EVALUATION OF THE CALANOID COPEPOD *PSEUDODIAPTOMUS PELAGICUS* AS A FIRST FEED FOR FLORIDA POMPANO, *TRACHINOTUS CAROLINUS*, LARVAE

By

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To my Family, for their enduring patience and unconditional love

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TABLE OF CONTENTS

LIST OF TABLES

Table page

LIST OF FIGURES

Figure page

Abstract of Thesis Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Master of Science

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Production of many marine fish species is currently impeded by poor performance during the larval phase. Traditional live feeds, such as rotifers, *Brachionus* spp., and brine shrimp, *Artemia* spp., can be nutritionally deficient, may not constitute the appropriate size range, and may not elicit a feeding response and therefore are inadequate for many marine fish species. An evaluation of copepods as a live feed for marine fish larvae is critical to the expansion and development of larval rearing techniques. Recently, advances in copepod culture have increased interest in their application as a live feed. In this thesis, the calanoid copepod *Pseudodiaptomus pelagicus* was evaluated as a first food for marine fish larvae. The development of culture techniques for *P. pelagicus* associated with marine fish larviculture, including batch cultures, mass-scale production, maintenance, nauplii collection, and microalgae cultures are discussed. Furthermore, five trials were conducted to evaluate the performance of Florida pompano, *Trachinotus carolinus,* larvae fed nauplii of *P. pelagicus*.

The Florida pompano is a highly prized marine fish species whose larviculture protocol currently includes a live feeds regime of rotifers and brine shrimp nauplii. Copepods have not been evaluated as a live food for Florida pompano larvae. In this evaluation, Florida pompano

larvae were fed five diets, which included *P. pelagicus* nauplii, and were compared to the standard reference diet (SRD), which consisted solely of rotifers. Dietary treatments were fed during the first 9 days post hatch (DPH) in both 13-L and 170-L tank systems. Phase feeding of copepods fed for the first day, the first three days, and mixed with rotifers for the entire trial were examined. Copepods fed exclusively during the entire trial and in a mesocosm were also examined. Significant increases in growth, survival, and resistance to prolonged durations of net stress resulted.

Florida pompano larvae fed copepod nauplii for the first day of feeding, at a density of 2–3 nauplii/mL, consistently had advantageous results when compared to larvae fed only rotifers. Beneficial results were also documented when larvae were fed nauplii for the first three days of feeding. But increased quantities of nauplii were needed to provide sufficient nutrients for growth and survival beginning on approximately 3 DPH. This density dependence was reflected in the results of larvae fed copepods for the entire trial, where survival was significantly higher than the other dietary treatments, but growth was significantly reduced. Larvae fed the mixed diet had similar survival as larvae fed the SRD (approximately 40%) however greater growth and resistance to stress occurred with larvae fed copepods, possibly reflecting a nutritional advantage to copepods in the diet. Resistance to net stress was greater for those larvae fed copepods during the trials, and survival was directly related to the duration copepods were fed. Larvae fed copepods were consistently alive after 10 minutes of net stress. Poor results were obtained from trials examining the use of a mesocosm feeding strategy. Refinement of stocking techniques and copepod preparation is discussed.

CHAPTER 1
INTRODUCTION

Expansion of marine fish production methods is critical to the advancement of the food, bait, and ornamental aquaculture industries. Currently, only a limited number of marine fish species are being produced with variable success. The major impediment to commercial production of currently grown species and success with candidate species is the utilization of an appropriate live feed during the first feeding phase of the larval cycle. This period is extremely crucial for marine fish larvae. A live feed with an adequate nutritional composition, constituting a suitable size range that stimulates a feeding response is necessary for the expansion of the number of species of marine fish cultured. Copepods have these characteristics and are the natural food of marine fish larvae. Therefore, improvements in growth, survival, and stress resistance should be observed in cultured marine fish larvae fed copepods.

An important dietary requirement for the optimal development of marine fish larvae is sufficient levels of highly unsaturated fatty acids (HUFAs), particularly docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (AA) (Sargent et al., 1999a). Improvements in growth, survival, and stress resistance are attained when suitable absolute amounts or ratios of these fatty acids are present in larval diets (Sargent et al., 1999b). The optimal levels are species-specific, however, an approximate ratio for DHA:EPA:AA of 10:5:1 is believed to be appropriate (Bell and Sargent, 2003). Gape is also a limiting factor in successful marine fish production. Many marine fish larvae have a small gape and are unable to consume traditional live feeds including rotifers, *Branchionus* spp., and brine shrimp nauplii, *Artemia* spp. (McKinnon et al., 2003; Chesney, 2005). Also, the ability of a live feed organism to elicit a feeding response is important to the successful development of aquaculture techniques for candidate species (Stottrup and Norsker, 1997).

Traditional Live Feeds

Traditionally, rotifers, *Branchionus* spp., and brine shrimp nauplii, *Artemia* spp., are fed to marine fish during the larval phase. Well documented culture protocols, recent improvements in nutritional supplementation, and readily available eggs make the use of these live feeds appealing to commercial producers (Rainuzzo et al., 1997; Lavens and Sorgeloos, 1999; Stottrup and McEvoy, 2003). Even though these live feeds are inadequate for many marine fish larvae, they are the best option available and some species can be successfully produced.

Rotifers

Rotifers (phylum Rotifera) are small metazoans with over 2000 species described; most inhabit freshwater lakes and ponds (Lubzens and Zmora, 2003). Two species, *Brachionus plicatilis* and *B. rotundiformis*, have been used to culture over 60 species of marine finfish larvae and 18 species of crustacean larvae (Dhert, 1996). First identified as a pest in pond cultures of eels in Japan during the 1950's, tests revealed there were advantages to feeding *B. plicatilis* to red sea bream, *Pagrus major*, larvae. This led to the mass culture of *B. plicatilis* for use as a live feed for other marine fish larvae (Dhert, 1996; Lubzens and Zmora, 2003). Rotifers propagate quickly under suitable conditions, with populations doubling over a few days (Lubzens and Zmora, 2003). Cultures can become quite dense with over 1000 rotifers/mL commonly maintained (Dhert, 1996; Lubzens and Zmora, 2003). This is an advantage for fish hatcheries with a large demand for live feeds during the larval phase. Bentley et al. (2008) estimated that 400 billion rotifers are needed to produce 10 million gilthead sea bream, *Sparus aurata*, fry. On average, 20,000 to 100,000 rotifers will be used per fish larva during a 20–30 day larval period (Lubzens and Zmora, 2003).

Typically, rotifers are grown as batch cultures at salinities from 15 to 20 g/L and temperatures of approximately 26°C, although specific culture characteristics vary between

species and strains (Dhert, 1996; Lubzens and Zmora, 2003). Prior to rearing fish larvae, rotifer production is increased through the use of high nutrient diets (Dhert, 1996; Lubzens and Zmora, 2003). Rotifer populations or resting eggs can be purchased, but typically a new culture is started from a subsample of another culture. Once a stock culture is established, the need to obtain new rotifers is reduced.

Although rotifers can be grown in sufficient numbers to satisfy a large hatchery, there are problems with their adequacy as a live feed. For example, they do not have the proper nutritional composition for marine fish larvae (Stottrup, 2000; Hamre et al., 2008). Rotifers do not have appropriate levels of DHA, EPA, or AA, nor do they have the ability to elongate shorter chain fatty acids, therefore they must be enriched prior to feeding to marine fish larvae (Rainuzzo et al., 1997; Sargent et al., 1997; Stottrup, 2000). Current enrichment techniques allow them to retain potentially adequate levels of HUFAs for several hours, provided they are kept at 10°C to reduce their metabolism (Rainuzzo et al., 1997; Sargent et al., 1997). Once rotifers are placed in the larval tank, metabolism resumes and those nutrients are only available to the fish larvae for a short period of time (Rainuzzo et al., 1997; Sargent et al., 1997).

The quantity of rotifers within a larval system must be constantly monitored to ensure that appropriate densities are maintained (Dhert, 1996; Lubzens and Zmora, 2003). If rotifers propagate beyond the grazing pressure of fish larvae, not only will the fish larvae consume a nutritionally inadequate prey item, but the water quality can quickly become detrimental to the survival of the fish larvae (Dhert, 1996; Lubzens and Zmora, 2003). Rotifers also constitute a small size range (90–350 microns) which may not be adequate for smaller fish larvae (Lubzens and Zmora, 2003; McKinnon et al, 2003). Evidence further suggests that rotifers are not easily

digested (Schipp et al, 1999) and their slow movement may not elicit a feeding response by marine fish larvae (Chesney, 2005).

Brine Shrimp

Brine shrimp (phylum Arthropoda) are the most widely used form of live feed for marine fish larvae in the world with over 2000 metric tons of cysts marketed annually (Van Stappen et al., 1996; Dhont and Van Stappen, 2003). Brine shrimp, *Artemia* spp., can be split into two different types, zygogenetic species and parthenogenetic species (Dhont and Van Stappen, 2003). Zygogenetic species reproduce by the fusion of male and female gametes and parthenogenetic species reproduce by development of an unfertilized female gamete. Currently seven species of zygogenetic types and many strains of a parthenogenetic species are acknowledged (Dhont and Van Stappen, 2003). All are referred to as *Artemia*. The production of dormant cysts is a key characteristic of all species, and this makes *Artemia* an appealing live feed for fish and crustacean larvae because of availability (Van Stappen et al., 1996; Dhont and Van Stappen, 2003). *Artemia* cysts are stored dry until needed; optimal hatching is initiated in 15–35 g/L salinity water (Dhont and Van Stappen, 2003). Nauplii of the instar I and II stages are the most common stages of *Artemia* fed to larval fish, and they develop within 24 and 36 hours after hatching, respectively (Van Stappen et al., 1996; Dhont and Van Stappen, 2003). The ability of cysts to be stored for long periods of time and relative predictability of hatching success makes *Artemia* a suitable live feed for many hatcheries and marine fish species (Van Stappen et al., 1996; Dhont and Van Stappen, 2003).

Artemia, however, are a nutritionally deficient live feed for most developing marine fish larvae (Rainuzzo et al., 1997; Sargent et al., 1997; Stottrup, 2000). Like rotifers, they have insufficient levels of DHA, EPA, and AA (Rainuzzo et al., 1997; Sargent et al., 1997; Stottrup, 2000). The instar I, a non-feeding stage, is incapable of enrichment but all other life stages can

be enriched prior to feeding to marine fish larvae (Rainuzzo et al., 1997; Dhont and Van Stappen, 2003). Although these enrichments temporarily ameliorate their nutritional composition, the improved fatty acid levels are often inconsistent (Stottrup, 2000; Palmtag et al., 2006). Newly hatched *Artemia* nauplii are usually too large (~450 microns) for most first feeding marine fish larvae, often only being fed during subsequent stages of larval development (Van Stappen et al., 1996; Dhont and Van Stappen, 2003; Lee, 2003). Typically they are fed after the rotifer feeding phase and up to the transition to an artificial diet (Sorgeloos et al., 2001; Lee, 2003). Complications with the hatching and growth characteristics of *Artemia* can also be an impediment to the successful culture of larvae. Decapsulation, hatching, and molting all produce membranes and molts which if not dealt with properly, can quickly deteriorate water quality of the system (Van Stappen et al., 1996; Dhont and Van Stappen, 2003). With advancements in artificial larval diets and the reduced availability of *Artemia* cysts from wild populations (Van Stappen et al., 1996; Dhont and Van Stappen, 2003), the use of *Artemia* may decrease in the future.

Rotifers and *Artemia* have advantages as live feeds for marine fish larvae. Both are relatively easy and predictable to culture in appropriate numbers and feeding protocols are well documented for many currently cultured marine fish species (Lavens and Sargeloos, 1999; Stottrup and McEvoy, 2003). Enrichment techniques are constantly being improved to provide not only essential nutrients, but also antibiotics and other forms of prophylactic treatments for fish larvae (Lavens and Sargeloos, 1999; Stottrup and McEvoy, 2003). However, their use does have limitations and they are not effective for the majority of marine fish species. The bottleneck to the production of most marine fish species is the early larval stage and the

inadequacy of rotifers as a first food for larvae, therefore culture of these species will likely require the feeding of copepods.

Copepods as Live Feeds

Copepods (phylum Arthropoda) are one of the most ubiquitous groups of marine organisms, with over 21,000 species currently described, and are a major component of the marine zooplankton community (Smithsonian Institution, 2008). It is well documented that in the wild, copepods constitute a major link in the nutrient pathway from primary producers to fish larvae serving as a supplemental or primary diet for first feeding marine fish larvae (Hunter, 1981; Delbare et al., 1996; Stottrup, 2003). Their role in the marine trophic system is essential to the survival of many marine fish species. To date, a limited number of researchers have investigated the efficacy of using copepods to culture various fish species. Ten orders of the Copepoda are currently recognized, but only four have been explored as live feeds for fish and crustacean larvae. These include Poecilostomatoida, Cyclopoida, Harpacticoida, and Calanoida (Lee et al., 2005).

Poecilostomatoida

The Poecilostomatoida have an unclear symbiotic relationship with marine bivalves and only one member, *Pseudomyicola spinosus*, has been evaluated for use as a live feed (Ho, 2005). Populations of *P. spinosus* were maintained on strips of mussel, *Mytillus* sp., meat and eggs sacs were collected daily (Ho, 2005). Once hatched, nauplii were cultured to adulthood (Ho, 2005). Theoretically, copepod populations could be maintained on mussel, *Mytilus* sp., beds and pelagic nauplii harvested daily and fed to marine fish larvae. However, evaluations of the symbiotic relationship and the potential effects of copepod production on mussel beds should be understood before evaluating this copepod species as a marine fish larval food. Members from the other three orders have been explored much more extensively as a live feed.

Cyclopoida

A few Cyclopoida have been grown for use as live feed, mainly belonging to the genera *Apocyclops* and *Oithona* (Stottrup, 2006). Farhadian et al. (2009) increased survival of black tiger prawn, *Penaeus monodon*, post larvae by feeding a mixed diet of *Apocyclops dengizicus* and *Artemia*. *Apocyclops panamensis* was fed to red snapper, *Lutjanus campechanus*, larvae and *Apocyclops royi* was fed to grouper, *Epinephelus coioides*, larvae all with inconclusive results (Stottrup, 2006). Survival of striped patio, *Eugerres brasilianus*, larvae was doubled through the use of *Oithona oculata* (Hernandez Molejon and Alvarez-Lajonchere, 2003). *Oithona* spp. has also been harvested in wild zooplankton communities and fed to grouper, *E. coioides*, larvae and produced increased survival and larvae displayed preferential feeding on copepod nauplii (Toledo et al., 1999). Members of this order show potential for mass-scale culture as they can be cultivated in high densities, have appropriate nutritional composition, accept a variety of diets, and reach maturation quickly (Shansudin et al., 1997; Stottrup, 2006).

Harpacticoida

Copepods of the order Harpacticoida are primarily benthic or epibenthic (Stottrup, 2003). *Tisbe*, *Tigriopus*, and *Euterpina* are the three most commonly produced genera for feeding fish or crustacean larvae (Stottrup, 2003). *Tisbe biminiensis* was fed to larvae and post larvae of the white shrimp, *Litopenaeus vannamei*, and improved feed consumption during the mysis 2 and 3 stages (de Lima and Souza-Santos, 2007). *Tisbe holothuriae* nauplii fed to first feeding turbot, *Psetta maxima*, larvae improved growth and survival as both a supplemental and primary diet (Stottrup and Norsker, 1997). Stottrup and Norsker (1997) also noted an increased rotifer consumption rate for those larvae fed a mixed diet as opposed to larvae fed solely rotifers. *Tisbe* spp. were also successfully used as a supplemental diet for yellowtail clownfish, *Amphiprion clarkii*, larvae increasing growth, survival, and the level of insulin-like growth factors I and II,

which promote cartilage and muscle growth (Olivotto et al., 2008). *Tigriopus japonicus* has been fed to numerous fish species and significantly improved growth and survival in black sea bream, *Mylio macrocephalus* (Stottrup, 2003). Mahi mahi, *Coryphaena hippurus*, larvae fed *Euterpina acutifrons* showed improved growth, survival, and resistance to stress and these results were associated with increased levels of DHA in the copepods (Kraul et al., 1993; Stottrup, 2003).

Some harpacticoid copepods have potential for mass-scale production because they can be grown in dense cultures (100/mL), withstand a wide range of environmental parameters, accept a variety of diets, reach maturity quickly, and have available life stages for feeding marine fish larvae (Fleeger, 2005; Stottrup, 2003). In addition to the genera *Tisbe*, *Tigriopus*, and *Euterpina*, *Amphiascoides atopus* has recently been mass produced with daily yields of 2–8 million copepod nauplii for 4 months of continuous culture (Fleeger, 2005; Sun and Fleeger, 1995). Sun and Fleeger (1995) documented that two species of crustacean, the white shrimp, *L. vannamei*, and the grass shrimp, *Palaemonetes pugio*, and the darter goby, *Gobionellus boleosoma*, can be successfully cultured on *A. atopus*. *Nitokra lacustris* is another harpacticoid copepod that appears suitable for mass-scale production (Rhodes, 2003). Although harpacticoid copepods can be grown in high densities, their nauplii are often benthically oriented and display negative phototactic behavior; this limits their presence in the water column as available prey for pelagic fish larvae (Payne and Rippingale, 2001a). For these reasons, development of culture systems for copepods produced to feed marine fish larvae have primarily consisted of calanoid copepods (Payne and Rippingale, 2001a).

Calanoida

Copepods from the order Calanoida are predominantly pelagic filter feeders and are concentrated in the water column (Stottrup, 2003). Numerous genera of calanoid copepods have

been investigated for mass-scale culture as a potential food organism for marine fish larvae, these genera include *Acartia*, *Gladioferens*, *Pseudodiaptomus*, *Eurytemora*, *Temora*, *Centropages*, *Bestiolina*, *Parvocalanus*, *Labidocera*, and *Calanus* (Evjemo et al., 2003; McKinnon et al., 2003; Lee et al., 2005).

One of the most researched species of copepod in terms of culture techniques is *Acartia tonsa* (Lee et al., 2005; Marcus and Wilcox, 2007). Parameters such as temperature, salinity, diet, and egg production have been investigated (Marcus and Wilcox, 2007; Peck and Holste, 2006; Stottrup and Jensen, 1990). *A. tonsa* broadcasts eggs into the water column, which can be collected and stored either in freshwater for up to 35 days, or cold (2–3°C) for up to a year (Drillet et al., 2006; Hojgaard et al., 2008). Ogle et al. (2005) developed a mass-scale production system for *A. tonsa* that has been used to feed red snapper, *L. campechanus*, larvae since 1998, significantly increasing survival of larvae at day 7 post hatch from approximately 3.0% to 16.5%. Southern flounder, *Paralichthys lethostigma*, larvae fed a mixed diet of *A. tonsa* nauplii and *B. plicatilis* had increased length and height and more than double the survival and weight gain then those larvae fed solely *B. plicatilis* or *B. rotundiformis* (Wilcox et al., 2006). *A. tonsa* has also been used as a live feed with spotted seatrout, *Cynoscion nebulosus* (Lemus et al., 2008), cod, *Gadus morhua*, ladyfish, *Elops saurus*, and *Fundulus* spp. (Stottrup, 2003).

Other *Acartia* spp. have also been cultured to serve as a live feed for larval fish. *Acartia clausi* fed to fourteen day-old seabass, *Lates calcarifer*, larvae significantly improved growth and survival (Rajkumar and Kumaraguru vasagam, 2006). Grouper, *E. coioides*, larvae fed zooplankton collected from the wild, which consisted primarily of *Acartia tsuensis* and *Pseudodiaptomus* spp., had improved growth and survival (Toledo et al., 1999) and grouper larvae showed preference for *Acartia tsuensis* nauplii over rotifers (Doi et al., 1997). *Acartia*

sinjiensis were fed to red snapper, *Lutjanus argentimaculatus*, larvae with mixed results (Stottrup, 2003). Research with *A. sinjiensis* investigated temperature, salinity, and photoperiod effects on egg production, hatching, and early life stage development to elucidate mass-scale culture techniques (Camus and Zeng, 2008; Milione and Zeng, 2008).

The culture techniques and efficacy of *Gladioferens imparipes* as a live feed are also well documented (Payne and Rippingale, 2001a). In 500-L batch culture systems, 439,000 nauplii were produced per day (0.88 nauplii/mL) and 1000-L semi-continuous populations yielded up to 520,000 nauplii per day for 184 days (0.5 nauplii/mL) (Payne and Rippingale, 2001a). The effects of diet, salinity, cold storage, and enrichment techniques of *G. imparipes* have all been explored (Payne and Rippingale, 2000a; Payne and Rippingale, 2001b). Nauplii can be stored at 8°C for 12 days with 99% survival, allowing sufficient numbers to be collected prior to feeding to fish larvae (Payne and Rippingale, 2001b). Nauplii were enriched for 6 hours in a mixture of Tahitian strain *Isochrysis galbana* (T-ISO) and *Nannochloropsis occulata* to increase their HUFA content (Payne and Rippingale, 2001b; Payne et al., 2001). *G. imparipes* nauplii were fed to juvenile pipefish, *Stigmatopora argus*, with improved growth and 99% survival (Payne et al., 1998). West Australian seahorse, *Hippocampus subelongatus*, juveniles were fed copepod nauplii and enriched *Artemia*, and results showed length, weight and survival were all significantly greater for larvae fed copepods (Payne and Rippingale, 2000b). When fed a mixed diet (1:1) of *G. imparipes* nauplii and rotifers, West Australian dhufish, *Glaucosoma hebraicum*, larvae displayed significantly greater growth and survival during a 25 day experiment when compared to larvae fed only rotifers (Payne et al, 2001). Pink snapper, *Pagrus auratus*, larvae were fed a mixed diet (4:1) of rotifers and copepod nauplii, a diet consisting of copepod nauplii for the first six days then transitioned to a diet of rotifers, and a diet of only rotifers. Results

showed both treatments fed copepods yielded significantly greater growth and survival when compared to larvae fed only rotifers (Payne et al., 2001).

Pseudodiaptomus spp. also demonstrate characteristics that make them suitable for mass-scale culture and for use as a live feed (Chen et al., 2006; Ogle et al., 2005; Puello-Cruz et al., 2009; Rhyne et al., 2009; Toledo et al., 1999). *Pseudodiaptomus annandalei* exhibits a wide salinity tolerance, and the highest reproduction occurs at salinities from 5–25 g/L (Chen et al., 2006). Sheng et al. (2006) evaluated the feeding behavior of larval three-spot seahorse, *Hippocampus trimaculatus*, and found larvae preferred *P. annandalei* nauplii over rotifers during the first 3 days post hatch. Larval seahorse selectively grazed on copepodites from 4 to 10 days post hatch, then began to eat adult copepods (Sheng et al., 2006). *P. annandalei*, in a mixture of wild caught zooplankton, was also fed to grouper, *E. coioides*, larvae and improved growth and survival and larvae displayed feeding preference for copepod nauplii even at low prey densities (Doi et al., 1997). Puello-Cruz et al. (2009) compared different algal diets for *Pseudodiaptomus euryhalinus*, and the highest production occurred in those fed the diatom *Chaetoceros muelleri*.

Pseudodiaptomus pelagicus

First described by Clarence L. Herrick in 1884, specimens of *Pseudodiaptomus pelagicus* collected from the Mississippi Sound were used to classify the genus *Pseudodiaptomus* (Walter, 1989). Early descriptions of *P. coronatus* and *P. americanus* are now believed to be descriptions of *P. pelagicus* (Walter, 1989).

Taxonomic Information: *Pseudodiaptomus pelagicus* Herrick, 1884

Kingdom: Animalia

Phylum: Arthropoda

Subphylum: Crustacea

Class: Maxillopoda

Subclass:	Copepoda	
Infraclass:	Neocopepoda	
Superorder:	Gymnoplea	
Order:	Calanoida	
Family:	Pseudodiaptomidae	(ITIS, 2009)

P. pelagicus is an estuarine calanoid copepod native to the eastern Atlantic and Gulf coasts of the United States, from Massachusetts to Mexico (Walter, 1989). Females (1.30–1.57 mm) and males (0.92–1.13 mm) often spend their mature life stage copulating as paired units (Jacobs, 1961; Walter, 1989). *P. pelagicus* are maternal egg brooders producing asymmetrical ovisacs with the right ovisac containing 2–6 eggs and the left containing 9–25 eggs (Rhyne et al., 2009; Walter, 1989). Upon hatching in the second naupliar stage, nauplii molt through five more naupliar stages reaching the first copepodite stage by approximately 6 days at a temperature of 20.3°C (Jacobs, 1961; Grice, 1969). Growth continues through six copepodite stages and they reach adulthood by approximately 18 days at 20.3°C (Jacobs, 1961; Grice, 1969). Increases in temperature significantly increase mean development time with growth from nauplii to adult taking approximately 10 days at 28°C (Rhyne et al., 2009). Adults spend the majority of their time on substrate or the walls and bottom of culture tanks, while nauplii appear to lack the ability to cling to substratum and are found in the water column (Jacobs, 1961; Rhyne et al., 2009).

Populations of *P. pelagicus* maintained at the University of Florida's Indian River Research and Education Center (IRREC) were isolated from South Florida waters in 2003 and have been kept in continuous culture for 6 years (Rhyne et al., 2009). An optimal temperature range of 28–30°C was determined; this range reduces mean development time, increases survival, and promotes a shortened brood interval (Rhyne et al., 2009). Sex ratio and brood size

were not affected by temperature (Rhyne et al., 2009). *P. pelagicus* can survive in a wide range of environmental parameters, tolerate heavy aeration, sedimentation and suspended solids, can be cultured on a variety of microalgae diets, and can reach densities of 5/mL; these make *P. pelagicus* an ideal candidate for mass production (Rhyne et al., 2009).

Stress Resistance

A key component to expanding the number of marine fish cultured through the larval stage is an increased resistance to stress. Diets with the appropriate levels of HUFAs, specifically DHA, EPA, and AA have been shown to increase stress resistance in marine fish larvae (Kanazawa, 1997; Bell and Sargent, 2003). Copepods constitute the appropriate composition of HUFAs for marine fish larvae (Sargent et al., 1997), and can be manipulated by feeding different microalgaes (van der Meeren et al., 2008). Therefore, the use of copepods as a live feed during the larval stage of production may increase stress resistance of marine fish larvae.

Stress resistance in marine fish larvae has been evaluated via prolonged exposure to air (Kraul et al., 1993; Ako et al., 1994), responses to bacterial inoculation (Chair et al., 1994; Yang et al., 2008), handling and transfer (Koven et al., 2001), salinity stress (Brinkmeyer and Holt, 1998; Nhu et al., 2009), temperature stress, exposure to low levels of dissolved oxygen (Kanazawa, 1997), and low pH (Wasielesky et al., 1997). A larvae's ability to withstand these stressors is directly related to nutrition.

HUFA's are extremely important in improving the stress resistance of marine fish larvae (Coutteau et al., 1997; Bell and Sargent, 2003; Tocher et al., 2008). Kanazawa (1997) fed larval red sea bream, *Pagrus major*, and juvenile marbled sole, *Limanda yokohamae*, a diet containing DHA at 1–2% of the diet, and phospholipids (soybean lecithin) at 5% of the diet. A series of stress tests was instituted including exposure to air, increased water temperature, and low

dissolved oxygen levels. This diet increased growth and survival of the larvae, and larvae fed this diet had improved resistance to stress in all tests (Kanazawa, 1997).

Ako et al. (1994) displayed an increased resistance to air exposure for striped mullet, *Mugil cephalus*, larvae that had been fed enriched *Artemia* nauplii, although growth and survival of the larvae were not significantly different between dietary treatments. Enriched *Artemia* nauplii had higher levels of n-3 HUFAs including DHA (Ako et al., 1994). Red drum, *Sciaenops ocellatus*, larvae fed a diet with a DHA:EPA ratio of 3.78 outperformed diets with lower DHA:EPA ratios in a hypersaline (70g/L) stress challenge (Brinkmeyer and Holt, 1998). A diet fortified with 3% EPA and DHA improved stress tolerance to air exposure in black sea bream, *Acanthopagrus schlegeli*, larvae compared to a commercial diet (Om et al., 2001). Increased tolerance of cobia, *Rachycentron canadum*, larvae to hypersaline stress tests was significantly correlated with elevated levels of DHA present in the live feed and fish larvae at various periods of the larval cycle (Nhu et al., 2009). AA in the diet of gilthead seabream, *Sparus aurata*, larvae improved resistance to handling stress and increased survival during the transition period onto a diet of *Artemia* (Koven et al., 2001).

Kraul et al. (1993) demonstrated the benefits of feeding the harpacticoid copepod *Euterpina acutifrons* to larval mahimahi, *Coryphaena hippurus*. Multiple tests were conducted comparing stress resistance of larvae fed diets consisting of copepod nauplii and *Artemia* enriched with different concentrations of SuperSelco, a commercial live feed enrichment (Kraul et al., 1993). To determine resistance to stress, larvae (15–18 days post hatch) were blotted dry and held out of water in netting for 60 or 120 seconds, after which they were returned to tank water and survival was ascertained after 25 minutes (Kraul et al., 1993). Larvae fed *Artemia* with increased levels of enrichment, and therefore higher levels of DHA, showed a greater

resistance to the stress tests. However, larvae fed copepod nauplii had significantly less mortality (3–4 times) than any of the *Artemia* fed treatments in all of the experiments, even though their DHA level was similar to that of the enriched *Artemia* (Kraul et al., 1993). It was postulated that DHA from the copepod nauplii, as a phospholipid, is more effectively incorporated by larval mahimahi than DHA from enriched *Artemia*, as a triglyceride. It was also suggested that the higher amino acid levels found in the copepod nauplii might have led to higher incorporation of DHA by the fish larvae.

Florida Pompano Larviculture

The Florida pompano, *Trachinotus carolinus*, is a highly prized commercial and recreational marine fish species with a distribution along the south Atlantic and Gulf coasts of the United States (Muller et al., 2002). Low harvests of wild populations coupled with rising market demand have increased interest in developing culture techniques for this species (Muller et al., 2002; Weirich et al., 2006). Like most marine fish species, 5–10% survival through the larval phase impeded the development of production techniques. However, recent advances in Florida pompano larviculture and rotifer enrichment techniques have increased survival (~35%) of larvae during the first feeding phase (Cavalin and Weirich, 2009).

Since 2004, experiments evaluating the larval performance of Florida pompano have been conducted by the United States Department of Agriculture, Agricultural Research Service, Center for Reproduction and Larviculture, located on the campus of Harbor Branch Oceanographic Institute at Florida Atlantic University in Fort Pierce, FL (USDA-ARS). Researchers have defined a protocol for first feeding Florida pompano larvae that includes rotifers and *Artemia* (Weirich and Riley, 2005). Enriched rotifers are fed to larvae beginning on 2 days post hatch (DPH) and transitioned onto *Artemia* instar I nauplii on 10–12 DPH. Larvae are then transitioned onto *Artemia* metanauplii on 15–17 DPH and finally transitioned onto a

formulated diet by 22–25 DPH. Greenwater culture conditions are maintained only while feeding rotifers. In Cavalin and Weirich (2009), various commercial enrichments and microalgae diets were evaluated to determine the most efficient and effective enrichment method for rotifers fed to Florida pompano larvae. Results showed larvae fed rotifers enriched with commercial enrichments had higher survival and growth than those fed rotifers enriched with microalgae diets. Furthermore, larvae fed rotifers enriched with the commercial enrichment Ori-Green (Skretting, Italy) had higher survival and only slightly less growth when compared to other commercial enrichments. Since Ori-Green requires much less time for enrichment, it was determined to be the most cost effective and adopted as a standard rotifer enrichment protocol.

Objectives

The purpose of this thesis is to develop suitable culture techniques for *P. pelagicus* and evaluate its efficacy as a live feed for marine fish larvae. An examination of batch cultures, mass-scale culture, nauplii collection and microalgae culture was conducted and is thoroughly discussed. Furthermore, five experiments were conducted to evaluate various feeding regimes with *P. pelagicus* as a primary and supplemental diet for first feeding Florida pompano larvae, and were compared to the standard reference diet of rotifers developed by the USDA-ARS. Differences in survival, growth (notochord length and body depth), and stress resistance (resistance to air exposure and salinity stress) were measured to determine the effect of feeding copepod nauplii to Florida pompano larvae.

CHAPTER 2
MICROALGAE AND COPEPOD CULTURE

Introduction

The efficient transfer of nutrients to higher trophic levels is extremely important to successful larval culture of marine fish species. Nutritional components including essential amino acids and fatty acids are critical to the development and survival of marine fish larvae. Research must be conducted in order to understand the specific nutritional requirements of each fish species produced. Furthermore, development of a live feed program, which includes suitable prey species at each trophic level, is important to the successful rearing of most species of marine fish.

The presence of microalgae in larval rearing systems has improved the performance of over 40 species of marine fish larvae (Muller-Feuga et al., 2003), although the mechanisms behind these improvements are not yet fully understood. Hypotheses include direct and indirect supply of nutrition, water quality improvement, light contrast, stimulation of feeding behavior and physiological processes, regulation of opportunistic bacterial populations, and improvements in the quality of live prey including copepod nauplii and rotifers (Muller-Feuga et al., 2003). Combinations of these benefits likely contribute to the successful culture of marine fish larvae.

Water Treatment

Microalgae and copepods were cultured with a 2:1 mixture of natural seawater (32–35 g/L salinity) and well water (0–4 g/L salinity) at the University of Florida's Indian River Research and Education Center (IRREC). Prior to use, all water was chlorinated (150 mg/L) with sodium hypochlorite for 24 hours, then aerated for 24–48 hours until 0 mg/L chlorine was detected. Water was then pumped into 20,000-L storage silos. From the storage silos, water was pumped into the hatchery facility after passing through both 50 and 5 micron mesh bag filters. Seawater

was stored in a 2500-L polyethylene tank and freshwater was stored in a 1350-L polyethylene tank. Sodium hypochlorite was added to both seawater and freshwater (4 mg/L chlorine) when stored in tanks for longer than one week. Seawater and freshwater were mixed in a 2500-L polyethylene tank (mixed tank) to a salinity of 22–25 g/L. The mixed tank water was then continuously circulated through an 80 Watt UV sterilizer (Emperor Aquatics Inc., PA) at rate of 170–220 L/minute which provided a sterilization intensity of 30,000–90,000 microWatts/cm^2. Prior to use, any chlorine detected in the water was neutralized with sodium thiosulfate. As water left the mixed tank, it passed through both 1.0 and 0.5 micron cartridge filters.

Microalgae Culture Techniques

Tahitian strain *Isochrysis galbana* (T-ISO) and *Thalassiosira weissflogii* (TW) were fed to copepods during these experiments. All microalgae culture techniques used were adapted from Anderson (2005). Fritz f/2 nutrient media solutions A and B (Fritz Industries Inc., TX) were provided to both T-ISO and TW microalgae cultures following manufacturer instructions, except twice the suggested amount was supplied to TW to elicit sufficient growth. Sodium metasilicate (Fritz Industries Inc., TX) was also provided to facilitate the formation of the cell wall in TW, a diatom (Andersen, 2005). All aeration provided to microalgae was continuously mixed with CO_2 at a rate of approximately 70–120 mL/minute and passed through a 0.20 micron in-line air filter. All water used to grow microalgae was autoclaved (Consolidated Stills and Sterilizers, MA) and maintained at 21°C and a salinity of 22–25 g/L unless otherwise noted. Once water was autoclaved, sterile procedures were used to reduce the potential for contamination of the microalgae (Andersen, 2005).

Small (1-L) flasks were used to maintain stock cultures of each microalgae species. Stock cultures were a sterile culture used to preserve the microalgae species within the facility and to inoculate larger cultures. Stoppers equipped with a 0.20 micron filter were inserted in the flasks

to allow exchange of air, but prevent entrance of contaminants. Cultures were exposed to light levels from 1000 to 2000 lux and no aeration was provided.

Small glass bottles (4-L) were used to grow microalgae for small scale experiments and to inoculate larger cultures. A two-holed rubber stopper was inserted into the bottle; each hole had a 6-mm diameter glass tube inserted. One tube extended to the bottom of the bottle and was used for aeration. The second was short and extended from the bottom to the top of the stopper and allowed air to escape. Algae were exposed to light levels from 1000 to 2000 lux. Maximum densities for T-ISO (15–20 million cells/mL) and TW (1–2 million cells/mL) were achieved before being used to inoculate larger cultures.

Glass carboys (20-L) were used to grow microalgae for experiments, maintenance of copepod and rotifer populations, and to inoculate larger cultures. A two-holed rubber stopper with glass tubes, in a similar design to those used for the 4-L cultures, provided aeration to the cultures. Cultures were exposed to light levels from 3500 to 4500 lux. Maximum densities for T-ISO (15–20 million cells/mL) and TW (1–2 million cells/mL) were achieved before being used for experiments, feeding, or to inoculate larger cultures.

Bags (450-L) were used to grow large quantities of T-ISO for experiments and maintenance of copepod and rotifer cultures. The details of the system and bag design cannot be discussed due to a contractual agreement with an industry partner. T-ISO bags were exposed to gradually increasing light levels from 300 to 18000 lux and attained densities up to 30 million cells/mL. TW was not grown in volumes larger than 20-L.

Pseudodiaptomus pelagicus Culture Techniques

Since 2005, a population of *Pseudodiaptomus pelagicus* has been maintained at IRREC in Fort Pierce, FL. This population was isolated in 2003 from the Indian River Lagoon and is maintained at Algagen LLC (Vero Beach, FL).

Water Quality

Water for copepod cultures came from the mixed water tank, and prior to use was heavily aerated for 24–48 hours. This was done to avoid a possible adverse response by the copepods to any chemical present, including plasticizers, originating from the polyethylene storage tanks. One chemical detected in water samples taken from the water treatment system was diphenyl sulfone. Diphenyl sulfone is an acaricid, a mite pesticide, also used in the production of some polyethylene tanks. Although an evaluation of copepods exposed to this chemical and others present in the water treatment system was not conducted, deleterious effects on the performance of copepod cultures have been noted when using water directly from some polyethylene tanks.

Rhyne et al. (2009) evaluated effects of temperature on various culture parameters of *P. pelagicus* and defined an optimal range for survival and fecundity to be between 28–30°C. In studies evaluating copepod performance at various salinities, an optimal range between 22–25 g/L was observed, although *P. pelagicus* can tolerate 10–42 g/L salinity (Ohs et al., submitted). The results of these experiments were used to maintain copepod cultures. No studies have been conducted on the performance of *P. pelagicus* cultures in response to other water quality parameters. Dissolved oxygen was maintained between 5–6 mg/L, although occasionally levels of 3 mg/L were detected with no deleterious effects observed. The pH was maintained between 7.6 and 8.2. Total ammonia-nitrogen (TAN) was maintained below 0.7 mg/L and nitrite was maintained below 0.13 mg/L. *P. pelagicus* cultures were subjected to surface light levels of 90–240 lux and a photoperiod of 24 light and 0 dark.

Diet

P. pelagicus cultures were fed diets consisting of T-ISO, TW, or a 1:1 mixture of T-ISO and TW. Dietary effects on *P. pelagicus* fed the various microalgae diets has been evaluated (Ohs et al., unpublished data). Results showed adults fed monoalgal diets consisting of TW produced on average 3–4 times more nauplii than adults fed T-ISO, the algae which has been fed to *P. pelagicus* for over five years (Ohs pers. comm.). Therefore, a combination of T-ISO and TW was used to enhance nauplii production in trials 4 and 5. *P. pelagicus* cultures were fed T-ISO to a density of 200,000–300,000 cells/mL. The 1:1 mixture, consisting of equal volumes of T-ISO (10–12 million cells/mL) and TW (1–2 million cells/mL), was fed to a density of 75,000–125,000 cells/mL. This algal density was sufficient for *P. pelagicus* cultures with a density of 0.25–1.00 individuals/mL and was adjusted accordingly. All new culture water was inoculated with an appropriate amount of microalgae prior to the addition of copepods.

Equipment

Aeration was provided to each *P. pelagicus* population with an air pump and airline tubing (4-mm inner diameter) weighted down with a porcelain electrical fence insulator. In tanks larger than 55-L, a coarse silica air stone was used to maintain dissolved oxygen concentrations. Airstones that create fine bubbles were avoided to prevent air from getting trapped under the carapace of the copepods. Weighted sponge filters (Aquarium Technology Inc., GA) were also used in each copepod culture tank larger than 55-L to act as a mechanical and biological filter. By design, air gently bubbled and pulled water through the sponge which filtered suspended particulate matter from the water. Sponge filters have been observed to reduce the frequency and magnitude of bacterial blooms within copepod culture tanks. When cleaned, the sponge filters were taken out of the culture tanks, placed in seawater, and the collected particulate matter was squeezed from them. At that point they were either placed back in the culture tanks or soaked in

tap water and replaced with new ones. A 200-watt heater (Visi-therm, OH) was also placed in each culture tank to maintain temperature.

A variety of sieves were used with the *P. pelagicus* cultures. A sieve was constructed from a PVC or acrylic pipe with a diameter of 10 to 20 cm cut into 15 to 20 cm lengths. A ring of the PVC or acrylic pipe of similar diameter and 2 to 3 cm high was also used. The ring was cut to fit snuggly inside the 15 to 20 cm section. Nylon screen of the appropriate mesh size was then cut and fit tightly inside one end of the pipe with the ring holding it in place. The ring held the screen tightly in place so the mesh size was not compromised and the screen was taut. The screen was then hot glued around the edge to avoid loss of copepods. A variety of nylon mesh screen diameters were utilized. A 240 micron nylon mesh screen (240 sieve) was used to retain paired (copulating) adults and larger females. A 200 micron nylon mesh screen (200 sieve) was used to retain all adults. A 150 micron nylon mesh screen (150 sieve) was used to retain all adults and copepodites fed T-ISO. A 140 micron nylon mesh screen (140 sieve) was used to retain all adults and copepodites fed a mixture of T-ISO and TW. A 50 micron nylon mesh screen (50 sieve) was used to retain the entire population.

Floating airlifts were constructed to passively separate nauplii from the rest of the *P. pelagicus* population. Airlifts consisted of 19-L plastic buckets with two openings cut out which removed 2/3 of the area of the sides of the bucket, the openings were covered with 50 micron nylon mesh screen and were hot glued in place. The bucket had a 5-cm hole removed from the bottom center and a bulkhead was attached. A PVC pipe extending from the bottom of the bucket was secured by inserting it into the bulkhead. A window was cut out of the PVC pipe and 150 or 140 micron nylon mesh screen covered the window and was secured with hot glue. A 150 micron nylon mesh screen was used for copepod populations reared solely on T-ISO and a

140 micron nylon mesh screen was used for copepod populations reared on a mixture of T-ISO and TW. A styrofoam ring was cut to fit around the outer perimeter of the bucket, under the handles, which allowed the airlift to float freely in the copepod culture tanks. Inside the bucket a standpipe was inserted into the bulkhead and extended above the surface of the water by 2–3 cm when floating. A small ceramic airstone was placed inside the internal standpipe which created a current that pulled copepod nauplii (<140–150 microns) through the screened pipe and concentrated them in the bucket. The copepodite and adult populations did not pass through the screens and were not disturbed by this nauplii harvest method.

Contamination

Copepods from the culture tanks were regularly inspected, under a microscope, for health issues or contamination. Contamination within populations of *P. pelagicus* was either tolerated or immediately addressed, depending on the severity and the species. A stalked ciliate, *Vorticella* sp., has been observed in the copepod culture tanks. Attempts to grow *Vorticella*-free cultures of *P. pelagicus* were not successful. However, there has been no observed negative effect of the *Vorticella* sp. on the copepod population. Smaller ciliates are commonly observed in *P. pelagicus* populations but they are consumed by the copepods. Presence of some ciliates may improve the health of a copepod population by reducing the bacterial load. Rotifers, *Brachionus plicatilis*, contaminated the copepod population once, requiring three attempts to eradicate them by physical separation. The entire *P. pelagicus* population was passed through a 240 sieve in an attempt to isolate a clean population of copepods because *B. plicatilis* is smaller than 240 microns. Although this method has worked for other copepod culturists, our attempt was successful for about two weeks before the rotifers reappeared. Rotifer contamination has to be dealt with immediately as they will quickly reproduce and outnumber the copepods, greatly reducing the performance of the population. Furthermore, rotifer production of cysts and asexual

life stages increases the difficulty in removing them entirely and long term from the copepod population.

All equipment was routinely soaked in 20 mg/L chlorine for 24–48 hours. Sponge filters, sieves, filter bags, and airlifts were soaked in 20 mg/L chlorine for 1–2 hours and then rinsed with tap water and allowed to dry. Routine cleaning will help reduce the potential of contamination, even though in culture tanks larger than 19-L some contamination is likely.

Airlift Efficiency

Two sizes of airlifts were used at IRREC, a small (7.5-L collection bucket) and a large (19-L collection bucket) size. Both had the same design and differed only in the size of bucket used. A simple experiment was conducted to see which size airlift, small or large, harvested the greater percentage of copepod nauplii after one hour of airlift operation. For this comparison, 200-L culture tanks were used.

Three evaluations were conducted with the small airlift. For each evaluation, collected nauplii were maintained in approximately 10-L of culture water with mild aeration. The harvested nauplii were then homogenized with increased aeration. After homogenization, 5, 2-mL samples were taken from the 10-L of collected nauplii. These volumetric samples were used to estimate nauplii density and subsequent total number of nauplii harvested by the airlift. A 100 % water change was then performed on the culture tank. The copepod population was passed through a 150 sieve to collect the remaining nauplii that were not harvested by the airlift. The collected nauplii were then homogenized with aeration. After homogenization, 5, 2-mL samples were then taken from the collected nauplii and were used to estimate density and subsequent total number of nauplii not harvested by the airlift. The estimated number of nauplii harvested was then divided by the estimated number of nauplii in the tank and multiplied by 100 to attain the percentage of nauplii that were collected with the airlift.

When examining the large airlift, four evaluations were conducted. For each evaluation, collected nauplii were maintained in approximately 4-L of culture water with mild aeration. The harvested nauplii were then homogenized with increased aeration. After homogenization, 3, 2-mL samples were taken from the 10-L of collected nauplii. These volumetric samples were used to estimate nauplii density and subsequent total number of nauplii harvested by the airlift. A 100 % water change was then performed on the culture tank. The copepod population was then passed through a 150 sieve to collect the remaining nauplii that were not harvested by the airlift. The collected nauplii were then homogenized with aeration. After homogenization, 3, 2-mL samples were then taken from the remaining nauplii and used to estimate density and subsequent total number of nauplii not harvested by the airlift. The estimated number of nauplii harvested was then divided by the estimated number of nauplii in the tank and multiplied by 100 to attain the percentage of nauplii that were collected with the airlift.

There was a significant difference between the percentage of nauplii harvested with the two sizes of airlifts (T-TEST, $T_5 = 7.86$; $p = 0.0005$). The mean percentage of nauplii harvested by the large airlift ($90.3 \pm 4.7\%$) was significantly greater than the mean percentage of nauplii harvested by the small airlift ($60.6 \pm 2.5\%$). Although this represents a small number of evaluations with each airlift size, the data shows the large airlift was more efficient at nauplii collection and was the preferred method to harvest nauplii from a mixed age population.

Pseudodiaptomus pelagicus Cultures

Copepods were maintained in stock, 200-L, and mass-scale cultures. Each was designed for different types of production goals and research purposes. Based on need, all or only one scale of copepod culture may be maintained. Techniques involved with each culture type apply to the culture techniques previously mentioned unless otherwise noted.

Stock Cultures

Stock cultures of *P. pelagicus* were grown in small containers (1–5 L), in static conditions to maintain a long term population and to provide starter cultures for larger populations. Water was maintained at 22–24°C and a salinity of 20–25 ppt with light aeration. Low feeding rates, 50,000–100,000 cells/mL of T-ISO, were provided every other day, and 100% weekly water changes were used to reduce growth and fecundity but maintain population vigor. Temperature was manipulated by placing the container in a water bath or temperature-controlled room. The stock population was kept young by occasionally removing adults to reduce crowding.

To perform a water change, the air supply was shut off and the air line was removed from the container. Approximately 10–20 minutes after removing aeration, unwanted debris settled and the copepods came to the surface. The copepods were decanted through a 50 sieve and all life stages were collected and placed into a sterile container with clean culture water to visually inspect the population. While collecting copepods, the sieve nylon mesh screen was always submerged in seawater under similar environmental conditions to reduce stress. If the culture was too dense, adults could be separated from all younger stages by passing through a 240 sieve prior to collection on a 50 sieve. Also, some of the population could be decanted off prior to collection on a 50 sieve. The population collected on the 50 sieve was easily returned to new water by using a squirt bottle, containing culture water, to gently concentrate and transfer the population off of the screen. The copepods were then returned to the water bath or temperature controlled room and gentle aeration was provided with a new, clean air line. Separated populations may be used to establish other cultures or discarded.

200-L Cultures

The 200-L, intermediate-size copepod populations at IRREC were batch cultured at 28–30°C and a salinity of 22–25 g/L in 200-L cylindrical, flat-bottom polyethylene tanks. These

served as an individual population (roughly 100,000–600,000 individuals) or to inoculate a mass-scale population. Copepod nauplii were harvested from tanks and used to inoculate new tanks. This allowed for greater control of the growth rate, feed efficiency, and nauplii production of each tank's population. Furthermore, multiple tanks at various stages of development could be maintained. At 28°C, 100,000–150,000 nauplii fed T-ISO would reach adulthood in 9 to 10 days and produce 70,000–120,000 nauplii/day for approximately 10 days. Under the same conditions, nauplii fed a 1:1 mixture of T-ISO and TW would reach adulthood in 7–8 days and produce 150,000–370,000 nauplii/day for approximately 9 days. At 30°C versus 28°C, time to adulthood was accelerated by approximately 1 day, but no effect on nauplii production was observed. Adult *P. pelagicus* have a high escape response, remain attached to substratum, and do not homogenize in the culture tanks making estimations of adult density difficult.

The 200-L tanks (97-cm high x 55-cm diameter) had a 2.54-cm bulkhead inserted in the bottom approximately 8-cm from the edge. A 2.54-cm PVC ball valve was fitted to the bulkhead with a 2.54-cm PVC street 90 elbow fitted on the opposite end of the ball valve. The rate of water flow leaving the 200-L tank could be controlled by the valve. Also, an internal standpipe was inserted in the tank to prevent water from entering the drain pipe and valve and creating an anaerobic area. A collection sump, consisting of a 19-L plastic bucket with a bulkhead inserted 15-cm from the top, was used during a water change. An outlet pipe, attached to the 2.54-cm PVC street 90 elbow, led into the collection sump. The end of the outlet pipe was positioned below the bulkhead of the collection sump so it would be submerged when the collection sump was full of water. A 40 micron mesh filter bag (80-cm long x 15-cm diameter) with a drawstring was tied to the end of the outlet pipe to collect the copepod population and transfer them to a new tank.

To perform a water change, the air supply to the sponge filter was shut off and it was removed from the tank. The air supply to the air stone was decreased but it was not removed. This reduced the amount of debris suspended in the water while maintaining dissolved oxygen concentrations. The outlet pipe was attached to the valve on the bottom of the tank which led into the collection sump. The 40 micron mesh bag was tied to the end of the outlet pipe in the collection sump. The collection sump was filled with the appropriate salinity seawater until the mesh bag was submerged, alleviating damage to the copepods collecting in the mesh bag. The valve was first opened completely and then turned back to ¼ open, eliminating any residual air in the outlet pipe. The flow of the water moved the copepods quickly but with limited injury from encountering the mesh bag. Once drained, the copepods were concentrated in the mesh bag by bobbing the bag several times in the water and rolling the top as it was brought out of the water. The copepods were concentrated at the bottom of the bag. The population was emptied from the mesh bag into a new tank and the sponge filter and air stone, heater, and feed rate were adjusted accordingly. If needed, the separation of life stages can occur during the water change procedure, similar to the methods described in the stock culture section. If desired, the population would be emptied from the mesh bag into a 19-L plastic bucket containing clean culture water and then sieved for isolation of the target life stage.

Mass-Scale Cultures

Mass-scale populations of *P. pelagicus* were attained in an 1800-L cylindrical, conical-bottom, polyethylene tank. The tank was provided mild aeration with two coarse airstones and three sponge filters were suspended in the tank. This population was grown to produce >1 million copepod nauplii per day to feed to marine fish larvae with reduced labor and use of water. At 30°C, approximately 750,000 copepods of a mixed age population were fed a 1:1 mixture of T-ISO and TW to a density of 100,000–200,000 cells/mL, and this population

produced approximately 1 million nauplii/day over an eight day period, gradually decreasing from 1.75 to 0.80 million nauplii/day during the eight days.

The mass scale system consisted of an 1800-L tank and a 1600-L sump. The 1800-L tank (118-cm high x 173-cm diameter) had a 3.81-cm bulkhead inserted into the center of the bottom of the tank. A 3.81-cm PVC pipe was connected from the bottom of the tank to a 2.54-cm ball valve positioned over a 1600-L sump (90-cm x 152-cm diameter). A 2.54-cm ball valve was also present at the lowest point in the PVC pipe, for purging the pipe. The rate of water flow leaving the 1800-L tank could be controlled by the valve over the sump. Also, an internal standpipe was inserted into the bulkhead and prevented water from entering the pipe and creating an anaerobic environment. During a water change, a collection device was used inside the sump to harvest copepod nauplii for a larval rearing experiment. The collection device consisted of a 75-L polyethylene container placed inside another 110-L polyethylene container. The 75-L container had 4 sides cut out and 50 micron nylon mesh screen was hot glued to cover and seal the openings. The 75-L container fit loosely inside the 110-L container which had a hole drilled in the side, 20-cm from the top. The hole allowed water to drain from the 110-L container into the sump and prevented the 75-L container inside it from over flowing, therefore concentrating the collected copepod nauplii. An outlet pipe was attached from the 2.54-cm ball valve over the sump leading into the collection device with the end of the outlet pipe submersed during harvest. An internal standpipe (60-cm high x 15-cm diameter) with windows cut out had 140 micron nylon mesh screen hot glued to cover the openings (140 standpipe) and was designed to allow harvest of the copepod nauplii while retaining the adult and copepodite populations within the 1800-L culture tank. Harvesting nauplii during a water change required the use of a small light suspended approximately 70-cm above the water surface illuminating the culture around the

internal standpipe. *P. pelagicus* nauplii are positively phototactic and gathered near the light during harvest.

To perform a water change, the internal standpipe was replaced with the 140 standpipe. The purge valve was opened and 15-L was removed and allowed to settle. The air flow to both the sponge filters and air stones were turned off and the light was suspended over the top of the 140 standpipe. After ten minutes, the valve over the sump was completely opened and then turned back to ¼ open, eliminating any residual air in the outlet pipe. The flow of the water moved the copepods quickly but limited injury from encountering the collection device was observed. As the collection device harvested nauplii, culture water would overflow into the sump. By design, the 1800-L tank would not completely drain with the 140 standpipe in place; the copepod population was retained in approximately 200-L of culture water. Once drained, the nauplii were transferred to 19-L plastic buckets and maintained under mild aeration for feeding to fish larvae. During the marine fish larval feeding experiments, the 1800-L tank was filled with new 22 to 25 g/L salinity water and the sponge filter and air stone, heaters, and feed rate were adjusted accordingly.

Conclusion

Observations

During 17 months of maintaining populations of *P. pelagicus* at IRREC, many observations were made about copepod performance. Once, when the copepod population was contaminated with rotifers, we sieved the population through a 240 sieve. This, undoubtedly, was a stressful event for the copepod population. However, within 30 hours the remaining adults had produced enough copepod nauplii to conduct a marine fish larval feeding experiment. This same effect was observed in Rhyne et al. (2009) when nauplii production was greatest on day 1 of a 10 day experiment examining effects of temperature on 10 pairs of *P. pelagicus* adults.

Again stocking the experiment was more than likely a stressful event for the copepods. Increased nauplii production was repeatedly observed following exposure to seemingly stressful events. However, multiple continued stressful events do not have this effect and will reduce the health of the copepod population. A change in density must also be considered when examining this response.

A 200-L tank normally produced approximately 70,000–400,000 nauplii/day depending on algal diet and adult density. Two to three times a year each tank produced approximately 1–4 million nauplii/day over a 2–3 day period. One hypothesis is a reaction to astronomical influences such as the moon or seasonal cycles. However, as exact dates of these events were not recorded it was impossible to compare production to these cycles. Also, numerous factors affecting copepod performance were not controlled during this time, so these are only observations.

Techniques to increase nauplii production of *P. pelagicus* were practiced in order to efficiently conduct marine fish larval rearing trials. In Rhyne et al. (2009), an optimal temperature of 28–30°C was suggested, however, advantages were seen in nauplii production of copepods kept at <26°C and increased to 30°C over a 24 hour period. By doing so, the metabolic demand of the population was believed to be reduced prior to the increase in temperature which triggered increased nauplii production. It was also observed that low adult densities (<5 individual/mL) produced more copepod nauplii than cultures with high adult densities (>5 individuals/mL). Increased nauplii production was also observed when the adult copepod density was maintained >5 individuals/mL and then reduced to < 5 individuals/mL over a 24 hour period. Counterintuitively, given that *P. pelagicus* are benthic oriented, the addition of substrate in the form of plastic mesh within the 200-L tanks had no observed effect on copepod

performance or nauplii production. However, these techniques and findings were based on anecdotal information and controlled experimental studies need to be conducted to confirm these observations.

Future Studies

Little information is available pertaining to the maintenance of *P. pelagicus* populations. First an examination into the tolerances and optimal ranges of various water quality and environmental parameters should be conducted. Dissolved oxygen, pH, TAN, nitrite, light intensity, and photoperiod all should be evaluated to determine optimal culture conditions for *P. pelagicus*. Although diet studies with live microalgae have been conducted at IRREC, studies examining commercial enrichments and microalgae pastes should be conducted. As contamination is a continual threat especially in larger populations (>55 L), evaluation of copepod performance subjected to various chemical treatments will help elucidate efficient procedures for the eradication of those contaminants. Furthermore, an evaluation of chemicals dissolved in the water originating from polyethylene tanks and the effects these chemicals have on *P. pelagicus* populations should be conducted.

Rapid increases in temperature, decreases in salinity, decreases in density, and increases in feed density seem to increase levels of nauplii production; these should be evaluated in replicated studies. Observed increases in nauplii production as a response to stress also should be evaluated. Once evaluated, the synergistic effects of these parameters and changes can be used to induce increased nauplii production.

CHAPTER 3
TRIAL 1

The goal of this study was to examine the benefits of feeding nauplii of the calanoid copepod *Pseudodiaptomus pelagicus* to larval Florida pompano, *Trachinotus carolinus*. Three treatment diets were evaluated to ascertain advantages in development and performance compared to larvae fed the standard reference diet (SRD). Two copepod diets, fed during the first three days of feeding, and a mesocosm feeding regime were provided to Florida pompano larvae spawned from F1 broodstock. Net stress and salinity stress experiments were also performed to observe advantages to Florida pompano larvae fed copepods.

Material and Methods

Water Treatment

At the University of Florida's Indian River Research and Education Center (IRREC), larval fish were cultured in natural seawater (32–35 g/L salinity). Prior to use, seawater was chlorinated (150 mg/L) with sodium hypochlorite for 24 hours, then aerated for 24–48 hours until 0 mg/L chlorine was detected. Seawater was then pumped into 20,000-L storage silos. From the storage silos seawater was pumped into the hatchery facility and passed through both 50 and 5 micron mesh bag filters. Seawater was stored in a 3500-L polyethylene tank and continuously circulated through an 80 Watt UV sterilizer (Emperor Aquatics Inc., PA) at rate of 170–220 L/minute which provided a sterilization intensity of 30,000–90,000 microWatts/cm^2. Prior to use, if chlorine was detected in the water it was neutralized with sodium thiosulfate. As water left the tank, it would pass through both 1.0 and 0.5 micron cartridge filters.

Spawning and Egg Incubation

Florida pompano eggs were acquired from the United States Department of Agriculture, Agricultural Research Service, Center for Reproduction and Larviculture, located on the campus

of Harbor Branch Oceanographic Institute at Florida Atlantic University in Fort Pierce, FL (USDA-ARS). Volitional spawning of F1 broodstock using a gonadotropin-releasing hormone analogue (GnRHa) was achieved following procedures described by Weirich and Riley (2007). Eggs were transported to IRREC and incubated under static conditions in two 200-L circular, conical-bottom fiberglass tanks (trials 1, 2, and 5) or one 55-L circular, conical-bottom polyethylene tank (trials 3 and 4). Incubation occurred at a temperature of 26°C and a salinity of 35.1 g/L with light aeration. Hatching occurred 30–37 hours post-fertilization. Larvae were volumetrically quantified following homogenization with increased aeration and were stocked into the experimental system within 8 hours post-hatch.

Larval Rearing and Experimental Design

A 7-day larval rearing trial was conducted in a flow through system consisting of 28, 13-L cylindrical, flat-bottom fiberglass tanks with black walls and a white bottom. An artificial photoperiod of 14 hours light and 10 hours dark was maintained during the experiment. Newly hatched larvae, 0 days post hatch (DPH), were volumetrically stocked into each tank at a density of 50 individuals/L. The initial water flow was 27 mL/minute, gentle aeration was provided, and daytime surface light levels were maintained below 150 lux (Milwaukee Model SM700, NC) for the first two days. Beginning on 2 DPH, tanks were inoculated daily with T-ISO to a density of 100,000–180,000 cells/mL. At this time water flow increased to 37 mL/minute, daytime surface light levels ranged from 1000–1550 lux, and aeration was increased slightly. At 4 DPH, aeration was again increased slightly and water flow was adjusted to 55 mL/minute for the duration of the trial. In each replicate tank temperature and salinity (YSI Incorporated Model 30-10-FT, OH), dissolved oxygen (YSI Incorporated Model 550 A, OH), and pH (Hach Model sensION1, CO) were monitored daily. Total ammonia-nitrogen (TAN) and nitrite-nitrogen (NO_2-N) were also measured daily with a Hach DR/4000U Spectrophotometer.

Dietary Treatments

Four dietary treatments were examined in this study, a standard reference diet (SRD) consisting solely of enriched rotifers, a 'one day' diet, a 'three day' diet, and a mesocosm treatment (Table 3-1). Each diet was replicated six times and four tanks were used to monitor unfed larvae. The 'one day' experimental diet consisted of feeding larvae copepod nauplii on 2 DPH at a rate of 2.14 nauplii/mL/day and then switching to the SRD from 3–6 DPH. The 'three day' experimental diet consisted of feeding larvae copepod nauplii on 2 DPH at a rate of 2.14 nauplii/mL/day, on 3 DPH at a rate of 2.50 nauplii/mL/day, and on 4 DPH at a rate of 3.00 nauplii/mL/day and then switching to the SRD from 5–6 DPH. The mesocosm experimental diet consisted of a mixture of *P. pelagicus* adults (>240 μm) stocked into each tank on 0 DPH at a density of 1.34 individuals/mL. Stocked adult copepods produced nauplii during the experimental trial and replicate populations were only fed algae. The mesocosm treatment populations were fed T-ISO at a density of 100,000–150,000 cells/mL for the first two days until the entire system was inoculated with T-ISO. For all treatments, when copepod nauplii were fed, an internal standpipe with 50 micron nylon mesh screen was used; when rotifers were fed, an internal standpipe with 240 micron nylon mesh screen was used. From 2 DPH to 6 DPH larvae were fed diets consisting of enriched S-strain rotifers, *B. plicatilis*, with a body width of 117.5 ± 19.8 microns (Cavalin and Weirich, 2009) and *P. pelagicus* nauplii with a body width of 93.2 ± 13.7 microns. Enriched rotifers were fed to larvae four times daily (0900, 1300, 1700, and 2100) at a rate of 2.5 rotifers/mL/day (Cavalin and Weirch, 2009).

Rotifer Culture and Enrichment

Rotifers were cultured at 26°C and a salinity of 20 g/L in a 120-L cylindrical, conical-bottom, fiberglass tank. Rotifers were fed 7.5 L of T-ISO (10–15 million cells/mL) and Culture Selco (INVE Aquaculture Inc., UT), a supplemental yeast-based rotifer diet, twice daily during

the trial according to manufacturer instructions. Approximately 5–10 million rotifers were harvested daily for enrichment prior to feeding to fish larvae. Rotifers were enriched with Ori-Green (Skretting, Italy) in 10-L buckets following manufacturer instructions for 3 hours at 27–28°C. After the enrichment period, the rotifers were rinsed with 20 g/L seawater, placed in new 20 g/L salinity water (10-L) and the temperature was reduced to approximately 15°C by placing frozen water bottles into the bucket. Once the temperature was reduced, the bucket was placed in a refrigerator at 5–10°C for cold storage. Enriched rotifers were fed within 24 hours of cold storage.

Copepod Culture

Copepods were batch cultured at 28–30°C and a salinity of 22–25 g/L in twelve 200-L cylindrical, flat-bottom polyethylene tanks. Aeration was kept moderate and sponge filters were used within each tank. Each tank was fed 1–2 L of T-ISO (15–20 million cells/mL) daily during the trial. Nauplii were harvested daily by sieving populations through a 150 micron nylon mesh screen and the adults not passing through the sieve were placed in new culture water. Harvested nauplii were then placed in a graduated bucket containing 22 g/L salinity water, volumetrically quantified, and then volumetrically fed to fish larvae.

Sample Collection and Morphometric Analysis

Sample collection and morphometric analysis techniques described by Cavalin and Weirich (2009) were used for this study. On 0 DPH, 60 yolk-sac larvae were randomly selected from the incubation tank for morphometric analysis. Thereafter, 10 larvae from each experimental tank were randomly sampled on 3 and 6 DPH for morphometric analysis. Larvae were euthanized via cold water immersion (~4°C), placed on a glass slide, and photographed using a dissecting microscope at 40-X magnification with a high resolution digital camera (Sony Model DCRA-C171, CA). Photographs were stored as JPEG Images at 6.1 megapixels. A 0.01-

mm micrometer was photographed prior to each larval series for calibration purposes. SigmaScan Pro 5.0 image analysis software (SPSS Science, IL) was used to measure photographed larvae. Standard length (SL; the distance parallel to the longitudinal axis of the body from the tip of the snout to the distal end of the hypural bone) and body depth (BD; the distance perpendicular to the longitudinal axis of the body from the insertion of the first dorsal spine to the ventralmost point on the base of the body) were measured and recorded. When measuring yolk-sac larvae, BD was considered the distance perpendicular to the longitudinal axis from the dorsal crest through the midpoint of the yolk-sac to the ventralmost point of the body. Larvae from each tank were counted to determine survival at the conclusion of the larval rearing trial.

Stress Resistance Analysis

On 7 DPH, a sample of larvae from all treatment replicates were combined and placed into separate 110-L aquaria, each representing a dietary treatment. The aquaria contained water from the larval culture system and were maintained at 26°C and a salinity of 35 g/L, with gentle aeration. Larvae were than randomly selected from the aquaria for net and salinity stress experiments. Larvae from the mesocosm dietary treatment were excluded from the stress resistance analysis due to low survival.

Net Stress

To simulate net stress, units were constructed consisting of a 0.5-L container which fit tightly into a 1.0-L container. The bottom of the 0.5-L container was removed and a 150 micron nylon mesh screen was hot glued to cover the opening. Five larvae were randomly selected from their respective aquaria with a 0.5-L screen-bottomed container and held out of water for 30, 60, 120, or 240 seconds. Each dietary treatment was replicated four times for each time interval. After their respective duration, larvae were submerged into the 1-L containers containing culture

system water (salinity of 35 g/L) and were maintained in a temperature controlled water bath at 26°C. Larval survival was recorded one hour after being submerged. Survival was defined as any movement detected within one minute of observation.

Salinity Stress

Prior to the salinity stress experiment, larvae were transported 1 mile from the IRREC hatchery facility to the IRREC aquaculture laboratory in buckets with gentle aeration. Five randomly selected larvae from their respective dietary treatment were then pipetted into a 1.0-L container containing 5, 15, 35, or 55 g/L salinity water with gentle aeration and held in water baths at 26°C. Treatment diets were replicated four times for each salinity treatment. Larval survival was recorded 2 hours and 18 hours after exposure. Survival was defined as any movement detected within one minute of observation. Salinities were acquired by mixing appropriate amounts of artificial sea salt with deionized water three days prior to the salinity stress experiments.

Fatty Acid Analysis

Larvae were collected at 0 DPH from the incubation tank and at the conclusion of the trial from each experimental treatment for fatty acid analysis. Copepod nauplii and enriched rotifers were also collected for fatty acid analysis at the conclusion of their use in the culture experiment. Larvae and live feed samples were rinsed with deionized water, placed in plastic vials, and stored in a freezer (-80°C) until analyzed.

Statistical Analysis

All statistical analyses were performed with SAS version 8.02 software (Cary, NC). Percentage data were arc-sine-square-root transformed prior to analysis. Treatment means of all dependent variables were subjected to one-way analysis of variance according to the General Linear Model (PROC GLM) procedure of SAS. A least significant difference test (LSD) was

used to compare treatment means for larval growth, survival, and stress resistance when the ANOVA was significant. When two treatments were compared a Studentized T-test (PROC TTEST procedure of SAS) was conducted to detected differences in the means. All statistical tests were considered significant when $p \leq 0.05$.

Results

Water Quality

All water quality parameters were recorded within normal limits (Table 3-2).

Growth

At 0 DPH, larvae had a mean SL of 2.77 ± 0.22 mm (mean ± SD) and a mean BD of 0.89 ± 0.06 mm. At 3 DPH, mean SL of larvae fed the mesocosm (2.96 ± 0.19 mm), SRD (2.85 ± 0.36 mm), 'one day' (2.86 ± 0.25 mm), and 'three day' (2.87 ± 0.18 mm) treatments were not significantly different (ANOVA, $F_{3,196} = 2.23$; $p = 0.0861$) (Figure 3-1). The mean BD of larvae was significantly different between dietary treatments (ANOVA, $F_{3,196} = 3.39$; $p = 0.0192$). Larvae fed the SRD had a significantly greater BD (0.77 ± 0.08 mm) than the 'one day' (0.73 ± 0.06 mm) and mesocosm (0.74 ± 0.05 mm) treatments (Figure 3-2). The BD of larvae fed the 'three day' treatment (0.76 ± 0.06 mm) was not significantly different from any of the dietary treatments.

At 6 DPH, mean SL of larvae was significantly different between dietary treatments (ANOVA, $F_{3,196} = 12.94$; $p < 0.0001$). The SL of larvae fed the 'three day' treatment had a significantly greater SL (3.54 ± 0.22 mm) than larvae from any of the treatment diets (Figure 3-1). SL of larvae fed the SRD (3.36 ± 0.26 mm) was significantly greater than both the 'one day' (3.22 ± 0.52 mm) and mesocosm (3.21 ± 0.12 mm) treatments. The mean BD of larvae was significantly different between dietary treatments (ANOVA, $F_{3,196} = 2.23$; $p < 0.0001$). Larvae fed the 'one day' treatment had a significantly greater BD (1.00 ± 0.13 mm) than larvae from any

of the treatment diets (Figure 3-2). BD of larvae fed the 'three day' (0.93 ± 0.08 mm) and SRD (0.91 ± 0.11 mm) treatments were both significantly greater than the mesocosm treatment (0.78 ± 0.06 mm), but were not different from each other.

Survival

At 7 DPH, there were significant differences in survival between treatments (ANOVA, $F_{3,196} = 12.94$; $p < 0.0001$). Survival of larvae fed the 'one day' treatment (38.6 ± 7.8%) was significantly greater than survival from the SRD (20.8 ± 10.5%) and mesocosm (6.5 ± 2.8%) treatments (Figure 3-3). Survival of larvae fed the 'three day' treatment (29.0 ± 4.6%) was significantly greater than survival from the mesocosm treatment. No significant differences were detected in the survival from the 'three day' treatment when compared to the 'one day' and SRD treatments.

Stress Resistance

Net Stress

No significant differences were detected in survival of larvae fed the various experimental diets following 30 (ANOVA, $F_{2,9} = 0.50$; $p = 0.6224$), 60 (ANOVA, $F_{2,9} = 1.21$; $p = 0.3417$), 120 (ANOVA, $F_{2,9} = 0.54$; $p = 0.6013$), and 240 (ANOVA, $F_{2,9} = 3.66$; $p = 0.0687$) seconds of exposure to net stress (Figure 3-4).

Salinity Stress

At the 2 hour time interval, no significant differences were detected in the survival of larvae from any of the experimental diets for the 15 g/L (ANOVA, $F_{2,9} = 2.48$; $p = 0.1389$) and 35 g/L salinity treatments (ANOVA, $F_{2,9} = 0.40$; $p = 0.6798$). There was a significant difference between treatments in the 5 g/L salinity treatment (ANOVA, $F_{2,9} = 4.89$; $p = 0.0366$). Survival of larvae fed the SRD (90.0 ± 11.6%) and 'one day' (85.0 ± 19.1%) treatments exposed to the 5

g/L salinity treatment were significantly greater than survival of larvae fed the 'three day' treatment (50.0 ± 25.8%) (Figure 3-5).

At the 18 hour time interval, no significant differences were detected in the survival of larvae from any of the experimental diets for the 15 g/L (ANOVA, $F_{2,9} = 1.44$; $p = 0.2857$) and 35 g/L salinity treatments (ANOVA, $F_{2,9} = 0.91$; $p = 0.4355$). There was a significant difference between treatments in the 5 g/L salinity treatment (ANOVA, $F_{2,9} = 6.44$; $p = 0.0184$). Survival of larvae fed the SRD (90.0 ± 11.6%) and 'one day' (85.0 ± 19.1%) treatments exposed to the 5 g/L salinity treatment were significantly greater than survival of larvae fed the 'three day' treatment (45.0 ± 25.0%) (Figure 3-6). No larvae were alive in the 55 g/L salinity treatment for any of the experimental diets tested at the 2 hour or 18 hour time intervals.

Discussion

Improvements in growth, survival and resistance to stress were noted for larvae fed copepods compared to those larvae fed the SRD. A 'one day' diet, which consisted of feeding *P. pelagicus* nauplii to pompano larvae for the first day of exogenous feeding, resulted in nearly double the survival and increased growth in BD at 6 DPH when compared to larvae fed the SRD. The 'three day' diet also resulted in higher survival and a significant increase in the SL when compared to larvae fed the SRD. When examining the net stress resistance of pompano larvae, no significant differences were detected between the dietary treatments, though increases in sample sizes may have displayed significant differences.

At 6 DPH larvae from the mesocosm treatment had the lowest SL and BD, and recorded the lowest survival of larvae from any of the treatment diets (6.5 ± 2.8%). At an assumed 1:1 sex ratio and an estimated nauplii production rate of 9.5 ± 1.9 nauplii/female/day (Rhyne et al., 1999), tanks stocked at a density of 1.34 individuals/mL should have produced roughly 6.4 nauplii/mL/day. However, fewer nauplii (0.11/mL) than expected were sampled from the

mesocosm treatment tanks during the experimental trial. This observation coupled with the low growth and survival of larvae from the treatment tanks suggests that poor nauplii production occurred. One explanation may be that an insufficient amount of the algae T-ISO was supplied to the adult copepods through "greening the culture water" in order for them to produce sufficient numbers of nauplii. In Rhyne et al. (2009), T-ISO was fed to adult *P. pelagicus* at densities of 200,000–300,000 cells/mL. During this experiment, T-ISO densities were maintained from 100,000–180,000 cells/mL. Another explanation is that a 1:1 sex ratio of copepods was not achieved at stocking. Only females were counted from samples of the populations used to stock the experimental system, because a 1:1 sex ratio is typical for *P. pelagicus* under these culture conditions (Rhyne et al., 2009). However, during the experiment schools of male copepods were observed near the tops of the mesocosm treatment tanks and antagonism was observed by unpaired males upon paired adults (the reproductive state). An elevated number of males within the treatment tanks could have further reduced the amount of T-ISO available for paired adults and increased antagonism likely reduced the amount of energy available for reproduction.

Directly following this experiment, the condition of the copepod cultures quickly deteriorated yielding low survival and low nauplii production. The cultures became cloudy and food consumption was reduced. It was deduced that high levels of bacteria were present in the cultures, likely from injury and damage to the copepods during the repeated sieving of the populations. This method of nauplii collection should not be used for feeding larval marine fish or the health of copepod populations will decline.

The results of this study provided information on Florida pompano larviculture techniques. However, further studies feeding *P. pelagicus* nauplii to larval Florida pompano are warranted.

Replicated feeding regimes of the 'one day' and 'three day' diets provided in this study examined on wild-caught broodstock are necessary to confirm advantages of feeding copepods to larval Florida pompano. Also, acquiring information on the advantages of feeding copepods to pompano larvae up to the *Artemia* transitioning period (9 DPH) and using a larger system designed for commercial production is warranted. Further, refining a mesocosm technique for *P. pelagicus* is needed to examine the most cost efficient methods of providing nauplii to larval marine fish species.

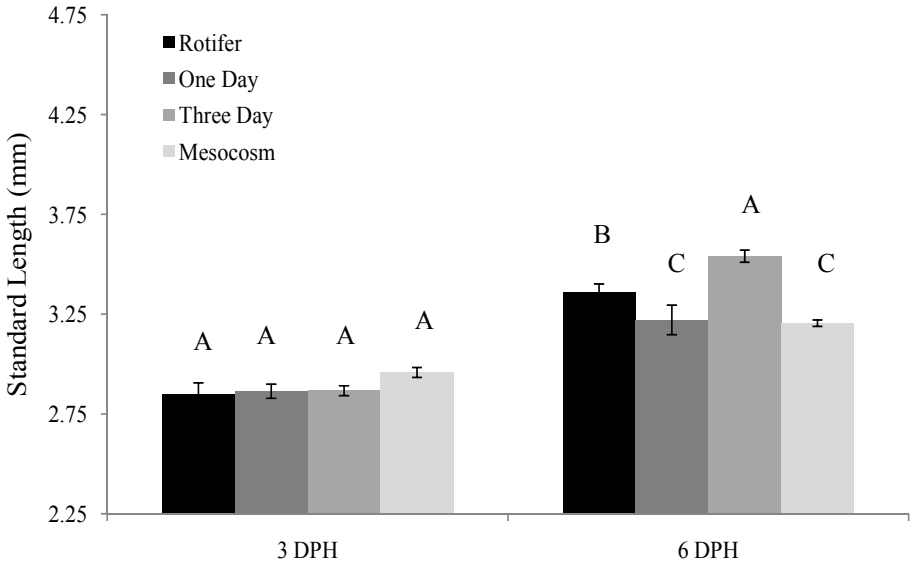

Figure 3-1. The mean standard length (mm) of Florida pompano larvae (*Trachinotus carolinus*) fed different dietary treatments during trial 1. Values were recorded on 3 and 6 days post hatch (DPH). Standard error bars and LSD multiple comparisons test results are displayed. Bars with different letters are statistically different ($p \leq 0.05$).

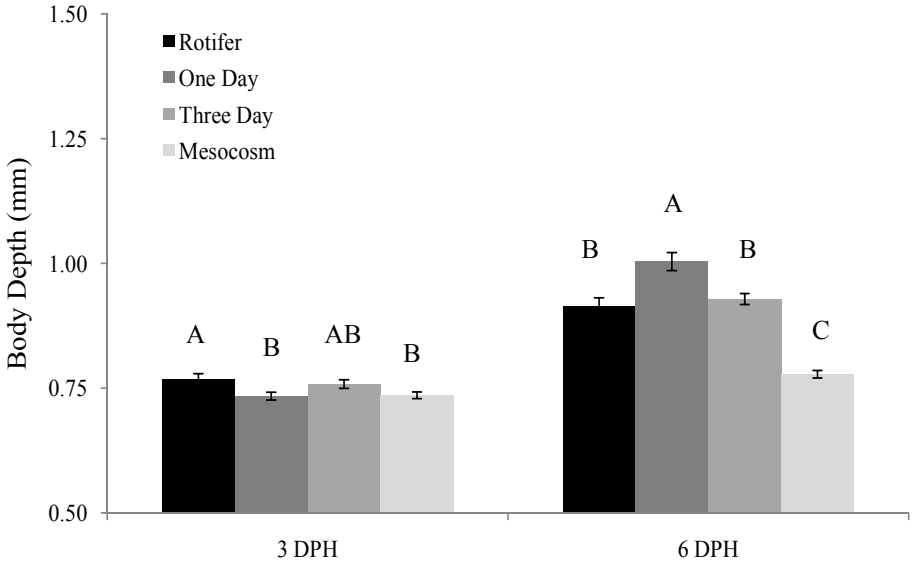

Figure 3-2. The mean body depth (mm) of Florida pompano larvae (*Trachinotus carolinus*) fed different dietary treatments during trial 1. Values were recorded on 3 and 6 days post hatch (DPH). Standard error bars and LSD multiple comparisons test results are displayed. Bars with different letters are statistically different ($p \leq 0.05$).

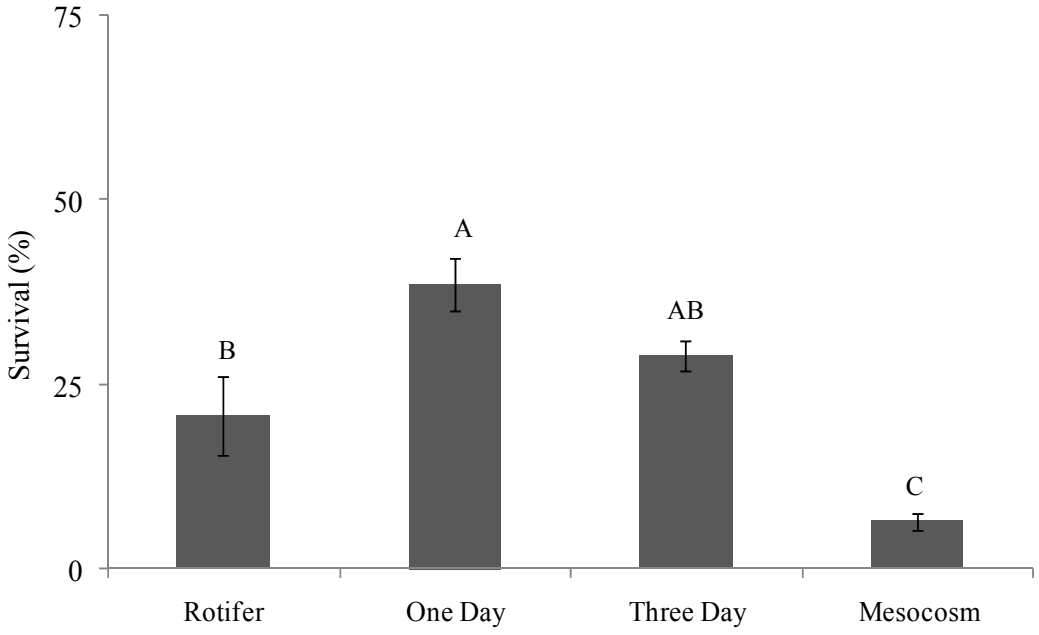

Figure 3-3. The mean survival of Florida pompano larvae (*Trachinotus carolinus*) fed different dietary treatments during trial 1. Standard error bars and LSD multiple comparisons test results are displayed. Bars with different letters are statistically different ($p \leq 0.05$).

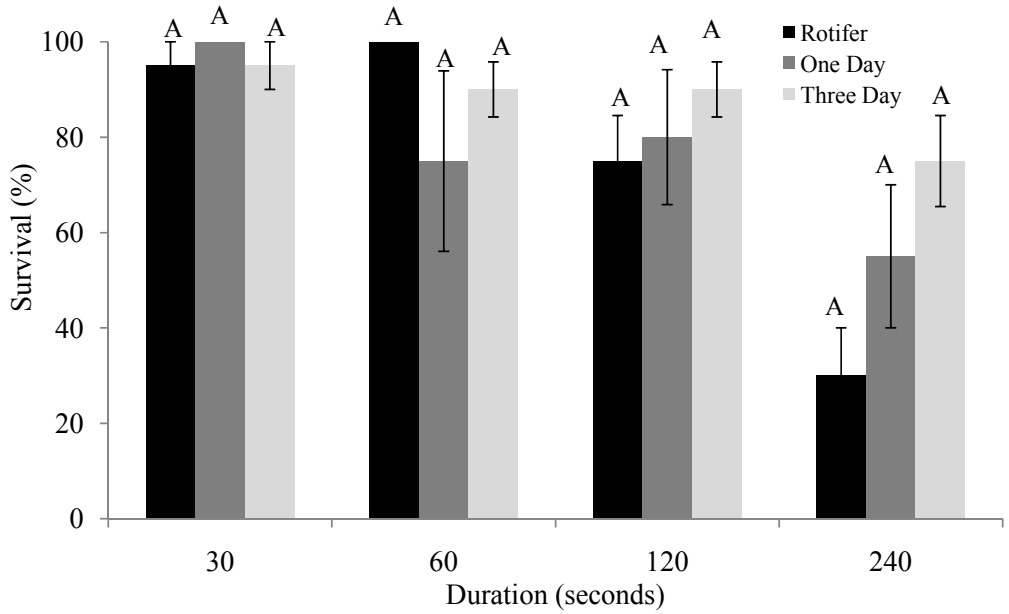

Figure 3-4. The mean survival of Florida pompano larvae (*Trachinotus carolinus*) fed different dietary treatments after exposure to net stress during trial 1. Exposure durations were 30, 60, 120 and 240 seconds. Standard error bars and LSD multiple comparisons test results are displayed. Bars with different letters are statistically different ($p \leq 0.05$).

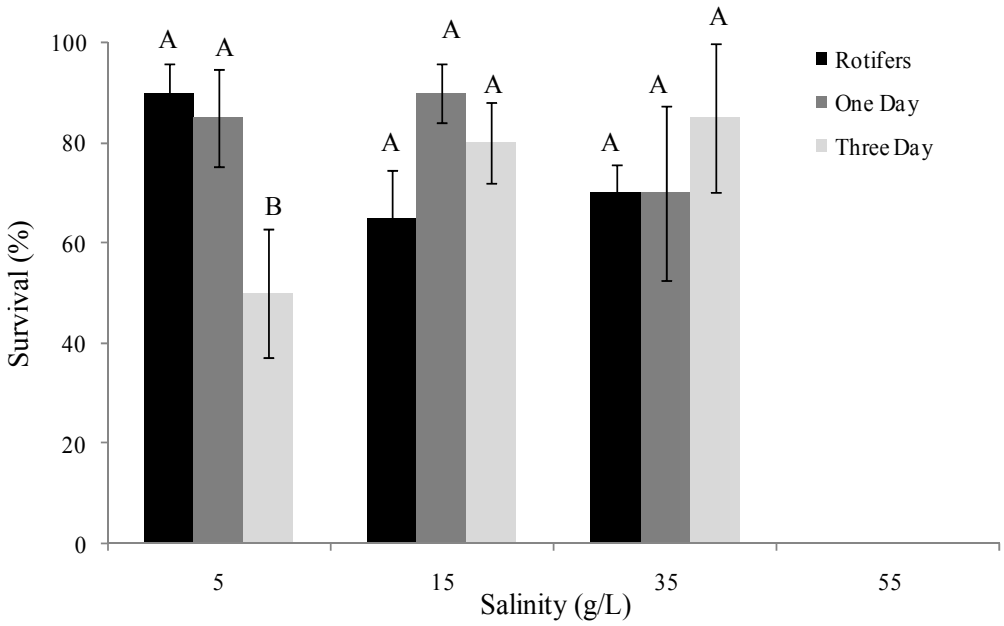

Figure 3-5. The mean survival of Florida pompano larvae (*Trachinotus carolinus*) fed different dietary treatments after 2 hours of salinity stress during trial 1. Salinities were 5, 15, 35, and 55 g/L. Standard error bars and LSD multiple comparisons test results are displayed. Bars with different letters are statistically different ($p \leq 0.05$).

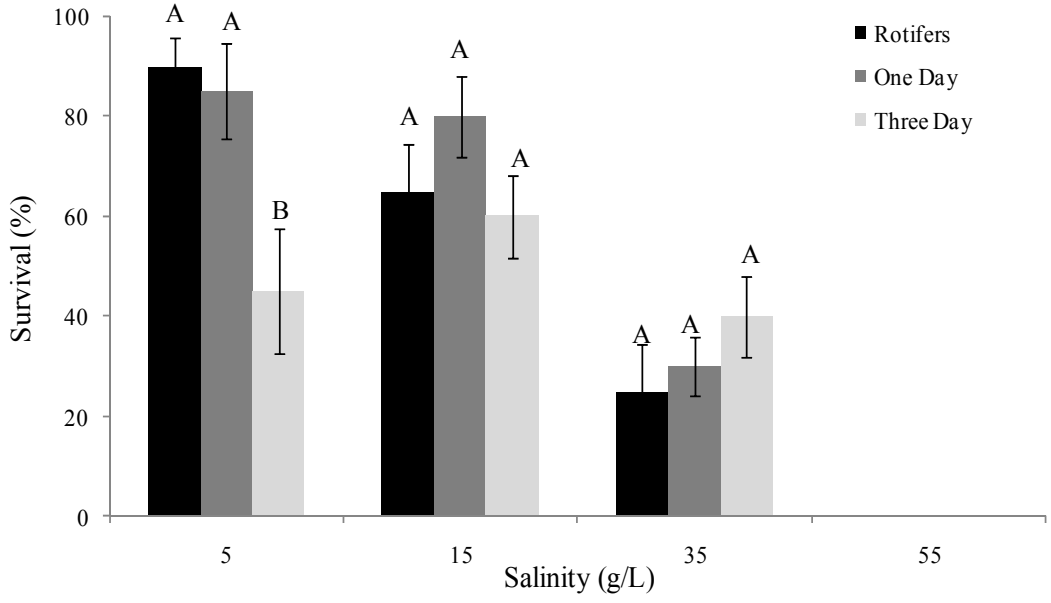

Figure 3-6. The mean survival of Florida pompano larvae (*Trachinotus carolinus*) fed different dietary treatments after 18 hours of salinity stress during trial 1. Salinities were 5, 15, 35, and 55 g/L. Standard error bars and LSD multiple comparisons test results are displayed. Bars with different letters are statistically different ($p \leq 0.05$).

Table 3-1. Dietary treatments fed to Florida pompano larvae (*Trachinotus carolinus*) during the experimental trials. Values are given as the number of live feeds (CN = copepod nauplii, R = rotifers) fed per milliliter per day. The day post hatch (DPH) that the feed was fed is also provided in parenthesis. Stocking density for mesocosm treatments is also given (CA = copepod adults). Following provision of 'one day' and 'three day' treatments, larvae were fed the standard reference diet (SRD) for their respective trial.

	One Day	Three Day	Copepod	Mix	Mesocosm	SRD
Trial 1	2.14 CN (2)	2.14 CN (2) 2.50 CN (3) 3.00 CN (4)			1.34 CA /mL	2.50 R X 4 (2-6)
Trial 2	2.43 CN (2)	2.43 CN (2) 3.10 CN (3) 3.41 CN (4)				2.50 R X 4 (2-6)
Trial 3	1.25 CN (2)		3.50 CN (2) 3.60 CN (3) 4.00 CN (4) 4.00 CN (5) 4.00 CN (6) 3.78 CN (7)		1.25 CA /mL	2.50 R X 4 (2-7)
Trial 4		2.50 CN (2) 3.00 CN (3) 4.50 CN (4)	2.50 CN (2) 3.00 CN (3) 4.50 CN (4) 6.25 CN (5) 7.80 CN (6) 8.00 CN (7) 7.80 CN (8)	2.00 R X 4 (2-8) 0.50 CN X 4 (2-8)		2.50 R X 4 (2-8)
Trial 5	1.11 CN (2)					2.50 R X 4 (2-5) 3.00 R X 4 (6-9)

Table 3-2. Water quality variables measured during the experimental trials. Variables including temperature, salinity, dissolved oxygen (DO), pH, total ammonia-nitrogen (TAN), and nitrite-nitrogen (NO_2-N) were measured. Values are given as the mean ± standard deviation and the range. The number of samples (n=) for each trial is given.

	Temperature (C)	Salinity (g/L)	DO (mg/L)	pH	TAN (mg/L)	NO_2-N (mg/L)
Trial 1 n=168	26.1 ± 0.8 24.2 – 27.2	35.1 ± 0.3 34.4 – 35.6	5.77 ± 0.27 5.02 – 6.25	8.03 ± 0.05 7.94 – 8.13	0.10 ± 0.02 0.03 – 0.18	0.0068 ± 0.0045 0.0020 – 0.0493
Trial 2 n=161	24.9 ± 0.9 22.7 – 26.8	35.3 ± 0.3 34.8 – 35.6	6.04 ± 0.22 5.42 – 6.54	8.07 ± 0.05 7.94 – 8.26	0.14 ± 0.13 0.00 – 0.74	0.0151 ± 0.0008 0.0001 – 0.0407
Trial 3 n=196	25.8 ± 0.6 24.7 – 26.8	36.0 ± 0.2 35.2 – 36.3	5.32 ± 0.28 4.17 – 5.79	8.07 ± 0.04 7.87 – 8.16	0.04 ± 0.09 0.00 – 0.61	0.0075 ± 0.0032 0.0012 – 0.0231
Trial 4 n=224	27.6 ± 0.6 26.2 – 28.5	33.9 ± 1.3 31.6 – 36.0	6.11 ± 0.18 5.46 – 6.55	8.22 ± 0.05 8.02 – 8.30	0.03 ± 0.06 0.00 – 0.40	0.0114 ± 0.0107 0.0018 – 0.0099
Trial 5 n=96	30.7 ± 0.7 28.9 – 32.5	35.9 ± 1.0 34.6 – 37.2	5.49 ± 0.48 3.83 – 6.63	8.14 ± 0.08 8.00 – 8.13	0.11 ± 0.10 0.00 – 0.35	0.0042 ± 0.0221 0.0169 – 0.0892

CHAPTER 4
TRIAL 2

The goal of this study was to examine the benefits of feeding nauplii of *Pseudodiaptomus pelagicus* to larval Florida pompano, *Trachinotus carolinus*, during the first three days of feeding. Treatment diets similar to trial 1 were fed, however, an increased rate was used to ascertain advantages in development, performance and solidify the findings of trial 1. Dietary treatments were provided to Florida pompano larvae spawned from wild-caught broodstock. The duration of net stress exposure was extended and two salinity stress resistance experiments were conducted to determine the effects of diet on Florida pompano larvae.

Material and Methods

Material and Methods from trial 1 were repeated unless otherwise noted.

Spawning and Egg Incubation

Volitional spawning of wild-caught broodstock supplied larvae for this trial.

Larval Rearing and Experimental Design

Experimental tanks were inoculated daily with Tahitian strain *Isochrysis galbana* (T-ISO) to a density of 120,000–260,000 cells/mL.

Dietary Treatments

Three dietary treatments were examined in this study, a standard reference diet (SRD) consisting solely of enriched rotifers, a ‘one day’ diet, and a ‘three day’ diet (Table 3-1). Each dietary treatment was replicated six times and four tanks monitored unfed larvae. The ‘one day’ experimental diet consisted of feeding larvae copepod nauplii on 2 days post hatch (DPH) at a rate of 2.43 nauplii/mL/day and then switching to the SRD from 3–6 DPH. The ‘three day’ experimental diet consisted of feeding larvae copepod nauplii on 2 DPH at a rate of 2.43 nauplii/mL/day, on 3 DPH at a rate of 3.10 nauplii/mL/day, and on 4 DPH at a rate of 3.41

nauplii/mL/day and then switching to the SRD from 5–6 DPH. Six tanks monitored larvae fed copepod nauplii on 2 DPH at a rate of 2.43 nauplii/mL/day and not fed again for the entire trial.

Copepod Culture

Copepods were batch cultured in eight 200-L cylindrical, flat-bottom polyethylene tanks. Copepod nauplii were harvested twice daily from each tank via floating airlifts ('Equipment section of Chapter 2) within each tank.

Stress Resistance Analysis

Net Stress

Larvae were held out of water for 30, 120, 240, 360, or 600 seconds.

Salinity Stress

1-L containers: Larvae were exposed to 1, 35, or 100 g/L salinity water. Larval survival was recorded 30 minutes after exposure. Salinities were attained by mixing appropriate amounts of >100 g/L salinity solution with deionized water one day prior to the salinity stress experiment. A >100 g/L salinity solution was obtained by continuously boiling treated seawater.

30 mL containers: One randomly selected larva was removed from 35 g/L salinity and was transferred into a 30 mL container comprising 100 g/L seawater at 26°C. 10 larvae were selected from each dietary treatment. Time to death for each larva was recorded in seconds.

Results

Water Quality

All water quality parameters were recorded within normal limits (Table 3-2).

Growth

At 0 DPH, larvae had a mean standard length (SL) of 2.41 ± 0.22 mm (mean ± SD) and a mean body depth (BD) of 0.81 ± 0.07 mm. At 3 DPH, mean SL of larvae was significantly different between dietary treatments (ANOVA, $F_{2,157} = 4.12$; $p = 0.0181$). The SL of larvae from

the ‘one day’ treatment (2.49 ± 0.14 mm) was significantly greater than the SL of larvae fed the SRD (2.40 ± 0.20 mm) (Figure 4-1). The ‘three day’ (2.44 ± 0.14 mm) treatment was not significantly different from any of the treatment diets. The mean BD of larvae was significantly different between dietary treatments (ANOVA, $F_{2,157} = 37.27$; $p < 0.0001$). The BD of larvae fed the ‘one day’ (0.79 ± 0.06 mm) and ‘three day’ (0.79 ± 0.06 mm) treatments was significantly greater than the BD of larvae fed the SRD (0.71 ± 0.05 mm) treatment, but were not significantly different from each other (Figure 4-2).

At 6 DPH, mean SL of larvae was significantly different between dietary treatments (ANOVA, $F_{2,157} = 14.35$; $p < 0.0001$). The SL of larvae fed the ‘one day’ treatment (3.73 ± 0.37 mm) was significantly greater than the SL of larvae fed the ‘three day’ (3.48 ± 0.45 mm) and SRD (3.29 ± 0.44 mm) treatments (Figure 4-1). Larvae fed the ‘three day’ treatment also had a significantly greater SL than larvae fed the SRD treatment. The mean BD of larvae was significantly different between dietary treatments (ANOVA, $F_{2,157} = 13.69$; $p < 0.0001$). The BD of larvae fed the ‘one day’ (0.98 ± 0.11 mm) and ‘three day’ (0.95 ± 0.13 mm) treatments were significantly greater than larvae fed the SRD treatment (0.88 ± 0.09 mm), but were not different from each other (Figure 4-2).

Survival

At 7 DPH, the mean survival of larvae was significantly different between dietary treatments (ANOVA, $F_{2,13} = 12.12$; $p = 0.0011$). The survival of larvae fed the ‘three day’ treatment (71.5 ± 5.1%) was significantly greater than survival from the ‘one day’ (63.5 ± 7.6%) and SRD (53.6 ± 5.0%) treatments (Figure 4-3). Survival of larvae fed the ‘one day’ treatment was also significantly greater than survival from the SRD treatment.

Stress Resistance

Net Stress

No significant differences were detected in the mean survival of larvae fed the experimental diets following 30 (ANOVA, $F_{2,9} = 1.75$; $p = 0.2280$), 120 (ANOVA, $F_{2,9} = 3.98$; $p = 0.0579$), and 360 (ANOVA, $F_{2,9} = 1.80$; $p = 0.2200$) seconds of exposure to net stress. At the 240 second duration, the mean survival of larvae was significantly different between dietary treatments (ANOVA, $F_{2,9} = 5.06$; $p = 0.0338$). The mean survival of larvae fed the 'three day' (65.0 ± 34.2%) and 'one day' (60.0 ± 16.4%) treatments were significantly greater than larvae fed the SRD (15.0 ± 19.2%) (Figure 4-4). At the 600 second duration, mean survival of larvae was significantly different between dietary treatments (ANOVA, $F_{2,9} = 21.50$; $p = 0.0004$). The mean survival of larvae fed the 'three day' treatment (30.0 ± 10.0%) was significantly greater than larvae fed the 'one day' (0.00 ± 0.00%) and SRD (5.0 ± 10.0%) treatments, which were not significantly different from each other.

Salinity Stress

1-L containers: No larvae were alive in the 1 and 100 g/L treatments. All larvae were alive in the 35 g/L treatment at the 30 minute time interval for all of the experimental diets tested. Therefore, no statistical analysis was performed.

30-mL containers: The mean time to death of larvae was not significantly different between dietary treatments (ANOVA, $F_{2,27} = 1.71$; $p = 0.2003$). The time to death of larvae fed the 'three day' treatment was greater (764.5 ± 330.2 seconds) than larvae fed either the 'one day' (669.4 ± 189.8 seconds) or SRD (577.8 ± 89.7 seconds) treatments (Figure 4-5).

Discussion

Significantly greater growth, in SL and BD, and survival were detected for pompano larvae fed copepod treatment diets when compared to larvae fed the SRD. Survival of larvae fed the

'three day' treatment during trial 2 was significantly higher than both the 'one day' and the SRD treatments. In trial 1, however, no significant differences were detected when survival from the 'three day' treatment was compared to both the 'one day' and SRD treatments. One explanation for the increased performance of larvae fed the 'three day' treatment in trial 2 is the increased feeding rate from 2–4 DPH. During trial 2 larvae were fed 0.30 nauplii/mL more on 2 DPH, 0.60 nauplii/mL more on 3 DPH, and 0.41 nauplii/mL more on 4 DPH. This increased feeding rate likely supplied more nutrients during the first 3 feeding days and significantly increased survival in the 'three day' treatment during trial 2. Another explanation is the decreased water temperature recorded during trial 2, which was on average 1.2°C cooler than water temperatures recorded during trial 1. A reduced water temperature could have decreased the metabolism of the larvae, subsequently reducing the amount of feed organisms needed to maintain sufficient growth and survival. Also, copepod nauplii fed during trial 2 were supplied continuously throughout the day as airlifts were continually harvesting nauplii to meet the required feed densities. During trial 1, nauplii were fed two times, 0900–1000 and 1600–1700. Although internal standpipes with 50 micron nylon mesh kept copepod nauplii inside the experimental tanks, their ability to find refuge between feedings was increased during trial 1. In trial 2, nauplii were supplied to experimental tanks up to 8 times a day with little time for nauplii to seek refuge before being consumed. This continuous feeding strategy may have influenced the significantly greater results seen in all morphometric and survival data for both copepod fed treatments.

Net stress resistance of pompano larvae fed copepod treatment diets was significantly higher than larvae fed the SRD at the 240 and 600 second durations. Remarkably, the 'three day' treatment had at least one larva from every replicate still alive in the 600 second treatment duration after one hour, while only one larva from combined replicates of both the 'one day' and

SRD treatments was alive. The salinity stress experiments conducted in 1-L containers produced poor results. More than likely, the extreme salinities (1 and 100 g/L) examined and the 30 minute time interval were too great to detect differences in tolerance due to dietary nutrition. The salinity stress experiments conducted in 30 mL containers produced results with high variation.

On 0 DPH a high level of rotifer contamination was noticed in the copepod populations. All tanks containing copepod populations were immediately sieved through a series of 240 sieves. This allowed all rotifers and younger life stages of copepods to be flushed from the population, while copepod adults were retained on the sieves and placed into new culture tanks. Nauplii were harvested via airlift and fed to fish larvae roughly 36 hours after sieving the populations. Surprisingly, sufficient quantities of copepod nauplii were harvested to complete this larval trial, although a reduced number of copepod adults were apparent. The use of airlifts during this larval trial provided an efficient and passive method for the collection of nauplii that had no observed negative effect on the condition and health of the population.

The results of this study confirm the increases in growth, survival, and resistance to stress that can be attributed to feeding copepods to first feeding Florida pompano. However, acquiring information on the advantages of feeding copepods to pompano larvae up to the *Artemia* transitioning period (9 DPH) and developing appropriate feeding regimes for pompano larvae is warranted.

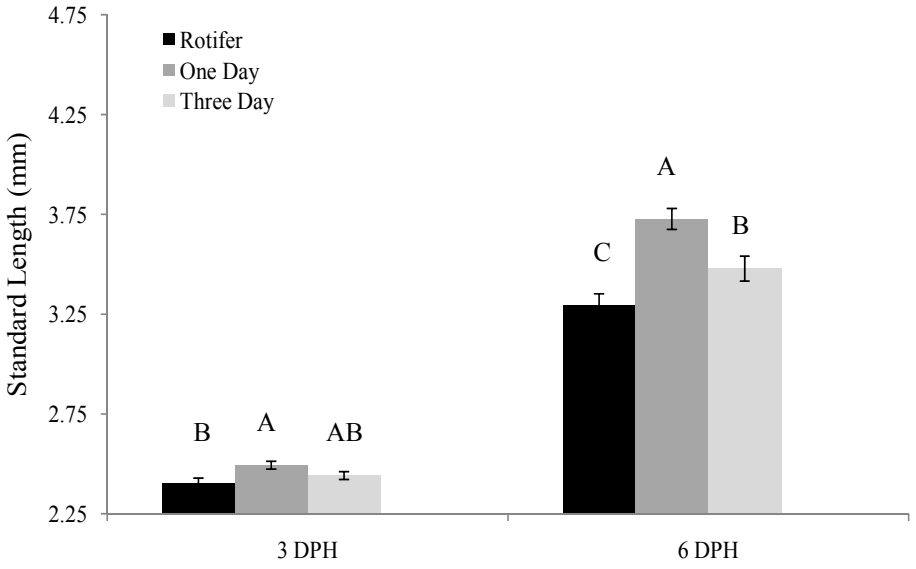

Figure 4-1. The mean standard length (mm) of Florida pompano larvae (*Trachinotus carolinus*) fed different dietary treatments during trial 2. Values were recorded on 3 and 6 days post hatch (DPH).Standard error bars and LSD multiple comparisons test results are displayed. Bars with different letters are statistically different ($p \leq 0.05$).

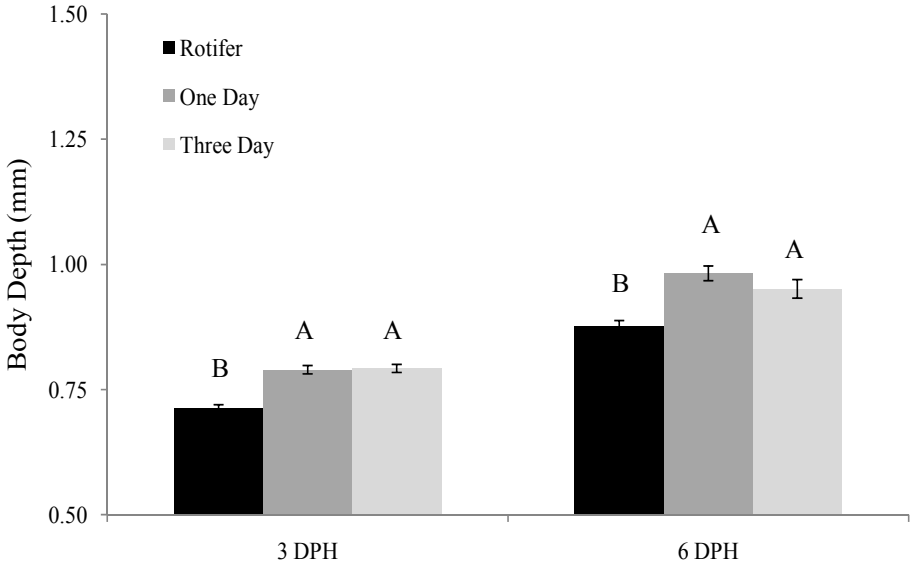

Figure 4-2. The mean body depth (mm) of Florida pompano larvae (*Trachinotus carolinus*) fed different dietary treatments during trial 2. Values were recorded on 3 and 6 days post hatch (DPH). Standard error bars and LSD multiple comparison test results are displayed. Bars with different letters are statistically different ($p \leq 0.05$).

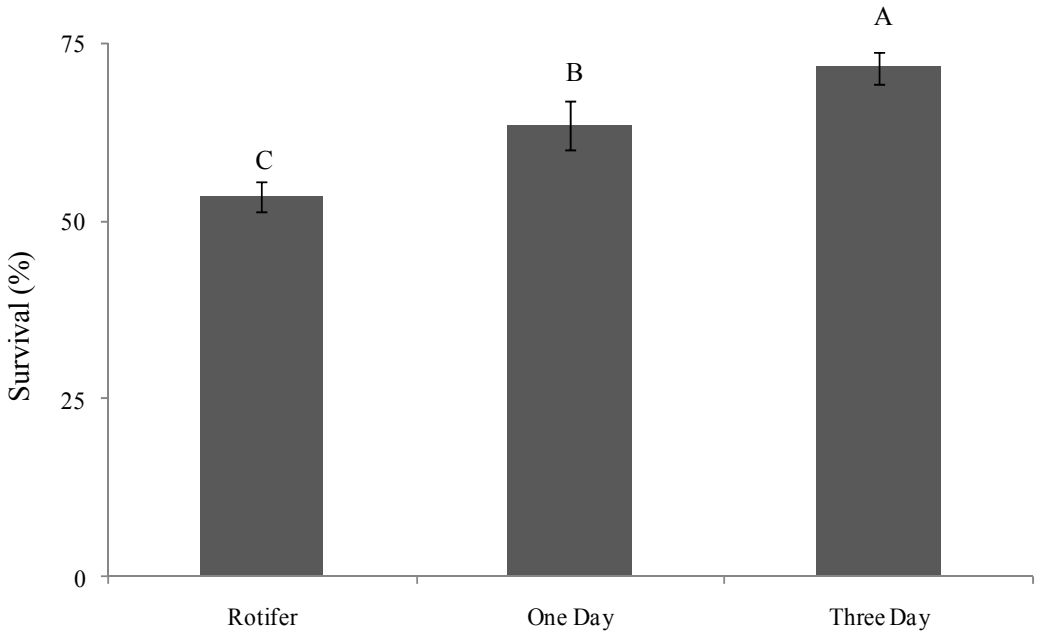

Figure 4-3. The mean survival of Florida pompano larvae (*Trachinotus carolinus*) fed different dietary treatments during trial 2. Standard error bars and LSD multiple comparisons test results are displayed. Bars with different letters are statistically different ($p \leq 0.05$).

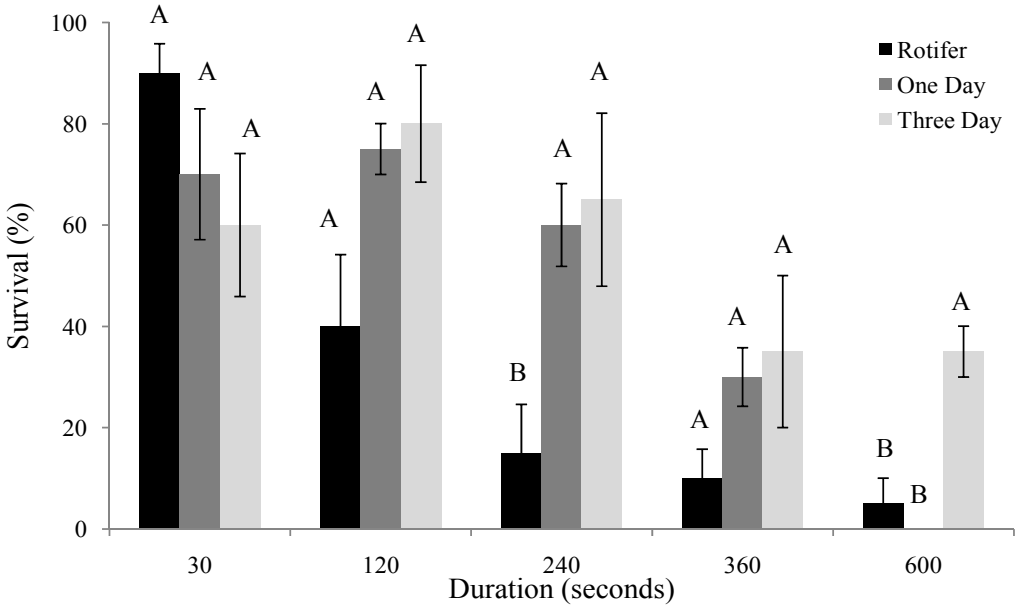

Figure 4-4. The mean survival of Florida pompano larvae (*Trachinotus carolinus*) fed different dietary treatments exposed to net stress during trial 2. Durations were 30, 120, 240, 360, and 600 seconds. Standard error bars and LSD multiple comparison test results are displayed. Bars with different letters are statistically different ($p \leq 0.05$).

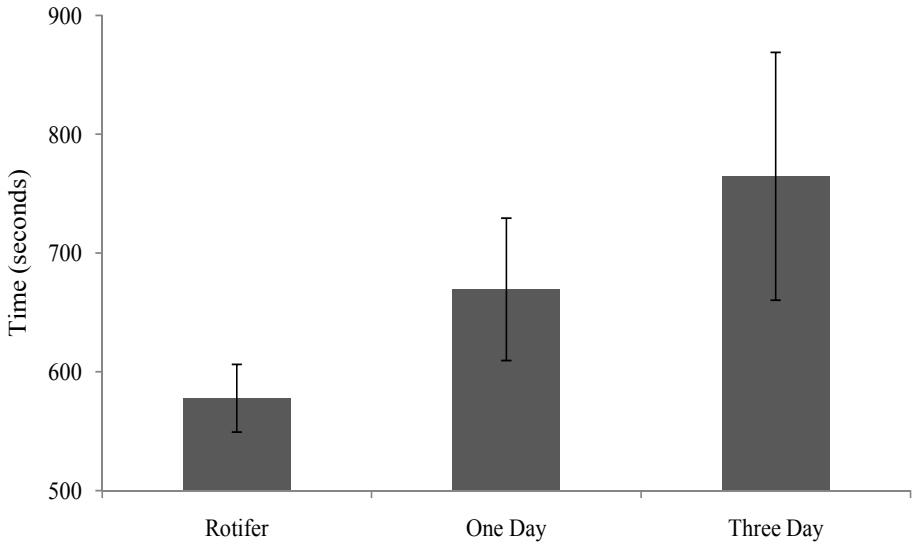

Figure 4-5. The mean time to death of Florida pompano (*Trachinotus carolinus*) larvae fed different dietary treatments exposed to 100 mg/L salinity seawater during trial 2. No significant differences in survival were detected ($p = 0.2003$). Standard error bars are displayed.

CHAPTER 5
TRIAL 3

The goal of this study was to examine the benefits of feeding nauplii of *Pseudodiaptomus pelagicus* to larval Florida pompano, *Trachinotus carolinus*, up to the *Artemia* transitioning period, 9 days post hatch (DPH). A mesocosm treatment was also examined to develop feeding strategies for marine fish larvae and a 'one day' dietary treatment was fed to determine the minimum duration of feeding copepod nauplii to larval Florida pompano necessary to have an effect.

Material and Methods

Material and Methods from trial 1 were repeated unless otherwise noted.

Spawning and Egg Incubation

Volitional spawning of wild-caught broodstock supplied larvae for this trial.

Larval Rearing and Experimental Design

An 8-day larval rearing trial was conducted. Experimental tanks were inoculated daily with Tahitian strain *Isochrysis galbana* (T-ISO) at a density of 100,000–170,000 cells/mL.

Dietary Treatments

Four dietary treatments were examined in this study, a standard reference diet (SRD) consisting solely of enriched rotifers, a 'copepod' diet, a 'one day' diet, and a mesocosm treatment (Table 3-1). The 'copepod' experimental diet consisted of feeding larvae copepod nauplii on 2 DPH at a rate of 3.50 nauplii/mL/day, on 3 DPH at a rate of 3.60 nauplii/mL/day, on 4 DPH at a rate of 4.00 nauplii/mL/day, on 5 DPH at a rate of 4.00 nauplii/mL/day, on 6 DPH at rate of 4.00 nauplii/mL/day, and on 7 DPH at a rate of 3.78 nauplii/mL/day. The 'one day' experimental diet consisted of feeding larvae copepod nauplii on 2 DPH at a rate of 1.25 nauplii/mL/day and then switching to the SRD from 3–7 DPH. The mesocosm experimental diet

consisted of 16,000 *P. pelagicus* copepodites (collected as nauplii 8 days prior) stocked into each experimental tank on -2 DPH. Copepodites were initially stocked into the experimental tanks at half volume (6.5-L) and kept under static conditions with gentle aeration. Tanks were maintained with ambient temperature and a salinity of 22 g/L. 35 g/L salinity seawater was dripped into the mesocosm treatment tanks beginning on -1 DPH, to slowly acclimate *P. pelagicus* copepodites to larval rearing conditions. By 0 DPH, mesocosm treatment tanks were filled with 35 g/L salinity seawater, and were suitable for larval stocking. Once filled (13-L), the copepod density within the mesocosm treatment tanks was 1.25 copepodites/mL. After inoculation, the mesocosm treatment was fed T-ISO daily to a density of 200,000–300,000 cells/mL until the entire system was inoculated with T-ISO. From 2 DPH to 7 DPH larvae were fed diets consisting of enriched S-strain rotifers, *Brachionus plicatilis*, and *P. pelagicus* nauplii.

Copepod Culture

Copepods were batch cultured in six 200-L cylindrical, flat-bottom polyethylene tanks with mild aeration and no sponge filters. Nauplii were harvested twice daily from each tank via floating airlifts ('Equipment' section of Chapter 2) within each tank.

Sample Collection and Morphometric Analysis

Ten larvae from each experimental tank were sampled on 3 and 7 DPH for morphometric analysis.

Results

Water Quality

All water quality parameters were recorded within normal limits (Table 3-2).

Growth

Morphometric data were not analyzed.

Survival

Survival data were not analyzed.

Discussion

The survival of all copepod fed treatments began to decline on 4 DPH and was severely reduced by 8 DPH, when the trial was discontinued. The health of pompano larvae was evaluated and an examination of the copepod culture techniques and larval feeding rates was warranted to explain the low survival experienced by larvae fed the copepod treatment diets. Also, a description of observations made regarding the copepod performance in the mesocosm treatment is discussed.

Upon visual inspection of the treatment tanks, a notably higher survival rate for larvae fed the SRD was observed when compared to the copepod fed treatments. Although these larvae were not counted, no deviation from the mean survival (30–40%) was apparent. Conversely, when larvae fed the 'copepod' treatment were counted a survival of 3.75 ± 3.97% (Mean ± SD) was recorded. Survival of the other copepod fed treatments, although not counted, appeared similar to the survival of larvae fed the 'copepod' treatment.

Therefore, at the conclusion of trial 3, samples of pompano larvae fed the 'copepod' treatment, copepods, T-ISO, and water from the treatment system were sent to the University of Florida's Fish Health Diagnostic Laboratory for analysis. Upon evaluation, a *Vibrio* sp. infection was detected in the gills of Florida pompano larvae fed copepods and likely caused increased mortality in those treatments (Figure 5-1).

Since the conclusion of trial 2, some changes were made pertaining to the population of copepods maintained at the University of Florida's Indian River Research and Education Center (IRREC). During the first two trials, copepod populations became contaminated with rotifers. Afterwards, unsuccessful attempts were made to maintain rotifer-free populations of copepods.

Therefore, the contaminated populations were bleached and a new population was acquired from Algagen LLC (Vero Beach, FL). Prior to the acquisition of the new population, all copepod culture equipment was either thrown away or soaked for days in a 25 mg/L solution of sodium hypochlorite. From this time forward, a stock population of copepods was maintained in a biosecure room, while copepods grown for fish larval rearing were maintained inside the hatchery. Copepod culture conditions for trial 3 were identical to those of trials 1 and 2 except no sponge filters were used in any of the copepod culture tanks.

Additionally, larvae in the 'copepod' treatment were overfed during this trial in an attempt to identify an appropriate feeding level for Florida pompano larvae during the first 9 DPH. However, this quickly became a problem. As the survival in the experimental tanks declined, the concentration of copepod nauplii within each tank grew (>7 nauplii/mL). Larvae experienced frequent repeated physical interactions with copepod nauplii and this may have had deleterious effects. Also, elevated numbers of copepod nauplii drastically reduced the amount of algae within 'copepod' treatment tanks, decreasing the contrast needed to visualize prey and likely affected the amount of indirect nutrition received. Continual provision of a low prey density (2–4 nauplii/mL) is recommended for future studies with Florida pompano larvae fed copepod nauplii.

Upon observation of the mesocosm treatment results, a suitable ratio of males to females was achieved during this trial when compared to trial 1. Most adults appeared to be paired (the reproductive state) and the presence of schooling males was reduced or absent in this trial. In trial 1, adults were taken from established populations and stocked into the experimental system. For this trial, copepod nauplii were harvested prior to the larval trial and reared together as a cohort. Once stocked into the experimental system, copepodites were acclimated from 22 g/L

salinity to 35 g/L salinity over a 30 hour period. By 2 DPH, copepodites had matured to adults and began to reproduce. However, samples of the culture water indicated the concentration of nauplii in the experimental tanks was still low (0.16 nauplii/mL). Copepod nauplii production may have, again, been reduced by insufficient amounts of live T-ISO within the experimental tanks. Algal densities similar to those of trial 1 were initiated for this trial and fell below those suggested for optimal nauplii production by *P. pelagicus* (Rhyne et al., 2009). Future studies evaluating a mesocosm should include provision of sufficient algal densities and use copepods fully acclimated to larval rearing conditions.

Although this trial was not completed, valuable information was obtained regarding the condition of copepod cultures fed to marine fish larvae and copepod feeding rates for Florida pompano larvae. Furthermore, useful insight in the development of an appropriate mesocosm feeding regime for *P. pelagicus* was observed.

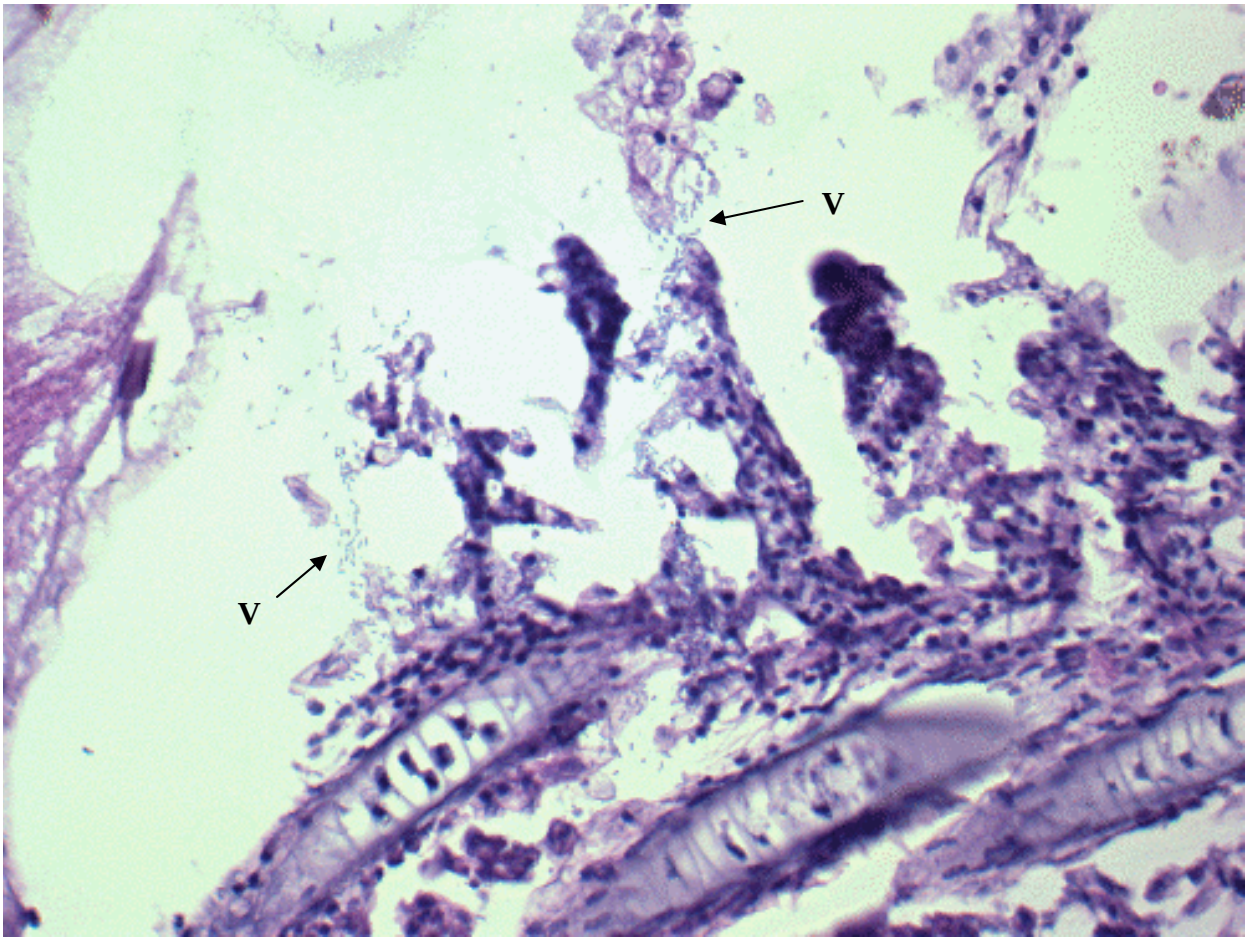

Figure 5-1. Histology section (hemotoxylin and eosin stain) displaying *Vibrio* sp. (V) within the gills of Florida pompano larvae (*Trachinotus carolinus*) fed copepods during trial 3. (Photo and evaluation courtesy of B. Denise Petty, University of Florida)

CHAPTER 6
TRIAL 4

The goal of this study was to examine the benefits of feeding nauplii of the calanoid copepod *Pseudodiaptomus pelagicus* to larval Florida pompano, *Trachinotus carolinus*, up to the *Artemia* transitioning period, 9 days post hatch (DPH). Florida pompano larvae were fed a copepod diet, consisting solely of copepod nauplii, and a mix diet, consisting of both rotifers and copepod nauplii, for the entire larval trial. Additionally, larvae were fed a diet of copepod nauplii for the first three days of feeding to confirm the results of trials 1 and 2. Net stress experiments were performed to detect differences in stress resistance of Florida pompano larvae fed dietary treatments.

Material and Methods

Material and Methods from trial 1 were repeated unless otherwise noted.

Spawning and Egg Incubation

Volitional spawning of wild-caught broodstock supplied larvae for this trial.

Larval Rearing and Experimental Design

A 9-day larval rearing trial was conducted. Experimental tanks were inoculated daily with Tahitian strain *Isochrysis galbana* (T-ISO) at a density of 200,000–380,000 cells/mL. At 7 DPH, aeration was increased slightly and water flow was adjusted to 72 mL/minute for the duration of the trial. In each replicate tank, temperature, salinity, dissolved oxygen, and pH were monitored daily (YSI Incorporated Model 556 MPS, OH).

Dietary Treatments

Four dietary treatments were examined in this study, a standard reference diet (SRD) consisting solely of enriched rotifers, a ‘copepod’ diet, a ‘three day’ diet, and a ‘mix’ diet (Table 3-1). Each dietary treatment was replicated seven times. The ‘copepod’ experimental diet

consisted of feeding larvae copepod nauplii on 2 DPH at a rate of 2.50 nauplii/mL/day, on 3 DPH at a rate of 3.00 nauplii/mL/day, on 4 DPH at a rate of 4.50 nauplii/mL/day, on 5 DPH at a rate of 6.25 nauplii/mL/day, on 6 DPH at rate of 7.80 nauplii/mL/day, on 7 DPH at a rate of 8.00 nauplii/mL/day, and on 8 DPH at a rate of 7.80 nauplii/mL/day. The 'three day' experimental diet consisted of feeding larvae copepod nauplii on 2 DPH at a rate of 2.50 nauplii/mLday, on 3 DPH at a rate of 3.00 nauplii/mL/day, and on 4 DPH at a rate of 4.50 nauplii/mL/day and then switching to the SRD from 5–8 DPH. The 'mix' diet consisted of feeding larvae a mixture of rotifers and copepods at a ratio of 4:1. Live feeds were fed to larvae four times daily (0900, 1300, 1700, and 2100), enriched rotifers at a rate of 2.0 rotifers/mL/day copepod nauplii at a rate of 0.5 nauplii/mL/day. From 2 DPH to 8 DPH larvae were fed diets consisting of enriched S-strain rotifers, *Brachionus plicatilis*, and *P. pelagicus* nauplii.

Rotifer Culture and Enrichment

Rotifers were cultured in a 950-L cylindrical, flat-bottom, fiberglass tank. Approximately, 20–25 million rotifers were harvested daily for enrichment prior to feeding to fish larvae. Rotifers were enriched in two 19-L buckets.

Copepod Culture

Copepods were batch cultured in five 200-L cylindrical, flat-bottom polyethylene tanks and one 1800-L cylindrical, conical-bottom tank. Copepod populations were fed a 1:1 mixture of T-ISO (15–20 million cells/mL) and TW (1–2 million cells/mL). Each 200-L population was fed 1–2 L of the algal mix and the 1800-L population was fed 18–24 L. Copepod nauplii were harvested twice daily from each tank via floating airlifts ('Equipment' section of Chapter 2) within each tank.

Sample Collection and Morphometric Analysis

Larvae from each experimental tank were randomly sampled on 3, 6, and 9 DPH for morphometric analysis. Larvae were euthanized with buffered Tricaine-S (Tricaine Methanesulfonate; Western Chemical Inc., WA) and photographed. For calibration purposes, larvae were photographed on a Sedgewick-Rafter Cell S50 (PYSER-SGI Limited, Kent, UK) with a 1.0-mm square grid base.

Net Stress Resistance Analysis

On 9 DPH, ten larvae were randomly selected and held out of water for 180, 360, 540, or 720 seconds. Each dietary treatment was replicated five times for each time interval.

Results

Water Quality

All water quality parameters were recorded within normal limits (Table 3-2).

Growth

At 0 DPH, larvae had a mean standard length (SL) of 2.97 ± 0.14 mm (mean ± SD) and a mean body depth (BD) of 0.90 ± 0.08 mm. At 3 DPH, the mean SL of larvae was significantly different between dietary treatments (ANOVA, $F_{3,276} = 12.57$; $p < 0.0001$). The mean SL of larvae fed the 'copepod' (3.29 ± 0.16 mm), 'mix' (3.24 ± 0.15 mm), and 'three day' (3.26 ± 0.22 mm) treatments were significantly greater than larvae fed the standard reference diet (SRD) treatment (3.10 ± 0.21 mm), but were not significantly different from each other (Figure 6-1). The mean BD of larvae was significantly different between dietary treatments (ANOVA, $F_{3,276} = 34.91$; $p < 0.0001$). The mean BD of larvae fed the 'mix' (0.75 ± 0.06 mm), 'three day' (0.75 ± 0.06 mm), and 'copepod' (0.74 ± 0.06 mm) treatments were significantly greater than larvae fed the SRD treatment (0.66 ± 0.05 mm), but were not significantly different from each other (Figure 6-2).

At 6 DPH, the mean SL of larvae was significantly different between dietary treatments (ANOVA, $F_{3,276} = 3.03$; $p = 0.0300$). The mean SL of larvae fed the SRD (3.92 ± 0.35 mm) and 'mix' (3.91 ± 0.38 mm) treatments were significantly greater than larvae fed the 'three day' treatment (3.78 ± 0.31 mm), but were not significantly different from each other (Figure 6-1). The mean SL of larvae fed the 'copepod' treatment (3.82 ± 0.31 mm) was not significantly different from any of the other dietary treatments. The mean BD of larvae was not significantly different for larvae fed the 'mix' (0.99 ± 0.14 mm), SRD (0.98 ± 0.13 mm), 'copepod' (0.95 ± 0.12 mm), and 'three day' (0.94 ± 0.12 mm) treatments (ANOVA, $F_{3,276} = 1.97$; $p = 0.1180$) (Figure 6-2).

At 9 DPH, the mean SL of larvae was significantly different between dietary treatments (ANOVA, $F_{3,276} = 16.65$; $p < 0.0001$). The mean SL of larvae fed the 'mix' (4.56 ± 0.52 mm) treatment was significantly greater than larvae fed the SRD (4.40 ± 0.46 mm), 'three day' (4.25 ± 0.29 mm), and 'copepod' (4.08 ± 0.37 mm) treatments (Figure 6-1). The mean SL of larvae fed the SRD treatment was significantly greater than larvae fed the 'three day' and 'copepod' treatments and the mean SL of larvae fed the 'three day' treatment was significantly greater than larvae fed the 'copepod' treatment. The mean BD of larvae was significantly different between dietary treatments (ANOVA, $F_{3,276} = 18.20$; $p < 0.0001$). The mean BD of larvae fed the 'mix' (1.24 ± 0.21 mm) treatment was significantly greater than larvae fed the SRD (1.18 ± 0.21 mm), 'three day' (1.11 ± 0.14 mm), and 'copepod' (1.03 ± 0.14 mm) treatments (Figure 6-2). The mean BD of larvae fed the SRD treatment was significantly greater than larvae fed the 'three day' and 'copepod' treatments and the mean BD of larvae fed the 'three day' treatment was significantly greater than larvae fed the 'copepod' treatment.

Survival

At 9 DPH, the mean survival of larvae was significantly different between dietary treatments (ANOVA, $F_{3,24} = 7.58$; $p = 0.0010$). The mean survival of larvae fed the 'copepod' treatment (57.3 ± 15.4%) was significantly greater than mean survival from the SRD (45.5 ± 9.5%), 'mix' (37.8 ± 2.4%), and 'three day' (34.0 ± 7.6%) treatments (Figure 6-3). The mean survival of larvae fed the SRD treatment was significantly greater than larvae fed the 'three day' treatment. The mean survival of larvae fed the 'mix' treatment was not significantly different from larvae fed the SRD or 'three day' treatments.

Net Stress Resistance

After the 180 second duration of exposure to net stress, mean survival was significantly different between dietary treatments (ANOVA, $F_{3,16} = 3.63$; $p = 0.0359$). The mean survival of larvae fed the 'copepod' treatment (80.0 ± 12.2%) was significantly greater than mean survival of larvae fed the 'three day' (56.0 ± 19.5%) and SRD (54.0 ± 11.4%) treatments (Figure 6-4). The mean survival of larvae fed the 'mix' treatment (76.0 ± 18.2%) was significantly greater than mean survival of larvae fed the SRD treatment, but no significant differences were detected when compared to the 'copepod' or 'three day' treatments. There were no significant differences between the 'three day' and SRD treatments.

After the 360 second duration of exposure to net stress, mean survival was not significantly different between larvae fed the 'mix' (52.0 ± 19.2%), 'copepod' (46.0 ± 15.2%), 'SRD' (42.0 ± 13.0%), and 'three day' (38.0 ± 14.8%) treatments (ANOVA, $F_{3,16} = 0.72$; $p = 0.5542$).

After the 540 second duration of exposure to net stress, mean survival was not significantly different between larvae fed the 'copepod' (56.0 ± 32.1%), 'three day' (36.0 ± 20.7%), 'mix' (32.0 ± 16.4%), and 'SRD' (28.0 ± 21.7%) treatments (ANOVA, $F_{3,16} = 1.41$; $p = 0.2775$).

After the 720 second duration of exposure to net stress, mean survival was significantly different between dietary treatments (ANOVA, $F_{3,16} = 15.31$; $p < 0.0001$). The mean survival of larvae fed the 'three day' treatment (52.0 ± 16.4%) was significantly greater than mean survival of larvae fed the 'copepod' (28.0 ± 16.4%), 'mix' (12.0 ± 11.0%), and SRD (0.0 ± 0.0%) treatments (Figure 6-4). The mean survival of larvae fed the 'copepod' treatment was significantly greater than survival of larvae fed the SRD treatment. There were no significant differences between the mean survival of larvae fed the 'mix' treatment and mean survival of larvae fed the 'copepod' or SRD treatments.

Discussion

Variable results were obtained from providing Florida pompano larvae with copepod diets for the entire 9 day trial. Improvements in growth, survival and resistance to stress were detected for treatments fed copepods compared to those larvae fed the SRD. However, the benefits of those improvements were inconsistent between the copepod fed treatments.

The 'copepod' treatment had the greatest survival for any of the dietary treatments examined. At 9 DPH, however, the recorded growth of those larvae (SL and BD) was significantly lower than any of the other dietary treatments, although reduced growth at both 3 and 6 DPH had not yet become apparent. One explanation for this decreased growth beyond 6 DPH for larvae fed the 'copepod' treatment is an insufficient feeding rate at the observed level of survival. One replicate in the 'copepod' treatment recorded much lower survival (23.8%) than any other replicate in the treatment. The next lowest survival recorded by a replicate from the 'copepod' treatment was 56.0%. When morphometric data, taken at 9 DPH, from larvae fed the 'copepod' treatment with 23.8% survival (23 C) was compared to the replicate with the lowest survival from the SRD treatment (35.4%; 35 R) significant differences in both mean SL (T-TEST, $T_{18} = 3.06$; $p = 0.0068$) and mean BD (T-TEST, $T_{18} = 2.53$; $p = 0.0211$) were detected.

The SL of 23 C (4.58 ± 0.37 mm) was significantly greater than the SL of 35 R (4.00 ± 0.49 mm) and the BD of 23 C (1.22 ± 0.15 mm) was significantly greater than the BD of 35 R (1.03 ± 0.19 mm). Further, when we examined replicates with similar survival rates from both the 'copepod' treatment (56.0%; 56 C) and the SRD treatment (57.0%; 57 R) no significant differences in SL (T-TEST, $T_{18} = 0.01$; $p = 0.9949$) or BD (T-TEST, $T_{18} = -0.10$; $p = 0.9188$) were detected. Since most of the other replicates from the SRD treatment had a lower survival than 57.0% and morphometric means detected when comparing 56 C and 57 R more closely resembled those recorded in the 'copepod' treatment, it can be deduced that a higher feeding rate for the 'copepod' treatment past 6 DPH could have resulted in greater growth in SL and BD. In addition, cannibalism was not observed in any of the replicate tanks for the entire trial.

Similarly, upon examination of larval growth fed the 'three day' treatment a significant decrease when compared to both the 'mix' and SRD treatments at 9 DPH is observed. At 6 DPH, a significant decrease in SL for larvae fed the 'three day' treatment was detected when compared to the SL of larvae fed both the 'mix' and SRD treatments. However, BD at 6 DPH and morphometric data at 3 DPH were not significantly different among any of the dietary treatments. Again decreases in growth after 6 DPH for a dietary treatment fed copepods was observed. When comparing 9 DPH morphometric data from replicates with similar survival from the 'three day' (37.0%; 37 T) and SRD (38.0%; 38 R) treatments, no significant differences are detected for SL (T-TEST, $T_{31.9} = -1.97$; $p = 0.0572$) or BD (T-TEST, $T_{30.7} = -1.85$; $p = 0.0742$). However, the relatively low p-value for both SL and BD, infers that the differences are small and significant differences may be detected with an increased sample size. In addition, unlike the 'copepod' treatment, the 'three day' treatment recorded the lowest survival at the conclusion of the trial. Therefore, a sudden mortality across all replicates would have had to

occur just prior to ascertaining survival to see the low growth associated with an insufficient feeding rate for the 'three day' treatment at 9 DPH. Nutritional disadvantages cannot be ruled out in the reduced growth of larvae fed copepods past 6 DPH.

For the first time in any of the larval rearing trials, *Thalassiosira weissflogii* (TW) was used in the diet of *P. pelagicus*. Dietary effects on *P. pelagicus* fed microalgae diets comparing T-ISO, TW, *Chaetoceros gracilis*, *Rhodomonas lens*, and *Tetraselmis suecica* have been conducted (Ohs et al., unpublished data). Adults fed monoalgal diets consisting of TW produced on average 3–4 times more nauplii than adults fed T-ISO, the traditional diet fed to *P. pelagicus*. Since trials 4 and 5 were simultaneously conducted, elevated numbers of nauplii were required to meet the demand of the treatments for both trials. However, published literature indicates an insufficient ratio of docosahexaenoic acid (DHA): ecosapentaenoic acid (EPA) : arachidonic acid (AA) for marine fish larviculture in *Acartia tonsa*, a marine calanoid copepod, fed monoalgal diets of TW (Stottrup and Jensen, 1990; Stottrup et al., 1999). Furthermore, cod, *G. morhua*, larvae fed *Acartia tonsa* nauplii reared on TW had the lowest growth of any of the treatment diets examined (St. John et al., 2001). With this in mind, our populations of *P. pelagicus* were fed a 1:1 mixture of T-ISO (15–20 million cells/mL), an algae high in DHA (Reitan et al., 1997), and TW (1–2 million cells/mL) in the hopes of both boosting nauplii production, but without the adverse effects on fish larvae from the low DHA found in a monoalgal TW diet. However, low levels of DHA may also account for the decreased growth seen in the 'copepod' and 'three day' treatments.

Conversely, the 'mix' treatment had the greatest growth for any of the dietary treatments examined during trial 4 with respect to SL and BD. Even though mean survival of the 'mix' treatment was significantly lower than the 'copepod' treatment, no significant differences were

detected when compared to those larvae fed the SRD treatment. By feeding a mixture of enriched rotifers and copepod nauplii to pompano larvae for the entire trial, disadvantages in growth and survival from a potential insufficient feeding rate and possible differences in the nutritional composition of live feeds were avoided.

Pompano larvae fed copepod treatment diets showed significantly higher survival compared to larvae fed SRD treatments at the 180 and 720 second duration of net stress exposure. Remarkably, 14 of 15 replicates fed copepods had at least one larva alive in the 720 second treatment duration after one hour, but none of the larvae fed the SRD treatment were alive.

The results of this study reflect the advantages in growth, survival, and resistance to stress that can be attributed to the addition of copepods in the diet of Florida pompano during the first 9 DPH. A better understanding of a feeding regime for pompano larvae that involves copepods has been achieved. Benefits are apparent when larvae are fed copepods for the first 1–2 days of feeding and then are switched to the SRD. However, acquiring information on the advantages of feeding copepods to pompano in a system designed for commercial production is warranted.

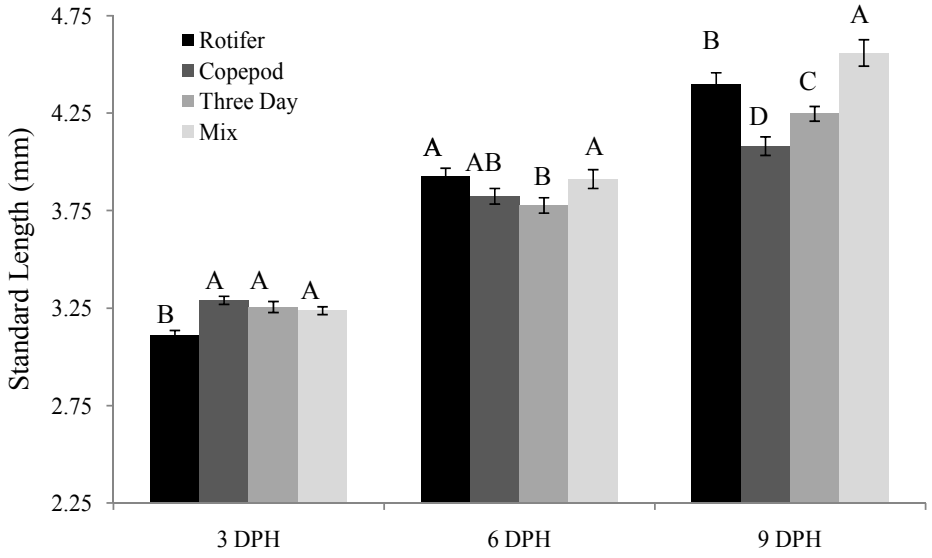

Figure 6-1. The mean standard length (mm) of Florida pompano larvae (*Trachinotus carolinus*) fed different dietary treatments during trial 4. Values were recorded at 3, 6, and 9 days post hatch (DPH). Standard error bars and LSD multiple comparisons test results are displayed. Bars with different letters are significantly different ($p \leq 0.05$).

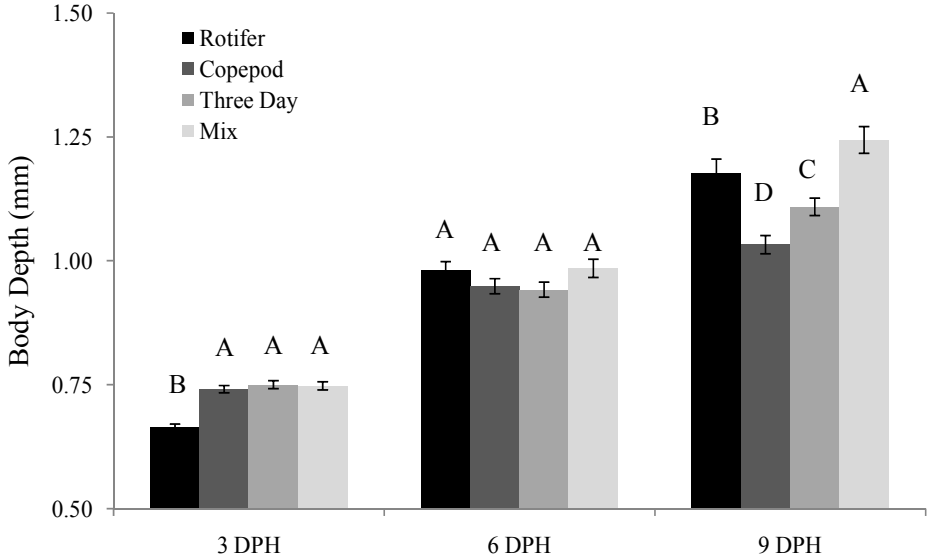

Figure 6-2. The mean body depth (mm) of Florida pompano larvae (*Trachinotus carolinus*) fed different dietary treatments during trial 4. Values were recorded at 3, 6, and 9 days post hatch (DPH). Standard error bars and LSD multiple comparisons test results are displayed. Bars with different letters are significantly different ($p \leq 0.05$).

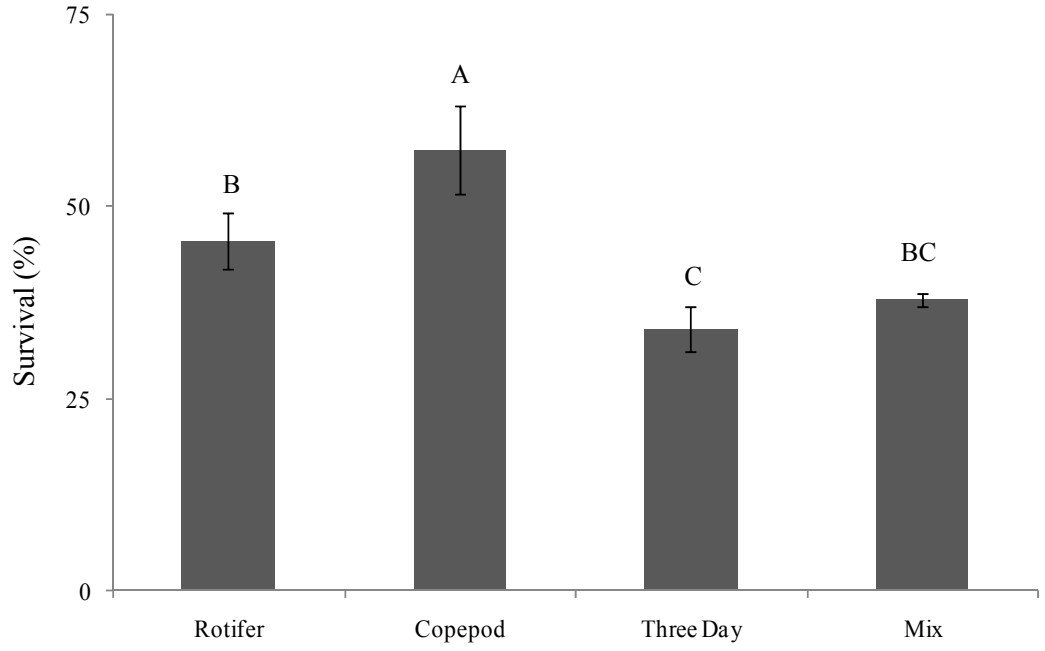

Figure 6-3. The mean survival of Florida pompano larvae (*Trachinotus carolinus*) fed different dietary treatments during trial 4. Standard error bars and LSD multiple comparisons test results are displayed. Bars with different letters are statistically different ($p \leq 0.05$).

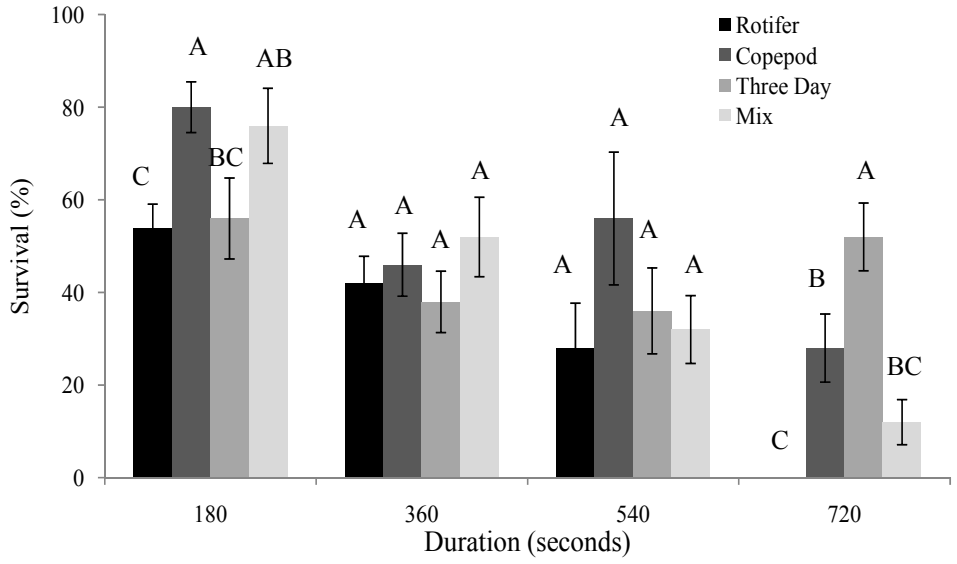

Figure 6-4. The mean survival of Florida pompano larvae (*Trachinotus carolinus*) fed different dietary treatments exposed to net stress during trial 4. Durations were 180, 360, 540, and 720 seconds. Standard error bars and LSD multiple comparisons test results are displayed. Bars with different letters are significantly different ($p \leq 0.05$).

CHAPTER 7
TRIAL 5

The goal of this study was to examine the benefits of feeding nauplii of *Pseudodiaptomus pelagicus* to larval Florida pompano, *Trachinotus carolinus*, in a system designed to simulate commercial production. Commercial facilities often do not adhere to the strict guidelines required for scientific inquiry, and so an investigation of pompano production in a greenhouse under ambient Florida weather conditions was conducted. Florida pompano larvae were either fed the standard reference diet of rotifers (SRD) or copepods for the first day of feeding. Net stress experiments were performed to detect differences in stress resistance of larvae fed the dietary treatments.

Material and Methods

Material and methods from Trial 1 were repeated unless otherwise noted.

Spawning and Egg Incubation

Volitional spawning of wild-caught broodstock supplied larvae for this trial. Incubation occurred at ambient greenhouse temperatures (27.8–32.1°C) and a salinity of 34.7 g/L.

Larval Rearing and Experimental Design

A 10-day larval rearing trial was conducted in two recirculating aquaculture systems each consisting of 8, 170-L cylindrical, conical-bottom fiberglass tanks with black walls and bottom. A natural photoperiod of approximately 14 hours light and 10 hours dark with ambient daytime surface light levels below 6430 lux (Milwaukee Model SM700) recorded during the larval rearing trial. The initial water flow was 354 mL/minute and gentle aeration was provided. Beginning on 2 DPH, tanks were inoculated daily with *Nannochloropsis oculata* paste (Algagen LLC, FL) to a density of 450,000–1,200,000 cells/mL and water flow was increased to 472 mL/minute. At 4 DPH, aeration was increased slightly and water flow was adjusted to 708

mL/minute. At 7 DPH, aeration was again increased slightly and water flow was adjusted to 945 mL/minute for the duration of the trial. In each replicate tank, temperature, salinity, dissolved oxygen, and pH were monitored daily (YSI Incorporated Model 556 MPS, OH). Temperature from one randomly selected replicate tank was also recorded every 30 minutes during the entire trial with a StowAway® Tidbit® temperature logger (Onset Computer Corporation, MA).

Dietary Treatments

Two dietary treatments were examined in this study, a SRD consisting solely of enriched rotifers and a 'one day' diet (Table 3-1). Each dietary treatment was replicated six times and two tanks from each system contained no larvae to reduce water quality problems associated with biomass. The SRD consisted of enriched rotifers fed to larvae four times daily (0900, 1200, 1600, and 1900) at a rate of 2.5 rotifers/mL/day from 2–5 days post hatch (DPH) and a rate of 3.0 rotifers/mL/day from 6–9 DPH. The 'one day' experimental diet consisted of feeding larvae copepod nauplii on 2 DPH at a rate of 1.11 nauplii/mL/day and then switching to the SRD from 3–9 DPH. From 2 DPH to 9 DPH larvae were fed diets consisting of enriched S-strain rotifers, *Brachionus plicatilis*, and *P. pelagicus* nauplii.

Rotifer Culture and Enrichment

Rotifers were cultured in a 950-L cylindrical, flat-bottom, fiberglass tank. Approximately, 20–25 million rotifers were harvested daily and were enriched prior to feeding to fish larvae. Rotifers were enriched in two 19-L buckets.

Copepod Culture

Copepods were batch cultured in five 200-L cylindrical, flat-bottom polyethylene tanks and one 1800-L cylindrical, conical-bottom tank. Copepod populations were fed a 1:1 mixture of Tahitian strain *Isochrysis galbana* (T-ISO) (15–20 million cells/mL) and *Thalassiosira weissflogii* (TW) (1–2 million cells/mL). Each 200-L population was fed 1–2 L of the algal mix

and the 1800-L population was fed 18–24 L. Copepod nauplii were harvested twice daily from each tank via floating airlifts ('Equipment' section of Chapter 2) within each tank.

Sample Collection and Morphometric Analysis

Larvae from each experimental tank were randomly sampled on 3, 6, and 9 DPH for morphometric analysis. Larvae were euthanized with buffered Tricaine-S (Tricaine Methanesulfonate; Western Chemical Inc., WA) and photographed. For calibration purposes, larvae were photographed on a Sedgewick-Rafter Cell S50 (PYSER-SGI Limited, Kent, UK) with a 1.0-mm square grid base.

Net Stress Resistance Analysis

On 9 DPH, ten larvae were randomly selected from each replicate tank and gently poured into units ('Material and Methods' section of Chapter 3) containing culture system seawater maintained at ambient greenhouse temperature and a salinity of 34 g/L. Two replicates from each tank were tested. Larvae were then lifted out of 1-L containers with the screen bottomed 0.5-L containers and held out of water for 75, 180, 360, or 720 seconds.

Results

Water Quality

All water quality parameters were recorded within normal limits (Table 3-1).

Growth

At 0 DPH, larvae had a mean standard length (SL) of 2.91 ± 0.20 mm (mean ± SD) and a mean body depth (BD) of 0.80 ± 0.09 mm. At 3 DPH, mean SL of larvae was not significantly different for larvae fed the 'one day' (3.07 ± 0.20 mm) and SRD (3.02 ± 0.24 mm) treatments (T-TEST, $T_{98} = 0.97$; $p = 0.3344$). The mean BD of larvae was not significantly different for larvae fed the 'one day' (0.66 ± 0.05 mm) and SRD (0.65 ± 0.04 mm) treatments (T-TEST, $T_{98} = 1.08$; $p = 0.2816$).

At 6 DPH, mean SL of larvae was not significantly different for larvae fed the 'one day' (3.77 ± 0.25 mm) and SRD (3.77 ± 0.21 mm) treatments (T-TEST, T_{98} = -0.14; p = 0.8871). The mean BD of larvae was not significantly different for larvae fed the 'one day' (0.90 ± 0.09 mm) and SRD (0.87 ± 0.09 mm) treatments (T-TEST, T_{98} = 1.41; p = 0.1631).

At 9 DPH, mean SL (T-TEST, T_{98} = 2.11; p = 0.0377) and BD (T-TEST, T_{98} = 2.18; p = 0.0317) of larvae were significantly different between larvae fed the dietary treatments (Figure 7-1). The mean SL of larvae fed the 'one day' treatment (4.11 ± 0.30 mm) was significantly greater than the mean SL of larvae fed the SRD treatment (3.99 ± 0.31 mm). The mean BD of larvae fed the 'one day' treatment (1.05 ± 0.10 mm) was greater than the mean BD of larvae fed the SRD treatment (1.00 ± 0.13 mm) (Figure 7-2).

Survival

At 9 DPH, mean survival of larvae fed the SRD (29.3 ± 9.4 %) and 'one day' (24.6 ± 6.0%) treatments were not significantly different (T-TEST, T_8 = -0.90; p = 0.3955) (Figure 7-3).

Net Stress Resistance

After the 75 second duration of exposure to net stress, the mean survival of larvae fed the 'one day' (36.0 ± 25.0%) and SRD (25.0 ± 27.2%) treatments were not significantly different (T-TEST, T_{18} = -1.06; p = 0.3013).

No larvae were alive in any of the replicates from either dietary treatment at the 180, 360, and 720 second durations of exposure to net stress, so no statistical analysis was necessary.

Discussion

Improvements in growth were detected for larvae fed copepods compared to those larvae fed the SRD. At 9 DPH, larvae fed the 'one day' treatment had significantly greater SL and BD. An appropriate feeding rate for larvae fed the 'one day' diet on 2 DPH would have been 2–3 nauplii/mL. Unfortunately, not enough copepod nauplii were produced to meet that goal.

Since larvae used in trial 4 and 5 came from the same spawn and the same SRD feeding regime was used in both trials, a comparison of those larvae fed the SRD treatments can be made between the two systems and their effect upon larval performance. However, larvae fed the SRD treatment in trial 5 (170-L system, Greenhouse = G) did have an increased feeding rate from 6–9 DPH and survival from that system was recorded one day later than survival recorded for trial 4 (13-L system, Hatchery = H). That may explain why survival of larvae in H (45.5 ± 9.5%) was significantly greater than survival of larvae in G (29.3 ± 9.4%) (T-TEST, $T_{10} = 3.10$; $p = 0.0113$). Reduced survival during that specific 24 hour period may have been likely, as pompano larvae are routinely transitioned onto *Artemia* nauplii beginning on 9 DPH (Charles R. Weirich, pers. comm.). Since larvae were not fed *Artemia* nauplii during trial 5, a sufficient quantity of feed may not have been available. Furthermore, the elevated temperature (>26°C) may have accelerated development of larvae and decreased the time when provision of *Artemia* should have been initiated.

Another explanation is that survival was initially reduced prior to first feeding. Under the ambient conditions of the greenhouse, larvae were exposed to incubation temperatures above those experienced in the hatchery (26°C). This may have led to an accelerated depletion of yolk-sac reserves prior to first feeding. When the ratio of body depth to standard length (BD:SL) for 0 DPH larvae from both incubation systems was compared, significant differences were detected (T-TEST, $T_{118} = -4.42$; $p < 0.0001$). Larvae from the H incubation system (0.30 ± 0.03) had a significantly higher BD:SL ratio than larvae from the G incubation system (0.27 ± 0.04). Furthermore, the BD:SL of larvae from the incubation systems in trials 1, 2, and 4 were significantly greater than the BD:SL of larvae from the incubation system in trial 5 (ANOVA,

$F_{3,237} = 17.82$; $p < 0.0001$). Upon examination of photographs taken of larvae from both systems on 0 DPH, reduced yolk-sac reserves were seen in larvae from the G incubation system.

No significant differences in growth at 3 DPH were detected for the SL or BD of larvae grown in either H or G. However, SL and BD at 6 and 9 DPH for larvae grown in H were significantly greater than SL and BD for larvae grown in G. The increased feeding rate of 0.5 rotifers/mL/day for larvae in the G system may have had a negative effect on growth during this period.

Interestingly, when comparing survival of larvae from the two systems, a reduction in survival from the G system is observed. The 'one day' treatment in the G system may not have received sufficient nutrients due to a low feeding rate on 2 DPH. This would have subsequently led to a reduced performance in survival, growth, and stress resistance when compared to copepod fed treatments from the H system. These differences, however, can not be applied to the SRD treatments from both systems, which received identical diets during the larval trials.

During this trial, 1.1 million nauplii were used to feed the 'one day' treatment. This feeding rate (1.1 nauplii/mL/day) provided advantages in growth for Florida pompano at that larval density. To reach a rate of 2–3 nauplii/mL/day, approximately 2–3 million nauplii would have had to have been produced. Using current methods, it is possible for *P. pelagicus* populations to produce this quantity of nauplii. However, the consistency of that production is not known. Furthermore, as the feeding rate for Florida pompano increases, it would be difficult to produce appropriate amounts of copepod nauplii to satisfy their demand. We were able to feed copepod nauplii to Florida pompano larvae for one day, but continued feeding at that rate would have been difficult.

The results of this study showed the advantages in growth and resistance to stress attributed to the addition of copepods in the diet of Florida pompano on the first day of feeding compared to those larvae fed the SRD. Furthermore, these results do verify results obtained in the 13-L system and provide valuable reference to the number of copepods required to obtain beneficial effects. Although advantages in growth were seen for Florida pompano larvae fed the 'one day' treatment, an increased feeding rate may have further improved results. However, continuous copepod production at the required scale has not been achieved and further research is warranted.

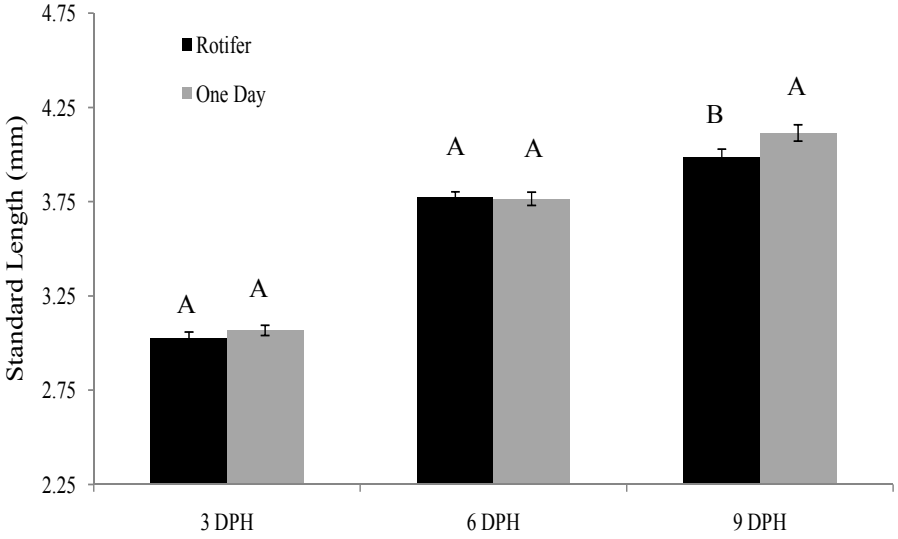

Figure 7-1. The mean standard length (mm) of Florida pompano larvae (*Trachinotus carolinus*) fed different dietary treatments during trial 5. Values were recorded at 3, 6, and 9 days post hatch (DPH). Standard error bars and student's t-test results are displayed. Bars with different letters are statistically different ($p \leq 0.05$).

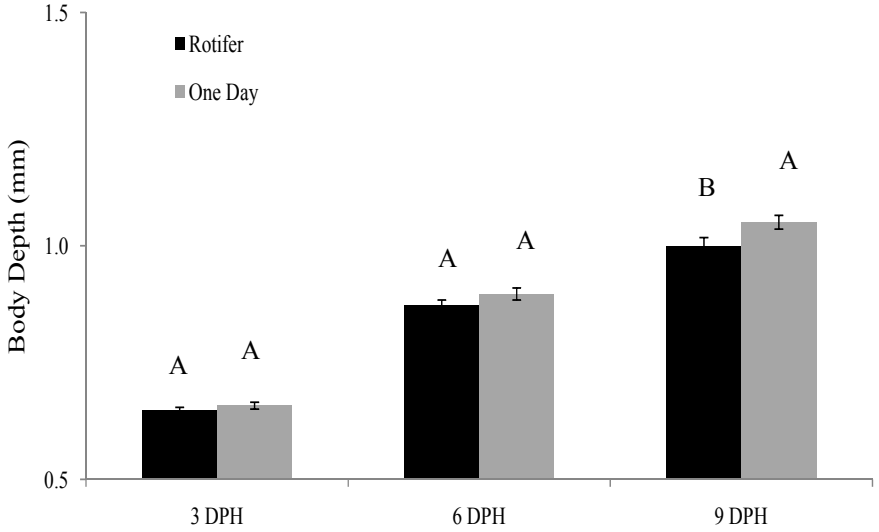

Figure 7-2. The mean body depth (mm) of Florida pompano larvae (*Trachinotus carolinus*) fed different dietary treatments during trial 5. Values were recorded at 3, 6, and 9 days post hatch (DPH). Standard error bars and student's t-test results are displayed. Bars with different letters are statistically different ($p \leq 0.05$).

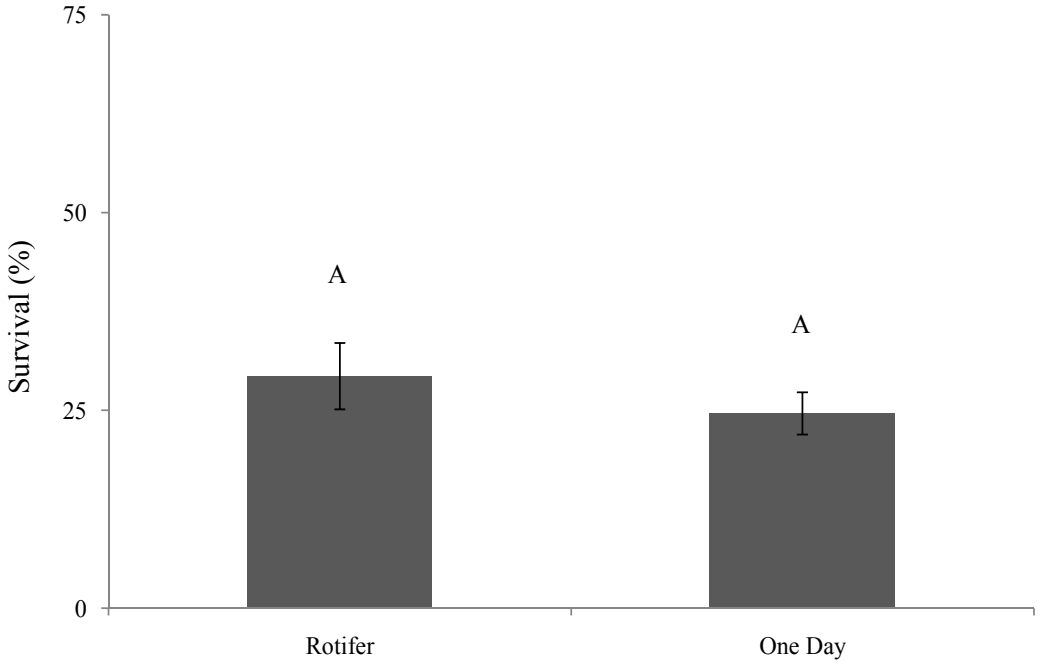

Figure 7-3. The mean survival of Florida pompano larvae (*Trachinotus carolinus*) fed different dietary treatments during trial 5. Standard error bars and student's t-test results are displayed. Bars with different letters are statistically different ($p \leq 0.05$).

CHAPTER 8
CONCLUSION

Five dietary treatments consisting of *Pseudodiaptomus pelagicus* fed to larval Florida pompano, *Trachinotus carolinus*, for various durations, at various concentrations and combinations were examined. Subsequent stress resistance of those larvae was examined at the conclusion of each trial through either net or salinity stress experiments. Furthermore, microalgae and copepod culture methods are summarized. A feeding regime for Florida pompano larvae which includes *P. pelagicus* has been elucidated and methods for production and harvest of *P. pelagicus* are identified.

Feeding copepod nauplii in addition to feeding rotifers, has traditionally proven beneficial for the production of marine fish larvae. Toledo et al. (1999) fed copepod nauplii (mainly *Acartia tsuensis*) to grouper, *Epinephelus coioides*, larvae from 2-6 days post hatch (DPH), switching to a rotifer diet from 7-18 DPH. When compared to larvae fed rotifers during the entire 18 day trial, improvements in feeding incidence, survival, and growth were detected (Toledo et al., 1999). First feeding turbot, *Psetta maxima*, larvae had higher growth and survival when fed *Tisbe holothuriae* nauplii for the first 3–6 DPH before switching to rotifers, when compared to rotifers for the entire 8 DPH (Stottrup and Norsker, 1997). Payne et al. (2001) fed pink snapper, *Pagrus auratus*, larvae nauplii of the copepod *Gladioferens imparipes* from 4–10 DPH and then switched to a rotifer diet for 15 more days. When compared to larvae fed rotifers for the entire 25 day period, increased growth from 6 DPH on was observed when copepods were fed (Payne et al., 2001). Survival and swim bladder inflation were also greater for larvae fed copepods (Payne et al., 2001).

Beneficial results were also seen during the course of the present study when copepod nauplii were fed to pompano larvae for either one or three days prior to transitioning to the

standard reference diet of rotifers (SRD). Three different feeding rates for each period of copepod provision were evaluated and results consistently displayed advantages to pompano larvae in growth, survival, and stress resistance. Pompano larvae fed the 'one day' diet during the course of this evaluation consistently outperformed larvae fed the SRD. Pompano larvae fed the 'three day' diet during trial 4 did not perform as well as larvae fed the SRD. However, this may have been due to nutritional deficiencies of the microalgae, *Thalassiosira weissflogii*, fed to the copepods during trial 4. Larvae fed the 'three day' diet did not perform as well as larvae fed the 'one day' diet during trial 1. But this may have been related to prey density and not providing adequate numbers of copepods. Results clearly indicate that providing copepod nauplii to Florida pompano larvae for at least the first day of feeding at a rate of 2–3 nauplli/mL/day improved growth, survival and stress resistance.

A mixed diet, consisting of enriched rotifers and copepod nauplii fed to pompano larvae at a ratio of 4:1 increased growth and stress resistance compared to larvae fed the SRD. In Payne et al. (2001) significant increases in growth were seen when pink snapper, *Pagrus auratus*, larvae fed a 4:1 ratio of enriched rotifers, *Branchionus* spp., and copepod nauplii, *Gladioferens imparipes*, compared to larvae fed only enriched rotifers. Southern flounder, *Paralichthys lethostigma*, larvae had significantly higher growth and survival when fed a 1:1 mixture of enriched rotifers, *B. plicatilis*, and *Acartia tonsa* nauplii when compared to larvae fed rotifers exclusively (Wilcox et al., 2006). Finally, yellowtail clownfish, *Amphiprion clarkii*, larvae fed a 1:1 mixture of rotifers, *B. plicatilis*, and *Tisbe* spp. nauplii resulted in significantly higher growth and survival when compared to those larvae fed only rotifers or only *Tisbe* nauplii (Olivotto et al., 2008). During trial 4, pompano larvae fed a 'mix' diet had greater growth and stress resistance than larvae fed the SRD. By feeding a 'mix' diet possible nutritional deficiencies of

enriched rotifers were avoided, and it is easier to maintain an appropriate density of live feeds within the larval rearing tanks when rotifers are included in the daily ration.

Larvae fed only copepods during the entire larval trial recorded the highest survival but had the lowest growth from any of the dietary treatments. This was likely due to an insufficient quantity of prey items available to the pompano larvae. Although some fish species, like red snapper, *Lutjanus campechiensis*, require copepod nauplii for development during the larval phase (Lemus et al., 2008); pompano larvae do not appear to require copepods. However, the clear benefits of providing copepod nauplii to larval Florida pompano have been identified. A mesocosm treatment was attempted two times and was not successful. However, valuable information was attained on the preparation of copepods for a mesocosm, including when and how many to stock into the larval rearing system. The advantages provided by this feeding strategy, especially greatly reduced labor, warrant a continued thorough examination.

Significant increases in resistance to net stress were also observed for those larvae fed copepods during this evaluation. The increase in survival was directly related to the duration of time larvae were fed copepods. The larvae fed the 'mix' diet also had significantly increased resistance when compared to larvae fed the SRD. Notably, 7–9 day old pompano larvae fed copepods and exposed to more than 600 seconds (10 minutes) of net stress were still alive one hour after being submerged in culture water. Similar resistance to stress was reported by Kraul et al. (1993) when mahimahi, *Coryphaena hippurus*, larvae were fed the harpacticoid copepod *Euterpina acutifrons*. In a similar net stress test, higher survival was recorded for larvae fed copepods at both the 60 and 120 second durations when compared to larvae fed enriched *Artemia* nauplii (Kraul et al., 1993). In that study, significantly higher levels of docosahexaenoic acid (DHA), provided by the copepods, were believed to have led to increased resistance to net stress.

In Kanazawa (1997), increased levels of dietary DHA were also reported to significantly improve net stress resistance in larval red sea bream, *Pagrus major*. As larvae are often netted for transport within the hatchery facility or to another location, their ability to withstand exposure to net stress is important to increased survival and subsequent production.

Populations of *P. pelagicus* were successfully cultured and the use of airlifts for nauplii collection was examined. Airlifts should be the method used for harvesting nauplii to feed to marine fish larvae to reduce stress on the adult and copepodite populations. Mass-scale culture of *P. pelagicus* was conducted and was shown to be very effective and labor efficient. Similar systems were developed by Payne and Rippingale (2001a) for *Gladioferens imparipes*. Their systems incorporated biofiltration and were kept in continuous culture for approximately 14 months. In the current study, the system was only used for 8 days. However, results provided estimates of nauplii production from this scale of system and can be equated to the nauplii demanded by marine fish larvae.

On a commercial scale, copepod nauplii, using current production methods, can only be fed to pompano larvae during the initial feeding phase. As larval feeding rates increase with growth, the feasibility of feeding copepods decreases because of the production necessary. Based on results of this evaluation, a diet including copepod nauplii beyond 3 DPH appears unnecessary. Therefore, a feeding regime which includes copepods fed to pompano larvae on 2–3 DPH at a rate of 2–3 nauplii/mL/day is recommended. Most commercial marine fish production facilities do not have systems to grow copepod populations. However, given the short duration of copepod provision necessary, simply purchasing and feeding copepod nauplii for the initial 2–3 DPH is a viable option.

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BIOGRAPHICAL SKETCH

Eric J. Cassiano was born in Buffalo, New York in 1975 and moved, at the age of 6 months old, to North Carolina. He spent his youth exploring the wilderness surrounding his house and developed an appreciation for wildlife. After high school, Eric continued those explorations through the United States, spending time in Oregon, Colorado, California, New York, and traversing the country numerous times before settling down to continue his academic career.

Eric received his Bachelor of Science in Marine Biology from Hawaii Pacific University in the spring of 2002. After a brief period with the Oregon Department of Fish and Wildlife working in fisheries science, he began working for the Oregon State University Molluscan Broodstock Program, where he was exposed to the culture of the Pacific Oyster, *Crassostrea gigas*. After a few years there, he began a position for the University of Florida where he focused on various aspects of the hard clam (*Mercenaria mercenaria*) production industry. Eric was then awarded a graduate assistantship and began his thesis studies under Dr. Cortney Ohs and Dr. B. Denise Petty for the University of Florida.

Eric has been blessed with a beautiful wife and a vivacious young boy, who both bring him joy and serenity. Furthermore, he wholeheartedly believes in the ideals of aquaculture as a means to preserve our natural aquatic systems while continuing to meet the demands of the food, bait, and ornamental markets.