RECIRCULATING AQUACULTURE SYSTEM INTEGRATION OF BIVALVE CULTURE FOR EFFLUENT NUTRIENT COMPOSITION REDUCTION

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A Thesis Submitted to
University of North Carolina Wilmington in Partial Fulfillment
of the Requirements for the Degree of
Master of Science

Center for Marine Science

University of North Carolina Wilmington

2006

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ABSTRACT

Closed recirculating aquaculture systems (RAS) are a technological innovation that reduces the amount of water needed for culture by treating and reusing up to 90% of the total water volume daily. Such systems consequently produce substantially smaller volumes of effluent, though it is enriched in nutrients relative to effluent from flow-through or open systems. For RAS to emerge as a viable culture strategy, an economical and efficient method must be developed to reduce effluent nutrient concentrations before discharge. As part of a larger project evaluating biofilter effects on RAS effluent, this study focused on the effect of bivalve culture on the composition of southern flounder RAS effluent. This system produces 1270 L/day of effluent, with nutrient and suspended solids concentrations 20-100x that of ambient levels. Two trials were conducted using this effluent as a nutrient base for bivalves stocked in an upwelling system for four weeks. Trial I utilized raw effluent nutrients for two densities of oysters (*Crassostrea virginica,* (average shell height ± standard error = 63.4 ± 1.7 mm). Trial II utilized effluent inoculated with microalgae, *Isochrysis galbana,* as the nutrient base for two densities of clams (*Mercenaria mercenaria*, average shell height ± standard error = 16.7 ± 0.2 mm). Effluent nutrient composition (total nitrogen, total phosphorus, and total suspended solids) was analyzed from samples (collected twice weekly) taken before and after bivalve filtration. Change in shell height was quantified by measuring subsamples at the start and end of each trial. Oysters did not have a significant impact (Wilcoxon-Mann-Whitney Test, $p>0.05$) on the RAS effluent nutrient compositions in this flow-through integrated system. Clams, also, did not have a significant impact (Wilcoxon-Mann-Whitney Test, $p>0.05$) on the RAS effluent nutrient compositions. Significant change in shell height was not exhibited in the oysters (Wilcoxon-Mann-Whitney and Welch-ANOVA Tests, $p>0.05$) over the time course of Trial I. Over the

course of Trial II, a significant change in shell height was exhibited within treatments (Low: $p=0.01$, High: $p=0.049$) and between clam densities ($p=0.01$). The magnitude of seawater dilution, high flow rates, and high suspended solids loads may have inhibited my detection of effluent composition reductions.

ACKNOWLEDGMENTS

I would like to thank my parents, Richard and Patsy Myers, for their continued support and encouragement. I would also like to thank my committee members, Ami Wilbur, Wade Watanabe, and Joan Willey for their guidance and assistance.

DEDICATION

I would like to dedicate this thesis to my grandmother, Martha Livingood, and my Aunt Mag, whose determination and love for life continue to inspire me.

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INTRODUCTION

Worldwide demands on food production are increasing due to declining fisheries stocks and an increasing world population that is approaching 8 billion people. In 2002, total aquaculture production was valued at $60 billion and aquaculture contributed almost a third of global fisheries (FAO, 2004). Aquaculture has expanded in the last thirty years and has the potential to enhance local food availability, alleviate poverty, and improve rural livelihoods (FAO, 2004). One drawback of many aquaculture methods is the discharge of nutrient-rich effluent into the environment. This discharge includes feed-derived wastes composed of dissolved components, such as nitrogen, phosphorus, and suspended solids (Losordo and Westers, 1994).

Dissolved components have the potential to contribute to environmental degradation. Aquaculture discharge can cause eutrophic conditions in receiving waters and can have an even greater negative impact when combined with nutrient-rich runoff from coastal development (Wu et al., 1994; Smith et al., 1999). Nutrient enrichment can cause phytoplankton blooms that eventually degrade, depleting the water of oxygen, therefore decreasing the amount of oxygen available to other aquatic organisms (Blackburn et al., 1988; Weston, 1990). Once oxygen is depleted in the water column, sediments can become anoxic, causing biological and chemical changes in the sediment and consequently altering benthic community structure (Boyd and Massaut, 1999). Anoxic sediments can become reducing and release ammonium, as well as methane and hydrogen sulphide into the water column (Pillay, 1992). These gases are capable of outgassing to aquatic animals, such as fish, causing gill damage and fish mortality (Landau, 1992).

Discharged aquaculture effluent also has the potential to change the species composition of aquatic species in receiving waters. The excess nutrients in effluent can cause increased microorganisms, like bacteria, as well as primary production, which can lead to changes in the composition and abundance of phytoplankton species (Pillay, 1992). If the phytoplankton species are toxic, blooms resulting from nutrient enrichment can also kill fish and contaminate shellfish (Wu, 1995). Macroalgae can also be affected by discharged effluent. The additional dissolved nutrients can enhance macroalgae growth and some waters can be dominated by filamentous algae (Schuenhoff et al., 2003).

Effluent produced in aquaculture farms may vary in nutrient concentrations and amount depending on the species cultured and culture practice. Nutrient and suspended solids concentrations are influenced by the culture density, as well as settling time. Other factors that can influence the composition of the effluent include water temperature and depth, food supply, digestibility of feed and feeding rate, and cleaning operations (Landau, 1992). However, to date, most of the available literature on effluent characterization comes from research conducted on cage culture.

The components of wastes in hatcheries or production farms include residual food, animal wastes and metabolic by-products. The resulting animal waste products usually contain organic carbon and nitrogen (carbohydrate, lipid and protein), ammonium, urea, bicarbonate, and phosphate (Pillay, 1992). Penczak et al. (1982) researched waste components discharged from rainbow trout cage culture and estimated it to contain 30% carbon, 4% nitrogen and 2% phosphorus. After accounting for nitrogen retention in the fish, they estimate 68% to 86% of the

consumed nitrogen has been excreted as soluble ammonium and urea (Penczak et al., 1982). The phosphates quantified in this study only included those in particulate form, although dissolved phosphate is also known to be discharged in aquaculture waste. One-half of the total phosphorus in feed was lost through the discharge (dissolved and particulate phosphorus) of a freshwater salmonid farm in Norway (Bergheim et al., 1984). However, when such a freshwater flow-through system is flushed adequately, fish farm effluent is diluted to concentrations similar to influent water (Warrer-Hansen, 1982).

These concerns about nutrient loading from aquaculture systems have led to an increasing interest in recirculating aquaculture systems (RAS). Closed RAS are a technological improvement because they can reduce the amount of water needed for culture by treating and reusing 50-90% of the total water volume daily (Goldburg and Triplett, 1997). Consequently, RAS produce substantial smaller volumes of effluent. However, the effluent discharged from these systems is concentrated and generally much more nutrient-enriched than flow-through or open systems (systems that use water once, and then discharge). True et al. (2004) researched effluent composition from two flow-through systems stocked with rainbow trout (*Oncorhynchus mykiss*) in Idaho, USA. Their research concluded that these flow-through systems yielded an average 0.09 mg/L total phosphorus and 1.34 mg/L for total suspended solids for particles greater than 53µm (True et al., 2004). Schulz et al. (2003) also studied flow-through systems stocked with rainbow trout in Germany and quantified average effluent concentrations. Total phosphorus (TP), total nitrogen (TN), and total suspended solids (TSS) were found to be 0.347 mg/L, 2.40 mg/L and 14.2 mg/L, respectively. Truesdale (personal communication) characterized effluent from a commercial scale recirculating aquaculture system in Wrightsville

Beach, North Carolina for three months. Truesdale found nutrient concentrations greater than estimated for flow-through systems and that exceed several magnitudes more than natural levels found in adjacent waters (Table 1). Truesdale's preliminary study on this same RAS system found, out of an average 2.37 kg of feed given, 26% were discharged as solids. An average 754 L of concentrated effluent was collected over a 24 hr collection. Of the total feed input, 1.17% of total phosphorus and 0.57% of total nitrogen was discharged as effluent. The effluent also contained 0.26% NH_4^+, 0.57% PO_4^{3-}, 0.021% NO_3^-, and 0.015% NO_2^- of the total feed input (Truesdale, personal communication). Thus, disposal of RAS effluent can contribute to environmental problems in the same way as more traditional aquaculture methods and treatment of this type of effluent may prove problematic. For RAS to emerge as a viable culture strategy, an economical and efficient method must be developed to mitigate the high concentrations of nutrients in the effluent (Boyd et al., 1998).

Wastewater treatment strategies have been implemented as one solution for reducing effluent nutrients, but they can be expensive for application and can be inappropriate in most aquaculture settings (Hopkins et al., 1993). Traditional methods of wastewater treatment include sedimentation, mechanical and biological filtration (Warrer-Hansen, 1982; Pillay, 1992). The selection of wastewater treatment strategy is determined by considering the aquaculture method, type and amount of effluent produced, and particle size.

One economical treatment incorporated into many traditional aquaculture systems is sedimentation. Sedimentation can occur in a designated pond or a constructed rectangular tank. The settling process involves the removal of suspended solids (consisting of mostly fecal matter

and uneaten feed) which are denser than the water in which they reside. Sedimentation can help reduce the effluent's solid load and can reduce a significant proportion of the phosphorus (30-84%), if it is bound to particulates (Bergheim et al. 1993).

The effectiveness of sedimentation can be highly variable and depends on the design, available surface area for settling and the flow or retention time of the effluent. Particulate size of the solids must also be considered, as smaller, less dense particles require lower current velocities for sedimentation than larger, denser particles (Pillay, 1992). Sedimentation works best in flow-through systems and pond culture because the effluent contains low concentrations of pollutants in large volumes of water. However, this can also present an environmental challenge because retaining the high volume of effluent for long enough to allow settling can occupy a large area of the farm. For example, a pond with a water use of $1200m^3/h$ would require an additional pond area of $500m^2$ designated for sedimentation in order to achieve the best results (Mantle, 1982). Retention time in sediment basins varies across culture settings and can significantly impact efficiency. In Asia, stagnant and semi-stagnant pond farms do not exchange their volume very often and thus allow a long retention time for effluent solids to settle (about one year or more), to the pond bottom. Then, after harvesting fish, the waste laden pond bottom sediments can be removed or reused as fertilizer for land crops. The sediment can also be dried and converted to fertilizer that can be placed back into the same pond when it is being prepared for the next crop (Landau, 1992). Alternatively, after draining, the remaining solid waste could be hauled to a landfill, but removal via a commercial hauler can cost $80/load, taking away from the overall profit of the facility (Yates et al., 2004).

Other effluent retention times may not be as long. For example, in Europe, a flow-through intensive culture pond can have a settling period range from 20-720 minutes, depending on the water flow (Alabaster, 1982). To increase efficiency, farms can reduce particle fragmentation, as larger particles tend to be heavier and settle out quicker. A salmonid hatchery wastewater treatment study found based on the waste particle size, it was necessary to require a period in excess of 3 days to remove over 60% of the suspended solids (Liao, 1970). Settling efficiency can be compromised if influent solids concentration is <10 mg/L because of resuspension (Henderson & Bromage, 1988).

Mechanical filtration of particulates is another way to treat effluent discharge. In its simplest form, this method uses a stationary filtering mesh to remove particulate matter. This method is used mostly (though not exclusively) with recirculating land based aquaculture since effluents in open water are usually very dilute and particulates can settle out of the water column. One commercially available filter, equipped with different size mesh on separate plates for sorting solids by filtering or transporting, has been recently studied. Results estimated phosphorus reduction to be somewhere between 40-80% because this nutrient is often bound to particles (Mäkinen et al. 1988). Major drawbacks to this method are clogging of the filter, high maintenance costs, and minimal impacts on dissolved nutrients.

Swirl concentrators (or sludge collectors) are another means of mechanical filtration. They are circular tanks with tangentially-directed water flow, which work to draw solids to the bottom of the tank. These are also generally seen in land-based farms that reuse water, such as RAS, because they are efficient at removing high particle loads from water to be returned to tanks.

One study found a swirl concentrator can reduce phosphorus loading from 60 to 10% (Mäkinen et al. 1988). When used in recirculating systems, swirl concentrators are commonly used in combination with other filtering mechanics such as a rotating screen (for smaller particle removal) and/or a foam fractionator which produces foam to create surface area, to remove even finer particles before reuse or release. However, larger particles remain in the swirl concentrator, creating a sludge effluent that is usually released into the environment. Similar mechanical filtration devices have been used to capture solid waste under cages in freshwater fish culture in open waters. These are effective, however, they were permanently fixed and restricted water flow through cages. Maintenance also proved to be somewhat difficult and stressed the fish (Pillay, 1992). Their use in marine waters is likely to be more difficult because of the corrosive nature of saltwater, fouling organisms, and tidal pressure. Hence, mechanical filtration is most efficient in removing large particles in reuse systems, but equipment can be costly and the remaining large particle sludge still poses a treatment problem.

One possible solution to marine wastewater management in RAS is an integrated aquaculture system. Integrated aquaculture systems link the cultivation of aquatic animals with additional farming systems to simultaneously reduce effluent nutrients while generating a second (or third) product. This is achieved through concurrent or sequential linkages, between two or more systems, where the waste nutrients from the first system, serves as food for the second. Integrated aquaculture can help to better utilize primary productivity and reduce nutrient discharges (Dunstan and Tenore, 1972). Several integrated systems have been shown to substantially reduce nutrient concentrations in some types of effluent from aquaculture. Jones et al. (2001) experimented with an integrated system to see if nutrient levels in shrimp effluent

from an open pond aquaculture system could be reduced by macroalgae and oysters. The effluent from the shrimp ponds was directed through tanks containing Sidney rock oysters (*Saccostrea commercialis*) and then through macroalgal cultures of *Gracilaria edulis.* This study showed that the end result of the integration of these "biofilters" (oyster and algal cultures) reduced TP by 14%, total Kjeldahl nitrogen (TKN) by 28% and TSS by 12% (170 mg/L reduced to 20 mg/L) (Jones et al., 2001). In 2003, Schuenhoff et al. studied the reduction of nutrients in an integrated biofilter system in a semi-recirculating system (50% of effluent recirculated). Biofilters included abalone (*Haliotis discus hannai*), sea urchin (*Paracentrotus lividus*), and seaweed (*Ulva lactuca*), and were used to reduce high nutrients in effluent produced by a sea bream (*Sparus aurata*) culture. They reported a 70% reduction of total ammonia nitrogen and 20% of available phosphate (Schuenhoff et al., 2003).

Such experiments using an algae component in an integrated system have used macroalgae as a final treatment. The macroalgae absorbs dissolved organic and inorganic nutrients remaining after effluent has been treated by the other components. Polyculture using various macroalgae, such as *Ulva* and *Gracilaria*, have effectively reduced such nutrients (Schuenhoff et al., 2003; Jones et al., 2001). Aquatic macrophytes such as water hyacinth and duckweeds have also been grown in discharge waters for nutrient removal, but the fast reproduction and dispersal of such plants make this type of treatment difficult to control and they cannot tolerate full strength seawater.

An alternative approach may be the incorporation of microalgae culture as an intermediate step. This may be beneficial to nutrient removal if the microalgae can package the nutrients

before exposure to another crop such as filter feeders. It is well-established that microalgae utilize dissolved nutrients (Hoff and Snell, 1987) and thus may be able to convert effluent nutrients into a form that is more accessible to filter feeders. This packaging could increase efficiency of the integrated system in removing dissolved nutrients. Truesdale (personal communication) observed TP and TN reductions in a large outdoor culture of natural microalgae grown in RAS effluent. By day 5, TP was reduced from 34 to 20 mg/L and TN was reduced from 157 to 80 mg/L suggesting that microalgae can reduce dissolved nutrients in effluent. Laboratory trials (Truesdale in prep) demonstrate that microalgae such as *Isochrysis galbana* and *Nannochloris sp*. are good candidates, for culture in effluent and have the advantage of being commonly used for bivalve feed (Hoff and Snell, 1987).

Bivalves are an ideal selection as the second stage of a microalgal/filter feeder integrated system because of their known efficiency of filtering plankton. It has been estimated that one mussel is capable of filtering between 2-5 liters of water/day and a raft of mussels can filter 70 million liters a day (Figueras, 1989). Oysters are also well known for their filtering efficiency of small organic rich particles, such as bacteria and very small phytoplankton (Gosling, 2003). Oysters can filter particles as small as 1 μm although their efficiency in removing smaller particles (1-3 μm) is substantially lower than for larger particles (Haven and Morales-Alamo, 1970). Oysters preferentially ingest organic material, reject inorganic material and preferentially ingest N-rich over C-rich particles (Newell and Jordan, 1983). Oysters can also take up dissolved organic and inorganic nutrients and (DOM) such as dissolved amino acids (Manahan, 1983; Dame, 1996). In particular, oysters can assimilate phosphate directly from the water column for carbohydrate metabolism, energy transfer and shell deposition (Newell and Jordan, 1983).

Retention percentages of plankton and detritus from filter feeding are estimated to be 35% - 40%. Pfeiffer and Rusch (2000) examined the feasibility of using a microalgae/clam integrated system. A continuous harvest of the microalgae (*Chaetoceros muelleri*) was grown using adjacent brackish river water as media, and was fed to a *Mercenaria sp.* clam culture. They reported higher seed clam growth rates than more traditional facilities are able to achieve.

Incorporating oysters in an integrated system has been shown to remove particles from effluent water. Lefebvre's 2000 experiment studied the feeding responses of the Pacific oyster (*Crassostrea gigas*) during exposure to a land-based fish farm effluent composed of uneaten feed, feces and phytoplankton flourishing from the culture's dissolved nutrients. He showed that a culture of Pacific oyster was able to remove particles (4-5 um size) at efficiency close to 100% (Lefebvre et al, 2000), demonstrating that bivalves could be practical animals for aquaculture water treatment. However, high cell densities and solid concentrations can inhibit filtering efficiencies. Clam broodstock typically consume 1-3 x 10^9 algal cells/clam/day, but an algal density of 750,000 cells/ml should not be exceeded since it actually inhibits feeding (Hadley et al., 1997). Feeding high cell densities to clams may cause them to reject available nutrients by producing copious, pseudofeces composed of algae. With this consideration, initial feeding should be about 100,000 cells/ml followed by lower densities of algae slowly added over a 24-hr period (Hoff and Snell, 1987). Increased concentrations of *Isochrysis galbana* (1.2 to 12 cells/µl) have been observed to decrease filtration in juvenile scallops, *Argopecten irradians*, by 56% and by 85% when the microalgae *Thalassiosira weissflogii* was used (Bricelj and Kuenster, 1989). Pseudofaeces production rose rapidly in the mussel, *Mytilus edulis*, when algae concentration increased from 50-100 cells/ µl (Gosling, 2003). However, it has been reported

that the clam, *Venerupis pullastra*, is able to decrease its filtration rate at elevated particle concentrations, therefore producing less pseudofeces, while maintaining a fairly consistent ingestion rate (Foster-Smith, 1975).

Based on the observed results with open and semi-open systems, the integration of bivalve culture in a RAS for the mitigation of effluent and removal of effluent particles may be a practical, economical, and an efficient means of reducing environmental impacts of aquaculture effluent. The addition of an intermediate step involving the cultivation of microalgae (hence packaging nutrients in algal cells) may increase nutrient reductions by bivalve filtration. However, such systems have yet to be evaluated, particularly in conjunction with RAS effluent.

In addition to the improvement in water quality discharged from RAS, a marine fish-microalgae-shellfish integrated system could increase the farm's overall profitability by producing additional crops in the form of bivalves which could be sold for consumption or to other facilities (Jones et al., 2001; van Rijn, 1996; Hardy, 1999). It is estimated that 50% of all harvested bivalves, worldwide, originate in hatcheries (Duerr et al., 1998). In 2002, worldwide aquaculture produced 12 million tons of bivalves (FAO, 2004). Oysters and clams are very popular in North Carolina, where in 2005 they made up one fourth of total landings for shellfish, with a combined value of 1 million dollars (NCDENR, 2006). Recently, there has been an increased demand for shellfish for consumption and for stocking in natural waters to improve water quality. The ability for the capture fishery to supply bivalves is unlikely to increase significantly from traditional fisheries because most natural stocks are being harvested to near maximum rates (Helm et al., 2004). This demand has put the pressure on seed production in

hatcheries. Future increases in bivalve production will require this increased seed supply to be inexpensive and reliable. Seed production in hatcheries is particularly crucial for bivalves like the hard clam because, unlike most bivalves, large quantities of their seed can not be easily harvested from nature (Helm et al., 2004). This has caused hard clam farmers to rely entirely on hatcheries for seed.

Hatcheries that succeed in producing large numbers of larvae for seed will need to have consistent and plentiful microalgae for bivalve feed until they reach a suitable size for stock in growout facilities. In the past this has been problematic (Duerr et al., 1998). The use of nutrient laden effluent could accelerate algae growth rates during this "nursery" culture stage allowing cultured bivalves to reach larger sizes quicker, and thus reducing the duration of this most expensive culture phase. Recycling concentrated, nutrient-rich effluent water for microalgae production to support bivalve culture could save facilities an average of 30% of operating costs (Lavens, 1996). For the continued growth of the shellfish aquaculture industry, economical methods need to be developed to support large-scale production of seed. An integrated system could allow facilities to accomplish this inexpensively while being environmentally friendly.

This study implements a pilot integrated culture system to evaluate the effectiveness of a microalgae/bivalve biofilter to reduce effluent nutrient concentrations of RAS effluent and support bivalve growth. It will also evaluate the effect of bivalve density on the efficiency of the biofilter.

METHODS

Integrated System

The RAS system (Figure 2) utilized two 11.3m^3 production tanks stocked with southern flounder (*Paralichthys lethostigma*) at UNCW Aquaculture Facility in Wrightsville Beach, NC. A sump was used for the collection of enriched effluent (average volume; March = 1068 L/day, April = 1402 L/day) and was connected to three 2000 L bioreactors used for effluent storage or algae culture (depending on trial). One bioreactor was gravity-fed to one 1000 L bivalve upwelling tank (Figure 2) containing 5 upwellers (45.7 cm tall, 0.3 m in diameter). Supplemental seawater was added to move nutrients through the upwelling system. Flow rates were based on those used at local commercial hatcheries. Two upwellers were stocked with a low bivalve density (Low 1 and Low 2), two with a high bivalve density (High 1 and High 2), and one remained empty for a control. Change in shell height was assessed by comparing the average height (mm) of bivalves at the beginning and end of each trial. An F-Test was used to compare oyster shell heights, while a Welch-ANOVA Test was used to compare clam shell heights. The statistical software JMP (Cary, NC) was utilized for shell height analysis.

Algae Culture

A 24-hr effluent collection (average = 1270 L) was pumped to one 2000 L bioreactor and inoculated with the golden-brown microalgae, *Isochrysis galbana* and allowed to bloom up over a five day period (Figure 1). Inoculation of each of the bioreactors was staggered, in order to provide a steady supply of algae. Bioreactors were then drained into the upwelling system at a rate that delivered 85% of the bioreactor volume over 48 hours.

Water Quality

The impact of bivalve filter feeding was evaluated by comparing the characteristics of the effluent composition before and after bivalve filtration. Effluent nutrient composition (total nitrogen (TN), total phosphorus (TP), and total suspended solids (TSS) was determined from triplicate 15-ml samples collected from immediately below (1) and from the outflow (2) of each upweller (Figure 2). Time between before and after samples was about one minute. All nutrient samples were analyzed by a Bran+Luebbe Continuous Flow Autoanalyzer III (CFA III) using standard methods (Bran+Luebbe, 2001). The nutrient concentrations estimated from the control upweller ("before" and "after" passing through upweller) were used to establish background levels. These were subtracted from density treatment nutrient estimates (from Low 1, Low 2, High 1, High 2) for "before" and "after" bivalve filtration, to yield density treatment effect means [(density treatment "before" – "after") – (control "before" – "after") = density treatment effect] . These means were tested using the null hypothesis that they did not differ significantly from zero (α=0.05) (Wilcoxon-Mann-Whitney test as implemented in the statistical software JMP Cary, NC).

Nutrient concentrations were set to a known standard on the Bran+Luebbe before each sample run. Concentrations of unfiltered TN and TP samples were analyzed by taking a totally automated measurement of total dissolved nitrogen (TDN) and total dissolved phosphate (TDP). This technique is based on the original CFA III method (G-219-98 Rev. 4) (Bran+Luebbe, 2001) and modified by including the application for simultaneous determination of total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) for a single sample (Dafner and Szmant, personal communication) and incorporating the oxidation reagent described by Valderrama

(1981). $PO^3_4^-$, NH_4^+, NO_3^-, and NO_2^- were analyzed for total constituents according to EPA marine water protocols (EPA, 1997). Orthophosphate was analyzed by an automated colorimetric method according to EPA method 365.5, which uses ammonium molybdate and antimony potassium tartrate reactions (EPA, 1997). Nitrate and nitrite was analyzed using an automated gas segmented continuous flow colorimetric method, using EPA marine water protocols (EPA method 353.4) (EPA, 1997). Nitrate and nitrite was determined by passing the sample through a copper-coated cadmium reduction column. Nitrate in the sample was reduced to nitrite in a buffer solution. TSS reduction of bivalves was assessed by filtering 5-ml of each water sample through a 0.20 μm, pre-dried Whatman filter paper. Weighing the sample-dried filter paper and subtracting the initial filter paper weight provided an estimate of TSS (Grasshoff et al., 2002). A Fisher Scientific Isotemp Oven 516G was used to dry filter papers for 24-hr at 105º C. Two trials were conducted to evaluate bivalve reduction in RAS effluent.

Trial I Conditions

The first trial was conducted from March to April of 2005. Average temperature was 15ºC (range: 13-19) and average salinity was 34 (range: 31-35). Full strength effluent was used as the nutrient source (1068 L) and was introduced (0.24 L/min) into a flow through (5.9 L/min) upweller system vault (1000 L) (Figure 2) stocked with oysters (*Crassostrea virginica,* average shell height (N = 60) ± standard error = 63.4 ± 1.7 mm). Oysters were collected from the dock in the intercoastal waterway behind the Center for Marine Science, in Wilmington, NC. The low density treatment consisted of two upwellers (Low 1 and Low 2) stocked with 200 oysters each, the high density treatment (High 1 and High 2) stocked with 468 oysters each, and one empty upweller served as a control. At this time dead were removed and all upwellers were randomly

repositioned to minimize position effects. Water quality parameters were monitored twice for 4 weeks.

Trial II Conditions

Trial II was conducted from July to August, 2005. Average temperature was 30ºC (range: 28-32) and average salinity was 32 (range: 30-33). Trial II was very similar to the first trial with a few modifications. This time I inoculated the full strength effluent (average volume = 1402 L) in the bioreactors with a commercially produced culture of golden-brown microalgae, *Isochrysis galbana*, to achieve a cell density of 1 x 10^6 cells/ml. An average of 267 L of seawater was added to effluent to increase the head pressure of the bioreactor and to give the culture fresh media. The initial inoculated *Isochrysis* was out competed by a *Nannochloris* species (green microalgae) which took over as the dominant species a few weeks into the trial (combined average cell density: 5,300,000 cells/ml). As this microalgae is also a popular feed for bivalves in aquaculture settings (Hoff and Snell, 1987) I continued to use the algae to feed the bivalves. These cultures were maintained for 5 days before being fed at a rate of 0.49 L/min (for an average 85% harvest) to a flow through upweller system stocked with clams (*Mercenaria mercenaria*, average shell height (N = 200) ± standard error = 16.7 ± 0.2 mm). Supplemental seawater was pumped to the upwelling system at a rate of 12.6 L/min**.** Each upwelling system consisted of two upwellers stocked at low density (Low 1 and Low 2) (N = 1463), two upwellers stocked at high density (High 1 and High 2) (N = 2945) and one empty upweller served as a control. The upwelling system was disassembled and sprayed thoroughly along with bivalves, with freshwater once a week. At this time dead were removed and all upwellers were randomly

repositioned to minimize position effects. Water quality parameters were monitored twice a week for four weeks.

OYSTER / EFFLUENT RESULTS

Average TP concentration in the effluent entering the upwelling vault was diluted by a factor of about 12 (2.94 to 0.242 mg/L) due to the addition of raw seawater needed to maintain the flow-through system. Average TN concentration was diluted by a factor of 13 (14.7 to 1.14 mg/L) (Table 3). However, it is interesting to note that average effluent TSS concentration was minimally effected by the dilution (5%: 1460 to 1380 mg/L) because of the supplemental raw seawater input (Table 2).

The cultivation of oysters had no significant effect on the nutrient composition of the diluted raw effluent (Table 2) (Wilcoxon-Mann-Whitney Test, $p>0.05$). On average, after adjusting for the control (Figure 4 and 6) the low density of oysters reduced TP and TN minutely (0.04 and 0.01 mg/L, respectively), while the high density seemed to add small amounts of TP and TN to the upwelling effluent (Table 2). Both densities added TSS to the upwelling effluent (Figure 8) with the low density adding almost 3 times that of the high density (Table 2). Over time, TP, TN, and TSS reductions and additions by oysters were irregular (Figure 3, 5, and 7).

The average initial shell height (± standard error [se]) in the low density was 63.3 ± 2.7 mm (N = 30) and the final shell height was 57.7 ± 2.8 mm (N = 30). The initial shell height (± se) in the high density was 63.4 ± 2.2 mm (N = 30), while the final shell height was 57.0 ± 2.8 mm (N = 30). Neither treatment, low or high density, exhibited a significant change in shell height

(mm) within (F-Test, $p>0.05$) or between densities ($p>0.05$) over the 4 weeks of the trial (Table 2). Mortality was evenly distributed over the course of the trial with the exception of Low 1 density where 1/3 of the mortality occurred during the first half of the trial, and 2/3 occurred during the latter half. An average of 16 oysters died in the low density (8% mortality) [Low 1: 18; Low 2: 13] while an average of 47 oysters died in the high density (10% mortality) [High 1:43; High 2: 50]. The average length of the dead was roughly the same for both densities (Low: 44.6 ± 4.3 mm (N = 16); High: 44.6 ± 2.1 mm (N = 47)).

CLAM / ALGAE RESULTS

Average TP and TN concentrations in the effluent grown algae entering the upwelling vault were diluted by a factor of 16 (1.61 to 0.099 mg/L) and 18 (11.3 to 0.638 mg/L), respectively, because of the addition of raw seawater to the inflow (Table 5). However, it is interesting to note that average TSS concentrations in the algae culture were not diluted at all by the addition of raw seawater upon entering the upwelling system, but the diluted effluent was 7% higher in TSS (1620 to 1742 mg/L) compared to the average found in the algae culture (Table 5).

The cultivation of clams had no significant effect on the nutrient composition of the effluent/algae mixture (Table 4) (Wilcoxon-Mann-Whitney Test, $p>0.05$). On average, after adjusting for the control (Figure 10 and 12) the low and high densities of clams increased the concentrations of TP and TN to the water exiting the upweller (Table 4). However, after adjusting for the control (Figure 14) TSS was reduced in both the low and high clam densities, with the high density being only slightly more effective (63.9 and 76.7 mg/L, respectively),

hough not significantly different (Table 4). Over time, there was not a regular pattern of nutrient fluxes in clam upwellers (Figure 9, 11, and 13).

The average initial shell height (± se) in the low density was 17.1 ± 0.3 mm (N = 100) and the average final shell height (± se) was 16.3 ± 0.2 mm (N = 200). The average initial shell height (± se) in the high density was 16.3 ± 0.3 mm (N = 100), while the average final shell height (± se) was 16.9 ± 0.2 mm (N = 200) (Table 4). Statistically, change in shell height within each density was found to be significantly different from 0 (Welch-ANOVA Test; Low: $p=0.01$, High: $p=0.049$), and significantly different between densities (Welch-ANOVA Test, $p=0.01$). The mortality was distributed evenly over the time course of the trial. An average of 40 clams died in the low density (2% mortality) [Low 1: 39; Low 2: 49] while an average of 202 clams died in the high density (6% mortality) [High 1: 179; High 2: 224]. The average shell height (± se) of the dead was roughly the same for both densities (Low: 16.4 ± 0.6 mm (N = 30); High: 16.2 ± 0.5 (N = 30)).

DISCUSSION

The effluent composition reduction differences were not found to be significantly different from zero in either trial (Wilcoxon-Whitney Test, $p>0.05$). The results of Trial I suggest that the oysters were ineffective in altering the nutrient composition of the effluent enriched source water that flowed through the upwellers and Trial II suggest that the clams were ineffective in altering the nutrient composition of effluent enriched source water even though the nutrients were "packaged" in microalgae.

Several factors may have contributed to my inability to detect an effect. One possibility is that the bivalves did not have enough time to effectively reduce nutrients in the effluent because flow rates were too high. The upwelling vault design and flow rates were modeled after upwelling systems found at local commercial facilities, which use high flow rates to maximize delivery of wild phytoplankton to support rapid growth. In order to achieve an optimal nutrient reduction of effluent and effluent-algae by bivalves, a slower flow rate may be needed. Most studies, that have demonstrated bivalve mediated nutrient reductions, do not use a flow-though design, but rather allow the bivalves to be in contact with the nutrients in a static system for a period longer time. This design has proven to work for observing nutrient reductions, however, does not allow for the removal of wastes and would be cumbersome to implement (increased time and labor in water exchanges) in a commercial hatchery for the purpose of mitigating effluent. The relationship of water flow and shell deposition is not fully understood and needs further study.

Another factor that may have contributed to my inability to detect an effect is the magnitude of the dilution of the effluent or effluent-algae upon introduction into the upwelling system. The phosphorus and nitrogen nutrient concentrations at input into the upwelling system (Table 3 and 5) are within the ranges reported for the source water (Table 1) suggesting that the effluent additions were ineffective in raising these nutrient concentrations above background. Algal concentrations (average: 204,000 cells/ml) were within limits for bivalve culture feeding (100,000 - 750,000 cells/ml) (Hoff and Snell, 1987; Hadley et al., 1997), however, no significant reduction in nutrients or TSS was observed. Higher cell densities may be needed in order to see an impact with respect to these reductions. Nutrient reductions have been observed in similar

nutrient ranges used in my two trials; however these studies observed bivalves with longer residence times with nutrient waters (Jones et al., 2001; Asmus et al., 1995). I should also point out that the relative concentration before and after exposure to the bivalves was highly variable across time and that variability may have inhibited my detection of an overall reduction effect.

The results of this study are not in keeping with Jones et al. (2001) who researched the effect of oyster biofilters on shrimp effluent (10 L) over a 24-hr period and found a 37% TP reduction (0.30 mg/L to 0.19 mg/L) in oyster treatments, when compared with a 30% addition in the control (0.30 mg/L to 0.39 mg/L). While our average TP concentrations before oyster filtration in trial I (0.267 mg/L) (Table 3) were similar to Jones et al. (0.30 mg/L), no significant reductions were seen.

Jones et al. (2001) also reported an average estimate of TN reduction of 10% (3.14 mg/L to 2.83 mg/L) in static oyster treatments compared with a 32% addition (3.14 mg/L to 4.15 mg/L) of TN in the control after 24 hours of exposure to 10 L of shrimp effluent. This is not in keeping with this study; however, the research of Jones et al. (2001) used a static setting with only 16 oysters compared with our flow-through system containing 200 oysters in the low density replicate and 468 in the high density replicates.

It is possible that concentrations of phosphorus and nitrogen may have been bound to the effluent's unsettleable particles (<5 um) and were too small to be effectively removed by the oysters in a flow through system (Cripps, 1995). Effluent particle size of 2-4 μm have been reported to be effectively reduced in oyster treatments, however, most of the particles were clay

and had a residence time of 24 hours (static system) with oysters (Jones et al., 2001). In my trials, there were also extremely high TSS concentrations of small particles in the supplemental seawater (90 times that of natural waters estimates) (Table 3) that may have been caused by resuspension at the pump in Bank's Channel before delivery to my system. If these particles were oligotrophic, this may help explain low nutrient impact by oyster treatments on the upwelling effluent dilution, but increased TSS exiting the system (Table 2).

Despite the fact nutrient concentrations were generously diluted, the TSS average concentrations within the vault were hardly diluted, if at all. In trial I, the average effluent TSS concentration upon entering the oyster upwelling system was only diluted by 5% (1460 mg/L to 1380 mg/L) (Table 3). This is due to the fact that the average TSS concentration in the supplemental seawater was extremely high (Table 3). Over time, there was no regular trend in TSS reductions (Fig. 7). Averaging all reductions, though variable, the low and high densities reduced TSS by 2% and 5%, respectively, while the control reduced 9% (Figure 8). It is suspected that these very high concentrations of TSS, consisting of mostly small particulates (< 3 μm), somewhat inhibited the filtering efficiency of the oysters. Loosanoff and Tommers (1948) observed a concentration of 100 mg/L reduced the pumping rate of oysters by up to 87% while Barille et al. (1997) found a seston concentration above 90 mg/L dramatically decreased oyster filtration.

While effluent particle size was not measured before being pumped over for bioreactor storage, there is no doubt that resuspension and the force needed to transport effluent across facility grounds broke down large effluent particles to multiple small suspended solids in both

trials. Microscope observations suggest that the particulate size in the integrated system ranged from 1.5 – 32 µm, where an estimated 90% were < 5 µm (Myers, unpublished data). A decrease in particle size leads to finer particles which settle slowly and are likely to stay in suspension, adding to the high TSS concentrations (Pillay, 1992). Haven and Morales-Alamo (1970) reported that *C. virginica* had a retention efficiency of less than 50% for particles smaller than 3 µm when compared with particles larger than 7 µm. Similarly, it has been reported for *C. virginica* that as algal concentrations increase, retention efficiencies for small particles decrease (Palmer and Williams, 1980). This combined with the possibility of oyster energy being put into spawning during the spring months, may help explain decreased clearance rates (Gosling, 2003). It is important to note, these small (< 3 µm) suspended particles may be of some nutritional value to the oysters because of the abundance of this size particle found in estuarine waters (Haven and Morales-Alamo, 1970). The clearance rate of *C. virginica* is quite variable and depends on many factors such as temperature, salinity, and particle concentration (Gosling, 2003).

There was no deposition of shell by the end of trial I (Table 2). Even though average shell height of oysters that died in both treatments were about the same (44.6 ± 4.3 mm (N = 16) and 44.6 ± 2.1 mm (N = 47), respectively) stocked oysters were not of the same cohort, so weight and height varied. The average estimates of final shell height, however, were smaller than the initial estimates. Measuring mortalities determined that high and small extremes shell heights were lost from the upwellers, yielding large standard errors (L: 44.6 ± 4.3 mm; H: 44.6 ± 2.1 mm) and skewing change in shell height results (yielded a negative increase in shell height). This is a result of selective mortality. Change in shell height rates are not best represented in this four week trial. Future integrated studies focusing on a positive change in bivalve shell height

may want to extend trial length during non-spawning months to increase the chances of seeing significant changes.

In trial II, the average final shell height in the low density was smaller than the initial, and larger in the high density. Again these differences may have been caused by selective mortality. The clams were so close in size throughout the trial, even a small variation in average shell height, could have caused the calculated significant differences in change in shell height (Within each treatment: Welch-ANOVA Test, Low: $p=0.01$, High: $p=0.049$), and between densities (Welch-ANOVA Test, $p=0.01$).

CONCLUSIONS

Oysters in this flow-through integrated system were ineffective at mitigating RAS effluent composition and clams were ineffective at mitigating RAS effluent-grown algae composition. Future studies may consider utilizing lower TSS loads (which could include such methods as filtration of source water) and effluent particle size higher than 3 µm for increased bivalve filtration efficiency. In order to achieve larger particulates, attention should be given to transport method of effluent in order to minimize fragmentation of solids, and a larger celled-microalgae, may be used as an intermediate step. Future studies should include an upwelling design that minimizes dilutions. It is highly recommended that future studies also configure their systems (where possible) to increase residence time of nutrients (slow flow-through) with bivalves and extend trial periods longer than four weeks. Higher nutrient concentrations (above ambient levels) may also be needed to detect significant impacts by biofilters in a flow-through system.

Lastly, an additional step may be added after bivalve filtration, such as macroalgal biofiltration or a settlement tank, in order to further reduce nutrient composition before discharge.

The results of this research contribute to a better understanding of the design needs for how to utilize wastes from RAS systems in order to minimize the environmental impacts of commercial scale marine finfish culture, improve the grower's profitability, and be economical. Bivalve seed and resulting marketable adults have great value and could be produced inexpensively if integrated systems are used in nursery grow-out facilities. This study highlights the need for more research and development work to make an integrated system sufficiently balanced to reduce optimal amounts of effluent components and remain economically viable. The increasing supply for seafood, including bivalves, will undoubtedly continue in the future and production will need to be increased to meet this demand. Expanding aquaculture technology in the U.S. will help supply seafood demand, improve global trade, and help conserve fish stocks.

	Effluent Elemental Concentrations		Natural Elemental Concentrations	
Effluent Parameter	mg/L	µM	mg/L	µM
Total Phosphorous	26 ± 1.6	838 ± 51.6	0.01 - 0.43	0.32 – 13.9
Total Nitrogen	108.8 ± 10.42	7771 ± 744.3	0.42 - 2.2	30 – 157.1
Total Suspended Solids	1391 ± 79.50	n/a	18.6	n/a

Table 1. RAS effluent composition based on characterizations of six separate 24 hour collections compared with ambient water (Bank's Channel, NC). RAS effluent concentrations provided by S. Truesdale (unpublished data UNCW, 2005) and ambient concentrations ranges provided by L. Cahoon, UNCW (2004).

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
I - BR1	**B**	→	→	→	**F**	**S**					
		I - BR2	**B**	→	→	→	**F**				
				I - BR3	**B**	→	→	→	**F**	**S**	**W**

Figure 1. Schedule of bioreactor maintenance for cultivation of algae (*I. galbana* and *Nannochloris sp.*) and sampling days for Trial II. (I= Inoculate algae; BR= Bioreactor; B= Bloom culture; F= Feed to clams; S= Sample; W=Without algae feed [to achieve a set weekly schedule]). Effluent collection began 24 hrs. before inoculation.

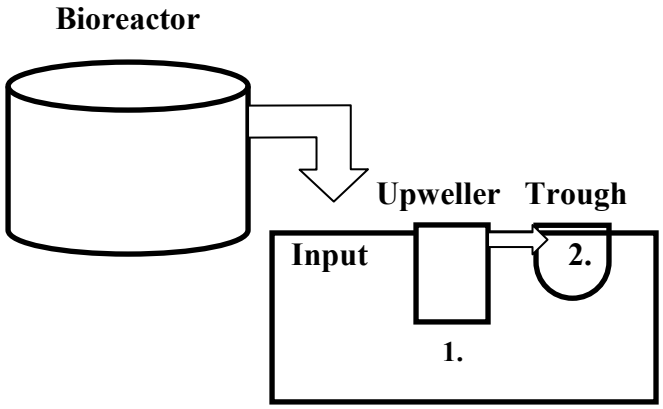

Figure 2. Location of water samples collected twice, weekly for length of trials. Samples taken: 1) "before" passing through upweller and 2) "after" passing through upweller.

Table 2. Average nutrient reductions from oyster culture for Trial I after adjustment for the control. Average shell height increase (mm) represents the average shell height of oysters at the beginning of the trial subtracted from the same estimate made at the end of the trial. Negative average shell height increase is the result of average final shell height being smaller than initial (selective mortality). Positive numbers indicate a nutrient reduction and negative numbers indicate a nutrient enrichment. SE=standard error. There were no significant differences within or between densities with respect to change in shell height (F-Test, $p>0.05$) or nutrient composition reduction (Wilcoxon-Mann-Whitney Test, $p>0.05$).

	Low Density		**High Density**	
	Mean	**SE**	**Mean**	**SE**
Average Shell Height Increase (mm)	-5.58	± 6.31	-6.56	± 1.31
Total Phosphorus Red. (mg/L)	0.04	± 0.02	-0.01	± 0.01
Total Nitrogen Red. (mg/L)	0.01	± 0.09	-0.01	± 0.09
Suspended Solids Red. (mg/L)	-139	± 134	-50.8	± 82.8

Table 3. Trial I: Parameter averages (mg/L) (± standard error) and ranges (mg/L) of effluent throughout the oyster integrated system in all treatments compared with parameter averages (mg/L) (± standard error) and ranges (mg/L) of bioreactor effluent and supplemental seawater. Input indicates at point of entry into bivalve vault. 1 and 2 refer to sampling "before" and "after" upweller (locations depicted in Figure 2). These averages represent eight sampling days.

TP (mg/L)	**Input**	**(1) "before"**	**(2) "after"**
Low	**0.246 ± 0.069** (0.050-0.568)	**0.291 ± 0.078** (0.046-0.577)	**0.247 ± 0.073** (0.043-0.573)
High	**0.245 ± 0.066** (0.052-0.566)	**0.261 ± 0.067** (0.044-0.556)	**0.263 ± 0.078** (0.045-0.646)
Control	**0.235 ± 0.061** (0.052-0.503)	**0.250 ± 0.068** (0.045-0.518)	**0.241 ± 0.067** (0.052-0.532)
Overall	**0.242 ± 0.004** (0.050-0.568)	**0.267 ± 0.012** (0.044-0.577)	**0.250 ± 0.007** (0.043-0.646)
TN (mg/L)			
Low	**1.16 ± 0.200** 0.389-1.94)	**1.25 ± 0.245** (0.363-2.30)	**1.06 ± 0.187** (0.339-1.82)
High	**1.11 ± 0.189** (0.427-1.94)	**1.19 ± 0.221** (0.405-2.02)	**1.07 ± 0.197** (0.360-1.87)
Control	**1.15 ± 0.197** (0.470-2.06)	**1.21 ± 0.219** (0.395-1.98)	**1.07 ± 0.190** (0.443-1.81)
Overall	**1.14 ± 0.014** (0.389-2.06)	**1.22 ± 0.018** (0.363-2.30)	**1.07 ± 0.006** (0.339-1.87)
TSS (mg/L)			
Low	**1310 ± 157** (433-1868)	**1496 ± 99.5** (1047-1884)	**1470 ± 147** (737-1848)
High	**1424 ± 138** (741-1815)	**1609 ± 130** (1025-1995)	**1495 ± 125** (944-1829)
Control	**1405 ± 184** (431-1874)	**1643 ± 118** (1033-1946)	**1478 ± 78.6** (1059-1809)
Overall	**1380 ± 35.0** (431-1874)	**1583 ± 44.5** (1025-1995)	**1481 ± 7.19** (737-1848)

	Bioreactor Effluent	**Supplemental Seawater**
TP (mg/L)	**2.94 ± 2.12** (0.682-5.79)	**0.084 ± 0.042** (0.026-0.135)
TN (mg/L)	**14.7 ± 1.97** (9.55-23.1)	**0.419 ± 0.028** (0.358-0.538)
TSS (mg/L)	**1460 ± 48.6** (967-1957)	**1665 ± 117** (601-2613)

Table 4. Average nutrient reductions from clam culture for Trial II after adjustment for the control. Average shell height increase (mm) represents the average shell height of clams at the beginning of the trial subtracted from the same estimate made at the end of the trial. Negative average shell height increase is the result of average final shell height being smaller than initial (selective mortality). Positive numbers indicate a nutrient reduction and negative numbers indicate a nutrient enrichment. SE=standard error. The high density exhibited a significant positive change in shell height within replicates (Welch-ANOVA Test, p=0.049) and was significantly different from the low density (Welch-ANOVA Test, p=0.01).

	Low Density		**High Density**	
Parameter	**Mean**	**SE**	**Mean**	**SE**
Average Shell Height Increase (mm)	-0.81	± 0.04	0.69	± 0.07
Total Phosphorus Red. (mg/L)	-0.001	± 0.003	-0.001	± 0.002
Total Nitrogen Red. (mg/L)	-0.01	± 0.06	-0.05	± 0.8
Suspended Solids Red. (mg/L)	63.9	± 71.2	76.7	± 33.4

Table 5. Trial II. Parameter averages (mg/L) (± standard error) and ranges (mg/L) of effluent grown algae throughout the clam integrated system in all treatments compared with parameter averages (mg/L) (± standard error) and ranges (mg/L) of bioreactor effluent grown algae and supplemental seawater. Input indicates at point of entry into the bivalve vault. 1 and 2 refer to sampling "before" and "after" upweller (locations depicted in Figure 2). These averages represent nine sampling days.

TP (mg/L)	**Input**	**(1) "before"**	**(2) "after"**
Low	**0.096 ± 0.016** (0.022-0.180)	**0.103 ± 0.017** (0.033-0.196)	**0.099 ± 0.018** (0.013-0.194)
High	**0.102 ± 0.017** (0.034-0.200)	**0.101 ± 0.018** (0.014-0.196)	**0.096 ± 0.017** (0.004-0.182)
Control	**0.098 ± 0.016** (0.030-0.182)	**0.103 ± 0.017** (0.028-0.194)	**0.097 ± 0.016** (0.018-0.180)
Overall	**0.099 ± 0.095** (0.022-0.200)	**0.102 ± 0.001** (0.014-0.196)	**0.097 ± 0.001** (0.004-0.194)
TN (mg/L)			
Low	**0.637 ± 0.091** (0.257-1.06)	**0.650 ± 0.087** (0.302-1.03)	**0.633 ± 0.094** (0.266-1.04)
High	**0.610 ± 0.102** (0.312-1.16)	**0.580 ± 0.109** (0.085-1.09)	**0.566 ± 0.086** (0.290-0.966)
Control	**0.668 ± 0.080** (0.316-0.99)	**0.634 ± 0.095** (0.187-1.01)	**0.574 ± 0.088** (0.262-0.993)
Overall	**0.638 ± 0.017** (0.257-1.16)	**0.621 ± 0.021** (0.085-1.09)	**0.591 ± 0.021** (0.262-1.04)
TSS (mg/L)			
Low	**1749 ± 76** (1362-2049)	**1805 ± 65** (1506-2007)	**1768 ± 85** (1429-2099)
High	**1746 ± 59** (1499-2010)	**1827 ± 67** (1585-2168)	**1777 ± 82** (1325-2157)
Control	**1731 ± 80** (1410-1993)	**1781 ± 74** (1448-2046)	**1808 ± 73** (1412-2059)
Overall	**1742 ± 5.3** (1362-2049)	**1804 ± 13** (1448-2168)	**1784 ± 12** (1325-2157)

	Bioreactor Effluent/Algae Mix	**Supplemental Seawater**
TP (mg/L)	**1.61 ± 0.218** (0.797-2.84)	**0.085 ± 0.015** (0.044-0.182)
TN (mg/L)	**11.3 ± 1.22** (7.18-17.0)	**0.597 ± 0.080** (0.398-1.09)
TSS (mg/L)	**1620 ± 102** (1081-2171)	**1765 ± 61.8** (1395-1995)

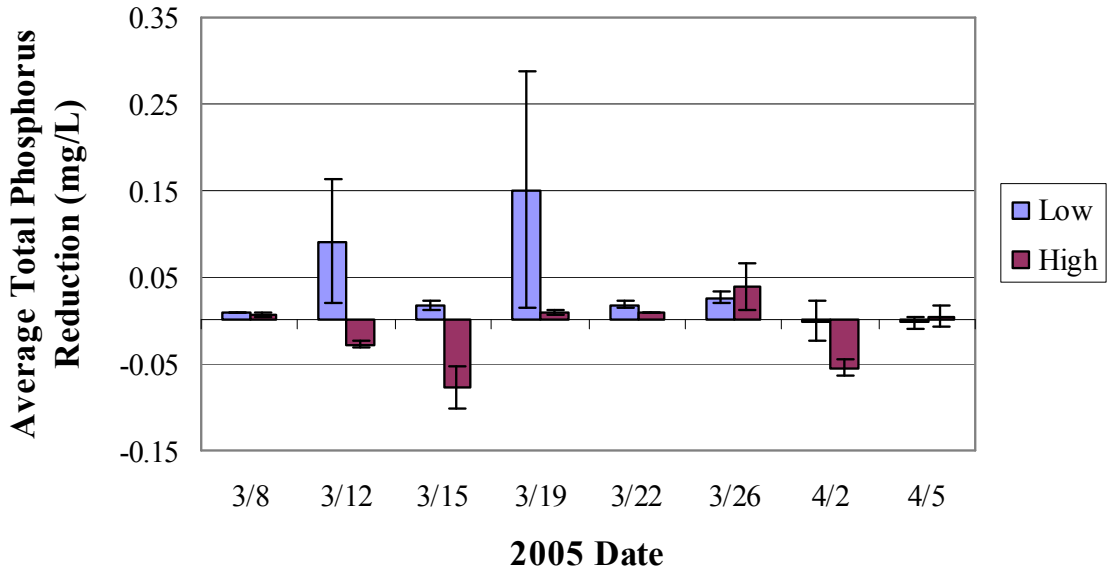

Figure 3. Average, total phosphorus reductions (mg/L) (N=6) by oyster treatments from below (1) and above (2) oysters (Figure 2). Positive averages indicate a nutrient reduction, while negative averages indicate a nutrient enrichment. Error bars indicate standard error.

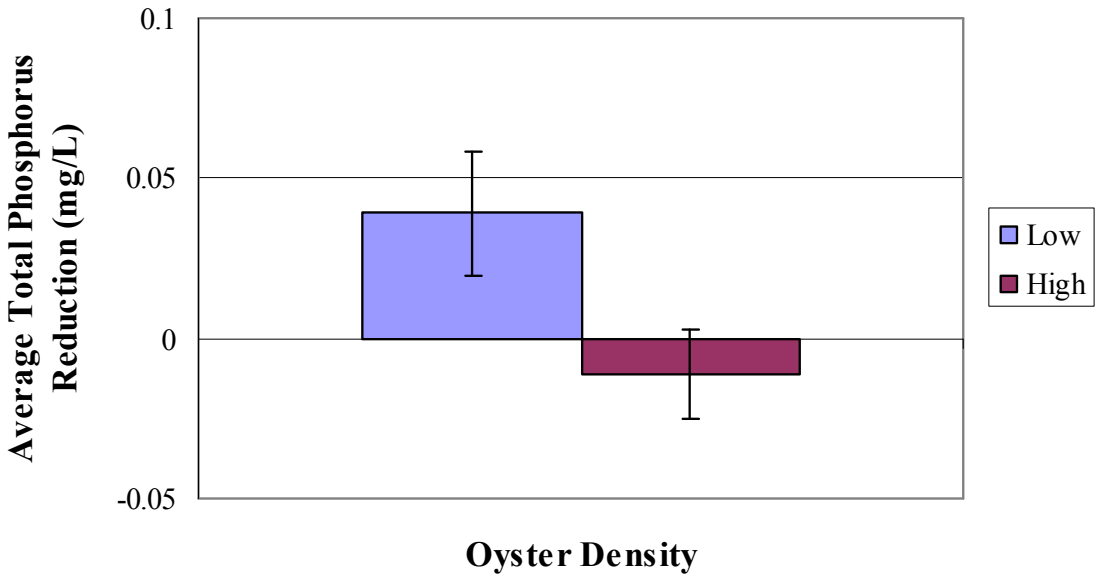

Figure 4. Overall average total phosphorus reductions (mg/L) by oyster treatments from below (1) and above (2) oysters (Figure 2). Positive averages indicate a nutrient reduction, while negative averages indicate a nutrient enrichment. Error bars indicate standard error. Data represents averages of eight sampling days.

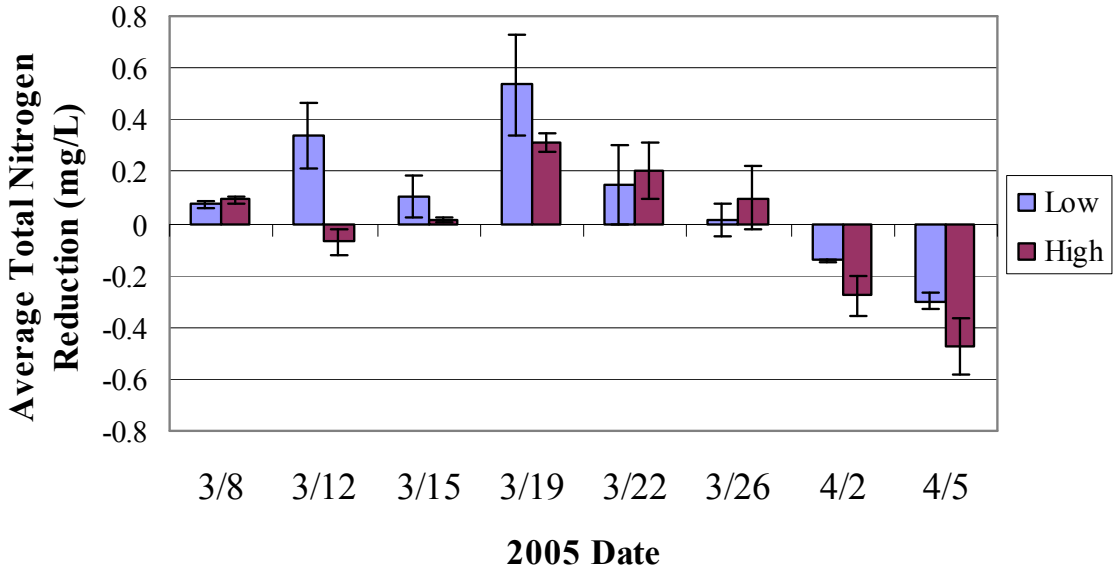

Figure 5. Average, total nitrogen reductions (mg/L) (N=6) by oyster treatments from below (1) and above (2) oysters (Figure 2). Positive averages indicate a nutrient reduction, while negative averages indicate a nutrient enrichment. Error bars indicate standard error.

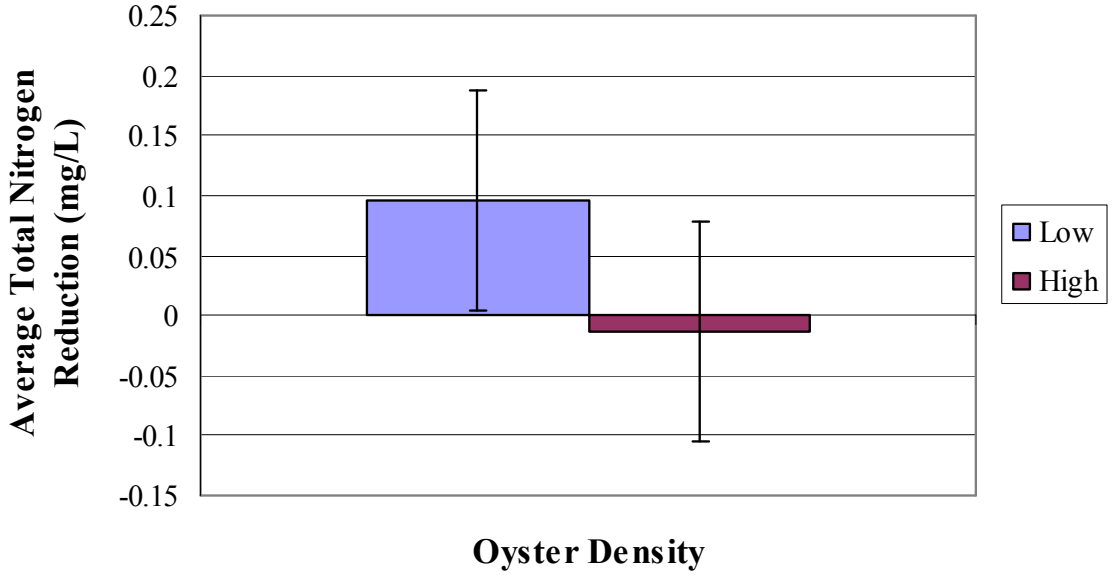

Figure 6. Overall average, total nitrogen reductions (mg/L) by oyster treatments from below (1) and above (2) oysters (Figure 2). Positive averages indicate a nutrient reduction, while negative averages indicate a nutrient enrichment. Error bars indicate standard error. Data represents averages of eight sampling days.

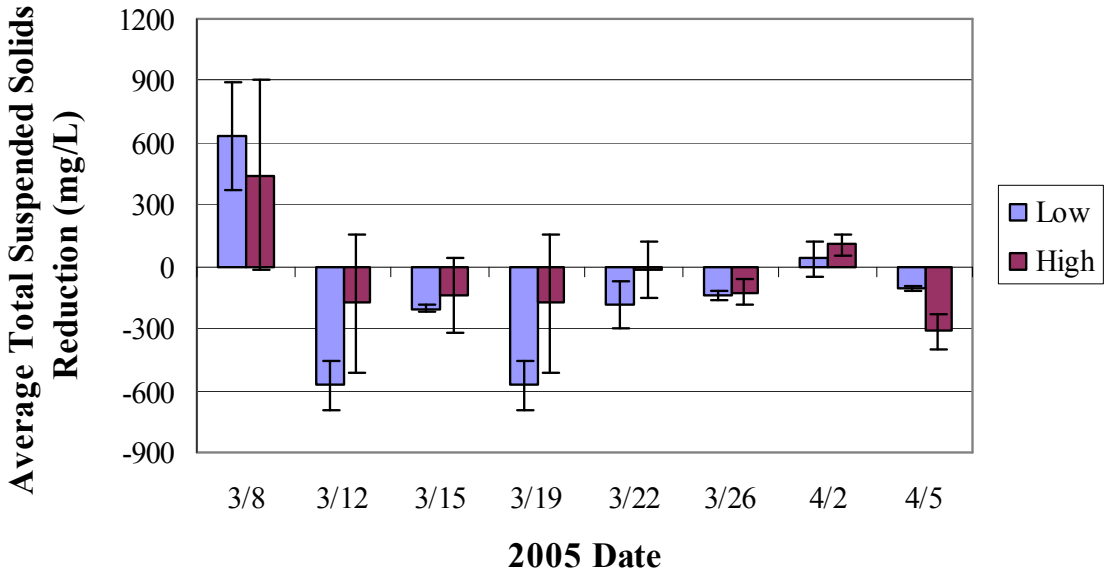

Figure 7. Average, total suspended solids reductions (mg/L) (N=6) by oyster treatments from below (1) and above (2) oysters (Figure 2). Positive averages indicate a nutrient reduction, while negative averages indicate a nutrient enrichment. Error bars indicate standard error.

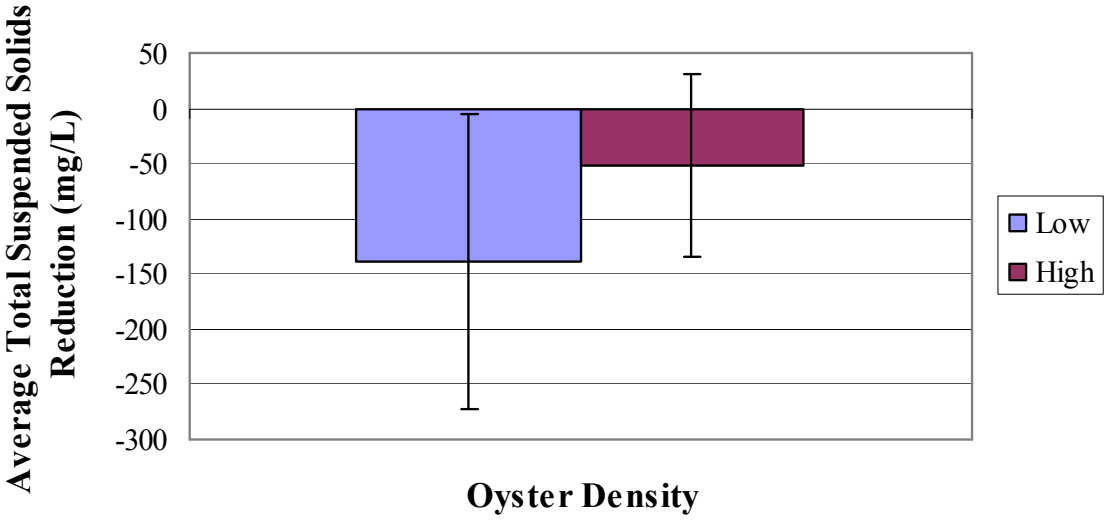

Figure 8. Overall average, total suspended solids reductions (mg/L) by oyster treatments from below (1) and above (2) oysters (Figure 2). Positive averages indicate a nutrient reduction, while negative averages indicate a nutrient enrichment. Error bars indicate standard error. Data represents averages of eight sampling days.

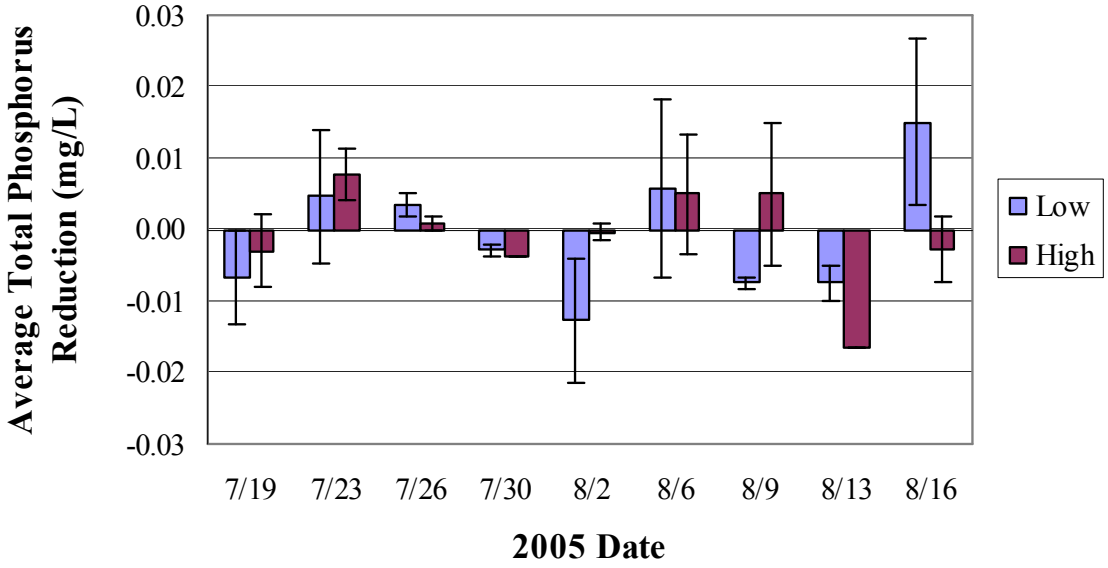

Figure 9. Overall average, total phosphorus reductions (mg/L) (N=6) by clam treatments from below (1) and above (2) clams (Figure 2). Positive averages indicate a nutrient reduction, while negative averages indicate a nutrient enrichment. Error bars indicate standard error.

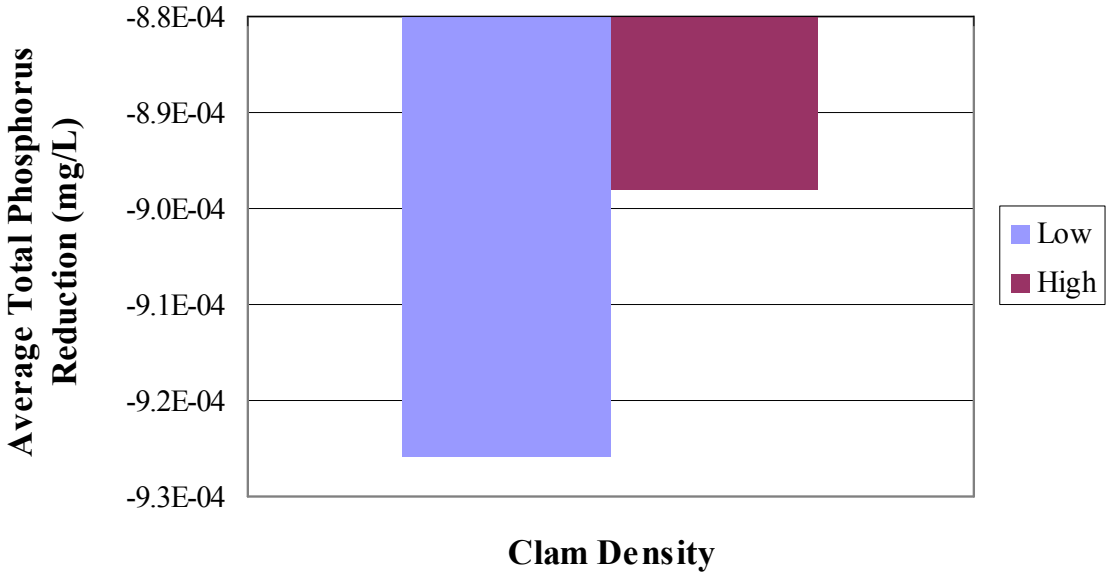

Figure 10. Overall average, total phosphorus reductions (mg/L) by clam treatments from below (1) and above (2) clams (Figure 2). Positive averages indicate a nutrient reduction, while negative averages indicate a nutrient enrichment. Error bars indicate standard error. Data represents averages of nine sampling days.

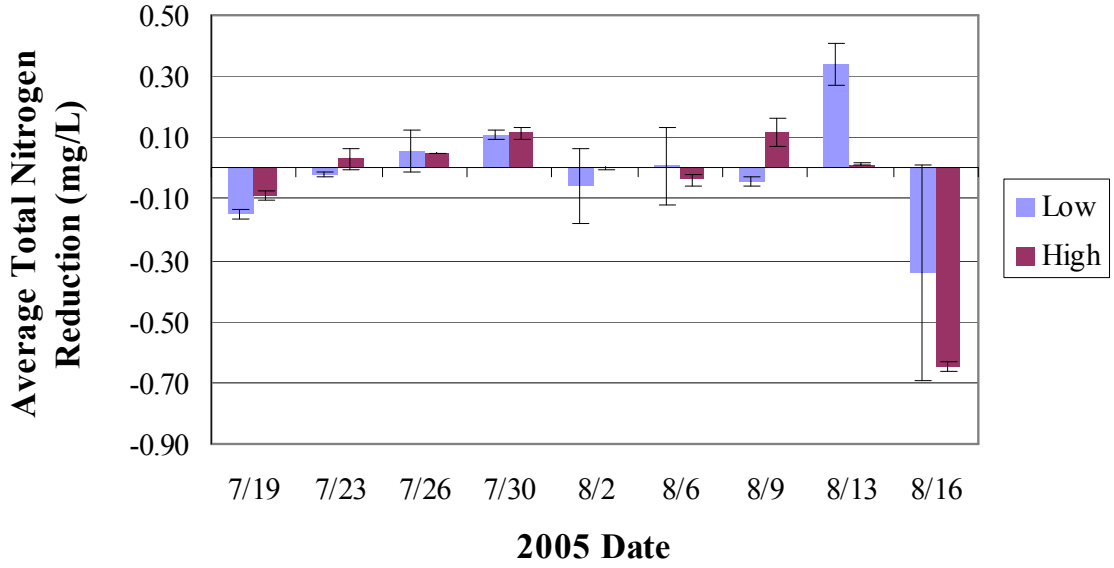

Figure 11. Average, total nitrogen reductions (mg/L) (N=6) by clam treatments from below (1) and above (2) clams (Figure 2). Positive averages indicate a nutrient reduction, while negative averages indicate a nutrient enrichment. Error bars indicate standard error.

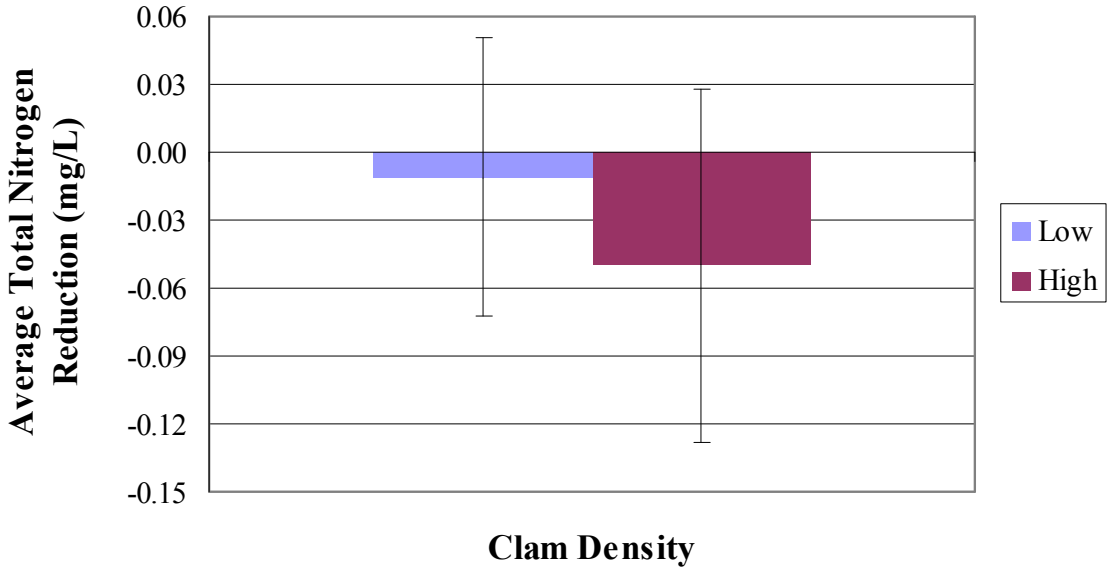

Figure 12. Overall average, total nitrogen reductions (mg/L) by clam treatments from below (1) and above (2) clams (Figure 2). Positive averages indicate a nutrient reduction, while negative averages indicate a nutrient enrichment. Error bars indicate standard error. Data represents averages of nine sampling days.

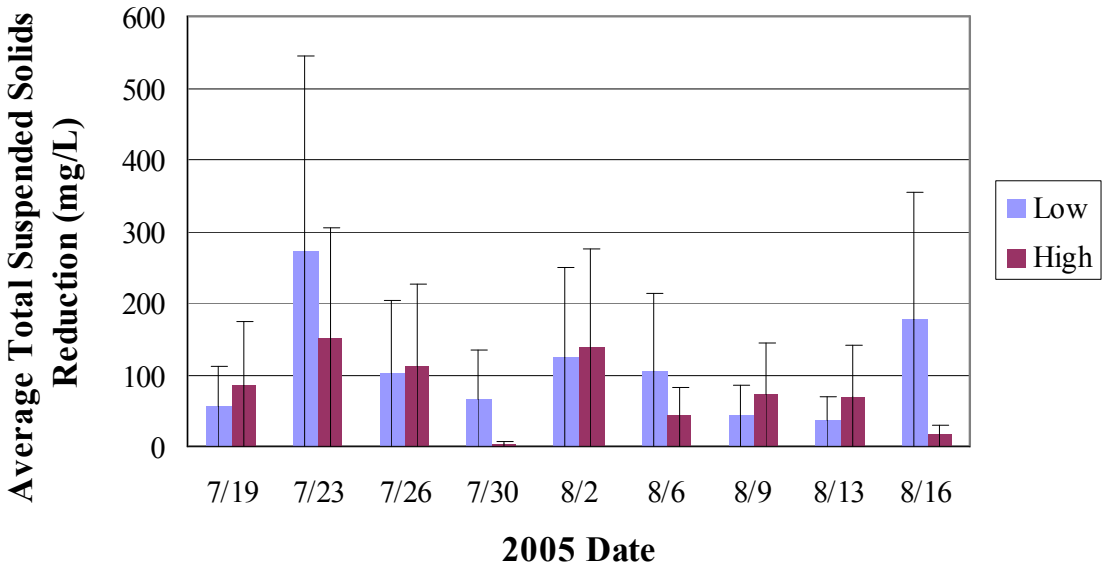

Figure 13. Average, total suspended solids reductions (mg/L) (N=6) by clam treatments from below (1) and above (2) clams (Figure 2). Positive averages indicate a nutrient reduction, while negative averages indicate a nutrient enrichment. Error bars indicate standard error.

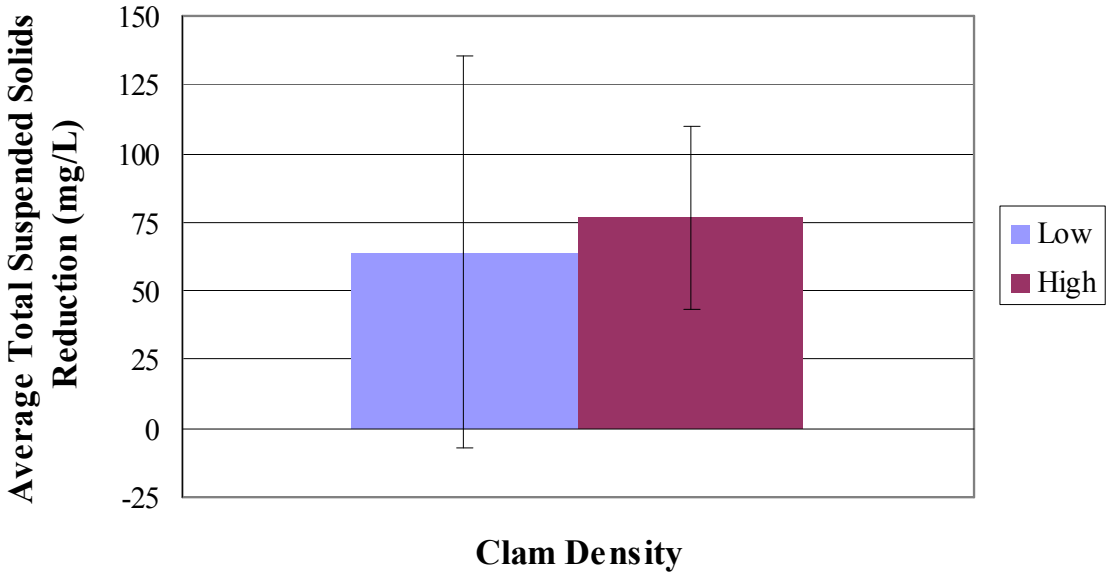

Figure 14. Overall average, suspended solids reductions (mg/L) by clam treatments from below (1) and above (2) clams (Figure 2). Positive averages indicate a nutrient reduction, while negative averages indicate a nutrient enrichment. Error bars indicate standard error. Data represents averages of nine sampling days.

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