

CLEARANCE RATES AND PARTICLE SELECTIVITY IN THE HARD CLAM,
Mercenaria mercenaria, FROM WARM WATER HABITATS

By

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The objective of this study was to examine the effects of temperature and phytoplankton concentration on the feeding selectivity and clearance rates of the hard clam *Mercenaria mercenaria*. My hypothesis was that temperature has an effect on the ability of the hard clam to preferentially ingest certain particles over others. Adult clams obtained from a commercial supplier were subjected to laboratory manipulated phytoplankton assemblages of three different algae (*Synechococcus* sp., *Isochrysis galbana*, and *Tetraselmis maculata*) of different sizes (2- μm , 5- μm , and 10- μm) at two temperatures (20°C and 30°C). One feeding treatment was conducted at two concentrations (10^5 cells/ml and 10^6 cells/ml). Clearance rates were determined from counting phytoplankton cells in water samples collected at the beginning and end of each feeding trial using a FacScan flow cytometer. Electivity indices were determined from initial water samples and clam pseudofeces using a FacScan flow cytometer.

Temperature, algal combination, and concentration all had significant effects on feeding selectivity of clams. Clams had greater selectivity at 20°C than at 30°C. In addition, clams showed a trend to select for larger particles over smaller particles. Selectivity was greater at the lower concentration than the higher concentration. Within each temperature/algal combination, there was a high amount of variability in electivity indices between replicates. Temperature, algal combination, and concentration had no effect on clearance rates. There was, however, an interaction effect between temperature and algal combination. Feeding history, adaptation of clams to their environment, seasonal changes in digestive enzymes, and/or other parameters like changes in water viscosity due to temperature may account for high variability in electivity indices and clearance rates between replicates. Results obtained have implications for future selectivity and clearance rates studies. In addition, this study provides important information for the future productivity of cultured clams in semi-tropical environments by demonstrating that feeding preferences may be different for clams from cooler environments.

CHAPTER 1 INTRODUCTION

The Suwannee River begins in the Okeefonokee Swamp and meanders through southern Georgia and north central Florida before it discharges into the Gulf of Mexico, draining 28,500 km² (Wolfe & Wolfe, 1985). Its estuary and the surrounding regions (also known as the Big Bend) are home to a highly productive nursery of fish and marine invertebrates (SRWMD, 1979). Oysters (i.e. *Crassostrea virginica*) are regularly harvested and clam farming (i.e. *Mercenaria mercenaria*) has exploded as a new aquaculture industry. In 2001, hard clam aquaculture comprised over 18% of Florida's \$99 million total aquaculture sales (USDA, 2002).

As the human population increases in northern Florida, anthropogenic activities are more likely to have a serious impact on the shallow estuarine waters along the Big Bend. Surface water runoff and ground water are contributing high concentrations of nutrients which, in turn, may cause phytoplankton blooms in the estuary (Phlips & Bledsoe, 1997; Bledsoe & Phlips, 2000). Maintaining a balance between the nutrients needed for productivity and excessive eutrophication is important for the stability of the Big Bend ecosystem, and especially for the growth and stability of the newly emerging clam farm industries. Understanding this balance and the effects of eutrophication on bivalves is essential.

Bivalves are adept suspension feeders and can modify seston in estuarine waters (Carlson *et al.*, 1984; Asmus *et al.*, 1990). On the other hand, seston quantity and composition can have effects on bivalve feeding behavior, particle selection, and

clearance rates (Bayne *et al.*, 1989, 1993; Navarro *et al.*, 1996). Studies on bivalves have shown an ability to sort particles based on size (Stenton-Dozey & Brown, 1992; Defosse & Hawkins, 1997) and quality (Arifin & Bendell-Young, 1997; Ward *et al.*, 1997). Furthermore, there appears to be variability among bivalve species in their ability to sort and preferentially ingest particles (Shumway *et al.*, 1985; Prins *et al.*, 1991; Ward *et al.*, 1998). Studies by Bayne *et al.* (1989;1993) have corroborated the idea that changes in food concentration can have an effect on clearance rates and, ultimately, the growth and productivity of bivalves. Baker *et al.* (1998) demonstrated that the diversity of a plankton assemblage is important in determining clearance rates of the zebra mussel *Dreissena polymorpha* as well as selectivity for individual species of phytoplankton within the assemblage.

While studies above have shown the composition of the seston to have an impact on clearance rates, temperature also has an effect. In a review of the physiological ecology of the hard clam, Grizzle *et al.* (2001) stated that temperature affected feeding rates. Feeding rates peaked at about 24-26°C, but fell abruptly at temperatures above 27°C. In this same review, temperatures between 20-24°C were shown to be optimal for clam growth with decreasing growth rates outside this range. This is important because feeding rates are thought to be the physiological control on growth rates.

While many studies have shown extremes in temperature to have a negative effect on clearance rates and growth of bivalves, there have been few, if any, studies to show what affect temperature may have on feeding selectivity. Selectivity studies are usually carried out at temperatures of 20°C or less (e.g., Shumway *et al.*, 1985; Bayne *et al.* 1989; Stenton-Dozey & Brown, 1992; Bayne *et al.* 1993; Arifin & Bendell-Young, 1997;

Defossez & Hawkins, 1997; Ward *et al.*, 1998). Surface waters in the Suwannee River estuary, however, commonly exceed 25°C between March and October and temperatures around 30°C are normal during summer months (T. Frazer, unpublished data; E. Philips, unpublished data; Jett, 2004). This challenges what we know about factors important to clam aquaculture success and our understanding of bivalve feeding physiology.

This study is part of a multi-faceted investigation determining the potential effects of coastal eutrophication on phytoplankton and bivalve communities of the Suwannee River estuary. The objective of this study was to examine the effects of temperature on particle selectivity and clearance rates of *Mercenaria mercenaria* feeding on bloom concentrations (10^5 and 10^6 cells/ml) of phytoplankton. Based on preliminary experiments, my hypothesis was that temperature would have an effect on the ability of hard clams to preferentially ingest smaller particles over larger particles. These effects may have a seasonal impact on the seston composition of the Suwannee River estuary.

CHAPTER 2 LITERATURE REVIEW

Description of the Study Area

The Suwannee River estuary is among the ten largest estuaries along the Gulf Coast of Florida. It is located between latitudes 29°13'N and 29°20'N and longitudes 83° 05' W and 83° 12' W (Siegal *et al.*, 1996). The estuary is home to a highly productive nursery of fish and marine invertebrates (SRWMD, 1979). The Suwannee River, which feeds the estuary, originates largely in the Okefenokee Swamp of southern Georgia and drains 28,500 km² of southern Georgia and north central Florida (Wolfe & Wolfe, 1985). It is fed, also, by the Alapaha, Withlacoochee, and Santa Fe Rivers. In addition, groundwater which emanates as spring discharges and diffuse seepage also feeds into the river. Groundwater contributions to riverflow are particularly important during low flow periods as a consequence of large-scale climate variation and reduced rainfall in the watershed. Due to the karst nature of the terrain, i.e. sinkholes, springs, and conduit systems in the underlying limestone, groundwater and surface water can have direct hydraulic and geochemical interactions (Katz *et al.*, 1997; Crandall *et al.*, 1999). These interactions are important because surface water and groundwater inputs contribute high concentrations of nutrients to the river which, in turn, discharges into the estuary (SRWMD, 2000b).

Sources of Nutrients to the Suwannee River Estuary

Nitrate concentrations in the river and loads to the Suwannee River Estuary have steadily increased over the years (Jones *et al.*, 1996, 1997; Pittman *et al.*, 1997). In fact,

from 2001 to 2003, nitrate loads have more than doubled from 2676 tons to 4,591 tons (SRWMD, 2001b; SRWMD, 2003). Increased nitrates can cause a variety of problems. In drinking water it can cause health concerns like methemoglobinemia or “blue baby” syndrome. Because nitrogen is a basic requirement for algae and other vegetation and can cause excessive growth, increased nitrogen loads are also an ecological concern. Studies have shown a positive correlation between nitrate concentration and growth of planktonic algae in this system (Phlips & Bledsoe, 1997; Bledsoe & Philips, 2000).

The years 1999 and 2000 were among the driest in the Suwannee River watershed since 1932. The annual mean stream flow was reduced to 28 to 52% of the long-term average (flow discharge of less than $142 \text{ m}^3 \cdot \text{sec}^{-1}$). As a consequence, elevated nutrient concentrations and extensive algal blooms occurred, with an appearance of some nuisance species like the red drift algae *Gracilaria* and filamentous brown algae *Ectocarpus* that are known to cause various water quality related problems (Bledsoe & Philips, 2000; SRWMD, 2001b).

Algal blooms can have an affect on the marine invertebrates and fish. For example, algal blooms can lead to extreme fluctuations in dissolved oxygen (DO) concentrations in the water column that can have damaging effects, sometimes fatal, on marine invertebrates and fish (Barica, 1980; Knights *et al.*, 1995). In addition, hypoxic and microxic conditions can affect the feeding behavior of young post-settlement oysters which can limit or delay recruitment into the adult oyster population (Baker and Mann, 1994).

Nitrogen enters the estuary in various ways: 1) the Floridian aquifer, 2) groundwater, and 3) the Suwannee River. The Floridian aquifer is impressive in that it is

one of the largest underground freshwater aquifers in the United States. The surrounding environment of the aquifer is composed of carbonate rock, such as marine dolomite and limestone (Rosenau *et al.*, 1977; SRWMD, 2001a). Sand or clay usually covers most of the carbonate rock. The aquifer extends from the southern portions of Alabama, Georgia, and South Carolina to the northeastern part of Florida to the Atlantic Ocean and the Gulf of Mexico. Weakly acidic rainwater dissolves the carbonate rock and creates cavities and caves in the aquifer. This type of terrain is known as a karst region and has many sinkholes and springs. In addition, it lacks a well-developed drainage system (SRWMD, 2001a).

Groundwater can enter the surface water system, e.g., the Suwannee River, through spring discharges or breaches in the underlying aquifers. The river, in turn, discharges into the estuaries. In addition, groundwater can enter estuaries as seepage along the coastline (Cable *et al.*, 1997). Spring water is usually a good indicator of the quality of the groundwater (SRWMD, 2001a). Human activities can have an effect on the quality and quantity of groundwater flow and these changes can have a significant impact on estuarine or coastal ecology because these areas receive large amounts of groundwater. Cable *et al.* (1996; 1997) have traced groundwater discharge by using ^{222}Rn and CH_4 and estimated that the seepage of groundwater into the northeastern coastal areas of the Gulf of Mexico (ca. 10 km^2) was equivalent to a first magnitude spring (i.e. a spring with $\geq 2.8 \text{ m}^3 \cdot \text{sec}^{-1}$ discharge).

Studies over a 12-year period (1970-1991) have shown a statistically significant increase in nitrate concentration in the Suwannee River (Ham & Hatzell, 1996). In the upper part of the river, the major source of this increase was due to transport of nitrates

by springs, while diffuse groundwater flow was the major source of transport in the lower portion (Pittman *et al.*, 1997). Augmenting this increase is the karst nature of the aquifer, which allows leaching of nitrates into the aquifer making it more susceptible to contamination (Kreitler & Browning, 1983).

Due to the karst topography of the terrain and increasing development along the Suwannee River, anthropogenic pollutants can also enter the estuary. High nitrate concentrations have been shown to come from numerous sources, highest among them being animal wastes, fertilizers, sewage effluent disposal, and residential and golf course landscapes (Jones *et al.*, 1996; 1997; Katz *et al.*, 1999). Katz *et al.* (1999) have shown that even though estimated nitrogen inputs from animal wastes have increased over the past 40 years in both Suwannee and Lafayette Counties, the nitrogen contributed by fertilizers is the highest input into the Suwannee River.

Because the average residence time of groundwater discharge from springs is on the order of decades, there is little to be done to reduce the present nitrogen load to the estuary (Katz *et al.*, 2001). While there are instances of denitrification by microorganisms, which can affect a decrease in the nitrogen load, especially in more eutrophic areas, studies have shown that the ability of ecosystems to regulate themselves can be exceeded by anthropogenic inputs into the ecosystems (Seitzinger & Nixon, 1985; Katz *et al.*, 1997). In the water year 2000, 2676 tons of nitrate-nitrogen was delivered to the Gulf of Mexico by Florida rivers. The vast majority, 2620 tons, was supplied by the Suwannee River (SRWMD, 2001b). Since an immediate reduction of nitrate-nitrogen is unlikely, we need to examine the effects of the increased nitrogen loading in the estuary to determine what impacts are likely to occur.

Characteristics of Algal Blooms

Phytoplankton are an important part of all estuarine and marine ecosystems and phytoplankton blooms are a naturally occurring phenomenon. Increased nutrient delivery to estuaries can result in an increase in the frequency and intensity of these blooms. Not all algal blooms are harmful, however. Blooms of some species may only cause water discoloration. Only when a phytoplankton species increases significantly in population size and has detrimental ecological and physiological effects on the surrounding area, it is considered a harmful algae bloom (HAB). Concentrations may vary depending on the species composition of the phytoplankton assemblages. For nontoxic species, biomass is the primary determinant of bloom conditions. For toxic species, the presence of a toxin in the water can determine the bloom status (Smayda, 1997).

While the “red tide” dinoflagellate, *Karenia brevis*, has been documented at least once in the Suwannee River estuary, it is not a common occurrence (Bledsoe & Phlips, 2000). Instead, typical bloom species include cyanobacteria and diatoms in the genera *Rhizoselenia*, *Thalassiosira*, *Cyclotella*, and another unidentified small centric diatom ranging from 3 to 10 μm in diameter (Bledsoe & Phlips, 2000; 2004).

Bivalves and their Effect on the Water Column

Bivalves are dominant suspension feeders in many estuarine ecosystems and are capable, in some cases, of maintaining phytoplankton at low levels. In a study on the freshwater clam, *Corbicula fluminea*, in the Potomac River, Cohen *et al.* (1984) found that the lowest concentrations of phytoplankton biomass were in the areas with the highest densities of clams. In the Chesapeake Bay, results from an estuarine model showed that resident bivalves could consume more than 50% of the primary production in shallow segments of the bay in spring and summer with 45% to 95% of the water

column being filtered by the bivalves during spring, summer, and fall (Gerritsen *et al.*, 1994). Werner and Hollibaugh (1993) also suggest that the clam, *Potamocorbula amurensis*, has a substantial impact on the phytoplankton biomass in northern San Francisco Bay. With a density of more than 2,000 clams m⁻² and an average clearance rate of 267 ml/h per clam, they calculated that the bivalves at a depth of 10-m could filter the water column 1.28 times per day, while those in shallower waters (1-m) could filter the water column 12.8 times per day. Based on these findings and those mentioned above, it is evident that bivalves have the capacity to greatly influence the abundance of phytoplankton, especially in enclosed systems.

Suspension Feeding in Bivalves

According to LaBarbera (1981), suspension feeding is comprised of three separate processes: 1) movement of water past suspension-feeding structures, 2) removal of particles from the water, and 3) transport of food particles to the mouth. All three processes are accomplished by means of both mucociliary and hydrodynamic processes (Ward, 1996).

Bernard (1974) related the pallial cavity (the latero-ventral space surrounding the visceral mass that includes the gills, labial palps, stomach, and rectum) of a bivalve to a “simple pump housed in a chamber (inhalant chamber) provided with a restricted inlet (inhalant aperture) and a larger exit (exhalant aperture)” in which the ctenidia (gills) “functions as a large diaphragm which is also porous to water.” Water is drawn through the inhalant siphon then encounters a partial obstruction, the ostial aperture, on its way through the ctenidium by water tubes and out the exhalant siphon (Bernard, 1974). The ostial aperture is the area that contains numerous pores (ostia) that connect the inhalant chamber with the water tubes, and comprises about 37% of the ctenidium (Jones *et al.*,

1992). This obstruction of the ostial aperture decreases the water speed as it approaches the ctenidium. Bernard (1974) also suggests that this allows preingestive selection of particles by allowing larger particles, like minerals or inorganic particles, to settle directly on the inhalant chamber mantle surfaces, according to the influence of gravity, and to be ejected. Once particles are captured on the ctenidium, they are transported by the activity of the frontal cilia. Mucus may play a role in the transportation of particles (Bernard, 1974; Beninger *et al.*, 1992; Ward, 1996; but see Jørgensen 1990). For most bivalves, the majority of particles $> 4 \mu\text{m}$ are completely retained on the ctenidium while smaller particles, $1 \mu\text{m}$ to $4 \mu\text{m}$, are retained with various efficiencies (Møhlenberg & Riisgård, 1978; Jørgensen, 1975). Some bivalves, like oysters, sort particles on the ctenidium. The rejected particles are excreted as pseudofeces (Ward *et al.*, 1997). The particles selected for ingestion are moved dorsally to the labial palps for further selection and then to the mouth for ingestion. The remains of digested particles are excreted as feces after passing through the gut (Beninger *et al.*, 1992; Ward *et al.*, 1997).

The concentration of the particles that the bivalve is exposed to appears to have an effect on the aforementioned feeding mechanisms. Beninger *et al.* (1992) found that as the concentration of particles increases, bivalves start to exhibit some ingestion volume control, among them being a reduction or a stoppage of movement of particles. In addition, bivalves start to exhibit lower selectivity where “good” particles are often rejected as pseudofeces (Beninger *et al.*, 1992).

The Effect of Temperature on Bivalve Clearance Rates

Several investigations have shown temperature to have a hyperbolic effect on clearance rates of bivalves (e.g., Hibbert, 1977; Grizzle *et al.*, 2001). Hence, clearance

rates increase as temperatures increase until an optimum temperature is reached after which clearance rates start to decrease. Optimum temperatures may vary between bivalve species. For example, for the penshell, *Atrina maura*, Leyva-Valencia *et al.* (2001) found clearance rates to be highest at 29°C when tested over a temperature range of 19-35°C. The clearance rates for *Crassostrea gigas* reach a maximum at 19°C (Bougrier *et al.*, 1995). For the catarina scallop, *Argopecten ventricosus*, clearance rates are greatest at 19-22°C (Sicard *et al.*, 1999).

Other investigations, however, have reported a different response. For instance, Sobral and Widdows (1997) showed that increasing temperature over the range 20°C to 32°C caused clearance rates to decrease in *Ruditapes decussatus*. Haure *et al.* (1998) showed clearance rates to increase for *Ostrea edulis* over a temperature range of 10-30°C. In fact, rates were maximum at 30°C. Doering and Oviatt (1986) also found clearance rates to have a linear relationship with temperature. On the other hand, some studies have shown clearance rates to be independent of temperature (Loosanoff, 1958; MacDonald *et al.*, 1996). Loosanoff (1958) showed pumping rates to be independent of temperature for adult oysters (*Crassostrea virginica*) between 16 and 28°C. Smaal *et al.* (1997) looked at clearance rates of mussels (*Mytilus edulis*) and cockles (*Cerastoderma edule*) and found that there was no relationship between temperature and clearance rates of mussels throughout the year. However, Smaal *et al.* (1997) found that temperature did have an effect on clearance rates for cockles.

The Effect of Diet Composition and Concentration on Bivalve Clearance Rates

There have been numerous experiments conducted to understand under what diet conditions bivalves perform best. These studies have determined that both seston quantity and quality may have an effect on bivalve feeding rates. In general, there is a

positive correlation between seston concentration and clearance rate (Albentosa *et al.*, 1996; Marsden, 1999; Hawkins *et al.*, 2001; Ellis *et al.*, 2002). Stenton-Dozey and Brown (1992) showed that *Venerupis corrugatus* displays an ability to alter its clearance rate in response to the quantity of the seston; as the seston concentration increased, the clearance rate of *V. corrugatus* increased. In another study, *Mytilus edulis* exhibited an increased rate of ingestion with increased particle concentration until the rate of ingestion reached an asymptotic value, which coincided with the threshold for pseudofeces production (Bayne *et al.*, 1989; 1993).

In some cases, however, clearance rates appear to be unaffected by increasing seston concentration (Cranford & Hargrave, 1994; Arifin & Bendell-Young, 1997). Rheault and Rice (1996), for example, found that clearance rates of *C. virginica* and *A. irradians irradians* did not vary significantly with “fourfold tidal variations in food concentration.” However, *A. irradians* did exhibit a reduction in clearance rates when the seston concentration was decreased by 88% compared to the original concentration. Cranford and Hargrave (1994) obtained similar results with ingestion rates (biodeposition rates) for *Placopecten magellanicus* using a new method for quantifying the feeding and absorption rates of suspension feeding bivalves. Arifin and Bendell-Young (1997) did not find a relationship between clearance rates and seston concentration, but did find that pseudofeces production was dependent on seston concentration which, as stated before, is another way for bivalves to regulate ingestion rates.

In a study examining the effects of seston quality on bivalve physiology, raft mussels (*Mytilus alloprovincialis*) exhibited maximum clearance rates and absorption efficiencies on mixed diets in which phytoplankton and sediment were provided in

similar proportions, with the phytoplankton being 30-40% of total particulate volume (Navarro *et al.*, 1996). Gatenby *et al.* (1996) found that cultured freshwater mussels (*Villosa iris* and *Pyganodon grandis*) grew best on mixed diets of algae and sediment rather than on algae alone. Both of the above studies suggest that sediment could play a vital role in enhancing absorption of microalgae or could even help with digestion in the stomach.

Generally, as seston quality increases, so does the clearance rate of a bivalve. In a study by Stenton-Dozey and Brown (1992), clearance rates were greatest during high tide when there was an abundance of particles greater than 9 μm and the organic content of available food was higher than at low tide. Likewise, Gardner (2002) found that clearance rates for three species of bivalves (*Aulacomya maoriana*, *Mytilus galloprovincialis*, and *Perna canaliculus*) increased in a linear fashion, with the highest clearance rates at high levels of organic matter in mixed diets.

Selective Feeding in Bivalves

Although there is some question as to how bivalves select particles for ingestion, it is generally accepted that bivalves can selectively ingest particles (Shumway *et al.*, 1985; Newell *et al.*, 1989; Defosse & Hawkins, 1997). Selectivity can be divided into two categories: 1) separation of inorganic particles from organic ones and 2) selection between organic particles (Bernard, 1974). For the first category, experiments involving variations in quantity and organic content of bivalve diets show that when presented with a mixed diet, the pseudofeces often contain intact, nonorganic particles (Newell *et al.*, 1989; Iglesias *et al.*, 1992; Bayne *et al.*, 1993; Arifin & Bendell-Young, 1997). This

preingestive selection of organic versus inorganic particles could be based on size (Bernard, 1974; Tamburri & Zimmer-Faust, 1996; Defosse & Hawkins, 1997).

Selectivity studies subjecting bivalves to organic particles of differing nutritional value have shown the pseudofeces to contain particles that are organic in nature, but not very nutritious, like detritus (Baker *et al.*, 1998; Ward *et al.*, 1998). In comparing selectivity between phytoplankton, the majority of the selection and clearance rate experiments have used few phytoplankton species, comparing preferential selection of nutritious versus less-nutritious phytoplankton. With regard to zooplankton, Wong *et al.* (2003) showed that rotifers in the micro- and mesozooplankton (140-210 μm range) can play a significant role in the diet of zebra mussels (*Dreissena polymorpha*). There are some experiments, however, involving various organic particles that indicate that there is also selection by bivalves between nutritious particles. Baker *et al.* (1998) showed that zebra mussels preferentially selected the cyanobacterium *Microcystis* over other phytoplankton that are typically found in the Hudson River. Shumway *et al.* (1985) showed that there is preferential selection of the cryptomonad flagellate, *Chroomonas salina*, over other nutritious particles in the majority of bivalves they tested. Further information is needed to understand particle selectivity.

Controversies Concerning Bivalve Feeding Mechanisms

There is still a question among scientists concerning the mechanisms of particle capture and selection in bivalves. Ward *et al.* (1998) found that the ctenidia of *Crassostrea virginica* are responsible for particle sorting and the labial palps perform an accessory function in particle selection or they regulate the volume of the ingested material. Ward *et al.* claim that it is essential for food particles to approach the gill

surface in a straight line at an angle of 30 degrees from the gill surface. They suggest that variations of this angle could have an effect on the interaction of particles with the gill filaments. A number of scientists disagree with the prerequisite of a low angle of approach (Beninger, 2000; Riisgård & Larsen, 2000). For instance, Riisgård and Larsen (2000) feel that because of the parallel arrangement of the lateral cilia, particles hit the ctenidium in a curved path between 70-90 degrees and that none hit below 40 degrees. They feel that the 30 degrees that Ward *et al.* (1998) stated is not a prerequisite of particle capture, but rather a result of the existing flow fields caused by ciliary activity on the ctenidia in the bivalve.

Another disagreement concerns the role of mucus in suspension feeding by bivalves. There are basically two hypotheses concerning the role of mucus. The first is that mucus only forms to entrap materials when the ingestive capacity of the bivalve is exceeded (Jørgensen, 1990). The second hypothesis is that mucus is present at all times and is a normal function of bivalve physiology (Beninger *et al.*, 1993; Ward, 1996). According to Bernard (1974), there appears to be two types of mucus in bivalves that are involved in entrapment and rejection of particles. The mucus for entrapment of food particles headed for ingestion appears to be light-colored and almost transparent, while the rejection mucus appears to be darker and contains more particles. Beninger *et al.* (1992) also has observed two distinct mucus types with the denser mucus being associated with those particles that have been rejected.

Because of their opaque shells, it is hard to determine what goes on during the normal functioning of living, intact bivalves. Different techniques for observation of bivalve physiological mechanisms have been employed, e.g., dissection with isolated gill-

filament preparations, the use of surgically altered animals, the use of confocal laser microscopy, and, most recently, the use of endoscopic techniques, have all been used to collect information. These methods have led to a different controversy involving the manner of data collection. Ward *et al.* (1998) have expressed some concerns about the data collected from techniques other than video endoscopy. These investigators feel that the manner of preparation of the organism for either methods of observation could change some critical interactions between the way particles interact with the gill filaments. However, some scientists disagree with this assessment (Beninger, 2000; Riisgård & Larsen, 2000; Silverman *et al.*, 2000). Beninger (2000) states that due to mechanical limitations “endoscopy cannot in fact access the underlying mechanisms for the processes of particle capture, transport, and selection, although it can... observe the net result of the processes and suggest where and what techniques to use to seek the sequence.” These scientists suggest that results from previously mentioned techniques and endoscopy are similar and that all techniques should be used to balance the weaknesses and strengths of the individual methods.

CHAPTER 3 METHODS AND MATERIALS

Test Subjects

Experimental Algae and Culture Protocols

Four algal species were used in this study: *Tetraselmis maculata* (a large green flagellate), *Isochrysis galbana*, (a small brown flagellate) and two strains of *Synechococcus* (a cyanobacteria; one strain forms long chains and one consists primarily of single cells). Algal cultures were supplied by Jose Nunez (*I. galbana* and *T. maculata*), Whitney Laboratory at the University of Florida, Dr. Edward Philips (*I. galbana* and both species of *Synechococcus*), Department of Fisheries and Aquatic Sciences at the University of Florida, and Dr. Gary Wikfors (*T. maculata*), Milford Laboratory. Algal species were chosen to represent different sizes, with *T. maculata* having cells that are ca. 10- μm , *I. galbana* ca. 5- μm , and *Synechococcus* spp. ca. 2- μm . *I. galbana* is the primary algae used in shellfish hatcheries, but *T. maculata* is also used. *Synechococcus* spp., and other picoplankton-sized blue-green algae, are not typically used in hatcheries (Hadley *et al.*, 1997) but are a major bloom forming taxa in coastal marine environments in Florida (Philips *et al.*, 1999; Bledsoe, 2003). Of the three, cyanobacteria are regularly found in the Suwannee River estuary (Bledsoe and Philips, 2000).

All algal species were cultured in 500-ml flasks containing 150 ml of sterilized L1-Si media at 28 ppt (Guillard and Hargraves, 1993) at a pH of 7.8-8.2. Cultures were kept at 25-28 °C on a 14:10 light/dark photoperiod at 30-60 $\mu\text{Einsteins}/\text{m}^2/\text{s}$ light flux except for the *Synechococcus* spp. which were kept at 20-30 $\mu\text{Einsteins}/\text{m}^2/\text{s}$ light flux. Light

intensity was measured using a quantum light meter with a *Li-Cor* data logger. Once cultures reached sufficient biomass they were transferred to either 1000-ml flasks or 2.8-L Fernbach flasks that contained 500 ml and 1.5 or 1.8 L of L1-Si, respectively. The 500-ml and 1000-ml flasks were swirled once a day. Fernbach flasks were aerated with 0.22- μm filtered air containing 1/32 psi of added CO_2 .

Phytoplankton Combinations

Experimental phytoplankton assemblages consisted of two of the algae species types combined at a 1:1 ratio based on numerical abundance (Table 1). Initial counts were determined by using either a fluorescent microscope or a Coulter Multisizer II fitted with a 100- μm orifice. Enough phytoplankton was added to the experimental water to bring the total experimental concentration to 10^5 cells/ml or 10^6 cells/ml.

Experimental Organism

Adults of the hard clam *Mercenaria mercenaria* were obtained from commercial suppliers operating out of Cedar Key, Florida, a coastal area influenced by the Suwannee River. Clams were allowed to purge in 0.2- μm filtered seawater (28 ppt) for 72 hours at the experimental temperature with no prior temperature acclimation. All water in the holding tanks was changed twice during this period. Shell length, height, and width were measured according to Fritz (2001). Data on size, weight, and number of animals used in each feeding trial are provided in Table 2.

Experimental Protocol

Between December 2003 and March 2004, feeding selectivity and clearance rates were examined through a series of laboratory feeding trials in static systems subjected to two different temperatures (20°C and 30°C). Particle selection and clearance rates were

measured following Baker *et al.* (1998). Ten individual clams were individually placed in 2-L clear glass Pyrex ® beakers containing the experimental phytoplankton assemblage in 28 ppt 0.2-µm filtered seawater. The experimental beakers were run concurrently with two control beakers, containing no clams. To lessen the occurrence of phytoplankton settling to the bottom, beakers were gently aerated throughout the experiment. Clearance rates were determined by analyzing 1.0-ml water samples at the start of each feeding trial and again after 45 minutes. Feeding selectivity was determined by examining the composition of the pseudofeces produced during the feeding trials (see below). At the end of each feeding trial, clams were measured, weighed wet, and then tissues were dried to a constant weight (60°C). Each feeding trial was replicated three times on different days.

Hurlbert (1984) wrote about the dangers of pseudoreplication in ecological field experiments; replicates that are spatially or temporally separated are not independent and the data should not be used as such. In the experimental design, time was not taken into account. For example, one replicate of IsoSyn at 30°C was performed in January, and another in February. The clams used for each replicate were from different batches and had been exposed to different environmental parameters. Therefore, according to Hurlbert (1984), the replicates were not “true” replicates of each temperature/algal combination but rather pseudoreplicates. In addition, the replicates were not performed randomly; feeding trials at 30°C were completed before the 20°C feeding trials.

Additional Experiments

Two additional types of feeding trials were conducted. To determine whether clams would prefer algae to which they had been acclimated, one batch of clams was split

into two groups. “Batch” refers to clams obtained at the same date and time and from the same area. Prior to purging and conducting feeding trials, the two groups of clams were kept in separate holding tanks for two weeks. One group of clams was fed *I. galbana* and the other group of clams was fed *Synechococcus* at a rate of 2% of their dry body weight every other day (<http://www.reedmariculture.com>). Feeding trials were conducted following the experimental protocol outlined above.

To eliminate time as a factor, a second type of feeding trial was performed. One batch of clams was obtained and split into two groups. The clams were allowed to purge for three days; one group at 20°C and one group at 30°C. Feeding trials for each group were subsequently performed following the experimental protocol described above. Ideally, this is how the experiment protocol should have been designed.

Calculation of Particle Selectivity

Phytoplankton species in the pseudofeces and water samples were identified and counted using a FACSCAN flow cytometer (BD BioSciences, San Jose, CA) at the University of Florida Flow Cytometry Core Lab. Samples were vortexed to disrupt aggregations immediately prior to analysis. Samples were shaken and filtered through a 40- μm mesh to remove large particles to prevent clogging of the flow cytometer. In the cytometer, cells flow singly through a 488 nm laser beam while their forward light scatter and red fluorescence emission above 650 nm are measured. Forward light scatter was used to eliminate small particles. Data was collected for one minute at the “high” flow rate (approximately 60 $\mu\text{L}/\text{min}$). Regions identifying phytoplankton were set based on forward light scatter and red fluorescence using CellQuest 3.3 software running on a

Macintosh computer (Apple Computer, Cupertino, CA). Counts of cells in each region were collected in a text file and imported into a Microsoft Excel spreadsheet.

To determine feeding selectivity, a modified electivity index (EI) was calculated. The equation for EI is based on Ivlev's index of electivity for freshwater fish and was modified as followed: $EI = -(P - S) / ((P + S) - (2 * P * S))$ where P is the ratio of a particular algal species in the pseudofeces and S is the ratio in the suspension (Ivlev, 1961; Bayne *et al.*, 1977). The electivity index can range from -1.0 to 1.0. If the EI is positive for a given algal species, then there is a preferential ingestion of that species. If the EI is negative, then it indicates a rejection of that algal species.

Since electivity indices are not continuous, the indices were arcsine transformed. The mean of the means for each replication within each temperature feeding trial (20°C and 30°C) was compared to zero using a one-sample t-test. For the feeding trials of IsoSyn, TetraIso, TetraSyn, and IsoSyn(Ch), the indices were compared using a 2-way ANOVA (temperature and feeding trial). To compare IsoSyn and IsoSyn6, a 2-way ANOVA (temperature and concentration) was used. Since there was only one replication for IsoSyn-AI, IsoSyn-AS, and IsoSyn-B, no statistical procedures were conducted. Instead the mean clearance rates were graphed. Feeding trials, statistical tests used, and p-values are summarized in Table 3 in the Results section.

Calculation of Clearance Rate

Clearance rates were also determined using a flow cytometer. The reduction in the concentration of particles over 45 minutes was used to calculate net clearance rates according to Coughlan (1969) as follows:

$CR = V * [(\ln(C_0) - \ln(C_1)) / t] - A$; where CR is clearance rate, V is the volume of the experimental suspension, C_0 is the initial concentration, C_1 is the concentration after time

t, and A is the average of the controls ($A = (\sum[(\ln(C_0) - \ln(C_1))]/n$, where n is the number of controls). Clearance rates were corrected for particle abundance changes in the controls and for the amount of time the bivalves were open. Clearance rates were standardized to 1-g of dry tissue mass using an allometric exponent for bivalves of 0.75 (Grizzle et al., 2001) as follows: $CR_{(1g)} = CR/b^{0.75}$; where CR is the uncorrected clearance rate of the experimental clam expressed in $L \cdot h^{-1}$ and b is the dry weight of the experimental clam expressed in grams.

The means for each replicate feeding trial at 20°C and 30°C involving mixed algal assemblages, i.e. IsoSyn, TetraIso, TetraSyn, and IsoSyn(Ch), were analyzed with a 2-way ANOVA. Clearance rates for IsoSyn and IsoSyn6 were compared also with a 2-way ANOVA. Since there was only one replication for IsoSyn-AI, IsoSyn-AS, and IsoSyn-B, no statistical procedures were conducted. Instead the mean clearance rates were graphed. Feeding trials, statistical tests used, and p-values are summarized in Table 4 in the Results section. Clearance rate data was not used if the particle concentration in the beakers declined below 40% of the initial concentration (Baker and Levinton, 2003).

Table 1. Algal assemblages and the date(s) of replication(s) of each feeding trial at two different temperatures. Unless stated otherwise, concentrations were 10^5 cells/ml.

Phytoplankton	Trial Abbreviation	Temperature	
		20°C	30°C
<i>I. galbana</i> + <i>Synechococcus</i> (no chains)	IsoSyn	I. 2/26/04	I. 1/29/04
		II. 3/04/04	II. 2/05/04
		III. 3/25/04	III. 2/19/04
<i>T. maculata</i> + <i>Synechococcus</i> (no chains)	TetraSyn	I. 3/04/04	I. 1/29/04
		II. 3/11/04	II. 2/12/04
		III. 3/18/04	III. 2/12/04
<i>T. maculata</i> + <i>I. galbana</i>	TetraIso	I. 3/04/04	I. 3/04/04
		II. 3/11/04	II. 3/11/04
		III. 3/18/04	III. 3/18/04
<i>I. galbana</i> + <i>Synechococcus</i> (Chain-forming)	IsoSyn(Ch)	I. 2/26/04	I. 1/15/04
		II. 2/26/04	II. 1/15/04
		III. 3/11/04	III. 1/22/04
<i>I. galbana</i> + <i>Synechococcus</i> (no chains) at 10^6 cells/ml	IsoSyn6	I. 3/04/04	I. 1/22/04
		II. 3/11/04	II. 2/05/04
		III. 3/18/04	III. 2/12/04
<i>I. galbana</i> + <i>Synechococcus</i> (no chains) (acclimated to <i>Synechococcus</i> -no chains)	IsoSyn-AS	I. 3/25/04	I. 2/19/04
<i>I. galbana</i> + <i>Synechococcus</i> (no chains) (acclimated to <i>I. galbana</i>)	IsoSyn-AI	I. 3/25/04	I. 2/19/04
<i>I. galbana</i> + <i>Synechococcus</i> (no chains) (Same batch, 2 temperatures) at 10^6 cells/ml	IsoSyn-B	I. 4/08/04	I. 4/08/04

Table 2. Mean size and weight, and actual number of animals that opened in each feeding trial. Numbers in parantheses are \pm SD.

	Trials	n	Height(mm)	Length(mm)	Width(mm)	Wet Weight(g)	Dry Weight(g)
20°C (0.47)	Experimental Clams	124	38.11 (0.19)	44.13 (0.21)	24.04 (0.19)	27.5 (0.40)	0.87 (0.01)
	IsoSyn-AI	10	39.41 (1.48)	46.11 (1.75)	26.37 (4.18)	31.2 (3.0)	1.01 (0.17)
	IsoSyn-AS	9	37.46 (1.75)	42.61 (2.00)	23.94 (0.89)	26.6 (2.8)	0.77 (0.08)
	IsoSyn-B	14	37.68 (2.18)	43.22 (1.81)	24.52 (0.76)	26.5 (3.0)	0.74 (0.12)
30°C (0.75)	Experimental Clams	150	37.43 (0.32)	43.50 (0.45)	23.31 (0.27)	26.18 (0.54)	0.76 (0.02)
	IsoSyn-AI	10	37.52 (3.59)	44.95 (1.75)	24.09 (1.06)	27.5 (2.7)	0.75 (0.10)
	IsoSyn-AS	9	38.70 (2.72)	45.46 (3.32)	24.03 (1.39)	28.1 (5.8)	0.82 (0.18)
	IsoSyn-B	19	38.42 (2.39)	44.66 (3.04)	25.06 (1.19)	29.4 (4.9)	0.80 (0.13)

CHAPTER 4 RESULTS

Electivity Indices

Based on results, clams sorted particles for ingestion or rejection. Out of eight algal combinations, three had EIs that were significantly different than zero (Fig 1). Specifically, there was preferential ingestion of *Isochrysis* in all three trials.

Algal combination had a significant effect on EIs (p-value = 0.000). Clams showed greater selectivity between *Synechococcus* (either chainforming or not) and *Isochrysis* than between *Tetraselmis* and *Synechococcus* or *Tetraselmis* and *Isochrysis* (Fig 1). In particular, IsoSyn(Ch) had much higher EIs than TetraIso (p-value = 0.0029) and TetraSyn (p-value = 0.0175). In addition, IsoSyn had significantly higher EIs than TetraIso (p-value = 0.0001). Although there was no interaction between algal combination and temperature, within the 20°C particle combinations, TetraIso had a significantly lower EI than IsoSyn (p-value = 0.0325). At 30°C, TetraIso had a significantly lower EI than either IsoSyn (p-value = 0.0021) or IsoSyn(Ch) (p-value = 0.0190) (Fig 1). Based on these results, there appears to be a pattern in which clams select the larger algal species (either *Tetraselmis* or *Isochrysis*) over the smaller *Synechococcus*.

Temperature had a significant effect on particle selectivity (p-value = 0.035); clams were more selective at 20°C than they were at 30°C (Fig 1). However, when a single batch of clams was used for both temperature experiments, there appeared to be no difference in EIs (Fig 2).

Cell concentration had a significant effect on electivity. Electivity indices were significantly greater at the lower concentration (p-value = 0.012) (Fig 3).

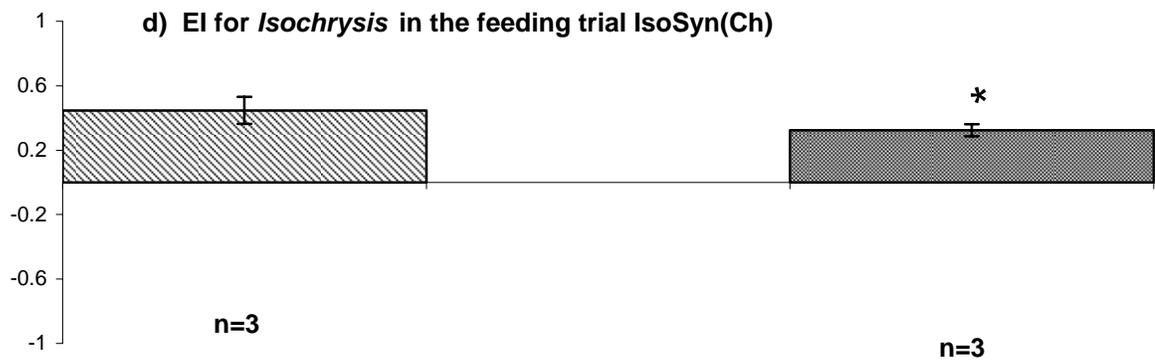
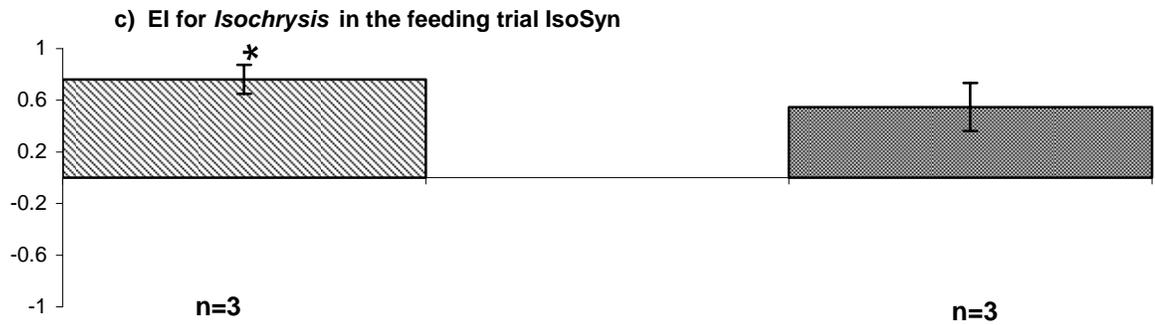
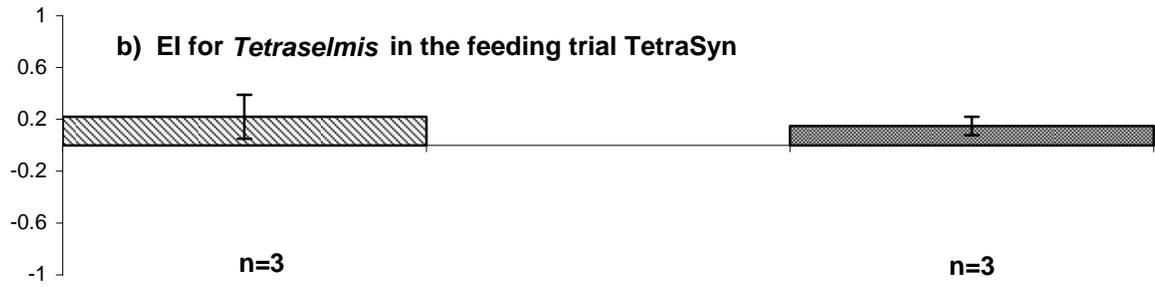
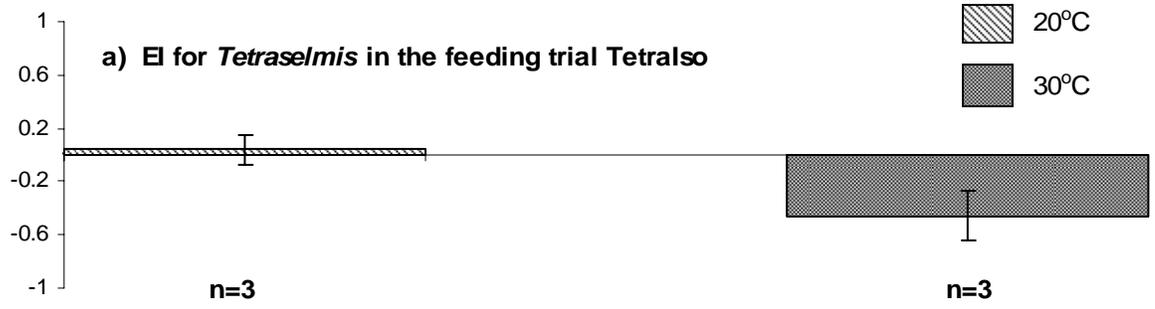
Within each temperature/algal combination, there was a high amount of variability in EIs between replicate trials (batches of clams) (Fig 4). For example, when three different batches of clams were fed *Tetraselmis* and *Synechococcus* at 30°C, one batch strongly selected for *Tetraselmis*, one batch slightly selected for *Tetraselmis*, and one batch selected for *Synechococcus* (Fig 4b). When the three batches were combined, this resulted in a small positive EI, but no active selection for either alga (Fig 1b). Prior feeding history did not appear to account for differences between batches (Fig 5). When a single batch of clams was split and two groups acclimated to different algae, there was no difference in their preference for those algae.

Clearance Rates

Temperature had no effect on clearance rate when clams were fed any of the four particle combinations (Fig 6). However, when a single batch of clams was used for both temperature experiments, mean clearance rates were greater at 30°C than at 20°C (Fig 7). Interestingly, there was a significant interaction (p-value = 0.0461) between temperature and algal combination due mainly to TetraSyn at 20°C having a lower clearance rate than TetraIso at 30°C. Cell concentration did not have a significant effect on clearance rates (Fig 8). However, temperature did have a significant effect when comparing IsoSyn and IsoSyn6, with clearance rates higher at 20°C than at 30°C (p = 0.003).

Prior feeding history may have had an impact on clearance rates. Clams acclimated to *Synechococcus* exhibited a higher mean clearance rates for a combination of *Isochrysis* and *Synechococcus* than did those clams acclimated to *Isochrysis* (Fig 9).

Figure 1. Electivity indices (means \pm SE) for *Mercenaria mercenaria* at two different temperatures, 20°C and 30°C, when fed different combinations of algae at a total concentration of 10^5 cells/ml. A positive EI indicates selection of an algal species. A negative EI indicates rejection of an algal species. Symbol (*) indicates which EIs were significantly different than zero ($p < 0.05$). a) Acceptance (+) or rejection (-) of *Tetraselmis* when *Isochrysis* is present. b) Acceptance or rejection of *Tetraselmis* when *Synechococcus* sp. is present. c) Acceptance or rejection of *Isochrysis* when *Synechococcus* is present. d) Acceptance or rejection of *Isochrysis* when the chainforming strain of *Synechococcus* is present.



Acceptance or rejection of *Isochrysis* when *Synechococcus* is present
in clams from a single batch (10^6 cells/ml):

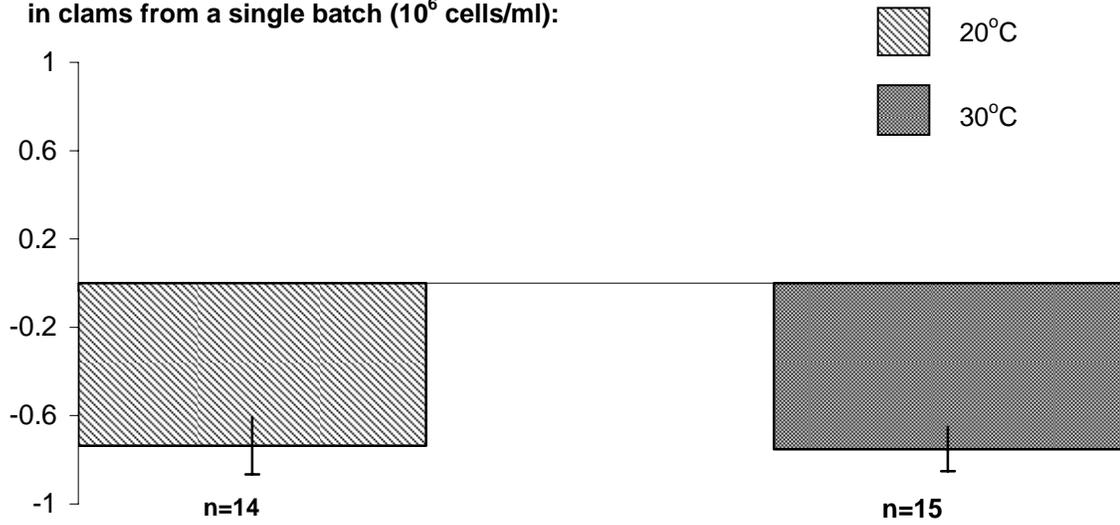


Figure 2. Electivity indices (means \pm SE) of *Mercenaria mercenaria* for *Isochrysis galbana* when *Synechococcus* sp. (nonchainforming) is present, at two different temperatures, in clams from a single batch (IsoSyn-B). Total cell concentration was 10^6 cells/ml. A negative EI indicates rejection of *Isochrysis*.

Acceptance or rejection of *Isochrysis* when *Synechococcus* is present at two concentrations a) 10^5 and b) 10^6 cells/ml:

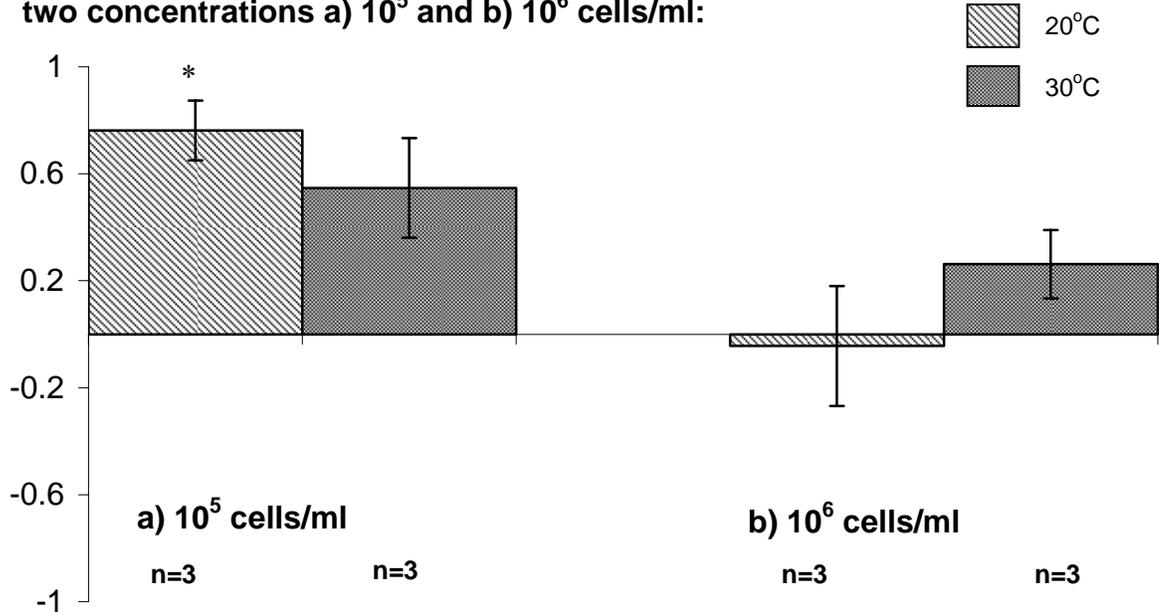


Figure 3. Electivity indices (means \pm SE) of *Mercenaria mercenaria* for *Isochrysis* when *Synechococcus* sp. (non-chainforming) is present, at two temperatures, 20°C and 30°C, and two cell concentrations a) 10^5 and b) 10^6 cells/ml. Symbol (*) indicates which replication(s) were significantly different than zero ($p < 0.05$).

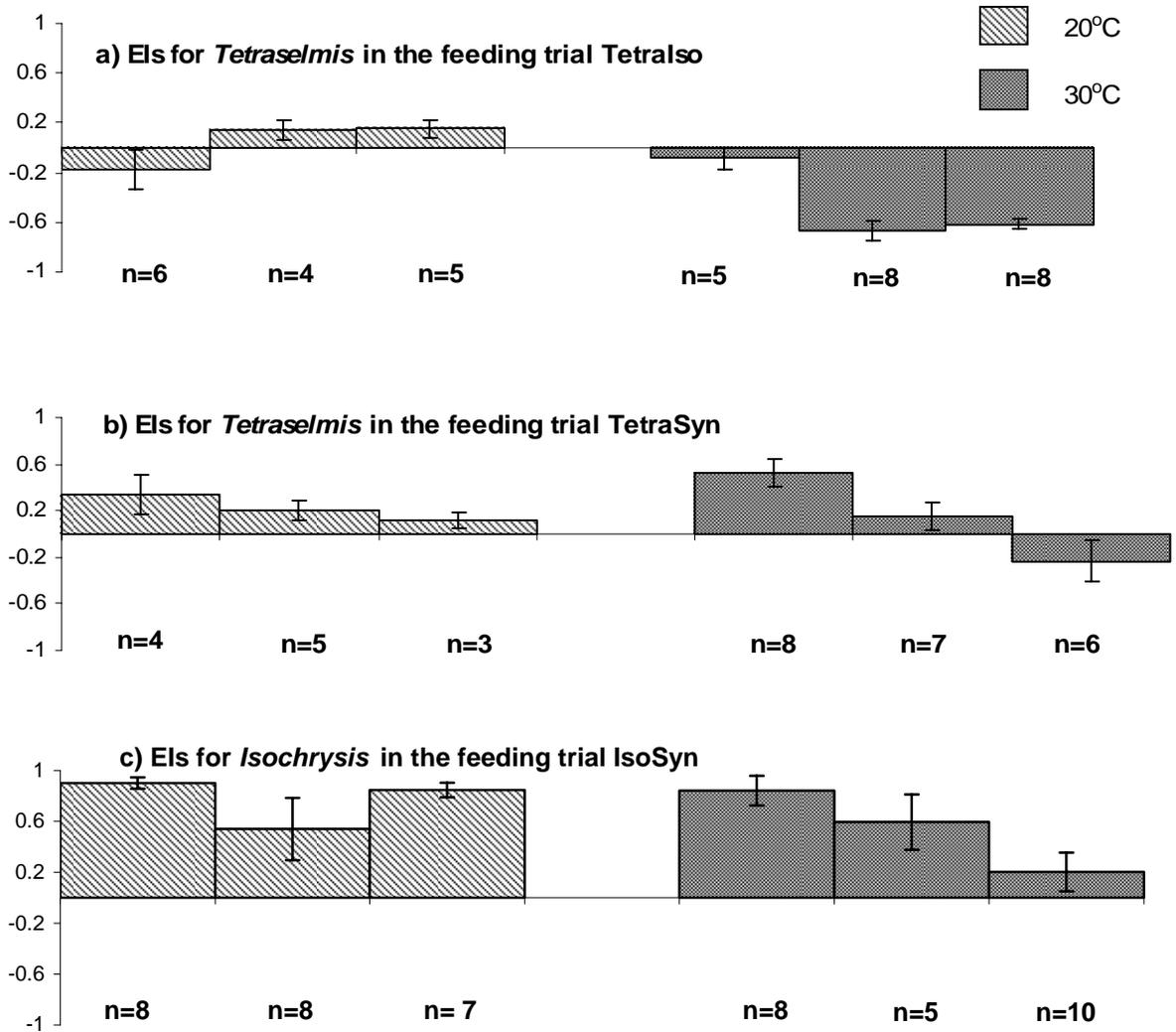


Figure 4. Mean replication (or batch) electivity indices (mean \pm SE) for *Mercenaria mercenaria* at two temperatures, 20°C and 30°C, when fed different combinations of algae. There were three replicates for each temperature. A positive EI indicates acceptance of an algal species. A negative EI indicates rejection of an algal species. a) Acceptance or rejection of *Tetraselmis* when *Isochrysis* is present at a total concentration of 10^5 cells/ml. b) Acceptance or rejection of *Tetraselmis* when *Synechococcus* sp. is present at a total concentration of 10^5 cells/ml. c) Acceptance or rejection of *Isochrysis* when *Synechococcus* is present at a total concentration of 10^5 cells/ml. d) Acceptance or rejection of *Isochrysis* when the chainforming species of *Synechococcus* is present at a total concentration of 10^5 cells/ml. e) Acceptance or rejection of *Isochrysis* when *Synechococcus* is present at a total concentration of 10^6 cells/ml.

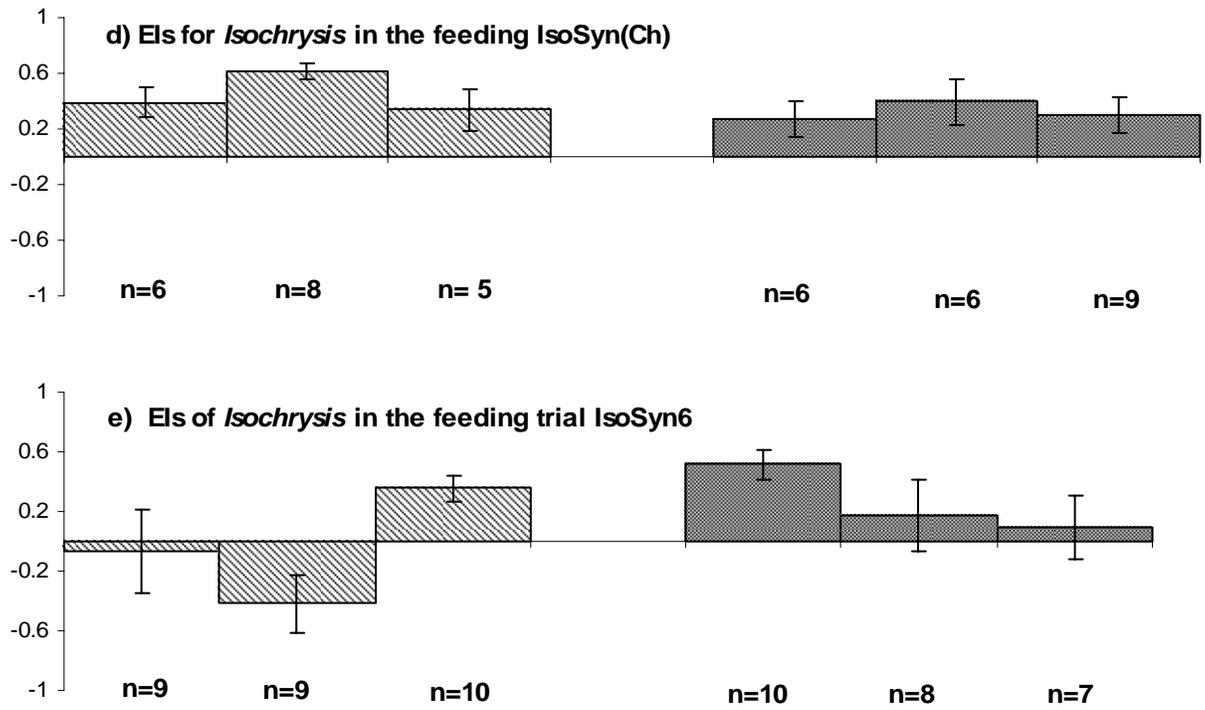


Figure 4. Continued.

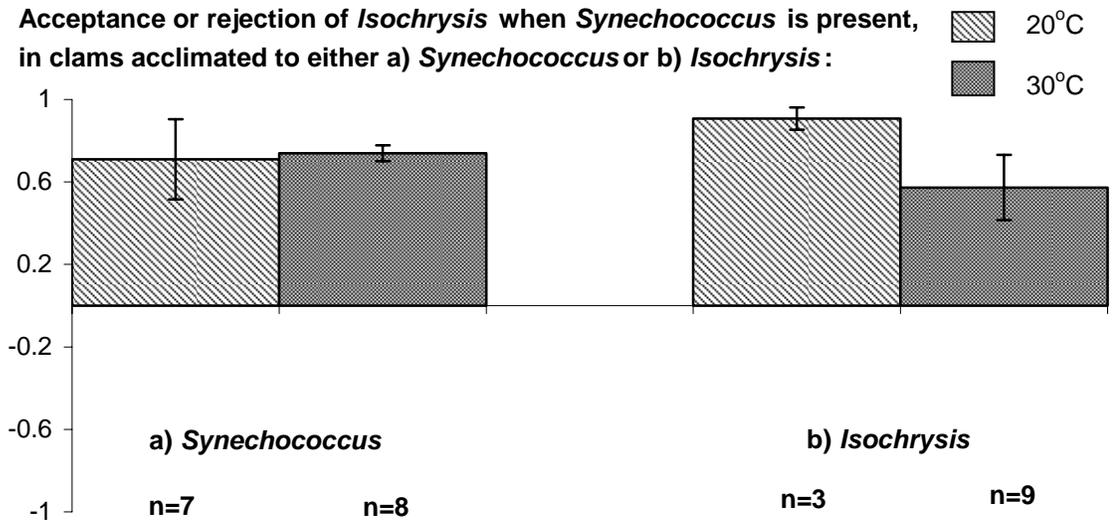


Figure 5. Mean electivity indices of *Mercenaria mercenaria* for *Isochrysis galbana* when *Synechococcus* sp. is present, at two temperatures, in clams acclimated for two weeks on either a) *Synechococcus* (IsoSyn-AS) or b) *Isochrysis* (IsoSyn-AI). Total cell concentration was 10^5 cells/ml. A positive EI indicates selection of *Isochrysis*. (Means \pm SE).

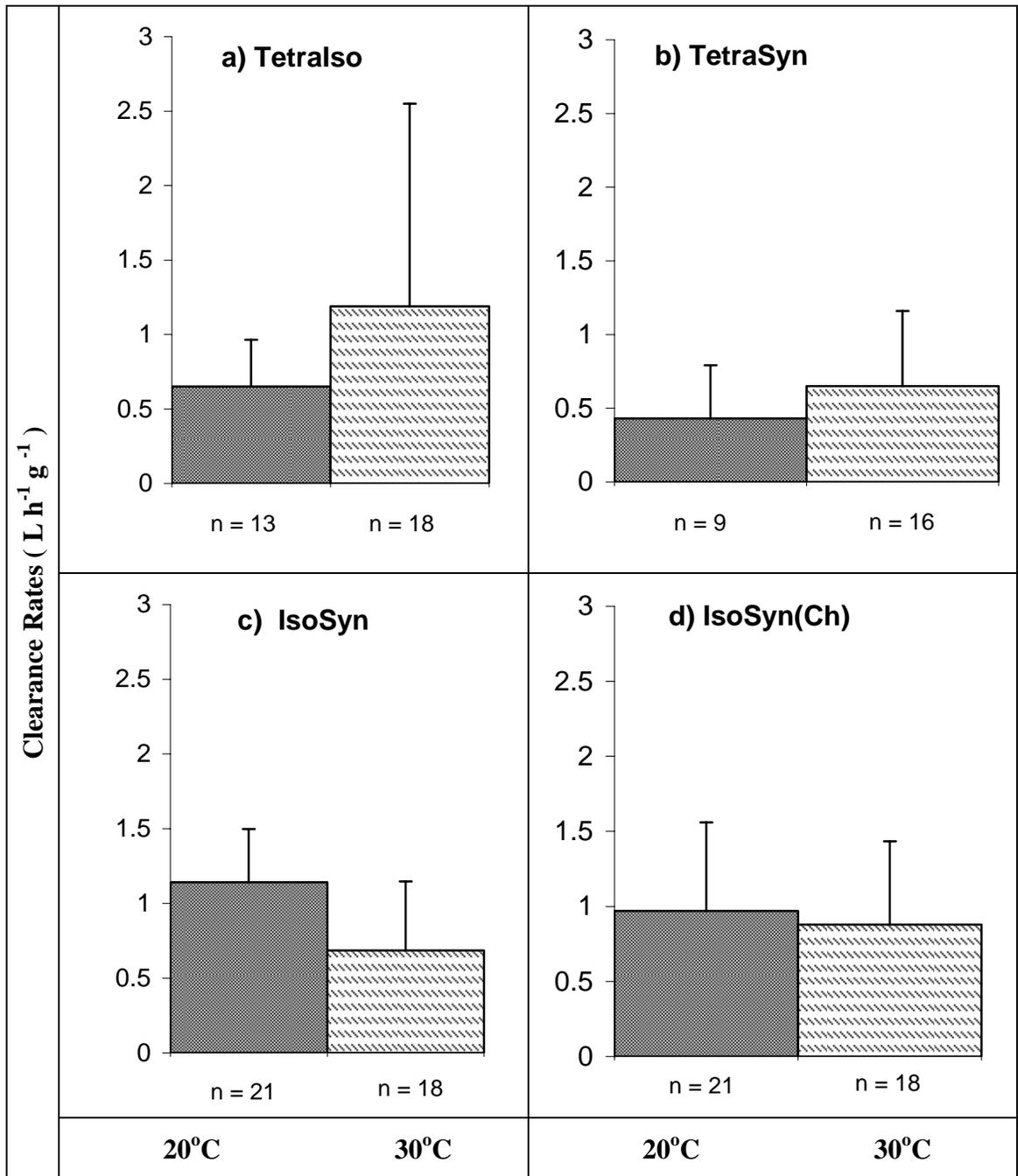


Figure 6. Clearance rates by *Mercenaria mercenaria* (means \pm SE) at two temperatures (20°C and 30°C) when fed algal suspensions at 10^5 cells/ml. All clearance rates were standardized to 1 gram of dry weight.

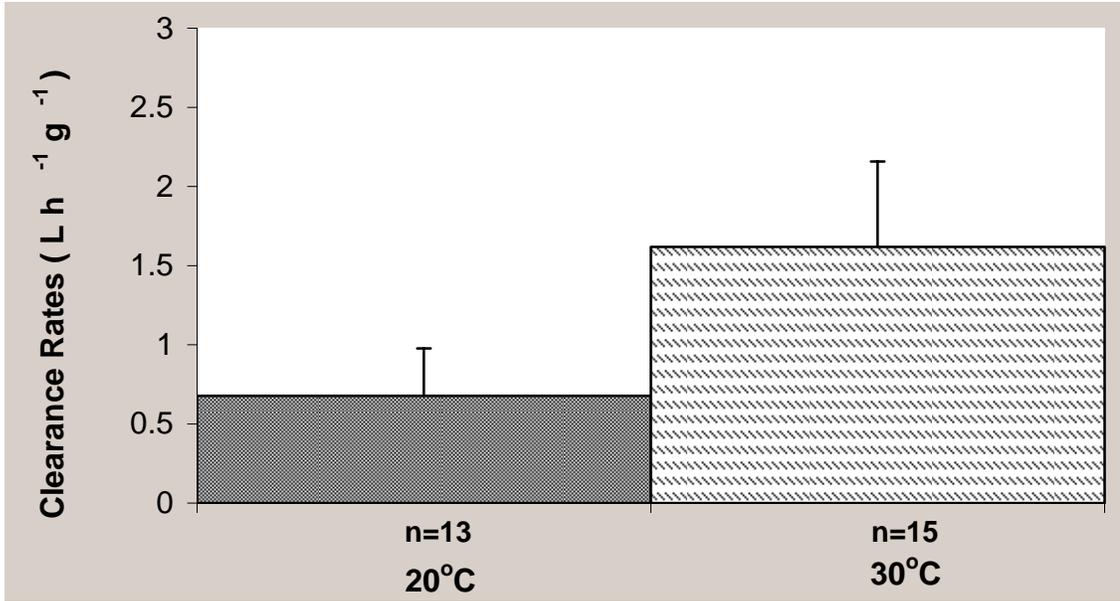


Figure 7. Clearance rates (means \pm SE) of *Mercenaria mercenaria* fed *Isochrysis galbana* and the nonchainforming strains of *Synechococcus* (IsoSyn-B) at two temperatures (20°C and 30°C). A single batch of clams was split between the two temperatures groups.

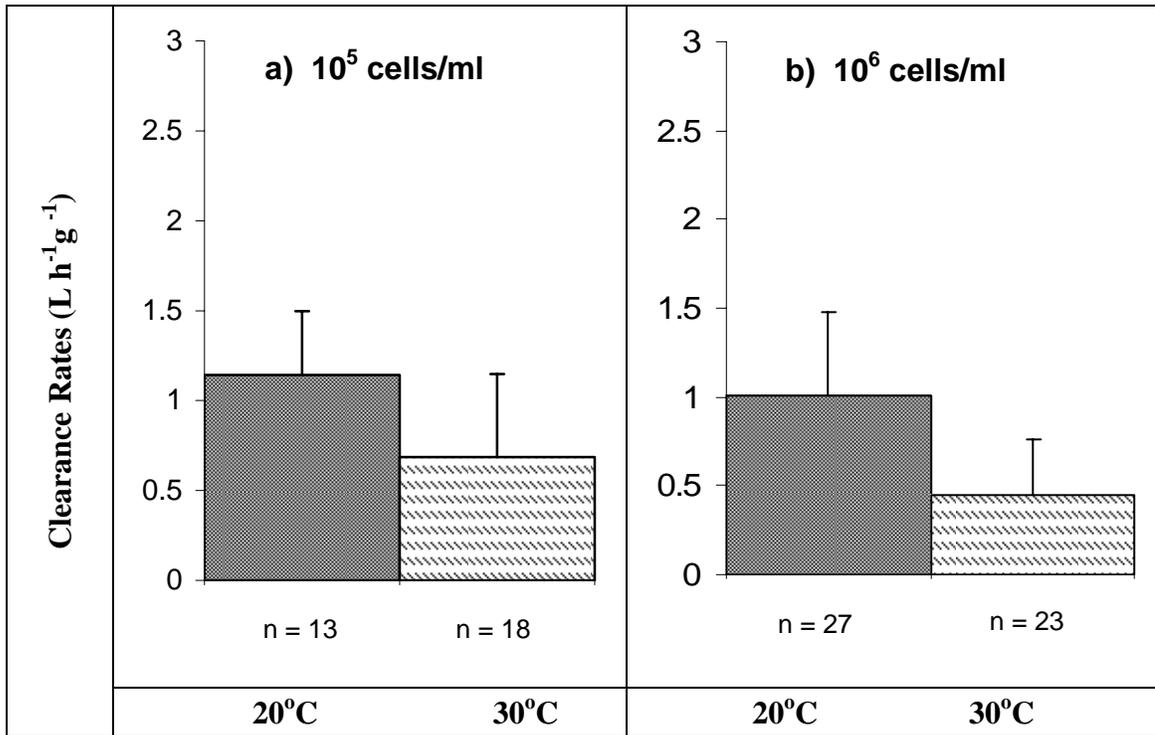


Figure 8. Clearances rates (means \pm SE) by *Mercenaria mercenaria* fed *I. galbana* and *Synechococcus* sp. at two temperatures (20°C and 30°C) and two concentrations: a) 10^5 cells/ml (IsoSyn) and b) 10^6 cells/ml (IsoSyn6).

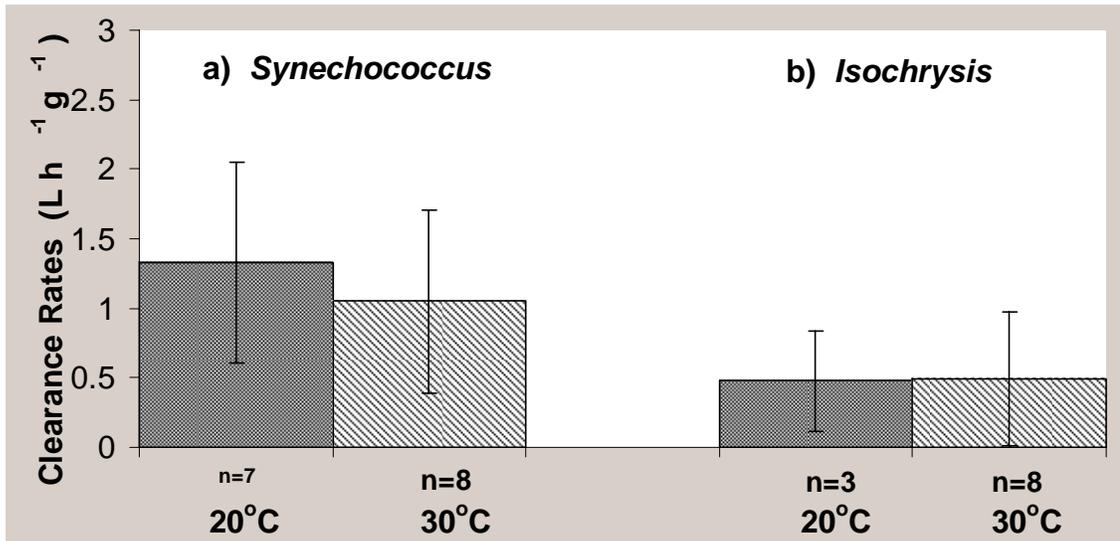


Figure 9. Clearance rates (means \pm SE) of *Mercenaria mercenaria* of the feeding trial *Isochrysis galbana* and the nonchain-forming species of *Synechococcus* (IsoSyn-AS and IsoSyn-AI) at two different temperatures (20°C and 30°C) when clams were acclimated to either a) *Synechococcus* or b) *Isochrysis*.

CHAPTER 5 DISCUSSION

This is the first study to conduct feeding selectivity experiments with bivalves at temperatures above 20°C . Most feeding selectivity studies have been conducted at temperatures between 12°C and 20°C (Shumway *et al.*, 1985; Bayne *et al.* 1989; Stenton-Dozey & Brown, 1992; Bayne *et al.* 1993; Arifin & Bendell-Young, 1997; Defosse & Hawkins, 1997; Ward *et al.*, 1998); appropriate given the cooler temperatures of collection sites. For example, summer water temperatures at northeastern locations such as Wells, Maine; Hudson River, New York; and Narragansett Bay, Rhode Island, reach no more than 16.5°C, 22.6°C, and 23.3°C, respectively (NERRS, 2004). In the mid-Atlantic, such as Chesapeake Bay, Bay Bridge, Maryland, temperatures reach about 27.5°C in August (NERRS, 2004).

In the shallow waters along Florida's Gulf coast, however, temperatures exceed 30°C in the summer and are routinely > 25°C for half the year (Jett, 2004; NERRS, 2004; Frazer, unpublished data; Philips, unpublished data). In the Suwannee River Estuary, near Cedar Key, Florida (a prominent site for clam aquaculture), water temperatures can also get up to 30°C (Jett, 2004). The warmest National Estuarine Research Reserve (NERRS, 2004) sites in Florida can be found in Rookery Bay, Naples (about 70 miles south of Charlotte Harbor which is another site for clam aquaculture), where waters reach 35.6°C (NERRS, 2004). Therefore, this study is important because it is the first to examine how high temperatures (30°C) may influence feeding selectivity of bivalves, and *Mercenaria mercenaria* in particular.

The results of this study indicate that temperature affects feeding selectivity of Florida hard clams. Clams exhibited greater selection at 20°C than they did at 30°C. This finding may be the result of the acclimatizing protocol. When acclimatizing the clams to the experimental temperature, they were immediately placed in either 20°C or 30°C water, depending on the feeding trial, and held for three days. Since the lower temperature trials were done in late February and March, the experimental water was within a typical range of water temperatures for that time of year in Cedar Key. In the feeding trials at 30°C, there was a 10-20°C difference between the collection water temperature and the experimental water temperature (www.floridaaquaculture.com). For fish, acclimation to the experimental water temperature should be gradual to reduce shock which could change physiological states like hormone levels or blood chemistry concentrations (Stickney and Kohler, 1990; Wedemeyer *et al.*, 1990). Fry (1971) suggests a gradual acclimation schedule of 1°C/day until the experimental temperature is reached. While intertidal organisms can withstand daily temperature variations of 20-30°C, subjecting the clams to a temperature change of more than 10°C have shocked the clams (Hochachka & Somero, 2002). Therefore, the lack of acclimation to 30°C may have rendered the clams less efficient in their selective abilities.

Another reason for the difference in selectivity between temperatures may have been a “batch effect.” Again, a “batch” refers to a quantity of clams collected at one time from the same area and exposed to the same environmental history. Since they were collected at different times of the year, the clams used in the low temperature trials may have been subjected to different environmental parameters than those used in higher temperature trials. In the batch experiment (IsoSyn-B), in which one batch of clams was

split between two temperatures, temperature did not appear to have an effect on selectivity (Fig 2). This suggests that the difference in selectivity between 20°C and 30°C in the main experiment may largely be due to the batch used rather than to the lack of acclimation. More study is needed, however.

In addition to temperature, algal combination had a significant effect on electivity indices of hard clams. At algal concentrations of 10^5 cells/ml, there was greater selection for larger particles (*Tetraselmis* at 10 μm and *Isochrysis* at 5 μm) over smaller particles (*Synechococcus* sp. at 2 μm and with chains) (Fig 1). *Tetraselmis* and *Isochrysis* are both well within the size range to be completely retained by a bivalve while *Synechococcus* is on the lower end of the size range (Jørgensen, 1975; Møhlenberg & Riisgård, 1978). Bass *et al.* (1990) examined the growth of *M. mercenaria* on picoplankton which included *Nannochloris* (ca. 3 μm) and two species of *Synechococcus* sp. (both ca. 1 μm) in length. They found that, while the picoplankton were filtered out of suspension by the clam, it was assimilated with low efficiency (17.6-31.1%) compared to the 4 μm *Pseudoisochrysis paradoxa* (86.5%). The results from this study, however, showed preferential ingestion of the smaller *Synechococcus* (2 μm) over the larger *Isochrysis* (5 μm) in four of the eight feeding trials (Fig 2; Fig 4) done at 10^6 cells/ml. Bass *et al.* (1990) worked with smaller picoplankton and lower concentrations (5×10^4 to 10^5 cells/ml) which could be why a difference was seen in this study. Although the mean values of replicates indicated no preference for *Synechococcus*, there was a high amount of variability in electivity indices between batches of clams (Fig 2). In addition, although statistics were not done in Fig 4, the electivity indices were high (0.7). Because of these conflicting results, further studies would be interesting.

Algal concentration had a significant effect on selectivity, with clams exhibiting active selection at the lower concentration and no sorting at the higher concentration. A study by Levinton *et al.* (2001) suggests that rate-limiting steps within a bivalve's digestive process may affect how a bivalve processes particles. As the gut becomes full, bivalves may start to increase rejection of nutritious particles as pseudofeces that they would otherwise ingest. As a result, bivalves may show no preferential ingestion between algal species when presented at high concentrations.

There was no significant effect of temperature on clearance rates when clams were fed any of the four algal combinations at 10^5 cells/ml (Fig 6). This is in agreement with findings reported by Smaal *et al.* (1997), who showed clearance rates for mussels (*M. edulis*) to be independent of temperature. In addition, MacDonald *et al.* (1996) showed clearance rates for *Placopecten magellanicus* to be independent of temperatures over the range of 0 to 15°C. In a field study, Paterson *et al.* (2003) examined growth in the rock oyster *Saccostrea glomerata* in Australia for a year and found that, although there was variation in temperature between study sites, growth (a good indicator of feeding rates) was independent of temperature. It is interesting to note that while statistics were unable to be done on the clearance rates for the batch experiment, the mean clearance rate for 20°C was higher than that at 30°C. Again this could be because of a batch effect.

However, when comparing the two different concentrations (10^5 and 10^6 cells/ml) of IsoSyn, temperature was found to be significant with higher clearance rates at 20°C than at 30°C (Fig 8). This is in agreement with studies of northeastern clams. Hamwi (1969) showed that temperature had an inverted parabolic effect on pumping rates, i.e. the rate at which water flows through the mantle cavity, with pumping rates at 30°C being

much less than the pumping rates at 20°C. Other bivalves are reported to react much the same way. Over the temperature range of 16-28°C, scallops (*Aropecten ventricosus circularis*) had maximum clearance rates at 19°C and 22°C (Sicard *et al.*, 1999). In the clam *Ruditapes decussates* (L.), increasing temperature has a negative effect on clearance rates, leading to a reduction in scope for growth (Sobral & Widdows, 1997). In contrast, cockles appear to increase clearance rates with increasing temperature (Smaal *et al.*, 1997). Levya-Valencia *et al.* (2001) showed that temperatures of 29°C or higher were optimum for clearance, ingestion, and growth rates for the penshell *Atrina maura*.

In contrast to other studies, concentration did not have a significant effect on clearance rates temperature was held constant. Bayne *et al.* (1989) showed that ingestion rates (equivalent to clearance rates when no pseudofeces are produced) of *Mytilus edulis* increased with increasing seston concentration. Tenore and Dunstan (1973) showed that although feeding rates of *M. mercenaria* increased with increasing food concentration, they were still lower than the feeding rates observed in *M. edulis* and *C. virginica*. This could be the result of a combination of reduction in pumping rate or filtering efficiency and an increase in pseudofeces production as concentrations increase. In addition, each species' response to increasing food concentration may reflect adaptation of bivalves to the areas where they were collected. Additionally, Tenore and Dunstan showed that feeding rates in the three bivalves exhibit a slight inverted parabolic effect in association with seston concentration. In contrast, Bricelj and Malouf (1984) found a negative relationship between seston concentration and clearance rates in *M. mercenaria*, with clearance rates decreasing as seston concentration increases. In this study, however, effect of concentration had no effect on clearance rates. Both concentrations used in the

present study were relatively high. Tenore and Dunstan (1973) suggest that hard clams may not be well suited for feeding at high particle concentrations, compared to other bivalves.

While there were no differences between clearance rate replicates, there was a high amount of variability among replicates or batches in the selectivity experiments. Differences between replicates may be due to a variety of parameters including feeding history, adaptation of clams to their environment, seasonal changes in digestive enzymes, and/or other factors, e.g., changes in water viscosity due to temperature.

Feeding history, for example, may have an impact on feeding selectivity and digestion. Bayne (1993) noted that bivalves can shift feeding preferences as an adaptative strategy to seasonal changes in food availability. In addition, Ibarrola *et al.* (1998) showed that there is an apparent seasonal pattern of digestive enzyme activity that may be affected by past feeding history and could potentially affect food selection. However, in the feeding trials IsoSyn-AI and IsoSyn-AS where clams were acclimated for two weeks on either *Isochrysis* or *Synechococcus* sp., there appeared to be no difference in the feeding selectivity between clams previously fed *I. galbana* and those previously fed *Synechococcus* (Fig 5). While the clams were fed 2% of their dry weight per day during the acclimation period, a ration generally recommended for bivalves (www.reedmariculture.com), this resulted in concentrations of only 317 or 6349 cells/ml *Isochrysis* and *Synechococcus*, respectively. Therefore, total particle concentrations may have been too low to have a significant acclimatory effect on the clams. Additional studies with higher cell concentrations are warranted.

Although the two-week study was inconclusive, bivalves may become adapted to exploit the specific suite of food available to them in the field. For example, bivalves from areas that are dominated by high bacterial counts had higher rates of clearance of bacteria compared to bivalves from other areas not dominated by bacteria (Wright et al., 1982; Berry & Schleyer, 1983). The high variability in selectivity between batches of hard clams in this study, with four out of eight feeding trials at 10^6 cells/ml able to select small particles (2 μm), suggests that these batches had adapted to high counts of small particles in the environment. There have been no studies to date that compare the clearance rates of clams from Cedar Key with clams from other areas along the Atlantic coast that may typically feed on other particle types and sizes.

The absorption efficiency of particular phytoplankton species is determined by digestive enzymes, and enzymatic activity may be influenced by season or food availability (Bayne *et al.*, 1993; Ibarrola *et al.*, 1998). For example, Seiderer and Newell (1979) reported that *Choromytilus meridionalis* changed the activity rate of α -amylase in response to changes in temperature and, coincidentally, with phytoplankton composition. Ibarrola *et al.* (1998) also found seasonal variation of digestive enzyme activities in the cockle *C. edule*, in northern Spain, where their spring/summer diet is predominantly living phytoplankton while in fall it consists mainly of kelp detritus. There is speculation that assimilation efficiency corresponds to selectivity; phytoplankton that are easily assimilated are selected for ingestion (Baker et al., 1998). It follows that if digestive enzymatic activity changes seasonally and in response to available food, then selectivity should change also. This offers a further explanation for differences in the ability of batches to select for the smaller algae, *Synechococcus* sp.

Temperature may affect the physiology of bivalves and, as a consequence, the ability to process certain food items. Temperature may also have an effect on the mechanical aspects of suspension feeding. For example, because temperature is inversely related to viscosity, suspension feeding echinoderm larvae (*Dendraster excentricus*) are more apt to ingest large particles when the water has a high viscosity (colder) (Podolsky, 1994). Podolsky (1994) suggested that changes in viscosity might also affect retention efficiencies of bivalves. Since waters in the Suwannee River Estuary change seasonally from cool ($\leq 11^{\circ}\text{C}$) to hot ($\geq 30^{\circ}\text{C}$) (Jett, 2003; Frazer, unpublished data; Phlips, unpublished data), water viscosity could play a role in what hard clams are able to filter and ingest.

In conclusion, temperature was found to have an effect on food selectivity, with Cedar Key hard clams exhibiting greater selection at 20°C than at 30°C . In addition, I found that temperature had almost no effect on clearance rates. However, due to the wide variability in results, more studies are needed to further test the effects of temperature and batch effect on particle selectivity and clearance rates for Cedar Key hard clams and bivalves in general. For example, it would be interesting to determine whether a batch effect is unique to Cedar Key hard clams, common to all hard clams, or common to all bivalves. In addition, it would be interesting to see how much the batch effect changed over a year and whether it corresponded to phytoplankton abundance or composition in the Suwannee River Estuary. It is documented that cyanobacteria like *Synechococcus* are common in Florida waters, especially in the summer (Phlips et al., 1999; Bledsoe, 2003) when the waters are warm, and bigger sized phytoplankton are common in the winter. It would make sense, then, that the clams tested in July 2003 would prefer the smaller sized

cyanobacteria and why they rejected it when the experiment was performed again in January through March 2004 (Figure 3). Temperature may have additional indirect effects by either affecting phytoplankton composition in the estuary or by being a conditioning factor for bivalves living in the area. This study is important in that it is one of the first to examine feeding selectivity of bivalves in association with changes in temperature. The results suggest important additional avenues of research which will be essential to improving aquaculture practices in warmer climates, especially for the growth and stability of the clam farms located in the vicinity Suwannee River Estuary.

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BIOGRAPHICAL SKETCH

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