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Physiological Ecology of the Cultured Hard Clam, Mercenaria mercenaria: A Case Study in Cherrystone Inlet, Virginia

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PHYSIOLOGICAL ECOLOGY OF THE CULTURED HARD CLAM, 

*MERCENARIA MERCENARIA*:

A CASE STUDY IN CHERRYSTONE INLET, VIRGINIA

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A Thesis
Presented to

The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment
Of the Requirements for the Degree of

Master of Science

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by

Elizabeth Darrow Condon

2005
APPROVAL SHEET

This thesis is submitted in partial fulfillment of

the requirements for the degree of

Master of Science

Approved, July 2005

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DEDICATION

To Rob, who spent many long nights with me in the lab;
and to Ian, who spent many long days with me in the field:

I love you both.
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ABSTRACT

This study combined laboratory, field, and modeling approaches to analyze the physiology of the cultured hard clam and compare it to the physiology of wild clams from previous studies. These results will be incorporated into a large-scale carrying capacity model for Cherrystone Inlet, Virginia. Laboratory-determined clam clearance rates were generally higher than in situ clearance rates, but both were highly variable. Clams were very sensitive to low food concentrations and low salinities, and generally exhibited an “all or nothing” feeding response during the laboratory studies. Egestion rates were lower for cultured clams than for past studies on wild clams; respiration rates were slightly lower for cultured clams; and excretion rates were comparable for cultured and wild clams.

In the field component of the study, clam clearance rates for field feeding experiments were lower on average than laboratory experiments for a similar same size range, temperature, and salinity. Clearance rates in the field were demonstrated to be predominantly reliant on chlorophyll $a$ and phaeophytin concentrations. Tidal energy was shown to be an important factor for resuspension of detritus from the benthos, which may be an important alternate food source for clams during months when phytoplankton production is limiting. Predator exclusion nets used in the clam growout practice may be inhibiting clam feeding processes and/or food availability.

Based on population data from the current study for the years 2003-2004 and physiological rates from previous studies, a simple bioenergetics model was constructed for the clam population in Cherrystone Inlet. Model results indicate that food (particulate organic carbon) is limited during months when clam metabolic rates are high but net primary productivity is relatively low. These effects are most prominent in the spring and fall, times of year when the water temperature is ideal for clam growth, but food limitation may be slowing growth rates. Despite these limitations, Cherrystone clams are still growing at a reasonable rate, reaching market size ($7/8"$ in width, which equals 40.05 mm in length) in approximately 20 months from planting. Clams in Cherrystone are estimated to play an important role in nitrogen cycling in the creek.

This study shows that existing laboratory estimates of physiological rates (feeding, biodeposition, respiration, and excretion) are probably accurate for the cultured hard clam feeding on natural seston; however, in situ factors affecting seston availability and clam feeding rates are multifaceted and are not well understood. Physical forcing on multiple scales, from tidal cycles to seasonal cycles, has a considerable impact on the physiology and ecology of clam growout sites. It is important to consider in situ factors when incorporating bivalve physiology into an ecosystem-scale model.
PHYSIOLOGICAL ECOLOGY OF THE CULTURED HARD CLAM,

*MERcenaria mercenaria*:

A CASE STUDY IN CHERRYSTONE INLET, VIRGINIA
The hard clam

*Mercenaria mercenaria* (Linnaeus, 1758) is an infaunal bivalve species, abundant in soft substrates throughout its native habitat on the east coast of North America (Ansell, 1968). The hard clam is naturally found in small patches to extensive beds at intertidal and subtidal depths; in relatively bare, coarse sediments as well as in eelgrass beds and among oyster beds (Harte, 2001). *M. mercenaria* is known to be a marine species, but is fairly tolerant of low salinities (Wells, 1961), as well as a wide range of temperatures (Harte, 2001). The hard clam can sustain itself during adverse conditions by closing its valves and respiring anaerobically for extended periods (Wells, 1961). The biology of the hard clam was reviewed recently by Kraeuter and Castagna (2001).

As suspension feeders, clams graze on natural seston in the water column by extending their siphons from below the sediment surface and pumping the water, and its contents, past its gills where food particles are removed. Clams are assumed to feed opportunistically, their diet consisting of water column phytoplankton, as well as resuspended benthic microalgae, detritus, and possibly bacteria and dissolved organic matter (Bayne and Hawkins, 1992). The hard clam’s gill cirri can retain particles larger than 4 μm in diameter with 100% efficiency, and particles from 2 – 4 μm with 50% efficiency. Below 2 μm, particle retention efficiency rapidly decreases (Riisgård, 1988), which excludes most free-living marine bacteria from being filtered efficiently. Of the suspended particles drawn into the mantle cavity by the incumbent siphon, those with
higher organic content can be selected for ingestion, while less nutritious particles are rejected as pseudofeces via the incurrent siphon, before entering the digestive tract. Presumably, particles with higher organic and nitrogen content (such as phytoplankton) would be selected in preference to less nutritious particles, such as detritus from macroalgae or vascular plants. A few models have been conceived for *M. mercenaria* physiology using phytoplankton as the clams’ sole food source (e.g., Doering and Oviatt, 1986). It is currently unknown what component of the clams’ energy budget may consist of alternate food sources, such as detritus (Grizzle et al., 2001). Langdon and Newell (1990) determined that it was unlikely that the oyster *Crassostrea virginica* or the mussel *Mytilus edulis* could survive by feeding solely on detritus from the marsh grass *Spartina alterniflora*. The carbon in macroalgal detritus is less refractory than the carbon from vascular plants, however, and macroalgae has been shown to be a more nutritious food source than vascular plant tissue, for the polychaete *Nereis diversicolor* (Olivier et al., 1997). It is currently unknown whether hard clams could survive using macroalgal detritus as an alternate food source to phytoplankton.

In comparison to another common mid-Atlantic species, the eastern oyster *Crassostrea virginica*, clams generally are found at higher salinities, and in deeper water. *M. mercenaria* has been found to have lower filtration rates (Tenore and Dunstan, 1973, Newell and Koch, 2004), biodeposition rates (Tenore and Dunstan, 1973), and excretion rates (Srna and Baggaley, 1976) than *C. virginica*. *M. mercenaria* is less sensitive to changes in temperature than *C. virginica* (Newell and Koch, 2004), and responds to high particle concentrations by closing the valves rather than producing copious quantities of pseudofeces (Tenore and Dunstan, 1973).
**Hard clam aquaculture**

Clams have been harvested in North America for their meat and shell since pre-colonial times, but commercial production did not become substantial until the late 1880s (MacKenzie Jr. et al., 2001). Hard clams are fished using a number of methods, including raking, hand tonging, patent tonging, and dredging. The hard clam fishery is notable for being more stable than other fisheries (e.g., the scallop fishery), due to the clams' longevity and relatively slow growth rates (MacKenzie Jr. et al., 2001). *Mercenaria* has been successfully reared in culture since the 1920s (Castagna, 2001), but commercial hatcheries did not start in earnest until the 1950s. Hard clam aquaculture is currently becoming a major industry in regions previously dominated by the oyster (*C. virginica*) fishery, which was decimated by overfishing and disease.

Currently, *M. mercenaria* culture consists of three phases: (1) hatchery, (2) nursery, and (3) grow out. Broodstock are selected, spawned, and larvae are reared in the hatchery. The nursery stage begins once larvae metamorphose, so that clams may be grown to a larger size before they are planted in the grow-out area (Castagna, 2001). On the eastern shore of Virginia, clams are planted and grown to market size on natural bottom in tidal creeks and bays under predator exclusion nets to avoid predation by crabs, rays, birds, and other large animals. In Cherrystone Inlet, a tidal tributary of the Chesapeake Bay on the western side of the Delmarva peninsula (Figures 1.1, 1.2), clam beds are planted at water depths shallow enough to be monitored and maintained on foot, generally less than one meter in water depth.

In recent years, the hard clam aquaculture industry in Cherrystone Inlet has reported slower growth rates in some grow-out areas, expressed as longer time to market size.
FIGURE I.1 Map of the Chesapeake Bay region, including study sites Cherrystone Inlet, Wachapreague, and Gloucester Point, Virginia
FIGURE I.2 Location of study sites in Cherrystone Inlet

A. Upstream
B. Midstream
C. Downstream
Growers have speculated that the creek may be reaching its carrying capacity due to food limitation of the clam population. An alternate hypothesis is that the predator exclusion nets may be having a detrimental effect on clam feeding, either by interfering with the suspension feeding process directly, or by modifying the benthic environment in more indirect ways that negatively affect clam growth. Yet another alternate hypothesis is that inbreeding of clams by aquaculturalists has reduced feeding rates or growth efficiencies. The effects of environmental factors such as temperature and salinity on clam physiological processes must also be considered, as well as whether feedbacks to the nitrogen pool via clam biodeposition (feces and pseudofeces production) and excretion have the potential to influence primary production. Determining whether putative decreases in clam growth rates are due to environmental (both natural and human-induced) or physiological factors is essential to the management of this industry.

**Physiology**

The physiology of wild clams has been studied previously, but because the cultured clams in this area have been selectively bred for faster growth, it is possible that the underlying physiology of the domesticated clams is different than the physiology of wild animals. Slower growth of clams could result from either: (1) decreased food availability, (2) lower energy acquisition (i.e., slower feeding rates for a given amount of food), or (3) less efficient energy utilization via increased egestion, respiration, or excretion. In order to determine whether changes in clam growth rates are due to environmental (number 1 above) or physiological factors (numbers 2 and 3), it is necessary to study the physiological ecology of this organism under both laboratory and
field conditions. Hard clams are not grown in their "natural" environment when grown at high densities under predator exclusion nets, immobilized by a combination of these nets and the fine sediments deposited by erosional processes influenced by local agricultural practices (Arnold et al., 2004). Predator exclusion nets act as a substrate for macroalgae and other organisms, and may affect sediment biogeochemistry and hydrodynamics of the benthic boundary layer. Perhaps most importantly, nets and their associated macroalgae may act as a physical barrier to seston flux, decreasing the availability of food to clams below them.

Clam growth rates may be affected by changes to at least one of the variables of the bioenergetics mass-balance equation, expressed by as:

\[ I = P + R + F + U \]

where \( I \) is ingestion, \( P \) is production or tissue growth, \( R \) is respiration, \( F \) is egestion (fecal production), and \( U \) is excretion. An increase in \( I \) or a decrease in \( F, R, \) or \( U \), could result in increased growth. Therefore it is important to study all components of the energy budget to determine influencing factors on growth rates.

The bioenergetics of wild \( M.\ mercenaria \) have not been studied as extensively as for some other bivalve species; nevertheless, data exist in the literature describing the physiological rates of this species over a range of environmental conditions. Very few, if any, data currently exist on the bioenergetics of cultured \( M.\ mercenaria \). Components of the energy budget are usually expressed as functions of clam size (shell length and/or
meat dry weight), and external temperature, recognizing the large influence of these factors on rate processes such as feeding and respiration (Hamwi, 1969). Other important environmental variables include seston concentration and composition (Tenore and Dunstan, 1973), suspended sediment concentration (Bricelj and Malouf, 1984), salinity and oxygen concentration (Hamwi, 1969), and water flow rate (Grizzle et al., 1992). Of these environmental factors, only temperature and salinity have been studied for *M. mercenaria* across a range approaching typical variability in nature (Grizzle et al., 2001). It is possible, however, to construct a bioenergetics model for the hard clam based upon basic relationships observed in previous studies and use these relationships to predict the response of a typical clam to the environmental conditions in an aquaculture grow-out area such as Cherrystone Inlet.

Most classical studies on bivalve physiology generally, and *M. mercenaria* physiology specifically, have been conducted in the laboratory under controlled conditions. Much of the existing knowledge about bivalve physiology comes from extensive studies on the blue mussel *Mytilus edulis* (i.e., Bohle, 1972; Bayne et al., 1976; Bayne and Newell, 1983; Famme et al., 1986; Riisgård, 1991; Clauesen and Riisgård, 1996), as well as many on other species of mussel (Griffiths and King, 1979; Berry and Schleyer, 1983; Charles and Newell, 1997); scallops (Bricelj et al., 1987; Bricelj and Shumway, 1991; Cranford, 1995) and oysters (Dame, 1972; Newell and Langdon, 1986; Newell et al., 2004).

Generalizations are often made on the feeding and respiratory physiology of all bivalves based on studies of *M. edulis* or other specific species of bivalve, making assumptions on the functions or ecological impacts of a species for which little specific
data exists. For example, there are few studies on the physiology of the hard clam *Mercenaria mercenaria*, so generalizations are often made using the oyster *Crassostrea virginica* as a model organism, or using average physiological rates for all bivalves (i.e., Powell et al., 1992). Assumptions that all bivalves feed alike may be true in the general sense, as most species are suspension feeders which attain most of their nutrition from phytoplankton food sources. However, more specific characteristics of the feeding mechanism, food sources, nutrient and oxygen metabolism, are very species specific. For example, life history has a large effect on feeding in bivalves. Many bivalves, including clams such as *M. mercenaria*, are infaunal suspension feeders, burrowing into the sediment and using the siphon to pull water full of suspended food particles through the gills where filtering takes place. Both mussels and oysters can attach themselves permanently to the surface of a substrate, using respectively byssal threads (mussels) to attach to structures or other mussels; or a cement-like substance (oysters) to attach to substrates or other oysters to form reefs. Scallops generally lie horizontally on the surface of the substrate, are very active, swimming by clapping their shells together. Other bivalves have unique life histories such as boring into wood, shell, or coral (Gosling, 2003); or using symbiotic zooxanthellae to aid in the fixation of carbon. The location of a bivalve in the water column and its locomotory abilities will have a large effect on the type and quantities of food available to it. A bivalve's diet is also affected by its morphology, most importantly the morphology of its gill structures (see Gosling, 2003 for review).
Ecology

Impacts of suspension-feeding bivalves on coastal and estuarine ecosystem processes have been well established in numerous systems throughout the world. In ecosystems where bivalves are found in large densities, they often represent a major functional component, consuming large quantities of primary producers and strongly coupling the benthic and pelagic environments (Dame, 1996). Bivalves can serve as keystone species in certain ecosystems by providing structural complexity (Jones et al., 1994), creating bioturbation (Levinton, 1995), and by modulating nutrient flux (Dame, 1993).

Commercially important bivalves, such as clams, oysters, and mussels, often grow naturally or are cultured in dense aggregations that can have significant impacts on primary production and nutrient dynamics. Suspension feeders facilitate benthic-pelagic coupling by consuming particulate organic matter from the water column, processing this material, and packaging organic waste into mucus-bound pseudofeces and feces (collectively termed biodeposits), which are deposited onto the benthos. Dense aggregates of particulate organic nitrogen (PON) are then available for the microbially-mediated processes of ammonification, nitrification and denitrification. The presence of bivalves increases flux of organic matter to the benthos, but the fate of this organic matter is dependent upon sediment conditions, such as oxygen availability. In the presence of oxygen, approximately 20% of deposited PON is nitrified and subsequently denitrified to N₂ gas (Newell et al., 2002), while 80% is ammonified, entering the dissolved inorganic nitrogen (DIN) pool. DIN may be subsequently taken up by phytoplankton, macroalgae, benthic microalgae, or other autotrophs. The presence of high densities of clams under predator exclusion nets may mediate these processes by: (1) increasing local
concentrations of sediment PON and ammonium due to high densities of bivalves, and
(2) affecting benthic oxygen concentrations by providing substrate for macroalgae
growth, causing high daytime oxygen concentrations due to photosynthesis and low
nighttime concentrations due to respiration. Subsequent effects of sediment hypoxia
include declining nitrification-denitrification and further buildup of ammonium in
sediments; and increased hydrogen sulfide concentrations in sediments. Hydrogen
sulfide is known to be toxic to most animals, including *Mercenaria mercenaria*
(Bergquist et al., 2003), which would likely decrease clam growth.

The influence, both quantitative and qualitative, of suspension-feeding organisms on
their food web, can include both direct and indirect effects. Suspension-feeding bivalves
graze phytoplankton and other water column autotrophs through “top-down control”
(Dame, 1996), but there are conflicting conclusions in the literature on the role bivalves
play in controlling phytoplankton abundance through “bottom-up” processes. Grazing is
often cited as a direct top-down control on primary producers (Sterner, 1986). However,
bivalves can also affect primary producer abundance via indirect bottom-up controls such
as nutrient and resource limitation or enrichment. Direct top-down effects may be seen in
regions of bivalve aquaculture, where bivalve densities are high and may be limiting to
primary production while reaching their own carrying capacity (Héral, 1993).

As mentioned above, high densities of bivalves can also affect their autotrophic food
sources indirectly, by mediating nitrogen fluxes between the water column and benthos.
Speculations have been made that high-density aquaculture areas could reach bivalve
carrying capacity when food is limited due to clam-induced nitrogen limitation of
phytoplankton growth (Kaspar et al., 1985). Theoretically, bivalve biodeposits can act to
sequester particulate nitrogen in sediments, slowing remineralization processes and limiting nitrogen availability to the water column, thus “stabilizing” estuarine ecosystems and replacing nutrient limitation control with suspension feeder grazing control of algal biomass (Herman, 1993). Alternatively, some studies have found that phytoplankton biomass is not reduced by bivalve grazing and that water column primary production actually may increase in treatments where bivalves are present (Doering and Oviatt, 1986). These positive effects on primary productivity may be a result of bivalve excretion of ammonium, which is a significant source of dissolved inorganic nitrogen (DIN) to the water column phytoplankton. In experimental mesocosms and raceways, clams have been found to increase total nitrogen, total DIN, and ammonium flux from the sediment to the water column (Doering and Oviatt, 1986; Tenore and Dunstan, 1973). Doering et al. (1987) also found that while DIN flux from the benthos was significant in the presence of clams, water column concentrations of DIN were low and relatively invariant. They hypothesized that DIN was probably utilized by phytoplankton in the water column, contributing to an observed doubling of system production. In a field study, Murphy and Kremer (1985) found that while the benthic flux of ammonium in a clam-dominated lagoon was seasonally variable, the net ammonium flux was more than the annual requirements of lagoon phytoplankton for net production.

In a region such as Cherrystone Inlet where clams are grown at much higher densities than in the wild, ammonium fluxes from the sediments would be expected to be greatly increased. In Hungar’s Creek, an inlet slightly north of Cherrystone with large amounts of clam aquaculture, clam beds were found to be a significant source of ammonium throughout the year in 2000 (Neikirk et al., 2001). Benthic microalgae, and high
macroalgal biomass associated with the clam nets (125 - 150 g DW m⁻²), were important removers of this dissolved inorganic nitrogen.

Doering et al. (1986) proposed that in bivalve-dominated systems, there can be "a stimulatory feedback effect from filter feeder to water column producer which tends to counter-act the potential negative effect of grazing on standing stock," which occurs through enhanced return of nutrients from the bottom. Kaspar et al. (1985) cited high rates of ammonium excretion from cultured mussel beds in New Zealand and emphasized the importance of mussel excretion as an immediately-available nitrogen source for primary production.

In addition to exerting control upon eutrophication and nutrient mineralization, suspension feeders can stimulate primary production by (1) enhancing log growth by keeping the phytoplankton population relatively younger so that phytoplankton do not senesce; (2) keeping phytoplankton densities low, thus increasing light availability to the living cells; and (3) shifting the size and/or species of phytoplankton within the community through selective pressure (Dame, 1993). These are examples of the mutualism-cybernetics theory of Odum and Biever (1984) who suggest that some plant-herbivore interactions are mutualistic, with herbivores stimulating the production of the plant species they feed upon. These types of autotroph-heterotroph mutualisms are considered to be "major subsystem controllers of the ecosystem" (Patten and Odum, 1981) or "keystone mutualists" (Gilbert, 1980). The importance of these feedbacks will obviously depend on residence times and physical processes of the water body in question, as well as the density of bivalves.
Another important characteristic of heavily fished or aquaculture-dominated ecosystems is that biomass is continually being removed by the harvest of bivalves, acting as a nitrogen sink from the system. The complex mutualistic nature of bivalve/phytoplankton dynamics is likely mediated by carbon and nitrogen flux through the system (Dame, 1996) and is related to the residence time of the embayment, the density of bivalves and whether the bivalves are being removed via harvesting.

**Modeling**

As much as suspension feeders control the abundance of phytoplankton, the ecological carrying capacity of bivalve populations is often constrained by phytoplankton production (Heip et al., 1995). Oyster reefs have been shown to demonstrate both top-down and bottom-up control of ecosystem function, depending on whether oysters are limiting phytoplankton growth or phytoplankton/food availability is limiting oyster growth (Ulanowicz and Tuttle, 1992; Dame and Libes, 1993). Carrying capacity models have been developed for different systems to predict the influences of phytoplankton abundance and physical characteristics of the ecosystem on bivalve production (Héral, 1993; Raillard and Ménesguen, 1994; Klepper et al., 1994). In an ecosystem box model of a macrotidal oyster aquaculture system in Marennes-Oléron Bay, France, Raillard and Ménesguen (1994) found that hydrodynamic processes have an overriding effect on renewal of food and oyster carrying capacity in the ecosystem. Utilizing relatively simple calculations of residence times and phytoplankton growth, the authors determined that significant accumulation of food is prohibited because the flushing time of water masses in the estuary is shorter than the doubling time of the phytoplankton. While this model describes phytoplankton growth and its limitations adequately, it does not consider
alternate food sources to the oyster population, such as benthic microalgae and detritus, which can be significant (Newell and Langdon, 1986; Newell et al., 2002).

The Powell et al. (1992) generalized bivalve feeding model calculates filtration rate as a function of biomass, temperature, salinity, and total particulate content. The model uses an assimilation efficiency of 0.75, which is typical of oysters feeding on phytoplankton under laboratory conditions (Tenore and Dunstan, 1973). This model’s predictive power may be improved by considering feeding on alternate food sources, which could lower assimilation efficiency.

Many existing bivalve feeding and carrying capacity models are based on either (1) theoretical predictions of physiological rate functions (i.e., Grant et al., 1993; Herman, 1993); or (2) laboratory-based estimates of feeding, respiration, and egestion rates (i.e., Doering and Oviatt, 1986; Powell et al., 1992), and are validated using field-based measurements of bivalve growth. It is apparent from field studies on bivalve growth that factors such as water current velocity (Grizzle and Morin, 1989), benthic turbulence (Muschenheim, 1987; Irlandi and Peterson, 1991), sediment type (Murphy and Kremer, 1985), and bivalve density (Crenshaw, Jr. et al., 1996), can have significant effects on bivalve feeding rates and assimilation efficiencies. Many of these environmental effects are difficult to describe empirically, so they are excluded from predictive models of bivalve growth. Likewise, terms such as “detritus” and “food quality” are nebulous and are not consistently measured using parameters that are significant to bivalve feeding. Carrying capacity models based upon long-term datasets of multiple environmental factors, including hydrodynamic and biogeochemical parameters, appear to most accurately depict environmental variability.
It is important when constructing a model of bivalve feeding and ecological effects, to base parameters on data for the particular species in question. Ideally, such a model will be combined with manipulative experiments to determine physiological rates under the range of conditions that would normally be seen in the study area, and that will be included in the model. This includes estimates of feeding, respiration, and excretion rates over seasonal temperatures, and using water with natural assemblages of seston and normally occurring concentrations of nutrients. Many past laboratory studies on *M. mercenaria* physiology attempted to quantify respiration or excretion rates in the laboratory using filtered seawater and starved animals (i.e., Hamwi, 1969; Srna and Baggaley, 1976). While the experiments were well controlled and information collected is useful, experiments were not conducted under normal conditions for the animals. It is important to supplement these data with experiments conducted using natural water and animals that are not starved, to find the range of physiological rates which may be expected for a species under natural conditions. It is difficult from a statistical standpoint to reproduce experiments using natural water which is changing in space and time, however, it is very important to determine how animals react physiologically under natural conditions.

**Study site**

Cherrystone Inlet is a small (6 km²) coastal embayment on the Chesapeake Bay side of the southern Delmarva Peninsula (Figures I.1; I.2). The inlet is shallow, averaging 1 m in water depth, with a narrow channel (maximum depth 3-5 m, Reay et al., 1995) and broad shoals. Sediments are predominantly sandy, with finer sediments in protected coves and
the upper creek (Reay et al., 1995). Deeper aphytic regions are dominated by heterotrophic activity, while shallow shoal areas support sizeable benthic microalgal communities (Reay et al., 1995). Eelgrass (*Zostera marina*) beds can be found in certain regions, particularly downstream, but are not widespread. Average water temperature ranges from 0 to 32 °C seasonally, and salinities generally range from 14 to 23 ppt (Reay et al., 1995). The clam aquaculture industry has thrived in recent years on the lower Delmarva peninsula, with the dockside value of hard clam aquaculture increasing from $4.5 to $11.0 million from 1992 – 1998 (U.S. Department of Agriculture, 2000). Given the potential ecological impacts of suspension feeding bivalves reviewed in this section, the implications of such a large expansion of the aquaculture industry are substantial.

This thesis is an attempt to analyze the feeding and overall physiology of the cultured hard clam in Cherrystone Inlet, and some of the potential impacts of high densities of these bivalves on their environment. Through a combination of laboratory and field experiments and simple modeling, the physiological ecology of *Mercenaria mercenaria* will be investigated. A large-scale modeling effort is currently underway for Cherrystone Inlet, which builds upon an existing model for the embayment incorporating hydrodynamics, water quality, and sediment resuspension (Kuo et al., 1998). These physical models will be combined with a land-use submodel, as well as a clam carrying capacity submodel, which incorporates detailed clam population data and clam physiology. Results from the current study will be used to help construct a bivalve physiology submodel to help predict carrying capacity for clams in the embayment and subsequent effects on water quality under different land use scenarios (Luckenbach and Wang, 2004).
Objectives

The objectives of this study are:

Chapter 1. Quantify components of the aquacultured clam *Mercenaria mercenaria* energy budget in the laboratory, for a range of clam sizes, and under ambient conditions for Cherrystone Inlet. Compare results from the current study to literature estimates of feeding, egestion, respiration, and excretion for wild *M. mercenaria*.

Chapter 2. Evaluate *M. mercenaria* feeding rates in the field (Cherrystone Inlet), using naturally occurring seston from under clam nets. Determine *in situ* controls on clam feeding, as well as the effects of predator exclusion nets on feeding rates.

Chapter 3. Construct a simple model based on hard clam growth and literature physiological rates to determine potential impacts of the hard clam *Mercenaria mercenaria* on seston, carbon, and nitrogen cycling in Cherrystone Inlet.
CHAPTER ONE

Laboratory Studies on the Physiology of the Cultured Clam,

*Mercenaria mercenaria*

Feeding, Biodeposition, Respiration, Excretion
INTRODUCTION

To determine the effects that cultured clams may be having on the water quality in Cherrystone Inlet, and to determine the exploitation carrying capacity of this embayment (standing stock at which the annual production of a marketable cohort is maximized), it is necessary to determine the energy budget of the cultured clam, Mercenaria mercenaria, and how this is affected by ambient conditions in the growout area. Many bivalve feeding and carrying capacity models have been based on laboratory estimates of bivalve physiology (i.e., Powell et al., 1992), but often the environmental forcing functions of the model (temperature, salinity, seston composition) differ from the conditions under which laboratory observations were made, especially when physiological rates are gleaned from the literature. It is unknown whether the cultured clam has comparable physiological rates to wild M. mercenaria, so it is necessary to determine estimates of feeding, biodeposition, respiration, and excretion in the cultured clam under local ambient conditions, to evaluate the suitability of using results from past laboratory studies in a carrying capacity model for M. mercenaria in Cherrystone Inlet.

Laboratory feeding studies

One of the earliest studies on Mercenaria mercenaria laboratory pumping rates was conducted by Coughlan and Ansell (1964), using a dye solution flowing into the clam’s inhalant siphon to directly measure pumping rates. They found a positive relationship between pumping rate and clam dry weight, as would be expected from allometry. As Coughlan and Ansell (1964) point out, pumping rate does not equal clearance or filtration...
rate unless there is 100% particle retention efficiency. Retention efficiency (RE) in *M. mercenaria* is approximately 100% for particles 5 \( \mu \text{m} \) or greater, but RE quickly decreases to 50% for 3 \( \mu \text{m} \) particles (Riisgård, 1988). For the following studies (see summary in Table 1.1), filtration rate or clearance rate was determined. The equation for clearance rate (CR, L h\(^{-1}\)) most commonly used in laboratory feeding studies on bivalve feeding in a flow-through system is:

\[
\text{CR} = f[(C_i - C_o)/C_o]
\]

where \( f \) is the water flow rate (L h\(^{-1}\)), \( C_o \) is the particle concentration in the feeding chamber outflow, and \( C_i \) is the particle concentration in the chamber inflow (particles L\(^{-1}\) or \( \mu \text{g} \) chlorophyll \( a \) L\(^{-1}\)). A comparison of past data on *Mercenaria mercenaria* clearance rates that have been reported in relation to body size (shell length or dry weight) is shown in Figure 1.1. Where equations were given in relation to shell length (SL), they were converted to relationships vs. dry weight (DW) using a SL-DW regression for Cherrystone clams made in January, 2004 (Appendix 3). Most equations for CR\(_{\text{DW}}\) in Figure 1.1.c were converted from CR equations by dividing CR by DW.

In a laboratory study on feeding rates of five species of bivalve including *Mercenaria mercenaria*, fed on cultured algae (*Isochrysis galbana*) in a flow-through system, Walne (1972) studied the effects of body size and water flow rate on filtration rate (clearance rate). He found significant positive correlations between clearance rate and dry meat weight; and clearance rate and shell length; and negative correlations between dry weight dependent clearance rate (CR\(_{\text{DW}}\)) and dry weight, and CR\(_{\text{DW}}\) and shell length (Table 1.1, Figure 1.1.c). His results for CR\(_{\text{DW}}\) were higher than the values converted from all other studies’ CR data for the same dry weights (Figure 1.1.c.)
### TABLE 1.1 Clearance rate relationships for *Mercenaria mercenaria* from previous studies

<table>
<thead>
<tr>
<th>Reference</th>
<th>Equation</th>
<th>Size range</th>
<th>Food source/suspension</th>
<th>Substrate</th>
<th>Temp</th>
<th>Flow Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coughlan &amp; Ansell 1964</td>
<td>PR = 2.595 DW&lt;sup&gt;0.73&lt;/sup&gt;</td>
<td>0.360-4.810 g DW,</td>
<td>Dye</td>
<td>Substrate</td>
<td>18-20 °C</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>30-83 mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walne 1972&lt;sup&gt;2,3&lt;/sup&gt;</td>
<td>FR&lt;sub&gt;DW&lt;/sub&gt; = -0.77 DW + 4.063</td>
<td>0.122-3.573 g DW</td>
<td><em>Isochrysis galbana</em></td>
<td>Chamber</td>
<td>20-22 °C</td>
<td>200 ml min&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>FR&lt;sub&gt;DW&lt;/sub&gt; = -0.82 DW + 4.344</td>
<td>mm SL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tenore &amp; Dunstan 1973&lt;sup&gt;4,5,6&lt;/sup&gt;</td>
<td>Log&lt;sub&gt;10&lt;/sub&gt;FR&lt;sub&gt;K&lt;/sub&gt; = 0.1154 FC + 0.1028</td>
<td>0.850 g DW, 0.454 mm SL</td>
<td><em>Skeletonema costatum</em></td>
<td>Raceways</td>
<td>20-22 °C</td>
<td>300 ml min&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hibbert 1977&lt;sup&gt;7,8&lt;/sup&gt;</td>
<td>Log&lt;sub&gt;10&lt;/sub&gt;FR = 0.892 Log&lt;sub&gt;10&lt;/sub&gt; L - A</td>
<td>43.4 - 88.1 mm SL</td>
<td>natural seston</td>
<td>Chamber</td>
<td>12 - 25 °C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Log&lt;sub&gt;10&lt;/sub&gt; A = -0.005 T + 0.241</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doering &amp; Oviatt 1986&lt;sup&gt;7&lt;/sup&gt;</td>
<td>FR = 0.033 L&lt;sup&gt;0.967&lt;/sup&gt;</td>
<td>32-107 mm SL</td>
<td>natural seston</td>
<td>Substrate</td>
<td>13.5 - 21 °C</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Riisgård 1988&lt;sup&gt;1,3&lt;/sup&gt;</td>
<td>FR = 1.24 DW&lt;sup&gt;0.80&lt;/sup&gt;</td>
<td>0.017 - 2.387 g DW</td>
<td>mix of natural seston and cultured algae</td>
<td>Beaker</td>
<td>28 °C</td>
<td>static system</td>
</tr>
<tr>
<td>Powell et al. 1992&lt;sup&gt;1,7&lt;/sup&gt;</td>
<td>“low gear” FR = -7.4437 x 10&lt;sup&gt;-2&lt;/sup&gt; + (1.3317 x 10&lt;sup&gt;-2&lt;/sup&gt; * L) + (1.7957 x 10&lt;sup&gt;-4&lt;/sup&gt; * L&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>Data combined from Hibbert 1977; Coughlan and Ansell 1964; Rice and Smith 1958</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Powell et al. 1992&lt;sup&gt;1,7&lt;/sup&gt;</td>
<td>“high gear” FR = -1.1994 + (0.12137 * L) + (8.1652 x 10&lt;sup&gt;-4&lt;/sup&gt; * L&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>Data combined from Hibbert 1977; Coughlan and Ansell 1964; Rice and Smith 1958</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newell &amp; Koch 2004&lt;sup&gt;9&lt;/sup&gt;</td>
<td>FR&lt;sub&gt;DW2&lt;/sub&gt; = 0.13</td>
<td>35.4 g DW(n = 20)</td>
<td>natural seston</td>
<td>Substrate, in tank</td>
<td>15 °C</td>
<td>static system</td>
</tr>
<tr>
<td></td>
<td>FR&lt;sub&gt;DW2&lt;/sub&gt; = 0.73</td>
<td>36 g DW (n = 20)</td>
<td></td>
<td></td>
<td>20 °C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FR&lt;sub&gt;DW2&lt;/sub&gt; = 0.83</td>
<td>36 g DW (n = 20)</td>
<td></td>
<td></td>
<td>25 °C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FR&lt;sub&gt;DW2&lt;/sub&gt; = 0.27</td>
<td>19.89 g DW (n = 20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>FR&lt;sub&gt;DW2&lt;/sub&gt; = 0.22</td>
<td>20.6 g DW (n = 20)</td>
<td></td>
<td></td>
<td>25 °C</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> PR or FR = pumping or filtration rate, L clam<sup>-1</sup> h<sup>-1</sup>

<sup>2</sup> FR<sub>DW</sub> = dry weight dependent filtration rate, ml g<sup>-1</sup> DW min<sup>-1</sup>

<sup>3</sup> DW = dry weight, g

<sup>4</sup> FR<sub>K</sub> = filtration rate, % C removed g<sup>-1</sup> DW

<sup>5</sup> Data from Tenore & Dunstan (1973) Figure 1 were replotted on logarithmic scale and regression line was calculated for current study.

<sup>6</sup> FC = food concentration, µg C L<sup>-1</sup>

<sup>7</sup> L = clam shell length, mm

<sup>8</sup> T = temperature, °C

<sup>9</sup> FR<sub>DW2</sub> = dry weight dependent filtration rate, L g<sup>-1</sup> DW h<sup>-1</sup>

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FIGURE 1.1 Comparison of clam clearance rates from past studies

Data were plotted for the range of clam sizes in Cherrystone Inlet in January 2004: 0.02 – 2.63 g dry weight. If necessary, clearance rate equations were converted from shell length (SL) to dry weight (DW) based measurements using a SL – DW regression determined in January 2004 for Cherrystone clams. Figure 1.1.a includes the lower clearance rate values from past studies; Figure 1.1.b compares the Powell et al. (1992) ‘low’ and ‘high’ models; Figure 1.1.c uses dry weight dependent clearance rates (CR_DW): if an equation for CR_DW was not provided by the authors, clearance rate equations were converted to CR_DW by dividing CR calculated in 1.1.a by DW.
Tenore and Dunstan (1973) conducted a comparative study on feeding and biodeposition rates of three bivalve species in flow-through raceways, which were also fed cultured algae (*Skelotonema costatum*). Feeding and biodeposition rates both increased with increasing food concentration (see Table 1.1 and Table 1.2). Assimilation efficiencies based on carbon consumption and biodeposition ranged from 71.2 - 77.3 %, and *M. mercenaria* feeding and biodeposition rates were lower than both the eastern oyster, *Crassostrea virginica*, and the blue mussel, *Mytilus edulis*. Assimilation efficiencies for oysters were higher than for clams, and equivalent for mussels and clams.

Potentially the most comprehensive study existing on various physiological rates of *Mercenaria mercenaria* in the laboratory was completed by Hibbert (1977). He measured pumping, respiration, and particle filtration rates, over the temperature range 12-25 °C. Filtration rate was measured using a flow-through system and local, naturally occurring seston from Southampton Water, England. The same five clams were used in all experiments, ranging from 43.4-88.1 mm in shell length. These data were combined with biomass and production data from the field to construct an energy budget for *M. mercenaria* on an intertidal mudflat in Southampton Water. The relationship between filtration or clearance rate (CR, L h⁻¹), clam length (L, mm) and temperature (T, °C) was summarized by the equations given in Table 1.1. Clearance rate was found to increase with increasing temperature and with increasing shell length, and larger individuals had reduced weight-dependent clearance rates (L g⁻¹ DW h⁻¹, Figure 1.1c) compared to smaller individuals, similar to the results of Walne (1972).

Hibbert (1977) found no relationship between biodeposition (pseudofeces + feces) rate and either clam size for the range of sizes measured, or with temperature. A mean value
<table>
<thead>
<tr>
<th>Reference</th>
<th>Equation</th>
<th>Size range</th>
<th>Food source/Suspension</th>
<th>Substrate</th>
<th>Temp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tenore &amp; Dunstan 1973&lt;sup&gt;1,2,3&lt;/sup&gt;</td>
<td>B = 2.0241e&lt;sup&gt;0.0022(FC)&lt;/sup&gt;</td>
<td>0.850 g DW, 0.454 mm SL</td>
<td><em>Skelotonema costatum</em></td>
<td>No substrate</td>
<td>19 - 21 °C</td>
</tr>
<tr>
<td>Hibbert 1977&lt;sup&gt;4,5&lt;/sup&gt;</td>
<td>F = 0.587 C</td>
<td>43.4 - 88.1 mm SL</td>
<td><em>natural seston</em></td>
<td>No substrate</td>
<td>12 - 15 °C</td>
</tr>
</tbody>
</table>

<sup>1</sup>Data from Tenore & Dunstan 1973 Figure 2 were replotted and regression line was calculated for current study.

<sup>2</sup>B = biodeposition rate, mg ash-free dry weight g<sup>-1</sup> clam DW day<sup>-1</sup>

<sup>3</sup>FC = food concentration, μg C L<sup>-1</sup>

<sup>4</sup>F = egestion rate, percent of POC consumed

<sup>5</sup>C = consumption rate, FR (L h<sup>-1</sup>) * immersion time * kcal m<sup>-2</sup>
of 59% of the particulate organic carbon (POC) consumption rate was therefore used in his energy budget. The advantages of using Hibbert’s (1977) results for modeling applications are two-fold: (1) naturally occurring seston was used as the food source for *M. mercenaria* instead of cultured algae, and (2) results are given for both clam size and temperature, allowing both of these important parameters to be incorporated into one predictive equation of filtration rate.

Doering and Oviatt (1986), Doering et al. (1986), and Doering et al. (1987) evaluated clams’ effects on carbon cycling and fluxes of inorganic nutrients and gases in experimental mesocosms, and measured clam feeding rates on natural seston for Narragansett Bay, Rhode Island, over a range of temperatures. Once again, a positive relationship was found between clearance rate and clam length (Table 1.1). Predictions of clearance rate using Doering and Oviatt’s (1986) equation based on clam size and water temperature are similar to those of Hibbert’s (1977) equation at 20 °C. As temperature and clam size increase, however, the two equations begin to diverge and Hibbert (1977) predicts higher clearance rates than Doering and Oviatt (1986) for the same clam size and water temperature.

Riisgård (1988) measured particle retention efficiency (RE) and clearance rates for six bivalve species, including *M. mercenaria*. Experiments were conducted in beakers using a variety of naturally occurring seston and cultured algae to obtain RE and CR for a size range of particles (see Table 1.1). He found a positive relationship between CR and clam size dry weight (Figure 1.1a). In a comparative study on *M. mercenaria* and *C. virginica* feeding rates for use in a water quality model, Newell and Koch (2004) combined 20 clams within a large tank and measured the change in particle concentrations to obtain a
gross particle clearance rate. Highest dry weight dependent clearance rates (CR$_{DW}$) were at 20 °C, followed by 25 and finally 15 °C (see Table 1.1). For the purposes of their model, they computed the average of their results for 20 and 25 °C and used a mean filtration rate of 0.5 L g$^{-1}$ DW h$^{-1}$ for $M$. mercenaria. $C$. virginica had much higher mean clearance rates than $M$. mercenaria overall (mean clearance rate = 6.4 L g$^{-1}$ DW h$^{-1}$), as was also noted by Tenore and Dunstan (1973).

Powell et al. (1992) constructed a feeding model for a “generalized” bivalve, based on laboratory data from a variety of sources, and on a variety of bivalve species. When feeding rates were plotted vs. bivalve size, they discovered two distinct groups of clearance rates, which they termed “high gear” and “low gear”. The differences in the higher and lower clearance rates could not be attributed to species, water temperature, or any other environmental factor. Powell et al. (1992) hypothesized that there is a physiological switch between high and low clearance rates, and cautioned against scaling up high observed feeding rates for use in carrying capacity or water quality models.

Overall, few observations have been made of $M$. mercenaria clearance rates, even under laboratory conditions. Most of the studies discussed here found similar trends such as clearance (filtration) rate increasing with body size and temperature, however, it is difficult to compare some of these results due to some differences in their experimental design. Water temperature and animal size are two major parameters than can control clam clearance rates. Food quantity (Tenore and Dunstan, 1973) and quality or type (see Grizzle et al. (2001) for review) can also have a large effect on clearance rates.

For the clam sizes found in Cherrystone Inlet, Hibbert (1977), Doering and Oviatt (1986), and the “low gear” model from Powell et al. (1992) all give very similar
calculated clearance rates (Figure 1.1.a). The results from Riisgård (1988) are similar to these three studies from 0.5 – 1.5 g DW, but at higher clam sizes, Riisgård’s (1988) relationship is much higher than the others.

Experimental protocols can have an effect on observed bivalve feeding rates, as discussed by Riisgård (1977). If feeding rates are being calculated using an indirect method (i.e., calculating clearance rates based on change in particle concentration), the use of a static system or a system with low flow rates can result in an under-estimation of bivalve clearance rates if particle concentrations become too low. At low particle concentrations, it is likely that bivalves re-filter the same water (Riisgård, 1977). At high flow rates in flow-through systems, particle concentrations may never become low enough to observe a change. It is important to choose an intermediate flow rate in a flow-through system so that a change in particle concentration can be seen, but bivalves are not re-filtering the water. Hildreth and Crisp (1976) criticized the use of the “standard” clearance rate equation, which uses the feeding chamber inflow particle ($C_i$) concentration to estimate particle concentration of ingested material. When this equation is used, clearance rate is dependent upon flow rate, and Hildreth and Crisp (1976) suggested the use of an alternate equation, using particle concentrations in the immediate vicinity of the animal in place of $C_i$. When this equation was used, there was no correlation between clearance rate and flow rate. Möhlenberg and Riisgård (1979) investigated this issue further and determined that for adequately high flow rates (> 50 ml min$^{-1}$), the two equations are essentially identical and that the original equation may be used.
Laboratory biodeposition studies

Very few studies exist on *M. mercenaria* biodeposition rates compared to the number of clearance rate studies. Biodeposition by clams includes both feces and pseudofeces. Pseudofeces are particles which have been filtered from the water column, but are rejected before entering the digestive tract and expelled through the inhalant siphon. This matter is considered “filtered” or “consumed”, but has not been “ingested” since it does not enter the gut. Pseudofeces production is a means for the clam to contend with high particle concentrations in the water column, as pseudofeces production is generally zero below 5 mg sediment L\(^{-1}\), increasing to 10-20% of the filtered food concentration as sediment increases to 40 mg L\(^{-1}\) (Bricelj and Malouf, 1984). Since it is problematic to discern pseudofeces from feces for hard clams since the inhalant and exhalant siphons are fused, most studies combine the two and call them “biodeposition”. If TSS or particle concentrations are high, biodeposition is not an accurate measure of egestion, since pseudofeces are included, and egestion is a measure of fecal production alone.

Biodeposition is important from the ecological perspective, however, since it is indicative of particulate matter flux from the water column to the benthos due to bivalve activity.

Tenore and Dunstan (1973) quantified carbon flux to the bottom via biodeposition for *M. mercenaria*, *C. virginica*, and *M. edulis*. All three species had a logarithmic increase in biodeposition rate with increasing food concentrations (*M. mercenaria* results are shown in Table 1.2), mostly due to increased pseudofeces production. *M. mercenaria* had the lowest biodeposition rate of the three species. Hibbert (1977) did not find any relationship between biodeposition rate and clam size or water temperature, so he used a mean value of 58.7% of consumed food in his energy budget for the hard clam (Table
1.2). In their mesocosm studies, Doering et al. (1986) used radioactive carbon labeling, and found that carbon sedimentation rates (fluxes to the benthos) were increased by 58% in treatments with clams; however, most of this carbon was respired or incorporated into clam tissue, not biodeposited.

**Laboratory respiration studies**

Hamwi (1969) completed a thorough study on *M. mercenaria* pumping and respiration rates at a variety of temperatures and salinities, and also reported temperature-salinity interactions. As with clearance rates, clam respiration rates increase with increasing body size, although the weight-dependent respiration rate (*Q*\(_0\)) decreases with increasing clam size, as expected from allometry (Table 1.3). Hamwi (1969) described a binomial relationship between oxygen consumption and temperature, with maximum respiration rates at 25 °C. Interacting effects of temperature and salinity indicate that highest respiration rates occur at a combination of 20 °C and 23 ppt (Hamwi, 1969; reviewed by Grizzle et al., 2001).

Loveland and Chu (1969) described results for *M. mercenaria* respiration and its dependence on clam size (Table 1.3). Hibbert (1977) determined oxygen consumption by measuring the change in O\(_2\) concentration between the inhalant and exhalent siphons and multiplying by the clam pumping rate to give ml O\(_2\) clam\(^{-1}\) h\(^{-1}\). He combined his results in an equation describing the effects of both clam length and temperature on O\(_2\) consumption (Table 1.3). Weight specific respiration decreased logarithmically with increasing clam size at 20 °C, as was also found by Loveland and Chu (1969).
TABLE 1.3 Relationship between *Mercenaria mercenaria* respiration rate and clam weight from previous studies

<table>
<thead>
<tr>
<th>Reference</th>
<th>Equation</th>
<th>Size range</th>
<th>Food source/Suspension</th>
<th>Substrate</th>
<th>Temp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loveland &amp; Chu, 1969&lt;sup&gt;1,2&lt;/sup&gt;</td>
<td>$\log_{10} Q_{O_2} = -0.3441 \log_{10} TWW - 1.0243$</td>
<td>4.95 - 284 g TWW</td>
<td>artificial seawater</td>
<td>Substrate</td>
<td>25 °C</td>
</tr>
<tr>
<td>Hamwi 1969&lt;sup&gt;1,2&lt;/sup&gt;</td>
<td>$Q_{O_2} = 20 - 203 \mu l O_2 g^{-1} TWW h^{-1}$</td>
<td></td>
<td>dyed seawater</td>
<td>Substrate</td>
<td>15 °C</td>
</tr>
<tr>
<td></td>
<td>$Q_{O_2} = 68 - 324 \mu l O_2 g^{-1} TWW h^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hibbert 1977&lt;sup&gt;3,4,5&lt;/sup&gt;</td>
<td>$\log_{10} O_2 cons = 1.016 \log_{10} L - A$</td>
<td>43.4 - 88.1 mm SL</td>
<td>natural seston</td>
<td>No substrate</td>
<td>12 - 25 °C</td>
</tr>
<tr>
<td></td>
<td>$\log_{10} A = -0.012 T + 0.478$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> $Q_{O_2}$ = weight dependent respiration rate, ml O$_2$ g$^{-1}$ TWW h$^{-1}$
<sup>2</sup> $TWW$ = clam total wet weight, g
<sup>3</sup> $O_2$ cons = oxygen consumption rate, ml O$_2$ clam$^{-1}$ h$^{-1}$
<sup>4</sup> L = clam shell length, mm
<sup>5</sup> T = temperature, °C
For ectothermic, poikilothermic animals such as bivalves, physiological rate processes can be expected to be strongly influenced by environmental temperatures. The dependence of physiological rates on temperature can be estimated by calculating a $Q_{10}$, the ratio of a rate function at one temperature to that at one 10 °C (or 10 K) below:

$$Q_{10} = \left(\frac{K_1}{K_2}\right)^{\frac{10(T_1-T_2)}}$$

where $K_1$ and $K_2$ are rates determined at high and low temperatures, $T_1$ and $T_2$. $Q_{10}$ values can be used to compare physiological responses by animals of different sizes, for different studies, or even for different species. $Q_{10}$ values can also be used to evaluate an animal's thermal acclimation, defined as any nongenetic adjustment by an organism in direct response to a change in a single factor in the environment (Crisp and Ritz, 1967).

An animal can be acclimated to a seasonal temperature regime and be exposed to a variety of temperatures throughout the day or tidal cycle (Dame, 1996). $Q_{10}$ values at or slightly higher than two (indicating a doubling of the rate process for every 10 °C) are observed when thermal effects on the rate process are within the species' "normal" range of temperatures (Hochachka and Somero, 2002). $Q_{10}$ values much lower than two, even less than one, are typical of high temperatures, indicating that increasing temperatures are damaging to the system and may have lethal effects. $Q_{10}$ values much higher than two may occur at low temperatures, which may be indicative of energy barriers to the process in question (Hochachka and Somero, 2002). A rate process with a $Q_{10}$ of 1 indicates complete acclimation to that range of temperatures. For example, the clearance rate of *Mytilus edulis* is independent of temperature between 10 and 20 °C, therefore the $Q_{10}$ is equal to 1 and *M. edulis* is considered to be completely acclimated to this temperature range (Bayne et al., 1977).
Hamwi (1969), Bricelj (unpubl., reported by Grizzle et al., 2001), and Hibbert (1977) reported results from which $Q_{10}$ values could be determined, for both clearance and respiration rates (Grizzle et al., 2001). Hamwi (1969) reports respiration $Q_{10}$ values below one from 20-25 °C and as low as 0.29 from 25-30 °C for respiration, indicating that increasing temperatures at these levels are most likely having lethal effects on the clams. Bricelj (unpubl.) and Hibbert (1977) both published data for $Q_{10}$ values much higher than these for the same temperature ranges: $Q_{10} = 2.89$ for 20-27 °C (Bricelj, unpubl.) and $Q_{10} = 3.38$ for 20-26 °C (Hibbert, 1977), indicating that clams were probably functioning normally. For the temperature range 10-20 °C, $Q_{10}$ values for oxygen consumption were generally at or near 2.5 (Hamwi, 1969; Bricelj, unpubl.; Hibbert, 1977), while $Q_{10}$ values for clearance rate for the same temperature range are generally slightly less than two (Hibbert, 1977; Doering and Oviatt, 1986). This may indicate that feeding processes may need slightly higher temperatures than respiration processes in order to function normally, or that feeding processes may simply be less sensitive to thermal effects than respiration. Newell and Koch (2004) noted that clam feeding rates were not as temperature sensitive as oyster (C. virginica) feeding rates. $Q_{10}$ values for M. mercenaria are almost always above 2, indicating that M. mercenaria does not completely acclimate to its temperature regime (Grizzle et al., 2001).

**Laboratory excretion studies**

Only one study has examined Mercenaria mercenaria’s direct excretion rates of ammonium ($\text{NH}_4^+$), and only one study has examined M. mercenaria excretion of organic nitrogen species such as urea, primary amines, and amino acids. Srna and Baggaley
(1976) conducted laboratory experiments with bivalves in static chambers filled with autoclaved artificial seawater, and measured NH$_3$ production by both $M$. mercenaria and $C$. virginica. They found a positive relationship between ammonia excretion rate and clam dry weight (see Table 1.4), and found that clam excretion rates were higher and more variable than those of $C$. virginica. This study also noted that nitrate flux was positive consistently in experimental chambers. Since it is unlikely that clams excrete nitrate, but the water was free of viable microbes, it was suggested that nitrifying bacteria in the bivalve gut may have been contributing to nitrate flux. This study provides the only known rate of nitrogen excretion by $M$. mercenaria, although the animals had been starved, and stress is known to have an effect on quantities and types of nitrogenous metabolites excreted (see Grizzle et al. (2001) for discussion).

Hammen (1968) observed excretion of NH$_4^+$, amino acids, and uric acid, and determined that each contributed respectively 66%, 30%, and 4% to the total nitrogen excretion of $M$. mercenaria, but did not determine rates for excretion processes. Hibbert (1977) assumed that nitrogen excretion is a relatively small component of the clam energy budget, and determined excretion by subtraction from the rest of his energy budget. Other studies such as Tenore et al. (1973) and Doering et al. (1987) have observed increased nitrogen fluxes to the water column in experimental treatments with clams using flow-through tanks and mesocosms, respectively, but it is difficult to include these observations in an energy budget for the hard clam since they were not made on a per-gram-dry-weight basis on clams alone. It is almost impossible to accurately quantify
TABLE 1.4 Relationship between *Mercenaria mercenaria* excretion rate and clam dry weight from previous study

<table>
<thead>
<tr>
<th>Reference</th>
<th>Equation</th>
<th>Size range</th>
<th>Suspension</th>
<th>Substrate</th>
<th>Temp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sma &amp; Baggaley 1976(^1)</td>
<td>$\log_{10} U = 0.94 \log_{10} DW + 1.33$</td>
<td>0.66 - 4.60 g DW</td>
<td>artificial seawater</td>
<td>no substrate</td>
<td>20 °C</td>
</tr>
</tbody>
</table>

\(^1\)U = excretion rate, \(\mu\)mol NH\(_4\)\(^+\) excreted day\(^{-1}\)
nitrogen excretion by clams alone without phytoplankton and bacteria present, since
starving the animals probably affects clams’ excretion rates.

Past studies give estimates of feeding (clearance and filtration), egestion, respiration,
and excretion rates for wild clams. These rates have been used in the past for bivalve
feeding (Powell et al., 1992) and water quality (Newell and Koch, 2004) models. The
current study will attempt to evaluate the applicability of these physiological rates for
cultured *M. mercenaria* in Cherrystone Inlet, Virginia.

*Mercenaria mercenaria* has been aquacultured for approximately 20 years in
Cherrystone Inlet, with aquaculturalists selecting breeding animals for fast growth. If
growth rates have declined in recent years as growers have observed, the reasons could
be physiological or environmental. Growth rates of cultured clams could be depressed
due to inbreeding, causing changes in the energy budget and leading to reduced feeding
and/or growth efficiencies. If this is the case, laboratory experiments would reveal
differences in the energy budget of the cultured clam, as compared to past studies on wild
clams. If differences in growth rates are due to environmental effects, such as localized
or large-scale food depletion, oxygen debt or sulfide toxicity, differences in feeding rates
would be observed in the field when compared to laboratory results.

**Objectives**

The objectives of the laboratory component of this study were the following:

1. Measure ecologically-relevant feeding, biodeposition, excretion, and respiration
   rates in the laboratory for cultured clams, over a range of animal sizes and water
   temperatures.
2. Compare physiological rates from the current study (cultured clams) to literature rates (wild clams) and determine:

(a) whether cultured clams from Cherrystone Inlet have significantly different physiological rates than wild clams under typical growing conditions, and,

(b) whether literature physiological rates on wild clams may be justifiably used in a physiological model for cultured *M. mercenaria*.

**MATERIALS AND METHODS**

Measurements of clam feeding, respiration, and excretion were made under ambient conditions to determine whether the bioenergetics of the cultured clams in Cherrystone Inlet are similar to those of wild *M. mercenaria* found in the literature.

**Feeding**

Summertime clam feeding was studied in the laboratory at Wachapreague, VIMS’ field laboratory on the Eastern Shore of Virginia (Figure I.1) from June – August 2003. Feeding trials were conducted using a flow-through system consisting of a 570 L reservoir and a 230 L head tank flowing by gravity into twelve individual 2.2 L feeding chambers (nine chambers with clams, three controls with no clams). Flow rates were controlled by valves on the chamber outflows, and were measured using graduated cylinders and a stopwatch.
Clams were collected each month during the field survey at the upstream site at Cherrystone Inlet (Figure I.2), returned to the laboratory, measured, and acclimated in a holding tank filled with Wachapreague water adjusted to ambient Cherrystone temperature and salinity. Naturally occurring algae was supplemented with cultured *Isochrysis galbana* to feed the clams twice each day (30 L per feeding, $2.175 \times 10^6$ cells ml$^{-1}$). Twenty-four hours before the feeding trial, 20 clams were chosen randomly and placed in filtered water to clear their guts. On the day of the trial, 1000-1500 L of water were collected from the upstream site and transported to Wachapreague, where water was pumped into the flow-through system. Water was filtered to remove larger grazers (200 μm bag filter) for August trials, and water was maintained at ambient Cherrystone temperature and salinity from the time of collection throughout feeding trials, using heaters and chillers.

Starved clams were observed for activity and nine clams were chosen haphazardly from those that had siphons extended. One clam was placed in each of nine flow-through chambers, with the three control chambers chosen randomly. All chambers were half filled with pre-combusted filter sand, to allow clams to burrow. Lengths and grow-out sites of clams were recorded. Flow rates to the chambers were checked and adjusted until flow into and out of each chamber was $30 \pm 3$ ml min$^{-1}$, which is estimated to be approximately half the average clam filtration rate for clams of this size (averaging 35 mm shell length), according to Hibbert (1977). Clams were allowed to bury and acclimate to their chambers for one hour, then allowed to feed for three hours, with flow rates being checked at 30 and 90 minutes. Clams were also observed for activity at these
times. Experiments were conducted under natural light conditions to determine clams’
daytime feeding activities.

After three hours, one 250 ml water sample was collected from each chamber outflow,
and three samples total were collected from chamber inflows. If feces could be seen,
feces samples were collected from each chamber and frozen. Clams were then placed in
filtered water in individual beakers to collect additional feces for 24 hours. This process
was repeated twice for three trials per month. Water temperature and salinity were kept
constant for clams for all three trials for the month, and all clams were maintained with
natural seston from the Wachapreague channel, supplemented with cultured *Isochrysis*,
until 24 hours before their respective feeding trial, when they were moved to a tank of
filtered water to clear their guts.

All feeding trial water samples were placed on ice and filtered immediately at the
Wachapreague lab. Two 10 ml subsamples from each water sample were filtered using
25 mm GF/F filters with a 0.7 μm pore size ,analyzed for chlorophyll *a* and phaeophytin
concentrations. Concurrently, three subsamples of each sample were read on the Coulter
counter for particle counts in the 2-4 and 4-8 μm size range. These particle size ranges
were chosen due to: 1) literature observations that clams can consume food particles
above 4 μm with 100% efficiency, and particles 2-4 μm with approximately 50%
efficiency (Riisgård, 1988); and 2) personal observation that Cherrystone water samples
consisted of 86% 2-4 μm particles and 13% 4-8 μm particles, totaling 99% of the < 50
μm particles in the water column. In June 2003, water samples were also analyzed for
particulate organic carbon and particulate nitrogen, total suspended solids, total volatized
solids, and dissolved inorganic nitrogen (*NH₄⁺*, *NO₃⁻*, and *NO₂⁻*).
Respiration and Excretion

Clam respiration experiments were conducted June - November 2004, and excretion experiments were conducted in conjunction with November respirometry experiments. Winter trials were conducted in the laboratory at Gloucester Point (Figure I.1), while summer trials were conducted in the laboratory at Wachapreague. Clams were maintained in running seawater tables pumping ambient water past clams at both locations. Since Wachapreague channel water generally has a higher salinity than Gloucester Point (lower York River) and Cherrystone Inlet water, clams were allowed to acclimate to local conditions for at least one month after being moved, before being used in respiration or excretion trials.

Respiration was determined by placing an individual clam in a 1 L static sealed chamber filled with whole seawater, measuring oxygen drawdown using polarographic oxygen sensors (Radiometer Instruments), and recorded in real-time using an analog to digital system (IOtech Daqbook 120), and the Dasylab version 7 (Dasytech, U.S.A.) computer software package to convert the voltage signal to percent $O_2$ saturation, and $O_2$ concentration in mg/L. Background respiration rates were determined immediately before or after clam respiration trials using whole seawater, with no clams added. Sensor drift was quantified periodically by filing the respiration chamber with oxygenated, deionized water and following the same protocol as for measuring respiration rates. Sensor drift was < 2-3% of background respiration rates for all trials. To compare respiration rates from the current study to results of Loveland and Chu (1969), analysis of covariance (ANCOVA) was conducted using MiniTab statistical software, GLM
procedure, with $\log_{10}Q_{O_2}$ as the response, data source (Loveland and Chu (1969) or current study) as the factor, and $\log_{10}$ total wet weight as the covariate.

Initial attempts were made to quantify excretion in June 2003, but it was impossible to determine a measurable change in DIN in the flow-through system. Consequently, excretion rates were determined simultaneously to respiration rates in November 2004 by measuring change in $\text{NH}_4^+$ in the static chamber from beginning to end of the respiration experiment (12 – 24 hours). Changes in $\text{NO}_3^-$, $\text{NO}_2^-$, and $\text{PO}_4^{3-}$ concentrations were also measured to determine effects of clams on cycling of these nutrients. Clams do not excrete $\text{NO}_3^-$, $\text{NO}_2^-$, or $\text{PO}_4^{3-}$, but may contribute to flux of these materials via egestion of POM and subsequent microbial mineralization of this material. Respiration rates were determined seasonally in the laboratory for ambient temperatures and salinities, using a range of clam sizes. $\text{NH}_4^+$ excretion rates and $\text{NO}_x$ and $\text{PO}_4^{3-}$ flux rates were also determined using a range of clam sizes, but since they were only conducted in November, the temperature range was limited to 14 – 21 °C. During winter 2004 trials, water, pseudofeces, and feces were also collected for analysis of chlorophyll $a$ and particulate carbon/particulate nitrogen to determine clam biodeposition rates and assimilation efficiencies.

**Analytical methods**

All water samples for feeding and excretion experiments were filtered before processing. Chlorophyll $a$ and phaeopigments were measured fluorometrically (Turner Designs TD-700 fluorometer) after filtration onto 25 mm GF/Fs and extraction using the acetone/ DMSO extraction method outlined by Shoaf and Lium (1976). For total
suspended solids (TSS) analysis, 100 ml of each water sample was filtered through a 47 mm pre-combusted and pre-weighed GF/F. TSS was analyzed according to the Standard Methods for the Examination of Water and Wastewater, 18th edition, Method 2540 D.

For particulate nutrient analysis (POC/PN), 50 ml of sample was filtered onto each of two replicate pre-combusted 25 mm GF/F filters and frozen, rinsed with ca. 5 ml 0.01N HCl in seawater, and the filters were dried in combusted glass vials at 60°C. Filters from 2004 samples were combusted using a Carlo-Erba Model EA1108 Elemental Analyzer; 2003 samples were combusted on an Exeter Analytical elemental analyzer, model CE-440, according to the methods by Menzel and Vaccaro (1964). Filtrate was collected in acid-washed bottles for dissolved nutrient analysis and frozen. Dissolved nitrate + nitrite (NO₃) was quantified by the SKALAR method (U.S.EPA, 1974), and dissolved ammonium (NH₄⁺) was quantified using a modified Berthelot-Phenol method (U.S.EPA, 1974). Blanks for DIN (NO₃ and NH₄⁺) analyses were run using deionized water.

Frozen biodeposits for assimilation efficiency calculations were thawed and emulsified, then filtered onto pre-combusted, pre-weighed 25 mm GF/F filters according to the method of Conover (1966). Samples were dried (60 °C), weighed, then combusted (500 °C) and re-weighed when cool.

Data analysis

Clearance rates for clams were calculated using the following formula with particle concentration (Coulter counter) and chlorophyll concentration data:

\[ CR = f[(C_i - C_o)/C_i] \]
where $f$ is the water flow rate (L h$^{-1}$), $C_o$ is the particle concentration (or chlorophyll concentration) in the feeding chamber outflow, and $C_i$ is the particle concentration (or chlorophyll concentration) in the chamber inflow (particles L$^{-1}$ or µg chlorophyll a L$^{-1}$). Particle flux rates (PFR, L h$^{-1}$) of control chambers were subtracted from clearance rates of clam chambers to give net clearance rates (net CR, L h$^{-1}$). From this point onwards, "CR" refers to net clearance rate, which is $[CR_{clam} - \text{mean PFR}_{control}]$.

Before statistical tests, data were tested for normality and homogeneity of variance, and transformed to meet test assumptions, if necessary. The most common transformations were log$_{10}$ and square root transformations. ANOVAs and general linear models were run for each parameter in June 2003 to determine differences between chamber inflows, control chamber outflows, and clam chamber outflows; as well as differences in these parameters over time (30, 90, and 180 minutes). Linear regressions were calculated for the effects of continuous variables on clearance rates. Calculated square-root transformed clearance rates ($CR^{1/2}$) were plotted vs. clam shell lengths and dry weight, and regressions were computed and tested for significance using SAS Version 9.0 statistical software. Clearance rate data were compared to past studies (Hibbert, 1977; Doering and Oviatt, 1986; Powell et al., 1992) using size parameters from the previous studies. Clearance rate vs. shell length was the most common relationship reported in previous studies; therefore, this relationship was used in the current study as well. To convert these values to tissue dry weights, total wet weights, or other size parameters, the regression equations in Appendix 3 may be used.
In June 2003, the effects of clams on chlorophyll $a$, particulate organic carbon (PC), particulate nitrogen (PN), total suspended solids (TSS), and total volatilized solids (TVS) were analyzed using ANOVAs and general linear models (GLMs). The effects of feeding trial length on these parameters were also determined, by taking samples at 30, 90, and 180 minutes, and comparing the above components over time using ANOVAs and GLMs.

Assimilation efficiencies (AE) were calculated by:

$$AE\% = 1 - \left[\frac{(Ash/AFDW_{food})}{(Ash/AFDW_{feces})}\right] \times 100$$

where $AFDW$ is the ash-free dry weight of the food (inflow water) or feces (g), and $Ash$ is the weight of the combusted fraction (Grizzle et al. (2001), after Conover, 1966).

Regressions for clearance rate vs. clam shell length, clam dry weight, temperature, salinity, particle concentration, chlorophyll concentration, and flow rate were computed, tested for significance, and compared to literature rates (Hibbert, 1977; Doering and Oviatt, 1986; Powell et al., 1992) where applicable.

Respiration rates ($mg\ O_2\ ind^{-1}\ h^{-1}$) were determined by plotting oxygen depletion (mg $L^{-1}$) over time (h) and determining the slope of the regression line to yield the respiration rate in mg $O_2\ L^{-1}\ h^{-1}$, and multiplying by the chamber volume to yield mg $O_2\ h^{-1}$. Net clam respiration (Net RR, $mg\ O_2\ h^{-1}$) was determined by subtracting the background respiration rate from the clam respiration rate for each experimental date. Regressions for size and temperature dependence for respiration rates were computed and compared to literature rates, such as Hibbert (1977), Loveland and Chu (1969), and Hamwi (1969).
using the size parameters reported in the past studies. Size parameters may be converted using regressions in Appendix 3. \( Q_{O2} \) (ml O2 g\(^{-1}\) wet weight h\(^{-1}\)) values were calculated by dividing respiration rate by clam total wet weight to compare to Loveland and Chu (1969). \( Q_{10} \) values were also calculated for respiration rates as in the Introduction section, and compared to literature \( Q_{10} \) values (Grizzle et al. (2001)).

Excretion rates (\( \mu \text{M NH}_4^+ \text{ h}^{-1} \)) were determined by calculating the difference in ammonium concentration (\( t_f - t_0 \)) over the length of the experiment for the clam respiration trials. Excretion rates of ammonium were compared to Srna and Baggaley (1976) for a range of clam sizes and water temperatures. Phosphate excretion rates (\( \mu \text{M PO}_4^{3-} \text{ h}^{-1} \)) during respiration trials were calculated in the same manner as DIN excretion rates.

**RESULTS**

**Feeding**

Mean (+/- SE) for all parameters measured during June feeding experiments (180 minutes) are reported in Table 1.5. Clearance rates (L h\(^{-1}\)) based on 2-8 \( \mu \text{m} \) particle counts were similar to those based on chlorophyll \( a \) concentrations, however, replicate particle counts were more consistent than replicate chlorophyll \( a \) concentrations, reducing error in clearance rate determinations. Clearance rates reported are therefore based upon 2-8 \( \mu \text{m} \) particle counts. During June trials, clams were observed to have siphons extended, actively feeding for the majority of the trials. Clearance rates were significantly higher for clam treatments than controls over all time intervals (Figure 1.2, two-way ANOVA, treatment: \( F_{1,12} = 31.85, p < 0.0001; \) Tukey's HSD \( \alpha = 0.05 \)), and the
TABLE 1.5 Observed parameters for laboratory feeding experiments, 2003

<table>
<thead>
<tr>
<th>Parameter</th>
<th>June 2003</th>
<th>July 2003</th>
<th>August 2003</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>9</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>Average net clearance rate (L h⁻¹)</td>
<td>2.56</td>
<td>0.18</td>
<td>0.03</td>
</tr>
<tr>
<td>Clearance rate range (L h⁻¹)</td>
<td>0.66 - 3.45</td>
<td>0 - 0.48</td>
<td>0 - 0.19</td>
</tr>
<tr>
<td>Average shell length (mm)</td>
<td>35.26</td>
<td>33.13</td>
<td>33.91</td>
</tr>
<tr>
<td>Average dry weight (g)</td>
<td>0.384</td>
<td>0.481</td>
<td>0.619</td>
</tr>
<tr>
<td>Average water temperature (°C)</td>
<td>30.6</td>
<td>29.9</td>
<td>28.0</td>
</tr>
<tr>
<td>Average salinity</td>
<td>15.0</td>
<td>15.7</td>
<td>10.0</td>
</tr>
<tr>
<td>Average inflow particle conc (particles mL⁻¹)</td>
<td>129142</td>
<td>16154</td>
<td>145509</td>
</tr>
<tr>
<td>Average inflow chlorophyll conc (µg L⁻¹)</td>
<td>13.96</td>
<td>3.35</td>
<td>31.96</td>
</tr>
<tr>
<td>Average flow rate (mL min⁻¹)</td>
<td>37</td>
<td>24</td>
<td>29</td>
</tr>
</tbody>
</table>
FIGURE 1.2 Clearance rates based on particle concentrations, June 2003 feeding trial

All clam treatments had significantly higher clearance rates than control treatments (two-way ANOVA, treatment: $F_{1,12} = 31.85$, $p < 0.0001$).
difference in clearance rates over time was also significant, with the 180-minute trial having the highest clearance rates (time: $F_{2,12} = 59.83$, $p < 0.0001$; Tukey's HSD $\alpha = 0.05$). Subsequently, all July and August 2003 trials were run for 180 minutes. The regression calculated for clearance rate vs. shell length for the June 2003 180-minute trial was not statistically significant at the $\alpha = 0.05$ level ($F_{1,7} = 3.45$, $p = 0.106$), but the $r^2 (0.330)$ indicates a slight positive relationship between clearance rate and shell length (Figure 1.3).

For the June 2003 trials, chlorophyll $a$ concentrations did not change significantly over time (one-way ANOVA, $F_{2,24} = 1.90$, $p = 0.171$), however clam treatment outflows contained significantly less chlorophyll $a$ concentrations than inflow and control chambers only for the 180-minute trial (Figure 1.4; one-way ANOVA, $F_{2,6} = 7.13$, $p = 0.026$, Tukey's HSD: clam < inflow, control, $\alpha = 0.05$). Particulate organic carbon (POC) was not significantly different for clam or control treatments, or for chamber inflows (Figure 1.5; two-way ANOVA, treatment: $F_{2,16} = 0.91$, $p = 0.419$), but POC did change significantly over time (time: $F_{2,18} = 5.83$, $p = 0.011$). A post-hoc one-way ANOVA with Tukey’s pairwise comparisons showed that the 180-minute trial had significantly more POC in outflows than the 90-minute trial (90 min < 180 min, $\alpha = 0.05$). No other pairwise comparisons were significant. The interaction between treatment and time for POC was not significant (two-way ANOVA, $F_{4,18} = 0.68$, $p = 0.613$). Similarly, particulate nitrogen (PN) was not significantly different between treatments (Figure 1.6; two-way ANOVA, treatment: $F_{2,18} = 0.79$, $p = 0.467$), but did change over time (time: $F_{2,18} = 6.60$, $p = 0.007$), with the 180-minute trial having the largest difference between clam and control and inflow PN concentrations. A post-hoc
FIGURE 1.3 Clearance rates based on particle concentrations vs. shell lengths, plotted by trial month

The June 2003 regression was not significant at the $\alpha = 0.05$ level ($F_{1,7} = 3.45$, $p = 0.106$), but the $r^2$ was 0.33. The July 2003 regression was non-significant and the $r^2$ was very low. The August 2003 regression was significant ($p = 0.036$), however, the $r^2 = 0.17$ and values are all close to zero.

June clearance rates were significantly higher than July and August (GLM for $CR^{1/2}$ and month: $F_{2,38} = 44.49$, $p < 0.0001$).
FIGURE 1.4 Chlorophyll $a$ in inflow, control and clam chambers, June 2003 feeding experiment

No significant differences were seen at $\alpha = 0.05$ over time (one-way ANOVA, $F_{2,24} = 1.90, p = 0.171$), but clam treatment outflows contained significantly less chlorophyll than inflow and control chambers (denoted by *, $F_{2,6} = 7.13, p = 0.026$, Tukey's HSD clam < inflow, control).
FIGURE 1.5 Seston particulate organic carbon concentrations, June 2003 feeding experiment.

There was no significant difference for treatment (two-way ANOVA, $F_{2,18} = 0.91$, $p = 0.419$), but there was a significant difference for time ($F_{2,18} = 5.83$, $p = 0.011$). Post-hoc one-way ANOVA for PC concentrations over time showed that the 180 minute trial had significantly more PC in chambers than 90 minutes (denoted by *). All other pairwise comparisons were non-significant.
FIGURE 1.6 Seston particulate nitrogen concentrations, June 2003 feeding experiment

PN was not significantly different between treatments (two-way ANOVA, treatment: $F_{2,18} = 0.79, p = 0.467$), but did change over time (time: $F_{2,18} = 6.60, p = 0.007$), with the 180 minute trial having the largest difference between clam and control and inflow PN concentrations. Post-hoc one-way ANOVA and Tukey’s HSD ($\alpha = 0.05$) showed that the 90 minute PN was significantly lower than 180 minute PN (denoted by *), but all other pairwise comparisons were non-significant.
one-way ANOVA and Tukey’s HSD (α = 0.05) showed that the 90-minute PN was significantly lower than the 180-minute PN, but all other pairwise comparisons were non-significant. There was no interaction between treatment and time ($F_{4,18} = 0.72, p = 0.588$). PC/PN ratios did not change significantly with treatment or time (Figure 1.7; two-way ANOVA, treatment: $F_{2,18} = 2.98, p = 0.076$; time: $F_{2,18} = 3.26, p = 0.062$; interaction: $F_{4,18} = 2.38, p = 0.090$).

The total suspended solid (TSS) concentrations were very high during the June trial (< 150 mgL⁻¹). TSS was not significantly different between treatments, but was different over time (GLM, treatment: $F_{2,19} = 0.61, p = 0.556$; time: $F_{2,19} = 8.21, p = 0.003$). A post-hoc one-way ANOVA and Tukey’s HSD showed that TSS at 180 minutes was significantly lower than at 30 and 90 minutes. (Figure 1.8). At 180 minutes, TSS concentrations in clam chamber outflows were significantly higher than TSS in inflow and control chamber outflows (one-tailed t-tests, $p = 0.03$ and $0.05$, respectively). There was no significant difference in total volatilized solids (TVS) between clam, control, or inflow chambers (GLM, treatment: $F_{2,19} = 0.05, p = 0.952$), or over time (time: $F_{2,19} = 0.14, p = 0.874$). Percent organic TSS was calculated by dividing TVS by TSS (Figure 1.9). Organic content of the water was very low: inflow seston was 19% organic, control outflow seston was approximately 17% organic; and clam outflow seston was approximately 17.5% organic. There were no significant differences between inflow, control, or clam chambers, and no significant difference over time (GLM, treatment: $F_{2,19} = 0.03, p = 0.975$; time: $F_{2,19} = 0.39, p = 0.685$).
FIGURE 1.7 Seston and biodeposit PC/PN ratios, June 2003 feeding experiment

There was no significant change in PC/PN ratios over time, or between control and clam chambers (one-tailed t-tests, $\alpha = 0.05$). Biodeposit (F) C:N ratio was lower than C:N for food (inflow chambers), indicating nitrogen enrichment of biodeposits.
FIGURE 1.8 Total suspended solids, June 2003 feeding experiment

TSS was not significantly different between treatments, but was different over time (GLM, treatment: $F_{2,19} = 0.61, p = 0.556$; time: $F_{2,19} = 8.21, p = 0.003$). Post-hoc one-way ANOVA and Tukey's HSD showed that TSS at 180 minutes was significantly lower than at 30 and 90 minutes (* below denoting difference from 30 and 90 min). At 180 minutes, TSS concentrations in clam chamber outflows were significantly higher than TSS in inflow and control chamber outflows (one-tailed t-tests, $p = 0.03$ and $0.05$, respectively, denoted by ** for difference from inflow and control chambers).
FIGURE 1.9 TSS organic content, June 2003 feeding experiments

The organic content of the TSS did not change significantly over time, or between treatments (GLM treatment: $F_{2,19} = 0.03$, $p = 0.975$; time: $F_{2,19} = 0.39$, $p = 0.685$)
In July and August 2003, clearance rates were calculated using particle counts, but relative changes in particulate matter were not assessed. Clams were observed for activity (extension of siphons), and little to no feeding activity was observed in July or August trials. Clearance rates were calculated for clam and control treatments, and treatments were compared for months (June, July, and August trials) using a GLM for \( CR^{1/2} \) with month as the model. Clearance rates were significantly different between months (Figure 1.5; \( F_{2,38} = 44.49, p < 0.0001 \)), with June > July, August (Tukey’s HSD, \( \alpha = 0.05 \)). In June, clearance rates were significantly higher for clam treatments than for control chambers (two-tailed t-test, \( t_{3,3} = 4.68, p = 0.021 \)), but in both July and August, clearance rates were not significantly different between clam and control treatments (two-tailed t-tests, July: \( t_{28,8} = 0.74, p = 0.238 \); August: \( t_{27,9} = 0.16, p = 0.438 \)). Net clearance rates (clam CR – control CR) were therefore near zero for the majority of July and August samples.

Individual clam clearance rates in the laboratory were highest during the June 2003 trial, averaging 2.56 L h\(^{-1}\) (see Table 1.5). Clearance rates in July and August were much lower, averaging 0.18 and 0.03 L h\(^{-1}\), respectively. Clam shell lengths and dry weights were similar for these three months (GLM shell length: \( F_{2,60} = 0.13, p = 0.879 \)), as was water temperature (range: 28 – 30.6 °C). Average inflow chlorophyll \( a \) concentration was significantly different between months (GLM: \( F_{2,25} = 21.17, p < 0.0001 \)), and highest during the August trials (32.0 \( \mu \)g L\(^{-1}\), compared to 14.0 and 3.35 \( \mu \)g L\(^{-1}\) for June and July, respectively). Particle concentration showed a similar trend, significantly different between months (GLM: \( F_{2,36} = 79.46, p < 0.0001 \)), with highest particle concentrations in August (\( 1.45 \times 10^5 \) particles mL\(^{-1}\)), followed by June (\( 1.29 \times 10^5 \) particles mL\(^{-1}\)) and July
(1.62 x 10^4 particles mL^{-1}). Average salinity was much lower during the August trials (10 ppt) than for June (15.0 ppt) and July (15.7 ppt).

Clearance rates (CR) were plotted vs. shell length (see Figure 1.3), and a linear regression was computed for CR^{1/2} vs. SL for June. As mentioned earlier, the July and August clearance rates for clam chambers were not significantly higher than for control chambers, so regressions for CR vs. clam size (SL or DW) for these months were disregarded. The June regression (CR^{1/2} = 0.648 + 0.0261 * SL) was not significant at the \( \alpha = 0.05 \) level (F_{1,7} = 3.02, p = 0.126, \( r^2 = 0.302 \)). When CR^{1/2} was plotted vs. clam tissue dry weight, there was a non-significant relationship (F_{1,7} = 1.35, p = 0.283, \( r^2 = 0.162 \)). Clearance rates were standardized for dry weight (CR_{DW} (L h^{-1} g^{-1} DW) = CR/DW), and compared for the three months using a GLM, and between pairs of months using t-tests. CR_{DW} were significantly different between all three months (F_{2,59} = 155.64, p < 0.0001), and between individual months (see Figure 1.10), with June having the highest CR_{DW} (5.7 ± 1.7 L h^{-1} g^{-1} DW), followed by July and August (1.9 ± 0.30 and 0.13 ± 0.03 L h^{-1} g^{-1} DW, respectively).

The effects of water temperature, salinity, inflow chlorophyll \( a \) concentration, inflow particle concentration, and flow rate on clearance rate were tested over all three months, using a combination of GLMs and regressions. Temperature and salinity both had significant effects on CR^{1/2} (temperature GLM: F_{5,57} = 11.67, p < 0.0001; salinity GLM: F_{2,60} = 20.96, p < 0.0001), as did inflow particle concentration (GLM: F_{6,56} = 55.08, p < 0.0001) and inflow chlorophyll \( a \) concentration (GLM: F_{6,56} = 55.08, p < 0.0001). The regression of CR^{1/2} vs. flow rate was also significant (Figure 1.11), CR^{1/2} = -0.478 + 0.0315 * Flow, F_{1,61} = 9.43, p = 0.003, \( r^2 = 0.134 \)).
FIGURE 1.10 Average dry weight dependent clearance rates (CR_{DW}) for summer 2003 months (error bars represent standard error)

Differences between all three months were significant, as denoted by asterices (*) (GLM, F_{2,59} = 155.64, p < 0.0001).
FIGURE 1.11 Effects of flow rate on dry weight dependent clearance rate, summer 2003

Flow rate had a significant effect on clearance rate (Regression for $CR^{1/2}$ vs. flow rate: $F_{1,61} = 9.43$, $r^2 = 0.134$)

$CR^{1/2} = -0.478 + 0.0313 \times \text{Flow}$
A multiple stepwise regression was conducted on the above variables to determine the best predictors of clearance rate ($CR^{1/2}$). The best single predictor of $CR^{1/2}$ was temperature, which explained 35% of the variance ($CR^{1/2} = -6.238 + 0.288 \times \text{Temp}$, $T = 5.73$, $p < 0.0001$). All of the remaining variables (salinity, particle concentration, and chlorophyll $a$ concentration) were correlated with temperature, so no larger multiple-factor model was used.

**Biodeposition**

Biodeposition (feces + pseudofeces) rates (mg AFDW g$^{-1}$ clam DW day$^{-1}$) were quantified during June 2003 feeding and November 2004 respiration trials. Biodeposition rates for June 2003 averaged 3.62 mg AFDW g$^{-1}$ clam DW day$^{-1}$. In the biodeposits collected from clam feeding chambers in June 2003, the average percent particulate organic carbon (POC) was 1.696 +/- 0.15 %, while the average percent particulate nitrogen (PN) was 0.289 +/- 0.026 % ($n = 9$). The C:N ratio of biodeposits was 5.87, indicating that biodeposits are more nitrogen-rich than the seston (Figure 1.6; control water POC/PN = 7.30; clam water POC/PN = 7.01; $n = 3$).

In November 2004, biodeposition rates ranged from 0 – 7 mg AFDW g$^{-1}$ clam DW day$^{-1}$, with percent carbon (by weight) ranging from 1.86 – 6.99 % (mean 3.80 % C); and percent nitrogen ranging from 0.27 – 1.05 % (mean 0.522 % N, Table 1.6). Particulate organic carbon biodeposition rates (mg POC h$^{-1}$) averaged 1.83 ± 0.97 mg POC h$^{-1}$, and particulate nitrogen biodeposition rates (mg PN h$^{-1}$) were 0.273 ± 0.152 mg PN h$^{-1}$.

Molar C:N ratios for biodeposits were fairly constant for all trials (Figure 1.12),
TABLE 1.6 Biodeposition rates, percent carbon and nitrogen, and C:N ratios, by weight

<table>
<thead>
<tr>
<th>Date</th>
<th>Biodeposition rate (mg AFDW g(^{-1}) clam DW day(^{-1}))</th>
<th>%C</th>
<th>%N</th>
<th>C:N biodep</th>
<th>C:N seston</th>
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</thead>
<tbody>
<tr>
<td>6/30/2003</td>
<td>3.62</td>
<td>1.70</td>
<td>0.29</td>
<td>5.9</td>
<td>*</td>
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<tr>
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<td>6.99</td>
<td>1.05</td>
<td>7.8</td>
<td>7.25</td>
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<td>7.5</td>
<td>8.51</td>
</tr>
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<td>0.48</td>
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</tr>
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<td>11/24/2004</td>
<td>0</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>7.77</td>
</tr>
</tbody>
</table>
FIGURE 1.12 Biodeposition rates for respirometry trials, November 2004

C:N ratios of biodeposits were relatively consistent (except for 11/16), despite differences in quantity of biodeposits.
averaging 8.64 ± 0.570. C:N ratios remained consistent between food and biodeposits for each trial (Figure 1.13), except for the November 9 trial, where biodeposits (C:N = 8.28) were relatively enriched in nitrogen relative to the water column (C:N = 13.87). Biodeposit percent C and N increased with increasing biodeposition rates (%C: $r^2 = 0.74$, %N: $r^2 = 0.70$; Figure 1.14).

Biodeposition rates (mg AFDW g$^{-1}$ clam DW day$^{-1}$) were plotted vs. food concentration (µg POC L$^{-1}$), and a positive exponential relationship was found (Figure 1.15, $r^2 = 0.95$).

**Respiration**

Net clam respiration rates for June – November 2004 ranged from 0 – 1.11 mg O$_2$ h$^{-1}$. There was a significant positive relationship between net respiration rate and clam dry weight (Figure 1.16, $F_{1,9} = 43.29$, $p < 0.0001$, $r^2 = 0.828$). Net respiration rates were normalized to clam dry weight (Net RR$_{DW}$, mg O$_2$ g$^{-1}$ DW h$^{-1}$), and regressed against water temperature. A polynomial regression was fit to the data, but this relationship was not statistically significant (Figure 1.17, $F_{1,9} = 0.35$, $p = 0.566$, $r^2 = 0.081$). Net RR$_{DW}$ was also regressed on salinity. The linear regression was non-significant (Figure 1.18, $F_{1,9} = 0.07$, $p = 0.795$, $r^2 = 0.008$). This relationship was most likely driven by the fact that the trials at higher salinities were conducted at Wachapreague during the summer months, when temperatures were also higher.
FIGURE 1.13  C:N by weight for food and biodeposits, clam respirometry trials, November 2004

C:N remains fairly consistent for food and feces, except for the November 9 trial, when biodeposits were enriched in nitrogen.
FIGURE 1.14 Biodeposit percent carbon and nitrogen vs. biodeposition rate

As biodeposition rate increases, so do the C and N content of the biodeposits.
Percent C (solid line) = 0.697 * (Biodeposition rate) + 0.734; $r^2 = 0.74$
Percent N (dashed line) = 0.098 * (Biodeposition rate) + 0.094; $r^2 = 0.70$
FIGURE 1.15 Biodeposition rates at different food concentrations, November respirometry trials

\[ y = 4 \times 10^{-5} e^{0.0052x} \]

\( R^2 = 0.9575 \)
FIGURE 1.16 Respiration rate vs. clam dry weight

Relationship is significant: $F_{1,9} = 43.29$, $p < 0.0001$, $r^2 = 0.828$.

Net respiration rate (mg O$_2$ h$^{-1}$) vs. dry weight (g)

Net RR = 0.3934 (DW) + 0.0324
FIGURE 1.17 Net dry weight dependent respiration rate vs. water temperature

Regression equation was not statistically significant (p = 0.566, $r^2 = 0.081$).

\[ y = -0.0108x^2 + 0.4663x - 4.4219 \]

\[ R^2 = 0.0811 \]
FIGURE 1.18 Dry weight dependent respiration rate vs. salinity

Regression equation was not significant ($F_{1,9} = 0.07$, $p = 0.795$, $r^2 = 0.0008$).

Net $RR_{dw} = -0.0040$ (sal) + 0.382
Respiration data were used to calculate $Q_{10}$ values for the temperature range 15.7 - 26.1 °C, and for included ranges (see Figure 1.19 and Table 1.7 for results and literature comparisons). The $Q_{10}$ value for the entire temperature range, 15.7 - 26.1 °C, was 1.67. For the higher end of the temperature range (23.9 - 26.1 °C), the $Q_{10}$ value was 0.64, while the $Q_{10}$ was 4.23 for the lower range (18.7 - 23.9 °C). To compare clam respiration rates to the literature, $Q_{O_2}$ (ml O$_2$ g$^{-1}$ total wet weight h$^{-1}$) values were calculated as in Loveland and Chu (1969). Log$_{10} Q_{O2}$ was plotted vs. log$_{10}$ total wet weight (TWW, g) to compare rates from the current study to values from Loveland and Chu (1969), Figure 1.20. Both the current study and Loveland and Chu (1969) results show a negative relationship between $Q_{O2}$ and TWW ($F_{1,10} = 10.29$, $p = 0.009$, $r^2 = 0.507$ for the current study; $F_{1,29} = 145.19$, $p < 0.0001$, $r^2 = 0.834$ for Loveland and Chu (1969).

Excretion

Dissolved inorganic nitrogen (nitrate, nitrite, and ammonium) concentrations were initially determined for the June 2003 feeding trials to attempt to quantify clam NH$_4^+$ excretion and N cycling in the flow-through system. For ammonium concentrations, no significant differences were seen between inflow, control, or clam chambers at 30 or 90 minutes (2-way ANOVA, treatment: $F_{2,18} = 0.86$, $p = 0.725$; time: $F_{2,18} = 0.86$, $p = 0.441$). No measurable changes were seen in any chambers for NO$_2^-$ or NO$_3^-$ concentrations. Therefore clam excretion rates were determined during November 2004 respiration trials, which were conducted using a static chamber filled with ambient unfiltered water.
TABLE 1.1 Temperature coefficients ($Q_{10}$) for respiration rates of *Mercenaria mercenaria*, current study and literature

<table>
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<tr>
<th>Reference</th>
<th>Data source</th>
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<td></td>
<td>20 - 26</td>
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</table>

$^a$ $Q_{10}$ values calculated by Grizzle et al., 2001

$^b$ reported by Grizzle et al., 2001
FIGURE 1.19 Temperature coefficients ($Q_{10}$) calculated for respiration rates, and literature comparisons

$Q_{10} = \left(\frac{K_1}{K_2}\right)^{10/(T_1-T_2)}$ where $K_1$ and $K_2$ = dry weight dependent net respiration rates at temperatures $T_1$ and $T_2$. 

![Bar chart showing temperature coefficients for different studies.](chart.png)
Regression is significant ($F_{1,10} = 10.29, p = 0.009, r^2 = 0.502$), and values are similar to Loveland and Chu (1969). Comparison of the two studies using analysis of covariance (ANCOVA) with study as the factor and TWW as the covariate, indicates no significant difference between the two study results ($p = 0.097$).
In November 2004, DIN (NH$_4^+$, NO$_3^-$, and NO$_2^-$) concentrations were measured at the start and end of respiration trials for clams and water alone, and excretion (flux) rates ($\mu$M h$^{-1}$) were calculated as $\Delta$ [DIN]/trial length. Excretion rates were small but measurable on most dates, with mean $\pm$ SE NH$_4^+$, NO$_3^-$, and NO$_2^-$ excretion being $1.43 \pm 1.02$, $0.027 \pm 0.028$, and $0.030 \pm 0.016 \mu$M h$^{-1}$, respectively. When regressed vs. clam dry meat weight (Figure 1.21), there were increasing trends for both NH$_4^+$ excretion and NO$_x$ (NO$_3^-$ + NO$_2^-$) flux. Data were log$_{10}$ transformed and regressions were calculated for log$_{10}$(excretion rate) vs. dry weight. Neither regression equation was statistically significant ($F_{1,4} = 3.58$, $p = 0.131$, $r^2 = 0.473$ for NH$_4^+$ and $F_{1,3} = 0.734$, $p = 0.455$, $r^2 = 0.196$ for NO$_x$); however, the relationship between excretion and clam dry weight was stronger for NH$_4^+$ than for NO$_x$.

Phosphate (PO$_4^{3-}$) fluxes ($\mu$M PO$_4^{3-}$ h$^{-1}$) were mainly negative, for both clam treatments and controls (Figure 1.22). Phosphate flux rates increased with increasing clam dry weight (Figure 1.23, $F_{1,4} = 3.72$, $p = 0.126$, $r^2 = 0.482$), indicating that clams were responsible for some amount of phosphate excretion, and that PO$_4^{3-}$ excretion rates were dry weight dependent. It is likely that phytoplankton and bacteria in the water column were utilizing PO$_4^{3-}$ faster than the clams could produce it. Nitrogen and phosphorus excretion results were not regressed against water temperature, since the temperature range was so narrow for November 2004 respirometry experiments (15-19 °C).
FIGURE 1.21 Nitrogen excretion/flux rate vs. clam dry weight, November respirometry experiments

Regressions were non-significant for NH$_4^+$ ($F_{1,4} = 3.58$, $p = 0.131$, $r^2 = 0.473$) and NO$_x$ ($F_{1,3} = 0.734$, $p = 0.455$, $r^2 = 0.196$).
FIGURE 1.22 Phosphate flux rates, November 2004 respirometry experiments

Negative fluxes indicate a decrease in PO$_4$ concentrations over the course of the experiment; positive fluxes indicate an increase in PO$_4$ concentrations. Clam and control treatments did not have significantly different results (paired t-test, $p = 0.353$).
FIGURE 1.23 Relationship between clam dry weight dependent PO₄ excretion rate and dry weight, November 2004 respirometry trials

Regression \( y = 0.1118x - 0.214 \) is significant \( (F_{1.4} = 3.72, \ p = 0.126, \ r^2 = 0.482) \).
DISCUSSION

Feeding

In June 2003, clam clearance rates were significantly higher than control clearance rates; with the 180-minute trial having the largest difference between the treatments. Clams were causing a significant change in chlorophyll $a$ concentrations after 180 minutes, although significant changes in particulate carbon and nitrogen concentrations (PC and PN) were not seen due to clams. It appears that particle fallout was significant in all treatments, as PC, PN, and TSS all decreased significantly over time. TSS % organic did not change over time, which indicates that organic and inorganic particles were falling out at equal rates, and no preferential removal of organic matter by grazers could be seen. These results indicate the importance of particle fallout in both clam and control chambers, and that calculations for clam clearance rate should account for controls.

June net clearance rates were significantly higher than those for July and August. In July and August trials, no significant difference was seen between clam and control treatments, so net clearance rates were essentially zero. The reasons for lack of feeding in July and August were probably two-fold: In July, chlorophyll and particle concentrations were very low; while in August, salinity was very low. All three of these parameters have significant effects on clearance rates.

Clearance rates from June 2003 feeding trials were compared to clearance rates from the literature (Hibbert, 1977; Doering and Oviatt, 1986; and Powell et al., 1992). Hibbert's (1977) study was the only one of the three which directly measured clam clearance rates. Doering and Oviatt (1986) used radioactively labeled carbon to
determine clearance rates. Powell et al. (1992) created a generalized bivalve feeding model incorporating many observations from the literature for a variety of bivalves, hypothesizing that bivalves have two feeding modes, termed “high gear” and “low gear”, which are not necessarily species specific.

Clearance rate results from June 2003 for the current study were closest to the Powell et al. (1992) “high gear” feeding rate (Figure 1.24). For this study’s range of temperatures and clam sizes, Hibbert (1977), Doering and Oviatt (1986), and Powell et al. (1992) “low gear” clearance rates are all very similar, exhibiting a similar dependence on clam size, and are all much lower than observed rates from June 2003. July and August 2003 clearance rates, which were close to zero, were considerably lower than any of the above literature estimates.

It should be noted that June 2003 feeding experiments were conducted at 30.6 °C, the ambient water temperature for Cherrystone Inlet at that time, which was higher than any literature feeding experiments. It is unlikely that water temperature alone explains the high observed clearance rates, however. July 2003 temperatures (29.9 °C) were almost identical to June 2003 temperatures, yet clearance rates were much lower in July (average CR = 0.18 L h⁻¹). July salinity (15.7 ppt) was also similar to June salinity (15.0 ppt), however, chlorophyll a concentrations were much lower in July (3.35 µg L⁻¹) than in June (13.96 µg L⁻¹). Particle concentrations mirrored chlorophyll a concentrations (see Table 1.5). Clam feeding rates have been shown to be very sensitive to food concentration (Tenore and Dunstan, 1973); therefore, it is likely that clams were feeding at low rates due to food concentrations being low. Chlorophyll a concentrations in the July
FIGURE 1.24 Comparison of June 2003 clearance rates to literature estimates for comparable temperatures and clam sizes

Rates from the current study appear closest to Powell “high gear” estimates.
experiments may have been an artifact of the experimental protocols in which water from Cherrystone Inlet was held overnight to wait for high tide before starting the feeding trial, mindful of the possibility that clams may exhibit an endogenous feeding rhythm. In June it had not been necessary to wait for the tidal stage. While water was being held in July, it is likely that zooplankton grazers were consuming phytoplankton at a high rate. In August 2003, attempts were made to remove larger grazers by filtering water with a 200 μm bag filter before feeding trials. This appeared to be successful, as the average chlorophyll \(a\) concentration during August trials was 31.96 μg L\(^{-1}\).

The most plausible explanation for low clam clearance rates in August 2003 is the extraordinarily low salinity of experimental water (10 ppt). Water was collected from Cherrystone at a spring low tide, after a period of high precipitation. Low salinity events are probably fairly common in Cherrystone Inlet, however, it was apparent that clams were not responsive under such conditions, as most clams did not extend their siphons in an attempt to feed or respire.

Overall, positive relationships were found between clearance rate and water temperature, salinity, and flow rate, corroborating findings by past studies. In an extensive review on suspension feeding, Winter (1978) summarized typical bivalve feeding responses to temperature increases. Typically, filtration rate increases with increasing temperatures up to an optimum temperature, which differs depending on the species. Above this optimum temperature, filtration rate decreases rapidly. Hibbert (1977) found highest filtration rates for \(M. mercenaria\) to be at 25 °C, but Newell and Koch's (2004) experimental results indicate higher filtration rates at 20 °C than at 25 °C. Growth rates of \(M. mercenaria\) are highest at 20-25 °C (review by Ansell, 1968; Laing et
(Grizzle et al., 1987), indicating that this range is most likely optimal for *M. mercenaria* feeding. In temperate areas, slow growth occurs in summer and winter, when temperatures are higher or lower than optimal; and fast growth occurs in spring and fall, when temperatures are in the optimal range (Grizzle et al., 2001). In Florida, slow growth occurs in summer and fall, corresponding to high water temperatures (see review in Arnold et al., 1991). It is surprising, therefore, that in the current study in Virginia, clearance rates would continue to increase with water temperatures as high as 30 °C.

Dry weight dependent clearance rate (CR_{DW}, L g^{-1} DW h^{-1}) data were used to calculate temperature coefficients, or \( Q_{10} \) values, for the temperature ranges 15.6 – 18.6 °C and 18.6 - 28.7 °C. For 15.6 – 18.6 °C, the \( Q_{10} \) value was 1.03, which is lower than most literature values for similar temperature ranges (see Table 8), and near 1, the value which is indicative of complete acclimatization by the animal. For the temperature range 18.6 – 28.7 °C, the \( Q_{10} \) value was 5.39, a very high value, reflecting that CR was continuing to increase at high temperatures.

Grizzle et al. (2001) state that salinities below 15 ppt generally have negative effects on *M. mercenaria* physiology, burrowing, growth, and long-term survival, citing Chanley (1958), and Castagna and Chanley (1973). Hamwi (1969) found a univariate relationship between pumping rate and salinity, with maximum *M. mercenaria* pumping rates at 28-30 ppt, decreasing to near zero at 15 ppt. The natural range of *M. mercenaria* in Chesapeake Bay is limited to regions with salinities above 12 ppt (17 ppt in summer) (Roegner and Mann, 1991). It is likely that *M. mercenaria* does not grow naturally in Cherrystone Inlet because the salinity is too low for reproduction and survival of larvae.
Results from the current study also indicate severe reduction of clearance rates below 15 ppt, although ambient salinities were not higher than 16 ppt for the study period.

In comparison to the current study results for flow rate effects on filtration rate (Figure 1.11), Walne (1972) found a positive relationship between filtration rate and flow rate in a laboratory flow-through feeding system for a variety of bivalves including *M. mercenaria*, noting that filtration rate per animal increased by about 25% with increasing flow rates up to about 70 ml min\(^{-1}\). Above this level, an increase in flow rate has an even greater effect (50%) on filtration rate. Flow rates in the current study were much lower than these, ranging from approximately 20-45 ml min\(^{-1}\), however, similar results were found: filtration rates were increased by about 18% with increasing flow rates.

Riisgård (1977) offered a mechanistic explanation for such observations in a flow-through feeding system. At low particle concentrations and low flow rates, bivalves are likely to be re-filtering the water, which would reduce apparent rates of filtration. Grizzle and Morin (1989) found in the field, increasing current speed had a positive effect on *M. mercenaria* growth, hypothesizing that increasing current speed also increases horizontal flux of particulate organic matter, making more food available to the clams. Walne (1972) also made observations of increased heart rate of bivalves with increasing flow rate, indicating that observed increases in filtration rate due to flow rate are likely physiological responses to the experimental conditions (increased feeding rates causing increased heart rates), not artifacts of the methods.
**Biodeposition**

Biodeposition rates (mg AFDW g\(^{-1}\) clam DW day\(^{-1}\)) showed a similar qualitative response to food (POC) concentration to the results of Tenore and Dunstan (1973), Figure 1.25. Both studies observed a logarithmic increase in biodeposition with increasing POC concentration, however, biodeposition rates from the current study were similar to Tenore and Dunstan's (1973) biodeposition rates for POC concentrations approximately 1000 µg L\(^{-1}\) lower. Clam biodeposits in both June 2003 and November 2004 were enriched in nitrogen relative to the water column. November biodeposits (average of 3.80 % C, 0.522 %N) had a higher percentage of both carbon and nitrogen than June biodeposits (average of 1.69 %C, 0.289 %N). There was a linear relationship between quantity of biodeposition and biodeposit percent carbon and nitrogen, with carbon and nitrogen content increasing at higher biodeposition rates (Figure 1.14). As feeding and biodeposition rates increase, gut residence time and assimilation efficiency decrease, and clams do not digest as much C or N from ingested food. If clams' C or N requirements are being met, excess C and N are egested in biodeposits. C:N ratios of biodeposits were often lower than seston C:N, seeming to indicate that clam egesta were more enriched in nitrogen than their food. It is probable that clams were being highly selective in the particles accepted for ingestion, particularly when TSS concentrations were extremely high in June 2003 feeding trials. If clams were rejecting large quantities of particulates in their pseudofeces, seston samples would not be indicative of material that is actually being ingested by clams.

Assimilation efficiencies based on organic content for November 2004 respirometry trials ranged from 17 – 65%. These were much lower than results reported by Tenore
FIGURE 1.25 Biodeposition rates at different food concentrations, comparison to Tenore and Dunstan, 1973

Rates are similar between two studies, despite differences in food concentrations.
and Dunstan (1973), (i.e., 71.2 – 77.3%) attained when the clams were fed cultured algae. When plotted against the organic content of the seston, it is apparent that assimilation efficiency increases with increasing organic content of food (Figure 1.26). These results were comparable to results from Hawkins et al. (1998) for *Crassostrea gigas* and *Mytilus edulis*, and appear to show a linear relationship for lower food organic content. At higher values for food organic content, Hawkins et al. (1998) found that this relationship is hyperbolic.

**Respiration**

Since Loveland and Chu (1969) reported respiration rate and clam wet weight data, it was possible to calculate $Q_{O2}$ values and compare their results to the current study using an analysis of covariance (ANCOVA). No statistical difference was found in the slopes of the two regression lines for the two studies ($p = 0.097$, Figure 1.20). Therefore respiration rates were as expected for this size range of clams, in comparison with the literature. Although there was not a statistically significant difference in respiration rates between the two studies, most respiration rates from the current study were lower than those of Loveland and Chu (1969) for wild clams. Slightly lower respiration rates for cultured clams would increase growth efficiency slightly, which could allow for increased growth rates in cultured clams. The increased variability in respiration rates from the current study compared to Loveland and Chu (1969), is probably due to the presence of phytoplankton, zooplankton, bacteria, and other small organisms in the unfiltered experimental water for the current study. Water was unfiltered to allow clam feeding while respiration rates were being measured.
FIGURE 1.26 Assimilation efficiency (AE) vs. organic content of ingested matter (OC), November 2004 respirometry experiments, compared to Hawkins et al. (1998) for *Crassostrea gigas* and *Mytilus edulis*

Current study: $AE = 4.103*OC - 0.3106$, $p = 0.48$, $r^2 = 0.2674$. 
The $Q_{10}$ value for 15.7 – 26.1 °C was 1.67, which was lower than literature $Q_{10}$ values from Hibbert (1977), and Bricelj (unpublished, reported by Grizzle et al. (2001)), but comparable to the value of 1.56 reported by Hamwi (1969) for the temperature range 20 – 25 °C (see Table 1.7 and Figure 1.19 for literature comparisons). These $Q_{10}$ values were slightly higher than 1, indicating that *Mercenaria mercenaria* does not fully acclimate its respiration to temperature below 25 °C, but is acclimating to these temperatures more than to higher temperatures. Between 23.9 and 26.1 °C, the $Q_{10}$ value of 0.64 was comparable to Hamwi’s (1969) $Q_{10}$ value of 0.29 for the temperature range 25 – 30 °C. It appears from the overall $Q_{10}$ value from the current study, that the clams were physiologically functioning normally from 15.7 – 26.1 °C. $Q_{10}$ values were very low for the upper end of this range (23.9 – 26.1 °C), indicating that clams are probably physiologically stressed and nearing lethal temperatures (Hochachka and Somero, 2002). These results corroborate results from growth studies such as Ansell (1968), indicating that temperatures above 20 °C are higher than optimal. The average mid-summer temperature at the southern end of *M. mercenaria*’s geographical range in Florida is 28 °C, indicating that temperatures higher than this are detrimental to hard clam survival and growth.

When weight-dependent clearance and respiration rates are plotted against water temperature (Figure 1.27), it is apparent that these rates increase at a similar rate for low temperatures, between ~15 and 20 °C. Above 20 °C, respiration rate increases at a slightly higher rate than clearance rate. At around 25 °C and above, respiration rate decreases while clearance rate continues to increase. These results were similar to those discussed in Grizzle et al. (2001). As temperature increases, energy expenditures by
FIGURE 1.27 Relationship between acclimatization temperature and weight-specific feeding rate and respiration rate, current study
increased respiration rates are offset by increased clearance rates. Since respiration rate increases at a faster rate than clearance rate between 20 and 25 °C, it would be expected that clam growth rate would decrease between these temperatures, as was observed by Bricelj (unpublished data, reported in Grizzle et al., 2001). Respiration rate begins to decrease with temperature near 25 °C as also observed by Hamwi (1969); however, clearance rate in this study continues to increase above 25 °C, where Hamwi observed decreased clearance rates mirroring respiration rates at these temperatures. It is possible that clams from Cherrystone Inlet are slightly better adapted to feed at higher temperatures than wild clams, after years of selective breeding. Alternatively, these results could be reflective of the unpredictability of an animal’s physiology at the upper limits of its thermal tolerance (Hochachka and Somero, 2002).

**Excretion**

Ammonium ($\text{NH}_4^+$) excretion data were converted to µmol day⁻¹, log transformed, and plotted vs. clam dry weight to compare results to the regression equation of Srna and Baggaley (1976). The resulting regression equation for the current study was almost identical to that of Srna and Baggaley (1976) when calculated over the same range of clam sizes (Figure 1.28). The relationship between $\text{NH}_4^+$ flux and clam dry weight was stronger than the relationship between $\text{NO}_x$ ($\text{NO}_3^- + \text{NO}_2^-$) flux and clam dry weight, as expected. Ammonium is the major N species of clam excretion (Hammen, 1968), and clams would not be expected to excrete nitrate or nitrite. $\text{NO}_x$ flux was most likely due to nitrification of excreted DON and PON from clam biodeposits. Srna and Baggaley (1976) also found positive flux (production) of nitrate in treatments with bivalves, but attributed this flux to nitrifying bacteria in the gut of the bivalves.
FIGURE 1.28 NH$_4^+$ excretion vs. dry weight, November 2004 respirometry trials

Data were converted to log (μmol day$^{-1}$) to compare to regression given by Sma and Baggaley, 1976. Regression lines are almost identical (current study is solid line, $y = 0.4486 \ln(x) + 1.352$, $r^2 = 0.65$; Sma and Baggaley is dashed line, $y = 0.4082 \ln(x) + 1.33$).
While net phosphate (PO$_4^{3-}$) fluxes were predominantly negative, indicating uptake from the water column, PO$_4^{3-}$ net flux increased with increasing clam dry weight (Figure 1.22). This indicates that clams were responsible for some amount of phosphate excretion, and that PO$_4^{3-}$ excretion rates were dry weight dependent. It is likely that phytoplankton and bacteria in the water column were taking up PO$_4^{3-}$ faster than the clams could produce it. Dame et al. (1989) found that in an oyster (*Crassostrea virginica*) reef, only a small percentage of the phosphate that was filtered was absorbed and subsequently excreted. Most of the phosphate filtered was egested in biodeposits. It is possible that *M. mercenaria* processes phosphate in a similar manner.

**CONCLUSIONS**

Major results from laboratory physiology experiments are summarized in Table 1.8. It appears that when all conditions are optimal for the clam (such as in June 2003), *Mercenaria mercenaria* feeding rates can reach levels as high as Powell et al.'s (1992) “high gear” model for bivalve feeding. When any condition is outside of the optimal range, clam feeding and respiration rates decline. Powell et al. (1992) hypothesized that differences between their “high gear” and “low gear” feeding models are not due to environmental factors, or methodological differences in feeding studies, but due to a physiological switch in the animal between two different, naturally occurring feeding rates. From the current study, it seems that all conditions being equal, some clams are more sensitive to environmental factors than others, keeping valves closed and siphons
### TABLE 1.8 Summary table of results from laboratory physiology experiments

<table>
<thead>
<tr>
<th>Physiological rate</th>
<th>Variable</th>
<th>Equation</th>
<th>F</th>
<th>p</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearance rate, L h⁻¹</td>
<td>shell length, mm</td>
<td>CR = 0.0759 * SL - 0.119</td>
<td>3.45</td>
<td>0.106</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>temperature, °C</td>
<td>CR¹/² = 0.305 (Temp) - 8.52</td>
<td>7.53</td>
<td>0.01</td>
<td>0.181</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CR¹/² = 0.494 (sal) - 8.32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>salinity, ppt</td>
<td></td>
<td>7.51</td>
<td>0.01</td>
<td>0.181</td>
</tr>
<tr>
<td></td>
<td>chlorophyll a, µg L⁻¹</td>
<td>CR¹/² = 0.0958 (chl) + 0.0893</td>
<td>63.16</td>
<td>&lt; 0.0001</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>particles ml⁻¹</td>
<td>CR¹/² = 1 x 10⁻⁵ (part) + 0.199</td>
<td>124.72</td>
<td>&lt; 0.0001</td>
<td>0.786</td>
</tr>
<tr>
<td>Biodeposition rate, mg AFDW g⁻¹ clam DW day⁻¹</td>
<td>POC, mg L⁻¹</td>
<td>log₁₀B = 8.12 log₁₀(POC) - 2.26</td>
<td>75.35</td>
<td>0.01</td>
<td>0.95</td>
</tr>
<tr>
<td>Net respiration rate, mg O₂ h⁻¹ R_DW, mg O₂ g⁻¹ DW h⁻¹</td>
<td>clam dry weight, g</td>
<td>R = 0.3934 (DW) + 0.0324</td>
<td>43.29</td>
<td>&lt; 0.0001</td>
<td>0.828</td>
</tr>
<tr>
<td></td>
<td>temperature, °C</td>
<td>R_DW = 0.0204(temp) - 0.013</td>
<td>0.35</td>
<td>0.566</td>
<td>0.038</td>
</tr>
<tr>
<td></td>
<td>salinity, ppt</td>
<td>R_DW = -0.0040(sal) + 0.382</td>
<td>0.07</td>
<td>0.795</td>
<td>0.008</td>
</tr>
<tr>
<td>NH₄⁺ excretion (V_NH₄⁺), µmol day⁻¹</td>
<td>clam dry weight, g</td>
<td>log₁₀V_NH₄⁺ = 1.03 log₁₀(DW) + 1.35</td>
<td>7.44</td>
<td>0.053</td>
<td>0.65</td>
</tr>
</tbody>
</table>
retracted while others are feeding at a fairly high rate. For many feeding studies, including the current study, individual clams were chosen for experiments from “active clams”, whose siphons were already extended. The difference between “high gear” and “low gear” feeding rates may be due to differences in percent time feeding between “feeders” and “non-feeders”. Because there is such a large difference between “high gear” and “low gear” estimates, it is not recommended to use these June 2003 feeding results for modeling estimates of feeding by the entire clam population, as they would most likely yield an overestimate of clam feeding.

At extreme salinity and temperature ranges, clams will remain closed and will not attempt to extend siphons to feed. Results from this study confirm previous studies’ conclusions that 20 °C is the ideal water temperature for hard clam feeding, respiration, and growth, and that salinities must be higher than 15 ppt for clams to extend siphons, feed, and respire. Clearance rate would be expected to have a univariate relationship with both water temperature and salinity. Feeding studies were not conducted over a full range of food (particle and chlorophyll a) concentrations, but clams appear not to feed below approximately 4 µg L⁻¹ chl a or 2 x 10⁴ particles ml⁻¹. These food concentrations are in the mid-range of those reviewed by Winter (1978), who found that mussel filtration rate decreases with increasing cell concentrations (10 - 40 x 10⁶ cells L⁻¹), such that the amount of algae consumed is constant. However, this is only true above a certain threshold cell concentration (10 x 10⁶ cells L⁻¹), below which filtration rate was assumed to be constant with increasing cell concentration. Results from the current study indicate that this threshold cell concentration may be higher for M.
mercenaria, and that below this level, filtration rate increases with increasing food concentration.

Biodeposition was not studied over the full range of temperatures, but biodeposition rates were on the same order of magnitude as those measured by Tenore and Dunstan (1973). Biodeposition increases with increasing food concentration. Assimilation efficiencies varied from 17 – 65%, which were much lower than those reported by Tenore and Dunstan (1973), however, the current study was conducted using natural seston, while Tenore and Dunstan (1973) used cultured Skelotonema costatum. Assimilation efficiencies were found to increase with increasing seston (food) organic content.

Respiration rates were similar to past studies, and increased with clam total wet weight, as was found by Loveland and Chu (1969). $Q_{10}$ values were near literature values, indicating that clams are probably physiologically stressed above approximately 23 °C. Respiration rates increase at a faster rate than clearance rates between approximately 20 and 25 °C, indicating that energy is being expended faster than it is being taken in at these temperatures, which would explain reduced clam growth rates at this temperature range (Ansell 1968, Bricelj, unpubl.). At very high water temperatures (30 °C), clam feeding rates did not decline in the predicted manner, possibly because animals were extremely stressed. It is unlikely that clams would be able to maintain these feeding rates for long periods of time.

Ammonium excretion rates increased as expected with increasing clam size, while NO$_x$ flux exhibited no dependence on clam size. Phosphate was likely to be excreted in small amounts which were not measurable due to uptake by phytoplankton.
Results from the laboratory component of this study indicate that cultured clams exhibit similar, if not identical, physiological responses as wild *M. mercenaria* for the ranges of conditions evaluated. Attempts were made to conduct experiments under ambient conditions, using transported water from Cherrystone Inlet, but these were not necessarily ideal conditions to measure physiological rates. It was problematical to maintain transported Cherrystone water long enough to repeat experiments under identical, controlled conditions. Sometimes it was not possible to measure rates under ambient conditions because they were outside the range of ideal conditions for the animal, so animals would not extend siphons to feed. Many of the environmental variables studied were not independent, which made multivariate analyses impossible. It was not possible to control all parameters that may have an effect on clam physiology, but measuring physiological rates under ambient conditions did give an accurate sense of how clams were being affected by local conditions in the creek. Average water temperatures in Cherrystone Inlet are generally higher than the optimal 20 °C from June – September, and while low salinity events below the 15 ppt cutoff for clam feeding are fairly rare, average annual salinities (approximately 21 ppt for 2001-2002) never reach the optimal 28 ppt (Chesapeake Bay Program, 2004). Conditions in Cherrystone Inlet may not be optimal for clam growth, but apparently they are adequate for a large percentage of the growing season. Observations of clam physiology at sub-optimal conditions will provide useful information for modeling efforts to predict growth rates, carrying capacity, and water quality for this embayment.
CHAPTER TWO

Field-based Studies on Feeding of the Cultured Clam, *Mercenaria mercenaria*

Clearance Rate and its Environmental Controls
2 Field-based Studies on Feeding of the Cultured Clam, *Mercenaria mercenaria*

Clearance Rate and its Environmental Controls
INTRODUCTION

Experiments on bivalve physiology for use in carrying capacity models are ideally conducted under natural conditions, to most accurately depict processes as they occur in situ. A few laboratory feeding experiments on Mercenaria mercenaria have been conducted using natural seston (e.g., Hibbert, 1977; Newell and Koch, 2004), under a range of temperature and salinity conditions that would be found in the animal’s natural habitat. However, some processes that are potentially important to bivalve physiology cannot be accurately duplicated in the laboratory. These include physical processes such as tides, wind, waves, and turbulence, as well as biological processes such as plankton processes, microbial processes, and competition. These processes occur on different time and space scales in nature than they would in the laboratory.

Often, there are processes affecting the bivalve energy budget in the field that researchers may not initially be able to predict. For example, early feeding studies focused on accurately determining the effects of food concentration on bivalve feeding rates using cultured algae (e.g., Riisgård and Møhlenberg, 1979; Kjørbe et al., 1981), but researchers realized that these studies possibly were not accurately depicting in situ feeding rates, since such a nutritious food source was not available to animals in the field (Grizzle et al., 2001). Phytoplankton is often mixed with inorganic material that must be sorted by the bivalve before consumption; as well as benthic microalgae, detritus, flagellates, bacteria, and many other potential food sources that make up what is termed “seston”. Assimilation efficiencies for such “mixed” food sources are likely to be much lower than for an algal monoculture. Inorganic components of the seston are likely to reduce bivalve growth rates due to production of pseudofeces (Bricelj and Malouf, 1984)
or reduction in feeding rates (Tenore and Dunstan, 1973). Detritus from vascular plants
(Tenore, 1983; Newell and Langdon, 1986), flagellates, and bacteria (Kreeger and
Newell, 1996; Langdon and Newell, 1990) are less nutritious and/or less available to
bivalves than planktonic food sources, and are not normally selected for ingestion by
suspension feeding bivalves (Langdon and Newell, 1990).

Researchers in the field of bivalve ecology now understand the importance of both
"physical and physiological influences on bivalve feeding behavior, set in the context of
response to the considerable spatial and temporal variability within the natural food
environment," (Bayne, 1993). A variety of studies analyzing bivalve physiological
processes in situ have been conducted in recent years. Studies on bivalve feeding and
food availability are difficult to conduct in the field because conditions can not be
controlled in the same manner as laboratory studies, and experiments must be designed
carefully to be statistically sound. A gradient of past laboratory – field studies exists,
from those feeding studies conducted under completely controlled conditions, to those
designed to observe in situ conditions and make correlations with bivalve feeding or
growth:

1. Laboratory feeding studies using controlled conditions, acclimated animals and
cultured algae or filtered water (i.e., Hamwi, 1969; Srna and Baggaley, 1976;
reviewed in previous chapter); flume studies observing effects of physical factors
in a laboratory setting (Grizzle et al., 1992; Butman et al., 1994);

2. Laboratory studies using natural seston and some combination of controlled and
ambient conditions (i.e., Hibbert, 1977; Newell and Koch, 2004);
3. Mesocosm studies using natural seston and attempting to replicate the natural environment as much as possible (i.e., Doering and Oviatt, 1986; Doering et al., 1986; Doering et al., 1987; Porter et al., 2004);

4. *In situ* studies using a device such as benthic ecosystem tunnels (Dame et al., 1984; Dame et al., 1989) or flux chambers (Boucher and Boucher-Rodoni, 1988) to measure changes in dissolved nutrients, particulate matter, and oxygen in response to bivalve metabolism;

5. *In situ* pumping of seston past bivalves in a separate enclosure, to facilitate observation of feeding (Hawkins et al., 1996; current study);

6. *In situ* studies tracking bivalve growth and making correlations with physical (currents, weather, resuspension, sediment type) and biological factors (chlorophyll/phaeophytin concentrations, particulate organic matter concentrations, C:N ratios) that have been simultaneously monitored (Grizzle and Morin, 1989; Irlandi and Peterson, 1991; Bock and Miller, 1994); and

7. *In situ* determination of components of the seston available for bivalve feeding on different time and space scales (Fréchette and Bourget, 1985; Muschenheim, 1987; Muschenheim and Newell, 1992; Judge et al., 1993; Huang et al., 2003).

Much of the work in the area of bivalve feeding *in situ* has focused on physical – biological interactions in the benthic boundary layer. A large body of work exists on the effects of flow, shear, and turbulence on bivalve feeding and growth and seston availability, indicating that these physical factors are very important in controlling food supply to the benthos. Many of these studies have analyzed aquaculture areas where
bivalves are grown at high densities and may be causing local seston depletion, which would decrease growth rates. Fréchette and Bourget (1985) sampled seston in situ and found that food (particulate organic matter, POM) is often depleted over mussel beds due to high rates of feeding, and stressed the role of mechanical energy in replenishing POM to these areas. Muschenheim (1987) also stressed the importance of seston flux, rather than concentration, to benthic suspension feeders. He conducted experiments using an apparatus to sample seston simultaneously at different heights to create a flux profile of organic seston which varied with flow rate, bottom roughness, and particle settling velocity. Muschenheim (1987) noted that the ability of a suspension feeder to reach less dense organic particles layered higher in the water column depends on the length of the animal's siphon and its pumping rate.

Based on results of a field study on clam growth, Grizzle and Morin (1989) hypothesized that horizontal seston fluxes are a major factor affecting individual growth of *M. mercenaria*, and created a statistical model (Grizzle and Lutz, 1989) to predict *M. mercenaria* growth over a range of tidal current/seston regimes and sediments. Grizzle and Lutz's (1989) “intermediate flow hypothesis” stated that intermediate flow rates produced highest growth rates in *M. mercenaria*. Turner and Miller (1991) tracked *M. mercenaria* growth in a laboratory flume during simulated storm events and found that, in contrast to Grizzle and Lutz (1989), horizontal seston flux alone was not able to predict clam growth, because growth was inhibited by large concentrations of inorganic matter in seston during resuspension events. Bock and Miller’s (1994) field study corroborated the findings that seston quality was more important than quantity to clam growth. They measured *M. mercenaria* shell growth in relation to weather and water column
parameters, and found that wave-induced resuspension of bottom sediments increased total suspended particulate matter, but reduced organic content, which had a negative effect on clam growth.

A variety of novel techniques and devices have been designed to sample seston available for bivalve feeding in situ. Continuing from Muschenheim’s (1987) designs for seston sampling devices, Muschenheim and Newell (1992) designed a Benthic Organic Seston Sampler (BOSS device) used to take simultaneous water samples at 10 heights within 0.5 m off the bottom. They used this to sample seston concentrations in and around mussel beds, and determined that over a mussel bed, chlorophyll $a$, total carbon, and total cell numbers were very uniform and low compared to upstream of the bed. Benthic diatoms which were prevalent upstream, were also virtually absent over the mussel bed. Mussels on the upstream edge of the bed had quantitatively and qualitatively different food than those further downstream, which would account for increased growth of mussels on the edges of beds, or the “edge effect” (Newell, 1990). Muschenheim and Newell (1992) also cited the importance of turbulence to replenish the food-depleted zone and prevent food limitation of mussel growth.

Newell and Gallager (1992) used Dame’s (1984) design for Benthic Ecosystem Tunnels (BEST) and time-lapse benthic video to observe mussel feeding over the course of a tidal cycle. They found tidal cycle variations in mussel shell gape (a proxy for filtration), seston consumed, oxygen consumed, and scope for growth, with maximum ingestion at high and ebb tides. Newell (1996) reported significant differences in mussel filtration rates between tidal stages, with mussels reducing shell gape for 2-3 hours in response to low ambient food concentrations at low tide.
Experimental approach

The approach of the current chapter builds upon knowledge of ecosystem influences on bivalve feeding behavior from past studies and on the laboratory studies from Chapter 1 to evaluate in situ influences on *M. mercenaria* feeding processes. It was necessary to determine feeding rates for cultured clams within their growout site, to evaluate whether laboratory-based feeding rates are suitable for use in water quality and carrying capacity models for this species, and to determine any important in situ influences on clam feeding that could not be seen in laboratory studies. Attempts made during 2003 feeding trials to simulate field conditions in the laboratory were not entirely successful, as properties of the seston transported from Cherrystone Inlet to the laboratory were constantly changing due to grazing and other plankton processes. Naturally occurring changes in seston flux due to physical influences, such as turbulence and tidal currents, could not be replicated in the laboratory. Based on the above studies, it is apparent that these parameters have important influences on clam feeding and should be included in any model which attempts to accurately depict clam physiological processes in situ.

Judge et al. (1993) pointed out the importance of sampling seston that is available for the hard clam by attempting to simulate incumbent siphon characteristics such as diameter, sampling height, and pumping rate, rather than isokinetic sampling (matching collection speed to the ambient current, which is generally faster than clam pumping rates). Isokinetic sampling would tend to over-sample the seston, including large particulates that normally would not be entrained in the current of a bivalve siphon. Judge et al.
(1993) sampled the seston, using intakes that were the same size and location in the water column as a clam siphon, and using pumping rates that were within the velocity range of clam pumping rates. Using this technique, they evaluated food available to clams within and outside of seagrass beds.

The current study combined the above seston sampling principles of Judge et al. (1993) with the “temporary field laboratory” of Hawkins et al. (1996) to measure feeding rates of *Mercenaria mercenaria* in response to local environmental conditions and seston characteristics. Seston was collected from the benthos using slow flow rates and clam siphon-sized tubing, and pumped into a flow-through feeding chamber to observe clam feeding rates *in situ*. Local environmental conditions during feeding studies were monitored, and seston characteristics (chlorophyll *a*, phaeophytin, and particle concentrations) were determined and correlations were made with clam clearance rates.

The influences of tidal currents on seston flux and bivalve clearance rates have been documented (Newell and Gallager, 1992; Newell, 1996; Huang et al., 2003), so feeding experiments in the current study were conducted over the course of the tidal cycle and correlations were made between tidal stage, feeding rates, and seston characteristics.

Shear and turbulence can have an effect on particle concentrations and seston flux (Muschenheim, 1987; Muschenheim and Newell, 1992), and reduction in current speeds by structures in the water column or bottom roughness can create vertical chlorophyll gradients (Judge et al., 1993), so the effects of predator exclusion nets on clam feeding rates are likely to be significant. These polyethylene nets, 4 m x 18 m, cover the clam beds and are held down by large sand bags. Various macroalgal species and other fouling organisms (e.g., tunicates, barnacles, oysters) grow persistently on the net substrate and
are cleaned off periodically by clam growers. Observations indicate that it is difficult for growers to keep up with the fouling growth, and clam nets were frequently observed as being covered with a thick carpet of macroalgae (Figure 2.1). Sediments under these nets generally appeared to be more fine-grained, and preliminary data indicates that they have a higher percent organic matter than sediments under “clean” nets (Condon, unpublished data). Hypoxia likely occurs fairly frequently under the algae-covered nets, as the sediments appeared to be sulfide-enriched. It is likely that flow rates are reduced by the nets or the algae fouling the nets, which leads to enhanced deposition of fine particles, and reduced dispersal of clam biodeposits. Experiments were therefore designed to test the effects of net presence on seston characteristics and clam feeding rates under the nets.

This first investigation of aquacultured clam feeding rates and their relevant environmental controls in situ will provide useful results on the applicability of laboratory feeding rates for use in suspension feeder carrying capacity models. It is also a primary investigation into the effects of predator exclusion nets on clam feeding rates and seston availability to the benthos, which will provide valuable information to the industry on human-induced controls on clam feeding and growth.

**Objectives**

1. Measure clam feeding rates in situ using Judge et al.’s (1993) recommendations for measuring food resources at a height applicable to the study organism and using sampling protocols with realistic pumping rates and incurrent tube diameters;
FIGURE 2.1 Photograph of macroalgae-covered clam nets at low tide
2. Whether clam feeding rates are correlated with seston characteristics at the bed level or tidal currents;

3. Determine whether predator exclusion nets affect bed level seston characteristics or clam feeding rates.

**METHODS**

Clam feeding experiments in the field were conducted during August and September 2004. Trials were conducted at the Cherrystone Inlet Upstream site (Figure 1.3), adjacent to Site 16 clam beds, and using clams from Site 16. Clams at this site had been planted in spring 2003, and were approximately 15-16 months old. Experiments were conducted throughout the tidal cycle, and water depth ranged from approximately 0.5 m (low tide) to approximately 1.5 m (high tide). Water temperature ranged from 21.8 – 28.5 °C and salinity ranged from 16.5 – 20.2 ppt throughout feeding trials.

Experimental feeding chambers were constructed to measure clam feeding rates *in situ*. The setup was designed to simulate clam feeding using a peristaltic pump to pull water from the creek sediment-water interface through a tube at a slow rate and push it into a chamber past a group of ten clams placed inside (Figure 2.2, Figure 2.3). The chamber dimensions were 50 x 16 x 10 cm, for a total volume of 8 L. Chambers were filled with sand to a depth of 6 cm, which gave a water volume of approximately 5 L. A pumping rate of 75 ml min⁻¹ was chosen, which was fast enough so that clams were not refiltering the water (Walne, 1972), yet slow enough to observe a difference in clearance rate between clam and control chambers, according to preliminary experiments. This
FIGURE 2.2 Experimental setup for \textit{in situ} clam feeding experiments

a. Peristaltic pump drawing water from creek benthos (see Fig. 3-2)
b. Feeding chamber inflow
c. Plexiglas\textsuperscript{®} baffles, to direct water flow
d. Chamber outflow
e. Outflow collection container
f. ISCO\textsuperscript{®} autosampler intake from collection chamber
FIGURE 2.3 Diagram of chamber inflow setup

a. predator exclusion net over clam bed
b. chamber intake, positioned towards the water column, 90° to the benthos
c. inflow tubing
pumping rate gave a filling time of 66.7 minutes for the chamber, so sampling times 1.5 – 3 hours apart would be adequate to observe changes in clearance rate over time.

Ten clams from the growout site were placed in the feeding chamber and the chamber was placed in a water bath, to which Cherrystone water was continuously pumped, to keep the clams at ambient temperature (Figure 2.4, Figure 2.5). The chamber inflow tube was placed on the creek bottom, oriented towards the water column as a clam siphon would be, and protruding approximately 1 cm into the water column (Figure 2.3). Water was drawn from the creek benthos, and into clam and control chambers. The control chamber was set up identically to the clam chamber, but with no clams inside the chamber, and with inflow tubing immediately adjacent to clam inflow tubing. Chamber outflows emptied into 1 L containers which were also kept at ambient temperatures in the water bath (Figure 2.6). Outflow samples were taken from each chamber automatically at set 1.5 - 3 hour intervals by an ISCO® autosampler, which kept each water sample on ice and in the dark until the end of the experiment (24 + hours). Ambient water temperature, salinity, dissolved oxygen, and pH were monitored continuously with a Hydrolab datasonde attached to the experimental setup. In this manner, clam feeding rates and environmental parameters could be monitored in a field setting, without human disturbance to the area.

Each trial was begun at low tide and ran for over 24 hours. To test the effects of predator exclusion nets on clam feeding rates, half of each trial (12-15 hours) was conducted with the inflow tubing under a net, within the clam bed ("Net" subtrials). The inflow tubing was uncovered and moved adjacent to the net for the other half of the trial ("No net" subtrials). Chamber intakes were moved at least 3 hours before the next
FIGURE 2.4 *In situ* feeding trials, experimental setup

a. PVC pole marking corner of clam plot #16
b. chamber intakes, under or adjacent to clam net
c. inflow tubing
d. peristaltic pumps, under cover
e. water bath containing clam and control feeding chambers
f. ISCO® autosamplers, intakes sampling from feeding chamber outflows inside water bath
FIGURE 2.5 In situ feeding setup

a. Inflow tubing from clam net
b. Peristaltic pumps for clam and control chambers
c. 12 V battery power source
d. Inflow tubing from pumps to clam and control chambers (in water bath)
e. Data sonde
FIGURE 2.6 Field feeding setup, water bath interior view

a. Clam feeding chamber
b. Control feeding chamber
c. Chamber outflow containers
sampling time. "Net" and "No net" subtrials alternated being conducted first, such that "Net" and "No net" subtrials each were conducted twice beginning at low tide and twice at high tide.

Water samples were returned to the laboratory immediately following each experiment, where samples were filtered for chlorophyll analysis and particle counts were made using the Coulter counter. Clearance rates were calculated using a similar formula as for laboratory trials:

\[ CR = f[(C_c - C_o)/C_o] \]

where \( f \) was the water flow rate (L h\(^{-1}\)), \( C_c \) was the particle concentration in the clam chamber outflow, and \( C_i \) is the particle concentration in the control chamber outflow (particles L\(^{-1}\)). The main difference in clearance rate calculations for the lab and field methods was that for the field, \( C_c \) represented the particle concentration in the control chamber outflow, rather than \( C_i \), the particle concentration in the clam chamber inflow, as it was impossible to measure inflow concentrations using this method without altering particle concentration or food availability. The assumption was made that inflow particle concentrations in control and clam chambers were identical, and that particle fallout occurred at a similar rate in control and clam chambers. The first assumption was tested in preliminary trials, where inflow concentrations in control and clam chambers were found to be statistically comparable (two-tailed t-test, \( t_{26} = 0.84, p = 0.797 \)).

Clam shell lengths and dry weights were measured and recorded for each trial. Data for environmental parameters were matched with feeding data for the same timepoint. Each sampling event was assigned a tidal cycle stage ("High", "Low", "Ebb", or "Flood"), according to the Tides and Currents software (Nautical Software Inc.)
predictions for Cape Charles Harbor. Predicted tides for Cape Charles Harbor were assigned to sampling events ½ hour after the predicted tide time, since the study site was approximately 10 miles upstream from Cape Charles Harbor. Data were pooled for both net intake locations ("Net" vs. "No net"), to test for differences in chl a at different stages of the tidal cycle. After testing data for normality and homogeneity of variances, data were transformed as required to meet test assumptions. Relationships between clam feeding rates and environmental parameters were determined using linear regression, analysis of variance (ANOVA), analysis of covariance (ANCOVA) and t-tests. In some cases, post-hoc tests (e.g., Tukey's HSD) were conducted on significant ANOVA results for pairwise comparisons of means. An α level of 0.05 was chosen to determine significance of results for all statistical tests.

Ecologically meaningful clam feeding rates were thus determined by allowing clams to feed on naturally occurring seston under natural temperature and salinity conditions, without confounding effects involved in transporting clams and water, or attempting to maintain water at Cherrystone conditions in the laboratory.

RESULTS

Since all trials were conducted at the same grow-out site, and clams were selected from that same site, all clams were the same age and very close in size: approximate age of clams was 15-16 months; mean shell length was 43.6 ± 0.13 mm; mean tissue dry weight was 0.764 ± 0.001 g. Means and ranges for environmental parameters are given in Table 2.1. All environmental variables (temperature, salinity, dissolved oxygen, pH, chlorophyll a concentration, phaeopigment concentration, and particle concentration)
TABLE 2.1 Environmental parameters for *in situ* clam feeding experiments, August – September 2004

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Mean ± SE</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water temperature</td>
<td>°C</td>
<td>25.9 ± 0.33</td>
<td>21.8 - 28.5</td>
</tr>
<tr>
<td>Salinity</td>
<td>psu</td>
<td>18.3 ± 0.26</td>
<td>14.4 - 20.2</td>
</tr>
<tr>
<td>Inflow chl a</td>
<td>µg L(^{-1})</td>
<td>13.0 ± 1.20</td>
<td>6.07 - 27.2</td>
</tr>
<tr>
<td>Inflow phaeopigments(^1)</td>
<td>µg L(^{-1})</td>
<td>13.6 ± 1.83</td>
<td>6.11 - 27.3</td>
</tr>
<tr>
<td>Inflow particle concentration</td>
<td>particles ml(^{-1})</td>
<td>3.43 x 10(^5) ± 1.30 x 10(^4)</td>
<td>2.17 - 4.70 x 10(^5)</td>
</tr>
<tr>
<td>Dissolved oxygen</td>
<td>mg L(^{-1})</td>
<td>6.14 ± 0.24</td>
<td>4.27 - 9.23</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>7.94 ± 0.027</td>
<td>7.62 - 8.12</td>
</tr>
</tbody>
</table>

\(^1\) not measured for all trials
were normally distributed, except for chlorophyll ($p = 0.015$) and temperature ($p < 0.005$). Chlorophyll data were square-root transformed for subsequent parametric tests (subsequently termed chl$^{1/2}$, and this transform succeeded in obtaining normality ($p = 0.051$). Transformation did not succeed in obtaining normality for temperature, so temperature was not included in parametric analyses.

Pearson’s correlation coefficients ($r$) were calculated for all remaining environmental variables (salinity, chlorophyll, phaeopigments, particle concentration, dissolved oxygen, and pH) to determine correlation between environmental factors. There was a significant correlation between chl$^{1/2}$ and salinity ($r = 0.542$, $p = 0.002$); chl$^{1/2}$ and pH ($r = 0.449$, $p = 0.041$); and dissolved oxygen and pH ($r = 0.633$, $p = 0.002$). Phaeophytin and chl $\alpha$ were strongly correlated ($r = 0.974$, $p < 0.0001$), and phaeopigment concentrations were correlated with salinity ($r = 0.542$, $p = 0.002$). All other tested interactions were non-significant.

The mean clam clearance rate for all trials was $1.01 \pm 0.12$ L h$^{-1}$, ranging from 0 - 2.57 L h$^{-1}$. Clearance rates were log$_{10}$ transformed to obtain normality and will be termed log$_{10}$CR hereafter. Clearance rates were significantly different for different trials (one-way ANOVA, $F_{3,24} = 6.76$, $p = 0.002$), with the major differences being between Trial 1 (8/19/04) and Trial 2 (8/25/04), and Trial 1 and Trial 3 (9/2/04) (Tukey’s HSD, 95% CI, Figure 2.7). There were no other significant differences between any other pairwise comparisons. The one-way ANOVA for log$_{10}$CR vs. mean shell length was non-significant ($F_{2,25} = 1.95$, $p = 0.163$), as was the one-way ANOVA for log$_{10}$CR vs. dry weight ($F_{2,25} = 1.95$, $p = 0.163$). Clams were comparable equal in size, so size did not have a significant effect on clearance rates.
CR was log$_{10}$ transformed to meet ANOVA assumptions, but non-transformed mean + SE shown here. There is a significant difference for Trial on mean log$_{10}$ clearance rate ($p = 0.002$). Post-hoc pairwise comparisons (Tukey's HSD, 95% CI) showed significant differences between log$_{10}$ CR for $2, 3 < 1$. All other pairwise comparisons were statistically equal.

a Means are statistically different (Tukey’s HSD)
Chlorophyll $\alpha$ concentrations were significantly different between trials (one-way ANOVA for chl$^{1/2}$ and trial, $F_{3,25} = 32.61$, $p < 0.001$), with all trials significantly different from each other (Tukey’s HSD, 95% CI, Figure 2.8). Patterns of chlorophyll concentrations between different trials were very similar to those for clearance rates (compare Figures 2.7 and 2.8): Trial 1 > Trial 4 > Trial 2 > Trial 3. Across all trials, clearance rate increased as chlorophyll $\alpha$ increased, with chl $\alpha$ explaining about 24% of the variation in clearance rate (Figure 2.9, regression equation: $\log_{10} \text{CR} = -0.600 + 0.153 \times \text{chl}^{1/2}$, $F_{1,26} = 8.15$, $p = 0.008$, $r^2 = 0.239$). Phaeopigment concentrations (phaeo) also had a positive effect on clam clearance rates, explaining about 41% of the variation in clearance rate (Figure 2.10, regression equation: $\log_{10} \text{CR} = -0.299 + 0.0140 \times \text{phaeo}$, $F_{1,13} = 8.91$, $p = 0.011$, $r^2 = 0.407$); however, since chl$^{1/2}$ and phaeo are strongly correlated, these effects are not additive.

As mentioned above, there was no correlation between inflow chlorophyll $\alpha$ and inflow particle concentration ($r = -0.003$, $p = 0.988$). There was also no significant relationship between particle concentration and clearance rate (regression equation: $\log_{10} \text{CR} = -0.341 + 0.000001 \times \text{Partconc}$, $F_{1,26} = 1.30$, $p = 0.265$, $r^2 = 0.048$). Particle concentration was not correlated with any other environmental parameter measured.

Salinity did not have a significant effect on clearance rate (regression equation: $\log_{10} \text{CR} = -1.03 + 0.0530 \times \text{Sal}$, $F_{1,26} = 1.99$, $p = 0.170$, $r^2 = 0.071$), despite having a positive correlation with chl$^{1/2}$ ($r = 0.542$, $p = 0.002$). Salinity was not positively correlated with any other environmental variable that was measured. Neither pH nor dissolved oxygen concentration had a significant effect on clearance rate (regression
FIGURE 2.8 Inflow mean chlorophyll \( a \) concentrations, by trial number

Note similar trends to mean clearance rates (Figure 2.7, above). Chl data was square root transformed for analysis (ANOVA, \( F_{3,25} = 32.61 \), \( p < 0.0001 \)), but non-transformed data are presented here.

\[
\begin{array}{c|c|c|c|c}
\text{Trial} & 1 & 2 & 3 & 4 \\
\hline
\text{Chlorophyll (mg L}^{-1} \text{)} & 25 & 15 & 10 & 15 \\
\end{array}
\]

Means are statistically different (Tukey’s HSD)
FIGURE 2.9 Linear regression for clearance rate vs. chlorophyll $a$ concentration

Plot is for non-transformed data. Regression equation: $\log_{10} CR = -0.600 + 0.153 \times \text{chl}^{1/2}$, $F_{1,26} = 8.15$, $p = 0.008$, $r^2 = 0.239$).
FIGURE 2.10  Linear regression for clearance rate vs. phaeophytin concentration

Non-transformed data are displayed. Regression equation: \( \log_{10} CR = -0.299 + 0.0140 \times \text{phaeo} \), \( F_{1,13} = 8.91, p = 0.011, r^2 = 0.407 \).
equation for pH: \( \log_{10} CR = -1.81 + 0.226 \, pH \), \( F_{1,26} = 0.17, p = 0.686, r^2 = 0.009 \); 
regression equation for DO: \( \log_{10} CR = -0.196 + 0.0311 \, DO \), \( F_{1,26} = 0.42, p = 0.525, r^2 = 0.02 \); however, dissolved oxygen and pH were correlated with each other (\( r = 0.633, p = 0.002 \)). When dissolved oxygen and pH were plotted vs. time of day that samples were taken, there was an apparent diel pattern to the data (Figure 2.11). Third-order polynomial curves were fit \( (\text{time})^2 - 4.02 \times (\text{time})^3 \); \( F_{3,24} = 3.83, p = 0.022, r^2 = 0.324 \). It was apparent that both dissolved oxygen and pH peaked at approximately 5 PM, and each had a minimum at approximately 5 AM.

**Net effects on clearance rates**

Average clearance rates were calculated for all treatments that had inflows under clam nets ("Net") vs. inflows that were taken from the water column ("No net"). Clearance rates averaged 1.244 ± 0.188 L h\(^{-1}\) for "No net" intakes, and 0.806 ± 0.107 L h\(^{-1}\) for "Net" intakes). "No net" clearance rates were higher than "Net" clearance rates, (Figure 2.12, one-tailed t-test, \( t_{26} = 2.52, p = 0.009 \)), with net presence reducing clam clearance rates by 35%. Chlorophyll concentrations were approximately 33% higher for "No net" than for "Net" intakes (Figure 2.13, one-tailed t-test, \( t_{26} = 1.79, p = 0.044 \)), but there was no significant difference in particle concentrations between "Net" and "No net" (one-tailed t-test, \( t_{26} = 2.58, p = 0.992 \)). Phaeophytin concentrations were not significantly different between intake locations (two-tailed t-test, \( t_{14} = -0.40, p = 0.693 \)). Temperature (\( t_{23} = -0.91, p = 0.371 \)) and salinity (\( t_{23} = -0.91, p = 0.371 \)) did not differ between net intake locations (two-tailed t-tests).
FIGURE 2.11 Diel patterns of dissolved oxygen and pH for clam beds (data pooled from all trials)

\[
\text{DO} = 6.77 - 16.4 \times \text{time} + 47.0 \times (\text{time})^2 - 32.5 \times (\text{time})^3; \\
F_{3,24} = 4.74, p = 0.010, r^2 = 0.372
\]

\[
\text{pH} = 8.10 - 2.36 \times \text{time} + 6.08 \times (\text{time})^2 - 4.02 \times (\text{time})^3; \\
F_{3,24} = 3.83, p = 0.022, r^2 = 0.324
\]
FIGURE 2.12 Clearance rates for clams with chamber intakes under predator exclusion nets ("Net"), and with intakes outside of nets "No net"

"No net" clearance rates were significantly higher than "Net" CR (one-tailed t-test, $t_{26} = 2.52$, $p = 0.009$), with CR being reduced by approximately 35% for "Net" intakes.
FIGURE 2.13 Average chlorophyll $a$ concentrations in “net” and “no net” clam chamber inflows for field feeding experiments

“No net” inflows had significantly more chlorophyll $a$ than “net” inflows (one-tailed t-test, $t_{26} = 1.79$, $p = 0.044$)
An analysis of covariance (ANCOVA) was conducted to compare the effect of intake locations ("Net" vs. "No net") on clam clearance rates, with chlorophyll concentration as the covariate. Results indicate that most of the influence of intake location on clam clearance rate was due to increased chlorophyll concentrations in "No net" inflows ($F_{1,25} = 5.21, p = 0.031$), rather than due to the inflow location itself ($F_{1,25} = 3.61, p = 0.069$).

**Tidal stage effects on food availability**

Feeding chamber inflow chlorophyll $a$ concentrations were highest during ebb and flood tides for the "no net" inflows (Figure 2.14). Chl $a$ was lower for high and low tides for "no net" inflows, but not significantly so (one-way ANOVA, $p = 0.18$). Tidal stage had no effect on chl $a$ for "net" inflows (one-way ANOVA, $p = 0.43$). Results indicate that for "No net" intakes, on a "moving" (ebb or flood) tide, there was significantly more inflow chl $a$ than on slack (high and low) tides (one-tailed t-test, $t_{11} = 1.89, p = 0.042$, Figure 2.15). For the "Net" intakes, there was no effect of tidal stage on chl $a$ concentrations (one-tailed t-test, $t_{11} = 1.55, p = 0.074$). At "moving" tidal stages, there was more chl $a$ for the "No net" intakes than for the "Net" intakes, but this was not significant at the $\alpha = 0.05$ level (one-tailed t-test, $t_{10} = 1.39, p = 0.097$).
FIGURE 2.14 Feeding chamber inflow chlorophyll $a$ concentrations were highest during ebb and flood tides for the “no net” inflows

Chl $a$ was lower for high and low tides for “no net” inflows, but not significantly so (one-way ANOVA, $p = 0.18$). Tidal stage had no effect on chl $a$ for “net” inflows (one-way ANOVA, $p = 0.43$).
FIGURE 2.15  Chl $a$ concentrations were higher at ebb/flood tides than at slack tides for “no net” intakes (one-tailed t-test for $\text{chl}^{1/2}$, $t_{11} = 1.89$, $p = 0.042$)

Tidal stage had no significant effect on chl $a$ concentrations for “net” intakes (one-tailed t-test for $\text{chl}^{1/2}$, $t_{11} = 1.55$, $p = 0.074$).
DISCUSSION

Clearance rates

The primary influence on clam clearance rates during these feeding trials was food concentration. Clams consistently increased their clearance rates in response to higher ambient food concentrations on the flooding tide. It is interesting that phaeophytin concentration ($r^2 = 0.410$) had a stronger effect on clearance rate than total chlorophyll $a$ concentration ($r^2 = 0.239$). One would normally expect that phaeophytin would be a less nutritious food source than total chl $a$ (which includes active chlorophyll and phaeopigments), and that clams would reduce clearance rates in the presence of a less-than-ideal food source. High phaeophytin concentrations in the vicinity of clam nets could be the result of either: (a) decaying macroalgae being grazed by microbes and possibly clams; or (b) high levels of clam feeding on phytoplankton resulting in high concentrations of degraded chlorophyll. Macroalgal detritus could be a food source for clams in Cherrystone Inlet, but this possibility must be investigated further.

Environmental parameters

Correlations between dissolved oxygen concentration and pH are as expected: as dissolved oxygen concentrations decrease due to respiration, carbon dioxide dissolves in seawater, making the water more acidic. There is a large diel gradient in oxygen concentrations at the benthos, which was very consistent from trial to trial. These results were similar to those of Breitberg (1990), who found that shallow Chesapeake Bay waters experience large diel fluctuations in dissolved oxygen, with daily minima in the
late night and early morning hours in July and August. In this area of Cherrystone Inlet at night during August – September, DO concentrations routinely decreased below 5 mg L\(^{-1}\), which corresponds to 50 – 60 % saturation. Hard clam oxygen consumption rates decline below 5 mg L\(^{-1}\) (Hamwi, 1969), and \textit{M. mercenaria} growth rates are reduced greatly below 4.2 mg L\(^{-1}\) (Morrison, 1971). The diel oxygen fluctuations in such a shallow tributary are likely due to local production and respiration: high rates of photosynthesis producing high concentrations of oxygen during daylight hours, and combined autotrophic and heterotrophic respiration using up this oxygen at night.

The correlation between pH and chlorophyll \(a\) is likely due to a covarying relationship between pH and DO. As chlorophyll \(a\) concentrations increase, production would be expected to increase, which would raise daytime DO levels via photosynthesis. As determined above, DO and pH are positively correlated, which would also make chl \(a\) and pH correlated. The correlations between salinity and chlorophyll \(a\), and salinity and phaeophytin are likely due to tidal seston flux, as discussed below.

\textit{Influence of tidal stage}

During the field experiments, when there was a moving tidal current (ebb and flood tides), more chlorophyll \(a\) was available than at slack (high and low) tides for the “No net” chamber intakes. The positive correlation between chlorophyll and salinity indicates that the major source of chlorophyll is a downstream, higher salinity area such as the Chesapeake Bay, or another source which is associated with the flood tide. On average, “flood” chlorophyll concentrations are slightly higher than “ebb” concentrations, but not significantly so. Two possible explanations are that the tide may have been bringing new
Phytoplankton to the region from downstream, or microphytobenthos and detritus may have been resuspended in the tidal currents, causing an increase in chl $a$ with the tide. The tidal current may be stronger on the flood tide than on ebb, which could cause increased resuspension. Whichever explanation is the case, it appears that the tidal current is the most important source of seston for the clams.

For all samples taken, phaeopigments contributed to a large proportion of total chlorophyll, and phaeophytin and chl $a$ were strongly correlated ($r = 0.974$, $p < 0.0001$). Phaeopigment concentrations were correlated with salinity ($r = 0.542$, $p = 0.002$), which supports the explanation that the tidal chlorophyll source was probably a detrital one, stemming from resuspension due to tidal currents. There was no significant relationship between “tide” designation and phaeophytin concentration; however, salinity is likely a better indicator of tidal stage than the “tidal stages” designated to each sampling event. The tidal designations given were estimates based on a model for a site more than 10 miles downstream, which did not take wind or other effects on the tidal current.

**Influence of nets and interacting effects**

There was significant local seston depletion under clam nets compared to “No net” intakes, which was a similar result to previous studies (Fréchette and Bourget, 1985; Muschenheim and Newell, 1992) who found local seston depletion in areas of bivalve aquaculture. Interestingly, there was no observable effect of the tidal stage on chlorophyll concentrations within the feeding chambers for “Net” subtrials. It seems that for clams under the nets, chlorophyll levels are always as low as at slack tide. The tidal current is bringing more chlorophyll to the area, but clams under the nets are not able to
acquire this additional food source. It is also possible that increased chlorophyll from the
tide was reaching the clams under the net, but it was consumed by the clams before being
pumped into feeding chambers. If this scenario were the case, clams must have been
feeding at a higher rate in response to increased food availability, or such consistent
levels of chl $a$ under the clam nets would not have been seen. The regressions of clearance
rate vs. chlorophyll $a$ concentration support these observations, since clam feeding rate
increases with increasing chl $a$. These findings are also similar to those of Newell
(1996), who observed decreased feeding activity of mussels in response to local seston
deployment. It is presumed, therefore, that high densities of clams are causing localized
food depletion under nets, which in turn causing decreased clam clearance and ingestion
rates.

There was no significant difference in phaeophytin concentrations between “Net” and
“No net” intakes, although the increased total chlorophyll seen for “No net” intakes
indicates higher relative contributions of phaeophytin to the total chlorophyll pool in
“Net” areas. This relationship would be expected, since clam nets are covered with
macroalgae, which would be a source of detritus and phaeophytin, and “No net” areas
would have more access to water column phytoplankton and active chl $a$. Additionally,
increased grazing by clams in “Net” areas would cause higher relative concentrations of
phaeophytin compared to total chl $a$ than in “No net” areas.

In summary, it is suggested that during late summer in Cherrystone Inlet, clams are
feeding at a higher rate when the tide is changing than at slack tide, due to increased food
availability at ebb and flood tides. These results support Grizzle and Morin’s (1989)
findings that horizontal seston flux has a major influence on clam growth. Increased
seston flux with flood tidal currents consisted predominantly of phaeophytin, which is indicative of a detrital seston source. Phaeophytin concentration had a larger influence on clam feeding rates than total chlorophyll concentration, which is contrary to Bock and Miller's (1994) conclusions that food quality is more important than quantity for hard clam growth. However, Bock and Miller's (1994) study based food “quality” on organic content rather than phaeophytin concentrations, and the current study did not analyze organic content of food.

It is likely that the tidal current is causing resuspension of detritus from the benthos, increasing its availability to suspension feeders. Detritus may contribute significantly to the hard clam energy budget during summer months when clam metabolic demands exceed primary production capabilities of the inlet (see Introduction). These results are somewhat surprising, as Langdon and Newell (1990) found that it was unlikely that *Spartina alterniflora* detritus could provide adequate nutrition for the mussel *Geukinsa demissa* or the oyster *Crassostrea virginica*, because they were not able to digest most of the carbon bound in cellulose. It is possible that macroalgal detritus is a more labile food source to the hard clam than *S. alterniflora*. A past study on the polychaete *Nereis diversicolor* found that the worm had higher assimilation efficiencies for macroalgal food sources than for vascular plants, which were correlated with the cellulose and lignin content of the plant (Olivier et al., 1994). Future studies would be necessary, however, to determine the availability of macroalgal detritus as a food source for cultured hard clams in Cherrystone Inlet.

Predator exclusion nets which are designed to protect growing clams from predation mortality, also have a negative effect on clam feeding rates by reducing chlorophyll
available to the clams. It may be possible for growers to abate these effects by cleaning macroalgae from the nets more frequently; however, this may also remove an alternate food source. Diel dissolved oxygen patterns indicate regular nighttime hypoxia events which could have a negative effect on clam growth according to EPA guidelines, and are likely to be associated with high macroalgal biomass on clam nets; however, these temporary hypoxia events do not appear to have a negative effect on short-term feeding rates and may not affect clam growth significantly in Cherrystone Inlet.

Further exploration is necessary to determine these effects on a seasonal basis, and to elucidate the role of alternate food sources in the hard clam energy budget. Field feeding studies such as the current one could be complemented by phytoplankton species identification, clam gut content analyses, and stable isotope analysis to determine predominant food sources for these hard clam populations. Up to date observations of nutrient cycling and primary productivity estimates would elucidate the effects of large-scale increases in clam aquaculture over the past 10 years on the biogeochemistry of Cherrystone Inlet. This in turn would aid in determining whether clams in the Inlet are food limited, and whether the Inlet is reaching carrying capacity.

The tidal influence on seston availability and concomitant changes in clam clearance rates observed in this field study could not have been duplicated in the laboratory. Physical factors and large scale influences on local seston and water quality characteristics have an important role in this system, from the basin level to the physiology of individual clams. These in situ influences on clam physiology are vital components to be included in bioenergetics and carrying capacity models for aquaculture sites. It is recommended that any bivalve feeding model utilizing laboratory observations
of physiological rates be calibrated to account for the effects of physical factors observed in the field.
CHAPTER THREE

Estimated Ecosystem Impacts of Aquacultured Clams,
Cherrystone Inlet, Virginia
INTRODUCTION

Cherrystone Inlet is a small (6 km²) coastal embayment on the Chesapeake Bay side of the southern Delmarva Peninsula (Figures 0.2, 0.3). The inlet is shallow, averaging 1 m in water depth, with a narrow channel (maximum depth 3-5 m, Reay et al., 1995) and broad shoals. Sediments are predominantly sandy, with finer sediments in protected coves and the upper creek (Reay et al., 1995). Deeper aphotic regions are dominated by heterotrophic activity, while shallow shoal areas support sizeable benthic microalgal communities (Reay et al., 1995). Eelgrass (*Zostera marina*) beds can be found in certain regions, particularly downstream, but are not widespread. Average water temperature ranges from 0 to 32 °C seasonally, and salinities generally range from 14 to 23 ppt (Reay et al., 1995).

Aquaculture industry estimates have given a standing stock estimate of clams *Mercenaria mercenaria* in Cherrystone Inlet of 45 x 10⁶ individuals, and an annual harvest of 20 x 10⁶ clams with an average shell length of 60 mm (Luckenbach and Wang, 2004). Luckenbach and Wang (2004) made estimates of clams' contributions to nitrogen metabolism in the creek using literature estimates of clam metabolism. They estimated 18,000 kg N yr⁻¹ removed by harvest of animals; 36,000 kg N yr⁻¹ removed to the atmosphere by denitrification of biodeposits; and 900 kg N day⁻¹ released to the water column by clam excretion.

In recent years, the hard clam aquaculture industry in Cherrystone Inlet has reported slower growth rates in some grow-out areas, expressed as a longer time to market size. Growers have speculated that the creek may be reaching its carrying capacity due to food
limitation of the clam population. An alternate hypothesis is that the predator exclusion nets may be having a detrimental effect on clam feeding, either by interfering with the suspension feeding process directly, or by modifying the benthic environment in more indirect ways that negatively affect clam growth. The nets and their associated macroalgae could reduce food availability to infaunal clams via reduction in water flow and seston flux around clam beds. Feedbacks to the nitrogen pool via clam biodeposition (feces and pseudofeces production) and excretion must also be considered, as well as the effects of environmental factors such as temperature and salinity on clam physiological processes. Sediment buildup of particulate organic nitrogen and ammonium due to clam biodeposition and excretion over time may lead to decreased sediment oxygen levels and increased sulfide toxicity, which both could impede clam growth rates. Determining whether putative decreases in clam growth rates are due to environmental (both natural and human-induced) or physiological factors is essential to the management of this industry.

Using accurate estimates of clam numbers and sizes in the creek, and empirical relationships for filtration and respiration rates, it is possible to model potential clam impacts on primary production in Cherrystone Inlet. Using model results, one may then evaluate potential food limitation and whether carrying capacity of the creek is being reached. Impacts are expected to vary seasonally, depending on clam physiology and seasonal productivity. Luckenbach and Wang (2004) made estimates of clams as sources and sinks for the nitrogen pool in Cherrystone. In this chapter I develop a model which evaluates clams' impacts on carbon and nitrogen cycling in the creek, and potential implications for the industry.
OBJECTIVES

The objective of this model is to determine potential feeding impacts of the hard clam population in Cherrystone Inlet on a monthly basis for the years 2003-2004. The model incorporates local clam population and water quality data, and published clam feeding and respiration rates. Potential impacts on the particulate carbon pool via feeding and respiratory demands; on nitrogen cycling via feeding and subsequent biodeposition, excretion, microbial processing of nitrogenous wastes, and harvest of clams; will be investigated.

METHODS

Clam population data

Clam population data were obtained from growers in Cherrystone Inlet for the years 2003-2004 (Arnold, 2004). Data collected include date, number and size of clams planted; and date, number, and size of clams harvested. The level of detail provided by growers varied significantly, so in some cases; assumptions were made (see Appendix 1). Numbers of clams at each site at the beginning of the project \(x_0\) were provided by growers. Subsequently, number of clams at site \(s\) and at month \(t\) was determined by:

\[ x_{s,t} = x_{t-1} + (p - h)_t \]

where \(t\) is time (months), \(h\) is the number of clams harvested, and \(p\) is the number of clams planted in a given month \((t)\). Number of clams in a given month \(t\) for all sites was determined by:
Clam growth rate estimates were made by taking three samples of 50 clams each for seven sample growout sites (see Appendix 1, #15), monthly from April 2003 – November 2004. Mean ± SE shell lengths were calculated for each site, and approximate age of clams for each site was estimated from grower data. Approximate age of clams and mean shell length were plotted for each site for the year June 2003 – May 2004. Growth curves were fit to the data by nonlinear least-squares regression (SigmaPlot, Systat Software, Inc.) to calculate a best fit line for the logistic, Gompertz, and von Bertalanffy curves as discussed by Devillers et al. (1998). Goodness of fit was evaluated using $r^2$ values for the model, and $p$ values for individual model parameters.

All three models estimated growth rates satisfactorily, with all $r^2$ values above 0.99, and all parameters of each model having $p < 0.005$. The Gompertz model ($r^2 = 0.9940$) was chosen over the von Bertalanffy ($r^2 = 0.9931$) and the logistic ($r^2 = 0.9927$) models. The Gompertz model was then used to predict clam shell length for all sites in the creek based on clam age data from growers (Figure 3.1). The average length $l_{s,t}$ of clams at site $s$ and month $t$ was calculated as:

$$\ln (l_{s,t}) = -3.968 \times e^{-\left[(t - 4.58)^{10.74}\right]}$$
FIGURE 3.1 Gompertz growth model, fit to Cherrystone clam size data (y) vs. clam age (x). $r^2 = 0.9940$.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coefficient</th>
<th>SE</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>52.89</td>
<td>1.102</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>b</td>
<td>0.74</td>
<td>1.028</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>$x_0$</td>
<td>4.58</td>
<td>0.632</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>
Weighted averages of shell lengths $\overline{l}_t$ were calculated for each month, 2003 – 2004 using the equation:

$$
\overline{l}_t = \frac{\sum_{s=1}^{118} (l_{s,t} * x_s)}{\sum_{s=1}^{118} x_s}
$$

Predicted shell lengths (mm) were converted to tissue dry weights (g) using a regression calculated in January 2004 for Cherrystone clams of a variety of sizes ($n = 200$; Appendix 3, Figure 5).

**Clam feeding rates**

A clearance rate function for cultured clams was determined by plotting clearance rate vs. water temperature from field experiments (Chapter 2), and calculating a polynomial regression based on average water temperature for each month ($w_t$):

$$
c_{s,t} \text{ (L individual h}^{-1}) = -0.011 * w_t^2 + 0.5623 * w_t - 6.095
$$

This relationship (Figure 3.2) is unimodal, with a maximum clearance rate of 1 L h$^{-1}$ at approximately 25 °C, and a clearance rate of 0 at approximately 15 and 35 °C. Average monthly water temperatures ($w_t$) for Cherrystone Inlet were obtained from the Chesapeake Bay Program Water Quality database (2005) for the years 2001-2002 (see Appendix 2). Monthly average feeding rates were scaled up for the entire creek by multiplying by the individual feeding rate by the total number of clams in the creek, giving a monthly population filtration rate in L day$^{-1}$. Percent of total creek volume filtered by clams per day was determined by dividing the filtration rate (L d$^{-1}$) by the mean sea level creek volume, 6,463,000 m$^3$. 
FIGURE 3.2 Relationship between clearance rate and water temperature for Cherrystone clams in situ
Carbon energy budget estimates

Ingestion

Monthly average available carbon was estimated by multiplying the monthly clearance rate by the monthly estimates of particulate organic carbon concentration (POC, g m⁻³) determined by Kuo et al. (1998) for Cherrystone Inlet in the year 1997. Monthly average ingested carbon was estimated by applying a selection efficiency of 0.9, assuming that on average, 10% of available carbon is rejected as pseudofeces. Resulting ingestion rates were given in kg POC month⁻¹.

Egestion

Assimilation efficiencies were calculated using the Conover (1966) method for November 2004 laboratory feeding experiments using natural seston. Average assimilation efficiency was 48%. Monthly egestion rates were therefore determined to be equal to 0.52 * ingestion.

Respiration

Respiration rates for Cherrystone clams were estimated using a respiration - temperature regression determined from laboratory data (Figure 1.7):

\[
r_r (\text{mg O}_2 \text{ g}^{-1} \text{ clam DW h}^{-1}) = -0.0108 \times w_t^2 + 0.4663 \times w_t - 4.422
\]

For each month, a weighted average clam dry weight \(w_t\) for the creek was utilized from the population sub-model above, and an average monthly water temperature was determined from Chesapeake Bay Program Water Quality data from 2001-2002 (Appendix 2). The above relationship is unimodal, with maximum respiration rates at approximately 22 °C, and respiration rates equaling zero at approximately 14 and 29 °C.
Since it is unlikely that respiration is zero constantly when water temperature is below 14 °C, a relationship from Hibbert (1977) was utilized for water temperatures < 14 °C. Metabolic carbon requirements were determined by converting $V_{O_2}$ to carbon respired according to the formula:

$$\text{Carbon respired (mg C h}^{-1}\text{clam}^{-1}) = V_{O_2} \times \frac{1 \text{ mmol O}_2 \times RQ \times 12 \text{ mg C}}{32 \text{ mg O}_2 \times \text{ mmol C}}$$

RQ or respiratory quotient (mmol O$_2$/mmol C) was assumed to be 1 as in Newell (1988), which is the value for pure carbohydrate metabolism. Respiration values were then scaled up by multiplying by number of clams in the creek for the month. Subtracting monthly estimates for respired C from monthly assimilated C allowed estimates of monthly amounts of C available for clam growth ($C_{growth}$). Daily metabolic carbon demands for all clams in the creek were also determined by using Doering and Oviatt’s (1986) estimate that respiration accounts for 47% of the clam energy budget. Daily carbon respiration estimates were scaled up by multiplying by the monthly number of clams and dividing by the surface area of the creek for a rate in g C m$^{-2}$ d$^{-1}$. These rates were then compared to monthly estimates of carbon production (gross primary production, GPP) for Cherrystone Inlet from 1991-1992, which ranged from 0.2 – 5.1 g C m$^{-2}$ d$^{-1}$ (Reay et al., 1995). Clam carbon demands were also compared to GPP adjusted (GPP$_{adj}$) for phytoplankton respiration, which was estimated to be equal to 10% of GPP (Cloern, 1987), such that GPP$_{adj} = 0.9 \times$ GPP.

_Nitrogen uptake and release_

Monthly harvest numbers and clam ages were obtained from clam growers (Arnold, 2004), and mean clam shell length at harvest was calculated using the Gompertz equation.
as above. Shell lengths (SL, mm) were converted to tissue dry weights (DW, g) as needed using a regression equation determined in January 2004 for Cherrystone clams (Appendix 3, Figure 5). Dry weights were converted to nitrogen content of dry tissue, calculated as 0.1 * DW (Hawkins et al., 1985), to obtain total nitrogen removal due to monthly harvest, kg N month\(^{-1}\).

Ingestion rates of particulate nitrogen (PN) were calculated by multiplying monthly average clearance rates by average PN concentrations given for Cherrystone Inlet by Kuo et al. (1998). Nitrogen biodeposition rates were calculated by applying an assimilation efficiency of 48% of ingested PN, then scaled up to the monthly number of clams in the creek. Total monthly particulate nitrogen (PN) from biodeposition (kg N month\(^{-1}\)) was then partitioned into nitrogen removal by burial and nitrification-denitrification of biodeposits; and N cycling by ammonification of biodeposits, according to results from Newell et al. (2002). Burial of biodeposits was estimated as 10% of biodeposition rates. Denitrification (kg N\(_2\) month\(^{-1}\)) was calculated as 20% of the remaining biodeposited N after burial, with ammonification (kg NH\(_4^+\) month\(^{-1}\)) accounting for the remaining 80% of biodeposited N after burial.

Clam excretion rates were calculated from the ammonia excretion – dry weight equation of Sma and Baggaley (1976): \(\log_{10} NH_3 (\mu mol \text{ day}^{-1}) = 0.94 \times \log_{10} DW + 1.33\), and scaled up for total number of clams in the creek and mean monthly dry weight, then converted to kg N month\(^{-1}\) to obtain monthly recycling of N by clam excretion.

Nitrogen uptake/removal by clams was calculated by summing nitrogen removal via clam harvest, and burial and denitrification of organic nitrogen in clam biodeposits.
Total nitrogen recycling by clams was calculated by summing direct DIN release by clam excretion of ammonium, and ammonification of organic nitrogen in clam biodeposits.

RESULTS

Clam population data

The total number of clams in Cherrystone Inlet ranged from a low of 78,749,623 clams in November and December 2004 (approximately 7.0 x 10^4 kg dry weight) to a high of 119,097,750 clams in October 2003 (7.5 x 10^4 kg dry weight; Figure 3.3). During the year July 2003 through June 2004, the total number of clams never dipped below 100,000,000 clams. The average estimated size of clams in the creek ranged from 37 - 49 mm, with the average size increasing slightly over time (Figure 3.4). The Gompertz growth model estimated clam size very well, as the linear regression line of observed vs. predicted clam size gave a ratio near 1:1 (Figure 3.5, observed = 0.9894 * predicted + 0.487, r^2 = 0.854, p < 0.0001)

Clam filtration rates

The individual clearance rates (L h^-1 clam^-1), weighted by number of clams, and averaged across growout sites and by month, ranged from 0.32 L h^-1 clam^-1 in January-February 2004, to 2.10 L h^-1 clam^-1 in June 2004 (Figure 3.6). These values were combined with the total number of clams to give monthly total population filtration, which ranged from 6.51 x 10^8 L d^-1 in December 2004, to a maximum of 5.29 x 10^9 L d^-1 in June 2004 (Figure 3.7). These values correspond, respectively, to 10.1% and 81.9% of the total creek volume filtered per day (Figure 3.8).
FIGURE 3.3 Total number of clams, Cherrystone Inlet, 2003-2004

Monthly estimates based on growers’ planting and harvest data.
FIGURE 3.4 Estimated average monthly size of clams in Cherrystone Inlet, 2003-2004
FIGURE 3.5 Regression for monthly observed clam size (seven sites, age classes 2000 – 2003, averaged for 3 nets/site) vs. predicted clam size (Gompertz model)

\[ y = 0.9894x + 0.487 \]

\[ R^2 = 0.8537 \]
FIGURE 3.6 Average monthly individual clearance rate, Cherrystone Inlet
FIGURE 3.7 Monthly filtration rate for all clams, Cherrystone Inlet
FIGURE 3.8 Estimated percent creek volume filtered per day by clams, 2003-2004
Carbon requirements

Scaled up estimates for carbon ingestion, egestion, assimilation, and respiration varied widely on a seasonal basis (Figure 3.9). Ingested C ranged from 1,875 – 28,900 kg month\(^{-1}\). Egested C was 975 – 15,000 kg month\(^{-1}\); and assimilated C was 900 – 13,900 kg month\(^{-1}\). Respiration rates were estimated for April - December, since data were not available for water temperatures < 14 °C (January – March). Respiration rates, averaged across cohorts and by month, ranged from 0.19 to 0.61 mg O\(_2\) g\(^{-1}\) DW h\(^{-1}\), corresponding to daily carbon respiration rates of 0.07 to 0.22 mg C g\(^{-1}\) DW h\(^{-1}\). Scaled up respiration estimates ranged from 3370 – 13,400 kg C month\(^{-1}\). Respiration accounted for 11 – 86% of the Cherrystone clam population energy budget, with annual average respiration equaling 38% of ingested C.

Assuming most assimilated carbon is used for respiration or growth, it was possible to calculate the amount of assimilated C available growth after respiration (C\(_{growth}\)). As much as 10,000 kg C was available for growth during August 2003; however, in certain months, negative values were estimated for C\(_{growth}\) (Figure 3.10). During November 2003, May 2004, and September – November 2004, no assimilated carbon was available for growth. Average values for ingestion, egestion, assimilation, respiration, and C\(_{growth}\) were calculated for 2003 and 2004 to construct population-wide carbon budgets. In 2003, egestion and respiration were 52% and 28% of ingested carbon, respectively, leaving 22% of ingested carbon available for clam growth (Figure 3.11a). In 2004,
FIGURE 3.9 Clam carbon budget for Cherrystone Inlet, 2003-2004

Egestion and assimilation each accounted for approximately 50% of monthly ingested C. Respiration accounted for 11 – 86% of monthly ingested C, demanding more C than was assimilated in certain months.
FIGURE 3.10 Carbon assimilated and respired by clams, and carbon available for clam growth, 2003-2004

Monthly $C_{\text{growth}}$ estimates = estimated monthly assimilated – respired C. In some months (November 2003; May 2004; September – November 2004), C available for growth was $\leq 0$. 
FIGURE 3.11 Carbon budgets for Cherrystone clam population, 2003 and 2004

In 2003, 52% of ingested C was egested, 28% was respired, and 22% of ingested C was available for growth (average of monthly values). In 2004, 52% of ingested C was egested, 49% was respired, and only 4% was available for clam growth.

a. 2003

b. 2004
egestion and respiration were 52% and 49% of ingested carbon, leaving only 4% of ingested C available for growth (Figure 3.11b).

Using respiration rates from Hibbert (1977) and assuming respiration is 47% of total carbon demands (Doering and Oviatt, 1986), this gives a range in total carbon requirements of 1.43 - 34.9 mg C d\(^{-1}\) clam\(^{-1}\) (Figure 3.12). To compare the total carbon requirements of the clams in Cherrystone Inlet to primary production rates, monthly Cherrystone gross primary production values from Reay et al. (1995) were used from the years 1990-91. These values ranged from a minimum of 0.2 g C m\(^{-2}\) d\(^{-1}\) in March to a maximum of 5.1 g C m\(^{-2}\) d\(^{-1}\) in August. Integrating the Cherrystone clams' carbon requirements over the surface area of the inlet (approximately 6.355 x 10\(^{6}\) m\(^2\) at mean high water), it was determined that between 2.7 (December 2004) and 80.2% (March 2004) of daily carbon production is utilized by the Cherrystone Inlet clam population (Figure 3.13).

**Nitrogen uptake and release**

Monthly permanent removal of nitrogen from the creek by clams ranged from 200 - 2200 kg N month\(^{-1}\), from a combination of burial of biodeposits, coupled nitrification-denitrification of biodeposits, and harvest of clam tissue (Figure 3.14). The majority of this nitrogen was removed to the atmosphere via nitrification-denitrification of biodeposits to N\(_2\) gas. Harvest accounts for between 0 and 52% of the total nitrogen removal each month, with a total estimated annual removal of N by harvest of 2360 kg N year\(^{-1}\) for 2003, and 5450 kg N year\(^{-1}\) for 2004. N removal via denitrification of biodeposits ranged from 189 - 2500 kg N month\(^{-1}\), and N removal by burial of
FIGURE 3.12 Estimated monthly respiration rates and total carbon requirements per clam in Cherrystone Inlet, 2003-2004, based on Hibbert (1977) and Doering and Oviatt (1986)
FIGURE 3.13 Total carbon requirements of clams in Cherrystone Inlet, 2003 and 2004 (based on Hibbert (1977) and Doering and Oviatt (1986)); and 1990-91 monthly values for gross primary production (Reay et al. 1995).
FIGURE 3.14 Estimated nitrogen removal by clams, Cherrystone Inlet, 2003-2004
FIGURE 3.15 Clam-induced nitrogen recycling, Cherrystone Inlet, 2003-2004
biodeposits ranged from 66 – 1390 kg month\(^{-1}\), totaling 22,600 and 23,400 kg N removed from the creek by clams annually for the years 2003 and 2004, respectively.

Monthly recycling of nitrogen by clams ranged from 1123 – 10,900 kg N month\(^{-1}\), from a combination of clam excretion and ammonification of biodeposits (Figure 3.15). Ammonification of clam biodeposits generally accounted for more monthly N recycling than excretion, especially in summer months; and annual totals of ammonified N were 5 – 10 times excreted N. Total annual release of N due to clam excretion was estimated to be 6785 kg N year\(^{-1}\) for 2003 and 9014 kg N year\(^{-1}\) for 2004. Annual release of N via ammonification of biodeposits was estimated at 52,000 kg N year\(^{-1}\) for 2003 and 46,200 kg year\(^{-1}\) for 2004.

Total nitrogen recycling by clams exceeded nitrogen removal by clams consistently throughout 2003 - 2004. During most months, nitrogen recycling exceeded removal by a factor of 3 (Figure 3.16).

**DISCUSSION**

It is apparent from these calculations that the cultured clam population in Cherrystone Inlet is now large enough to have a considerable impact on phytoplankton populations, and may be dominating carbon and nitrogen processes in the creek. Seasonal variability in the total creek filtration rate is due primarily to increasing water temperatures, which increase clam metabolic rates and carbon demand. Clam respiration rates and carbon demand show a similar seasonal trend to gross primary production rates (Reay et al., 1995), but with clam C demand increasing at a faster rate than GPP in early summer.
FIGURE 3.16 Estimated nitrogen recycling and removal by clams in Cherrystone Inlet, 2003-2004
Clam C demand also decreases later in the fall than GPP, and at a slower rate. Therefore, clams are demanding a high percentage (over 50%) of the gross primary production in Cherrystone Inlet in March and April, June, and September through November. In July and August, clams do not have much of an impact on the high production rates, even though clam metabolic rates are high. In November through February, GPP and clam metabolic rates are both relatively low.

These estimated impacts of clams on primary production are conservative, since they are made with respect to gross primary production rather than net primary production (NPP), which is equal to GPP – phytoplankton respiration. NPP is a better indicator of phytoplankton standing stock (food for clams) than GPP, but estimates of net primary production do not currently exist for Cherrystone Inlet. If phytoplankton respiration is estimated to be approximately 10% of GPP (Cloern, 1987), then NPP accounts for the other 90% of GPP. If a corrected value for GPP (GPP corrected for phytoplankton respiration or $GPP_c = 0.9 \times GPP$) is used to approximate NPP, it is apparent that even less phytoplankton is available for clam consumption throughout the year. Clam carbon demands are an estimated 3 - 90% of net primary production rates, depending on month (Figure 3.17). Clams are demanding over 50% of $GPP_c$ for five months of the year (March, April, June, September, and October).

Not all net primary production is available for clam consumption. Clams also must compete with micro- and mesozooplankton grazers, which are pelagic and likely to consume phytoplankton before it reaches the benthic clams. Zooplankton population estimates and grazing rates are unknown for Cherrystone Inlet, but numbers must be high.
FIGURE 3.17  Cherrystone Inlet gross primary production values (Reay et al., 1995), corrected for phytoplankton respiration (GPPc, see text); clam population carbon demands (from current model); monthly averages of percent GPPc required by clams.

Clams demand 2 – 80% of C production, with the highest C demand being in March, April, June, September, and October (greater than 50% of GPPc demanded by clams).
enough to support the sizeable blooms of *Mnemiopsis leidii* and *Beroe ovata*, known predators of micro- and mesozooplankton, observed throughout the year.

Cherrystone Inlet is at or near exploitation carrying capacity for hard clam aquaculture. This is especially apparent in the spring and fall months, when temperatures near 20 °C are ideal for clam growth (Ansell, 1968), but clam feeding demands are encroaching upon net primary production rates. During certain months, clams are demanding more of the net primary production than is available. This suggests that clam growth rates may not be reaching their full potential in Cherrystone Inlet during spring and fall months due to food limitation.

Estimates of nitrogen removal via harvest were lower than those given by Luckenbach and Wang (2004). The 2003 annual harvest of 16.3 x 10^6 clams was slightly lower than their previous estimate of 20 x 10^6 clams, but the 2004 harvest was much higher (57.3 x 10^6 clams). Mean clam size at harvest (43 mm shell height) was much lower than the 60 mm shell height estimated by Luckenbach and Wang (2004), giving lower estimates of nitrogen removed from the creek via clam harvest. Estimates of N removal via denitrification of biodeposits were also lower in the current study (11,600 – 13,000 kg year⁻¹) than in previous estimates (36,000 kg yr⁻¹) by Luckenbach and Wang (2004). It is unknown why these estimates differ, except that different studies were used to calculate biodeposition rates by clams (assimilation efficiencies from Chapter 1 for current study; Hibbert, 1977 for Luckenbach and Wang (2004) estimates). Hibbert’s (1977) estimates for biodeposition as a percentage of the clam energy budget are high compared to other studies (e.g., Doering et al., 1986), which may account for this discrepancy.
Estimates of N recycling via clam excretion for the two studies differed by an order of magnitude: 12 – 30 kg N day\(^{-1}\) for the current study, based on excretion rates from Chapter 1 of the current study; 900 kg N day\(^{-1}\) for Luckenbach and Wang’s (2004) estimates, based on excretion rate estimates by Hibbert (1977). Luckenbach and Wang (2004) did not include ammonification of clam biodeposits in their estimates of N release by clams, as the current study did, based on the results of Newell et al. (2002).

The end result is much lower estimates of nitrogen removal and recycling, by clams by the current study than by Luckenbach and Wang (2004). Nitrification-denitrification and ammonification of clam biodeposits account for more of the total clam-induced N flux than clam excretion and harvest. The impacts of increased nitrogen recycling to the water column are unknown at this time, but they are likely to have an impact on primary production. Cherrystone Inlet primary production rates (gross or net) have not been measured in recent years since the recent expansion of the clam aquaculture industry on the lower Eastern Shore. GPP estimates from Reay et al. (1995) were made in 1990-91, and the dockside value of hard clam aquaculture increased from $4.5 to $11.0 million from 1992 – 1998 (U.S. Department of Agriculture, 2000).

It is possible that DIN fluxes from hard clams could be fueling primary production in the water column, which in turn supports hard clam growth. However, it is equally possible that instead, these DIN fluxes are supporting the thick macroalgal mats which cover the clam nets throughout much of the growing season (Neikirk et al., 2001), and the benthic microalgal populations which cover sediment surfaces in the shallows. Clams may feed on detritus from decaying macroalgae, but this would not be as nutritious of a food source as water column phytoplankton (Tenore, 1983). Macroalgal populations on
the Eastern Shore of Virginia are known to go through boom-bust cycles throughout the
summer (McGlathery et al., 2001), and would not be a consistent food source for clams.
Benthic microalgae are only available to clams during resuspension to the water column,
which occurs erratically during periods of increased wind mixing. Clams may feed
opportunistically, relying on these alternate food sources when available. However, a
sustainable hard clam industry in Cherrystone Inlet will rely on water column
productivity as a consistent and nutritious food source. It is recommended that new
estimates of primary production be made to determine whether recent increases in the
clam population of Cherrystone Inlet have had an effect on NPP. Combining clam
population and physiology models with sophisticated hydrodynamic and water quality
models will elucidate the impacts clam metabolic processes are having on water quality
in this embayment.
THESIS SUMMARY

Based on population data from the current study for the years 2003-2004 and physiological rates from previous studies, Cherrystone Inlet is at or near carrying capacity for hard clam culture. Food (particulate organic carbon) limitation is an issue during months when clam metabolic rates are high but net primary productivity is relatively low. These effects are most prominent in the spring and fall, times of year when the water temperature is ideal for clam growth, but food limitation may be slowing growth rates. The estimated total nitrogen release to the water column exceeds estimated N uptake and removal by clams by a factor of three for most months of 2003-2004. A current study on N and C cycling in the inlet would be helpful to elucidate the biogeochemical effects of increased clam production since 1992.

Laboratory-determined clam clearance rates were generally higher than in situ clearance rates, but both were highly variable. Laboratory clearance rates under “ideal” conditions (June 2003) were near the Powell et al. (1992) “high gear” estimates for a generalized bivalve feeding rate. Clearance rates during July and August 2003 feeding trials were near zero. Clams were very sensitive to low food (chlorophyll a) concentrations and low salinities, and generally exhibited an “all or nothing” feeding response during the laboratory studies. If conditions were less than ideal (i.e., July and August 2003), clams simply would not extend their siphons to feed. This behavior may offer some explanation to the “high gear” vs. “low gear” debate: “high gear” estimates may have been made on “feeders” - animals chosen prior to experiments for exhibiting active feeding behavior. “Low gear” estimates may include “non-feeders” - animals who
do not extend siphons to feed a large proportion of the time - in clearance rate estimates, which would decrease average clearance rates.

The field component of the current study was designed to account for percent time feeding by including ten clams in a single feeding chamber for each trial, and measuring feeding rates repeatedly on the same group over time. Clams were not selected for feeding activity, and an average clearance rate for the group was obtained for each time point. This experimental design also allowed feeding rates to be analyzed at high clam densities, which is typical of conditions in the aquaculture environment, and is known to be a factor impeding clam growth (Peterson and Beal, 1989). Clam clearance rates for field feeding experiments were lower on average than laboratory experiments for a similar same size range, temperature, and salinity. Clearance rates in the field were demonstrated to be predominantly reliant on chlorophyll \( a \) and phaeophytin concentrations. Tidal energy was shown to be an important factor for resuspension of detritus from the benthos, which may be an important alternate food source for clams during months when phytoplankton production is limiting.

Summertime conditions in Cherrystone Inlet test the boundaries of clam physiology. Clams did not feed below salinities of 15 ppt, yet water collected from Cherrystone for 2003 laboratory experiments was 16 ppt or below for June, July, and August collection periods. Water temperatures in the shallow waters of the inlet peaked in the high 20's to 30 °C during most days of the summer. These temperatures were shown to be detrimental to clam physiology according to low \( Q_{10} \) values calculated from respiration rates. Benthic dissolved oxygen concentrations dipped below 5 mg L\(^{-1}\) at night on the clam beds, below which oxygen consumption declines and anaerobic metabolism
becomes increasingly important (Hamwi, 1969). Clams were shown to be very sensitive to seston concentration, which varied significantly from day to day, and was as low as 6 \( \mu g \text{ L}^{-1} \) under the clam nets in August. Despite these limitations, Cherrystone clams are still growing at a reasonable rate, reaching market size (7/8” in width, which equals 40.05 mm in length) in approximately 20 months from planting.

Laboratory biodeposition, respiration, and excretion responses to animal size, water temperature, and food concentration were similar to those of past studies. Biodeposition rates and assimilation efficiencies were lower for a given food concentration than those from past studies using cultured algae (Tenore and Dunstan, 1973), which is to be expected for a food source consisting of natural seston. Respiration rates from the current study were very similar to those from past studies, despite the fact that clams were fed during the current study and starved in past studies (Hamwi, 1969; Loveland and Chu, 1969). Slightly lower respiration rates by cultured clams may lead to slightly faster growth rates than would be expected for wild clams under current conditions in Cherrystone Inlet. Ammonium excretion rates were almost identical to those determined by Srna and Baggaley (1976).

Accurate assessments of the effects of clam bioenergetics on particulate matter and water quality parameters depends on the accurate estimation of both physiological and environmental controls of feeding. This thesis has shown that existing laboratory estimates of physiological rates (feeding, biodeposition, respiration, and excretion) are probably accurate for the cultured hard clam feeding on natural seston. However, \textit{in situ} factors affecting seston availability and clam feeding rates are multifaceted and are not well understood. Physical forcing on multiple scales, from tidal cycles to seasonal
cycles, has a considerable impact on the physiology and ecology of clam growout sites. Clam – water column interactions are much more complex than the simple model of carbon and nitrogen release and removal by clams presented in Chapter 1. To begin to understand and model bivalve – benthos – water column interactions, an interdisciplinary approach is required, including manipulative experiments, observational studies, and modeling exercises on multiple scales, and incorporating physical, chemical, and biological factors. Such projects will certainly be complex, but will lead to greater understanding of the entire ecosystem and the key role suspension feeding bivalves can play.
APPENDIX 1: CLAM POPULATION DATA ASSUMPTIONS (Arnold, Unpubl.)

1. When planting month not specified, month is assigned as follows:

<table>
<thead>
<tr>
<th>Season</th>
<th>Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>If spring</td>
<td>March</td>
</tr>
<tr>
<td>If summer</td>
<td>June</td>
</tr>
<tr>
<td>If fall</td>
<td>September</td>
</tr>
<tr>
<td>“Before 2002”</td>
<td>Assigned as 2001</td>
</tr>
<tr>
<td>Year only</td>
<td>Assigned June of that year</td>
</tr>
</tbody>
</table>
| Clams planted over a two-year period | ½ planted June of Year 1  
| | ½ planted June of Year 2 |

When month is specified, it is used instead of June.

2. When size at planting is not specified, assume Class 3 (see #4 below)

3. Assume clams are 7/8” at harvest.

4. Planting size classes are as follows:

<table>
<thead>
<tr>
<th>Size</th>
<th>mm</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 mm</td>
<td>6 mm</td>
<td>1</td>
</tr>
<tr>
<td>8-10 mm</td>
<td>8-10 mm</td>
<td>2</td>
</tr>
<tr>
<td>1/2 – 1/3</td>
<td>8.5 – 12.7</td>
<td>3</td>
</tr>
<tr>
<td>Buttons</td>
<td>&lt; 20 mm</td>
<td>5</td>
</tr>
<tr>
<td>15 mm</td>
<td>15 mm</td>
<td>6</td>
</tr>
</tbody>
</table>

Harvest sizes are as follows:

<table>
<thead>
<tr>
<th>Harvest Size</th>
<th>Shell width</th>
</tr>
</thead>
<tbody>
<tr>
<td>7/8”</td>
<td>22.225 mm</td>
</tr>
<tr>
<td>1”</td>
<td>25.4 mm</td>
</tr>
</tbody>
</table>

5. Assume 3000 clams/bushel.

6. Harvest size is based on shell width. Other sizes indicated are based on shell length.

7. Survival rate is based on grower reports. When not reported, a value of .66 is assigned

8. Numbers in green represent clams in spat bags to be used as source clams for planting. So, harvest numbers for these mean the grower removed the clams from the spat bags and planted them in another location.
9. When harvest date not specified, assume were clams harvested in the first month after age 1095 days (3 years), or first month after grower ceased providing information and clam age is greater than 1095 days.

10. Grower 1 could not be reached, for 6 weeks, for an update at the end of 2004. He will likely provide information if he can be contacted at some time in the future. Therefore, his records were not updated for 2004. They are highlighted in red.

11. Age indicates days spent in the creek, not days alive.

12. Harvest data in italics indicates that more clams were reported harvested than planted. Such harvest data is not included in monthly totals. Instead, monthly totals are based on data subsequently reported by grower.

13. Population numbers indicate totals at the first day of that month.

14. Specific dates on some column headings (e.g., 6/3/2003) indicate the date that clams were measured that month. Numbers represent mean (mm) shell length for three reps of 50 clams each.

Estimated growth rates from these sites are applied to other sites based on year class, location, and confidence in the data. The correlation between measured clam size and estimated size based on these growth rates, size at planting, and age is very poor for some cohorts.

15. Sites and year classes sampled for monthly monitoring of clam growth

<table>
<thead>
<tr>
<th>SITE</th>
<th>BED #</th>
<th>YEAR CLASS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Downstream</td>
<td>5</td>
<td>2000</td>
</tr>
<tr>
<td>Downstream</td>
<td>9</td>
<td>2001</td>
</tr>
<tr>
<td>Midstream</td>
<td>11</td>
<td>2001</td>
</tr>
<tr>
<td>Midstream</td>
<td>12</td>
<td>2002</td>
</tr>
<tr>
<td>Upstream</td>
<td>10</td>
<td>2001</td>
</tr>
<tr>
<td>Upstream</td>
<td>13</td>
<td>2002</td>
</tr>
<tr>
<td>Upstream</td>
<td>15</td>
<td>2003</td>
</tr>
</tbody>
</table>
APPENDIX 2: CHERRYSTONE INLET DATA

1. Water temperatures for Cherrystone used in clearance rate model were averaged from Chesapeake Bay Program’s biweekly data for 2001-2002:

<table>
<thead>
<tr>
<th>Month</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan</td>
<td>4.24</td>
</tr>
<tr>
<td>Feb</td>
<td>7.91</td>
</tr>
<tr>
<td>Mar</td>
<td>11.65</td>
</tr>
<tr>
<td>Apr</td>
<td>17.32</td>
</tr>
<tr>
<td>May</td>
<td>19.61</td>
</tr>
<tr>
<td>Jun</td>
<td>27.57</td>
</tr>
<tr>
<td>Jul</td>
<td>26.81</td>
</tr>
<tr>
<td>Aug</td>
<td>27.84</td>
</tr>
<tr>
<td>Sep</td>
<td>24.48</td>
</tr>
<tr>
<td>Oct</td>
<td>22.01</td>
</tr>
<tr>
<td>Nov</td>
<td>17.15</td>
</tr>
<tr>
<td>Dec</td>
<td>4.05</td>
</tr>
</tbody>
</table>

2. Gross primary production (GPP) data was taken from Reay et al. (1995) for the years 1990 – 1991:

<table>
<thead>
<tr>
<th>Month</th>
<th>GPP (g C m$^{-2}$ d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan</td>
<td>0.5 (est. same as Feb)</td>
</tr>
<tr>
<td>Feb</td>
<td>0.5</td>
</tr>
<tr>
<td>Mar</td>
<td>0.2</td>
</tr>
<tr>
<td>Apr</td>
<td>0.5</td>
</tr>
<tr>
<td>May</td>
<td>1.95 (avg of May 1990 &amp; May 1991)</td>
</tr>
<tr>
<td>Jun</td>
<td>1.5</td>
</tr>
<tr>
<td>Jul</td>
<td>3.3 (avg of Jun &amp; Aug 1990)</td>
</tr>
<tr>
<td>Aug</td>
<td>5.1</td>
</tr>
<tr>
<td>Sep</td>
<td>1.0</td>
</tr>
<tr>
<td>Oct</td>
<td>0.75</td>
</tr>
<tr>
<td>Nov</td>
<td>1.3</td>
</tr>
<tr>
<td>Dec</td>
<td>1.3 (est. same as Nov)</td>
</tr>
</tbody>
</table>
APPENDIX 3: CLAM MORPHOMETRICS

Shell lengths, heights, and weights; total wet weights, meat dry weights, and meat ash-free dry weights were determined in January 2004 for sites and year classes listed in Appendix 1 (# 15). Regressions for these parameters are shown below (Figures 2 – 6).

Shell length (SL) is defined as the anterior – posterior axis; shell height (SH) is the greatest distance from the umbo to the ventral margin; and shell width (SW) is the greatest thickness through both valves (Fritz, 2001; see Figure 1 below).

Figure 1. Outline of left valve of *M. mercenaria* showing principal valve (lower case) and measurement (upper case) axes (Fritz, 2001).
Figure 2. Shell height (SH, mm) vs. shell length (SL, mm):

\[ SH = 0.8835 \times (SL) - 0.4901; r^2 = 0.9892 \]

Figure 3. Shell width (SW, mm) vs. shell length (SL, mm):

\[ SW = 0.4751 \times (SL)^{1.0421}; r^2 = 0.9832 \]
Figure 4. Total wet weight (TWW, g) vs. shell length (SL, mm):

\[ TWW = 0.0002 \times (SL)^{3.0776}; r^2 = 0.9945 \]

Figure 5. Dry weight (DW, g) vs. shell length (SL, mm):

\[ DW = 2 \times 10^{-6} \times (SL)^{3.394}; r^2 = 0.9176 \]
Figure 6. Ash-free dry weight (AFDW, g) vs. shell length (SL, mm):

\[ AFDW = 7 \times 10^{-7} \times (SL)^{3.5953}; r^2 = 0.8408 \]
LITERATURE CITED


VITA

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