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## **Cultural eutrophication and the clam *Macoma balthica*: Evidence for trophic disruption and effects on blue crabs**

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**Cultural eutrophication and the clam *Macoma balthica*:  
Evidence for trophic disruption and effects on blue crabs**

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**A Dissertation  
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  
Doctor of Philosophy**


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**By**

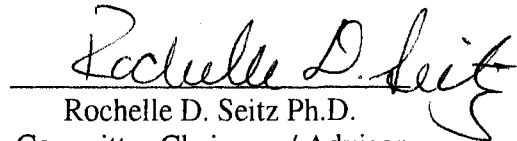
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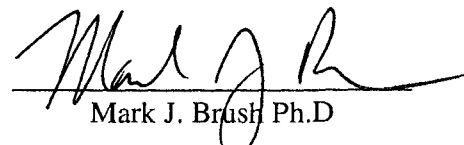
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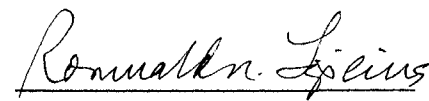
  
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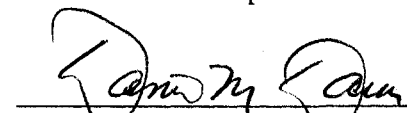
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## ABSTRACT

Cultural eutrophication (CE) is the allochthonous input introduction of a quantity of matter, such as sediments, organic material, or nutrients, into a water body over the pre-anthropogenic (natural) levels. In most coastal estuaries CE has come to refer primarily to an increase in the concentration of phyto-nutrients. CE has been identified as the cause of very graphic phenomena such as hypoxia and fish kills. In this work I examine the potential for CE to alter the composition of the primary producer community and potentially alter or disrupt the benthic food web, using *Macoma balthica* as an indicator species. A series of surveys and experiments identified that clams in areas with greater than average nutrient concentrations had lower health, slower growth, and greater non-predatory mortality than clams in less eutrophic areas. Primary production, as estimated from chlorophyll *a* concentration, was greater at higher nutrient locations while the health and growth of clams was lower. The phytoplankton community in the more eutrophic areas had a lower proportion of diatoms relative to dinoflagellates. A biochemical analysis of clam tissue indicated that the clams from the less nutrient rich sites had a greater proportion of Eicosapentaenoic acid (EPA) relative to other fatty acids. Diatoms are rich in EPA compared to dinoflagellates. Thus, we hypothesize that CE induced shifts from diatom-based production toward dinoflagellates may be limiting trophic transfer due to a lack of EPA. Using a series of models we were able to predict that trophic disruption could significantly reduce the scope for growth of the blue crab, *Callinectes sapidus*. Thus it is possible that the CE induced changes to primary producer community could disrupt the food web creating a trophic bottleneck.

**Cultural eutrophication and the clam *Macoma balthica*:  
Evidence for trophic disruption and effects on blue crabs**

## **Chapter 1**

**The potential for cultural eutrophication to disrupt flow through the benthic food web**

## INTRODUCTION

This dissertation documents my investigation into the relationship between cultural eutrophication (CE) and the effects it can have on the benthic food web. CE is the elevated introduction of organic matter allochthonously input into a water body over the pre-anthropogenic (natural) (Cole 1994, Nixon 1995). In coastal estuaries, cultural eutrophication has come to refer primarily to an increase in the concentration of phyto-nutrients (from anthropogenic sources, which can lead to an increase in primary production (Nixon 1995). In the 20<sup>th</sup> century, increasing population and shifts in land-use patterns increased the allochthonously input nutrients and made CE a ubiquitous phenomenon in coastal estuaries (Cloern 2001). Despite attempts to control the rate of CE (by improved sewage treatment and land-use management) it is still growing in scale, making increasing the need for improving our understanding the mechanisms by which CE alters the ecosystem (Valiela et al. 1992, Boesch et al. 2001).

In estuarine systems, most of the research concerning the effects of eutrophication has focused almost exclusively in the highly visible effects of increased nutrients, such as hypoxia and harmful algal blooms (HABs) (Boesch et al. 2001). Although the effects of hypoxic events and HABs such as mass die-offs and emigrations of macrofauna are highly dramatic, it is not well demonstrated that the effects result in lasting damage to the ecosystem (Diaz 2001, Rabalais et al. 2002, Kemp et al. 2005).

Instead of focusing on the more dramatic effects of eutrophication, my research examines the relationship between CE, the composition of the primary producer community and alteration of the benthic food web which could create a trophic

disruption. Trophic disruption is an alteration in the food web structure that reduces the rate of energy transfer through the food web. Cultural eutrophication could create a disruption in trophic transfer since it has been observed to shift the composition of the primary producer community toward one comprised of more opportunistic species that are less palatable or nutritious (Kemp et al. 2001, Grall and Chauvaud 2002, Sterner and Elser 2002). This less-nutritious community may not be able to fulfill the metabolic requirements of the primary consumers, thus preventing the increased primary production from moving up the food chain. Trophic disruption has been proposed as a solution to Rosenzweig's (1971) "paradox of enrichment" hypothesis (Muller-Navarra et al. 2004). Anthropogenically increased nutrients shift the bulk of the primary production from benthic macrophytes and microphytobenthos to a system dominated by phytoplankton, such as dinoflagellates and cyanobacteria which may have lower nutritional value (Cares et al. 1998, Marsh et al. 1989, Wacker et al 2002, Cloern 2001). These shifts in the primary-producer community are hypothesized to alter dynamics of higher trophic levels; however, a CE-induced trophic disruption is not well documented in estuarine systems (Charndra et al. 2005).

I documented a potential CE-induced trophic disruption in an estuary using traditional ecological techniques combined with biochemical analyses and modeling. Although it would be ideal to track the flow of material through the entire food web, this is not practical due to the sheer size and complexity of an estuarine benthic food web. In this work, I utilize an indicator species which is representative of the rest of the benthic primary consumer community (Seitz et al. 2006).

In this work, I used a population of baltic clam (*Macoma balthica*) in shallow-water coves along the mesohaline section of the York River, VA, as a proxy for the generalized effects of CE on benthic macrofauna. *M. balthica* is a small thin-shelled clam found commonly in the oligohaline to polyhaline waters of the tributaries of the Chesapeake Bay (Diaz & Schaffner 1990, Holland et al. 1987). *M. balthica* has been used in other estuarine systems as an indicator of macrofaunal response to stress and pollution (Shaw et al. 1976, Cain & Louma 1990). The experimental population used in this study is located in the southern part of their range. This potentially increases their sensitivity to stress and makes them more responsive to the subtle changes that CE may have on the food web, thus increasing my chances of detecting a significant effect (Hummel et al. 1996). They act both as a filter- and deposit feeder and thus are more likely to survive in areas that have reduced benthic primary production, due to eutrophication, than obligate deposit feeders which will allowed for the observation of sub-lethal effects. Most importantly for this study, the wide salinity tolerance of *M. balthica* means that they are found throughout the high oligohaline to polyhaline sections of riverine estuaries, which allowed me to explore the effects of variable phyto-nutrient conditions along a gradient using a single population.

The York River, like many riverine estuaries, displays a pattern of decreasing nutrient concentrations from the fall line to the river mouth along the estuarine axis (Boesch 2002). In most coastal river estuaries, nutrients tend to decline farther down the estuary along an estuarine gradient due to the dilution effect and consumption by primary producers (Ouboter et al. 1998). I was able to utilize test sites along this nutrient gradient,



which are assumed to represent differing intensities of CE, in order to explore the effect of nutrient induced shift in the food web on the reactions of *M. balthica*.

My results from this work are reported in five chapters written as individual journal manuscripts. In Chapter 2, I describe a three-year caged mark-and-recapture study used to examine the growth, survival, and health of *M. balthica* in four coves in the mesohaline portion York River. Two upriver coves with higher nutrient levels were compared with two downriver coves that had lower nutrient concentrations. The upriver coves had clams with significantly worse condition indices and greater non-predatory mortality, potentially indicating an effect of nutrients and food-web disruption.

Chapter 3 reports on a survey of ambient clam abundance and condition which was performed to confirm patterns of clam condition across a broader scale and to identify which forcing factors may have been responsible for the patterns observed in Chapter 2. Two additional coves, in the middle of the mesohaline section, were added to provide a test area with an intermediate amount of nutrients for use in confirming the patterns in clam abundance and for the determination of the most important forcing factor. I used an information-theoretic modeling approach to determine that the primary-producer community composition was most responsible for the patterns in *M. balthica* health along the York River axis. As hypothesized, the nutrient-rich upriver sites had less benthic microalgae and a greater proportion of dinoflagellates than the more downriver sites. The results of Chapter 3 support the hypothesis that nutrient conditions can affect dynamics of primary consumers through shifts in the composition of the primary-producer community, providing some evidence for CE-induced trophic disruption.

In Chapter 4, I explore the mechanism by which primary producer community composition could affect clam health through an analysis of the lipid composition of *M. balthica*. Because more primary production occurs upriver, more energy should flow into the primary consumers. My studies show this is not the case, most likely due to the type of primary production present in the more nutrient rich areas. By examining the lipid composition of *M. balthica*, I attempted to determine if the clams were lacking essential fatty acids not commonly found in the more opportunistic phytoplankton species, such as the polyunsaturated fatty acids (Pond et al. 1998, St. John et al. 2001). I focused on polyunsaturated fatty acids because they are one of the most important micronutrients that affect metazoan health (Cares et al. 1998, Marsh et al. 1989, Wacker et al 2002). No significant difference in the amount of bulk polyunsaturated fatty acids was detected among the sites; however, the ratios of the individual fatty acid components differed among sites. Upriver clams had lower eicosapentaenoic acid (EPA) levels than downriver clams. These results support the hypothesis that consumers in more eutrophic areas are not able to utilize the excess primary production because the dinoflagellate dominated primary producer community lacks sufficient EPA to meet the clam's metabolic requirements.

In Chapter 5, I combine the material from the previous chapters to explore the ramifications of the CE-induced trophic disruption on higher trophic levels. Through the use of bioenergetic models, I examined the effects of the intensity of CE-induced shifts in the health and density of *M. balthica* on the scope for growth of the blue crab (*Callinectes sapidus*), a common benthic predator of substantial fisheries importance. Due to the combined effects of reduced average clam size and the lower condition of

clams in the more eutrophic sites, the model predicted that crabs in higher nutrient conditions would have 40% lower scope for growth than crabs in more oligotrophic sites. This model predicted that the CE-induced changes to the food web could create a trophic disruption of energy flow reducing growth of higher trophic levels.

In Chapter 6, I present an integrated argument for how CE-induced trophic disruption occurs in the York River, VA, benthic community based on the results detailed Chapters 2 through 5. I also promote the concept that trophic disruption may be one explanation for Rosenzweig's (1971) "paradox of enrichment."

## AUTHORS NOTE

Although this work was designed to explore the potential for cultural-eutrophication-induced trophic disruption in an integrated manner, the following chapters were written to be independent manuscripts for journal submission. Because the chapters are written as separate journal manuscripts, some information is covered in multiple chapters. The following chapters are also written in the third person to represent my co-authors involvement.

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## **Chapter 2.**

**Growth and Condition of Baltic clam *Macoma balthica* in a subestuary of Chesapeake**

**Bay**



## ABSTRACT

The Baltic macoma (*Macoma balthica*) is one of the most common benthic organisms in the tributaries of the Chesapeake Bay. Though it is common, little work has been done to quantify growth and other life-history parameters for *M. balthica* near the southern extent of its western Atlantic range. Due to the morphological effects of rapid growth, the commonly applied ring-based ageing technique, or modal analysis of cohort frequencies, cannot be reliably applied to estimate age and growth in this species. Growth must be directly measured through mark and recapture studies to ensure accurate estimates. We performed a series of caged mark and recapture studies to estimate growth in four shallow-water coves in the mesohaline reaches of York River, VA. By combining the results from caging with a paired survey of ambient animals, we were able to estimate indices of mortality and condition, in addition to growth. We observed rapid growth rates (20.37 mm/year), in keeping with the trends observed in other studies of southern *M. balthica* stocks. In addition, growth decreased and condition indices worsened at the sites near the upriver sections of the river. Salinity or food availability were identified as the most likely forcing factors responsible for these trends, though this has not been confirmed, and the forcing factors are currently under further investigation.

## INTRODUCTION

The Baltic macoma (*Macoma balthica*) is a primarily deposit-feeding (facultative suspension-feeding) clam, commonly found in shallow coastal areas and bays throughout the northern hemisphere. Though usually considered a northern high-latitude species ranging up to ~70 °N, *M. balthica* is found as far south as Gironde estuary (~45°N) in Europe and the South Carolina coastal bays (~33°N) in North America (Kamermans et al. 1999). The Chesapeake Bay (~37°N) is near the southern extent of *M. balthica*'s range; however, the warmer conditions do not seem to negatively impact its abundance. In the shallow oligohaline to polyhaline reaches of the southern Chesapeake Bay tributaries, *M. balthica* is considered a biomass dominant of the benthic community (Holland et al. 1987, Seitz et al. 2006). It is considered ecologically important to the Chesapeake Bay ecosystem. *M. balthica* acts as a primary consumer linking the detrital and primary producers with higher trophic levels, facilitating benthopelagic coupling (Baird & Ulanowicz 1990). Additionally, it is a prey species for several commercially exploited species such as the blue crab (*Callinectes sapidus*) (Hines et al. 1999, Mansour 1992).

Though *Macoma balthica* is important in the ecology of the Chesapeake Bay, there have been few studies of its growth in the Bay (McErlean 1964, Beukema & Meehan 1985) relative to the number of studies conducted on the more northern populations (Beukema & Meehan 1985, Bukema & Cadee 1991, Madsen & Jensen 1987). Growth parameters from previously published studies may not appropriately applied to the Chesapeake Bay *M. balthica* population (Blachet 1980). Based on enzyme analysis, the American stocks are hypothesized to be a different sub-species or sibling

species than the northern European stocks, potentially imparting a different physiology and life-history, and potentially invalidating any parameters estimated based on European clams (Meehan 1985, Meehan et al. 1989). Though the taxonomic relationship between the stocks has not been confirmed using modern genetic markers, well-documented morphological variations indicate significant enough deviation to warrant a growth study focused on the Chesapeake Bay stock (Beukema & Meehan 1985, Kamermans et al 1999).

The number of growth studies for the southern stocks may be limited since the southern clams lack a reliably interpretable age markers. In the northern stocks, *Macoma balthica* commonly has distinct late summer growth rings allowing for fast and repeatable estimates of age and production. This is most likely caused by the biphasic nature of clam growth in the more seasonally variable northern waters. Rapid growth during warmer periods of high primary productivity, combined with slower growth during the colder months, create variable patterns of growth rings, which form darkened checks that act as easily discernable annual marks for back-calculating growth (Lammens 1967, Madsen & Jensen 1987). In areas where winter temperatures are constantly around or below 0°C, the annual growth rings are usually distinct enough to be used as an accurate age markers (Nichols and Thompson 1982). By interpreting these marks, age and relative growth rates can be estimated using methodologies developed for use with teleost otoliths (Smith 1994). Having this consistent age marker makes analyzing the population dynamics of the northern stocks relatively easy.

In the lower latitudes, the seasonal temperature disparity may not be enough to create distinct and interpretable annual growth rings (Glibert 1973). This appears to hold

true for the *Macoma balthica* population in the southern Chesapeake Bay. The shells of the southern clams exhibit distinct morphological differences from northern populations such as having lighter rings. The Chesapeake Bay clams also lack of coloration common in the northern stocks that tend to enhance the readability of the age rings (Beukema & Meehan 1985, Meehan 1985). In a pilot study, we attempted to enhance the rings of southern *M. balthica* though dying and etching; however, no enhancement techniques yielded clear and consistent seasonal marks.

In animals where there is no easy age marker, modal analysis is traditionally used to estimate growth (Lammens 1967, Sergerstral 1980). In modal analysis, distinct cohorts are identified from a survey of animal abundance and length histograms. Alternatively, through repeated sampling, the identified cohorts can be tracked and age and growth modeled through techniques such as Ford – Wollford plotting (Pitcher and Hart 1983). This technique can provide accurate results for species with large single spawning or settling events. Unfortunately, *Macoma balthica* in the Chesapeake have been observed to have both a spring (April-May) and fall (Sept.-Nov.) spawning and recruitment period, which creates an overlap in the year-zero classes (Shaw 1965). Even with this limitation, McErlean (1964) was able to estimate growth using frequency-based techniques using a hydraulic dredge to collect large numbers of individuals at close intervals (Delano 2004). Unfortunately, this collection methodology is prohibitively destructive to justify its use in the sensitive areas selected for this study. Additionally, McErlean (1964) did not observe clear modal progressions representing growth similar to that in other studies of *M. balthica*, potentially due to estimation error from multiple spawning events occluding cohort maxima.

Because the more efficient age- and growth-determining methods are not effective in the lower latitudes, growth must be directly measured and age estimated from mark and recapture techniques. Mark and recapture has been used to explore age and growth in *Macoma balthica* that do not express identifiable growth rings in North American stocks (Gilbert 1978, Nichols & Thompson 1982). The previous mark and recapture studies were performed in Massachusetts and the San Francisco Bay estuary, thus the estimates are most likely not applicable to the Chesapeake Bay populations. In the present work, we attempt to expand our knowledge of *M. balthica*'s age and growth dynamics in the southern Chesapeake Bay through a series of randomized surveys and caged mark-and-recapture experiments performed in the mesohaline section of the York River, Virginia. Caging the marked animals is necessary in the Chesapeake Bay due to the high predation pressure exerted by predators such as the blue crab (*Callinectes sapidus*). Though the caging may alter the growth of the clams, it maximized the recapture rate and allowed estimates of predatory mortality and non-predatory survival in addition to growth.

## METHODS

To determine growth, abundance, and survival of *Macoma balthica*, *in situ* caged growth trials were performed, along with paired and randomized sampling of the ambient uncaged clam population. Trials were performed in four test coves (Poropotank Bay [PR], Purtan Islands [PI], Cattlett Islands West [CW], and Timberneck Creek [TC]) along the mesohaline reach of the York River, VA (Fig. 1). Multiple test coves were used to examine the potential for local variation in the life history parameters. Sites were selected

to have similar biotic and abiotic conditions, such as sediment type, marsh edging, and tidal influence. Salinity was estimated to differ less than 4.2 ppt between sites, based on 10 years (1/1/1998 to 12/31/2007) of Chesapeake Bay Program monitoring data taken at sites near the most upriver and downriver sites (Fig. 1). Due to influence of tidal- and wind-influenced mixing, salinities overlap greatly in the long term (Table 1). Average temperatures did not vary greatly between upriver and downriver sites (Table 1). Long-term average dissolved nutrients, Chlorophyll *a*, and total suspended solids were all greater at the upriver monitoring station, most likely due to the effects of riverine input; however, all parameter estimates overlapped within one standard deviation (Table 1).

#### **Mark and recapture caged trials**

In all caged growth trials, a 50 cm x 50 cm x 20 cm (length x width x depth) cage constructed of one cm galvanized hardware cloth were used to contain 20 marked *Macoma balthica*. Clams were collected via suction sampling within 25 m of the cage locations. Clams were measured, marked with individual letters using indelible ink, and transplanted in the cage block within 20 minutes of collection. Only intact clams ranging in size from 8 to 33 mm length with rapid siphon withdrawal responses were used.

In 2004, two blocks of six cages each were placed in the four test coves (48 cages total). Cages were retrieved after four, eight, and twelve months to obtain seasonal estimates of growth. In the 2005 and 2006 trials, the experimental setup was modified to distribute the plots more evenly throughout the coves; four blocks of two cages were randomly distributed through each of the four coves (32 total cages). One of the two

cages in each block was sampled after six months of soak time while the other was sampled after twelve months. The four-month cage collection used in the first trial was eliminated as it did not provide a long enough duration for the size increase to be easily interpreted.

All cages were sampled using a suction apparatus to a depth of 30 cm with an attached three mm mesh bag. Upon return to lab, the sieved samples were sorted and all of the clams removed. The identifiable marked clams were measured and the observations compared to measurements of their initial size at the time of transplanting. For each clam that was not chipped or otherwise damaged, growth increments were calculated by subtracting the original size from the final size. Proportional growth was estimated by normalizing the growth increments to the initial size of the clam. Von Bertalanffy growth models (Equation 1) were fit to the growth increments using least squares.  $L_{\infty}$  is the predicted maximum size and  $k$  is a slope parameter predicting how quickly  $L_{\infty}$  is reached.  $L_{\infty}$  was fit to the data using least squares estimation, not set at maximum size.

$$\text{Clam size} = L_{\infty} (1 - e^{-k \cdot \text{Time}}) \quad (1)$$

Non-predatory mortality was estimated from the marked clams in the cage trials by calculating the proportion of live clams recovered compared to those transplanted. This calculation also included the few clams that had observable markings but that were not clear enough to determine the clam's individual identity for use in the growth estimation.

### **Ambient clam sampling**

Samples of the ambient clams were taken concurrently with the caged samples using a suction apparatus and 37-cm diameter cylindrical core to a depth of ~30cm, to provide an estimate of ambient abundance for comparison with the caged populations. All paired ambient clam samples were taken 0.5 m from the cage parallel with the shoreline. In the second and third trials, two additional unpaired ambient clam samples in addition to the paired samples were taken, at random locations in the test cove, to provide a more thorough estimate of within-cove variation. Ambient clam surveys were always paired with the mark-and-recapture experiment recovery. Two surveys of ambient clams were conducted in 2005 and 2006, in association with the six and 12 month growth trials. In 2004, ambient clams were surveyed at four, six and 12 months.

The uncaged samples were used to estimate clam abundance and predation pressure. Predation pressure of large predators (those excluded by the one cm mesh) can be estimated because the cages acted as predator-exclusion devices. Proportional predation pressure was calculated by subtracting the mean density of paired uncaged clams from the mean density of unmarked caged clams in each cage, and then dividing by the density of uncaged clams. This may not yield a true estimate of predation pressure, as it may be confounded by caging effects; however, it should provide an estimate of relative predation pressure on the ambient clam population among the test coves.

From each ambient-clam sample collected during the 2005 and 2006 trials, six intact clams were randomly selected for individual biomass determination. Ash-free dry



mass (AFDM) was estimated using the loss on ignition technique. A length-weight key was created by fitting the AFDMs to a power curve using nonlinear regression routine in Sigmaplot (Equation 2).

$$\text{Ash - free dry mass} = \alpha \bullet \text{Clam Length}^{\beta} \quad (2)$$

Condition index (CI<sub>2</sub>) was calculated by dividing the AFDM by the shell length (in mm) and multiplying the results by 1000, as an indicator of overall clam health (Wene & Steczynska-Jurewicz 1985).

All of the indices estimated above were evaluated using analysis of variance (ANOVA; MINITAB 15.1 software program). An ANOVA was conducted on all indices to determine if there were differences among the four test coves and also among collection times. For the indices based on whole sample plots such as abundance and average size, the four test coves were combined into two river position groupings (Upriver and Downriver), to have sufficient sample sizes and to reduce heteroscedasticity, facilitating parametric hypothesis testing. Assumptions of ANOVA were tested using the Anderson-Darling test for normality and Levene's test for equal variances.

Transformations were made when necessary to meet the assumptions of ANOVA. In cases where transformation could not make the data set meet the assumptions of ANOVA, Kruskal-Wallis non-parametric hypothesis tests were employed.

## RESULTS

### Ambient clams

The ambient clams samples paired with the retrieval of the mark-and-recapture cages in the four test coves were grouped into upriver and downriver locations to increase the sample size for analysis and to ease visualization of seasonal trends. *M. balthica* abundance did not vary significantly between river positions but did vary between sampling events (Kruskal-Wallis River Position: degrees of freedom [df] = 1, 168,  $H = 0.20$ ,  $p = 0.651$ ; Kruskal-Wallis Collection date:  $df = 6$ , 168,  $H = 59.68$ ,  $p < 0.0001$ ). Clam density decreased through the summer and fall followed by an increase in spring each year, caused by a large recruitment event (Figure 2). Clam size also reflected the effects of spring recruitment with a significant variation in average size of clam per sample with collection time (ANOVA  $df = 6$ , 160,  $F = 7.36$ ,  $p < 0.0001$ ). The average clam size dropped in the spring and then increased throughout the year (Figure 3). Average size was significantly greater at the downriver coves than the upriver coves for all collections excluding spring 2007 (Figure 2, Table 2).

To act as a proxy for overall animal health, 696 clams were used for condition index determination. Condition index was only run for clams collected along with the second and third mark-and-recapture trials which had an expanded number of uncaged samples added, due to availability of sufficient *Macoma balthica* for analysis. A significant difference in condition was observed among the four test coves with condition index lower at the upriver sites (Figure 4, ANOVA, data natural log transformed,  $df = 3$ , 693,  $F = 20.57$ ,  $p < 0.0001$ , Tukey's multiple comparisons: 98.95 individual confidence

level). Additionally, a significant difference was detected among collection dates using mixed model ANOVA to account for variation due to location (data natural log transformed, ANOVA  $df = 4,689$ ,  $F = 6.68$ ,  $p < 0.0001$ ). Condition index was greater in the spring (May) than in the fall (Oct. and Sept.), probably due to the loss of mass from the large fall spawning event (Figure 5). Because significant differences were observed in the condition index of *M. balthica* along the river axis, three length-weight keys were created: one for the upriver coves, one for downriver coves, and one for the river as a whole (Table 3).

### **Caged Trials**

Of 112 cages deployed, 88 were recovered intact. Cages were most likely lost due to storm damage, physical deterioration of the cages or markers, and damage due to fishing and boating activities. We recovered 536 marked live clams in the three growth trials. Labels were unreadable for nine clams and 88 were chipped and thus were unusable for growth estimation. Marked valves of dead clams were also found, accounting for most of the dead clams in intact cage blocks.

Growth was decreased at larger initial sizes, indicating an asymptotic growth pattern similar to the von Bertalanffy growth model (Figure 6). Growth rates, corrected for initial size, differed significantly among the test coves (ANOVA,  $df = 3, 435$ ,  $F = 6.250$ ,  $p < 0.0001$ ). Growth in the two downriver coves, TC and CW, was not significantly different from each other while growth in the two upriver coves, PI and PR, was significantly lower than that in the downriver coves and distinct from each other with

higher growth in PI (Tukey's multiple comparisons: 98.94% individual confidence level). Significant variation in growth with the seasons was also observed (ANOVA,  $df=2, 436$ ,  $F=12.85$ ,  $p < 0.0001$ ) with significantly greater daily growth occurring in the summer trials (collected in the fall,  $9.65 \pm 0.51$  mm/year) than in the trials that included fall and winter growth (Figure 7; Spring =  $4.56 \pm 0.39$ , Year =  $5.78 \pm 0.31$ , Tukey's multiple comparisons: 98.03 individual confidence level).

With respect to the variations observed in clam size and raw growth data, we fit von Bertalanffy growth models using least squares estimation to all growth data, summer only growth data, all data from the downriver coves, and to all data from the upriver coves (Table 4). Parameter estimates predicted faster growth downriver, reflecting the trend of decreased condition, and smaller average size. Growth parameters calculated for the summer months predicts faster growth to a larger maximum size is, as it eliminates the slower growth during the winter months.

Non-predatory mortality was also estimated from the marked animals as the percentage of survival in each cage. Mortality was significantly different between the test coves and appeared to increase moving upriver along the York River axis (Kruskal-Wallis  $df=3, 84$ ,  $H=50.81$ ,  $p < 0.0001$ , Figure 8). This estimate of non-predatory mortality is most likely increased by handling stresses and cage effects, and magnitudes may be inflated over natural levels.

The cages appear to have provided a refuge from predation with an average of  $86.2 \pm 26.4$  more unmarked clams inside the cages than outside of the cages at the end of the trials. When converted to proportional predatory mortality, no difference was observed between the two river positions (ANOVA  $df=1, 83$ ,  $F=2.28$ ,  $p=0.134$ ), nor

among collection times (ANOVA  $df = 6, 79, F = 1.5, p = 0.200$ ) indicating relatively equivalent predation pressure.

The length-weight equations were combined with the growth estimates to create a biomass-based growth model. This allowed us to further explore the combined effects of the significant differences in condition and growth rates between the upriver and downriver sections of the river. The model predicts a greater relative difference in mass based growth than the shell width simulation, with the upriver clams having for the majority of the simulation run (Figure 9). As growth asymptotes at the end of the simulation run the biomass appear to be converging; however, the likelihood of an upriver clam surviving to as large a size is low due to the high mortality rates in the upriver sites.

## DISCUSSION

The results of our three-year-long a mark-and-recapture caged trials produced estimates of growth that were used to parameterize von Bertalanffy growth models. The combined growth observations for the York River produced a model that predicts that the average 1mm *Macoma balthica* recruit will grow to be 19.17 mm after one year.

In our study, we observed a great variation in *Macoma balthica* growth between the upriver and downriver test locations. When we parameterized separate von Bertalanffy models for the upriver and downriver sections of the River we observed a significant variation in growth rates. A 1 mm clam downriver can be expected to achieve a final size of 20.37 mm while an upriver clam will only grow to an average of 17.23 mm

after a full year. In addition to the slower growth at the upriver sites, *M. balthica* condition index decreased 48.8% on average between the most upriver and downriver site. This reduced condition was reflected in the survival of the animals used in the mark-and-recapture trials. Mortality was 43.75% greater at the most upriver compared to the most downriver site. Condition index also showed a progression of worsening conditions from cove to cove as one moves further upriver. The York River *M. balthica* population seems to be expressing a strong response to a stressor that is present as a gradient in the York River.

The observed *Macoma balthica* growth follows the trends observed in other studies that account for temperature. Being near the southern limit of their range, the clams in the York River, VA, experience much higher temperatures than the more northerly areas where this species is thought to have evolved (Meehan 1985). The warmer average water temperatures appear to foster rapid growth compared to the northern stocks. The von Bertalanffy parameters fall within the trends of increased maximum size and slope parameter  $k$  with decreasing latitude (Figure 10). Though our values appear to follow the latitudinal trends, we do not achieve the asymptotic shell size ( $L_{\infty}$ ) noted from the last formal growth study in the Chesapeake Bay (McElean 1964). Our maximum size estimate is 3 mm lower (37 mm vs. 40 mm) than the one observed in the Patuxent River, MD, study. This discrepancy is caused by the earlier study fixing maximum size at the largest animal observed while we fit the value using least squares estimation.  $L_{\infty}$  was not fixed at the maximum size since it would cause the growth model to over predict the size of small clams. By fitting  $L_{\infty}$ , we sacrificed some accuracy in

predicting clams larger than 35 mm but we more accurately predict growth for the smaller clams which comprise a large percentage of the York River population.

The fast growth in the Chesapeake Bay southern *Macoma balthica* population is most likely due to the almost continuous growth, even in the winter months. We were able to detect a significant growth increment in animals that were collected in the spring versus those collected in the late fall after the water temperatures had dropped below 10 °C, the cut-off temperature for growth recorded for a Norwegian population of *M. balthica* (Lammens 1967). This growth in the winter may also help explain the ability of Chesapeake clams to spawn multiple times in a year (Shaw 1965). Though we are confident that there is substantial growth over the winter as a whole, given the frequency of collections used, we could not be sure whether growth was incessant over the winter months. With the spacing of our collection times (three months) it was impossible for us to detect a short cessation of growth, such as the one that occurs in the middle of winter for the San Francisco Bay, CA, population (Nichols & Thompson 1982).

Additionally, we noted that the trend of increasing maximum size with decreasing latitude is primarily driven by the North American *Macoma balthica* (Figure 11). The trend for the European clams is actually slightly negative (Figure 11). Our estimates fall in line more closely with the North American latitudinal trend, possibly due to the variation in shell morphology between the stocks observed by Beukema and Meehan (1985), which, combined with limited molecular biological analysis, suggest that the two stocks may not be conspecific (Meehan 1985, Väinölä 2003). There may be many subspecies of *M. balthica*, with different ecophysiological responses within the European populations along their latitudinal distribution (Hummel et al. 1998 a, Hummel et al.

2000). Thus, it is possible that the increased growth rate observed in our study may be because the North American stocks are a subspecies of the European *M. balthica*.

Though our growth predictions indicate a difference in physiology from the European stocks, the variation observed along the river axis may imply that the Chesapeake Bay *Macoma balthica* population could be a stock existing at the extreme range of its range. As with most species with a wide distribution, *M. balthica* has been observed to experience greater responses to stress than those individuals living in the middle of the range (Hummel et al. 1996). An animal living at the extremes of its range will be more greatly effected by small shifts in conditions due to its already stressed nature.

Though the speciation of the different *Macoma balthica* stocks has not been fully explored, the different patterns of growth and response to stress leads us to believe that extreme caution must be taken in applying and extrapolating life history parameters between the European and North American populations. Additional genetic studies to determine the true nature of the stocks are warranted.

The stressor that is causing such extreme shifts in growth and condition within the mesohaline section of the York River, VA cannot be easily identified. *Macoma balthica* growth and production has been correlated with a variety of environmental variables, such as recruitment variability, primary production, tidal level, sediment grain size, salinity, population density, inter-specific competition, temperature, and food quality and quantity (Beukema et al. 2002, Van de Meer et al. 2001, McErlean 1964, Vincent et al. 1994, Kamermans 1994, Gilbert 1973, Bukema & Cadee 1991). Some of these factors cannot explain the trends in our data since they do not greatly vary between sites or do



not occur along a gradient of the York River axis. We believe that the most likely forcing factors that vary along the axis of the York River and that could potentially control changes in the growth and condition of *M. balthica* are either salinity or food quantity and quality.

The most obvious forcing factor that may be affecting the growth, condition, and mortality of *Macoma balthica* along the river axis is salinity. Salinity has been linked to *M. balthica* growth but with mixed results (Gilbert 1973). *M. balthica* growth rates have been observed to increase, decrease, or be essentially unaffected by moderate salinity differences (McErlean 1964, Beukema & Cadee 1991, Madsen and Jensen 1987, Bachelet 1980). All of our sites are mesohaline with a long-term average differential in salinities between the sites of > 4.2 ppt, and *M. balthica* can be found in waters ranging from oligohaline to polyhaline in the Chesapeake Bay, we believe that salinity is only partially responsible for the patterns observed (Diaz & Schaffner 1990).

It is probable that food limitation may be controlling the growth and health of *Macoma balthica* in the York River. In the San Francisco Bay, the most comparable well-studied *M. balthica* population, clam growth seems to be controlled mainly by food availability more than any other forcing factor (Thompson & Nichols, 1988). In the York River, food availability follows an along-axis gradient due to the effects of cultural eutrophication. Due to the dilution effect and consumption by primary producers, thus the nutrient concentrations and intensity of cultural eutrophication decreases downriver (Boesch 2002, Ouboter et al. 1998). Though this trend should lead to increased food availability upriver, and thus the opposite trend than what we observed, the food quality available for deposit feeders may be lower upriver than the less-eutrophic downriver

sites. The excessive nutrients in the York River shift the composition of the primary producer guild away from benthic diatoms and toward pelagic dinoflagellates and cyanobacteria (Sin et al. 2000, Kemp et al. 2005). This switch in primary producers could affect *M. balthica* growth and condition in two ways. First, the loss of microphytobenthos reduces *M. balthica*'s ability to deposit feed which may impact juveniles the most as they have been observed to primarily act as benthic feeders (Olafsson 1986, Harvey & Louma 1984, Rossi et al. 2004). Second, the phytoplankton taxa that bloom in the increased nutrient conditions tend to be less palatable and nutritious and potentially even toxic compared to the micro algae associated with lower nutrient conditions (Olafsson 1986, Bukema & Cadee 1991, Smith 1998, Anderson et al. 2002).

The southern Chesapeake Bay provides an interesting environment in which to explore the growth and life history of the Baltic clam. Assuming that they are conspecific with the other Atlantic *Macoma balthica* stocks, our test location is near the southern part of their range where they are living at the extremes of their tolerances for stress. The overall stress created by the high-temperature waters exacerbates the effects of other forcing factors creating large variation in growth, condition, and mortality, which we detected as a pattern decreasing health of the *M. balthica* populations moving upstream in the York River. This can have implications for higher trophic levels, because the reduced growth and condition, and the higher mortality rate reduce the total biomass of *M. balthica* available to be passed up the food web. Thus it may be possible that the upriver areas would not support as large a population of predators as the downriver coves could. We believe that either salinity or eutrophication-induced shifts in the food quantity and quality has created these patterns. A follow-up study is currently underway to determine

if food, salinity, or another forcing factor can be identified as the stressor that is most responsible for different growth and abundance patterns observed in the York River.

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Table 1. Chesapeake Bay Program water-quality parameters for stations near the upriver (LE 4.1) and downriver (LE 4.2) test coves. Means  $\pm$  standard deviation for surface water samples taken from 1/1/1998 to 12/31/2007 are reported.

Parameter	Location		Units
	LE 4.1	LE 4.2	
Chlorophyll a	13.33 $\pm$ 8.45	10.59 $\pm$ 10.18	ug/L
Salinity	13.44 $\pm$ 4.21	17.61 $\pm$ 3.76	psu
Temperature	16.610 $\pm$ 0.781	16.31 $\pm$ 0.76	$^{\circ}$ C
Total Suspended Solids	25.84 $\pm$ 15.64	16.30 $\pm$ 12.81	mg/L
Dissolved Nitrogen	0.39 $\pm$ 0.12	0.34 $\pm$ 0.10	mg/L
Dissolved Phosphorous	0.03 $\pm$ 0.02	0.03 $\pm$ 0.018	mg/L



Table 2. ANOVA results for the difference between the clam widths of ambient *Macoma balthica* from the two upriver and two downriver sites.

Collection Time	DF	F value	P value
October 2004	221	20.60	<0.0001
May 2005	1277	83.66	<0.0001
October 2005	237	27.24	<0.0001
May 2006	249	213.77	<0.0001
Sept 2006	229	30.87	<0.0001
October 2006	221	18.15	<0.0001
May 2007	708	2.83	0.060

Table 3. Parameterization results for power fit on length-weight data for *Macoma balthica* based on ash free dry mass of uncaged paired samples.

Data Type	$\alpha$	B	R <sup>2</sup> value
All data	3.99E-06	3.2409	0.8537
Upriver	6.61E-07	3.8411	0.8107
Downriver	4.48E-06	3.1959	0.7639

Table 4. von Bertalanffy growth model parameters fit using least squares to various divisions of the mark and recapture data growth estimates for *Macoma balthica*.

Data Type	$L_{\infty}$	K
All data	31.830	0.890
Summer only	34.280	0.956
Downriver	31.938	0.984
Upriver	30.664	0.792

Figure 1. Site map for the four test covers along the York River axis with the Chesapeake Bay program long term monitoring station locations.

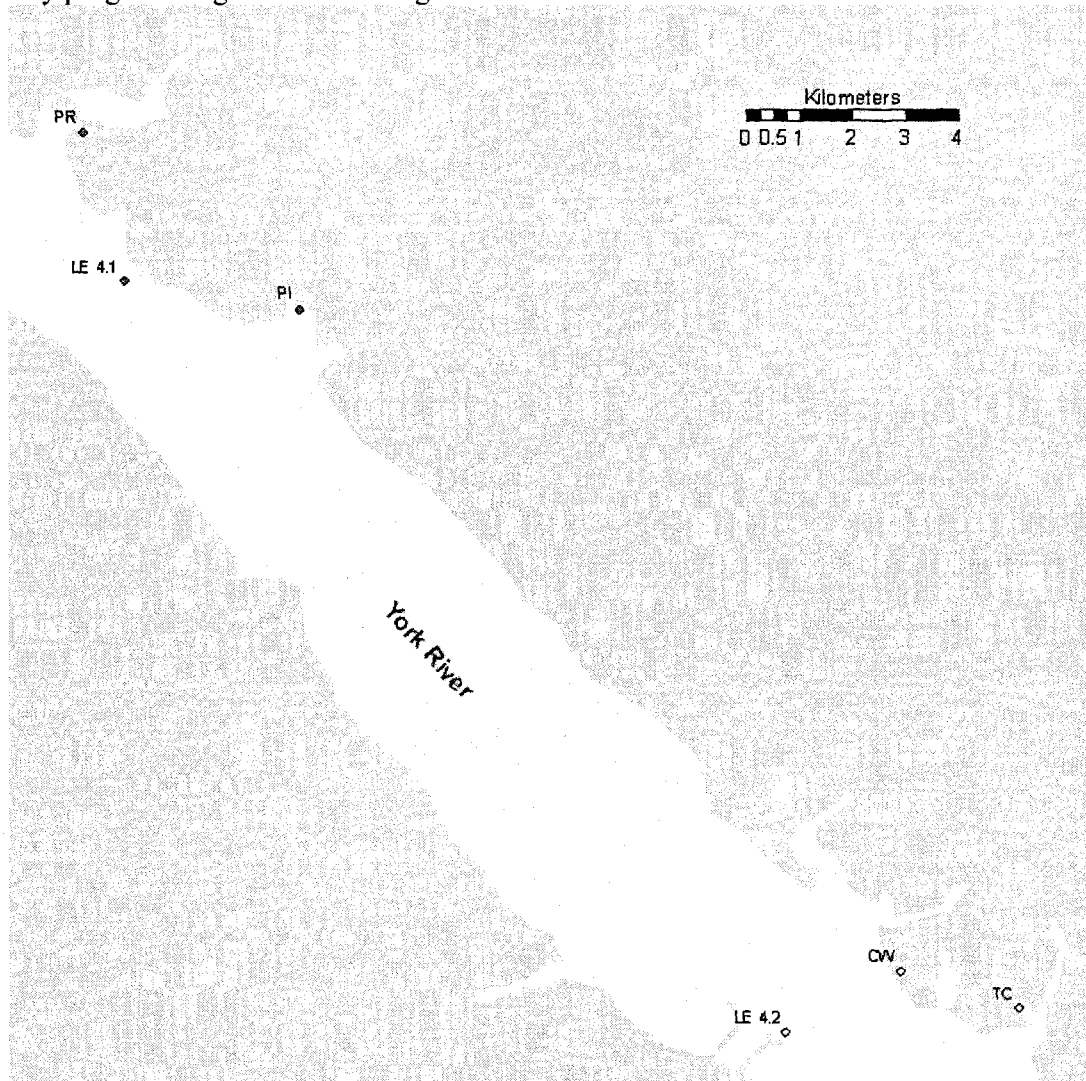


Figure 2. *Macoma balthica* uncaged abundance from paired samples taken with the recovery of the three mark and recapture trials. The four test coves were combined into river position groupings to provide sufficient samples for analysis and to ease visualization. Error bars are one standard error of the mean.

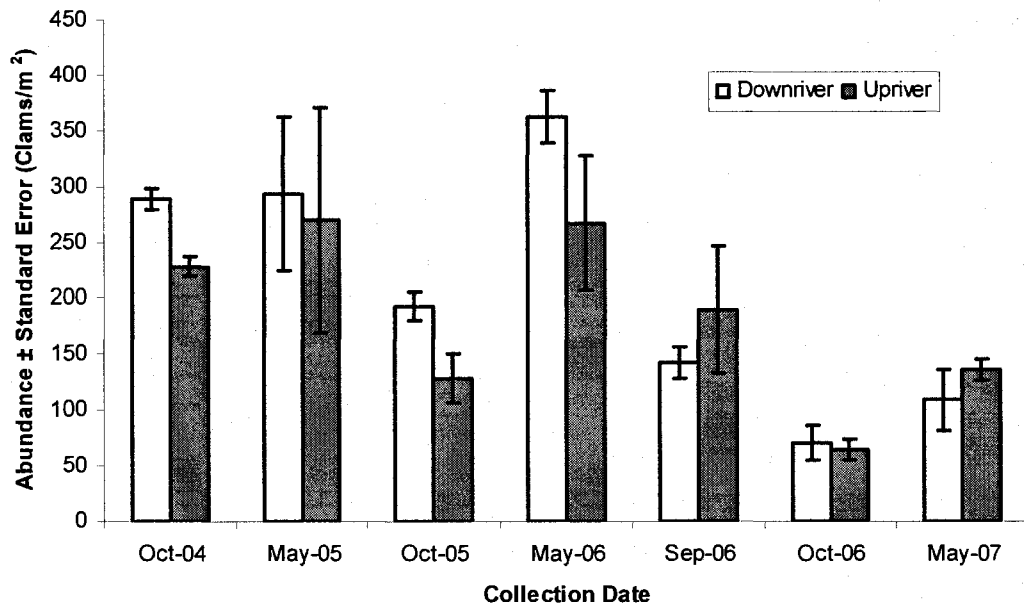


Figure 3. *Macoma balthica* average size as a function of river position and collection time. The four test coves were combined into river position groupings to provide sufficient samples for analysis and to ease interpretation. Error bars are one standard error of the mean.

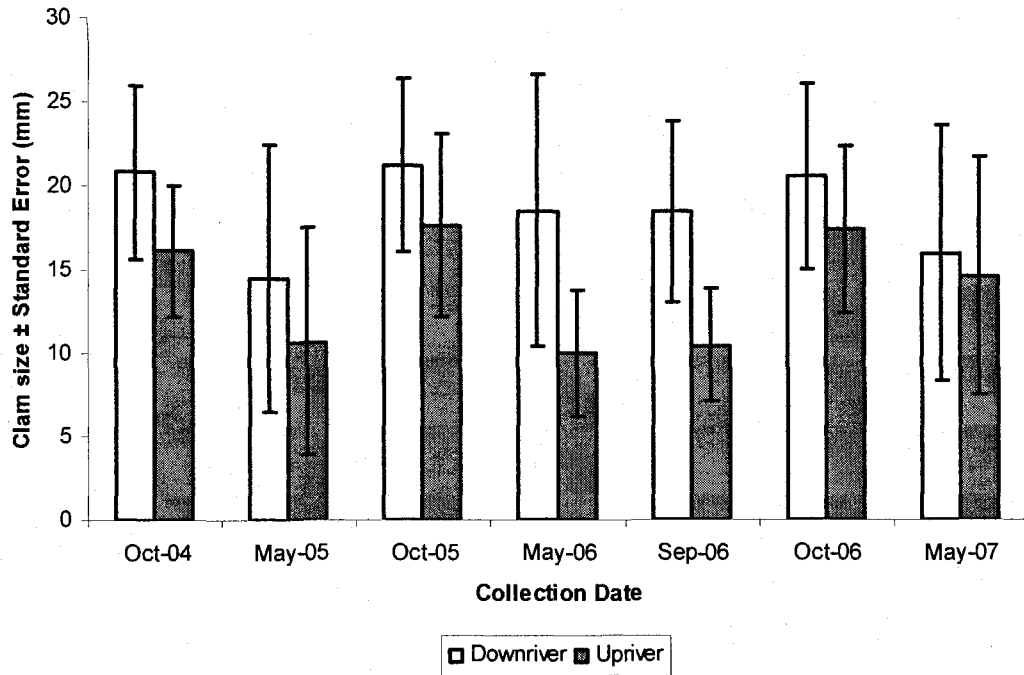


Figure 4. Condition index of ambient *Macoma balthica* in four test coves along the York River, VA, axis, collected as paired samples with the second and third mark and recapture trials. Locations are arranged from most downriver to most upriver and TC = Timberneck Creek (downriver), CW = Catlett West (downriver), PI = Purtan River (upriver), and PR = Poropotank River (upriver). Letters indicate significant differences. Error bars are 1 standard error of the mean.

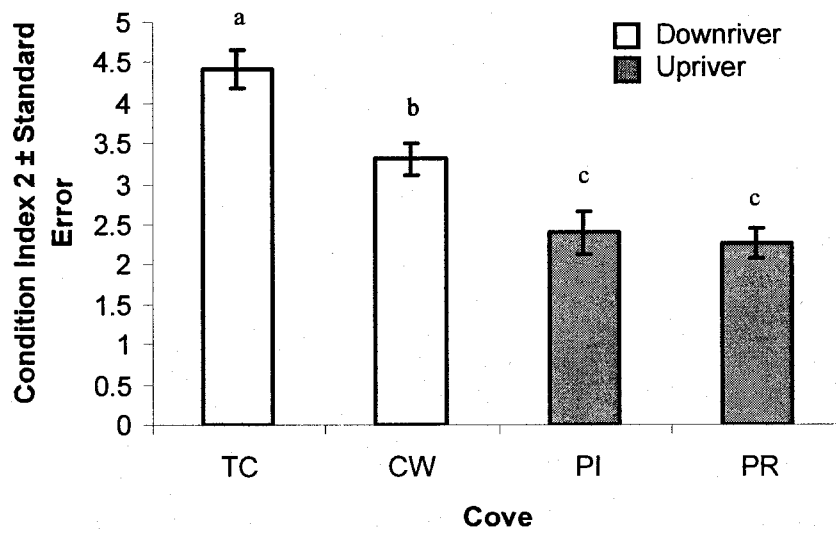


Figure 5. Mean condition index for *Macoma balthica* from paired ambient clam samples from upriver and downriver test coves, measured during the second and third mark and recapture trials. Error bars are one standard error of the mean.

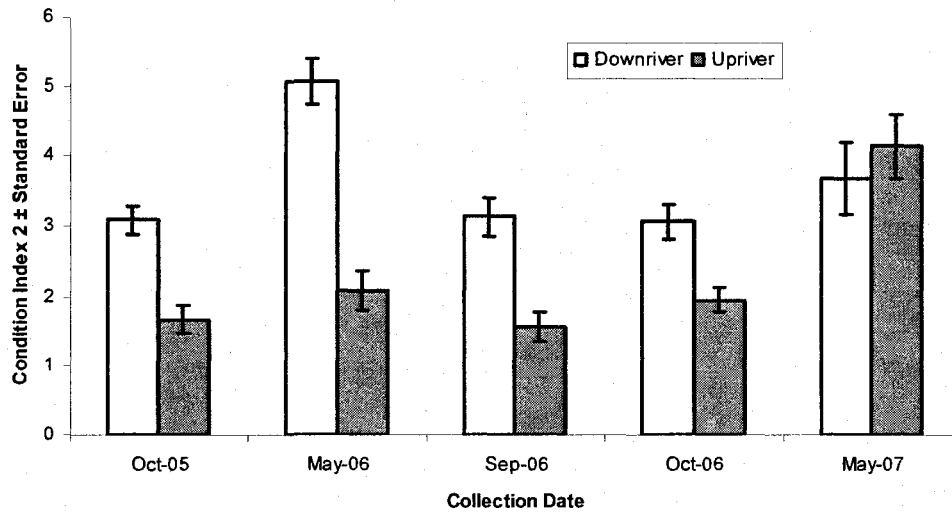




Figure 6. *Macoma balthica* mean annual growth rate from three mark and recapture trials combined as a function of binned size at deployment (a). Annualized *M. balthica* growth rates from three mark and recapture trials as a function of initial size for the four test coves (b). Locations are TC = Timberneck Creek (downriver), CW = Catlett West (downriver), PI = Purtan River (upriver), and PR = Poropotank River (upriver). Black slashes through the x-axis indicate that no data was collected in those trials, due to lack of *M. balthica* survival.

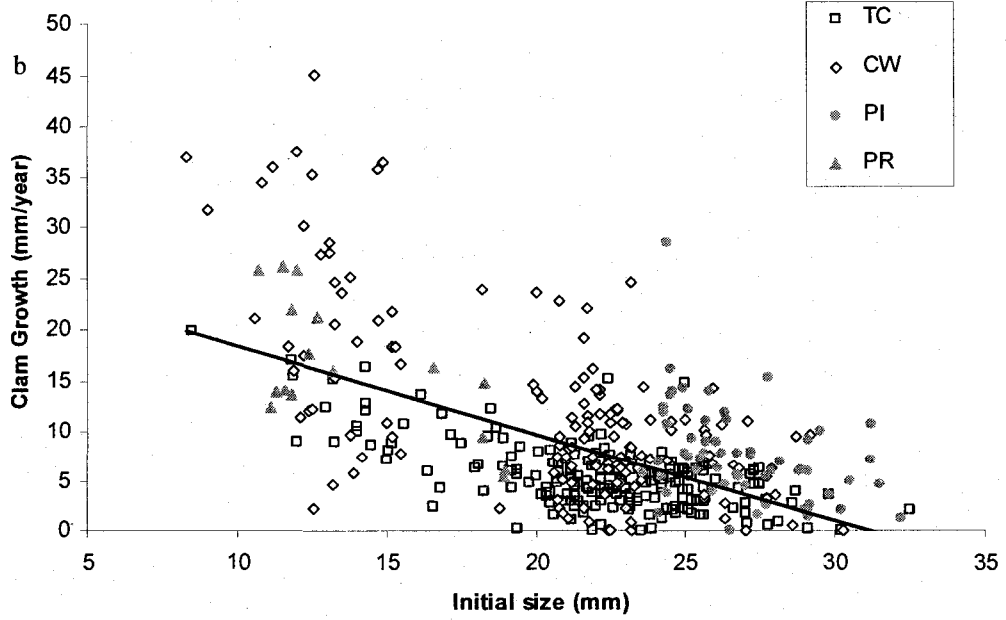
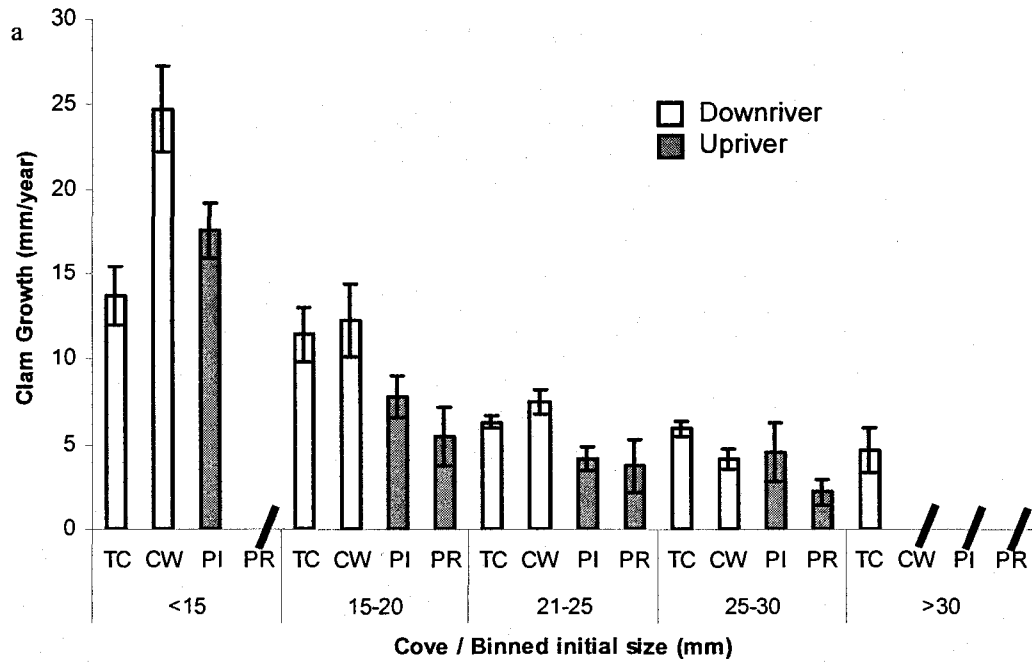


Figure 7. *M. balthica* annual growth rate from three mark and recapture trials combined as a function of initial size grouped by trial season. The fall trials were conducted from the spring through the summer growing season and were collected in fall. Spring samples were retrieved after overwintering. The year trials were collected after approximately one year of soak time.

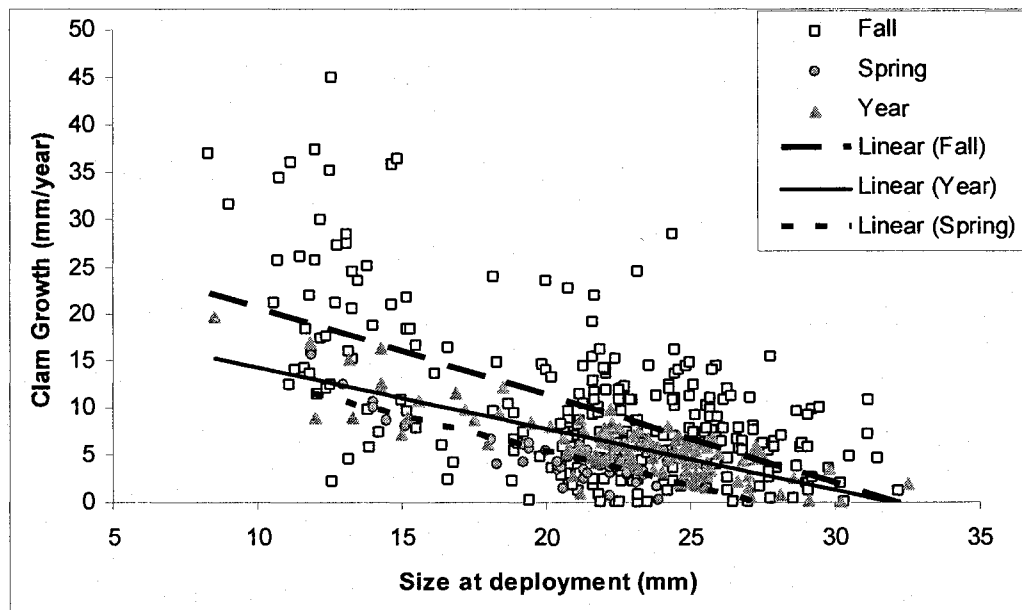


Figure 8. Mortality of *Macoma balthica* from three mark and recapture caged growth trials from four test coves along the river axis. TC is the most downriver cove. This is a proxy for non-predatory natural mortality though is probably inflated over the natural populations levels due to caging and handling effects. Error bars are one standard error of the mean.

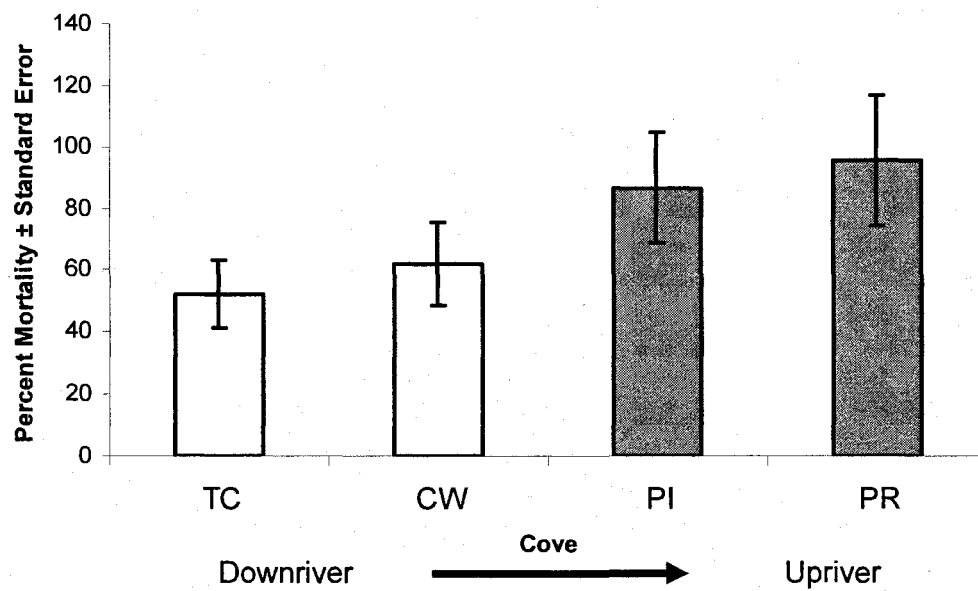


Figure 9. Results of a von Bertalanffy growth model parameterized for *Macoma balthica* from the upriver and downriver mesohaline reaches of the York River, VA. Thick lines are the predicted clam size starting from a 1mm clam. Symbols and thin lines are the predicted ash free dry mass of the animal.

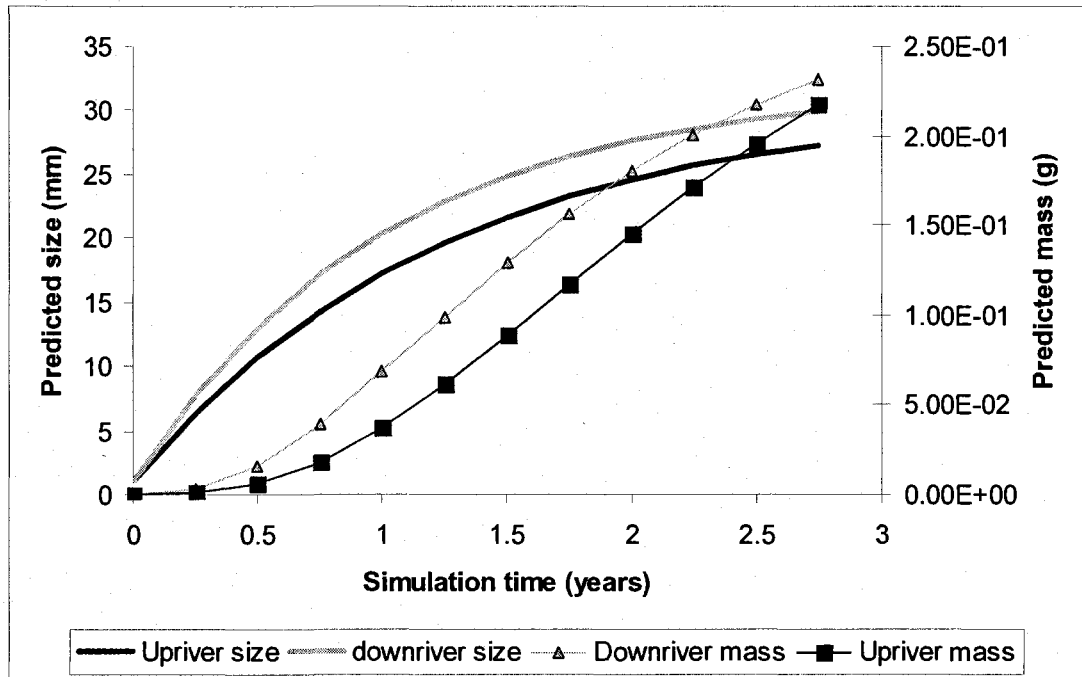


Figure 10. Von Bertalanffy parameters from various studies as a function of latitude. Grey diamonds are the previously published studies as collected by Blachlet (1980) with additional data from Mcerlan (1964). The large black squares are combined values for this study. Grey diamonds are European estimates. Open circles are parameters for North American *Macoma balthica*.

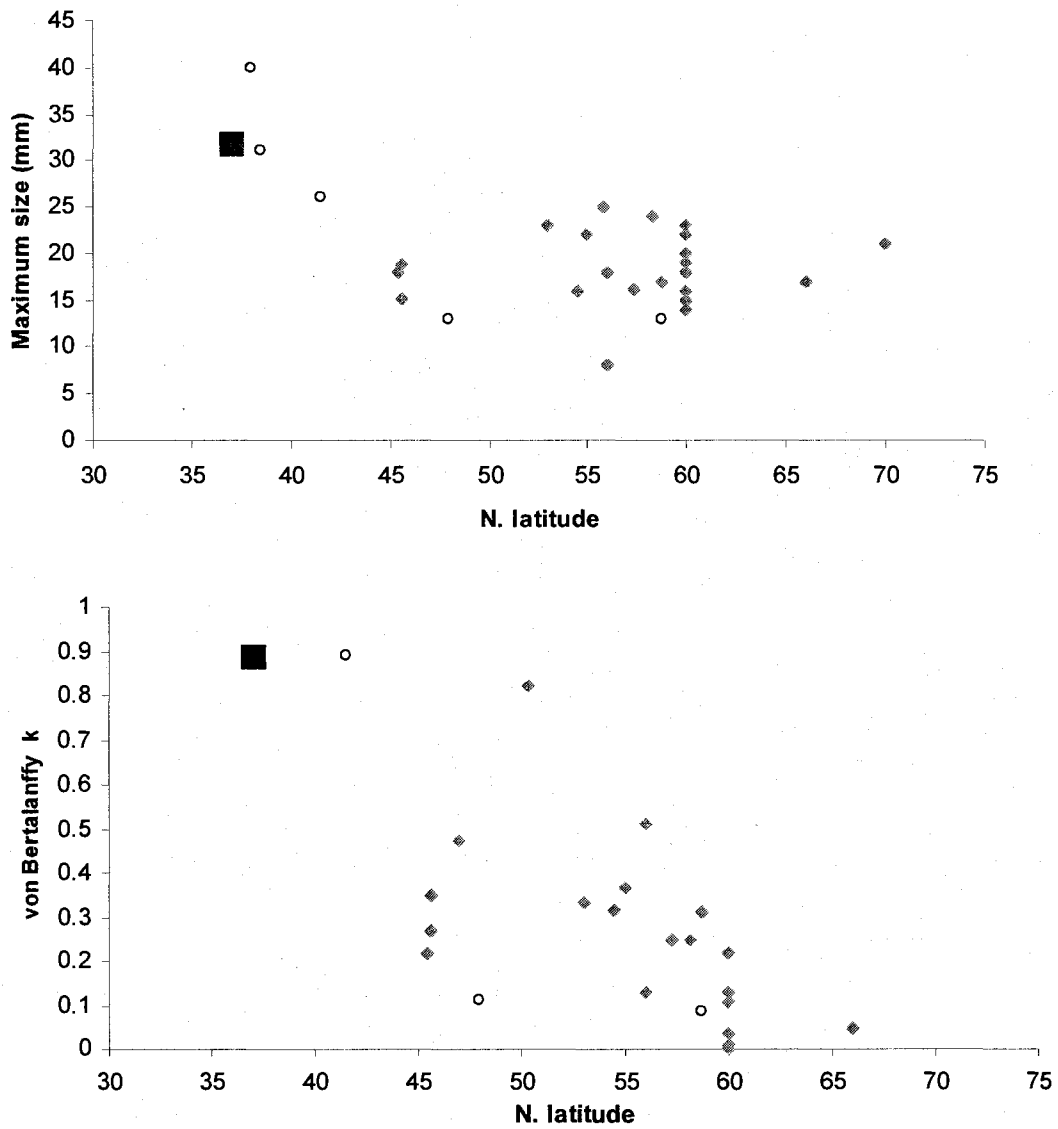
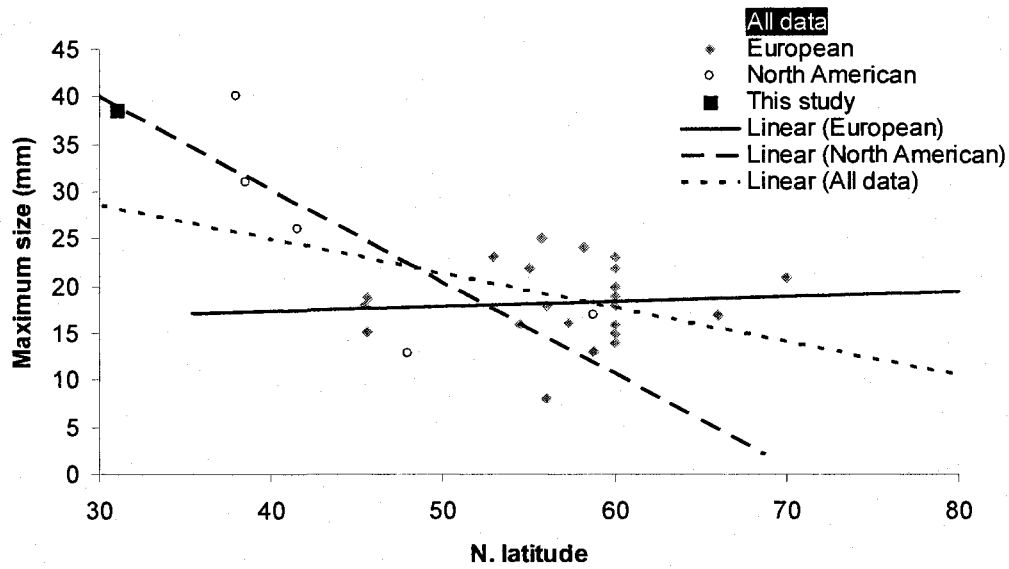


Figure 11. *Macoma balthica* maximum size as a function of latitude based on published studies collected by Blachlet (1980) with additional data from McErlan (1964). The large black square is the combined values for this study. Grey diamonds and the solid trend line are European estimates. Open circles and the large dashed line are the maximum size parameters for North American *M. Balthica*. The small dashed lines are all studies combined.



### **Chapter 3:**

Eutrophication-induced shifts in the primary producer community and resultant changes  
in *Macoma balthica* health



## ABSTRACT

*Macoma balthica* is a deposit-feeding clam commonly found in the oligohaline to polyhaline areas in the tributaries of the Chesapeake Bay. A previously performed three-year-long caged mark-and-recapture study uncovered a pattern of decreased growth, decreased health, and decreased stress-related survival for the clam sub-populations in the upper mesohaline section of the York River, VA, compared to downriver sub-populations. A follow-up field survey of ambient clam densities and health indices was performed in six shallow-water coves along the mesohaline reaches of the river. A suite of potential forcing factors was examined from long-term monitoring datasets to determine which stressors most affected clam health. Average clam health decreased along a gradient moving upriver along the river axis. Using an information-theoretic analysis of potential forcing factors, we identified several important forcing factors, with the composition of the primary producer community emerging as the most important factor affecting clam health. It appears that upriver locations, which have greater nutrient concentrations, have a greater proportion of opportunistic primary producers of low nutritional value compared to the less nutrient-rich waters downriver. We believe that the benthic food web upriver is disrupted by a cultural-eutrophication-induced shift in primary production toward less-nutritious taxa preventing the benthos from utilizing the full amount of available primary production.

## INTRODUCTION

The Baltic macoma, *Macoma balthica*, is a small thin-shelled clam commonly found in the shallow coastal bays and rivers of the northern hemisphere ranging from the Arctic to South Carolina in North America (Kamermans et al. 1999). In the tributaries of southern Chesapeake Bay, it is the biomass-dominant infaunal organism (Holland et al. 1987, Diaz & Schaffner 1990). The Chesapeake Bay lies near the southern extent of *M. balthica*'s range which may make the Bay's *M. balthica* population more responsive to stressors than the populations living in the middle part of the range (Hummel et al. 1996). Because clams are stressed by higher average water temperatures, compared to the areas in which they evolved, we may observe large shifts in population and individual dynamics in response to slight variations in habitat quality (Hummel et al 1996). We hypothesized that we would observe habitat-induced changes in *M. balthica* density, biomass, and condition for subpopulations in the York River sub-estuary of Chesapeake Bay.

We previously studied the growth, condition, and non-predatory mortality of *M. balthica* in the mesohaline York River, VA (Chapter 2; Brylawski et al., in prep). We noted a trend of decreasing growth rates, survivorship, and condition index moving upriver from the mouth. The study was comprised of a caged mark-and-recapture experiment paired with ambient benthic sampling in four coves in the mesohaline reaches of the York River. In the caging study, non-predatory mortality was significantly higher, condition index was lower, and growth was reduced at the two upriver sites compared to

the downriver sites, suggesting worsening habitat conditions moving up the river axis (Figure 1).

In this study, we seek to explain the trends observed in the previous study by examining potential forcing factors which may be controlling the along-axis variation in the health of *Macoma balthica*. To allow analysis by regression, two sites were added between the four original mark-and-recapture study areas, giving us a total of six sites grouped into upriver, midriver, and downriver zones. To more fully explore the cause of the trends in reduced condition with distance upriver, we measured a suite of potential forcing factors during three sampling events during the primary clam growing season (spring to fall).

The underlying variable controlling the health of *Macoma balthica* in the York River appears to follow a gradient of stress that increases in smooth function moving upriver along the river axis. In the previous work, we proposed that salinity stress and food availability were potentially responsible for the trends we observed; however, there is a large suite of potential forcing factors that can affect *Macoma balthica*'s health. *M. balthica* dynamics shift due to recruitment variability, primary production, tidal level, sediment grain size, salinity, population density, inter-specific competition, temperature, and food quality and quantity (Beukema et al. 2002, Van de Meer et al. 2001, McErlean 1964, Vincent et al. 1994, Kamermans 1994, Gilbert 1973, Bukema & Cadee 1991). We can eliminate several of these possible forcing factors because their intensity does not vary along a river- axis gradient. For this work, we measured or observed the following factors: salinity, sediment grain size, sediment organic carbon, benthic chlorophyll *a*, water column chlorophyll *a*, and the taxonomic groupings of phytoplankton species. In

addition, we concurrently collected samples of *M. balthica* for estimating population density and health. Long-term monitoring performed by the Chesapeake Bay Program was also used to provide extended data-sets for temperature, salinity, nutrient variations, and other water-quality parameters. These factors were used in a series of regression models which were evaluated using an information-theoretic approach to determine which parameters were most responsible for the patterns of *M. balthica* condition in the river.

## METHODS

### Survey Design

Six shallow sampling coves were selected within the mesohaline section on the north shore of the York River, VA (Figure 2). Test coves were required to have similar biotic and abiotic conditions such as fringing marsh, shoreline type, and average depth, to eliminate confounding variables that could mask the along-axis gradient effect. For data analysis, these test coves were grouped into the following three river zones: upper mesohaline (Poropotank Bay [PR] and the Purtan Islands [PI]), middle mesohaline (Jones Creek [MD] and Aberdeen Creek [AM]), and lower mesohaline (the Cattlett Islands [CT] and Timberneck Creek [TC]). The upriver and downriver test coves were retained from the mark and recapture study, while the midriver sites were added for this study. The midriver sites were approximately halfway between the existing sites and had similar conditions as other sites. These six coves were sampled in the spring (5/22/2007-

5/24/2007), midsummer (7/24/2007 – 8/1/2007), and fall (10/29/2007 – 11/30/2007) to explore seasonal variation in the *M. balthica* population and factors influencing variation.

### **Water-Column Sampling**

At one site per cove, water was collected from ~10 cm above the sediment surface using dark polycarbonate bottles. The salinity of the samples was determined using an optical refractometer. A 10 ml aliquot was taken and filtered onto a Whatman GFF filter and stored prior to chlorophyll *a* and phaeophytin extraction and analysis using flourometry (Arar & Collins 1997). Phytoplankton community composition was determined using the Haas (1982) protocol. A 10 ml aliquot was taken, placed into a 6% aqueous glutaraldehyde solution to preserve the phytoplankton, and used to make slides for determination of phytoplankton taxonomic group abundances using epifluorescent microscopy. Phytoplankton samples were stained using DAPI, Calcoflour white M2r, and Proflavine and filtered onto a black stained Poretics 0.2  $\mu$ m filter. Slides were viewed using an epifluorescent microscope. Sixty total fields of view per slide were observed and the phytoplankton classified according to taxa and counted. The ratio of dinoflagelates to diatoms was also calculated due the historical importance of this ratio on growth of *Macoma balthica* in Europe (Beukema & Cadee 1991).

### **Long-term water-quality parameters**

Because the duration of this study would only provide a snapshot of water quality based on the three sampling events (tidal stage, storm events, and wind can induce a short-term shift in water quality), we used the Chesapeake Bay Program's long-term monitoring program to supplement our water-quality data. We used 10 years (1/1/1998 to 12/31/2007) of approximately monthly sampling to obtain an accurate estimate of water-

quality parameters near our sites. Due to the proximity of the Chesapeake Bay program monitoring sites to the upriver and downriver sites the monitoring data should be representative of average conditions in our adjacent test areas. Because there is not a monitoring location near the midriver locations, linear interpolation based on river kilometer, was used to estimate the long-term water-quality parameters.

### **Sediment grain size and organic carbon**

At three sites within each of the six test coves, two sediment samples were taken using a 2.1 cm diameter syringe core to a depth of 5 cm. Samples were homogenized and frozen until use. One sample was used to determine particle size classes based on mass using a protocol modified from (Folk 1966). A 5 to 10 g wet-weight subsample was drawn from the homogenized sample and deflocculated in sodium hexametaphosphate for 8 hours. The sample was then wet sieved through a 64  $\mu\text{m}$  sieve to separate the sand and gravel components. The filtrate was used to estimate the silt and clay components using a settling tube pipetting method. The sand and gravel components were dried for 48 hours at 60°C and dry sieved through a 2 mm mesh to isolate the components. Individual components were estimated gravimetrically and the total percentage of sand and gravel was calculated to categorize the site.

The second 2.1 cm core was used for estimating the organic carbon by loss on ignition based on a protocol modified from (Konen et al. 2002). A 5 to 10 g wet weight subsample was drawn from the homogenized sample and dried for 72 hours at 60°C or

until the mass stabilized. The samples were then incinerated in a muffle furnace at 650°C for 8 hours and weighed again. The percent organic carbon was estimated by subtraction.

At the same sites, a 1.2 cm diameter syringe was used to sample to 1 cm depth for benthic chlorophyll *a* and phaeophytin extraction. The samples were stored frozen in the dark until processing. All samples were processed within 1 month of collection to avoid photopigment decay. Chlorophyll *a* corrected for phaeophytin was determined fluorometrically using protocol modified from Neubauer (2000).

### **Clam Sampling**

*Macoma balthica* was sampled at six sites randomly selected within each of the six sample coves using a 37 cm diameter cylindrical suction core to a depth of 30 cm, using a 3 mm mesh filter bag. To provide enough samples for analysis, the coves were aggregated into three river zones. All samples were stored on ice in the field and frozen upon return to the lab prior to before processing. The core samples were sorted twice to ensure removal of all *M. balthica*. All *M. balthica* collected were counted, and their shell lengths were measured from anterior to posterior at the widest part of the shell to the nearest one mm. When available, subsamples of six intact clams per suction core, ones lacking any cracks or chips in the shell, were selected for biomass estimation. Clams were also removed for biochemical analysis (Chapter 4; Brylawski et al., in prep.). In some cases, clam abundance was insufficient for biomass to be performed for every site. Biomass was calculated based on ash-free dry weights from the loss-on-ignition technique. Clams were oven dried for 72 hours at 60°C weighed and then burned in a muffle furnace for 8 hours at 650°C to remove the organic carbon. From the ash-free dry

weight, condition index (CI<sub>2</sub>) was calculated by dividing the ash free dry mass by the shell length (in mm) as an indicator of overall clam health (*sensu* Wene & Stczynska-Jurewicz 1985).

From the biomass data, length-weight keys were created for each of the three river zones and the river as a whole using a power function fit in SigmaPlot 7 (EQ1).

$$\text{Ash - free dry mass} = \alpha \cdot \text{Clam Length}^{\beta} \quad (1)$$

To provide the best available parameter estimates, we combined the data from this study with individual-based biomass and length values generated in our previous study (Chapter 2; Brylawski et al., in prep.). These length-weight keys were then used to estimate the ash-free dry mass for all of the clams collected in the macrofauna cores in this study.

#### **Data analysis and integration**

In this study, we considered the density, size structure, and condition index as the response variables and other measured variables were considered forcing factors. Both the response variables and forcing factors were analyzed using ANOVA (Minitab 15.2 software) to determine if there were differences among sites. To provide sufficient samples for the ANOVAs, data from two sub coves of each river zone were combined. Assumptions of ANOVA were tested using the Anderson-Darling test for normality and Levene's test for homogeneity of variances. Natural-log transformations were made when necessary to meet the assumptions of ANOVA. No hypothesis tests were performed on the Chesapeake Bay Program long-term water quality data; because linear interpolation was used to estimate the midriver values. In addition, hypothesis tests were



not performed on the chlorophyll *a* or salinity data measured in this study due to the small sample size.

To determine which forcing factor was most responsible for the trends in response variables observed, we utilized an information-theoretic (I-T) modeling approach. We constructed a series of non-linear regression models containing some or all of the potential forcing factors, which were selected based on hypothesized biological significance. Models were fit using least squares regression (Minitab 15.2 statistical package). The regression models were used to create Akaike's information criterion (AIC) scores, which represent a combined statistic for each model that incorporates model parsimony and goodness of fit (Burnham & Anderson 1998). AIC values corrected for small sample size (AICc) were calculated from the residual sum of squares values from the least square regressions using the methods of Anderson (2008). The AICc values were converted into model weights which were used to evaluate the plausibility of each model and, in turn, which of the potential forcing factors is driving the trends observed. Models with  $AIC < 4$  or  $w_i \geq 0.10$  in a model set were considered likely models.

Two separate I-T analyses were run one using the data from the 2007 study only and one using the data for the CI values from all surveys. The I-T analysis using the 2007 data was run using only the observed forcing factors averaged for river zone but with separate estimates for each of the three seasonal surveys. Because this study was only one year long with three collection periods, the potential sample sizes were limited. We predicted that there would be high variation in the indices of *Macoma balthica*'s health that could occlude the long-term trends. Thus the second analysis combined the results of

the current study with those of Brylawski et al. (in prep: Chapter 2) to increase the sample size and allow for greater focus on the effects of the along-axis forcing factor gradient. In the second I-T analysis, the long-term forcing factors as well as the ones observed in this study were used. An overall average value for the three river zones, combining the three individual seasonal sampling events, for the response variable and the forcing factors was used in this analysis to help eliminate short term variation that could occlude long term trends and to allow for a lag time in clam CI. A series of univariate models were run in the second I-T analysis to identify the most important forcing factors affecting *Macoma balthica* health in the mesohaline York River.

We focused on the combined multi-year condition index (CI) as the response variable for determining the forcing factor or factors responsible for the along river-axis trends for two reasons. First as an individual-based metric, CI is also the measure of clam health most robust to the effects of recruitment pulses, variation in predation pressure, and other random events that can reduce clam abundance and biomass (Wene & Stuczynska-Jurewicz 1985). Second, CI best represented the trends of all of the indicators of clam health in the previous study (Chapter 2), including those of non-predatory mortality and growth that were not measured in this work.

## RESULTS

### **Water-quality parameters**

Water-column parameters were estimated and averaged into the three river zones from the individual coves. No hypothesis tests were run for the water-column forcing

factors measured in this study due to the small sample sizes. Salinities followed the expected trend of increasing at the more downriver sites (Table 1). The greatest variation in salinity occurred in the spring, most likely due to the effect of the increase in freshwater flow at that time.

Water-column chlorophyll *a* was highest in the spring sampling event and decreased throughout the year with the upriver sites having greater concentrations than the downriver sites (Table 1). In all sampling events and coves, cyanobacteria dominated the phytoplankton community with dinoflagellates and diatoms also prevalent (Figure 3). In the ratio of dinoflagellates to diatoms, there was a trend of an increasing proportion of dinoflagellates at the more upriver sites, which was most pronounced in the spring and fall sampling events (Figure 4a). When the individual coves are not aggregated, this trend appears to be an exponentially increasing function with distance from the river mouth (Figure 4b).

The long-term water-quality parameters from the Chesapeake Bay Program monitoring sites were interpolated for our sites (Table 2). Of the long-term water-quality parameters, salinity varied the greatest, decreasing 31% (4.18 ppt) between the upriver and downriver sites. Chlorophyll *a* (21%, 2.74 ug/l), and dissolved phosphorous (7%, 0.00197 mg/l) were the next most variable parameters. Temperature (2%, 0.3°C) and dissolved nitrogen (1%, 0.004 mg/l) were the least affected by river zone.

#### **Sediment parameters**

Sediment grain size expressed as the percentage by mass of the sand and gravel portion did not vary significantly among river zones in any of three collection events

(Table 1, spring ANOVA:  $df = 2, 15, F = 0.44, p = 0.649$ ; midsummer ANOVA:  $df = 2, 15, F = 2.15, p = 0.151$ ; fall ANOVA:  $df = 2, 15, F = 1.76, p = 0.206$ ).

Sediment organic carbon was lowest at the downriver sites and highest in the midriver sites in all three sampling events (Table 1). Organic carbon varied significantly among river zones in all sampling periods except for the fall collection period when variability in the upriver zone was high (natural-log-transformed data, spring ANOVA:  $df = 2, 14, F = 7.65, p = 0.006$ ; midsummer ANOVA:  $df = 2, 15, F = 10.59, p = 0.001$ ; fall ANOVA:  $df = 2, 15, F = 1.52, p = 0.250$ ).

Benthic chlorophyll *a* did not vary significantly among river zones in any of three collection events (Table 1; spring ANOVA:  $df = 2, 15, F = 1.07, p = 0.368$ ; midsummer ANOVA:  $df = 2, 15, F = 2.19, p = 0.146$ ; fall natural-log-transformed ANOVA:  $df = 2, 15, F = 2.60, p = 0.107$ ). However, in the spring and midsummer sampling events there was a trend of decreased benthic chlorophyll *a* at the upriver sites.

#### ***Macoma balthica* density, average size, and health**

In each section of the river, the *Macoma balthica* population densities were high due to recruitment in the spring and declined throughout the midsummer and into the fall (Figure 5). There was no difference among river zones in the spring; however, there was a significant difference among river zones in both the midsummer and fall (ANOVAs for Natural-log-transformed data: spring  $df = 2, 33, F = 0.24, p = 0.785$ ; midsummer  $df = 2, 33, F = 4.58, p = 0.018$ ; fall  $df = 2, 33, F = 8.09, p = 0.001$ ). After the initial large spring recruitment, mortality at the upriver sites was greater than that downriver, leading to significantly lower densities upriver in the midsummer and fall. The midriver locations

experienced substantially greater mortality and had the lowest densities in the later collection events.

As with density, clam size among the three river zones did not differ significantly in the spring, but did differ significantly in the midsummer and fall (Natural-log-transformed data, spring ANOVA:  $df = 2, 708, F = 3.81, p = 0.023$ ; midsummer ANOVA  $df = 2, 346, F = 14.76, p < 0.001$ ; fall ANOVA:  $df = 2, 33, F = 17.80, p < 0.0001$ ). The average clam size increased throughout the year for the midriver and downriver zones but decreased for the upriver zone (Figure 6).

Condition index (CI) is a common body-mass-based proxy for overall animal health. In this study, 391 clams were used to estimate CI. The pattern of decreasing CI at the upriver coves did not hold true for all sampling events. In the spring sampling event there was not a significant difference in CI observed among the river zones (Figure 7a, spring ANOVA:  $df = 2, 169, F = 0.29, p = 0.751$ ). A significant difference among river zones was detected in the midsummer and fall, with CI lower in the upriver sites (Figure 7a; midsummer ANOVA:  $df = 2, 121, F = 5.46, p = 0.005$ ; fall ANOVA:  $df = 2, 70, F = 8.05, p = 0.001$ ). When individual coves are considered, there is a trend of decreasing condition at the more upriver sites (Figure 7b). When combined with the data collected from Brylawski et al. (in prep.; Chapter 2) to yield 958 total estimates of CI, the trend of decreasing condition at the more upriver sites becomes more obvious (Figure 8).

The same information used to estimate CI was used to fit a length-weight key for each river zone (Table 3). These length-weight keys were combined with the measures of ambient clam density and size to estimate the biomass of *Macoma balthica* in each sample. Biomass per core was highest at the downriver sites and decreased moving up the

river axis; however, the river zones are only significantly different from each other in the fall collection period (Figure 9; Square-root-transformed data, spring ANOVA:  $df = 2, 33, F = 0.46, p = 0.635$ ; midsummer ANOVA:  $df = 2, 33, F = 3.98, p = 0.03$ ; fall ANOVA:  $df = 2, 33, F = 8.4, p = 0.001$ ).

### **Data integration and forcing factors determination**

As with our previous study (Brylawski et al., in prep; Chapter 2), there was a general trend of decreasing indicators of health at the upriver sites (Table 4). Some of the potential forcing factors observed in this study also had similar along-river-axis patterns as the clam health indices (Table 4). From a qualitative examination of the potential forcing factors, salinity and the indices of food availability (e.g., Chlorophyll *a*, phytoplankton, nutrient compositions that may drive food trends) appear to be most responsible for driving the trends in clam health because the along-axis patterns correlate well.

From this qualitative examination, a series of models was created, based on the most probable factors contributing to clam density and health (CI), for use in the I-T analysis. I-T analysis using the 2007 survey data and the seasonalized forcing factor estimates did not yield conclusive results. The model that best fit the CI data combined all parameters except for water column chlorophyll *a* (Table 5). The second most probable model was comprised of all parameters tested. Due to the large number of parameters identified in the best fitting models it is impossible to determine the forcing factor that most influences clam health. The inability of the seasonalized I-T analysis to identify a dominant forcing factor is most likely due to combined effect of short-term

variation in forcing factors and a potential a lag effect between a forcing factor and clam condition. The response of *M. balthica* to stressors is most likely not instantaneous leading to a miscorrelation between the forcing factors and clam condition observed in the snapshot studies.

Our second I-T analysis, using mean annual values for forcing factors and condition index, evened out the short-term variation and the potential for lagged effects. In this analysis, only univariate regressions were run on the observed and long-term forcing factors and AIC analysis was used to estimate the most important parameter affecting clam condition index. Though all parameters are likely to have some influence on clam health, restricting the analysis to univariate models allowed us to identify which of the potential forcing factors was most responsible for the patterns in CI.

The results of the univariate I-T analysis indicated that the observed ratio of dinoflagellates to diatoms was the model that best explained the natural-log-transformed condition index data, followed by the observed water column chlorophyll *a* and grain size (Table 6). From the model probabilities, the ratio of dinoflagellates to diatoms was eight times more likely to explain the clam condition index than any other factor. There appears to be a strong negative correlation between the proportion of dinoflagellates and clam condition index (Figure 10).

## DISCUSSION

The goals of this work were to identify the forcing factors responsible for trends in the density, biomass, and health of *Macoma balthica* in a tributary near their southern range limit. In this follow-up to our previous three-year-long study, we were able to detect similar trends of worsening conditions at the upriver sites for the indicator

variables of biomass, clam size, and condition index. We hypothesized that bottom-up control (i.e., the composition of the primary-producer community) was most likely the principal forcing factor responsible for the patterns of clam health in the York River (add reference for bottom-up control).

Not all of the indicator variables displayed significant differences among sites at each sampling period due to low sample size, high variation, seasonal events (spawning), and annual variations. The variation among sampling periods seemed to be driven by the large spring recruitment, which increased the densities in the coves and produced greater numbers of recruits in the upriver coves (Figure 5, spring). This recruitment pulse also disrupted the river-zone patterns of clam size and condition index, and to some extent biomass (Figures 6, 7, and 9). By the midsummer and fall collections, the recruitment pulse seems to have been reduced by predation and other sources of mortality, creating the along-axis pattern seen in the combined dataset from the mark-and-recapture study.

Due to seasonal variation, the seasonalized Information Theoretic (I-T) analysis of forcing factor importance yielded a combination of many factors contributing to clam condition index (CI). To overcome the problems of seasonality, we elected to combine the datasets from this study with our previous study (Chapter 2; Brylawski et al. in prep.) for use in a second I-T analysis. This increased sample sizes and helped overcome the effects of individual clam variations, seasonality, and lag in response time of CI to environmental factors. From the second I-T analysis, the model including the observed ratio of dinoflagellates to diatoms was the factor that best explained clam CI (probability of 0.79). Areas with a greater proportion of diatoms to dinoflagellates had higher overall condition indices. Additionally, models with water-column chlorophyll *a* and grain size



were the next best predictors of clam health, though models with these factors had low weights (near 0.10), indicating that they eight times less likely than the diatom to dinoflagellate ratio to explain the CI data..

Interestingly, this analysis did not rank salinity as an important forcing factor though it has been well documented that salinity does affect *M. balthica*'s health, growth, and abundance (Gilbert 1973, King et al. 2005). Salinity may not have emerged as an important factor in this study because all sites were within the mesohaline section of the river, well within the range of common occurrence in the tributaries of the Chesapeake Bay (McErlean 1964, Diaz & Schaffner 1990, King et al. 2005). The long-term average salinities varied only by 4.2 ppt between the most upriver and downriver sites, which may not be significant enough to affect the health of the clams. Rather, the analyses show the importance of food availability on clam health and suggest that variation in nutrient concentrations disrupt the food web through a shift in the primary producer community to less-palatable food resources.

In the York River, the concentration of phytonutrients is highest upriver and decreases moving downriver a common trend in riverine estuaries (Table 2; Ouboter et al. 1998, Boesch 2002). The increased nutrients increase overall primary production which should be represented in an increase in growth and health of consumers; however, this is not the case in the York River. In our study, areas of highest primary production, as represented by high chlorophyll *a* values, are the areas with the lowest clam health. This paradox appears to be driven by the composition of the primary producer community.

With increased nutrient concentration, more opportunistic types of primary producers flourish (Nixon 1995). Phytoplankton, namely dinoflagellates and

cyanobacteria, are opportunistic and can take advantage of the increased nutrient conditions, forming large aggregations and outcompeting slower-growing species evolved for lower nutrient concentrations. These more-opportunistic species shade out the benthic macrophytes and microphytobenthos and replace the large diatoms cells (Cloern 2001).

We observed this replacement of diatoms by dinoflagellates in our study. At the higher-nutrient upriver sites, there was reduced benthic chlorophyll *a* during the spring and midsummer collections. The more upriver sites had greater proportions of dinoflagellates compared to the downriver sites, and when individual coves are examined, there is a clear pattern of decreasing dinoflagellates compared to diatoms moving downriver to the less nutrient-rich sites (Figure 4). On average, dinoflagellates do not provide as much nutrition for benthic animals such as *Macoma balthica*, as do the benthic microalgae and pelagic diatoms (Cares et al. 1998, Marsh et al. 1989, Wacker et al 2002, Pond et al. 1998). The primary-producer community we see at the nutrient-rich upriver sites is most likely acting as a lower-quality food for the clams, resulting in the lower clam growth, health, and predation-independent survival.

The relationship between *Macoma balthica* growth and food quality has been examined in the Wadden Sea (Beukema & Cadee 1991) where there is also a gradient of nutrient concentrations and shifts in primary producer communities due to the effects of cultural eutrophication. There was a positive relationship between the relative concentration of diatoms, compared to the rest of primary producer community, and clam growth (Beukema & Cadee 1991). The clams could not take advantage of increased

production in the more eutrophic sites because the food source was comprised of species other than diatoms.

Beukema & Cadee (1991) suggested that *Macoma balthica* grew better on a diet of diatoms due to size selection, but we believe that the relationship between diatom concentration and clam health and growth is due to the micronutrient composition (Amino Acids, Fatty Acids, Nucleic Acids) of the diatoms relative to that of the more opportunistic taxa. Diets lacking in essential micronutrients can reduce growth and production of organisms even if there is more than adequate calories provided (Sargent et al. 1990, Sterner & Schultz 1998). Essential micronutrients, such as vitamins or essentially fatty acids (EFAs), are molecules that metazoans cannot *de novo* synthesize in sufficient quantities to satisfy metabolic requirements. Limitations in the availability of essential micronutrients can limit consumer production even if macronutrients are in sufficient supply (Sargent et al. 1999, Hendricks et al. 2003). Although a lack of micronutrient can reduce growth, EFAs are the most commonly limiting nutrient in aquatic food webs (Muller-Navarra et al. 2000, Brett & Muller-Navarra 1997).

The EFAs are usually long chain (18+ carbons) polyunsaturated fatty acids (PUFAs) such as the  $\omega$ 3 fatty acids, which, in metazoans, are important membrane components that are needed for neural development (Sargent et al. 1999). In general, phytoplanktonic primary producers contain few EFAs compared to benthic producers which are less common in the more nutrient-rich sites (Pond et al. 1998). In addition, the concentration of EFAs is related to the type of phytoplankton. Diatoms are very rich in PUFAs with greater amounts of Eicosapentaenoic acid (EPA). Dinoflagellates have lower

over all PUFAs and more saturated fats. Their PUFAs have greater concentrations of docosahexaenoic acid than EPA (Pond et al. 1998, St. John et al. 2001).

*Macoma balthica* condition can correlate strongly with the concentration of PUFAs, especially EPA and DHA. Clams with higher ratios of EPA: DHA also have greater condition index (Jarbeski et al 1986). Thus, we believe that the nutrient-induced shift in primary producer community from one dominated by diatoms to one comprised primarily of dinoflagellates may be affecting *M. balthica*'s health and survival through the availability and ratios of EFAs. The clams at the upriver zone appear to be eating "junk food" phytoplankton (lacking the necessary EFAs) which may explain the strong relationship we detected between primary-producer community and clam health. To test for this link, in a subsequent study we are examining the lipid composition of clams collected in this study.

In this study, along with the preceding mark-and-recapture study, we have observed patterns of decreasing growth, health, and predation-independent survival which appears to be dependent on the composition of the primary producer community, which is historically controlled by the nutrient inputs. It is hypothesized that this may be one of the reasons why increased production from nutrient enrichments does not move up the food chain as energetic theory would suggest (Kemp et al. 2001, Grall & Chauvaud 2002, Sterner & Elser 2002).

Though the relationship between primary producer community composition and consumer health was observed along a nutrient gradient within a single river, similar shifts can occur through an estuary due to the effects of cultural eutrophication increasing the nutrient concentrations and shifting the ecosystem toward the disrupted state. With

the increase in the scale and intensity of cultural eutrophication, this disruption of the food web may have great negative ramifications for ecosystem health.

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Table 1. Mean observed forcing factor measurements collected during the three surveys from the three river zones. Values are means  $\pm$  one standard error. DR = downriver, MR = midriver, and UR = upriver.

Parameter	Spring			Midsummer			Fall		
	DR	MR	UR	DR	MR	UR	DR	MR	UR
Water column chlorophyll-a (mg/l)	13.22 $\pm$ 6.8	27.19 $\pm$ 15.37	23.57 $\pm$ 5.6	11.09 $\pm$ 4.2	5.5 $\pm$ 0.06	14.97 $\pm$ 1.11	3.53 $\pm$ 0.17	4.32 $\pm$ 1.7	8.87 $\pm$ 0.13
Water column salinity (ppt)	16.5 $\pm$ 0.5	14.75 $\pm$ 0.25	11.25 $\pm$ 1.25	19.75 $\pm$ 2.25	17.5 $\pm$ 0.5	18.75 $\pm$ 1.25	22.5 $\pm$ 0.5	21 $\pm$ 1	18.5 $\pm$ 1.5
Sediment % sand and gravel	27.22 $\pm$ 6.30	17.97 $\pm$ 3.19	24.28 $\pm$ 10.0	35.83 $\pm$ 6.42	21.04 $\pm$ 5.33	38.31 $\pm$ 7.20	34.86 $\pm$ 9.83	15.74 $\pm$ 2.60	41.64 $\pm$ 14.31
Sediment % organic carbon	4.81 $\pm$ 0.35	9.39 $\pm$ 0.50	6.83 $\pm$ 1.25	4.87 $\pm$ 0.49	9.06 $\pm$ 0.35	7.37 $\pm$ 0.82	4.90 $\pm$ 0.65	8.35 $\pm$ 0.31	6.83 $\pm$ 1.61
Sediment chlorophyll a (ug/cm <sup>2</sup> )	4.45 $\pm$ 0.68	3.94 $\pm$ 0.32	2.91 $\pm$ 1.07	6.08 $\pm$ 1.14	4.58 $\pm$ 0.51	3.64 $\pm$ 0.70	2.52 $\pm$ 0.22	6.42 $\pm$ 1.85	3.25 $\pm$ 0.93

Table 2. Water-quality parameter estimates ( $\pm 1$  standard error) from 10 years of Chesapeake Bay program monitoring data (1/1/1998-12/31/2007, LE 4.1 and LE 4.2) for the upriver and downriver sites. Estimates for the midriver test position have been estimated using linear interpolation based on river kilometer.

Parameter	Location			Units
	upriver / LE 4.1	midriver	downriver / LE 4.2	
Chlorophyll <i>a</i>	13.326 $\pm$ 8.452	11.654	10.590 $\pm$ 10.182	UG/L
Salinity	13.436 $\pm$ 4.206	15.060	17.613 $\pm$ 3.762	PPT
Temperature	16.610 $\pm$ 0.781	16.493	16.31 $\pm$ 0.756	$^{\circ}$ C
Total Nitrogen	0.687 $\pm$ 0.223	0.660	0.654 $\pm$ 0.071	MG/L
Total Phosphorous	0.078 $\pm$ 0.004	0.069	0.064 $\pm$ 0.006	MG/L

Table 3. Length-weight model parameters calculated for *Macoma balthica* for the York River for the three zones. Data from our previous study (Brylawski et al. in prep.; Chapter 2) was combined with this study to provide the most accurate estimates available.

River Position	a	B
DR	$3.4975 \times 10^{-6} \pm 1.5082 \times 10^{-6}$	$3.2653 \pm 0.1304$
MR	$2.7853 \times 10^{-7} \pm 1.8328 \times 10^{-7}$	$4.1109 \pm 0.1365$
UR	$4.1178 \times 10^{-7} \pm 3.2545 \times 10^{-7}$	$3.9825 \pm 0.3577$

Table 4. Combined results of hypothesis tests and examination for along-axis trends for the indicator variables and potential forcing factors observed in this study. Parameters with a “yes” in significant among sites were determined by ANOVA at an  $\alpha = 0.05$ . River-axis gradient results are from visual observation of trends from the graphical representations. “Down” indicates that the parameter is greater downriver and decreases upriver. “Up” indicates that the variable is greater upriver and decreases as some function moving downriver. “Marginal” indicates that a trend appears to be present but two of the river zones are grouped too closely for clear identification of a along axis trend. “None” in the river axis gradient column mean that no trend clear trend was discernable.

Parameter	spring / long term		midsummer		fall	
	Significant among sites	River axis gradient	Significant among sites	River axis gradient	Significant among sites	River axis gradient
Clam density	No	None	Yes	None	Yes	None
Clam size	No	Down	Yes	Down	Yes	Marginal
Clam Condition	No	None	Yes	Down	Yes	Down
Clam Biomass	No	Down	Yes	Down	Yes	Down
Sediment sand %	No	None	No	None	No	None
Sediment carbon %	Yes	None	Yes	None	No	None
Benthic Chl a	No	Down	No	Down	No	None
Water column Chl a	Not tested	None	Not tested	None	Not tested	Down
Dinoflagellates:						
Diatoms ratio	Not tested	Down	Not tested	Down	Not tested	Down
Observed salinity	Not tested	Down	Not tested	No	Not tested	Down
10-year Chl a	Not tested	Up				
10-year salinity	Not tested	Down				
10-year temperature	Not tested	Up				
10-year Nitrogen	Not tested	Up				
10-year Phosphorus	Not tested	Up				

Table 5. Results from the seasonal I-T analysis of the effect of the observed forcing factors on the condition index of *Macoma balthica*. Only the 10 most probable models are results are reported below. WC = water-column chlorophyll *a*, SC = sediment chlorophyll *a*, OC = sediment % organic carbon, GS sediment grain size (% sand and gravel), DD = dinoflagellate : diatom ratio, SL = observed salinity. Models are arranged from best to worst and models with bold are considered the most viable models in the set.

Parameters	AIC	$\Delta$ AIC	Model Probability
<b>GS,WC,SC,OC,DD,SL</b>	<b>2175.08</b>	<b>0.00</b>	<b>0.643</b>
<b>GS,SC,OC,DD,SL</b>	<b>2176.78</b>	<b>1.70</b>	<b>0.274</b>
GA,WC,DD,SL	2180.10	5.02	0.052
GS,OC,DD,SL	2182.07	6.99	0.019
GS,WC,SC,OC,SL	2184.30	9.23	0.006
GS,WC,SC,OC,DD	2185.94	10.86	0.003
GS,OC	2186.47	11.39	0.002
GS,WC,OC,SL	2217.81	42.74	3.37E-10
GS,WC,SC,DD,SL	2218.05	42.97	3E-10
GS,WC	2219.21	44.13	1.68E-10

Table 6. Results from the AIC-based information-theoretic analysis of least-squares regression models of *Macoma balthica*'s condition index, as observed in all surveys, as a function of the observed forcing factors and the 10-year aggregated water-quality information. Models are arranged from best to worst and the model with bold is considered the most viable model in the set.

Parameter	AIC	$\Delta$ AIC	Model Probability
<b>Observed Dinoflagellate :Diatom Ratio</b>	<b>6558.56</b>	<b>0.00</b>	<b>0.7916</b>
<b>Observed Water Column Chlorophyll a</b>	<b>6562.73</b>	<b>4.17</b>	<b>0.0986</b>
<b>Observed Grain Size</b>	<b>6562.73</b>	<b>4.17</b>	<b>0.0986</b>
10-year Water Column Chlorophyll a	6570.08	11.52	0.0025
10-year Total Phosphorus	6570.08	11.52	0.0025
10-year Salinity	6570.08	11.52	0.0025
10-year Total Nitrogen	6570.30	11.74	0.0022
Observed Salinity	6571.19	12.63	1.44E-03
Observed Sediment Chlorophyll a	6598.14	39.58	2.01E-09
Observed Sediment Organic Carbon	6677.68	119.12	1.07E-26

Figure 1. Condition index of ambient *Macoma balthica* from four coves in the mesohaline section of the York River, VA, as a function of distance from the river mouth. Based on 958 clams collected from 2005 - 2007 from Brylawski et al. (in preparation / Chapter 2). "SE" = standard error of the mean.

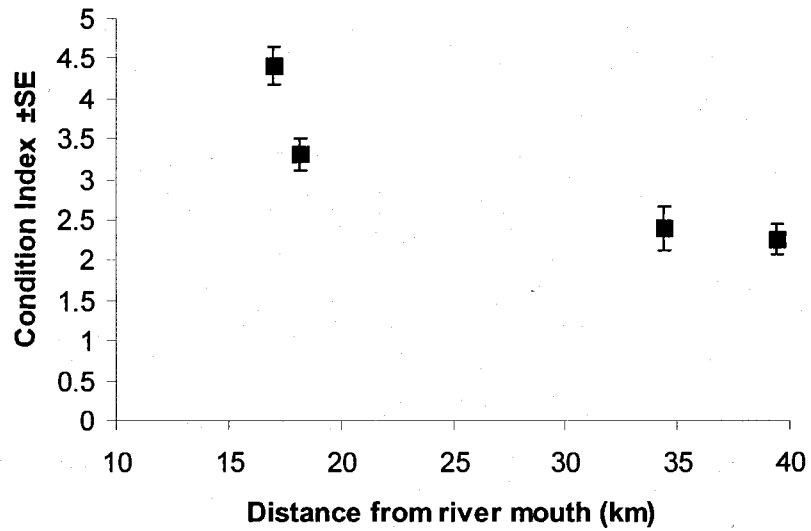




Figure 2. The locations of the six test covers used in this study along with the Chesapeake Bay Program long-term monitoring stations (CBP LE 4.1, CBP LE 4.2).

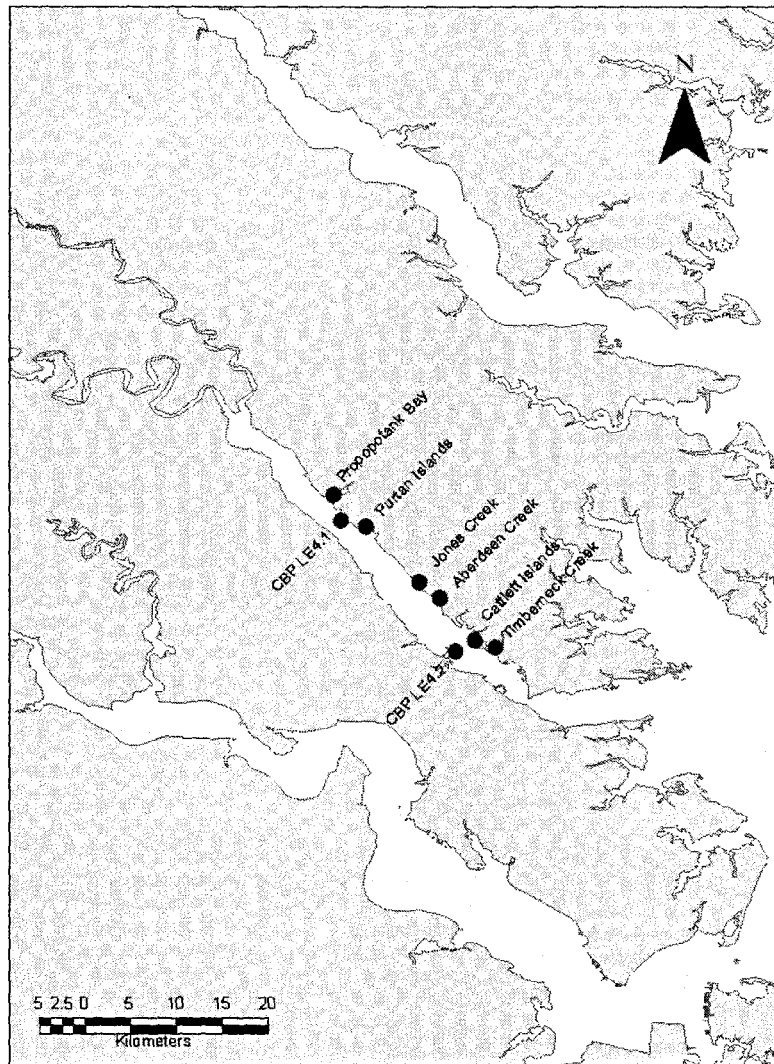


Figure 3. (a) Water column phytoplankton composition from the three river zones in the mesohaline section of the York River, VA, from the three sampling events. (b) Phytoplankton composition with the cyanobacteria removed.

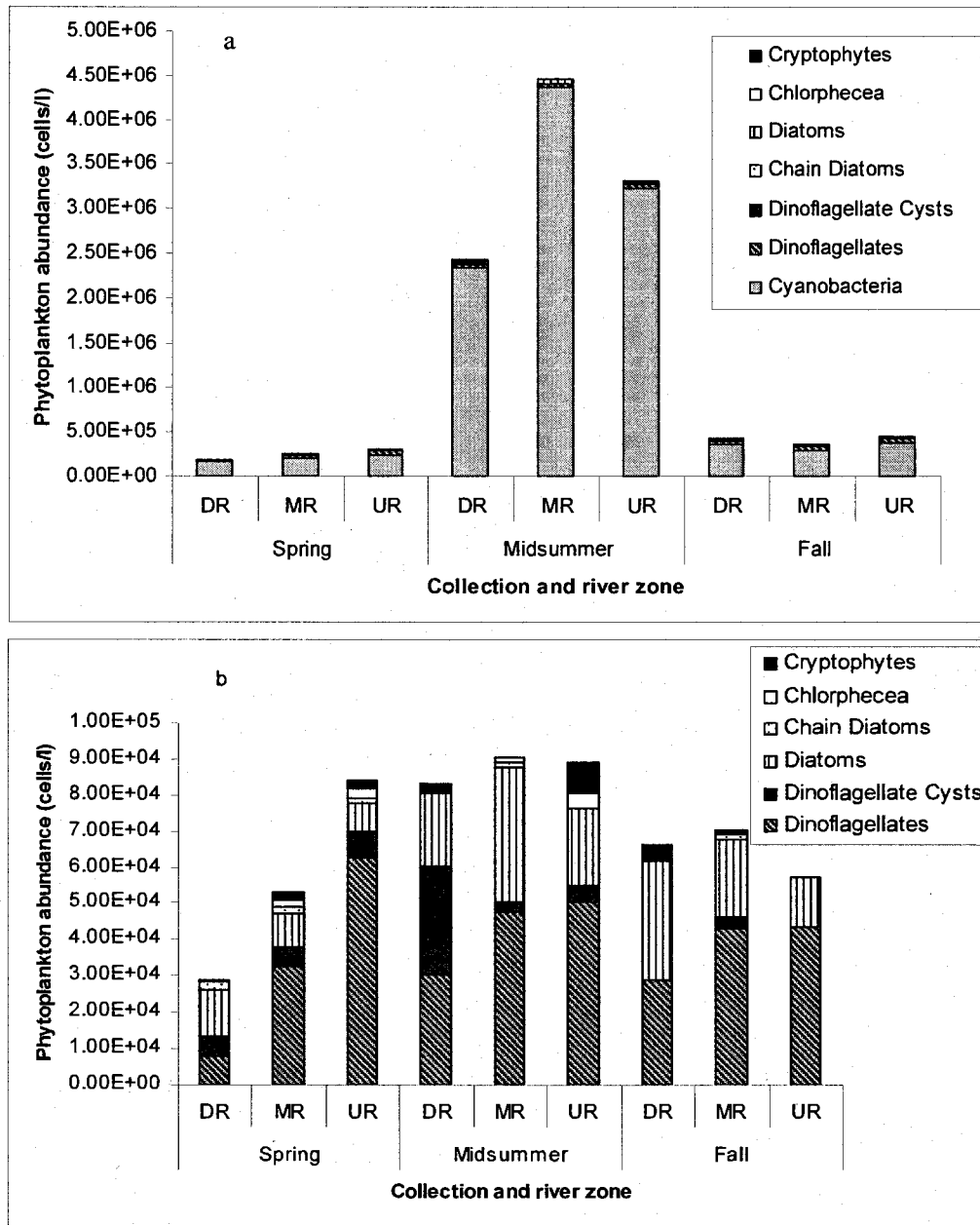


Figure 4. Proportion of Dinoflagellates to Diatom diatoms in the water column of the six test coves aggregated into the three river zones in the mesohaline section of the York River, VA for the three sampling events (a). The proportion of Dinoflagellates to Diatom diatoms in the six test coves as a function of distance from the river mouth with an exponential trend-line overlaid (b).

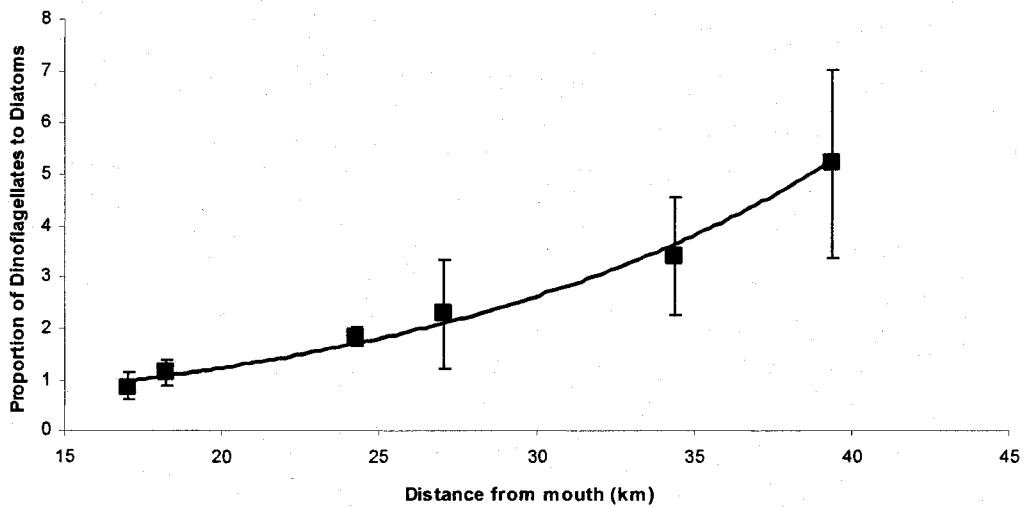
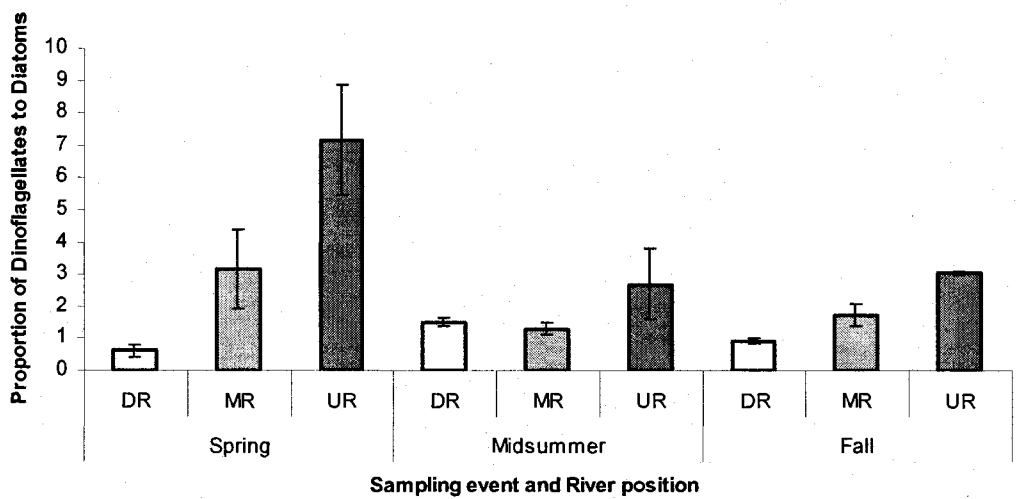


Figure 5. Density of *Macoma balthica* from the three river zones in the mesohaline section of the York River, VA for three sampling events in 2007. "SE" = standard error of the mean.

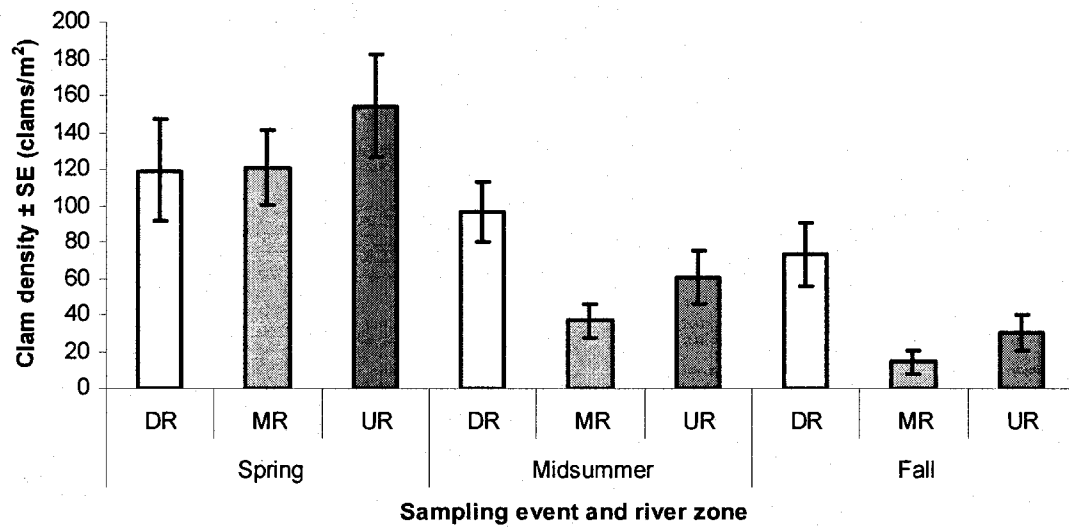


Figure 6. (a) Average size of *Macoma balthica* aggregated into the three river zones and sampling events. (b) Average size of *Macoma balthica* in each of the six individual coves. "SE" = standard error of the mean.

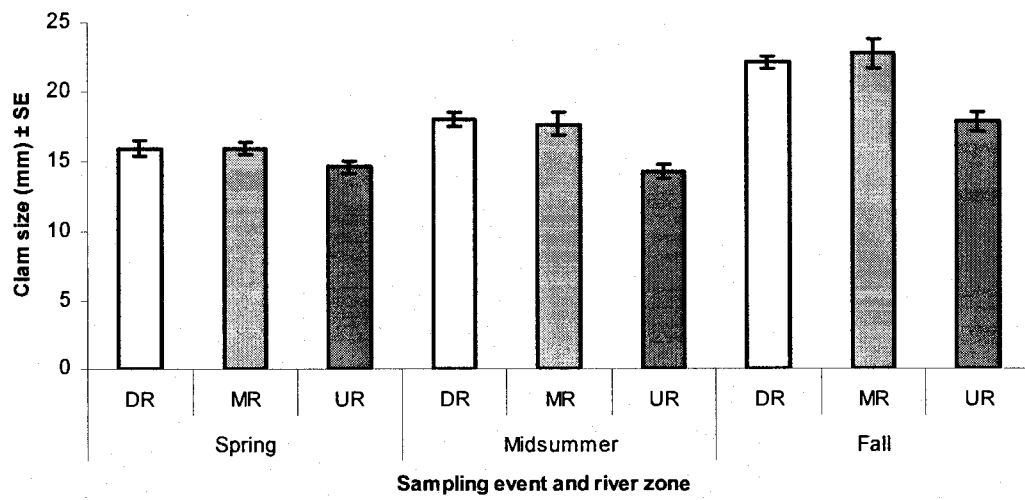


Figure 7. (a) Condition index of *Macoma balthica* aggregated into the three test river zones and sampling events. (b) Condition index of *Macoma balthica* in each of the six individual test covers. “ND” indicates that no data was procured due to insufficient numbers of clams collected. “SE” = standard error of the mean.

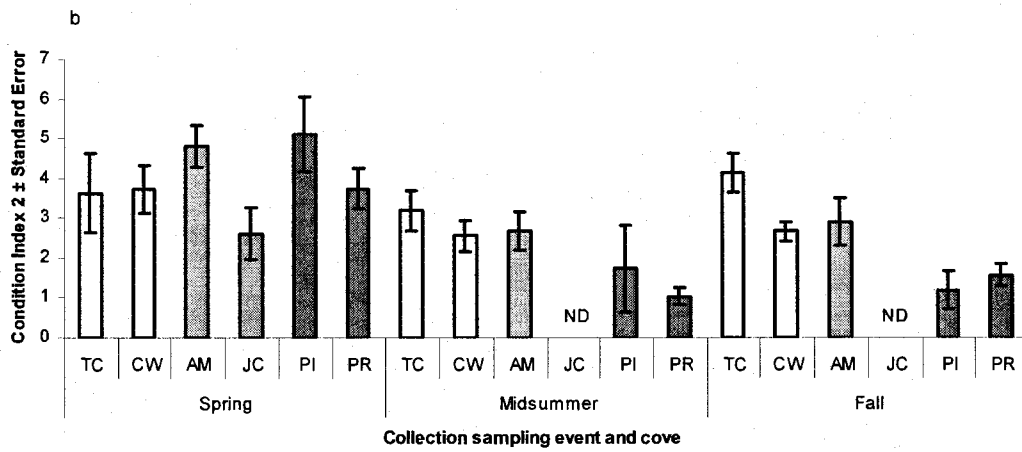
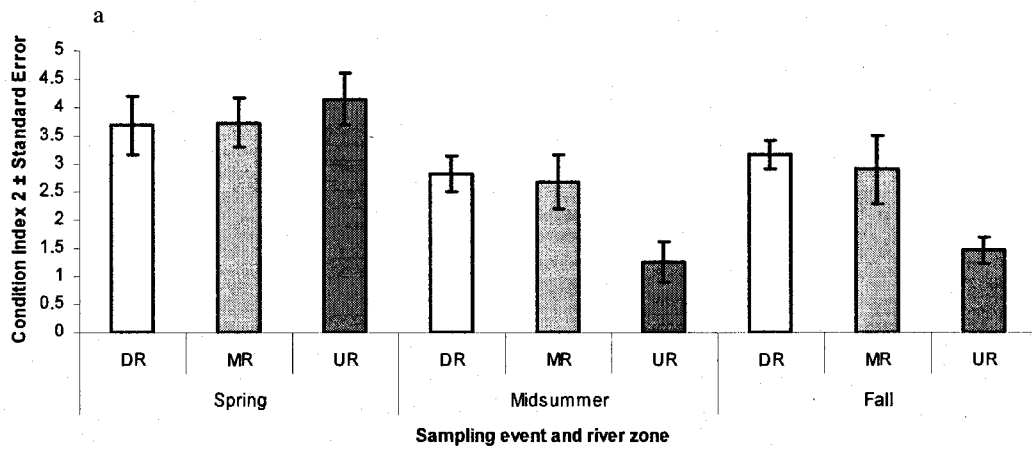


Figure 8. *Macoma balthica* condition index as a function of distance from the mouth of the York River. Black squares represent the average for the individual test covers. Grey circles are the average values for data pooled by river position. Data from this study and previous mark and recapture trials (Brylawski et. al in prep.: Chapter 2) are represented. "SE" = standard error of the mean.

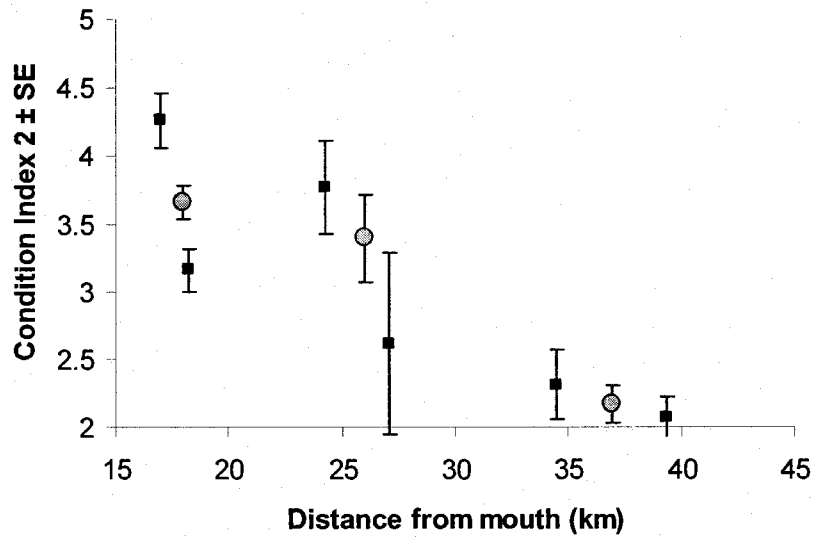


Figure 9. Average estimated biomass of *Macoma balthica* per core for the three test river zones and sampling events. Biomass was estimated by combining the specific length-weight key for each river position with the measured sizes of individual clams collected in core samples. "SE" = standard error of the mean.

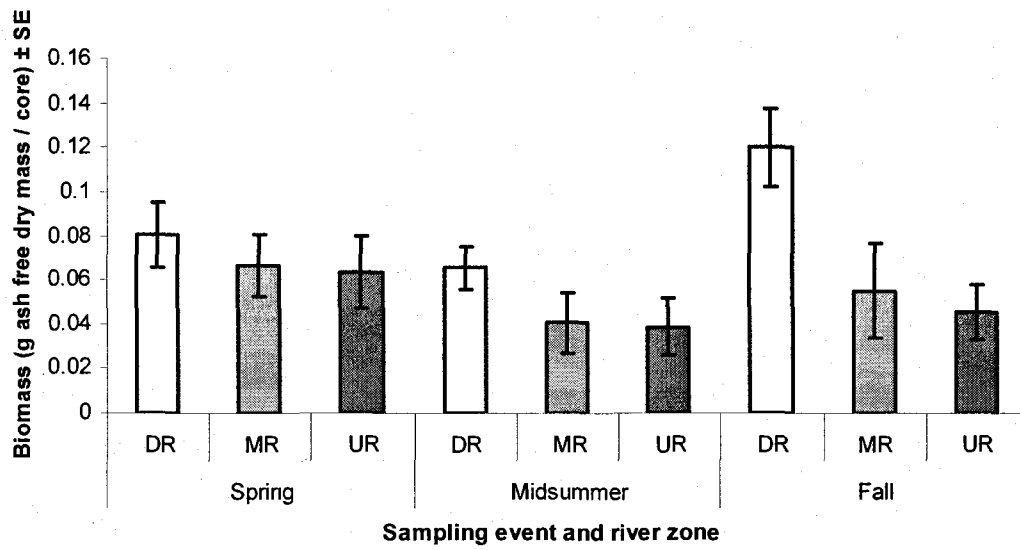
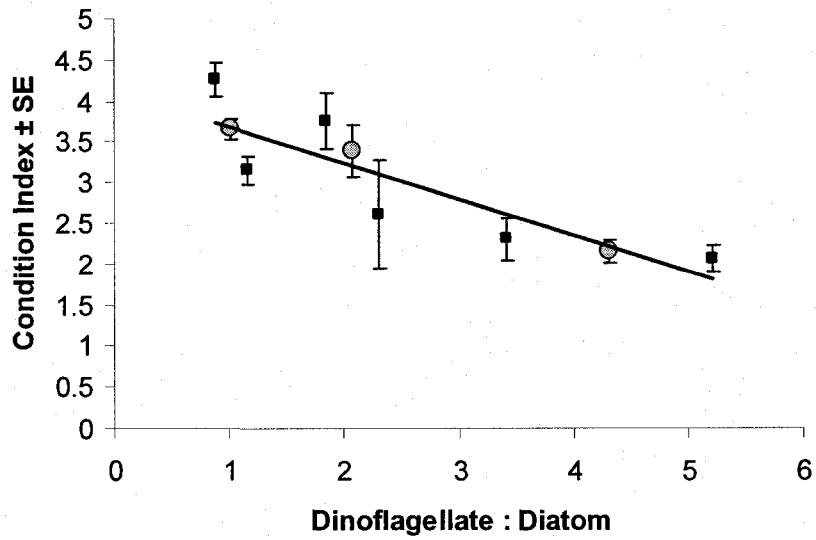




Figure 10. Average condition index of *Macoma balthica*, from all surveys, as a function of average Dinoflagellate: Diatom ratio. Black squares are for individual coves, grey circles are averages for three river zones. The trendline is for the individual coves data. "SE" = standard error of the mean.



## **Chapter 4.**

Variations in the fatty composition of *Macoma balthica* along an estuarine gradient in a  
Chesapeake Bay subestuary

## ABSTRACT

The Baltic macoma, *Macoma balthica*, is a biomass dominant clam which is an important forage species for several commercially exploited species in the Chesapeake Bay. The fatty acid composition for a southern Chesapeake Bay population of *M. balthica*, was determined for six locations spaced along an estuarine gradient at three times during the spring to fall growing season. Previously we observed a reduction in the condition index of clams coinciding with a shift in the primary producer community from domination by diatoms to dinoflagellates. This work attempted to determine if the fatty acid composition of the clams altered with the shift in the phytoplanktonic community. Total fatty acid concentration did not significantly vary with the season or among the test sites. The dominant fatty acid components were 16:0 Palmitic acid, 16:1 Palmitoleic acid, and 20:5 (n-3), eicosapentaenoic acid (EPA). There was a slight trend of increasing EPA and decreasing 22:6 (n-3), docosahexaenoic acid (DHA) moving downriver among test locations. A combined EPA/DHA ratio correlated strongly with variations in condition index, indicating the potential for stoichiometry of (n-3) fatty acids to play a role in regulating clam health.

## INTRODUCTION:

The Baltic macoma (*Macoma balthica*) is a thin shelled clam common to the estuaries of the northern hemisphere ranging from ~70 °N to ~45 °N (Kamermans et al. 1999). Though the Chesapeake Bay (~37°N) is near the southern extent of *M. balthica*'s range, the clams are highly abundant and are considered a biomass dominant of the benthic community (Holland et al. 1987, Seitz et al. 2006). Though this clam is not directly consumed by humans, it is an important forage species for several commercially exploited species such as the blue crab (*Callinectes sapidus*) and striped bass (*Morone saxatilis*) (Mansour 1992, Hines et al. 1999). It is also considered a key benthopelagic coupler, linking the detrital and primary producer pools with higher trophic levels (Baird & Ulanowicz 1990).

Due to its importance in the Chesapeake Bay trophic network there is interest in understanding its growth and life history for use in ecosystem modeling and management. In a previous study a series of studies examining the growth and condition of *M. balthica* in shallow water coves in the mesohaline section of the York River, VA, a sub-estuary of the Chesapeake Bay was performed (Chapter 2). Three year-long mark and recapture studies, were run in four coves located near the upper and lower extent of the mesohaline section of the river. Though the coves were selected to have similar biotic and abiotic conditions, such as sediment type, marsh edging, and tidal influence, a significant shift in indices of clam health and life history parameters was observed. We measured significantly lower growth rates, and greater non-predatory mortality, at the two locations located in the more upriver areas of the mesohaline region of the river. The

condition index of the clams (a measure of individual based biomass compared to shell width) was also significantly reduced at the upriver sites and increased moving downriver between coves. Due to the observed patterns in the clam parameters, we hypothesized that these trends were created by a forcing factor that altered as a gradient along the river axis. We hypothesized that either the effects of salinity, or eutrophication induced shifts in the primary producer community were most likely responsible for the patterns in clam health.

A follow-up field survey of clam condition index was performed in six shallow-water coves along the mesohaline reaches of the river in 2007 to confirm the results of the previous study and to determine the most important forcing factor controlling clam health in the mesohaline York River (Chapter 3). The four sites from the mark and recapture study were retained and an additional two sites were added to better explore the along river axis effects. The sites were grouped into three river zones (upriver, midriver, and downriver) consisting of two test coves. A suite of potential forcing factors were examined from long-term monitoring datasets to determine which stressors most affected clam health. The follow up study confirmed the pattern of decreasing clam health along the gradient moving upriver between the test coves along the river axis. Using an information-theoretic analysis of potential forcing factors, we identified that the composition of the primary producer community was the factor that was most closely related to clam health. The health of clams was determined to be dependant on the ratio of diatoms to dinoflagellates present in the phytoplanktonic community. With upriver areas that were dominated by dinoflagellates having significantly lower clam condition

than more downriver areas which have a greater proportions of diatoms, even though the upriver sites had a greater amount of total primary production.

In the present work we examine the local variation in fatty acid composition of *Macoma balthica* in six shallow-water coves spaced throughout the mesohaline section of the York River, VA, for three collection periods during the growing season. We sought to determine if this relationship between clam health and the composition of the primary producer community could be detected in the fatty acid profile of *M. balthica* collected in the 2007 survey. By examining the fatty acid composition of clams for telltale lipid biomarkers we were able to examine if *M. balthica* was capable of selectively feeding on diatoms that have been observed to promote better growth and health (Chapter 3, Beukema & Cadee 1991). Additionally, we hypothesized that through an examination of the clam fatty acid profile we may be able to determine if the clam's health is reduced due to essential fatty acid limitation. Since lipid limitation may act as a bottom-up control on the production and limit the trophic transfer efficiency could have broad reaching implications for the total ecosystem health, due to the key position of *M. balthica* in the Chesapeake Bay trophic network (Baird & Ulanowicz 1990, Brett & Muller-Navarra 1997).

#### METHODOLOGY:

##### ***Macoma balthica* collection**

Clams were collected from six shallow-water muddy coves in the mesohaline section of the York River as part of the forcing factor determination study (Chapter 3, Figure 1). These test coves were grouped into three river zones: (1) upriver (Poropotank

Bay [PR, 39.39 km from mouth] and the Purtan Islands [PI, 34.44 km from mouth]), (2) midriver (Jones Creek [JC, 27.08 km from mouth] and Aberdeen Creek [AM, 24.25 km from mouth]), and (3) downriver (the Cattlett Islands [CT, 18.21 km from mouth] and Timberneck Creek [TC, 17.01 km from mouth]) for some analyses. The two coves in each river zone were adjacent coves spaced no farther apart than 5 km. These six coves were sampled in the Spring (5/22/2007-5/24/2007), Midsummer (7/24/2007 – 8/1/2007), and Fall (10/29/2007 – 11/30/2007) to explore the effects of seasonality. The coves were selected to have similar abiotic and biotic conditions. The major variations between the coves was salinity (varied between 1 – 5.25 ppt between the most upriver and downriver sites depending on season), and the composition of the primary producer community (Diatom and cyanobacteria dominated downriver, and dinoflagellate and cyanobacteria dominated upriver, Chapter 3).

Clams were sampled at six locations within each cove using a 37-cm-diameter cylindrical suction core to a depth of 30 cm and filtered a 3-mm-mesh collection bag. Samples were immediately submerged in ice and frozen at -20°C upon return to lab. Within one week of collection, the samples were sorted and, when available, three clams of 20 – 30 mm length were removed from each core sample, creating a pool of 18 clams from each cove available for biochemical analysis. Due to low clam abundances in Jones Creek (JC) there were inadequate numbers of clams available for lipid analysis. In the JC site only 2 clams were available for fatty acid analysis, and no clams suitable for analysis were collected during the fall survey. The body tissue from the clams was separated from the shell and dried by lyophilization. All tools used in sample preparation and processing were metal or glass and pre-cleaned in acetone or incinerated in a muffle furnace to

reduce the potential for contamination of the lipid sample from plastics or other sources. The dried tissue was homogenized with a glass pestle and stored at  $-80^{\circ}\text{C}$  until use.

### **Lipid extraction**

Three clams from each cove were randomly selected for lipid extraction. Lipids were extracted using a protocol modified from Bligh and Dyer (1959). A 50 – 100 mg of homogenized dried tissue from each clam were processed using a single stage extraction using 1:1 HPLC-grade Chloroform : Methanol ( $\text{CHCl}_3:\text{MeOH}$ ). The lipid extract was then dried in a under a stream of nitrogen. The dried extract was massed in order to gravimetrically determine the total lipid density. The dried extract was resuspended in 1 ml of 1:1  $\text{CHCl}_3:\text{MeOH}$ , capped with nitrogen, and stored at  $-80^{\circ}\text{C}$  until needed.

### **Fatty Acid Composition Determination**

A 50  $\mu\text{l}$  subsample of lipid extract from each clam was spiked with 20  $\mu\text{g}$  of C23:0 internal standard. The samples were derivatized into Fatty acid methyl esters (FAMES) using 14% methanolic  $\text{BF}_3$  acid catalyst (Metcalf and Schmitz 1991). The fames were extracted using carbon disulfide and evaporated under nitrogen (Marty et al. 1992). The FAMES were resuspended in 1.5 ml of hexane prior to gas chromatography flame ionization detection (GC-FID) analysis. One  $\mu\text{l}$  of the FAME solution was manually injected into a Varian 3300 GC-FID using a DB-Wax column (25m x 0.32mm; 0.2 $\mu\text{m}$  film thickness; J&W scientific). The column was programmed to ramp from  $60^{\circ}\text{C}$  to  $150^{\circ}\text{C}$  at  $30^{\circ}\text{C min}^{-1}$  and then from  $150^{\circ}\text{C}$  to  $220^{\circ}\text{C}$  at  $2^{\circ}\text{C min}^{-1}$ . The injector temperature was set at  $230^{\circ}\text{C}$  and the detector at  $250^{\circ}\text{C}$ . Helium was used as the carrier gas at  $1.5\text{ml min}^{-1}$  at 20 psi. Hydrogen and compressed air were set at 30 and 300  $\text{ml min}^{-1}$  respectively.



The data were integrated and interpreted on the ChemStation software against known standards. The quantity of each fatty acid components were estimated based on the internal standard. Samples were post-processed to remove all trace level components ( $<10 \mu\text{g}/\text{sample}$ ). Unidentified components are not reported in this manuscript for brevity. The three clam samples from each of the six individual coves were combined into the three river zones to provide six samples per river location for some analyses. This aggregation was performed to facilitate interpretation, and to allow the results of the current work to be compared to the results of Chapter 3 which used combined observations in the statistical analyses.

The results of the biochemical analyses were combined with condition index values calculated for the mark and recapture and forcing factor determination study (Chapter 2,3). Condition index (CI) was calculated by dividing the ash free dry mass (as determined by oven drying and incineration) by the shell length (in mm) as an indicator of overall clam health (*sensu* Wene & Stczynska-Jurewicz 1985). This value was compared to the fatty acid profile values to determine if any shifts in fatty acid profiles would result in variations in clam health.

## RESULTS AND DISCUSSION:

Lipid concentrations were not significantly different among the collection sites (Figure 2, Kruskal-Wallis degrees of freedom [df] = 2, 50,  $H = 0.01$ ,  $p = 0.994$ ), nor was there a significant difference in the percent lipid per gram dry tissue weight between the three collection periods (Kruskal-Wallis  $df = 2$ , 50,  $H = 1.08$ ,  $p = 0.582$ ). Clams from the midriver sites had non-significantly elevated lipid concentrations in all surveys but the

fall 2007 collection (Figure 2), possibly reflecting the increased production in the area due to the nearby estuarine secondary turbidity maxima (Lin & Kuo 2001).

The total lipid concentration for *Macoma balthica* in the York River averaged for all coves and sampling events was  $8.99 \pm 0.39\%$  (standard error). Dutch Wadden Sea *M. balthica* populations have lipid concentrations ranging from 8.35- 36% lipid (Jarzebski et al. 1986, Wenne and Styczynska-Jurewicz 1987). The Chesapeake Bay *M. balthica* lipid concentrations are lower than the previously reported values, which may be related to the much greater growth rates of this population relative to the more northern populations, thus leading to lower energy storage rates (Chapter 2; Brylawski et al. in prep.). It is also possible that the variation in lipid storage is because the two populations are actually sibling species, as has been suggested by genetics studies (Meehan 1985, Väinölä 2003). It is also likely that the Chesapeake Bay population is being affected by some stressor, as these values are very close to those observed in a location within the Dutch Wadden Sea which is heavily impacted by anthropogenic eutrophication (Jarzebski et al. 1986).

The fatty acid composition did not vary qualitatively between collection period or river zone (Table 1, river zone Kruskal-Wallis  $df = 2, 47, H = 1.55, p = 0.460$ ; collection period Kruskal-Wallis  $df = 2, 47, H = 1.86, p = 0.394$ ). The dominant fatty acids of *Macoma balthica* were in order 16:0 Palmitic acid, 16:1 Palmitoleic acid, and 20:5 (n-3) eicosapentaenoic acid (EPA) (Table 2 and Table 3). Docosahexaenoic acid 22:6 (n-3) (DHA) was also a major constituent of the lipid profile. The European *M. balthica* lipid profile varied slightly from our results. The European clams were also dominated by 16:0 Palmitic acid, 16:1 Palmitoleic acid, but also had a large proportion of their fats in the

form of 20:0 arachidic acid, though the authors were not able to differentiate it in all cases from 20:1 forms (Jarzebski et al. 1986).

There were a number of unidentifiable components with concentrations greater than 10 µg per 50-100 ug dry tissue mass. These were grouped according to retention time, which are not presented in this document for brevity. The greatest percentage contribution in any one sample by an unknown component was 5.56%; however, no unidentified fatty acid component appeared in all samples, and the highest average percent contribution to the overall York River *M. balthica* fatty acid profile was 2.64%. Though none are a dominant component of the fatty acid composition, an attempt will be made in the future to identify these unidentified samples through additional post-processing of the chromatograms and potential reanalysis using gas chromatography mass spectrography.

A non-significant trend of increased unsaturated and decreased saturated fatty acid percent composition moving toward the river mouth was observed (Figure 3). This trend was especially discernable in the summer sampling period. The relative compositions of individual fatty acids also shifted depending on the collection point. One of the most marked changes along the river axis was in the EPA, Palmitoleic acid, and DHA concentrations. Moving downriver, the percent composition of EPA and Palmitoleic acid increased markedly, while DHA decreased slightly (Figure 4).

Palmitoleic acid is a monounsaturated fatty acid commonly used in phospholipid membranes. It is able to be synthesized by higher animals including clams from precursors such as palmitic acid (de Moreno et al. 1977). EPA and DHA are long chain polyunsaturated fatty acids. They are considered essential fatty acids (EFAs) since can

not be synthesized by higher animals in sufficient amounts to meet their metabolic needs they must be procured from food sources. Both EPA and DHA are important membrane components. The length of the carbon chain and the number of double bonds alters the permeability and flexibility of membranes which is important in the formation of neural, pancreatic, hepatic, and other specialized tissues. Specifically, DHA is an important component of membrane phospholipids in neural tissue. Lack of DHA in food sources has led to retinal and spinal abnormalities in fish. In addition to being an important membrane component EPA also acts as precursor for eicosanoids, intracellular signaling agents involved with anti-inflammatory and immune responses. A lack of EPA in the diet can lead to inflammatory diseases in higher animals (Gurr et al. 2002)

Since EPA and DHA are some of the most common fatty acids observed in the York River *M. balthica*, are not able to be synthesized by the clams, and their concentrations alter according to river position we elected to utilize them for comparing to the trends in clam health observed in chapter 2 and 3. By combining the EPA amount with the DHA to form a EPA:DHA ratio, the along river axis trend is more easily visualized. There is a marked trend in decreased EPA:DHA moving upriver (Figure 5a). There is also a strong trend between the EPA:DHA ratio and the condition index of clams as observed in the same locations (Figure 5b, Chapter 3). Clams with a greater EPA:DHA ratio have a greater condition index. This trend was also observed in the European clam stock. The condition index of the European clams was not affected by total lipid concentration but did vary with the percentage of EPA. This trend is exemplified by converting the data to an EPA:DHA ratio, with clams with a greater EPA:DHA ratio having a higher condition index (Jarzebski et al. 1986).

We think that the relationship between the EPA:DHA ratio and condition may be due to the composition of the primary producer community on which the clams are feeding. In general, diatoms contain greater amounts of EPA, while dinoflagellates have relatively greater amounts of DHA (Pond et al. 1998, St. John et al. 2001). In the forcing factor study it was observed that that the ratio of dinoflagellates : diatoms also varied along the river axis with greater number of diatoms in the downriver locations compared to the midriver and upriver sites (Chapter 3). We hypothesized that this shift in species composition was caused by variations in phytonutrient stoichiometry created by the effects of cultural eutrophication.

Combining the results of this work with the parallel study, there is a strong relationship between the EPA:DHA ratio and the dinoflagellates : diatom ratio. Thus, it is possible that the shifts in the primary producer community composition can be detected in the fatty acid composition of the clams (Figure 6). The close regression relationship appears to indicate that the clams are not able to selectively feed on diatoms preferentially over dinoflagellates.

Diatoms have been thought to be a better food source for *M. balthica* since it has been observed that clam growth alters dependant upon the concentration of diatoms with greater proportions of diatoms in the phytoplankton community leading to faster growth. Clam growth observed in a European population decreased over a 25 year period during a period of eutrophication that shifted the primary producer community away from domination by diatom species (Beukema and Cadee 1991). It is possible that clams are less able to capture and assimilate dinoflagellates and other phytoplankton relative to diatoms; however, the lack of a significant difference in total lipid concentrations

contradicts this idea (Figure 2). If clams were unable to effectively utilize dinoflagellates as a prey source their total lipid concentration should be reduced indicating a relative lack of energetic reserves due to food limitation. Since there appears to be similar proportions of total lipid to body mass regardless of sampling location, condition index, and prey type, it is more likely that some micronutrient component that makes diatoms a preferred prey.

It may be possible that diatoms contain the preferred type of EFAs that promote clam health and growth. Diatoms contain relatively high proportions of EPA along with palmitoleic 16:1(n-7), and palmitic acid 16:0 ( St. John et al. 2001). Of the common biomarkers both EPA and palmitoleic acid correlate with clam condition index. However, since EPA is essential and palmitoleic acid can be upgraded from other fatty acids it is more likely that EPA is the limiting micro nutrient created by a reduction in the number of diatoms upriver sites though there is a greater overall abundance of phytoplankton at those locations (Chapter 3). Since *M. balthica* is an important prey species and benthopelagic coupler, a reduction in clam health caused by essential fatty acid limitation could create a disruption in trophic network, leading to alteration in the efficiency of energy transfer to higher trophic levels. Additional studies using fatty acid additions to the diets will be necessary to confirm if EPA is limiting clam health and to explore how EFA limitation could alter the trophodynamics of the Chesapeake bay trophic network.

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Table 1. *Macoma balthica* average number of fatty acid components by type ( $\pm$  standard error) from the three river zones and three collection periods (spring, summer, and fall). DR = downriver, MR = midriver, and UR = upriver.

	Spring			Summer			Fall		
	DR	MR	UR	DR	MR	UR	DR	MR	UR
Saturated	5.00±0.00	5.20±0.37	5.25±0.25	5.00±0.00	5.00±0.00	5.00±0.26	4.67±0.22	5.33±0.33	4.83±0.17
Monounsaturated	5.00±0.37	6.00±0.32	4.50±0.50	5.50±0.50	4.80±0.73	5.33±0.56	5.67±0.61	5.67±0.33	4.83±0.40
Polyunsaturated	6.83±0.98	7.80±0.58	6.50±0.65	6.67±0.42	6.40±0.81	7.17±0.75	6.33±0.67	5.33±0.88	6.00±0.86
Unidentifiable	10.17±1.54	15.00±1.67	7.75±2.13	8.17±1.40	6.40±2.23	10.33±2.03	11.33±2.74	10.00±3.21	7.00±1.21
Total	27.00±2.29	34.00±2.19	24.00±2.97	25.33±2.12	22.60±3.68	27.83±3.32	28.00±3.17	26.30±4.33	22.67±2.02

Table 2 *Macoma balthica* average fatty acid concentrations (mg lipid/ g dry tissue)  $\pm$  standard error from the six test coves and three collection periods. Only the ten highest concentration identifiable non-trace components are represented. TC = Timberneck Creek, CW = Cattlett Islands, AM = Aberdeen Creek, JC = Jones Creek, PR = Poropotank Bay, and PI = Purtan Islands. The data are ordered from the highest fatty acid concentration in the TC samples to the least in each sampling period.

Spring						
Fatty acid	TC	CW	AM	JC	PI	PR
16:0	9.17±0.45	9.17±1.01	11.5±1.20	11.0±11.0	12.2±0.70	6.39±0.89
16:1(n-7)	4.20±1.83	5.63±3.15	8.81±0.78	13.3±1.78	5.00±1.33	1.83±0.12
20:5(n-3)	3.53±2.48	6.71±1.01	8.52±0.32	10.8±0.03	1.60±0.69	1.35±0.30
18:0	2.02±0.26	1.26±0.51	2.18±0.34	2.63±0.68	2.34±0.14	1.75±0.28
22:6(n-3)	1.74±1.36	4.43±0.38	5.45±0.63	8.03±2.45	2.15±0.34	2.65±0.67
14:0	1.47±0.15	1.43±0.13	2.24±0.28	3.01±1.97	2.57±0.02	1.07±0.08
18:1(n-9)	1.36±0.20	1.71±0.59	3.05±0.38	3.98±1.71	3.01±0.63	1.43±0.05
18:2(n-6)	0.96±0.34	1.81±0.23	2.22±0.57	4.50±2.44	1.64±0.78	0.76±0.05
15:0	0.83±0.13	0.67±0.24	1.53±0.38	6.86±4.66	1.61±0.80	0.25±0.03
20:1(n-9)	0.67±0.28	1.14±0.08	1.13±0.23	1.41±0.38	0.80±0.10	0.49±0.00

Summer						
Fatty acid	TC	CW	AM	JC	PI	PR
16:0	6.68±3.25	7.96±3.37	9.94±7.45	13.9±2.07	10.3±2.52	16.4±3.62
16:1(n-7)	2.71±1.18	5.55±2.81	3.00±2.42	5.50±0.93	4.05±1.90	4.11±3.74
18:0	1.53±0.86	1.67±0.74	2.55±1.45	2.83±0.15	2.33±0.24	3.46±0.42
20:0	1.03±0.51	1.08±0.46	1.72±0.79	1.70±0.08	1.02±0.41	2.03±0.26
14:0	0.97±0.48	1.32±0.62	1.63±1.32	2.34±0.55	1.57±0.43	2.95±0.97
15:0	0.86±0.24	0.99±0.49	1.94±1.13	1.87±0.42	1.44±0.33	1.17±0.62
20:5(n-3)	0.76±0.44	4.30±3.48	0.99±0.68	2.25±0.63	2.78±1.95	1.65±0.40
18:1(n-9)	0.75±0.67	1.39±0.61	0.32±0.32	2.53±0.28	1.97±0.54	2.42±0.67
22:5(n-3)	0±0	0±0	0±0	0.79±0.79	0±0	0±0
17:00	0±0	0±0	0±0	0±0	0±0	0.16±0.16

Fall						
Fatty acid	TC	CW	AM	JC	PI	PR
16:00	10.7±3.25	3.89±1.97	8.88±2.13	No data	6.05±1.83	8.01±0.69
16:1(n-7)	6.27±2.44	4.31±1.74	5.74±2.02	No data	3.18±1.67	5.83±0.92
20:5(n-3)	4.58±0.97	4.68±0.37	4.71±0.91	No data	3.11±0.61	4.57±0.72
18:0	2.40±0.31	2.06±0.46	2.27±0.41	No data	1.67±0.40	2.29±0.02
22:6(n-3)	2.39±0.11	1.94±0.15	2.36±0.21	No data	2.22±0.68	2.30±0.40
15:0	2.05±1.31	2.29±0.93	2.12±1.08	No data	0.85±0.30	0.62±0.01
18:1(n-9)	2.03±0.38	1.57±0.53	1.21±0.27	No data	1.05±0.53	1.72±0.20
14:0	1.44±0.53	0.92±0.38	1.30±0.48	No data	0.89±0.54	1.67±0.32
16:4(n-3)	1.42±1.09	2.01±0.87	1.89±1.10	No data	0.56±0.14	0.14±0.14
20:1(n-9)	1.26±0.20	1.36±0.69	0.82±0.09	No data	0.98±0.34	1.93±0.23

Table 3 Average percent compositions ( $\pm$  standard error) of fatty acids from *Macoma balthica* for the three aggregated river zones and the three surveys. Only identifiable components non-trace fatty acids are listed. DR = downriver, MR = midriver, and UR = upriver. The data are order from the highest percent composition to the least for the DR site for the spring survey.

FA	Spring			Summer			Fall		
	DR	MR	UR	DR	MR	UR	DR	MR	UR
16:1(n-7)	10.9 $\pm$ 2.76	14.4 $\pm$ 0.64	9.28 $\pm$ 1.29	13.6 $\pm$ 0.64	8.57 $\pm$ 1.86	7.65 $\pm$ 1.68	10.9 $\pm$ 1.39	12.8 $\pm$ 2.04	11.8 $\pm$ 1.97
20:5(n-3)	10.4 $\pm$ 2.29	13.3 $\pm$ 1.17	4.39 $\pm$ 0.77	5.16 $\pm$ 2.41	3.56 $\pm$ 0.95	4.21 $\pm$ 1.32	11.4 $\pm$ 1.30	11.5 $\pm$ 1.89	11.6 $\pm$ 1.35
22:6(n-3)	6.17 $\pm$ 1.61	8.73 $\pm$ 0.59	7.67 $\pm$ 1.83	3.52 $\pm$ 1.17	2.86 $\pm$ 0.64	4.56 $\pm$ 0.94	5.69 $\pm$ 1.00	6.05 $\pm$ 1.22	7.19 $\pm$ 1.35
18:0	4.63 $\pm$ 1.44	3.23 $\pm$ 0.29	6.20 $\pm$ 0.68	5.55 $\pm$ 0.55	7.56 $\pm$ 1.02	6.34 $\pm$ 0.62	5.35 $\pm$ 0.43	5.51 $\pm$ 0.43	5.83 $\pm$ 0.22
18:1(n-9)	3.86 $\pm$ 0.76	4.63 $\pm$ 0.54	6.26 $\pm$ 0.42	3.55 $\pm$ 0.82	3.62 $\pm$ 1.15	4.62 $\pm$ 0.26	4.11 $\pm$ 0.30	2.90 $\pm$ 0.36	3.56 $\pm$ 0.75
14:0	3.62 $\pm$ 0.45	3.35 $\pm$ 0.56	5.19 $\pm$ 0.80	3.91 $\pm$ 0.18	4.22 $\pm$ 0.58	4.34 $\pm$ 0.65	2.45 $\pm$ 0.38	2.94 $\pm$ 0.74	3.22 $\pm$ 0.77
18:2(n-6)	3.35 $\pm$ 0.73	3.98 $\pm$ 0.77	3.28 $\pm$ 0.49	1.23 $\pm$ 0.24	0.62 $\pm$ 0.28	1.53 $\pm$ 0.59	0.84 $\pm$ 0.21	0.17 $\pm$ 0.17	0.99 $\pm$ 0.33
16:0	23.2 $\pm$ 3.65	14.9 $\pm$ 3.74	27.0 $\pm$ 2.59	26.6 $\pm$ 2.19	27.0 $\pm$ 2.22	27.3 $\pm$ 3.06	17.7 $\pm$ 3.83	20.9 $\pm$ 1.14	20.0 $\pm$ 0.82
20:0	2.22 $\pm$ 0.38	1.83 $\pm$ 0.39	4.24 $\pm$ 0.67	3.84 $\pm$ 0.41	5.17 $\pm$ 0.88	2.82 $\pm$ 0.63	3.43 $\pm$ 1.31	5.03 $\pm$ 0.42	5.62 $\pm$ 0.24
20:1(n-9)	2.07 $\pm$ 0.26	1.69 $\pm$ 0.18	1.89 $\pm$ 0.05	2.20 $\pm$ 0.46	2.14 $\pm$ 0.69	1.45 $\pm$ 0.46	3.11 $\pm$ 0.57	2.10 $\pm$ 0.42	4.00 $\pm$ 0.38
18:4(n-3)	2.00 $\pm$ 0.66	2.85 $\pm$ 0.63	2.72 $\pm$ 0.90	1.41 $\pm$ 0.76	0.27 $\pm$ 0.18	1.02 $\pm$ 0.60	0.37 $\pm$ 0.17	0.21 $\pm$ 0.21	0.59 $\pm$ 0.20
15:0	2.00 $\pm$ 0.46	5.18 $\pm$ 2.90	2.25 $\pm$ 0.87	4.27 $\pm$ 0.78	5.11 $\pm$ 0.64	3.19 $\pm$ 0.79	4.41 $\pm$ 0.85	4.36 $\pm$ 1.06	2.17 $\pm$ 0.34
14:1	1.58 $\pm$ 0.20	1.88 $\pm$ 0.25	2.10 $\pm$ 0.15	1.94 $\pm$ 0.20	1.50 $\pm$ 0.51	1.90 $\pm$ 0.41	1.35 $\pm$ 0.16	1.54 $\pm$ 0.18	2.21 $\pm$ 0.48
16:3(n-3)	1.27 $\pm$ 0.30	0.85 $\pm$ 0.14	0.84 $\pm$ 0.30	1.61 $\pm$ 0.20	1.60 $\pm$ 0.38	1.40 $\pm$ 0.26	1.89 $\pm$ 0.27	1.76 $\pm$ 0.26	1.31 $\pm$ 0.15
20:4(n-6)	1.10 $\pm$ 0.27	1.15 $\pm$ 0.23	0.99 $\pm$ 0.35	1.46 $\pm$ 0.72	0.15 $\pm$ 0.15	0.85 $\pm$ 0.18	1.04 $\pm$ 0.74	0.73 $\pm$ 0.73	0 $\pm$ 0
16:4(n-3)	1.03 $\pm$ 0.27	1.85 $\pm$ 0.44	1.33 $\pm$ 0.98	2.47 $\pm$ 0.54	3.28 $\pm$ 0.94	2.33 $\pm$ 0.90	3.46 $\pm$ 0.86	3.74 $\pm$ 1.27	1.19 $\pm$ 0.41
18:1(n-11)	0.94 $\pm$ 0.72	0.42 $\pm$ 0.06	0.13 $\pm$ 0.13	1.13 $\pm$ 0.75	2.60 $\pm$ 1.33	0.47 $\pm$ 0.15	0.19 $\pm$ 0.08	0.09 $\pm$ 0.09	0.07 $\pm$ 0.07
18:3(n-3)	0.74 $\pm$ 0.24	2.22 $\pm$ 1.16	1.03 $\pm$ 0.37	0.27 $\pm$ 0.18	0.17 $\pm$ 0.11	0.62 $\pm$ 0.36	0.26 $\pm$ 0.12	0.13 $\pm$ 0.13	0.19 $\pm$ 0.12
18:1(n-7)	0.47 $\pm$ 0.21	0.83 $\pm$ 0.06	0.20 $\pm$ 0.20	0.62 $\pm$ 0.21	0.40 $\pm$ 0.18	0.42 $\pm$ 0.14	0.87 $\pm$ 0.19	0.77 $\pm$ 0.13	1.83 $\pm$ 0.69
20:3(n-6)	0.26 $\pm$ 0.18	0.14 $\pm$ 0.14	0 $\pm$ 0	0.40 $\pm$ 0.32	0.04 $\pm$ 0.04	0.08 $\pm$ 0.08	0 $\pm$ 0	0 $\pm$ 0	0.13 $\pm$ 0.13
22:5(n-3)	0.12 $\pm$ 0.12	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0.81 $\pm$ 0.81	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
20:4(n-3)	0 $\pm$ 0	0.06 $\pm$ 0.06	0 $\pm$ 0	1.94 $\pm$ 1.64	1.12 $\pm$ 0.61	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	1.48 $\pm$ 0.68
20:1(n-11)	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0.67 $\pm$ 0.50	0 $\pm$ 0	1.88 $\pm$ 1.45	1.61 $\pm$ 0.82	0.40 $\pm$ 0.40	0 $\pm$ 0
17:0	0 $\pm$ 0	0.36 $\pm$ 0.36	0.21 $\pm$ 0.21	0 $\pm$ 0	0 $\pm$ 0	0.08 $\pm$ 0.08	3.28 $\pm$ 3.28	0.50 $\pm$ 0.50	0 $\pm$ 0
Saturated	35.67	28.85	45.09	44.17	49.06	44.07	36.62	39.24	36.84
Monounsaturated	19.82	23.85	19.86	23.71	18.83	18.39	22.14	20.6	23.47
Diunsaturated	3.35	3.98	3.28	1.23	0.62	1.53	0.84	0.17	0.99
PUFA (n-3)	21.73	29.86	17.98	16.38	13.67	14.14	23.07	23.39	23.55
PUFA (n-6)	1.36	1.29	0.99	1.86	0.19	0.93	1.04	0.73	0.13

Figure 1. The York River, VA sub-estuary showing the six test coves.

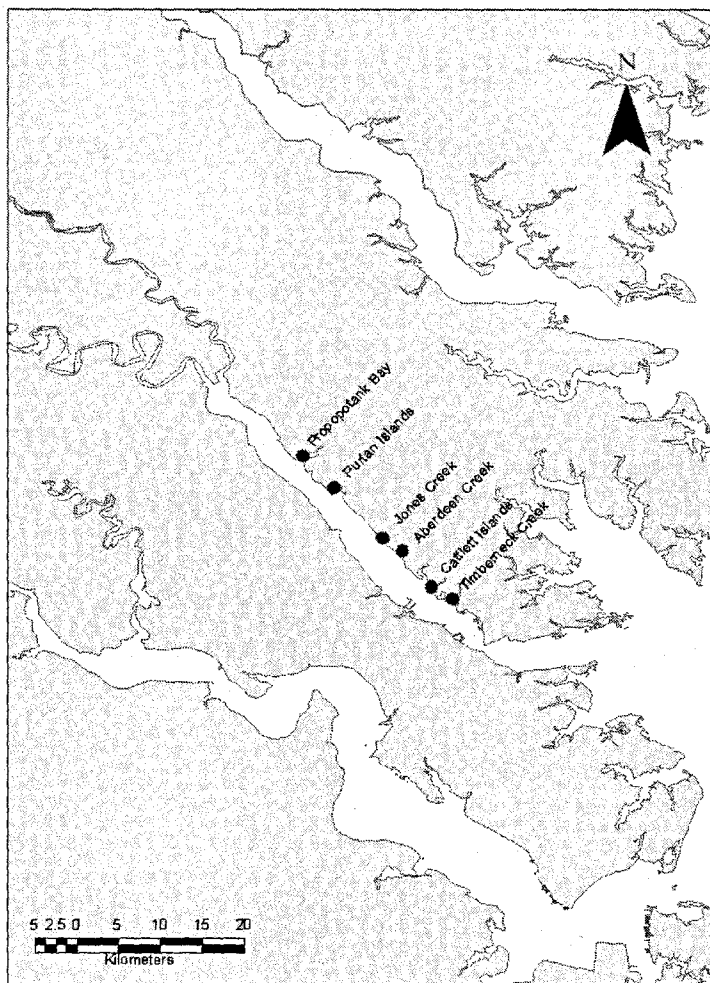


Figure 2. *Macoma balthica* total lipid percent per gram dry tissue mass by river zone from the three collection periods, spring, summer, and fall. Error bars are one standard error. DR = downriver, MR = midriver, and UR = upriver.

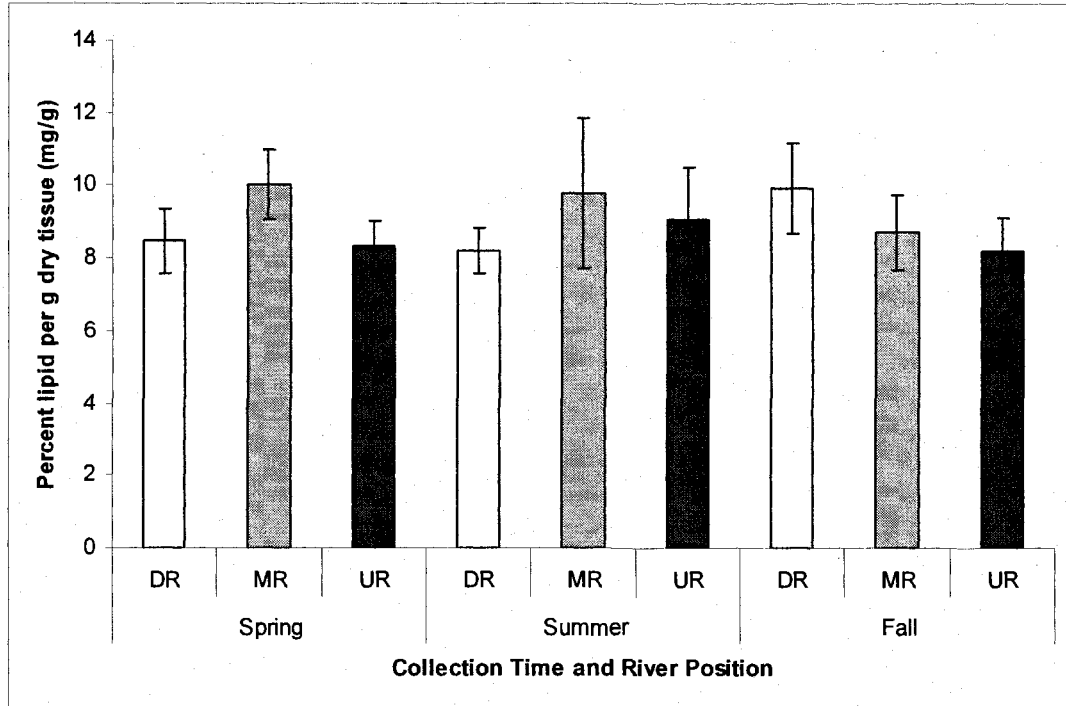


Figure 3. Mean ratio of saturated to unsaturated fatty acids from *Macoma balthica* for each of the three river zones by mean distance from the York River mouth and collection period.

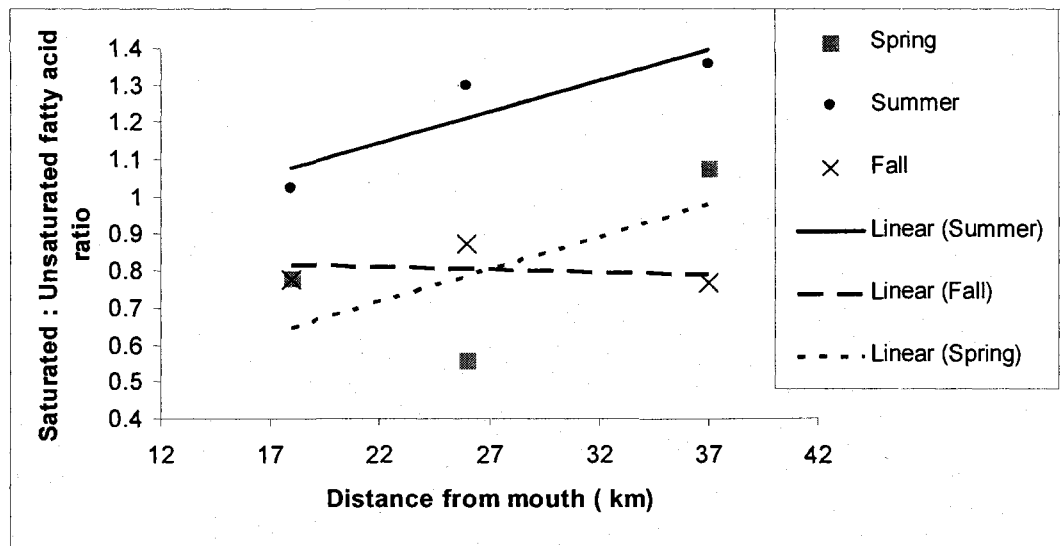




Figure 4. Mean composition of EPA 20:5(n-3), Palmitoleic acid 16:1 (n-7), and DHA 22:6(n-3)  $\pm$  standard error as a function of dry tissue weight from *Macoma balthica* in the York River, Virginia, as a function of distance from the river mouth. Data are from all three seasonal surveys combined.

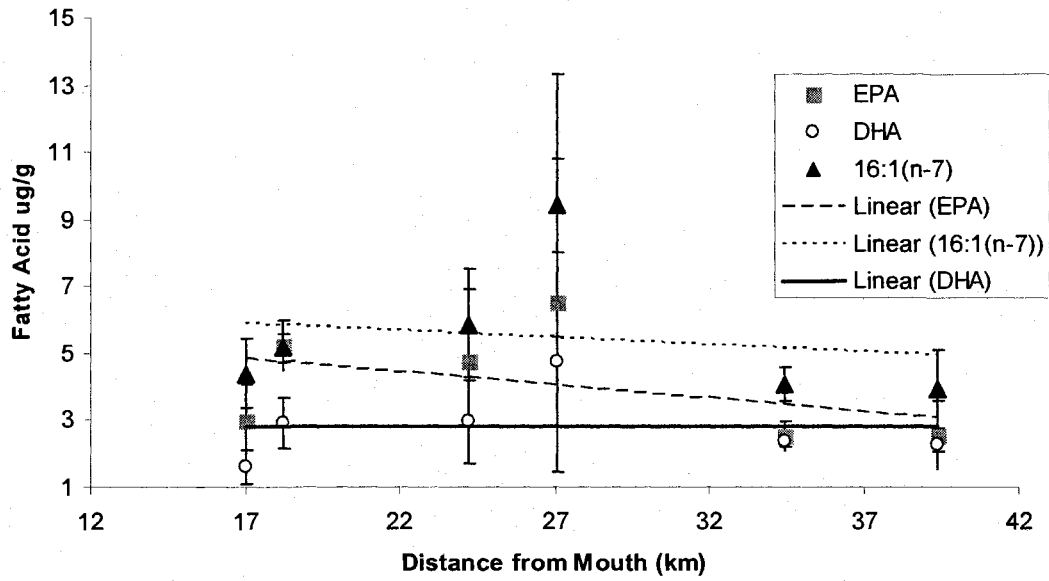


Figure 5. Mean ratio of EPA to DHA of *Macoma balthica* (a) as a function of distance from mouth and (b) as a function of average clam condition index. Data are means of each of the three individual river zones. Error bars are one standard error.

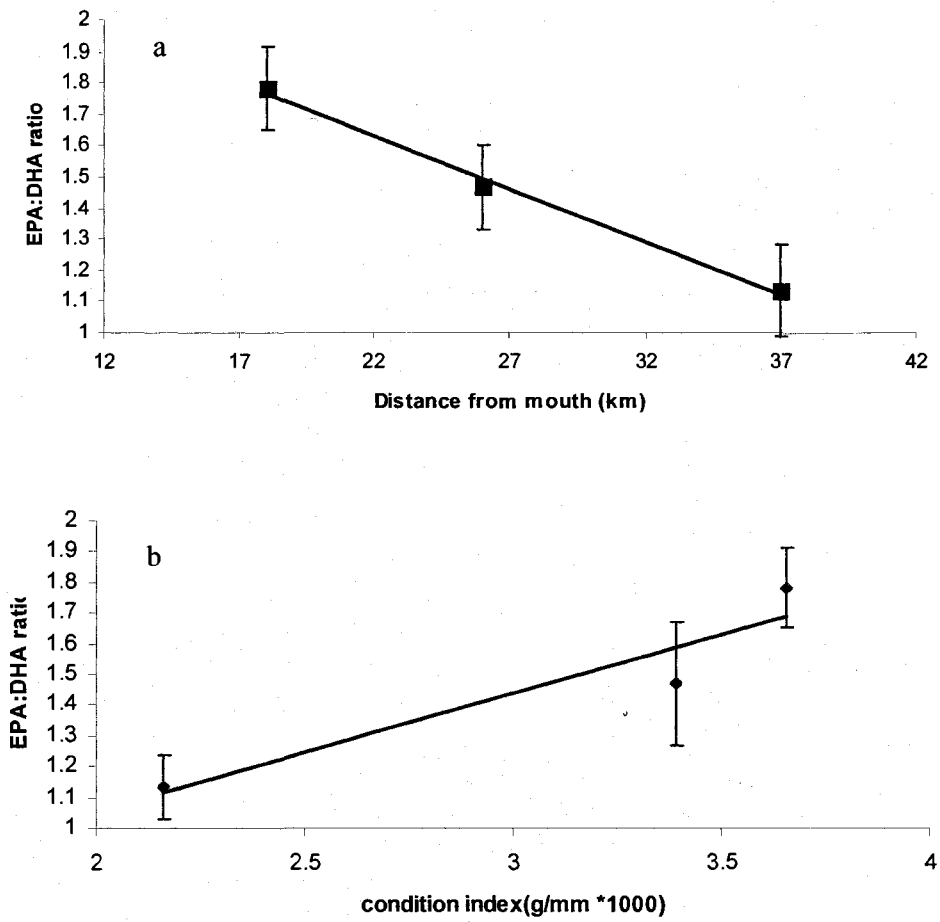
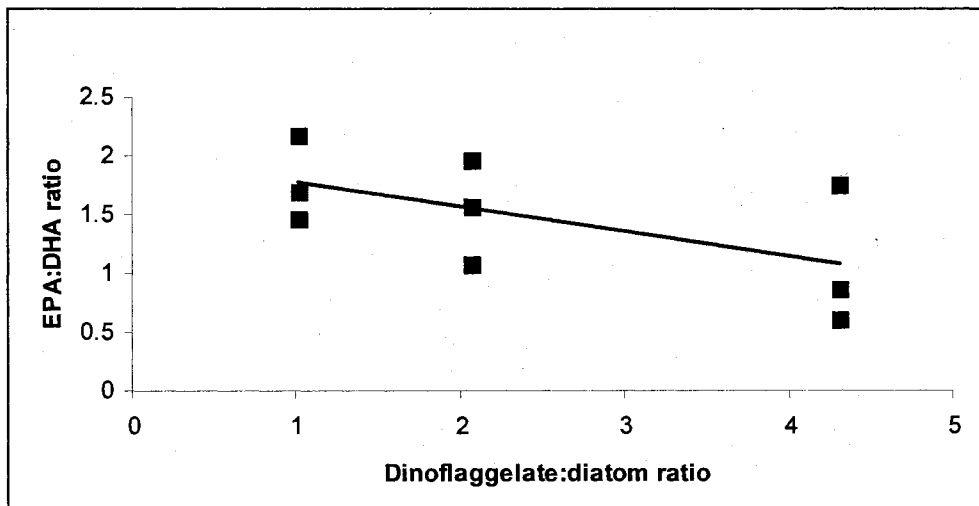


Figure 6. The observed EPA:DHA ratio in the clam tissue as a function of Dinoflagellate:Diatom ratio from the water column samples (modified from Chapter 3).



## **Chapter 5**

**Implications of prey quality for the scope for growth of blue crabs and for stock-enhancement habitat selection**

## ABSTRACT

Due to sharp declines in the blue crab (*Callinectes sapidus*) populations in the Chesapeake Bay, we are examining the potential to enhance the spawning stock using hatchery reared animals. To successfully implement a stock enhancement program potential nursery habitats must be identified. Prey resource availability has been suggested as one of the most important forcing factors defining a good nursery habitat; however, the work to date has focused on food quantity alone. In this work we integrate the results from a survey of prey resources in potential nursery habitats in the York River, VA, with a mathematical model to examine if prey quality can significantly affect the scope for growth of crabs. Prey quality is the individual-based variation in the energy delivered from a single prey item. The model was run for six test coves in the river that have significant variations in the abundance and individual specific biomass of *Macoma balthica*, an indicator species for the benthic community and an important prey species for the blue crab. The model predicted that scope for growth could be greatly reduced by the combined effects of prey quantity and quality, such that areas with similar abundance but lower quality prey could reduce crab scope for growth by more than 40%. The results of this modeling exercise indicate that multiple surveys of both prey abundance and quality should be performed in order to accurately quantify the suitability of an area to act as a nursery habitat for enhancement.

## INTRODUCTION:

The blue crab (*Callinectes sapidus*) is an estuarine-dependent predator that is highly valuable both ecologically and economically in the Chesapeake Bay (Norse 1977). Ecologically, the blue crab plays an important role in the estuarine food web (Hines et al. 1990), is a dominant benthic predator on shellfish, and is an important scavenger. The blue crab is prey for several fishes, such as the recreationally and commercially fished moronids, possibly acting as a link between the benthic and pelagic ecosystem (Baird and Ulanowicz 1989). The blue crab also plays a role in promoting ecosystem stability and resilience through mechanisms that are still being explored, such as its potential to act as a natural defense against invasive species (Harding 2003).

The blue crab also plays a large socioeconomic role in the eastern estuaries of the United States. The blue crab fishery is the largest crustacean fishery in the world and has a dockside value in excess of 130 million dollars per year in the US alone (NMFS Data 2003). The socioeconomic value of the blue crab fishery is greater than dockside values indicate, because there is an extensive support network of meat packers, boat builders, fishing gear manufacturers, etc., which has developed solely based on the capture of crabs and other aquatic animals (Warner, 1977).

Given that blue crabs play a important role in the Chesapeake Bay economy and culture, there is great concern for the fishery, as landing are at a stable but historically low level (Miller et al. 2005). The reason for the reduction in the crab stock is not known, but it is hypothesized that it was depressed due to several years of over-exploitation. As of the most recent bay-wide stock assessment the blue crab is not currently considered to be over-fished; however, the stock has not rebounded to the abundances recorded in the

1980s (Miller et al. 2005). This may be due to a depensatory mechanism caused by severely reduced spawning stock biomass maintaining the population in an alternative stable state. Due to hysteresis, the stock may not be able return the historical abundances with only a reduction in the effort to non over-fished levels. The spawning stock abundance has declined by 81% since the 1980s indicating the potential for recruitment limitation, which may not be overcome by effort restrictions alone (Lipcius and Stockhausen 2002, Miller et al. 2005).

The potential recruitment limitation in the Chesapeake Bay blue crab population has created interest in starting a large-scale restoration plan that includes stock enhancement using hatchery-reared animals (Secor et al. 2002, Zamora et al. 2005). The theory is that by supplementing the stock with hatchery-reared animals placed in recruitment-limited areas, the depensatory mechanism created by the low spawning stock abundance can be overcome (Blankenship and Leber, 1995).

For stock enhancement to be effective and efficient it is imperative to identify the quality of potential nursery habitats into which hatchery-reared animals can be released. Specifically, we aim to examine the secondary nursery habitats that are used by larger juveniles after dispersing from seagrass beds, which are primary nursery habitats (Lipcius et al. 2005). We will examine whether secondary nursery habitats have enough prey to support thousands of additional hatchery-reared crabs from enhancement. Juvenile blue crabs feed on clams (~ 50% of the diet), polychaetes, clams, amphipods, and other small benthic macrofauna (Lipcius et al. 2007). In areas with greater abundance of prey resources, specifically bivalves, blue crab growth is higher (Seitz et al. 2005).

Numerous factors can affect the quality of a nursery habitat for blue crabs; however, the ultimate limit on how many additional animals an area can support is dependent on the prey resources (Seitz et al. 2008). Additionally, the abundance and species composition of benthic macrofauna is closely tied to the level of environmental stress, which allows the benthic community composition to be used as an index of biological integrity (Weisberg et al. 1996). Due to the direct connection with carrying capacity and environmental stress, the abundance of these benthic species may be usable as a habitat suitability index for stock enhancement (Seitz et al. 2008). Previously, studies exploring the potential for benthic resources as a predictor of nursery habitat quality have focused on prey quantity which may not tell the whole story regarding the suitability of a nursery habitat to support additional blue crabs.

Prey resource availability is actually composed of two parts, prey abundance and prey quality. Prey quality is the nutritional content of an individual animal that is available to be passed up the food chain, as measured in energy, matter (carbon units, or micronutrients). It is possible for areas with equal or greater abundances of prey items to have a significantly lower nutritional content per prey item, which could make an area appear suitable for enhancement when it may not be.

To explore the interplay of benthic prey abundance and quality, we combined the results from a multiyear study of the prey resources in several shallow water coves in the York River, VA, with a blue crab scope-for-growth model. The York River, a sub-estuary of the southern Chesapeake Bay, contains a number of marsh-fringed coves that have been identified as secondary blue crab nurseries which may be suitable for enhancement with hatchery-reared blue crabs (Seitz et al. 2003, 2008; Lipcius et al. 2005). In surveys



of benthic resources, six shallow-water coves in the mesohaline section of the river were selected to act as test areas for this study (Figure 1). These test coves were grouped into three river zones to allow easier statistical and model interpretation: (1) upriver (Poropotank Bay [PR] and Purtan Islands [PI]), (2) midriver (Jones Creek [JC] and Aberdeen Creek Mainstem [AM]), and (3) downriver (Cattlett Islands [CT] and Timberneck Creek [TC]). These test coves were selected to have similar conditions such as average depth, temperature regimes, and fringing marsh. Because the coves were located at different distances from the river mouth, the coves had variation in nutrient input patterns which may affect the prey quality through cultural-eutrophication-induced trophic disruption (Chapter 3; Brylawski et al., in prep.). We hoped that by using test coves with many similar conditions we could detect the subtle differences that prey quality could have on the suitability of each habitat.

In these coves, we performed a three-year mark-and-recapture study as well as randomized surveys to assess the variations in the prey density, health, average size, growth, individual-based biomass, and non-predatory mortality of an indicator species, the Baltic macoma (*Macoma balthica*). We focused on a single prey species to make data collection and modeling more feasible. *M. balthica* was predicted to be a good indicator organism for exploring the interplay of prey quality and quantity on blue crab nursery habitat suitability, because they are commonly found in the shallow coves previously identified as potential nursery habitats and they comprise large proportion of the blue crab's diet (Holland et al. 1997, Ebersole and Kennedy 1995, Lipcius et al. 2005, 2008). Blue-crab growth has been correlated with the abundance of *M. balthica* indicating their importance to blue crabs (Seitz et al. 2005). Additionally, the abundance of clams

correlates with the abundance of the rest of the benthic macrofaunal community, so they may be used as an indicator for overall benthic abundance (Seitz et al. 2008).

In the surveys, *M. balthica*'s condition, an index of individual-based biomass and animal size, varied greatly between the upper and lower mesohaline sections of the York River. The upriver sites had lower average clam size and reduced individual-based biomass on average than downriver sites even though clam abundance and total population biomass was greater during some surveys (Chapter 2 and 3). Thus it is possible that the upriver sites may not be optimal habitats for enhancement, because prey quality may be reduced. To evaluate the interplay of food quality and quantity on crab-enhancement nursery habitats, we combined the results from field studies with a model designed to estimate relative shifts in a blue crab's scope for growth, a theoretical estimate of how much a crab could grow with the resources available (Brylawski and Miller 2003). By estimating scope for growth we hoped to be able to determine if significant differences in prey abundance and quality can transfer up to the next trophic level and thus affect each habitat's utility as a potential nursery.

## METHODS

### **Modeling Approach**

A two-part model was created to test the effect of *Macoma balthica* abundance and quality on the scope for growth of blue crabs. A simple foraging model was combined with a bioenergetic model of crab growth.

The bioenergetic section of our model is based on Wisconsin fish bioenergetics model as modified for the Fish Bioenergetics 3.0 computer program (Hewett and Johnson 1987, Hanson et al. 1997). The Wisconsin model had been previously adapted for the Chesapeake Bay blue crab population (Brylawski and Miller, 2003). In this work, we have implemented the model using Stella 8.0, an object-based modeling program, which allows for the addition of the foraging sub-model (Figure 2). The bioenergetic model used all of the parameters and physiological function forms from Brylawski and Miller (2003), except for the consumption section of the model. In the Wisconsin model, consumption is based on a combined function of temperature, an allometric relationship, and a theoretical maximum consumption rate (Cmax). Cmax is usually estimated from *ad libitum* laboratory studies (Hanson et al. 1997). While this provides a good estimate for situations where food is not limiting, it would not be appropriate for this implementation because we seek to determine the combined effects of food quantity and quality on blue crab scope for growth.

In this model, we estimate Cmax using a foraging model to simulate the maximum feeding rate under field conditions. The foraging model was based on a type III functional response which was determined to be most appropriate for blue crabs feeding on *M. balthica* (Eq1, Gotelli 1998, Seitz et al. 2001).

$$\text{Clams} * \text{crab}^{-1} \text{day}^{-1} = \frac{6.656 * \text{clam density}^2}{36.756^2 + \text{clam density}^2} \quad (1)$$

The functional-response equation was used to estimate the number of simulated clams that should be drawn from a *M. balthica* population pool, dependent upon the

density of clams set as the initial simulation conditions. The density of the *M. balthica* pool was set dependent upon the ambient conditions to be simulated. The maximum clam consumption per day number was combined with estimates of the average clam size and the power curve length-weight key parameterized for each of the three test zones to get an estimate of the mass of clams a crab could consume on a simulated day (See chapter 3 for estimation, EQ2). The  $\alpha$  and  $\beta$  parameters, of the length-weight key, were calculated using least squares regression for each river position using data from individual based biomass measurements from the three seasonal surveys performed in 2007 (Chapter 3).

$$\text{Ash free dry mass} = \alpha \cdot \text{Clam Length}^{\beta} \quad (2)$$

The cove-specific length-weight keys remained constant through the simulations while the average clam density and size was set dependent on the area and season simulated. The combined effects of clam size and the area-specific length-weight key simulate the effect of food quality variation among habitats. The mass-based  $C_{\max}$  was then transformed into Joules using an energy density estimate for *Macoma balthica* of 22.88 J/mg, for integration into the bioenergetics sub-model (Beukema and deBruin 1979).

In this model implementation, the *M. balthica* pool was continuously refilled to keep a constant density. Additionally, only a single crab in the crab “population” was modeled. These assumptions were made to keep the model as simple as possible while still meeting the goals of the modeling, which was focused on individual-based crab growth rather than clam population dynamics.

Though we attempted to make this model as predictive as possible by employing the best information available, it should be used only for comparison of relative shifts in scope for growth, not to generate values for use in management without further

modification, parameter refinement, and validation. The model should be internally consistent and thus usable for exploring the relative variation in scope caused by shifts in prey resource availability among habitats.

### **Simulations**

With this model we can predict blue crab scope for growth based on four variable forcing factors that are altered to create the different simulation conditions. The temperature history can be altered to represent different temperature regimes, which can have a great effect on the model dynamics (Hanson et al. 1997, Brylawski and Miller 2003). Average clam size and abundance were varied to simulate the conditions in the test areas at the different collection times. The length-weight key was shifted to represent the variation in clam health and nutritional value for each area simulated.

The data used for the starting parameters for the simulations came from surveys of *M. balthica* health, individual-based biomass, and abundance performed between 2004 and 2007 (Chapter 2-3). For the simulation runs we used the 2007 surveys that were performed at three points: Spring (5/22/2007-5/24/2007), Summer (7/24/2007 – 8/1/2007), and Fall (10/29/2007 – 11/30/2007).

To verify that the model was reacting as expected and comparable to the Fish Bioenergetics 3.0 version of the model, a series of preliminary simulation runs was performed using both fixed and variable temperature histories and fixed hypothetical clam abundances (100 clams/m<sup>2</sup>) and average clam size (15 mm), and the upriver length-weight key with a starting crab mass of 1g (Table 1). A sensitivity analysis was then run varying clam sizes, abundances, and variations in the  $\alpha$  and  $\beta$  components of the length-

weight key. Except for the parameter that was being examined, the conditions were held constant at those used for the stability analyses. Only one parameter was varied at a time all others were held constant in each sensitivity analysis run. One hundred simulations were run using a range of input parameters for a length of 100 simulated days (Table 2). The parameters within the bioenergetics section of the model were not examined in the sensitivity analysis since they have been previously examined during the parameterization of the model detailed in Brylawski and Miller (2003). The sensitivity analysis results were also used to explore the ecological implications of changes in the clam population. Specifically, we were interested in exploring the relative importance of clam abundance, average size, and individual based biomass on the scope for growth of blue crabs. Once model stability and reasonable behavior was assured, a series of experimental simulations were run. The models produced hypothetical growth trajectories from which the final mass was considered the scope for growth over that time period.

Simulations of the conditions observed in the 2007 surveys were run for the three river zones for the three collection periods as well as an overall average condition for the growing season. One-year simulations were run using the conditions for the three river positions and a temperature history for 2001 for a continuous monitoring station located at the Virginia Institute of Marine Science (Tables 1 and 3); the 2001 temperature history was the most recent dataset available that had been compiled into the daily averages and that was free of extended periods of missing observations. The few missing observations occurring during days when the monitoring station was out of service were estimated using linear interpretation. The temperature history was held constant throughout all simulations and the differences among simulations, not the accuracy of the individual

predictions, was our focus. Year-long simulations were repeated for the six independent test coves conditions from the three 2007 abundance surveys (Table 4).

A seasonally variable simulation was also run for the three aggregated test areas. The spring survey conditions were used to simulate growth for 90 days starting with March 1 in the temperature history. The crab size results from the spring simulations were then fed into a midsummer simulation which ran for another 90 simulated days. Respectively the midsummer results became the starting crab values for a 90 day fall simulation of conditions observed in the fall 2007 survey.

Percent differences from the highest predicted value were calculated for the scope for growth estimates from the simulation runs to ease interpretation of the model results.

## RESULTS

The sensitivity analysis on the model was most reactive to shifts in the  $\beta$  parameter of the clam length-key key followed by the mean clam size over the range of simulated values (Table 2, Figure 3). The  $\beta$  parameter, however, does not result in a great shift in predicted crab mass until it gets near the extreme of the range beyond the parameters beyond the values used in the other simulations. The sensitivity analysis demonstrates that the model is more sensitive to clam size, and the resulting energy per animal, than clam abundance.

In the simulations of crab growth, using the averaged river-zone data, the downriver sites had the greatest growth followed by the midriver and the upriver sites for all of the surveys' initial conditions except for the spring simulation (Figure 4, Table 5). In the spring simulation, the midriver zone had the greatest predicted scope for growth.

The scope for growth simulations using the initial conditions averaged for the individual coves, resulted in predictions that varied greatly, depending upon the season survey being simulated (Figure 5, Table 6). In all simulations, the most downriver cove, Timberneck creek, had the greatest scope for growth and Jones Creek a midriver site had the lowest. The spring simulation resulted in no apparent pattern in scope-for-growth along the river axis; however, the summer and fall simulations resulted in a decrease in crab scope-for-growth with distance upriver (except for Jones Creek).

The seasonal simulations resulted in the downriver zone having the greatest scope for growth followed by the midriver and upriver sites (Figure 6, Table 5). The midriver site had a greater scope-for-growth prediction in the spring section of the simulation. In the summer simulation, a pattern of the more downriver sites having greater scope for growth was established and maintained throughout the fall.

## DISCUSSION

With recent improvements in hatchery technology it has become possible to produce large numbers of blue crabs for use in stock enhancement (Zamora et al. 2005). For stock enhancement to be successful, nursery habitats which are recruitment limited and can support additional crabs must be identified (Davis et al. 2005, Seitz et al. 2008). It has been proposed that we can predict the quality of a potential nursery habitat by quantifying the prey species abundance (Seitz et al. 2008). We hypothesized that prey abundance information alone may not provide a complete picture of prey resources, and that the nutritional quality of the prey may also need to be considered in selecting nursery habitats.



In this work, we used modeling to determine if variations in prey quality could significantly affect the scope for growth of the blue crab in similar habitats that have been identified as potential nurseries areas for crab stock enhancement. In this work we use *Macoma balthica* as an indicator organism of the entire benthic community, though the early stages of the hatchery-reared crabs may not be able to feed directly on the clams. *M. balthica* was selected as an indicator organism since their dynamics are indicative of the rest of the benthic community and crab growth correlates with their abundance (Seitz et al. 2005, Seitz et al. 2008). Additionally, high *M. balthica* abundances may decrease the predation pressure on the hatchery-reared crabs by providing ample alternative prey (Seitz et al. 2008).

Surveys of *M. balthica* abundance and individual-based biomass in several similar potential nursery sites uncovered significant variations in quality of the prey, which was not always reflected in the density of clams observed in the snapshot studies (Chapters 2 and 3). We predicted that prey quality, in addition to prey density, would play an important role in determining blue crab growth rates. If prey quality can significantly affect a crab's scope for growth, a potential nursery habitat with abundant prey of poor quality might not have suitable prey resources for crabs. We examined this by using a two-part model, one sub-model predicted crab foraging based upon prey abundance while the second sub-model predicted the amount of material that could be transformed into new crab tissue through a bioenergetic framework.

Sensitivity analysis of this model showed that crab growth was more effected by clam size and average tissue weight per clam than the density of clams in the prey pool. This is most likely caused by the limitations of the functional response form. Beyond a

threshold density, a crab cannot increase its consumption rate any more due to handling time. Clam size creates more variation in the model since it is directly linked to the energy obtained from each prey item.

The combined effects of the forcing factors led to predictions of greater growth in the more downriver coves compared to the most upriver coves in the majority of the simulations. In the summer and fall simulations, as well as the seasonal simulation, based on the three river zones, crab growth was predicted to be at least 60% greater at the more downriver sites compared the upriver ones. The crab growth estimates at the midriver sites were predicted to be between the upriver and downriver growth estimates. This pattern of better conditions downriver related directly to the estimates of clam health observed in the test coves in the surveys (Chapters 2 and 3). The spring simulation did not show this along-river pattern of better growth moving downriver, as the midriver site had the greatest growth estimate. In the spring simulation, the difference between the highest and lowest predicted growth values was only 18.2%, which was the smallest deviation observed in any of the simulations. This is most likely caused by the effect of the large spring recruitment pulse.

*M. balthica* in the Chesapeake Bay tributaries have two recruitment pulses, one in the spring and in the fall, with the spring recruitment being greater (Shaw 1965). This large recruitment pulse reduced the average individual clam size of the population and greatly increased the density (Chapters 2 and 3). This recruit domination evened out the abundance and average size of clams in the coves making the blue crab growth estimates similar. By the summer survey, the recruit pulse was reduced by predation and non-predatory mortality, resulting in the along-river axis patterns in clam abundance, mean

size, and the resulting scope-for-growth estimates. This recruit domination may make areas look like good nursery habitats in the spring, but they actually were predicted to support lower scope for growth over the entire growing season

This along-river-axis pattern of decreasing scope for growth was also present in the simulations using the individual coves conditions. In all simulations growth at the most upriver site was predicted to be at least ~60% lower than the most downriver sites. There was a general trend of decreasing growth moving progressively upriver with a few exceptions to the pattern caused by between-cove variations not due to the effect of river position. One of the consistent exemptions was the prediction from Jones Creek, which produced the lowest scope for growth in all simulations; the scope for growth in Jones Creek was constrained in the spring simulation by a very small average clam size and in the other seasons by low clam density (Table 4).

In all, Jones Creek and the upriver sites do not appear to be suitable habitats for supporting blue crab stock-enhancement activities though some of them appeared to be so in the preliminary surveying stages. For example, upriver sites appeared to be a adequate nursery habitat in the spring survey, but the prey resources degraded later in the season. Therefore, care must be taken in selecting nursery habitats and single snapshot surveys may not be sufficient for quantifying habitat quality.

Our prediction that the upriver sites would support lower crab growth is counter to the trends in the published literature. A caging study performed in the York River observed higher blue crab growth in upriver muddy coves in the York River versus coves near the mouth (Seitz et al. 2005). In the Seitz et al. 2005 study, the downriver areas compared were considerably closer to the mouth (0 to 10 km from the mouth) and well

into the polyhaline section of the river, whereas our study was performed within the mesohaline section of the river. In the previous study, the sites near the mouth had virtually no *M. balthica* indicating that they were different than the downriver sites we observed probably due to the effects of salinity and other stressors not included in our simulations. We suggest that additional crab-growth field trials in the lower mesohaline sections of the river be conducted to confirm or refute the results of our model simulations.

The results of these simulations predict that the upriver coves may be worse nursery habitats than the downriver coves based on crab growth estimates. However, these results may be misleading for the small hatchery crabs that will be released. A crab remaining in an upriver cove throughout its life would experience reduced growth due to the combined effects of reduced prey quantity and quality. However, it is unlikely that a crab would remain in the cove once prey resources became reduced in the summer. Thus the upriver coves may be adequate habitats for enhancement for spring releases of crabs, assuming that crabs would emigrate when the food resources became limiting. However, because the clams appear to be stressed in the upriver coves, there may be some stressor, unobserved in this study, that could reduce the potential for crab survival. We conclude that it would be best to enhance the coves in the lower mesohaline of the York River.

In conclusion, prey quality can be as important or more important than prey abundance for blue crab growth. Also, prey abundance can shift drastically throughout a growing season. Thus, it is necessary to perform multiple surveys of prey abundance and quality to select the best overall nursery habitat to improve the chance for success in blue crab stock-enhancement activities.

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Table 1. Length-weight model parameters ( $\pm$  standard error) calculated for *Macoma balthica* for the York River for the three zones (Chapter 3 / Brylawski et al. in preparation). DR = downriver, MR = midriver, and UR = upriver.

River Position	$\alpha$	$\beta$
DR	$3.4975 \times 10^{-6} \pm 1.5082 \times 10^{-6}$	$3.2653 \pm 0.1304$
MR	$2.7853 \times 10^{-7} \pm 1.8328 \times 10^{-7}$	$4.1109 \pm 0.1365$
UR	$4.1178 \times 10^{-7} \pm 3.2545 \times 10^{-7}$	$3.9825 \pm 0.3577$

Table 2. Sensitivity analysis set up parameters and results. Low and high values were the extreme parameter values from 100 simulations. The mass shift is the difference between the results using the high and low parameter values. The percentage shift is the percentage shift in the mass relative to a 1% shift in the input parameter. L-W  $\alpha$  and L-W  $\beta$  are parameters for the *Macoma balthica* length-weight model.

Parameter	Low Value	High value	Mass Shift	Percentage Shift
Clam abundance (Clams/m <sup>2</sup> )	10	150	6.91	0.61
Clam size (mm)	4	40	112.49	12.61
L-W $\alpha$	$1 \times 10^{-7}$	$5 \times 10^{-6}$	46.15	1.01
L-W $\beta$	1	5	58.72	14.93



Table 3. Average *Macoma balthica* size and density from three surveys (spring, summer, and fall) and an annual average at three river zones in the mesohaline section of the York River, VA, used in the model simulation runs.

Survey	River Zone	Clam mean size (mm)	Clam density (clams/m <sup>2</sup> )
Spring 2007	Downriver	15.93	52.94
Spring 2007	Midriver	15.92	105.39
Spring 2007	Upriver	14.60	149.51
Summer 2007	Downriver	17.96	122.55
Summer 2007	Midriver	17.67	35.29
Summer 2007	Upriver	14.26	53.43
Fall 2007	Downriver	22.09	75.49
Fall 2007	Midriver	22.72	29.90
Fall 2007	Upriver	17.83	18.63
2007 average	Downriver	18.17	96.48
2007 average	Midriver	16.87	57.41
2007 average	Upriver	14.91	81.67

Table 4. Clam size and density values used in the simulations of blue crab scope for growth taken from each cove surveyed in 2007 study. TC = Timberneck Creek, CW = Cattlett Islands, AM = Aberdeen Creek, JC = Jones Creek, PR = Poropotank Bay, and PI = Purtan Islands.

Survey	Cove	Clam length (mm)	Clam density (Clams/m <sup>2</sup> )
Spring 2007	TC	21.10	54.44
Spring 2007	CW	15.07	184.44
Spring 2007	AM	19.31	101.11
Spring 2007	JC	12.90	141.11
Spring 2007	PI	18.09	104.44
Spring 2007	PR	13.36	204.44
Summer 2007	TC	20.66	53.33
Summer 2007	CW	16.77	140.00
Summer 2007	AM	18.17	62.22
Summer 2007	JC	11.94	11.11
Summer 2007	PI	15.81	35.56
Summer 2007	PR	12.88	85.56
Fall 2007	TC	26.77	40.00
Fall 2007	CW	19.67	106.67
Fall 2007	AM	22.00	27.78
Fall 2007	JC	19.50	1.11
Fall 2007	PI	20.63	10.00
Fall 2007	PR	17.02	50.00

Table 5. Percentage difference in the predicted blue crab scope for growth from the simulations using initial conditions from the three surveys performed in 2007, the grand average of all surveys, and the seasonal simulation for the three aggregated river positions. "Highest" indicates that the scope for growth was the greatest in that river position; all other values are the percentage deviation from the highest value.

Survey	River zone		
	DR	MR	UR
Spring	-6.02%	Highest	-18.15%
Summer	Highest	-43.73%	-60.15%
Fall	Highest	-30.45%	-78.43%
Average	Highest	-34.53%	-47.35%
Seasonal	Highest	-14.14%	-59.63%

Table 6. Percentage difference in the predicted blue crab scope for growth from the simulations using initial conditions from the three seasonal surveys performed in 2007 by cove. "Highest" indicates that the scope for growth was the greatest in that cove, all other values are the percentage deviation from the highest value. Cove abbreviations are as in Table 4.

Survey	Cove					
	TC	CW	AM	JC	PI	PR
Spring	Highest	-42.10%	-4.54%	-69.54%	-19.74%	-64.03%
Summer	Highest	-22.71%	-25.39%	-94.69%	-62.48%	-68.77%
Fall	Highest	-30.50%	-49.70%	-98.65%	-87.17%	-63.50%

Figure 1. Locations of the six test covers used in this study along with the Chesapeake Bay Program long term monitoring stations (LE 4.1, LE 4.2).

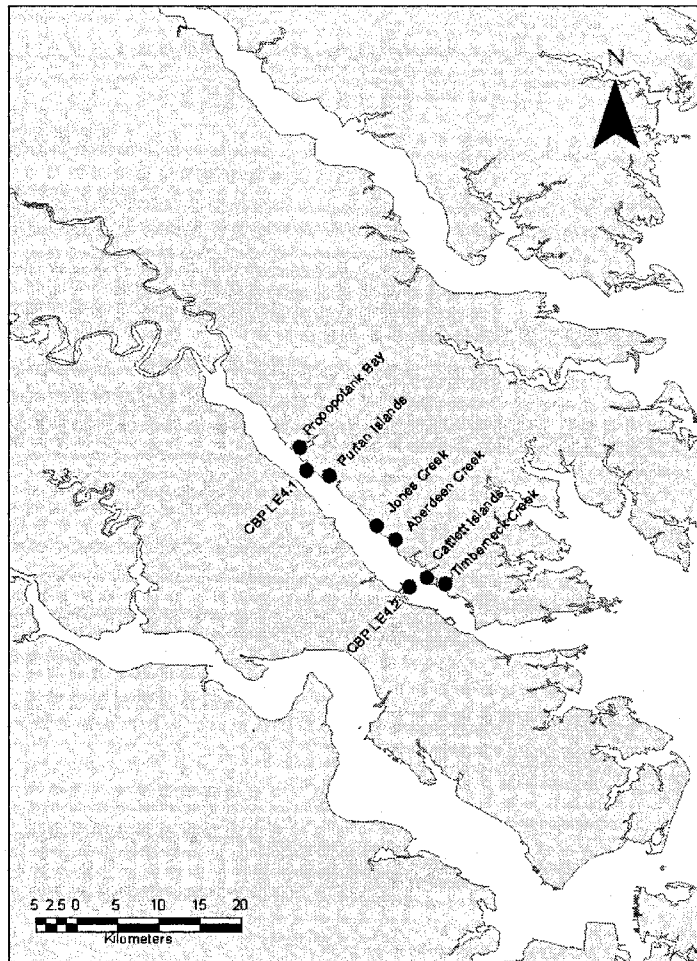


Figure 2. The Stella 8.0 diagram for the blue crab – *Macoma balthica* model system, showing the two foraging (top panel) and bioenergetic (bottom model) sub-models. See Brylawski and Miller (2003) for abbreviations and bioenergetic model details.

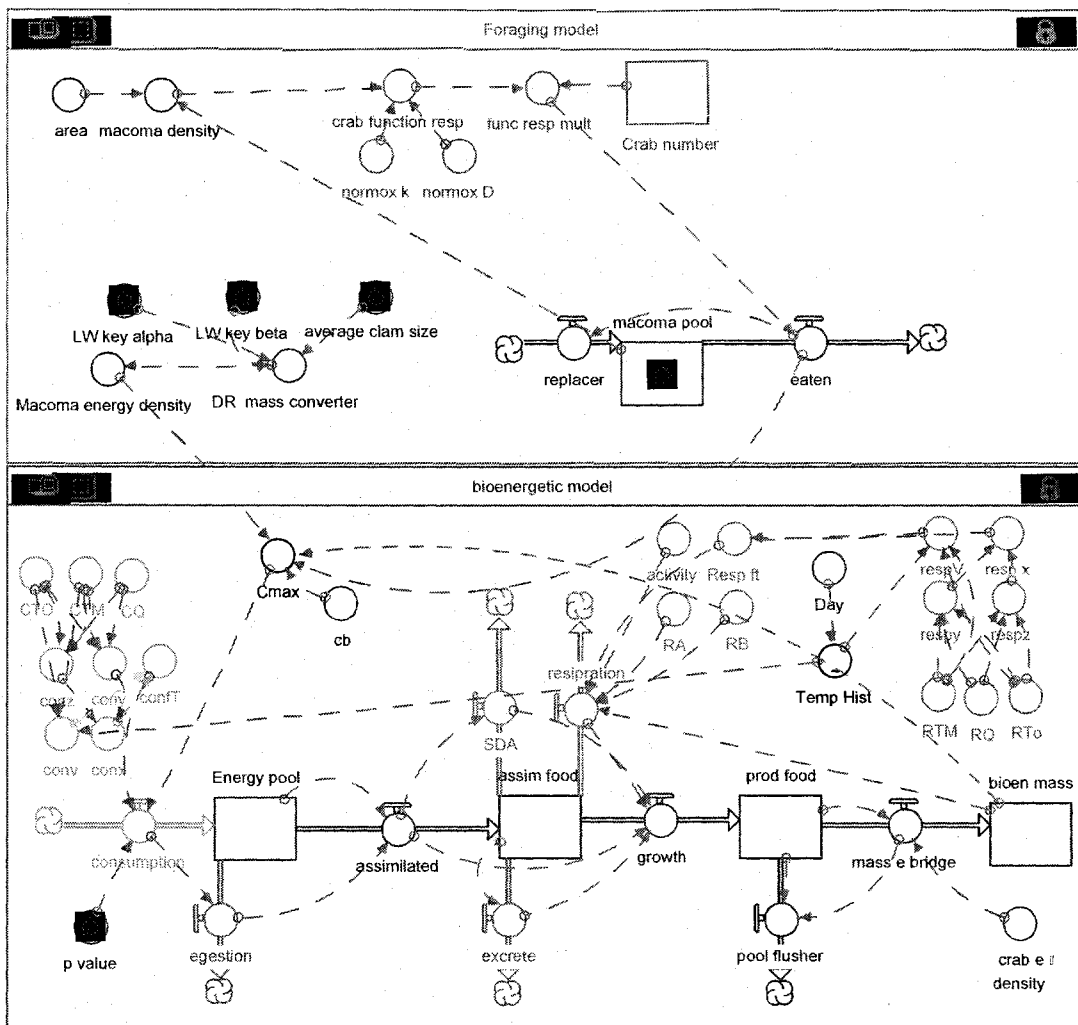


Figure 3. Final mass estimates from the 100 day-long sensitivity analysis simulations as a function of varied initial parameter. (A) clam mean density varied, (B) Clam average size varied, (C) Length-weight key  $\alpha$  parameter varied, and (D) Length-weight key  $\beta$  parameter varied.

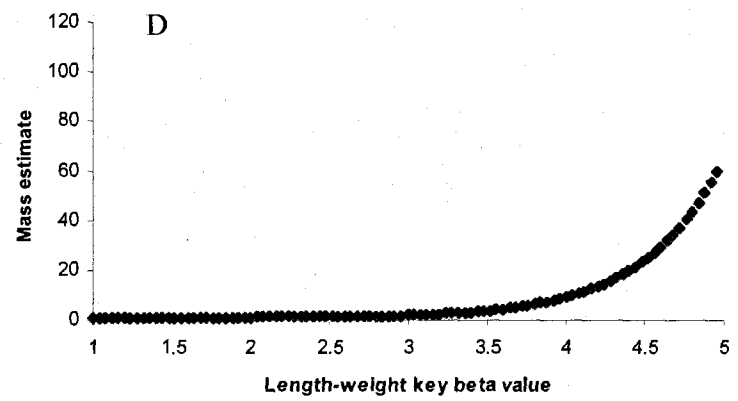
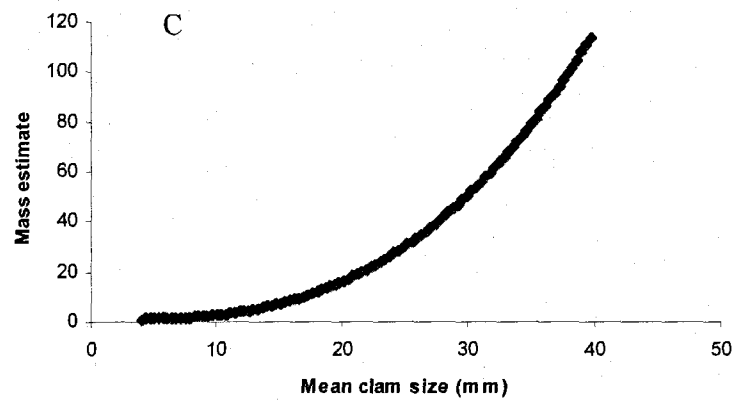
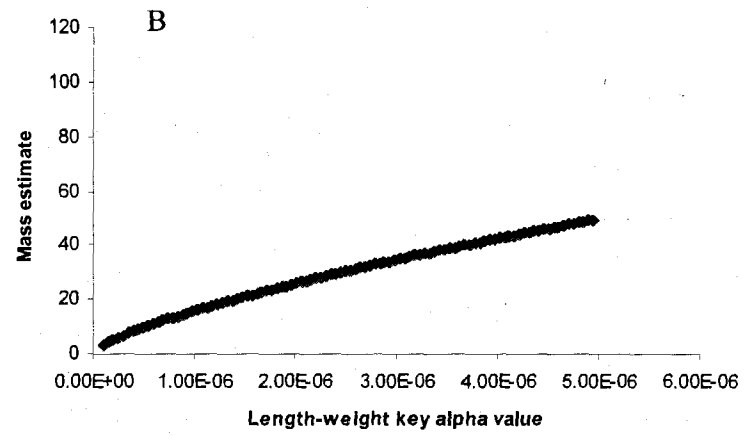
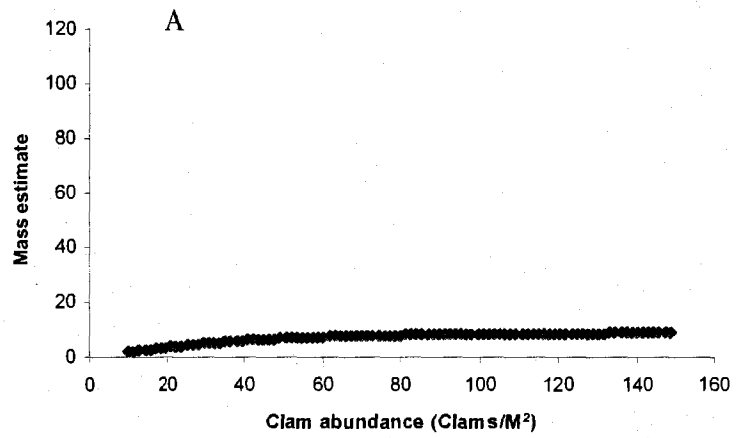




Figure 4. Predicted growth trajectories from year long simulations of crab growth at the three aggregated river positions growth using initial conditions from the: (A) spring 2007 survey, (B) fall 2007 survey, (C) midsummer 2007 survey, and (D) overall averages.

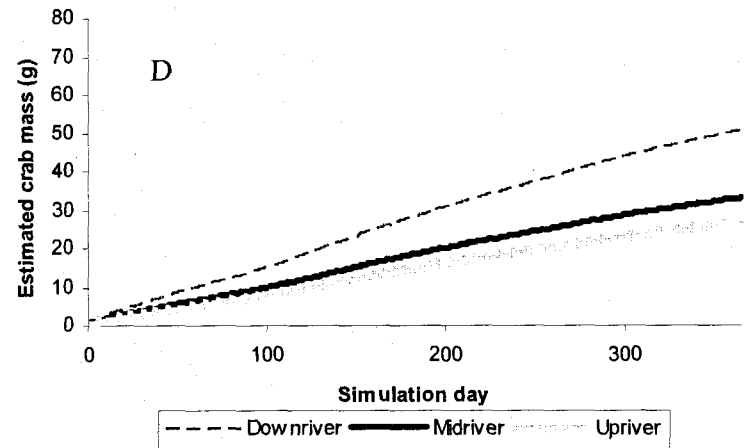
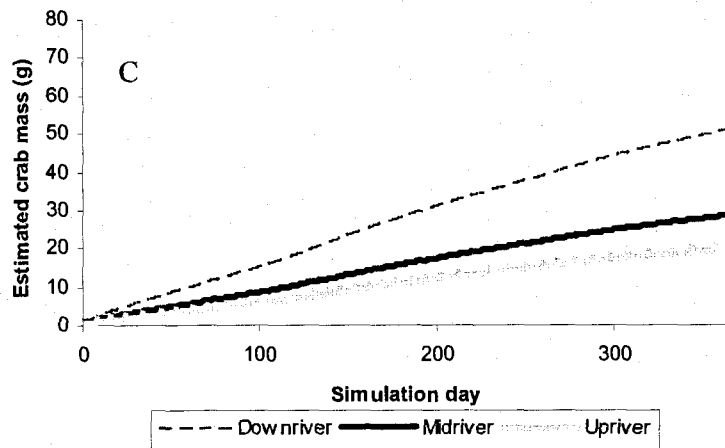
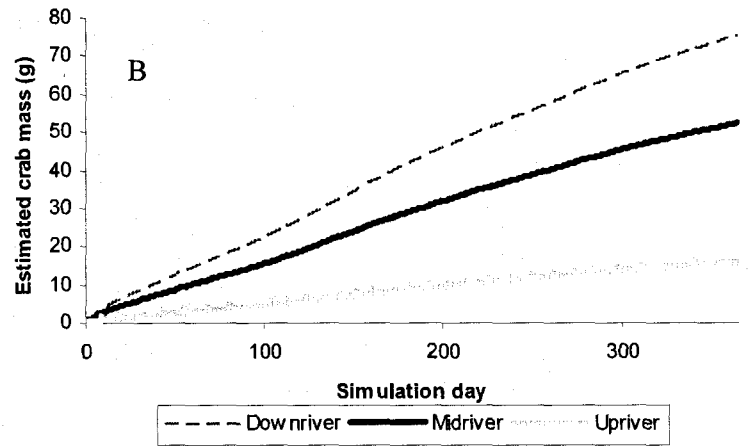
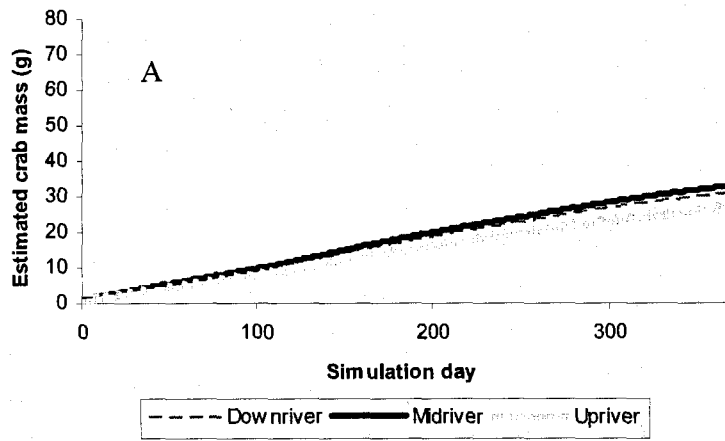
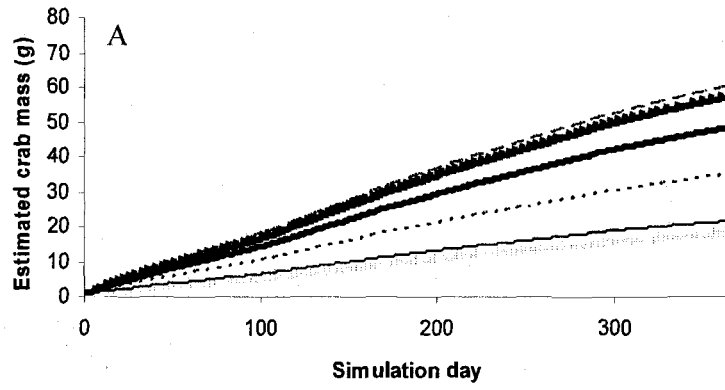
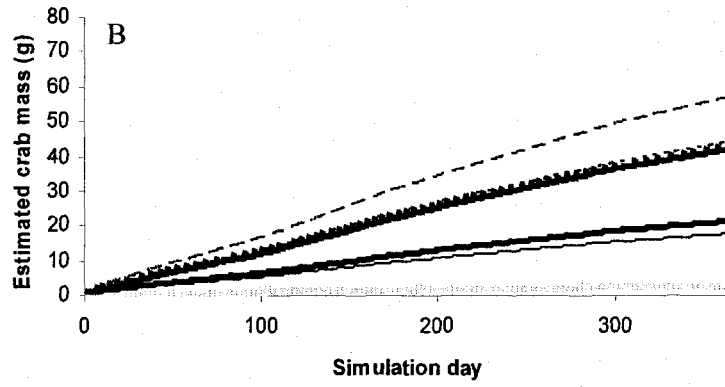


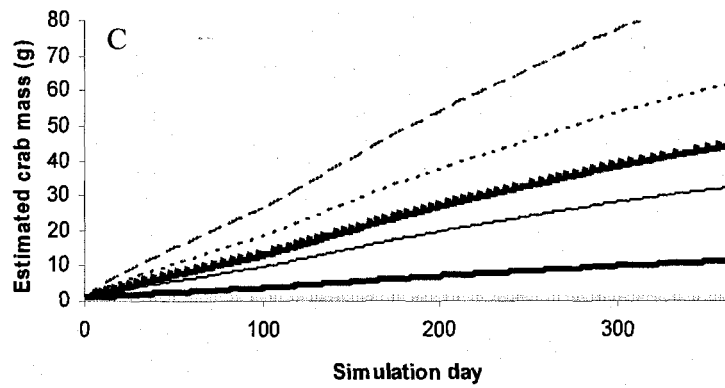
Figure 5. Predicted growth trajectories from year long simulations of blue crab scope for growth in the six separate experimental coves using initial conditions from the: **(A)** spring 2007 survey, **(B)** midsummer 2007 survey, **(C)** fall 2007 survey. Coves are listed in legend from left to right as most downriver to most upriver. TC = Timberneck Creek, CW = Cattlett Islands, AM = Aberdeen Creek, JC = Jones Creek, PR = Poropotank Bay, and PI = Purtan Islands.



--- TC    ..... CW    ——— AM    ..... JC    ——— PI    ——— PR

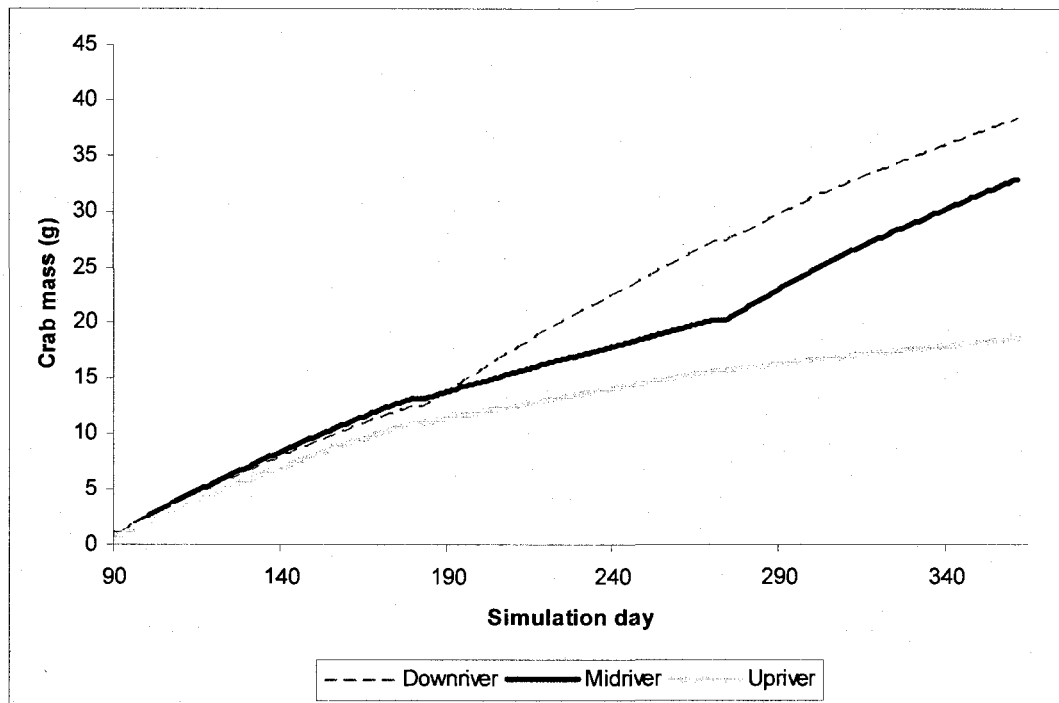


--- TC    ..... CW    ——— AM    ..... JC    ——— PI    ——— PR



--- TC    ..... CW    ——— AM    ..... JC    ——— PI    ——— PR

Figure 6. Growth trajectories for a seasonal simulation of blue crab growth using initial conditions from the 2007 surveys varied every 90 days to simulate seasonal progression for the three river zones. The short horizontal sections at 181 and 272 simulated days are a modeling artifact caused by the switching of the model to the summer and fall conditions.



## **Chapter 6.**

### **Fatty acids and the paradox of enrichment**

## SUMMARY

In 1971 Rosenzweig predicted, through Lotka-Volterra style models, that an increase in the carrying capacity of prey species could decimate consumer populations instead of increasing them. This phenomenon has been called the “paradox of enrichment”. The obvious effects of cultural eutrophication (CE), such as hypoxia induced fish kills, have commonly been used as an example of the paradox. I believe that the paradox can be partially explained by a CE-induced disruption of the food web. CE shifts the primary-producer community composition, which creates micronutrient limitation in higher trophic levels. I examined this hypothesis in a model system comprised of a population of primary consumers, *Macoma balthica*, in areas of the York River, VA, that experience varying nutrient conditions. A series of surveys and experiments identified that clams in areas with greater average nutrient concentrations had lower health, slower growth, and greater non-predatory mortality than clams in less-eutrophic areas. Primary production, as estimated from chlorophyll *a* concentrations, was greater at higher-nutrient locations while the health and growth of clams was reduced, indicating that a “paradox of enrichment” may be occurring. The phytoplankton community in the more eutrophic areas had lower proportions of diatoms relative to dinoflagellates. In a biochemical analysis of clam tissue, the healthier clams from the less nutrient-rich sites had greater proportions of Eicosapentaenoic acid (EPA) relative to other fatty acids. Diatoms are rich in EPA compared to dinoflagellates. Thus, I hypothesized that CE-induced shifts from diatom-based production toward dinoflagellate-based production may be limiting trophic transfer due to a lack of EPA. A *Callinectes sapidus* growth model predicted that these effects could telegraph up the food chain.

## BACKGROUND

The “paradox of enrichment” describes the counterintuitive destabilization of classic Lotka-Volterra style models due to an unsuccessful attempt to increase the carrying capacity of the prey species (Rosenzweig 1971). In his work, Rosenzweig (1971) cautions against increasing the supply of limiting nutrients into a system in an attempt to increase fisheries yield, as it may destroy the stable state and lead to the “decimation of the food species that are wanted in greater abundance”. Though not an intentional increase in prey production, the process of cultural eutrophication (CE) is similar to the suggested intentional boosting of carrying capacity that Rosenzweig warns may lead to disastrous results. Thus, the “paradox of enrichment” has come to be closely associated with CE (Jensen and Ginzberg 2005).

CE is the elevated introduction in the quantity of matter, such as sediments, organic material, and nutrients, allochthonously input into a water body over the pre-anthropogenic (natural) (Cole 1994). In most coastal estuaries, CE refers primarily to an increase in the concentration of phyto-nutrients. CE commonly increases the rates of primary production; however, only a limited portion is passed up the food web relative to what is predicted by ecosystem models (Kemp et al. 2005, Nixon 1995). The failure of material to pass up the food chain has been attributed to the obvious ramifications of CE, namely hypoxia (Diaz 2001, Diaz 2001, Rabalais et al. 2002, Jensen & Ginzberg 2005). Though low oxygen effects can dramatically remove biomass, they apparently do not have long-lasting destabilizing effects on overall ecosystem function (Diaz 2001, Kemp et al. 2005). Instead, hypoxia is most likely due to a disruption in the food web, caused by shunting of the excess primary production into the microbial loop, leading to the



consumption of dissolved oxygen. Though it may aggravate the destabilization of the food web, I believe hypoxia to be a symptom rather than a root cause of the “paradox of enrichment”. The underlying reason why the increased primary production from CE does not flow smoothly up the food web is not fully understood; however, it is hypothesized that it may be caused by shifts in the type of primary producers to less palatable, vulnerable, inedible, or even toxic prey types (Roy & Chattopadhyay 2007).

CE shifts the type of primary production in coastal estuaries away from benthic microalgae, primarily diatoms, to a community dominated by pelagic microalgae, namely dinoflagellates and cyanobacteria; this could prevent the uptake of the increased production (Sin et al. 2000, Cloern 2001, Kemp et al. 2005). I believe that the mechanism preventing the increased primary production from moving up the food web is micronutrient limitation induced by a shift in the primary-producer community to one associated with higher nutrient conditions that is of poor quality for higher trophic levels.

Brett and Muller-Navarra (1997) proposed that variations in the micronutrient composition (Amino Acids, Fatty Acids, Nucleic Acids) are responsible for reshaping the food web and reducing trophic transfer in eutrophic conditions. The lack of a single micronutrient can cause a reduction in growth and production of consumers even if macronutrients and all other micronutrients are in ample supply (Sargent et al. 1990, Sterner and Schultz 1998). If the primary producers do not contain the correct stoichiometric ratios of micronutrients, the consumers may not be able to meet their metabolic demands and be able to take advantage of the increased primary production from CE, thus creating a disruption in trophic flow.

Essential micronutrients, such as vitamins or some fatty acids, are most likely to be limiting in aquatic food webs since they cannot be *de novo* synthesized by consumers (Sargent et al. 1999, Hendriks et al. 2003). Essential fatty acids (EFAs) may be limiting in aquatic food webs (Brett and Muller-Navarra 1997, Muller-Navarra et al. 2000). EFAs are usually long chain (18+ carbons) polyunsaturated fatty acids (PUFAs), such as the  $\omega$ 3 fatty acids (Sargent et al. 1999). EFA deficiency has been observed to reduce growth and production of aquatic consumers provided an otherwise sufficient diet (Marsh et al. 1989, Cares et al. 1998, Wacker et al. 2002). The concentration and stoichiometric ratios of EFAs have been observed to shift due to nutrient-induced changes in primary producer community composition in mesocosm experiments; however, this has yet to be directly documented in a natural estuarine system (Pond et al. 1998).

This CE-induced trophic disruption may have been indirectly documented in the Dutch Wadden Sea for the Baltic clam, *M. balthica*. *M. balthica* is a small thin-shelled clam commonly found in the shallow coastal bay and rivers of the northern hemisphere (Kamermans et al. 1999). In the Wadden Sea, *M. balthica* growth was strongly correlated with the proportion of diatoms in the primary-producer community relative to the other microalgae taxa (Beukema & Cadée 1991). There was also a reduction in the number of diatoms, relative to the more opportunistic dinoflagellates, during a period of increasing cultural eutrophication in the sea (Beukema & Cadée 1991). Diatoms in general contain greater proportions of PUFA compared to dinoflagellates. Diatoms commonly have a greater proportion of their PUFA in the form of Eicosapentaenoic acid (EPA, 20:5(n-3)), while dinoflagellates have greater amounts of docosahexaenoic acid (DHA, 22:6 (n-3)) (Pond et al. 1998, St. John et al. 2001). In a separate Wadden Sea study, *M. balthica*'s

condition index was strongly related to its ratio of EPA:DHA, with EPA concentration driving overall clam health (Jarbeski et al. 1986). These two studies taken together propose a causal agent linking CE to the health of *M. balthica* though a shift in the availability of EPA.

In this work, I document an example of CE-induced trophic disruption via the mechanism of EFA limitation in an estuarine ecosystem. I designed a multipart study comprised of field surveys and experiments combined with analyses of potential forcing functions and the lipid composition of an indicator organism. The results of this study were combined to extrapolate the potential effect of CE-induced trophic disruption on a secondary consumer through the use of a scope-for-growth bioenergetic model for blue crabs.

## RESEARCH OVERVIEW

I utilized *M. balthica* as our indicator organism due to its wide distribution, great abundance, ease of collection, and general tolerance to stress. The mesohaline section of the York River, VA, a sub-estuary of the Chesapeake Bay, was selected as the test area for this study. The York is fed by two rivers whose watershed is comprised of a mix of agricultural and residential areas. There is also a paper processing plant at the head of the York. The combined effects of runoff and the point sources of nutrients have created the classic pattern of nutrient enrichment common in riverine estuaries where the highest nutrient concentrations are found in the upper oligohaline region and decrease moving downriver, due to effects of dilution with seawater and consumption by primary producers (Ouboter et al. 1998, Boesch 2002). This nutrient gradient allowed us to

explore the potential for CE-induced trophic disruption in one riverine system by selecting test sites spaced along the river axis with differing nutrient conditions.

Six coves were selected within the mesohaline section of the river to act as test areas. The test coves had similar biotic and abiotic conditions such as fringing marsh, shoreline type, and average depth, to eliminate confounding variables. These test coves were grouped into the following three river zones for data analysis: upriver (Poropotank Bay [PR] and Purtan Islands [PI]), midriver (Jones Creek [JC] and Aberdeen Creek Mainstem [AM]), and downriver (Cattlett Islands [CT] and Timberneck Creek [TC]). The upriver sites had the highest overall nutrient concentrations and were considered the highly culturally eutrophic test areas, whereas the midriver sites and downriver had proportionally lower levels of nutrient loadings (Table 1).

#### ***M. balthica* growth and non-predatory mortality**

In the first part of this study (Chapter 2, Brylawski et al., in prep.), I aimed to determine if the paradox of enrichment may be occurring in the York River by observing the dynamics of *M. balthica* in the upriver and downriver zones. From 2004 to 2006, I performed a series of one-year mark-and-recapture trials, to determine if growth of *M. balthica* was affected by river position and thus the intensity of CE. Due to the high predation pressure, I used enclosures over the test plots which may have affected total growth but should not have affected the relative growth rates. Use of the cages also allowed the estimation of non-predatory mortality. Paired collections of ambient clams were taken at the time of cage collection and used to estimate condition index and to create an area-specific length-weight key. Condition index is a proxy for overall clam

health and was calculated by dividing the ash-free dry mass (AFDM) by the shell length (in mm) and multiplying the results by 1000. Animals with low condition index are thought to have reduced biomass due to food limitation or stressor-induced autocatabolism (Wene & Stczynska-Jurewicz 1985).

Growth estimates were used to parameterize von Bertalanffy growth models. The area-specific length-weight keys were used to transform the growth models from size-based to mass-based. Estimated growth was lower at the upriver more eutrophic sites (Figure 1). Condition was also reduced at the upriver sites, which is reflected in a reduced length-weight relationship. Non-predatory mortality was also significantly greater in the upriver, more eutrophic sites (Figure 2).

The lower growth and higher mortality at the sites with greater CE may indicate that the paradox of enrichment affects the York River *M. balthica* population. However, I cannot conclusively attribute the effects to CE because salinity also varies among sites, though salinity change is most likely a minor stressor as it changes less > 4.2 psu among locations based on 10-year average salinity measurements (Table 1).

### **Determination of most influential forcing factor**

To determine which forcing factor was most responsible for the reduced health and growth of clams at the upriver sites, I conducted three surveys in 2007 of ambient clam abundance and potential forcing factors (Chapter 3, Brylawski et al., in prep.). In addition to the four sites used in the mark-and-recapture study (Chapter 2), two midriver sites were also used. I measured salinity, sediment grain size, sediment organic carbon,

benthic chlorophyll *a*, water column chlorophyll *a*, and the taxonomic groupings of phytoplankton species along with the abundance, condition, and biomass of clams.

An overall condition index was calculated for all six locations and the three aggregated river zones. Data from the forcing factor surveys were combined with the condition index values observed in the ambient clams from the 2004-2006 studies. Condition index decreased with distance from river mouth (Figure 3). I created a series of regression models for the potential forcing factors and calculated Akaike's information criterion scores (AIC) to determine the dominant forcing factor(s). The forcing factor that most closely matched the trends in condition index was the Diatom:Dinoflagellate ratio, which had a much greater model probability than salinity and the other potential forcing factors (Table 2).

Condition index was higher in areas with a greater proportion of diatoms and reduced in areas that were more dominated by dinoflagellates. The number of diatoms was lower in the upriver zone while the nutrient input was great, supporting the hypothesis that nutrient conditions drive shifts in the primary-producer community. Thus, I believe that CE-induced shifts in the primary-producer community composition are responsible for decreasing clam condition and growth rates, which provides evidence for disruption of trophic flow. These results suggest that the primary producer community in the more eutrophic sites was less usable by the clams for either mechanical (handling or gape limitation) or nutritional reasons. Because *M. balthica* are effective filter and deposit feeders, I hypothesized that clam condition was reduced due to differences in the nutritional content of the primary producers in more eutrophic locations.

### Fatty acid composition of *M. balthica*

I hypothesized that the relationship between the diatom:dinoflagellate ratio and condition index may be caused by a shift in the fatty acid stoichiometry of the primary producers. A series of biochemical analyses was run to determine if clams from the sites used in the forcing-factor surveys had observable differences in fatty acid composition (Chapter 4, Brylawski et al., in prep.). I examined the fatty acid composition of the clams rather than the primary producer community to eliminate the effects of active selection of particles by the clams.

There was an observable trend of increased saturated fats in the more upriver sites. The ratio of EPA to DHA decreased moving away from the river mouth (Figure 5a). Condition index also declined with decreased EPA:DHA ratio (Figure 5b). The EPA:DHA ratio appears to be strongly related to the diatom:dinoflagellate ratio indicating a possible link between the primary producer community composition and EFA stoichiometry (Figure 6). However, no differences in the total lipid density in the clam tissue were detected among the different river zones. This indicates that there is most likely not a limitation in the total amount of fatty acids provided by the primary producers in the more eutrophic sites, though the types of lipids (EPA vs. DHA) is important.

The primary-producer community in the more eutrophic upriver locations appears to be shifted toward taxa that do not provide sufficient EPA to meet the metabolic demands of *M. balthica*. This lack of EPA is reflected in the lower condition, slower growth, and higher non-predatory mortality in the more eutrophic areas. Thus, the increase in primary production due to CE may not be fully utilized by consumers due to a

shift in the stoichiometry of essential fatty acids, potentially explaining how the “paradox of enrichment” phenomenon occurs in the York River.

### **Modeling effects on higher trophic levels**

In the experiments, surveys, and biochemical analyses, I determined that clams in areas with greater nutrient loadings have reduced health most likely due to shifts in the primary-producer community and facilitated through the mechanism of EFA limitation. To determine if the reduction in clam health could affect other trophic levels, I created a model to examine the effect of clam condition on the growth of the blue crab, *Callinectes sapidus*. I created a two-part model by combining a foraging model with a bioenergetic model of crab growth (Chapter five, Brylawski et al., in prep.).

The model was used to predict the scope for growth of crabs based on the abundances and individual biomass of clams in each of the three river zones from the 2007 forcing factor surveys. A simulation predicting the growth of crabs throughout the entire growing season estimated that variation in prey resources could lower blue crab growth by more than 40% (Figure 7). Thus, it appears that the CE-induced constriction of trophic flow may be able to transfer up the ecosystem and reduce the potential production of higher trophic levels as predicted by the paradox of enrichment hypothesis.

### **CONCLUSIONS**

In this research, I have observed the potential for Cultural Eutrophication (CE) to reduce the health and growth of multiple trophic levels. There is evidence that CE shifts the primary-producer community to one dominated by phytoplankton of limited



nutritional quality. Though CE increased the total production of the phytoplankton pool, *M. balthica* was not able to fully utilize the extra production due to a lack of EFAs. Lack of EFA in the eutrophic phytoplankton community may lead to reduced health and increased mortality of *M. balthica*. Modeling indicated that this trophic disruption may significantly alter the dynamics of higher-trophic-level animals (secondary consumers).

Though our study provides evidence that CE can alter and potentially disrupt the food web, additional studies should be run to confirm these results and to determine if this pattern is present in systems other than the York River. In addition, laboratory and field-enrichment experiments could confirm if micronutrient limitation is occurring. However, the evidence presented in this dissertation appears to make it a viable hypothesis explaining “the paradox of enrichment” in the York River.

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Table I. Water-quality parameter estimates ( $\pm 1$  standard error) from 10 years of Chesapeake Bay Program monitoring data (1/1/1998-12/31/2007, LE 4.1 and LE 4.2) for the upriver and downriver sites. Estimates for the midriver test position have been estimated using linear interpolation based on river kilometer.

Parameter	Location			Units
	upriver / LE 4.1	midriver	downriver / LE 4.2	
Chlorophyll a	13.326 $\pm$ 8.452	11.654	10.590 $\pm$ 10.182	UG/L
Salinity	13.436 $\pm$ 4.206	15.060	17.613 $\pm$ 3.762	PPT
Temperature	16.610 $\pm$ 0.781	16.493	16.31 $\pm$ 0.756	$^{\circ}$ C
Total Nitrogen	0.687 $\pm$ 0.223	0.660	0.654 $\pm$ 0.071	MG/L
Total Phosphorous	0.078 $\pm$ 0.004	0.069	0.064 $\pm$ 0.006	MG/L

Table 2. Results from the AIC-based information-theoretic analysis of least-squares regression models of *Macoma balthica*'s condition index, as observed in all surveys, as a function of the observed forcing factors and the 10-year aggregated water-quality information (Chapter 3, Brylawski et al in prep.). Models are arranged from best to worst and the model with bold is considered the most viable model in the set.

Parameter	AIC	$\Delta$ AIC	Model Probability
<b>Observed Dinoflagellate :Diatom Ratio</b>	<b>6558.56</b>	<b>0.00</b>	<b>0.7916</b>
Observed Water Column Chlorophyll <i>a</i>	6562.73	4.17	0.0986
Observed Grain Size	6562.73	4.17	0.0986
10-year Water Column Chlorophyll <i>a</i>	6570.08	11.52	0.0025
10-year Total Phosphorus	6570.08	11.52	0.0025
10-year Salinity	6570.08	11.52	0.0025
10-year Total Nitrogen	6570.30	11.74	0.0022
Observed Salinity	6571.19	12.63	1.44E-03
Observed Sediment Chlorophyll <i>a</i>	6598.14	39.58	2.01E-09
Observed Sediment Organic Carbon	6677.68	119.12	1.07E-26

Figure 1. Results of a von Bertalanffy growth model parameterized for *Macoma balthica* from the upriver and downriver zones of the York River, VA. Thick lines are the predicted clam size starting from a 1 mm clam. Symbols and thin lines are the predicted ash-free dry mass of the animal.

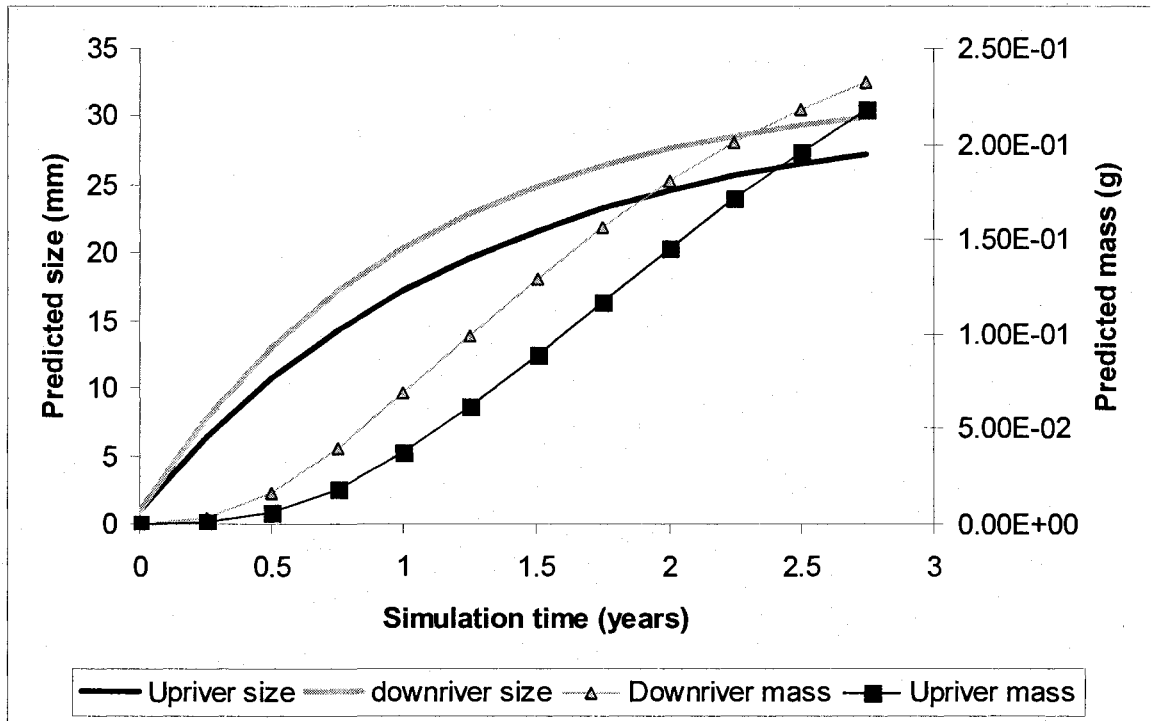


Figure 2. Mortality of *Macoma balthica* from three mark-and-recapture caged growth trials from four test coves along the river axis. This was a proxy for non-predatory natural mortality though was probably inflated over the natural population levels due to caging and handling effects. TC was the most downriver cove and coves are listed from left to right as downriver to upriver. Error bars are 1 standard error of the mean. TC = Timberneck Creek, CW = Cattlett Islands, PR = Poropotank Bay, and PI = Purtan Islands.

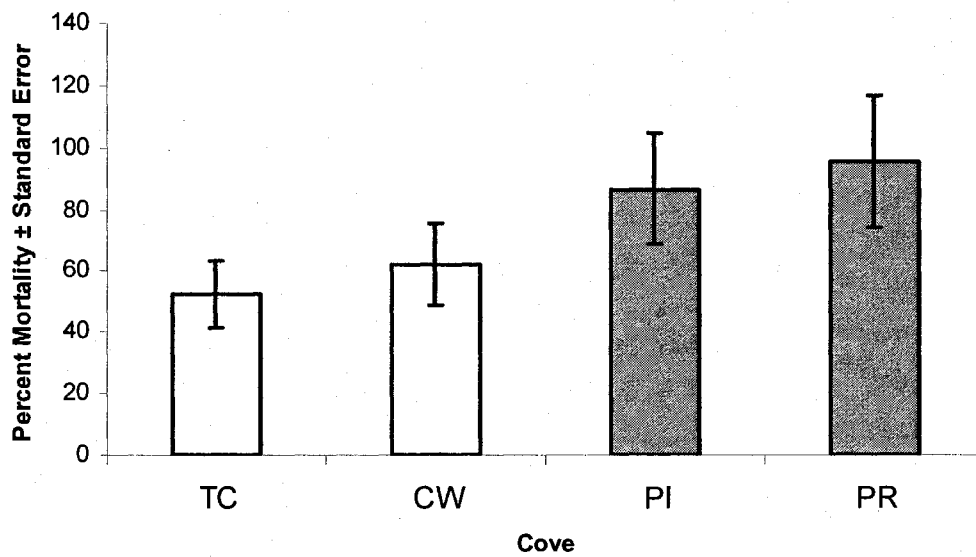




Figure 3. *Macoma balthica* condition index as a function of distance from the York River mouth. Black squares represent the average for the individual test coves. The grey circles are the average values for the three aggregated river positions. Data from all surveys of ambient clams were combined.

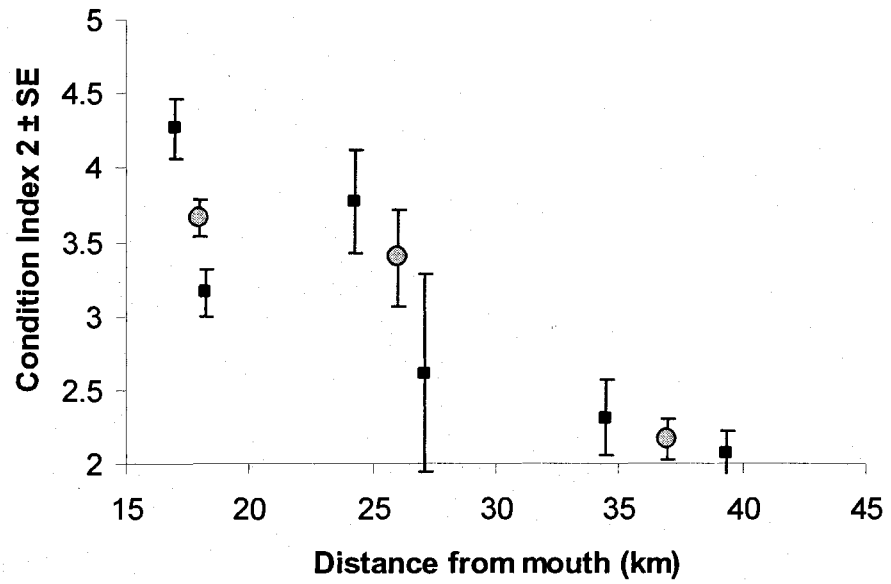


Figure 4. (a) Mean proportion of Dinoflagellates to Diatoms in the water column at the three river positions in the mesohaline section of the York River, VA, for the three sampling events. (b) The Proportion of Dinoflagellates to Diatoms at the six test covers for the sampling events vs. distance from the river mouth with an exponential trend-line overlaid.

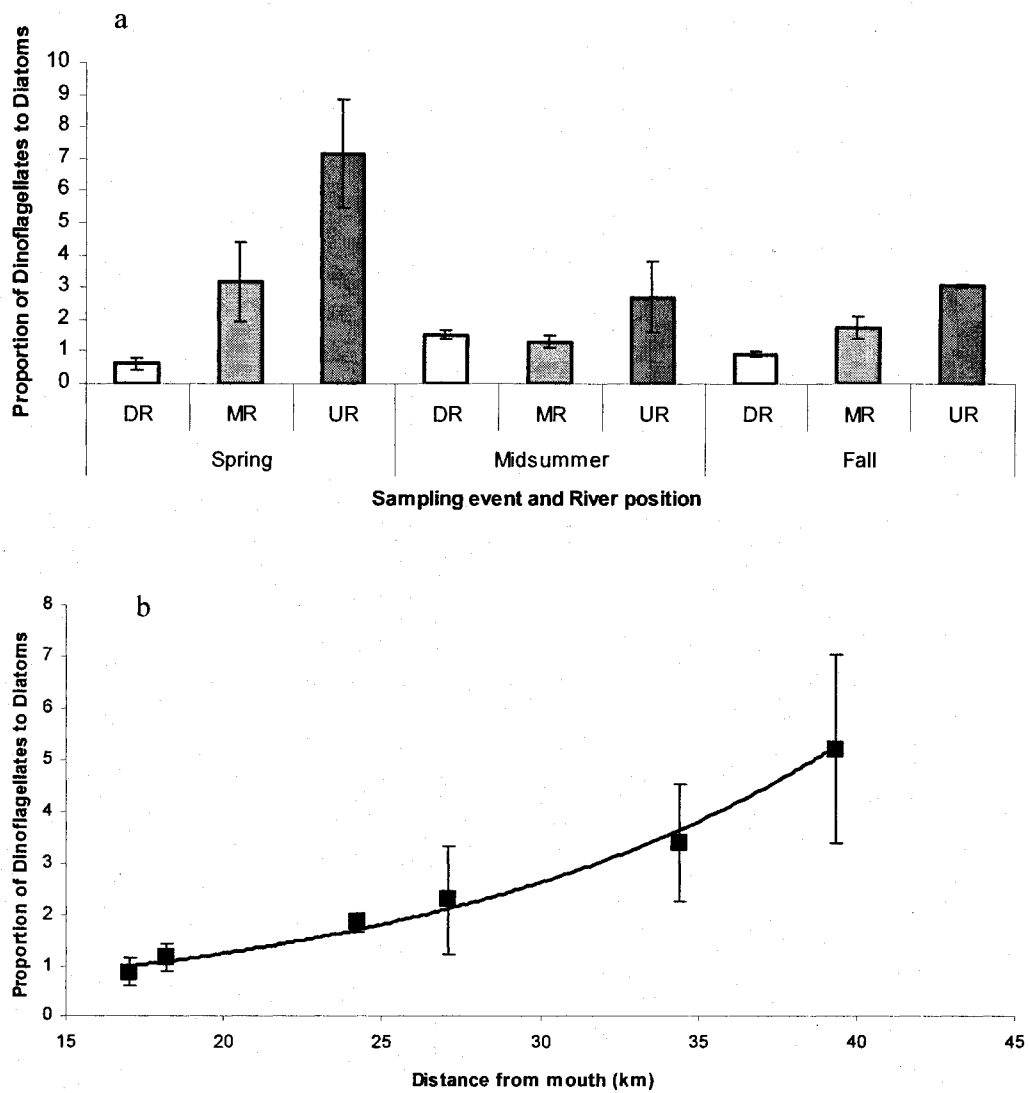


Figure 5. (a) Ratio of EPA to DHA of *Macoma balthica* as a function of distance from mouth in the York River, and (b) as a function of average clam condition index.

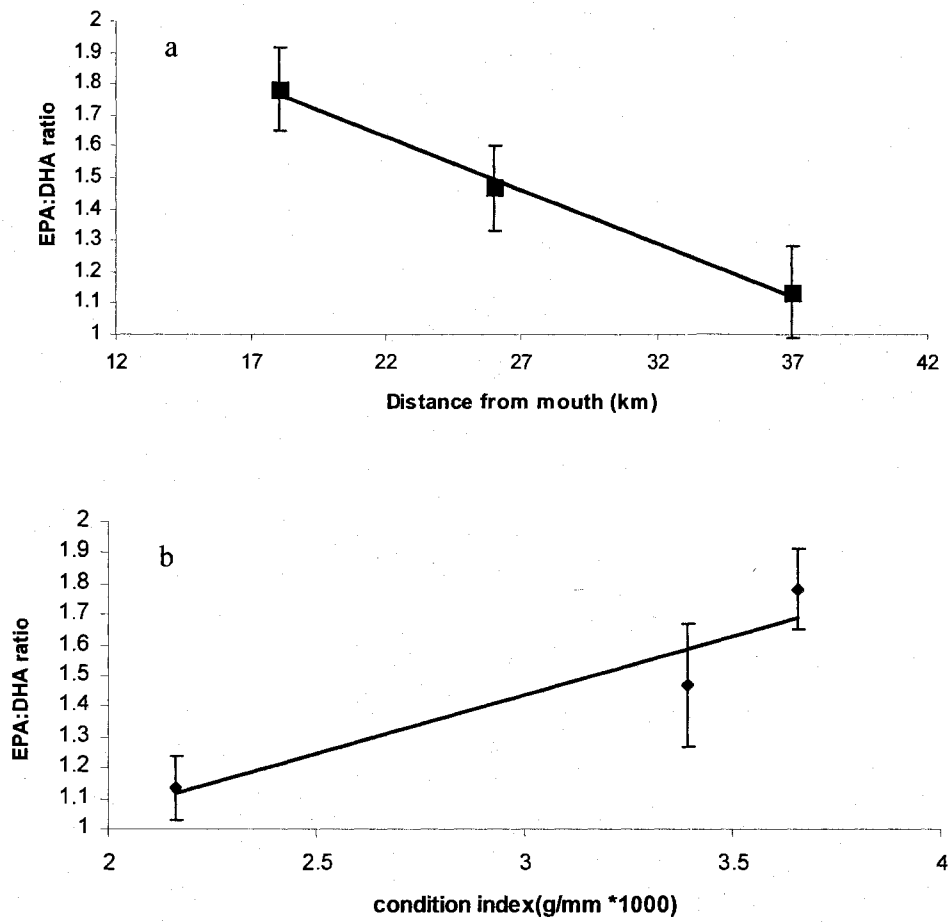


Figure 6. *Macoma balthica* tissue EPA:DHA ratio as a function of Dinoflagellate:Diatom ratio as observed in the York River, VA.

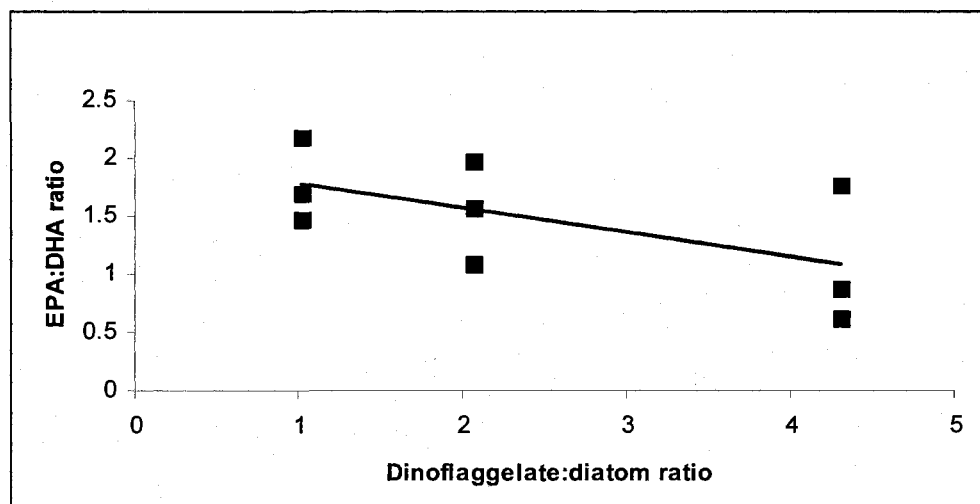
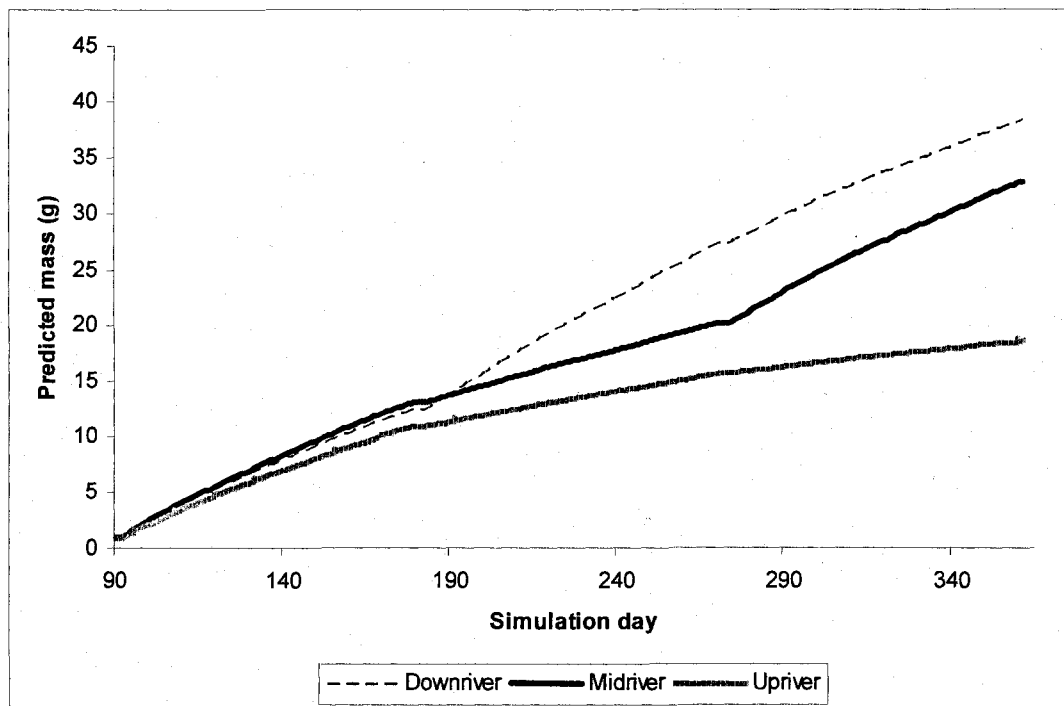


Figure 7. Growth trajectories for a seasonal simulation of blue crab growth using initial prey resource conditions from the 2007 surveys of *Macoma balthica* abundance and individual-based biomass. The simulations varied every 90 days to simulate seasonal progression. The short horizontal sections at 181 and 272 simulated days are a modeling artifact caused by the switching of the model to the summer and fall conditions.



## Vita

Born in Maryland, September 21 1976. Graduated from Eleanor Roosevelt High School, Greenbelt, MD in 1994. Received a B.A. in biology from St. Mary's College of Maryland, St. Mary's City, Maryland in 1998. Completed a M.S. in Marine Ecological Estuarine Science from the Chesapeake Biological Laboratory, University of Maryland Center for Ecological Science, Solomon's, Maryland in 2002. Entered a Ph.D. program at the Virginia Institute of Marine Science, The College of William and Mary in 2003.