FRESHWATER CLAMS AS A TREATMENT MECHANISM FOR PHOSPHORUS IN AGRICULTURAL WASTEWATER

By

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To my parents, Captain Roy “Luke” Riley (United States Navy, Retired) and Linda C. Riley,

Thank you for all of your love, support and confidence,
I couldn’t have done this without you both, I love you

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The objective of this study was to determine the potential of using a recirculating raceway system to remove phosphorus-containing material from agricultural wastewater streams. The focus of the research was on the biological and physical characteristics of *Corbicula* populations and monitoring of various water quality parameters within the system, with special emphasis on phosphorus dynamics. A prototype raceway system was designed and constructed at the University of Florida Dairy Research Unit at Hague, Florida to test the adaptability and phosphorus removal capacity of the clams in wastewater treatment.

The ability of freshwater clams to capture, sequester and retain phosphorus-containing material from varying amounts of fertilizer additions was demonstrated in this study. Clam biomass contained an average phosphorus concentration of 0.299 mg P/g of whole clam DW (SE = 0.005), similar to other bivalves. Tagged clams recaptured alive over the course of the study showed growth rates of up to 0.117 mm/day in shell length (0.0024 g clam DW/day), yielding phosphorus removal rates up to 0.0079 mg P/individual/day. Overall, raceway clam populations were subject to high mortality and were unable to demonstrate significantly long-term removal of total phosphorus, dissolved phosphorus or chlorophyll *a* from overlying source water. High

temperatures and possible impacts from amphipod infestations may have affected clam populations.

Even though some clams in this study did survive and grow, use of *Corbicula* culture for phosphorus treatment in Florida agriculture operations may require creative solutions to temperature and parasite problems. Despite these issues, the raceway-based recirculation system design demonstrated in this study provided a dependable, easy to construct and reusable platform for testing aquaculture potential of a variety of organisms in wastewater treatment conditions at large scale. The ultimate goal of this study was to provide an effective biological remediation mechanism for removal of phosphorus from dairy waste streams; however, toxicity of dairy effluent, even at high dilutions, may prohibit application of clam-based aquaculture systems without additional treatment mechanisms.

CHAPTER 1
INTRODUCTION

Phosphorus (P) produced in waste management of agricultural operations can result in degradation of water quality in surface and groundwater flows. An addition of nutrients to aquatic systems by human activities has resulted in the growth of nuisance macrophytes, alga bacteria and periphyton in natural and man-made systems (Sharpley 1994, Johnson et al. 2004, Dao et al. 2006, Wetzel 2001, Phlips et al. 2002, Timmons et al. 2002). This unwanted growth can also cause contamination of drinking water supplies, degradation of aquatic habitat for desirable species, fouling of engineered water systems, limitation of navigation and other negative impacts to commercial and recreational activities (Sharpley 1994, Johnson et al.. 2004, Dao et al. 2006). In warmer climates such as Florida, aquatic plant growth is magnified due to the nearly year-round growing season (Scinto and Reddy 2003, DeBusk et al. 2004). The focus of the current study was application of a clam-based biofiltration approach to remove of particulate phosphorus from agriculture wastewater streams, with special emphasis on dairy systems.

Excess nutrients can enter surface waters from both point and non-point sources associated with concentrated farming activities (Sharpley et al. 1994, Knight et al. 2000, Johnson et al. 2004, Dao et al. 2006). Manure and wastes produced in dairy operations are much different than other land-based agriculture operations since much of the waste products generated have high water content due to milk parlor operations and bedding waste handling (NRCS 1999, Wilkie 2003). High liquid fractions in dairy wastes are encouraged by the removal of solids through settling and through microbial decomposition by anaerobic digestion (Wilkie 2003). Management practices that target nutrients such as phosphorus from animal manure include oxidation ponds, facultative lagoons and storage ponds in conjunction with land application,

constructed wetlands and composting (NRCS 2006). In order to manage Florida dairy wastewaters, liquids are stored in short-term retention ponds prior to land application that supplies water and fertilizer to forage crops (Wilkie 2003).

There are severe limitations to the technologies currently available to reduce, effectively and economically, nutrient levels in agricultural wastewater streams. Some nutrients are bound in the terrestrial environment, but the remainder end up in surface water bodies (Sharpley et al. 1994) and can also enter groundwater (Johnson et al. 2004). Phosphorus is of special concern because it is the primary limiting factor for growth of algae and other plants in most freshwater systems (Wetzel 2001), and it cannot be removed by volatilization, as in the case of other important nutrients, such as nitrogen and carbon (Wetzel 2001, Timmons 2002). Dairy wastes subjected to anaerobic digestion can pose some special problems for aquatic environments because of their high liquid content (Wilkie et al. 2004) and high dissolved phosphorus content, making them readily available for uptake by aquatic macrophytes and algae (Sooknah and Wilkie 2004).

In response to the special demands of nutrient removal from wastewater streams, recent research has focused on integration of aquaculture systems. Use of aquaculture for wastewater treatment is designed to convert phosphorus into a solid form that can be harvested as a potentially useful commodity. In order to be successful, these culture systems must be capable of promoting growth and reproduction of the organisms that are the end product of the process. However, organisms capable of desirable water treatment functions, such as filtration of particulate phosphorus-containing matter, in the natural environment may not conform to aquaculture conditions. Therefore, the experimental system design should provide as many

natural habitat conditions for the target organisms as possible, including hydrology, food resources, substrate and temperature.

Unlike production aquaculture, organisms in wastewater treatment systems are selected for their ability to manipulate water quality parameters within source water and not necessarily for their specific value as a consumer commodity. Development of experimental and eventually commercial wastewater treatment systems is therefore driven more by public opinion on environmental issues through government legislation than by consumer product demand and profit normally associated with traditional aquaculture species. However, like traditional aquaculture systems, wastewater designs must also be cost effective, not too land intensive and have low water demands to be economically and environmentally feasible.

Various aquatic organisms have been evaluated for potential applications in the treatment of agricultural wastewater, including dairy. Plant-based systems have received the most attention; however, some animal based systems have also been proposed. Aquatic macrophyte systems remove phosphorus from dairy effluents in small and large-scale systems (Reddy and Smith 1987, Sooknah and Wilkie 2004, Lansing and Martin 2006, Wood et al. 2007). Periphyton-based systems similar to the Algal Turf Scrubber (ATS) technology developed by Adey and Hachney (1989) have been demonstrated to remove phosphorus from dairy wastewaters at experimental scales (Pizarro et al. 2002) (Mulbry and Wilkie 2001). Algae suspended in large outdoor tanks containing dairy wastewater have also demonstrated phosphorus treatment potential (Sooknah and Wilkie 2004), as have phytoplankton in laboratory flasks (Lincoln et al. 1993, Lincoln et al. 1996).

Freshwater, pond-scale studies of aquaculture-based wastewater treatment have focused mostly on fish (Greer and Ziebell 1974, Dempster et al. 1995, Van Rijn 1996, Drapcho and

Brune 2000, Prein 2002, Azim et al. 2003, Ghaly 2005, Sindilariu 2007) and to a lesser extent on filter-feeding bivalves (Busch 1974, Buttner and Heidinger 1980, Buttner 1986). Use of bivalves in polyculture with other organisms has also been demonstrated as a treatment mechanism in freshwater aquaculture operations in fish polyculture ponds (Buttner and Heidinger 1980, Buttner 1986, Soto and Mena 1999). Use of bivalves for wastewater treatment is more pronounced in the mariculture industry, where more elaborate systems have been used in conjunction with phytoplankton and/or seaweed to remove nutrients generated from finfish and shrimp culture (Shpigel 1993, Shpigel and Neori 1996, Lefebvre et al. 2000, Jones et al. 2001, Mazzola and Sara 2001).

Freshwater clams were proposed and for use nutrient reduction by Greer and Ziebell (1972) and Stanley (1974) who made calculations based on available literature; however, no large-scale systems have been tested. Little is known about phosphorus sequestration by these organisms or their potential for large-scale culture using dairy wastewater. Freshwater clams may provide an ideal P-removal vector for dairy wastewater because of their ability to remove and sequester phosphorus from the overlying water column (Fuji 1979). In a typical clam-based system, dissolved phosphorus from wastewater is converted to a particulate form through a phytoplankton intermediary and is coupled with other wastewater particulates to feed clam populations (Greer and Ziebell 1972, Stanley 1974). Filter feeding allows the clam to pump overlying water through its siphon and into the mantle cavity where particulates are then removed by the gills and converted to biomass (McMahon and Bogan 2001). Sequestered phosphorus in clam biomass and sediment depositions can be periodically removed at harvest intervals to remove phosphorus permanently from the treatment system.

One of the clam species that has been the focus of past efforts in treatment systems is the well known invasive, *Corbicula*. This organism is a recent invader to North America as described in McMahon and Bogan (2001), and is a significant contributer to fouling in power plants and industrial raw water systems Williams and McMahon (1986). The success of this organism as a biofouling agent is due to its high reproductive fecundity stemming from self-fertilization no complex life cycle needing water-born gametes and intermediate hosts, unlike freshwater mussels that possess all of these traits (McMahon and Bogan 2001, McMahon 2002). A statewide distribution of *Corbicula* has been noted in most Florida waterways (Blalock and Herod 1999), so the clam can be considered as a naturalized species instead of a potential invasive species. Its high reproductive potential makes the clam an ideal candidate for propagation under aquaculture conditions, since it should repopulate rapidly following harvest of only a few individuals.

Corbicula can be found throughout most of North American are all expected to be very similar due to reproductive characteristics such as hermaphrodism and self-fertilization that result in the production of exact copies of the parent lineage as examined in McMahon and Bogan (2001). Exact taxonomic determination of clams in the genus *Corbicula* is subject to intense debate and ontogenetic variation can lead to improper usage of different species designations such as *fluminea* and *japonica*, that are commonly used in the literature to describe most *Corbicula* clams found in freshwater environments. For this reason, clams in this study are only referred to as *Corbicula*.

The goal of this study was to analyze the phosphorus removal potential of an engineered raceway system containing populations of the freshwater clam *Corbicula*. The potential for using freshwater clams as a mechanism for phosphorus removal was based on its ability to

remove and sequester phosphorus through active biofiltration. This study had the following objectives:

- Design, construct and operate a large-scale raceway-based treatment system for examining the performance of freshwater clams as a P removal mechanism
- Determine the adaptability of the freshwater clam, *Corbicula,* to wastewater treatment conditions
- Determine the ability of clam raceways to remove and sequester phosphorus containing material from agricultural waste streams

In this study, a raceway-based recirculating system was developed in order to study phosphorus removal rates using clam populations under simulated wastewater conditions in raceway systems. Design and construction of systems that can evaluate the performance of organisms in wastewater aquaculture at a commercial level are critical for developing nutrient management strategies for future applications. Systems must account for problems not only with the aquaculture practices, but with the conditions unique to dairy wastewater effluent that may be remedied by dilution (Sooknah and Wilkie 2004). The following questions were posed for investigation in this study:

- What are the growth rates of clams and survival and recruitment rates of clam populations exposed to different concentrations of nutrients?
- Does the physiological condition of raceway clam vary over time and does it correlate with nutrient addition, environmental parameters or mortality events?
- Are clam raceway systems able to capture, sequester and retain P-containing material from varying concentrations of dairy wastewater effluent?
- How do seasonality, clam population dynamics, temperature, algal density and P availability affect the removal of P-containing material by clam raceway systems?
- How is P sequestered and allocated by clams into soft tissue and shell biomass?
- Is this technology suitable for use as a mechanism for P-removal by agricultural operations in Florida?

CHAPTER 2
RACEWAY-BASED RECIRCULATING WASTEWATER TREATMENT SYSTEM DESIGN AND CONSTRUCTION

Introduction

Most biologically-based wastewater treatment systems involve use of ponds, tanks or raceways as steps in the removal of solids, nutrients and contaminants (Buttner 1986, Shpigel 1993, MacMillan et al. 1994, Shpigel et al. 1997, Jara-Jara et al. 1997, Jones and Preston 1999, Jones et al. 2002, Sooknah and Wilkie 2004). The focus of this design effort was removal of nutrients using filter-feeding bivalves as the active agent in the final stage of a process beginning with conversion of soluble nutrients into particulate forms via production of plankton. The design had to meet several key criteria in terms of both experimental and operational demands. From an experimental standpoint, the system had to incorporate the ability to deal with multiple treatment groups in a replicated manner. Operationally, the system had to be of sufficient size to provide a reasonable measure of potential success in real life applications.

Many elements of traditional aquaculture system designs were incorporated into the design process of the raceway-based treatment systems used in this study. Aquaculture system hydrology flows either flow-through or recirculating water flow regimes, depending upon the extent of water reuse and residence time (Van Rijn 1996). Recirculating systems offer the distinct advantages of lower water consumption and confinement of wastes, reducing potential harm to the natural aquatic environment (Timmons et al. 2002). These attributes make recirculting systems ideal for study of wastewater treatment mechanisms because they do not involve discharge into the environment.

Raceway-based aquaculture systems have been used to cultivate a variety of aquatic organisms including fish, bivalves, algae and plants (Shpigel and Neori 1996, Adey et al. 1993). The most common large-scale raceways are usually associated with the production of finfish,

such as salmonids (Timmons et al. 2002). These structures typically range in size from 3-5.5 m in width, 24-46 m in length and 0.8-1.1 m deep (Timmons et al. 2002). This larger scale limits construction materials most often to concrete, plastic, or earthen structures with plastic liners (Sindilariu 2007, Van Rijn 1996). Reinforced fiberglass panels have also been used in finfish culture to construct raceways using a modular design as an alternative to concrete (Vantaram 2004). Raceways used in the commercial rearing of Quahog clams (*Merceneria merceneria*) employ long, sand-bottomed, flow-through plastic troughs to raise juveniles prior to placement in estuarine farm sites for grow out (Lorio and Malone 1995). In addition to widespread commercial applications, raceways have also been used in a variety of experimental aquaculture systems targeting organisms associated with biofiltration or bioaccumulation, such as bivalves, benthic microalgae and macrophytes (Shpigel 1993, Craggs et al. 1996).

The majority of experimental raceway designs using bivalves are involved in wastewater remediation. In these systems, commercially valuable bivalve species are commonly cultured as a secondary commodity on the effluent of primary culture organisms such as finfish and shellfish (Buttner 1986, Shpigel 1993, MacMillan et al. 1994, Shpigel et al. 1997, Jara-Jara et al. 1997, Jones and Preston 1999, Jones et al. 2002, Zhou et al. 2006). Examples of raceways for the culture of bivalve species include: 14.4 L fiberglass tanks (Shpigel et al. 1997), 34 L plastic tanks (Jones and Preston 1999), 340 L plastic tanks (Huchette et al. 2003), 1500L concrete tanks (Jones et al. 2002), 1500 L fiberglass tanks (MacMillan et al. 1994), 2240 L fiberglass tanks (Jara-Jara et al. 1997), 2 m^3 V-bottom fiberglass tanks (Shpigel 1993), and 15,000 m^3 concrete tanks (Zhou et al. 2006).

A variety of raceway designs have also used algae or higher plants as the active treatment agent. Possibly the most notable vegetative raceway system is the Algal Turf Scrubber, which

consists of an artificial stream used to culture periphytic algae. Periphyton is grown on plastic mesh screens placed in shallow rectangular flumes. Source water is supplied using a pulse-flow regime down the length of the raceway (Adey et al. 1993). This design has been adapted for use at various size scales from small-scale laboratory systems (Mulbry and Wilkie 2001, Pizzarro et al. 2002, Wilkie and Mulbry 2002, Kebebe-Westhead 2003), to 1021 m^2 raceways consisting of landfill liners between concrete sidewalls used for tertiary treatment of municipal wastewater (Craggs et al. 1996).

Drawing on many elements of the aforementioned aquaculture technologies, a two stage treatment system was designed for this study. The first stage involved growth of phytoplankton in ponds supplemented with either nutrients from a dairy wastewater stream or inorganic fertilizer. The ponds served as the source of water for a series of recirculating raceways. The freshwater clam was used as the primary agent for nutrient removal from the source water, through filtration of plankton and conversion into harvestable biomass.

The raceway design was chosen for this application since it mimics the small stream environment widely occupied by *Corbicula* in North Florida (Blalock and Herod 1999). Like a stream system, water is constantly supplied to the raceways, and the channel-like shape (width to length ratio= 1 : 7.5 in this study) induces a plug-flow hydrology that has little back mixing. This hydrology is maintained by recirculating water between the source ponds and raceways in this system, while replenishing food particles and dissolved oxygen and removing waste products. A coarse sand substrate was chosen for this application since it is an intermediate aggregate size preferred by *Corbicula* in small stream environments (Blalock and Herod 1999, Schmidlin and Baur 2007).

The raceway design can be constructed of a variety of materials and is scalable, adaptable to a variety of hydrologic regimes and can be easily manipulated for sampling, cleaning and maintenance. Raceways are also versatile, in that the structure can be used to culture a variety of aquatic organisms, including other bivalves, fish, algae and plants using a variety of substrates. The design used in this study is easy to disassemble and transport for reuse at different effluent source locations, can be assembled in remote locations and can be used in short-term evaluations of source waters without leaving a significant footprint. The modular components used in this design can be prefabricated and assembled quickly on site without intensive labor requirements needed to construct other large-scale systems.

Integrating *Corbicula* into wastewater streams via phytoplankton production has been suggested by Stanley (1974); however no studies have been performed to evaluate the viability of using large-scale engineered systems that emulate features that may be applicable in a full-sized wastewater treatment system. The study of *Corbicula* biofiltration potential has been limited to small- and medium-scale applications such as laboratory-based bench scale flow chambers (Lauritsen 1985), aerated 37 L aquaria with sand substrate (Beaver et al. 1991, Brock 2000), 150 L aquaria (Greer and Ziebell 1972) and 515 L rectangular fiberglass tanks containing mesh trays (Haines 1977). Cultivation in larger systems has focused on shallow ponds used to produce monoculture *Corbicula* as a food crop in Taiwan (Phelps 1994). Use of *Corbicula* in a pond-based polyculture scenario has been studied in catfish rearing ponds using benthic sediments and suspended cages as substrates (Buttner and Heidinger 1980, Buttner 1986). The clams have also been cultured in cages suspended within power plant discharge canals (Mattice 1977). The aquaculture system described in this study is meant to be an intermediate size scale

between the smaller experimental systems and the much larger pond and canal systems used in previous *Corbicula* studies.

Investigations using experimental large-scale engineered systems are an important step in developing commercial size treatment systems, especially when targeting an organism like *Corbicula* that has not been traditionally cultured at such a scale. Predicting the adaptability of these organisms to large-scale culture scenarios cannot be accomplished sufficiently from small-scale experiments since the behavior of the organism in these systems may not coincide with observations in larger systems. The systems designed for this study provide an opportunity for replication of treatment groups without sacrificing the structural elements of real-world treatment systems. These systems were designed using the following basic considerations necessary for the implementation of any experimental, biological-based water treatment system:

- Must be capable of maintaining environmental conditions necessary to promote survival, growth and reproduction of the target organism such as hydrology, substrate, food resources, waste removal and aeration
- System design intended for scientific manipulation must be able to conform to desired experimental treatments and replication for statistical analysis
- System must be scalable to provide an adequate surface area for the desired outcome in a real-world treatment application
- System design and operation must be applicable to various land topographies and source water body layouts found at different site locations
- Design must maximize energy efficiency by using gravity flow to reduce pumping requirements

To test the efficacy of the basic pond-raceway design, two recirculating systems were designed and constructed at different locations in northern Florida. The first system was completed in October 2002 at the Sam Mitchell Aquaculture Demonstration Facility in Blountstown, Florida and consisted of nine raceways supplied by two source ponds. This system was operated from November 2002 until January 2003 when the entire facility in Blountstown

was closed permanently due to university budget cuts. Parts of this system were excavated, dismantled and transported to the Dairy Research Unit in Hague, FL where a second system was constructed and operated from June 2003 to October 2004. The Hague facility consisted of 3 separate systems, each with 2 ponds and 3 raceways. Multiple ponds were used in both locations to provide an alternative to sustain phytoplankton populations in case of an unfavorable pond condition, while multiple raceways were used for statistical rigors. Differences in the topography and source pond design for each location required the use of different water delivery system configurations; however, the individual raceway design remained the same for both locations.

Multiple raceways were assembled at the Blountstown and Hague facilities for this study using different source pond layouts and water delivery configurations. The water delivery configurations used for these two systems were: 1) Source water was gravity-fed through the raceways and pumped back to the source pond and 2) Source water was pumped to the raceways and gravity-fed back to the source pond. The gravity-fed source water option was applied to the Blountstown system, while the Hague system incorporated the pump-fed source water configuration due to the elevations of the available areas for raceway construction in relation to the source ponds.

Raceway Design

The central components of the raceway system were closed-ended rectangular tanks. Raceways were formed from a 1.0 m wide by 7.4 m long channel, assembled from 10 individual panels constructed with pressure-treated wood framing and plywood backing. These frame sections were joined together using galvanized lag screws and a framing board (3.8 cm (1.5”) thick x 8.9 cm (3.5”) wide) was placed across the width of the raceway at the bottom of each

framing section joint to help maintain the rectangular shape. The basic layout and components of the raceways used in this study are illustrated in Figure 2-1.

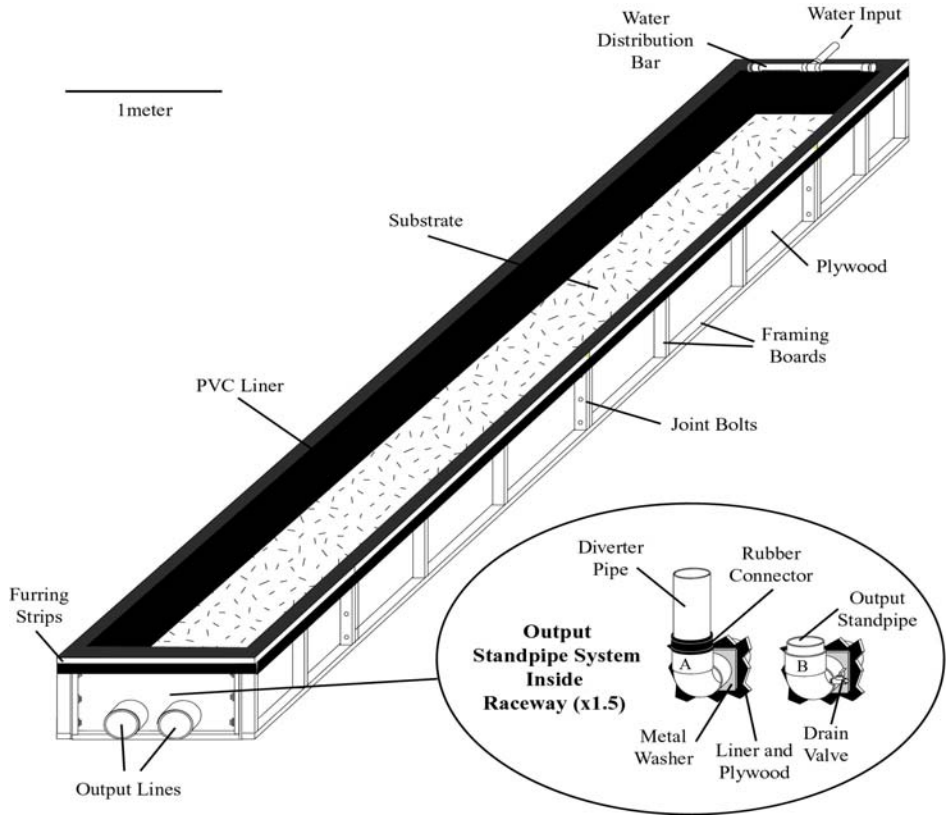

Figure 2-1. Components and design of individual raceways.

A custom made, black 20-mil thick ABS-PVC liner was then placed inside the assembled raceway and attached using treated wood furring strips fastened to the outside of the framing. The substrate was added before the furring strips were attached to minimize stretching as a result of sand settling. Coarse grade SiO_2 filtration sand (0.6-1.0 mm particle size), available from Feldspar, Incorporated in Edgar, Florida, was used as a substrate. Some slack was left in the liners around the raceway sides in order to allow for expansion and contraction with changes in temperature. A 0.31 m thick layer of fill dirt was placed around each raceway frame before the substrate was added to help support the raceway frames. Raceways were filled with sand to a

depth of 0.20 m so the substrate surface would be level with the raceway drain valves located on each output standpipe elbow (Figure 2-1). Fill dirt placed around each of the raceways was used in conjunction with the interior sand substrate in order to stabilize the sides framing and prevent buckling. The finished raceway dimensions and capacities are listed below in Table 2-1.

Table 2-1. Raceway dimensions and capacities as tested, available capacities adjusted for standpipe presence.

Specification	Parameter	Value
Inner Dimensions (empty)	Width	1.0 m
	Length	7.4 m
	Height	0.6 m
	Available Volume	4200 L (4.2 m^3)
Substrate Capacity	Depth	0.2 m
	Available Substrate Surface Area	7.2 m^2
	Volume	1432 L (1.4 m^3)
Water Capacity	Depth	0.2 m
	Available Volume	1432 L (1.4 m^3)

The supply plumbing to each raceway consisted of a 10.2 cm (4") inside diameter (ID) poly-vinyl-chloride (PVC) line stemming from the 15.2 cm (6") ID main supply line from each source pond. Supply lines had a brass gate valve that allowed selection of the desired source pond. After the valve, supply lines are reduced to 5.1 cm (2") ID, and the two were joined into a single raceway input line equipped with a brass gate valve to regulate input flow and a Pitot-tube type flow meter to help balance input water flow between the raceways in the system. Source water was fed into the raceway through a slotted water distribution bar that dissipated the energy of the falling water over the width of the raceway, initiating a laminar-type plug-flow hydrological pattern. Threaded caps were used at the ends of the distribution bars to allow easy cleaning of slotted portions to prevent blockage from biofouling.

Water exited the raceways via a standpipe system that acted as a type of weir structure to govern water column height and channel output water to the appropriate source pond. The

standpipes were made from 15.2 cm (6") ID PVC 90^{o} elbows with threaded male adapters on the output ends. The threaded ends passed through holes cut in the back plywood panels of the raceways and the liners. The threaded female adapters on the outside of the panel were tightened to hold the standpipe system in place, and a bulkhead fitting was formed over the liner where output lines passed through by using an aluminum washer on the inside of the raceway to prevent leakage around the pipes.

For this study, raceway water depth was maintained at 0.20 m; however, depth could be adjusted by extending the standpipe height using additional segments of pipe. Raceway output water was routed to the desired source pond by placing a 20 cm (8") long removable diverter pipe with a rubber "no-hub" connector over one of the output standpipes to divert water to the appropriate source pond return plumbing. Small, 1.9 cm (3/4") ID, drain valves were added to each of the 90^{o} standpipe elbows level with the substrate surface inside the raceway to allow removal of overlying water for periodic substrate sampling or observation, and they could be used to drain the raceway for batch-fed experiments.

The raceways had a maximum input flow rate of 303 Liters per minute (LPM) (80 gallons per minute (GPM)) due to the gravity flow capacity of the 15.2 cm (6") diameter output standpipe. This flow rate limited theoretical retention time to no less than 6.3 minutes at the 0.20 m raceway water depth used in this study. Raceway flow rate was maintained at 227 LPM (60 GPM) in this study yielding a retention time of 9.5 minutes.

The one-meter width was chosen since it is approximately the limiting distance for accessing the entire bottom area by hand from the sides of the raceways. Raceway length was estimated from target stocking amounts of 7,000 to 10,000 adult clams per raceway at population densities similar to the high densities (> 1,000 clams/m^{2}) sometimes found in naturally

occurring *Corbicula* populations (McMahon and Bogan 2001). Ultimately, the exact length and height dimensions of the raceways were determined according to the dimensions of the standard size for the plywood used in the raceway side framing, to expedite construction. In this case, the 7.4 m length is a result of using 3 lengths of a standard sheet of plywood, while the 0.6 m raceway side height equals half of the width of a standard sheet of plywood.

The low width to length ratio of the raceway design makes the bottom area more physically accessible than circular tanks of the same volume or surface area. When widths of 1m or less are used, bottom area can be manipulated easily by hand provided both sides of the raceway are accessible. By scaling width and length, the amount of culture area can be expanded without losing the plug-flow hydrology. Multiple raceways can also be employed to increase the scale of the culture area as well as to conform to the statistical demands of experimental research.

Blountstown Facility

The raceway system constructed at the freshwater fish aquaculture farm in Blountstown, FL site ($30^{o}35.5$' North, $85^{o}02.6$' West) consisted of 2 source ponds supplying a group of 9 raceways arranged side-by-side. Raceways were positioned at the same elevation as the source pond bottoms with ponds located to the north and west. Source ponds had an approximate area of 0.20 ha (0.5 acre), and water depth was maintained at 1.5 m, yielding an estimated volume of 3084 m^3 (108,900 ft^3). A 15.2 cm (6") ID PVC supply line was installed through each pond berm using a concrete anti-seep collar. Source water from the ponds was gravity fed to the raceways, and the flow rate was regulated using a brass gate valve installed on each supply line. A gravity fed inflow was chosen to minimize operational and equipment costs associated with pumping water both to and from the raceways. Flow rate of the incoming water was determined using a 15.2 cm (6") ID turbine-type, in-line flow meter for each raceway supply line.

Maximum system flow rate was limited to 950 LPM at 1.5 m source pond depth by the gravity-fed design.

The design of the plumbing for the entire raceway set is illustrated in Figure 2-2. Supply lines from the ponds were connected to a 15.2 cm (6") ID PVC manifold for each. Each manifold was plumbed with a single 10.2 cm (4") ID PVC feed line for each raceway with a 10.2 cm (4") ID brass gate valve installed on each raceway feed line to select for the desired source pond inflow. Raceway feed lines from the manifolds were then reduced to 5.1 cm (2") ID PVC lines and joined together to form the raceway input plumbing.

The output plumbing from each raceway consisted of two separate 15.2 cm (6") ID PVC output lines that joined corresponding manifolds made of 20 cm (8") ID PVC. Each manifold emptied into a separate 4,542 L (1,200 gallon) concrete sump tank buried underground. Vertical vent pipes were installed on the ends of the manifolds to prevent a suction effect in the standpipes for the raceway furthest from the sump tanks. Each sump tank was equipped with a 120-volt, 5-horsepower centrifugal pump rated at 787 LPM (208 GPM) that was cycled using a float switch. The pump suction lines drew water from the bottom of the sumps using a 10.2 cm (4") ID PVC line equipped with a PVC foot valve to prevent the need for priming of the pumps at the startup of each run cycle. A 10.2 cm (4") ID PVC outflow line leading to each pond was plumbed from each pump and an array of 10.2 cm (4") ID brass gate valves was used to divert water to the desired pond. This plumbing system was designed so that both source ponds could be used simultaneously by supplying several raceways without any mixing of the two water bodies.

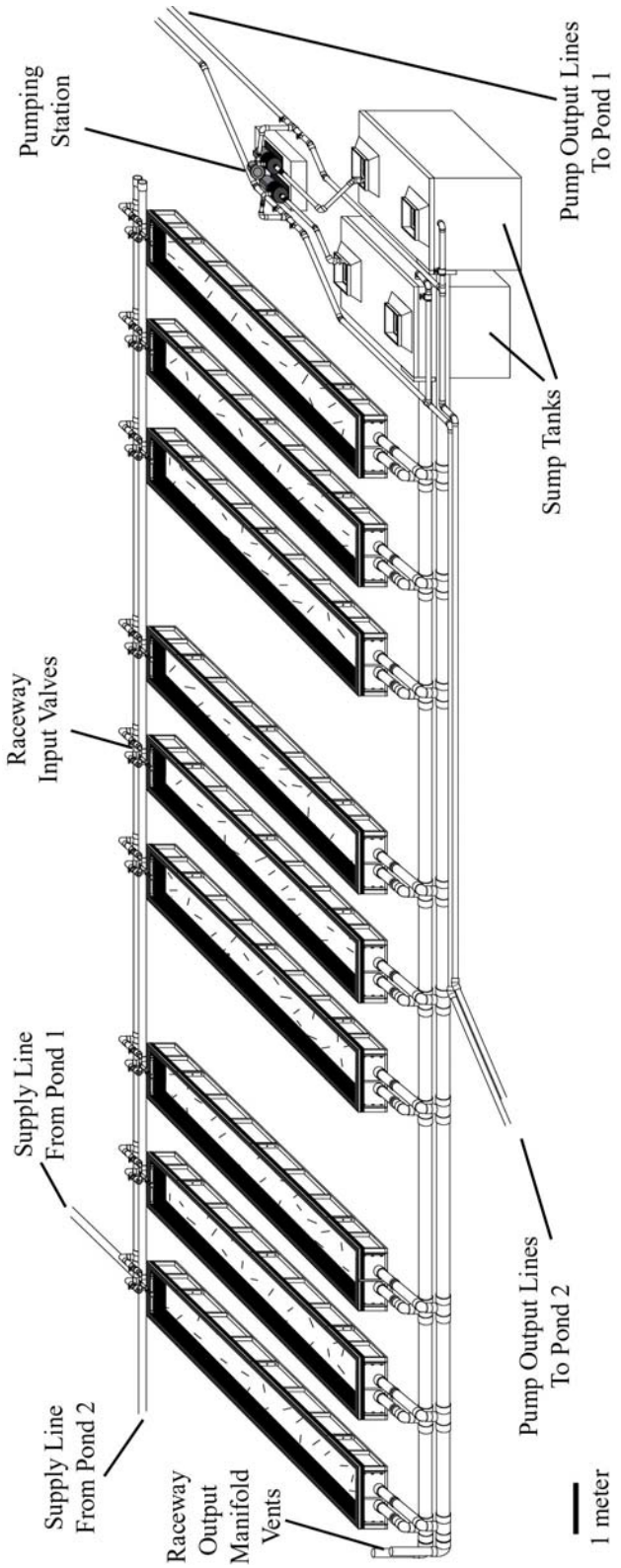

Figure 2-2. Blountstown system plumbing diagram.

Hague Facility

The raceway system constructed in Hague, FL (29°48.0' North, 82°25.1' West) was made of three independent test systems, each consisting of two source ponds and three raceways. Source ponds had an approximate area of 0.05 hectare, and depths were maintained at 1.9 m, yielding an estimated volume of 970 m^3. Each pond set was fitted with aeration supplied by a 1.5-horsepower continuous-duty centripetal blower, and a 5.08 cm (2") ID PVC main line reduced to a polyethylene line extending to the center of the ponds. A 1.9 cm (¾") ID brass ball valve was installed at each pond to balance the airflow to weighted 15.2 cm (6") long, stone diffusers on the pond bottoms.

In each triplicate test system, raceways were positioned near the banks of the source ponds at the north end of each pond set. Source water was supplied by continuously pumping from the south end of the ponds to the raceways where it exited through a standpipe and was returned to the north end of the pond by gravity-feed. A diagram of the plumbing used for each raceway system at the Hague facility is shown in Figure 2-3. A single 120-volt, 5 HP centrifugal pump rated at 787 LPM (208 GPM) was located on a concrete pad between the ponds on the south end of each treatment pond pair. The suction side of each pump was plumbed using 7.6 cm (3") ID PVC pipe with a PVC foot valve at the pond end to prevent loss of prime. A strainer made from plastic 64 mm (¼") mesh screen was installed over the intake to prevent the passing of large particles that might have been harmful to the pump. A pair of brass 7.6 cm (3") ID gate valves was used to isolate the desired supply pond. The pump intakes were suspended from steel-framed piers to one meter above the bottom of the pond bottom. A union was also added to each suction line at the pier to enable removal of the submersed portion for regular cleaning of the strainer screen.

Water output from each pump was delivered to the raceways using a 10.2 cm (4") ID PVC line and regulated through a set of overpressure relief valves located near the raceways that sent the overpressure water back to the source pond through a 10.2 cm (4") ID PVC return line. The relief system was necessary because the continuous-duty pump configuration introduces water at a constant flow. Therefore, in order to reduce the amount of flow to the raceways without cycling the pump, some water volume must be relieved from the pump output line. Each pump was also fitted with a 75-pounds per inch^2-rated pressure relief valve as an emergency feature in the event of a line blockage. After the overpressure water was relieved, the source water passed through a 10.2 cm (4") ID turbine flow meter before entering a 15.2 cm (6") ID PVC raceway supply manifold similar to the one used in the Blountstown system.

The manifold is reduced to a 10.2 cm (4") ID PVC fitting at each raceway and further reduced to a 5.08 cm (2") ID PVC raceway feed line that empties into the distribution bar. Each raceway feed line flow was measured using a Pitot-tube flow meter located between the valve and the spreader bar. These flow meters, along with the valves at each raceway, were used to regulate the flow balance between individual raceways, while the flow meter and relief valves before the manifold regulated available water input to the raceway set.

As in the Blountstown system, the standpipe plumbing from each raceway consisted of 2 separate 15.2 cm (6") ID PVC output lines that joined to manifolds made of 20 cm (8") ID PVC corresponding to the source pond receiving the outflow. Vertical vent pipes were installed on the ends of the manifolds to prevent a suction effect in the standpipes for the raceway fartherest from the outflow, as used in the Blountstown system.

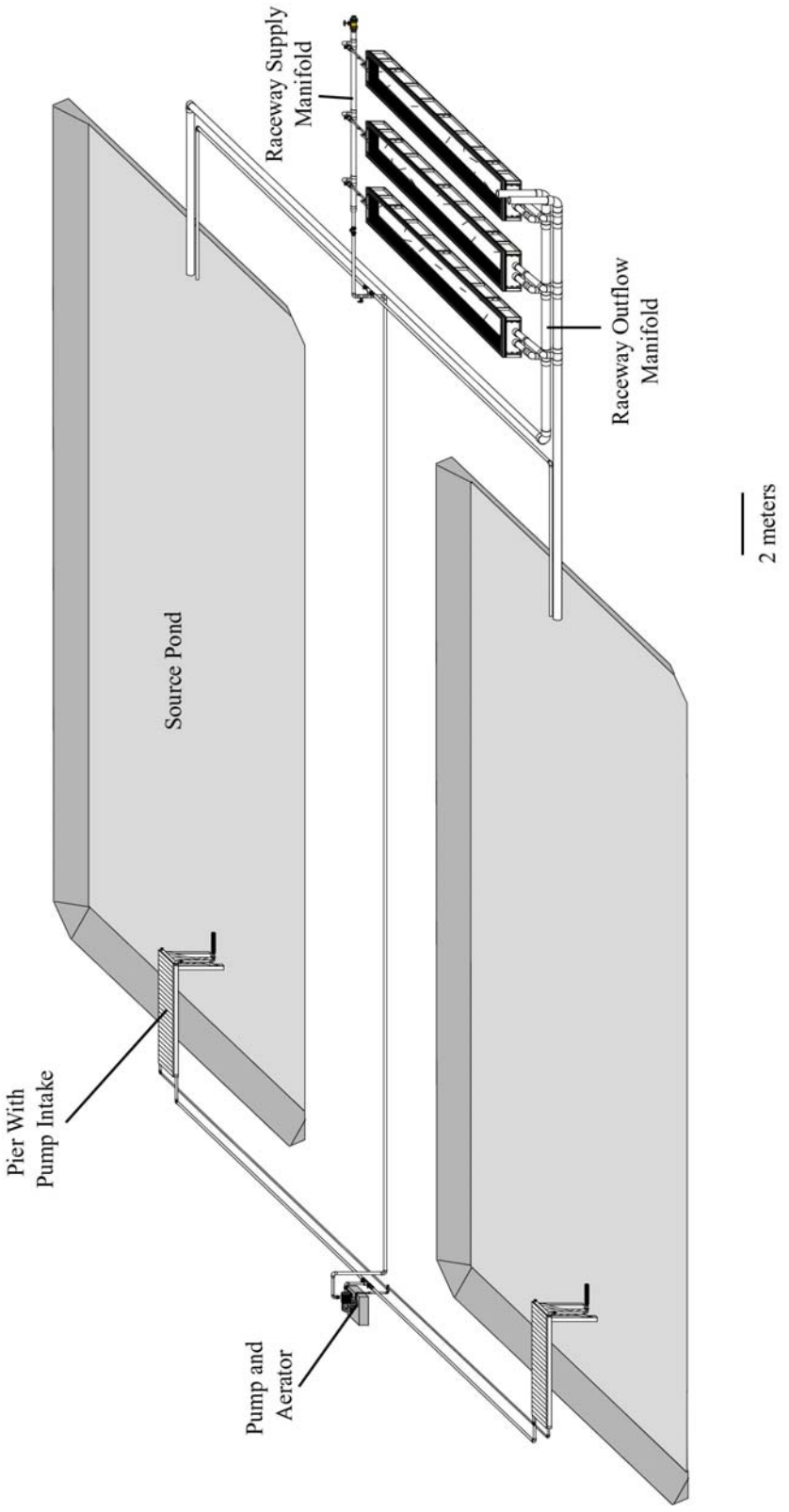

Figure 2-3. Hague system plumbing diagram.

System Design Summary

The Blountstown facility source ponds were three times larger than the Hague ponds; however, only two ponds were available as opposed to the six at the Hague facility. The Hague facility allowed for three different pond treatments to be performed simultaneously since the raceways were distributed into three separate pond/raceway systems each consisting of three raceways and two source ponds. This layout allowed for an alternate source pond in the event of a catastrophic event such as phytoplankton population crash or water quality issues. The Blountstown system could only support two simultaneous pond treatments and only one if an alternate pond was to be incorporated. Using nine raceways per pond treatment at the Blountstown system increased the ability to test different raceway conditions with more statistical rigor than the three raceways per pond treatment. Consequently, the Hague system had a 1 : 224 raceway set volume to pond volume ratio, similar to the 1 : 236 ratio found in each Blountstown pond/raceway set when all nine raceways were being fed from a single source pond.

The gravity inflow/pump outflow configuration constructed at Blountstown may be the more energy efficient choice; however, in the event of pump failure, the source pond will continue to flow, flooding the raceway site and evacuating the source pond. In order to prevent this catastrophic failure, the only options are large self-actuating valves for the main input lines or an emergency power generator, both of which are expensive propositions. A pneumatic or mechanical self-actuating valve triggered by a battery-back up float switch circuit from the sump tanks is the ideal mechanism since pump failure may occur without power outage.

Another drawback of the gravity-fed raceway inflow is that flow rate is dependent on water depth in the source ponds. In the Blountstown system, the input flow rate to the raceway varied with changes in pond depth. As a result of the main input flow variation, the individual raceway

flow rates would become unequal, requiring constant adjustment of the individual raceway input valves and pond refill rate to maintain consistent flow regime. This constant monitoring was not needed at the Hague facility since the pump input delivered a constant, inflow volume balanced among the raceway set. Consequently, the maximum input flow rate available to the raceway set was limited by the source pond depth and the input flow line diameter in the gravity-fed design, whereas a larger capacity pump can be installed to increase flow in the pump-fed design to achieve the individual raceway maximum of 227 LPM.

The raceway-based system design used in this study provided important elements of flexibility, which allows for a broad range of applications. Integration of raceways into various source and receiving water system configurations can be accomplished by modifying the water delivery component to adapt to the layout of the water bodies, area topography and raceway hydrological demands. The most energy efficient design is the gravity inflow/gravity outflow configuration that eliminates costs and energy consumption associated with multiple pumps; however, this can only be applied in a flow-through system with the proper raceway elevations. In most circumstances, at least one pump will be required to feed and/or evacuate the raceway systems. Other components, such as settling ponds, could also be integrated into the source water stream to remove excess particulates (Krom et al. 1995, Shpigel and Fridman 1990 Van Rijn 1996).

Changing input flow rate or adjusting the water depth by modifying standpipe height or substrate volume allows for manipulation of raceway hydrology. Changes in these settings could be used to adjust water retention time or linear velocity in constantly flowing systems. Water retention time is often shortened in large commercial raceway systems either to maintain high dissolved oxygen concentrations or to avoid ammonia build-up (Timmons et al. 2002).

Retention time can be markedly increased to increase exposure of the water for treatment purposes by using a batch-fed regime where raceway inflow is stopped and raceway water is circulated or agitated to mix and re-aerate it. In this study, retention time was increased to six hours using a batch-fed configuration that incorporated a 1/3 horsepower submersible pump to circulate and aerate raceway water. Another concern with long retention times is heat exchange, since temperature increases in shallow raceways are due in part to radiant heat exposure from the sun as well as evaporative heating/cooling.

The raceway structures in this study incorporated a modular design allowing for transport and reuse at multiple sites. Portability makes the raceways ideal for application in the scientific arena since: 1) experiments are often short term, 2) systems must be cleared from the site at the end of the study and 3) sites may be located in remote areas. Another modular design has been applied to reinforced fiberglass panels by Vantaram (2004) as a lightweight and less permanent alternative to concrete. The Vantaram (2004) system requires no bracing around the sides of the raceway to maintain the structural integrity such as in metal, fiberglass or wood-framed raceways that need buttresses or dirt to maintain the desired shape. The raceway design used in this study required soil backfill for structural support. This made each raceway fully accessible from all sides, allowing for easy and comfortable physical manipulation, cleaning and sampling, an advantage in the experimental systems. The advantage of this design over metal, fiberglass and the Vantaram-type panels is that the materials can be readily purchased locally and assembled without molding structures, curing time, special tools and health concerns over solvents and dust, all of which impact budgetary demands.

The raceway-based systems constructed in this study were chosen and developed as a low-cost, less permanent and easy to assemble alternative to other raceways constructed from

concrete, fiberglass or plastic. The raceway system designs employed here are versatile enough to be applied to other organisms targeted for large-scale water treatment/biofiltration studies in both fresh and saltwater conditions, a variety of locations and effluent sources.

CHAPTER 3
ADAPTABILITY OF *Corbicula* TO TREATMENT RACEWAYS

Introduction

The potential for using the freshwater clam *Corbicula* in dairy wastewater treatment ultimately depends on the ability of the organism to adapt successfully to that environment. Clam-based treatment raceways are essentially production aquaculture systems designed to function using phytoplankton biomass grown on wastewater as the main food source. Growth and harvest of clam tissue biomass represent the accumulation and removal of wastewater derived nutrients. As in traditional aquaculture systems, growth, recruitment and health of the clams in the treatment raceway population are important elements in assessment of the potential success of large-scale systems.

Studies based on the production of filter-feeding organisms as a mechanism for wastewater treatment has been applied mainly in the mariculture industry, where commercially desirable species are cultured using finfish and shellfish farm effluents in both natural and engineered systems (Shpigel and Blaylock 1991, Jakob et al. 1993, Shpigel et al. 1997, Lin et al. 2001). No large-scale commercial markets for freshwater filter feeders currently exist. The freshwater clam *Corbicula* may be an ideal candidate for such a wastewater treatment system in freshwater due to its high filtration and growth rates under eutrophic conditions (Greer and Ziebell 1972, Mattice 1977, Buttner 1986, Beaver et al. 1991, Brock 2000). *Corbicula* is also known for its ability to form high-density populations (Gardner 1976) and maintain high rates of reproduction (Rodgers et al. 1977, McMahon and Williams 1986, McMahon and Bogan 2001). In freshwater aquaculture applications, *Corbicula* utilizes uneaten feed and feces, in addition to phytoplankton, produced from the polyculture of other organisms (Buttner 1986).

Sustained aquaculture of freshwater *Corbicula* for human consumption has been reported in open pond-based polyculture systems (Villadolid and Del Rosario 1930, Miller and McClure 1931, Ingram 1965, Chen 1976). These open systems utilize constant exchange of water from natural bodies that can contain juveniles and are therefore not dependent upon reproduction from individuals within the system. Little is known about large-scale *Corbicula* aquaculture practices using engineered wastewater treatment systems.

Other freshwater species of bivalves have also been considered for wastewater treatment including *Lampsilus clairbornensis* (Swingle 1966), *Diplodon chilensis* (Soto and Mena 1999) and *Eliptio complanata* (Stuart et al. 2001); however, *Corbicula* may be better suited for aquaculture since it does not require an intermediate fish host for reproduction and can self-fertilize (Kraemer et al. 1986, McMahon and Bogan 2001). This reproductive advantage over freshwater mussels indicates that *Corbicula* populations should in theory be self-sustaining and should not require re-stocking from external sources or polyculture with proper fish intermediates to balance recruitment with biomass removed by mortality and harvest.

An application of *Corbicula* aquaculture to large-scale treatment of agriculture effluents using phytoplankton as an intermediary has been proposed by Greer and Zeibell (1972), Stanley (1974) and Olszewski et al. (1977). Greer and Ziebell (1972) used *Corbicula* in short-term aquarium-based filtration experiments (16 days) to evaluate the clam's treatment potential for waters enriched with inorganic nitrogen and phosphorus. However, the short duration and small scale of this study may not accurately reflect the organism's ability to provide treatment over longer time periods and in a larger system needed for real-life applications. Long-term studies utilizing *Corbicula* for wastewater aquaculture have targeted freshwater finfish effluent (Habel 1970, Busch 1974, Buttner and Heidinger 1980, Buttner 1986) and municipal wastewater

(Haines 1977), but not agricultural wastewaters. In addition to nutrient reduction, *Corbicula*-based systems decreases turbidity (Habel 1970, Busch 1974, Haines 1977, Buttner 1986) and increase dissolved oxygen at dawn (Buttner 1986). The extent that these treatment actions can be performed is primarily a function of the amount of biomass present and is strongly influenced by population dynamics.

In this study, a large-scale raceway-based treatment system was used to examine the adaptability of *Corbicula* to the raceway conditions that may be encountered in northern Florida agriculture operations. It was hypothesized that *Corbicula* populations introduced to treatment raceways would survive, grow and reproduce under wastewater conditions. Changes in clam population were compared to raceway water quality, nutrient concentrations and phytoplankton biomass parameters to assess the impact of environmental conditions and food availability on population dynamics. Assessment of population parameters along with monitoring of environmental conditions was used to determine the potential for the successful application of *Corbicula* in the large-scale treatment of agricultural effluents in northern Florida.

Methods

Growth of clams in the treatment raceways was assessed as a function of changes in shell size, tissue biomass and health, while survival and recruitment of juvenile clams was used to assess changes in the numbers of individuals in the raceways over time. Tagging studies are more precise, but they introduce additional handling stress resulting from tag application. Population sampling limits handling stress but is imprecise and requires an independent confirmation of growth from tagging studies. The combination of these two methods was intended to provide a measure of quality assurance/quality control.

Raceway-based Treatment System

The previously described (Chapter 2) raceway-based recirculating system constructed at the University of Florida Dairy Research Unit in Hague, Florida was used to test the adaptability of *Corbicula* populations to large scale culture under exposure to simulated agricultural wastewater conditions. Three independent pond/raceway systems were constructed in June 2002 to compare source waters with low, medium and high levels of nutrients. Each pond/raceway system consisted of two earthen source water ponds and three wood-framed, PVC-lined raceways. Table 3-1 shows the numerical designations for the ponds and raceways in each nutrient addition treatment group.

Table 3-1. Source pond and raceway numerical designations for the treatment systems at the Hague site.

Nutrient addition treatment	Source pond	Raceways
Low	1, 2	1 – 3
Medium	3, 4	4 – 6
High	5, 6	7 – 9

Source ponds

Source ponds had an approximate area of 0.05 hectares (ha), with depths of approximately 2 m and volumes approximately 1000 m^3. Ponds were enriched with a blend of nitrogen and phosphorus fertilizer or anaerobically digested dairy farm wastewater, to simulate possible water conditions associated with tertiary wastewater treatment. The low nutrient addition treatment received no external nutrient addition. A 5 % and 10 % addition of anaerobically digested dairy farm effluent was added to Pond 3 from the medium nutrient group and Pond 5 from the high nutrient addition treatment group, respectively. For effluent physical and chemical characteristics see Wilkie et al. (2004). Effluent was pumped from the digester to the source ponds and metered through a 2.54 cm (1") turbine-type flowmeter.

Pond 4 in the medium nutrient addition treatment was dosed with 1.1 kg of triple super phosphate ($9Ca(H_2PO_4)_2$, N-P-K = 0-45-0) and 6.8 kg ammonium nitrate (NH_4NO_3, N-P-K = 15-0-0) resulting in a total addition of 0.23 kg of phosphorus and 1.02 kg of nitrogen per pond in October 2002, one month prior to clam stocking. Pond 6 in the high nutrient addition treatment was dosed with 2.2 kg of triple super phosphate (0.46 kg P) and 13.6 kg of ammonium nitrate (2.04 kg N) in January 2002, also one month before introduction of clam populations.

Nutrient additions were designed to enhance phytoplankton biomass, which was the putative source of particulate nutrition for the clams. Fertilizer loading levels were targeted at increasing phosphorus and nitrogen levels by 0.23 mg/L TP and 1020 µg/L TN in the medium nutrient treatment source pond and 0.46 mg/L TP and 2040 µg/L TN in the high nutrient treatment source pond. Fertlizer was introduced to ponds by placing it into a burlap bag suspended in the water column by a 0.5 m x 0.5 m floating frame constructed from 5.08 cm (2") ID PVC pipe.

Neither of the ponds supplied with dairy effluent were exposed to the clam raceways due to excessively high ammonia levels (2.0 mg/L or greater as NH_3-N), which represented a direct threat to the health of the clams. NH_3-N levels in the effluent ponds and input and output water from each operating raceway were monitored monthly using Aquacheck® brand Ammonia Nitrogen (NH_3-N) Test Strips (Hach Incorporated, Colorado, USA) commonly used for aquaculture and aquarium applications. Levels of NH_3-N in the raceways supplied by ponds 4 and 6 never reached the 0.25 mg/L (as NH_3-N) minimum value of the test kit.

Source ponds were circulated through the raceways for 10 to 20 days prior to clam addition. Supply ponds were aerated at night throughout the study to help with mixing, maintenance of nighttime dissolved oxygen and evaporative cooling. Floating macrophytes were

cleared by hand from each source pond several times at the start of the experiment. In addition, six juvenile triploid grass carp ranging between 15 and 20 cm in length were stocked in each pond in June 2002 to help reduce vegetation in ponds. No fish mortality was evident in ponds not exposed to dairy wastewater effluent. In contrast, ponds treated with wastewater all experienced 100 % fish mortality in less than one week after effluent addition. Fish mortality events corresponded with high ammonia levels (> 0.25 mg/L NH_3-N).

Raceways

Raceways were 1 m in width by 7.4 m in length, with an available substrate surface area of 7.2 m^2, after subtracting standpipe area. Water depths were maintained at 0.2 m, yielding a raceway water capacity of 1.4 m^3 each. Source water inflow to the raceways was maintained at 227 liters per minute (LPM) (60 GPM) during normal operating conditions. The flow rates yielded retention times of approximately 9.5 minutes, with a linear velocity of 1.17 m/min. Flow rates were calculated assuming near laminar flow through the raceway structure. Raceways were filled to 0.2 m depth with a coarse grade SiO_2 filtration sand (0.6-1.0 mm particle size), that was purchased from Feldspar Incorporated in Edgar, Florida.

Aquatic plants such as *Chara sp*. and filamentous algae (mainly *Spirogyra sp*.) growing on the raceway substrate and liners were removed by hand from each raceway at least weekly to reduce fouling from increased water retention time. The control of plants, especially *Chara*, on the substrate made accurate estimation of clam biodeposit sedimentation impossible. This was due to the constant resuspension of sediment deposits associated with disturbance from removal of the vegetation that was often rooted below the sediment surface. Filamentous algae tended to utilize the sides of the raceways where PVC liners were submersed.

Water analysis

Source water ponds and raceways were monitored once per week at 6:00 and 18:00 for temperature, dissolved oxygen, pH, chlorophyll *a*, total nitrogen and total phosphorus. Measurements in the ponds were taken at the end of sampling piers near the intake pipe leading to the raceways. Monitoring intervals were changed to monthly in June 2003, after 75 % or more of the clams were assumed dead from numbers of cumulative dead clams found on the sediment surface. Temperature and dissolved oxygen was measured using a YSI model DO550, and pH was measured using a Fisher model AP63 meter. Water temperature, dissolved oxygen and pH readings were compared using a paired t-test (Microsoft Excel©) at the raceway input and output, as well as between nutrient addition treatments.

Water samples were collected from the sampling piers in each pond for nutrients, using a pole sampler designed especially for this experiment. The sampler used a plunger-type mechanism to collect a 1 L water sample from in front of the intake pipe. When the unit was lowered to the desired depth, the plunger was actuated by the operator via a spring-loaded handle at the opposite end of the pole. After the sample was collected, the plunger was released, sealing a 1 L plastic (Nalgene Incorporated, USA) bottle and raised for retrieval. The sample bottle was unscrewed from the sampler and capped for transport to the laboratory.

Water samples collected from the source ponds were analyzed at the laboratory for phosphorus, nitrogen and phytoplankton biomass in terms of chlorophyll *a*. Total phosphorus (TP) and total dissolved phosphorus (TDP) were determined using the potassium persulfate digestion method (APHA 1998) with a Hitachi spectrophotometer. TDP determination involved pre-filtering through a 0.7 μm glass fiber filter. Total nitrogen was determined using potassium persulfate digestion method (APHA 1998) with colorimeteric analysis performed using a Bran-Luebbe auto analyzer. Phytoplankton biomass was estimated using chlorophyll *a* (chl *a*),

measured by filtering 250 mL of water onto a 0.7 μm glass fiber filter, followed by an ethanol extraction (Sartory and Grobbelaar 1984) and spectrophotometric determination (APHA 1998) using a Hitachi spectrophotometer. Microscope observations of phytoplankton species composition were obtained periodically to describe dominant organisms with help from Mary Cichra at the University of Florida Fisheries and Aquatic Sciences Department. Data obtained from these phytoplankton species observations were not assessed quantitatively.

Clam Population Dynamics In The Raceway Environment

The adaptability of clams to the raceway environment was determined by assessing survival, recruitment, growth and health. Clams were sampled at the time of stocking and at specified intervals over a period of 440 days to evaluate population density, shell size and tissue biomass. A tagging study was employed to validate survival and growth determined from monitoring of raceway populations. A three-month interval was chosen for sampling time duration der to reduce handling stress, while retaining the ability to assess changes in clams on a seasonal basis. The raceway stocking and sampling time schedule is shown in Table 3-2.

Table 3-2. Raceway (RW) stocking and population sampling schedule for the low, medium and high nutrient addition treatment systems. Time interval 0 corresponds to the time of stocking, and treatments were not stocked during the same season due to the time required to obtain the large numbers of clams needed.

Year	Season	Time interval Low nutrient RW 1-3	Med. nutrient RW 4-6	High nutrient RW 7-9
2002	Summer	0		
2002	Fall	1	0	
2003	Winter	2	1	0
2003	Spring	3	2	1
2003	Summer	4	3	2

Shell length was used as the primary indicator of clam size, since it can be measured quickly, is not subject to the variability exhibited by soft tissue, and is stable over time. Shell

length was defined as the greatest distance anterior to posterior measured perpendicular to the hinge line using a caliper measured to the nearest 0.01 mm. Shell length may be the best measurable size variable compared to height and width in *Corbicula* because it is the largest size variable, making it less sensitive to measurement error. Shell length also encompasses areas of the shell that are less susceptible to erosion. Other studies of *Corbicula* have used shell length as a descriptor of size, including Mattice and Wright (1986) and McMahon and Williams (1986).

In order to confirm that length provides the most dependable measurement of clam size, an allometric analysis was performed on a sample of 500 clams obtained from the Santa Fe River (29°51.1' North, 82°37.9' West) in March 2002. Each clam was measured for shell length, width and height. A regression analysis using SAS (PROC REG) (SAS Institute©, Cary, NC) yielded shell length as the measurement with the highest R-square value ($r^2 = 0.96$) compared to the width and height ($r^2 > 0.94$), thus making this size variable the most consistent over the size range used in this study (shell lengths 9.4 mm to 28.4 mm). Length may also be the preferred variable because shell erosion was apparent in the umbo region in larger clams, thereby affecting height and width measurement values. Measurement error was determined by repeating the length measurements three times on twenty randomly selected clams from the allometric analysis. The maximum variance for the mean shell length was $\pm$ 0.1 mm.

Stocking clam raceways

Clams for stocking the raceways were obtained from populations in three different natural water bodies under permit number FNC-04-022 issued by the Florida Fish and Wildlife Commission. Clams for the low nutrient group (Raceways 1-3) were collected in June 2002 from a 0.5 km stretch of the Santa Fe River near the State Road 49-bridge in Gilchrist County, Florida (29°54.2' North, 82°52.0' West). By November 2002, the rising water level of the river

made further clam excavation impossible; therefore, animals for Raceways 4-9 were collected from lakes located in Lake County, Florida that had accessible populations of clams. Clams for the medium nutrient group (Raceways 4-6) were collected from the southwest shore of Lake George (29°12.2' North, 81°35.7' West) in November 2002. Clams for the high nutrient group (Raceways 7-9) were obtained from the west shore of Lake Dalhousie (28°54.0' North, 81°36.8' West) in February 2003. Possible adaptability issues with ontogenetic differences in the populations obtained from different locations were not addressed due to the difficulties inherent in locating and obtaining such high numbers of clams from systems in a timely manner.

All three collection sites had coarse sand sediments similar to the substrate used in the raceways. Clams were excavated by shoveling bottom material into weighted baskets made from plastic mesh with 0.635 cm^2 (¼ in^2) perforations. Clams were also excavated by hand using trowels or a commercial clam rake modified for the small size of the *Corbicula* by affixing similar plastic mesh on the inside of the collection basket. Periodic excavation of bottom sediment using a 0.25 m^2 PVC sampling quadrat was used to determine population densities for the clams in their natural habitat. Densities ranged from 48 and 864 clams/m^2 with a mean of 272 clams/m^2 (standard error (SE) = 23, n = 47 observations) for all locations combined.

After excavation, clams were enumerated and divided into mesh bags. The clams were then placed into coolers packed with wet newspapers and kept out of direct sunlight to help minimize heat stress and dessication. They were transported directly to the aquaculture facility in Hague, FL and scattered evenly throughout each raceway. Stocking of each raceway took up to 15 days involving 2 to 6 people working per day. Raceways 1–3 were stocked from June 17 to 28, 2002, Raceways 4–6 from November 4 to 14, 2002, and Raceways 7-9 from February 11 to 26, 2003.

Stocking densities were estimated using a volumetric method of enumeration. The method employed a 0.5 L plastic container that was used to transfer the clams collected in the field to the mesh shipping bags. Ten mesh bags of clams were added to each raceway. Each mesh bag contained approximately 1,000 clams. This method was chosen as opposed to counting each individual or bulk weighing in order to minimize handling stress, time and equipment needed to enumerate the large numbers of clams needed for this study.

Shell length measurements were taken on 270 clams per raceway, selected at random from mesh basg just before stocking. A sample of clams was obtained from each mesh bag by scooping a sample from the middle of the bag using the 0.5 L plastic container mentioned above. A total of 27 clams were selected for the biomass analysis per raceway by keeping the tenth clam out of every 270 clams sampled for shell length determination.

Clam raceway population sampling

Sample sites within the raceways were defined using a submersible quadrat grid and sampling sleeve. The quadrat grid consisted of an aluminum frame divided inside into 10 cm x 10 cm squares using 2 mm thick nylon line. In order to define 100 cm^2 surface areas more accurately for excavation, a 10 cm x 10 cm ID square metal tube, 20 cm in length was used as a sampling sleeve. The sleeve was inserted into the raceway sediment, and clams were extracted to a depth of approximately 10 cm.

The entire grid measured 1.2 m x 0.9 m. Grids were deployed at the input, middle and output of each raceway to cover the length of each raceway. The 10 cm wide area was not sampled to reduce the risk of puncturing the raceway liner. Areas around the raceway edges, under the input spreader bars, around the outflow standpipes were not sampled. Placement of grid in all three sections allowed for 85 % coverage of the raceway bottom, providing a total of

567 possible quadrat locations per raceway. The grid rested on stainless steel pegs buried in the raceway sediment to ensure repeatability in placement.

The number of sampling quadrats needed per event was determined in December 2001 using a power analysis with equations from Sokal and Rolhf (1995) and performed using Microsoft Excel® on a population of 500 clams introduced to the Blountstown raceway system. Clams were distributed over a 1 m^2 area of active raceway substrate and left for 5 days. A 100 cm^2 PVC square quadrat was used to divide sample areas into 10 cm x 10 cm increments. Quadrats were excavated along a 1m transect in the middle of the raceway, and the number of live clams recorded. The power analysis on the clam density data yielded a sample size of at least 19 quadrats to achieve a 95 % confidence interval for clam density. A sample number of 27 quadrats per raceway were chosen since the sample grid consisted of 9 sample coordinates over the width of the raceway and 3 grid placements per raceway.

Clam sampling followed a stratified random design without repetition. One length division was chosen at random for each width division within the grid to stratify the sample areas over the width of the raceway. Stratification of raceway sampling allowed for spatial analysis of data over both the length and width of the raceways. Quadrat positions were repeated within each grid placement at the input, middle and output regions of the raceways.

Live clams in each quadrat were counted, measured for shell size and returned to the same location in the raceway. Clams used in the biomass analysis were selected from clams included in the density analysis by retaining the third clam excavated from each sample quadrat. In the event that there were fewer than 3 clams, the first clam excavated was kept for biomass analysis. This procedure yielded a maximum possible sample size of 27 clams per raceway at each time interval. This sample size was chosen to minimize the impact on raceway populations due to the

destructive nature of biomass determination. Individuals for biomass determination were placed in numbered plastic bags and frozen until analysis.

In order to estimate raceway population densities using the spatial technique, the clam densities obtained during each sampling event were used to calculate the average number of clams per 100 cm^2 quadrat sampled (n = 27 quadrat samples per raceway). Average quadrat densities were then converted to the number of clams per m^2 and multiplied by 7.2 m^2 of available substrate area per raceway to obtain the estimated number of clams per raceway.

Tagged clams

Clams used in the tagging study were obtained from a random sampling of individuals at the time of stocking. The clams were marked with EZ-Code® brand wire markers (Thomas & Betts Incorporated, USA) which are self-adhesive numerical tags applied to the shells that minimized the handling stress to the animals. This type of tag was chosen because it caused less damage and reduced the risk of injury when compared to engraving (Mattice and Wright 1986, McMahon and Williams 1986, Lemarie et al. 1995) or insertion of passive integrated transponder tags into the shell cavity (Kurth et al. 2007). The pre-applied adhesive expedited the tagging process by reducing drying time and increasing tag readability of the liquid adhesive traditionally used for affixing numerical tags (Lemarie et al. 1995), brass washers (Toll et al. 2003), coded wires (Layzer and Heinricher 2004) or monofilament tethers that anchor the animals to the substrate (Foe and Knight 1986).

This type of tag was chosen because of their small size, readability, adhesive strength and low cost compared to traditional numerical bee tags used in bivalve research. These vinyl decals are generally used in electrical applications and have 4 mm-tall black numbers with a white background. Tags were trimmed to 5 mm x 5 mm squares before application to towel dried shells. Tagged clam shell lengths were measured as described earlier and placed into the

raceways using the sampling grid described above. Using the randomly generated coordinates that were sampled over the study, one tagged clam was placed in each location using at least 33 locations at the input, middle and output sections of each raceway.

In order to test the durability of the tags, a sample of 20 live clams was obtained from the Santa Fe River (29°51.1' North, 82°37.9' West) in December 2001. Tags were affixed and clams placed in a 1 L plastic (Nalgene Incorporated, USA) bottle along with 200 mL of coarse sand and 200 mL of water. The mixture was capped and shaken vigorously by hand for 15 minutes, after which the clams were removed and rinsed with water for inspection. Only one clam lost its tag and was removed while the remaining numbered clams were placed back in the bottle. Then the clams were again shaken for fifteen minutes and removed for inspection. Tags on six of the clams had come off during this treatment, and the shells of all of the clams exhibited chipping around the margins. All tags remained legible after both treatments. The conditions that these clams were exposed to are certainly harsher than the raceway environment because of the lack of significant water movement to produce the same tumbling effect but neglects dissolution and bacterial decay that may also account for loss of adhesive strength with longer exposure times in aquatic systems.

A total of 108 clams were randomly selected from each raceway at the time of stocking for the application of tags. Specimens were tagged and distributed in a stratified random fashion by using the sampling grid to place 36 clams in the input, middle and output sections of each raceway. Shell length, clam number and initial quadrat coordinates were recorded for each tagged clam at stocking and at each 3-month sampling interval when found alive. Tagged clams that were later found dead were not used even though growth may have been evident by comparing measurements of final shell size to that at stocking.

Clam survival

Clam survival was assessed by counting live individuals in the raceways at stocking and at designated sampling intervals. Total densities were determined at the end of the study by counting the live and dead clams remaining in the raceway, along with cumulative counts of dead clams removed from each raceway over the course of the study. Raceway population densities at stocking were estimated using the volumetric technique and at each sampling interval using the spatial technique, as described above. Clam stocking estimates were later verified using information on cumulative counts of dead clams removed over the course of the study, along with counts of dead and live clams at the end of the study.

In the case of visible clam mortality events, dead clam shells were removed and counted. Counts of dead clams removed from the substrate surface were used as an overall indication of population mortality. No attempts were made to evaluate the dead shells buried in the substrate after the events because the removal of dead clam shells caused rususpension of sediment deposits.

Biomass changes

Changes in clam biomass were assessed using tissue weight and length data taken from clams obtained at stocking and at each designated sampling interval. Tissue weight to shell length relationships were developed from a subset of clams collected at stocking and sampling intervals. Sample clams were frozen prior to dry weight (DW) analysis (Copar and Yess 1996). Freezing provided an alternative to live shucking, since gaping of frozen clams occurs naturally when the clam is removed from the freezer and placed at room temperature for about 15 minutes. Shucking can also chip shell valve edges resulting in measurement error in shell-dependent variables.

Clam dry weights were determined for meat and shell of freshly thawed clams. Soft tissues and shell material were separated and placed into individual dried and pre-weighed aluminum drying dishes. Whole clam, soft tissue and shell wet-weights were recorded and placed in a drying oven at 80 ^{o}C for a period of 24 hours. This period of time was used due to the results of preliminary tests to establish a drying time needed to attain constant weight in clams sampled from the Santa Fe River (29^{o}51.1' North, 82 ^{o}37.9' West) in June 2002. After 24 hours, the dried tissue samples were removed from the oven and allowed to cool to room temperature in a dessicator. Meat and shell tissue dry weights were recorded to the nearest 0.001 g and tissues stored in a dessicator before ashing.

Dried shell and meat tissues of selected individuals were ashed to obtain ash weight, from which ash free dry weight (AFDW) was calculated. Dried meat tissue was collected from the drying dishes and placed into ceramic crucibles. Shell was prepared for ashing using a stainless steel grinding device powered by a rotary hammer. The device consisted of a 7.62 cm (3") tall x 2.54 cm (1") ID chamber made by welding a segment of pipe onto a 7.62 cm^2 (3")^2 x 0.635 cm (¼") thick plate. A plunger made from a stainless steel billet was machined on one end to accept a standard 3/8" square drive adapter attached to a Hilti T52 (Hilti Incorporated, Germany) rotary hammer. Each specimen was placed in the chamber and the piston lowered down on top of the shell. The rotary hammer was engaged for 10 seconds or less, long enough to pulverize the shell. The resulting powder was collected in pre-weighed ceramic crucibles, dried for 24 hours at 80 ^{o}C and weighed to the nearest 0.001 g. Shell biomass recovered from the grinding device averaged 94.9 % (SE = 0.3, n = 238) of the original shell DW. Tissue samples were then placed in a muffle furnace at 550 ^{o}C for 6 hours and cooled to room temperature in a dessicator prior to

weighing. The ash values were used to calculate percentage ash composition and AFDW (Wetzel 2001).

Dry weight values were primarily used to assess clam biomass since there was less variability and a larger sample size (n = 453) than for AFDW (n = 238). However, AFDW/DW relationships did provide insight into organic content and biomass allocation. The coefficient of variation was very similar for DW (0.54) and wet weight (0.52) for the animals in this study (n = 453). Wet weight measurements taken from whole frozen clams at the time of analysis were also used to help describe biomass allocation.

Shell, meat and whole clam DW measurements were performed on the low nutrient group at stocking, interval 1 and interval 2, the medium nutrient raceways at stocking and at interval 1, and on the high nutrient raceways at stocking. The AFDW of shell, meat and whole clams were taken from the low nutrient raceways at stocking and at interval 1 and the medium nutrient raceways at stocking only. The DW and AFDW analysis were discontinued after these time periods due to the establishment of strong linear regression relationships ($r^2 > 0.90$) between biomass and shell.

Tissue biomass allocation was used to evaluate biomass distribution in shell and meat, as well as to examine biomass. Biomass allocation was also used to examine water and ash content of shell and meat tissue, for comparison with other clam studies. Clam wet and dry weights of shell and meat were used to calculate percent shell tissue and percent water content for the whole clam. In order to understand variability in clam tissue biomass allocation, an ANCOVA (SAS PROC MIXED procedure, SAS Institute©, Cary, NC) was performed using both the percent shell tissue and the percent water content as the response variables and nutrient level as the factor with covariates shell length, time interval, nutrient level and season. The least squared means

(LSMEANS) procedure was applied to percent shell tissue and the percent water content (Microsoft Excel©) for pair-wise comparisons. Pair-wise comparisons of the means were performed using Tukey's method to control the experiment-wise error rate. Data from individual raceways within each nutrient addition treatment were pooled in this analysis since no blocking effect was found in either analysis.

The mean percentage of shell tissue and water content was determined for each nutrient addition treatment. Mean ash content was determined for clam, shell and meat tissues for each nutrient addition treatment. The coefficient of variation was calculated for the DW, and AFDW values determined for whole clam biomass to indicate the least variable biomass parameter. Coefficient of variation was calculated by dividing the standard deviation by the mean for each variable in the sample population.

A relationship between tissue DW and shell length was used to provide a means of estimating biomass using measurements of shell size. Tissue DW and shell length were transformed using natural logarithm (ln) to best fit the polynomial regression calculated by the SAS PROC REG procedure (SAS Institute©, Cary, NC). An ANCOVA (SAS PROC MIXED procedure, SAS Institute©, Cary, NC) was performed on the DW and shell length data using the natural logarithm (ln) of the values for the clam, shell and meat tissue DW. Tissue DW values were used as the response variables versus shell length, while nutrient level was the factor with covariates time and season. Non-significant effects were removed from the ANCOVA model. The tissue DW and shell length values were also analyzed using a correlation procedure (SAS PROC CORR, SAS Institute©, Cary, NC).

Clam biomass was estimated using shell length to DW relationships developed in the regression analyses. Raceway clam biomass was calculated using the regression equations to

convert shell measurements to tissue biomass. Changes in clam biomass over time were plotted for each nutrient addition treatment at each sampling interval to assess biomass production and clam growth.

Reproduction and recruitment

Clam populations were assessed for reproduction and recruitment by determining whether juveniles were present in the system at any time. Adult clams were used to stock the raceways, and therefore, any clams found with a shell length less than the smallest clam measured at stocking should indicate successful reproduction and recruitment. In this study, juvenile clams were defined as having a shell length less than 5 mm. Source ponds were also drained at the end of the study to check for the presence of clams that may have been released as juveniles from the raceway populations but did not successfully recruit to the raceway. Water samples from the source ponds used for phytoplankton analysis were also inspected for juveniles suspended in the water column.

Health

Changes in clam health were tested to evaluate the physiological condition of clams in the raceway environment over time. The goal was to relate changes in condition to changes in raceway environmental parameters. Other studies have used percent meat content to assess population health (Haines 1977). A similar approach to the condition indices was used in this study, which also relies on the amount of meat and shell tissues present in the clams.

Condition index (CI) was estimated using gravimetric and volumetric indices, based on dry meat : dry shell weight and dry meat : shell cavity volume, respectively. Both indices are defined by the ratio of a sensitive numerator- tissue dry weight, to relatively insensitive denominators-shell weight and shell cavity volume. The resulting values were compared for

each index and used to describe changes in raceway clam populations. CI is a measure of the nutritive status of the clam (Rainer and Mann 1992).

Clams were collected at stocking and during the seasonal time intervals described in the biomass sampling section. Meat DW and shell DW, along with shell cavity volume values obtained from biomass sampling, were used to calculate the gravimetric ($CI_{(WT)}$) and volumetric ($CI_{(VOL)}$) indices . The following relationships, after Rainer and Mann (1992), were used to estimate CI values:

- $CI_{(WT)}$ = (dry meat weight (g) x 100 / dry shell weight (g))
- $CI_{(VOL)}$ = (dry meat weight (g) x 100 / shell cavity volume (mL))

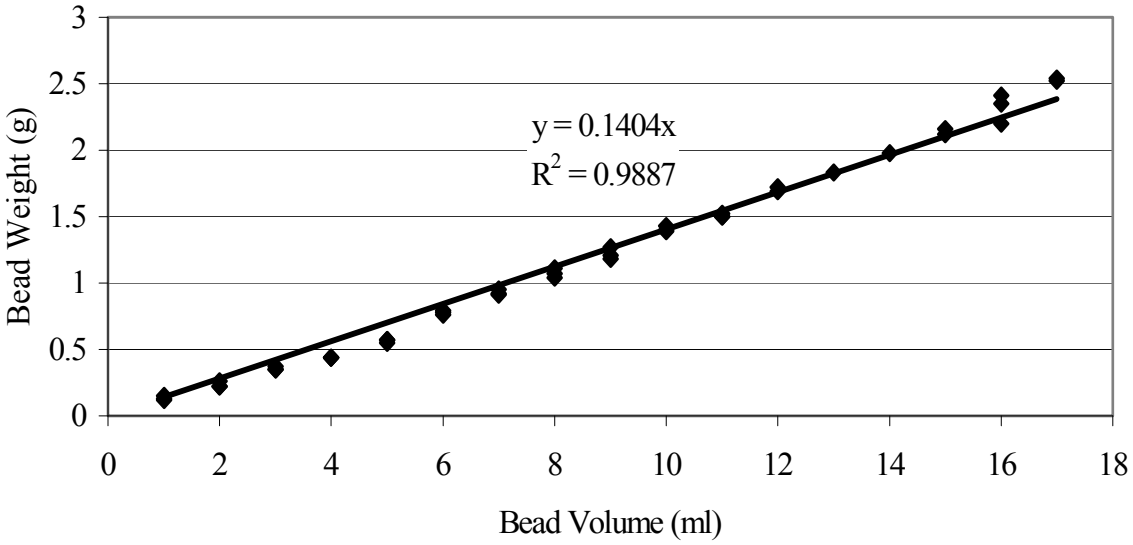

Figure 3-1. Linear regression relationship of glass sphere volume to glass sphere weight used to estimate clam shell cavity volume for the volume-based condition index calculation.

Shell cavity volume was calculated by filling the empty shell valves with 100 µm diameter glass beads and weighing the beads. This method was selected since the small size of *Corbicula* makes it very difficult to assess volume accurately using the water displacement method (Rainer and Mann 1992). In order to obtain a weight to volume conversion factor, a volumetric analysis

was performed by weighing glass beads. Three samples of beads were weighed at each 1 mL increment up to 18 mL (n = 54), and a linear regression relationship was developed between bead volume and weight (Figure 3-1) using the PROC REG procedure (SAS Institute©, Cary, NC). The regression equation from this analysis yielded the following conversion formula:

- Clam Shell Valve Cavity Volume (mL) = Bead Weight (g) / 0.1414

A dry weight and shell volume analysis was then performed on a sample of 20 clams obtained from the Santa Fe River (29°51.1' North, 82 °37.9' West) to investigate possible differences in volume between the two valves of each clam. Three bead weight samples were taken from the two shell valves of each clam, and a paired, two-sample t-test for means was performed on the measurements using Microsoft Excel©. The two-sided p-value was not significant (p = 1), therefore, no difference in shell cavity volume was found between the 2 shell valves in each animal. Therefore, by modifying the above conversion factor, the following equation was used to calculate the shell cavity volume for each clam:

- Clam Shell Cavity Volume (mL) = 2 x (Bead Weight (g) / 0.1414)

Two different comparative analyses were performed on $CI_{(WT)}$ vs $CI_{(VOL)}$ values to determine the best index for assessing clam health. A linear regression analysis was performed on each index versus length and $CI_{(WT)}$ vs $CI_{(VOL)}$ using the SAS PROC REG procedure, (SAS Institute©, Cary, NC), and the coefficient of variation was calculated for both $CI_{(WT)}$ and $CI_{(VOL)}$. Variation was similar for the two indices; although, $CI_{(WT)}$ had the lower coefficient value.

An ANOVA (SAS PROC MIXED procedure, SAS Institute©, Cary, NC) was then performed using the condition values for both indices as the response variable and shell length as the factor to determine the effect of clam size on condition for each index. Shell length was subsequently removed from the model, and an ANCOVA was performed with the $CI_{(WT)}$ and

$CI_{(VOL)}$ indices as response variables, nutrient addition treatment as the factor with covariates time interval, raceways within each nutrient level and season. The least squared means (LSMEANS) procedure was applied to $CI_{(WT)}$ and $CI_{(VOL)}$ indices (Microsoft Excel©). Pair-wise comparisons of the means were performed using Tukey's method to control the experiment-wise error rate.

Results

Raceway System Environmental Parameters

Air temperatures at the racewaty site at the Dairy Research Unit in Hague, FL ranged from 2 to 41 ^{o}C (Figure 3-2). Recorded air temperatures varied diurnally as much as 15 ^{o}C. Raceway water temperatures ranged from 10.1 to 32.6 ^{o}C (Figure 3-3) and displayed a similar seasonal pattern as air temperature. However, water temperatures only differed diurnally by a maximum of 2 ^{o}C. Water temperatures reached 30 ^{o}C or greater just after the beginning of the experiment in July 2002 through October 2002 and from May through the end of the study in August 2003.

Raceway dissolved oxygen (DO) averaged 8.79 mg/L (SE = 0.07) and ranged from 6.10 to 12.03 mg/L (Figure 3-4). DO was higher in the afternoons by up to 4.26 mg/L with greater diurnal differences in the warmer months. Higher DO values were observed during the November 2002 to April 2002 period corresponding to lower air and water temperatures.

Raceway pH averaged 7.76 (SE = 0.02) and ranged from 6.87 to 8.81. Diurnal fluctuations in pH ranged from –0.76 to 0.89. Raceway pH was significantly higher ($p > 0.05$) in the low and high nutrient addition treatments (Figure 3-5); however, water temperature and DO values did not differ significantly between the low, medium and high nutrient addition treatments. No significant differences ($p > 0.05$) were detected at the input and output of each raceway or between raceways in each nutrient addition treatment for the water temperature, dissolved oxygen and pH parameters.

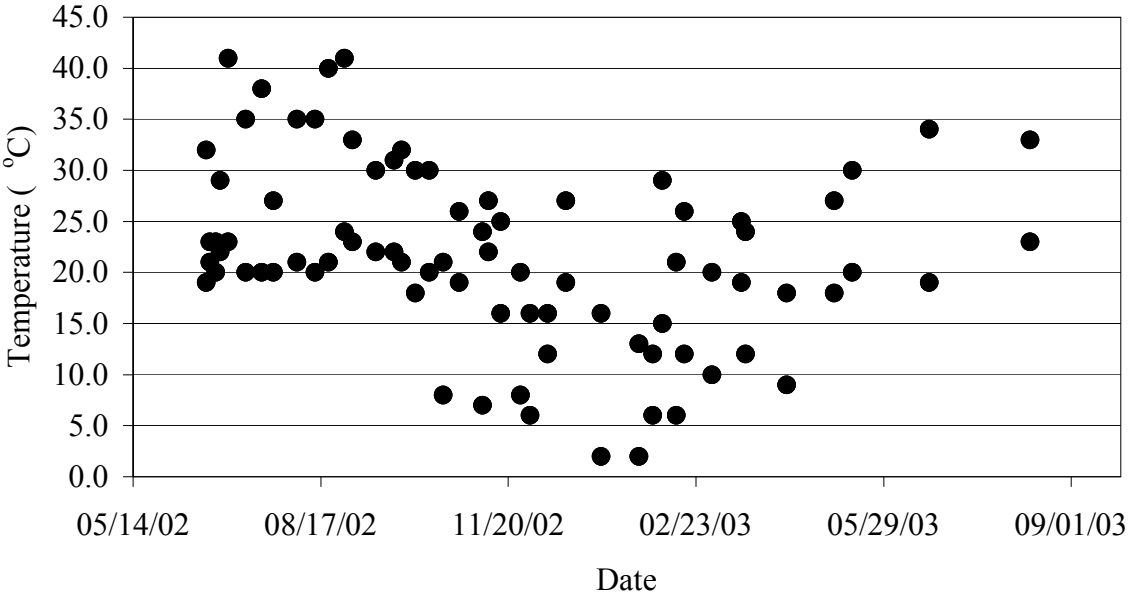

Figure 3-2. Air temperature readings at the Dairy Research Unit in Hague, FL over the study period. Air temperatures varied both seasonally and diurnally with afternoon values exceeding 40 °C in the summer of 2002.

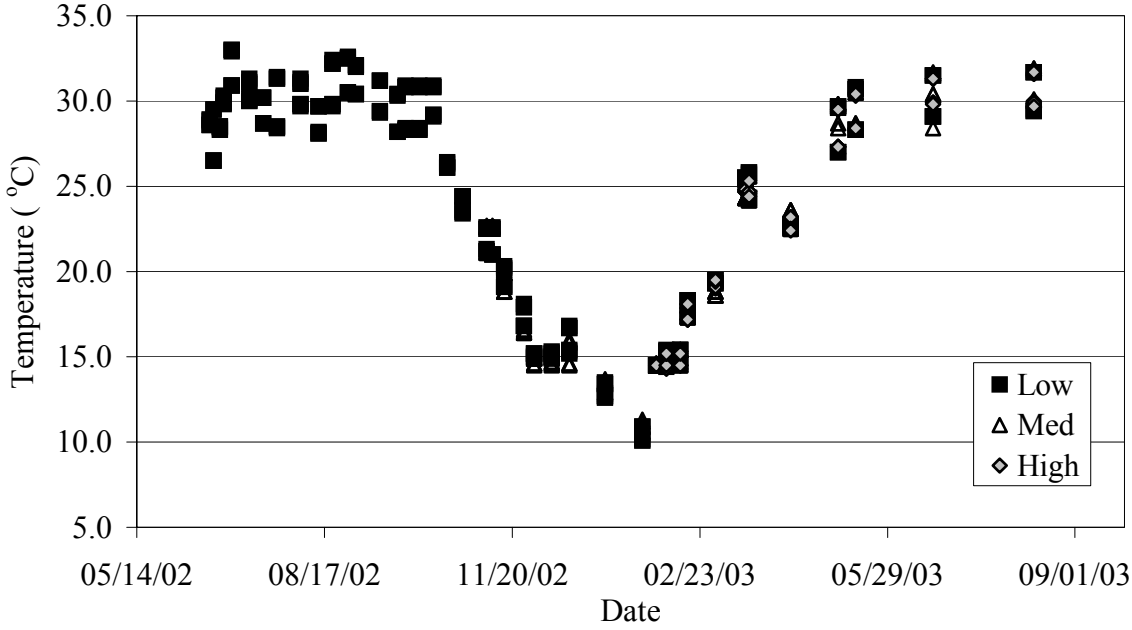

Figure 3-3. Input water temperatures in the low, medium and high nutrient addition treatments. Water temperatures fluctuated both diurnally and seasonally, often exceeding 30 °C in the afternoons and did not differ between treatments.

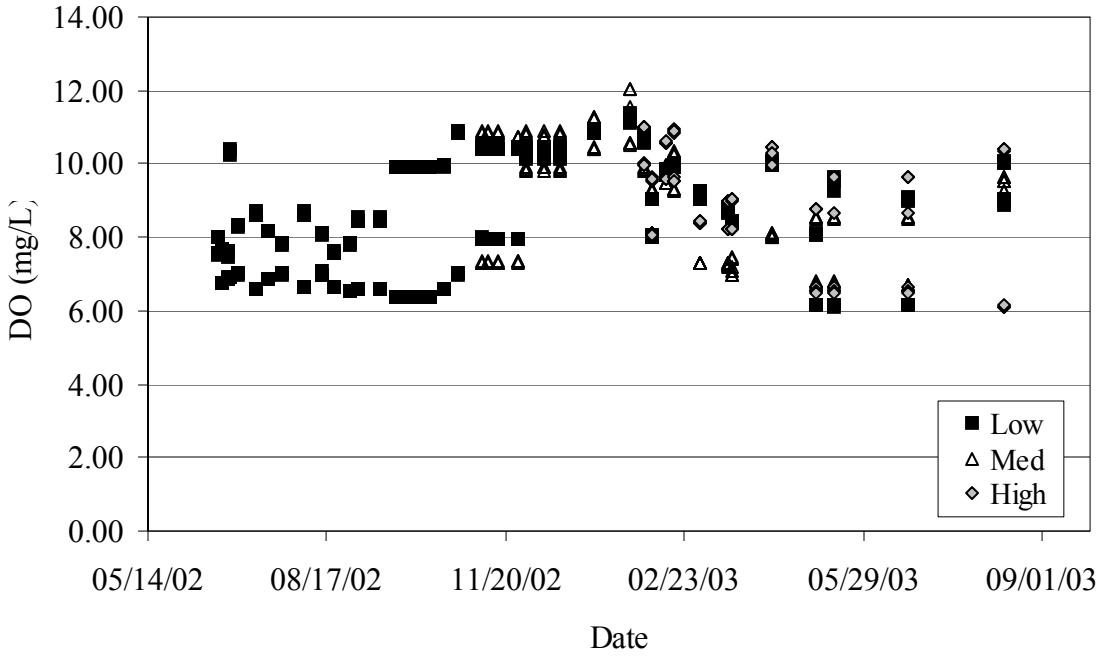

Figure 3-4. Raceway dissolved oxygen (DO) readings in the low, medium and high nutrient addition treatments. Values did not differ between treatments.

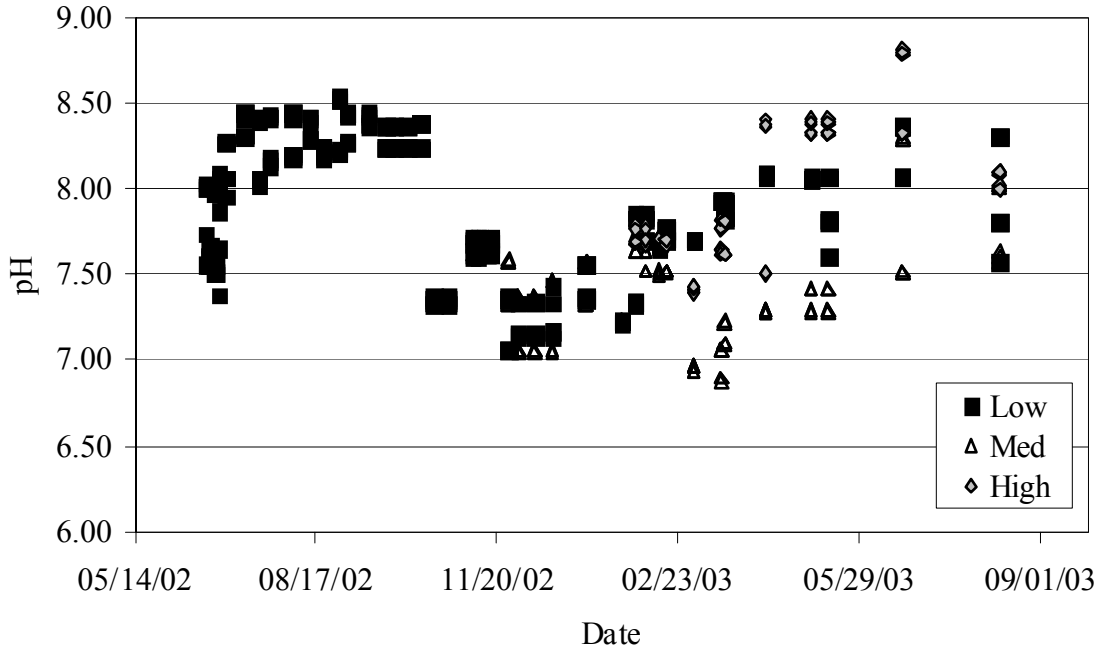

Figure 3-5. Raceway pH in the low, medium and high nutrient addition treatment systems. Raceway pH values fluctuated both sesasonally and diurnally and tended to be elevated in the low and high nutrient addition treatments.

Increases in phosphorus and nitrogen levels in the source ponds did not correspond to fertilizer addition or clam mortality events. Source pond total phosphorus (TP) ranged between 0.061 and 0.211 mg/L in the low nutrient addition treatment, 0.047 and 0.471 mg/L in the medium nutrient addition treatment and 0.043 and 0.386 mg/L in the high nutrient addition treatment (Figure 3-6). Source pond total dissolved phosphorus (TDP) ranged from 0.002 to 0.091 mg/L in the low nutrient addition treatment, 0.007 to 0.223 mg/L in the medium nutrient addition treatment and 0.028 to 0.300 mg/L in the high nutrient addition treatment (Figure 3-7). A major portion of the source pond TP was made up of the dissolved form as TDP in all of the nutrient addition treatments as TDP followed a similar pattern as TP with sharp increases in the spring 2003.

Source pond total nitrogen (TN) ranged from 0.177 to 9.034 µg/L in the low nutrient addition treatment, 0.328 to 12.069 µg/L in the medium nutrient addition treatment and 1.066 to 2.786 µg/L in the high nutrient addition treatment (Figure 3-8). Total N values fluctuated in the low and medium nutrient addition treatments and only slightly increased in the high nutrient addition treatment over the experimental period.

Chlorophyll *a* (chl *a*) ranged from 3.218 to 27.511 mg/m^3 in the low nutrient addition treatment, 4.505 to 26.397 mg/m^3 in the medium and 19.789 to 147.299 mg/m^3 in the high (Figure 3-9). Chlorophyll *a* in all treatments displayed an increase after February of 2003 with peaks from April to August of 2003. Phytoplankton communities in the source ponds were dominated by diatoms in the low nutrient addition treatment, diatoms and cyanophytes in the medium and chlorophytes in the high. Diatoms were present in all ponds throughout the study period.

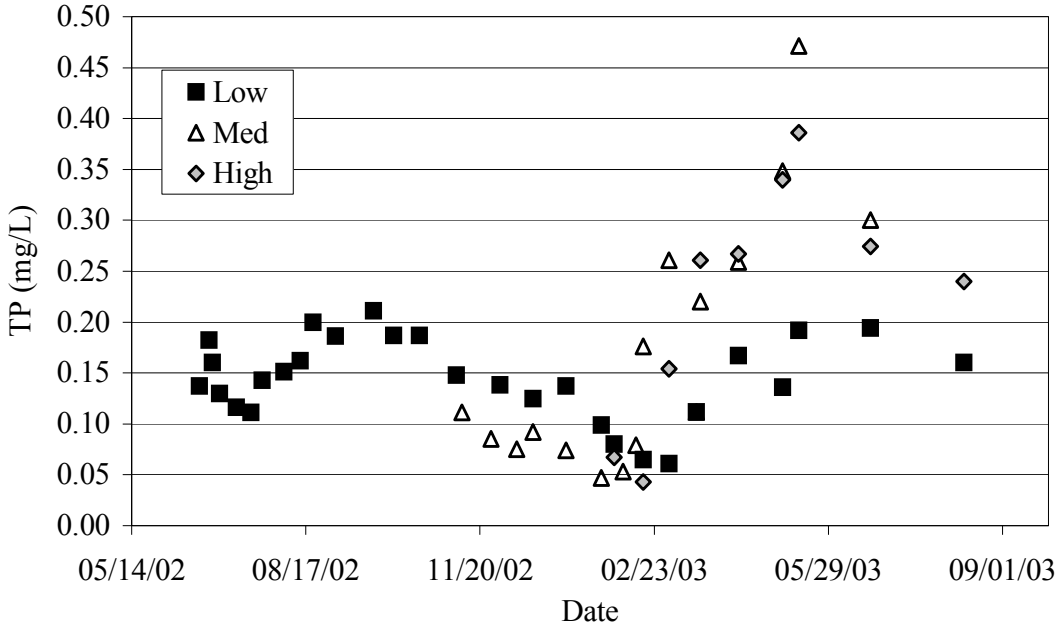

Figure 3-6. Total phosphorus (TP) in the low, medium and high nutrient addition treatments. TP increased during the summer of 2003 in the medium and high treatments while values fluctuated with no apparent seasonality in the low treatment.

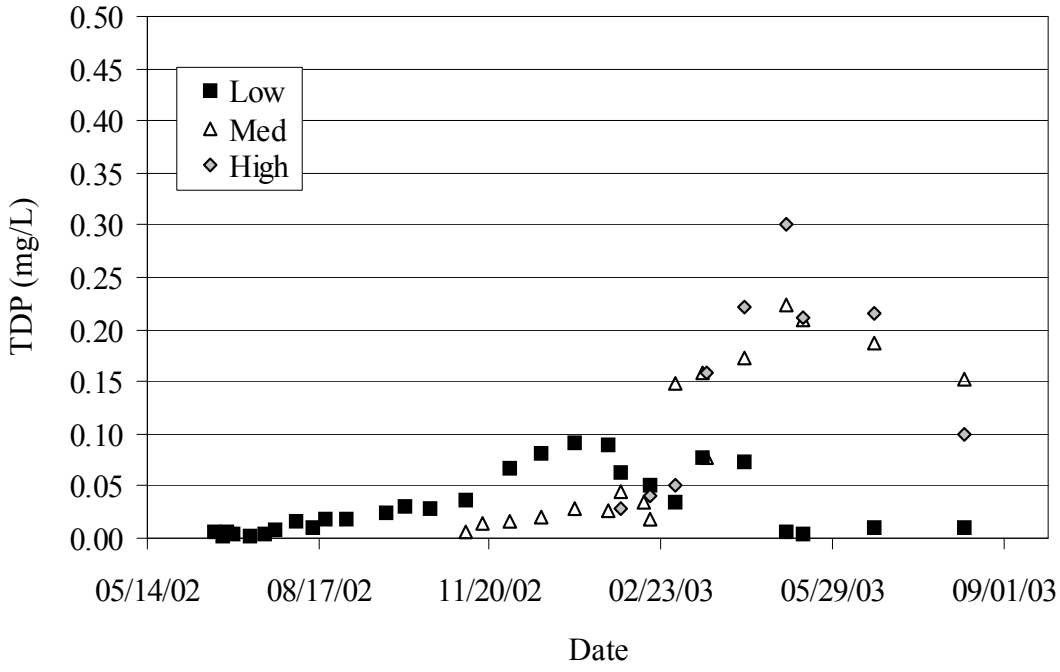

Figure 3-7. Total dissolved phosphorus (TDP) in the low, medium and high nutrient addition treatments. Total phosphorus was comprised largely of TDP therefore patterns were similar to trends in TP.

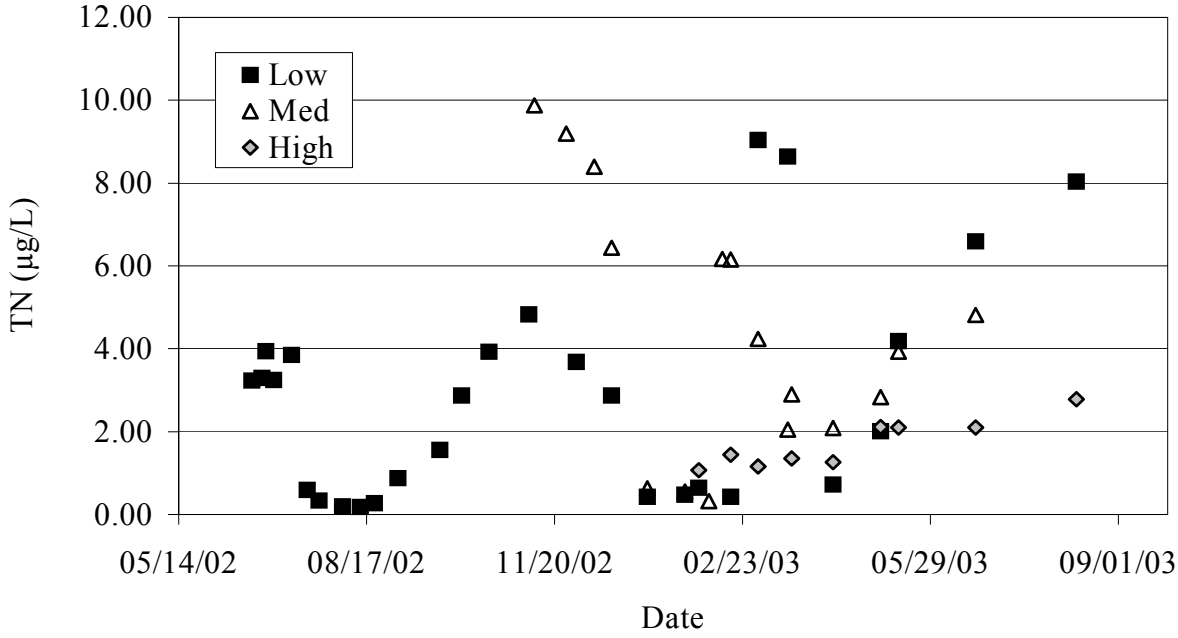

Figure 3-8. Total nitrogen (TN) in the low, medium and high nnutrient addition treatment source water. TN fluctuated in the low and medium treatments and steadily increased in the high nutrient addition treatment.

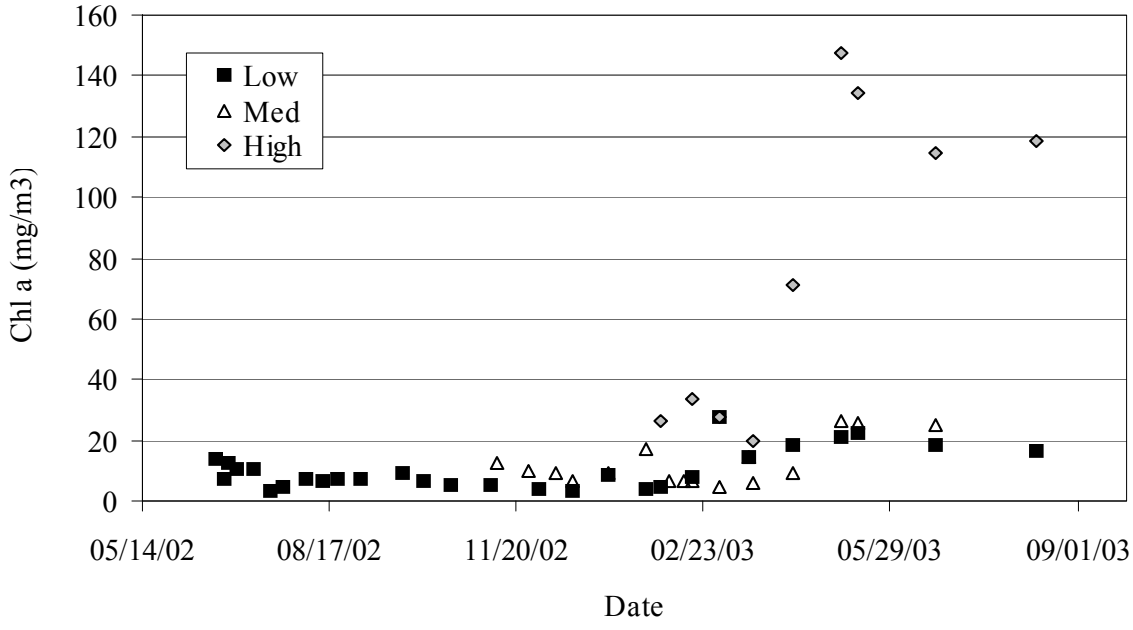

Figure 3-9. Chlorophyll *a* (chl *a*) in the low, medium and high nutrient addition treatment source ponds. All treatments show an increase in chl *a* beginning in Febuary 2003.

Clam Population Dynamics In Treatment Raceways

Survival

Clam stocking densities were estimated at between 6,336 to 10,686 individuals per raceway using the volumetric method (Table 3-3), which was near the target stocking density of 10,000 clams per raceway. The number of live clams in each raceway declined at each sampling interval for all raceways in each of the nutrient addition treatment systems (Table 3-4). All nine raceways had decreasing densities and low numbers of surviving live clams at the end of the study period (Table 3-5). The actual number of clams stocked was reduced by 99 % by the end of the study in all raceways; however, a small number of clams survived (Table 3-5).

Table 3-3. Number of clams stocked in each raceway estimated using the volumetric method. The estimated number of clams per raceway (RW) and standard errors (SE) were calculated from the mean number of clams measured per transfer cup and the number of full cups added to each raceway. The number of clams sacrificed for biomass analysis was subtracted to yield the estimated clams/raceway.

RW	Cups/RW	Mean clams/cup	SE	n	Estimated clams/RW	SE
1	52	206	4	10	10,686	208
2	51	207	6	10	10,547	306
3	51	190	4	10	9,670	204
4	50	192	3	10	9,575	150
5	50	196	7	10	9,790	350
6	50	196	4	10	9,775	200
7	99	64	6	10	6,336	594
8	69	102	12	10	7,004	828
9	109	78	13	10	8,448	1417

The actual number of clams stocked in each raceway was overestimated using the volumetric method of estimation at the time of stocking. Therefore, the values given in Table 3-3 were not used in any further analysis. The final live clam densities for each raceway estimated by the spatial method (Table 3-4) were more similar to the actual number of live clams found in each raceway at the end of the study (Table 3-5).

Table 3-4. Number of live clams found alive at each sampling interval estimated using the spatial technique. Clams per raceway (RW) and standard errors (SE) were calculated using the average number of clams found per 100 cm^2 quadrat sampled, (n = 27 samples per raceway) converted to the number of clams per m^2,which was multiplied by the 7.2 m^2 of available substrate area per raceway.

	Summer 2002		Fall 2002		Winter 2002		Spring 2003	
RW	Clams/ RW	SE	Clams/ RW	SE	Clams/ RW	SE	Clams/ RW	SE
1	4000	306	560	150	427	110	320	117
2	4400	340	907	179	1013	259	880	208
3	4267	295	827	166	613	251	80	44
4	n/a	n/a	4282	387	1973	292	400	129
5	n/a	n/a	4128	286	2747	356	640	155
6	n/a	n/a	4377	563	2827	354	400	111
7	n/a	n/a	n/a	n/a	2476	294	27	27
8	n/a	n/a	n/a	n/a	3225	369	133	67
9	n/a	n/a	n/a	n/a	4665	424	107	74

There were two indications that the spatial technique may have also overestimated the actual number of clams per raceway at each time interval: 1) raceway densities estimated at the end of the study were much higher than the actual number of live clams found in each raceway at the end of the study and 2) the increase in clam density in raceway 9 from stocking (Table 3-5) from the first sampling interval (Table 3-4) could not be substantiated since 123 dead clams had been removed from the raceway during that time period and no indications of reproduction were observed. The number of live clams in each nutrient addition treatment (Figure 3-10) was calculated using the actual number of clams stocked (Table 3-5), the actual number of live clams found at the end of the study (Table 3-5) and the number of clams at each sampling interval in between (Table 3-4).

No attempt at biomass harvest was made due to the high losses of clams over the study period. Clams were only removed from the raceways as needed for periodic mortality counts and at seasonal sampling intervals for biomass determination. There were no indications of predation by wildlife as evidenced by the lack of obvious disturbance to the raceway substrate and lack of

tracks that would have been left by mammalian predators around the raceway site. Large birds such as cattle egrets often congregated on the sides of the raceways; however, predation of clams in the raceway by avian predators was not directly observed.

Table 3-5. Actual number of live clams stocked in each raceway and at the end of the study. Actual number of clams stocked was determined from cumulative counts of live and dead clams over time as well as live and dead clams present at the end of the study. The number of clams removed represents losses due to mortality and destructive sampling but not predation.

Raceway	Actual clams stocked	Clams removed	Live clams present at finish
1	7083	7047	65
2	9251	9210	47
3	6794	6771	23
4	6356	6301	55
5	4663	4601	62
6	4478	4434	38
7	4880	4871	9
8	5340	5323	17
9	6338	6319	19

Clam survival could not be accurately verified using periodic counts of dead shells removed from the substrate surface of each raceway because dead clams were also found buried in the substrate, making them inaccessible for enumeration until the entire raceway could be excavated at the end of the experiment. The cumulative number of dead clams collected on the substrate surface after large mortality events (Figure 3-11) reflects trends in reductions of raceway live clams (Figure 3-10). Dead clams removed over the course of the study and at the end of the study accounted for more than 90 % of the total number of clams stocked.

Growth

There were no clear patterns in shell size distributions at stocking or at each sampling interval; therefore, a cohort-based analysis of changes in shell size within the raceway population could not be performed. Instead, the average shell length of the individuals found over time in each raceway is given (Table 3-6**)**. No consistent long-term trends in shell size data were observed

over time in raceways 1-6. All raceways showed some increase in mean shell length at the first sampling interval, and clams in raceways 7-9 showed a continued increase in mean shell length at Interval 2; however, any trends present must be viewed with caution due to the decreasing sample size and high mortality rates.

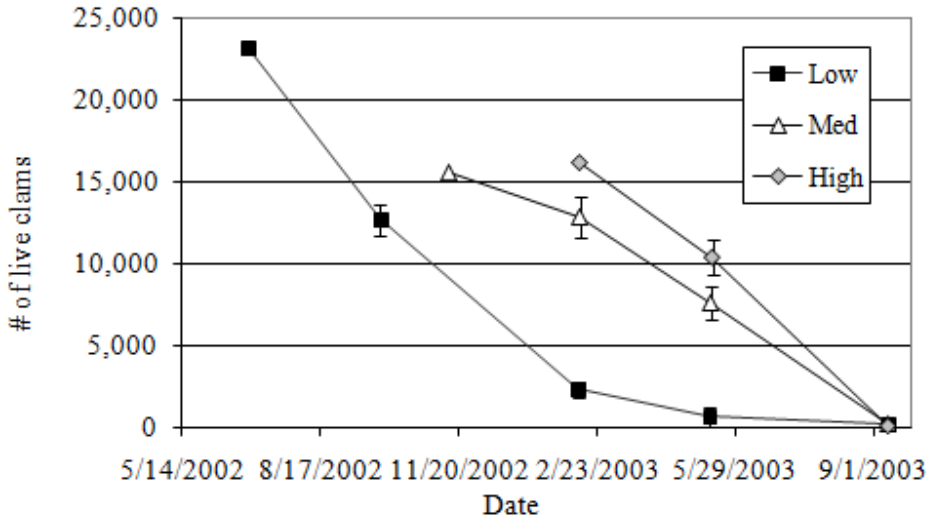

Figure 3-10. Number of live clams in each nutrient addition treatment. No increases in live clam density were found in any of the nutrient addition treatments.

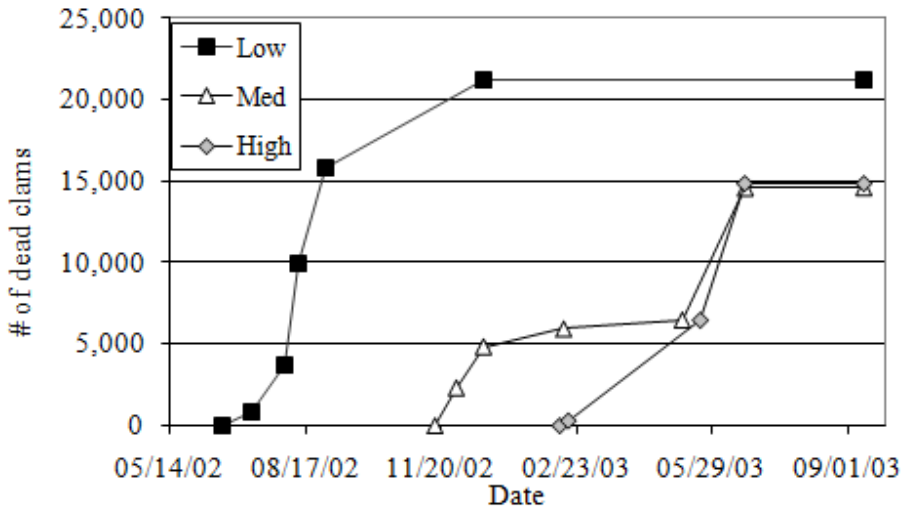

Figure 3-11. Cumulative number of dead found on the substrate surface in the low, medium and high nutrient addition treatments. Mortality was greater during the warmer periods in all three nutrient addition treatments.

Table 3-6. Mean shell lengths measured from clams in each raceway (RW) both at stocking and at each sampling interval. Mean shell length displayed a constant increase in the high nutrient addition treatment raceways; however, decreases in sample sizes (n) over time in each raceway prevented positive identification of any discernable trends in shell size distributions and mean shell length over time.

RW	Shell Length	Time Interval				
		Stocking	Interval 1	Interval 2	Interval 3	Interval 4
1	Mean	18.6	20.3	20.1	19.5	21.2
	SE	0.2	0.2	0.5	0.6	0.5
	Range	10.9-30.6	16.6-30.0	13.2-24.1	14.4-23.3	18.7-23.3
	n	270	150	21	16	12
2	Mean	18.0	20.4	15.9	16.4	20.1
	SE	0.2	0.2	0.7	0.5	0.4
	Range	8.0-27.1	8.9-28.7	10.2-24.6	11.0-23.6	11-24.1
	n	270	165	34	38	35
3	Mean	18.0	20.8	17.5	17.4	19.6
	SE	0.2	0.2	0.8	0.4	0.3
	Range	8.5-28.5	13.2-24.1	8.8-24.4	15.0-22.4	19.1-20.0
	n	270	160	31	23	3
4	Mean	19.2	20.0	22.5	22.6	
	SE	0.2	0.2	0.2	0.8	
	Range	9.21-28.9	10.6-29.0	16.3-29.2	13.9-25.6	
	n	270	200	74	15	
5	Mean	19.5	19.8	22.6	20.0	
	SE	0.2	0.2	0.3	1.0	
	Range	12.1-27.4	12.8-33.5	10.0-28.0	9.7-24.8	
	n	270	206	102	24	
6	Mean	19.1	18.9	22.0	20.3	
	SE	0.2	0.2	0.3	1.2	
	Range	11.1-26.5	11.7-31.3	8.4-30.0	10.2-25.1	
	n	270	203	105	15	
7	Mean Length	22.2	25.8	31.4		
	SE	0.5	0.6	0		
	Range	10.2-34.1	16.4-34.3	31.4		
	n	270	130	1		
8	Mean	24.9	25.0	29.2		
	SE	0.4	0.4	1.5		
	Range	10.3-38.8	17.3-33.3	25.0-33.0		
	n	270	168	5		
9	Mean	22.7	26.5	27.0		
	SE	0.4	0.4	1.1		
	Range	10.3-34.0	5.8-41.3	23.7-28.7		
	n	270	222	4		

Of the 972 total tagged clams introduced to the raceways, only 25 were recaptured alive over the course of the study. All of the recaptured live tagged clams showed increased shell length (Figure 3-12). The largest increases in shell length were observed in clams recovered from the high nutrient group, and clams in the medium nutrient addition treatment appeared to have a larger increase in shell growth during warmer months (Table 3-7). The range in shell length for the individuals sampled for biomass analysis was 10.2 to 33.7 mm (Table 3-8). The high nutrient addition treatment contained individuals with the largest shell size (31-33.7 mm) compared to the low and medium nutrient addition treatment.

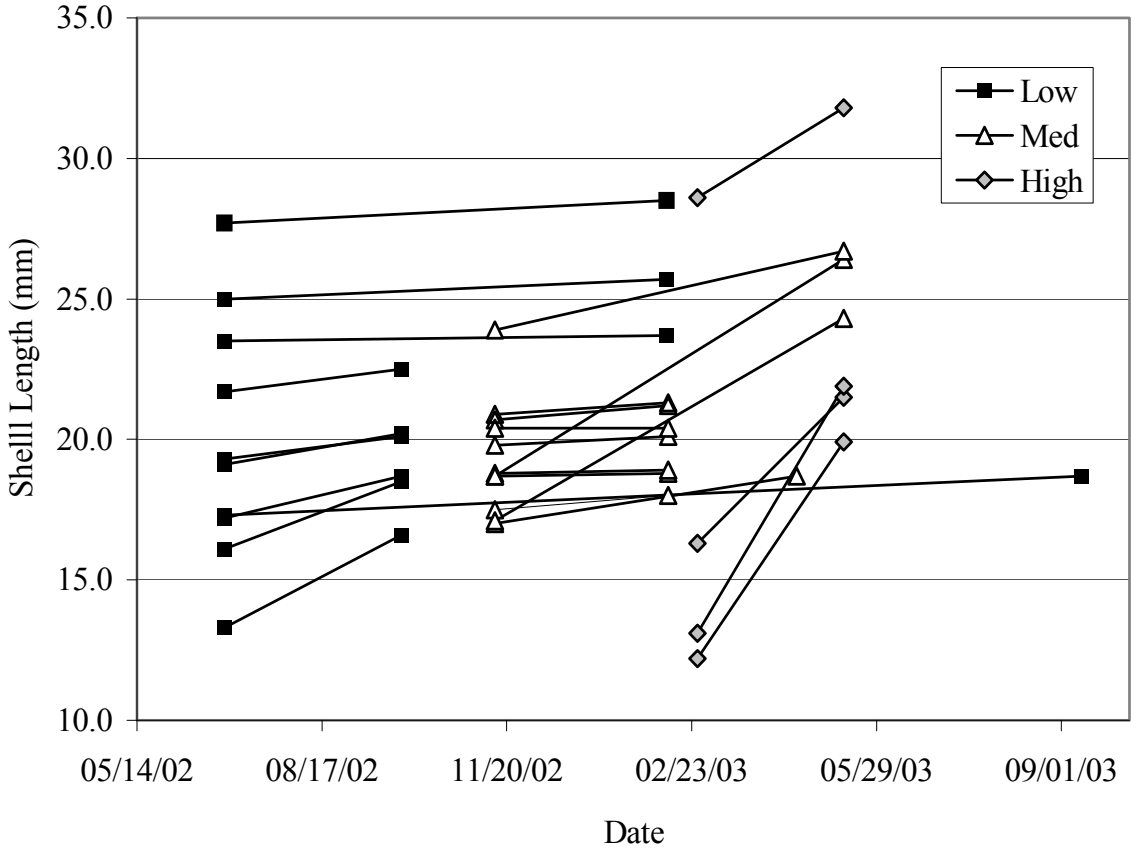

Figure 3-12. Changes in shell lengths of tagged clams captured alive in each nutrient addition treatment. Growth was observed in all live tagged clams recovered with the largest increases found in the high and medium nutrient groups.

Table 3-7. Shell growth rates for tagged clams in each nutrient addition treatment for each seasonal time interval. Growth rates were calculated from initial and recovered shell lengths of live, tagged clams. Shell growth rates were similar for live, tagged clams found at any time in the low nutrient addition treatment, while growth rates during the spring were much higher than the winter for the medium nutrient addition treatment.

Nutrient treatment	Season	Mean growth rate (mm/day)	SE (mm/day)	Growth rate range (mm/day)	Initial shell size range (mm)	n
Low	All	0.0119	0.0036	0.0009-0.0363	13.3-27.7	10
Med	Winter 2002	0.0031	0.0008	0-0.0056	17.5-20.5	7
Med	Spring 2003	0.0556	0.0158	0.0258-0.0856	17.0- 23.9	4
High	Spring 2003	0.0830	0.0168	0.0427-0.1173	12.2-28.6	4

Table 3-8. Shell size information on clams sampled for tissue biomass analysis from each nutrient addition treatment raceway system. The largest individuals were found in the high nutrient addition treatment.

Nutrient treatment	Average length (mm)	SE (mm)	Shell size range (mm)	n
Low	19.9	0.2	10.2 – 30.4	210
Med	20.3	0.2	13 – 28.4	162
High	24.8	0.7	12.6 – 33.7	81

The mean water content of whole clams was 31.5 % (SE = 0.4) with a range of 25.0 – 45.1 %, n = 453 observations. Water content did not vary significantly ($p > 0.05$) with shell length, between raceways, over time or between nutrient addition treatments based on the ANOVA analysis. The wide range of water content values observed prevented the use of tissue wet weights to assess biomass even though clam wet weight values were highly correlated with the whole clam DW values (corr coeff = 0.90, n = 453 observations).

Ash content of the meat tissue was much lower than the shell tissue, and shell ash made up a greater portion of the total clam ash than meat tissue (Table 3-9). Ash content did not vary significantly ($p > 0.05$) with shell length, between raceways, over time or between nutrient addition treatments based on the ANOVA analysis.

Table 3-9. Mean and range of ash content values for meat, shell and total clam tissues pooled for all clams sampled. Shell tissue had higher ash content and made up a greater portion of the total clam ash than meat tissue.

Tissue	Mean ash (%)	SE (%)	Ash content range (%)	n
Clam	94.71	0.06	90.45 – 97.39	238
Shell	97.48	0.02	96.06 – 98.23	238
Meat	13.74	0.55	2.50 – 17.00	238

Overall, clams sampled in this study allocated a mean of 95.9 % (SE = 0.1) of total clam DW biomass as shell tissue with a range of 88.4 – 99.7 %, n = 453. Meat biomass accounted for the other 4.1 % of the dry weight on average (SE = 0.1) with a range of 0.3 to 11.6 %, n = 453. The amount of DW biomass allocated to shell tissue did not vary significantly ($p > 0.05$) with shell length, between individual raceways within each nutrient addition treatment, over time in each nutrient level or between nutrient addition treatments. Dry weight values for the meat, shell and whole clam varied significantly ($p < 0.05$, n = 453) with shell length. Correlation analysis showed a strong relationship between shell and whole clam tissue biomass and shell length (Table 3-10).

Table 3-10. Results of the meat, shell and total clam tissue dry weight (DW) to shell length correlation analysis. Shell and whole clam tissue biomass had a stronger correlation to length than to meat tissue

	Length	Meat DW	Shell DW	Clam DW
Length	1	x	x	x
Meat DW	0.626	1	x	x
Shell DW	0.966	0.599	1	x
Clam DW	0.967	0.626	0.999	1

No statistically significant differences ($p > 0.05$) in the length-weight regression relationships were found between raceways, over time or seasonality in each nutrient addition treatment using the ANOVA. However, significant differences ($p > 0.05$) were found in each nutrient level for all meat, shell and whole clam tissue. Tissue DW and shell length values were

then pooled for the clams in each nutrient addition treatment, and length was removed in the ANOVA. Regression relationships between shell length and DW are presented in Table 3-11 for each tissue type and each nutrient addition treatment. Intercepts and slopes of the regression lines for ln (clam DW) and ln (shell DW) for the high nutrient group were significantly different ($p < 0.05$) than the low and medium nutrient levels. This relationship did not differ significantly ($p > 0.05$) between the low and medium nutrient addition treatments; however, high nutrient addition treatment did differ significantly ($p = 0.03$) from the low and medium levels.

Table 3-11. Dry weight (DW) biomass vs length regression relationships, significance differences and variability for whole clam, shell and meat tissues from each nutrient addition treatment. This relationship did not differ significantly between the low and medium nutrient addition treatments The relationship for the high nutrient addition treatment was significantly different from both the low and medium nutrient addition treatments.

Tissue	Nut. level	Regression Equations * denotes significant difference ($p < 0.05$)	r^2	n
Clam	Low	ln (clam DW) = -3.6884 + 0.2893 (length) -0.0038 (length^2)	0.9394	243
	Med	ln (clam DW) = -3.6723 + 0.2873 (length) -0.0038 (length^2)	0.9614	161
	High	ln (clam DW) = -4.9450* + 0.3949*(length) -0.0059*(length^2)	0.9738	81
Shell	Low	ln (shell DW) = -3.8920 + 0.3042 (length) -0.0041 (length^2)	0.9421	243
	Med	ln (shell DW) = -3.9270 + 0.3056 (length) -0.0042 (length^2)	0.9642	161
	High	ln (shell DW) = -5.0245* + 0.3990*(length) -0.0060*(length^2)	0.9718	81
Meat	Low	ln (meat DW) = -4.0291 + 0.0190 (length) + 0.0021 (length^2)	0.2903	243
	Med	ln (meat DW) = -2.8659 – 0.0716 (length) + 0.0042 (length^2)	0.2447	161
	High	ln (meat DW) = -6.7690* – 0.2476 (length) -0.0029 (length^2)	0.8895	81

Shell length values were plotted against the actual and predicted DW values derived from the regression equations (Table 3-11). These relationships are plotted for the whole clam, shell and meat tissues in Figure 3-12, Figure 3-13 and Figure 3-14, respectively. The mean percentage shell tissue calculated using the regression equations for the predicted whole clam biomass values was similar to the tissue DW and wet weight (WW) values.

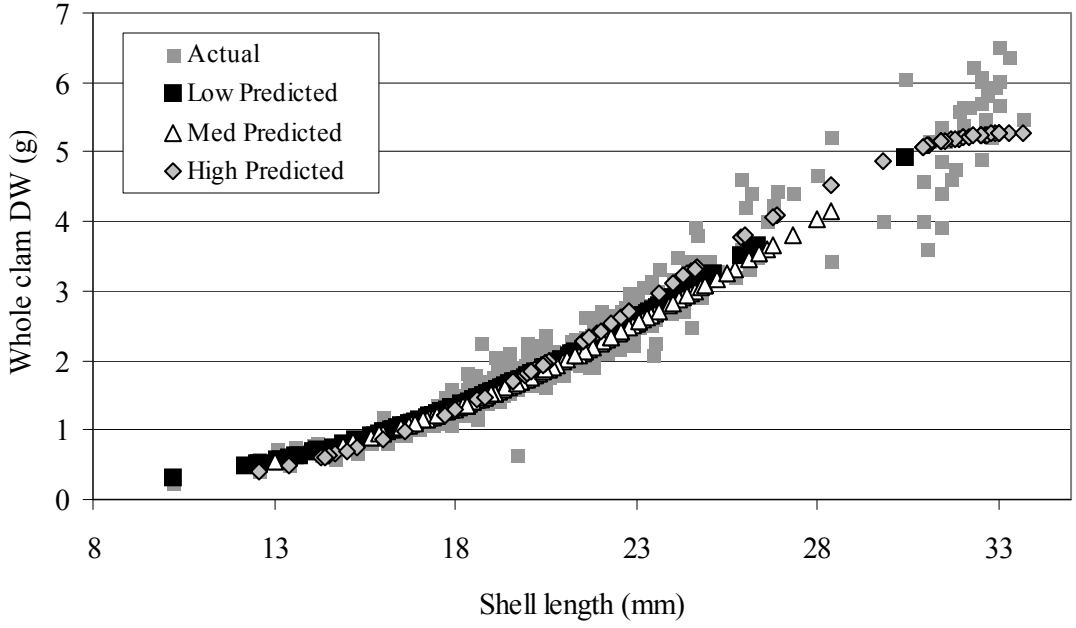

Figure 3-12. Regression relationships for shell length vs actual and predicted (Table 3-11) whole clam dry weight (DW) values for each nutrient addition treatment. This relationship for the high treatment was significantly different than both low and medium nutrient addition treatments.

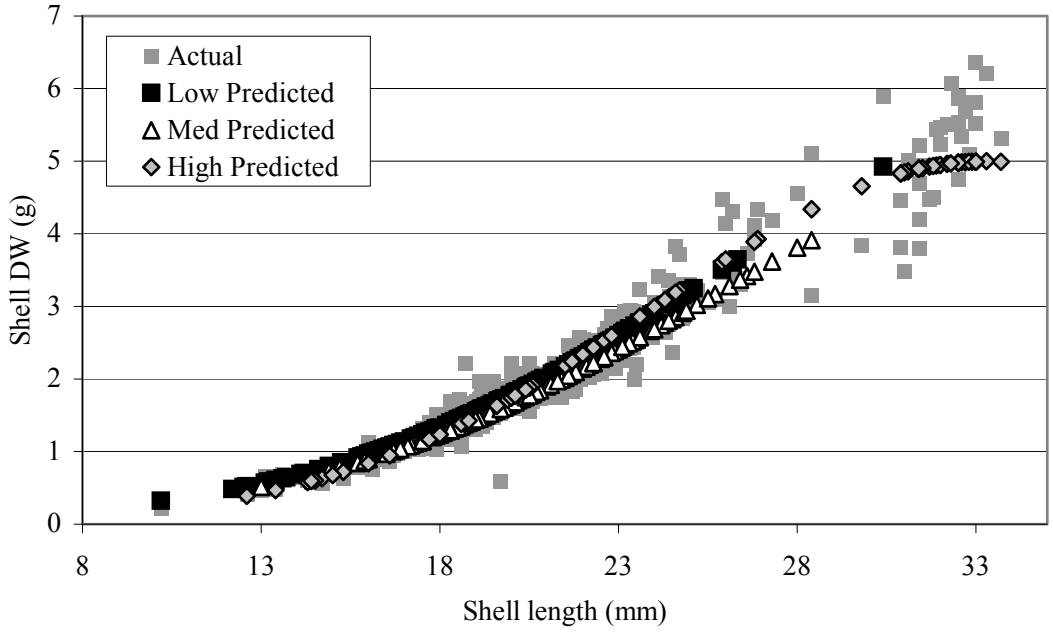

Figure 3-13. Regression relationships for shell length vs actual and predicted (Table 3-11) shell dry weight (DW) values for each nutrient addition treatment. The relationship was significantly different in the high nutrient addition treatment than both the low and medium nutrient addition treatments.

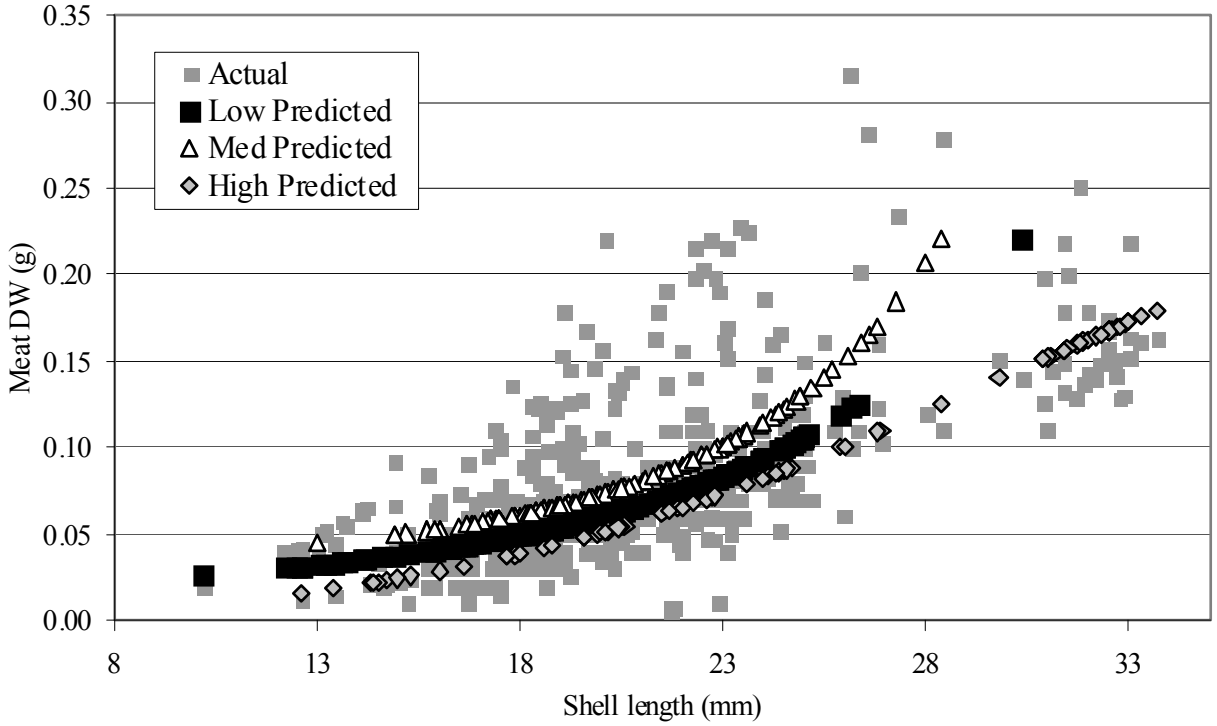

Figure 3-14. Regression relationships for shell length vs actual and predicted (Table 3-11) meat dry weight (DW) values for clams. Relationships for the high nutrient addition treatment were significantly different than the low and medium nutrient addition treatments.

These regression relationships can only be applied to clams in the 10.2 mm to 33.7 mm shell length range for the high nutrient addition treatment and the 10.2 – 30.4 mm range for the medium and low treatments that were used to calculate them (Table 3-8). Interpolation of the equations (Table 3-11) for clams beyond 34 mm upper limit results in reduced or logarithmically increased tissue dry weight estimates due to the shape of the extended regression line.

Biomass at stocking and at proceeding time intervals was estimated using length vs weight relationships, similar to Joy and McCoy (1975). Calculations of biomass were made for each nutrient addition treatment (Figure 3-15) using average shell length (Table 3-6). Shell length to dry weight regression equations (Table 3-11) and live clam densities (Figure 3-10) were similar to those found by Cataldo et al. (2001) who were not able to discern population cohorts. It was

acceptable to apply the regression equations to calculate dry weight biomass from the average shell lengths at each interval since shell length values fell within the ranges required by each nutrient addition treatment level (Table 3-8). Estimated population biomass decreased markedly from stocking to the end of the study for each nutrient addition treatment.

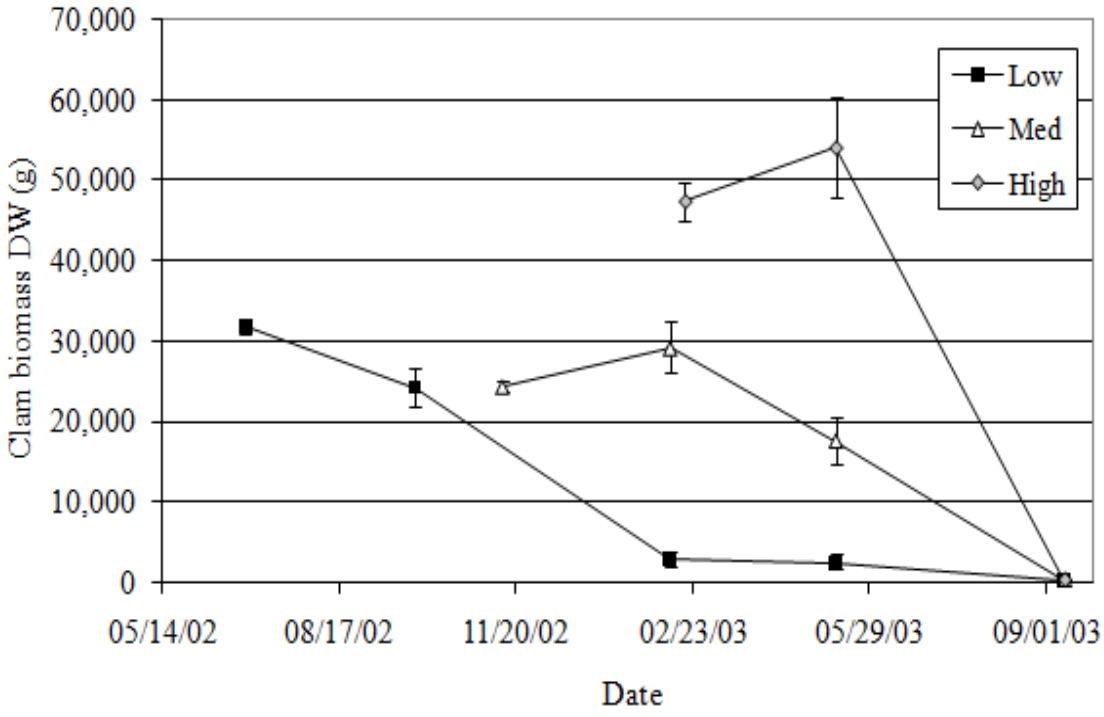

Figure 3-15. Estimated clam dry weight (DW) biomass over time in the low medium and high nutrient addition treatments. Increases in estimated DW biomass may not be indicative of tissue production due to the variability in the clam density measurements.

Reproduction and recruitment

No evidence of successful reproduction and recruitment was found in any of the nutrient addition treatments, as indicated by declines in clam populations (Figure 3-10), the lack of individuals less than 7 mm in shell length (Table 3-6), and no evidence of juveniles in any of the water samples used in the phytoplankton speciation survey.

Health

Linear regression analysis yielded a correlation coefficient of 0.85 between the condition indices based on $CI_{(WT)}$ and $CI_{(VOL)}$, indicating that they were very similar. The $CI_{(WT)}$ index had a slightly lower coefficient of variation (0.53) than $CI_{(VOL)}$ (0.56) (n = 452). Shell length was not a significant factor ($p > 0.05$) in the values of either index, so it was removed from the analysis.

Results for the ANOVA for both indices were similar, showing significantly higher condition values for both indices ($p = 0.01$) in the medium nutrient level, but only at sampling Interval 1. $CI_{(WT)}$ and $CI_{(VOL)}$ ranged from 0.302 to13.210 and 1.435 to 15.831 per individual, respectively (Table 3-12). Condition indices calculated for clams in the medium nutrient addition treatment at interval 1 tended to be higher than all other nutrient addition treatment/interval combinations. This trend was probably due to slightly higher meat dry weight values in the medium nutrient addition treatment at interval 1. Consequently, meat DW values were not significantly different at interval 1 compared to the other intervals within the medium nutrient addition treatment; therefore, the CI is apparently highly susceptible to differences in meat tissue quantity that may not be apparent using the regression analysis.

Table 3-12. Mean, standard error (SE) and range of condition indices values ($CI_{(WT)}$ and $CI_{(VOL)}$) calculated for the medium nutrient addition treatment at Interval 1 compared to values calculated at all other treatment/interval combination. Values for both indices tended to be lower in all other treatment/interval combination.

	Condition index values				
Population	Index	Mean	SE	Range	n
Medium nutrient	$CI_{(WT)}$	7.758	0.238	2.798-13.1280	81
Interval 1	$CI_{(VOL)}$	8.931	0.262	3.406-14.9269	81
All other	$CI_{(WT)}$	3.601	0.080	0.302-10.033	372
combinations	$CI_{(VOL)}$	4.581	0.131	1.435-15.831	372

Amphipod infestation

Amphipods (*Hyallela azteca*) began appearing in dead clam soft tissue and empty shell valves removed from the raceways on July 26, 2002 (38 days after introduction of clams). A sample of five live clams on the substrate surface was randomly removed for inspection on August 9, 2002 and yielded one clam with an amphipod. The clams were immediately shucked to reveal six additional live amphipods enclosed in the shell cavity of one clam.

A notched clam specimen was removed from the sediment surface. The clam was shucked, treated with a 90 % ethanol solution and examined under a dissecting scope (10x – 30x) fitted with a digital camera to reveal nine amphipods. Amphipods were identified and photographed by Gary Warren at the Florida Fish and Wildlife Conservation Commission in Gainesville, Florida. Amphipods were ruled out as a direct cause of the chipping due to their lack of hard mouthparts capable of damaging clam shell material (Covich and Thorpe 2001).

In an attempt to quantify the extent of the amphipod infestation in the raceway clam populations, collections from the following three categories of clams were made: fresh dead clams, live clams on top of the sediment and live clams buried in the sediment. Fresh dead clams are defined as having gaped valves with soft tissue intact. Six live clams were removed from the sediment surface on August 9, 2002, and a sample of 10 live clams was excavated from the substrate on August 18, 2002. All clams were collected at random from the same raceway. Amphipods contained in fresh dead clams were counted immediately after removal from the raceway, and any shell notching was noted. Live clams were placed in Ziploc bags, spun tight, sealed and wrapped with a rubber band to prevent clams from opening during transport to the laboratory for shucking and amphipod count. All clams sampled had a shell length greater than 20 mm.

The observations showed that 100 % of the fresh dead clams sampled contained live amphipods. Amphipod abundances in these clams averaged 12 individuals per clam and ranged from 2 to 23 individuals per clam, and 36 % of the dead shell valves sampled had notches. All live clams taken from the substrate surface were notched, and only 50 % of these individuals contained amphipods. Amphipod abundances within the shell cavities in these live, notched clams ranged from 4 to 9 individuals per clam. None of the live clams found beneath the raceway substrate surface had notches, and only one of these clams contained amphipods within the shell cavity. This live clam, however, had the highest number of amphipods recorded per live clam sampled, nineteen.

After the initial observations on amphipod invasion and shell chipping in raceway clams were made, a small scale experimental system was constructed in an indoor laboratory at Alee Academy in Eustis, FL to test the repeatability of this phenomenon in smaller-scale aquaculture environments. A total of 9 27-L glass aquaria were stocked with between 10, 20 or 30 clams (200 to 600 clams/m^2) and 50, 75 or 100 amphipods (1075 to 2150 amphipods/m^2) collected by hand from clam raceways and transported in large aerated coolers to the laboratory in Eustis, Florida. Lights were maintained on a 12 hour-on/12 hour-off cycle and water temperature was regulated at 23-25 ^{o}C, using submersible heaters. Sand for the aquarium substrate was obtained from the same retailer as the Hague, FL facility. Aquaria were fed with 1 gram per week per aquarium of dried, powdered *Spirogyra sp.* algae obtained from the Hague raceways. No indications of shell chipping, penetration of live clam shell cavities or high clam mortality were recorded. The lack of clam/amphipod interaction in these small-scale systems indicates that the Hague observations could be an isolated incident or that the negative interactions are more likely to occur at larger scale or higher air temperatures.

Discussion

Use of the freshwater clam, *Corbicula*, as the primary active agent in wastewater treatment raceway systems yielded mixed results. Significant growth and phosphorus sequestration was observed in tagged clams, which survived through the study period. Growth in tagged clams appeared to be substantial during warmer periods in the medium and high nutrient addition treatment systems, with growth rates ranging from 0.043 to 0.117 mm/day (Table 3-7). These rates are similar to those reported for other freshwater bivalves (Table 3-13).

Table 3-13. Individual shell length and biomass dry weight (DW) growth rates reported for *Corbicula* and other bivalves occupying different fresh and saline environments. Some values were not reported by the reference author and are noted as (n/a).

Organism	Shell Length Growth (mm/day)	Biomass Growth (g DW/day)	Environment Type	Reference
Corbicula	0.117	0.0024	Agriculture nutrient treatment raceways	This Study
Corbicula	0.058	0.0023	Laboratory at optimum temp	Foe & Knight (1986)
Corbicula	0.180	n/a	Natural river	McMahon & Williams (1986)
Corbicula	0.085	n/a	Power plant effluent canals	Mattice & Wright (1986)
Corbicula japonica	n/a	0.0077	Natural estuary	Fuji (1979)
Dreissena polymorpha	0.080	n/a	Natural lakes and rivers	McMahon & Bogan (2001)
Dreissena polymorpha	0.095	0.0150	Great Lakes	Bitterman et al. (1994)
Elliptio complanata	0.001	n/a	Natural lakes	Anthony et al. (2001)
Venerupis pullastra	0.189	0.0189	Mariculture effluent treatment raceways	Jara-Jara et al. (2000)
Tapes semidecussatus	n/a	0.0422	Mariculture effluent treatment ponds	Shpigel & Fridman (1990)
Ruditapes decussatus	0.333	0.0083	Mariculture effluent treatment raceways	Jara-Jara et al. (1997)
Ruditapes philippinarum	0.377	0.0730	Natural estuary mariculture	Nizzoli et al. (2006)

Despite the positive results with tagged clams, the long-term performance of the broader clam population in the raceways was poor, and dairy wastewater effluent could not tested due to the high ammonia levels present in the effluent addition ponds throughout the study. The latter problem highlights an important issue in the use of an animal-based system for wastewater treatment: ammonia toxicity. From a broader perspective, the high rates of mortality of clams in the raceway systems focus attention on a range of environmental and design issues that must be dealt with in future research efforts, such as temperature, food availability, dissolved oxygen, the effect of multiple environmental stressors, parasitism and predation, depressed reproduction in captivity and issues with enumeration of raceway clam biomass.

Ammonia Concerns

Use of *Corbicula* or other freshwater bivalves in large-scale raceway-based systems as a phosphorus treatment mechanism for dairy wastewater may be limited foremost by ammonia toxicity concerns that could skew normal water usage and land requirements. Ammonia in the toxic, unionized form ammonium (NH_4^+), is a serious concern in the aquatic environment. Levels of ammonium of 0.2 mM as ammonium-nitrogen (NH_4^+-N) negatively affect other bivalves and finfish (Patrick et al. 1968, Epifanio and Srna 1975). Amounts of the toxic form are directly proportional to pH and temperature and increases in ammonia-nitrogen (NH_3-N) are generally used to gauge ammonia toxicity.

Ammonia levels harmful to *Corbicula* and other freshwater mussels have been suggested by Cherry et al. (2005) to be 0.11 to 0.8 mg/L total ammonia nitrogen (NH_3-N). Levels of 0.041 to 0.158 mg/L NH_3-N are harmful to bivalves in marine aquaculture systems and may even be harmful at concentrations as low as 0.006 mg/L NH_3-N (Harris et al. 1998). The presence of ammonia concentrations in excess of 0.25 mg/L NH_3-N in the source ponds receiving

anaerobically digested dairy effluent in this study indicate that even effluent addition rates as low as 5 % to 10 % of source pond volume are not acceptable for production of *Corbicula*.

High ammonia levels in commercial aquaculture systems decimate stocks if not managed correctly. This is especially true in recirculating systems where the lack of water exchange necessitates removal of ammonia to achieve safe levels (Timmons et al. 2002). Keeping ammonium within a tolerable range will be critical to using *Corbicula* as a wastewater treatment mechanism (Haines 1977). Additional treatments would need to be added to the dairy wastewater stream to manage ammonium. Coupling vegetation with *Corbicula* into polyculture systems has also been proposed for nutrient uptake in agricultural waste streams (Stanley 1970, Greer and Ziebell 1974, Mattice 1977). This type of system may provide an additional ammonia management strategy in future applications; however, no large-scale experiments have been developed to demonstrate the applicability of such technologies.

Anaerobically digested dairy effluents may also present additional ammonia concerns compared to non-digested effluents, since digester systems convert nitrogen bound in solids to ammonia by microbial degradation, which adds to the ammonia already present in the barn and milking operation wastewaters (Wilkie et al. 2004). Potential problems with introduction of harmful levels of ammonia were circumvented in this study by simulating algal concentration under dairy wastewater conditions using nitrogen and phosphorous fertilizer applied at different loading rates.

Among the raceways supplied by pond water of different nutrient concentrations, all were subject to high rates of clam mortality over time, particularly in summer. It is possible to form several hypotheses about the environmental factors responsible for these losses other than ammonia, including high temperature, limited food availability, low dissolved oxygen and

parasite interactions. It is useful to explore each of these considerations in an effort to find possible solutions and future directions for research and development.

Temperature

Temperature probably had the most impact on growth of *Corbicula* populations in the clam raceways. Tagged clams recaptured alive in this study showed growth in water temperatures between 10 and 30 ^{o}C with higher growth rates occurring at temperatures above 18 ^{o}C. Growth rate in *Corbicula* increases with temperature as Mattice (1977) observed a maximal growth rate of clams in power plant canals at 24 ^{o}C. Other studies using lab experiments by Mattice (1977) and Foe and Knight (1986) suggest that temperatures above 25 ^{o}C cause a decrease in growth. Buttner and Heidinger (1980) observed the highest growth rates in the temperature range of 11.2 to 24.7 ^{o}C and minimal growth at 4.2 ^{o}C. Conversely, exposure to temperatures exceeding 30 ^{o}C coincided with increased population mortality in this study.

McMahon and Williams (1986) suggest an upper lethal limit of 36 ^{o}C for naturally occurring populations of *Corbicula*. Habel (1970) reports 98 % mortality when temperature exceeds 35 ^{o}C, and Busch (1974) observed no growth and high mortality when temperatures exceed 32 ^{o}C in polyculture ponds shared with catfish. Buttner (1986) suggests that temperatures above 30 ^{o}C be avoided when culturing *Corbicula*, which is supported by the results of this study since water temperatures greater than 30 ^{o}C were observed through much of the study (Figure 3-2), and losses of biomass (Figure 3-11) were consistent with exposure to water temperature above 30 ^{o}C.

Site selection based on temperature may be the key to successful application of *Corbicula* to wastewater treatment. High temperatures have been implicated in limiting success of other *Corbicula*-based experimental wastewater treatment systems in arid and tropical areas prone to high temperatures such as Arizona (Greer and Ziebell 1972) and the US Virgin Islands

(Haines1977). Pond-based experiments examining use of *Corbicula* for aquaculture effluent treatment in temperate Illinois have also reported problems with high temperatures (Habel 1970, Busch 1974, Buttner 1986). Implementation of *Corbicula*-based treatment systems may be restricted to locations where water temperatures do not exceed 30 ^{o}C as suggested by Greer and Ziebell (1972) and may require geographical locations even farther north or in traditionally colder climates than Florida or even Illinois.

Corbicula-based systems may be better suited to application in colder areas because there are no real effective techniques for cooling water in systems of this scale or larger. Source ponds at the Hague site were aerated at night to induce evaporative cooling, but water temperatures still reached 30 ^{o}C and above in the afternoon throughout the spring, summer and fall. Industrial-sized water chillers are not a practical solution due to equipment and energy costs that would exceed the value of the treatment potential. Shading of open water areas may help reduce heat buildup, but it also lowers light availability for phytoplankton productivity. Shading of small raceways with plywood coverings has been used by Haines (1977) to prevent nuisance vegetation growth in *Corbicula* systems and greenhouse netting is also commonly used in commercial aquaculture and agriculture operations, but the effects that covering of raceways may have on clam populations through changes in temperature, oxygen exchange and disruption of possible circadian rhythms are not known. Heat exchange with cold water drawn deep from aquifers or surface water reservoirs may provide solutions, however, the energy required for pumping and lack of specific locations with available cold water sources may prohibit implementation.

Application of *Corbicula* to systems in colder climates may result in limited biomass production at temperatures below 4.2 ^{o}C (Buttner and Heidinger 1980) but cold-related

mortalities as well (McMahon and Williams 1986). Ice cover can depress dissolved oxygen below tolerable levels in northern areas (McMahon and Bogan 2001). Temperatures in this study did not fall into the low temperature ranges (< 5 ^{o}C) identified by McMahon and Williams (1986) as lethal; however lower clam growth rates were evident during the winter months.

Waste heat generated from power plants has been proposed by Mattice (1977) for encouraging biomass production in *Corbicula* aquaculture in colder climates, however, this resource may not be applicable to dairy operations, since locations of power plants would determine available sites for clam raceways. Land use requirements for coupling power plants and dairy farms may be the ultimate barrier to waste heat utilization of this type due to the potential contamination by agriculture practices in the vicinity of surface water reservoirs needed for power plant cooling towers. The need for transfer of wastewater over long distances in the volumes produced by typical dairy farms (average milking herd of 359 cows = wastewater production of 502 m^3/day at Dairy Research Unit in Hague, FL (Wilkie et al. 2004), decreases the attractiveness of this type of waste heat usage at any appreciable scale in dairy wastewater treatment.

Food Availability

Food limitation may also have been a problem in the survival of clams in this study. Phytoplankton availability has been implicated as a limiting factor for *Corbicula* biomass production in natural systems (Foe and Knight 1986, Mattice and Wright 1986), and low particle concentrations have been linked to poor performance in *Corbicula*-based wastewater treatment systems (Haines 1977). Chlorophyll *a* levels remained below the 100 to 300 µg/L range reported by Cohen et al. (1986) to sustain growth and survival in natural systems. Source pond phytoplankton production was probably limited to factors other than phosphorus availability.

As expected, increased in source pond water phosphorus in the high nutrient addition treatment system corresponded with an increase in phytoplankton biomass; however, phytoplankton biomass remained low in the medium nutrient addition treatment system (Figure 4-3). Utilization of phosphorus by phytoplankton is the first stage in this clam raceway system; however, performance of this phase was poor since there was a large portion of dissolved phosphorus in all of the systems indicating that phytoplankton production was probably not limited by phosphorus. In order to identify possible limiting factors, study of nutrient dynamics beyond nitrogen and phosphorus may be needed in large-scale systems when utilizing phytoplankton incubation ponds for clam raceway source water.

Phosphorus and nitrogen levels in source pond water after the one-month incubation period following fertilizer additions were similar to pre-addition levels in both fertilizer addition treatments. Source pond total phosphorus concentrations of 0.23 and 0.46 mg P/L were expected in the medium and high nutrient addition treatments, repectively, if the fertlilizer P would have been retained in the water column. These total phosphorus levels were not achieved until February 2003 after the clam raceways had been operational for at least one month. Initial investigation of phytoplankton productivity with wastewater addition, as demonstrated in clam-based treatment system scaling by Borges et al. (2005), should be employed to enhance large-scale clam raceway food availability prior to clam exposure to identify and solve potential problems.

Although *Corbicula* can utilize a wide variety of particles as a food (Silverman et al. 1995, McMahon and Bogan 2001), the potential for its use in phosphorus treatment depends on the extent that phosphorus is transformed into a particulate form for clam uptake. Phytoplankton species management could potentially impact clam food resources since food value, and algal

phosphorus content can vary with speciation and environmental conditions (Wetzel 2001). This may be ideal in theory, but it may not be practical at a large scale due to the susceptibility of the outdoor systems to influx of local genetic material. Maintaining desired phytoplankton composition in large-scale wastewater treatment through inoculation may not be effective in governing algal speciation in outdoor, larger-scale systems as suggested by Greer and Ziebell (1972) and Haines (1977). Management of phytoplankton nutrients in large-scale clam raceway systems may also require additional engineering solutions such as pond liners to minimize losses of nutrients due to the interaction with sediments and groundwater.

Dissolved Oxygen

Low dissolved oxygen levels associated with receiving waters downstream from municipal wastewater treatment plant discharges have also been associated with high mortality and low growth rates in *Corbicula* (Belanger 1991). In this study, dissolved oxygen never fell below 6.0 mg/L, which is well over the 3.0 mg/L minimum suggested by Buttner (1986) for *Corbicula* under aquaculture wastewater conditions. Maintenance of adequate dissolved oxygen may be attributed to the design of the raceway input spreader bar that was installed to help volatilize ammonia and aerate incoming raceway water.

Multiple Stressors

Temperature alone may not have been responsible for the poor survival of clams in raceways since other chemical and physical stressors in the environment can decrease the threshold temperature for survival. For example, Buttner (1986) attributed *Corbicula* biomass losses to temperatures up to 33 ^{o}C, along with depressed oxygen levels (< 40 % saturation) at temperatures above 25 ^{o}C. Haines (1977) attributes lack of growth, high mortalities and temperature stress, in combination with the possible presence of ammonium, at 30-32 ^{o}C in municipal wastewater conditions. Other studies have attributed no growth along with substantial

stocked biomass loses (Habel 1970, Haines 1977), to a combination of high temperatures and low dissolved oxygen (Buttner and Heidinger 1980, Buttner 1986). Interactions of these concerns, along with possible amphipod interactions observed in this study, may also inhibit implementation of *Corbicula*-based systems in dairy wastewater treatment.

Parasites and Predation

Potential problems with amphipods that infested clam raceways at the Hague, Florida facility were unforeseen and may have contributed to the limited adaptability of freshwater clams to the large-scale raceway aquaculture systems. The previously undescribed phenomenon of *Corbicula* infestation by the amphipod *Hyalella azteca* may have contributed to population declines, decreased filtration and lack of growth. This phenomenon may have been the result of the substrate choice and other environmental parameters unique to the raceway systems that could be encountered in other engineered systems.

Negative impacts on *Corbicula* may be due to direct parasitic interactions or tissue damage from movement and occupation within the shell cavity; or possibly its incurrence of stress from constant valve closure stimulated by amphipod movements over the raceway substrate. Repetitive valve closure as a result of the clam's defensive response to outside stimuli, such as the constant activity of an amphipod infestation, may deplete energy reserves and limit its ability to respire, expel waste products and obtain food (Gainey 1978). Shell chipping appeared to be related directly to the ability of the amphipod to invade clam shell cavities. There was, however, evidence that amphipod invasion of shell cavities can also occur in the absence of a chipped shell area; therefore, amphipod invasion needed to be verified by shucking live, freshly collected organisms.

The chipping of shell material was probably related to the amphipod infestation even though the breech in shell tissue may not have been the only entrance mode. Chipping was

probably caused as a result of repetitive valve closure and the inability of the clams to expel sand grains as the valves were closing. Shell margin chipping was not evident in the systems exposed to fertilizer addition, which may have caused a difference in substrate characteristics with the accrual of soft sediments as a result of settleable material such as phytoplankton and clam wastes over the sand substrate. This change in sediment structure may have helped to deter shell chipping and the phenomenon of inter-cavity invasion by amphipods, since live tissue samples from these raceways did not contain amphipods. Clams undergoing physiological stress, such as the mortality events in this study indicated, may be more susceptible to shell cavity invasion by amphipods, even without the occurrence of shell chipping. This idea is supported by the low number of live clams without evidence of shell chipping that were found containing amphipods.

Engineered systems are still in their experimental infancy and may be subject to unforeseen parasitism and predation by organisms that are not normally pests in the natural environment as systems achieve larger and larger scale. Negative interactions between invertebrates and bivalves are more common in the mariculture industry, where organisms are maintained in high densities using either closed engineered (Dame 1996, Lin et al. 2001) or open-water natural systems (Hickman 1992, Haines et al. 1994, Dame 1996). Application of bivalve-based biofilter technology in the freshwater environment may be susceptible to similar problems that are much less understood at this time due to the lack of large-scale culture experience beyond the marine environment.

Parasitic and/or amensal interactions have been described between naturally occurring and cultured open-water marine bivalves by invertebrates such as pea crabs and other crustaceans (Hickman 1992, Haines et al. 1994, Dame 1996, Mercado-Silva 2005). In estuarine populations of *Corbicula*, attachment of barnacles has led to declines in clam health and increased mortality

(Foe and Knight 1986). Lin et al. (2001) reported mortality due to parasitic interactions between Pyramidellid snails and the giant clam *Tridacna derasa* in an engineered mariculture effluent treatment system. Oysters (*Crassostrea spp.*) in both natural and culture systems are susceptible to negative effects of shell-boring polycheates (Wargo and Ford 1993, Debrosse and Allen Jr 1994). Other microscopic parasites have also have a detrimental effect on oysters, including the endoparasite known as MSX (Wargo and Ford 1993), however, no effects from organisms of this nature have been documented in *Corbicula*.

Observations and negative effects of inter-shell cavity parasites in naturally occurring freshwater clams and mussels have been reported in a variety of systems. Parasitism of the freshwater clam *Pisidium amnicum* in Finish lakes by tremetodes (*Bunodera luciopercae*, *Palaeorchis crassus* and *Phyllodistomum elongatum*) has been reported by Holopainen et al. (1997). Parasitism of freshwater unionid mussels by unionicolid mites in the United States has been reported by Edwards and Dimock, Jr. (1988) and Fisher et al. (2000). Freshwater mussels inhabiting Chilean salmon farms can be preyed upon by freshwater crabs and shrimp when the valves of the mussels are open (Soto and Mena 1999), but these predators did not inhabit the inside of the shell valves as did the amphipods in this study. *Corbicula* populations inhabiting natural systems in the United States may be impacted by parasitism of soft tissue by the oligochaete *Chaetogaster limnaei*, as described by Eng (1976). *Corbicula* stocked in fish polyculture ponds have been subject to predation by terrestrial mammals and fish (Chen 1976, Buttner and Heidinger 1980), but no reports of amphipods negatively affecting *Corbicula* populations have been made.

This is likely the first observed report of the effects of *Hyalella azteca* presence as a possibly negative aspect in *Corbicula* populations as well as infiltration of the mantle cavity area

by amphipods. Understanding the *Corbicula* and *Hyallela* interaction observed here will require further study in order to accurately describe, understand and document the extent of the interaction and the effect that it may have on the animal's health and survival. The extent that amphipods may affect freshwater clam and mussel populations under culture conditions can be magnified by substrate choice as well as other environmental stressors such as temperature that can pre-stress populations prior to amphipod infestation. Potential problems with amphipods identified here would need to be solved either by polyculture with other organisms as suggested by Soto and Mena (1999) and Lin et al. (2001) or pesticides tolerable to clam populations. Even with fish polyculture, invertebrate pests may still be problematic in freshwater bivalve-based treatment systems as indicated by Soto and Mena (1999). Incidental parasites and other pests would need to be managed in any future freshwater bivalve-based wastewater treatment system, which is difficult since organisms such as *Hyalella azteca* that occur with the clam in the natural environment may have an undescribed impact on the target organisms, especially under large-scale monoculture conditions.

Fish/clam polyculture may also help eliminate potential problems with amphipods in clam raceways through predation, provided that the fish do not impact clam populations as well. Other problems from nuisance organisms encountered in the raceways, such as biofouling from plant growth, may also be solved through polyculture of clams with other organisms such as herbivorous snails that have been used to control biofouling by macroalgae in clam-based mariculture systems (Lin et al. 2001).

In natural and engineered systems, the presence of larger organisms may affect survival and growth of *Corbicula*; for example, predation of *Corbicula* in culture ponds by muskrats has been reported by Buttner and Heidinger (1980), but no indications of such disturbances were

apparent at the Hague site. Chen (1976) reported predation of *Corbicula* by fish in large-scale polyculture systems. In estuarine populations of *Corbicula*, attachment of barnacles has led to declines in clam health and increased mortality (Foe and Knight 1986). Parasitism of freshwater bivalves mussels by unionicolid mites in natural water bodies has been reported by Edwards and Dimock, Jr. (1988) and Fisher et al. (2000), but no negative relationships between freshwater bivalves and amphipods has been reported in the literature.

Reproductive Success

An important part of *Corbicula* biomass production is the clam's ability to reproduce and recruit new individuals to the raceways. The lack of juveniles (defined as having a shell length less than 7 mm) observed in the raceways and water samples in this study indicates that reproduction and subsequent recruitment did not take place. The 5 mm shell length threshold was chosen, as opposed to the 10 mm size used by Buttner (1986), since the presence of clams 7 to 10 mm were recorded during stocking and significant growth in the raceways was questionable.

Clams were expected to repopulate the raceways readily as a result of *Corbicula*-specific attributes such as high fecundity in monoecious individuals, brooding of larvae with no need for intermediate hosts, rapid growth (Byrne et al. 2000) and their ability to recruit new individuals in a wide variety of habitats (Sickel 1986). The lack of adapted predators has also been attributed to recolonization success in naturally occurring populations of *Corbicula* (Sickel 1986). This is advantageous for raceway-based monocultures in closed systems that can be engineered to keep larger predators out, such as fish, raccoons and birds that may prey on smaller individuals with softer shells (Buttner 1986). Smaller omnivorous predators such as flatworms or amphipods are more difficult to control with mechanical methods and may prevent clam recruitment in raceways even if successful reproduction and larvae expulsion into the water column takes place.

The lack of reproduction or recruitment in clam raceways is odd since clams readily repopulate even obscure engineered systems such as power plant reactor plumbing (Hakenkamp and Palmer 1999). These systems are subject to influx of larvae from natural population reproduction where clam raceways are closed to such influx of genetic material. Clams have also been reported to reproduce successfully at pond-scale in polyculture with fish for treatment of aquaculture wastewater (Buttner 1986). Even with a lack of influx of natural genetic material, high temperatures and the presence of potential clam predators (catfish), culture ponds exhibited clam reproduction and recruitment (Buttner 1986)

As McMahon and Bogan (2001) pointed out, successful *Corbicula* reproduction may not be achievable in engineered culture or experimental systems especially at smaller scales. The lack of evidence supporting the idea that successful incidents of clam reproduction took place in the raceways supports this theory; however, the clam raceway systems in this study were presumed to be of large enough scale so reproduction is not inhibited. Environmental stressors such as high temperatures, ammonia levels, amphipod infestation and food limitation that may have been responsible for growth limitation may have also reduced the clam's reproductive capacity.

Clam Stock Assessment Issues

Another problem with comparison and implementation of *Corbicula* raceways at large scale may be that population sampling can prevent an accurate assessment of phosphorus sequestration via raceway clam biomass and settled waste products. In this study, live clam density values estimated from seasonal spatial sampling differed greatly from clam density estimations using actual live counts at the last sampling event of the study period. Clam biomass losses due to mortality were not verified in this study since dead clams were not measured. Measurement error in the raceway population spatial sampling technique probably resulted in an over estimation of live clam density even though 3.8 % of each raceway was sampled during

each event. In the raceway design used in this study, there is an open, uniform area that maximizes conditions for growth with far less spatial variability in biomass accrual and hydrologic conditions than expected in natural systems. Any attempt at large-scale aquaculture of *Corbicula* should pay particular attention to clam population monitoring and disturbance of culture substrate due to the variability encountered with the methodology used in this study. Methods for assessing clam populations at stocking also need to be addressed in order to estimate population growth and biomass phosphorus sequestration accurately.

Clam size can have a strong negative relationship on *Corbicula* growth rate (Buttner and Hiedinger 1980, Foe and Knight 1986, McMahon and Williams 1986). This relationship is similar to the von Bertalanfy growth model used to describe differences in growth rate with age in clam of the saltwater clam *Mya arenia* (Brousseau 1979). However, age is difficult to estimate in *Corbicula* due to the absence of growth rings related to annual shell deposition as found in saltwater clams, such as *Mya arenia* (Brousseau 1979). No conclusions could be made on size/growth relationships since no discernable trends were found in population shell length distributions, and low recovery of live tagged individuals did not allow for statistical analysis of growth rate trends.

Counts of dead clams on the substrate surface may be the best indication of mortality and actual clam biomass than the volumetric and spatial estimations used in this study. The cumulative number of dead clams collected over time and excavated at the end of the study accounted for up to 90 % of the clams stocked in the raceways. Stocking densities were grossly over-estimated by the volumetric method and other techniques should be considered for future clam stocking estimation. Raceway clam densities estimated by the spatial technique may have also overestimated the actual live clam biomass compared to the cumulative number of dead

clams found at each sampling time. Since there was no indication of reproduction by clams, it is assumed that the number of dead clams is the best reflection of actual live density remaining in the raceway.

General Conclusion

Overall clam population biomass phosphorus sequestration did not occur due to high mortality, even though significant growth rates were observed for tagged clams. Timing of noticeable mortality events indicated that high seasonal temperature was the major factor limiting the ability of clam raceway populations to adapt to treatment raceways. Water temperatures in the range of 28 to 30 ^{o}C and above have been implicated as the limiting factor to success of *Corbicula* applications in most other waste treatment studies and in natural populations (Greer and Ziebell 1972, Haines 1977, Mattice 1977, Buttner and Heidinger 1980, Buttner 1986). Major population declines took place when water temperatures reached this level, regardless of the level of nitrogen and phosphorus addition and chlorophyll *a* in source pond water.

Bivalve-based treatment of dairy-derived wastewater phosphorus would require, at the very least; scaling, study and implementation of additional treatment technologies in order to reduce high levels of nitrogenous wastes common in dairy operations. Other than obvious issues with implementing mechanisms untested at large scale, *Corbicula*-based treatment of dairy or any other agriculture-based wastewater stream will need to manage predation and parasitism from unforeseen organisms, as well as environmental parameters, before applications at any appreciable scale could possibly take place.

Other environmental factors present in the raceways, including stress from the potential problems due to infestation by amphipods (*Hyalella azteca*), may have affected phosphorus removal and sequestration potential as well. Consequently, interactions reported between

Corbicula and *Hyalella* in this study are the first to recognize amphipods as having a potential predatory or parasitic role in clam population dynamics. The negative interactions observed in this study between amphipods and clams are typical of aquaculture and mariculture systems and will need to be managed in future systems. Possible management strategies borrowed from mariculture systems include hand removal, which is not applicable to the small size and numbers of amphipods, top-down predation by snails or other invertebrates, polyculture with higher order predators such as fish, or development of pesticides for this application.

CHAPTER 4
PHOSPHORUS REMOVAL AND SEQUESTRATION IN CLAM RACEWAYS

Introduction

For large-scale, clam-based raceway systems to be successful in removing and retaining phosphorus from wastewater, they must operate within the physiological tolerances of the animal to ensure survival, growth and reproduction. In addition to concerns over adaptability of clams to a particular raceway environment, the system must demonstrate an ability to remove phosphorus at rates compatible with the needs of wastewater treatment systems. Naturally occurring populations of filter-feeding bivalves, such as *Corbicula*, can remove phosphorus from the water column and sequester it into shell and meat biomass (Fuji 1979). Wastewater nutrient management in agriculture, aquaculture, municipal and surface water sources by freshwater bivalves, utilizing a phytoplankton intermediary, has been proposed by Stanley (1974), Haines (1977), Mattice (1977) and Greer and Ziebell (1979).

In addition to phosphorus removal (Soto and Mena 2004), freshwater bivalves have been used to lower turbidity (Habel 1970, Busch 1974, Haines 1977, Buttner 1986), nitrogenous waste levels (Buttner 1986), particulate protein concentrations (Haines 1977) and seston biomass (Mattice 1977). Actual investigations into wastewater treatment potential of bivalve-based systems has been largely limited to the mariculture industry, where species that are commercially desirable as a food commodity have been targeted for profitable production (Shpigel and Fridman 1990, Jones and Iwana 1991, Shpigel and Blaylock 1991, Jakobworks et al. 1993, Shpigel et al. 1993, Shpigel and Neori 1996, Shpigel et al. 1997, Jones and Preston 1999, Neori et al. 2000, Jones et al. 2001). These studies focused on management of water clarity parameters, nitrogenous compounds and removal of wastewater-generated suspended solids including phytoplankton intermediaries, as opposed to phosphorus removal. In mariculture and

aquaculture waste systems, undigested feed and feces from a primary species, such as fish or shrimp, has also been used as food for bivalves (Jakobworks et al. 1993, Jones et al. 2001). Some bivalve-based treatment systems in the mariculture industry utilize multiple stages of biofiltration including filter-feeding organisms and harvested macrophytes (seaweed) (Shpigel and Neori 1996, Neori et al. 2000, Jones et al. 2001, Kinne et al. 2001).

Adaptation of mariculture technologies to freshwater waste streams from agriculture, aquaculture and municipal wastewater treatment facilities requires use of freshwater organisms, such as *Corbicula*, that are far less desirable commercially than other bivalves. These organisms are potentially well-suited for wastewater treatment applications because of their high filtration, growth and reproductive abilities that can contribute to phosphorus uptake and sequestration. Other freshwater bivalves, including the mussel *Diplodon chilensis* (Soto and Mena 1999), to reduce phosphorus in small aquarium-type systems in wastewater streams generated by freshwater aquaculture; however, freshwater mussel species are typically sexually dimorphic and require a fish as an intermediate host for larval development. These traits can limit the geographical distribution and biomass production potential of mussels. *Corbicula*, on the other hand, can rapidly repopulate an area as a result of a number of life history traits including high fecundity, hermaphroditic sexuality, self-fertilization and marsupial incubation of larvae (McMahon 1983, McMahon and Bogan 2001). *Corbicula* are also able to reproduce over longer time spans due to their bivoltine (occurs twice annually) reproductive effort periodicity, as opposed to most mussel species, which tend to be univoltine (occurs only once each year) (McMahon 1983, McMahon and Bogan 2001). High population densities achievable by *Corbicula*, exceeding 1000 individuals/m^2 (McMahon 1983), can minimize the space needed for treatment, compared to freshwater mussels.

Utilizing *Corbicula* for treatment of phosphorus from agricultural wastewater effluents has been proposed by Greer and Ziebell (1972), Stanley (1974) and Mattice (1977). Similar to bivalve-based treatment systems used in the mariculture industry (Shpigel 1993, Shpigel and Neori 1996, Lefebvre et al. 2000 and Mazzola and Sara 2001), this approach uses a phytoplankton intermediary to convert dissolved phosphorus into particulate matter that can be used for food. Wastewater effluent is used to generate phytoplankton biomass, which in turn provides clams with an unlimited food source. Sparcity of food resources has been indicated as a limitation to *Corbicula* growth in natural systems (Foe and Knight 1985), however, wastewater effluent addition decreases the likelihood that phosphorus or nitrogen becomes a limiting factor in phytoplankton production. It is necessary to recognize that source water supply redundancy must be designed into any engineered system to handle catastrophic events such as phytoplankton population crashes or harmful algae blooms, which may occur under normal operation. Even though dairy effluent addition can support algal growth (Wilkie and Mulbry 2002), phytoplankton production can be limited by a range of factors commonly found in aquatic systems such as micronutrient limitation, seasonality of light availability and temperature.

Corbicula utilizes a wide variety of phosphorus-containing particulates. Greer and Ziebell (1972) observed that *Corbicula* is not only able to remove phosphorus added to source waters from municipal wastewater effluent through consumption of phytoplankton biomass, but can also remove dissolved forms of phosphorus when they are converted to colloidal hydroxyl-apatite at high pH levels. This phenomenon appears to require elevated dissolved phosphorus concentration (5 to 15 mg/L PO_4^{3-}), along with a pH $\geq$ 9, which can be achieved through addition of lime and the diurnal reduction in CO_2 due to phytoplankton metabolism (Greer and Ziebell 1972). Although sequestration of colloidal phosphorus has been observed only in the laboratory,

achieving removal of colloidal phosphorus from wastewater streams by *Corbicula* adds to the organism's appeal as a phosphorus removal mechanism.

In the Fuji (1979) model for *Corbicula* in a natural population, phosphorus consumed by clams is sequestered within the system either by sedimentation of feces and pseudofeces or ingestion and incorporation into new clam tissue and shell. Filtration by *Corbicula* is characterized by high rates of phytoplankton clearance, up to 500 mL/day/clam in hypereutrophic lake water (Beaver et al. 1991). Like growth rate, filtration rate is primarily dependent upon temperature and size (McMahon and Williams 1986), but can also be governed by particle concentrations (Haines 1977). Fuji (1979) suggested that, of the total amount of phosphorus consumed annually by filtration, 62 % is ingested, while the rest is rejected and deposited as pseudofeces. The particulate phosphorus ingested from the water column can be converted directly into soluble form as metabolic waste product excretions by the clams (Lauritsen and Mozley 1989). The rest of the total amount of phosphorus ingested is exported from the system through mortality (13 %), ejection of gametes (9 %), excretion (8 %) and juveniles that were exported from the system by water currents and therefore not recruited into the population (1 %) (Fuji 1979).

Phosphorus (P) content (mg P/individual) of clam biomass increases with body weight over time in size-based age groups (Fuji 1979). Phosphorus content for clams in each age group also varies with the time of the year due to ejection of gametes. Fuji (1979) estimate phosphorus content of clams between 0.011 g and 3.093 g total clam dry weight to be from 0.008 mg P to 1.500 mg P per individual, yielding biomass concentrations in the range of 0.506 mg P/g to 0.800 mg P/g of total clam dry weight biomass. Using phosphorus content of clam biomass derived from tissue analysis, Fuji (1979) estimated that clam populations can sequester 130 mg P/m^2 in

one year as biomass growth (without accounting for mortality) in a population originally containing 65 mg P/m^2. Data from Fuji (1979) showed amounts of phosphorus in meat tissue (8.293 - 22.667 mg P/g meat dry weight) was up to 14 times higher than in shell tissue biomass.

In this study, a large-scale raceway-based wastewater treatment system was used to assess the phosphorus removal potential of *Corbicula* populations. The system was based on phytoplankton as an intermediary to convert dissolved nutrients into particulate form (Greer and Ziebell 1972, Stanley 1974 and Haines 1977, Mattice 1977). Source ponds that fed the raceways in this system acted as incubation ponds for phytoplankton. The potential for using a clam-based raceway system was assessed by the two most important mechanisms used to determine phosphorus treatment capacity. First, direct removal of phosphorus from the overlying water within the raceway was measured in terms of change in phosphorus concentration in the water column. Second, the capacity of the raceways to retain and sequester phosphorus as clam biomass was determined by changes in clam growth over time. *Corbicula* populations were exposed to high, medium and low wastewater nutrient addition treatments to test the ability of the clam systems to remove and sequester phosphorus over time in systems of different nutrient load.

Methods

Raceway-based Treatment System

A raceway-based recirculating water treatment system constructed at the University of Florida Dairy Research Unit in Hague, FL (See Chapter 2) was used to test the P-removal and sequestration potential of *Corbicula*. Three independent pond/raceway systems were constructed in June 2002 to compare source waters with low, medium and high levels of nutrients. Each nutrient addition treatment group utilized two earthen source water ponds in conjunction with

three wood-framed, plastic-lined raceways. Table 4-1 shows the numerical designations for the ponds and raceways in each nutrient addition treatment group.

Table 4-1. Source pond and raceway numerical designations for the treatment systems at the Hague site.

Nutrient addition treatment	Source Pond	Raceways
Low	1, 2	1 – 3
Medium	3, 4	4 – 6
High	5, 6	7 – 9

Source water ponds

Source ponds had an approximate area of 0.05 hectares (ha), with depths of approximately 2 m and volumes approximately 1000 m^3. Ponds were enriched with a blend of nitrogen and phosphorus fertilizer or anaerobically digested dairy farm wastewater, to simulate possible water conditions associated with tertiary wastewater treatment. The low nutrient addition treatment received no external nutrient addition. A 5 % and 10 % addition of anaerobically digested dairy farm effluent was added to Pond 3 from the medium nutrient group and Pond 5 from the high nutrient addition treatment group, respectively. For effluent physical and chemical characteristics at the University of Florida Dairy Research Unit, see Wilkie et al. (2004). Effluent was pumped from the digester to the source ponds and metered through a 2.54 cm (1") turbine-type flowmeter.

Pond 4 in the medium nutrient addition treatment was dosed with 1.1 kg of triple super phosphate ($9Ca(H_2PO_4)_2$, N-P-K = 0-45-0) and 6.8 kg ammonium nitrate (NH_4NO_3, N-P-K = 15-0-0) resulting in a total addition of 0.23 kg of phosphorus and 1.02 kg of nitrogen per pond in October 2002, one month prior to clam stocking. Pond 6 in the high nutrient addition treatment was dosed with 2.2 kg of triple super phosphate (0.46 kg P) and 13.6 kg of ammonium nitrate (2.04 kg N) in January 2002, also one month before introduction of clam populations.

Nutrient additions were designed to enhance phytoplankton biomass, which was the putative source of particulate nutrition for the clams. Fertilizer loading levels were targeted at increasing phosphorus and nitrogen levels by 0.23 mg/L TP and 1.02 mg/L TN in the medium nutrient treatment source pond and 0.46 mg/L TP and 2.00 mg/L TN in the high nutrient treatment source pond. Fertlizer was introduced to ponds by placing it into a burlap bag that was suspended in the water column from a floating frame (0.5 m x 0.5 m) constructed from 5.08 cm (2") ID PVC pipe.

Neither of the ponds supplied with dairy effluent were exposed to the clam raceways due to excessively high ammonia levels (2.0 mg/L or greater, as NH_3-N), which represented a direct threat to the health of the clams. Ammonia (NH_3-N) levels in the effluent ponds and input and output water from each operating raceway were monitored monthly using Aquacheck® brand Ammonia Nitrogen (NH_3-N) Test Strips (Hach Incorporated, Colorado, USA) advertised for use in aquaculture and aquarium applications. Levels of NH_3-N in the operating raceways never reached the 0.25 mg/L (as NH_3-N) minimum value of the test kit.

Source ponds were circulated through the raceways for 10 to 20 days prior to clam addition. Supply ponds were aerated at night throughout the study to help with mixing, maintenance of nighttime dissolved oxygen and evaporative cooling. Floating macrophytes were cleared by hand from each source pond several times at the start of the experiment. In addition, six juvenile triploid grass carp ranging between 15 and 20 cm in length were stocked in each pond in June 2002 to help reduce vegetation in ponds. No fish mortality was evident in ponds not exposed to dairy wastewater effluent. In contrast, ponds treated with wastewater all experienced 100 % fish mortality in less than one week after effluent addition. Fish mortality events corresponded with high ammonia levels (> 0.25 mg/L as NH_3-N).

Raceways

Raceways were 1 m in width by 7.4 m in length with an available substrate surface area of 7.2 m^2, after subtracting standpipe area. Water depths were maintained at 0.2 m, yielding a raceway water capacity of 1.4 m^3 each. Source water inflow to the raceways was maintained at 227 liters per minute (LPM) (60 GPM) during normal operating conditions. The flow rates yielded retention times of approximately 9.5 minutes, with a linear velocity of 1.17 m/min. Turnover time for each supply water pond was approximately 24 hours. Flow rates were calculated assuming near laminar flow through the raceway structure. Raceways were filled to 0.2 m depth with a coarse grade SiO_2 filtration sand (0.6-1.0 mm particle size), that was purchased from Feldspar Incorporated in Edgar, Florida.

Nuisance aquatic plants such as *Chara sp*. and filamentous algae (mainly *Spirogyra sp*.) growing on the raceway substrate and liners were removed by hand from each raceway at least weekly to reduce fouling. The control of nuisance plants, especially *Chara*, on the substrate made accurate estimation of clam biodeposit sedimentation impossible. This is due the constant resuspension of sediment deposits associated with disturbance from removal of the vegetation that was often rooted below the sediment surface. Filamentous algae tended to utilize the sides of the raceways where PVC liners were submersed.

Water quality monitoring

Source water ponds and raceways were monitored weekly in the morning and evening for temperature, dissolved oxygen, pH, chlorophyll *a*, total nitrogen and total phosphorus. Measurements in the ponds were taken at the end of sampling piers near the intake pipe leading to the raceways. Monitoring was carried out monthly after 75 % or more of the clams were assumed dead. Temperature and dissolved oxygen was measured using a YSI model DO550, and pH was measured using a Fisher model AP63 meter by submerging the probe tips to mid-water

column depth at the raceway input and output. Water temperature, dissolved oxygen and pH readings were compared using a paired t-test (Microsoft Excel©) at the raceway input and output, as well as between source ponds in the nutrient addition treatments.

Water samples were collected from the sampling piers in each pond for nutrients using a pole sampler designed especially for this experiment. The sampler used a plunger-type mechanism to collect a 1 L water sample from in front of the intake pipe. When the unit was lowered to the desired depth, the plunger was actuated by the operator via a spring-loaded handle at the opposite end of the pole. After the sample was collected, the plunger was released, sealing a 1 L plastic bottle (Nalgene Incorporated, USA) bottle and raised for retrieval. The sample bottle was unscrewed from the sampler and capped for transport to the laboratory.

Water samples collected from the source ponds were analyzed at the laboratory for phosphorus, nitrogen and phytoplankton biomass in terms of chlorophyll *a*. Total phosphorus (TP) and total dissolved phosphorus (TDP) were determined using the potassium persulfate digestion method (APHA 1998) with a Hitachi (Japan) spectrophotometer. TDP determination involved pre-filtering through a 0.7 μm glass fiber filter. Total nitrogen was determined using potassium persulfate digestion method (APHA 1998) with colorimeteric analysis performed using a Bran-Luebbe (Germany) auto analyzer. Phytoplankton biomass was estimated using chlorophyll *a* (chl *a*). Samples were measured by filtering 250 mL of water onto a 0.7 μm glass fiber filter, followed by an ethanol extraction (Sartory and Grobbelaar 1984) and spectrophotometric determination (APHA 1998) using a Hitachi (Japan) spectrophotometer. Microscope observations of phytoplankton species composition were obtained periodically to describe dominant organisms with help from Mary Cichra at the University of Florida Fisheries

and Aquatic Sciences Department. Data obtained from these phytoplankton species observations were not assessed quantitatively.

Raceway clam populations

Clams for stocking the raceways were obtained from populations in three different natural water bodies under permit number FNC-04-022 issued by the Florida Fish and Wildlife Commission. Clams for the low nutrient group (Raceways 1-3) were collected in June 2002 from a 0.5 km stretch of the Santa Fe River near the State Road 49-bridge in Gilchrist County, Florida (29°54.2' North, 82°52.0' West). By November 2002, the rising water level of the river made further clam excavation impossible. Therefore, animals for Raceways 4-9 were collected from lakes located in Lake County, Florida that had accessible populations of clams. Clams for the medium nutrient group (Raceways 4-6) were collected from the southwest shore of Lake George (29°12.2' North, 81°35.7' West) in November 2002. Clams for the high nutrient group (Raceways 7-9) were obtained from the west shore of Lake Dalhousie (28°54.0' North, 81°36.8' West) in February 2003.

All three collection sites had coarse sand sediments similar to the substrate used in the raceways. Clams were excavated by shoveling bottom material into weighted baskets made from 0.635 cm (¼") plastic mesh. Clams were also excavated by hand using trowels or a commercial clam rake modified for the small size of the *Corbicula* by affixing ¼" plastic mesh on the inside of the collection basket. Periodic excavation of bottom sediment using a 0.25 m^2 PVC sampling quadrat was used to determine population densities for the clams in their natural habitat. Densities ranged from 48 and 864 clams/m^2 with a mean of 272 clams/m^2 (standared error (SE) = 23, n = 47 observations) for all locations combined.

After excavation, clams were enumerated and divided into mesh bags. The clams were then placed into coolers packed with wet newspapers and kept out of direct sunlight to help

minimize heat stress and dessication. They were transported directly to the aquaculture facility in Hague, FL and scattered evenly throughout each raceway. Stocking of each raceway took up to 15 days involving 2 to 6 people working per day. Raceways 1–3 were stocked from June 17 to 28, 2002, Raceways 4–6 from November 4 to 14, 2002, and Raceways 7-9 from February 11 to 26, 2003. Ten mesh bags of clams were added to each raceway. Each mesh bag contained approximately 1,000 clams. This method was chosen as opposed to counting each individual or bulk weighing in order to minimize handling stress, time and equipment needed to enumerate the large numbers of clams needed for this study.

Clam populations in the raceways were monitored for density, size and biomass at approximately 3-month intervals following stocking, as described in Chapter 3. A tag and recapture study was also employed to verify clam growth (Chapter 3). Live clam density, shell size and growth data from clam population surveys and tag and recapture data were used to assess clam biomass, phosphorus removal and changes in clam phosphorus sequestration.

P Removal From Source Water By Clam Raceways

The ability of the clam raceway system to remove phosphorus and P-containing material from overlying source water was estimated from three components: phosphorus removal from water within a single raceway pass (through-flow): phosphorus removal from water recirculated within the raceways and phosphorus removal from water in the source ponds through sequestration of phosphorus in clam biomass over time.

Raceway through-flow trials

P removal from water using raceway through-flow measurements was only assessed in the low nutrient raceways beginning after stocking in July 2002. Raceway flows were maintained at 227 LPM (60 GPM) from the time of stocking until August 2002, when input flow rates were changed to increase raceway water retention time (Table 4-2). The low flow condition of 151

LPM (40 GPM) and a higher flow condition of 227 LPM yielded linear water velocities of 0.78 m/min and 1.17 m/min and water retention times in the raceway of 9.5 minutes and 6.3 minutes, respectively.

Table 4-2. Raceway source water input flow rates for the period of July 1 to August 24, 2002. Flow rates were lowered in August to increase water retention time for raceway water phosphorus removal trials using through-flow conditions

Raceway	Raceway Inflow Rate (LPM)			
#	7/1-7/31	8/5-8/8	8/12-8/15	8/19-8/22
1	227	227	227	151
2	227	227	189	151
3	227	227	151	151

Samples were taken from the input and output of each raceway to estimate the changes in TP concentrations resulting from each pass of water over the clam population in the raceways. The values were used to estimate instantaneous phosphorus removal rates from each raceway. Environmental parameters including water temperature, DO and pH were also sampled at the raceway input and output at 6:00, 12:00 and 18:00 hours.

The initial experiment was designed to provide baseline data on amount and variability of phosphorus removal from raceways on a short-term basis. The sample technique involved taking a water sample from the raceway input and output at each sample time of day. In this phase, samples were captured from falling water under the input water distribution bar and from within the output standpipe of each raceway. One liter of sample water was collected at each input and output using plastic bottles (Nalgene Incorporated, USA). Samples were taken from each raceway daily at 6:00, 12:00 and 18:00 for five, 3-day periods from July 1-31, 2002 (high flow) and three, 3-day periods from August 5-24, 2002 (variable flow).

The differences between TP and TDP concentrations at the input and output end of the raceways were converted to area-specific removal or addition values by multiplying by 200 L/m^2. Removal rates were then calculated using the retention time estimated for each flow rate.

Negative values indicate an uptake of P, while positive values indicate a phosphorus addition from the raceways to the water column. Rates larger than 120 mg P /m^2/hr or smaller than –120 mg P/m^2/hr were considered outliers and removed from the analysis to normalize the data.

The July and August through-flow TP removal data were analyzed using an ANOVA (SAS PROC MIXED procedure, SAS Institute©, Cary, NC). The model was a randomized complete block design (Cox 1996) with raceway as the block, where time of day and sampling period (the 5 different 3-day trials) were the factors with covariates inflow DO and water temperature. Another ANOVA (SAS PROC MIXED procedure, SAS Institute©, Cary, NC) was performed on the August data in order to determine the significance of flow rate. This analysis consisted of a randomized complete block design with three factors, flow rate, time of day and raceway number, and no covariates.

Observations from Raceway 2 from August 12-15 were excluded from the analysis due to a low sample size, since it was the only instance where input flow rate was not 151 or 227 LPM. Another ANOVA (PROC MIXED, SAS Institute©, Cary, NC) was performed using a randomized complete block design with month as the block to determine if the P-removal values were significantly different for each month. A pair-wise comparison of the means was performed using Tukey's method to control the experiment-wise error rate in all ANOVAs. Uneaqual variances for each time period were assumed in the ANOVA due to the significantly low variability (x^2, $p = 0.033$) in the 6:00 samples for the month of July. Sample variability was assumed equal over both flow conditions for the flow rate comparison analysis (x^2, $p = 0.27$); however, unequal variance was assumed in ANOVA models due to significant differences in variability between each month (x^2, $p < 0.001$) and sampling technique (x^2, $p = 0.002$).

In another set of phosphorus removal tests, auto-samplers were employed to increase the sampling frequency over each 24-hour test period. This methodology allowed for a larger sample size spread over the entire day and night. A pair of Teledyne-Isco, Inc. auto-samplers was used to collect water samples from the input and output of raceways 1 through 3, in August 2002. Samplers were programmed to capture water at 2-hour time intervals over a 24-hour period.

Samples were collected at two-hour time intervals beginning at 6:00 and ending at 6:00 the following day, over two, four-day time periods (August 5-8 and August 21-24). Each of the three raceways was only sampled for one 24-hour interval during each 4-day time period. During the first sampling interval, raceway flows remained at 227 LPM; flows were changed to 151 LPM for the second interval. DO, water temp, air temp and pH were sampled at 6:00, 12:00 and 18:00.

An ANOVA (PROC MIXED, SAS Institute©, Cary, NC) was performed on the August auto-sampler data using a randomized complete block design with two factors, flow rate and time of day, and raceway number as the block. Pair wise comparisons of means were performed using Tukey's method to control the experiment-wise error rate.

Phosphorus removal rates obtained from August samples and auto-sampler techniques were compared to determine any differences in the two sampling methods. An ANOVA (PROC MIXED, SAS Institute©, Cary, NC) was performed on the 6:00, 12:00 and 18:00 data using a randomized complete block design with two factors, flow rate and time of day and sampling method as the block. A pair-wise comparison of the means was performed using Tukey's method to control the experiment-wise error rate. Only data collected at 6:00, 12:00 and 18:00 sampling

periods were used in the analysis, since there were far more 24-hour values. Samples taken at 189 LPM flow rate were also withheld from the comparisons.

Raceway water recirculation

To provide additional information on phosphorus uptake rates, a second method was applied to extend the probability of exposure to the clams. This involved recirculation of water within the raceway to extend the total exposure period. Phosphorus removal was measured in terms of total phosphorus (TP), total dissolved phosphorus (TDP) and chlorophyll *a* (chl *a*).

A submersible pump placed within a plastic collar was used to simulate standpipe water flow characteristics. This unit was placed just before the output standpipes, and water was piped to the input water distribution bar at about 189 LPM within the normal operation range of 151 to 227 LPM. Raceway recirculation was started 10 minutes prior to water sampling. Periphyton and dead clams were removed prior to recirculation. Water samples for nutrient analysis were taken hourly at the raceway input using an ISCO auto sampler. The auto sampler intake line was submerged approximately 3 cm from the water surface. Water temperature, DO and pH measurements were taken at the raceway input at the beginning and end of each recirculation time period.

Raceways 1-3 and 7-9 were utilized in this experiment. Raceways 4-6 were excluded from the study due to high clam mortality. Clam populations were stable in raceways 1-3 and 7-9 although 7-9 had higher densities of clams. Raceway recirculation tests were conducted during two separate experimental periods, April 11, 2003 to June 10, 2003 and June 20, 2003 to June 27, 2003. Tests were carried out for 6-hour intervals in the morning from 6:00 to 12:00 hours and again in the evening from 18:00 to 24:00 hours.

To investigate the impact that particle deposition may have on phosphorus removal, a control group was set up by covering the bottom of the raceways with weighted plastic covers to

eliminate interaction between the clams and the overlying water column. The control tests were only run for 3 hours so clams would not be impacted.

P removal potential was estimated from regression relationships using TP, TDP and chl *a* values in normally operating and covered raceways. Slope values formed by regression lines were used to calculate removal rates according to the following formulae:

- TP and TDP:
  Regression slope (mg/L/hr) * 200 (L/m^2) = removal rate ($mg/m^2/hr$)
- Chl *a*:
  Regression slope ($mg/m^3/hr$) * 0.20 (m^3/m^2) = removal rate ($mg/m^2/hr$)

Six-hour recirculation TP, TDP and chl *a* removal values from the uncovered raceways were used to perform an ANOVA (PROC MIXED, SAS Institute©, Cary, NC) using a randomized complete block design, with slope of the line formed by TP, TDP and chl *a* values over each 6-hour time period as the block, and month (April/May) and nutrient enrichment (high/low) as the factors to determine if removal values were greater than zero. Slopes from each am/pm time period were pooled since raceways within each treatment were not factored into the ANOVA, due to the lack of significant differences ($p > 0.05$) found between am and pm trials or raceways within each treatment. A pair-wise comparison of the means was performed using Tukey's method to control experiment-wise error rate.

TP, TDP and chl *a* values from the three-hour recirculation experiments in the covered raceways were used to perform a similar ANOVA (PROC MIXED, SAS Institute©, Cary, NC) using a randomized complete block design with slope of the line formed by TP measurements over each 3-hour time period as the block and nutrient enrichment (high/low) as the factors. Slope values from all raceways in each triplicate for each sample interval were pooled for each

nutrient addition treatment as in the uncovered raceways. A pair-wise comparison of means was also performed on the covered data using Tukey's method to control experiment-wise error rate.

An ANCOVA for repeated measures (PROC REPEATED, SAS Institute©, Cary, NC) with a covariance structure AR-1 model was then utilized to determine any covariance between the TP, TDP and chl *a* slopes for the low and high nutrient levels. This procedure was chosen due to differences in variance between the nutrient addition treatments and lack of a significant difference between the am and pm time periods in each nutrient addition treatment from the uncovered analysis found in the ANOVA; therefore, time can be treated as a continuous variable.

Sequestration of phosphorus by clams in treatment raceways

Another method used to estimate phosphorus removal from the treatment water was determination of increases in clam biomass over time, in combination with analysis of the phosphorus content of clam tissue. Clams were randomly selected from raceways (as described in Chapter 3) for the dry weight (DW) and ash-free dry weight (AFDW) analysis. Tests were performed using the low and medium nutrient addition treatment raceways in June and November 2002. A total of 26 clams were sampled per raceway, yielding a sample size of 79 clams per nutrient addition treatment group.

Shell length, wet weight, meat and shell DW and AFDW values were measured for each clam using methods described in Chapter 3. Phosphorus content of the meat and shell tissues in the selected clams was determined using an ignition and hydrochloric digestion method (Andersen 1976). In this method, tissue material was dried, finely ground and ignited at 550 ^{o}C until a constant weight is achieved. The ash was then dissolved by adding 1N HCl to achieve a pH < 2, heated and diluted with DI water to neutralize the solution prior to phosphorus determination using inductively-coupled plasma spectrometer (Copar and Yess 1996).

Meat tissue samples were prepared by dissolving the ashed material in 1 mL of 1N HCl, heating and diluting to 50 mL. For shell material, up to 1 g DW of shell ash material was taken from each clam, dissolved in 3 mL of 1N HCl, heated and diluted to 50 mL. Phosphorus analysis was carried out on the sample solutions using an inductively coupled plasma spectrometer and was performed by the Analytical Research Laboratory of the Soil Science Department at the University of Florida. Phosphorus concentrations in the sample solutions were then converted to mg P per g of DW for the meat, shell and total clam tissues using the ash DW and tissue DW values obtained prior to digestion with the following formulae:

$$\text{Meat} = \frac{[\text{P}]\text{ in sample solution}}{1000} \times \frac{\text{50 mL dilution volume}}{\text{sampled meat ash DW}} \times \text{sampled meat ash DW}$$

$$\text{Shell} = \frac{[\text{P}]\text{ in sample solution}}{1000} \times \frac{\text{50 mL dilution volume}}{\text{sampled shell ash DW}} \times \frac{\text{crushed shell ash DW}}{\text{crushed shell DW}}$$

$$\text{Clam} = \frac{(\text{meat mg P/g meat DW} \times \text{meat DW}) + (\text{shell mg P/g shell DW} \times \text{shell DW})}{(\text{total meat DW} + \text{total shell DW})}$$

Meat, shell and clam phosphorus concentrations were then compared to shell length values using two different regression analyses (SAS PROC MIXED, SAS PROC GLM), where meat, shell and clam phosphorus concentrations were the response variables with length and length^2 as factors to obtain the best-fit regression line. A polynomial regression was chosen since both length and length^2 factors were significant ($p < 0.05$). Both analyses yielded no significant ($p > 0.05$) relationships between any of the tissue phosphorus concentrations and shell length. An ANOVA (SAS PROC MIXED procedure, SAS Institute©, Cary, NC) was then performed using the meat, shell and clam tissue phosphorus concentrations as response variables and nutrient addition treatment as the factor with covariates, 3-month time interval, individual raceways within each nutrient level and season in each analysis. The least squared means (LSMEANS) procedure was applied to meat, shell and clam tissue phosphorus concentrations (Microsoft

Excel©) for pair-wise comparisons. Pair wise comparisons of the means were performed using Tukey's method to control the experiment-wise error rate.

Data from individual raceways within each nutrient addition treatment were pooled in the analysis since no blocking effect was found. No relationship between meat, shell and clam phosphorus concentrations (mg P per g dry weight) and shell length could be established using the regression analysis; therefore, shell length as a factor was also removed from the ANOVA. A group of 12 outliers, defined as phosphorus concentration values higher than the range of 3 standard deviations away from the mean, were removed from the analysis.

Sequestration of phosphorus by raceway clam populations was estimated using population DW biomass estimates for each nutrient addition treatment regime both at stocking and each sampling interval. Estimated clam biomass at each time interval was multiplied by the average phosphorus concentration of the clams, yielding the amount of phosphorus allocated in the total raceway clam biomass. Clam biomass phosphorus values were applied to raceway population and tagged clam results to assess removal of phosphorus by clams.

Results

Raceway Environmental Conditions

Air temperatures at the racewaty site at the Dairy Research Unit in Hague, FL ranged from 2 to 41 ^{o}C (Figure 3-2). Recorded air temperatures varied diurnally as much as 15 ^{o}C. Raceway water temperatures ranged from 10.1 to 32.6 ^{o}C (Figure 3-3) and displayed a similar seasonal pattern as air temperature. However, water temperatures only differed diurnally by a maximum of 2 ^{o}C. Water temperatures reached 30 ^{o}C or greater just after the beginning of the experiment in July 2002 through October 2002 and from May through the end of the study in August 2003.

Raceway dissolved oxygen (DO) averaged 8.79 mg/L (SE = 0.07) and ranged from 6.10 to 12.03 mg/L (Figure 3-4). DO was higher in the afternoons by up to 4.26 mg/L with greater

diurnal differences in the warmer months. Higher DO values were observed during the November 2002 to April 2002 period corresponding to lower air and water temperatures.

Raceway pH averaged 7.76 (SE = 0.02) and ranged from 6.87 to 8.81. Diurnal fluctuations in pH ranged from –0.76 to 0.89. Raceway pH was significantly higher ($p > 0.05$) in the low and high nutrient addition treatments (Figure 3-5); however, water temperature and DO values did not differ significantly between the low, medium and high nutrient addition treatments. No significant differences ($p > 0.05$) were detected at the input and output of each raceway or between raceways in each nutrient addition treatment for the water temperature, dissolved oxygen and pH parameters.

Increases in phosphorus and nitrogen levels in the source ponds did not correspond to fertilizer addition or clam mortality events. Source pond total phosphorus (TP) ranged between 0.061 and 0.211 mg/L in the low nutrient addition treatment, 0.047 and 0.471 mg/L in the medium nutrient addition treatment and 0.043 and 0.386 mg/L in the high nutrient addition treatment (Figure 3-6). Source pond total dissolved phosphorus (TDP) ranged from 0.002 to 0.091 mg/L in the low nutrient addition treatment, 0.007 to 0.223 mg/L in the medium nutrient addition treatment and 0.028 to 0.300 mg/L in the high nutrient addition treatment (Figure 3-7). A major portion of the source pond TP was made up of the dissolved form as TDP in all of the nutrient addition treatments as TDP followed a similar pattern as TP with sharp increases in the spring 2003.

Source pond total nitrogen (TN) ranged from 0.177 to 9.034 µg/L in the low nutrient addition treatment, 0.328 to 12.069 µg/L in the medium nutrient addition treatment and 1.066 to 2.786 µg/L in the high nutrient addition treatment (Figure 3-8). TN values fluctuated in the low

and medium nutrient addition treatments and only slightly increased in the high nutrient addition treatment over the experimental period.

Chlorophyll *a* (chl *a*) ranged from 3.218 to 27.511 mg/m^3 in the low nutrient addition treatment, 4.505 to 26.397 mg/m^3 in the medium and 19.789 to 147.299 mg/m^3 in the high (Figure 3-9). Chl *a* in all treatments displayed an increase after February of 2003 with peaks from April to August of 2003. Phytoplankton communities in the source ponds were dominated by diatoms in the low nutrient addition treatment, diatoms and cyanophytes in the medium and chlorophytes in the high. Diatoms were present in all ponds throughout the study period.

Raceway clam populations

Raceway live clam biomass, based on population shell length and survivorship data decreased over time in all the nutrient addition treatment raceways (Figure 3-13). Populations were subject to large mortality events during experimental periods (Figure 3-10), as described in Chapter 3.

Phosphorus Uptake In Clam Raceways

Raceway through-flow input/output measurements

Temporal patterns in low nutrient addition raceway TP concentrations during the through-flow trials from July 1-August 21, 2002 (Figure 4-1), were similar to patterns of TP concentrations in the source ponds (Figure 3-6). Raceway input TP values varied up to 0.07 mg/L per day with no apparent diurnal trends. Input TP was slightly higher in August 2002 than in July 2002, but did not differ significantly ($p < 0.05$) between the three triplicate raceways.

The results of the ANOVA showed that neither phosphorus removal (negative input/output delta TP) nor addition (positive input/output delta) were significantly different than zero ($p > 0.05$) for the different times of day, flow rates, months or sampling techniques. By contrast, time of day did have a significant effect ($p = 0.02$) on the magnitude of the rate of change in TP

concentrations in July trials. The ANOVA showed that the 6:00 sampling time had an estimated phosphorus removal significantly ($p = 0.001$) higher than the 12:00 and 18:00 hours of day; however, this was probably due to the lower variability for the 6:00 sampling time.

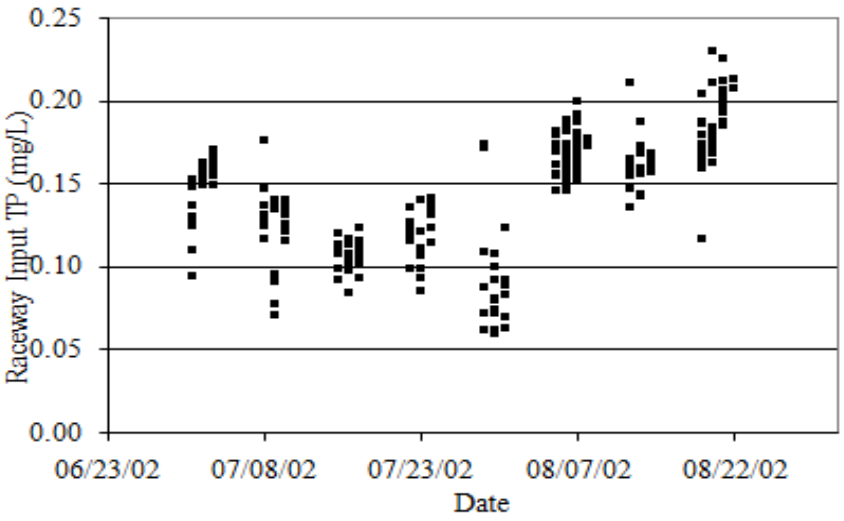

Figure 4-1. Raceway input total phosphorus (TP) in the low nutrient addition treatment during the through-flow trials for July and August of 2002.

Time of day did not significantly affect changes in TP between the input to the output regardless of flow rate, month, input TP or sampling technique. Input temperature, DO, and sampling date also had no effect ($p > 0.05$) on the change in TP in any of the trials. Change in TP observations did not significantly differ ($p < 0.05$) between raceways or between grab sampling and ISCO auto-sampler techniques. Due to the lack of significant differences in experimental variables, changes in TP (ΔTP) were pooled for all through-flow trials.

Raceway ΔTP values ranged between –0.585 and 0.119 mg/L, yielding a removal rate range of -116.206 mg P/m^2/hr to an addition rate of 99.035 mg P/m^2/hr (Figure 4-2). The 25th percentile for removal had ΔTP values ranging between - 116.206 and -11.427 mg P/m^2/hr ($n = 255$). ΔTP values appeared to be normally distributed in all trials with a slight kurtosis; however, the ΔTP frequency data is not kurtotic enough (Figure 4-2) to be considered

unreasonable for assuming normality for the ANOVA analyses. The results of these trials determined that a raceway water retention time longer than 10 minutes is needed to evaluate significant removal of TP by the clam raceways.

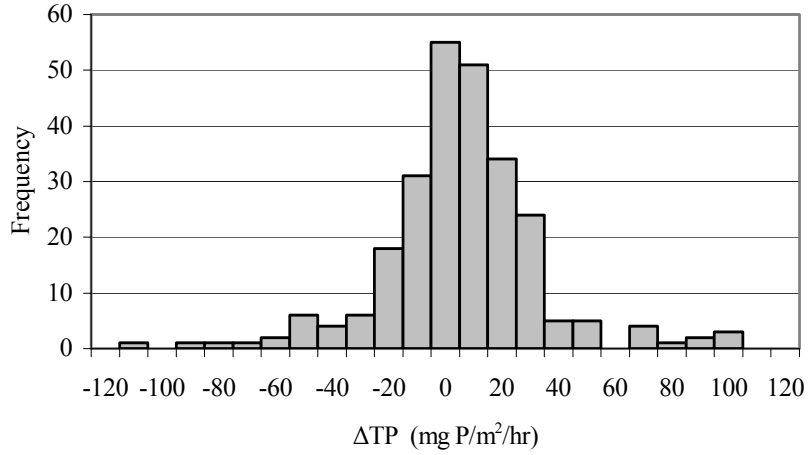

Figure 4-2. Frequency distribution of changes in total phosphorus (TP) from the input to the output in the low nutrient addition treatment raceways from July through August 2002.

Raceway recirculation measurements

Raceway input TP values at time 0 of each recirculation trials decreased significantly ($p < 0.001$) from April to May 2003 in the both high and low nutrient addition treatments (Table 4-4). Input TP was significantly higher ($p < 0.001$) in the high nutrient addition treatment compared to the low (Table 4-4). Initial TP values measured at time 0 of the recirculation trials (Table 4-4) were higher than source pond TP values (Figure 4-1), in both the low and high nutrient addition systems.

Table 4-4. Monthly mean input total phosphorus (TP) concentrations in raceways and standard error (SE), at time 0 in the recirculation trials for each nutrient addition treatment pond groups.

Month	Nutrient treatment	Time 0 mean TP (mg/L)	SE (mg/L)	n
April	High	1.229	0.024	6
April	Low	0.853	0.044	6
May	High	0.245	0.013	6
May	Low	0.067	0.030	6

Regardless of TP level at time zero, slopes of the regression lines calculated for TP values calculated for the April-May trials did not indicate any significant decrease or addition of phosphorus by the raceways. The ANOVA analysis showed that none of the mean TP slopes calculated for the six-hour recirculation period were significantly different from zero in any of the treatments during the two-month period. TP slopes calculated for the covered raceways did not differ significantly ($p > 0.05$) from zero as well.

Raceway TDP values at time 0 of each recirculation trials decreased significantly ($p < 0.01$) from April to May 2003 in both the high and low nutrient addition treatment pond groups (Table 4-5). Input TDP was significantly higher ($p < 0.01$) in the high nutrient addition treatment pond group compared to the low (Table 4-5). Initial TDP values measured at time 0 of the recirculation trials (Table 4-5) were higher than source pond TDP values (Figure 4-2), in both the low and high nutrient addition systems.

Table 4-5. Raceway system monthly mean input total dissolved phosphorus (TDP) at time zero in the recirculation trials for each nutrient addition treatment. TDP at time zero decreased in low and high nutrient addition treatments from April to May 2003.

Month	Nutrient treatment	TDP mean (mg/L)	SE (mg/L)	n
April	High	1.119	0.024	6
April	Low	0.631	0.014	6
May	High	0.044	0.003	6
May	Low	0.045	0.003	6

The analysis showed a significant difference ($p = 0.05$) in TDP over time for recirculation trials. Significant TDP decrease was observed in both the high and low nutrient addition treatment systems during April 2003, while TDP increased during the 6-hour recirculation trials conducted in May 2003 (Table 4-6).

Table 4-6. Total dissolved phosphorus (TDP) removal rates calculated from TDP slopes in the recirculation trials for each nutrient addition treatment system during April and May 2003. TDP removal was observed during April but not in May for both treatments.

Month	Nutrient treatment	TDP intercept (mg/L)	SE (mg/L)	TDP slope (mg/L/hr)	TDP removal ($mg/m^2/hr$)	n
April	High	1.227	0.146	-0.172	-22.566	6
April	Low	0.648	0.066	-0.001	-0.131	6
May	High	0.033	0.009	0.002	0.262	6
May	Low	0.044	0.009	0.0001	0.123	6

Slopes of the regression lines formed by TDP values were not significantly different from zero ($p > 0.05$) in any of the covered raceway trials in the low and high nutrient addition treatments. TDP values at time 0 of the covered raceway trials were significantly ($p < 0.01$) higher in the high nutrient system compared to the low (Table 4-7); however, TDP values at time zero in the covered raceways (Table 4-7) were much higher than source pond TDP values (Figure 4-3).

Table 4-7. Raceway total dissolved phosphorus (TDP) values at time 0 for covered raceways in the low and high nutrient addition treatments. Raceway TDP at time 0 was much higher than source pond TDP values in Figure 4-3.

Month	Nutrient treatment	Mean TDP at Time 0 (mg/L)	SE (mg/L)	n
April	High	0.615	0.008	3
May	Low	0.554	0.008	3

Raceway chl *a* values at time 0 of each recirculation trials decreased significantly ($p < 0.01$) from April to May of 2003 in the low nutrient addition treatment pond group but not the high (Table 4-8). In April, chl *a* in the low nutrient addition treatment pond group was significantly ($p < 0.05$) higher than the high nutrient addition treatment group (Table 4-8). Input chl *a* was significantly higher ($p < 0.01$) in the high nutrient addition treatment compared to the

low in May. Chl *a* values in the low nutrient addition treatment pond raceways at time zero during the April 2003 recirculation trials (Table 4-8) were higher than the source pond chl *a* values during the same month (Figure 4-8). Chl *a* values in the high nutrient addition treatment raceways at time 0 (Table 4-8) were lower than source pond values in both April and May 2003 (Figure 4-3).

Table 4-8. Raceway chlorophyll *a* (chl *a*) values at time 0 for raceways in the low and high nutrient addition treatments during April and May 2003. Chl *a* at time 0 was much higher in the low nutrient raceways during April.

Month	Nutrient treatment	Chl a mean (mg/m^3)	SE (mg/m^3)	n
April	High	76.682	2.440	6
April	Low	112.452	2.785	6
May	High	72.050	5.075	6
May	Low	19.598	1.095	6

The ANOVA performed on the 6-hour recirculation trial data showed a significant ($p = 0.02$) reduction in chl *a* over time, and there were no significant interactions of time with any other variabe. This means that the chl *a* slopes were the same in each experimental treatment groups even though chl *a* values at time zero were significantly ($p < 0.01$) different between nutrient addition treatments during each month. A mean chl *a* removal rate of –0.190 $mg/m^2/hr$ was estimated from the mean slope (-1.450 $mg/m^3/hr$) formed for chl *a* values over the 6-hour recirculation time for nutrient addition treatments and months by the ANOVA.

Chl *a* values in the covered raceway trials involving the low nutrient group were much higher at time zero (Table 4-9) than in the source ponds (Figure 4-3). Conversely, source pond chl *a* values (Figure 4-3) were higher than values at time zero (Table 4-9) in the high nutrient addition treatment. No chl *a* slopes were significantly different from zero ($p < 0.05$) in any of

the covered raceway treatments; therefore, it is assumed that removal of chl *a* in the raceways was a result of exposure to clam populations.

Table 4-9. Raceway chlorophyll *a* (chl *a*) values at time 0 for covered raceways in the low and high nutrient addition treatments. Chl *a* at time 0 was much higher in the low nutrient raceways.

Month	Nutrient treatment	Chl a mean (mg/m^3)	SE (mg/m^3)	n
April	High	54.291	2.831	3
May	Low	108.280	2.831	3

Significant reductions in raceway water chl *a* were detected after 6 hours of water recirculation; however, these removal values are may not represent removal under normal operation. TDP removal was only significant in the high treatment over the April 2003 period and was estimated at nearly 22 mg $P/m^2/hr$. Otherwise, TDP removal rates were no different than 0, indicating that this high mean removal rate based on 3 trials may have been coincidental. A chlorophyll *a* removal rate of 0.190 mg chl *a* $/m^2/hr$ was estimated from recirculation trials in both low and high nutrient addition treatments since no difference in chl *a* removal rates could be found between them. Some difference in removal rates was expected since both live clam biomass and initial chl *a* were considerably higher in the high nutrient addition treatment compared to the low during the recirculation trials.

Sequestration Of Phosphorus By Clams In Treatment Raceways

Shell length and DW biomass characteristics of the clam population sampled for this analysis are shown in Table 4-10. Ash content and AFDW values for the sampled clam population are given in Table 4-11. Shell contained the majority of the total clam ash indicating a larger inorganic content as opposed to meat tissue.

Table 4-10. Mean shell length, clam wet weight (WW), meat and shell tissue dry weights (DW) and condition index (CI) values for the sample population of clams used to determine clam biomass phosphorus content. Much of the clam DW biomass was contained in shell tissue.

Parameter	Shell length (mm)	Clam WW (g)	Meat DW (g)	Shell DW (g)	Clam DW (g)	$CI_{(wt)}$	$CI_{(vol)}$
Mean	20.5	3.056	0.064	1.887	1.951	3.42	4.64
SE	0.2	0.079	0.002	0.047	0.050	0.07	0.16
Range	14.9-30.4	1.200-9.280	0.010-0.220	0.731-5.900	0.773-6.041	1.00-0.22	1.14-15.83
n	228	228	228	228	228	228	228

Table 4-11. Mean and range of ash content values for meat, shell and total clam tissues pooled for all clams sampled. Shell tissue had higher ash content and made up a greater portion of the total clam ash than meat tissue.

Tissue	Mean ash (%)	SE (%)	Ash content range (%)	n
Clam	94.71	0.06	90.45 – 97.39	228
Shell	97.48	0.02	96.06 – 98.23	228
Meat	13.74	0.55	2.50 – 17.00	228

Phosphorus allocation in clam biomass

No significant differences in phosphorus concentrations for the meat, shell and clam tissues could be found between the 2 nutrient addition treatments at stocking or between time intervals for the low treatment using the ANOVA. No discernable relationships in phosphorus concentrations for each tissue type (meat, shell and whole clam) were found between the tested variables (shell length, meat DW, shell DW, clam DW, condition indices, meat AFDW, shell AFDW, clam AFDW) in the ANOVA correlation. Values for tissue phosphorus concentrations were then pooled for all clams used in the experiment to obtain the meat, shell and clam tissue phosphorus concentration data (Table 4-12).

The values in Table 4-13 indicate that clams of shell lengths between 13.9 and 30.4 mm contain from 0.143 to 1.411 mg P per individual. They also indicate that the majority of the total

phosphorus is allocated into meat tissue even though shell material constitutes the majority of the total clam DW (Table 4-10).

Table 4-12. Summary statistics for phosphorus concentrations [P] found in meat, shell and clam tissue types pooled for all clams sampled. Phosphorus was found in much higher concentrations in the meat tissue than shell.

Tissue type	Mean tissue [P] (mg P/ g DW)	SE tissue [P] (mg P/ g DW)	Range tissue [P] (mg P/ g DW)	n
Meat	7.657	0.103	2.650 – 12.853	228
Shell	0.053	0.001	0.022 – 0.136	228
Clam	0.299	0.005	0.141 – 0.606	228

Table 4-13. Amounts of phosphorus (P) contained in meat, shell and clam tissues along with percentages of total clam phosphorus allocated to meat and shell tissues for individual clams. The majority of the total clam phosphorus is a result of meat phosphorus and not shell phosphorus.

Parameter	Amount of P contained in meat (mg)	Amount of P contained in shell (mg)	Amount of P contained in clam (mg)	% of Total clam P allocated to meat	% of Total clam P allocated to shell
Mean	0.480	0.095	0.575	82.2	17.8
SE	0.014	0.003	0.016	0.4	0.4
Range	0.087 – 1.137	0.042 – 0.347	0.143 – 1.411	50.7 – 93.1	6.9 – 49.3
n	228	228	228	228	228

No statistically significant conclusions related to seasonality and clam phosphorus concentration could be assessed due to the drastically decreasing sample sizes at each sampling interval after stocking as a result of the high overall population mortality that took place in all of the raceways. Shell phosphorus values may have been slightly higher than expected since internal cavity area was not cleaned of residual pallail fluid and meat from shucking. Phosphorus detected from the inner clam shell was not accounted for in the analysis and is considered minimal, therefore no further investigation is needed

Treatment raceway clam population phosphorus

No significant reductions in net phosphorus were documented in the raceways, probably due to high overall clam mortality. The amount of phosphorus contained in the total raceway live clam populations was calculated using the mean clam tissue phosphorus concentration values in Table 4-12 and the clam population DW biomass estimation in Figure 3-13, as shown in Figure 4-3. High clam population mortality prevented accurate assessment of raceway clam population phosphorus on a temporal scale.

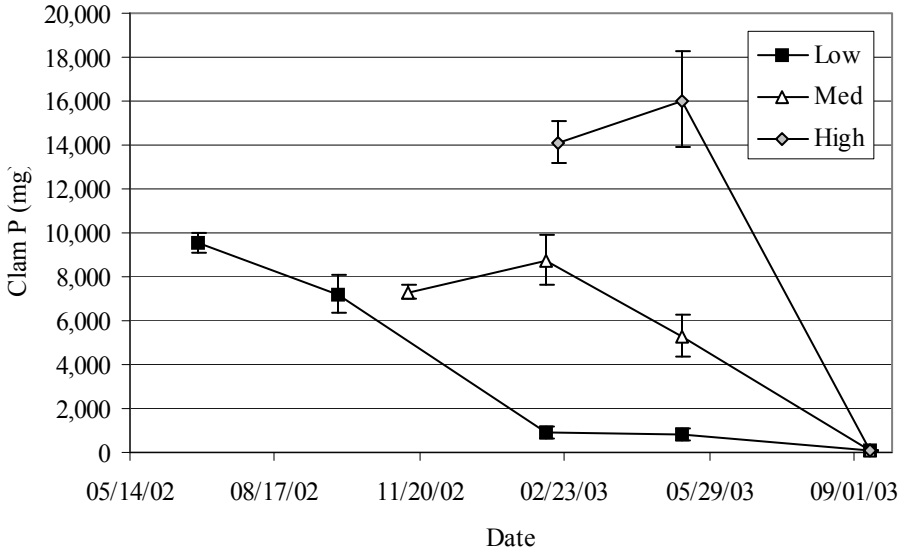

Figure 4-3. Amount of phosphorus (P) sequestered in clam biomass for the low, medium and high nutrient addition treatments over the study period. Raceway clam phosphorus decreased with clam mortality and biomass loss, and no significant accumulation of phosphorus occurred over any of seasonal time periods.

In the high nutrient addition treatment pond raceways from February to May 2003 (Table 3-7), clams measuring 12.2 to 28.6 mm in shell length grew from 0.042 to 0.118 mm/individual/day (Figure 3-11). This equates to a phosphorus sequestration rate of 0.0022 to 0.0079 mg P/individual/day calculated from the clam DW to shell length relationship for the high nutrient addition treatment pond group (Table 3-11) and the clam biomass phosphorus concentration of 0.299 mg P/g DW (SE = 0.005) (Table 4-12). Individual tagged clams in the

medium nutrient addition treatment were able to sequester from 0.0015 to 0.0071 mg P/individual/day during February to May 2003 time period. Due to low individual clam growth rates (0.026 to 0.086 mm/day) during winter (November 2002 to February 2003) (Table 3-7), phosphorus sequestration potential was limited to less than 0.0004 mg P/individual/day.

Discussion

The goal of this phase of the clam treatment raceway study was to evaluate phosphorus removal potential and limitations. The three major components of the evaluation incuded, 1) distribution of phosphorus taken up by clams, 2) rate of phosphrous removal by the clam raceways and 3) sustainability of phosphorus removal. The results of the study revealed some promising best-case scenarios for the system in terms of phosphorus removal, but also a number of major challenges in terms of sustainability.

Distribution of Phosphorus Taken Up By Clams

Clams allocate phosphorus in both meat and shell biomass. Mean clam biomass phosphorus concentration determined from this study was 0.299 mg P/g of clam DW (SE = 0.005, range 0.141 to 0.606, n = 228). Phosphorus concentration did not differ with clam size or between populations of stocked clams from the Santa Fe River and Lake George. Clam biomass phosphorus was allocated in greater amounts to clam meat tissue, as opposed to shell, as suggested by Fuji (1979). Meat tissue had much higher average concentrations of phosphorus (7.657 mg P/ g meat DW, SE = 0.103) than shell (0.053 mg P/g shell DW, SE = 0.001), and as a result, more clam phosphorus was sequestered in meat than shell biomass.

On an individual clam basis, clams contained 0.143 – 1.411 mg P/individual based on clam phosphorus concentration and dry weight values. This is slightly higher than values reported by Fuji (1979) for *Corbicula* in an estuarine lagoon (0.008- 1.500 mg P/individual). Clam meat phosphorus ranged from 0.087 to 1.137 mg P/individual in this study, similar to the range of

0.007 to 1.433 mg P/individual reported by Fuji (1979). Shell phosphorus ranged from 0.042 to 0.347 mg P/individual in this study, slightly higher compared to values ranging from 0.001 to 0.067 mg P/individual shell found by Fuji (1979). The relationship between shell size and individual clam phosphorus was suggested to be exponential by Fuji (1979). In this study, individual clam phosphorus to clam size followed a curvilinear regression relationship developed in Chapter 3 for clam biomass to clam size. Decreasing slope with increasing size in clams larger than 28 mm in shell length was most likely due to shell erosion evident in the larger clam specimens from Lake Dalhousie, FL as explained in Chapter 3. Other studies of biomass phosphorus in *Corbicula* (Fuji 1979) and saltwater *Manilla* clams, *Ruditapes* (Nizzoli et al. 2006) have suggested no effect of clam size or sample location on biomass phosphorus concentration; however, these studies did find that biomass phosphorus concentration differs seasonally. It was suggested that increases in clam phosphorus concentrations during warmer months are related to increases in phytoplankton availability and reproductive activity, which did not occur in the clam raceways.

Oysters contain around 1.067 mg P/g DW (Newell 2004), which is higher than observed for *Corbicula* in this study (Table 4-14). Saltwater *Manilla* clams cultured in an open estuary (Nizzoli et al. 2006) had similar meat phosphorus concentration as clams in this study; however, phosphorus concentrations in clam shell material in this study (0.053 mg P/g shell DW) were on the low end of the range observed for *Manilla* clams. Phosphorus concentrations reported by Yamamuro et al. (2000) for the shells of other saltwater clams (*Musculista*, *Ruditapes* and *Anadara*), are also higher (0.101 to 0.880 mg P/g shell DW) than phosphorus concentrations reported here. The large difference between the values reported by Yamamuro et al. (2000) and

other studies may be due to chemical absorption from the overlying water column, since Yamamuro's values were based on analysis of dead shell material.

Table 4-14. Comparison of meat and shell phosphorus concentrations [P] in dry weight (DW) biomass of *Corbicula* versus other fresh and saltwater clams.

Organism	Meat [P] (mg P/g of meat DW)	Shell [P] (mg P/g of shell DW)	Total [P] (mg P/g of total DW)	Total P per individual (mg)	Reference
Corbicula (freshwater)	2.650- 12.853	0.022- 0.136	0.141- 0.606	0.143- 1.411	This study
Corbicula (estuarine)	8.293- 22.667	0.024- 0.056	0.506- 0.800	0.008- 1.500	Fuji (1979)
Manilla clams	3.000	> 0.150	0.373	n/a	Nizzoli et al. (2006)
Hardshell clams	1.100 ± 0.250 (SE)	n/a	n/a	n/a	Capar &Yess (1996)
Softshell clams	1.400 ± 0.500 (SE)	n/a	n/a	n/a	Capar & Yess (1996)
Eastern oysters	1.100 ± 0.300 (SE)	n/a	n/a	n/a	Capar & Yess (1996)
Pacific oysters	2.000 ± 0.550 (SE)	n/a	n/a	n/a	Capar & Yess (1996)
Saltwater clams	n/a	0.101 - 0.880	n/a	n/a	Yamamuro et al. (2000)

Even though meat contains higher amounts of P, it is more mobile after death than shell material and can more easily return to the overlying water column or substrate (Fuji 1979, Nizzoli et al. 2006). Shell phosphorus, therefore, may be a better long-term sequestration product if phosphorus is in fact incorporated into the organic portions of the shell material and not just loosely bound to shell surfaces. Elements bound in clam shells may require additional steps to degrade since the material is so tightly bound. The higher levels of phosphorus sequestered into shell biomass in this study may, in part, have been due to meat biomass and fluid retained by the shell during processing (McMahon and Bogan 2001).

Estimates of Phosphorus Uptake Rates

Estimated clam biomass phosphorus sequestration rates from the tagged clam study ranged from 0.0022 to 0.0079 mg P/individual/day. Estimated annual phosphorus sequestration potential of 0.803 to 2.884 mg P/individual/yr calculated from daily rates may be overestimated due to lower winter growth of clams. Therefore, an individual clam sequestration rate between 0.201 to 0.721 mg P/individual/yr may be a more accurate estimation of clam phosphorus sequestration, since it is based on longer-term growth estimates. Using the phosphorus sequestration rate for clam population biomass based on tagged clam growth, a theoretical clam population of 16,560 individuals per 21.6 m^2 of raceway area in northern Florida should be able to sequester on average 354 mg P/m^2/yr, with a range of 154 to 553 mg P/m^2/yr, assuming a 3-month cessation in growth during winter.

Clam raceway phosphorus sequestration would be expected to increase beyond rates estimated for tagged clam growth if successful reproduction and recruitment occur and clams are not limited by environmental conditions in the raceway. High reproductive capacity of the animals in large natural systems suggests, that under ideal conditions, raceways should be able to reach population densities of over 2000 clams/m^2 as reported for natural systems by Gardner et al. (1976), Eng (1979), Sickel (1986) and McMahon and Bogan (2001). This is roughly 3 times the stocked density of 766 clams/m^2.

Theoretically, the ideal raceway population would be expected to follow similar phosphorus dynamics as presented in the Fuji (1979) model, who suggested that an estuarine *Corbicula* population of 65 mg P/m^2 can sequester clam biomass phosphorus at an annual rate of about 130 mg P/m^2/yr as growth and recruitment when not limited by food availability. The stocked clam densities in this study represent a total phosphorus level of 14,000 mg P (648 mg P/m^2), translating into 28,000 mg P (1296 mg P/m^2) of harvested phosphorus annually using the

Fuji (1979) model. This value is much higher than the tagged clam phosphorus removal rates given in this study, since it does not account for mortality, which would ideally be minimized by strategic harvest of clam biomass that would also maintain younger clams capable of higher growth rates. The Fuji (1979) model lends itself well to application in an ideal clam raceway calculation since food resources did not appear to be limited and phosphorus sequestration was influenced by seasonality as it is in the natural environment.

Adding to the phosphorus removal potential, phosphorus accumulated in raceways from clam waste would be periodically removed with raceway sediment, thereby minimizing losses to the surrounding environment. The stocked clam population densities used in this study would be expected to produce nearly 4000 mg P/m^2/yr in biosolids as feces and pseudofeces according to the Fuji (1979) model. Ideally, a *Corbicula*-based raceway could be designed and operated to maximize removal of clam wastes and other solids settling on the substrate along with growth of clam biomass. A similar approach in bivalve-based mariculture waste treatment systems has been employed by Shpigel et al. (1993), Shpigel et al. (1996) and Neori et al. (2000) in which settling ponds are used to increase treatment potential. Settling of organic constituents may lead to other problems such as dissolved oxygen demand and ammonia production due to decay (Dame 1996). Dissolved phosphorus loss from accumulated sediments has also been observed in freshwater bivalve-based treatment systems (Soto and Mena 1999). Harvest and substrate removal/replacement times would need to be optimized to minimize these stressors.

An annual phosphorus uptake rate of at at least 5300 mg P/m^2/yr is expected for the *Corbicula* –based raceway system in this study under ideal conditions and solids management. The theoretical clam-based raceway system would also be expected to lose phosphorus at a rate of 1166 mg P/m^2/yr as excreted wastes based on the Fuji (1979) model, that may be more

difficult to capture and are often lost in natural systems with outflowing water. Based on these theoretical values, clam raceway P removal potential would be expected to be approximately 4100 mg P/m^2/yr if solids capture simply by management of settling through optimizing raceway hydrology or the addition of a settling stage following clam raceways.

Comparison Of Phosphorus Removal By Clam Raceways And Other Systems

Estimates for phosphorus sequestration by clams at the stocking rates used in this study are 154 to 553 mg P/m^2/yr based on tagged clam growth rates. The clam phosphorus sequestration values in this study were lower than potential values of a theoretical cultured population of *Corbicula*, estimated by Mattice (1977) to be between 1200 and 1400 mg P/m^2/yr (Table 4-15). The latter range is based on annual clam wet weight biomass growth rates of naturally occurring populations and potential mariculture density assumptions by Mattice (1977), converted to phosphorus removal capacity using average % water and phosphorus content values. However, the biomass phosphorus accumulation range estimated by Mattice (1977) may be unrealistically high due to population biomass density assumptions of over 10,000 clams/m^2, which may not be possible even under ideal culture conditions.

In another study, estimated clam biomass phosphorus sequestration for *Corbicula* exposed to municipal wastewater by Greer and Ziebell (1974) was calculated using biomass phosphorus content values, yielding 877 mg P/m^2/yr (Table 4-15). However, the small scale of the experimental system used by Greer and Ziebell (1974), short time duration of the study and lack of data given on the actual surface area in the experimental culture, casts some doubt on the value of these estimates for large scale systems. Estuarine populations of *Corbicula* have been estimated to sequester phosphorus at 134 mg P/m^2/yr (Fuji 1979), while other phosphorus sequestration values calculated by Fuji (1979) for *Corbicula* populations from other studies

range from 13 to 250 mg P/m^2/yr compared to some other freshwater bivalves that ranged from 37 to 77 mg P/m^2/yr.

Rates reported by Nizzoli et al. (2006) indicate that phosphorus removed from an estuary through harvest of *Manilla* clams can range from 1300 to 2600 mg P/m^2/yr using 3-month growth intervals between harvests (Table 4-15). Phosphorus sequestration may be possible using raceway-based *Manilla* clam culture in saltwater systems and could be increased significantly by capture of waste materials lost to tidal outflow and sediment deposition in natural systems. Phosphorus sequestration rates for bivalve culture systems are much less than the 98,000 mg P/m^2/yr rate given by Dame (1996) for an intertidal oyster reef community containing many different organisms; therefore, this value is not indicative of bivalve phosphorus removal potential. It is difficult to compare phosphorus sequestration in *Corbicula* to mariculture effluent phosphorus treatment systems using other bivalves since treatment potential in these systems is usually assessed on the basis of nitrogen and phytoplankton removal.

Table 4-15. Estimated annual phosphorus removal in various biological treatment systems applied to different effluent types using systems of varying design and scale. Estimated phosphorus removal by the *Corbicula*-based system in this study is similar to other harvested systems, while harvested aquatic plant-based systems are capable of much higher removal rates than animal-based systems.* *denotes phosphorus removal rate based on periodic harvest of system*

Effluent type	Target organism	P removal (mg/m^2/yr)	System type and scale	Reference author(s)
Agriculture N and P fertilizer	*Corbicula*	154 to 553	Raceway tagged clam growth	Measurements from this study
Agriculture N and P fertilizer	*Corbicula*	4100	*Large-scale raceways, theoretical	[a] This study, ideal conditions
Nutrient enriched water	*Corbicula*	877	Theoretical aquaculture	Greer and Ziebell (1974)
Nutrient enriched water	*Corbicula*	1200-1400	Theoretical aquaculture	Mattice (1977)

Table 4-15 Continued.

Estuary, natural	Brackish water clams, *Corbicula* and *Musculista*	17-60	Natural tidal estuary	Fuji (1979), Yamamuro et al. (2000)
Estuary mariculture	Manilla clam, *Ruditapes*	1300-2600 as biomass	*Natural tidal estuary	Nizzoli et al. (2006)
Estuary, natural	Oyster reef ecosystem	98,000	Natural tidal estuary	Dame et al. (1989) in Dame (1996)
Tertiary-treated municipal wastewater	Freshwater finfish, *Tilapia* and catfish	194 to 5840 biomass & wastes	*Large-scale pond culture	[b] Hallock and Ziebell (1970), [b] Greer and Ziebell (1974), [b] Bunting (2007)
Agriculture runoff-enriched surface water	Periphyton	36,550 to 47,450	*Large-scale algal turf scrubber	[a] Adey (1993)
Tertiary-treated municipal wastewater	Periphyton	91,250 to 266,450	*Large-scale algal turf scrubber	[a] Craggs et al. (1996)
Diluted dairy wastewater	Periphyton	29,200 to 120,000	*Small-scale algal turf scrubber	c Wilkie and Mulbry (2002), Pizzaro et al. (2002)
Diluted dairy wastewater	Assorted aquatic plants and algae	58,000	*Med.-scale circular tanks	a Sooknah and Wilkie (2004)
Aquaculture wastewater	Lettuce grown hydroponically	365,000	*Med.-scale conveyor belt	a Adler et al. (2003)
Agriculture runoff-enriched surface water	Periphyton dominated, mixed vegetation	320	Large-scale wetland-type raceways	a DeBusk et al. (2004)
Dairy effluent	Corn silage forage crops	6900	*Land application	d Wilkie and Mulbry (2002)
Dairy effluent	Grassland forage crops	18,800 to 59,000	*Land application	Johnson et al. (2004)
Surface water systems	Natural wetland vegetation	146 to 803,000	General Wetlands	Dodds (2003)

[a] P removal based on annual P removal values for target organisms from seasonal-based experiments at an appreciable scale
[b] P removal estimated using fish P content from Greer and Ziebell (1974) and annual fish production rates from Hallock and Ziebell (1970) and Greer and Ziebell (1974)
[c] Lower P removal value from Wilkie and Mulbry (2002), higher value from Pizzaro et al. (2002), includes Kebede-Westhead et al. (2003) P removal estimate under the same conditions
[d] P removal estimated by reference author using annual values from other studies

Even under ideal culture conditions, estimated removal of phosphorus by *Corbicula* and other bivalves pales compared to those determined for aquatic periphyton and macrophyte-

dominated systems (Table 4-15). Harvested systems appear to be capable of higher phosphorus removal rates, especially in plant-based treatment systems. Natural wetlands show the largest phosphorus removal values; however, they also have the largest range, with values as low as 146 mg $P/m^2/yr$ being reported by Dodds (2003) (Table 4-15). Wetlands are subject to capacity limitations after several years whereas harvested systems are theoretically more sustainable. Periodic harvesting increases phosphorus removal potential of the organisms by minimizing losses due to mortaility, maintaining high growth rates and increasing the longevity of the system.

Vegetative systems may be better suited for management of phosphorus in a farm-scale system due to their ability to withstand a wider variety of environmental stressors and decreased water use compared to clam raceways as seen in this study. Vegetative systems have been added to bivalve-based systems in the mariculture industry to provide treatment following exposure to bivalve populations (Shpigel et al. 1993, Shpigel et al. 1996, Neori et al. 2000). Periphyton may also be used to remove phosphorus following exposure to *Corbicula*-based systems since harvested types of these systems have been successful in rapidly sequestering phosphorus from agricultural applications especially at low water phosphorus concentrations (Scinto and Reddy 2003). Raceway systems used in this study may be applicable in vegetative-based biofilters as well.

Problems With Measuring Short-term Phosphorus Uptake

There were no indications that the raceway systems were able to provide a noticeable reduction in water phosphorus or chlorophyll *a* during the normal through-flow operation at a range of 151 to 227 L/minute. Reduction of phosphorus by the clam raceways was not observed even after being converted to a temporary recirculation mode to increase retention time to 6 hours from 9.5 minutes and 6.3 minutes. High temperature, low dissolved oxygen and ammonia

toxicity concerns prevented longer retention times by recirculation in an attempt to show phosphorus reduction especially during daylight hours. There was no significant reduction of total phosphorus levels in the covered raceways, indicating that raceway water phosphorus reduction may not be affected by clams or settling in the raceway systems.

The most plausible explanation for the lack of large-scale phosphorus uptake may be the obvious environmental and physiological stress that clams were undergoing over the entire study period including during the through-flow and recirculation trials. High clam mortality was evident throughout the study, especially in warmer months when water phosphorus removal trials were taking place even though condition indicies did not indicate any decreases in clam health over time. Haines (1977) attributes similar mortality and decreased water treatment capacity in *Corbicula* cultured on municipal wastewater to high temperature and possibly ammonia. Also, potential stress from amphipod infestation may have severely affected the raceway clam population's ability to filter feed and, therefore, remove phosphorus-containing material as described in Chapter 3.

Similarly, Kinne et al. (2001) was unable to show that TP was lowered significantly in a medium-scale raceway system using oysters to treat shrimp farm effluent, possibly because of soluble phosphorus excretion meeting phosphorus uptake or ammonia concerns. Conversion of particulate phosphorus to dissolved phosphorus by excretion of soluble phosphorus by *Corbicula* waste products as described by Lauritsen and Mozley (1989) may not explain the lack of phosphorus removal in this system. The extent of phosphorus conversion within the raceways is unknown since significant TDP addition was never detected during any recirculation trial, and TP removal was no different in raceways containing substantial amounts of clams compared to those with depleted stocks. Clam density is expected to limit treatment capacity for raceway

clam populations. However, raceway systems in the high nutrient addition treatment contained approximately 460 clams/m^2 (10,000 clams per raceway) more than the low nutrient system, but still did not provide a noticeable phosphorus removal.

Chlorophyll *a* uptake values in the recirculation trials may have been induced by disturbance of substrate materials related to switching raceway hydrology from through-flow to recirculation for experimental purposes. Disturbance of raceway substrate did occur in the recirculation trials as indicated by the higher TP, TDP and chl *a* values as opposed to source pond levels at the same time. Haines (1977) attributed increased treatment potential of *Corbicula*-based systems to increased particle concentration. Therefore, any disturbance and increase in particle availability may result in removal by clam populations as well as settling. The apparent significant chlorophyll *a* removal seen in both high and low nutrient addition treatments may have been due to such a disturbance. However, removal rates were probably not due to settling, since covered raceways failed to provide any definitive indication of chlorophyll *a* uptake. Given the large differences in clam population density between the high and low nutrient addition treatments during the recirculation trials, actual removal of chlorophyll *a* due to clam filtration is also doubtful. Potential problems encountered with the raceway recirculation modification suggests that phosphorus uptake was probably not achievable under normal through-flow operating conditions by the clam raceways, as expected.

Problems such as high temperature, low dissolved oxygen and dangerous levels of ammonia may result with the six-hour retention times, as seen in this study. Raceway system hydrology can also be modified to accommodate full or partial raceway water recirculation as demonstrated by the additional pump placement in this study to extend retention time. Water retention time would need to be managed closely with real-time monitoring of temperature,

ammonia and dissolved oxygen to avoid levels that may negatively affect clam populations. System water retention time greater than 10 minutes and less than 6 hours is recommended to improve treatment potential, while minimizing environmental stress. More conclusive testing of TP, TDP and chl *a* uptake by clam populations at large scale is needed before reliable short-term removal rate estimates and system operating tolerances can be assessed.

Dairy Application Demands And Issues

Issues with water consumption may limit widespread use of this technology since digested dairy wastewater effluent addition less than 5 % (by volume) was needed in clam raceway source water ponds to overcome ammonia concerns. Sooknah and Wilkie (2004) have demonstrated that a 1 : 1 dilution of anaerobically digested wastewater enhances biological uptake of phosphorus and nitrogen in aquatic plant-based systems, substantially decreasing amounts of ammonia in effluent water. Application of these kinds of vegetative systems may provide pre-treatment of phosphorus and harmful nitrogenous compounds prior to clam raceway addition to increase feasibility at a large scale. Coupling of vegetative and clam-based systems in this way would lower water demand, while decreasing ammonia and utilizing less treatment surface area than required by clam systems alone.

Application of clam raceway technology in a real-world scenario is perhaps best analyzed on an individual farm basis. The University of Florida Dairy Research Unit (DRU) in Hague, Florida is described by Wilkie et al. (2004) as having an average milking herd of 359 cows, a wastewater production of 502 m^3/day and a daily freshwater water usage of 52.25 m^3/day. A large scale, fixed film anaerobic digester is in place at the DRU and is not expected to change the amounts of P-loading to wastewater handling systems that are restricted primarily to a secondary settling lagoon until land application. Normal digester outflow would be similar to values

reported by Sooknah and Wilkie (2004) for water quality in a system accepting digester effluent from the DRU, including 24 mg/L TP and 136 mg/L total ammonia nitrogen (TAN).

Phosphorus and ammonia levels in the macrophyte system output are expected to be 0.24 to 6.0 mg/L TP with a significant portion of phosphorus as dissolved phosphorus and 0.29 to 3.53 mg/L TAN (Sooknah and Wilkie 2004). Insertion of air-stripping or biofilm filtration systems similar to ones used in the aquaculture industry (Timmons et al. 2001) or bacteria-based systems under assessment for dairy wastewater, as indicated by Sooknah and Wilkie (2004), could possibly be inserted following macrophyte treatment to lower TAN levels. Another possible solution to the ammonia problem may be another vegetative treatment phase. However, these biological-type systems may not be as dependable as mechanical ones used in commercial aquaculture due to seasonality and temperature dependence of biological systems. Application of aquaculture technology should help to decrease TAN and increase DO levels with little or no excess water demand except for increased evaporation with increased treatment surface area.

The continuous growth of nuisance plants evident during raceway operation may indicate the need for a vegetative system to be incorporated into the clam system design to get a noticeable decrease in raceway water phosphorus. Fuji (1979) suggested that clam deposition of organic materials provides a food source for plant growth in detrital systems; therefore, harvested plant growth in polyculture with *Corbicula* may provide a way to increase phosphorus sequestration potential of the raceway system. Use of vegetative-type filters along with bivalves has been demonstrated in marine mariculture wastewater treatment systems to remove dissolved phosphorus excreted from bivalves (Jones et al. 2001, Borges et al. 2005) and has been suggested for *Corbicula* culture in agriculture wastewater (Stanley 1974); but no applications of this technology coupling have been demonstrated using dairy effluent. Nuisance plant growth did

not appear to be inhibited by the high water temperatures encountered in this study, unlike clam populations, adding to the appeal of plant-based systems.

Using the 1:1 dilution and phosphorus removal potentials suggested by Sooknah and Wilkie (2004) for an aquatic macrophyte-based system at the DRU, I would expect freshwater usage to increase from 502 m^3/day, to 554 m^3/day just for the macrophyte system. This would increase wastewater volume to 105 m^3/day and require an active system capable of sequestering 655 mg TAN/m^2/day (239,075 mg TAN/m^2/yr). This value is based on TAN removal of 99.6 % from diluted wastewater containing 68 mg/L TAN and over a 31-day period using a 0.5 m x 0.36 m raceway at a water depth of 0.3 m (Sooknah and Wilkie 2004). The resulting macrophyte system sized for TAN removal at the DRU would need 68 m^2 of treatment area per day of retention. With the suggested hydraulic retention time of 31 days to remove incoming TAN, raceway treatment area would need to be at least 2116 m^2 to meet the TAN treatment needs for *Corbicula* systems using anaerobically digested wastewater at the DRU.

Removal of ammonia would be the primary goal of vegetative treatment prior to clam raceway introduction; however, macrophyte systems have been suggested for treatment of phosphorus as well (Sooknah and Wilkie 2004). The macrophyte system phosphorus removal potential would be 58,000 mg P/m^2/yr removing 96.5 % of the incoming phosphorus under ideal conditions as indicated by Sooknah and Wilkie (2004). Assuming that ammonia levels could be maintained without removal of phosphorus or further dilution, phosphorus loading from the macrophyte system is expected to be up to 230 kg P/yr for the 105 m^3/day (38,325 m^3/yr). Wastewater would exit the macrophyte system at 0.24 to 6.0 mg/L TP, considerably higher than the range tested in this study; however, within expectations of 3 mg/L values tested by Greer and Ziebell (1972) at aquarium scale using *Corbicula*. Under this maximum phosphorus loading rate

expected in the macrophyte filter outflow, a clam raceway system having up to 38,325 m^2 of treatment area would be needed to remove the remaining phosphorus in the system.

Losses of phosphorus around 1166 mg $P/m^2/yr$ would be expected from the 38,325 m^2 of clam raceway system area and would require additional treatment prior to discharge into surface waters since effluent TP concentration would be around 0.05 to 1.24 mg/L at a volume of 105 m^3/day in a properly managed clam system under these circumstances. This value may be improved by increasing the retention time or deceasing water usage. Application of clams or macrophytes at such a scale is preposterous considering the limited size scale demonstrated by Sooknah and Wilkie (2004) and the lack of large-scale success of *Corbicula*-based raceways at the DRU that prevents an accurate system sizing from being made.

Other large-scale harvested aquatic plant systems such the Algal Turf Scrubber® (ATS) raceways described by Craggs et al. (1996) may also need to be employed prior to introduction to clam systems, in addition to macrophyte-based raceways to lower TP to an optimum level. TP in clam raceway influent should be between the 0.04 and 3 mg/L range shown by Greer and Ziebell (1972) to have some phosphorus removal potential as determined in aquarium-based studies. Small-scale algal turf scrubber systems have been used by Pizzaro et al. (2002), Wilkie and Mulbry (2002) and Kebede-Westhead et al. (2003) on diluted dairy wastewater at the DRU to remove an estimated 29,200 to 120,000 mg $P/m^2/yr$. Assuming the lower phosphorus removal value of 29,200 mg $P/m^2/yr$, the system would need to have at least 7,876 m^2 of treatment area, much larger than the largest ATS wastewater treatment system tested by Craggs et al. (1996) at 1,018 m^2, but not impossible for large scale implementation at the DRU. Using ATS systems to treat dairy wastewater at a quartenary or lower level, as suggested by these calculations, is

certainly more feasible than clam raceways requiring a treatment area 1,774 times larger than the area afforded by the 3-raceway system tested in this study.

Concerns over salinity and DO levels in dairy wastewater remediation (Sooknah and Wilkie 2004) may also impact clam raceways. *Corbicula* can tolerate at least some salinity in the natural environment (Deaton 1980, McCorkle and Dietz 1980), however low tolerance for dissolved oxygen levels in the 3-5 mg/L range reported by Belanger (1985), suggests that oxygen may be the more critical factor. Dissolved oxygen levels of less than 3 mg/L and ammonia levels from 0.29 to 3.53 mg/L TAN in various macrophyte system effluents reported by Sooknah and Wilkie (2004) will need to be addressed prior to application of clam systems since they are not within the tolerable ranges for *Corbicula*.

Sustainability

Application of clam raceway systems as a dairy wastewater treatment mechanism will need further investigation, primarily since raceway systems were not able to operate under dairy effluent addition at 5 or 10 % by volume due to high ammonia concerns. Keeping both ammonia and temperature within a tolerable level will be critical to implementing *Corbicula* as a wastewater treatment mechanism. High levels of undesirable materials such as ammonia in source water can be addressed by dilution of effluents; however, increased constraints on freshwater usage may limit the use of clam raceways alone to solve the phosphorus management of any operating dairy, especially in Florida. Successful implementation of clam raceways or other high water demand/low aereal phosphorus sequestration potential for the treatment of dairy wastewater on a large scale may be limited unless coupled with other technologies such as harvested plant systems as seen in the mariculture industry by Shpigel et al. (1993), Shpigel et al. (1996) and Neori et al. (2000).

Corbicula-based raceway systems may be more applicable to aquaculture waste scenerios since ammonia, dissolved oxygen and temperature are typically monitored and managed in commercial aquaculture systems. While salt tolerance in *Corbicula* affords the animal some expansion into mariculture effluent phosphorus treatment, species selection in these systems will probably be limited to saltwater species traditionally cultured in larger scale as a food crop, such as oysters (Jones et al. 2001). Bivalve-based systems may be able to perform for longer durations between harvest efforts by utilizing other species due to the shorter life spans found in *Corbicula* (normally about 2 years, from McMahon and Bogan 2001).

The lack of marketability for *Corbicula* cultured in wastewater conditions may also be an obstacle to implementation since phosphorus treatment effectiveness is not only gauged by phosphorus removal and sequestration potential, but also cost effectiveness, energy costs and water consumption. *Corbicula* has been proposed as a desirable protein source by Iritani et al. (1979) and has been recommended as a product to offset operational costs in clam-based systems by (Mattice 1977), Greer and Zeibell (1972) Stanley (1974), and Haines (1977). Even though a market for live *Corbicula* or *Corbicula*-based products may exist in some places (Chen 1976, Phelps 1994), clams cultured on dairy or municipal wastewater may not be acceptable in any market due to human health concerns. Biomass produced on wastewater can sequester toxins such as metals (Marcussen 2007) and pesticides (Barber 2006), although worries over choliform bacteria may be unfounded (Islam 2004). Stanley (1974) cautions about potential problems with poisoning from cyanobacteria toxins in wastewater aquaculture systems as well. The lack of market for *Corbicula* as a food resource domestically is due in part to the filter-feeding ability of freshwater bivalves that captures various harmful organisms easily transferred to humans such as *Cryptosporidium* (Graczyk et al. 2003 and Izumi et al. 2004), *Giardia* (Graczyk et al. 2003) and

Cyclospora (Graczyk e al 1998). Other species such as pearl oysters can provide profit opportunities other than food or aggregate in mariculture waste treatment systems as suggested by Gifford et al. (2004), and the organisms could possibly be adapted to raceway culture conditions.

In an attempt to use raceway biomass in a positive way, dead clam shells removed from the raceways in this study were spread out as an aggregate over areas surrounding the raceways that were covered with crushed lime rock to prevent weed growth. Clam shells are typically removed from aggregate sources excavated from river bottoms in the Applachicola River in Florida and may not be as desirable an aggregate choice compared to gravel. Problems with decaying soft tissue may prevent use of clam aggregates without pre-treatment to reduce odors.

Substrate removed from the raceways is rich in clam biosolids and could potentially be composted for use as a soil amendment (Greer and Ziebell 1977). This compost may be more valuable than the clams themselves, therefore, composting both clams and substrates simultaneously may be the best alternative for phosphorus sequestered by *Corbicula* raceway systems. Products from vegetative-based phosphorus treatment systems are often equally unwanted and ultimately the most valuable as a soil amendment by composting the material to offset operational costs in plant and animal-based treatment systems as suggested by Greer and Ziebell (1974).

Even if an economically feasible use could be found for *Corbicula*-based treatment raceway by-products, and adequate phosphorus removal on a large scale could be established in a wastewater treatment stream, implementation of a system that utilizes such an invasive animal can be met with negative responses. Introduction of *Corbicula* has been implicated in different large-scale habitat modifications in natural water bodies that may not be viewed as acceptable in

all surface water systems (Ingram 1959). Other studies indicated concerns over native mussel displacement due to the high colonization success and reproductive capacity of *Corbicula* (Kraemer 1979, Darrington 2002, Cooper et al. 2005).

Inevitably, no matter what size and component design of a biologically-based system, land and water demands, or its usefulness and desirability, environmental conditions must allow clams to survive, grow and reproduce to sequester phosphorus effectively into shell and meat tissue at a large scale. Potential problems with high temperatures, ammonia, food availability, seasonality, phytoplankton production, amphipod infestations, system performance evaluation, biofouling by vegetation, available land area and water usage need to be solved before a reliable large-scale *Corbicula*-based raceway treatment system can be implemented into any wastewater stream in Florida.

CHAPTER 5
SUMMARY

The three primary goals of this dissertation research project were; 1) To design, construct and implement an experimental raceway system for removal of particulate phosphorus from wastewater streams on a size scale that represents real-life applications 2) To test suitability of the freshwater clam *Corbicula* as the primary active agent in phosphorus removal within a raceway environment and 3) To determine if P-removal capabilities of the system are adequate to deal with phosphorus loads anticipated for dairy wastewater streams.

Raceway Function and Attributes

The raceway-based systems constructed in this study were chosen and developed as a low-cost, portable and easier-to-assemble alternative to other raceways constructed from concrete, fiberglass or plastic. The advantage of the modular raceway design was the culture tanks are easily scalable by length, width, depth and quantity of units to meet surface area needs of the application, desired hydrologic regimes and experimental design criteria. The design also maximizes physical accessibility to the bottom area for stocking, sampling, harvest and maintenance. The raceway systems constructed at both Blountstown and Hague, FL sites operated without failure or leaking over the study period and provided an ideal platform for the water treatment experiments incorporated in the current study.

Adaptability of Clams to Raceway Conditions

The tag and recapture study was the primary measure of growth potential of *Corbicula*. Individual tagged clam shell growth rates based on length ranged from around 0.001 mm/day on an annual basis in the low nutrient addition treatment group to up to 0.118 mm/day in the high nutrient addition treatment group in spring. Temporal patterns in tagged clam growth rates showed seasonality prossibly due in part to changing water temperature, level of nutrient

addition, source water phosphorus levels and possibly phytoplankton availability. Tagged clams grew in all nutrient addition treatment groups without a consistent correlation to chlorophyll *a* concentration, suggesting that either clams utilized non-chlorophyll *a* containing food resources, such as bacteria and suspended detritus, or phytoplankton biomass in the source ponds was sufficient to sustain growth.

Despite the growth observed in the surviving tagged clams, the overall clam population did not adapt well to raceway conditions over extended periods, with over 90 % mortality during a one-year period. Timing of mortality indicated that high summer temperatures in the raceways may have been the major factor responsible for the severe losses. Water temperatures in the range of 30 ^{o}C and above have been implicated as a limiting factor in the success of *Corbicula* in other applied studies and in natural populations (Greer and Ziebell 1972, Mattice 1977, Haines 1979, Buttner and Heidinger 1980, Buttner 1986). Major population declines took place when water temperatures reached this level in all systems, regardless of the level of nitrogen and phosphorus addition and chlorophyll *a* in the source water.

Other environmental factors present in the raceways, including increased ammonia levels encountered during periodic execution of 6-hour recirculation trials, and potential stress from infestation by amphipods (*Hyalella azteca*), may have also contributed to mortality and affected phosphorus removal and sequestration potential as well. Interactions observed between *Corbicula* and *Hyalella* in this study are the first to recognize amphipods as having a potential predatory or parasitic role in clam population dynamics; however, the intricacies of this interaction are not yet understood.

P-removal Capacity

Clam raceway systems were able to demonstrate phosphorus removal and sequestration potential as evidenced by significant shell growth in tagged clams. Individual clams selected for

analysis were between 14.9 and 30.4 mm shell length (0.773 – 6.041 g total clam DW). Clams contained an average of 0.299 mg P/g DW (SE = 0.005, range 0.141 to 0.606, n = 228), derived from shell and meat values combined, which equates to 0.143 to 1.411 mg P/individual. The concentration of phosphorus (mg P/g DW) in the shell and meat tissues did not change with shell size, location collected or exposure time in the raceways. Meat tissue had a much higher average concentration of phosphorus (7.657 mg P/ g meat DW, SE = 0.103) than the shell (0.053 mg P/g shell DW, SE = 0.001). As a result, the majority of the total clam phosphorus was sequestered in meat as compared to shell biomass, even though shell biomass comprised the majority (approximately 82%) of the total clam DW biomass.

Based on the tagged clam study, phosphorus sequestration potential was estimated to range from 0.803 to 2.884 mg P/individual/yr for adult clams. *Corbicula* biomass phosphorus sequestration potential estimated in this study was similar to values given by Fuji (1979) for biomass phosphorus sequestration in natural populations. Ideally, raceway clam populations would be able to sustain estimated clam biomass phosphorus sequestration rates along with successful reproduction and recruitment as seen in the Fuji (1979) model. An similar raceway system as tested in this study under ideal conditions would be stocked with 648 mg P/m^2, and would be expected to sequester 28,000 mg P/yr (1296 mg P/m^2/yr) as clam biomass. In a clam culture scenario, this live biomass could be harvested along with most of the 708 mg P/m^2/yr expected to be produced by normal mortality and excretion retained in the raceway sediment.

Ideally, a *Corbicula*-based raceway would be designed and operated to maximize sequestration of particulate clam waste phosphorus and other solids settling along with growth of clam biomass. Phosphorus removal potential may be higher in an engineered system equipped to deal with environmental variation and capable of further enhancing growth rate and treatment

area through sustainable harvest. By retaining and harvesting settled particulates that would otherwise be lost in natural systems and maximizing growth and reproduction rates, an annual phosphorus removal rate of at least 4100 mg P/m^2/yr is expected for the *Corbicula*–based raceway system as tested in this study. This phosphorus removal and sequestration potential is higher than potentials calculated for other freshwater bivalve and finfish populations.

Future Applications

Limitation of reproductive success by *Corbicula* held in captivity in this and other studies (McMahon and Bogan 2001) suggests that implementation of *Corbicula* systems at large scale may not be attractive if juveniles need to be restocked periodically from natural populations. Perhaps partially open systems such as power plant discharge canals that are open to a natural water body part of the year would allow for inflow of juveniles to repopulate raceways after harvest or mortality events may be needed to maintain adequate stocks. Other solutions for sustaining clam stocks in raceway-based systems such as genetic selection for traits that support growth and reproduction outside of the normal tolerances has been proposed for *Corbicula* by Sickel (1986) however this would take an exceptionally long time to develop, if at all, given homogeneity and genotypic plasticity of *Corbicula*.

Clam raceway, phosphorus treatment potential of anaerobically digested dairy effluent could not be assessed directly, due to toxicity concerns over total ammonia nitrogen levels present at > 2 mg/L NH_3-N present in source ponds at 5 % and 10 % (volume of source pond volume) wastewater addition levels tested. Low tolerance to ammonia toxicity in *Corbicula* (Cherry et al. 2001) and to ammonia in combination with high temperatures have been implicated in limiting the success of clam-based treatment systems utilizing municipal sewage treatment plant effluentsby Haines (1979). Ammonia toxicity is of great concern in all aquaculture systems (Harris et al. 1998), especially recirculating ones where technologies

utilizing treatment by volatilization, sequestration or conversion have been developed to encourage higher density cultures of fish and other aquatic organisms (Timmons et al. 2002). Lack of ammonia management technologies tested at large scale for application in clam raceway systems will be an important goal in future development of freshwater animal aquaculture applications for dairy waste treatment. Bivalve-based treatment of dairy-derived wastewater phosphorus would require implementation and integration of additional treatment technologies in order to reduce high levels of nitrogenous wastes common in dairy operations.

Application of vegetative systems such as wetlands, managed aquatic plant systems and biofilm systems may be able to provide needed treatment of ammonia prior to clam raceway addition in order to increase feasibility. Coupling of vegetative and clam-based systems in this way would lower water demand while decreasing ammonia and utilizing less treatment surface area than required by clam systems alone. Using vegetative-type filters along with bivalves has also been demonstrated in marine mariculture wastewater treatment systems to remove dissolved phosphorus from the outflow of bivalve-based systems (Jones et al. 2001, Borges et al. 2005) and has been suggested for *Corbicula* culture in agriculture wastewater by Stanley (1974).

Consideration should be given to source pond geology, depth, sediment permeability and use of clay or plastic liners in future systems to limit or promote possible exchange of pond water nutrients and heat with the surrounding environment to maintain tolerable environmental conditions. Seasonality of system function is a central issue not only from the standpoint of summer high temperatures, but from growth and survival, issues under low winter temperatures.

Despite the lack of *Corbicula* success on a large scale, the raceway-based recirculation system design demonstrated in this study provided a dependable, easy to construct and reusable platform for testing aquaculture potential of organisms in wastewater treatment conditions at

large scale. The design should be used as a standard system to assess other organisms besides *Corbicula* for comparative purposes since systems can be easily constructed to accommodate a variety of operating parameters. The raceway system designs employed here are versatile enough to be applied to other organisms such as bivalves, fish, algae and high plants; targeted for large-scale water treatment/biofiltration studies in both fresh and saltwater conditions and a variety of locations, as well as effluent sources.

From a comparative standpoint, it is important to make the observation that the phosphorus removal capacities of many animal-based systems, which typically range from 17 to 5840 $mg/m^2/yr$, are low compared to the best-case estimates for algae and other plant based systems, which range from 320 to 365,000 $mg/m^2/yr$. However, this comparison is somewhat misleading, since the function and structure of the two systems are different in several important aspects. Plant/algae systems provide a mechanism for removal of particulate and dissolved nutrient forms as opposed to animal-based systems that focus on particulates. Also, plant/algae systems are energetically dependent on sunlight, while animal systems can be independent of such a direct requirement. In addition, the end products of plant/algae and animal systems are fundamentally different and subject to separate end-use issues and opportunities. Ultimately, many treatment needs may be best addressed by integration of animal and plant/algae systems, thereby allowing for optimal processing of soluble and particulate wastes and production of a wide range of valuable goods and services, such as food, feed, biodiesel, building materials and chemicals.

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BIOGRAPHICAL SKETCH

Lance W Riley was born at the US Navy Submarine base in Guam, Mariannas Islands in 1974 to Capt. (USN) Roy "Luke" Riley and his lovely wife, Linda Cline Riley. The family eventually moved to Gold Hill, North Carolina, and Lance graduated from Mount Pleasant High School, Mount Pleasant, NC in 1992. Lance received his Bachelor of Science degree in Environmental Biology at the University of North Carolina at Charlotte in 1998. He then earned his Master of Science degree in Environmental Engineering Sciences with a Graduate Certificate in Wetlands at the University of Florida in 2000. Lance is currently employed at the University of Florida Fisheries and Aquatic Sciences Department where he performs analytical and field research using laboratory experiments and in-situ monitoring of waterways throughout Florida.