those that would be realized without human mediated nutrient additions. Similarly, due to potential lack of inorganic fertilizer assimilation by INO pond autotrophs, it remains unknown whether INO treatment taxa isotopic signatures would differ from those of 'no applied nutrient' control treatment ponds.

It was assumed, that for each trophic level occurring within the INO ponds from primary producer (small plankton assemblage) to swordtails, that the nitrogen isotope signature of the subsequent trophic level would become further enriched in ¹⁵N (~ + 3.03 ‰ Δδ¹⁵N per trophic level; Chapter 2). INO trophic guild nitrogen isotope signatures did not become enriched to the point where they differed from those of their non-INO counterparts, until applied inorganic fertilizer nitrogen had reached the top predator that we isotopically examined, and had become sequestered within the INO large harvest fish (Figure 4-11).

These observations (pond taxa isotope signatures/lack of cyanobacteria growth) support the hypothesis that the primary inorganic nitrogen pool utilized within the INO ponds originated

from the applied inorganic fertilizer. They also suggest that the somewhat arbitrarily selected plankton size assemblages (via mesh sizes chosen by the author) partly coincided with actual trophic assemblages.

To some degree within all ponds, applied nutrients were consumed or assimilated by trophic groups (plankton), which in turn may have been consumed by higher trophic level heterotrophs (fish, zooplankton). In general, applied nutrients and lower trophic groups were assimilated or consumed by successive trophic groups higher up the food web. This was indicated by the carbon and nitrogen isotope signatures (Figures 4-38 – 4-42) of these various trophic groups (applied nutrients/plankton assemblages), and trophic distances estimates derived from isotope signature difference values between trophic groups (Table 4-4; e.g., feeds and feed treatment swordtails).

Interesting further research would have been to continue monitoring the isotopic signatures of plankton size assemblages after the cessation of applied pond nutrients. To observe if, and when, isotopic signatures again converged to a common set point value, or remained on different deterministic trajectories (D. Canfield, pers. comm.).

Harvest Fish and Applied Nutrient Isotope Differences

Carbon isotope signature differences between large harvest fish and their applied nutrients were lower within the two feed treatments relative to the cottonseed meal fertilizer treatment (applied inorganic fertilizer did not contain carbon). However, carbon isotope signature differences between small harvest fish and their applied nutrients did not differ among treatments. This may have been due to the high variation in carbon isotope signature results, relative to the low carbon isotope signature enrichment magnitudes typically observed between successive trophic levels ($\sim 0.5\text{‰ } \Delta\delta^{13}\text{C}$ per trophic level) within an ecosystem. Unfortunately,

these factors combine to make trophic relationship and distance estimates, based upon carbon isotope signature differences, broad and open to interpretation. This is cited as a major limitation inherent to using carbon isotope signature results alone, as an analytical tool in feeding habit studies, stressing the need for trophic relationship conclusions to be based upon both carbon and nitrogen isotope signature results. Carbon isotope signature results are better suited for tracing food web linkages and identifying potential nutrient sources, rather than taxa trophic positions (DeNiro and Epstein 1978, Minigawa and Wada 1984, Vander Zanden and Rasmussen 1999). Greater nitrogen isotope signature differences between trophic guilds, relative to differences measured for carbon isotope signature, are expected, given that nitrogen isotope signature enrichment is typically on the order of six to seven times that observed for carbon isotope enrichment for a single trophic level increase (DeNiro and Epstein 1981, Peterson and Fry 1981, Fry 2006).

The nitrogen isotope signature differences between large harvest fish and the two applied fertilizer treatments were much greater than those occurring within the two feed treatments, and more so within the INO than within the CSM ponds. These nitrogen isotope signature results were the basis for the conclusion that applied feeds were directly consumed by fish and possibly by larger zooplankton, and a basic pattern of parallel nutrient movement occurred within the feed treatment ponds. Applied inorganic fertilizer nutrient was moving serially (becoming further enriched with each trophic level) within the INO pond food web. Additionally, INO treatment nutrients were moving serially [via a trophic cascade: inorganic N_2 → phytoplankton → small zooplankton (rotifers, nauplii) → large macro-zooplankton (copepods, daphnids) → swordtails] among pond taxa to a greater degree than nutrients within the CSM ponds. This was indicated by the larger nitrogen isotope signature differences between INO treatment taxa and the applied

inorganic fertilizer, as well as the higher overall variation in nitrogen isotope signatures among the INO treatment taxa (Figures 4-29 – 4-30, 4-43). High protein cottonseed meal may have been acting as both a directly ingestible (and assimilated) feed for swordtails (pers. obs.), and via its decay, as a green manure which was enhancing swordtail live food production (via CSM food web). Due to the intermediate nitrogen isotope signature differences between small and large CSM swordtails and their applied organic basal nutrient relative to those of the other three treatments, a nutrient movement pattern intermediate to parallel and serial appears to be occurring within the CSM pond food webs. Based upon applied nutrient and pond taxa isotope signature values, functional trophic processes occurring within treatment ponds were dependent upon the form of the applied pond nutrient. These patterns appeared to apply more for large fish than for smaller fish and fry, which may have been more reliant upon live planktonic and epiphytic foods occurring within the ponds, regardless of treatment (e.g., processed feed treatment small fish).

Static isotope signature food web models assume isotopic equilibrium between predator and prey groups, and do not take into account isotopic signature changes occurring within both prey and predators (Ben-David and Schell 2001). Predator and prey isotopic signature changes can result from such factors as seasonal plankton community changes (e.g., plankton community succession), predator and prey reproductive status, prey switching due to changes in prey abundance and nutritional quality, and ontogenetic changes in predator feeding behavior (e.g., predator mouth gape), among others (Holbrook and Schmitt 1992, 1994, Cailliet et al. 1986). Unfortunately, dynamic isotope signature food web models, which are more robust in accurately predicting and explaining stable isotope signatures and their changes over time, are also more complex and require additional analyses that were not available in this study.

Isotopically Derived Fish Diet Scenario Issues

The CSM medium plankton assemblage carbon and nitrogen isotope signatures placed it directly upon a line (collinear) midway between the carbon and nitrogen isotope signature coordinates of the CSM large zooplankton assemblage and applied cottonseed meal (Figure 4-41). This made it difficult to determine whether CSM harvest fish were primarily consuming prey from the medium size plankton assemblage or roughly equal proportions of large zooplankton and the applied cottonseed meal, as both of these dietary strategies would result in similar large harvest fish isotopic signatures (Fry and Sherr 1984, Phillips 2001, Phillips and Gregg 2001, 2003, Phillips and Koch 2002). Direct observation of CSM large swordtails feeding directly upon applied cottonseed meal and that large swordtails and large macro-zooplankton aggregations co-occur at the pond surface within the central portions of the CSM ponds, tends to support the conclusion that large swordtail diet within the CSM ponds consist primarily of roughly equal proportions of large macro-zooplankton and directly ingested (and partially assimilated) cottonseed meal.

INO swordtail diet estimations derived from INO taxa isotope signature results, also might have encountered the isotopic collinearity problem (Figure 4-42), due to the isotopic resemblance of the INO medium plankton assemblage to that of a roughly equal mixture of INO large zooplankton and small plankton. An INO swordtail diet consisting primarily of medium plankton or one consisting of roughly equal parts large zooplankton and small plankton would be isotopically similar. However, the presence of isotopic collinearity among the small, medium, and large plankton assemblages within the INO treatment appeared to be a 'moot' point as the isotopic plot positions of small and large swordtails were not in close proximity to the INO small and medium plankton assemblage plot positions (Figure 4-42).

When given a choice between equally valid (isotopically) alternative diets, the determination of which diet is more likely must rely upon supplemental, non-isotopic information, e.g., direct observation of feeding behavior, gut content analysis, lipid analysis, etc. Unfortunately, not all of these methods were feasible given the biology and anatomical characteristics of the model animal, as well as a limited research budget.

Plankton Assemblage Trophic Identity Issues

The plankton size assemblages analyzed for the study, were chosen due to a combination of commercially available filter mesh sizes, information gathered from researchers in the scientific field (M. Cichra, pers. comm.), and from the published literature (Pennak 1989, APHA/AWWA 1999). Mesh sizes were not chosen according to known size thresholds for ‘phytoplankton’ versus ‘zooplankton’ assemblages, which did not actually exist due to the large overlap in phytoplankton and zooplankton cell sizes (pers. obs.; Chapter 5). The plankton assemblage derived from the largest mesh size consisted primarily of large macro-zooplankton (with some phytoplankton present; Chapter 5), while the plankton size assemblage resulting from the smallest mesh sizes consisted primarily of phytoplankton, and the intermediate ‘medium’ plankton size assemblage consisted largely of a mixture of larger phytoplankton, rotifers, zooplankton eggs, and copepod nauplii. This may have been the primary reason that clear-cut isotopic thresholds/functional trophic guilds (e.g., 1°, 2°, 3° consumer) were not observed between the different plankton size class assemblages, as there were no discernable size class ‘cut-off’ thresholds for trophic guilds and the fact that assemblages changed over time (Chapter 5). Additionally, cursory microscopic examination of samples from the medium plankton assemblage, revealed that many of the individuals present in the medium assemblage were smaller, younger zooplankton of the same taxa as those found in the large assemblage [e.g., copepod nauplii (medium plankton assemblage), and adult copepods (large plankton

assemblage)], further blurring the taxonomic, trophic and isotopic divisions among the three plankton size assemblages.

In contrast, isotopic signature values of fish, pond nutrients, and to a lesser degree the large zooplankton assemblage, appeared to be more clearly defined and assumed values expected for trophic groups from simple predator-prey systems. This was probably due to the more compartmentalized feeding ecology of a single taxon (fish) versus the multiple taxa (and likely trophic positions) within the different plankton size assemblages. The gross definitions of large zooplankton, medium zooplankton, and small phytoplankton used in this study were actually a gradation of organism sizes from varying trophic levels. Additionally, many taxa of the large zooplankton assemblage were likely consuming organisms from multiple trophic levels (i.e., primary producers, primary consumers, and secondary consumers). As a result, actual trophic positions (1° producer, 1° and 2° consumers, etc.) were not as clearly defined and closely associated with plankton size assemblages (ecologically relevant trophic levels) as desired. Potential omnivory, cannibalism and predation from multiple trophic levels within the three pond plankton size assemblages were systematically ignored to simplify pond food web structure and hypothesis testing.

Realized swordtail trophic positions (i.e., actual food web position) were almost certainly lower than their potential trophic positions (i.e., strict food chain with swordtail as top predators), possibly due to the same trophic processes previously mentioned and believed to be occurring within the pond plankton assemblages: omnivory and zooplanktivory from multiple trophic levels, and possible cannibalism.

Although only qualitatively assessed, it was interesting to note that small size plankton assemblage filtrates were usually a light to dark green color. In contrast, medium size plankton

assemblage filtrates were usually a dark brown color, and large plankton filtrates were composed of conspicuous red macroscopic organisms, usually copepods and daphnids, when filtrates were viewed on a bright white filter disk background (Figure 4-3).

A primary reason isotopically derived swordtail trophic distance estimates were non-integer values, may have been due to their omnivorous dietary habits (realized trophic position; Vander Zanden and Rasmussen 1999, Kling and Fry 1992). If isotopic signatures upon which trophic distance estimates were based, resulted largely from swordtail omnivory, then the simplified trophic food chain model (small phytoplankton → medium phytoplankton/zooplankton (rotifers/larger algae) → large zooplankton (copepods/daphnids) → small and large harvest fish) was largely invalid (potential trophic position; Vander Zanden and Rasmussen 2001, Vander Zanden and Vadeboncoeur 2002). The more complex, but still simplistic food web model was much more likely to have occurred within the INO ponds (Figure 4-45), and to a lesser degree within the CSM ponds (Figure 4-44). Additionally, the presence of zooplankton and swordtail cannibalism would cause a further departure from the simple linear food chain, requiring a more complex food web model to describe the trophic interactions occurring within the INO and possibly non-INO ponds. Unfortunately, the degree of cannibalism occurring within the INO ponds was unknown, and therefore its effects upon the isotopic signatures of INO large harvest fish (as well as non-INO large fish) are unknown.

Juvenile Swordtail Live Food Dependence

It is my belief that swordtails within all four nutrient ponds obtained a significant portion of their juvenile nutrition from live foods. This was supported by the casual visual observation that fry and juvenile fish were generally absent from surface waters, especially in the deeper sections of the ponds where floating applied feeds and cottonseed meal tended to concentrate. Ontogenetic swordtail micro-habitat differences may have required fry and juvenile fish to be

more dependent upon smaller live prey (due to mouth gape limitations and predation pressures) associated with plant cover that occurred only along the pond perimeter. Large aggregations of macroscopic zooplankton (e.g., daphnids) were often observed at the surface in deeper sections of the ponds some distance from the pond perimeters (pers. obs.). Unfortunately, a thorough survey of pond plankton and epiphytes, and their occurrence with vegetative cover, distance from shore, and depth was not performed.

The hypothesis of greater juvenile dependence upon live foods also was supported by the relative isotopic signature values of small and large harvest fish (i.e., non-INO small fish $\delta^{15}\text{N} >$ large fish $\delta^{15}\text{N}$) that may be explained as residual nitrogen isotope signals resulting from greater fry and juvenile fish dependence upon live foods. Within all pond treatments, this juvenile (ontogenetic) dietary isotopic signal, produced from smaller planktonic and epiphytic prey, may have been present within small harvest fish, and present as a latent weaker isotopic signal within all large harvest fish groups.

If trophic guilds within the PRO, UNP, and CSM ponds were largely dependent upon applied nutrients and not the interdependence of living pond organisms (trophic cascade: trophic guilds/predation,), two expected outcomes were: (1) some pond trophic guilds would have roughly equal nitrogen isotope signatures that would have been expected to have isotopic signatures that reflected trophic dependence ($\sim 3.4 \text{‰ } \Delta\delta^{15}\text{N}$ per trophic level) of one guild upon another (e.g., PRO large fish and large zooplankton $\delta^{15}\text{N}$ values were nearly equal), and (2) because fry and juvenile fish were believed to have a greater dependence upon live feeds relative to larger fish (mouthgape/predation pressure), smaller fish would be isotopically enriched relative to larger fish within the non-INO treatments. If small non-INO fish were isotopically enriched in comparison to large non-INO fish, then the estimated trophic positions of small fish

also would be higher than large fish within the non-INO treatments, due to the derivation of taxa trophic position estimates from taxa nitrogen isotope signature differences relative to their applied basal nutrient. This was the general pattern observed for non-INO small and large harvest fish, and more so within the two feed treatments than within the CSM pond treatment.

Again, parallel applied nutrient movement among phytoplankton, zooplankton, and omnivorous swordtails, and greater juvenile swordtail live food dependence, may have been responsible for producing trophic level estimates ($\delta^{15}\text{N}$ based) greater than unity for the two feed and cottonseed meal small harvest fish groups (Table 4-4). As non-INO small fish grew and entered the large fish size class, estimated small fish trophic distances to their applied nutrients (> 1.0), became less pronounced (near unity). Serial nutrient movement within the INO treatment ponds produced a monotonous increase in nitrogen isotope signature and trophic distance estimate ($\delta^{15}\text{N}$ based) as lower to higher (e.g., INO large zooplankton $\delta^{15}\text{N} <$ small swordtail $\delta^{15}\text{N} <$ large swordtail $\delta^{15}\text{N}$) trophic level organisms were isotopically measured.

Applied Nutrient Isotopic Signature Influences upon Pond Taxa

Based upon the initial alternate hypothesis, it was expected that applied feeds would be directly consumed by and promote fish production, and that applied fertilizers would need to be assimilated by various pond taxa before becoming available in forms (live prey) capable of promoting fish production. If correct, this alternate hypothesis predicts that nitrogen isotope signature differences between harvest fish and their applied nutrient treatments would be greater for the CSM and INO treatments than for the two feed treatments, as applied nutrients were utilized (and consumers became isotopically enriched) and moved serially within the food webs of the two fertilizer treatments. This is what was actually observed for the fish and isotopically analyzed pond plankton taxa within the various pond nutrient treatments (Figures 4-30 – 4-31).

In fact, nitrogen isotope signature difference patterns were identical for both small and large harvest fish size classes within all four treatments, supporting the contention that harvest fish within the four treatments, were obtaining their nutrition from the applied nutrients, and that the food webs of the two fertilizer treatments were longer than those of the two feed treatments.

Although “do fish assimilate feeds?”, and “do fertilizers promote biomass production, which fish then utilize?” were simple questions, which had obvious expected outcomes for the pond trial and from many decades of previous aquaculture pond practice and research, actually answering these questions with any degree of certainty was quite difficult (Boyd and Bowman 1997, Coman et al. 2003, Lochmann and Phillips 1996).

Biases Using the ED and IS Dietary Estimate Methods

Based upon CSM taxa isotopic signature profiles (Figure 4-41) and the two diet estimation methods (Table 4-1; ED and IS), there existed two isotopically plausible CSM large harvest fish diet scenarios: (1) primarily CSM medium plankton, or (2) roughly equal proportions of applied cottonseed meal and CSM large zooplankton; assuming equal assimilation efficiencies of the medium plankton and mixed large plankton and cottonseed meal diets. From direct observations of CSM large harvest fish feeding upon applied cottonseed meal at the pond surface, it appears much more likely that CSM large harvest fish consume (diet scenario 2) roughly equal proportions of large zooplankton and cottonseed meal.

CSM taxa isotope signatures appear to have produced erroneous CSM large harvest fish ED and IS diet estimates. Within the CSM treatment, the collinear isotopic signature plot positions of cottonseed meal fertilizer, and the medium and large plankton assemblages, may have incorrectly indicated that the CSM medium plankton assemblage was the primary prey of CSM large harvest fish (Phillips 2001, Phillips and Gregg 2001, 2003).

Without other (non-isotopic) fish dietary information, such as direct observations of fish feeding behavior, the diet consisting primarily of CSM medium plankton could not have been eliminated as a plausible CSM large harvest fish diet. Again, illustrating a major limitation of relying solely upon isotope signature based diet estimation methods (Euclidean distance/IsoSource), and the need for corroborating direct observation and/or other methods to determine fish diet and feeding behavior [e.g., gut content analysis, potential prey availability information, controlled prey choice/selection experiments, controlled feeding experiments (Chapter 6), etc.]. Additionally, a large visual predator such as the swordtail would be expected to preferentially predate upon larger macro-zooplankton, rather than smaller prey, when larger prey are present. Although cottonseed meal was indicated to be an important CSM large harvest fish dietary component using the ED and IS diet estimates (Table 4-1), without actual feed assimilation data for cottonseed meal fed to swordtails in a controlled setting (e.g., cottonseed meal feed efficiency estimates), it is not entirely certain that ingested cottonseed meal was assimilated by swordtails to an appreciable degree (Elliot and Persson 1978, Diana 1981).

Regardless of potential CSM treatment diet estimation biases due to nutrient and plankton assemblage isotopic signatures, both the IS and ED CSM large harvest fish diet estimates produced similar results (Table 4-1; ~ 55-65% medium plankton, ~15-20% large zooplankton, ~15-20% cottonseed meal). The finding that the IsoSource and Euclidean distance diet estimation methods produced similar results, even though they used different calculation methods (linear algebra versus coordinate distances), tends to indicate that the ED and IS diet estimation methods had high precision, although the accuracy of both methods appears poor when taxa have collinear isotopic signature values (Phillips 2001, Ben-David and Schell 2001).

Supplemental information such as direct observation of CSM treatment large fish actively consuming applied cottonseed meal and their microhabitat preference for surface waters in deeper areas of the ponds, proved invaluable in differentiating between two 'isotopically' plausible dietary scenarios for CSM treatment large harvest fish. INO treatment ED and IS diet estimations determined that large harvest fish primarily utilized large zooplankton, which was expected as the applied inorganic fertilizer was not directly usable by pond heterotrophs (Table 4-1). It also was not unexpected that visual predators like swordtails would be concentrating on larger macrozooplankton relative to smaller zooplankton and phytoplankton (Jones et al. 2007).

Small Harvest Fish ED and IS Diet Estimation Issues

Small harvest fish ED and IS diet estimations were much more varied than their large harvest fish counterparts within all pond treatments (Table 4-1). Additionally, small harvest fish diet composition estimates differed greatly between the ED and IS estimation methods, which may indicate an inherent limitation of using stable isotopes to estimate diets among smaller/younger individuals following diet switching within a consumer population (Fry 2006). A small difference in diet among individual fry or among fry from different replicate ponds from the same treatment will have a disproportionately larger isotopic signature effect upon smaller individuals. This is due to the lower predator to prey mass ratio of smaller fish relative to larger fish, and a greater prey isotopic tracer signal within a smaller predator. Additionally, larger/older fish are more likely to be in isotopic equilibrium with their prey sources, due to temporal variations in diet being averaged over a longer time (greater fish age) and less isotopic dilution of a larger fish's greater elemental pool/biomass due to diet changes (T. Frazer, pers. comm.). Rather than providing reasonable estimates of subpopulation diets within given treatments, small harvest fish isotopic analyses may have only produced confounding diet estimations results.

Alternately, or in conjunction with lower small harvest fish weights being responsible for higher variation among small harvest fish ED and IS diet estimates, plankton and epiphytes consumed by smaller fish within the experimental ponds may have been much more varied, both taxonomically and trophically (1° producers, 1° and 2° consumers, etc.) than food items consumed by larger fish within these ponds. Within the shallower areas where smaller fish were known to occur, greater trophic variation among the planktonic and epiphytic community was highly plausible, considering the large amounts of vegetative structure and surface area available, in contrast to the more exposed and homogenous deep water areas of the pond frequented by larger fish. For small fish, a more ecologically diverse and speciose live food base would tend to consist of organisms of greater trophic diversity (Paine 1980). Greater trophic and taxonomic variation in prey items (e.g., omnivory) would likely be correlated with greater isotopic variation in prey, leading to greater isotopic variation in any potential predators and isotopically based (i.e., ED and IS) diet estimates (Fry and Peterson 1984, Phillips and Gregg 2003).

Within the present study, the ED and IS diet estimation methods only utilized two elements, which can produce dissimilar and inaccurate diet estimations when potential prey isotopic signatures are similar or prey signatures are collinear when plotted on a Cartesian plane (Phillips 2001, Ben-David and Schell 2001). When potential prey isotopic plots are collinear along the side of a predator's isotopic resource polygon (e.g., Figures 4-39 – 4-42, 4-53), it can be difficult to isotopically discern a mixed diet composed of prey groups that are isotopically collinear to, or between vertices, and a diet composed primarily of a prey group that is collinear to, and between these two prey groups (Figures 4-46, 4-53). Unfortunately, due to isotopically similar mixtures of plausible prey organisms/nutrient sources, multiple plausible dietary scenarios can occur, which can lead to large diet estimation errors (Phillips and Gregg 2003).

Small Harvest Fish Dietary Estimate Issues

In summary, small harvest fish ED and IS diet estimate prey/nutrient importance ranking differences appeared to be the result of three potential factors: (1) isotopic similarities of prey/nutrient groups, (2) prey/nutrient group(s) isotope signatures laying along an isotopic gradient between other potential prey/nutrient groups (isotopically collinear prey/nutrient groups), and (3) low individual small harvest fish weights. The first of these factors did not appear to be an issue, as the four applied nutrients and their respective plankton assemblages appeared to be isotopically separated (Figures 4-39 – 4-42) to varying degrees. The second factor was likely an issue within both the CSM and INO treatments. Both the CSM and INO treatments had applied nutrient and/or plankton taxa that had collinear isotopic plot positions, which can produce isotopically similar composite diets from differing proportions of collinear potential nutrient sources. However, the collinear plankton assemblages were not in close proximity to the isotopic plot position of either swordtail size class. The third factor may have been occurring within the INO treatment and to varying degrees within the three non-INO treatments. Small harvest fish may not have reached isotopic equilibrium (lower fish biomass/prey ratios) with their diets, resulting in higher levels of isotopic variation among individual fish within a pond and among fish from different ponds, producing ambiguous small harvest fish dietary estimates using the ED and IS methods.

Plankton Size Assemblage and Trophic Position/Isotopic Signature Issues

Collinear isotopic plot positions for different plankton assemblages were likely an experimental artifact resulting from plankton sieve selections. Plankton size assemblages appeared to be roughly correlated with functional trophic groups (1° producer, 1°, 2° and 3° consumers, etc.) along a trophic (and isotopic) continuum from primary producer to herbivore to zooplanktivore (excluding omnivores). Microscopic examination of the three plankton size

assemblages indicated that these assemblages were not discrete trophic groups, but existed as mixed (especially medium) trophic groups (phytoplankton and zooplankton), except for the possible exception of the large plankton assemblage, which consisted primarily of large macrozooplankton (Chapter 5). Plankton size and trophic gradients, likely produced the gradual isotopic gradients (collinear isotopic signature plots) observed among the CSM (Figure 4.41; small and medium plankton assemblages), and INO (Figures 4-42; small, medium, and large plankton assemblages) fertilizer treatments.

As previously mentioned, three or more isotopically collinear plankton assemblage and/or applied nutrient plot positions, occurring along the side of a potential resource polygon (Figure 4-39-4.42), made diet estimations difficult using the ED and IS methods. Although, the use of stable carbon and nitrogen isotope signatures to answer biological and geological research questions is now commonplace and has been widely applied within many different scientific fields (geology, ecology, anthropology, oceanography, climatology, etc.), the utilization of additional elements and their isotopes (i.e., $^{32}\text{S}/^{34}\text{S}$ and $^{16}\text{O}/^{18}\text{O}$), has only recently become routine and affordable, allowing greater precision and accuracy in isotopic diet estimations (Fry 2006). By utilizing additional elements such as sulfur, hydrogen and oxygen, potential food source candidates can be plotted in three and even four dimensions, allowing implausible diets to be more easily eliminated than by the use of two-dimensional (e.g., $\delta^{13}\text{C}$, $\delta^{15}\text{N}$) isotopic signature plots (Figures 4-39 – 4-42).

An additional isotopic signature plot position issue was that INO small and large harvest fish carbon and nitrogen isotope plot positions were outside the food resource polygon connecting the large zooplankton, medium plankton, and small plankton (Figure 4-42), indicating that an important food source(s) may have been omitted (Phillips 2001, Saito 2001,

Fry 2006). Due to time and budget restrictions, emergent plant material and associated epiphytes and macroinvertebrates were not isotopically analyzed. These may have been important nutrient sources for fry and juvenile swordtails living in the INO treatment ponds (possibly within all treatments), and may warrant further study to determine if emergent plants and associated epiphytes and macroinvertebrates are important nutritional sources in the early life history of swordtails.

Another concern in estimating fish and plankton assemblage trophic positions and trophic differences using isotopic signature data within outdoor ponds, was the major assumption that indoor feed assimilation efficiencies and isotopic enrichment rates for carbon and nitrogen (Chapter 2; $\Delta\delta^{13}\text{C}$ 0.64 ‰, $\Delta\delta^{15}\text{N}$ 3.03 ‰ per trophic level) were comparable to the assimilation efficiencies and isotopic enrichment rates occurring within outdoor ponds for swordtails, applied nutrients, and various live foods. Many factors including temperature, photoperiod, predator characteristics (activity level, growth rates, dietary history, size/age, specific dynamic activity rates), food availability, diet proximate composition, diet protein to energy ratios, and prey handling time may have affected assimilation, and therefore isotopic composition.

Further research investigating swordtail live feed assimilation rates and isotopic enrichment rates using cultured live foods fed defined or partially defined nutrients (including the nutrients used in this study) in controlled feedings trials in laboratory or outdoor mesocosms, would be extremely useful in further characterizing and modeling intensive aquaculture pond trophic dynamics. This would give a more realistic approximation of aquaculture pond culture conditions, and a better understanding of tropical aquaculture pond trophic dynamics.

Small Plankton and Nutrient Isotope Signature Differences

Although it was hypothesized that feed nutrients were generally moving in parallel within the feed treatment pond taxa, carbon isotope signature differences between the PRO and UNP small plankton assemblages and their respective applied feeds did not support this conclusion. The large $\delta^{13}\text{C}$ signature differences between feed treatment small plankton assemblages and their respective applied feeds indicated that phytoplankton within these treatments were not directly utilizing the applied feeds (via ingestion) as a carbon source. Feed treatment small plankton assemblages consisted primarily of autotrophic phytoplankton that utilized dissolved inorganic carbon compounds (DIC: CO_2 , HCO_3^- , CO_3^{2-}), some of which may have been derived from remineralized and highly recycled applied feed nutrients (designed for heterotrophic consumption) released during respiration, excretion, egestion, and decay. This may have been responsible for the carbon isotope signature similarity of the two feed treatment small plankton assemblages, and their isotopic dissimilarity with the INO treatment small plankton assemblage. Photosynthetic fixation of available DIC causes autotroph carbon to be highly fractionated in favor of the lighter ^{12}C isotope as physical (e.g., diffusive fractionation) and enzymatic fractionation (i.e., ribulose biphosphate carboxylase kinetics- RuBisCo) processes are strongly biased toward isotopically lighter carbon compounds (O'Leary 1988). Nevertheless, the DIC source that is utilized by autotrophs, still influences the carbon isotope signature of the resulting primary production ($\sim -20\text{‰}$ $\Delta\delta^{13}\text{C}$ freshwater algae and inorganic carbon source; Peterson and Fry 1987). In addition to autochthonous, non-anthropogenic allochthonous DIC inputs, and remineralized allochthonous applied nutrient DIC, these carbon fractionation processes were likely responsible for the large carbon isotope signature differences observed between the non-INO small plankton assemblages and their respective applied nutrients.

Due to the presence of small plankton assemblage carbon isotope signature differences among treatments (Figure 4-28), feed and cottonseed meal application magnitudes were apparently sufficient to isotopically influence phytoplankton within the treatment ponds. Although DIC pool carbon isotope signature values were not measured for the four pond treatments, the fact that small plankton assemblage carbon isotope signatures differed among treatments implied that the dissolved inorganic carbon pools used for phytoplankton primary production also differed isotopically among treatments.

Although the outdoor pond trial lacked a ‘no nutrient’ control treatment, INO treatment taxa carbon isotope signature values, were likely close to actual baseline taxa carbon isotope signature values that would have been observed within a no applied nutrient control treatment, because the dissolved inorganic carbon, fixed and driving production within the INO pond food web was not intentionally human derived.

Further research investigating dissolved inorganic carbon pool sources, input rates, and their carbon isotope signatures would be extremely useful. Including such information as the amounts and isotopic signatures of aqueous carbonates and carbon dioxide entering the pond as groundwater and well water used to maintain pond volume losses due to seepage and evaporation; as well as autochthonous DIC derived from pond substrates, and decaying organic matter (detritus, humus). Again, carbon isotope signature information for the various plankton assemblages and fish in the absence of anthropogenic nutrient applications would be extremely important and helpful for making further inferences and conclusions about tropical pond aquaculture trophic systems, and their responses to different aquaculture management strategies. Fortunately, INO treatment taxa carbon isotope signature values served as de facto ‘no nutrient’ control treatment baseline taxa carbon isotope signatures.

Similar to non-INO small plankton assemblage carbon isotope signatures, non-INO small plankton nitrogen isotope signatures also appear to have been influenced by remineralized and recycled allochthonous applied nutrients. With the same general pattern of nitrogen isotope signature differences among treatment taxa, i.e., non-INO treatment small plankton assemblage nitrogen isotope signature values did not differ, but all differed from that of the INO small plankton assemblage (Figure 4-28).

The nitrogen isotope signature influences of applied nutrients were much more direct than that of their carbon isotope signatures, with only a minimal nitrogen isotope signature difference occurring between small plankton assemblages and their respective applied nutrient treatments (Figure 4-38) relative to the often large carbon isotope signature difference between small plankton assemblages and their respective applied nutrients (again, excluding the INO treatment which did not contain carbon). Dissolved inorganic nitrogen originally derived from applied nutrients designed for animal consumption (feeds) or as a combination animal feed and slow nutrient release fertilizer (cottonseed meal), appear to have influenced the nitrogen isotope signature of the small plankton assemblages within the non-INO treatments (Figure 4-28). Alternately, applied inorganic nitrogen fertilizer may have lowered the INO small plankton assemblage nitrogen isotope signature to a value significantly lower than those within the non-INO treatments. Additionally, both processes may have been occurring simultaneously, inorganic fertilizer application lowered INO primary producer $\delta^{15}\text{N}$ signature, and non-INO treatment applied nutrients were remineralized and present in concentrations sufficient to influence non-INO primary producer $\delta^{15}\text{N}$ signatures. Regardless of the actual mechanisms by which food web taxa were isotopically influenced by applied nutrients, applied nutrients

isotopically influenced the autotrophic phytoplankton communities within the outdoor treatment ponds.

Unfortunately, because all ponds received nitrogen from their applied nutrient treatments, it could not be determined if all applied nutrients isotopically ($\delta^{15}\text{N}$) influenced their respective taxa, or if only certain applied nutrients affected the isotopic signatures of their pond taxa. Autochthonous or unidentified allochthonous (e.g., groundwater DIN) nitrogen also may have made a large, but unmeasured contribution to all pond DIN pools. These issues again stressed the need for a ‘no nutrient applied’ control treatment to obtain baseline taxa nitrogen isotope signature values as well as baseline ‘extensive aquaculture’ fish production rates.

Baseline extensive aquaculture pond DIN pool concentration data, would have been extremely valuable in determining the overall contribution to pond DIN pools made by the addition of applied nutrients (Figure 4-46; via subtraction) and those contributions derived from autochthonous and non-anthropogenic allochthonous sources within the various treatments. Additionally, absolute contributions to plankton and fish production, resulting from non-anthropogenic sources and from applied nutrients (via subtraction) could have been determined with the presence of ‘no nutrient’ control treatment ponds.

The INO small plankton assemblage nitrogen isotope signature ($1.19\text{‰ } \Delta\delta^{15}\text{N}$) was only slightly enriched relative to its applied fertilizer (INO $-0.08\text{‰ } \delta^{15}\text{N}$), although this nitrogen isotope enrichment rate was approximately twice that observed within the non-INO treatments, it was close to the isotopic enrichment rate (depending on phytoplankton growth rates) measured for marine phytoplankton assimilating inorganic nitrogen in the western North Pacific Ocean (Wada 1980). Again, this was not surprising given that most autotrophs undergo little nitrogen isotope signature fractionation relative to their nitrogen source unless nitrogen is greatly in

excess of metabolic need and/or autotroph growth rates are extremely low (Fogel and Cifuentes 1993, Evans et al. 1996, Finlay and Kendall 2007). Other investigators have found larger phytoplankton nitrogen isotope enrichment rates, but these studies were done under laboratory settings where high nutrient concentrations were employed, and high inorganic nutrient concentrations ('luxury uptake') have been known to strongly influence (generally increase) phytoplankton isotopic signature enrichment rates (Waser et al. 1998, 1999, Needoba et al. 2003). In particular, high nitrogen availability often results in a greater nitrogen isotope enrichment magnitude between algae and the exploited inorganic nitrogen pool, due to higher nitrogen turnover rates within algal cells (Pennock et al. 1996, Cole et al. 2004, Needoba 2004, Finlay and Kendall 2007).

As mentioned earlier, fertilizer treatment ponds (CSM and INO) had higher total ammonia nitrogen (TAN) levels than those of the two feed treatments (Figure 4-46). The greater nitrogen isotope signature difference between the INO small plankton assemblage and the inorganic fertilizer may have been due to the greater availability of dissolved inorganic nitrogen (TAN) within the INO ponds relative to TAN concentrations within ponds of the two feed treatments (Figure 4-46).

Interestingly, although CSM pond DIN concentrations did not differ from those observed within the INO treatment ponds (Figure 4-46), greater nitrogen isotope fractionation was not observed between the CSM small plankton assemblage and applied cottonseed meal fertilizer. This lack of a large nitrogen isotope signature enrichment magnitude occurring between CSM small plankton and cottonseed meal may have been due to higher phytoplankton concentrations within the CSM ponds (Figure 4-49), which may have reduced the per capita DIN availability for individual phytoplankton cells. Due to nitrogen limitation, CSM phytoplankton may have had to

assimilate all available DIN, thereby undergoing little nitrogen turnover and therefore little isotope fractionation relative to their nitrogen sources occurred. CSM treatment pond DIN was believed to be primarily derived from the metabolism and decay of applied cottonseed meal protein.

Many factors can affect the rate at which nitrogen is taken up by algae and can affect the nitrogen balance and overall isotopic signatures of biological materials, including phytoplankton surface area to volume ratios, nitrogen availability, nitrogen chemical form (NH_4^+ , NO_3^- , NO_2^- , urea) within the aqueous environment, current and past history of nutrient availability, algal characteristics (species, cell size and age, growth rates, genetic differences), photoperiod, and temperature (Fogg and Thake 1987, Wada and Hattori 1978, Montoya et al. 1991, Montoya and McCarthy 1995, Valisneria 199X, Hein et al. 1995).

Interestingly, freshwater lentic phytoplankton nitrogen isotope signature values have been reported as varying from 3 to 8 ‰ $\delta^{15}\text{N}$, influenced by factors including phytoplankton population growth rates, algal species, and season (spring and summer months) in a Japanese eutrophic lake (Wada and Hattori 1976, Yoshioka et al. 1994), from 4.2 to 6.0 ‰ $\delta^{15}\text{N}$ in an oligotrophic lake located along the Sierra Nevada foothills along the California-Nevada border (Estep and Vigg 1985), and ~ 3 to 7.8 ‰ $\delta^{15}\text{N}$ in carp polyculture ponds in Southern China (est. Fig. 1; Gu et al. 1996). All reported values agreed well with the small plankton assemblage nitrogen isotope results for the PRO, UNP and CSM treatments (Figure 4-28; range 4.29 to 4.38 ‰ $\delta^{15}\text{N}$), which was surprising given the variety of climates/seasons, water body nutrient levels (oligotrophic to eutrophic), phytoplankton species and investigator plankton sieve size choices.

Again, baseline nitrogen isotope signatures of the various plankton assemblages and fish, in the absence of allochthonous nutrient application, would have been useful to better determine

actual food web processes occurring at lower trophic levels (e.g., whether dominant pond DIN source(s) were anthropogenic or non-anthropogenic). Additionally, how fish production (Chapter 3) and large plankton assemblage compositions and production rates (Chapter 5) were altered by nutrient additions, and how these factors would have been affected by the absence of anthropogenic nutrient addition, would have been extremely valuable information.

Baseline taxa nitrogen isotope signatures also may have provided greater insights into pond trophic dynamics and led to more meaningful and robust conclusions. Due to the lack of nitrogen isotopic signatures for autochthonous and non-anthropogenic allochthonous DIN pools, as well as the DIN pool sizes (concentration) being utilized by phytoplankton (small plankton assemblage) within the four pond treatments - not resulting from human invention. Conclusions regarding what trophic processes [e.g., identifying autochthonous and non-anthropogenic allochthonous DIN input source(s) and their relative contributions to total DIN] were occurring at the base of the food web within the four pond treatments were somewhat conjecture.

Additionally, because baseline fish production values were lacking, the impact of applied nutrients within the different treatments must be primarily assessed from relative empirical measurements of fish production, rather than absolute fish production measurements compared against baseline fish production in the absence of applied nutrients. Unfortunately, due to limited pond replicate availability, and the typically high variation in fish production that occurs among pond replicates (even within the same treatment), ponds that could be assigned to a 'no applied nutrient' control treatment were not available for the outdoor pond study.

Plankton Assemblage Experimental Artifact and Model Species Issues

Several experimental design modifications and additions could have been made to the outdoor pond study, which likely would have facilitated interpretation of the experimental data, giving a better understanding of aquaculture pond trophic dynamics, and an increased ability to

evaluate aquaculture pond management practices. Plankton size assemblages, resulting from sieve selection, certainly produced experimental artifacts that divided pond plankton assemblages into convenient, but artificial assemblages that were not grouped into actual functional trophic groups but rather by similar gross particle size. Physically separating large zooplankton into species or genus groupings prior to isotopic analyses would have been extremely useful in producing true trophic guilds, although this would have greatly increased the time and expense of analyses.

Poeciliid swordtails were not the ideal experimental model animal choice, due to their iteroparous reproductive life history (~ 24-30 days per brood), relatively small brood size (~ 30 fry per brood) and early age of first reproduction, which has been reported as early as six to eight weeks of age (Wischnath 1993, Tamaru et al. 2001). Continuous reproduction of pond broodstock and sexually mature older fry created numerous fry age/size cohorts with mixed genetic histories. Unfortunately, mixed age cohorts and continuous reproduction made individual fish growth rate and fry survival rate calculations impossible. Generalist dietary habits also made trophic estimates problematic, as did potential cannibalism due to large size disparities between broodstock (and older pond spawned fish) and smaller juveniles and natal fry (Jones et al. 1998, Tamaru et al. 2001).

A more ideal experimental model fish species would have had life history characteristics such as: highly fecund egg layer (high recruitment), first age of reproduction occurring at greater than three months, and highly specialized dietary habits (e.g., zooplanktivore specialist). Common ornamental fishes such as the zebra danio (*Danio rerio*), or redbelly dano (*Epalzeorhynchus bicolor*) would have been better model animals to characterize trophic dynamics occurring within tropical aquaculture ponds within the experimental design of the

present study. A large number of eggs/fry resulting from a limited number of female broodstock would have been desirable to limit the genetic and size/age variation among the fry/eggs used to randomly stock the individual treatment ponds, and any potential maternal dietary history effects (i.e., maternal nutrient isotopic signature effects). Another advantage of having only a single age cohort would have been greatly reduced potential cannibalism rates due to lower fish size disparities (Jones et al. 1998, 2007). A single age/size cohort also would have greatly aided in the determination of individual fish (somatic) growth rate calculations. Short pond trial duration, lack of reproduction within the ponds, and a single fry cohort, also would have greatly aided fry survival rate estimates for obvious reasons. Additionally, the presence of ontogenetic changes in fish trophic position estimates would likely have either occurred in a predictable pattern, or may have been entirely absent due to a lack of intraspecific predation pressures and the necessity for microhabitat differences among fish of different size/age classes due to cannibalism and/or food availability issues.

Large Zooplankton Assemblage Taxa and Abundances

Weekly large (> 200 μm) plankton assemblage taxa were enumerated, and identified to order, and farther when possible. Large zooplankton volumes and densities were calculated based upon taxa numbers and taxa geometries derived from photomicrographs. Differences in large zooplankton densities, volumes and major taxa (highest %IRI) did not greatly differ among pond treatments, but frequently differed among sampling dates within treatments. Additionally, trends in total zooplankton density and volume, major taxa %IRI, and major taxa density did not greatly differ among treatments, but were similar among treatments.

Large Zooplankton Densities

As previously mentioned, large zooplankton assemblage density and volume differences were generally absent among the four nutrient treatments for the ten weekly samples. Large

zooplankton density and volume differences among samples were more a function of time within pond treatments, rather than a function of nutrient treatment differences. Plankton densities and volumes early in the study were significantly lower than later densities and volumes due to the time required for large plankton communities to develop following pond draining and sterilization.

Time-integrated large zooplankton density and volume averages did not differ among the four treatments, indicating that large plankton standing stocks, and thus live food availabilities, probably did not differ for swordtails among the four treatments. However, this does not indicate whether swordtails within the different treatments were utilizing large zooplankton to differing degrees, since actual plankton production rate (i.e., Δ mg large zooplankton C/day) and swordtail predation rate (i.e., Δ mg large zooplankton/fish/day) values were lacking. Large zooplankton production rate may have been higher within a given treatment, but if predation upon the zooplankton was higher within this treatment, large zooplankton standing stock measurements alone would not indicate whether swordtail dependence upon large zooplankton communities differed among treatments.

The presence of a 'no fish' control treatment would have been useful in providing baseline large zooplankton standing crop information in the absence of fish predation. At a minimum, this would have allowed a gross estimate of swordtail predation rates upon large zooplankton via subtraction, and then integration over the 10 weeks. Also, the addition of a 'no fish and no nutrient' control treatment would have been useful in determining the baseline large zooplankton production rates (again via subtraction) in the absence of both fish predation and anthropogenic nutrient addition.

Unexpectedly, pond nutrient treatment was not a significant factor in determining large zooplankton volume, in contrast to sampling date, which was the primary factor determining large zooplankton standing stock volume (Figure 5-7). It was initially expected that ponds within the two fertilizer treatments would have had greater live food productivity relative to their feed treatment pond counterparts, due to fertilizer applications being intended to stimulate phytoplankton production and subsequent zooplankton production. However, greater swordtail dependence upon live foods within the fertilizer treatments, may have reduced CSM and INO large zooplankton standing stock/biomass levels below that of feed ponds. Not surprisingly, large zooplankton densities increased as a function of time, zooplankton standing stocks increased as water temperatures and photoperiods increased, and as nutrients were continuously applied on a daily (feeds and cottonseed meal), weekly or bi-weekly basis (inorganic liquid fertilizer).

Large plankton assemblage maximum density means occurred roughly midtrial for the PRO, UNP and CSM treatments, although PRO mean densities did not statistically differ among the ten weekly samples. INO large plankton assemblage density means were bimodal with a second larger peak occurring near the end of the study (Figure 5-11). Again, decreasing large zooplankton standing stocks during the latter half of the study may have been due to a combination of factors, including increased zooplanktivory, and lower phytoplankton prey availability. Lower phytoplankton prey availability also may have resulted from photoinhibition and/or self-shading by the phytoplankton community. Phytoplankton primary production also may have been reduced by higher water temperatures or higher night time pond respiration rates (hypoxia) that were suboptimal for primary production. However, phytoplankton photoinhibition does not appear to be likely due to the generally stable or increasing chlorophyll

[a] during the latter half of the twelve week trial (Figures 4-45 - 4-48). Phytoplankton self-shading may have occurred, as prominent algal blooms were present within the majority of the treatment ponds during the study (pers. obs.). Although slight decreases in chlorophyll [a] occurred during the study, this may have been due to physical (density independent) factors such as warmer water temperatures, and increased solar insolation (slight photoinhibition), as well as biological factors (density dependent) such as shading and increased nighttime respiration and possible hypoxia stress that may have resulted in smaller phytoplankton standing stocks. Zooplankton herbivory did not appear to be the likely cause for the slight decrease in phytoplankton during the last weeks of the study, due to lower large zooplankton densities starting in the last third of the 12-week trial (Figure 5-2-5.5).

Interestingly, large zooplankton assemblages did not dramatically differ among the four pond nutrient treatments over the 12-week study. Of the large zooplankton taxa that regularly occurred within the weekly samples (%IRI > 0.5%), the pattern that taxa colonized newly established ponds was highly consistent regardless of treatment. The three major large zooplankton taxa (highest %IRI rank) displayed predictable %IRI and abundance patterns (density: number/liter): copepods were the dominant group by %IRI and often by number (density), followed closely by *Filinia* rotifers, and daphnid %IRI and densities consistently ranked third.

Similar to large zooplankton assemblage standing stocks, large zooplankton assemblage taxa compositions did not appear to be correlated with any particular nutrient treatment. Time had a much greater influence upon large zooplankton assemblage taxa compositions. Not surprisingly, large zooplankton abundances and assemblage species diversity increased as the 12-week outdoor pond study progressed.

Ponds typically had large numbers of copepods and *Filinia* rotifers, which closely mirrored (inversely proportional) each other in %IRI (especially pooled %IRI; Figures 5-14-5-17), possibly due to predation of copepods upon *Filinia* rotifers. Daphnids first appeared within ponds about mid-study and apparently increased at the expense of both copepods and *Filinia* rotifers, probably due to competitive exclusion of copepods and direct predation upon *Filinia* rotifers, based on the relative sizes of these taxa.

Density counts of the three highest %IRI taxa indicated the same pattern of importance as %IRI within the large zooplankton assemblage community. Large numbers of copepods and daphnids usually resulted in subsequent decreases in *Filinia* rotifer numbers, and copepod numbers decreased as daphnid numbers increased (Figures 5-21 – 5-36).

Due to the extremely small amounts of biological material needed for isotopic analysis (Chapter 4; 600-800 μg), pooled samples separated into individual taxa, could easily be performed for future studies. Combined with time series isotopic analysis of fish predators, this would allow the determination of whether fish predators switch dietary taxa as prey abundances change, which would be expected for a generalist omnivore like the swordtail. Additionally, stable isotope signature analysis of individual zooplankton taxa could resolve the suspicion that copepods and daphnids are directly consuming *Filinia* rotifers to a significant degree.

Deterministic Large Plankton Assemblage Similarity Indices

As expected, similar large plankton assemblage taxon %IRI values resulted in high large plankton assemblage Morisita's (MSI) and percent (PSI) similarity indices values between treatments and sampling dates within treatments (Figures 5-38, 5-45). Pairwise treatment MSI trajectories displayed much greater variation among sampling dates than their PSI counterparts, and noticeably oscillated during the first six to nine weeks of the study, before plateauing to fairly constant and high values during the last few weeks (Figure 5-38).

PSI value trajectories displayed much lower variation among sampling dates than weekly MSI values, and all but one treatment pair combination were similar ($PSI \geq 60\%$). This made the PSI virtually useless, in that it did not provide any new information regarding large zooplankton assemblage communities and any differences that may have existed between them, nor how these communities may have changed over time. Additionally, the higher PSI critical value ($PSI \geq 80\%$) used by Silver (Silver 1975), did not greatly reduce the utility of the percent similarity index.

The simplified Morisita's similarity index was much more sensitive to changes in large zooplankton assemblage composition differences between treatment groups or sampling dates within treatments, changing both temporally as the study progressed and possibly in direct response to large zooplankton community perturbations (i.e., diuron herbicide application on 19 April 2006). PSI did not appear to noticeably change due to large zooplankton community changes and development over time, nor in response to diuron herbicide application, with the possible exception of the PRO and CSM PSI time series values (Figure 5-47). These results indicate that MSI was superior to PSI in determining plankton assemblage similarities within the current study (Krebs 1998).

Bootstrap Large Plankton Assemblage Similarity Indices

As expected, bootstrap similarity indices typically displayed lower variation among estimates as draw pool size and iteration numbers increased (Figures 5-52 – 5-55, 5-68 - 5-73). To observe the effects of increased draw pool size on bootstrap similarity index estimates, draw size pools were increased to 20 resampled replicates per iteration, which resulted in moderate curve smoothing as variation among resampled PSI mean values decreased (Figures 5-68 – 5-73). However, draw pool size should not be increased greatly beyond the actual number of

replicates within the original dataset, as bootstrap generated statistical parameters (e.g., mean) may become suspect when draw size is greatly increased (Simon 1997).

Interestingly, bootstrap MSI and PSI time series values, generated using taxon %N, closely resembled each other, unlike the deterministic MSI and PSI time series values generated using %IRI values. Also, unlike their deterministic %IRI generated PSI counterparts, bootstrap generated PSI time series values appeared to be sensitive to large plankton community disturbances (e.g., diuron application) as were both the deterministic %IRI and bootstrap %N generated MSI estimates. This may have been due to the use of %N for bootstrap derived estimates and the more complex %IRI for deterministic estimates. Unfortunately, bootstrap MSI and PSI estimates using %IRI values could not be generated due to the use of percent frequency of occurrence (%FO) within the %IRI, and the resulting lack of multiple datasets (replicates) from which to randomly draw (with replacement) for the generation of resampled population parameters (i.e., means, standard errors, and 95 % CI). The primary rationale for using resampling techniques to generate bootstrap similarity index estimates and error measures (Appendix 5B; resampling program) was an attempt to more objectively identify within-treatment differences in similarity values among sampling dates other than by the use of arbitrarily chosen 'critical' threshold values, 0.65 for the simplified Morisita's similarity index and 60% for the percent similarity index (Zaret and Rand 1971, Cailliet and Barry 1978, Krebs 1998, Lindquist 1998).

Large zooplankton assemblage communities did not correlate with pond nutrient treatments but did change in predictable patterns over time. Additionally, large zooplankton assemblages did not display greater similarities with similar pond nutrient types (i.e., feeds versus fertilizers). Over the course of the 12-week study, large zooplankton communities largely

stabilized among the different treatments. Both MSI and PSI pairwise treatment large zooplankton assemblage similarity values leveled off during the last three to four weeks of the study.

Large plankton assemblage biomass (density and volume) parameters and community analyses indices (%IRI, MSI, PSI) did not appear to correlate with differences in swordtail biomass production (Chapter 3) due to the lack of any clear differences in large zooplankton biomass or large zooplankton community composition with pond nutrient treatment type. It was initially believed that large zooplankton assemblage biomass and/or community composition indices would be correlated with applied pond nutrient treatments and swordtail harvest biomass differences among treatments. However, interesting and predictable trends in large zooplankton biomass and community composition did occur among weekly sampling intervals within treatments, allowing some limited insight into ecosystem functioning within the large zooplankton communities occurring within the different pond treatments.

The experimental design of the outdoor pond study was not intended to quantitatively measure the degree to which swordtails exploited available live foods (ration requirements) within the different pond treatments (e.g., serial capture and gut content analysis over diel periods; Elliott and Persson 1978, Diana 1979). Swordtail daily ration estimates from future studies might be combined with information from the current study (Chapter 4, isotopically derived diet estimates; Chapter 5, large zooplankton taxa %IRI) to provide better quantitative estimates of applied nutrient and live food resource usage. Additionally, information regarding large zooplankton and swordtail spatial distributions and microhabitat usage (depth, distance from shore, association with vegetation, etc.) within individual ponds and pond nutrient treatments, would be useful in further determining food availability differences for swordtails

due to microhabitat associations (including ontogenetic microhabitat changes) of swordtails and their potential large zooplankton prey. The integrated water column plankton sampling method employed in the current study (Chapter 4; Figure 4-2, PVC pipe sampler) attempted to account for typically patchy distributions of plankton by pooling subsamples collected from each side of the rectangular ponds. It is unlikely that this adequately accounted for actual plankton availability within the ponds, as large zooplankton aggregations were often seen at the surface midway between the pond perimeter/shallows and the floating airline bisecting individual ponds. Adult fish were often seen congregating in association with the floating airline and air stone ganglia.

Unfortunately, swordtail anatomy (pharyngeal jaws) prevented a thorough and direct survey of prey taxa exploited by swordtails within the experimental treatments. In future studies, selection of a species that lacks pharyngeal teeth may allow improved identification of gut content prey items (Stone et al. 2006, Lochmann and Phillips 1996). Suitable fish predator species selection allowing direct gut content analysis combined with potential prey isotopic analyses might provide a clearer and more robust interpretation of aquaculture pond trophic dynamics among differing applied nutrients, nutrient types (i.e., feeds, fertilizers), and pond management scenarios (e.g., twice daily feeding versus weekly and bi-weekly pond fertilization).

Future research using large zooplankton assemblage community taxa analysis and detailed stable isotope signature information of individual major large zooplankton community taxa and fishes might indicate which live prey taxa (if any) are preferred and/or provide the highest nutritional quality to the target aquaculture species. Possibly allowing aquaculture pond management plans to be designed that differentially enhance the production of pond live food taxa/communities that best contribute to commercial fish species production (Schroeder 1978,

1983, 1987, Lochmann and Phillips 1996, Kruger et al. 2001). Also, stable isotope and community analyses of the benthos may be necessary to properly describe and model tropical pond aquaculture systems, as pond benthos may make a sizable contribution to pond food webs and fish production (Fry and Parker 1979, Cifuentes et al. 1988, Yusoff and McNab 1989, Vander Zanden and Vadeboncoeur 2002, Melville and Connolly 2003, Tyler et al. 2003).

24-Hour Large Zooplankton Assemblage Captive Feeding Trials

Due to the pharyngeal jaw anatomy within swordtail buccal cavities, swordtail gut content analysis was not feasible as ingested prey is typically reduced to a homogenous bolus. It was therefore necessary to employ more indirect methods, by comparing large zooplankton biomass and community compositions in the presence and absence of a single swordtail predator after 24 hours, and with a sample that was immediately fixed with preservative following collection. Captive feeding trials were conducted within 1-liter samples from the four nutrient treatments over a 24-hour period. Three treatments were employed for this portion of the study: one liter 'no fish' for 24 hours, one liter 'single fish' for 24 hours, one liter immediately preserved upon collection.

Large Plankton Assemblage Biomass

Large plankton assemblage biomass (density and volume) did not differ among pond nutrient treatments within individual feeding trial treatments (e.g., PRO Initial and UNP Initial), but did differ among feeding trial treatments (Initial, NF, Fish) within certain pond nutrient treatments (i.e., UNP and INO). When pond nutrient treatments were pooled, biomass within the Fish treatment was significantly lower than those of the two control treatments. Two feeding trial control treatments were necessary to account for the effects of time/trial duration which likely included such factors as intra-plankton predation, and possible physiological stressors (temperature, DO, starvation, etc.) effects upon large plankton assemblage biomass (NF and

Fish). Time and fish presence, both reduced large plankton biomass, lower biomass resulting from the 24-hour duration of the trial alone (NF), was likely due to both intra-plankton predation and physiological stressors, but only fish presence (Fish) resulted in significantly lower biomass measurements (unpooled data: PRO and UNP treatments only). This finding indicated that the presence of a single swordtail fish predator over a 24-hour period was sufficient to lower large plankton biomass, most likely as a direct result of fish planktivory.

When densities of the three major large plankton taxa were examined, taxa densities were lower within the Fish experimental treatment than within those of the two other treatments. Major taxa densities within the Fish were not significantly lower across all pond nutrient treatments, but copepods were significantly lower for the INO, and daphnid densities were significantly lower for the UNP and INO treatments.

When pond nutrient treatments were pooled, copepod and daphnid densities decreased among the three feeding trial treatments in the following order: Initial, NF, and Fish. The Fish treatment had significantly fewer copepods and daphnids than the two control treatments, which did not differ from each other. This indicates that swordtails were an effective predator, given the experimental conditions for the captive feeding trial (24-hour trial duration, 1-L pond water sample, one swordtail predator, no aeration, etc.).

Among all captive feeding trial replicate large plankton assemblages, *Filinia* numbers were much lower than those of copepods and daphnids, which may have been why rotifer density analysis provided inconsistent results among pond nutrient treatments and among the three feeding trial treatments. Significant differences in rotifer density only occurred among the captive feeding treatments within the PRO pond treatment (Figure 6-42). Not surprisingly, pooled *Filinia* density averages also did not differ among feeding trial treatments. Again, this

was likely due to the small rotifer densities present among the different experimental treatments. Low *Filinia* numbers may have been due to seasonal effects, as the feeding trials occurred in mid-September 2007, whereas the outdoor pond trials occurred from late March to mid June 2006.

Interestingly, daphnids were preferred prey for swordtails, as indicated by changes in relative copepod and daphnid %N and %IRI values among the Initial, and both 24-hour incubation treatment groups [NF (no-fish) and Fish]. Copepod % number and % volume were markedly higher than daphnid % number and % volume within the Fish treatment relative to these parameters within the Initial and NF treatments. Additionally, copepod %IRI was much higher following fish predation than prior to (Initial) or in the absence of fish predation (NF), although absolute copepod numbers were still reduced within the Fish treatment large plankton assemblages, likely as a result of fish predation. These taxa differences were much more apparent within pooled pond nutrient treatment comparisons of feeding trial data.

Pooling data is not an ideal strategy for data analyses and several limitations and problems associated with data pooling must be addressed. Simply increasing sample size can produce a statistically significant difference even when the difference has no biological meaning (Zar 1984, Krebs 1998). However, this usually entails increasing sample sizes to extremely high numbers (>100), while pooling data for these experiments only increased sample size from four to sixteen. Because the 24-hour feeding trial experimental design was constructed without the consideration that data might be pooled among pond nutrient treatments, pooling treatment groups that were not biologically equivalent was a possibility; to partially compensate, significance level (α) can be lowered when pooling data in an attempt to increase the rigor of conclusions based upon analyses of pooled data, however, this increases the probability of a Type II (not rejecting a false

null H_0) statistical error (Ott et al. 2000). Adjusting the significance level was not deemed necessary, as Initial large plankton assemblages derived from different pond nutrient treatments, qualitatively resembled each other (community composition, taxa %IRI, % numbers, and % volumes), indicating that fundamental differences in large plankton communities did not appear to exist among pond nutrient treatments. Additionally, the objective of the 24-hour captive feeding trial was not to compare large plankton assemblage biomass and communities among pond nutrient treatments *per se*, but to compare these parameters in the presence and absence of swordtail predation.

Pooled copepod densities displayed the same significance pattern observed when pooled large zooplankton volumes were compared among the three feeding trial treatments. Copepod densities, in replicates incubated with fish for 24 hours, decreased to the point that this treatment had significantly fewer copepods than either the Initial or NF (no-fish) 24-hour controls. Although single fish were able to significantly reduce copepod densities (assumed via grazing) in 1-L incubation jars after 24 hours, the actual density of fish within the ponds almost certainly differed from that used for the 24-hour captive feeding trial (i.e., one fish per liter). Additionally, fish and zooplankton are not evenly distributed within ponds; larger fish were routinely observed aggregated in schools near the surface, with smaller fry and juveniles among the emergent vegetation along the perimeter of the pond. Macrozooplankton were observed in large aggregations at the pond surface midway between the pond center and shore. Again, the primary purpose of the 24-hour captive feeding trial was to evaluate and validate the potential for swordtails to produce a detectable grazing/predation pressure upon captive zooplankton assemblages.

Bootstrap estimates of large plankton community MSI and PSI, using large plankton taxa % number values, were more erratic than their deterministic %IRI MSI and PSI counterparts. For example, large numbers of extremely small *Pediastrum spp* within the UNP Fish experimental treatment, and low *Pediastrum spp.* density in its INO Fish treatment counterpart (Figures 6-6, 6-12), were likely responsible for the observed significant difference between Initial and Fish MSI values of the UNP and INO treatments that otherwise did not appear to greatly differ in taxa composition or relative taxa % numbers or % volumes.

Interestingly, MSI values differed among pond nutrient treatments within a given captive feeding trial treatment-pair comparison for some pond nutrient treatments (e.g., Initial and NF UNP, and Initial and NF INO), whereas no differences among pond nutrient treatments within feeding trial treatments were found for PSI. Again, PSI may be less sensitive to slight taxa % number differences among large plankton assemblage comparisons relative to MSI (Chapter 5 Discussion/Conclusion). This suggests that the use of % number was inferior to % IRI for many of the reasons described by Cortes (Cortes 1997, E. Cortes, pers. Comm.) in that large numbers of small taxa can skew MSI and PSI values disproportionately to their importance within the population being analyzed (i.e., large plankton community).

The captive feeding trial experiments were designed to qualitatively evaluate potential swordtail predation effects upon large plankton community taxa under controlled conditions. It was shown that a single swordtail can reduce the overall taxa densities, bio-volumes, and alter community composition (i.e., feeding preferentially upon daphnids) within a small volume of pond water within a 24-hour period. To better quantify swordtail feeding behavior, several simple experimental trials also could be performed. Different potential prey taxa handling time comparisons, and diet preference/dietary choice trials could be performed in simple aquaria, or

feeding behavior choice trials could be performed within Y-shaped aquaria in which predators are allowed to choose from a variety of potential prey (Mills et al. 1984, DeVries et al. 1998). Additionally, potential large zooplankton palatability issues may arise from the anthropogenic application of different pond nutrients, which may influence swordtail prey/food source choice.

Larger pond water samples (e.g., 2 L) and/or longer trial duration (> 24 hrs.) may have more clearly demonstrated potential swordtail predation upon the large phytoplankton and zooplankton community. To adequately investigate potential swordtail predation upon pond large plankton communities, a fully orthogonal trial employing different potential predation magnitudes (numbers of fish in a container, e.g., 1, 2, 4) and trial durations (e.g., 12, 24, 36, 48 hrs.) would better describe (and quantify) the potential impact of swordtail predation upon the large plankton community and the magnitude of predation per fish. This combined with more detailed baseline investigations of large zooplankton abundance and distribution within ponds, and swordtail population size structure, abundance and distribution within the ponds, would give a clearer picture regarding the potential trophic effects and limitations of swordtail predation upon the large zooplankton community.

In floating pond mesocosms, or pond side aquaria with continuous pumping of filtered pond water into aquaria, may have produced environmental conditions (temperature, dissolved oxygen) more similar to those actually occurring within the outdoor ponds. The reduction or elimination of physiological stressors such as low dissolved oxygen, ammonia, nitrite and high temperatures, etc., may have increased the similarity of the large plankton communities within the two control treatments. Additionally, reduction of large community taxa differences between the two control treatments, and increasing the number of replicate ponds sampled (from four to six), would likely have increased the statistical power of the various analyses. Increasing the

likelihood that pooling of replicates among pond nutrient treatments would not have been necessary.

Daily ration estimates, combined with gastric evacuation rate measurement of swordtails (and various swordtail size classes) would also allow rough estimates of swordtail carrying capacity and the proportion of potential swordtail production attributable to live foods and the proportion derived from direct anthropogenic nutrient additions (intended for direct consumption by fish), given that bioenergetic needs of swordtails, and potential swordtail food calorimetrics were known (Winberg 1956, Elliott and Persson 1978, Eggers 1979).

APPENDIX A
LARGE PLANKTON ASSEMBLAGE %N, %V, AND %FO

Large Zooplankton Assemblage Community Taxa Parameters

Legend Key to Graphs:

COP - CYCLOPIDAE copepod	EPH - Ephemera insect
CLD - Filinia rotifer	NON-LHC - 'rare' copepod
DAP - Moina macrocopa (CLADOCERA)	FLS - terrestrial floral stamen
TLR - rotifer (Brachionus havanaensis)	DPP - Pediastrum spp. algae
BIN - big insect	SEED - terrestrial grass seed
CLR - rotifer (Keratella spp.)	BBROT - brown ball rotifer
MITE - water mite (HYDRACARINA)	SPBLL - spore ball
LNE - nematode	ARTH - arthropod (misc)
SCLR - spiked rotifer (Keratella spp.)	IHT - insect head
OST - ostracod	RR - round rotifer
CST - bryozoan statoblast	GSR - grape seed rotifer
ELM - copepod egg mass (unattached)	PHR - " Papal Tiara" rotifer
VLUKE - large single egg	SBT - Spikey Ball Thing
VSUKE - small single egg	SN - snail
NAUP - copepod nauplii	NLR - Synchaeta spp. rotifer

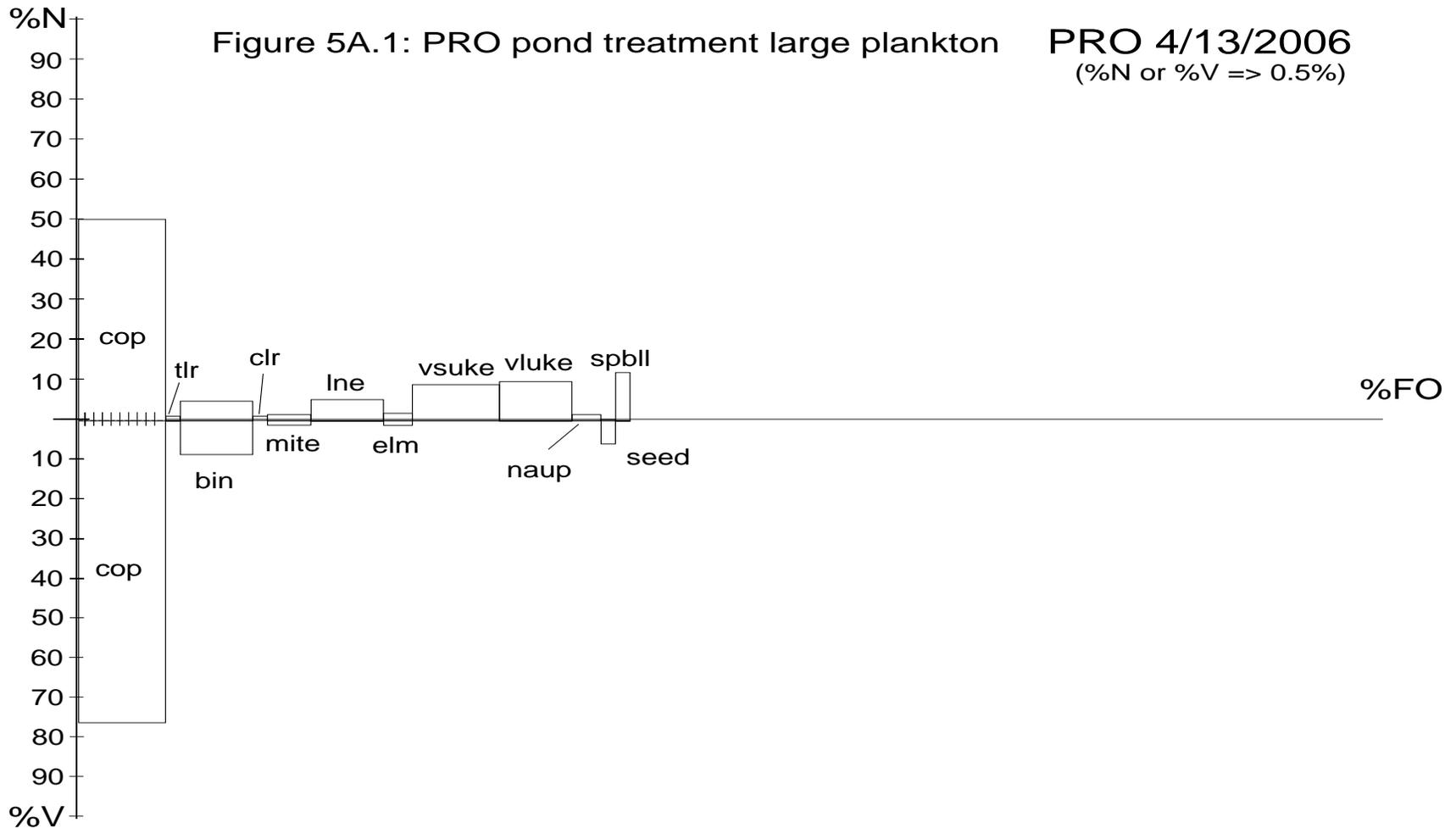


Figure 5A-1. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for processed feed (PRO) treatment 13 April 2006; taxa data pooled among six replicate ponds (0.015 hectares).

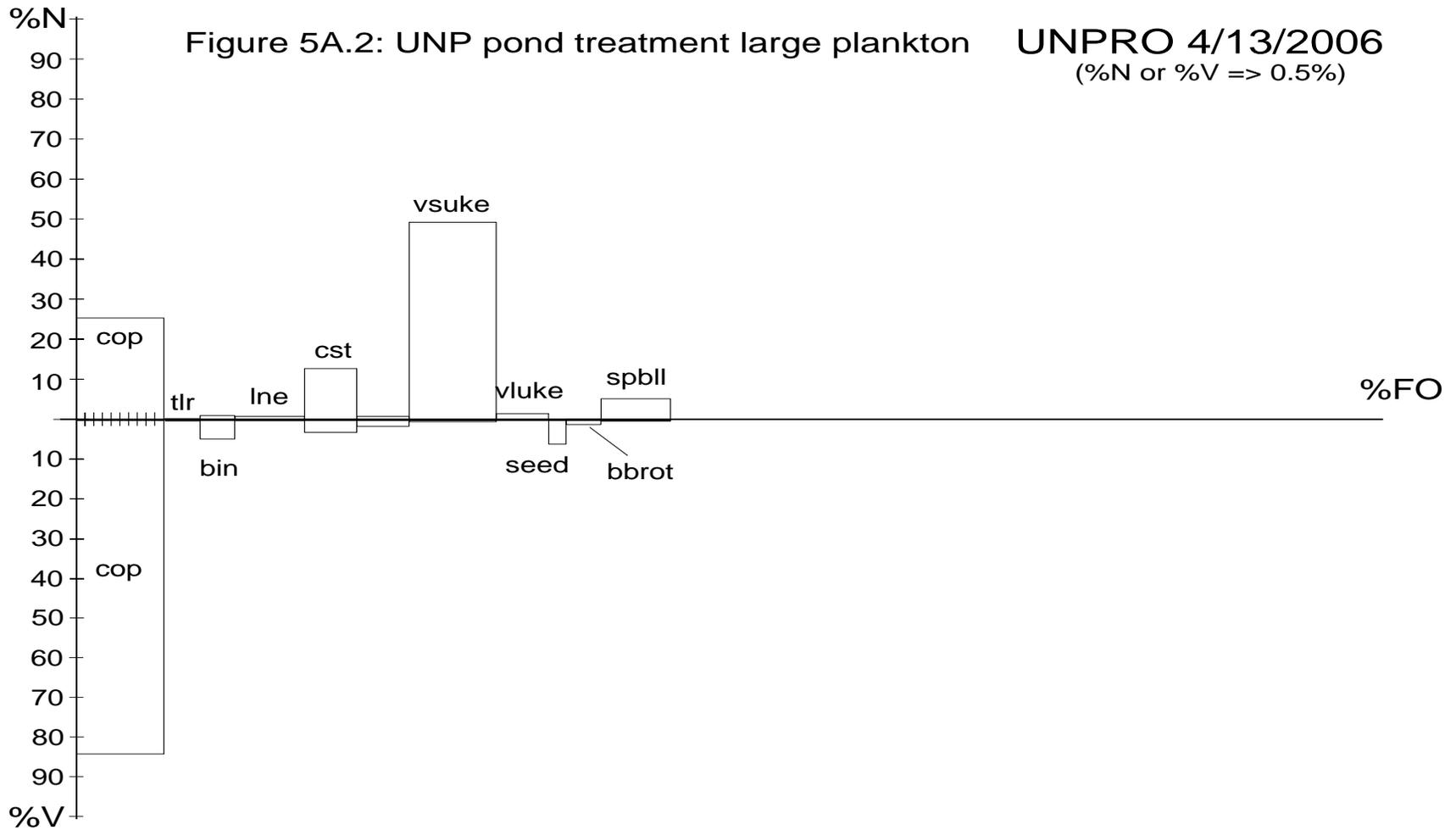


Figure 5A-2. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for unprocessed feed (UNP) treatment 13 April 2006; taxa data pooled among six replicate ponds (0.015 hectares).

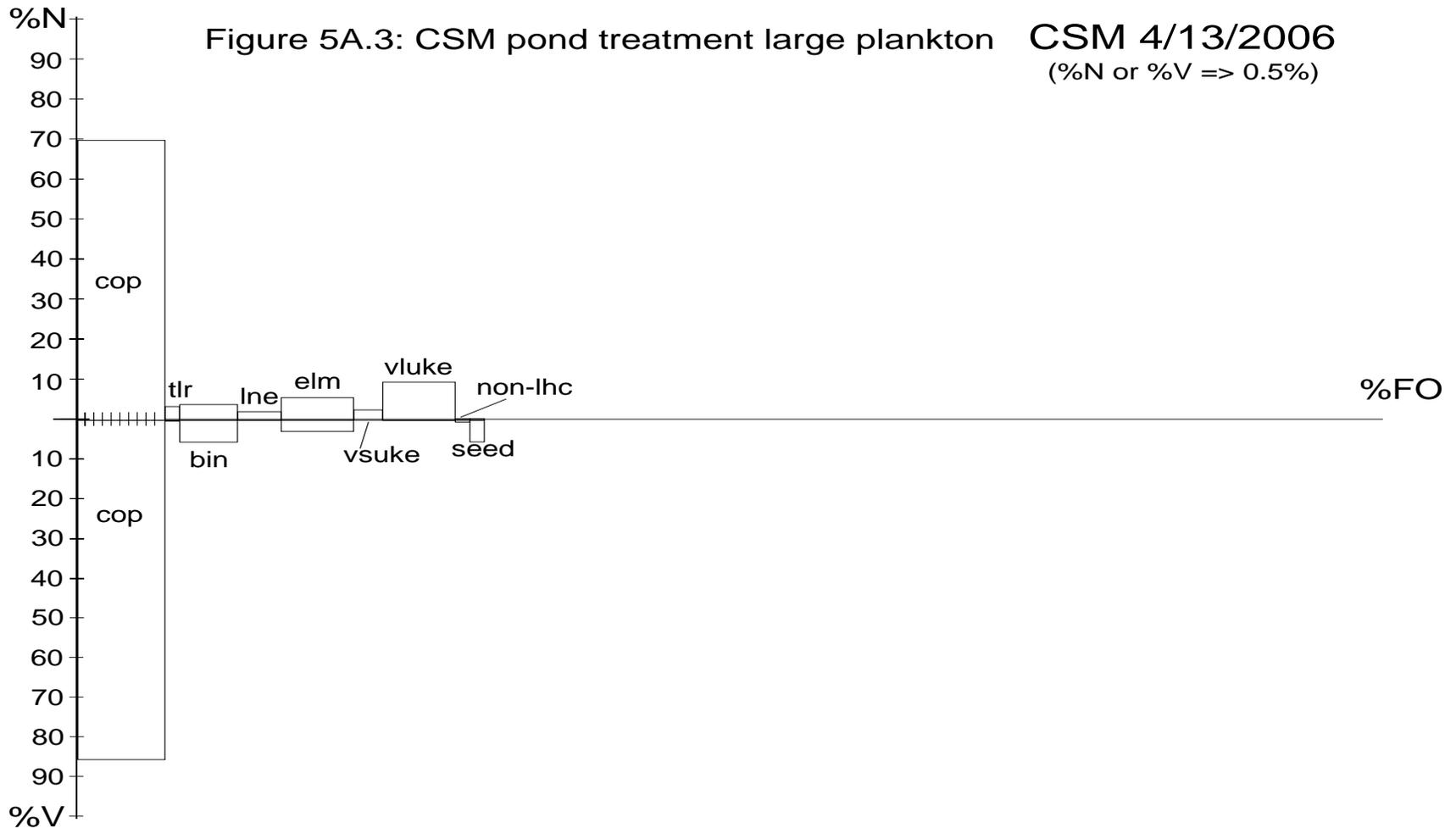


Figure 5A-3. Large zooplankton (> 200 μ m) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for cottonseed meal fertilizer (CSM) treatment 13 April 2006; taxa data pooled among six replicate ponds (0.015 hectares).

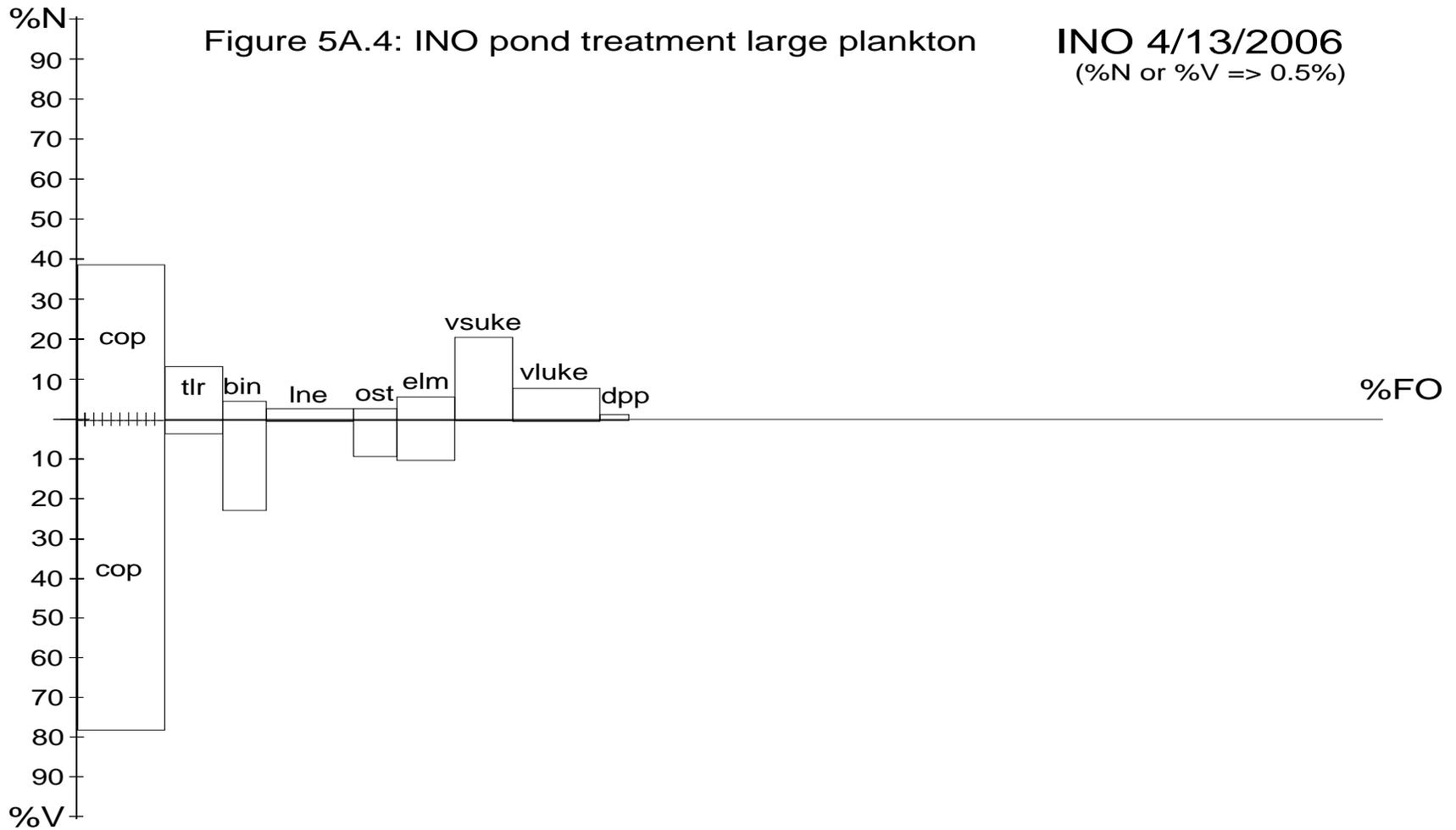


Figure 5A-4. Large zooplankton (> 200 μ m) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for inorganic fertilizer (INO) treatment 13 April 2006; taxa data pooled among six replicate ponds (0.015 hectares).

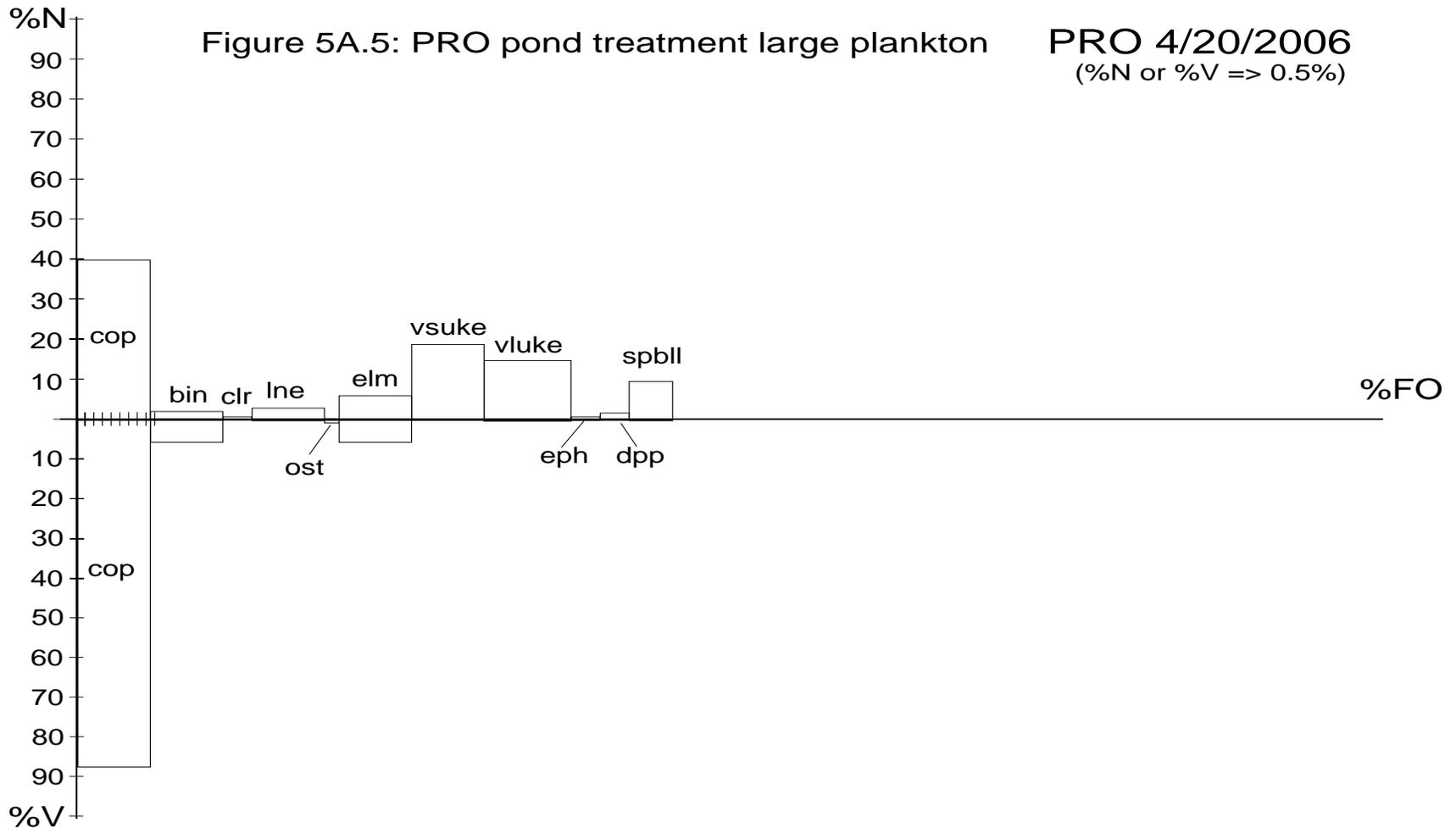


Figure 5A-5. Large zooplankton (size > 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for processed feed (PRO) treatment 20 April 2006; taxa data pooled among six replicate pond (0.015 hectares).

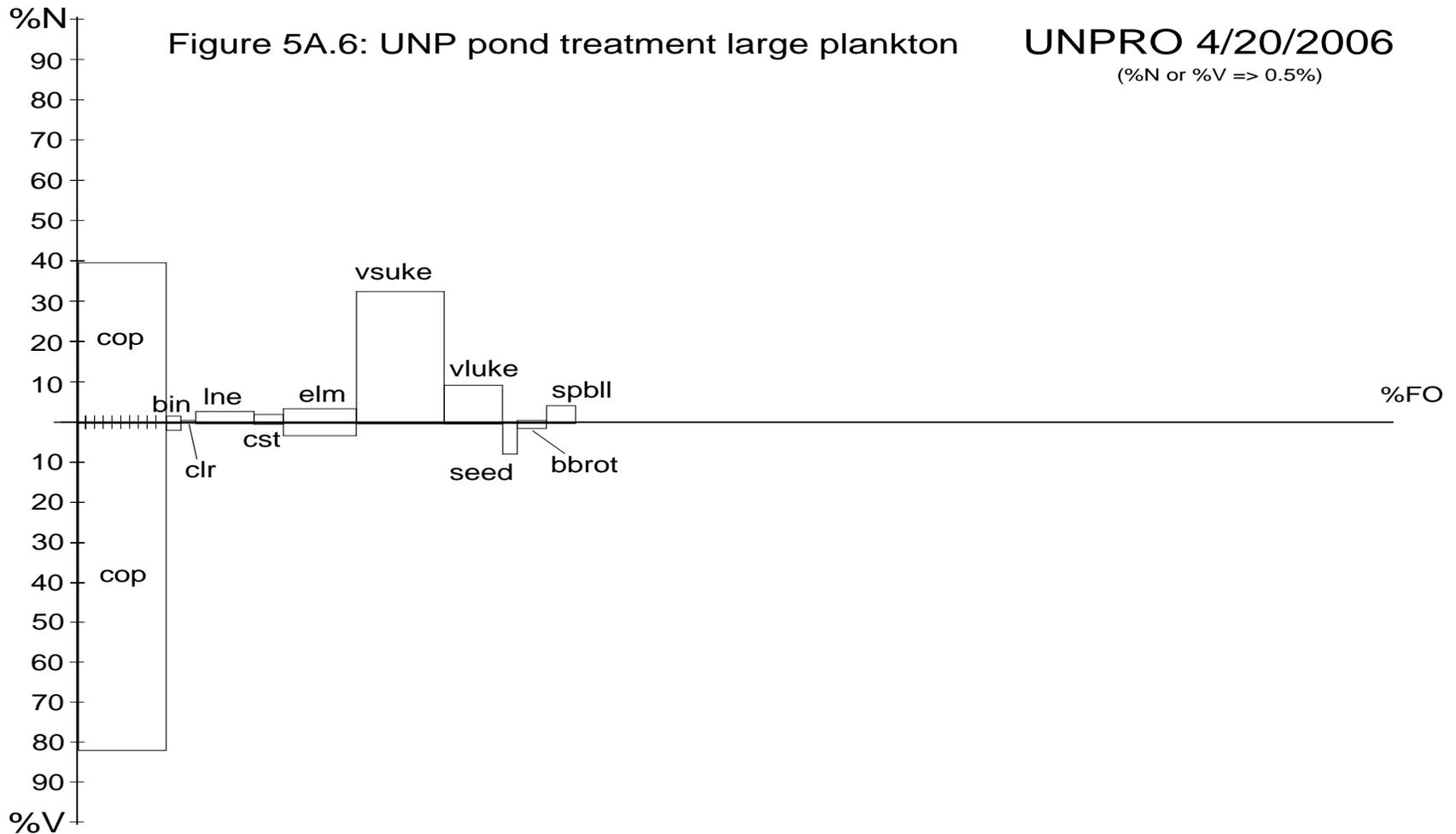


Figure 5A-6. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for unprocessed feed (UNP) treatment 20 April 2006; taxa data pooled among six replicate ponds (0.015 hectares).

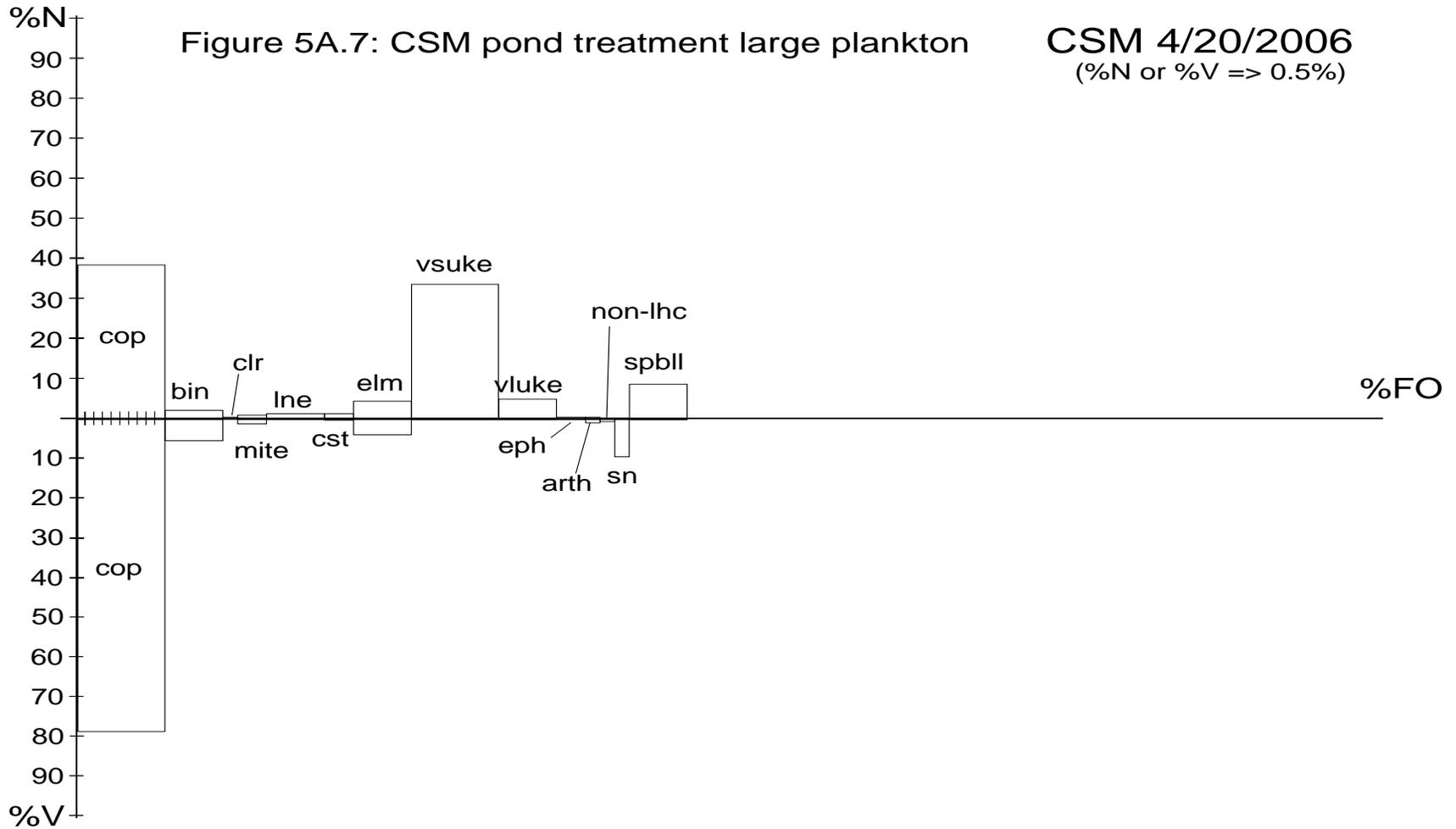


Figure 5A-7. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for cottonseed meal fertilizer (CSM) treatment 20 April 2006; taxa data pooled among six replicate ponds (0.015 hectares).

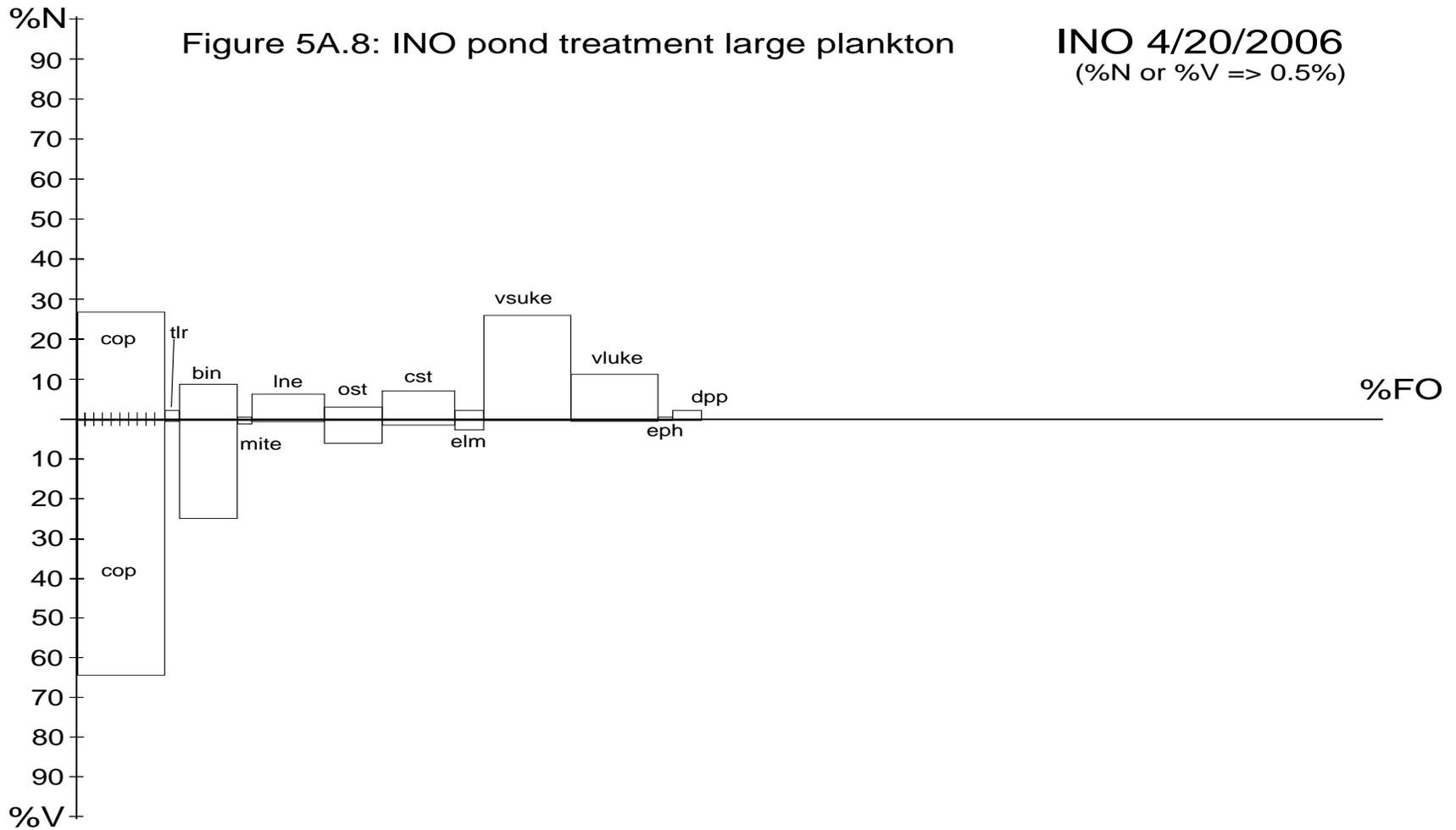


Figure 5A-8. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for inorganic fertilizer (INO) treatment 20 April 2006; taxa data pooled among six replicate ponds (0.015 hectares).

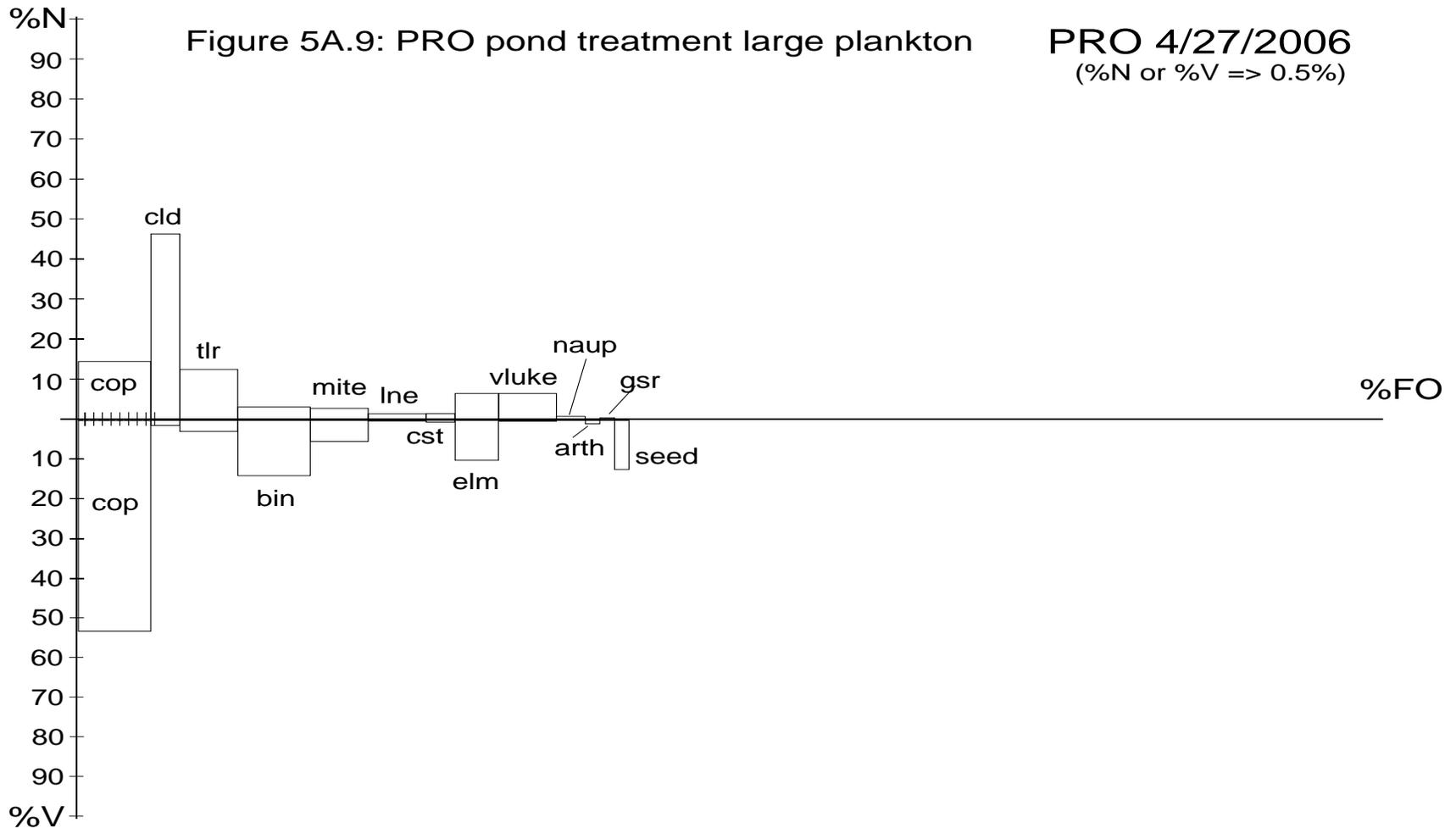


Figure 5A-9. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for processed feed (PRO) treatment 27 April 2006; taxa data pooled among six replicate ponds (0.015 hectares).

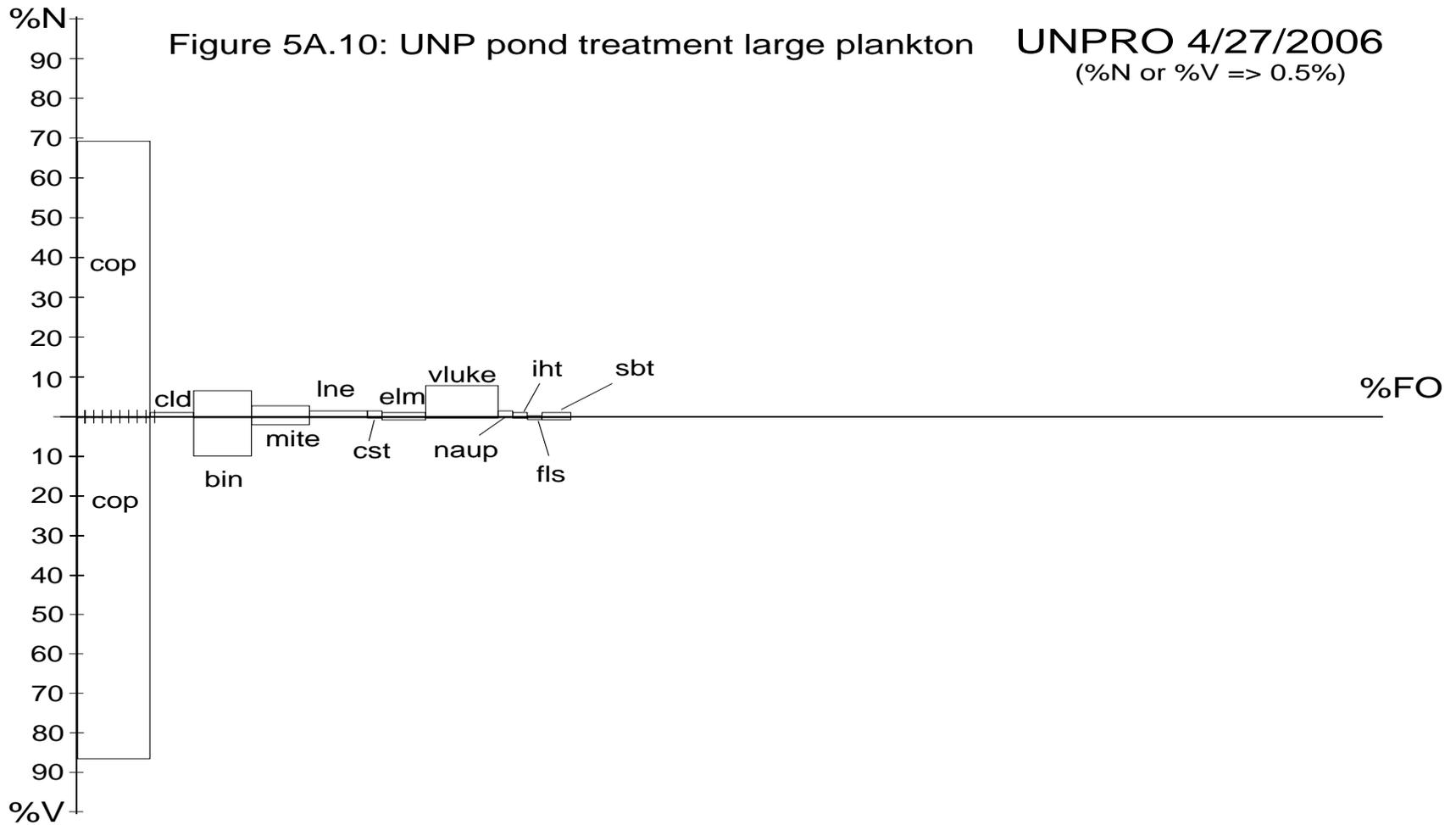


Figure 5A-10. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for unprocessed feed (UNP) treatment 27 April 2006; taxa data pooled among six replicate ponds (0.015 hectares).

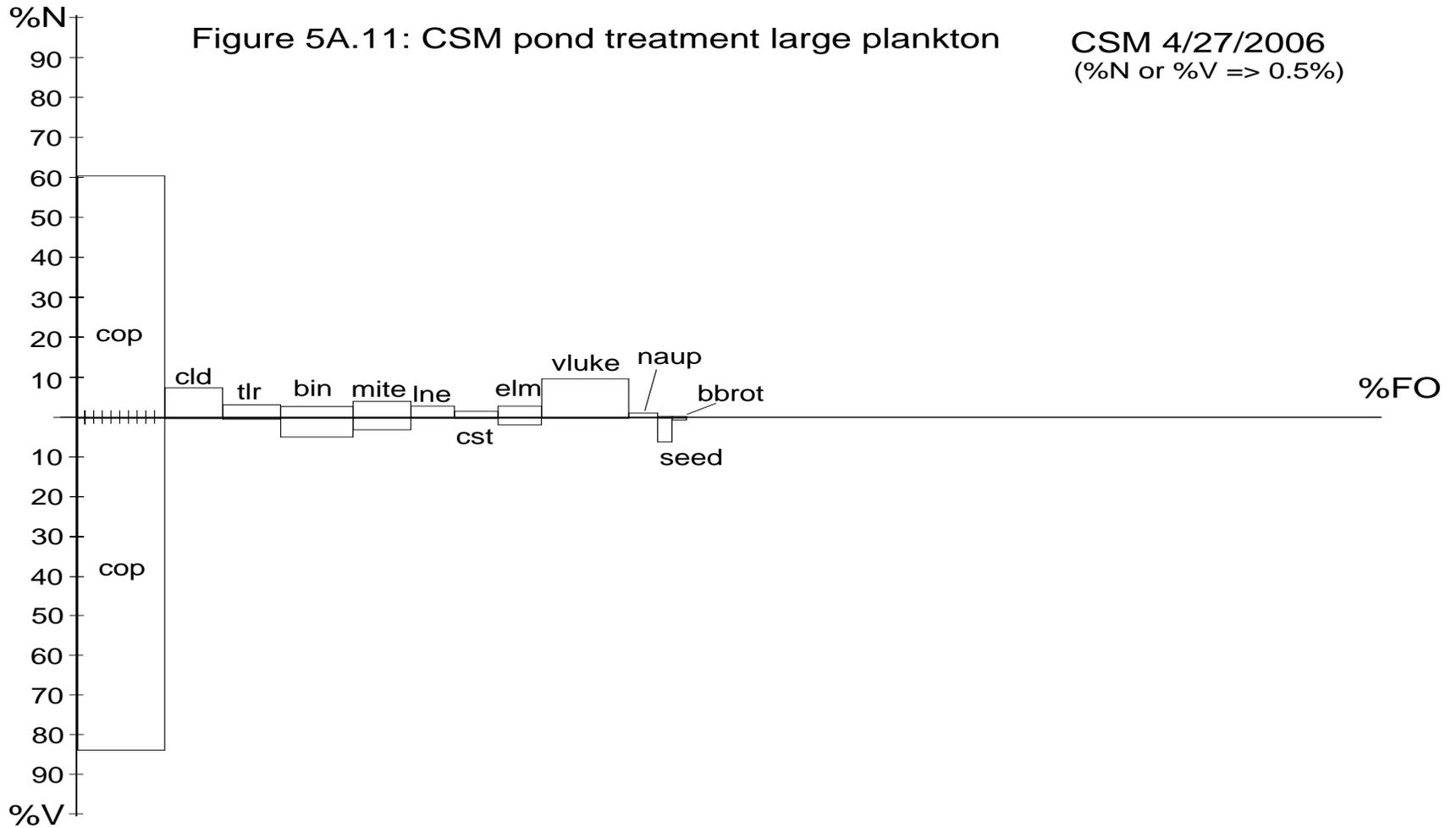


Figure 5A-11. Large zooplankton (> 200 µm) assemblage community taxonomic parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for cottonseed meal fertilizer (CSM) treatment 27 April 2006; taxa data pooled among six replicate ponds (0.015 hectares).

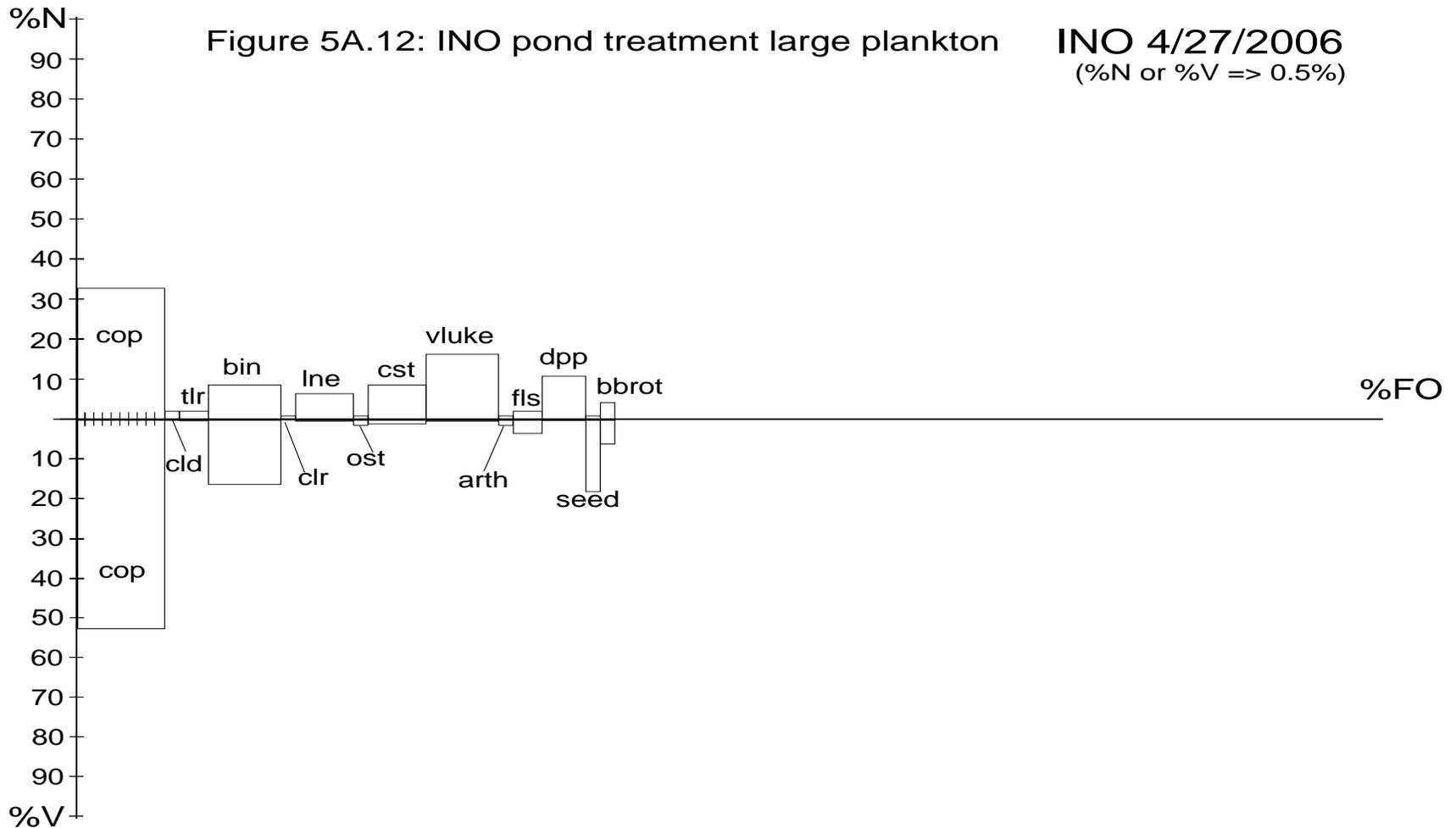


Figure 5A-12. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for inorganic fertilizer (INO) treatment 27 April 2006; taxa data pooled among six replicate ponds (0.015 hectares).

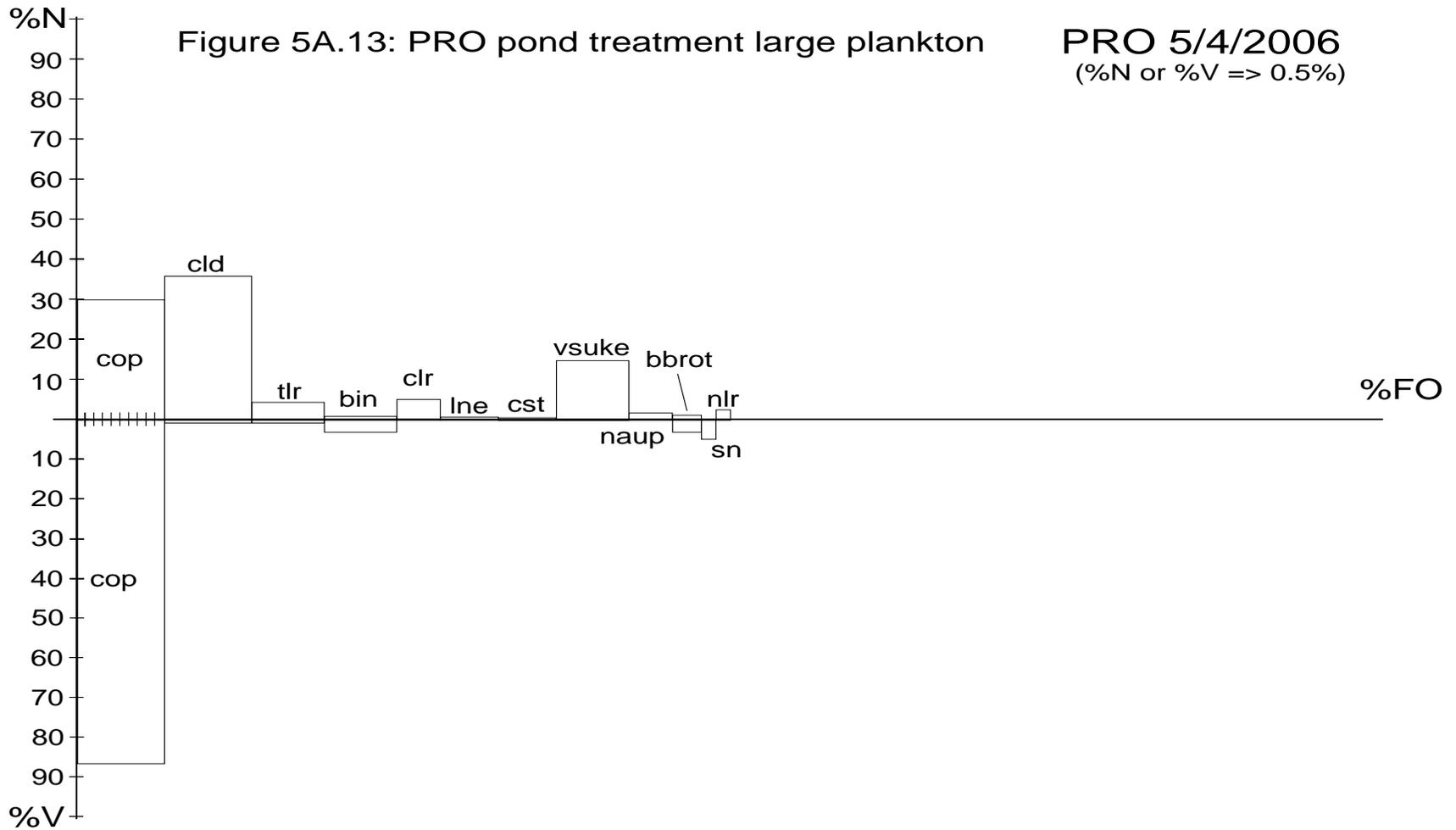


Figure 5A-13. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for processed feed (PRO) treatment 4 May 2006; taxa data pooled among six replicate ponds (0.015 hectares).

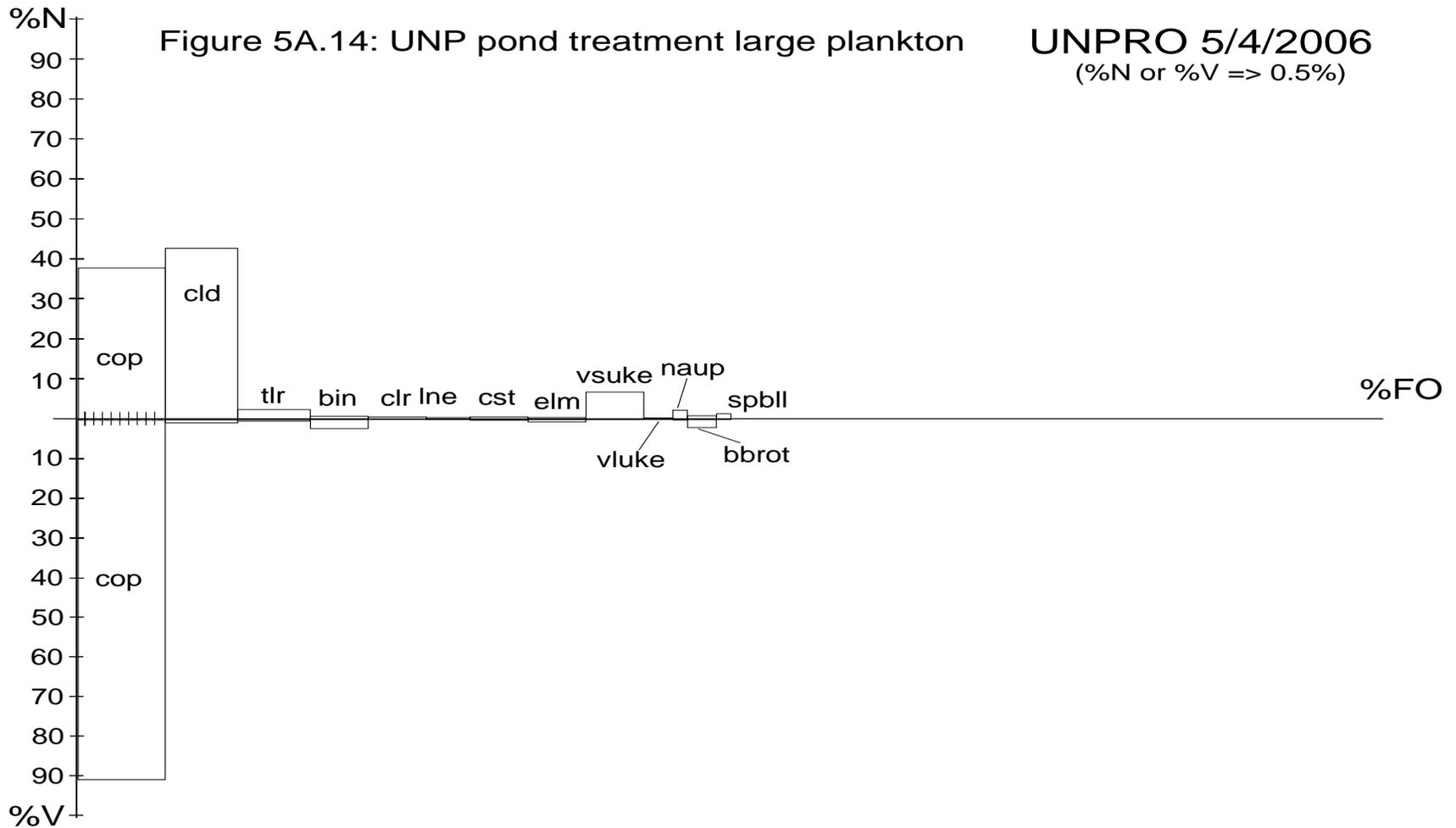


Figure 5A-14. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for unprocessed feed (UNP) treatment 4 May 2006; taxa data pooled among six replicate ponds (0.015 hectares).

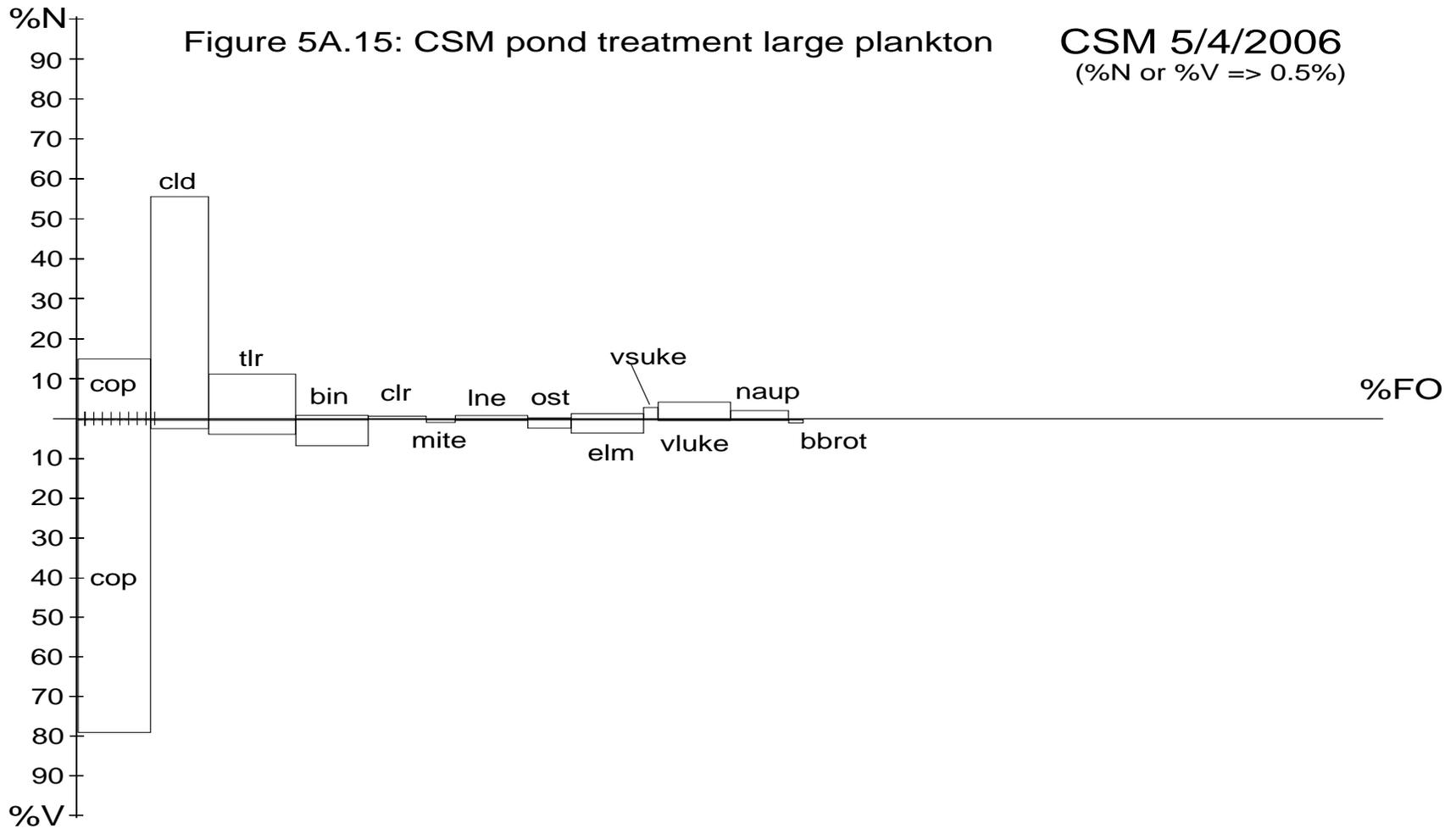


Figure 5A-15. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for cottonseed meal fertilizer (CSM) treatment 4 May 2006; taxa data pooled among six replicate ponds (0.015 hectares).

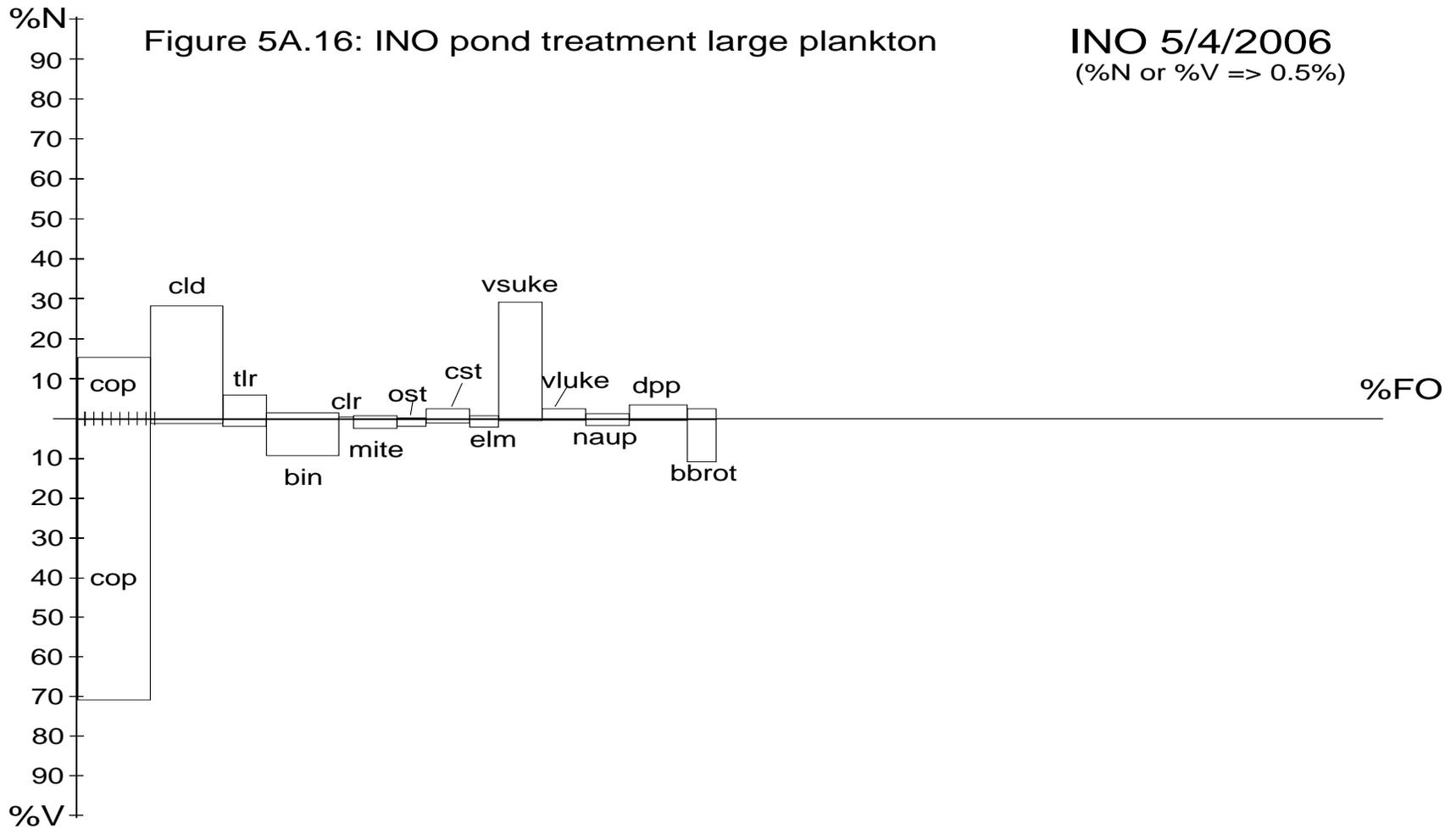


Figure 5A-16. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for inorganic fertilizer (INO) treatment 4 May 2006; taxa data pooled among six replicate ponds (0.015 hectares).

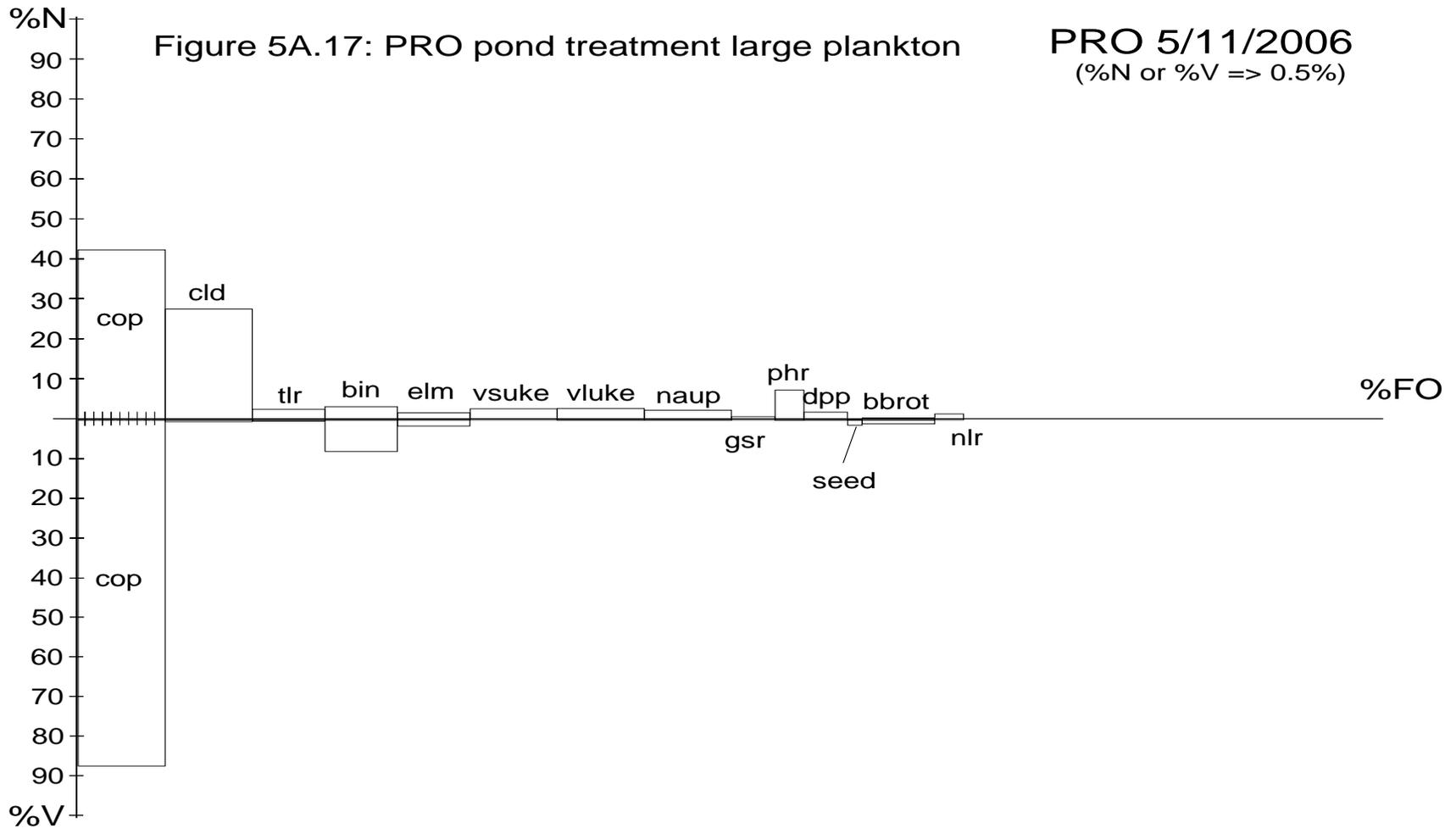


Figure 5A-17. Large zooplankton (> 200 µm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for processed feed (PRO) treatment 11 May 2006; taxa data pooled among six replicate ponds (0.015 hectares).

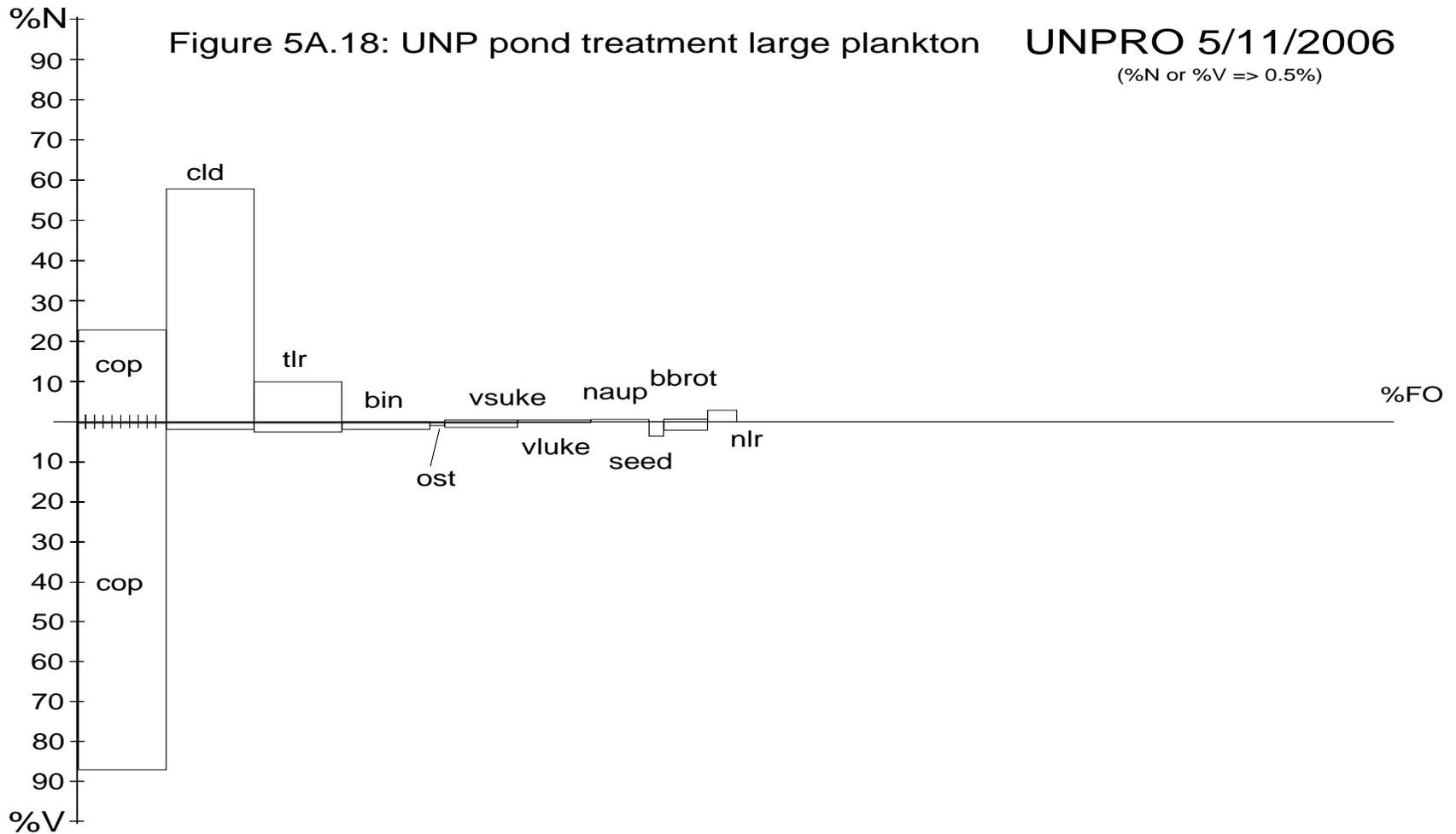


Figure 5A-18. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for unprocessed feed (UNP) treatment 11 May 2006; taxa data pooled among six replicate ponds (0.015 hectares).

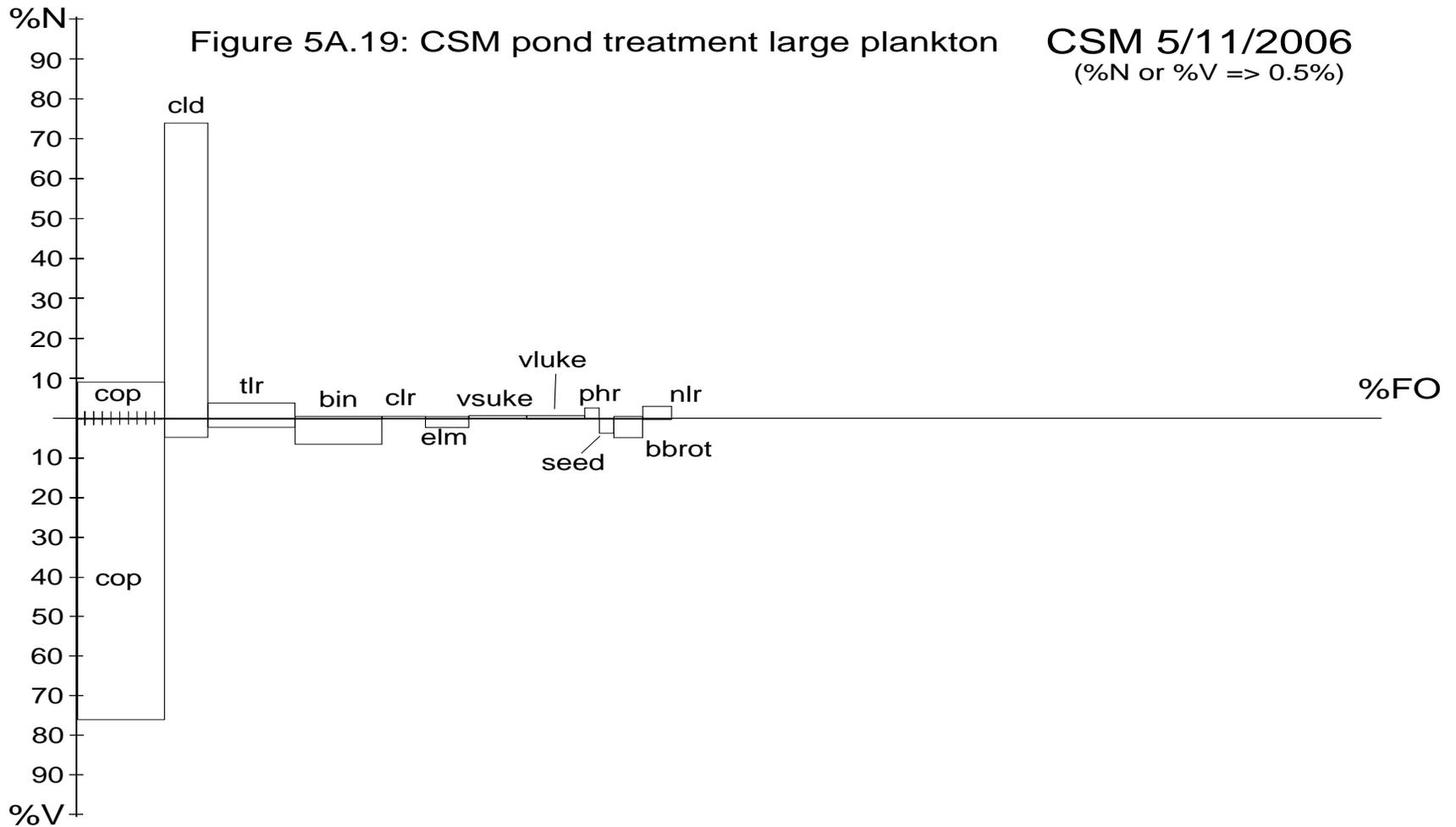


Figure 5A-19. Large zooplankton (> 200 µm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for cottonseed meal fertilizer (CSM) treatment 11 May 2006; taxa data pooled among six replicate ponds (0.015 hectares).

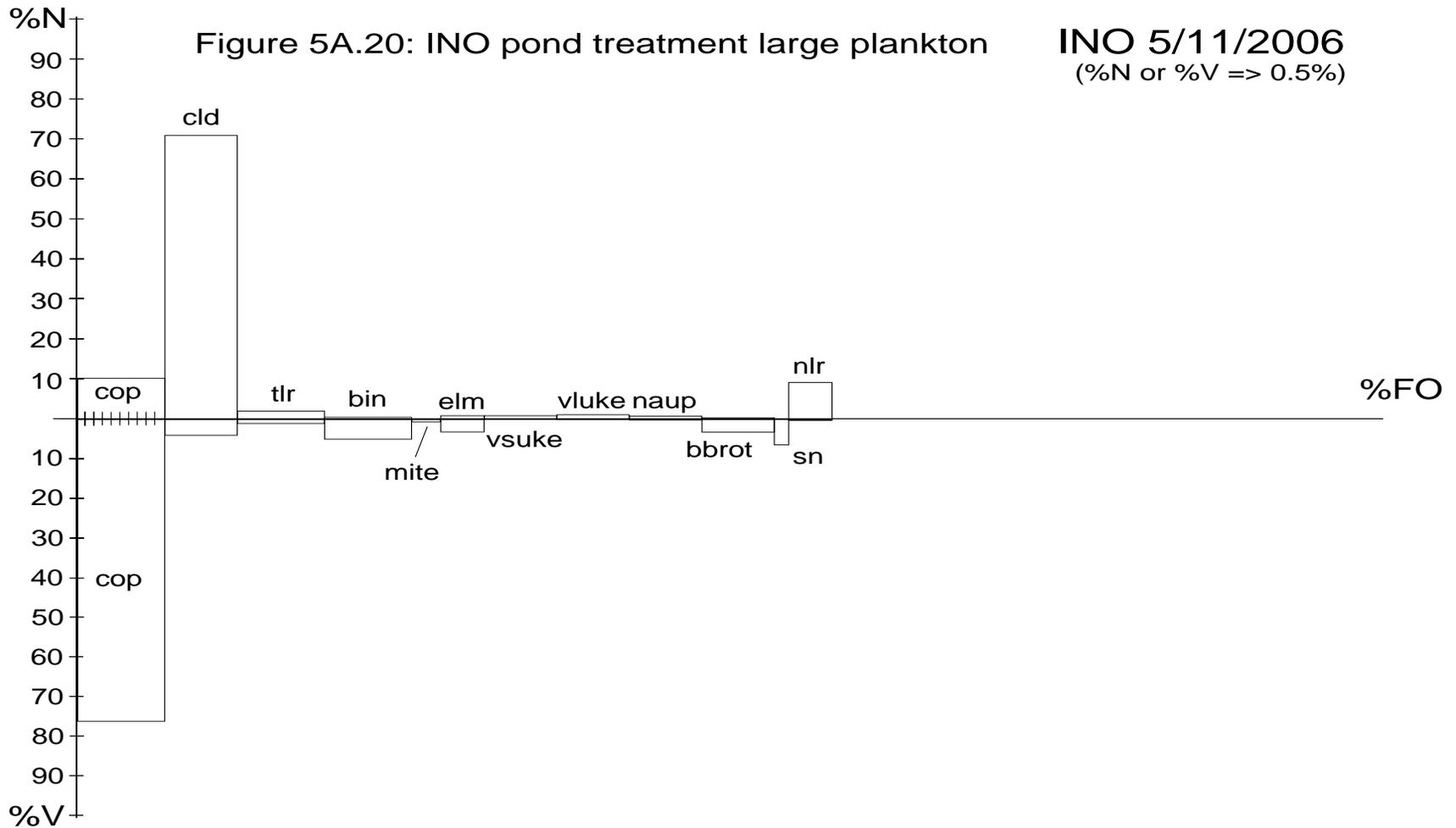


Figure 5A-20. Large zooplankton (> 200 µm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for inorganic fertilizer (INO) treatment 11 May 2006; taxa data pooled among six replicate ponds (0.015 hectares).

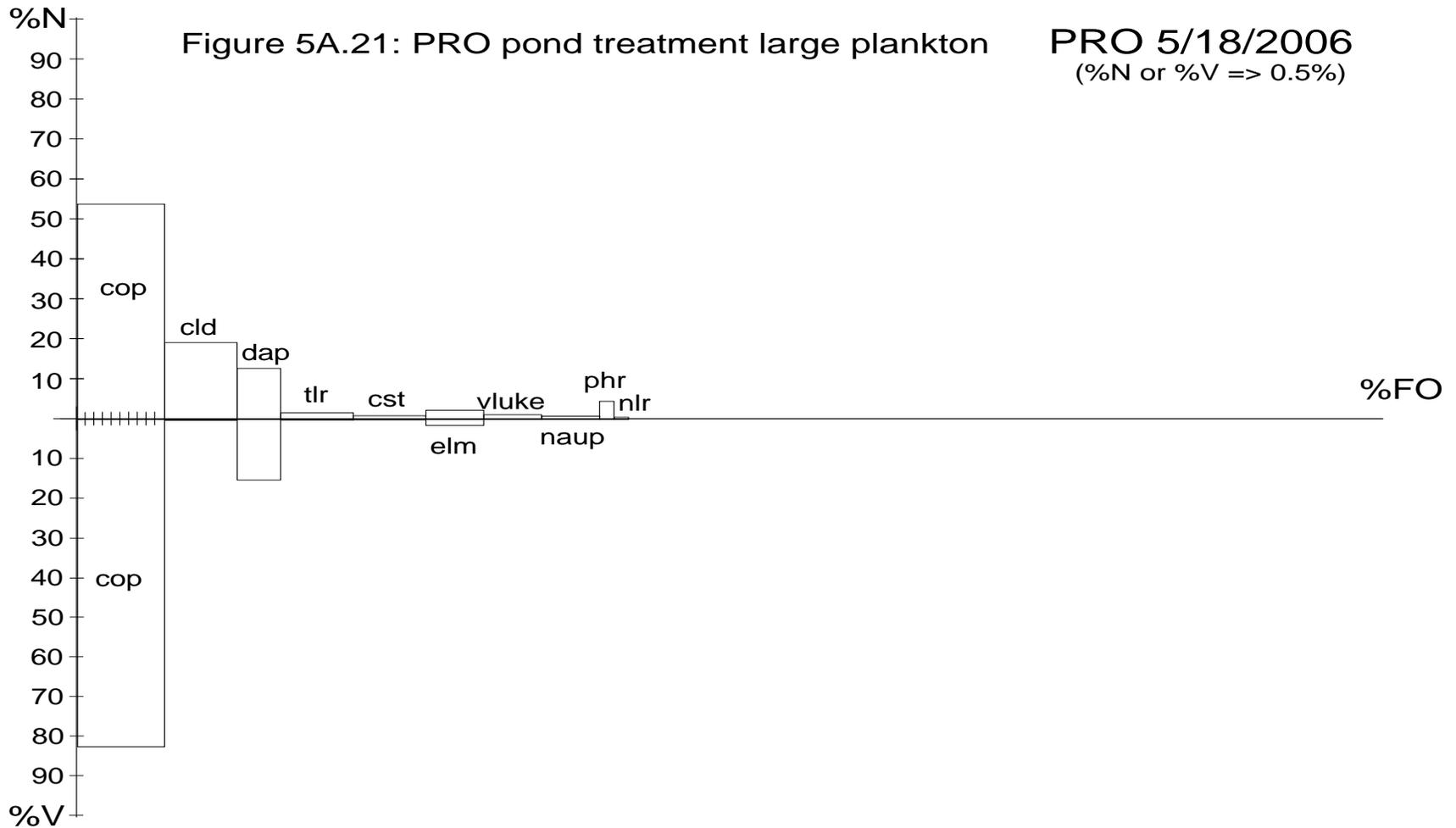


Figure 5A-21. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for processed feed (PRO) treatment 18 May 2006; taxa data pooled among six replicate ponds (0.015 hectares).

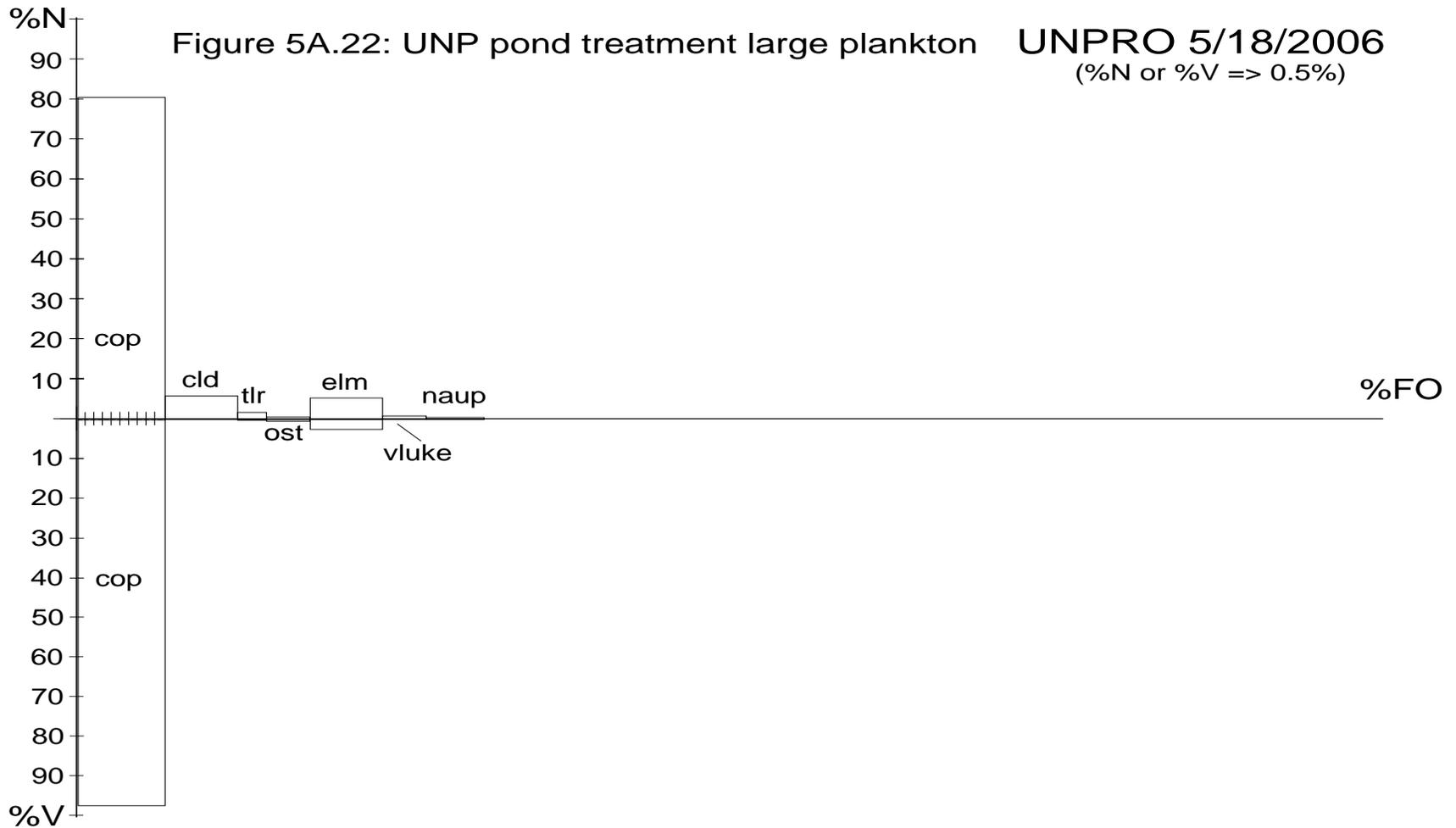


Figure 5A-22. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for unprocessed feed (UNP) treatment 18 May 2006; taxa data pooled among six replicate ponds (0.015 hectares).

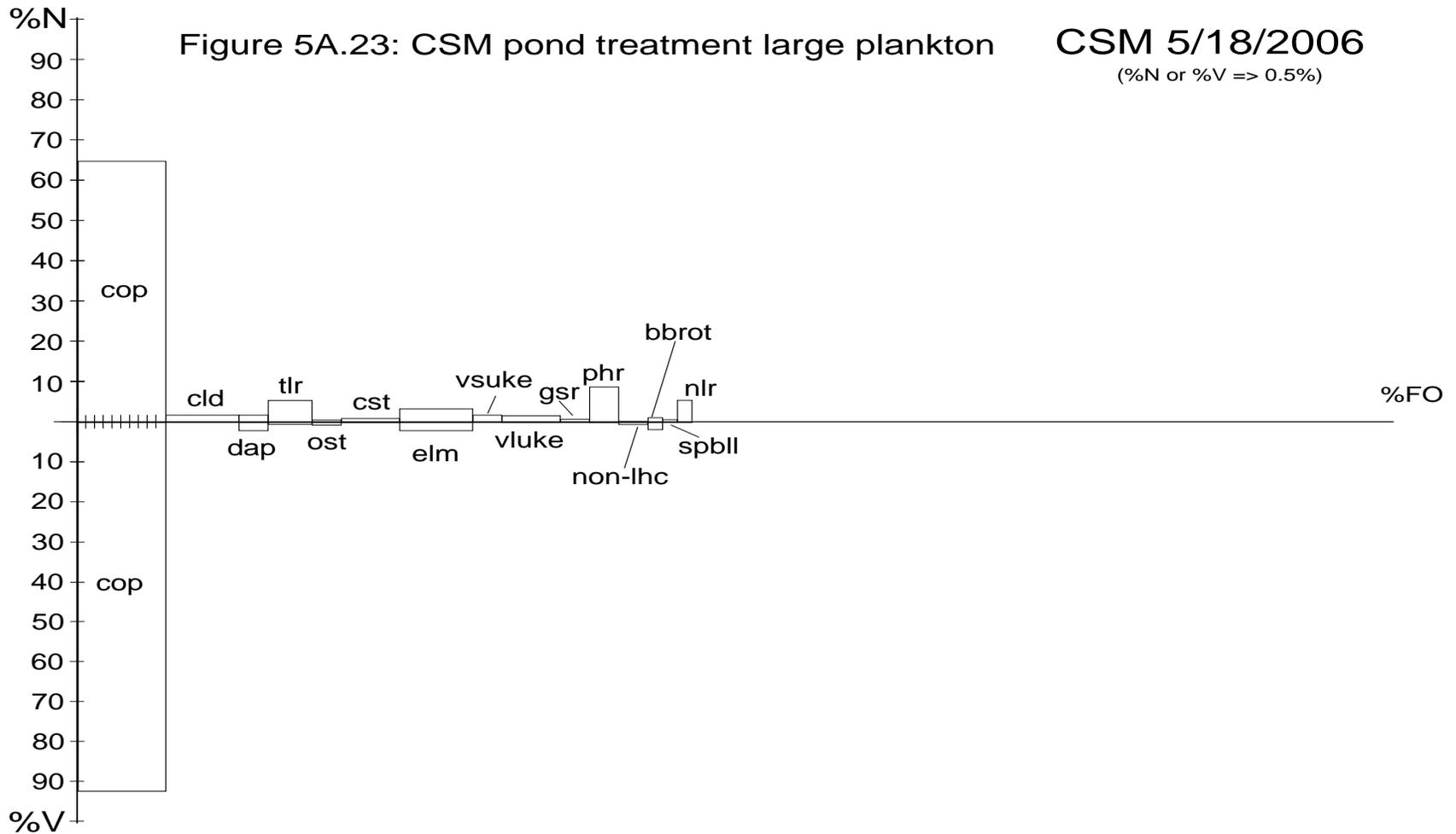


Figure 5A-23. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for cottonseed meal fertilizer (CSM) treatment 18 May 2006; taxa data pooled among six replicate ponds (0.015 hectares).

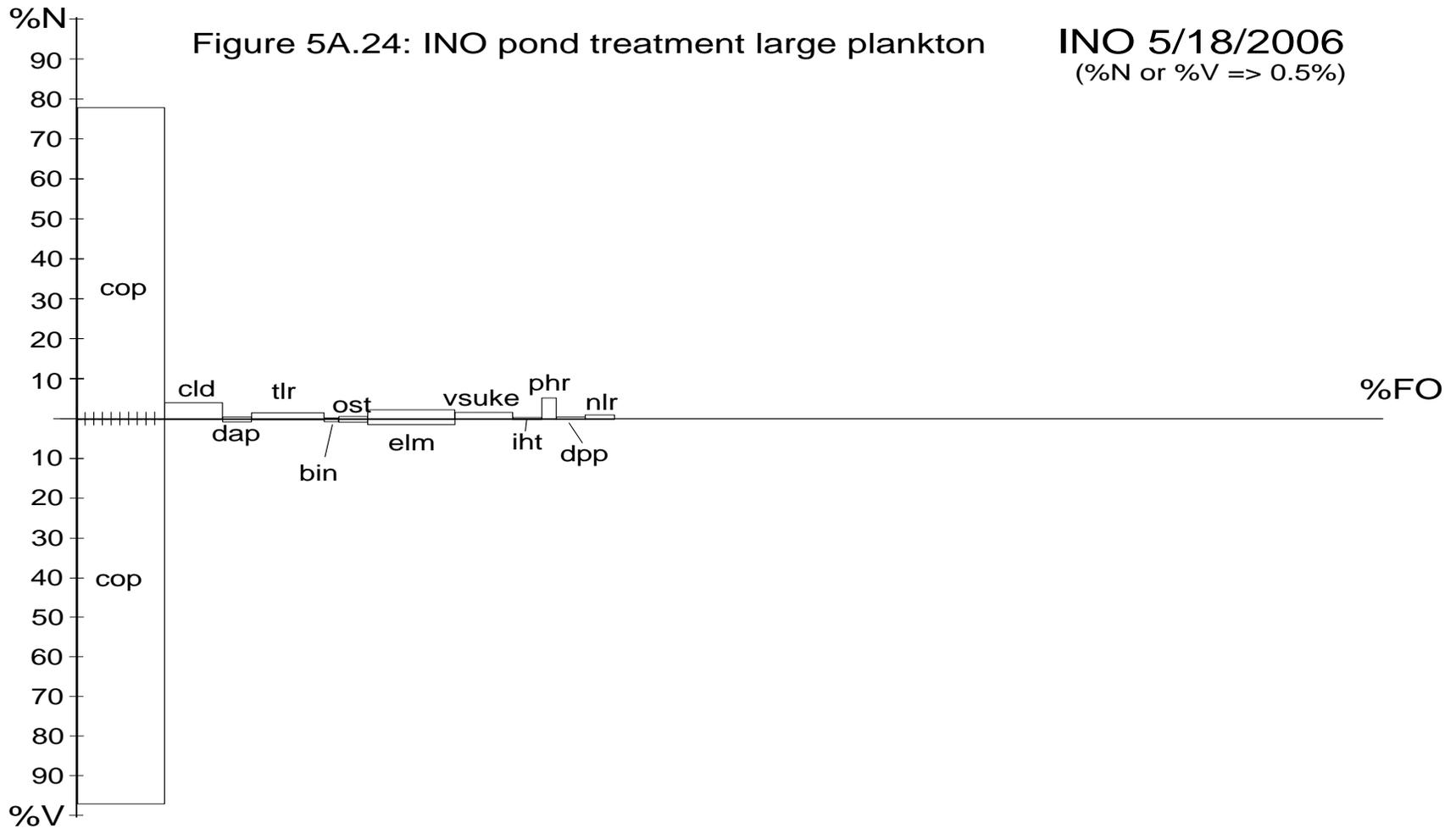


Figure 5A-24. Large zooplankton (> 200 µm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for inorganic fertilizer (INO) treatment 18 May 2006; taxa data pooled among six replicate ponds (0.015 hectares).

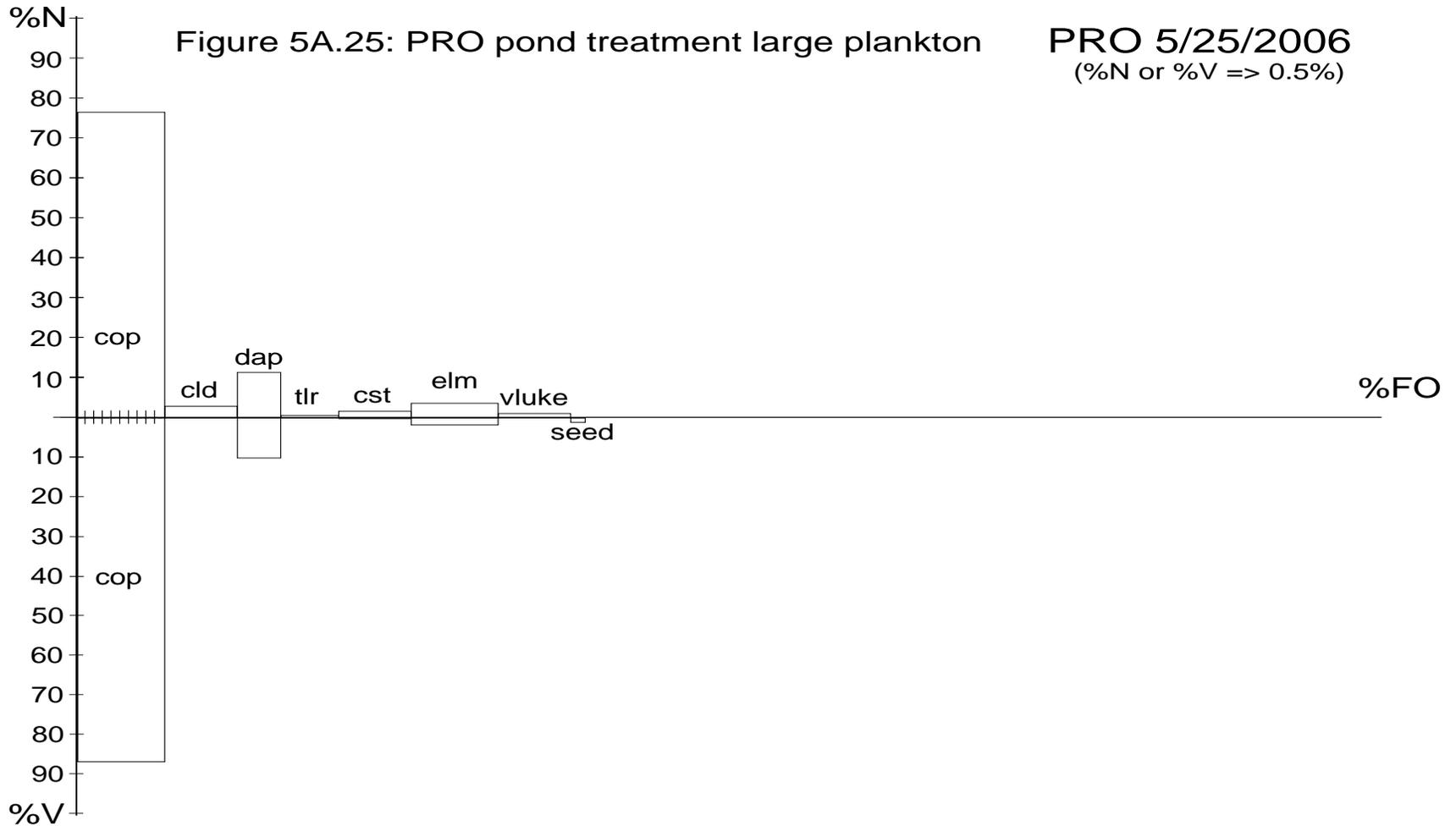


Figure 5A-25. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for processed feed (PRO) treatment 25 May 2006; taxa data pooled among six replicate ponds (0.015 hectares).

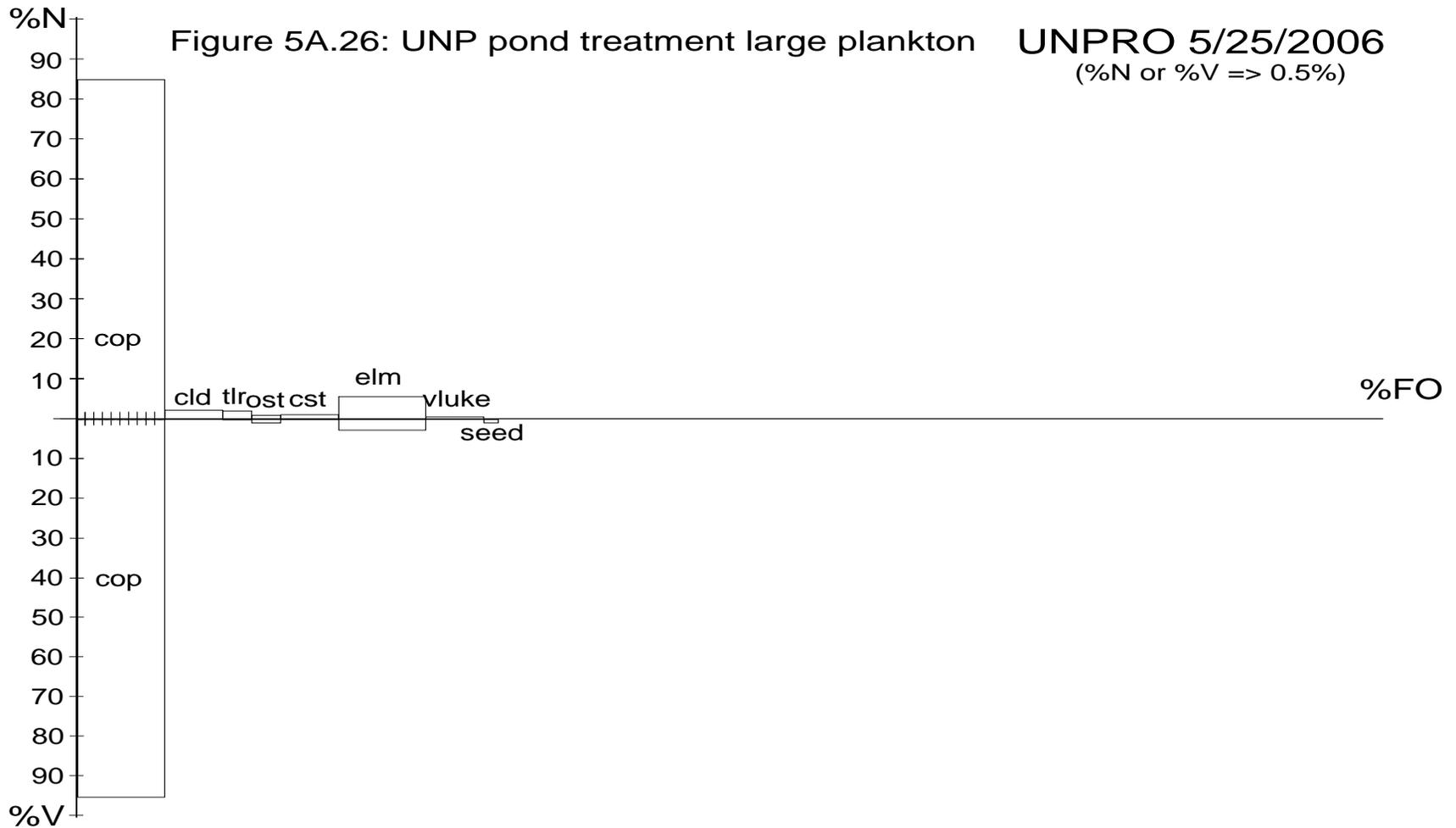


Figure 5A-26. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for unprocessed feed (UNP) treatment 25 May 2006; taxa data pooled among six replicate ponds (0.015 hectares).

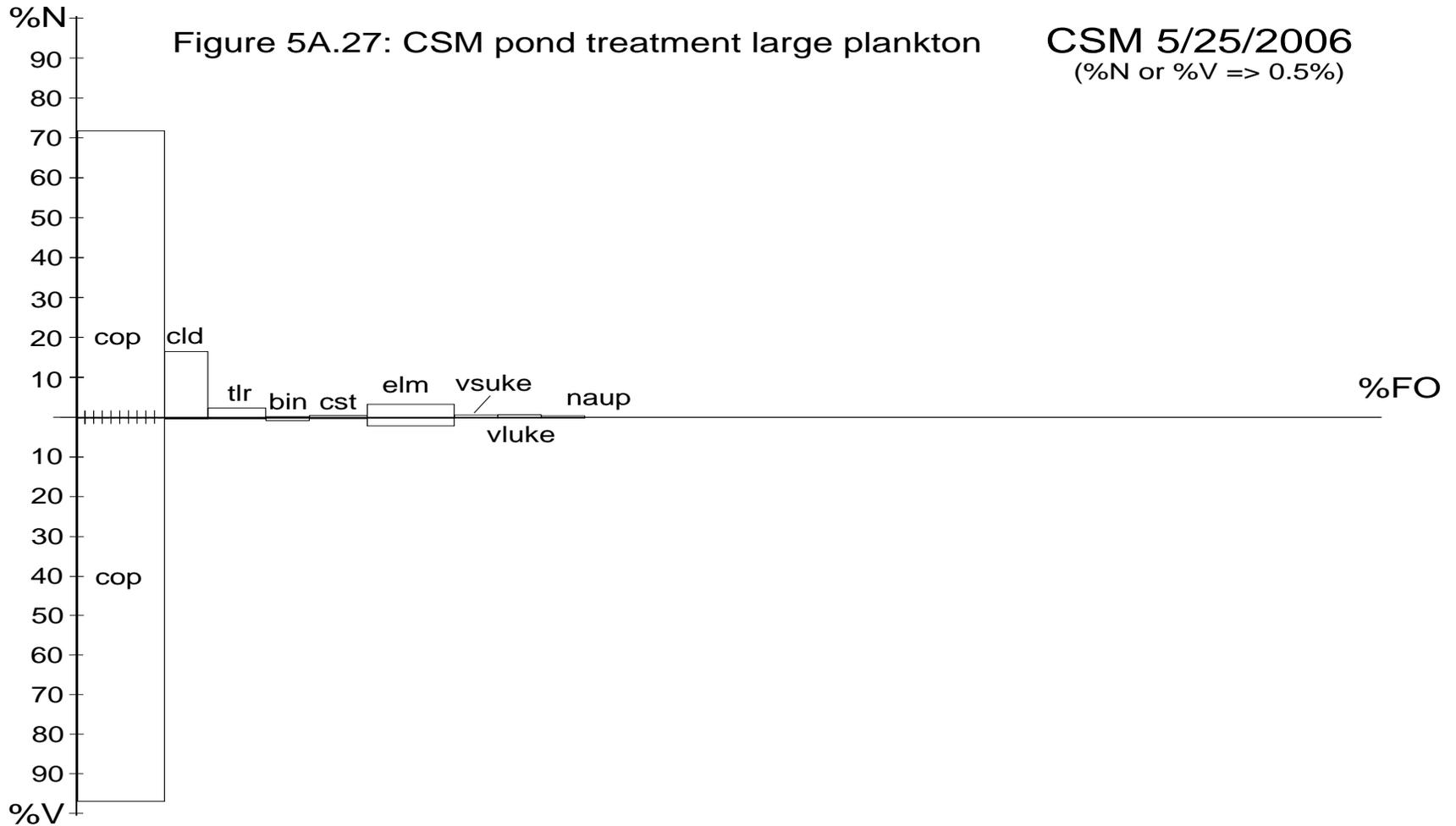


Figure 5A-27. Large zooplankton (> 200 μm) assemblage community taxonomic parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for cottonseed meal fertilizer (CSM) treatment 25 May 2006; taxa data pooled among six replicate ponds (0.015 hectares).

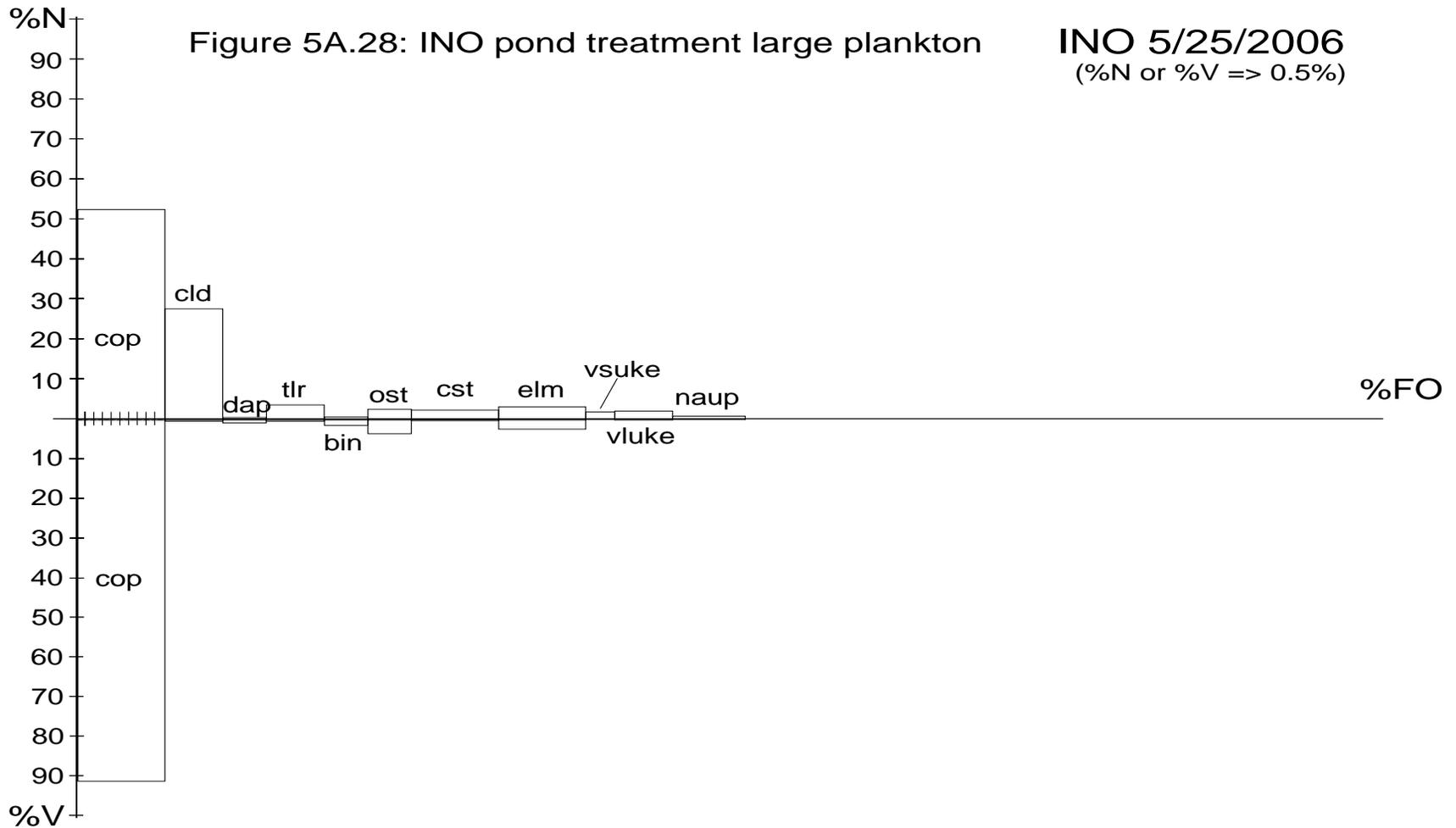


Figure 5A-28. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for inorganic fertilizer (INO) treatment 25 May 2006; taxa data pooled among six replicate ponds (0.015 hectares).

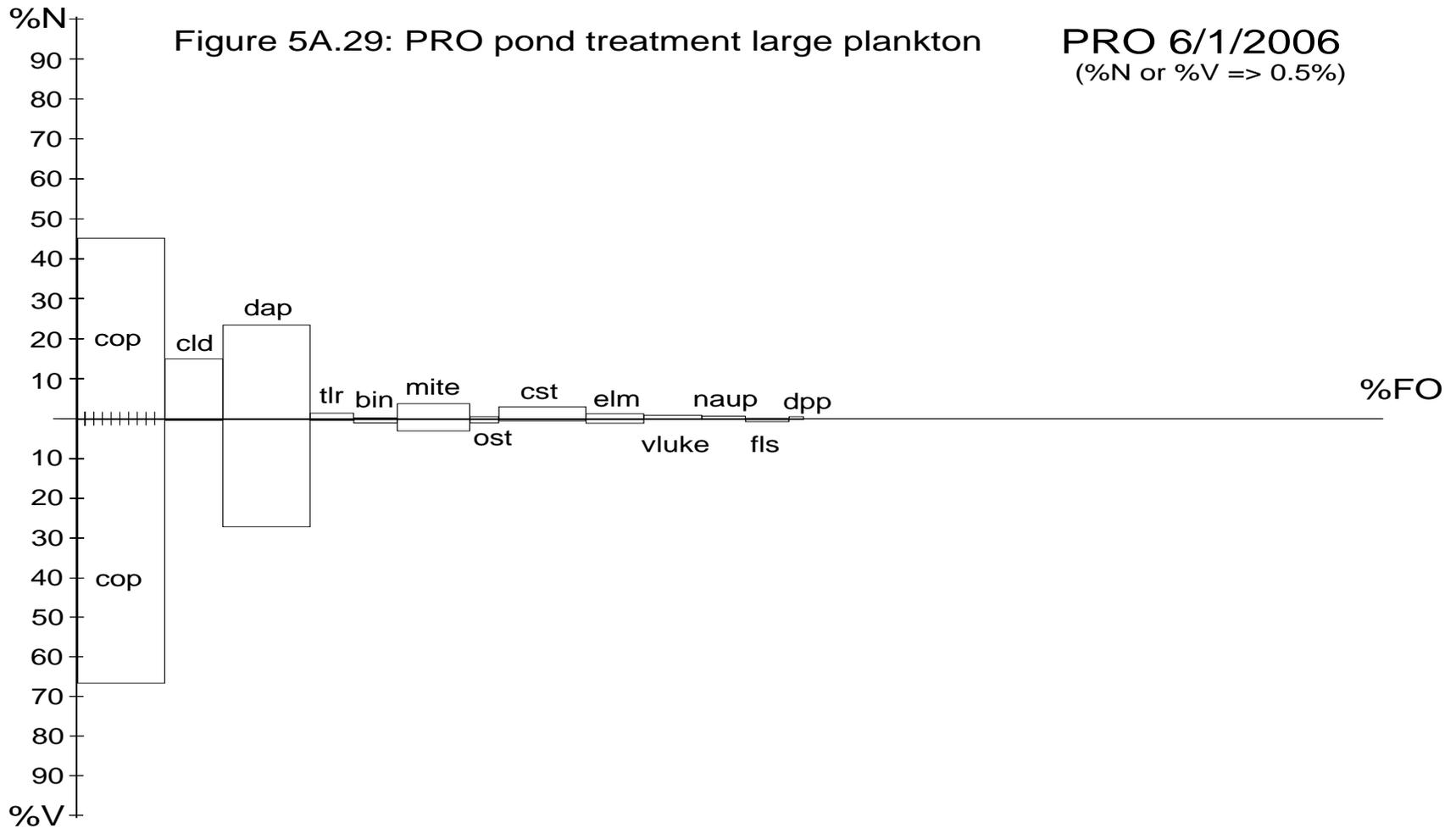


Figure 5A-29. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for processed feed (PRO) treatment 1 June 2006; taxa data pooled among six replicate ponds (0.015 hectares).

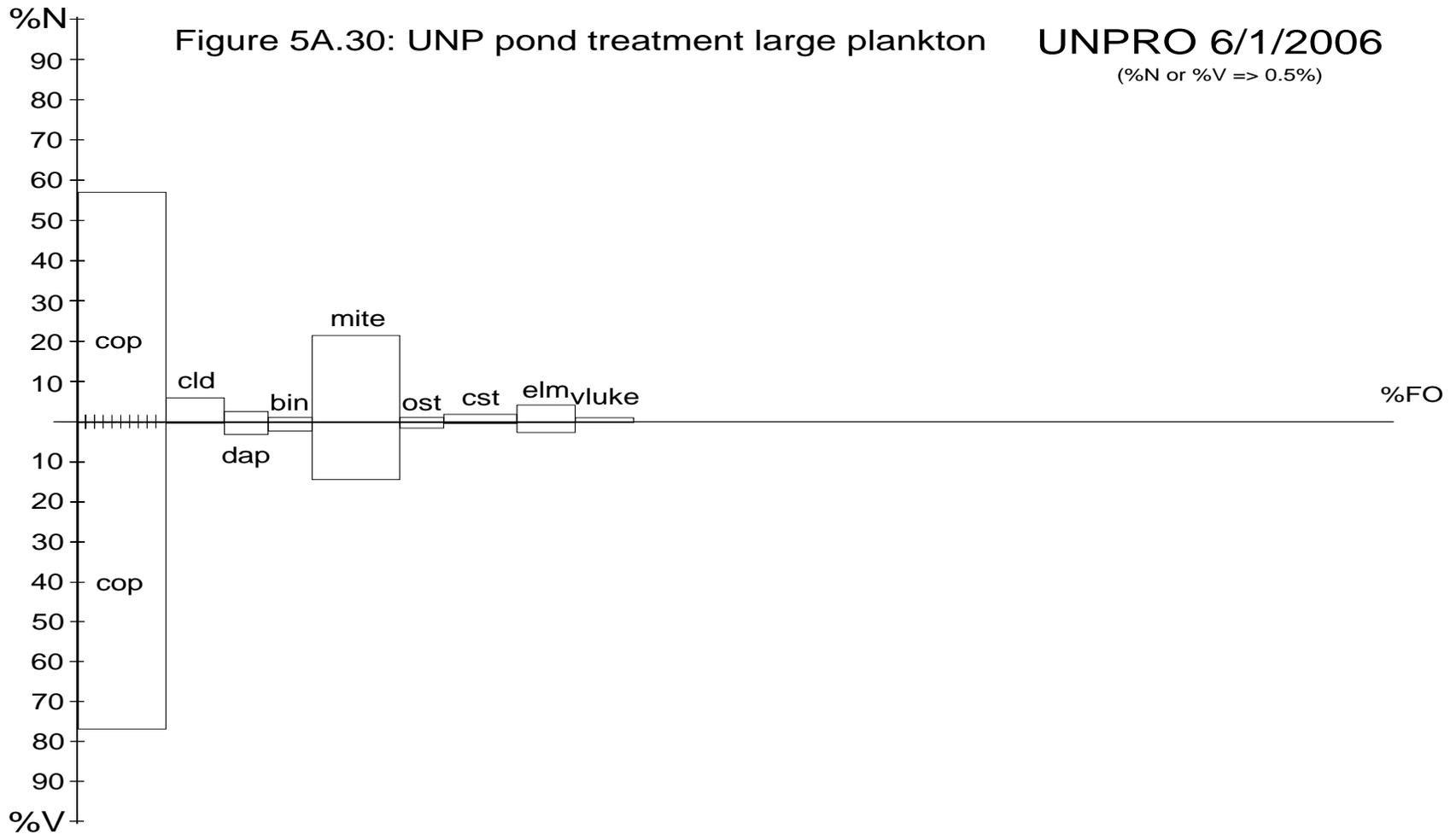


Figure 5A-30. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for unprocessed feed (UNP) treatment 1 June 2006; taxa data pooled among six replicate ponds (0.015 hectares).

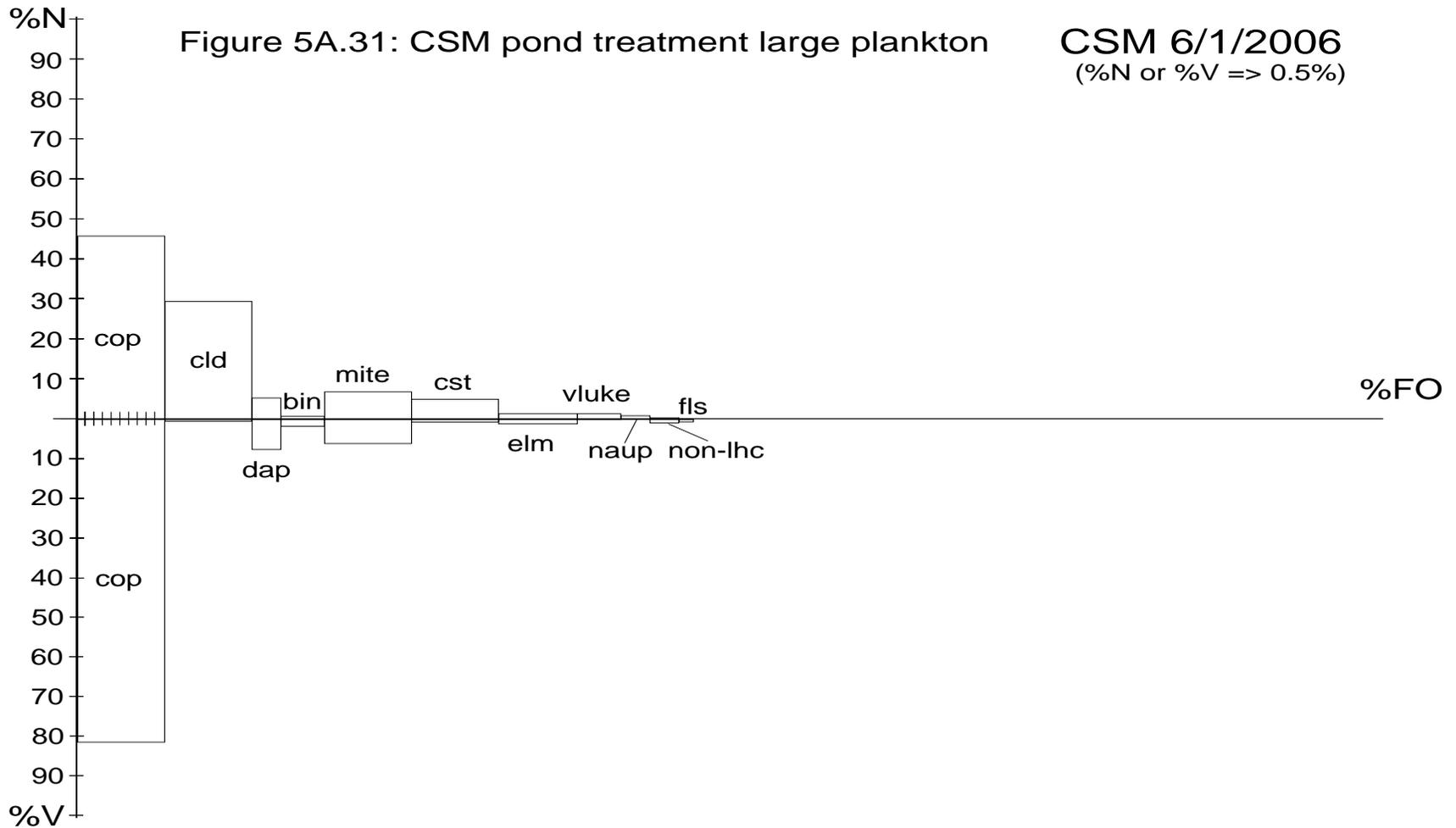


Figure 5A-31. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for cottonseed meal fertilizer (CSM) treatment 1 June 2006; taxa data pooled among six replicate ponds (0.015 hectares).

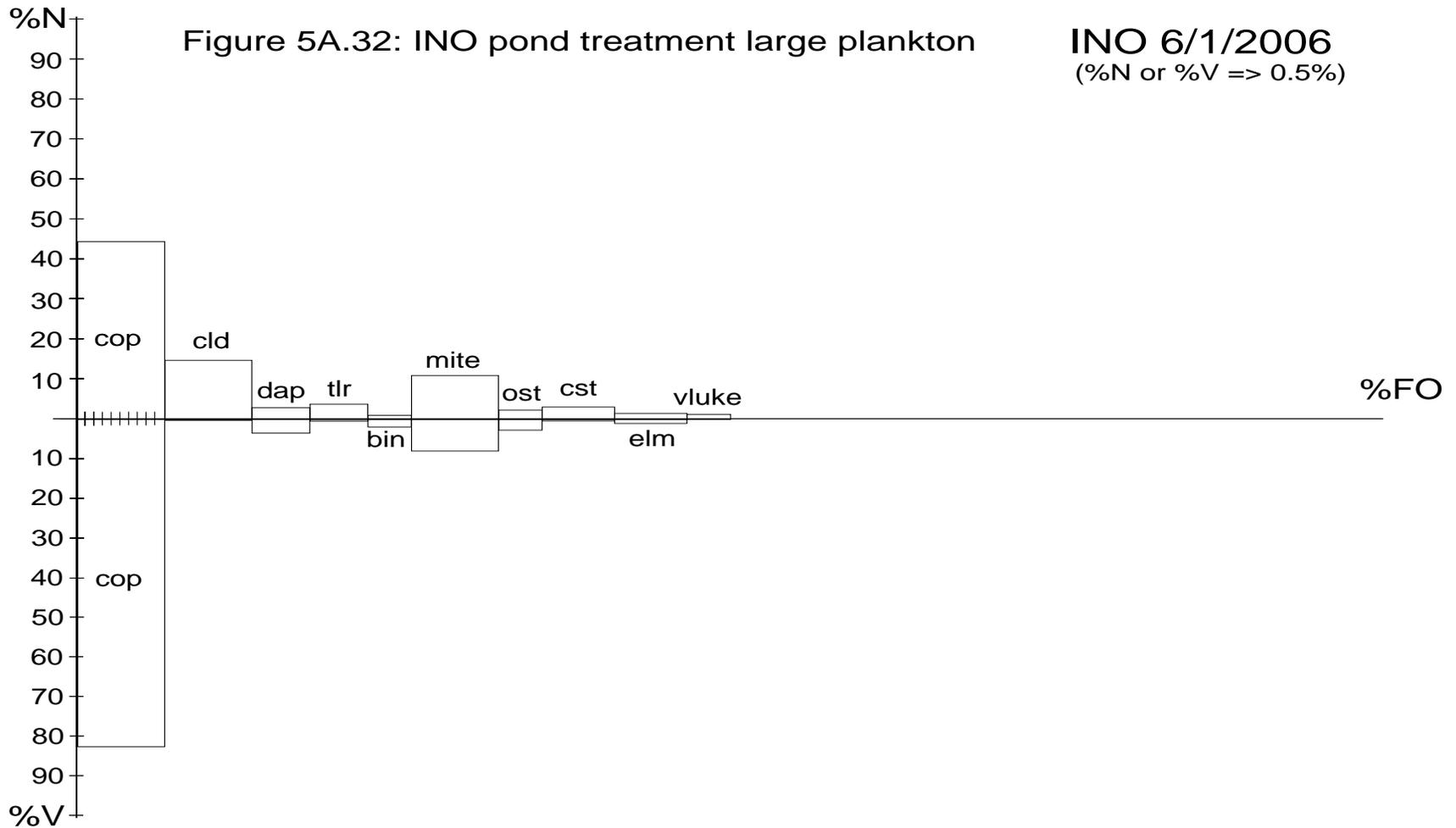


Figure 5A-32. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for inorganic fertilizer (INO) treatment 1 June 2006; taxa data pooled among six replicate ponds (0.015 hectares).

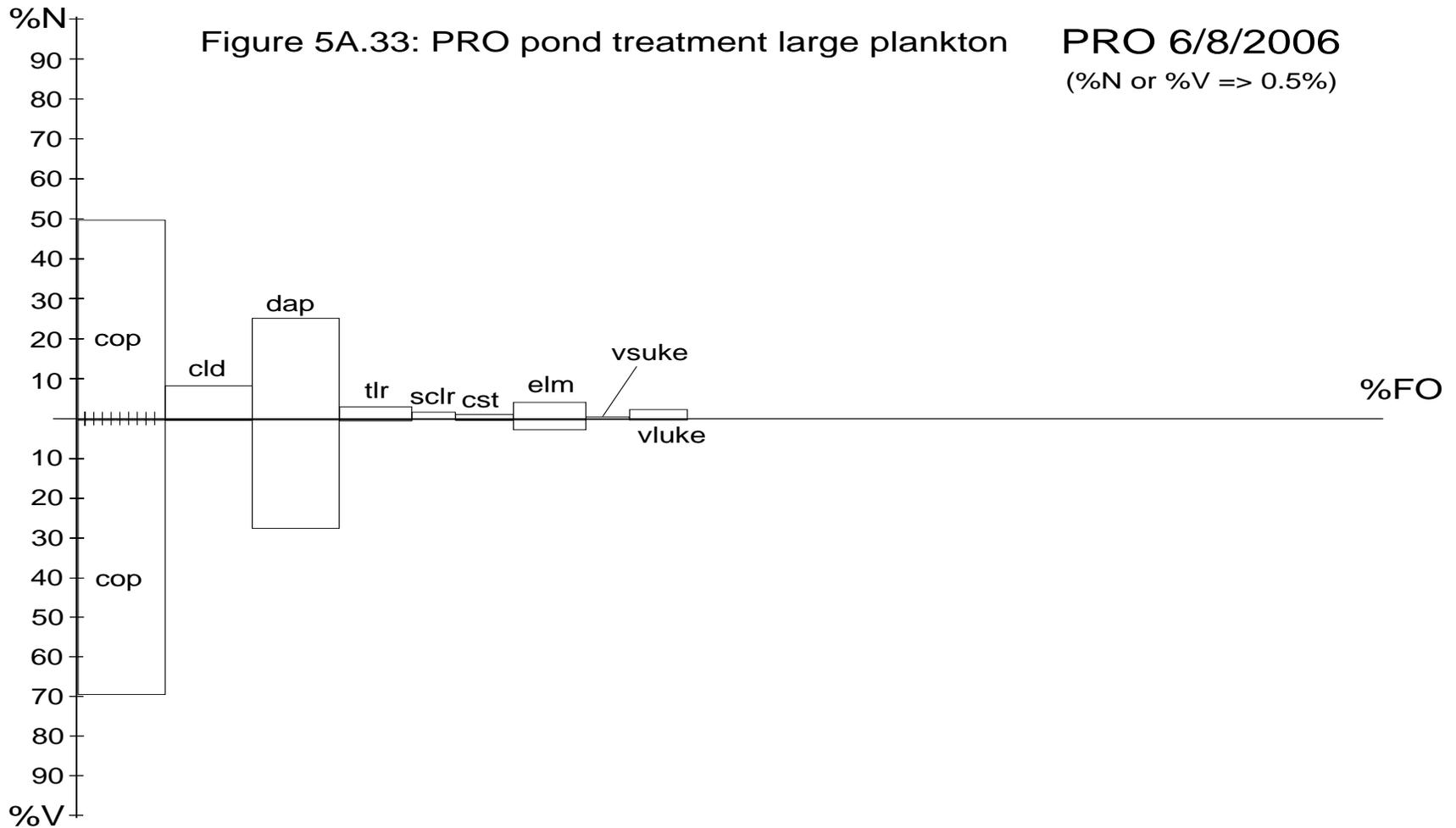


Figure 5A-33. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for processed feed (PRO) treatment 8 June 2006; taxa data pooled among six replicate ponds (0.015 hectares).

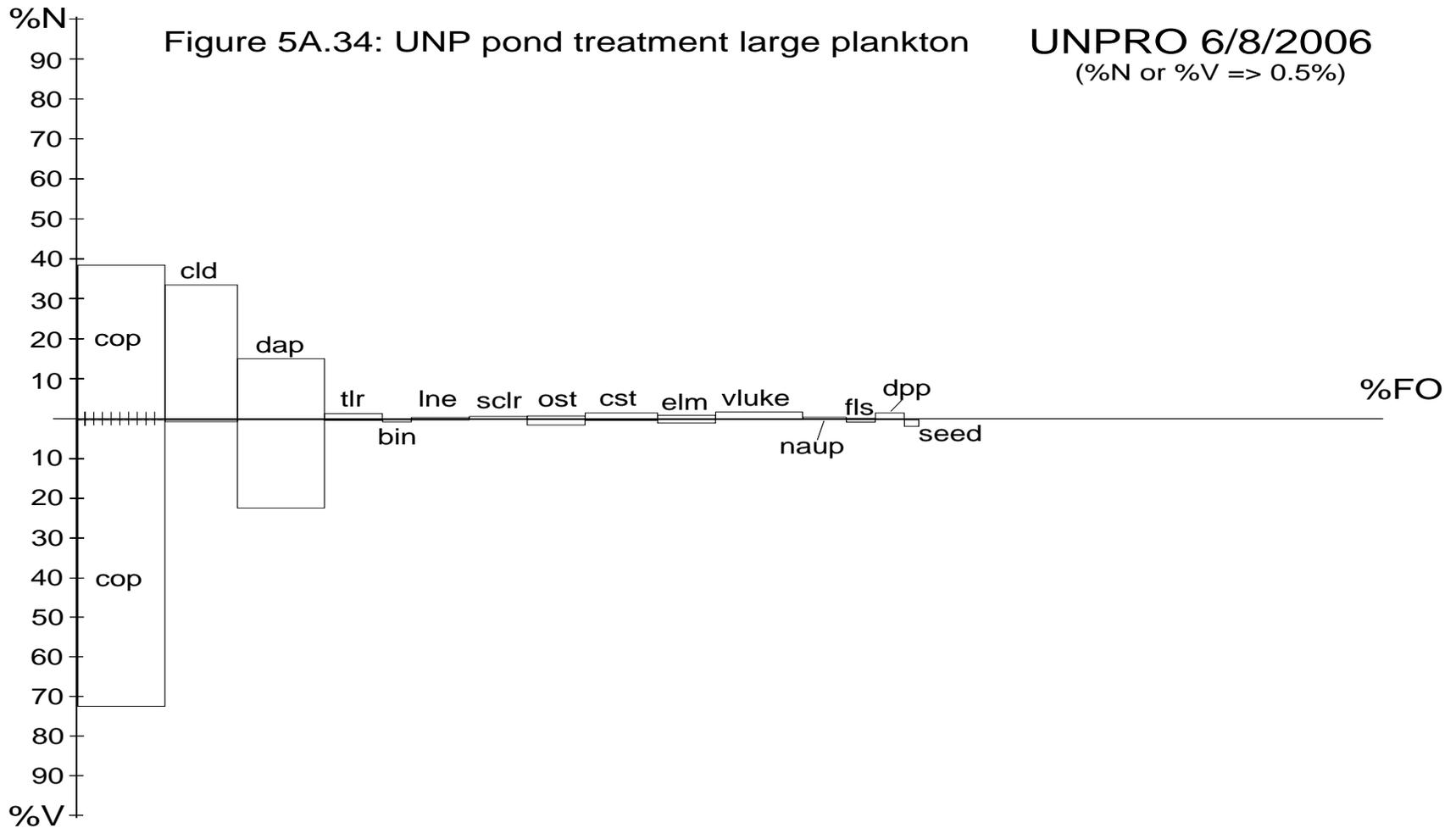


Figure 5A-34. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for unprocessed feed (UNP) treatment 8 June 2006; taxa data pooled among six replicate ponds (0.015 hectares).

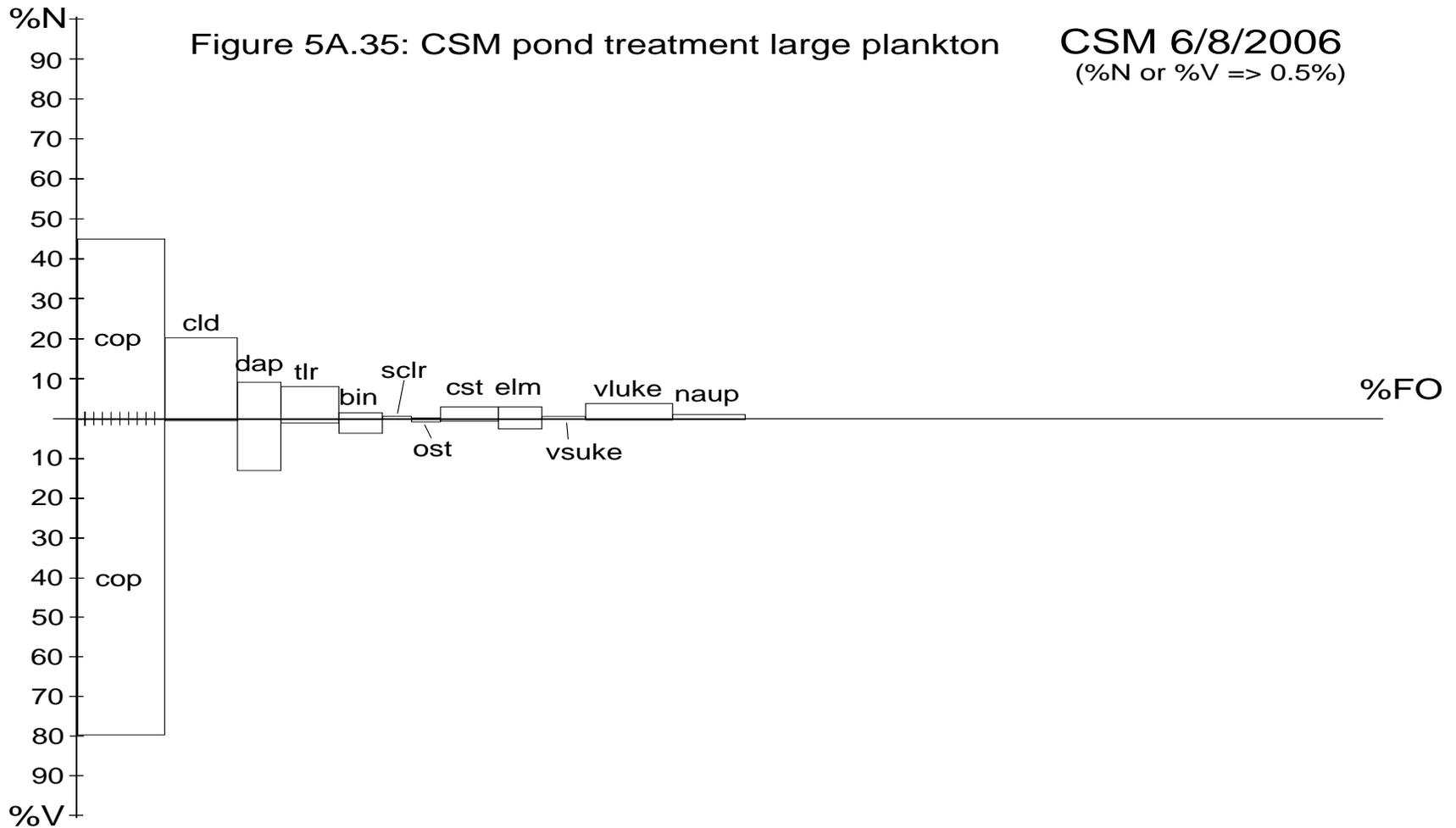


Figure 5A-35. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for cottonseed meal fertilizer (CSM) treatment 8 June 2006; taxa data pooled among six replicate ponds (0.015 hectares).

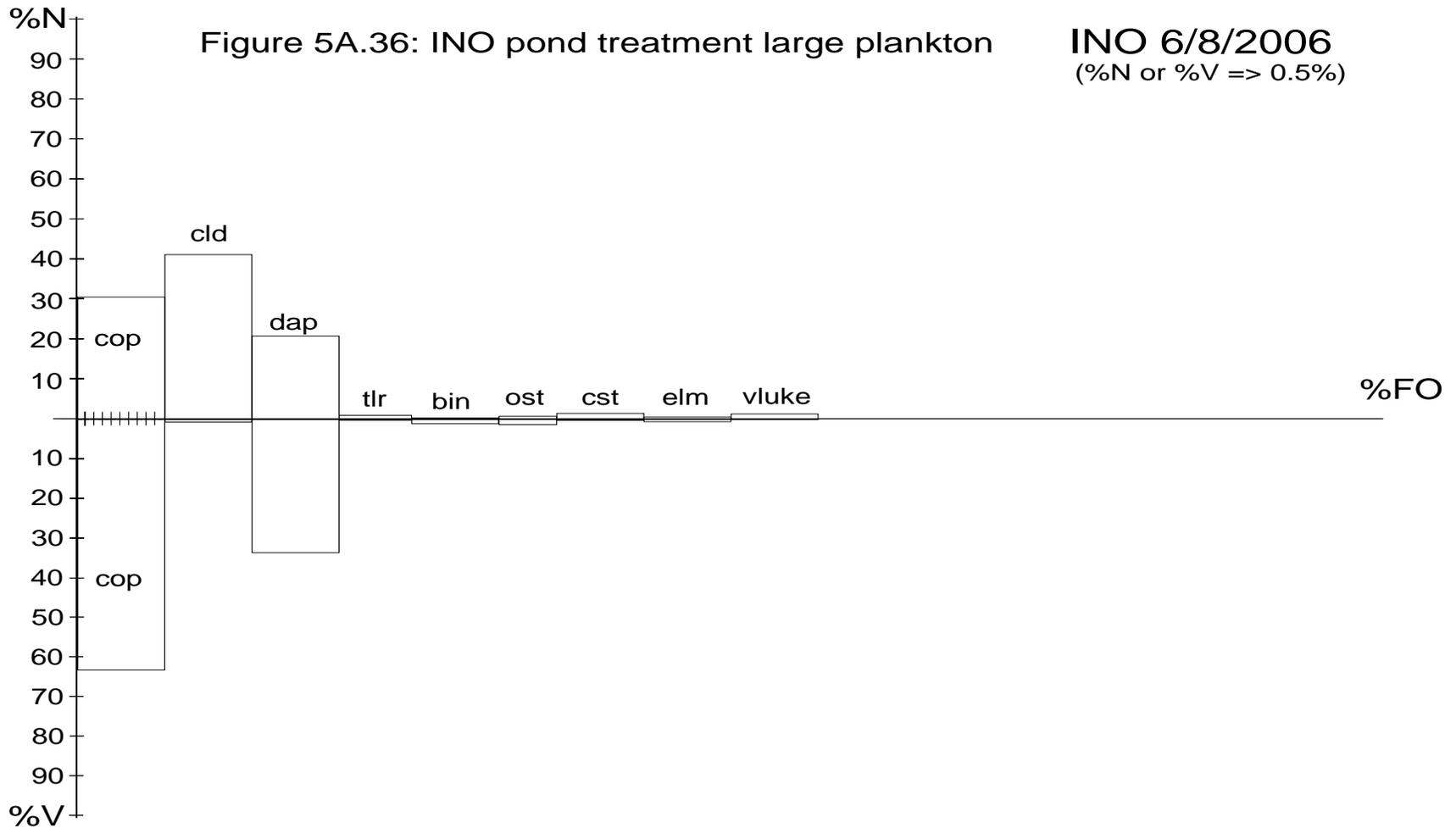


Figure 5A-36. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for inorganic fertilizer (INO) treatment 8 June 2006; taxa data pooled among six replicate ponds (0.015 hectares).

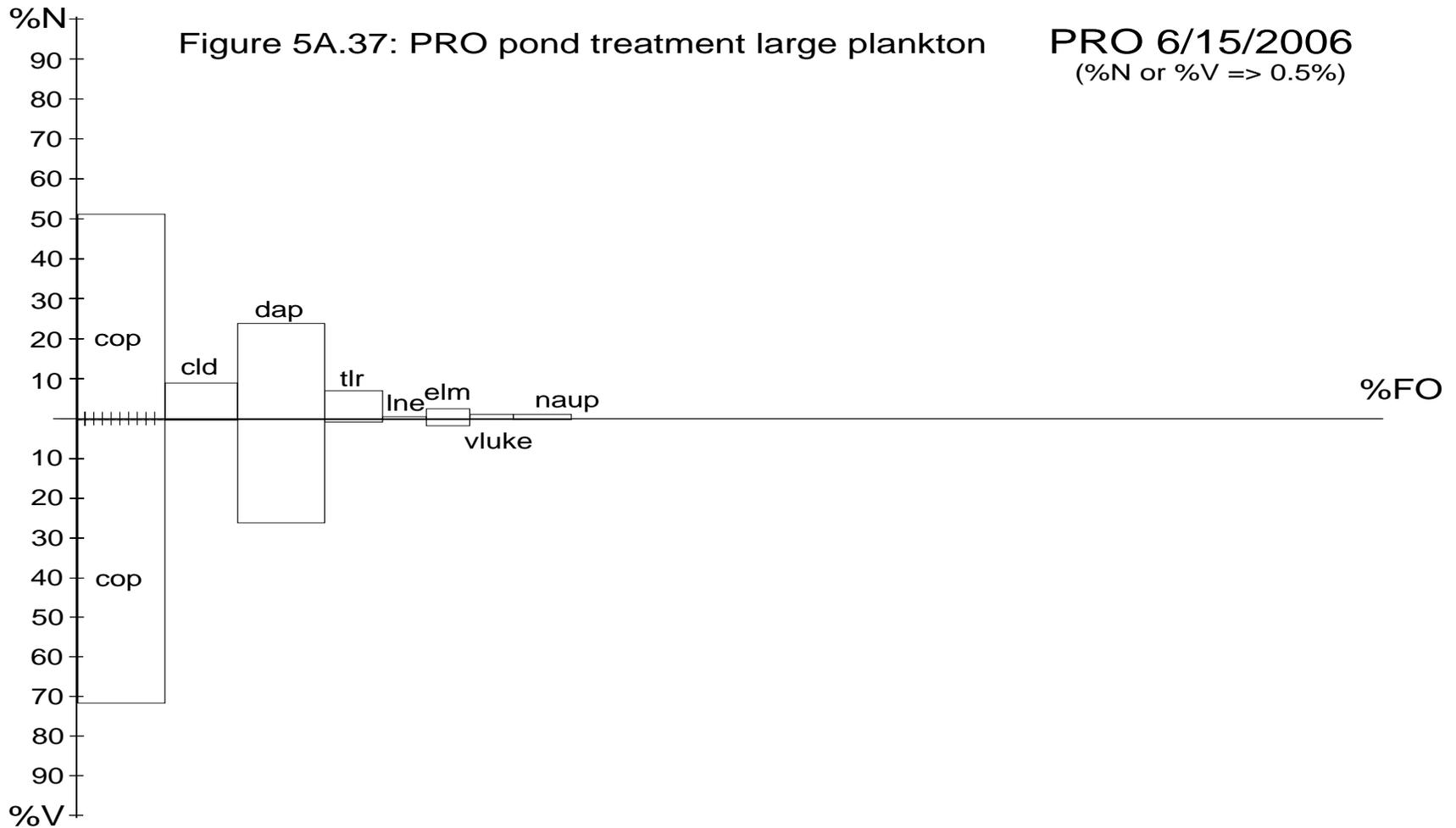


Figure 5A-37. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for processed feed (PRO) treatment 15 June 2006; taxa data pooled among six replicate ponds (0.015 hectares).

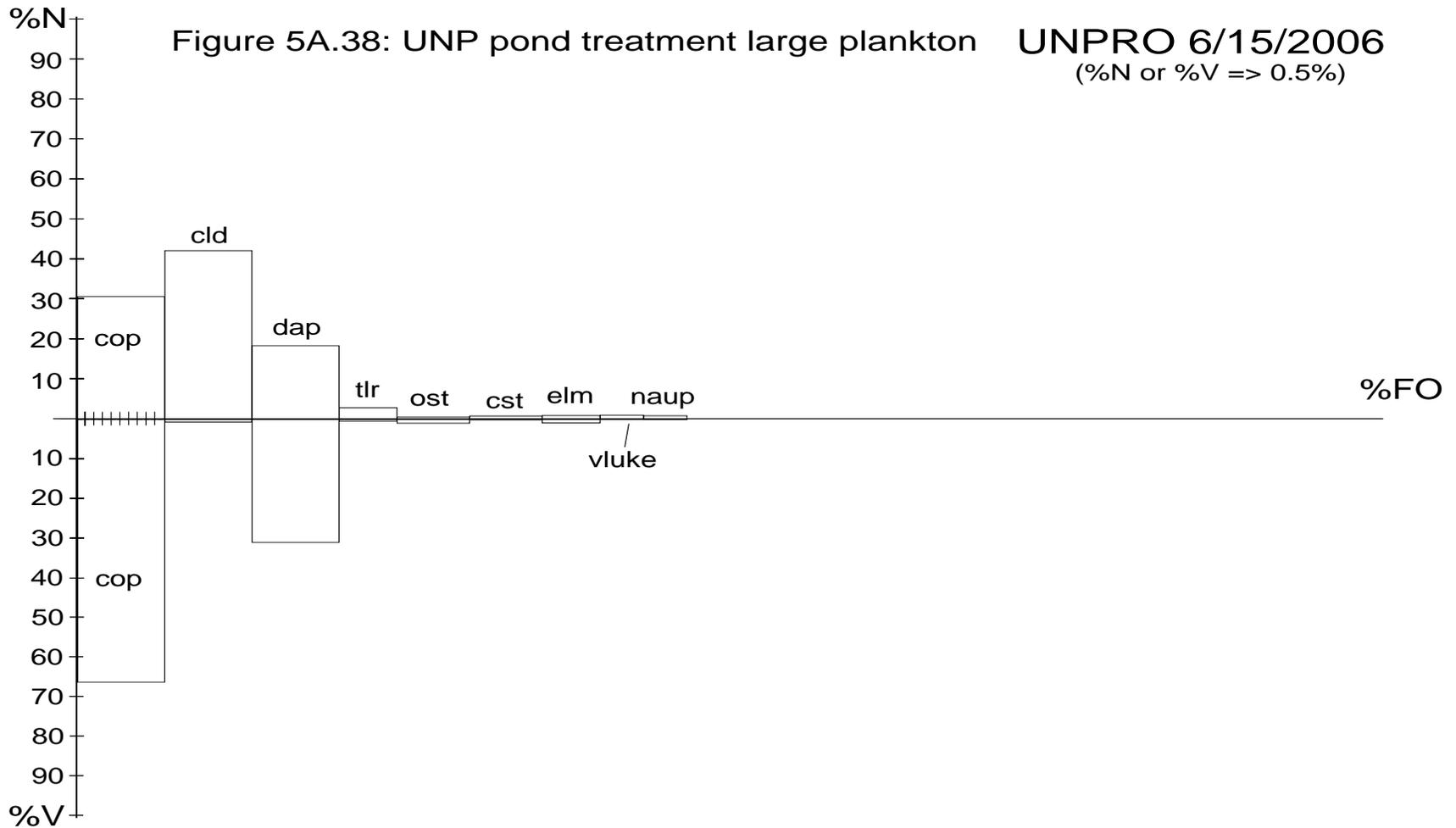


Figure 5A-38. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for unprocessed feed (UNP) treatment 15 June 2006; taxa data pooled among six replicate ponds (0.015 hectares).

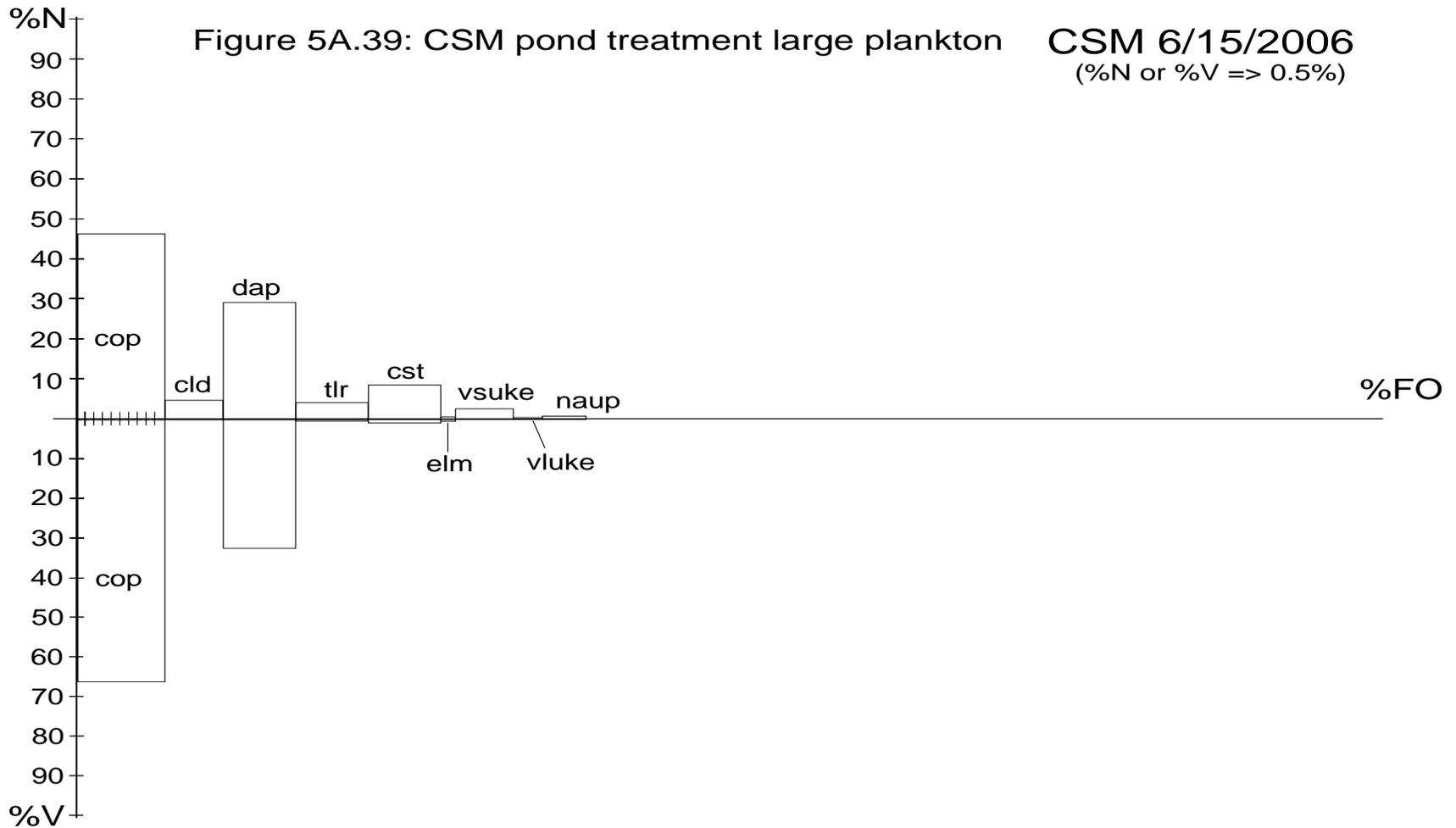


Figure 5A-39. Large zooplankton (> 200 µm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for cottonseed meal fertilizer (CSM) treatment 15 June 2006; taxa data pooled among six replicate ponds (0.015 hectares).

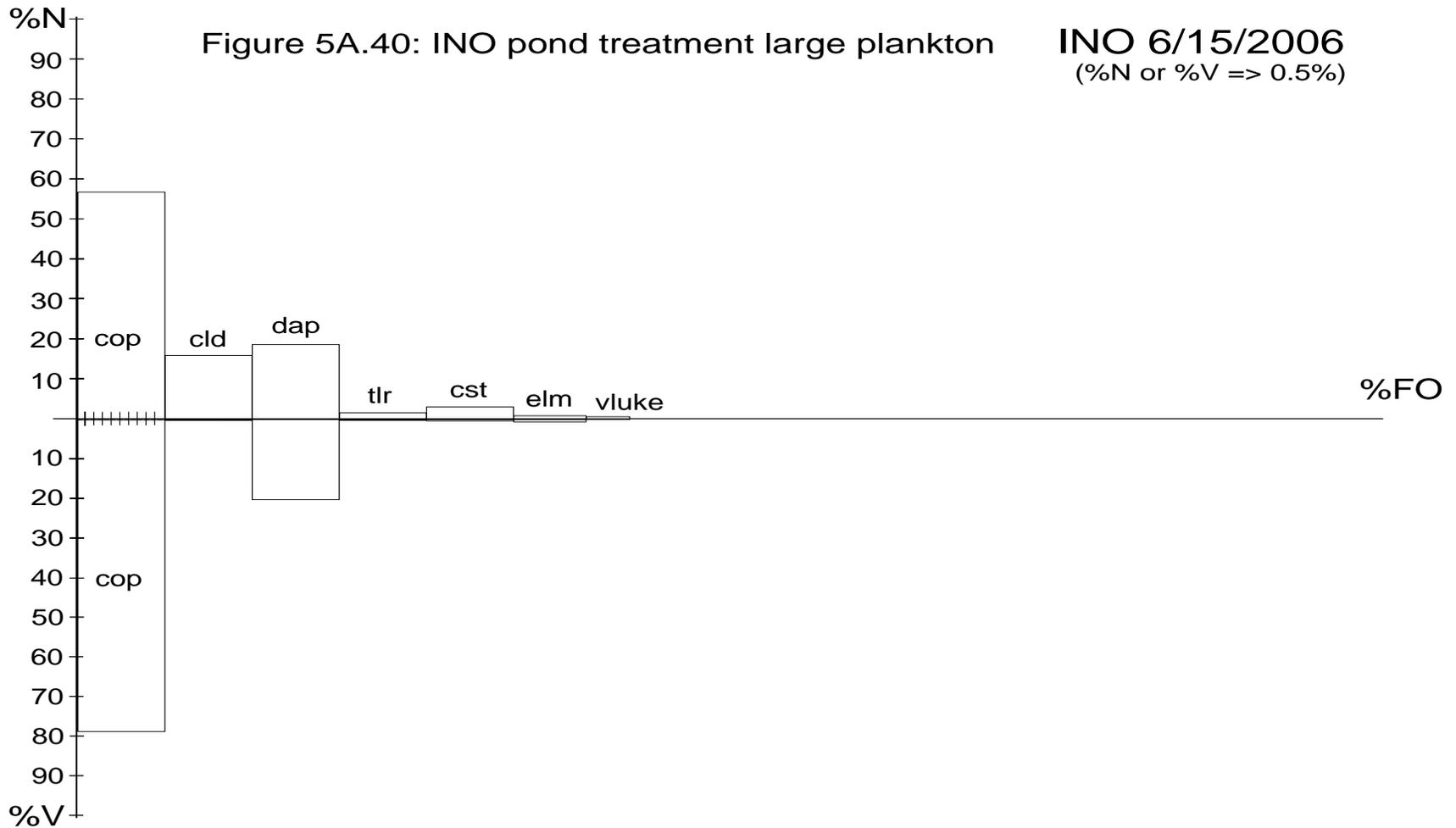


Figure 5A-40. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for inorganic fertilizer (INO) treatment 15 June 2006; taxa data pooled among six replicate ponds (0.015 hectares).

PRO 4/13/2006 - not pooled
 (%N or %V => 0.5%; +/- 1 SE)

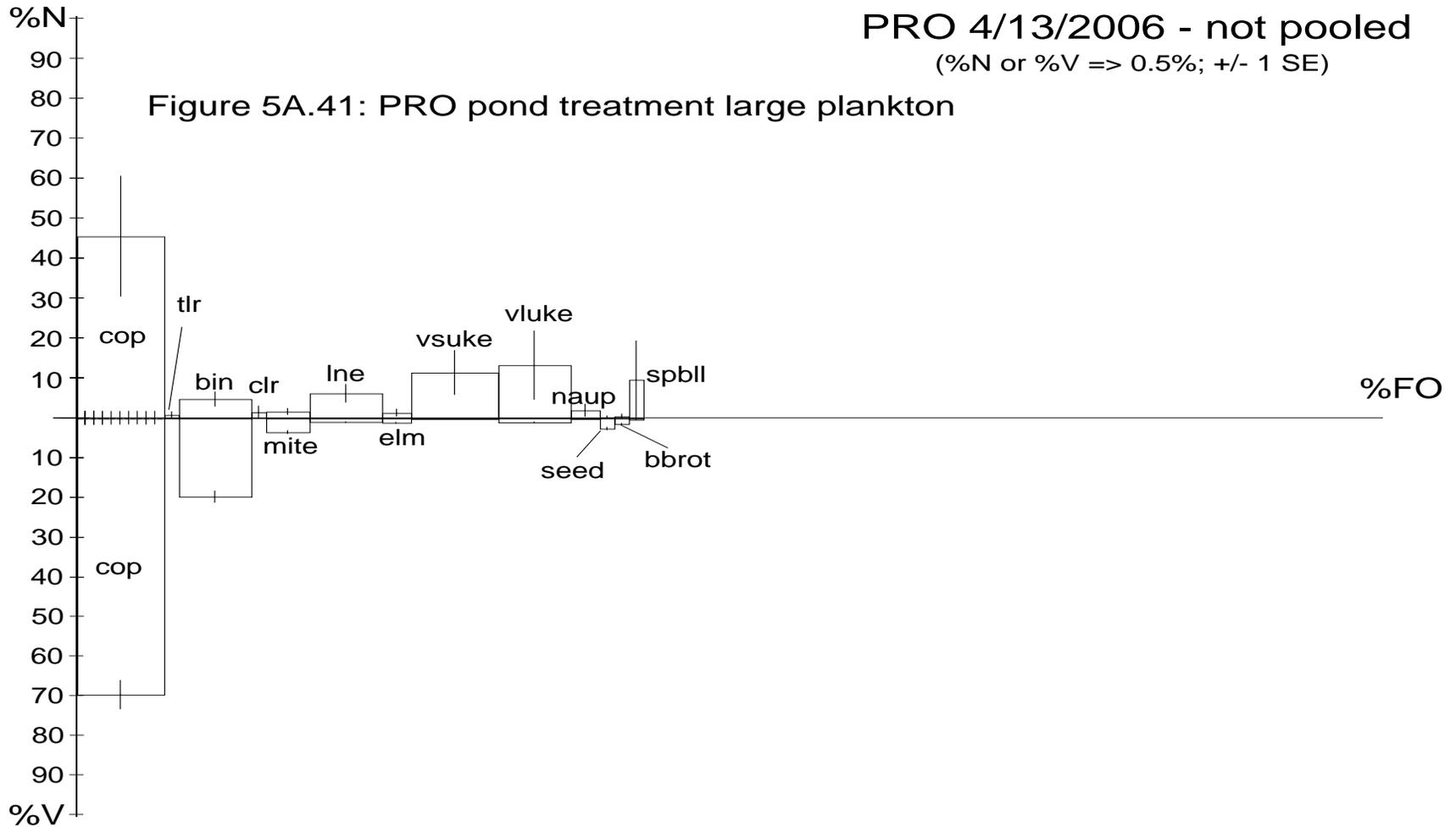


Figure 5A-41. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for processed feed (PRO) treatment 13 April 2006; taxa %N (± 1 SE) and % V (± 1 SE) for six replicate ponds (0.015 hectares).

UNPRO 4/13/2006 - not pooled

(%N or %V => 0.5%; +/- 1 SE)

Figure 5A.42: UNP pond treatment large plankton

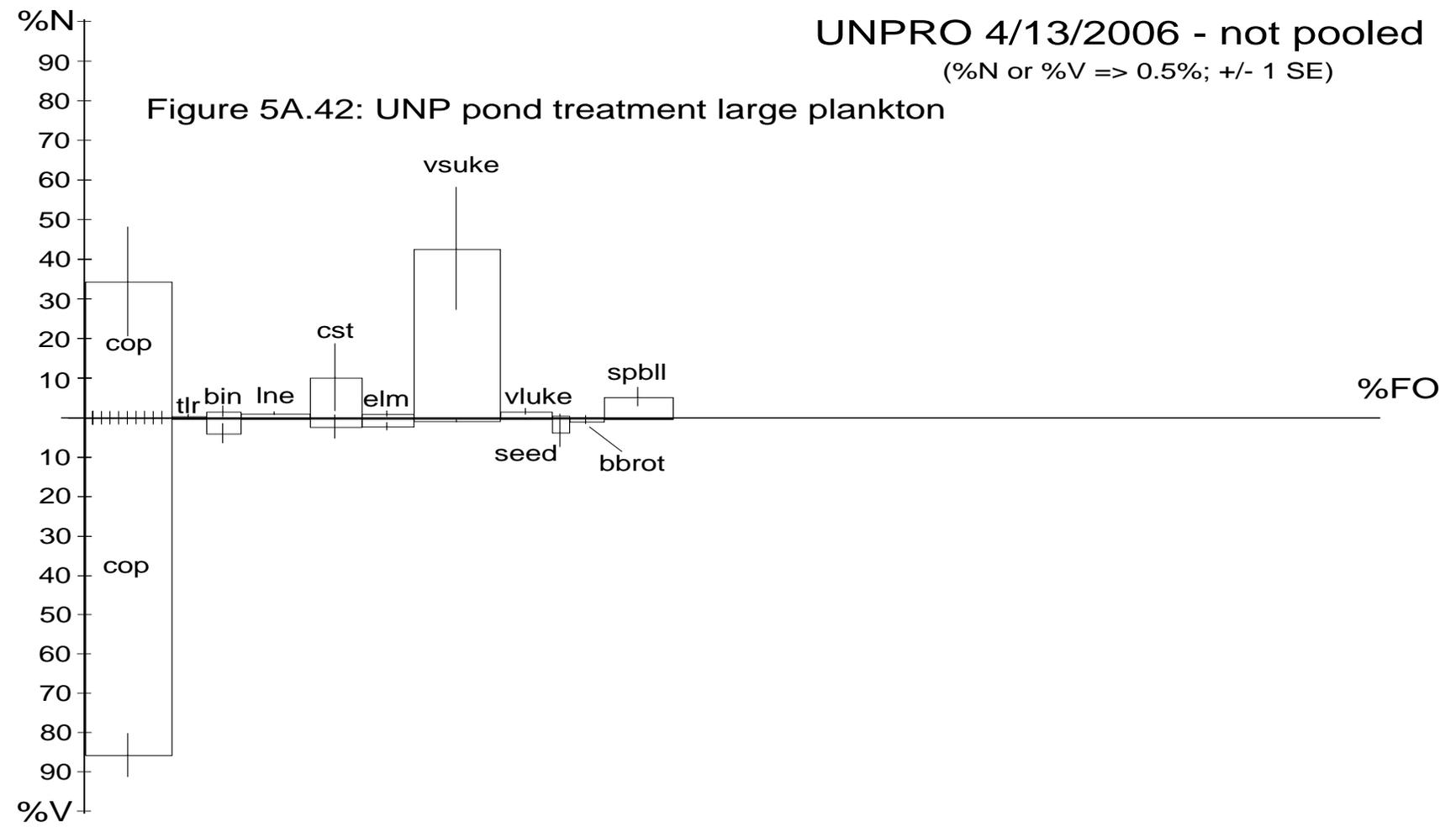


Figure 5A-42. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for unprocessed feed (UNP) treatment 13 April 2006; taxa %N (± 1 SE) and % V (± 1 SE) for six replicate ponds (0.015 hectares).

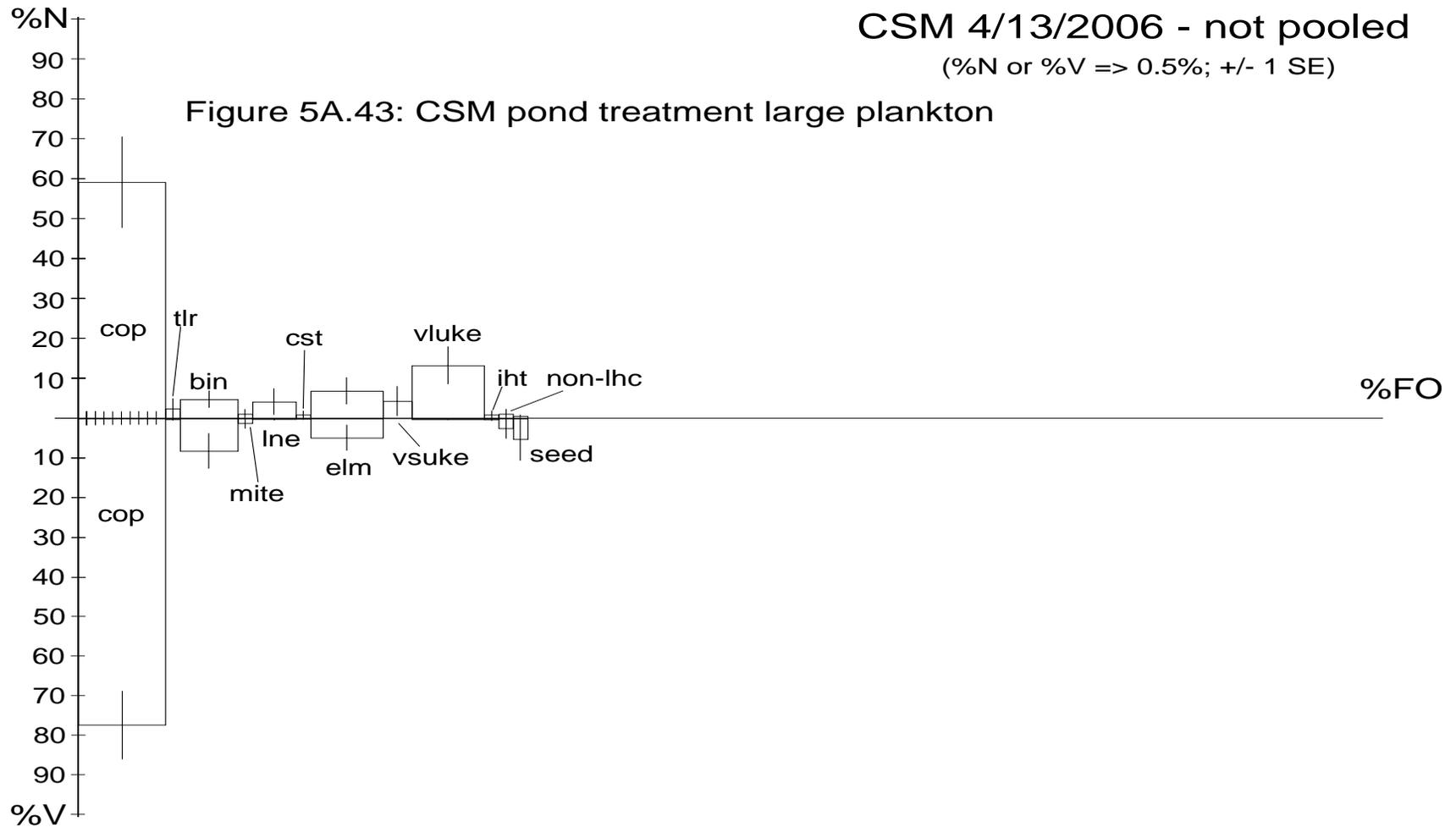


Figure 5A-43. Large zooplankton (> 200 μ m) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for cottonseed meal fertilizer (CSM) treatment 13 April 2006; taxa %N (\pm 1 SE) and % V (\pm 1 SE) for six replicate ponds (0.015 hectares).

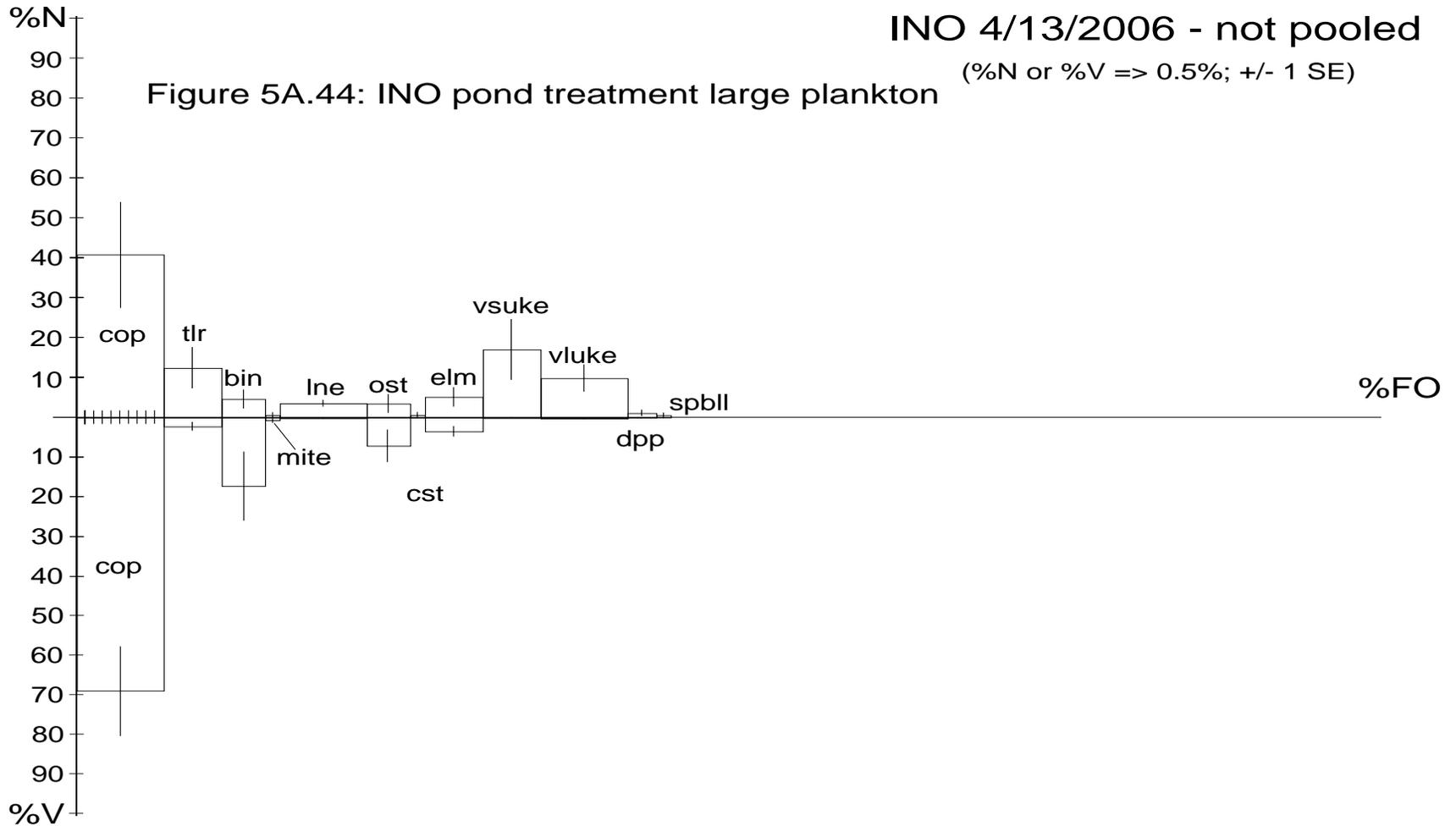


Figure 5A-44. Large zooplankton (> 200 μ m) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for inorganic fertilizer (INO) treatment 13 April 2006; taxa %N (\pm 1 SE) and % V (\pm 1 SE) for six replicate ponds (0.015 hectares).

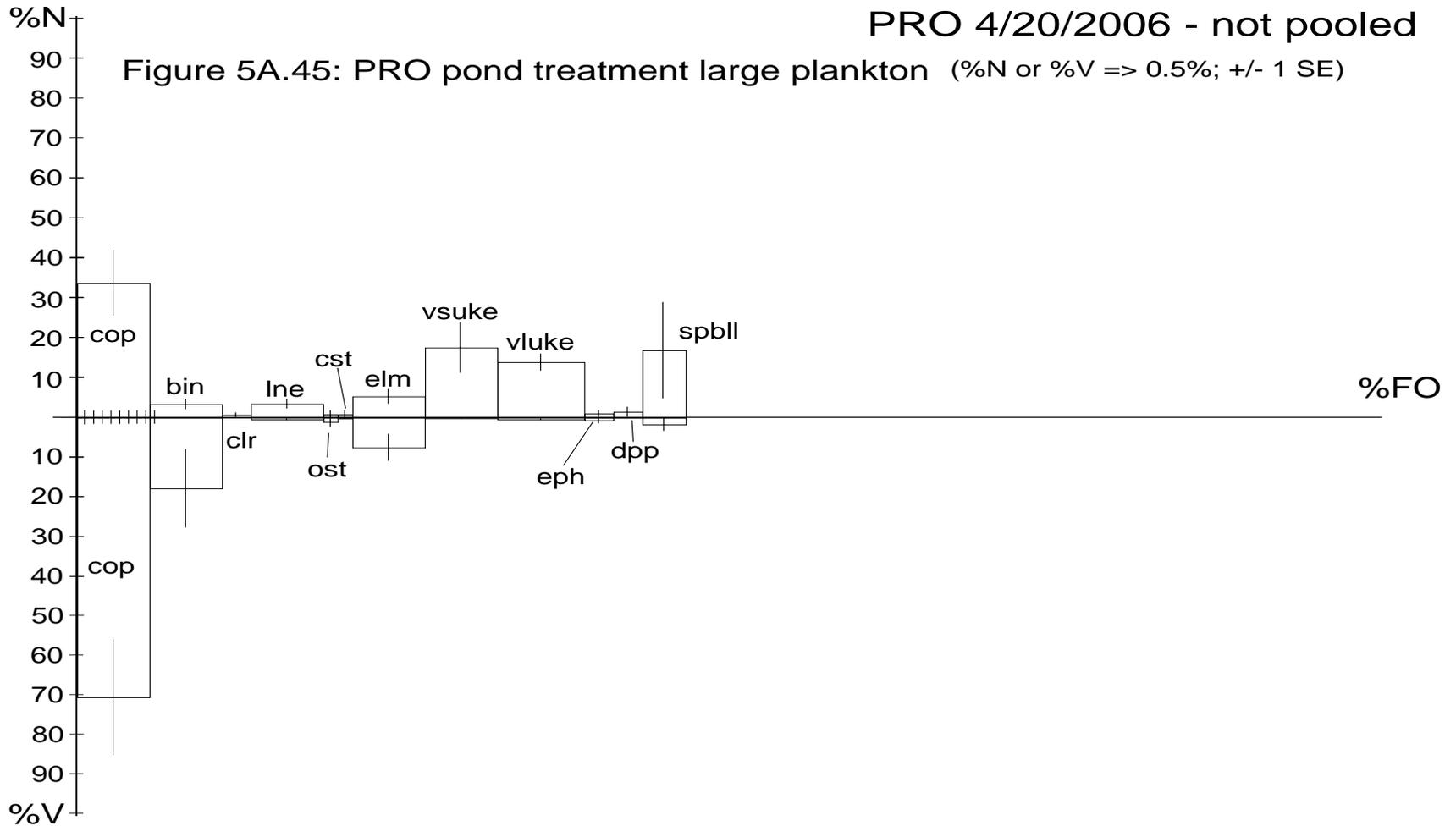


Figure 5A-45. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for processed feed (PRO) treatment 20 April 2006; taxa %N (± 1 SE) and % V (± 1 SE) for six replicate ponds (0.015 hectares).

UNPRO 4/20/2006 - not pooled

(%N or %V => 0.5%; +/- 1 SE)

Figure 5A.46: UNP pond treatment large plankton

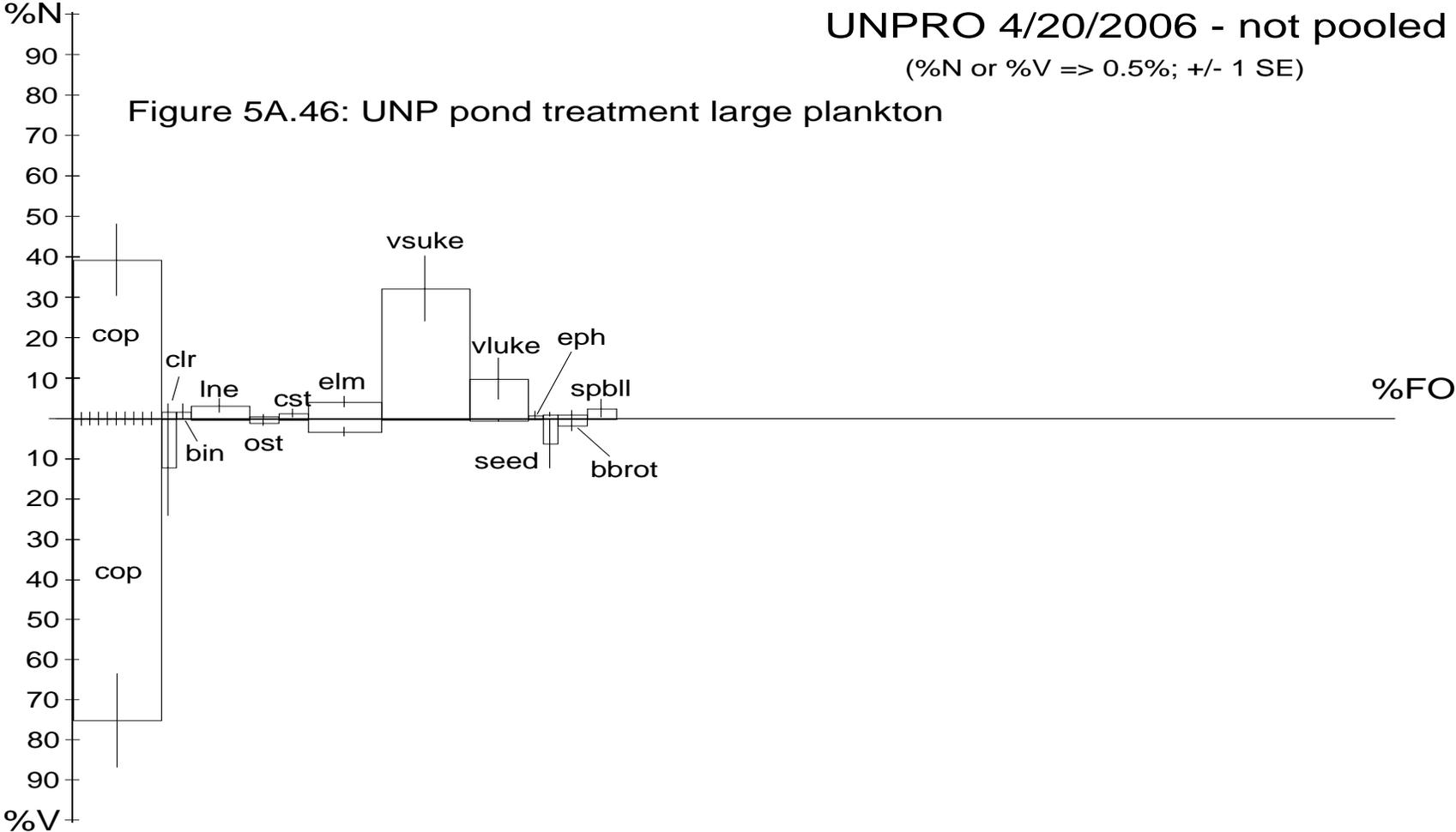


Figure 5A-46. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for unprocessed feed (UNP) treatment 20 April 2006; taxa %N (± 1 SE) and % V (± 1 SE) for six replicate ponds (0.015 hectares).

UNPRO 4/20/2006 - not pooled

(%N or %V => 0.5%; +/- 1 SE)

Figure 5A.46: UNP pond treatment large plankton

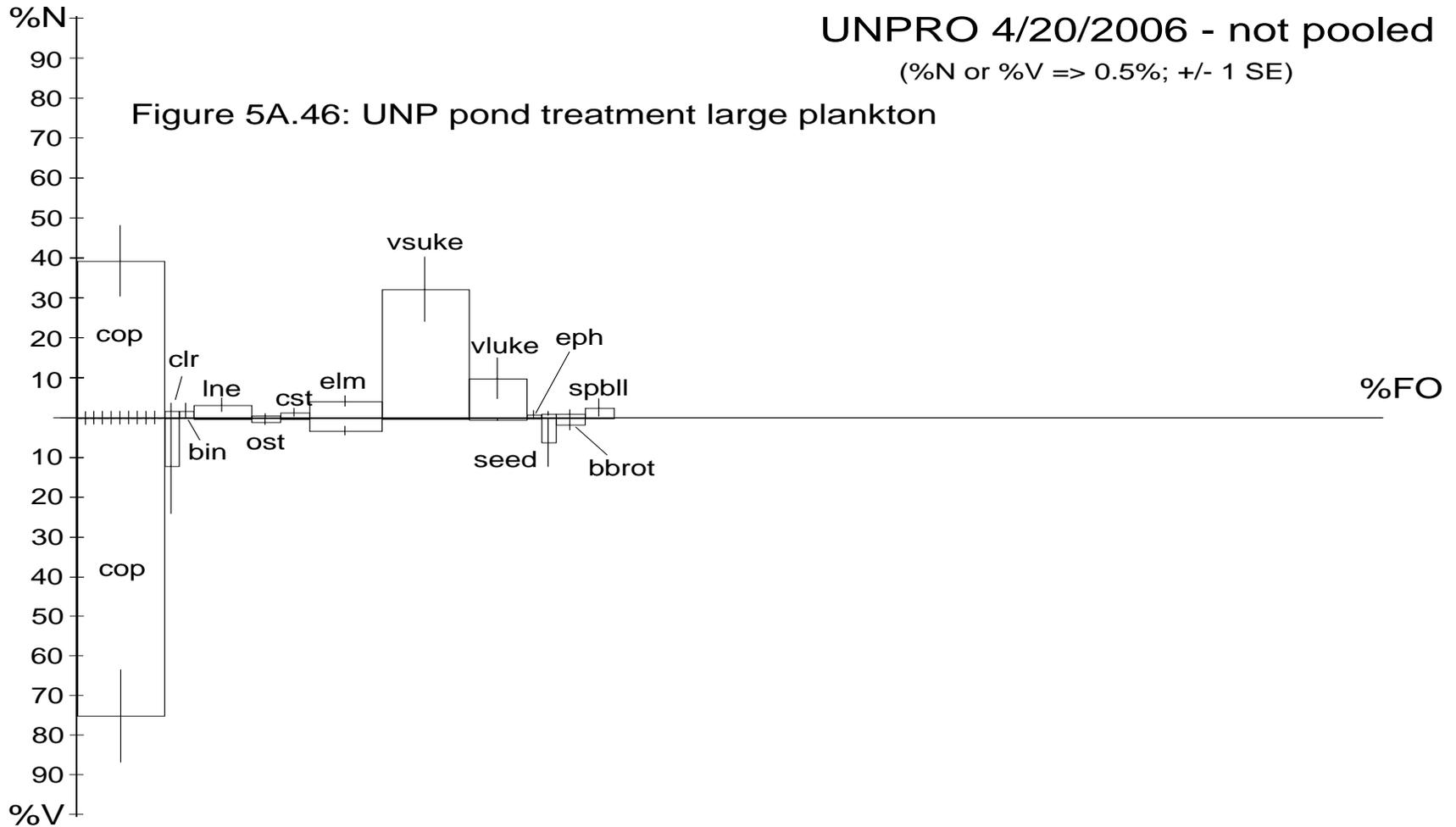


Figure 5A-47. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for cottonseed meal fertilizer (CSM) treatment 20 April 2006; taxa %N (± 1 SE) and % V (± 1 SE) for six replicate ponds (0.015 hectares).

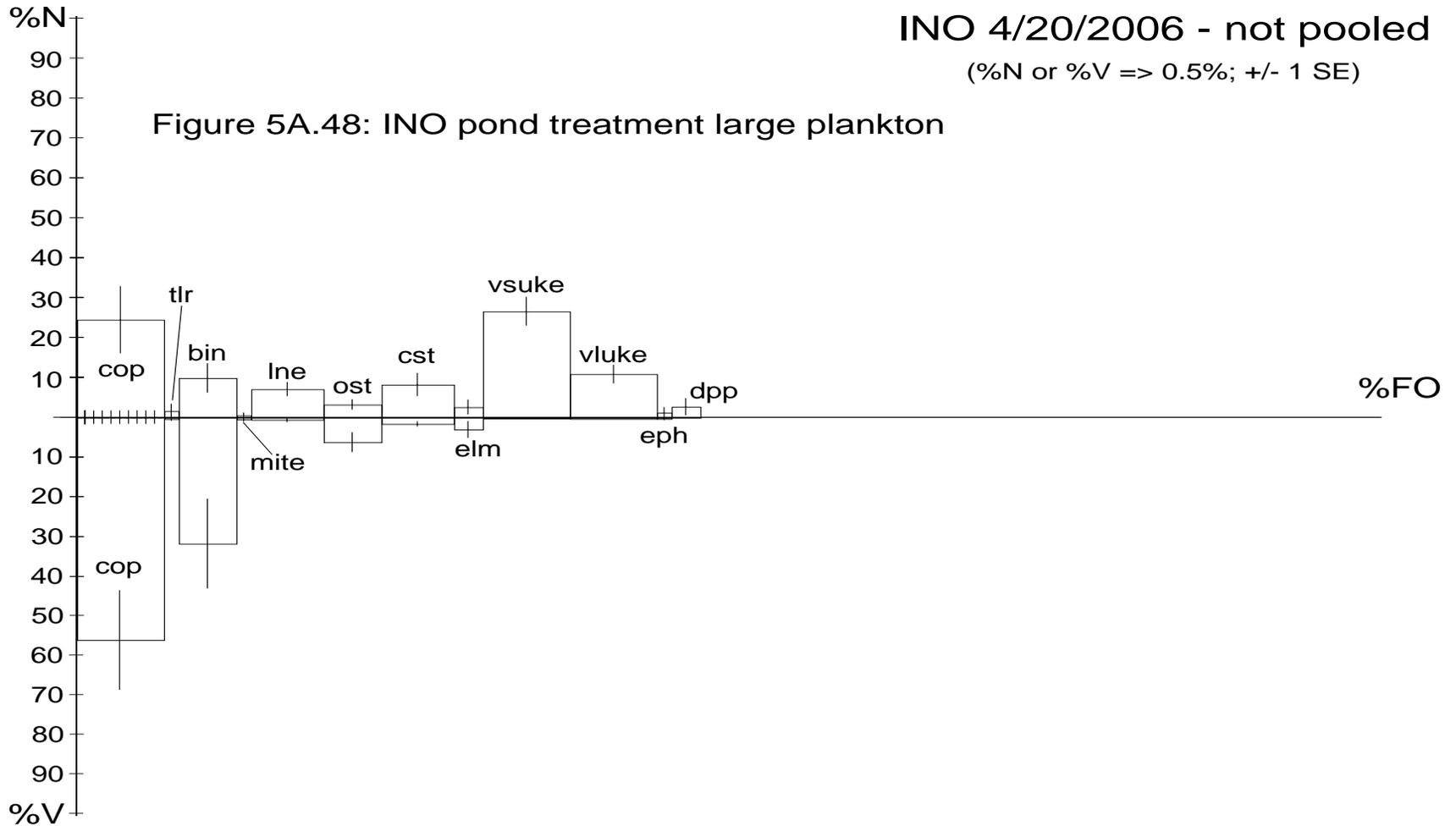


Figure 5A-48. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for inorganic fertilizer (INO) treatment 20 April 2006; taxa %N (± 1 SE) and % V (± 1 SE) for six replicate ponds (0.015 hectares).

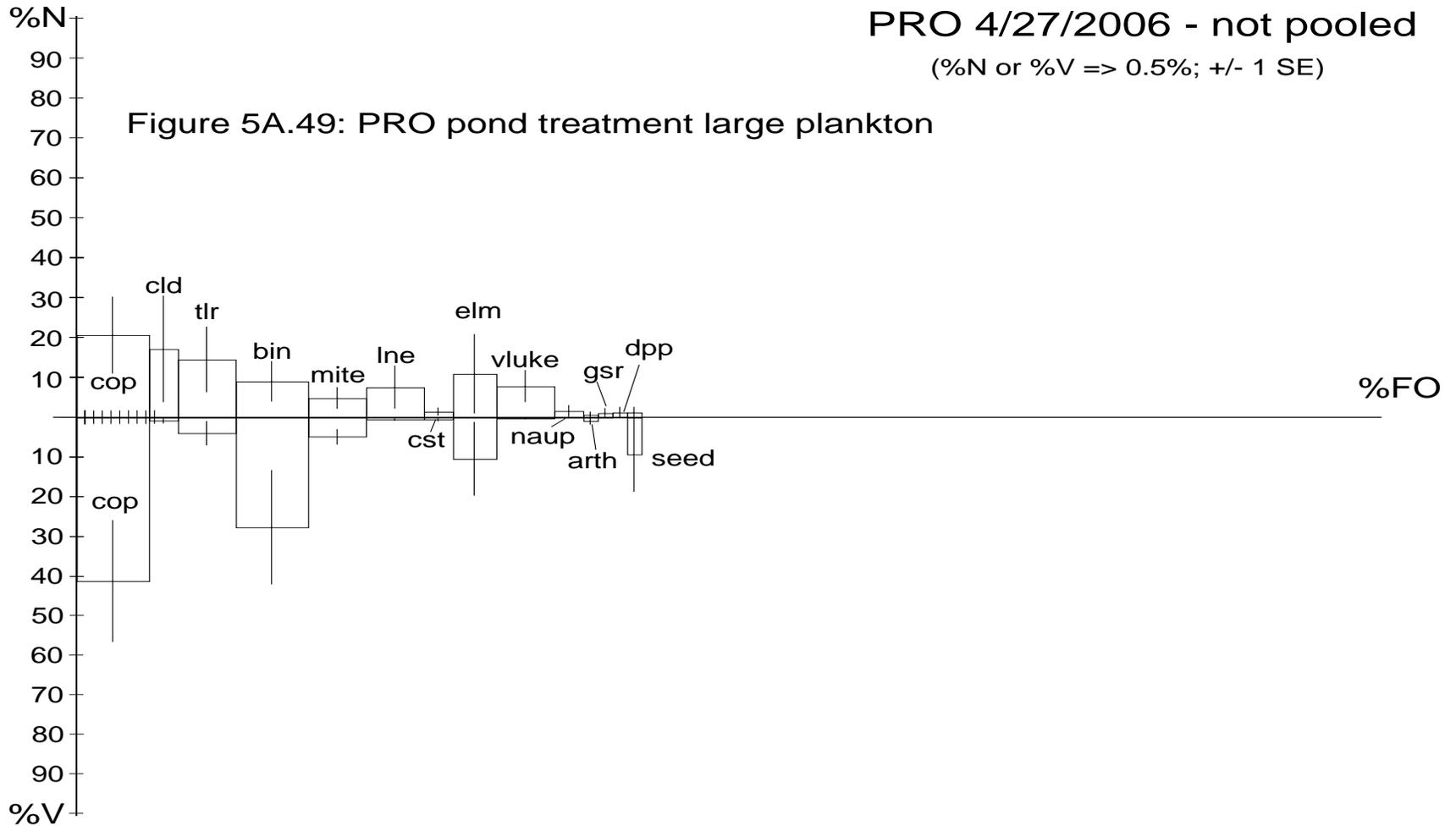


Figure 5A-49. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for processed feed (PRO) treatment 27 April 2006; taxa %N (± 1 SE) and % V (± 1 SE) for six replicate ponds (0.015 hectares).

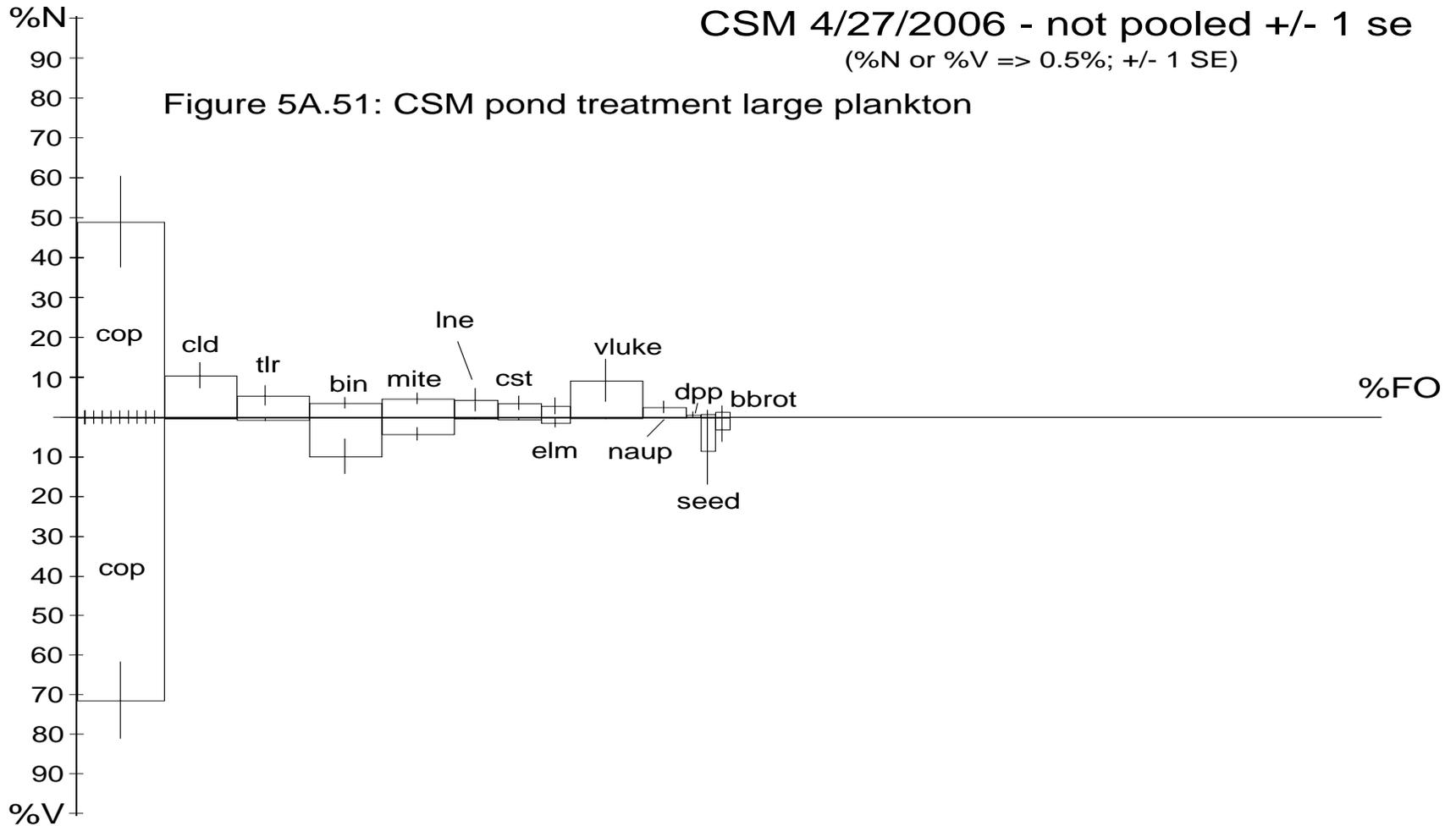


Figure 5A-51. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for cottonseed meal fertilizer (CSM) treatment 27 April 2006; taxa %N (± 1 SE) and % V (± 1 SE) for six replicate ponds (0.015 hectares).

INO 4/27/2006 - not pooled

(%N or %V => 0.5%; +/- 1 SE)

Figure 5A.52: INO pond treatment large plankton

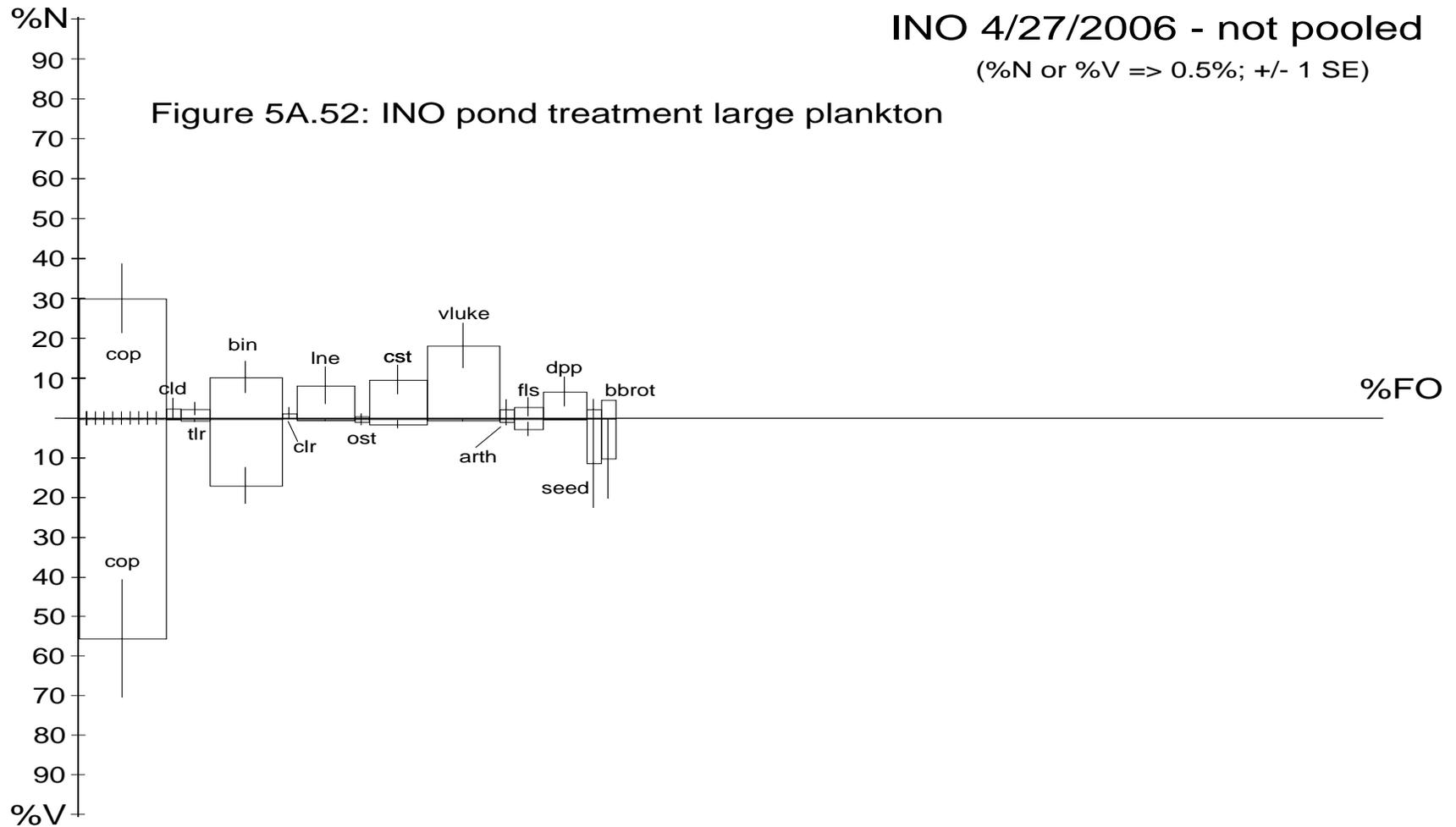


Figure 5A-52. Large zooplankton (> 200 μ m) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for inorganic fertilizer (INO) treatment 27 April 2006; taxa %N (\pm 1 SE) and % V (\pm 1 SE) for six replicate ponds (0.015 hectares).

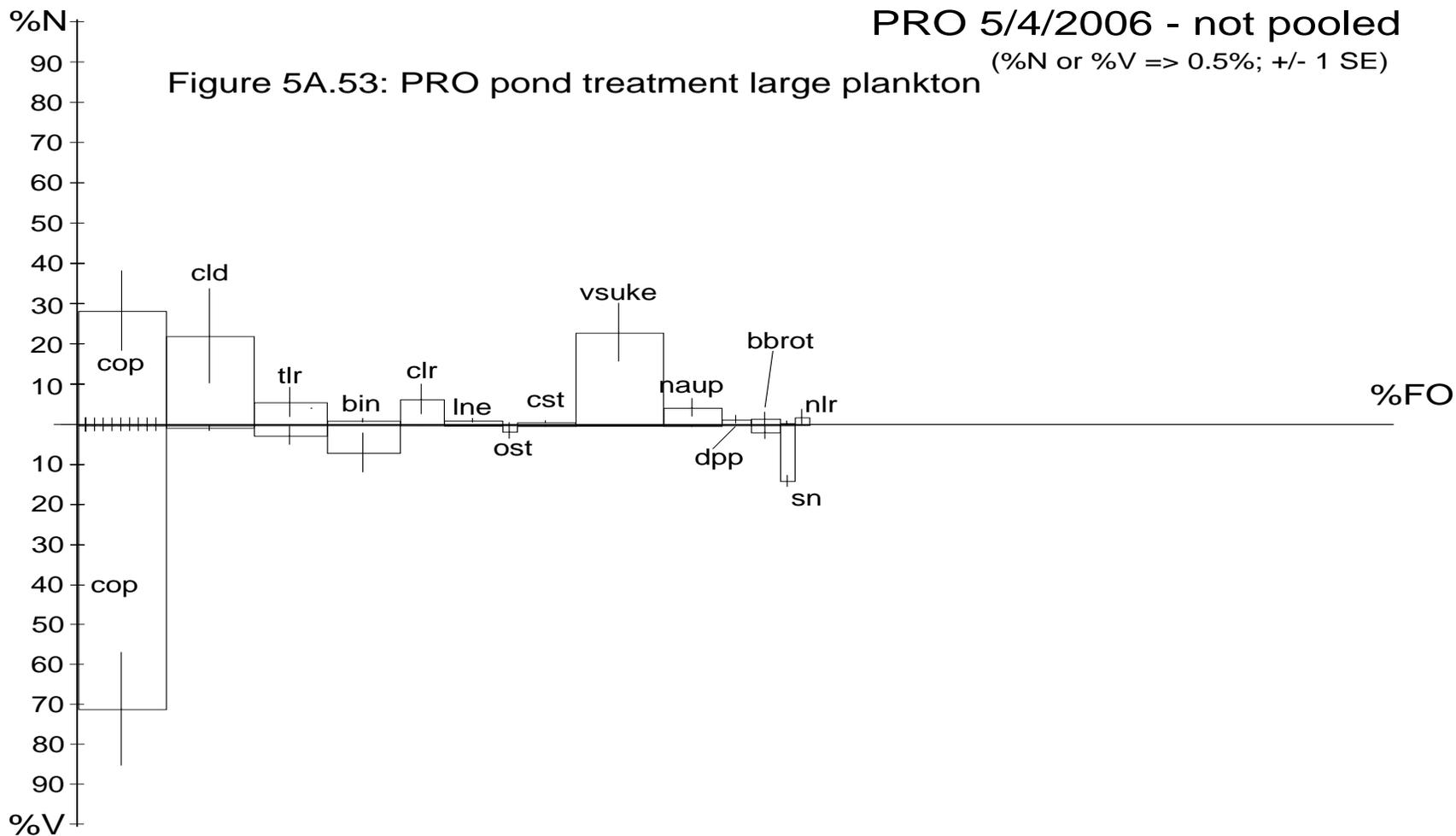


Figure 5A-53. Large zooplankton (> 200 μ m) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for processed feed (PRO) treatment 4 May 2006; taxa %N (\pm 1 SE) and % V (\pm 1 SE) for six replicate ponds (0.015 hectares).

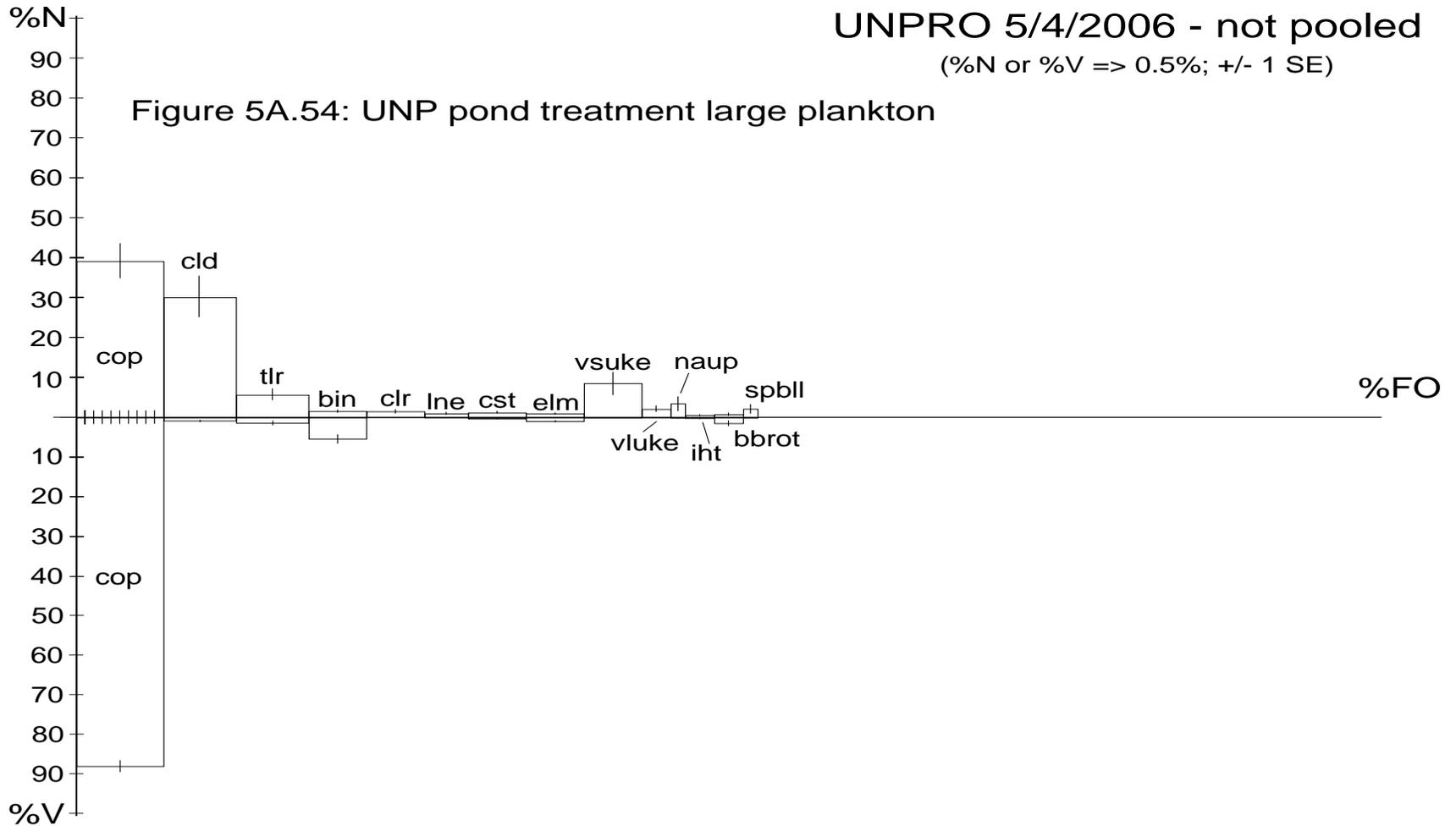


Figure 5A-54. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for unprocessed feed (UNP) treatment 4 May 2006; taxa %N (± 1 SE) and % V (± 1 SE) for six replicate ponds (0.015 hectares).

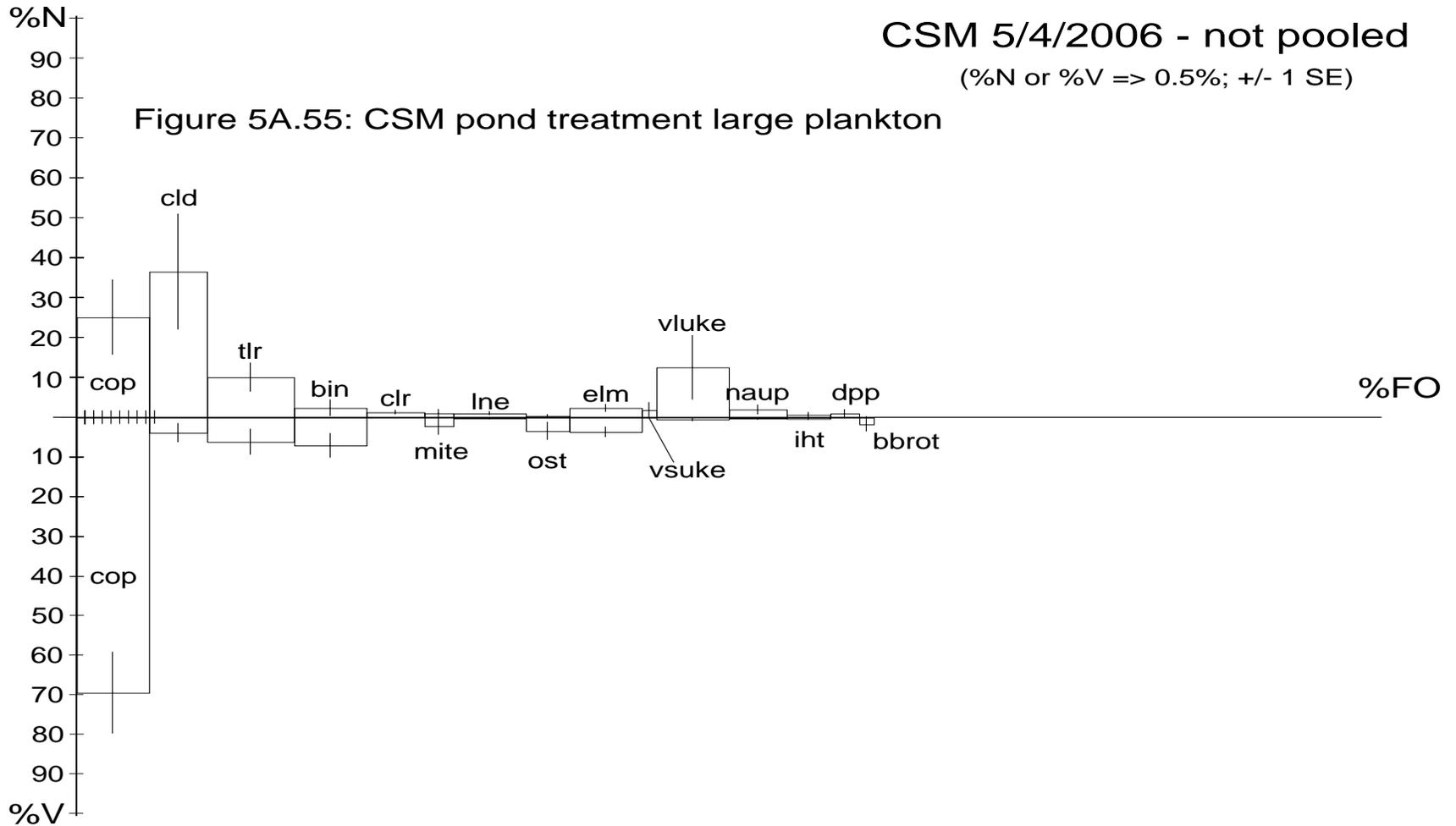


Figure 5A-55. Large zooplankton (> 200 μ m) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for cottonseed meal fertilizer (CSM) treatment 4 May 2006; taxa %N (\pm 1 SE) and % V (\pm 1 SE) for six replicate ponds (0.015 hectares).

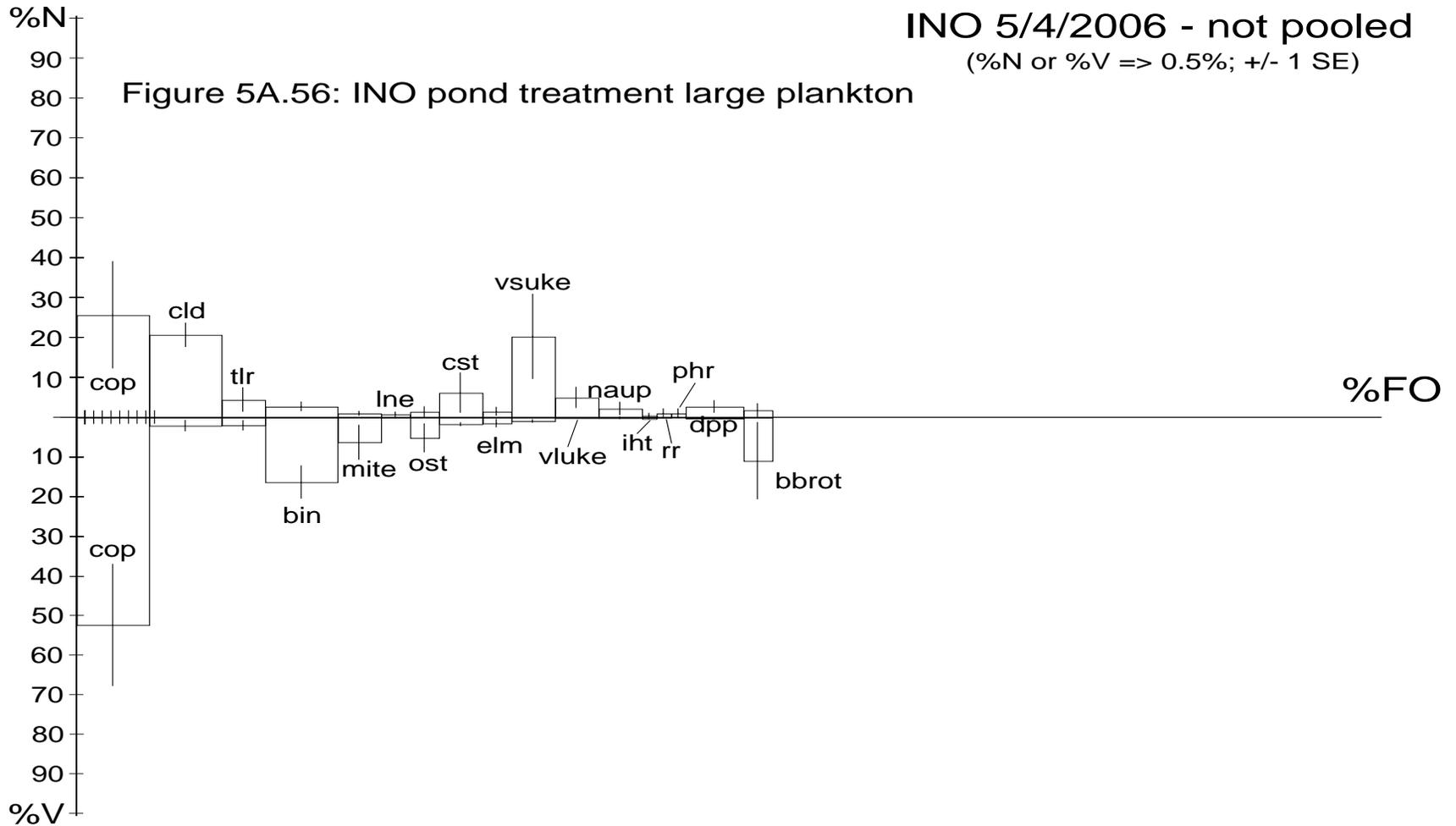


Figure 5A-56. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for inorganic fertilizer (INO) treatment 4 May 2006; taxa %N (± 1 SE) and % V (± 1 SE) for six replicate ponds (0.015 hectares).

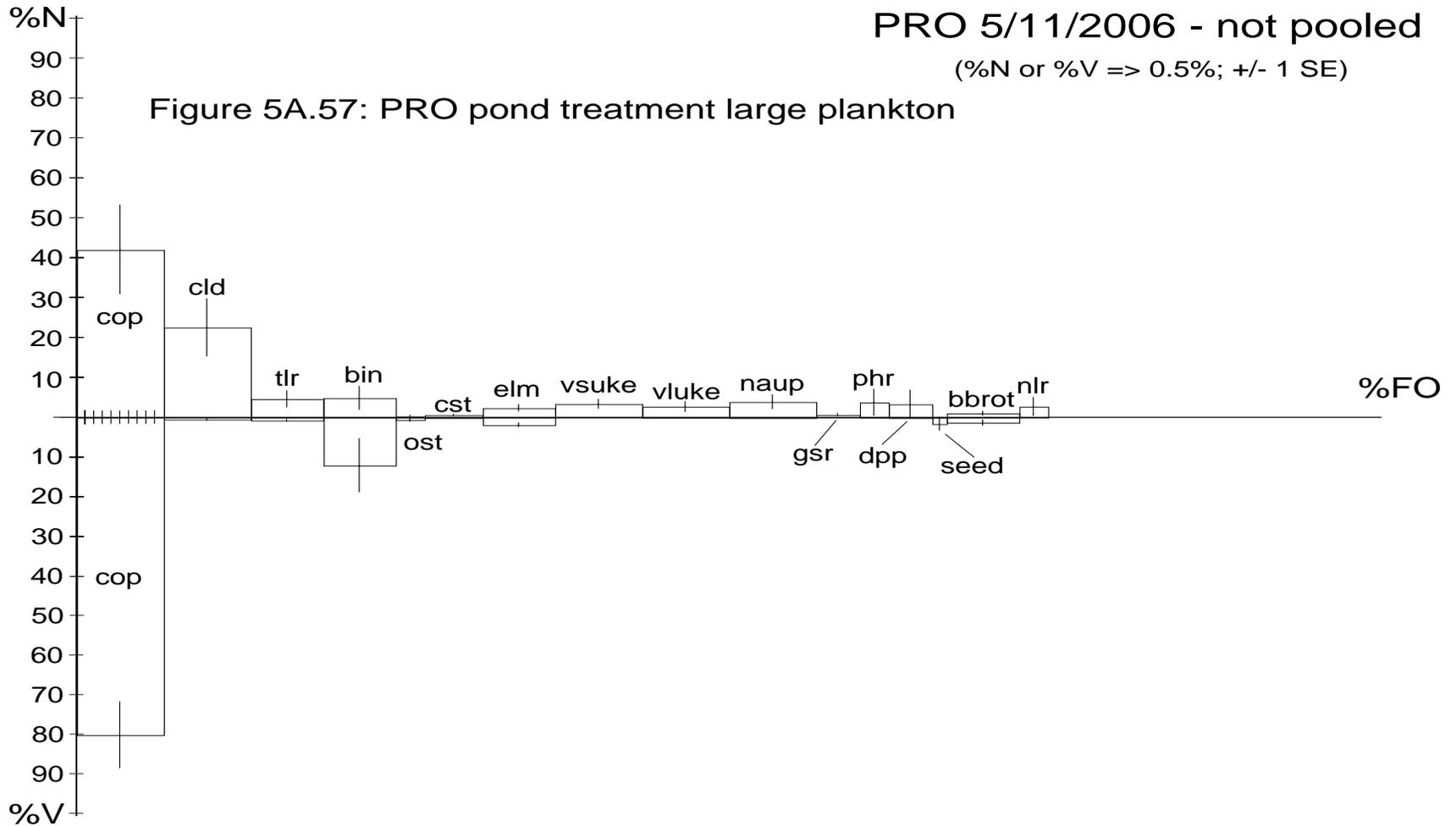


Figure 5A-57. Large zooplankton (> 200 μ m) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for processed feed (PRO) treatment 11 May 2006; taxa %N (\pm 1 SE) and % V (\pm 1 SE) for six replicate ponds (0.015 hectares).

UNPRO 5/11/2006 - not pooled

(%N or %V => 0.5%; +/- 1 SE)

Figure 5A.58: UNP pond treatment large plankton

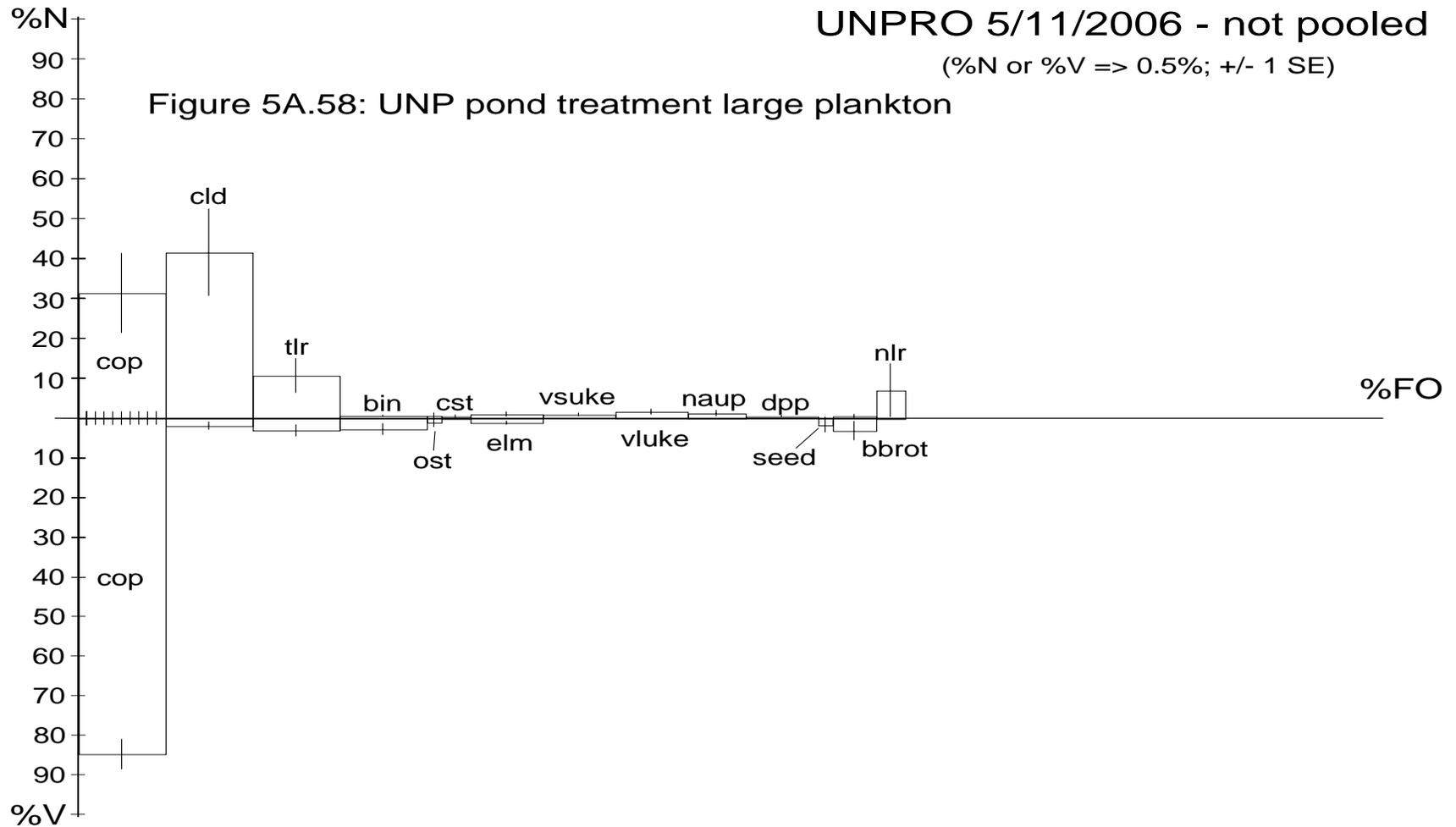


Figure 5A-58. Large zooplankton (> 200 μ m) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for unprocessed feed (UNP) treatment 11 May 2006; taxa %N (\pm 1 SE) and % V (\pm 1 SE) for six replicate ponds (0.015 hectares).

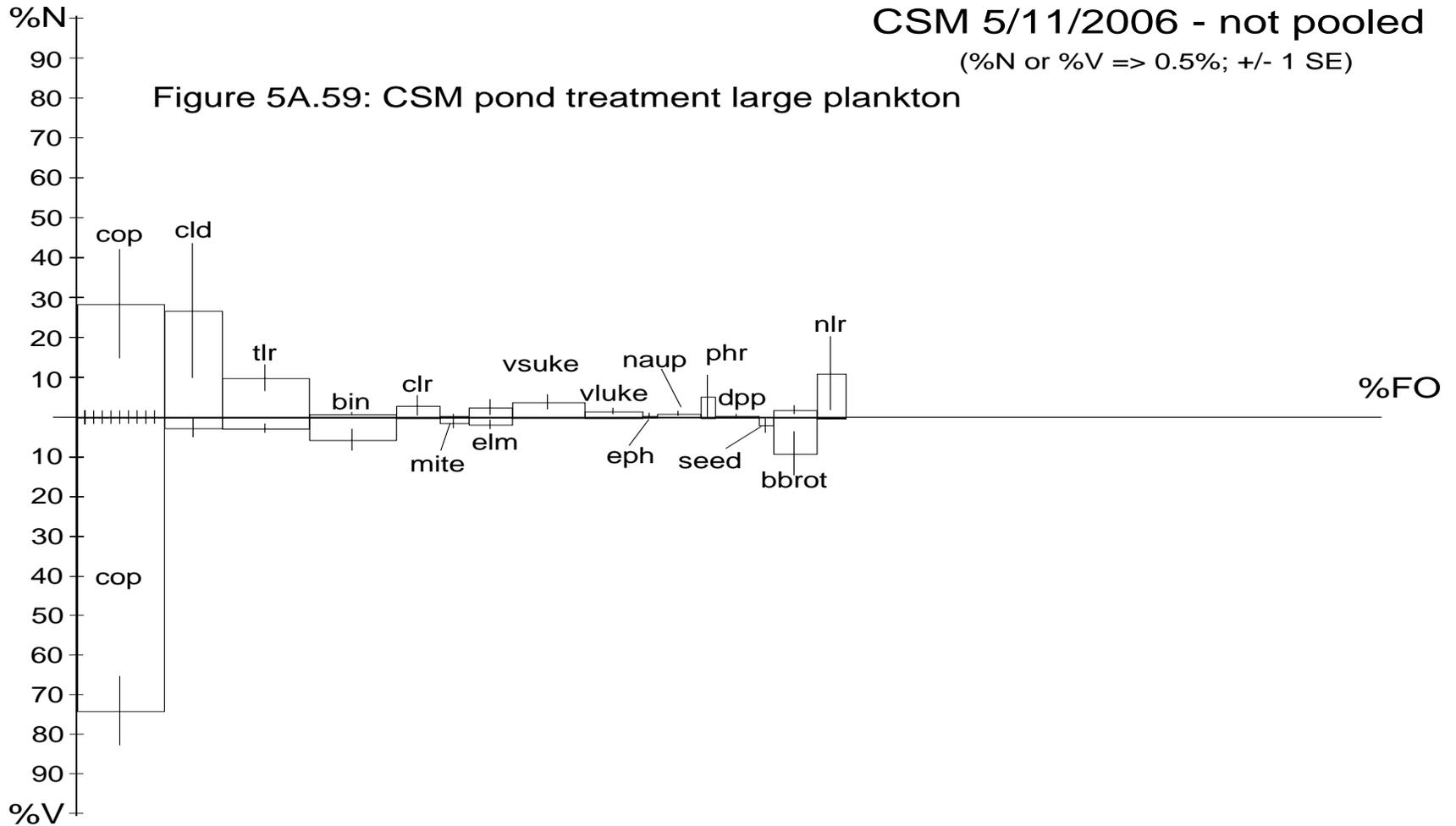


Figure 5A-59. Large zooplankton (size > 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for cottonseed meal fertilizer (CSM) treatment 11 May 2006; taxa %N (± 1 SE) and % V (± 1 SE) for six replicate ponds (0.015 hectares).

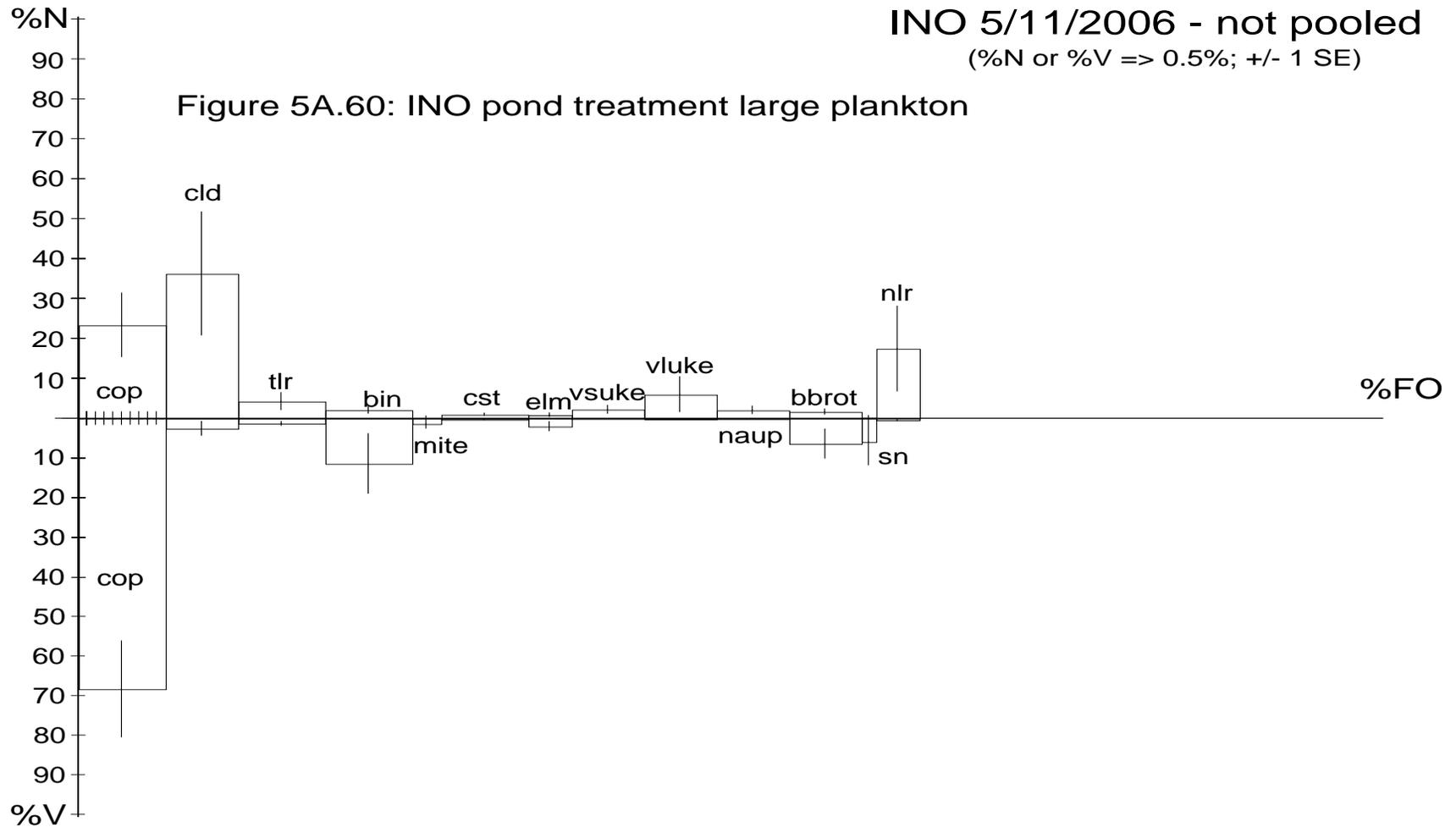


Figure 5A-60. Large zooplankton (> 200 μ m) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for inorganic fertilizer (INO) treatment 11 May 2006; taxa %N (\pm 1 SE) and % V (\pm 1 SE) for six replicate ponds (0.015 hectares).

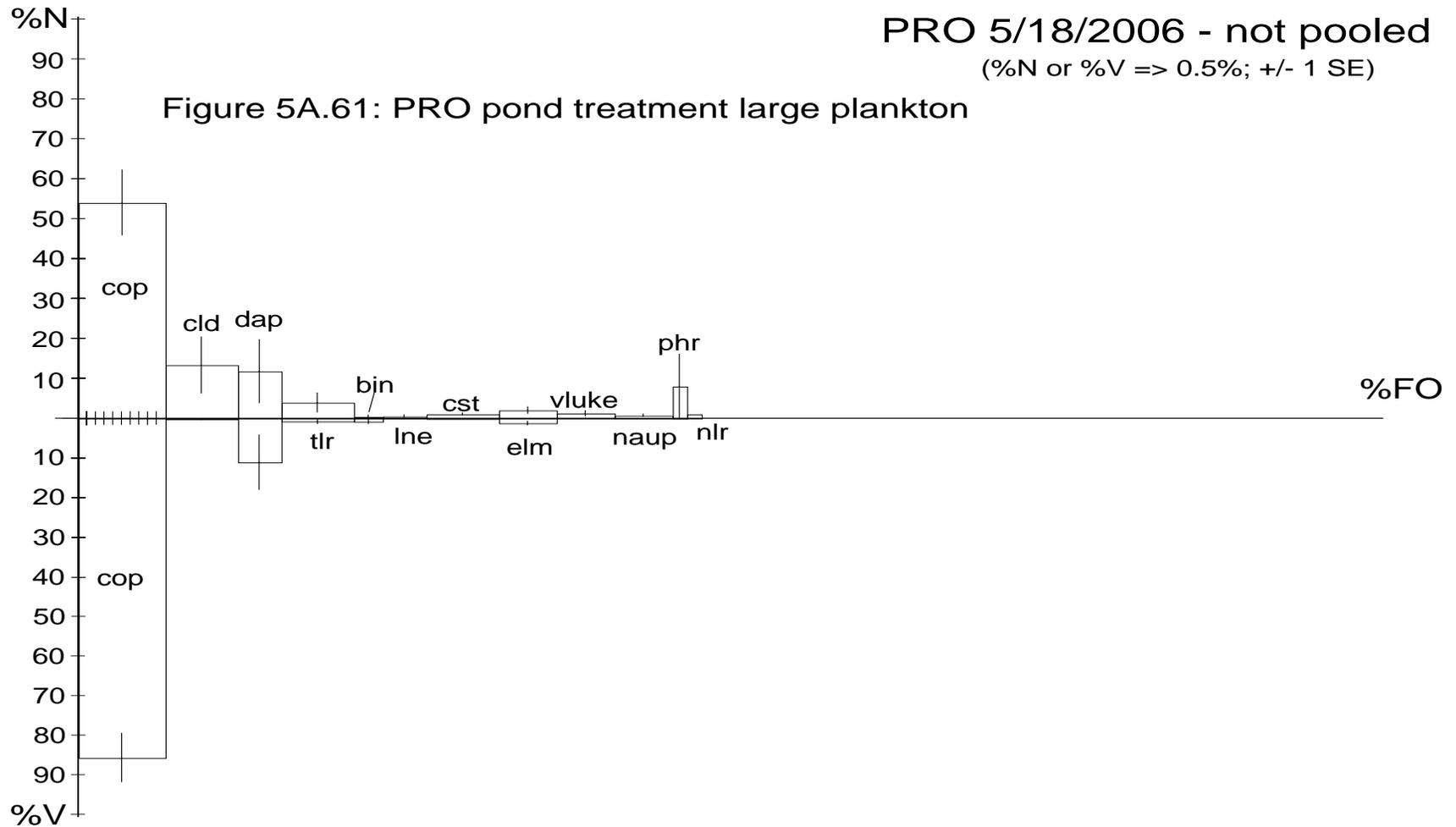


Figure 5A-61. Large zooplankton (> 200 μ m) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for processed feed (PRO) treatment 18 May 2006; taxa %N (\pm 1 SE) and % V (\pm 1 SE) for six replicate ponds (0.015 hectares).

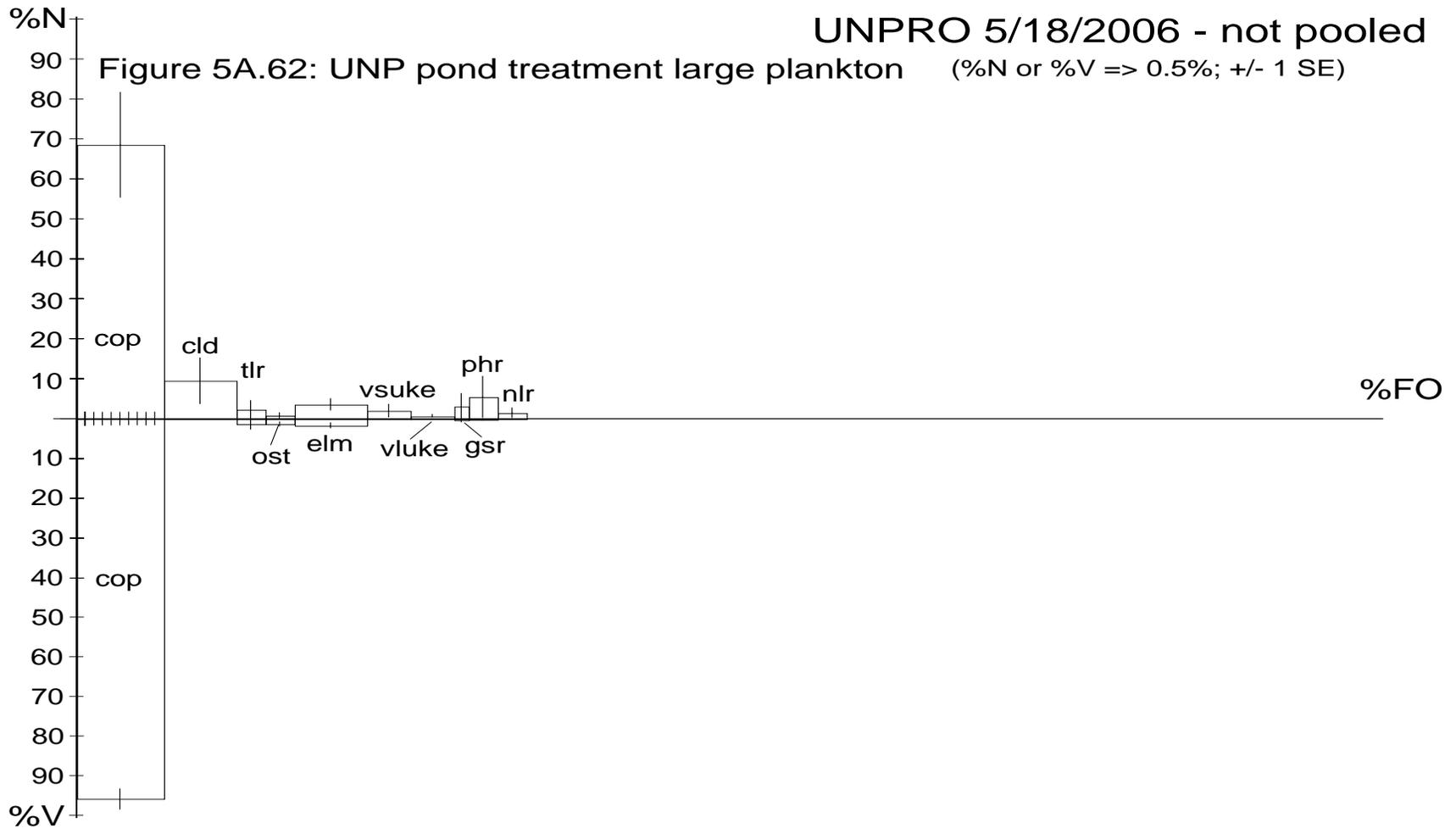


Figure 5A-62. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for unprocessed feed (UNP) treatment 18 May 2006; taxa %N (± 1 SE) and % V (± 1 SE) for six replicate ponds (0.015 hectares).

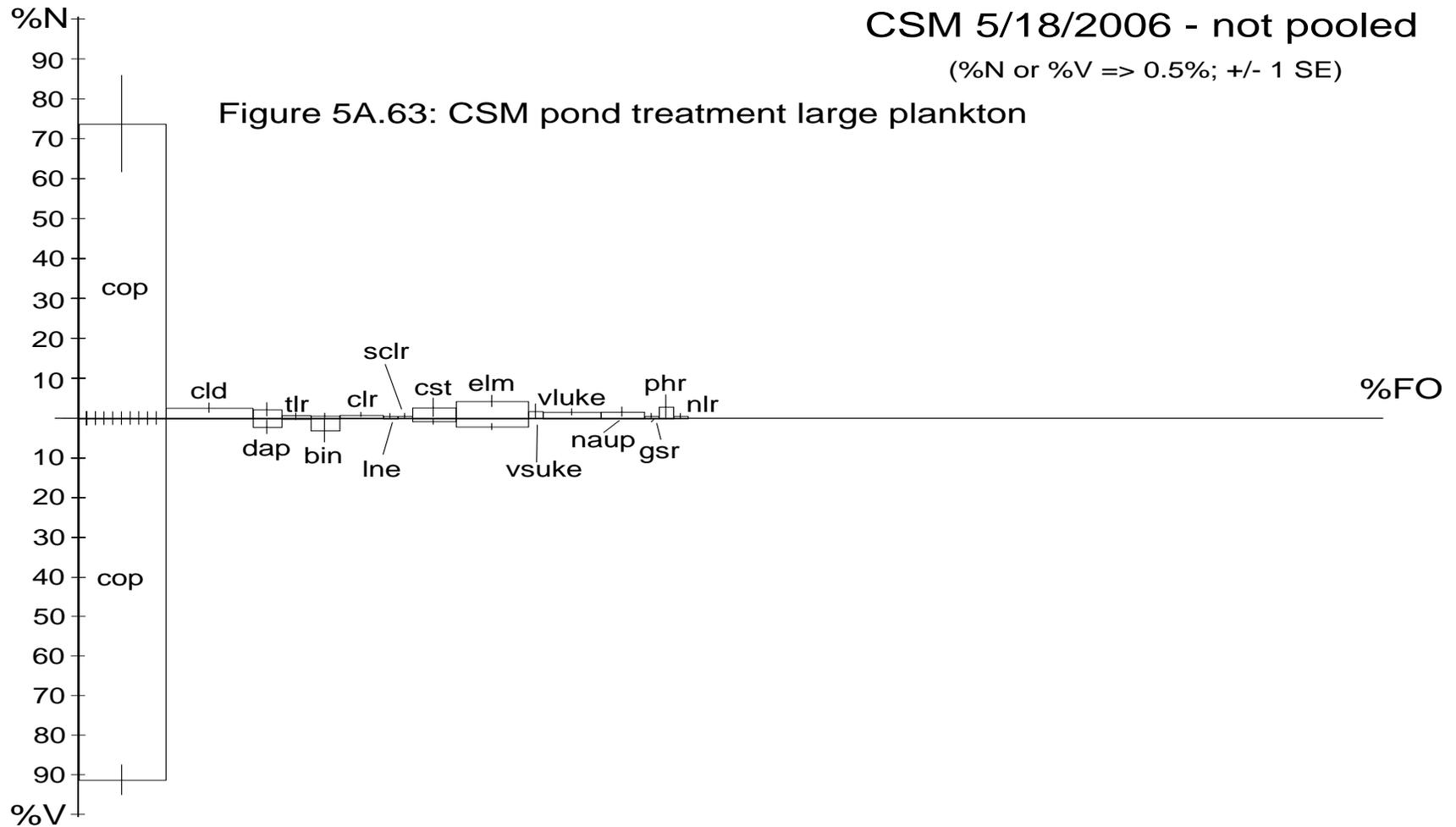


Figure 5A-63. Large zooplankton (> 200 μ m) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for cottonseed meal fertilizer (CSM) treatment 18 May 2006; taxa %N (\pm 1 SE) and % V (\pm 1 SE) for six replicate ponds (0.015 hectares).

INO 5/18/2006 - not pooled
 (%N or %V => 0.5%; +/- 1 SE)

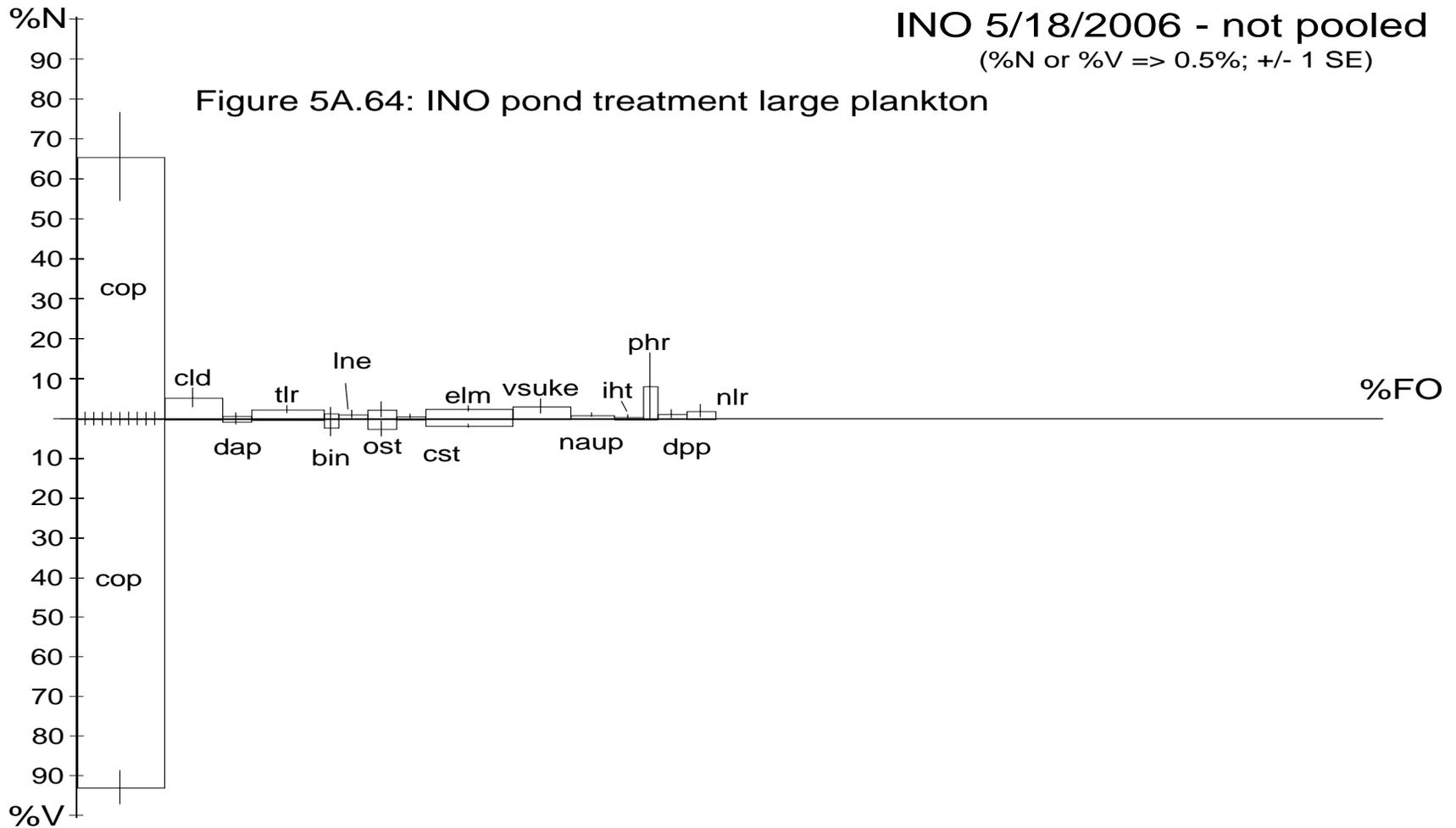


Figure 5A-64. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for inorganic fertilizer (INO) treatment 18 May 2006; taxa %N (± 1 SE) and % V (± 1 SE) for six replicate ponds (0.015 hectares).

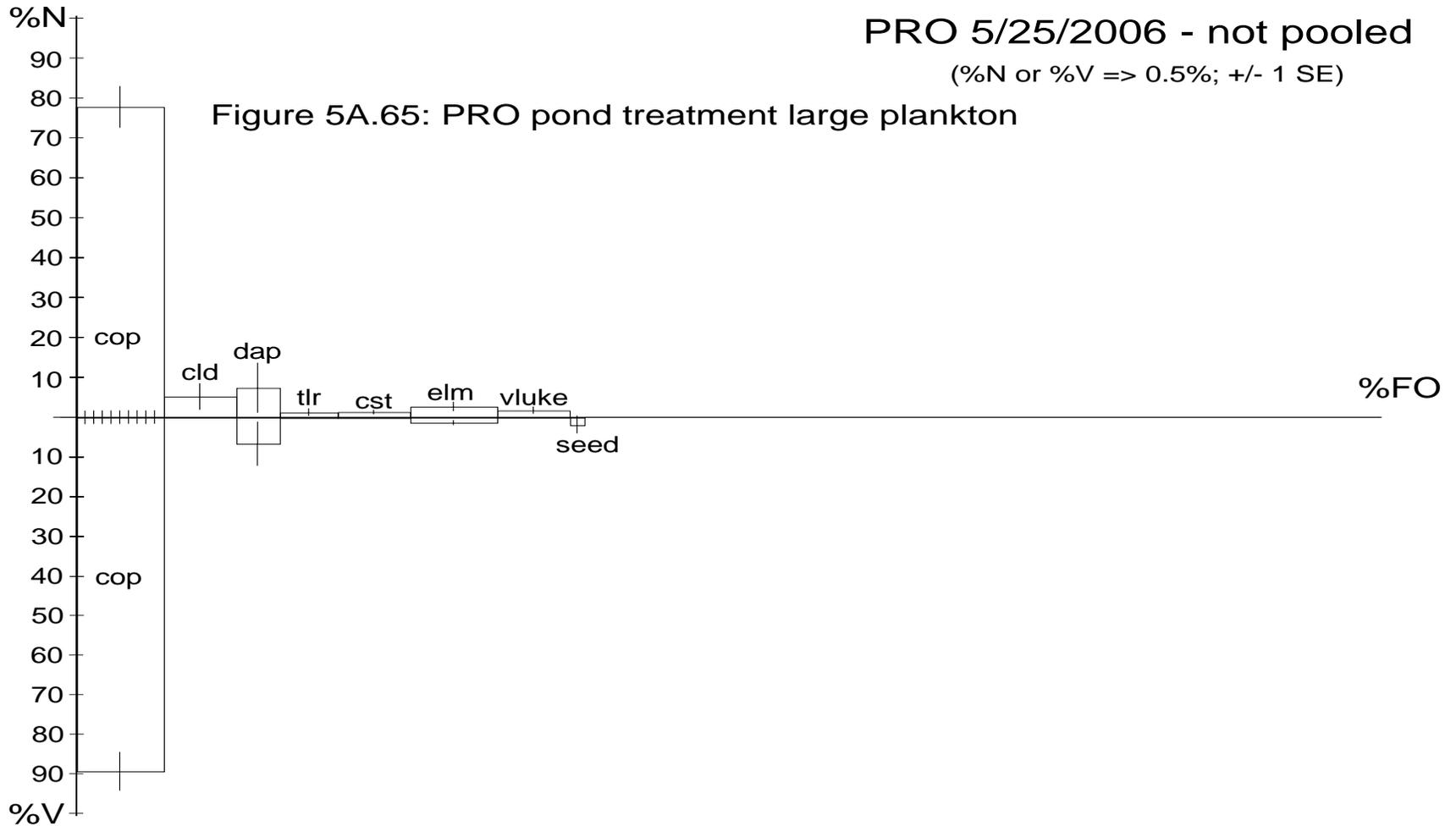


Figure 5A-65. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for processed feed (PRO) treatment 25 May 2006; taxa %N (± 1 SE) and % V (± 1 SE) for six replicate ponds (0.015 hectares).

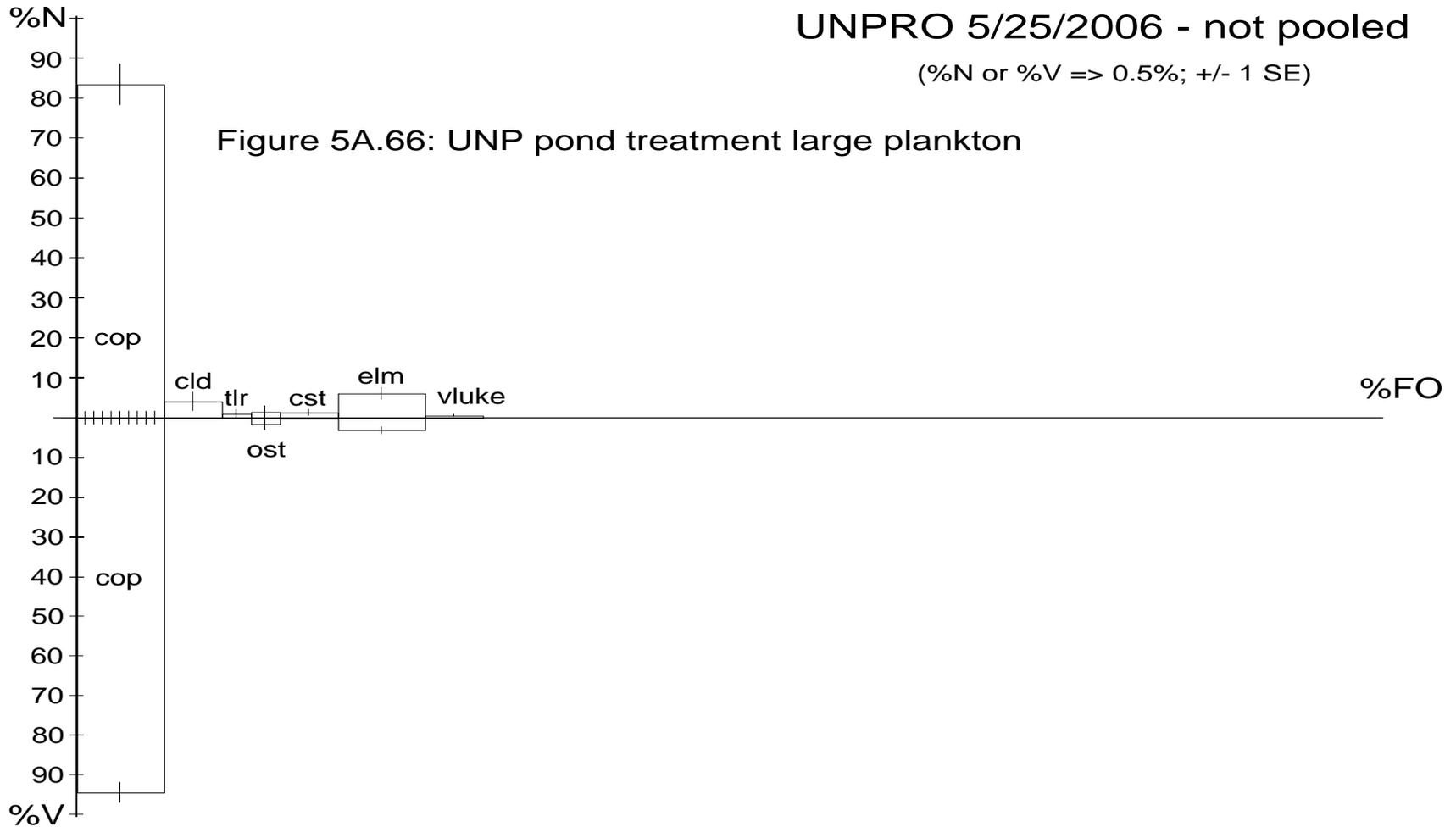


Figure 5A-66. Large zooplankton (size > 200 μ m) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for unprocessed feed treatment 25 May 2006; taxa %N (\pm 1 SE) and % V (\pm 1 SE) for six replicate pond (0.015 hectares).

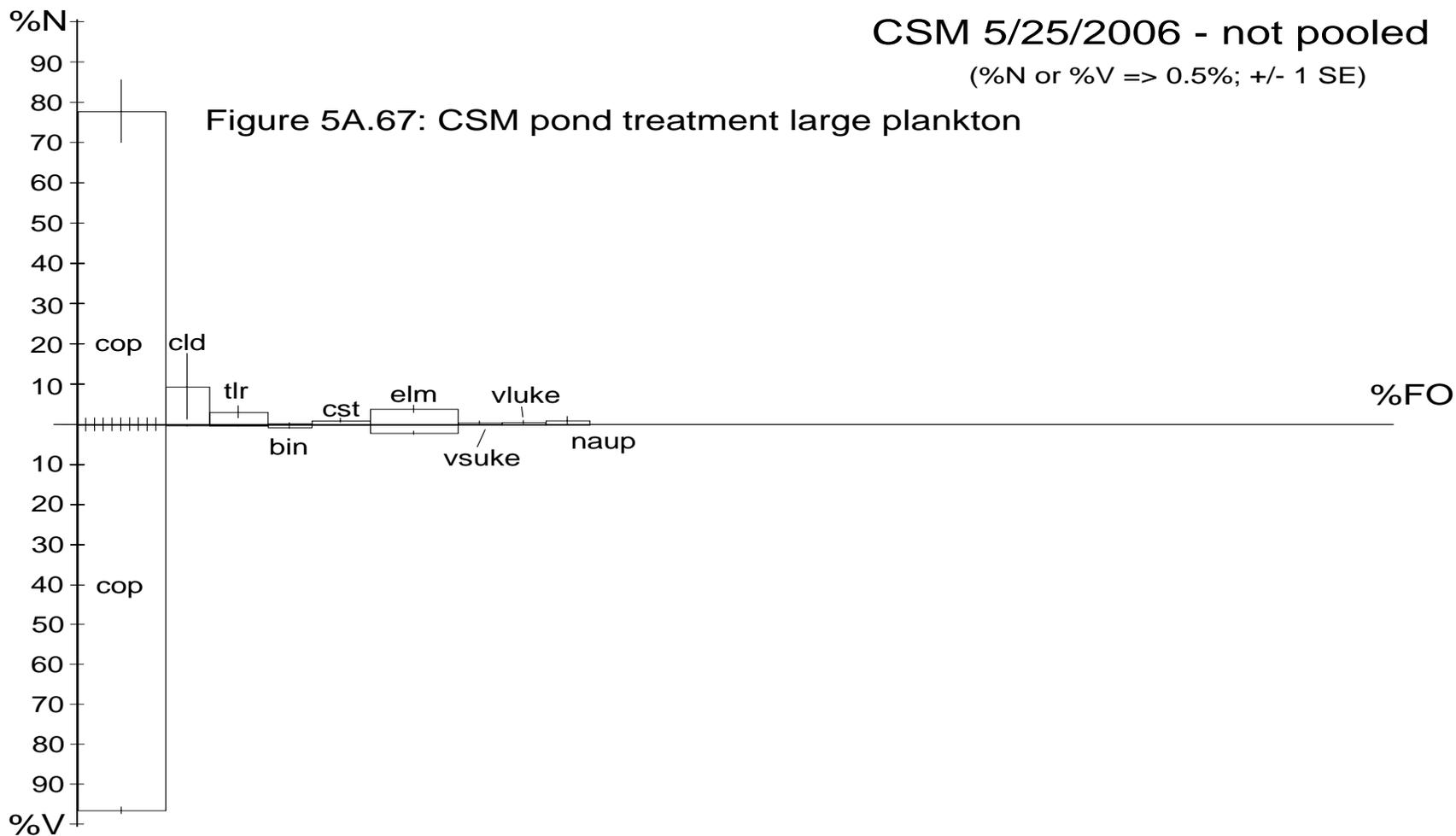


Figure 5A-67. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for cottonseed meal fertilizer (CSM) treatment 25 May 2006; taxa %N (± 1 SE) and % V (± 1 SE) for six replicate ponds (0.015 hectares).

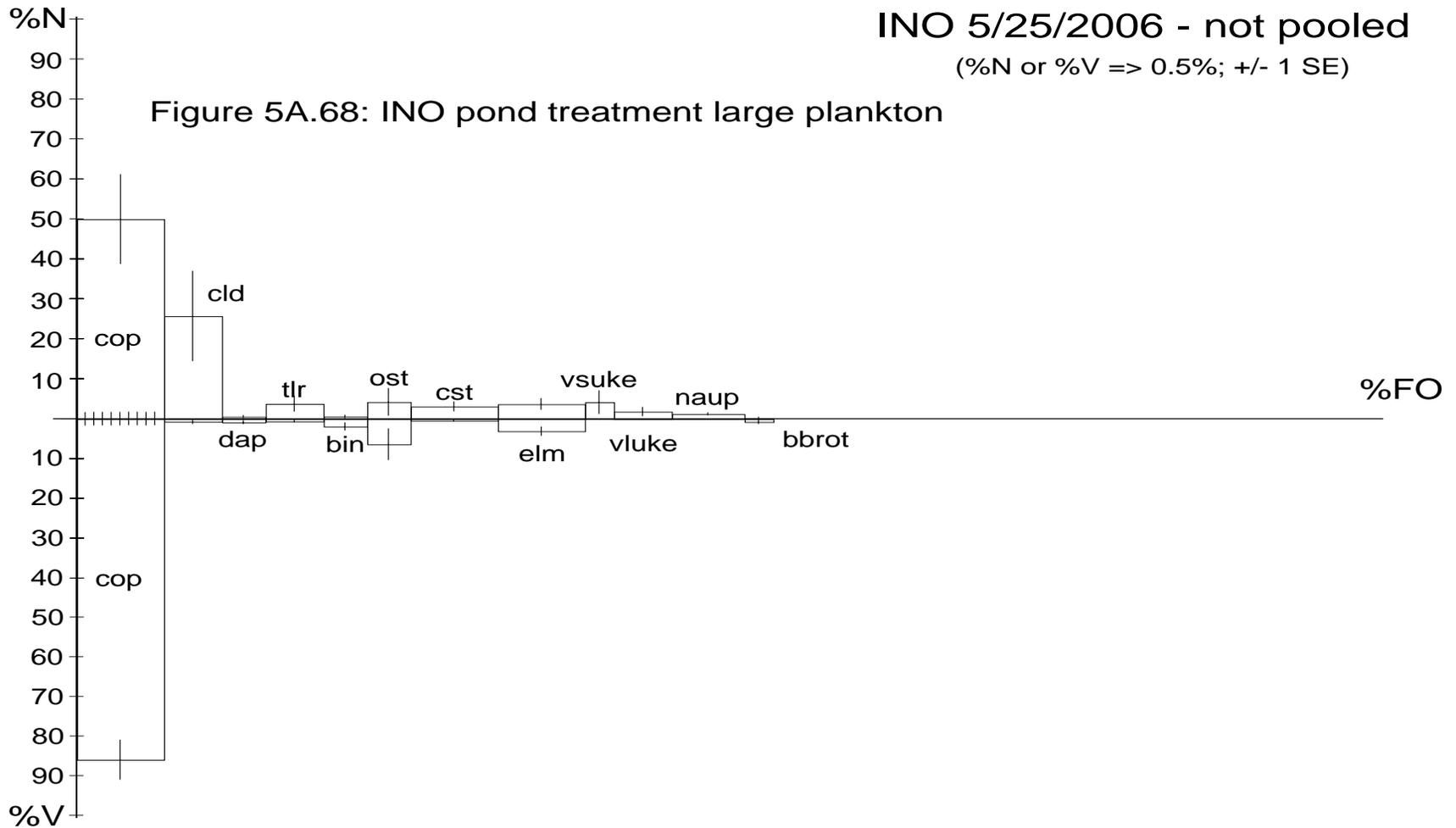


Figure 5A-68. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for inorganic fertilizer (INO) treatment 25 May 2006; taxa %N (± 1 SE) and % V (± 1 SE) for six replicate ponds (0.015 hectares).

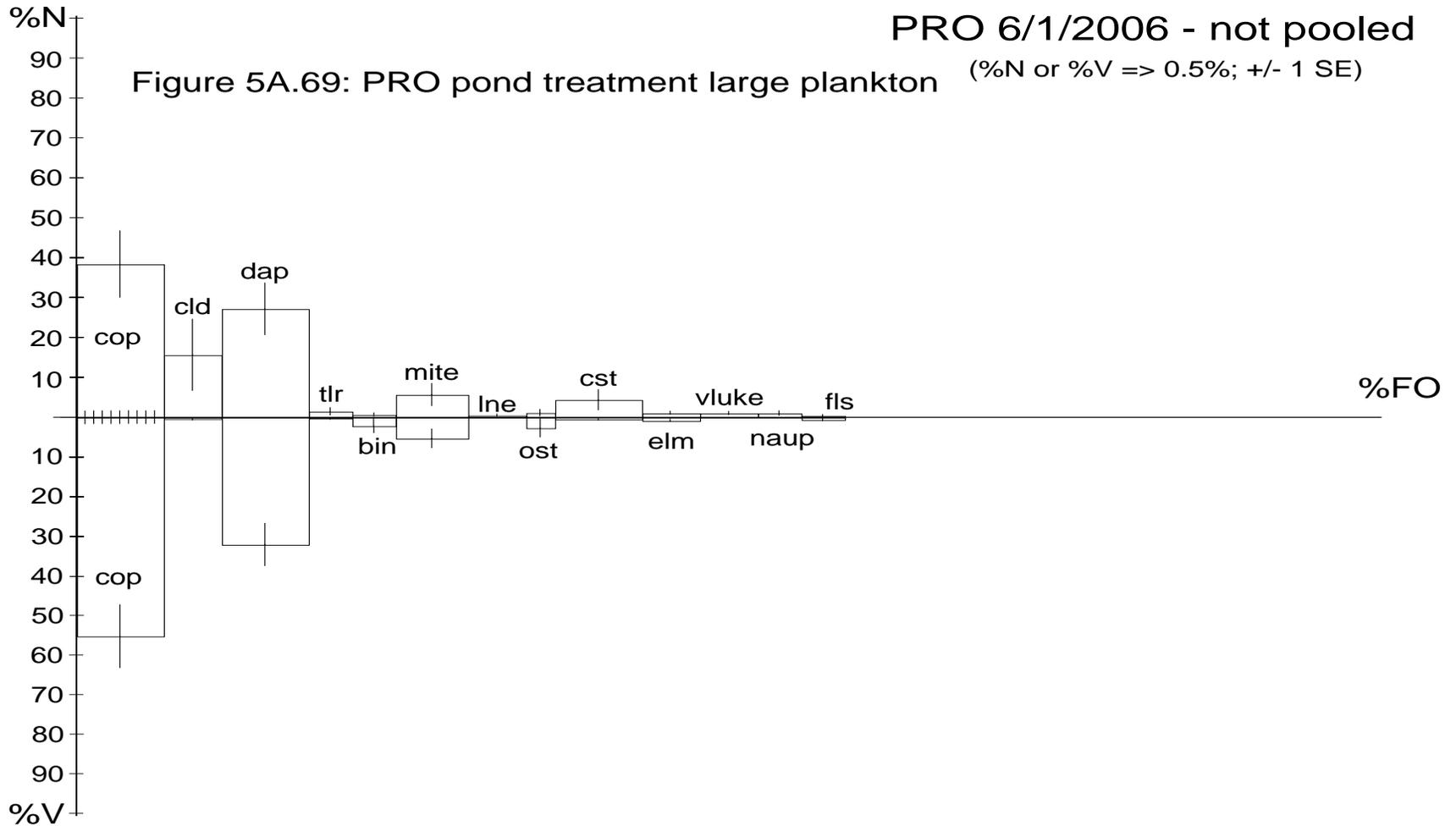


Figure 5A-69. Large zooplankton (> 200 μ m) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for processed feed (PRO) treatment 1 June 2006; taxa %N (\pm 1 SE) and % V (\pm 1 SE) for six replicate ponds (0.015 hectares).

UNPRO 6/1/2006 - not pooled

(%N or %V => 0.5%; +/- 1 SE)

Figure 5A.70: UNP pond treatment large plankton

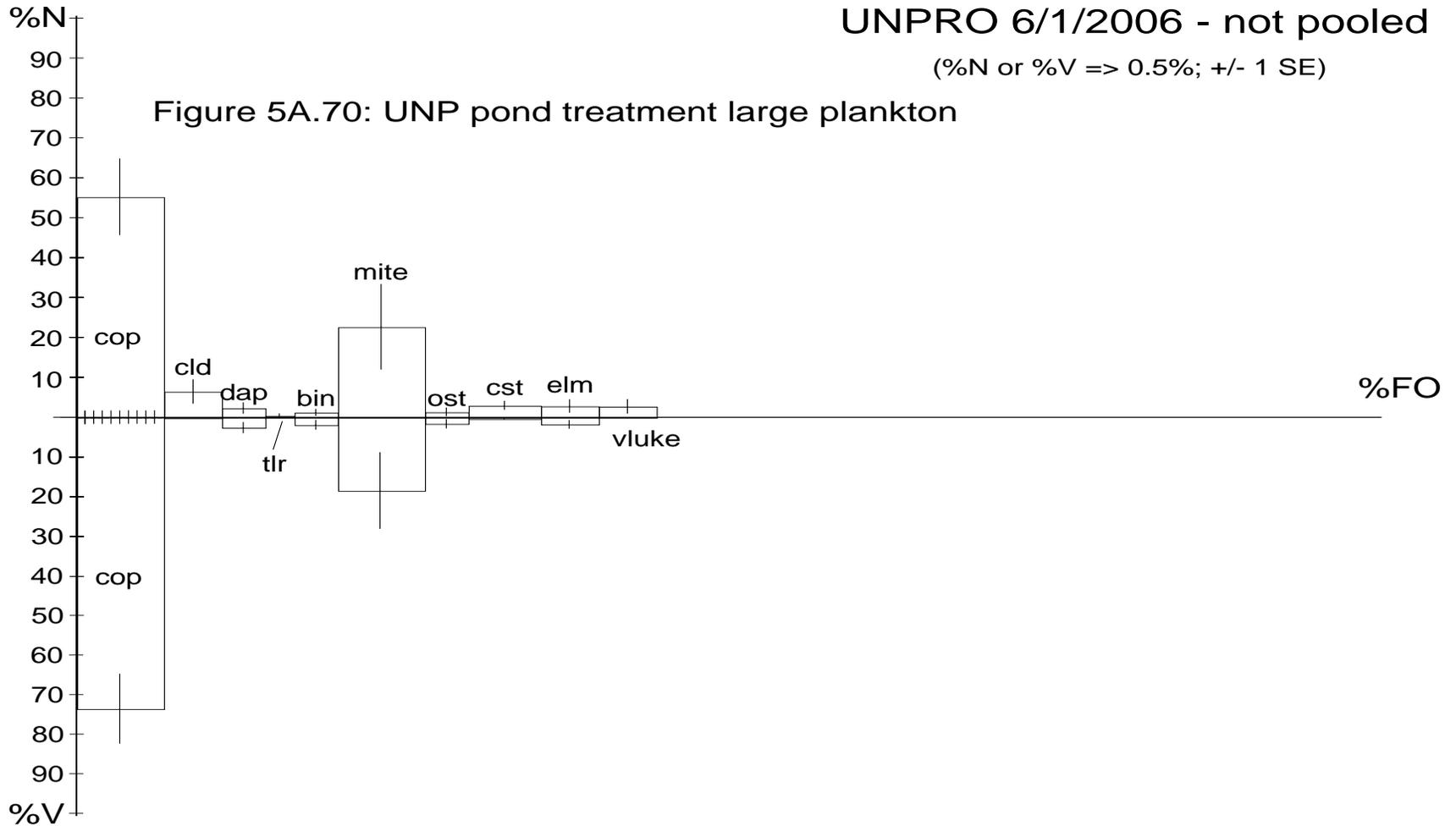


Figure 5A-70. Large zooplankton (> 200 μ m) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for unprocessed feed (UNP) treatment 1 June 2006; taxa %N (\pm 1 SE) and % V (\pm 1 SE) for six replicate ponds (0.015 hectares).

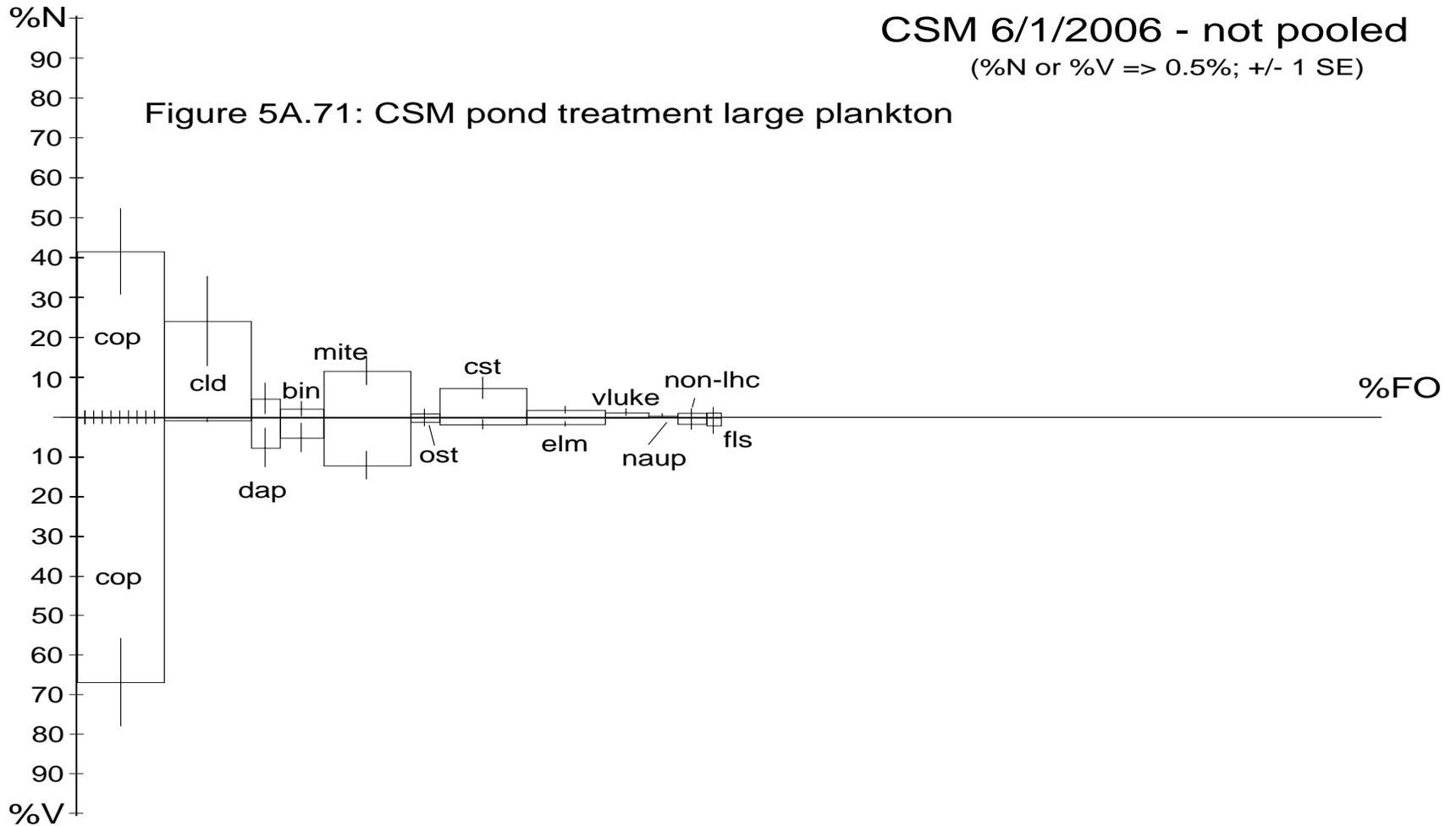


Figure 5A-71. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for cottonseed meal fertilizer (CSM) treatment 1 June 2006; taxa %N (± 1 SE) and % V (± 1 SE) for six replicate ponds (0.015 hectares).

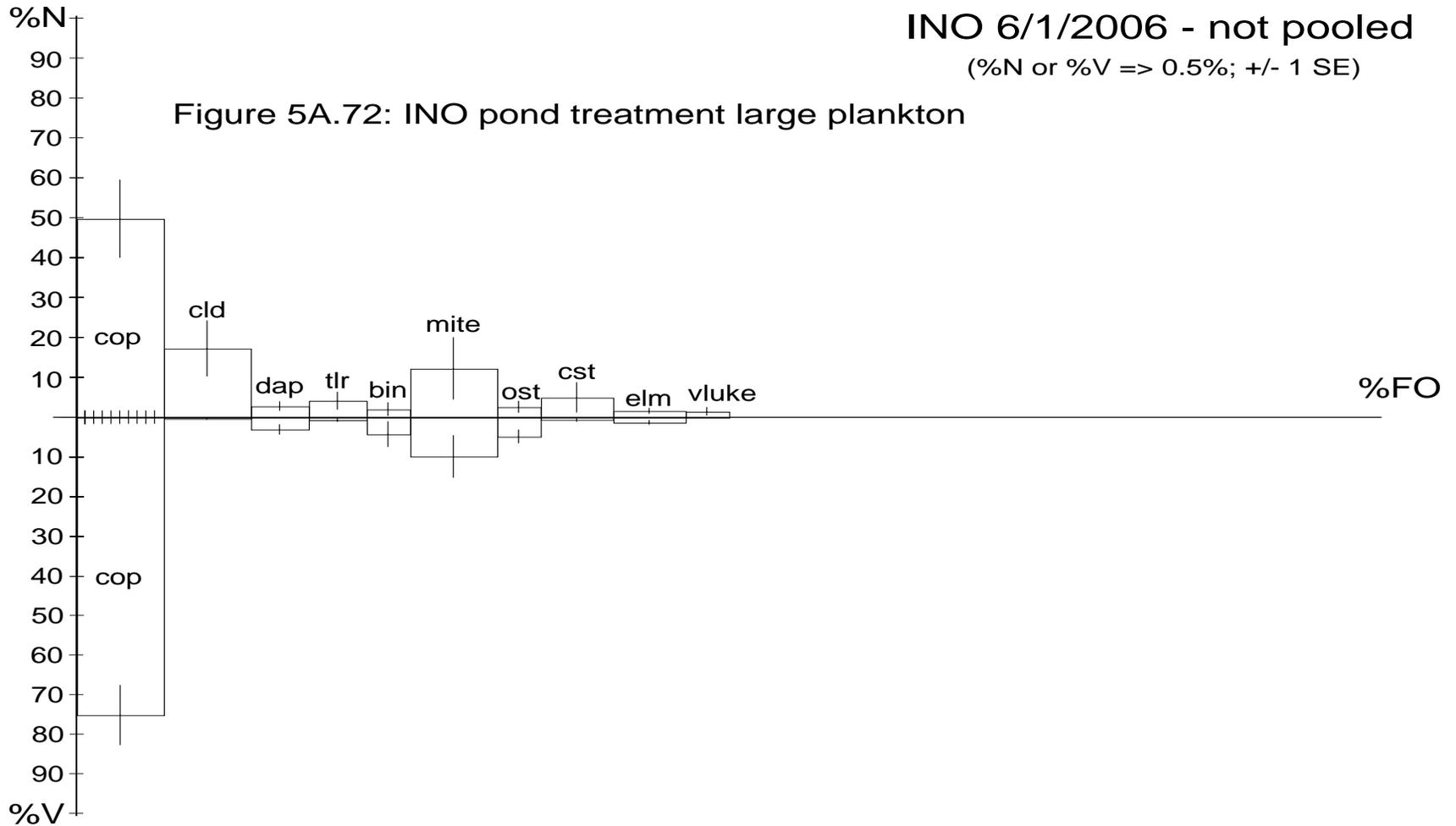


Figure 5A-72. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for inorganic fertilizer (INO) treatment 1 June 2006; taxa %N (± 1 SE) and % V (± 1 SE) for six replicate ponds (0.015 hectares).

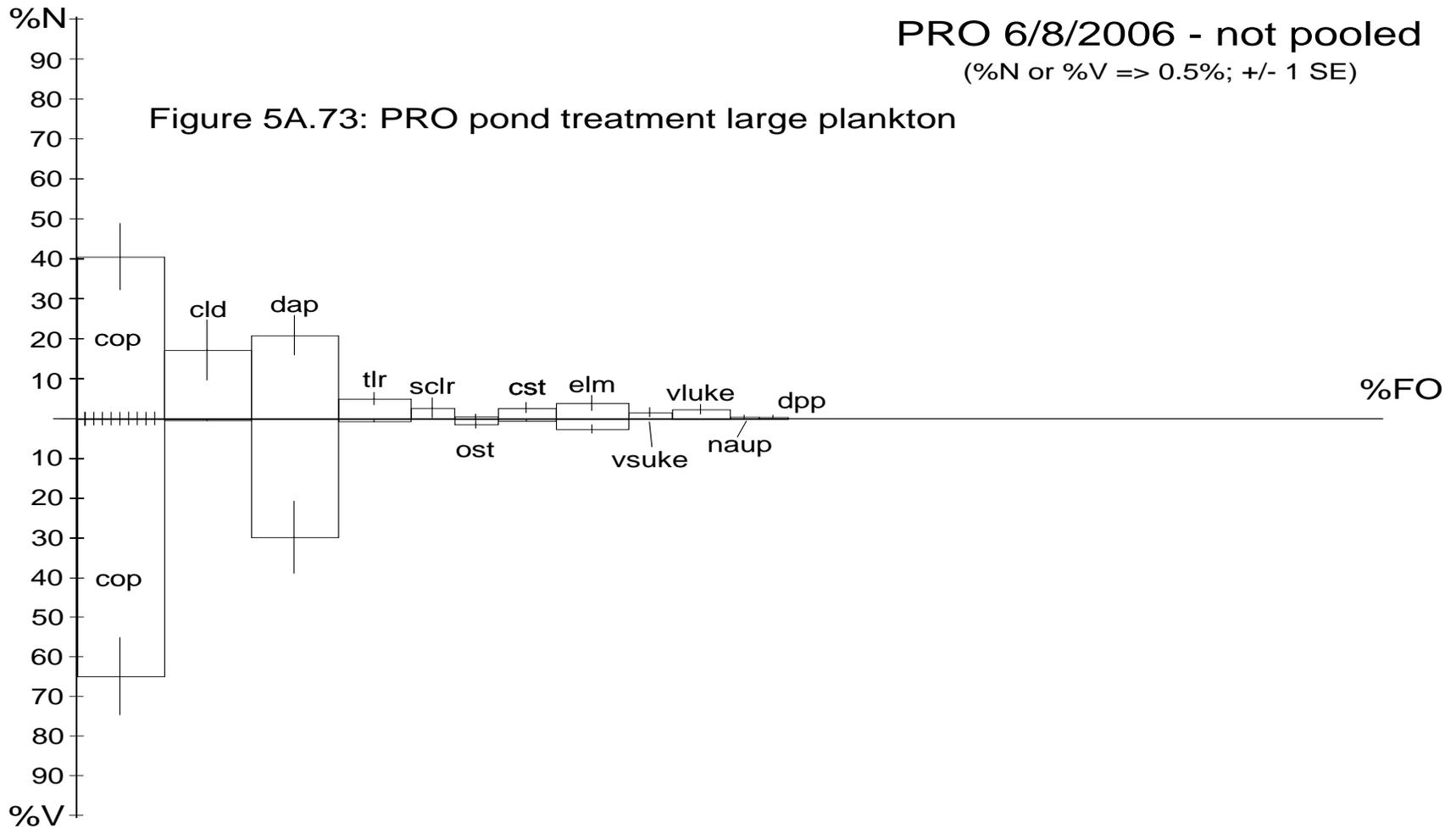


Figure 5A-73. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for processed feed (PRO) treatment 8 June 2006; taxa %N (± 1 SE) and % V (± 1 SE) for six replicate ponds (0.015 hectares).

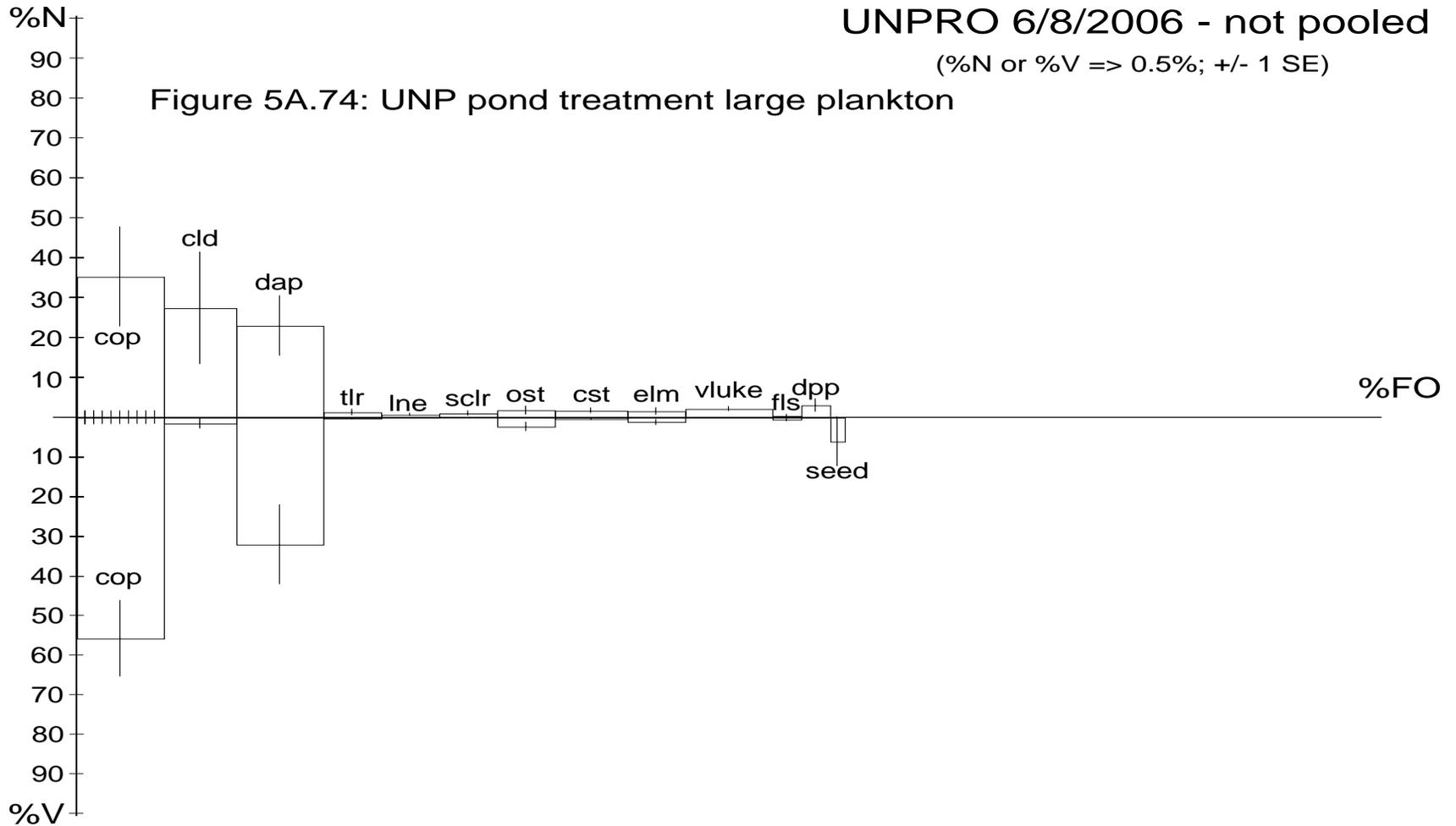


Figure 5A.74. Large zooplankton (size > 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for unprocessed feed treatment 8 June 2006; taxa %N (± 1 SE) and % V (± 1 SE) for six replicate pond (0.015 hectares).

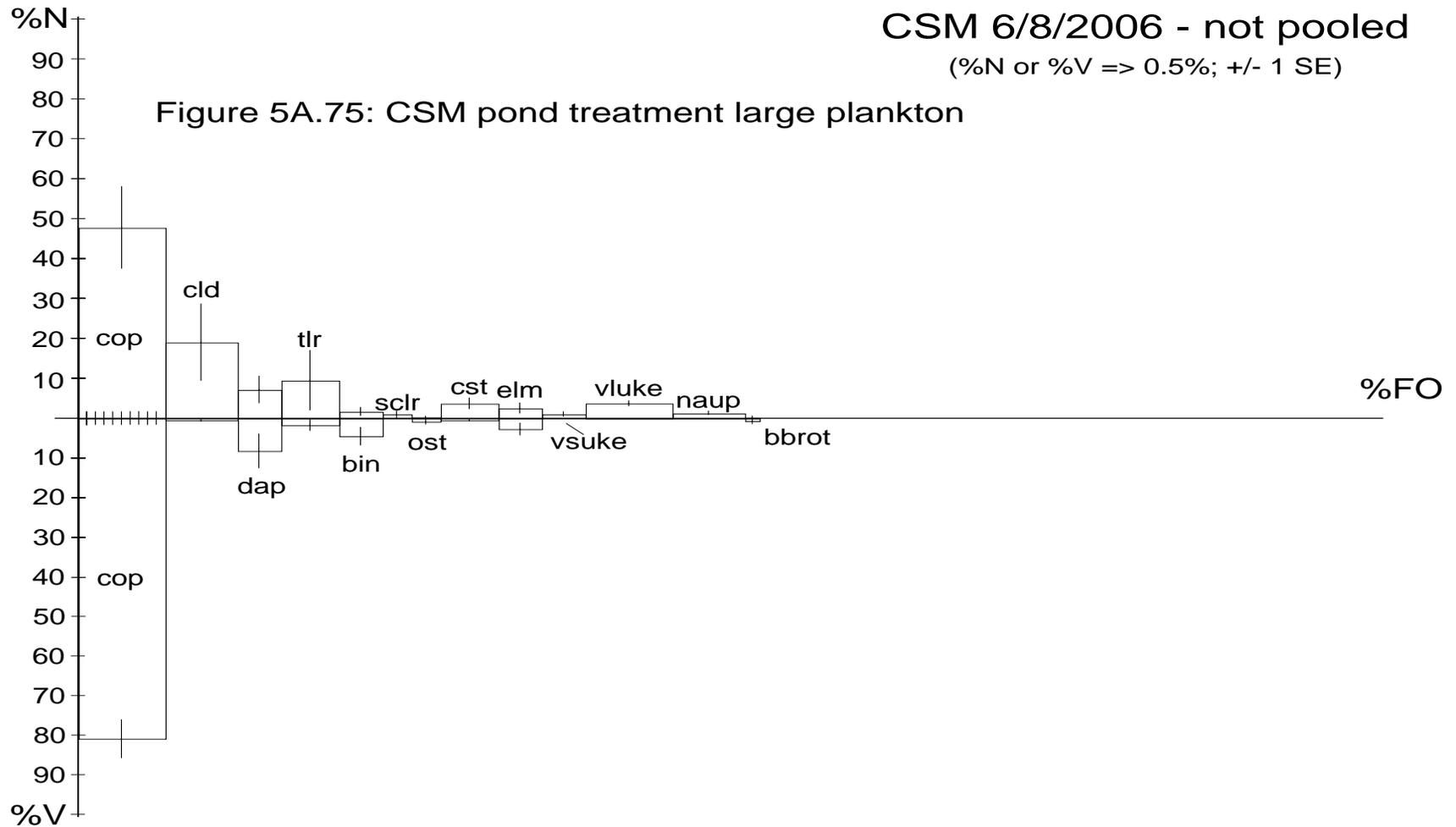


Figure 5A-75. Large zooplankton (> 200 μ m) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for cottonseed meal fertilizer (CSM) treatment 8 June 2006; taxa %N (\pm 1 SE) and % V (\pm 1 SE) for six replicate ponds (0.015 hectares).

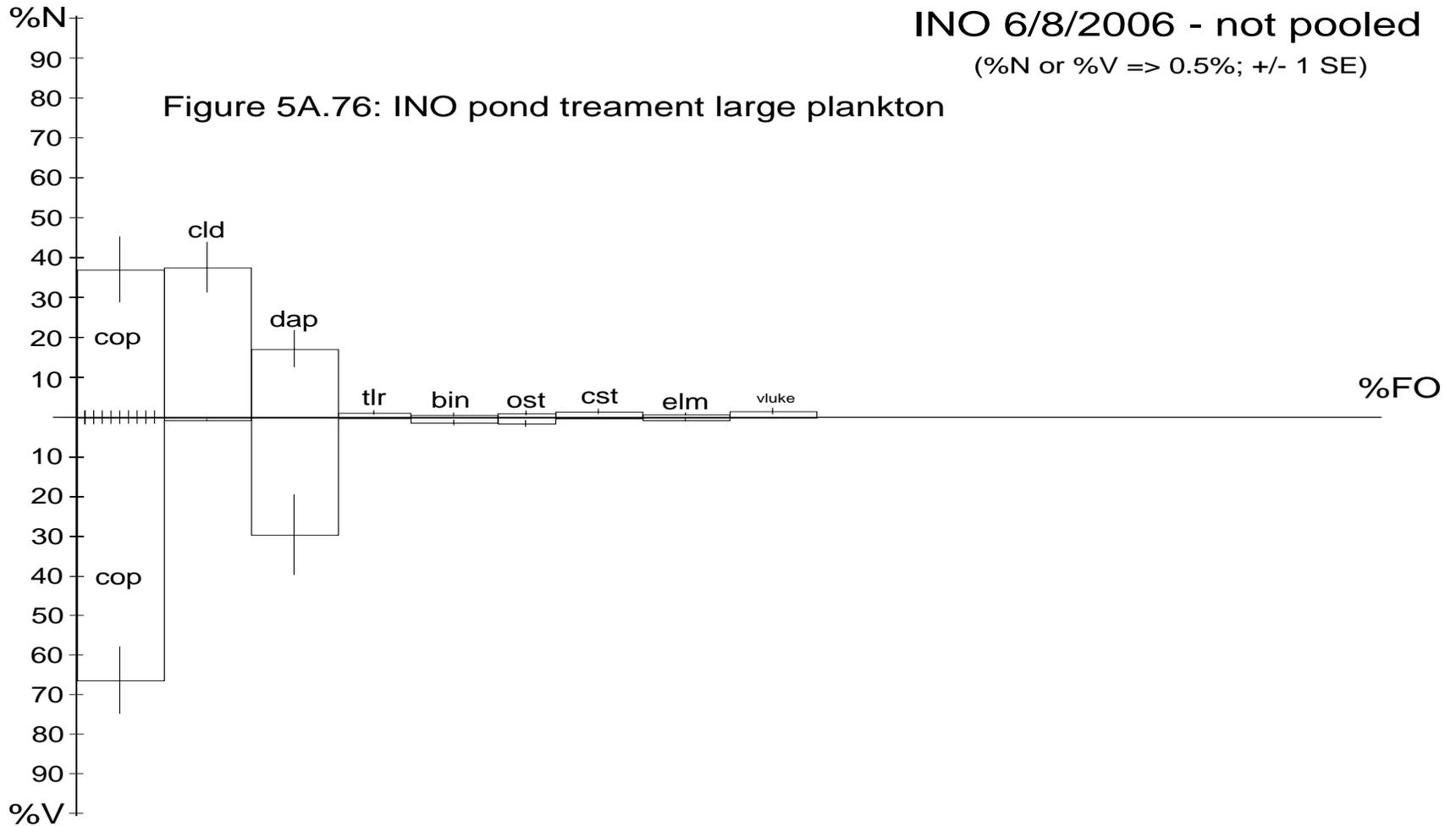


Figure 5A-76. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for inorganic fertilizer (INO) treatment 8 June 2006; taxa %N (± 1 SE) and % V (± 1 SE) for six replicate ponds (0.015 hectares).

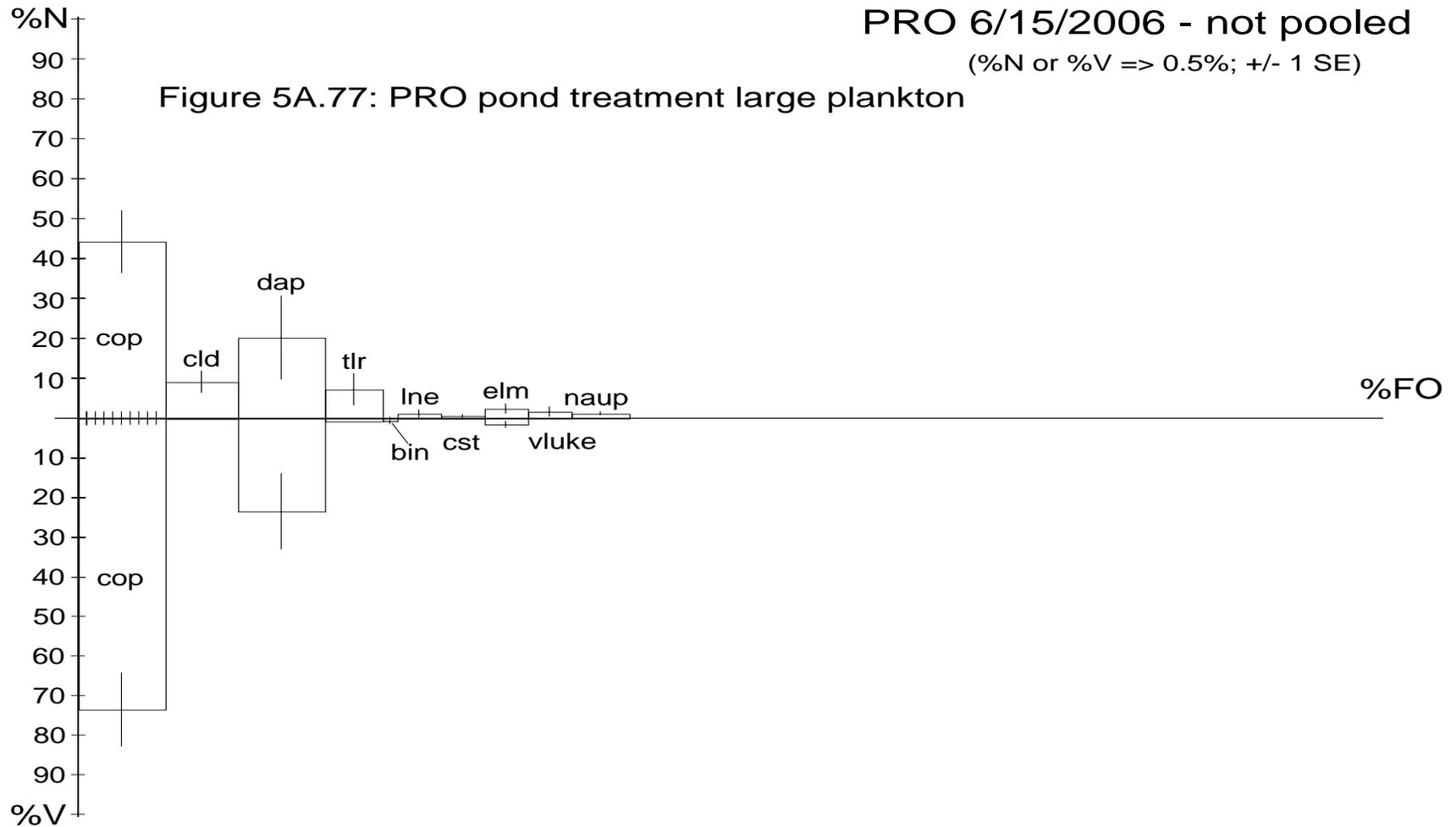


Figure 5A-77. Large zooplankton (> 200 μ m) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for processed feed (PRO) treatment 15 June 2006; taxa %N (\pm 1 SE) and % V (\pm 1 SE) for six replicate ponds (0.015 hectares).

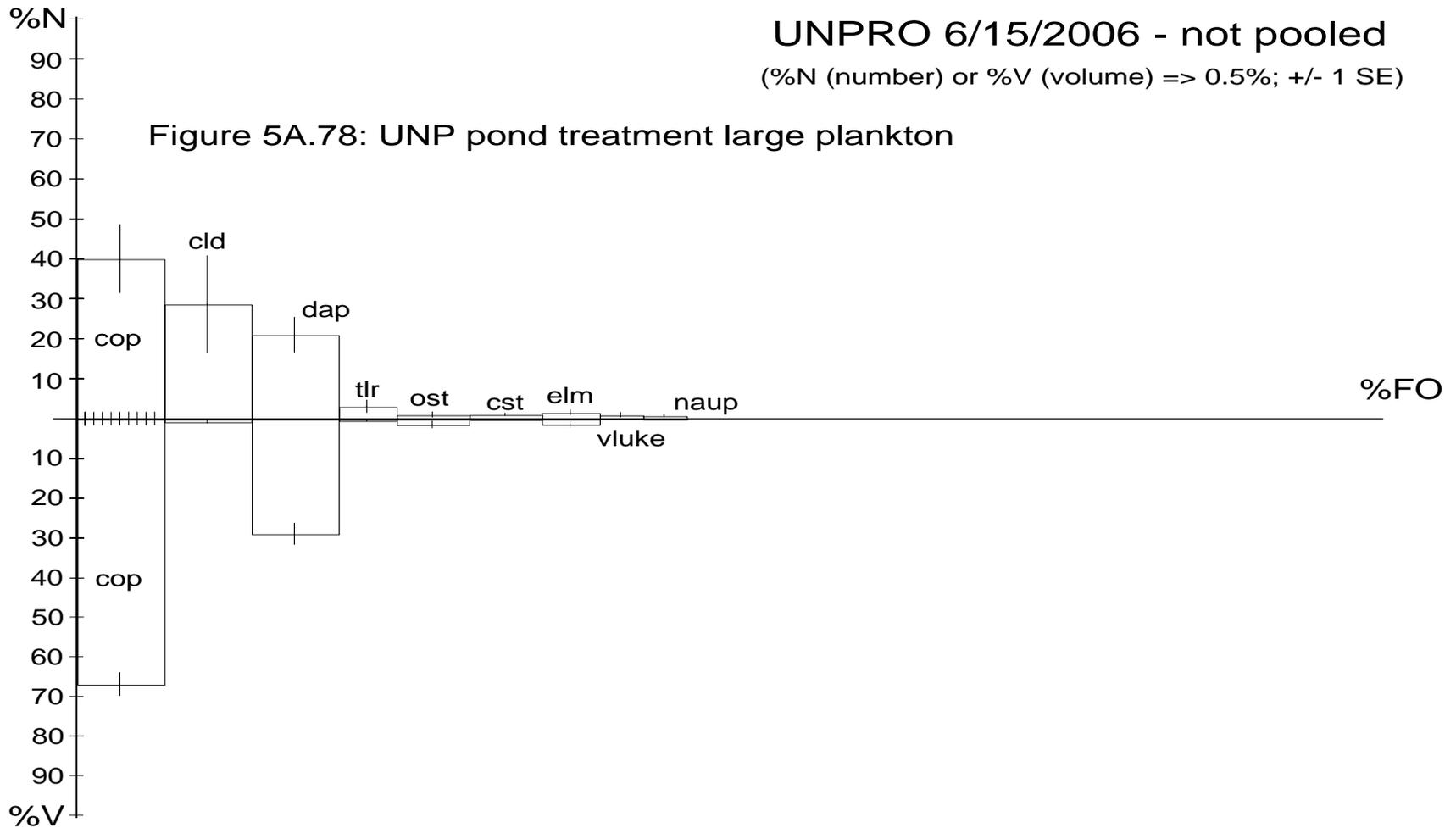


Figure 5A-78. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for unprocessed feed (PRO) treatment 15 June 2006; taxa %N (± 1 SE) and % V (± 1 SE) for six replicate ponds (0.015 hectares).

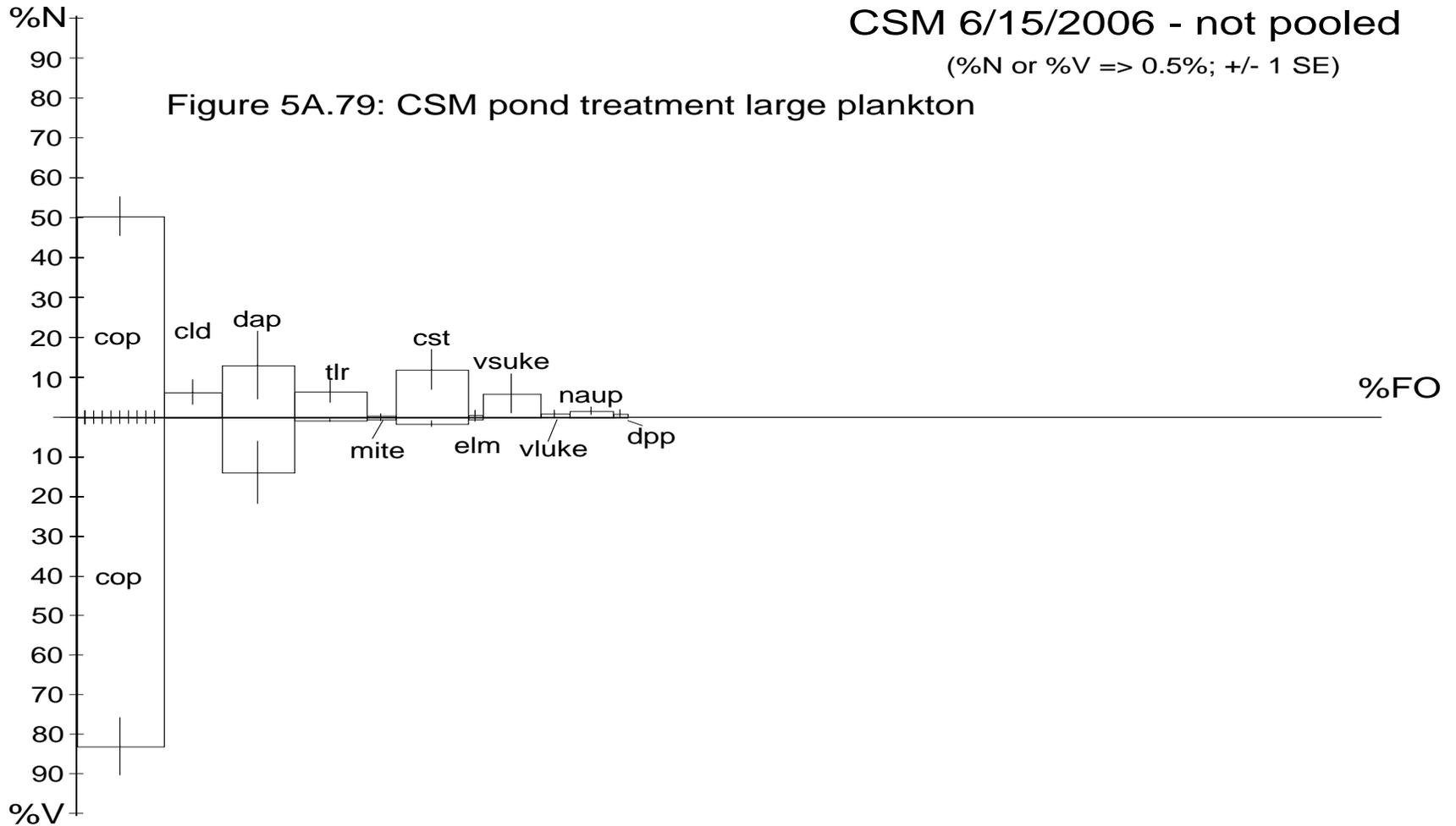


Figure 5A-79. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for cottonseed meal fertilizer (CSM) treatment 15 June 2006; taxa %N (± 1 SE) and % V (± 1 SE) for six replicate ponds (0.015 hectares).

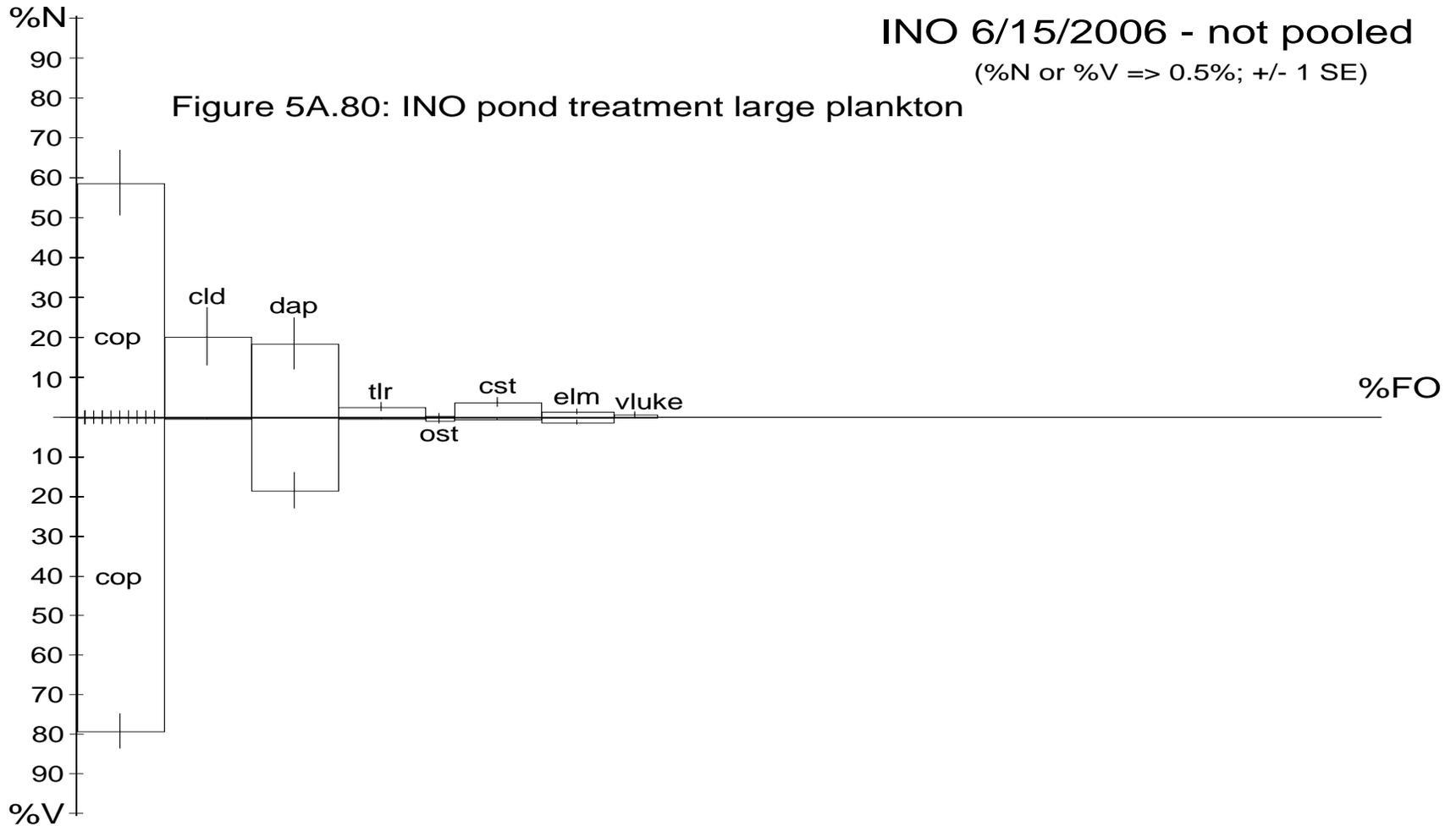


Figure 5A-80. Large zooplankton (> 200 μm) assemblage community taxa parameters [% number (%N), % volume (%V), % frequency of occurrence (%FO)] for inorganic fertilizer (INO) treatment 15 June 2006; taxa %N (± 1 SE) and % V (± 1 SE) for six replicate ponds (0.015 hectares).

APPENDIX B
BOOTSTRAPPING SIMILARITY INDEX PROGRAM

Bootstrap Simplified Morisita's Similarity and Percent Similarity Index Estimator with Error
Measures BASIC Computer Program (written by J.S. Kao).

```

10  CLS      ' Similarity Index Bootstrap Program, Written by Jon S. Kao (12/12/2008).
20  RANDOMIZE TIMES$("milliseconds") ' seed random # generator w/ # milliseconds past midnight
    DefaultDir$ = "C:\\"
    gtvar = 0
    DIM psidrawdev(50)
    DIM pnum(50)
    DIM catnum(120)
    DIM zoo1(200,200)
    DIM zoo2(200,200)
    DIM zoo1$(200,200)
    DIM zoo2$(200,200)
    DIM preypct1(150)
    DIM preypct2(150)
    DIM Pdraw(45)
    DIM psitotal(6000)
    DIM psiiter(6000)
    DIM psivariter(6000)
    DIM psistdeviter(6000)
    DIM psisterriter(6000)
    DIM psiarraytrials(6000)
    DIM Mpreypct1(150)
    DIM Mpreypct2(150)
    DIM Mdraw(45)
    DIM MORiter(6000)
    DIM MORvariter(6000)
    DIM MORstdeviter(6000)
    DIM MORsterriter(6000)
    DIM MORarraytrials(6000)
    DIM Mcrossprod(150)
    DIM Pdraw(50)
    DIM tval(1000)
30  PRINT      ' Treatment Comparison Module
40  PRINT "Welcome to the Similarity Index Bootstrap Program "
50  PRINT:PRINT "for calculating Simplified Morisita's Index and Percent Similarity Index"
60  PRINT:PRINT "                Written by Jon S. Kao"

70  PRINT:PRINT:PRINT "95% Confidence Intervals calculated for a two-tailed Student's t critical value, alpha
= 0.05"

80  PRINT:PRINT:PRINT "enter pond treatment comparison:"
90  PRINT
100 TS(1)= "(1) PRO vs. UNP":TS(2)="(2) PRO vs. CSM":TS(3)="(3) PRO vs. INO":TS(4)="(4) UNP vs.
CSM":TS(5)="(5) UNP vs. INO":TS(6)="(6) CSM vs. INO"
120 PRINT TS(1):PRINT TS(2):PRINT TS(3):PRINT TS(4):PRINT TS(5):PRINT TS(6)
130 PRINT
140 INPUT "enter a number 1-6, followed by <enter> "; comp
150 IF comp <> int(comp) THEN 140
160 IF comp < 1 OR comp > 6 THEN 140

```

```

170 PRINT

200 GOSUB [date_check_module]

210 GOSUB [read_t_values] ' read t values into array for correct 95% CI calculation

220 ' input "true replicate" pond data: pond numbers, # prey categories and zooplankton counts for 230 '
each category

240 FOR treats = 1 TO 2 ' treatment groups one and two
260 PRINT

300 GOSUB [treatment_array] ' tells user what treatments are being compared, does nothing else

320 PRINT
380 INPUT "enter number of ponds: "; pnum(treats) ' number of ponds = pnum
400 IF pnum(treats) <> int(pnum(treats)) THEN 380

420 IF pnum(treats) <= 0 THEN 380
460 PRINT
500 PRINT "you entered "; pnum(treats); " ponds"
520 PRINT
540 cn = 0
600 INPUT "enter total # of prey categories: "; catnum(treats)
640 IF catnum(treats) <> int(catnum(treats)) THEN 600
680 IF catnum(treats) <= 0 THEN 600
700 PRINT
720 PRINT "you entered "; catnum(treats); " categories"

740 GOSUB [data_file_check]

760 PRINT
770 IF dfile$ = "y" THEN 1120 ' manual zooplankton number input bypass
780 FOR x = 1 TO pnum(treats) ' create dimensional arrays for # of ponds
800 FOR y = 1 TO catnum(treats) ' create dimensional array within pond # x for entire zooplankton suite
820 PRINT " how many prey category # "; y
840 PRINT " are present for : pond # "; x
860 PRINT
880 IF treats = 2 THEN 980
900 INPUT "enter number of zooplankter taxa found: "; zoo1(x,y)
920 PRINT ' zooplankton numbers inserted into arrays zoo1(x,y) and zoo2(x,y)
940 IF zoo1(x,y) <> int(zoo1(x,y)) OR zoo1(x,y) < 0 THEN 900

960 GOTO 1060

980 INPUT "enter number of zooplankter taxa found: "; zoo2(x,y)
1000 PRINT
1040 IF zoo2(x,y) <> int(zoo2(x,y)) OR zoo2(x,y) < 0 THEN 980
1060 PRINT
1080 NEXT y
1100 NEXT x
1120 NEXT treats
1140 df = min(pnum(1),pnum(2)) - 1 ' calculate degrees of freedom for t value calculation: (smaller number of
ponds -1)

1160 GOSUB [calc_95CI] ' choose Student's t-score for 95% CI calculation

```

```

1190 ' line 1200 is starting point after iteration run
1200 PRINT ' manual selection of # of draws and iterations, or pre-selected draw and iteration values
1300 PRINT "would you like to select the number of draws and iterations manually?"
1400 PRINT
1500 PRINT " or would you like to use pre-selected values: "
1600 PRINT: PRINT " 4, 6, 8, 15 and 20 draws; 10, 50, 100, 200, 500, and 1000 iterations"
1700 PRINT
1800 INPUT "please press <m> or <p>, followed by <enter> : ";manpre$
1900 IF manpre$ = "m" THEN 2200
2000 IF manpre$ <> "p" THEN 1800

2100 GOTO 43000

2200 ' module to perform Simplified Morisita's Index or Percent Similarity Index
2220 PRINT
2260 PRINT "you chose manual bootstrap program execution."
2300 ' PRINT "Are we performing"
2320 ' PRINT "(1) Simplified Morisita's Index"
2360 ' PRINT "(2) Percent Similarity Index": PRINT
2400 ' INPUT "enter 1 or 2 followed by <enter> :";index
2440 ' IF index <> int(index) or index < 0 THEN 2400
2480 ' if index <> 1 and index <> 2 then 2400
2520 ' PRINT:PRINT "Your Choice: "
2560 ' IF index = 1 then 2640
2600 ' Print: Print "Percent Similarity Index"

2620 ' GOTO 3000

2640 ' PRINT: PRINT "Morisita's Index"
3000 PRINT ' select the number of draws module
3100 INPUT "enter the number of draws (typically 3 - 20) to be taken per iteration: ";draws
3200 IF draws <> int(draws) THEN 3100
3240 IF draws <= 2 THEN 3100
3280 IF draws < 45 THEN 5000
3300 PRINT
3320 PRINT " too many 'replicates' drawn!, choose a smaller number!"
3350 PRINT

3380 GOTO 3100

5000 PRINT ' select the number of iterations to perform module
5100 INPUT "enter the number of iterations to be performed: "; iter ' iter a.k.a. trialnum
5200 IF iter <> int(iter) THEN 5100
5300 IF iter <= 1 then 5100
9000 ' IF index = 1 then 30000 ' goto Morisita's Index module if index = 1

10000 ' Percent Similarity Index Module
10050 PRINT ' start trial (iteration loop), typically 500 or more iterations
10100 FOR trials = 1 TO iter
10200 ' set initial 'draw' variables to zero
10300 PSItot3 = 0
        PSItot = 0
        psidrawsum = 0
10400 FOR pull = 1 to draws ' run single draw of treatment comparisons
10500 ' select one "true" pond replicate from each of 2 treatment groups in a two way comparison

```

```

10600 ' get n1 (random pond # among treatment 1) and n2 (random pond # among treatment 2)

10700 gosub [random_number_selection_II]

10800 ' converts numbers of zooplankton (from randomly selected replicate) into percent of total zooplankton
10820 ' and inserts these values into two arrays: preypct1(intn1) <-- treatment 1 and preypct2(intn2) <-- treatment
2

10850 gosub [generate_percentages]

10880 ' sets prey category number to either treatment one or two, which ever is less
10900 IF catnum(1) < catnum(2) THEN 10950 ELSE 11000
10950 cn = catnum(1)

10970 GOTO 11100

11000 cn = catnum(2)
11100 ' run through every category for treatment one and two, which ever is the smaller is added into variable
PSItotal

11200 ' PSI calculation module
11300 FOR y = 1 TO cn ' run through each zooplankton category
11400 IF preypct1(y) < preypct2(y) THEN PSItot = PSItot + preypct1(y) ' zooplankton category percent of
treatment one is placed into PSItot
11500 IF preypct1(y) > preypct2(y) THEN PSItot = PSItot + preypct2(y) ' zooplankton cateogry percent of
treatment two is placed into PSItot
11600 ' PSItot is sum of minimum percentages for two compared treatments, aka PSI value treat 1 v. treat 2
11700 NEXT y
12000 ' PSI value from single treatment comparison, a draw of 'x' of these comparisons are averaged into draw
average
12200 PRINT "single comparison PSI: ";print using("###.###",PSItot) ' display single, truncated PSI value
12700 Pdraw(pull) = PSItot 'place individual PSI values of each draw into array Pdraw(x)
12800 PSItot = 0 ' set single comparison value to zero after storing value in array, next loop generates new
PSItot
12900 next pull

13000 ' subroutine [draw_deviation] calculates and prints the draw PSI average, variance, std dev and std error.

13500 gosub [PSI_draw_deviation]

13700 ' subroutine [grand_total_stats]

13900 gosub [grand_total_stats_PSI]

14400 next trials ' # of iterations loop

14600 ' subroutine [grand_total_stats_II]

14700 gosub [grand_total_stats_II]

14800 print
14900 bsPSImean = psiitermean/iter
15000 print "the overall PSI (bootstrap) mean is : ";PRINT USING("###.###",bsPSImean)
15100 PRINT
15200 bsPSIvar = psiitervariance/iter
15300 print "the overall PSI (bootstrap) variance is : ";PRINT USING("#####.#####",bsPSIvar)

```

```

15400 PRINT
15500 bsPSIstdev = psiiterstddeviation/iter
15600 PRINT "the overall PSI (bootstrap) standard deviation is : ";PRINT USING("#####.#####",bsPSIstdev)
15700 PRINT
15800 bsPSIsterror = psiiterstderror/iter
15900 PRINT "the overall PSI (bootstrap) standard error is : ";PRINT USING("#####.#####",bsPSIsterror)
16000 bs95PSILL = bsPSImean - tscore2*bsPSIsterror
16100 PRINT
16200 PRINT "the overall PSI (bootstrap) 95% LL is "; PRINT USING("##.##",bs95PSILL)
16300 bs95PSIUL = bsPSImean + tscore2*bsPSIsterror
16400 PRINT
16500 PRINT "the overall PSI (bootstrap) 95% UL is "; PRINT USING("##.##",bs95PSIUL)
16600 PRINT
16700 PRINT "the Student's t score for ";df;" degrees of freedom is : ";tscore2
21700 fname$ = G$+"Manual_SimIndex"+sdate$+".txt"
21800 PRINT: PRINT " your output file will be : ";fname$
21900 PRINT
22000 OPEN fname$ FOR APPEND AS #1
23700 PRINT #1, "PSI: ";
23800 PRINT #1, " draws: ";
23840 PRINT #1, draws;
23860 PRINT #1, ", iter: ";
23900 PRINT #1, iter;
24000 PRINT #1, ", mean: ";
24100 PRINT #1, bsPSImean;
24200 PRINT #1, ", std err: ";
24300 PRINT #1, bsPSIsterror
24400 PRINT #1, ""
24500 PRINT #1, " CI LL: ";
24600 PRINT #1, bs95PSILL;
24700 PRINT #1, ", CI UL: ";
24800 PRINT #1, bs95PSIUL;
24900 PRINT #1, ", tscore: ";
25000 PRINT #1, tscore2;
25100 PRINT #1, " alpha = 0.05, two-tailed. "
25200 CLOSE #1

25300 ' GOTO 39000

30000 ' Morisita's Index module
30100 PRINT
30200 FOR trials = 1 TO iter 'start iteration loop, typically 500 or more iterations
30300 ' set initial 'draw' variables to zero
30400 MORtot3 = 0
MORtot = 0
MORdrawsum = 0
30500 FOR pull = 1 to draws ' set number of replicate comparisons to be made for each iteration
30600 ' select one "true" pond replicate from each of 2 treatment groups in a two way comparison

30700 gosub [random_number_selection_II] ' get n1 (random pond # among treatment 1) and n2 (random pond #
among treatment 2)

30800 ' converts numbers of zooplankton (from randomly selected replicate) into percent of total zooplankton
30900 ' and inserts these values into two arrays: preypct1(intn1) <-- treatment 1 and preypct2(intn2) <-- treatment
2

```

```

31000 gosub [generate_percentages]

31600 Gosub [Morisita_Draw_Module]

31700 PRINT "single comparison Morisita's Index: ";:print using("###.##",MorisitaDraw) ' display single, truncated
Morisita's value
31900 Mdraw(pull) = MorisitaDraw 'place individual Morisita's Index values of each draw into array Mdraw(n)
array
32000 MorisitaDraw = 0 ' reset MorisitaDraw value to zero after value is stored in array Mdraw(n), loop to
next value
32100 next pull

32200 ' subroutine [draw_deviation] calculates and prints the draw MOR average, variance, std dev and std error.

32300 gosub [MOR_draw_deviation]

32400 ' subroutine [grand_total_stats]

32500 gosub [grand_total_stats_MOR]

32600 next trials ' loop to next iteration of size iter

32700 ' subroutine [grand_total_stats_II], generate bootstrap statistical values

32800 gosub [grand_total_stats_II_MOR]

32900 PRINT
33000 bsMORmean = MORitermean/iter
33100 print "the overall Morisita's Index (bootstrap) mean is : ";:PRINT USING("###.##",bsMORmean)
33200 PRINT
33300 bsMORvar = MORitervariance/iter
33400 print "the overall Morisita's Index (bootstrap) variance is : ";:PRINT USING("#####.#####",bsMORvar)
33500 PRINT
33600 bsMORstdev = MORiterstddeviation/iter
33700 PRINT "the overall Morisita's Index (bootstrap) standard deviation is : ";:PRINT
USING("#####.#####",bsMORstdev)
33800 PRINT
33900 bsMORsterror = MORiterstderror/iter
34000 PRINT "the overall Morisita's Index (bootstrap) standard error is : ";:PRINT
USING("#####.#####",bsMORsterror)
34100 bs95MORLL = bsMORmean - tscore2*bsMORsterror
34200 PRINT
34300 PRINT "the overall Morisita's (bootstrap) 95% LL is ";: PRINT USING("###.##",bs95MORLL)
34400 bs95MORUL = bsMORmean + tscore2*bsMORsterror
34500 PRINT
34600 PRINT "the overall Morisita's (bootstrap) 95% UL is ";: PRINT USING("###.##",bs95MORUL)
34650 PRINT
34700 PRINT:PRINT " your output file will be : ";fname$
34750 PRINT
34800 OPEN fname$ FOR APPEND AS #1
34850 PRINT #1, ""
34900 PRINT #1, "Morisita: ";
34920 PRINT #1, " draws: ";
34940 PRINT #1, draws;
35000 PRINT #1, ", iter: ";
35100 PRINT #1, iter;

```



```

46400 IF preypct1(y) > preypct2(y) THEN PSItot = PSItot + preypct2(y) ' zooplankton category percent of
treatment two is placed into PSItot
46500 ' PSItot is sum of minimum percentages for two compared treatments, aka PSI value treat 1 v. treat 2
46800 NEXT y

47000 ' PSI value from single treatment comparison, a draw of 'x' of these comparisons are averaged into draw
average
47100 PRINT "single comparison PSI: ";print using("###.##",PSItot) ' display single, truncated PSI value
47200 Pdraw(pull) = PSItot 'place individual PSI values of each draw into array Pdraw(x)
47300 PSItot = 0 ' set single comparison value to zero after storing value in array, next loop generates new
PSItot
47400 next pull

47500 ' subroutine [draw_deviation] calculates and prints the draw PSI average, variance, std dev and std error.

47700 gosub [PSI_draw_deviation]

47800 ' subroutine [grand_total_stats]

47900 gosub [grand_total_stats_PSI]

48000 next trials ' # of iterations loop

48100 ' subroutine [grand_total_stats_II]

48200 gosub [grand_total_stats_II]

48300 print
48500 bsPSImean = psiitermean/iter
48600 print "the overall PSI (bootstrap) mean is : ";PRINT USING("###.##",bsPSImean)
48700 PRINT
48800 bsPSIvar = psiitervariance/iter
48900 print "the overall PSI (bootstrap) variance is : ";PRINT USING("#####.#####",bsPSIvar)
49000 PRINT
49100 bsPSIstdev = psiiterstddeviation/iter
49200 PRINT "the overall PSI (bootstrap) standard deviation is : ";PRINT USING("#####.#####",bsPSIstdev)
49300 PRINT
49400 bsPSIsterror = psiiterstderror/iter
49500 PRINT "the overall PSI (bootstrap) standard error is : ";PRINT USING("#####.#####",bsPSIsterror)
49600 bs95PSILL = bsPSImean - tscore2*bsPSIsterror
49700 PRINT
49800 PRINT "the overall PSI (bootstrap) 95% LL is "; PRINT USING("###.##",bs95PSILL)
49900 bs95PSIUL = bsPSImean + tscore2*bsPSIsterror
50000 PRINT
50100 PRINT "the overall PSI (bootstrap) 95% UL is "; PRINT USING("###.##",bs95PSIUL)
50110 PRINT
50115 PRINT "the Student's t score is ";tscore2;" for ";df;" degrees of freedom"
50120 fname$ = G$+"SimIndex"+sdate$+".txt"
50140 PRINT: PRINT " your output file will be : ";fname$
50160 PRINT

50180 OPEN fname$ FOR APPEND AS #1
50200 PRINT #1, T$(comp);
50210 PRINT #1, " ";
50220 PRINT #1, sdate$
50230 PRINT #1, " "

```

```

50240 PRINT #1, "Draws: ";
50260 PRINT #1, draws
50280 PRINT #1, ""
50300 PRINT #1, "PSI: ";
50400 PRINT #1, " iter: ";
50500 PRINT #1, iter;
50600 PRINT #1, ", mean: ";
50700 PRINT #1, bsPSImean;
50800 PRINT #1, ", std err: ";
50900 PRINT #1, bsPSIsterror
51000 PRINT #1, "  CI LL: ";
51100 PRINT #1, bs95PSILL;
51200 PRINT #1, ", CI UL: ";
51300 PRINT #1, bs95PSIUL;
51400 PRINT #1, ", tscore: ";
51500 PRINT #1, tscore2;
51600 PRINT #1, ", alpha = 0.05, two-tailed. "
51700 PRINT #1, "          "
51800 CLOSE #1

53000 ' Morisita's Index module
53100 PRINT
53200 FOR trials = 1 TO iter 'start iteration loop, typically 500 or more iterations
53300 ' set initial 'draw' variables to zero
53400 MORtot3 = 0
          MORtot = 0
          MORdrawsum = 0
53500 FOR pull = 1 to draws ' set number of replicate comparisons to be made for each iteration
53600 ' select one "true" pond replicate from each of 2 treatment groups in a two way comparison

53700 gosub [random_number_selection_II] ' get n1 (random pond # among treatment 1) and n2 (random pond #
among treatment 2)

53800 ' converts numbers of zooplankton (from randomly selected replicate) into percent of total zooplankton
53900 ' and inserts these values into two arrays: preypct1(intn1) <-- treatment 1 and preypct2(intn2) <-- treatment
2

54000 gosub [generate_percentages]

54100 Gosub [Morisita_Draw_Module]

54200 PRINT "single comparison Morisita's Index: ";;print using("###.###",MorisitaDraw) ' display single, truncated
Morisita's value
54300 Mdraw(pull) = MorisitaDraw 'place individual Morisita's Index values of each draw into array Mdraw(n)
array
54400 MorisitaDraw = 0 ' reset MorisitaDraw value to zero after value is stored in array Mdraw(n), loop to
next value
54500 next pull

54600 ' subroutine [draw_deviation] calculates and prints the draw MOR average, variance, std dev and std error.

54700 gosub [MOR_draw_deviation]

54800 ' subroutine [grand_total_stats]

54900 gosub [grand_total_stats_MOR]

```

```

55000 next trials ' loop to next iteration of size iter

55100 ' subroutine [grand_total_stats_II], generate bootstrap statistical values

55200 gosub [grand_total_stats_II_MOR]

55300 print
55400 bsMORmean = MORitermean/iter
55500 print "the overall Morisita's Index (bootstrap) mean is : ";PRINT USING("##.##",bsMORmean)
55600 PRINT
55700 bsMORvar = MORitervariance/iter
55800 print "the overall Morisita's Index (bootstrap) variance is : ";PRINT USING("####.####",bsMORvar)
55900 PRINT
56000 bsMORstdev = MORiterstddeviation/iter
56100 PRINT "the overall Morisita's Index (bootstrap) standard deviation is : ";PRINT
USING("####.####",bsMORstdev)
56200 PRINT
56300 bsMORsterror = MORiterstderror/iter
56400 PRINT "the overall Morisita's Index (bootstrap) standard error is : ";PRINT
USING("####.####",bsMORsterror)
56500 bs95MORLL = bsMORmean - tscore2*bsMORsterror
56600 PRINT
56700 PRINT "the overall Morisita's (bootstrap) 95% LL is "; PRINT USING("##.##",bs95MORLL)
56800 bs95MORUL = bsMORmean + tscore2*bsMORsterror
56900 PRINT
57000 PRINT "the overall Morisita's (bootstrap) 95% UL is "; PRINT USING("##.##",bs95MORUL)
57020 PRINT
57040 PRINT "the Student's t score is ";tscore2;" for ";df;" degrees of freedom"
57060 PRINT

57100 OPEN fname$ FOR APPEND AS #1 ' add analyses to text file
57200 PRINT #1, "Morisita: ";
57300 PRINT #1, " iter: ";
57400 PRINT #1, iter;
57500 PRINT #1, ", mean: ";
57600 PRINT #1, bsMORmean;
57700 PRINT #1, ", std err: ";
57800 PRINT #1, bsMORsterror
57900 PRINT #1, " CI LL: ";
58000 PRINT #1, bs95MORLL;
58100 PRINT #1, ", CI UL: ";
58200 PRINT #1, bs95MORUL;
58300 PRINT #1, ", tscore: ";
58400 PRINT #1, tscore2;
58500 PRINT #1, ", alpha = 0.05, two-tailed. "
58700 PRINT #1, " "
58800 CLOSE #1
58900 NEXT bigruns
58950 RESTORE ' reset data pointer to beginning data value

59000 PRINT ' do you wish to continue module
59100 INPUT "do you wish to continue? press <y> or <n>, followed by <enter> : ";z$
59200 IF z$ = "y" THEN 59500
59300 PRINT
59400 PRINT "goodbye." : END

```

```
59500 PRINT
59600 PRINT "Do you wish to continue with the same data set?"
59700 INPUT "press <y> or <n>, followed by <enter> : ";dset$
59800 RESTORE
```

```
59900 IF dset$ = "y" THEN 1200
60000 CLS
62000 GOTO 60
```

' various subroutines

[treatment_array]

```
100000 if comp <> 1 or treats <> 1 then goto 100100
100050 print "please enter data for PRO treatment"
100080 return
100100 if comp <> 1 or treats <> 2 then goto 100200
100150 print "please enter data for UNP treatment"
100180 return
```

```
100200 if comp <> 2 or treats <> 1 then goto 100300
100250 print "please enter data for PRO treatment"
100280 return
```

```
100300 if comp <> 2 or treats <> 2 then goto 100400
100350 print "please enter data for CSM treatment"
100380 return
```

```
100400 if comp <> 3 or treats <> 1 then goto 100500
100450 print "please enter data for PRO treatment"
100480 return
```

```
100500 if comp <> 3 or treats <> 2 then goto 100600
100550 print "please enter data for INO treatment"
100580 return
```

```
100600 if comp <> 4 or treats <> 1 then goto 100700
100650 print "please enter data for UNP treatment"
100680 return
```

```
100700 if comp <> 4 or treats <> 2 then goto 100800
100750 print "please enter data for CSM treatment"
100780 return
```

```
100800 if comp <> 5 or treats <> 1 then goto 100900
100850 print "please enter data for UNP treatment"
100880 return
```

```
100900 if comp <> 5 or treats <> 2 then goto 101000
100950 print "please enter data for INO treatment"
100980 return
```

```
101000 if comp <> 6 or treats <> 1 then goto 101100
101050 print "please enter data for CSM treatment"
101080 return
```

```
101100 if comp <> 6 or treats <> 2 then goto 101180
```

```
101150 print "please enter data for INO treatment"
101300 return
```

```
[random_number_selection_II]
```

```
110000 ' module to generate random numbers to select which replicate is chosen in treatment comparison
110200 seed1 = time$("milliseconds")
110300 seed1$ = str$(seed1)
110400 seeda = val(right$(seed1$,2))
110500 if seeda <=0 then 110200
110600 randomize seeda
110700 n1 = rnd(seeda/100)+1 ' replicate for treatment one chosen
110800 n1 = n1 - int(n1)
110900 n1int = int(n1 * 1000)
111000 if n1int > pnum(1) or n1int = 0 then 110700 ' random number generated up to 'true replicate' sample size
111100 for t = 1 to 5000: next t
111200 seed2 = time$("milliseconds")
111300 seed2$ = str$(seed2)
111400 seedb = val(right$(seed2$,2))

111500 if seedb <= 0 then 111200
111600 randomize seedb
111700 n2 = rnd(seedb/100)+1 ' replicated for treatment two chosen
111800 n2 = n2 - int(n2)
111900 n2int = int(n2 * 1000)
112000 IF n2int > pnum(2) OR n2int = 0 THEN 111700 ' random number generated up to 'true replicate' sample
size
112100 RETURN
```

```
[generate_percentages]
```

```
117100 totprey1 = 0
totprey2 = 0
117200 FOR y = 1 TO catnum(1)
117300 totprey1 = totprey1 + zoo1(n1int,y) ' counts all prey of category one; aka cat sum treatment one
117400 NEXT y
117500 FOR y = 1 TO catnum(2)
117600 totprey2 = totprey2 + zoo2(n2int,y) ' counts all prey of category two; aka cat sum treatment two
117700 NEXT y
118250 for y = 1 to catnum(1)
118280 preypct1(y) = ((zoo1(n1int,y))/totprey1)*100 ' generate and store percentage value of prey category y,
treat 1
118300 next y
118600 for y = 1 to catnum(2)
118700 preypct2(y) = ((zoo2(n2int,y))/totprey2)*100 ' generate and store percentage value of prey category y,
treat 2
118900 next y
119000 return
```

```
[PSI_draw_deviation]
```

```
130900 psidrawsum = 0
131000 for pulls = 1 to draws
131100 psidrawsum = psidrawsum + Pdraw(pulls)
131200 next pulls
131250 psidrawavg = psidrawsum /draws ' average PSI for pull of size 'draw' replicates
132000 for pulls = 1 to draws
```

```

132100 psidrawdevsum = psidrawdevsum + (Pdraw(pulls)-psidrawavg)^2
132200 next pulls
132400 for pulls = 1 to draws
133500 psidrawdevtot = psidrawdevtot + psidrawdev(pulls) ' sum of squares of deviatons of ind. PSI from mean
PSI of draw
133600 next pulls
133800 PRINT "PSI draw mean = ";;PRINT USING("###.##",psidrawavg)
133900 psidrawvar = psidrawdevsum/(draws-1)
134000 print "PSI draw variance = ";;PRINT USING("#####.#####",psidrawvar)
134100 psidrawdev = sqr(psidrawvar)
134200 print "PSI draw standard deviation = ";;PRINT USING("#####.#####",psidrawdev)
134300 psidrawsterr = psidrawdev/sqr(draws)
134400 print "PSI draw standard error = ";;PRINT USING("#####.#####",psidrawsterr)
134500 psidrawsum = 0
134700 psidrawdevsum = 0
140000 return

```

[grand_total_stats_PSI]

```

150100 ' module to place individual draw stats into overall PSI 'iterations' array and stats
150200 psiiter(trials) = psidrawavg
150300 psivariter(trials) = psidrawvar
150400 psistdeviter(trials) = psidrawdev
150500 psisterriter(trials) = psidrawsterr
160000 return

```

[grand_total_stats_II]

```

170000 ' module to run through all iteration arrays and generate overall 'bootstrap' stats
170050 psiitermean = 0
psiitervariance = 0
psiiterstddeviation = 0
psiiterstderror = 0
170100 for trials = 1 to iter
170200 psiitermean = psiitermean + psiiter(trials)
170300 psiitervariance = psiitervariance + psivariter(trials)
170400 psiiterstddeviation = psiiterstddeviation + psistdeviter(trials)
170500 psiiterstderror = psiiterstderror + psisterriter(trials)
170800 next trials
180000 return

```

[MOR_draw_deviation]

```

200000 MORdrawsum = 0
MORdrawdevsum = 0
Mprey1sum = 0
Mprey2sum = 0
Mcrosssum = 0
200100 for pulls2 = 1 to draws
200200 MORdrawsum = MORdrawsum + Mdraw(pulls2) ' Mdraw(x) array contains individual draw
comparisons... 4, 6, 8, etc.
200300 next pulls2
200400 MORdrawavg = MORdrawsum /draws ' average PSI for pull of size 'draw' replicates
200500 for pulls2 = 1 to draws
200600 MORdrawdevsum = MORdrawdevsum + (Mdraw(pulls2)-MORdrawavg)^2
200700 next pulls2

```

```

210200 PRINT "Simplified Morisita's Index draw mean = ";PRINT USING("##.##",MORdrawavg)
210300 MORdrawvar = MORdrawdevsum/(draws-1)
210400 print "Morisita's Index draw variance = ";PRINT USING("####.####",MORdrawvar)
210500 MORdrawdev = sqrt(MORdrawvar)
210600 print "Morisita's Index draw standard deviation = ";PRINT USING("#####.#####",MORdrawdev)
210700 MORdrawsterr = MORdrawdev/sqrt(draws)
210800 print "Morisita's Index draw standard error = ";PRINT USING("####.####",MORdrawsterr)
220000 return

```

[grand_total_stats_MOR]

```

225100 ' module to place individual draw stats into overall PSI 'iterations' array and stats
225200 MORiter(trials) = MORdrawavg
225300 MORvariter(trials) = MORdrawvar
225400 MORstdeviter(trials) = MORdrawdev
225500 MORsterriter(trials) = MORdrawsterr
300000 return

```

[grand_total_stats_II_MOR]

```

310000 ' module to run through all iteration arrays and generate overall 'bootstrap' stats
310100 MORitermean = 0
      MORitervariance = 0
      MORiterstddeviation = 0
      MORiterstderror = 0
310300 for trials2 = 1 to iter
310400 MORitermean = MORitermean + MORiter(trials2)
310500 MORitervariance = MORitervariance + MORvariter(trials2)
310600 MORiterstddeviation = MORiterstddeviation + MORstdeviter(trials2)
310700 MORiterstderror = MORiterstderror + MORsterriter(trials2)
310800 next trials2
311000 return

```

[Morisita_Draw_Module]

```

318810 Mprey1sum = 0
      Mprey2sum = 0
      Mcrosssum = 0
      Mnumsum1 = 0
      Mnumsum2 = 0
      Mnumsumsum1 = 0
      Mnumsumsum2 = 0

318820 for y = 1 to catnum(1)
318840 Mpreypct1(y) = preypct1(y)*(preypct1(y)-1)
318880 Mprey1sum = Mprey1sum + Mpreypct1(y)      ' Mprey1sum aka Morisita's numerator
318900 next y
319000 for y = 1 to catnum(2)
319020 Mpreypct2(y) = preypct2(y)*(preypct2(y)-1)      ' Mprey2sum aka Morisita's numerator
319040 Mprey2sum = Mprey2sum + Mpreypct2(y)
319060 next y
319080 for y = 1 to catnum(1)
319100 Mnumsum1 = Mnumsum1 + preypct1(y)      ' cat sum treatment 1
319200 next y
319300 for y = 1 to catnum(2)
319400 Mnumsum2 = Mnumsum2 + preypct2(y)      ' cat sum treatment 2
319500 next y
319600 Mnumsumsum1 = Mnumsum1*(Mnumsum1-1)
319700 Mnumsumsum2 = Mnumsum2*(Mnumsum2-1)

```

```

320000 Lambda1 = Mprey1sum/Mnumsumsum1      ' aka Morisita's denominator treatment 1
320100 Lambda2 = Mprey2sum/Mnumsumsum2      ' aka Morisita's denominator treatment 2
320200 if catnum(1) < catnum(2) then 320800
320300 for y = 1 to catnum(2)
320400 Mcrossprod(y) = preypct1(y) * preypct2(y)
320500 Mcrosssum = Mcrosssum + Mcrossprod(y)
320600 next y

320700 goto 321200

320800 for y = 1 to catnum(1)
320900 Mcrossprod(y) = preypct1(y) * preypct2(y)
321000 Mcrosssum = Mcrosssum + Mcrossprod(y)
321100 next y
321200 MorisitaDraw = (Mcrosssum*2)/((Lambda1+Lambda2)*(Mnumsum1*Mnumsum2))
323000 return

[date_check_module]
330000 INPUT "enter sample date month: <mm>, followed by <enter> : ";smonth
330100 IF smonth < 1 or smonth > 12 THEN 330000
330200 IF smonth <> int(smonth) THEN 330000
330300 INPUT "enter sample date day: <dd>, followed by <enter> : ";sday
330400 IF sday < 1 or sday > 31 THEN 330300
330500 IF sday <> int(sday) THEN 330300
330600 INPUT "enter sample date year: <yyyy>, followed by <enter> : ";syear
330800 IF syear <> int(syear) THEN 330600
331000 smonth$ = str$(smonth)
331200 sday$ = str$(sday)
331400 syear$ = str$(syear)
331500 IF len(syear$) <> 4 THEN 330600
331700 sdate$ = smonth$ + sday$ + syear$
331800 IF comp = 1 THEN G$ = "PU"
331900 IF comp = 2 THEN G$ = "PC"
332000 IF comp = 3 THEN G$ = "PI"
332100 IF comp = 4 THEN G$ = "UC"
332200 IF comp = 5 THEN G$ = "UI"
332300 IF comp = 6 THEN G$ = "CI"
333000 RETURN

[data_file_check]
335000 PRINT
335100 PRINT "do you want to read data from a text file?"
335200 PRINT
335300 INPUT "please press <y> or <n>, followed by <enter> : ";dfile$
335400 IF dfile$ = "n" THEN 340000
335500 IF dfile$ = "y" THEN 336000

335700 GOTO 335300

336000 PRINT
336050 IF treats = 2 THEN 337750
336100 PRINT "please load file with data from first treatment of treatment pair : "
336150 PRINT T$(comp)
336200 FILEDIALOG, "Open", "c:\*.txt", fname$
336300 IF fname$ = "" then 336200
336400 PRINT

```

```

336500 OPEN fname$ FOR INPUT AS #2
336600 FOR x = 1 to pnum(treats)
336700 FOR y = 1 to catnum(treats)
337000 zoo1$(x,y) = inputto$(#2,",")
337050 FOR t = 1 TO 400: next t
337100 zoo1(x,y) = val(zoo1$(x,y))
337300 NEXT y
337400 NEXT x
337500 CLOSE #2
337600 FOR t = 1 to 5000: next t
337700 IF treats = 1 THEN 340000
337750 PRINT
337800 PRINT "please load file with data from second treatment of treatment pair : "
337900 PRINT T$(comp)
338000 FILEDIALOG, "Open", "c:\*.txt", fname2$
338100 IF fname2$ = "" then 336200
338300 OPEN fname2$ FOR INPUT AS #3
338500 FOR x = 1 to pnum(treats)
338700 FOR y = 1 to catnum(treats)
338900 zoo2$(x,y) = inputto$(#3,",")
339100 for t = 1 to 100: next t
339300 zoo2(x,y) = val(zoo2$(x,y))
339500 NEXT y
339700 NEXT x
339900 CLOSE #3
340000 RETURN

```

[read_t_values]

```

360000 FOR RT = 1 to 35
360500 READ tscore
360550 IF tscore = 0 THEN 360700
360600 tval(RT) = tscore
360700 NEXT RT
360900 RETURN

```

[calc_95CI]

```

' calculate t-score based upon degrees of freedom (i.e. smaller pond treatment number of replicates - 1)
370500 IF df <= 30 THEN hdf = df
380000 IF df > 30 AND df < 40 THEN hdf = 31
380100 IF df >= 40 AND df < 60 THEN hdf = 32
380200 IF df >= 60 AND df < 100 THEN hdf = 33
380400 IF df >= 100 AND df < 130 THEN hdf = 34
380500 IF df >= 130 THEN hdf = 35
390000 tscore2 = tval(hdf) ' set t-score based upon array holding t-scores and the degrees of freedom
39500 RETURN

```

' t-score array: alpha = 0.05, two-tailed

```

DATA 12.706, 4.303, 3.182, 2.776, 2.571, 2.447, 2.365, 2.306, 2.262, 2.228
DATA 2.201, 2.179, 2.160, 2.145, 2.131, 2.120, 2.110, 2.101, 2.093, 2.086
DATA 2.080, 2.074, 2.069, 2.064, 2.060, 2.056, 2.052, 2.048, 2.045, 2.042
DATA 2.021, 2.000, 1.980, 1.960, 0

```

```
' automatic preset number of iterations and draws (not manual)
DATA 10, 4, 10, 6, 10, 8, 10, 15, 10, 20
DATA 50, 4, 50, 6, 50, 8, 50, 15, 50, 20
DATA 100, 4, 100, 6, 100, 8, 100, 15, 100, 20
DATA 500, 4, 500, 6, 500, 8, 500, 15, 500, 20
DATA 1000, 4, 1000, 6, 1000, 8, 1000, 15, 1000, 20
DATA 0, 0 'end of data
```


Morisita, 50, 0.68623326, 0.11613755, 0.45860366, 0.91386285

(5) UNP vs. INO,

PSI , 50, 57.2556306, 7.4335738, 42.685826, 71.8254353

Morisita, 50, 0.68424005, 0.88926879e-1, 0.50994337, 0.85853674

(5) UNP vs. INO,

PSI , 50, 56.5456242, 6.32544941, 44.1477434, 68.9435051

Morisita, 50, 0.68632794, 0.76004842e-1, 0.53735845, 0.83529743

(5) UNP vs. INO,

PSI , 100, 60.1864029, 14.4598765, 31.845045, 88.5277609

Morisita, 100, 0.79337088, 0.12171307, 0.55481327, 1.03192849

(5) UNP vs. INO,

PSI , 100, 58.6007076, 11.018936, 37.0035929, 80.1978222

Morisita, 100, 0.73957487, 0.12044588, 0.50350094, 0.97564879

(5) UNP vs. INO,

PSI , 100, 58.5079084, 9.89758171, 39.1086482, 77.9071685

Morisita, 100, 0.74588278, 0.10739447, 0.53538962, 0.95637594

(5) UNP vs. INO,

PSI , 100, 58.361429, 7.65176116, 43.3639772, 73.3588809

Morisita, 100, 0.69685134, 0.85044149e-1, 0.53016481, 0.86353788

(5) UNP vs. INO,

PSI , 100, 59.4303341, 6.57935342, 46.5348014, 72.3258668

Morisita, 100, 0.68755745, 0.74509769e-1, 0.54151831, 0.8335966

(5) UNP vs. INO,

PSI , 500, 61.4148541, 12.3721985, 37.165345, 85.6643633

Morisita, 500, 0.77519512, 0.13241533, 0.51566108, 1.03472916

(5) UNP vs. INO,

PSI , 500, 59.1547502, 11.6211914, 36.377215, 81.9322853

Morisita, 500, 0.73502015, 0.12419932, 0.49158948, 0.97845082

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BIOGRAPHICAL SKETCH

Jon S. Kao was born in Waterloo, Iowa. The youngest of three children, he spent his childhood in Cedar Falls, Iowa, and summers with his mother in Milwaukee, Wisconsin. Jon graduated from Cedar Falls High School in 1983 and then spent two months with his mother, sister and brother, traveling through China before heading off to college. He attended the University of Iowa, and in 1990 earned his B.A. majoring in biology and botany. Pursuing his childhood dream to be a marine biologist, Jon was faced with a major dilemma, as the state of Iowa was surprisingly devoid of opportunities to study marine biology. He soon packed up most of his worldly belongings and moved to California to attend graduate school studying marine biology. As a budding ichthyologist, he exhaustively studied the diet and feeding physiology of the leopard shark (*Triakis semifasciata*) at Moss Landing Marine Laboratories, and earned his M.S. in Marine Science in 2000. He then again gathered all of his worldly possessions to drive 2000+ miles to study aquaculture as a Ph.D. student at the University of Florida, at the former Department of Fisheries and Aquatic Sciences (currently the program in Fisheries and Aquatic Sciences in the School of Forest Resources and Conservation).

Upon completion of his Ph.D. program, Jon, his veterinarian significant other Shari, and their large brood of dogs and cats, will again make a continent wide trek back to America's west coast to start a commercial fish and livestock farm of modest, but hopefully comfortable means.