HYPOXIA AND *MACOMA BALTHICA*:

ECOLOGICAL EFFECTS ON A KEY INFAUNAL BENTHIC SPECIES

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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

William Christopher Long 2007

APPROVAL SHEET

This dissertation is submitted in partial fulfillment of

The requirements for the degree of

Doctor of Philosophy

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Approved, September 2007

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Dedication

To my dear wife, Jordana:

You are marvelous.

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ACKNOWLEDGMENTS

This dissertation would not have been possible without the assistance, support, and encouragement of a vast number of people. First, I must recognize and thank my advisor, Rochelle Seitz, who has guided me through this phase of my development as a scientist. Additional thanks goes to my committee members, all of whom have been integral to this process: to Steve Kaattari for his oversight in all things immunological, to Sebastian Schreiber for being the happiest mathematician I know (and a darn good modeler), to Rom Lipcius for statistical advice, to Bob Diaz for sharing his vast knowledge of hypoxia, and to Donna Wolcott for breaking every known physical law in getting manuscripts read, criticized, and returned, and for her gentle encouragement.

Thanks too, are due to the many fellow students and staff who helped in the field and laboratory. In especial, Bryce Brylawski was considerably involved in the planning, design, execution, and writing of nearly every part of this dissertation, and, additionally, helped maintain my insanity by providing many hours of conversation in the lab while picking dead worms out of detritus and listening to bad fantasy novels on CD. I also thank Mike Seebo, whose mad SCUBA skills and willingness to dive in zero visibility mean that he has probably spent far more time groping for me in the dark than he would have wished; Katie Knick, who taught me everything I know about identifying polychaetes; and all the members of the Conservation Ecology group for their willingness to get down and dirty. I am also most grateful for the support of the Kaattari Lab, especially to Chris Earhart and Erin Bromage for help with making and using monoclonal antibodies, and to Mary Ann Vogelbein for helping me find everything in the lab.

I also thank the many places that funded this research. I was supported by both a VIMS Dean's Fellowship and an EPA GRO fellowship during my tenure here. Further financial support was provided by a NOAA National Sea Grant, Essential Fish Habitat Program, by the Commonweath of Virginia, a grant to the Blue Crab Advanced Research Consortium from the NOAA Chesapeake Bay Program, a VIMS student research grant, and a VIMS GSA grant.

A special thanks goes to my family for their moral support and encouragement. My dear wife Jordana deserves particular recognition; she has not only been the most supportive wife imaginable, but has also gotten muddy doing boxcoring, nursed our firstborn while writing down DO measurements on Saturday mornings, and provided extensive criticism for every word I've written from the title of my prospectus to the last word of this dissertation.

Finally, I recognize my Lord and Savior Jesus Christ, for whom and through whom I do all things.

ABSTRACT

Hypoxia, low dissolved oxygen, is an important environmental stressor in estuarine systems. In this dissertation, I examine the effects of hypoxia on the macrobenthic communities of the York and Rappahannock Rivers, Chesapeake Bay, USA, and in particular its effects on the infaunal clam *Macoma balthica*. A survey of the macrobenthic community was performed using box-coring before and after hypoxia in 2003 and 2004 in both rivers. Hypoxia was associated with a change in the macrobenthic community towards smaller, shorter-lived, opportunistic species; and a substantial decrease in biomass. *M. balthica* recruited into all areas of the river but suffered local extinction in hypoxic areas, demonstrating that these areas represent sink habitats. I developed an enzyme-linked immunosorbent assay to quantify fecundity in *M. balthica* and used it determine the effect of hypoxia on clam fecundity. In laboratory experiments performed in 2005 and 2006, *M. balthica* migrated toward the sediment surface and decreased egg production in response to hypoxia. In field caging experiments, performed during the summers of 2005 and 2006, episodic hypoxia caused a three-fold increase in the rate of predation on *M. balthica*, suggesting that the behavioral responses of *M. balthica* to hypoxia make it more vulnerable to predation. These results further suggested that hypoxia may change the functional response of epibenthic predators to *M. balthica* from a stabilizing type III to a destabilizing type I or II. Using the results of the previous experiments, I constructed a density-independent model of the *M. balthica* population, which predicted that increasing the spatial extent and duration of hypoxia could cause the population to decline toward extinction. A second model, which incorporated density-dependent predation, predicted that, under mild hypoxic conditions, trophic transfer of biomass from *M. balthica* to predators could be enhanced, but that increasing the severity of hypoxia would decrease trophic transfer. The model further predicted that increasing hypoxia would decrease the resilience of the *M. balthica* population to disturbance, making functional extinction of the population more likely. This body of work underscores the negative effects of hypoxia on the levels of the individual, the population, and the ecosystem.

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CHAPTER 1:

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WORSENING EFFECTS OF HYPOXIA ON MACROBENTHOS COMMUNITY STRUCTURE IN CHESAPEAKE BAY TRIBUTARIES

ABSTRACT

We assessed the effects of hypoxia (DO < 2.0 mg l^{-1}) on the macrobenthic communities in the York and Rappahannock Rivers, Chesapeake Bay, by box-coring before and after hypoxic episodes in 2003 and 2004. Hypoxia was severe in both years and was associated with a decrease in biomass and a shift in community structure toward more opportunistic species in both the York (which has not been documented previously) and the Rappahannock. Rapid recovery was observed in the Rappahannock due to strong recruitment and increasing benthic biomass. Recovery in the York was less robust but still detectable, suggesting that complete recovery could occur within 2-3 years in the absence of further hypoxia. Analysis of the frequency of hypoxia in the York over the last 22 years, unfortunately, indicates that it is increasing. Previous work from ~20 years ago could not detect substantial changes in the macrobenthic community structure due to hypoxia in the York; however, this study did. We conclude that hypoxia has become a more important environmental problem in the York than it has been in the past and that it may be capable of chronically depressing the benthic community. Hypoxia likely has a negative effect on the estuarine food web, as a decrease in macrobenthic biomass could decrease the quantity of food available to benthic predators.

KEY WORDS: Hypoxia, Benthic community, macrobenthos, low dissolved oxygen, environmental stressor.

INTRODUCTION

Development of hypoxia

Hypoxia, or a dissolved oxygen (DO) concentration below 2.0 mg l^{-1}, is a serious ecological problem in aquatic systems ranging from freshwater lakes to coastal shelves around the world (see Diaz & Rosenberg 1995, Diaz 2001, and Gray et al. 2002 for affected areas). Almost 46% of US estuaries are affected by hypoxia (Diaz 2001, Gray et al. 2002), which is exacerbated by land-based nutrient runoff.

The Chesapeake Bay is one of the most productive estuarine systems that suffer from hypoxia. The annual cycle of hypoxia in the Chesapeake is predictable and well understood (Officer et al. 1984, Kemp et al. 2004). The yearly spring freshet carries nutrients into the Bay, resulting in large blooms of phytoplankton. As the phytoplankton die, they sink through the water column and concentrate near the bottom, leading to an increase in respiration of decomposers. Coupled with a system strongly stratified by freshwater inputs and surface-water heating, this increase in respiration reduces the concentration of dissolved oxygen below the pycnocline, leading to hypoxia or anoxia in the deeper or more strongly stratified areas of the Bay (Seliger et al. 1984, Officer et al. 1984).

Hypoxia and the benthic community

Seasonal hypoxia affects the benthic macrofauna in a manner similar to the way periodic fires affect prairie floral species: stressors keep longer-lived ‘climax community’

species from dominating the habitat. Many macrobenthic species are adapted to mild hypoxic conditions of short duration (less than 2 weeks) and low intensity (DO > 1.0 mg l^{-1}), but increasing severity of hypoxia can defaunate whole regions (Llansó 1992, Diaz & Rosenberg 1995, Kemp et al. 2004). In Chesapeake Bay, areas that experience mild hypoxia, such as the York River, historically have shown no change in the structure of the macrobenthos as compared with similar normoxic areas, which usually includes a mix of long- and short-lived species dominated by bivalves in the mesohaline regions (Boesch & Rosenberg 1981, Dauer et al. 1992, Diaz et al. 1992). In contrast, areas experiencing severe hypoxia, such as the Rappahannock River and the Chesapeake Bay mainstem, show large differences in the macrobenthic communities in association with hypoxic compared to normoxic areas (Dauer et al. 1992, Llansó 1992, Kemp et al. 2004). In such regions, diversity and total biomass tend to be lower, and the community is likely to be dominated by short-lived opportunistic species, such as euryhaline annelids (Dauer et al. 1992), instead of longer-living species, such as large bivalves.

The longer-lived bivalve *Macoma balthica* is the biomass-dominant species in the mesohaline regions of the Chesapeake (Holland et al. 1977, Holland et al 1987), and it is an important food source for the blue crab, *Callinectes sapidus*, since bivalves compose up to 50% of the blue crab's diet (Holland et al. 1977, Baird & Ulanowicz 1989, Hines et al. 1990). As such, *M. balthica* is an important species in the trophic transfer of energy between primary producers and commercially harvested species. This clam is known to be moderately tolerant of hypoxia (Borsuk et al. 2002) and can survive up to three weeks of hypoxia (Seitz et al. 2003).

Study aims

The York and the Rappahannock Rivers offer an interesting opportunity to look at the effects of hypoxia. Both rivers have similar macrobenthic communities and both experience periodic hypoxia, but hypoxia in the Rappahannock tends to be more severe and longer lasting (Kuo and Neilson 1987). Hypoxia in the Chesapeake has been increasing in intensity and duration (Haggy et al. 2004). Diaz (2001) listed the Rappahannock as a declining system and the York as a stable system. The benthic community in these rivers serves as the food base for the largest fishery in the Bay, the blue crab, and, as such, represents an important natural resource. We designed this study as a large scale survey of the benthos to establish the extent and effects of hypoxia in the system. By comparing the current data with that published ~20 years ago, we could establish whether hypoxia and its effects have worsened.

MATERIALS AND METHODS

Our study sites were the middle and lower reaches of the York and Rappahannock Rivers (Fig. 1). Both rivers are partially mixed mesohaline tributaries of Chesapeake Bay, and both experience summer hypoxia, with the Rappahannock having longer and more intense hypoxia than the York (Kuo & Neilson 1987). To look at trends in dissolved oxygen in the York River over the last two decades, we downloaded and examined water-quality data from the Chesapeake Bay Program's water quality website (Chesapeake Bay Program 2006). We used bottom dissolved oxygen data from 1984-2005 at sites within our study areas: LE4-1, LE4-2, and LE4-3 (lat/long N 37°25'7.8'/W 76°41'28.7", N 37°17'25.6"/W 76°34'41.2", and N 37°14'2.1"/W 76°25'51.2").

We analyzed bottom dissolved oxygen concentrations from 1984-2005 for the three York River sites that are monitored by the Chesapeake Bay Program. Our analysis was restricted to May-September, when hypoxia occurs. We calculated the percent of hypoxic measurements (DO < 2.0 mg l^{-1}) recorded during each summer for each of the sites to estimate the frequency of hypoxia, and we regressed the frequencies against year.

We sampled the infaunal community in 2003 and 2004 using a box-core to establish the current effects of hypoxic on community structure. We used a random, stratified design to sample the rivers, dividing the rivers into three depth strata (< 3 m, 3 to 6 m, and > 6 m) and randomly selecting sites in each stratum. In 2003, we sampled 10 sites per stratum in the York and 5 in the Rappahannock (due to logistical constraints), and in 2004, we sampled 10 sites per stratum in both rivers. Stratification by depth was used because depth is a good proxy for hypoxia (Powers et al. 2005); deep sites are more

likely to experience hypoxia than shallow, and we wanted to ensure that we sampled a sufficient number of hypoxic sites. Benthic box-core samples were taken in the York before hypoxia on June 19, 2003, and May 25, 2004, and after hypoxia on October 14, 2003, and November 11, 2004 (See Fig. 2 for timeline of all sampling events). The Rappahannock was sampled with a box-core before hypoxia on June 24, 2003, and June 7, 2004, and after hypoxia on February 10, 2004, and November 19, 2004. The sites were revisited during hypoxia in July of 2003, and in June, July, and August of 2004, when the bottom temperature, salinity, and dissolved oxygen were measured with a DO probe (YSI Model 85, Yellow Springs Instruments, Dayton, Ohio, USA).

In 2003, two rounds of sampling were planned, one in June before hypoxia, and one in October after hypoxia. Unfortunately, Hurricane Isabel hit the Virginia Institute of Marine Science in the fall of 2003 and destroyed many of our samples from the June (before hypoxia) sampling. These samples had been partially processed; samples had been sorted to remove all macrofauna but only the bivalves had been identified, measured, and recorded. The hurricane effects led us to push back the second round of sampling in the Rappahannock River until February 2004, to quantify system recovery after recruitment. In 2004, we managed to avoid major natural disasters and sampled both rivers in the late spring and early fall.

In 2003, each site was sampled with a 25 x 25 cm Gray O'Hara box-core. Sub-cores were taken from each sample; one 10 cm diameter core down to 15 cm was taken and sieved on a 0.5 mm mesh screen to quantify smaller infauna, and a 2.5 cm diameter core was taken from the top 5 cm for grain-size analysis. The remaining sediment was sieved through a 2 mm mesh. All samples were frozen and transported back to the lab for

processing. Bottom temperature, salinity, and dissolved oxygen were measured, as well as water depth and core penetration depth. Samples with < 15 cm penetration were not used in 2 mm analyses, and samples with < 5 cm were not used for the 0.5 mm analysis, to ensure that we adequately sampled the biota in each size class (Hines & Comtois 1985). In 2004, the same procedure was used, except that three replicate box-core samples were taken at each site. Only the first core had sub-cores taken from it, while the last two were sieved in entirety through a 2 mm mesh.

The 2 mm samples were sorted and identified to the lowest taxonomic level (LTL) possible (usually species). The 0.5 mm samples were stained with Rose Bengal dye before sorting. These samples also were identified to the LTL. Wet, dry, and ash-free dry mass (AFDM) were determined for each of the major taxonomic groups (bivalves, crustaceans, and annelids) for each sample. Grain-size analysis was conducted using standard wet sieving and pipetting (Folk 1974).

The community-structure data from the 2 mm samples and the 0.5 mm samples were converted into density of individuals m^{-2} and combined before analysis. The data were square-root transformed and a non-metric multi-dimensional scaling (nMDS) analysis from a Brey-Curtis similarity matrix was performed on each, using Primer v6.1.6 software (Clark & Warwick 2001). In nMDS analysis, the stress value is a calculation of the proportion of information lost by the plot (analogous to $1 - r^2$ for regression analysis). Stress values of < 0.20 are generally useful representations of the data. Stress values < 0.25 and > 0.2 may be useful, but must be interpreted with caution and with more attention paid to other analyses, such as ANOSIM and SIMPER (Clark and Warwick 2001). Since many of the sites in the Rappahannock River in 2003 had core penetrations

between 5 and 15 cm and we could not use the 2 mm sections, we did an additional separate analysis of the 0.5 mm samples. Differences in community structure between hypoxic sites and non-hypoxic sites were analyzed using an analysis of similarity (ANOSIM). ANOSIM is a multivariate analysis analogous to an analysis of variance (ANOVA) which determines if there is a statistical difference in community structure between two (or more) areas (Clark and Warwick 2001). The 'global R' statistic is analogous to ANOVA's 'F' statistic. Where a significant difference between hypoxic and normoxic sites was found, a similarity percentages (SIMPER) analysis was performed to determine which species most contributed to that difference. Since we had data on the bivalve community from both before and after hypoxia in 2003, we analyzed that subset separately. We excluded sites where no bivalves were found (similarity matrixes cannot be calculated by Primer if there are multiple sites where no species were found) to look at changes over time, using nMDS and a 2-way ANOSIM with river and hypoxia level as factors.

The biomass data were separated into 2 mm and 0.5 mm sections and analyzed separately. All bivalves > 2 mm were considered to be part of the 2 mm section even if they were sampled in the 0.5 mm core. Polychaetes and other annelids were only analyzed for the 0.5 mm samples. In 2003, the samples were regressed against the minimum dissolved oxygen observed during the summer using the least sum of squares method. In 2004, because we had more observations of dissolved oxygen, we used the average dissolved oxygen. We examined several curves and best fit curve was generally a logistic growth curve (F-test).

RESULTS

Hypoxia in the rivers

Hypoxia was more extensive in the Rappahannock than in the York (Fig. 1) and less extensive in 2003 than in 2004. In 2003, 44% of the stations in the Rappahannock experienced hypoxia, whereas 16% of stations in the York experienced hypoxia. In 2004, 47% of the Rappahannock and 27% of the York sites experienced hypoxia. Also, in 2003, hypoxia in the Rappahannock was more intense than the York, with an average DO at hypoxic stations of 0.5 mg l^{-1} versus 1.3 mg l^{-1} in the York (1-way ANOVA, $F_{1,21}$ = 12.17, p = 0.002). In 2004, the trend was reversed, with the York having an average of 0.3 mg l^{-1}, and the Rappahannock 0.7 mg l^{-1}. For the three Chesapeake Bay Program benthic monitoring sties, there was an increase in the frequency of hypoxia in the York between 1984 and 2005 of about 0.1, or 10% (Chesapeake Bay Program data; regression; p = 0.009). There was a lot of scatter in the data and the r^2 was low (0.1), but this is likely due to the low spatial and temporal coverage of the data (an average of one measurement $month^{-1}$ at each of the three sites). Despite these limitations, these data are a useful time-series of dissolved oxygen and the only long-term DO data set available from this region.

Community structure

After hypoxia in 2003, small polychaetes and crustaceans were associated with sites that experienced summer hypoxia (hypoxic sites) in the York, while large bivalves and crustaceans were common in normoxic sites (Fig. 3, Table 1). In 2003, nMDS analysis of the macrobenthic community in October, after hypoxia, separated hypoxic

and normoxic sites in the York River in both the whole core and the 0.5 mm sub-core (Figs 4a, 4b). A similar separation was not found in the Rappahannock (Figs 4c, 4d) by February, which was long after hypoxia and after spring recruitment. The non-significant difference between the hypoxic and non-hypoxic sites in the Rappahannock was consistent in both the whole core (Fig. 4c; 8 sites) and the 0.5 mm sub-core (Fig. 4d), where there were more sites that could be analyzed (14 sites). The average dissimilarity between the hypoxic and normoxic sites in the York River was over 85%, and 13 taxa, including amphipods, polychaetes, and clams, contributed substantially to the dissimilarity (SIMPER analysis; Table 1).

Although recruitment of bivalves was strong in both rivers, hypoxic areas had higher mortalities of the biomass dominant species. In the spring, before the onset of hypoxia, nMDS and ANOSIM showed no difference in the bivalve community between hypoxic sites and normoxic sites for both rivers (Fig. 5a). After hypoxia, there was a significant difference (Fig. 5b); hypoxic areas were dominated by *Mulinia lateralis*, whereas nomoroxic areas were dominated by *Macoma balthica* and *M. mitchelli*. There was no significant difference in either the density or community structure of bivalve recruits between the hypoxic and normoxic sites in the early spring in the Rappahannock.

In 2004, patterns in community structure were similar to those in 2003. In the York, nMDS and ANOSIM separated the hypoxic sites from the normoxic sites both before and after hypoxia (Fig. 6a, 6b). In the Rappahannock, there was no significant difference between hypoxic and normoxic sites in June, similar to the findings from February, but after hypoxia they were separated (Fig. 6c, 6d).

The species that were associated with normoxic sites included the bivalves *Macoma balthica* and *Macoma mitchelli* and the amphipod *Leptocheirus plumulosus* (Table 1, Fig. 7). Some of the larger infaunal polychaetes, as well as other crustaceans, such as Mysidacea spp. and *Cyathura polita*, were only abundant in normoxic sites. The polychaete species that was consistently characteristic of normoxic areas was *Neanthes succinea*. The species associated with hypoxic areas included small, typically opportunistic polychaetes, including Spionidae spp., Capitellidae spp., *Glycinde solitaria*, and Leitoscoloplos sp. (Table 1, Figs. 3 & 7). Small crustaceans in the families Caprellidae and Cumacea were also abundant in hypoxic areas. In the York after hypoxia, some larger species of polychaetes, such as *Pectinaria gouldii* and *Amphitrite ornata,* were abundant at hypoxic sites.

Community biomass

The effect of hypoxia on the community structure was mirrored in the community biomass. In the York River in 2003 after hypoxia, sites that experienced a minimum dissolved oxygen level below 3 mg l^{-1} had lower biomass than sites that remained above 3 mg l^{-1} in both the 2 mm samples and the 0.5 mm samples, though the regression analysis was only significant for the 0.5 mm samples (Fig. 8a & b). The Rappahannock samples collected in February, longer after the hypoxic period and after the fall recruitment, showed no effect of hypoxia in either the 2 mm or 0.5 mm samples (Figs 8c & d).

A similar reduction of biomass in hypoxic areas also occurred in 2004. The York showed a significant effect of summer DO both before and after hypoxia in the 2 mm

samples (Figs 9a & b; before: $p = 0.003$, $r^2 = 0.23$; after: $p = 0.03$, $r^2 = 0.13$.). The biomass dropped in all areas, but hypoxic areas suffered a greater percent loss. In the Rappahannock, there was no significant effect of summer DO on biomass before hypoxia (we performed regressions analysis both with and without the strong outlier and it did not affect the results), but afterwards there was a strong negative effect (Figs 9c & d; after hypoxia: $p = 0.004$, $r^2 = 0.29$). Bivalves made up about 80-95% of the biomass during all time periods and the majority of the bivalve species were either *Macoma balthica* or *Macoma mitchelli*.

DISCUSSION

Community structure

Hypoxia was associated with lower macrobenthic biomass and a macrobenthic community dominated by opportunistic species. In both the York and Rappahannock, there was a significant difference in macrobenthic community structure in our hypoxic versus our normoxic sites. In contrast, no significant effects of hypoxia were detected in the previous two studies examining the effects of hypoxia on the macrobenthic community in the York River (Dauer et al. 1992, Diaz et al. 1992), though Holland et al. (1987) documented a Bay-wide trend of replacement of larger bivalves with opportunistic species from 1971-1984. There are several possible explanations for the difference between our results and the previous results. Our study had a larger sample size than either of the other two York River studies (Dauer et al. [1992] had only three stations in the York River), which increased the spatial resolution and power of our study. However, a likely reason for the differences in the two sets of results is an increase in the frequency of hypoxia in the York River during the intervening ~20 years between our study and previous published work. This conclusion is supported by the increase in the frequency of hypoxia over the last two decades, and similar demonstrations for the Chesapeake Bay over time (Hagy et al. 2004, Kemp et al. 2005). Moreover, the community in hypoxic areas in the York shifted to a community similar to that in the more severely chronic hypoxic areas of the Rappahannock River (Dauer et al. 1992, Llansó 1992) and other areas of the Bay (Holland et al. 1987), comprised of small, short-

lived opportunistic polychaetes Spionidae spp., Capitellidae spp., *Glycinde solitaria*, and *Leitoscoloplos* sp., and small crustaceans. A few larger polychaetes, *Pectinaria gouldii* and *Amphitrite ornata,* were associated with hypoxic sites in the York. Because polychaetes are generally tolerant of hypoxia, these could be individuals that survived hypoxic events. However, this is unlikely given that they were found after hypoxia but not before. Both these species spawn and recruit in the late summer and fall, so it is more likely that they recruited into the hypoxic sites after the relaxation of hypoxia (Scott 1909, Franz & Harris 1988).

In both rivers, normoxic areas were dominated numerically by *Leptocheirus plumulosus*, *Neanthes succinea*, and *Macoma balthica* and *M. mitchelli*. Approximately 90% of the biomass was *Macoma* sp. These species are all well-characterized members of the benthos of the Chesapeake Bay associated with non-degraded environments. *L. plumulosus* is a highly productive (Holland et al. 1987), pollution-sensitive amphipod that is widely used in assays of sediment toxicity (Schlekat et al. 1992). *N. succinea* is a medium-sized polychaete that responds negatively to environmental degradation (Lerburg et al. 2000). Although it is tolerant of organic enrichment, it is not tolerant of hypoxia (Detwiler et al. 2002). *M. balthica* and *M. mitchelli* are the biomass-dominant species in the mesohaline regions of Chesapeake Bay (Holland et al. 1977, 1987) and are moderately tolerant of hypoxic conditions (Borsuk et al 2002, Seitz et al. 2003). All three of these species are important links in the Chesapeake Bay food web, as they are heavily consumed by benthic predators (Virnstein 1977, Holland et al. 1987, Baird & Ulanowicz 1989).

There seems to be good potential for recovery from hypoxic conditions in both rivers. Recolonization began with the fall recruitment such that, by Feburary 2004, the hypoxic sites in the Rappahannock were no longer distinguishable from adjacent normoxic areas as is observed in other areas (Holland et al. 1987). Larvae of benthic organisms recruit into areas that go hypoxic, and will even settle during a hypoxic event (Sagasti et al. 2003). The lower biomass (and therefore lower competition) may actually increase recruitment into hypoxic areas (Rhodes & Young 1970, Woodin 1974). When we sampled the Rappahannock again in June, a larger sample size still did not show any strong differences between the communities in hypoxic and normoxic areas. Much of the recovery was attributable to the recruitment of bivalves in the Rappahannock; the number of *M. balthica* recruits alone exceeded 1000 m^{-2} at many formerly hypoxic sites in February 2003. There was no difference in the bivalve populations between sites that experienced hypoxia and those which remained normoxic in the York before hypoxia in 2003, similarly indicating that recruitment was strong, though after hypoxia, bivalves were essentially absent from the hypoxic areas by the fall. Additionally, samples taken in the 1970s found densities of *M. balthica* in the now-hypoxic areas of the river similar to those we found in normoxic areas, which shows that they can live in these areas (Boesch & Rosenberg 1981). In the spring of 2004, there was still a measurable difference between the hypoxic and normoxic areas in the York. Much of this difference was numerical, however; *M. balthica*, *L. plumulosus*, and *N. succinea* were all present in hypoxic areas, but they were at lower densities than in normoxic areas. This partial recovery in hypoxic areas is similar to that observed in other systems, such as the Neuse

River, where recovery was complete after a mildly hypoxic year, but only partial after a severely hypoxic year (Powers et al. 2005).

Biomass followed a pattern typical of hypoxic systems (e.g., Lim et al. 2006, Montagna & Ritter 2006), with lower biomass in sites that experienced hypoxia. In the Rappahannock, biomass increased over the fall of 2003 and spring of 2004 in areas that went hypoxic so that there was no difference between those areas and normoxic areas when we sampled in the spring of 2004, indicating strong recovery. In contrast, in the York River the biomass in areas that typically go hypoxic was still lower than in normoxic areas in the spring of 2004. This difference between the Rivers is not due to differences in production in hypoxic areas, however, as similar amounts of biomass accumulate in the hypoxic areas on both rivers during the fall, winter and spring, indicating similar rates of recovery. Rather, because biomass in normoxic areas is lower in the Rappahannock than in the York, the rate of recovery in hypoxic areas as a proportion of biomass in normoxic areas is higher in the Rappahannock. It is interesting that the Rappahannock, which has a greater extent of hypoxia than the York, has lower biomass in normoxic areas. It is possible that as the extent of hypoxia increases, it causes negative feedback through a decreased supply of larvae into all areas of the river (Pulliam and Danielson 1991), though this hypothesis remains untested. Since so much of the biomass in the systems is comprised of bivalves and these were all but extinct in hypoxic areas, the pattern of continued low biomass through the end of spring is not surprising. Full recovery of the system could be attained within the time it takes to establish a mature bivalve population, which for *M. balthica* is about 3 years (pers. obs.).

Implications for the estuarine food web

Such a decrease in biomass and shift in community structure due to hypoxia probably has an effect on the food web, since the benthic community serves as prey for many epibenthic predators (e.g. Virnstein 1977, Baird & Ulanowicz 1989). Direct mortality of benthic infauna due to hypoxic stress may result in biomass being transferred into the microbial loop rather than to a higher trophic level (Baird et al. 2004, Altieri & Whitman 2006). While our study does demonstrate a decrease in biomass in hypoxic areas, it cannot differentiate between direct mortality and predatory mortality.

The behavioral response of benthic fauna when faced with hypoxia (e.g. reduced burial depth, exposure on sediment surface, extension of siphons or palps in the water column) may make them more vulnerable to predation (Jørgensen 1980, Dauer et al. 1992, Diaz et al. 1992, Seitz et al. 2003, Long et al. in review). Whether predators are able to take advantage of this is still under debate. Predators generally move out of hypoxic areas (e.g. Phil et al. 1991, Eby & Crowder 2004, Bell & Eggleston 2005). However, predators have been observed feeding in mildly hypoxic areas (Rahel & Nutzman 1994, Nestlerode & Diaz 1998). Phil et al. (1992) documented a shift in the diets of benthic predators to include more infaunal species during and immediately after hypoxia, and caging experiments performed in the York suggest that episodic hypoxia increases predation on *M. balthica* (Long & Seitz in review). These studies were done in mildly hypoxic areas; more intense hypoxia may exclude predators or decrease predation (Bell et al. 2003, Seitz et al. 2003, Motagna & Ritter 2006).

CONCLUSIONS

Hypoxia, a contributor to habitat degradation, causes a decrease in the ecosystem services provided by the organisms within a habitat. In the case of infauna in the York and Rappahannock Rivers, the organisms provide two main ecosystem services (along with other secondary services such as nutrient transformation, bioturbation, etc.). One ecosystem service is filtering (McCay et al. 2003); many infaunal organisms remove particulates from the water through their filter or suspension feeding. This, in turn, increases the clarity of the water, which has positive effects on other species, particularly submerged aquatic vegetation (Kemp et al. 2004). Benthic organisms also provide food for higher trophic levels, including harvestable species like the blue crab, spot, and flounder. Both of these main functions are determined primarily by abundance and biomass, and secondarily by species composition. Hypoxia has an effect on both of these ecosystem services, decreasing the biomass and shifting the species composition away from larger species to smaller ones that are less-preferred prey items for megafaunal species. Determining the value of the two main services diminished by hypoxia is beyond the scope of this research, but is a worthwhile effort at which future research could be aimed. Finally, our data suggest that hypoxia in the York River is worsening, both in its frequency and effects, and that greater action to reduce nutrient inputs may be warranted.

ACKNOWLEDGEMENTS

I would like to thank K Delano, B Brylawski, K Knick, K Erickson, R Burke, J Falls, D Lambert, and M Seebo for help with the field sampling, K Knick and B Brylawski for help with the laboratory work, and R Diaz, D Wolcott, and J Long for help with the revision of this manuscript. WC Long was funded by an EPA GRO fellowship. Funding was provided by NOAA National Sea Grant, Essential Fish Habitat Program, by the Commonweath of Virginia, and by a grant to the Blue Crab Advanced Research Consortium from the NOAA Chesapeake Bay Program.

Table 1: SIMPER analysis of York and Rappahannock River benthic communities after hypoxia in 2003 and 2004. c = crustacean, p = polychaete, b = bivalve, o = other.

York 2003	**Average Abundance**		**Average**	**%**
Species	**Hypoxic**	**Normoxic**	**Dissimilarity**	**Contribution**
Leptocheirus plumulosus (p)	49	620	14	17
Cumacea spp. (c)	99	140	10	11
Glycinde solitaria (p)	99	22	8	9
Neanthes succinea (p)	0	140	8	9
Pectinaria gouldii (p)	99	0	6	7
Capitellidae spp. (p)	49	50	6	7
Caprellidae spp. (c)	123	6	5	6
Spionidae spp. (p)	49	39	5	6
Macoma balthcia (b)	0	26	4	5
Leitoscoloplos sp. (p)	25	11	3	3
Macoma mitchelli (b)	0	9	2	3
Phoronis spp. (o)	0	45	2	3
Mediomastus ambiseta (p)	0	34	2	3
Sabellaria vulgaris (p)	25	0	2	2
Average Dissimilarity	87		Total	90

York 2004	**Average Abundance**		**Average**	**%**
Species	**Hypoxic**	**Normoxic**	**Dissimilarity**	**Contribution**
Amphitite ornata (p)	98	5	12	15
Macoma balthica (b)	2	94	10	12
Leptocheirus plumulosus (c)	0	127	7	9
Neanthes succinea (p)	11	47	6	8
Leitoscoloplos sp. (p)	17	19	6	7
Glycinde solitaria (p)	20	10	5	6
Pectinaria gouldii (p)	25	0	4	5
Macoma mitchelli (b)	1	13	4	5
Capitellidae spp. (p)	5	9	4	5
Cumacea spp. (c)	8	27	4	5
Phoronis spp. (o)	6	18	4	4
Cyathura polita (c)	0	15	3	4
Mulinia lateralis (b)	4	1	2	2
Tunicates (o)	23	0	2	2
Glycera americana (p)	4	0	2	2
Average Dissimilarity	81		Total	91

Table 1 continued:

Rappahannock 2004 Species	Average Abundance		Average	%
	Hypoxic	Normoxic	Dissimilarity	Contribution
Leptocheirus plumulosus (c)	3	143	13	19
Macoma mitchelli (b)	17	85	10	14
Macoma balthcia (b)	4	40	7	11
Glycinde solitaria (p)	25	0	6	8
Capitellidae spp. (p)	23	3	6	8
Neanthes succinea (p)	41	56	5	7
Macoma balthica recruits (b)	12	20	5	7
Cumacea spp. (c)	9	20	4	6
Mulinia lateralis recruits (b)	2	14	3	5
Cyathura polita (c)	0	11	3	4
Mulinia lateralis (b)	2	3	2	3
Average Dissimilarity	68		Total	92

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Figure 1

Map showing extent of hypoxia in study areas in the early-summer of 2004. York River is the left inset, and the Rappahannock River the right. Sites are white triangles, and DO contours are calculated using inverse distance weighing interpolation (ESRI ArcMap v9.1 software).

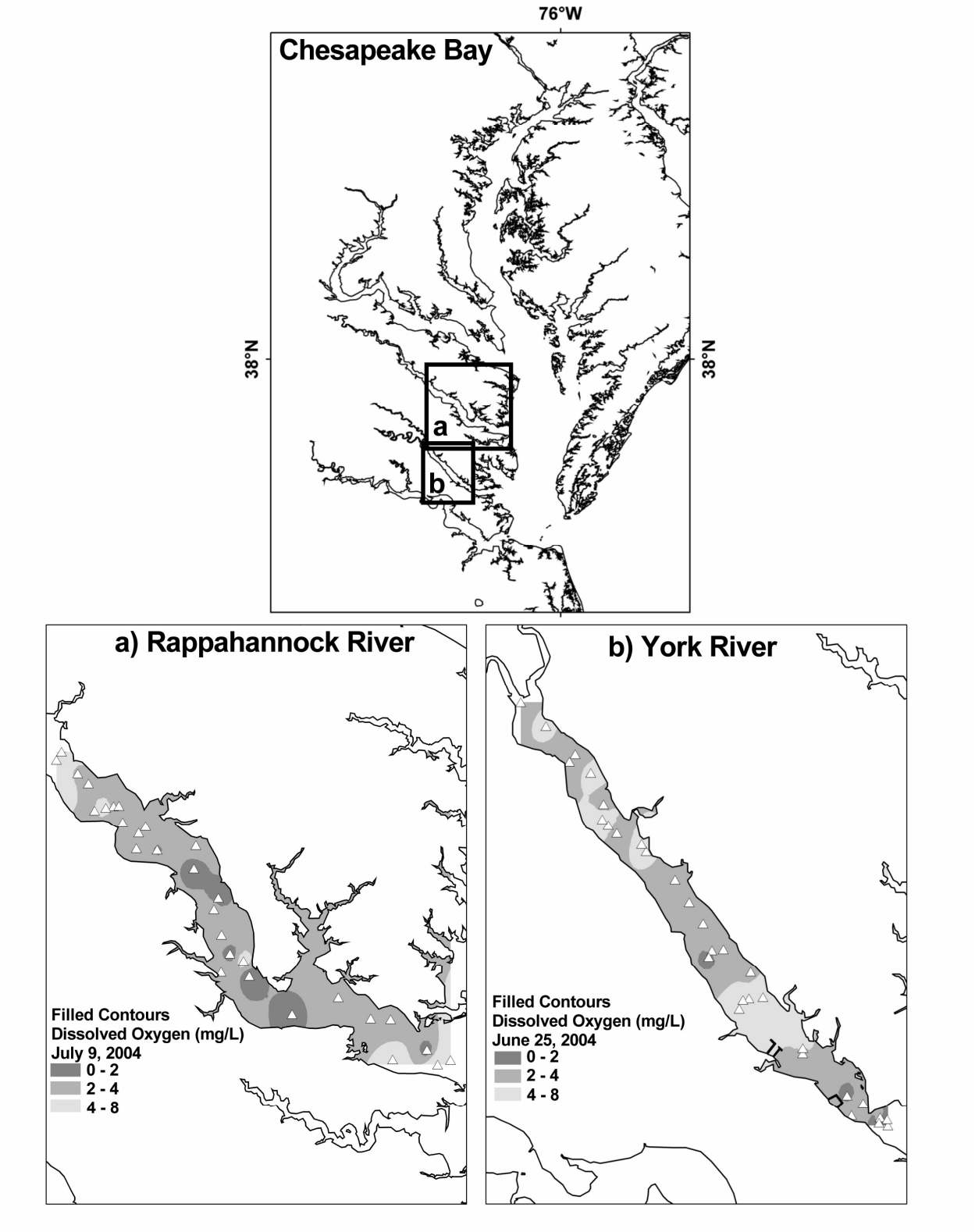
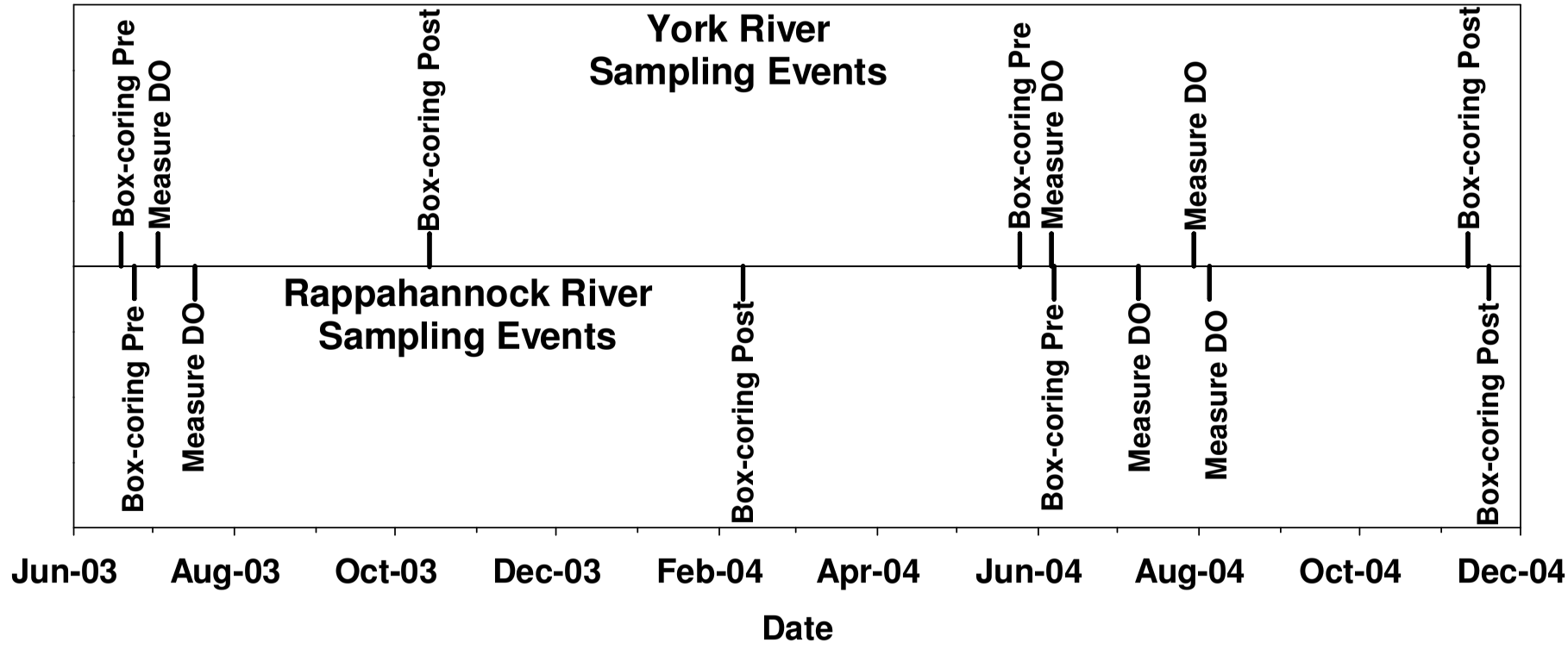

76°W
Chesapeake Bay
38°N
38°N
a
b
a) Rappahannock River
Filled Contours
Dissolved Oxygen (mg/L)
July 9, 2004
0 - 2
2 - 4
4 - 8
b) York River
Filled Contours
Dissolved Oxygen (mg/L)
June 25, 2004
0 - 2
2 - 4
4 - 8

Figure 2



Timeline of sampling events in the York and Rappahannock Rivers. At time marked 'Measured DO' the bottom temperature, salinity, and DO were measured at each site.

Figure 3

Density of selected taxa in hypoxic and normoxic sites (mean + SE) in York River after hypoxia in 2003, arranged in order of abundance in hypoxic sites. Similar patterns were observed in 2004 for both the York and the Rappahannock. (see Table 1).

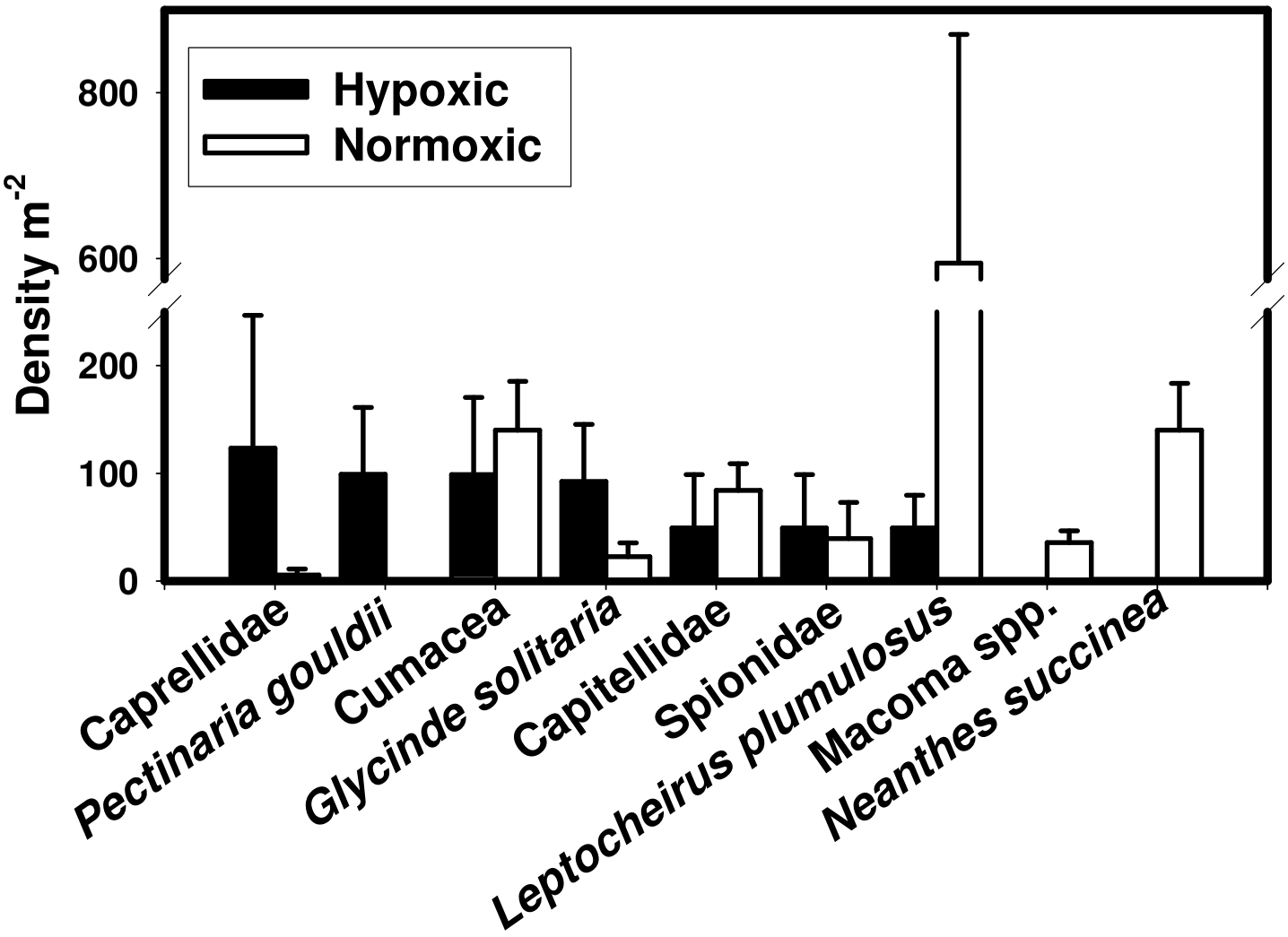

Hypoxic
Normoxic
Density m-2
800
600
200
100
0
Caprellidae
Pectinaria gouldii
Cumacea
Glycinde solitaria
Capitellidae
Spionidae
Leptocheirus plumulosus
Macoma spp.
Neanthes succinea

Figure 4

nMDS plots of community structure data after hypoxia in 2003 for (a) York River whole core, 2 mm and 0.5 mm combined (b) York River 0.5 mm-sieved sub-core, (c) Rappahannock River whole core, and (d) Rappahannock River 0.5 mm sub-core. Global R and p values are from ANOSIM analysis comparing differences between hypoxic (black triangles) and normoxic (gray triangles) sites. nMDS plots are a two-dimensional representation of a similarity matrix. The distance between two points is proportional to the similarity in community structure between the two sites represented.

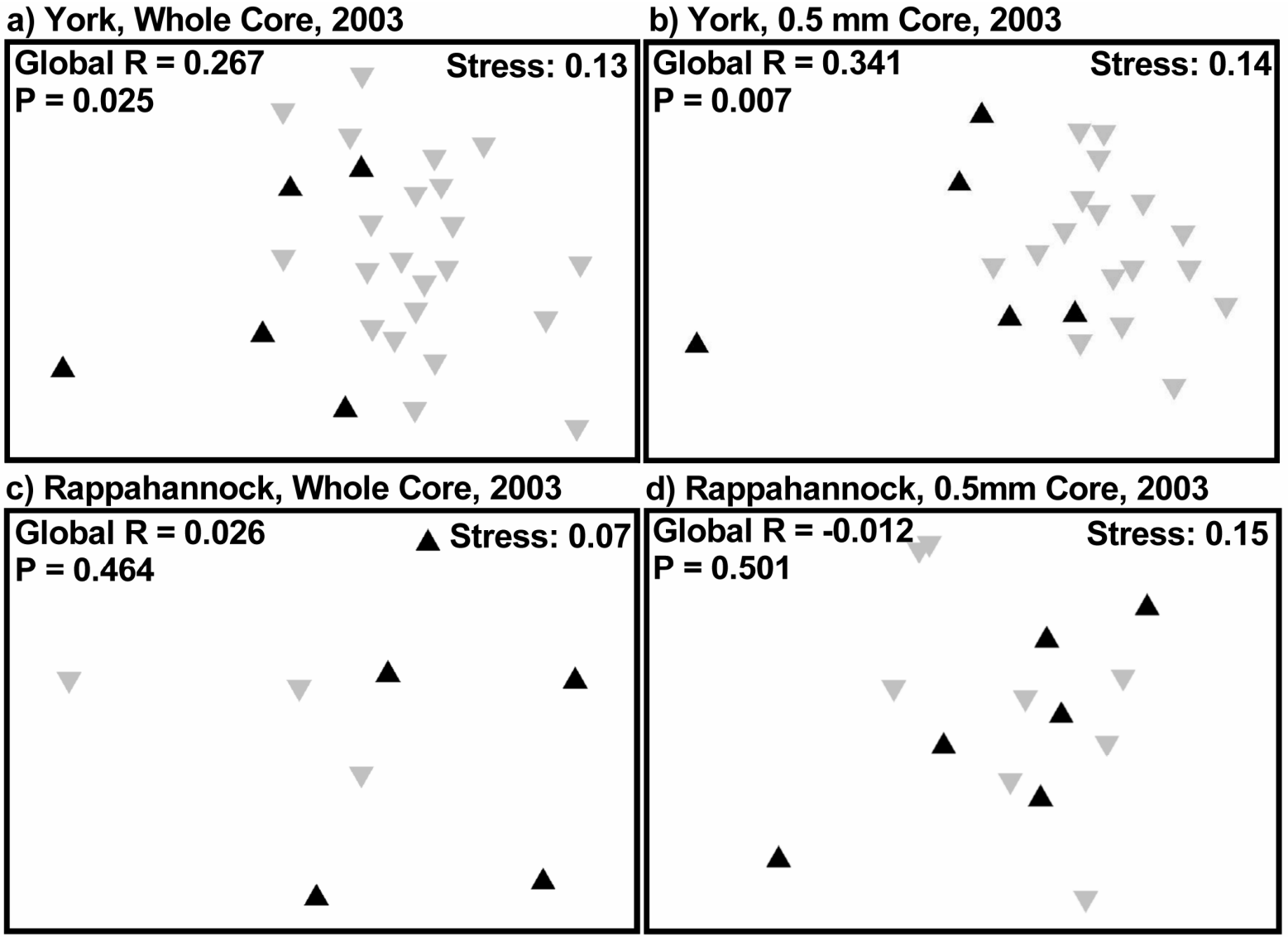
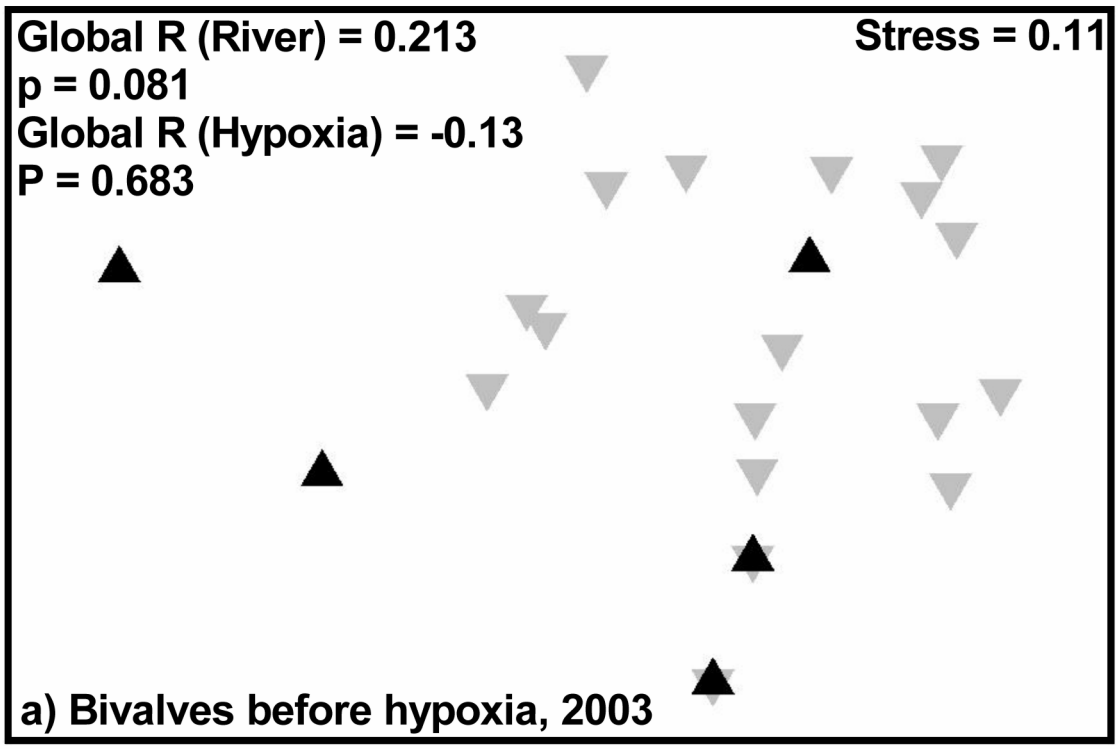

a) York, Whole Core, 2003
Global R = 0.267
P = 0.025
Stress: 0.13
b) York, 0.5 mm Core, 2003
Global R = 0.341
P = 0.007
Stress: 0.14
c) Rappahannock, Whole Core, 2003
Global R = 0.026
P = 0.464
Stress: 0.07
d) Rappahannock, 0.5mm Core, 2003
Global R = -0.012
P = 0.501
Stress: 0.15

Figure 5

nMDS plot of York and Rappahannock bivalve community structure data (a) before and (b) after hypoxia in 2003. Global R and p values are from ANOSIM analysis comparing differences between hypoxic (black triangles) and normoxic (gray triangles) sites.


Global R (River) = 0.213
Stress = 0.11
p = 0.081
Global R (Hypoxia) = -0.13
P = 0.683
a) Bivalves before hypoxia, 2003

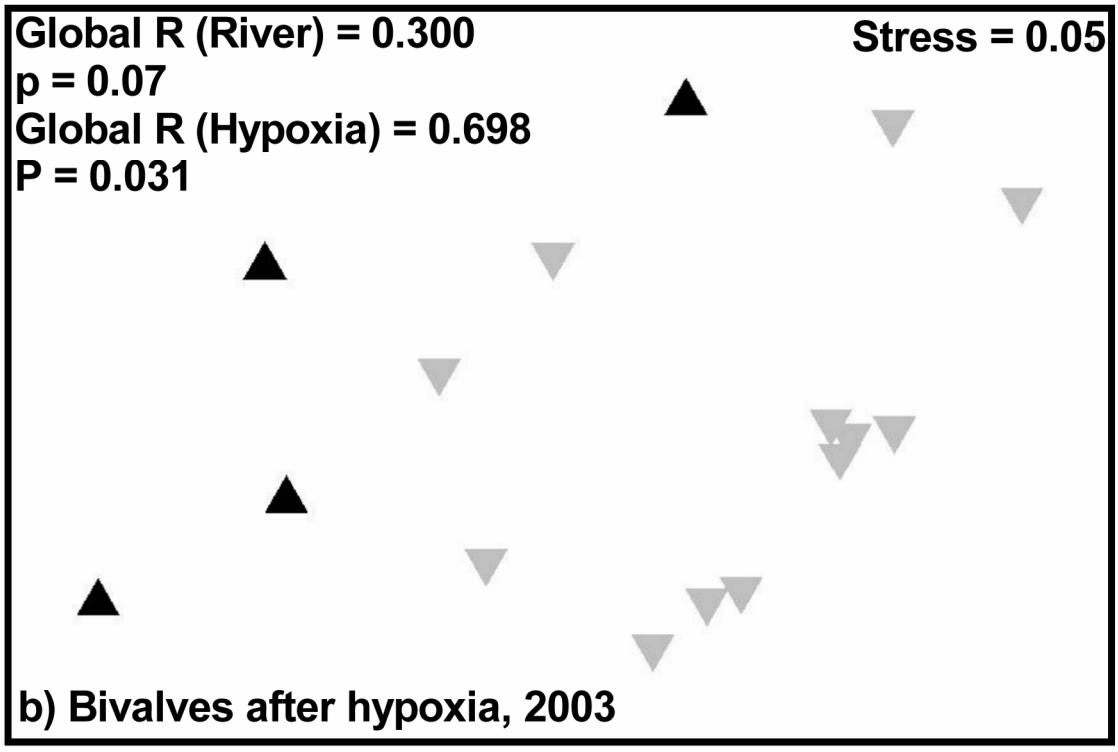

Global R (River) = 0.300
Stress = 0.05
p = 0.07
Global R (Hypoxia) = 0.698
P = 0.031
b) Bivalves after hypoxia, 2003

Figure 6

nMDS plot of community structure (2 mm and 0.5 mm cores combined) for the York (a) before and (b) after hypoxia in 2004, and for the Rappahannock (c) before and (d) after hypoxia in 2004. Global R and p values are from ANOSIM analysis for differences between hypoxic (black triangles) and normoxic gray triangles) sites.

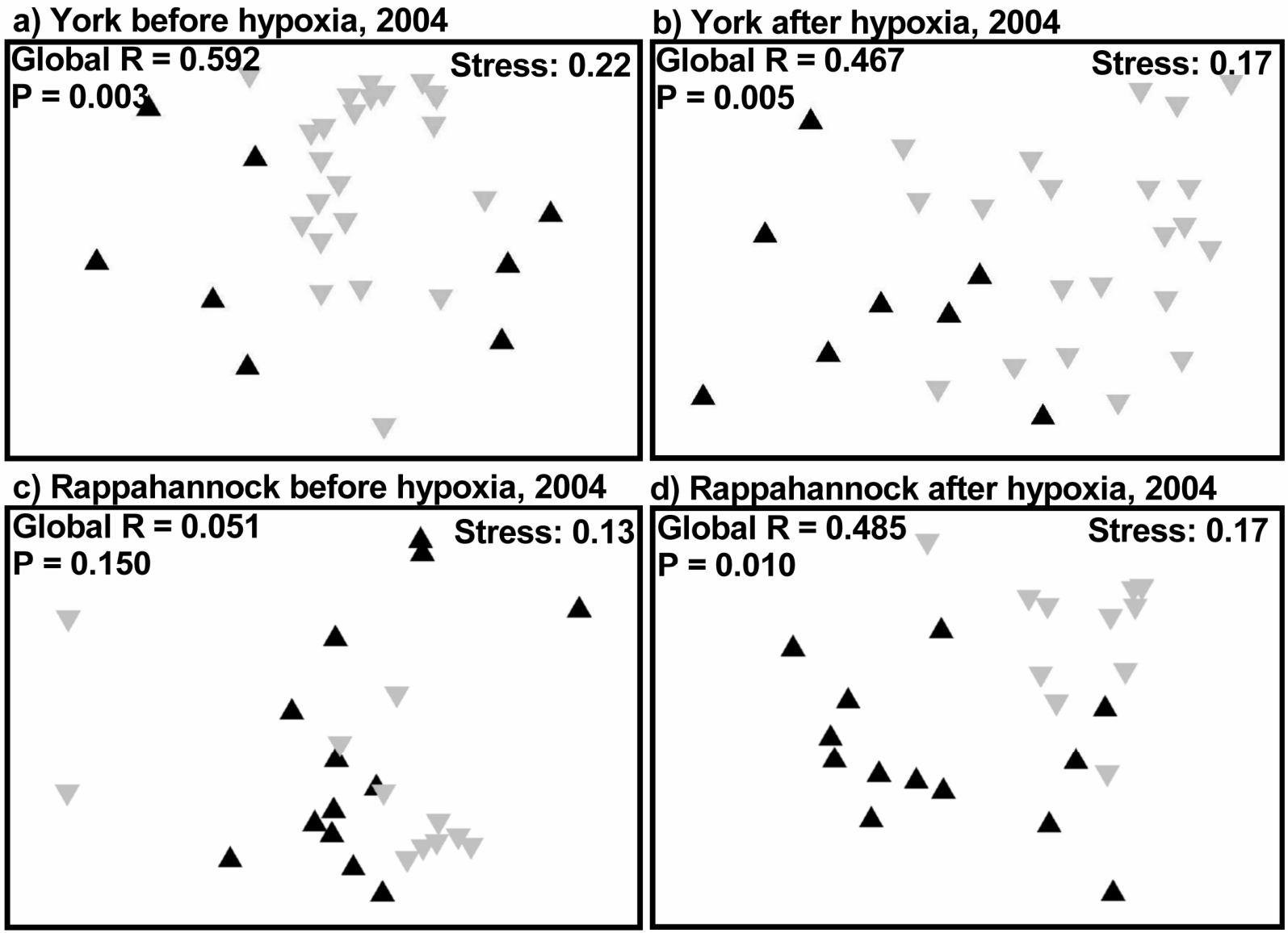
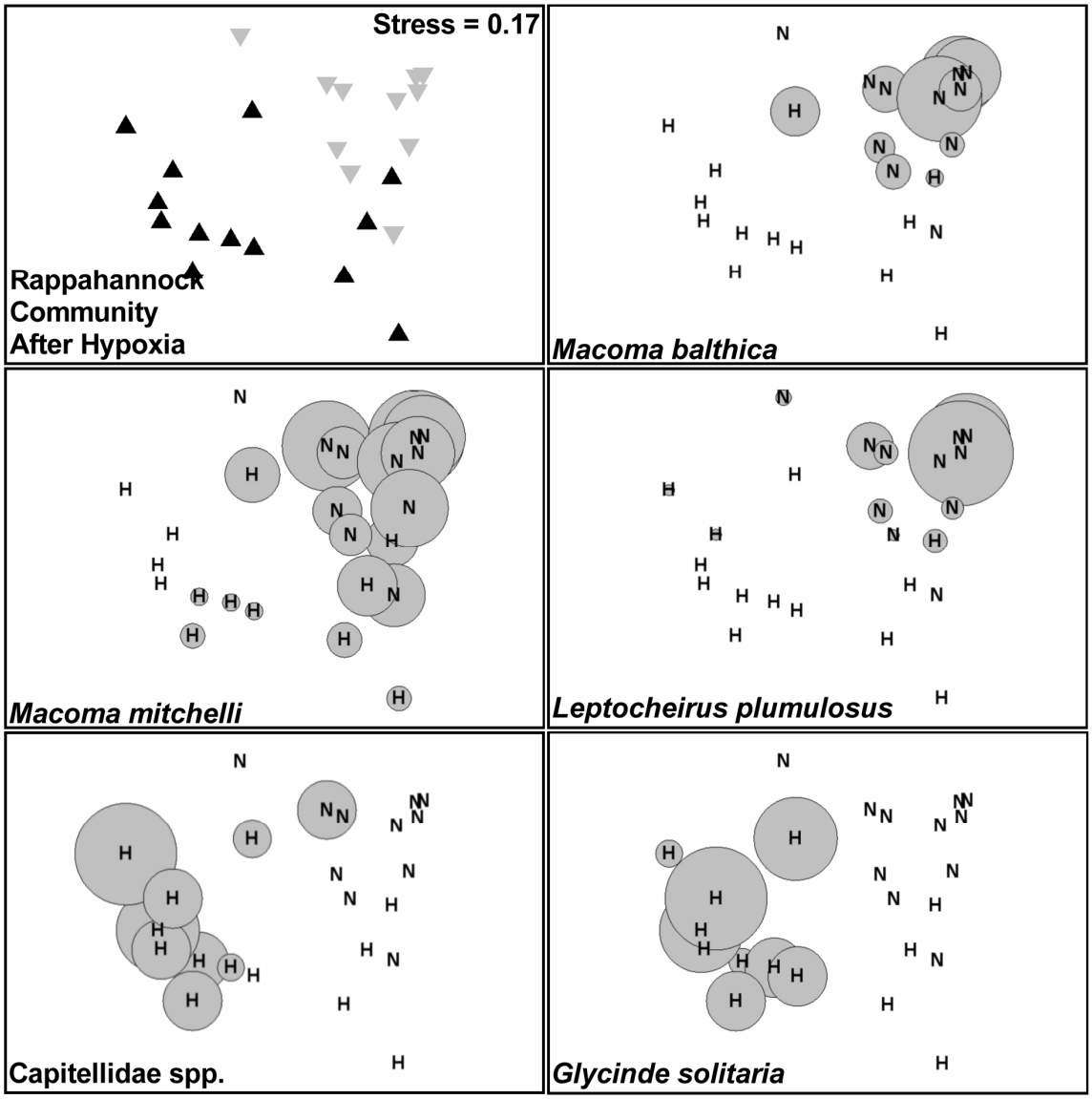

a) York before hypoxia, 2004
Global R = 0.592
P = 0.003
Stress: 0.22
b) York after hypoxia, 2004
Global R = 0.467
P = 0.005
Stress: 0.17
c) Rappahannock before hypoxia, 2004
Global R = 0.051
P = 0.150
Stress: 0.13
d) Rappahannock after hypoxia, 2004
Global R = 0.485
P = 0.010
Stress: 0.17

Figure 7



nMDS plots for the Rappahannock after hypoxia in 2003 showing the distribution of selected species in hypoxic (black triangles) and normoxic (gray triangles) sites. The size of the circle is proportional to the relative density of each species at each site and the letter indicates if the site is hypoxic (H) or normoxic (N). The stress value is the same for each plot.

Figure 8

AFDM plotted against minimum observed dissolved oxygen. (a) York River 2 mm samples. (b) York River 0.5 mm samples (Line is best fit logistic growth equation; $p = 0.001$; $r^2 = 0.18$) (c) Rappahannock River 2 mm samples. (d) Rappahannock River 0.5 mm samples. York samples were taken shortly after hypoxia in October, 2003, whereas Rappahannock samples were taken after the fall and spring recruitment events following hypoxia in February, 2004. Note that y-axis scales change among plots.

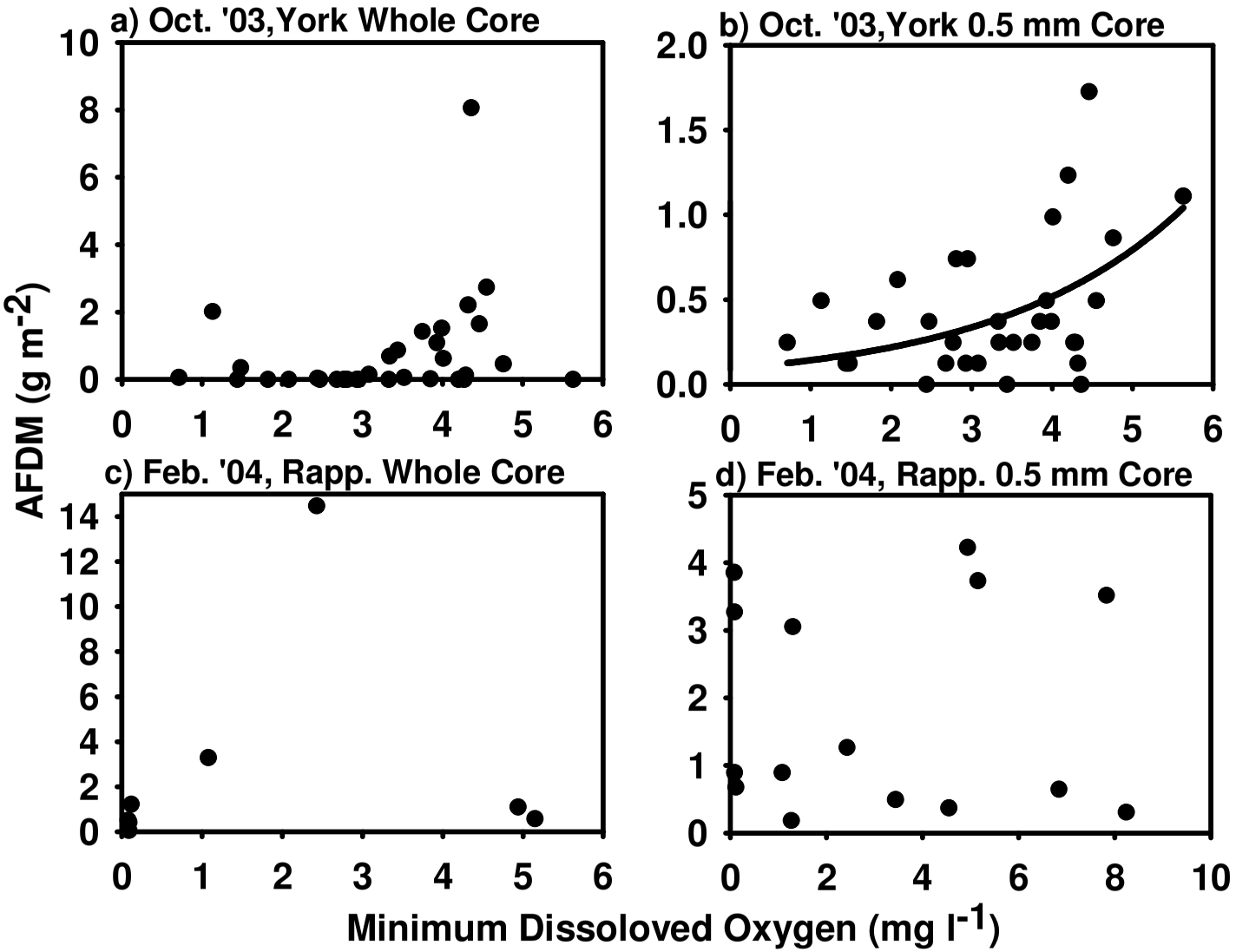
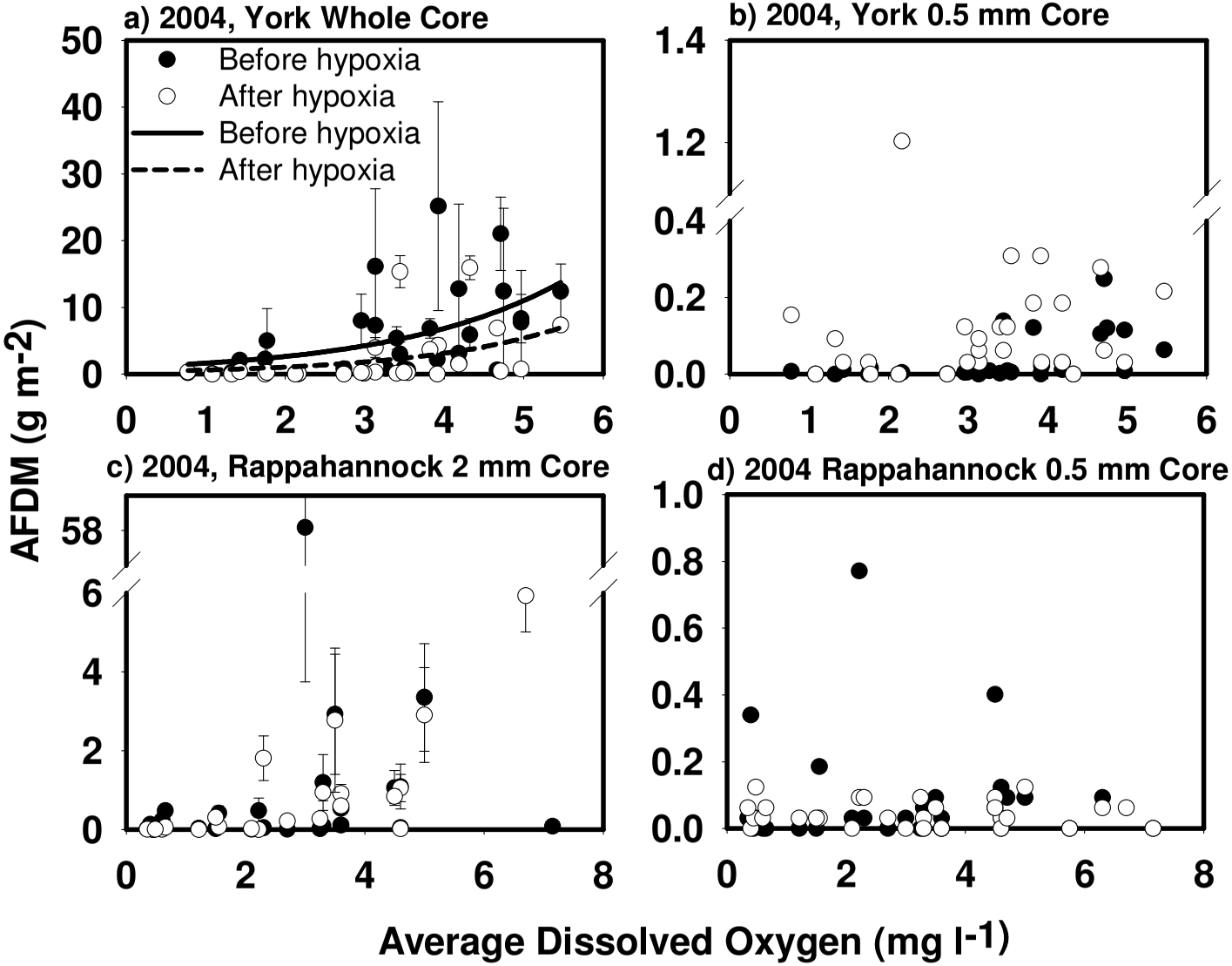

a) Oct. '03,York Whole Core
b) Oct. '03,York 0.5 mm Core
c) Feb. '04, Rapp. Whole Core
d) Feb. '04, Rapp. 0.5 mm Core
AFDM (g m-2)
Minimum Dissoloved Oxygen (mg l-1)

Figure 9

AFDM plotted against average observed dissolved oxygen for the York and Rappahannock before (solid circles) and after (hollow circles) hypoxia in 2004; (a and c) 2 mm samples (mean ± SE); (b and d) 0.5 mm samples. Trend lines show best-fit logistic growth equations (see text for statistics). Note that y-axis scales change among plots.


a) 2004, York Whole Core
Before hypoxia
After hypoxia
Before hypoxia
After hypoxia
b) 2004, York 0.5 mm Core
c) 2004, Rappahannock 2 mm Core
d) 2004 Rappahannock 0.5 mm Core
AFDM (g m-2)
Average Dissolved Oxygen (mg l-1)

CHAPTER 2:

QUANTIFYING FECUNDITY IN *MACOMA BALTHICA* USING AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

ABSTRACT

Monoclonal antibodies specific to an HSP-70-like protein expressed in the eggs of *Macoma balthica* were used to develop an enzyme-linked immunosorbent assay to quantify fecundity in females. The assay was specific to egg tissue; no egg protein was measured in male tissue. The concentration of HSP-70 increased as the gonads matured, necessitating the determination of a calibration curve for future experiments. Egg production was positively correlated with body mass index (BMI) and clams with a BMI lower that 1.4 did not produce eggs. The estimated number of eggs produced per clam was similar to that observed in eastern North Atlantic populations in clams of similar size. This assay effectively quantifies eggs at any stage of gonadal development where eggs can be distinguished microscopically, and it is easier and cheaper to perform than other techniques of similar precision.

KEY WORDS: *Macoma balthica*, fecundity, egg production, ELISA, monoclonal, antibodies

INTRODUCTION

The tellenid clam, *Macoma balthica*, is a small, thin-shelled clam that is common in estuaries in both the eastern and western North Atlantic. In the Chesapeake Bay, it is the biomass-dominant organism in mesohaline, muddy habitats, comprising up to 85% of the biomass (Holland et al. 1977). In the Chesapeake, *M. balthica* is an important link in the food web, as it is preyed upon by a variety of piscine and crustacean predators, comprising approximately 50% of the blue crab (*Callinectes sapidus*) diet alone (Hines et al. 1990). In eastern North Atlantic estuaries, it is an important food supply for migratory shorebirds (Stillman et al. 2005). It is considered an indicator of ecosystem health, as it is relatively sensitive to stressors such as hypoxia (Seitz et al. 2003, Powers et al. 2005).

The reproductive cycle of *M. balthica* has been well studied, although it varies among populations. In the Chesapeake, *M. balthica* spawn in both the fall and spring (Shaw 1965), whereas in Massachusetts and in the eastern North Atlantic they only spawn in the early spring (Gilbert 1978, Honkoop & van der Meer 1998). *Macoma balthica* invests heavily in eggs, producing large, energy-rich eggs with high lipid content that can account for over 30% of their mass (Honkoop et al. 1999). Reproductive output varies with water temperature (Honkoop & van der Meer 1998), food quality (Hendriks et al. 2003), and the body mass index (BMI) of the clam (Beukema et al. 2001).

Measuring fecundity in *M. balthica* and in other bivalves is difficult, and various methods have been used. Visual examination and ranking of gonadal fullness is an easy but highly qualitative measurement (e.g. Glibert 1978, de Goeij & Honkoop 2003).

Tissue sectioning and manual counting of eggs under a microscope is more quantitative but labor intensive (e.g., Kang et al. 2003a). A common technique used with *M. balthica* is induction of spawning via temperature shock followed by collection and counting of eggs, which is quantitative but also labor intensive (e.g., Honkoop et al. 1999, Beukema et al. 2001).

Recently, enzyme linked immunosorbent assays (ELISAs) have been developed to quantify egg production in a variety of bivalves, including *Crassostrea virginica* (Choi et al. 1993), *Crassostrea gigas* (Kang et al. 2003b), and *Saxidomus purpuratus* (Park et al. 2003). ELISAs allow for rapid, simultaneous, quantitative assessments of a large number of samples. In this study, we develop a sandwich ELISA, using monoclonal antibodies to quantify egg production in *M. balthica*.

MATERIALS AND METHODS

Production of antibodies and purification of egg protein standard

Monoclonal antibody 7A4, specific to a *Macoma balthica* egg-specific HSP70-like protein (mb-HSP70), was produced (Bromage et al. in review) and stored at 1 mg antibody ml^{-1} at -20°C. Gravid *M. balthica* were collected from the York River, Chesapeake Bay, and stored at -20°C before use. An egg protein standard was made by incising and squeezing the gonads to collect eggs. The eggs were then homogenized in phosphate-buffered saline (PBS; 10 mM Na_2HPO_4, 150 mM NaCl, pH 7.2) and centrifuged at 15,000 g for 1 hr. at 4°C. The supernatant was collected, and the protein concentration was determined with a BCA protein assay kit (Sigma) using bovine serum albumin as the standard. The egg protein standard was stored at 1.25 mg protein ml^{-1} in 1 ml aliquots at -20°C. In this paper, concentrations of mb-HSP70 will be expressed in 'units', where one unit of mb-HSP70 is equal to the concentration of mb-HSP70 in 1 ml of 1.0 mg ml^{-1} egg protein standard.

Enzyme-linked immunosorbent assay (ELISA)

ELISAs were run in 96-well polystyrene micro-plates and all incubations were executed at 37°C. One hundred µl of 7A4 at 2 µg ml^{-1} in citrate buffer (10 mM sodium citrate, pH 4.0) were added to each well and incubated for 1 hr. The wells were then blocked with 240 µl of tris-buffered saline with 0.1% tween (TTBS; 50 mM Tris, 150 mM NaCl, 1 mM EDTA, pH 8.0) with 1% bovine serum albumin, and incubated for 1 hr.

One hundred µl of each undiluted sample together with 1:5 serial dilutions were analyzed in duplicate. Serial dilutions of the egg standard were also run on every plate. The plates were incubated for 1 hr. before being washed three times with TTBS. One hundred µl of biotinylated 7A4 (b-7A4) at 2 µg ml^{-1} in TTBS was incubated for 1 hr. in each well, after which the plates were washed three times with TTBS. One hundred µl of 1:500 dilution in TTBS of 0.125 mg ml^{-1} strepavidin conjugated horseradish peroxidase was incubated in each well for 1 hr. The plates were washed three times in TTBS and 100 µl 2,2'-azino-bis (3-ethylbenzthiazoline-6 sulfonic acid), ABTS solution (200 µl 1% ABTS, 4.8 ml citrate buffer, 10 µl 30% H_2O_2), was added to each well. The optical density (OD) at 405 nm was read using a plate reader (Multiskan MCC EX, Thermo Electron Co.). Multiple readings were done and the maximum rate of change in OD in each well was calculated. A non-linear regression was performed for each sample to find the least sum-of-squares fit for equation 1,

Equation 1: $$rate = \frac{R_{\max}}{1 + (\frac{C}{C_{50}})^{s}}$$

where '*rate*' is the rate of OD change in each well, '*C*' is the sample concentration, 'R_{max}' is the maximum rate, 'C_{50}' is the concentration at which the rate is 50% of R_{max}, and '*s*' is the slope coefficient. The concentration of mb-HSP70 at the C_{50} is the same among samples, thus knowing the concentration of mb-HSP70 at the C_{50} in the standard solution and the level of dilution at the C_{50} for each of the samples allows the calculation of the concentration of mb-HSP70 in the undiluted sample (Ottinger et al. 2001).

Verifying specificity of an enzyme-linked immunosorbent assay (ELISA)

Ripe *M. balthica* were collected from the York River and stored at -20°C before use. The clams were sexed (Gilbert 1978), and the following tissues were excised; (1) female somatic tissue (including samples of mantle, siphon, abductor muscle, foot muscle, and viscera), (2) female gonadal tissue including eggs, (3) male gonadal tissue. Each tissue was homogenized in PBS and centrifuged at 15,000 g for 1 hr. The supernatant was collected and centrifuged a second time at 15,000 g for 1 hr. for further clarification of the sample and the supernatant was collected. Protein concentration was determined for each extract using BCA assay as above, and the extracts were stored at -20°C. The concentration of mb-HSP70 in each sample was determined by ELISA and standardized by dividing the concentration of mb-HSP70 by the total concentration of protein.

Quantification of eggs at different stages of gonadal development and in different years

Gravid female *M. balthica* were collected in the York River on 10/23/03, 9/30/05, 10/30/05, and 11/29/05 and stored at -20°C until used. Each clam was opened, its gonads incised, and samples of eggs extracted. The eggs were placed in PBS and gently suspended by repeated pipetting. The concentration of eggs in each sample was determined with a hemocytometer. The samples were centrifuged at 15,000 g for 5 minutes and the pellet was homogenized. The homogenates were centrifuged at 15,000 g for 30 minutes and the supernatant was collected and stored at -20°C. The concentration of mb-HSP70 in each sample was determined by ELISA. Total protein concentration

was determined for each sample with a BCA assay as above and the amount of protein per egg was calculated and analyzed with a 1-way analysis of variance (ANOVA) with date (10/30/05, and 11/29/05) as the factor. The concentration of mb-HSP70 was regressed (linear, least-squares) against the known concentration of eggs for each sampling period. The ratio of mb-HSP70:total protein (units mb-HSP70 per mg total protein) was calculated for the egg samples in October and November of 2005. These were compared to those calculated for the male gonadal and female somatic tissues (previous section) with a one-way ANOVA and a Tukey's test. All samples were log(N + 10^{-4}) transformed to achieve homogeneity of variance prior to analysis.

Relationship between fecundity and body mass index (BMI)

Ripe female *M. balthica* collected on 10/30/05 and 11/29/05 were used to determine the relationship between fecundity and BMI. The shell length was recorded and the clams were shucked and homogenized in 15 ml PBS. Homogenates were centrifuged at 3000 g for 1 hr., after which 1 ml of supernatant was removed and the concentration of mb-HSP70 was determined by ELISA. The number of eggs produced by each clam was determined using the relationship previously determined between the concentration of mb-HSP70 and egg concentration. The rest of the sample was dried at 65°C and the dry mass determined. The BMI (dry mass /length^3 in mg cm^{-3}; Honkoop & Beukema 1997), also known as the condition index (Bonsdorff & Wenne 1989), was calculated for each clam. An Analysis of Covariance (ANCOVA) was conducted on egg production with date as a factor and BMI as a covariant. Linear regression was used to determine the relationship between BMI and egg production.

RESULTS

The ELISA was specific for female gonadal tissue (Fig. 1). The non-linear regression was an excellent fit in every case, with R^2 values > 0.98 and *p* values < 0.0001. Small concentrations of mb-HSP70 were detected in female somatic tissues, but the concentrations were two orders of magnitude lower than in female gonadal tissue (Fig. 1). Mb-HSP70 was below reliable detection limits in male gonads, and our best estimate was four to six orders of magnitude lower than in female gonads. Western blot analysis (not shown) showed a weak reaction in the stacking gel layer of the poly-acrylimide gel, and nothing at 74 kDa (Bromage et al. in review), indicating that the reaction is likely a non-specific binding of the antibody to large protein aggregates and that mb-HSP70 is not actually present in males.

Clams collected on 9/30/05 were at a very early stage of gonadal development; no eggs could be distinguished microscopically, and ELISA revealed extremely low levels mb-HSP70 in the gonads. At all other time periods (10/23/03, 10/30/05, 11/29/05), the mb-HSP70 concentration was positively correlated with egg concentration (Fig. 2), though the relationship varied among all time periods. Clams collected on 11/29/05 were fully mature; some had already spawned, as evidenced by empty gonads (Gilbert 1978). In 2005, total protein per egg increased from 0.016 µg egg^{-1} ±0.002 (SE) in October to 0.030 µg egg^{-1} ±0.005 (SE) in November (ANOVA; N = 27; $F_{1,26} = 4.37$; *p* = 0.047). The ratio of mb-HSP70:total protein differed among tissue types (Figure 3; ANOVA; N = 40; $F_{4,35} = 455.73$, $p < 0.0005$), and was low in male gonads and in female

gonads prior to egg development, intermediate in somatic tissue from gravid females, and highest in female gonads during egg development (Fig. 3).

The number of eggs in female *M. balthica* was influenced by BMI (ANCOVA; N = 40; $F_{1,38}$ = 11.82; $\underline{P}$ = 0.001), but not by month ($F_{1,38}$ = 1.227; $\underline{P}$ = 0.276). Clams that had already spawned contained very low levels of mb-HSP70, as expected, and were not included in the analyses. BMI was a good predictor of egg production and clams with a BMI below 1.4 mg cm^{-3} did not produce eggs (regression analysis; Fig. 4).

DISCUSSION

We have developed a simple and rapid assay for the quantification of eggs in female *Macoma balthica* once egg production begins. ELISAs are a highly quantitative, reliable means to measure concentrations of antigens (e.g., Choi et al. 1993). The ELISA developed in this study has the added benefit of utilizing monoclonal antibodies, unlike other, similar assays that used polyclonal antibodies to quantify bivalve eggs (Choi et al. 1993, Kang et al. a&b, 2003, Park et al. 2003). Although polyclonal antibodies have similar utility in ELISAs, assays are only reliably comparable if they all use the same batch of antibodies. As polyclonal antibodies can only be produced in limited amounts, sharing between laboratories and comparison of results are likewise limited. This limitation does not exist with monoclonal antibodies; they are produced by an immortal hybridoma cell line that can be used in any laboratory to produce the same antibody, making direct comparison of results possible.

The ELISA in this study is specific to egg tissue in female *M. balthica*. A very small concentration of mb-HSP70 may be present in somatic tissue of gravid females, but this result could be due to the inclusion of a small amount of gonadal tissue in the sample, possible due to leakage from the gonads during freezing and thawing or because gonads in gravid *M. balthica* envelop most other tissues (Gilbert 1978), making dissection difficult. Regardless, the concentration of mb-HSP70 in these tissues is low enough to not influence the results. Male *M. balthica* and females prior to egg development had concentrations of mb-HSP70 below the reliable limit of this assay. This low level of

reactivity in the ELISA, six to seven orders of magnitude lower than that of egg tissue, was likely due to non-specific binding of the antibody to protein aggregates and not to the presence of mb-HSP70.

The concentration of the mb-HSP70 measured by this ELISA was linearly related to egg-concentration in samples and increases with the maturity of the gonads. This necessitates calibration of the ELISA, whenever it is used to quantify eggs, by the establishment of an egg concentration to mb-HSP70 concentration curve, as we do here. As this can be reliably done with ten samples; it is not labor intensive. Without this calibration step, samples taken at the same time and from the same area may be compared with each other, but they cannot be compared with samples from another time or place.

The number of eggs in each clam did not change between October and November but the total amount of protein in each egg increased as well as the fraction of mb-HSP70 in that protein. This indicates that once eggs are produced and discernable under a light microscope, they increase in size but not in number, so the assay can be used at any point during oogenesis without over or under-estimating the fecundity of a given clam. In November, there were some clams that had high BMIs but produced few eggs. Many of these clams were small (16-19 mm), and some may have already partially spawned, explaining the trend. There are many potential roles of HSP70 in *M. balthica* that would explain its increase with egg maturity. HSP70 proteins are involved in the folding of newly synthesized proteins and in the transport of proteins across membranes and thus are important during oogenesis (Billoud et al. 1993, Bukau & Horwich 1998). Additionally, an HSP70, not expressed in non-reproductive adult tissues, has been linked

to meiosis during oogenesis and is essential in mitosis during embryogenesis in the sea urchin *Paracentrotus lividus* (Sconozo et al. 1999, Geraci et al. 2003).

Egg production in *M. balthica* from the Chesapeake increases with clam BMI, and clams with a BMI lower than ~1.4 mg cm^{-3} do not appear to invest energy in egg production. This is similar to *M. balthica* from the eastern North Atlantic, except that clams from the Chesapeake have a lower BMI (range in Chesapeake: 1-8, this study; range in eastern North Atlantic: 4-14, Beukema et al. 2001) and are likewise capable of reproduction at a lower BMI (1.4 in Chesapeake, this study; 5.5 in eastern North Atlantic, Honkoop et al. 1999). This is likely due to differences in growth patterns; *M. balthica* in the Chesapeake attain a maximum shell length that is approximately double that observed in Europe. However, *M. balthica* in both areas produce a similar number of eggs at the same size. Our smallest fecund clams (shell length 16-19 mm) produced between 7,000 and 60,000 eggs, which is very similar to 15mm clams from the Baltic that produced between 10,000 and 90,000 eggs (Honkoop & van der Meer 1998). Many of the clams that had high BMIs but produced fewer eggs than expected were these small clams.

CONCLUSIONS

We present here a new method for quantifying egg production in *M. balthica*. Our technique is accurate, as well as simpler, and cheaper to perform than other methods, and will allow for meaningful comparisons among clams from different systems. This method has been tested thus far only on clams from Chesapeake Bay and is likely applicable to all western North Atlantic populations of *M. balthica*. As there are differences between the western and eastern populations (Meehan 1985), the assay still needs to be verified for clams from eastern North Atlantic populations.

ACKNOLEDGMENTS

I would like to thank B. Brylawski and K. Knick for help in field collection of clams, and J. Long and D. Wolcott whose comments on previous drafts substantially improved the document. W.C. Long was supported by an EPA GRO fellowship. This project was supported by the Commonwealth of Virginia and a grant from the Graduate Student Association of the Virginia Institute of Marine Science.

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Figure 1:

ELISA curves for somatic and gonadal tissues from adult *M. balthica*. The standard line is plotting both against its dilution in µl of original solution (along with the other samples) as well as the concentration of mb-HSP70. The horizontal dotted line indicates the rate at the C_{50} from the standard curve fit. The dashed vertical lines indicate the approximate C_{50} values for each curve based on the standard C_{50}. The C_{50} for each sample has the same concentration of mb-HSP70 as the C_{50} in the standard, so the dilution factor (in µl of original sample) can be used to calculate the concentration of mb-HSP70 in the undiluted sample: standard (1.0 µl, 1.25 units mb-HSP70), female somatic (6.6 µl, 0.19 mb-HSP70), female gonadal (0.038 µl, 33.4 mb-HSP70), male gonadal (unreliable fit, ~200 µl, ~0.006 mb-HSP70).

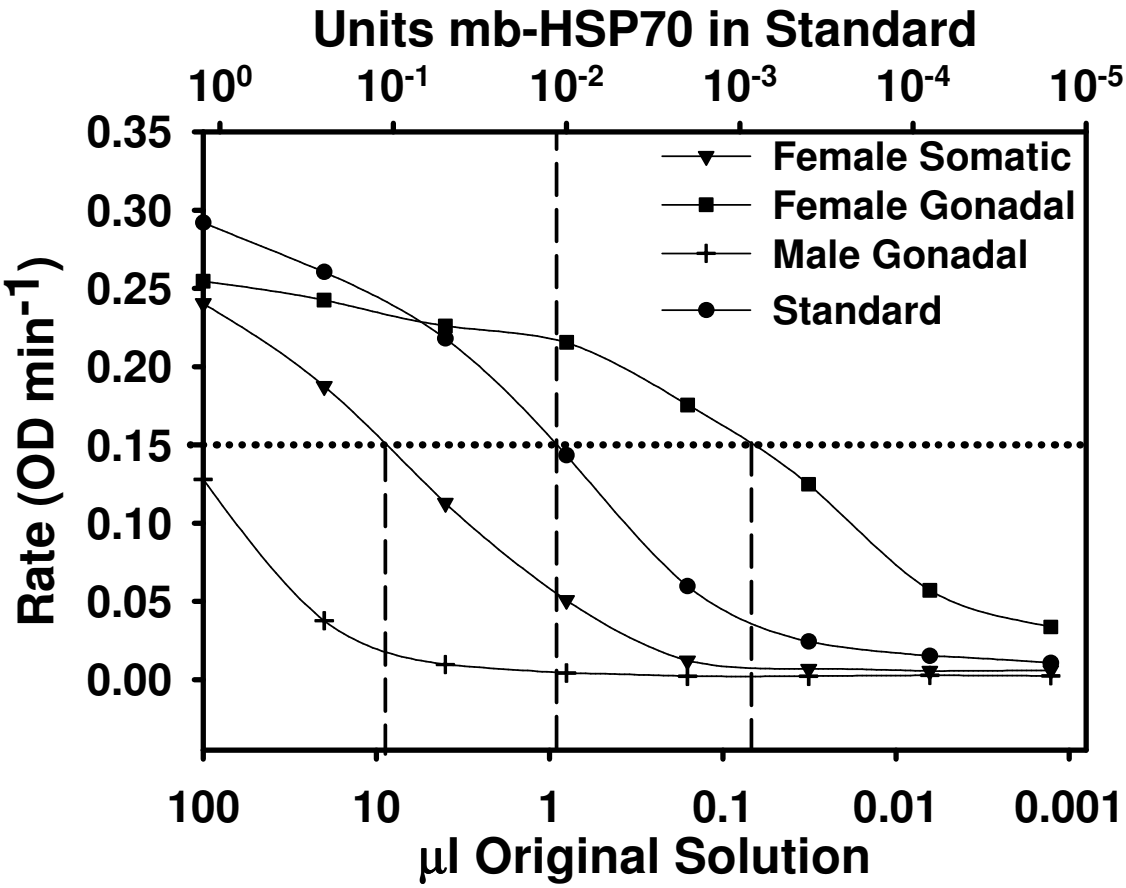

Units mb-HSP70 in Standard
10^0
10^-1
10^-2
10^-3
10^-4
10^-5
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Rate (OD min^-1)
Female Somatic
Female Gonadal
Male Gonadal
Standard
100
10
1
0.1
0.01
0.001
µl Original Solution

Figure 2

Relationship between egg concentration and mb-HSP70 concentration at three different times A) Oct. 2003, B) Oct. 2005, C) Nov. 2005. Equation and trend line represent least squares fit ($\underline{P} > 0.0001$ for all three).

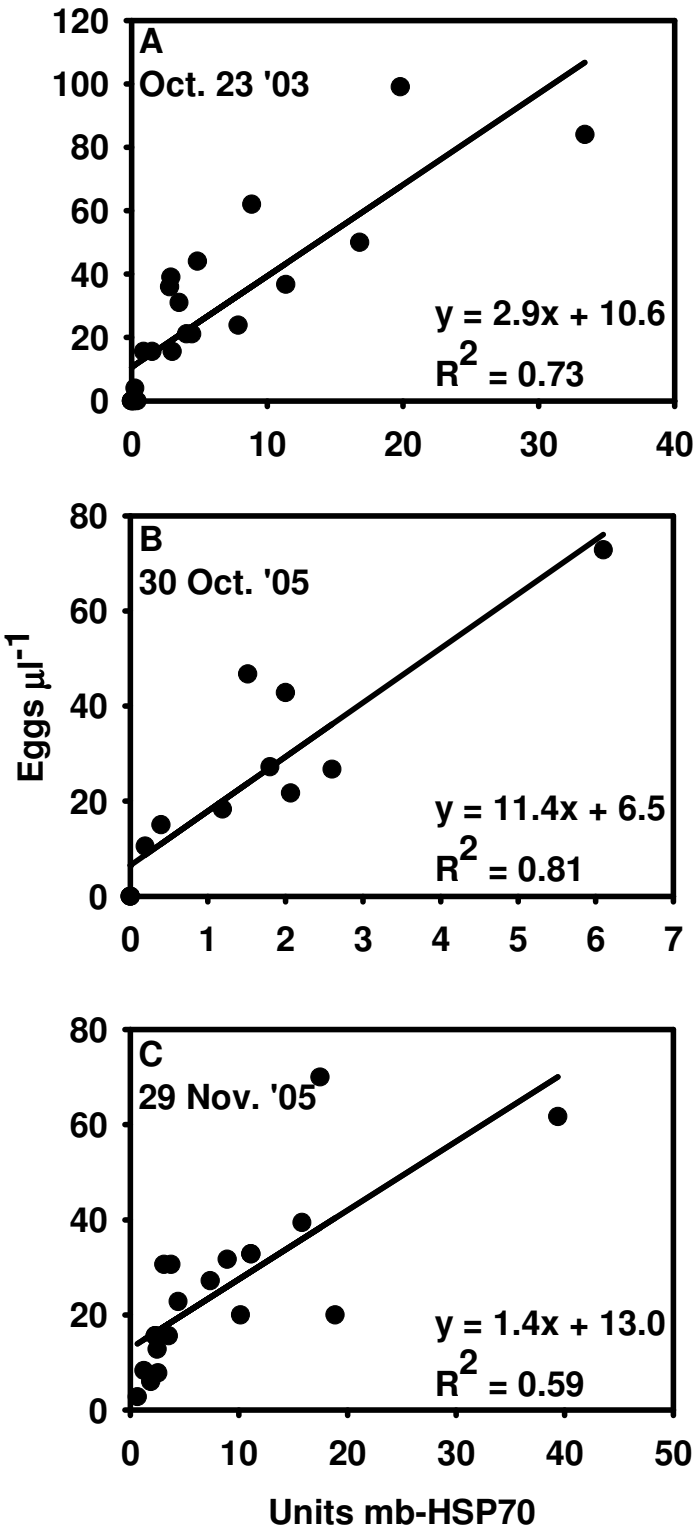
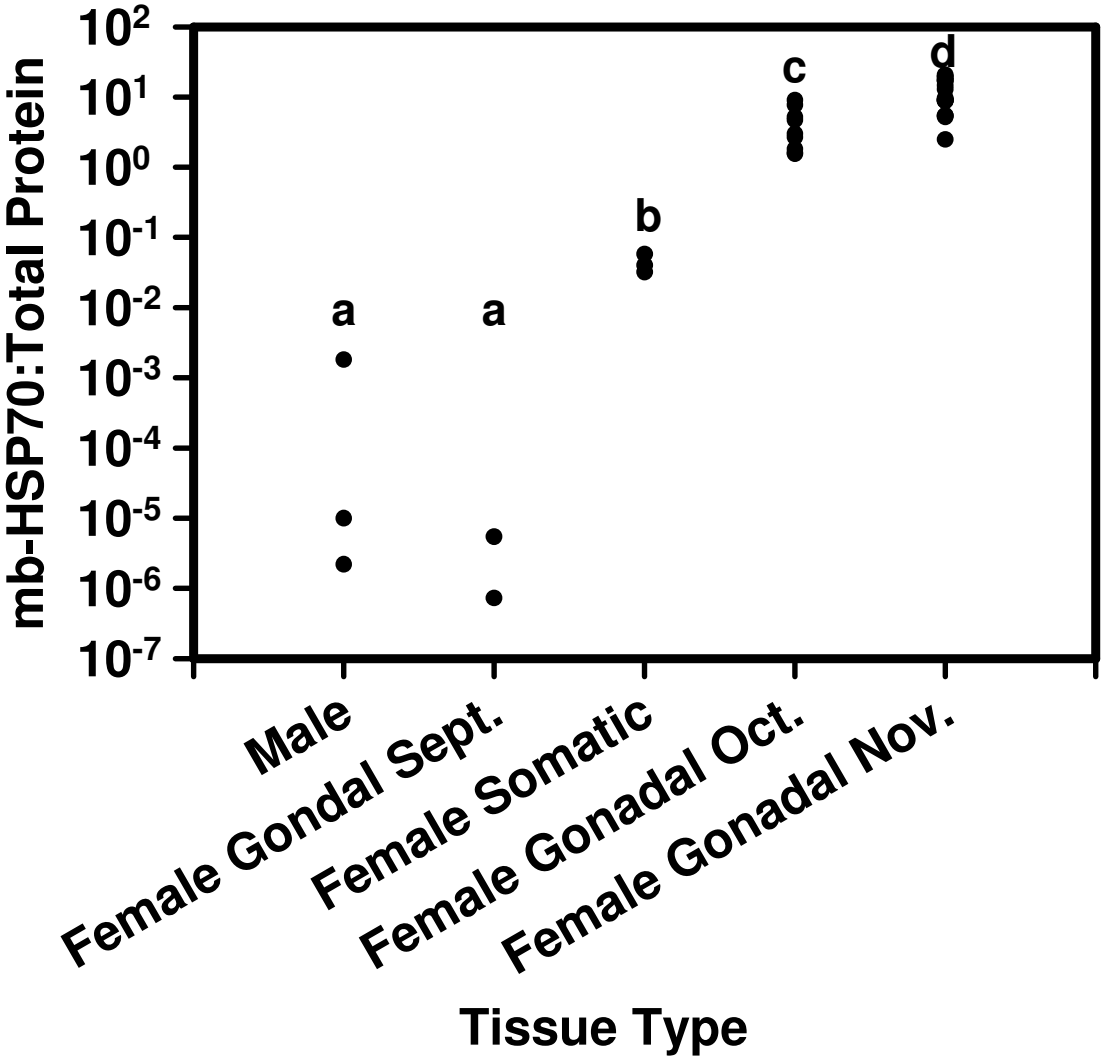
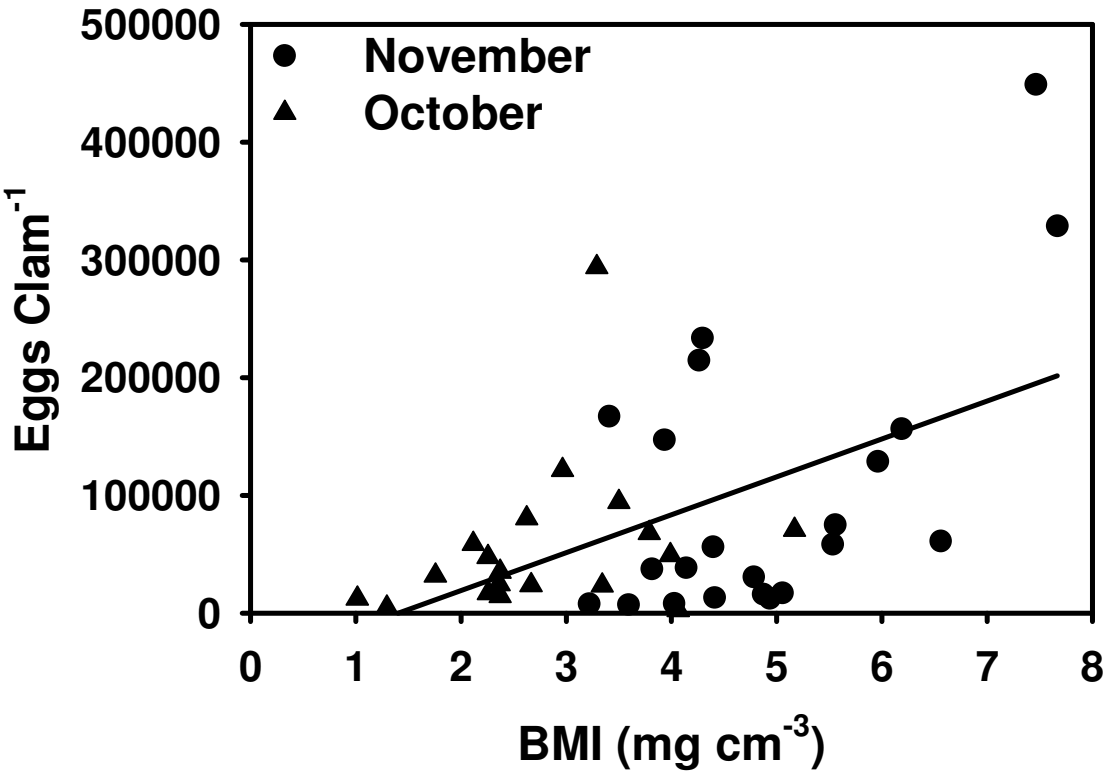

A
Oct. 23 '03
y = 2.9x + 10.6
R2 = 0.73
B
30 Oct. '05
y = 11.4x + 6.5
R2 = 0.81
C
29 Nov. '05
y = 1.4x + 13.0
R2 = 0.59
Eggs µl-1
Units mb-HSP70

Figure 3



Ratio of mb-HSP70:total protein in *M. balthica* tissues. Samples with different letters over them differ at the 0.05 level (Tukey's test).

Figure 4



Relationship between BMI and egg production in *M. balthica* in 2005. Line is least squares regression ($\underline{P} = 0.001$; $R^2 = 0.26$; Equation: Eggs = 32000*BMI – 45000).

CHAPTER 3:

BEHAVIORAL EFFECTS OF LOW DISSOLVED OXYGEN ON THE BIVALVE *MACOMA BALTHICA*

ABSTRACT

Hypoxia, a dissolved oxygen concentration below 2 mg l^{-1}, is a significant anthropogenic stressor in estuarine ecosystems. Sedentary organisms are unable to avoid hypoxic areas and typically are highly stressed. We tested the effects of hypoxia on the behavior and mortality of Macoma balthica, the Baltic macoma, using four levels of dissolved oxygen in flow-through tanks. We used five replicates of each of four treatments: (1) Hypoxic (DO < 2.0 mg O_2 l^{-1}), (2) Moderately hypoxic (DO 2.5 ± 0.5 mg O_2 l^{-1}), (3) Nearly normoxic (DO 3.5 ± 0.5 mg O_2 l^{-1}), (4) Normoxic (DO > 4.0 mg O_2 l^{-1}). Dissolved oxygen in hypoxic treatments was lowered using a novel fluidized mud-bed, designed to mimic field conditions more closely than the common practice of solely bubbling nitrogen or other gasses. Our method for lowering the DO concentrations for a laboratory setup was effective. In addition, we calculated a lethal concentration for 50% of the M. balthica population (LC_{50}) at 1.7 mg O_2 l^{-1} for our 28-day experimental period. M. balthica decreased its burial depth under hypoxic and moderately hypoxic (~ 2.5 mg O_2 l^{-1}) conditions within four days of the onset of hypoxia. Since reduced burial depth makes the clams more vulnerable to predators, our results suggest that the sub-lethal effects of hypoxia could change the rate of predation on M. balthica in the field.

Key Words: Behavior; Bivalves; Burial depth; Hypoxia; Eutrophication; Macoma balthica

INTRODUCTION

In the 20th century, shifts in land-use patterns have increased the rate of input of allochthonous phyto-nutrients to estuarine systems (Cloern, 2001; Kemp et al., 2005). This cultural eutrophication has had many serious ecological consequences, such as the development of hypoxia, dissolved oxygen concentrations (DO) below 2.0 mg l^{-1}, in bottom waters (Diaz and Rosenberg, 1995; Gray et al., 2002). Nutrient inputs into an estuary can cause algal blooms that increase the input of fresh organic matter to the benthic community. Hypoxia develops when organisms on or near the bottom, primarily microbes, respire and consume oxygen at a faster rate than it is replenished by mixing with surface waters.

As hypoxia develops in benthic waters, it can have substantial effects on benthic community structure and function (Diaz and Rosenberg, 1995). Motile animals, including predators with low tolerances for hypoxia, escape the area, seeking refuge in nearby more oxygenated habitats (Pihl et al., 1991; Das and Stickle, 1994; Bell and Eggleston, 2005; Powers et al., 2005). The benthic community is acutely affected, as many species cannot move to avoid hypoxic waters; reductions in community biomass, abundance, and diversity are commonly observed in hypoxic areas (Rosenberg, 1977; Gaston, 1985; Dauer et al., 1992; Diaz et al., 1992; Llansó, 1992; Powers et al., 2005).

Sub-lethal effects of hypoxia are common. Organisms respond by decreasing metabolism and thus oxygen consumption (Wu, 2002). This, in turn, can lead to diminished growth and reproduction (Nilsson, 1999; Condon et al., 2001; Grove and

Breitburg, 2005). In addition, behavioral changes are often observed that either increase oxygen supply or decrease oxygen demand. Bivalves and polychaetes extend siphons or palps, decrease burial depth, or even float up above the benthic boundary layer into higher DO waters (Brafield, 1963; Rosenberg et al., 1991; Taylor and Eggleston, 2000; Seitz et al., 2003). Some polychaetes and anemones will elongate, increasing their surface-area-to-volume ratio to facilitate oxygen diffusion. Reduced feeding and movement also is commonly observed (Sagasti et al., 2001).

These sub-lethal responses may increase an organism's vulnerability to predation. For example, the extension of siphons and palps farther into the water column and decreasing burial depth make detection by predators more likely for species that use burial depth to obtain a refuge from predation, such as the Baltic macoma, Macoma balthica (Piersma et al., 1995; De Goeij et al., 2001). Whether predators are capable of taking advantage of these stressed prey is a matter of debate, since predators may leave hypoxic areas (e.g., Pihl et al., 1991; Bell and Eggleston, 2005). Predation on tethered Glycera in field experiments was DO dependant with predation occurring at low rates under hypoxic conditions (Nestlerode and Diaz 1998). In laboratory experiments, hypoxia changed the predator-prey relationship, likely due to a change in both predator and prey behavior (Breitburg et al., 1994; Breitburg et al., 1997; Taylor and Eggleston, 2000; Sagasti et al., 2001; Seitz et al., 2003).

M. balthica is a small, thin-shelled clam that is common in estuarine systems on the East Coast of the U.S. north of South Carolina. Shell lengths are typically <40 mm. In Chesapeake Bay, this species comprises over 85% of the infaunal biomass in many areas in mesohaline muddy habitats, (Holland et al., 1977; Hagy, 2002). It is an

important species in the food-web, since it is preyed upon by numerous fish and crustaceans, comprising ~50% of the diet of the blue crab, Callinectes sapidus (Hines et al., 1990). M. balthica is an excellent model organism for studying the effects of hypoxia, since it is common in areas that experience hypoxia and is easier to manipulate than other benthic species, such as crustaceans and polychaetes. Tolerant of hypoxia, it can survive for an average of 15 days under near anoxic (0 mg O_2 l^{-1}) conditions (Brafield, 1963) and extends its siphons further into the water column in response to hypoxia (Seitz et al., 2003). This clam avoids predation by burying deep in the sediment, down to 35 cm (Hines and Comtois, 1985), which makes it a good organism for examining the effect of hypoxia on burial depth.

Prior experiments on the effects of hypoxia on M. balthica used two levels of DO, hypoxic and normoxic (e.g., Seitz et al., 2003). We designed this experiment to look at the effects over a range of DO concentrations observed in the field, including moderate hypoxia (2-3 mg O_2 l^{-1}) and near normoxia (3-4 mg O_2 l^{-1}). We also designed a system that uses natural sediment oxygen demand (SOD) to reduce DO and that generates a continuous supply of low DO water, allowing test organisms to be held in a flow-through system. The goal was to remove confounding factors of toxicity inherent in recirculating water systems and to generate low DO water with a chemical composition more typical of field conditions during hypoxic events. In most previous experiments the DO was reduced by bubbling gases, such as nitrogen (e.g. Seitz et al. 2003), or hydrogen sulfide (e.g., Brafield, 1963) through the water. Our objectives in this study were to examine the effect of low DO on M. balthica burial depth and mortality, and to test the effectiveness of our mechanism for the supply of low-DO water.

MATERIAL AND METHODS

Fluidized-mud reactor

To produce and distribute low-DO water for experiments, we designed a system that minimized our reliance on nitrogen and allowed us to conduct the experiment in a flow-through system. DO was lowered using two large tanks connected in series that acted as fluidized-mud reactors (described in detail below; Fig. 1). At the bottom of each tank, a perforated array of poly-vinyl chloride (PVC) pipe was embedded in ~15 cm of crushed oyster shell. Above this, we layered ~50 cm of mud collected from shallow coves in the York River, Chesapeake Bay, enriched with a small quantity (approximately 50 grams) of Osmocote slow-release fertilizer to stimulate microbial growth. Unfiltered seawater from the York River was dispensed into the first tank, where it was distributed by the pipe array and flowed up through the mud, where SOD reduced the DO. The water drained by gravity from the first reactor into a second identical reactor, after which it was stored in a 400-l distribution tank covered with a sheet of plastic to reduce air exchange. When we ran the system at maximum flow and allowed it to come to equilibrium, the DO in the distribution tank stabilized at ~0.8 mg O_2 l^{-1} (measured with a DO probe, YSI Model 85, Yellow Springs Instruments, Dayton, Ohio, USA). The flow from the reactors was slightly less than what we needed for the experiment, so we supplemented it with additional unfiltered river water. This necessitated bubbling a small amount of nitrogen though the water to maintain hypoxic conditions; however, the amount used was approximately an order of magnitude less than what was necessary in

previous experiments without the addition of the reactors. Slowly bubbled nitrogen had the added benefit of filling the headspace and reducing the diffusion of oxygen into the tank. A second distribution tank, for normoxic water, was filled with unfiltered seawater and air was bubbled through it.

Experimental setup

We conducted our experiment in 40-l, transparent, plastic tanks. Each tank was filled with 20 cm of mud collected from shallow coves in the York River where Macoma balthica are abundant. Larger bivalves and other macrofauna were removed by sieving the mud through a 2-mm mesh sieve. Twenty tanks were randomly assigned five replicates of each of four treatments: (1) Hypoxic (DO < 2.0 mg O_2 l^{-1}), (2) Moderately hypoxic (DO 2.5 ± 0.5 mg O_2 l^{-1}), (3) Nearly normoxic (DO 3.5 ± 0.5 mg O_2 l^{-1}), and (4) Normoxic (DO > 4.0 mg O_2 l^{-1}). DO levels were maintained by manually adjusting the relative amounts of hypoxic and normoxic water from the distribution tanks. Holes were drilled in the lids of the tanks and sealed with corks so that the DO and temperature could be monitored without fully opening the tank lids.

We collected M. balthica with shell lengths > 20mm from the York River via suction sampling (Eggleston et al.,1992). We transported the clams back to the lab and allowed them to recover for at least 24 hours in a flow-through system prior to use. Twenty clams were transplanted in each tank. Only healthy clams with whole shells and a quick siphon-withdrawal reflex were used. We attached a monofilament line to the center of each clam shell with cyanoacrylate glue and ran the line through a pinhole in the lid of the tank. A mark was made on the line with permanent ink 35 cm above the clam,

and after the clams acclimated, the relative burial depth was estimated by measuring the distance from the top of the lid to the mark and adjusted for the distance from the lid to the sediment surface to calculate the absolute depth. Due to the curvature of the lids, topology of the sediment surface, and the fact that our clams were not always directly under their holes, our estimate of absolute burial depth was only precise to ±3 cm, and tended to overestimate. However, since we calculated the change in burial depth as the difference between the distance from the lid to the mark on two different day, thus eliminating the other sources of error, it was precise (±2 mm). Clams were acclimated in the tanks under normoxic conditions for at least 24 hours to allow them to recover from handling and to bury to natural burial depths. Clams that died or did not bury before the experiment began were replaced. The day we started the experimental treatment, any dead or unburied clams were removed without replacement. Before the experiment, four of the tanks did not receive adequate water flow and had high clam mortalities (these were tanks at the end of the flow lines where water-pressure was lowest, and we prevented additional mortalities by increasing flow to the distribution tanks); these tanks were excluded from analyses.

After the acclimation period, the water flow in each of the tanks was adjusted to lower the DO in the tanks to their nominal treatment range. DO and temperature were monitored daily, and the flow to each tank was adjusted at the same time if necessary to maintain the DO within the nominal range. Burial depth of each clam was monitored daily for the first week and, after that, every two-three days. Dead clams were identified if they gaped and continued to do so when handled; they were removed. The DO was kept within the nominal experimental ranges for 28 days before returning it to normoxia.

The mud from each tank was sieved and the clams that were still alive were counted and measured. Proportional survival was calculated for each tank.

Data analyses

The DO measurements were analyzed with a nested Analysis of Variance (ANOVA; tank nested within DO treatment). The data were square-root transformed to reduce heteroscedasticity. Change in burial depth (initial burial depth minus burial depth on each subsequent day) was analyzed for each day up through day 10 with a nested ANOVA (tank nested within DO treatment). Burial depth after day 10 was not analyzed, as the number of clams remaining in the lowest DO treatments was too low. Where an effect of treatment was found, a Tukey's pair-wise multiple comparison test was performed. Survival in each tank was plotted against the average DO and analyzed with a non-linear least-squares regression.

RESULTS

There was a significant difference in dissolved oxygen among the treatments (nested ANOVA; $F_{3,656} = 799.42$; $\underline{p} < 0.0005$), and the mean for each treatment fell within the nominal range and differed from the other means (Tukey's test, $p < 0.0005$; Fig. 2). The overall burial depths were, on average on day 1, 126 mm for all treatments. By day 10, average burial depth decreased (i.e., clams moved up toward the sediment-water interface) by approximately 20 mm in our Hypoxic treatment (< 2.0 mg O_2 l^{-1}) and by 10 mm in our Moderately hypoxic treatment (~ 2.5 mg O_2 l^{-1}), while increasing slightly (i.e., clams moving away from the sediment–water interface) in our Nearly normoxic and Normoxic treatments. There was a significant effect of DO treatment on change in burial depth on days 4 through 10 (nested ANOVAs; $\underline{p} < 0.05$; Fig. 3), and a significant effect of tank (nested within DO treatment) on days 2 and 4. In both Hypoxic (DO < 2.0 mg O_2 l^{-1}) and Moderately hypoxic (2.5 ± 0.5 mg O_2 l^{-1}) treatments, the burial depth decreased beginning almost immediately (on day 2) and reached an asymptote at around day 6. The Hypoxic and Moderately hypoxic treatments both differed from the Normoxic treatment (DO > 4.0 mg O_2 l^{-1}) on days 5 through 10 (Tukey's test). Clam survival decreased in a non-linear fashion with decreasing DO, and we estimated a lethal concentration for 50% of the population (LC_{50}) of 1.7 ± 0.5 (SE) mg O_2 l^{-1} for our 28-day experiment (Fig. 4).

DISCUSSION

Our method for lowering the DO concentrations for a laboratory setup was effective and has several benefits. First, the mechanism for lowering the DO is similar to that in the field; DO concentrations are reduced by SOD. In previous laboratory experiments, the DO was reduced by displacement through bubbling a gas or mixture of gases, usually nitrogen (e.g., de Zwaan and Babarro, 2001) or hydrogen sulfide (Brafield, 1963), through the water. This difference between field and laboratory DO development may confound the results. Second, as our design used a flow-through system, it avoided the use of stagnant or re-circulated water (e.g., Modig and Ólafsson, 1998; Grove and Breitburg 2005) and the resulting high abundance of bacteria known to lower survival times in lab experiments (de Zwaan and Babarro, 2001; de Zwaan et al., 2001). Finally, our design was cost effective, as it substantially reduced the amount of gas necessary for maintaining hypoxic conditions.

Our 28-day LC_{50} for M. balthica of 1.7 mg O_2 l^{-1} is comparable to experimentally determined values in the literature (de Zwaan and Babarro, 2001 [Lethal time for 50% of the population, LT_{50}, 9.0 days under anoxia]; de Zwaan et al. 2001 [LT_{50} of 4.5 days under anoxia]; Seitz et al., 2003 [LT_{50} of 16 days at 1.0 mg O_2 l^{-1}]), as well as to models of the survival of M. balthica (Borsuk et al., 2002 [21 day LC_{50} 1.5 mg O_2 l^{-1}]). Thus, M. balthica is apparently quite tolerant of hypoxia in the lab; however, M. balthica populations suffer heavy mortality in the field under moderate to severely hypoxic conditions, comparable to the range used in this experiment (e.g., Llansó, 1992; Buzzelli

et al., 2002; Powers et al., 2005, Long et al., in review). This suggests that another form of mortality is driving the pattern observed in the field.

Our results, combined with those of prior experiments, suggest that sub-lethal exposure to hypoxic conditions may result in increased predation risk. We demonstrate that M. balthica responds to hypoxia by vertically migrating upward in the sediment. Clams in both our Hypoxic (DO < 2.0 mg O_2 l^{-1}) and our Moderately hypoxic (DO ~ 2.5 mg O_2 l^{-1}) treatments moved towards the sediment surface by approximately 20 and 10 mm, respectively. Although no vertical migration was observed in previous similar experiments (Seitz et al., 2003), this is likely due to differences in sediment type; Seitz et al. (2003) used sand and we used mud. M. balthica is known to bury deeper in mud (its preferred habitat), down to 35 cm, than in sand, down to 20 cm (Hines and Comtois, 1985), allowing substantial scope for change in mud. Changes in M. balthica's burial depth of as little as 2 cm, the change observed in our Hypoxic tanks, can decrease predator handling time by 66% (Piersma et al., 1995). In addition, this vertical migration may be increased in field situations by siphon cropping. M. balthica extends its siphons into the water column in response to hypoxia (Seitz et al., 2003), which can lead to higher rates of siphon cropping (Peterson and Skilleter, 1994; Skilleter and Peterson, 1994) and decreased burial depth (de Goeij et al., 2001), thus, further increasing the vulnerability of clams to predation.

The time scale of the behavioral changes we observed is much shorter than that for lethal effects. Siphon extension occurs within a day (Seitz et al., 2003) and burial depth reduction begins within two days and decreases substantially within four days (this study), whereas significant mortality under moderately hypoxic conditions (1-2 mg O_2 l^{-1})

does not occur for one to two weeks (Borsuk et al., 2002), or longer (Seitz et al., 2003). In many systems where hypoxia is episodic, such as the York River (Diaz et al., 1992), the Neuse River (Powers et al., 2005), and parts of the Baltic Sea (Modig and Ólafsson, 1998), hypoxic conditions seldom last for more than a week at a time before returning to normoxia. Since M. balthica is highly tolerant of episodic hypoxia of this type (Modig and Ólafsson, 1998), we suggest that predators taking advantage of stressed prey may be a major source of mortality in these systems.

Our knowledge of predator behavior in these systems suggests that post-hypoxic foraging occurs. Motile predators can rapidly reinvade areas after relaxation of hypoxic conditions (e.g., Pihl et al., 1991; Nestlerode and Diaz, 1998; Bell and Eggleston, 2005; Long and Seitz, in prep). They may take advantage of stressed benthic organisms following hypoxia, as their diet shifts to include a higher percentage of deeper-burying organisms, including M. balthica (Pihl et al., 1992). Our results suggest that this increase in predator foraging may be due to the demonstrated vertical migration of M. balthica in response to hypoxia in systems where this species is common.

ACKNOWLEDGMENTS

We would like to thank K. Knick, J. Long, and M. Seebo for help with the experiment. Comments by R. Diaz, J. Long, and D. Wolcott improved earlier versions of this paper. C. Long is supported by an EPA GRO fellowship. Funding for this project was provided by the Commonwealth of Virginia. This is contribution XXXX from the Virginia Institute of Marine Science.

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Figure 1

Illustration of fluidized mud-bed reactor and experimental setup. Darker water indicates lower DO and arrows indicate direction of water flow. Four experimental tanks are shown on the bottom level, however, multiple replicate tanks for each treatment were used, and treatments were randomly interspersed.

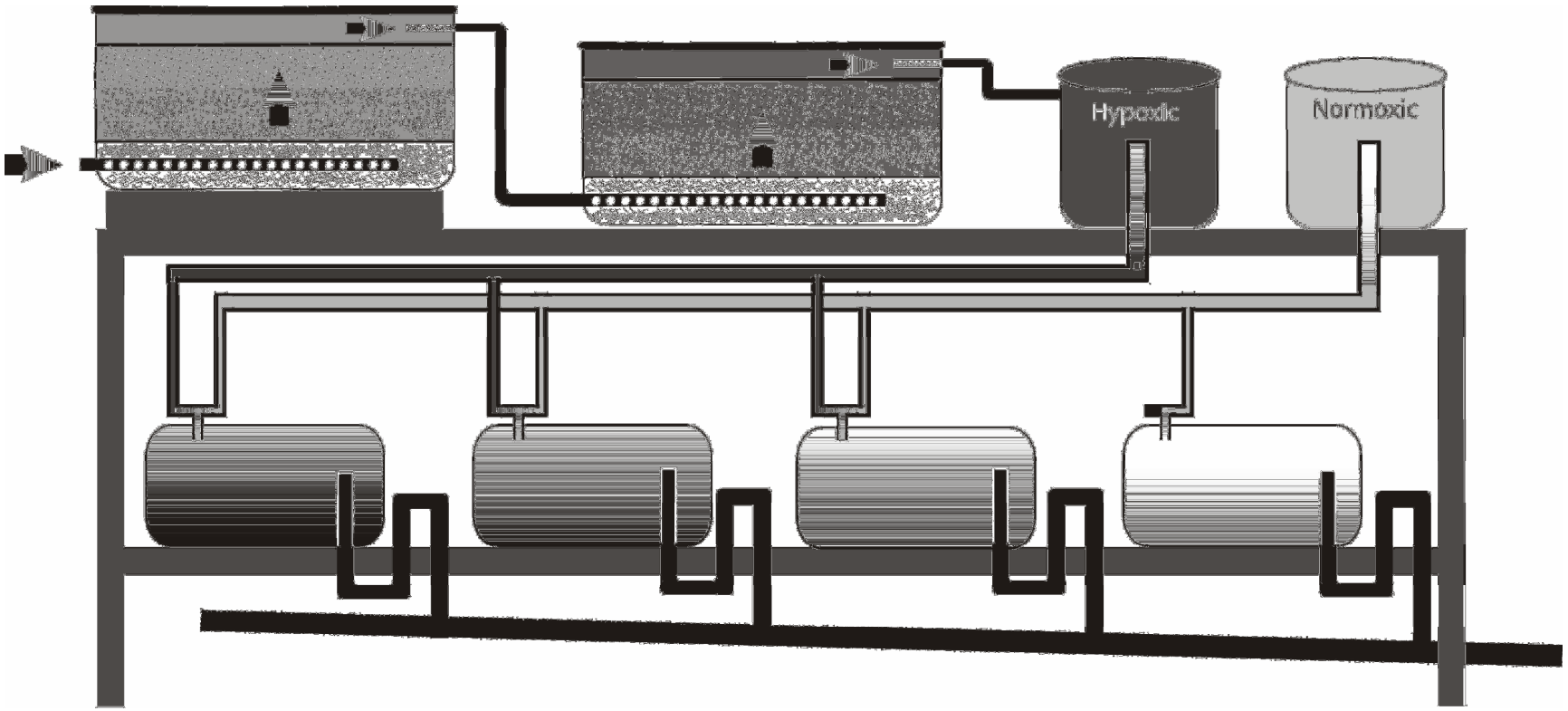
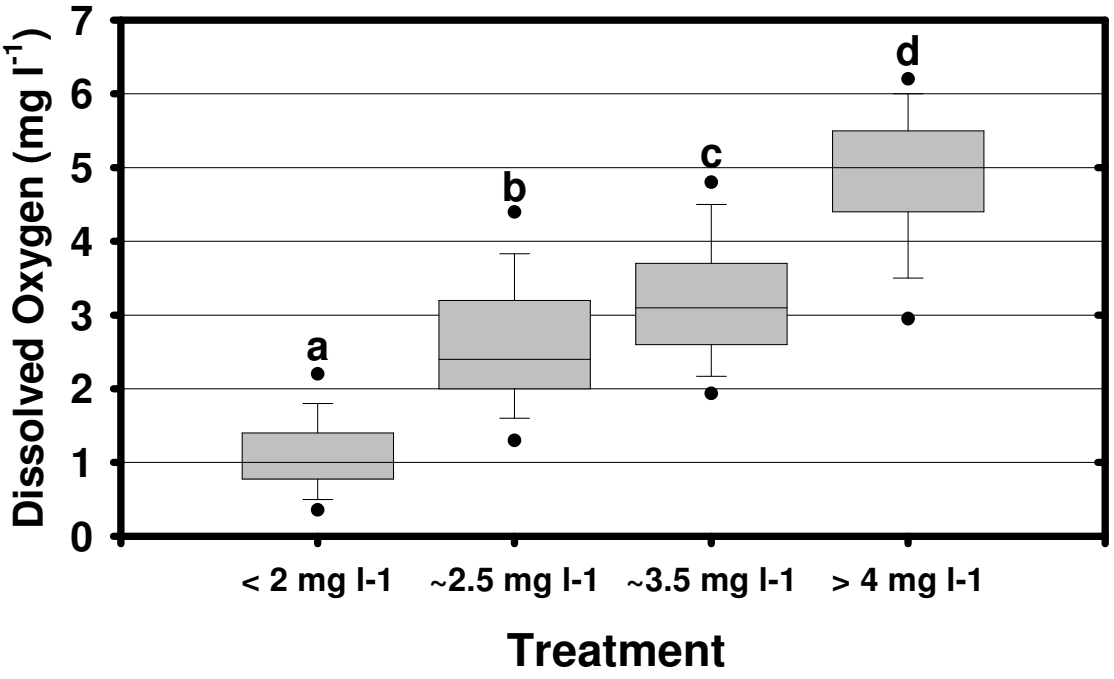
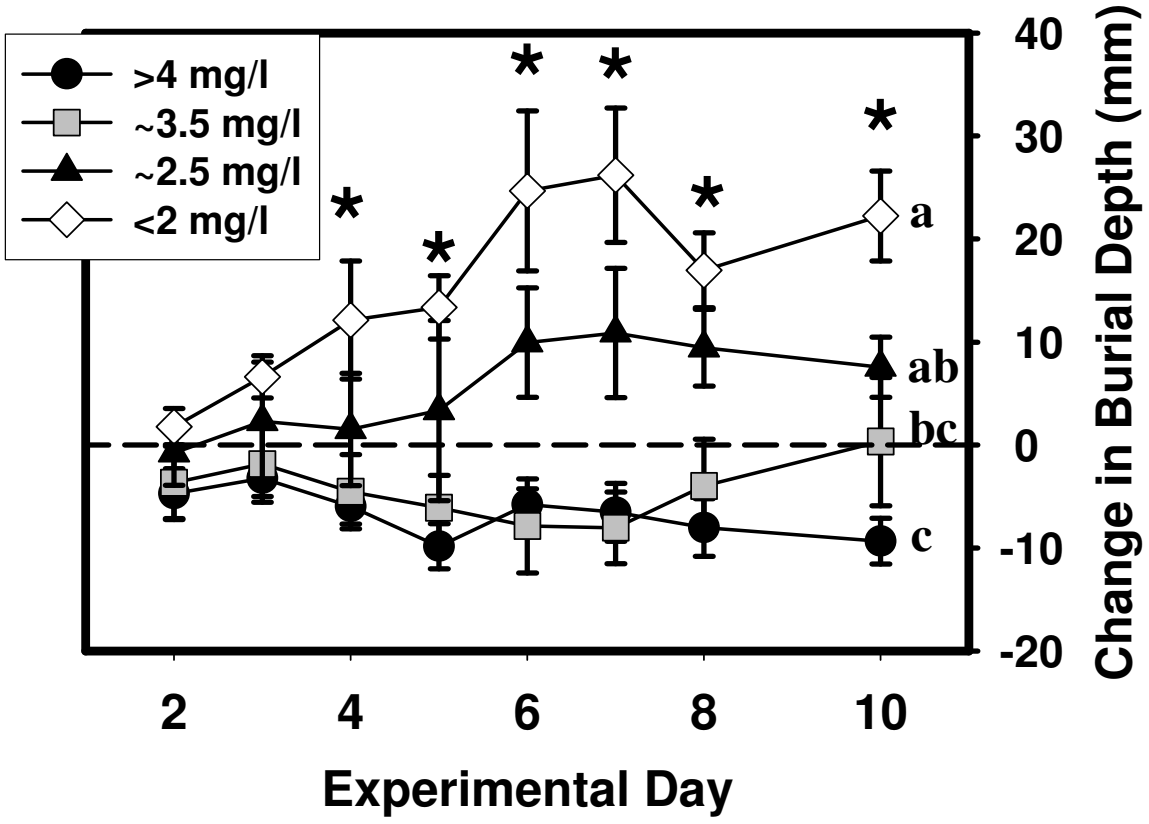

Hypoxic
Normoxic

Figure 2



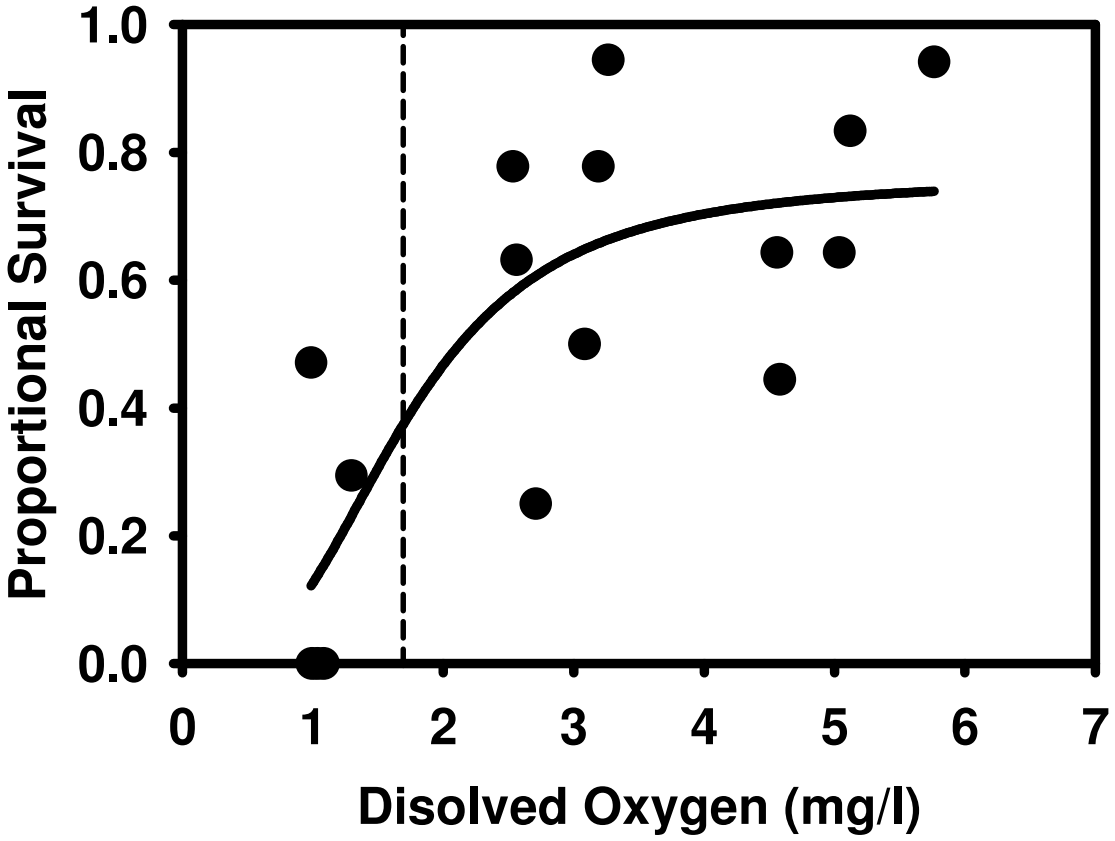
Box-plots of DO observations for each of the four treatment levels. Horizontal lines represent the median DO, boxes the 25th and 75th percentiles, whiskers the 10th and 90th percentiles, and dots the 5th and 95th percentiles. Boxes with different letters over them differ ($p < 0.0005$; Tukey's test).

Figure 3



Mean (± 1 SE) change in burial for each DO treatment. Hypoxic treatments were initiated on day one. Positive numbers indicate movement towards the water-sediment interface. Asterisks indicate a significant effect of DO treatment on burial depth. Treatments with different letters next to them differ at the 0.05 level (Tukey's test; shown for day 10 only). Dashed line indicates no net vertical migration.

Figure 4



Effect of DO on M. balthica survivorship. Proportional survival at the end of the 28 day experiment is plotted against average DO for each tank. Vertical dashed line indicates LC_{50}. Trend line is from best fit equation: $y = \frac{0.76}{1+(\frac{x}{1.71})^{-3}}$, ($R^2 = 0.55$, p = 0.0021).

CHAPTER 4:

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TROPHIC INTERACTIONS UNDER STRESS: HYPOXIA ENHANCES FORAGING IN AN ESTUARINE FOOD WEB

ABSTRACT

Ecosystem-level effects of stressors are critical to understanding community regulation, and environmental stress models are useful in describing such effects. Hypoxia is an important stressor in aquatic ecosystems that usually decreases abundance and biomass of benthic fauna. In field surveys, predator abundance is low in hypoxic areas, and in lab experiments, predators reduce their feeding rates under hypoxic conditions, leading to the prediction that consumer stress models, rather than prey stress models, apply to the systems. We tested predictions from these models with manipulative field experiments wherein we varied predator access to marked *Macoma balthica* clams at deep and shallow sites in the York River, Chesapeake Bay, before (June) and during (August) hypoxic episodes. In June, DO in deep and shallow sites was normoxic (> 2 mg l^{-1}) for most of the experiment. In August, the shallow zone remained normoxic, while the deep zone experienced several hypoxic episodes. During hypoxia, predation rates in hypoxic sites were more than twice those in normoxic sites, whereas mortality due to physical stress did not differ between time periods or depths. Ambient clam densities were lower at the deep sites than at the shallow sites, and in August than in June. We conclude that hypoxia increased the susceptibility of benthic prey to predation, enhancing infaunal secondary production available to predators, but concurrently reducing the resilience of the benthic community. These findings are inconsistent with the predictions of consumer stress models, indicating that prey stress models are more appropriate in this system.

KEY WORDS: environmental stress models, food web, hypoxia, predation, predator-prey, *Macoma balthica*.

INTRODUCTION

Predation and hypoxia

Environmental stress is a major determinant of community structure. Species respond differently to the same stressor, such that an increase in the magnitude of a stressor is expected to shift the outcome of interactions between species to favor the one with greater tolerance. This expectation has been expressed in consumer stress models (CSMs), which predict that a stressor will reduce predation when consumers are less tolerant of a stressor than their prey (Menge & Sutherland 1987). Alternatively, prey stress models (PSMs) predict that a stressor will increase predation when consumers are more tolerant of or resilient to the stressor than their prey (Menge & Olson 1990).

Anthropogenic eutrophication of estuaries has had widespread effects on these ecosystems (Kemp et al. 2005), including development of hypoxic, or oxygen-depleted, bottom waters (Diaz & Rosenberg 1995). Hypoxia (here defined as dissolved oxygen [DO] concentrations < 2 mg l^{-1}) is an important stressor of benthic communities, and its effects are well documented in many systems (e.g., Diaz et al. 1992, Powers et al. 2005). Typically, abundance, biomass, recruitment, and diversity decrease, and there is a shift from large, long-lived species to small, opportunistic species. The magnitude of these effects generally increases with the severity of hypoxic stress.

Hypoxia has a multitude of non-lethal effects. Metabolism, and thus oxygen demand, decreases (Wu 2002), reducing growth and reproductive output (e.g., Grove & Breitburg 2005). Infaunal organisms migrate vertically in the sediment, stretching their

siphons or palps above the benthic boundary layer into higher DO concentrations, and they may expose themselves on the surface or float in the water column (Brafield 1963, Rosenberg et al. 1991, Taylor & Eggleston 2000, Seitz et al. 2003). Almost all species decrease oxygen demand by decreasing activity and feeding rates (Sagasti et al. 2001).

These effects, especially the behavioral responses, potentially increase the availability of benthic fauna to their predators. The closer proximity of infaunal prey to the sediment surface and extension of siphons and palps decrease predator searching time. However, the responses of predators to hypoxia may preclude their ability to take advantage of stressed prey. Many predators in these systems are highly mobile, and have a much lower tolerance for hypoxia than do the sessile prey (Das & Stickle 1993, Seitz et al. 2003). Field studies on predator abundance show a migration of motile predators out of hypoxic areas, often followed by a re-invasion shortly after hypoxia relaxes (Pihl et al. 1991, Das & Stickle 1994, Bell & Eggleston 2005, Powers et al. 2005). Almost universally, laboratory experiments show a decrease in predation rate under hypoxic conditions (Breitburg et al. 1994, Breitburg et al. 1997, Sagasti et al. 2001, Seitz et al. 2003), mostly due to a decrease in predator activity.

Either CSMs or PSMs could apply to hypoxic systems. Some authors (Sagasti et al. 2001, Powers et al. 2005) argue that, because the predators have lower tolerances for hypoxia than their prey and avoid hypoxic zones, hypoxia is likely to act as a refuge for prey species, as predicted by CSMs. Others suggest that PSMs are more appropriate and that predators consume prey stressed by hypoxia, either during a hypoxic episode (Rahel & Nutzman 1994) or immediately afterwards, before the prey have time to recover (Nestlerode & Diaz 1998). Foraging can occur during hypoxia; fish in a freshwater lake

foraged in hypoxic waters (Rahel & Nutzman 1994), and predation on tethered polychaetes in the York River, Chesapeake Bay, occurred at low levels during hypoxia (Nestlerode & Diaz 1998). Gut contents of predators in the York River shifted to include larger and deeper-burrowing prey items after hypoxic events (Pihl et al. 1992). These studies suggest that PSMs are appropriate but the studies did not quantify predation so they could not distinguish between PSMs and CSMs. Thus far, the only field study to definitively support CSMs was a caging study in Narragansett Bay, Rhode Island (USA) showing that mussels suffered no predation during hypoxia (Altieri & Witman 2006). In our study, we tested the effects of hypoxia in a soft-sediment community, using a manipulative caging experiment to quantify predation under hypoxic and normoxic conditions, to address the question of whether PSMs or CSMs are more appropriate for the York River system.

Study organisms

The thin-shelled clam *Macoma balthica* (hereafter *Macoma*) was the experimental prey species. *Macoma* is a deposit feeder and facultative suspension feeder that is the biomass-dominant macrofaunal species in mud habitats of Chesapeake Bay, comprising over 85% of the biomass in some habitats (Holland et al. 1997). Its shell length is typically < 40 mm, and it contributes greatly to energy flow and benthic-pelagic coupling (Baird & Ulanowicz 1989). *Macoma* is tolerant of hypoxia, with an LT (lethal time) 50 % of 15 d under near-anoxic conditions (Henriksson 1969). In response to hypoxia, *Macoma* migrates upward in the sediment (Brafield 1963, W. C. Long and R. D. Seitz unpublished manuscript) and extends its siphons into the water column to reach normoxic

waters (Seitz et al. 2003). In recent experiments *Macoma* moved 20-25 mm upwards in the mud during hypoxia over a period of 6 d (W. C. Long and R. D. Seitz unpublished manuscript). Siphon extension is measurable within 24 h (Seitz et al. 2003) and vertical migration within 72 h (W. C. Long and R. D. Seitz unpublished manuscript) of exposure to hypoxia (DO ~ 1.0 mg l^{-1}). As *Macoma* avoids predation by burying down to 40 cm in the sediment (Hines & Comtois 1985), a decrease in burial depth with hypoxia is likely to make this species more vulnerable to predation (Clark et al. 1999 a,b, De Goeij et al. 2001, Seitz et al. 2001).

Predators of *Macoma* in the York River include the blue crab, *Callinectes sapidus* (Seitz et al. 2001), which is a key link in the food web (Baird & Ulanowicz 1989) and includes up to 55 % of its diet as clams (Hines et al. 1990). Three benthic piscine predators, the Atlantic croaker (*Micropogonias undulatus*), spot (*Leiostomus xanthurus*) and hogchoker (*Trinectes maculatus*), nip *Macoma* siphons (Pihl et al. 1992, Hines et al. 1990, Powers et al. 2005). Siphon nipping can force *Macoma* to migrate vertically, making it more susceptible to other predators (De Goeij et al. 2001). These predators, however, have low tolerances for hypoxia (Das & Stickle 1993) and generally avoid hypoxic areas (Pihl et al. 1991, Bell & Eggleston 2005).

MATERIALS AND METHODS

We conducted this study in the York River, a tributary of Chesapeake Bay (Fig. 1), which is one of the largest eutrophic estuaries in the world and which suffers from seasonal hypoxia (Officer et al. 1987, Kemp et al. 2005). Hypoxia in the York River is episodic and primarily tidally driven; it tends to develop during neap tides, lasts about a week, and then dissipates when the spring tides mix oxygen-rich waters down from the surface (Haas 1977, Kuo & Neilson 1987).

At our field sites hypoxic waters regularly develop in deeper areas during the summer (Pihl et al. 1991). In 2005, we haphazardly chose four replicate sites in both shallow (3-4 m), and deep (10-12 m) water (Fig. 1); environmental factors such as sediment type (all sites were mud or sandy mud), temperature, and salinity, were similar among all sites. At each site, SCUBA divers established three 50 x 50 cm plots marked with a PVC frame: (1) caged, (2) uncaged, and (3) partially caged. We transplanted 40 *Macoma* (shell lengths 10-35 mm), collected from the York River and marked with red permanent ink, into each plot. This resulted in a density of 160 m^{-2}, which is within the natural range (Seitz et al. 2005). We placed a full cage made from galvanized steel hardwire cloth (1-cm mesh) over each plot for a minimum of 24 h to allow the clams to acclimate and bury (Seitz et al. 2001). After acclimation, we removed the cage on the uncaged plot, and replaced the cage on the partially caged plot with a partial cage. The partial cage had a 25 x 25 cm hole in the center of the top and a 25 x 7 cm hole in each of the sides. The partial cages allowed predator access but they may have excluded larger

piscine predators. The cages were 14 cm high and were inserted 7 cm into the sediment so the side holes were flush with the sediment surface.

We left the plots undisturbed for approximately 28 d before they were re-sampling with a suction apparatus to a depth of 40 cm (Eggleston et al. 1992). We counted and measured marked *Macoma*, and calculated percent recovery for each plot. We identified unmarked ambient bivalves in each of the plots to species. The experiment was performed once in June, under normoxic conditions, and once in August-September, under episodic hypoxic conditions. Two sites, one shallow and one deep, were unexpectedly destroyed during the experiment.

We used a continuous water-quality recorder (model DataSonde 3, Hydrolab, Austin, Texas, USA) to record bottom DO, temperature, and salinity every five minutes. The recorder was placed at the most downriver deep site (Fig. 1) and was downloaded and serviced weekly. We used it for the full period of the June experiment and during one week of the August experiment, after which it was permanently damaged. Once, during the course of the experiments, we applied a linear correction factor to the raw DO data when there was significant drift in the readings after deployment. DO measurements were smoothed by applying a running five-point average (Fig. 2a and c). Spot measurements (Figs 2b and d) were made every 3-4 d at each of the sites using a DO probe (YSI Model 85, Yellow Springs Instruments, Dayton, Ohio, USA).

We calculated predation based on the recovery of marked *Macoma*. Recovery in the caged plots averaged 87 % in June and 85 % in August. Marked undamaged shells of *Macoma* were counted as recovered for the purpose of calculating predation, because they represented non-predatory mortality. The predation rate was calculated using

$S = Ne^{-pt}$ where S is the number of recovered clams, N is the initial number, p is the instantaneous predation rate per day, and t is the time elapsed. The rate of predation, p, was calculated for both the uncaged and partially caged plots in each site. The recovery of clams in the caged plot was used as the initial number, N, to account for sampling error (i.e. non-recovered clams). In two instances, the caged plots were not used, once because a failure of the suction sampler resulted in a lost sample, and once because a blue crab had been inadvertently included in the cage, as indicated by a high abundance of marked shell fragments due to predation and the low recovery of live *Macoma* (13 %). In both of these cases, the mean recovery from caged plots within that depth and time period was used as N instead. The rate of non-predatory mortality was calculated with the same equation using the recovery of dead marked whole *Macoma* shells in the partially caged plot as 1-S and the recovery in the caged plots as N.

Predation and ambient bivalve densities were analyzed with an Analysis of Variance (ANOVA) with Time period (pre- or post-onset of hypoxia), Depth (deep or shallow), and Plot (uncaged or partially caged) as factors and Site (nested within Depth and Time) as a blocking factor. Non-predatory mortality was analyzed with a 2-way ANOVA with Time and Depth as factors. Where a significant interaction effect was observed, a Student-Newman-Keuls (SNK) post-hoc multiple comparison test was performed.

Predators were sampled by the Virginia Institute of Marine Science (VIMS) Juvenile Finfish and Blue Crab Trawl Survey, which takes monthly trawls at sites in the Virginia portion of the Chesapeake Bay and its tributaries. The Survey uses a 9-m semi-balloon otter trawl (38.1-mm stretch-mesh body, 6.35-mm mesh cod-end liner). Each

month, one five minute trawl was performed at the sampling site (Fig. 1). All animals were identified to species, counted, and measured.

RESULTS

In June, before hypoxia, DO in deep and shallow sites was normoxic (> 2 mg l^{-1}) for most of the experimental period (Figs. 2a and b). DO did not differ between deep and shallow sites (one-way ANOVA; $F_{1,32} = 0.87$; $p = 0.357$; N = 34; Deep = 6.1 mg l^{-1} ± 0.58 [SE]; Shallow = 6.8 mg l^{-1} ± 0.35 [SE]). During August, DO in the shallow zone remained normoxic, but the deep zone experienced several hypoxic episodes (Figs. 2c and d). DO in the deep sites was significantly lower than that in the shallow sites (one-way ANOVA; $F_{1,56} = 25.98$; $p < 0.0005$; N = 58; Deep = 3.0 mg l^{-1} ± 0.30 [SE], Shallow = 5.1 mg l^{-1} ± 0.28 [SE]). During both time periods, the deep sites were about 1^{o} C cooler than the shallow sites and 1 ppt more saline, as expected due to the stratification of the system.

Recovery rates of marked *Macoma* were generally lowest in open plots, intermediate in partial cages, and highest in full cages. Predation rates differed significantly by the interaction between time period (pre- or post-onset of hypoxia) and depth (ANOVA; $F_{1,10} = 5.06$; $p = 0.048$; N = 28). Predation was significantly higher at the deep sites after hypoxia than in the shallow sites after hypoxia (Fig. 3a; SNK; $p < 0.05$) or in the deep sites before hypoxia (Fig. 3a; SNK; $p < 0.01$). There was a non-significant trend (SNK; $p < 0.1$) for higher predation at the shallow sites after hypoxia as compared to before. A significant interaction existed between Plot (uncaged and partially caged) and Time (ANOVA; $F_{1,10} = 12.97$; $p = 0.005$; N = 28), with uncaged plots having significantly higher predation rates than partially caged plots in August (Fig. 3b; SNK; P

< 0.01), but not in June. No major fouling occurred on the cages over the course of the experiment.

Ambient clams were significantly less dense in the deep sites than in the shallow sites (Fig. 3c; ANOVA; $F_{1,21} = 25.90$; $p < 0.0005$; N = 44), and less dense in August than in June ($F_{1,21} = 13.23$; $p = 0.002$). Non-predatory mortality was 0.055 day^{-1} ± 0.014 (SE) and did not differ by Depth (2-way ANOVA; $F_{1,10} = 0.00$; $p = 0.974$; N = 14), Time ($F_{1,10} = 1.04$; $p = 0.332$), or interaction ($F_{1,10} = 2.08$; $p = 0.180$).

Predator density and composition changed between the June and August experiments (Fig. 4). In May and early June predator density was low and dominated by *Callinectes sapidus*, *Micropogonias undulatus*, and *Trinectes maculatus*. In August and September the predator assemblage was dominated by *Leiostomus xanthurus* and *T. maculatus*, which resulted in a near doubling of the predator abundance from early June to early August. *Callinectes sapidus* were also abundant in September. There were no clear trends in the size of piscine predators; the mean length of *M. undulatus* was 238 mm and the monthly means varied from 205 mm in September to 278 in June. Mean length of *T. maculatus* was 109 and varied from 92 mm in August to 123 mm in May and September. *Leiostomus xanthurus* were only abundant in late summer and had a mean length of 116 mm. *Callinectes sapidus* increased slightly in size between May (mean carapace width = 51 ± 11 mm [SE]) and September (mean carapace width = 75 ± 13 mm [SE]).

DISCUSSION

Patterns of dissolved oxygen concentrations in the summer of 2005 were similar to those observed during strong hypoxic years in the York River (Pihl et al. 1991). Our time series of DO corresponded well with the tidal regime, with a twice daily cycling of about +/-0.5 mg l^{-1} around a daily mean, as well as a longer cycle that correlated with the neap-spring tidal cycle (Haas 1977). Although there were hypoxic excursions lasting only 1-2 h during our nominally normoxic experiment, this duration of hypoxia is not long enough to cause behavioral changes in *Macoma*; in laboratory experiments *Macoma* extended their siphons after about 24 h of exposure to hypoxia (Seitz et al. 2003), and vertically migrated after 48-72 h (W. C. Long and R. D. Seitz unpublished manuscript). In contrast, during the August experiment, there were at least two hypoxic episodes at the deep sites, one around 17 August, and one around 1 September. During the first episode DO dropped to less than 1.2 mg l^{-1} and lasted at least 4 d, which is long enough for *Macoma* to exhibit behavioral responses to hypoxia. During the second episode, severe hypoxia occurred as the DO dropped below 1 mg l^{-1}.

In our experiment, predation varied with time period, depth, and DO. Predation in the shallow areas increased slightly from June to August. This is not surprising given that predator abundance also increased over this period. In the deep areas, however, predation was low in June (with normoxia) and high in August (with episodic hypoxia), and it was higher than the nearby shallow areas in August. This is counter to expectations based on laboratory and field studies where predators have much lower tolerance for hypoxia than

Macoma (Henriksson 1969, Bell & Eggleston 2005) and feed at a lower rate under hypoxic conditions (Seitz et al. 2003).

We suggest that the pattern of higher predation in deep zones during hypoxia derives from the predators' optimal foraging behavior. In June, there is no hypoxia to stress either the predators or the prey, and at that time, the prey populations are denser in the shallow zones (Fig. 5a), likely because of the annual hypoxic conditions that occur in deeper areas and cause mortality of infauna there (e.g., Powers et al. 2005). Lower densities in the deep zone would increase searching time for the predators; thus, they preferentially forage in the shallow areas (Clark et al. 1999a,b). For example, blue crabs forage with tactile probing and will leave an area if few prey items are detected, but will continue to search in an area if multiple prey are encountered (Clark et al. 1999a,b). Throughout the summer, predation reduces prey densities in the shallows (Holland et al. 1977). When hypoxia develops, the prey species in the deep zone become stressed and exhibit behaviors that make them easier to find and thus more vulnerable to predation (e.g., clams extending siphons and reducing burial depths; Seitz et al. 2003, W. C. Long and R. D. Seitz unpublished manuscript). Therefore, during periods of episodic hypoxia, although prey densities in the deep areas are lower than those in shallow areas, a predator's searching time is much lower due to the increased susceptibility of prey to encounters, and predators can exploit the prey in this area at a higher rate (Fig. 5b). These results therefore support Prey Stress Models (PSMs) rather than Consumer Stress Models (CSMs), whereby prey are more stressed than predators, allowing predators to increase the rate of predation.

In our experiments, we could not distinguish whether predation occurred during hypoxia or shortly after each hypoxic episode because of the episodic nature of hypoxia in this system and the length of our experiments. Two distinct hypoxic episodes occurred during August, and the predators could have been foraging at any time during the 28-d experiment. Because most predators avoid hypoxic areas, foraging probably occurred soon after hypoxia but before the prey recovered (Pihl et al. 1992); however, our data do not rule out the alternative of predation during hypoxia (Rahel & Nutzman 1994, Nestlerode & Diaz 1998). Our results indicate that hypoxia is the driving force of the enhanced predation and prey mortality in the deep zone, regardless of when that predation takes place.

Caging artifacts differed by time period, but not by depth. Partial cages may have provided limited protection against predation, explaining the lower predatory mortality in the partially caged plot as compared to the uncaged plot. Some predators, especially large fish such as Atlantic croaker, *Micropogonias undulatus*, with lengths > 15 cm, may not have been able to access the clams in the partial cages, feeding only in the uncaged plots. Blue crab, *Callinectes sapidus*, had access to partial cages, as we found molted exoskeletons in the partial-cage plots, and blue crabs were at high densities during late August and early September during our hypoxic experiments. The major change in the predator assemblage between June and August was the increase in the densities, but not the sizes, of all predators, especially fish predators. The temporal patterns in predator density may thus explain the greater caging artifacts in August, when more fish predators would be feeding preferentially in uncaged plots instead of partially caged plots. Caging

artifacts differed between deep and shallow areas, indicating that our conclusions regarding depth differences were robust.

Based on our finding that predation rates were higher during periods of episodic hypoxia than under normoxic conditions, a shift in our concept of hypoxia's effects on trophic dynamics and energy flow is necessary. Previously, it had been assumed that a CSM applies to this system because benthic infauna suffer mortality during hypoxia and predators avoid hypoxic areas; it has been concluded that the majority of the mortality in hypoxic areas is caused by hypoxic stress rather than by predation (Sagasti et al. 2001, Powers et al. 2005). Under this assumption, the energy from animals that die directly from hypoxic stress would enter the microbial loop, rather than being transferred to predators. Thus, hypoxia would have a net negative effect on energy transfer to higher trophic levels and fishery species (Baird et al. 2004, Altieri & Witman 2006).

In our study, predation increased significantly during episodic hypoxia, whereas non-predatory mortality did not increase. In laboratory experiments, *Macoma* can survive for more than 4 d under mild hypoxia, such as they experienced here, so we did not expect to see an increase in non-predatory mortality (Henriksson 1969, Seitz et al. 2003). As predation increased during periods with hypoxia and the majority of the biomass was passed up to higher trophic levels rather than into the microbial loop, episodic hypoxia can have a positive effect on trophic transfer to predators, as predicted by PSMs. This enhanced flow of secondary production likely depends on the prey species. Species that rely on shell strength, such as *Mercenaria mercenaria*, or aggregation, such as *Mytilus edulis* (Altieri & Witman 2006), to increase the handling time reduce the encounter rate of predators probably do not exhibit increased

vulnerability during hypoxia, because behavioral changes during hypoxic conditions should not affect their primary defense (Vermeij 1987). In contrast, species such as *Macoma* and *Mya arenaria* (Taylor & Eggleston 2000), which rely on burial depth to avoid predation, are more likely to be more vulnerable to predators under hypoxia because they migrate to the sediment surface where they are easily detected.

These results also indicate that the spatial and temporal scales of hypoxic episodes determine trophic effects (Diaz & Rosenberg 1995, Eby & Crowder 2004), as observed in other consumer-prey interactions (Orrock et al. 2003). Our hypoxic sites were in close proximity (100s of meters) to shallow normoxic sites, where predators congregate during hypoxia (Lenihan et al. 2001, Eggleston et al. 2005). Predatory density and predation pressure can be elevated on the outside edge of hypoxic patches during hypoxic episodes (Lenihan et al. 2001). Similarly, reinvasion and predation by predators in hypoxic areas is likely to be most intense along the inside edge of hypoxic patches after hypoxia relaxes (Clark et al. 1999b, Eggleston et al. 2005). If a hypoxic patch is large (>> 1000 m in diameter), predators may not be able to exploit vulnerable prey in central areas before the prey recover. Although our study allows inference regarding changes in predator-prey interactions during episodic hypoxia (< 1 wk), it may also apply to systems where hypoxia lasts weeks or months. In the Rappahannock River of Chesapeake Bay, extended periods of hypoxia can be preceded by one or more short episodes of hypoxia (Llansó 1992), which would give predators a chance to prey upon much of the infaunal biomass. Furthermore, our study does not preclude active foraging by predators during hypoxia (Rahel & Nutzman 1994, Nestlerode & Diaz 1998). Ultimately, if hypoxia is severe enough, benthos within a hypoxic zone will be killed, either by physiological stress (Seitz

et al. 2003, Powers et al. 2005, Altieri & Witman 2006) or by predation, and the relative importance of each is probably influenced by the duration and spatial extent of hypoxia.

Hypoxia has long been recognized as a severe environmental degradation that devastates benthic communities (Diaz & Rosenberg 1995). We demonstrate that, under certain conditions, decreases in the benthos can be primarily attributed to enhanced predation on stressed prey and not to mortality from hypoxic stress. Regardless of the proximal cause, this decrease in abundance and biomass may lead to a reduction in net annual benthic production. However, the impact on production in higher trophic levels in the short term is probably not as negative as has been thought previously, and may be positive. Indeed, there has been no observed decrease in fisheries yield in the Chesapeake Bay attributable to hypoxia, despite an increase in the spatial and temporal extent of hypoxia over the past few decades (Kemp et al. 2005). Moreover, the yield of some fisheries in the Gulf of Mexico increased during four decades of increasing hypoxia, suggesting that any effects of hypoxia on the nekton are masked by compensatory forces (Chesney & Baltz 2001).

Though our findings demonstrate that the effects of periodic hypoxia may increase predation and thus transfer of secondary production to upper trophic levels, this does not imply that hypoxia and the associated eutrophication are insignificant. In the Neuse River, habitat compression and the resulting increase in predator density can cause an increase in cannibalism in blue crabs (Eggleston et al. 2005), and this may have a greater effect on predator populations than does food limitation (Aumann et al. 2006). However, these studies do not account for increased availability of prey due to hypoxia.

The effect of a stressor on consumer-prey interactions sometimes can be predicted based on the relative tolerance of the species to that stressor (e.g., Altieri & Witman 2006), but, as in this study, this is not always the case (e.g., Thomson et al. 2002). In the York River system, subtle changes in prey behavior (e.g., reduced burial depth) to hypoxic stress have a substantial effect on trophic dynamics as predation increases during hypoxia. The results are therefore consistent with Prey Stress Models rather than Consumer Stress Models, and they highlight the need to measure ecological processes directly through field experiments to confirm or refute inferences. Further work on the effects of hypoxia on food web interactions is essential.

ACKNOWLEDGMENTS

This work was supported by a grant from the NOAA National Sea Grant, Essential Fish Habitat Program, and the Commonwealth of Virginia. W.C. Long was supported by an EPA GRO fellowship. I thank B Brylawski for help in the field and lab and M Seebo for help in the field and with SCUBA diving. We thank R Diaz, R Lipcius, D Wolcott, B Brylawski, J Long and two anonymous reviewers, whose comments on earlier drafts substantially improved this manuscript. Symbols in Figure 4 were courtesy of the Integration and Application Network (*ian.umces.edu/symbols*), University of Maryland Center for Environmental Science. Predator abundance data was graciously provided by the VIMS Juvenile Finfish and Blue Crab Survey (http://www.fisheries.vims.edu/trawlseine/mainpage.htm).

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Figure 1

Map of Chesapeake Bay (left inset), the York River (middle inset), and our study sites at Gloucester Point (large frame).

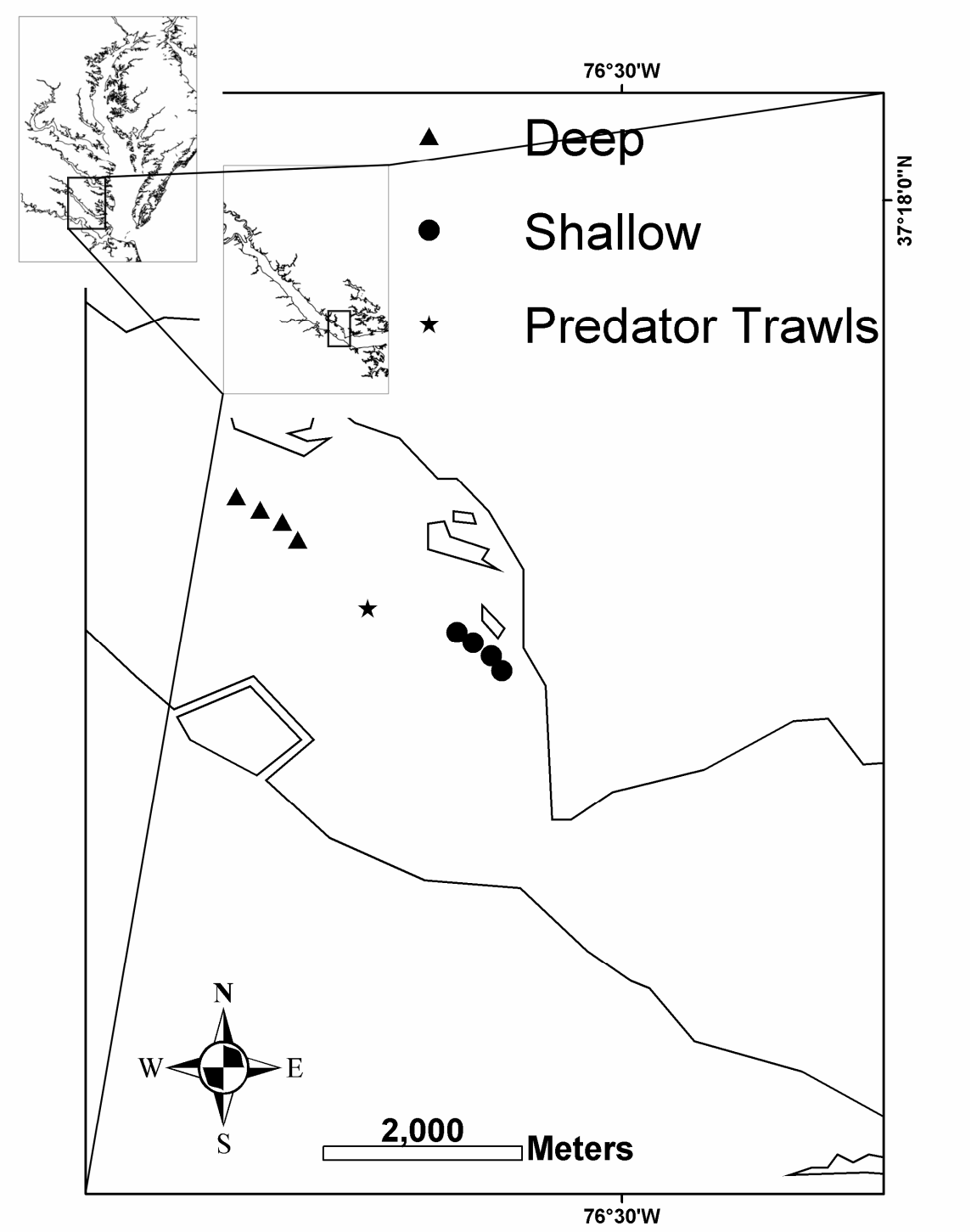

76°30'W
37°18'0"N
Deep
Shallow
Predator Trawls
N
W
E
S
2,000
Meters
76°30'W

Figure 2

Dissolved oxygen concentrations at study sites in the York River. Readings under dashed line at 2 mg l^{-1} are considered nominally hypoxic. Continuously recorded Datasonde measurements taken at deep site 1 from (a) the June experiment before hypoxia, and (b) the August experiment during hypoxia (arrows indicate the approximate beginning and end of hypoxic episode from 14-18 August) Lunar phase is indicated with circles. YSI measurements taken at both deep and shallow study sites during (c) the June experiment, and (d) the August experiment. Hypoxia is indicated by arrows.

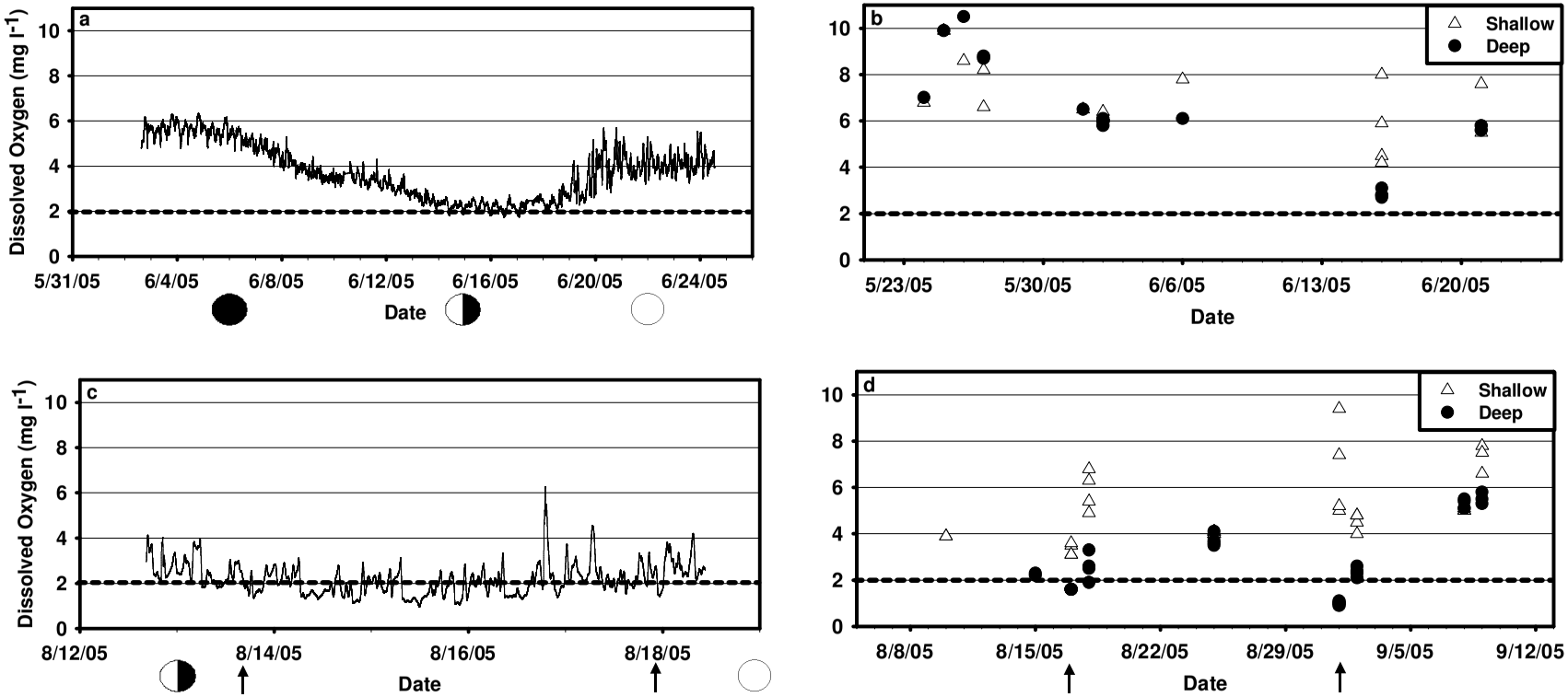

a
Dissolved Oxygen (mg l-1)
10
8
6
4
2
0
5/31/05
6/4/05
6/8/05
6/12/05
6/16/05
6/20/05
6/24/05
Date
b
Shallow
Deep
5/23/05
5/30/05
6/6/05
6/13/05
6/20/05
Date
c
Dissolved Oxygen (mg l-1)
8/12/05
8/14/05
8/16/05
8/18/05
Date
d
Shallow
Deep
8/8/05
8/15/05
8/22/05
8/29/05
9/5/05
9/12/05
Date

Figure 3

(a) Predation rate (N =10) and (b) caging artifacts (expressed as a mortality rate; N=10) at each site during each experiment. Error bars are + 1 standard error. Bars with different letters above them differ at the 0.05 level (SNK). (c) Ambient bivalve densities at both depths during each experiment. Error bars are + 1 standard error. Levels within a factor marked with an asterisk differ at the 0.05 level (ANOVA, N=28).

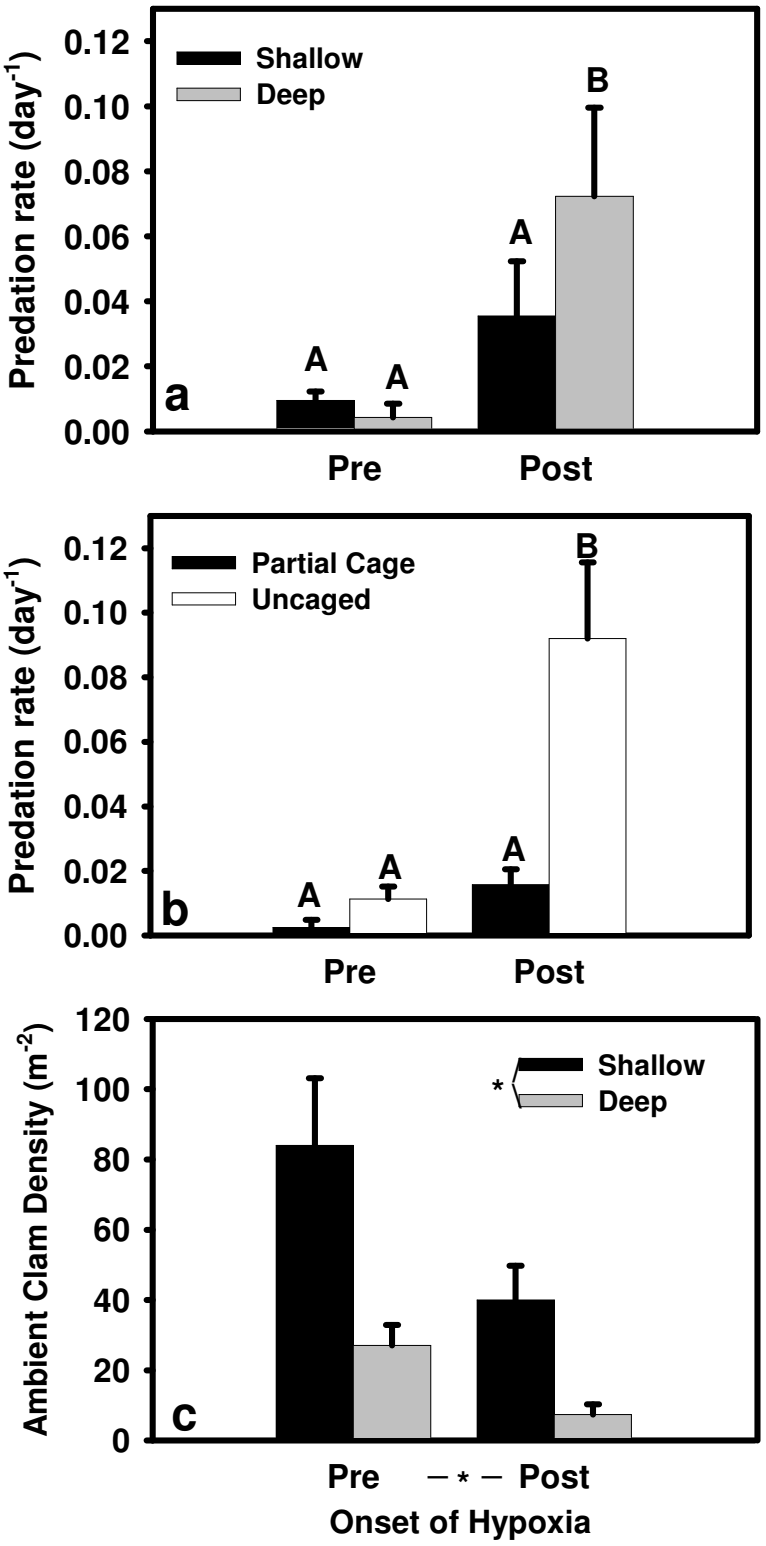
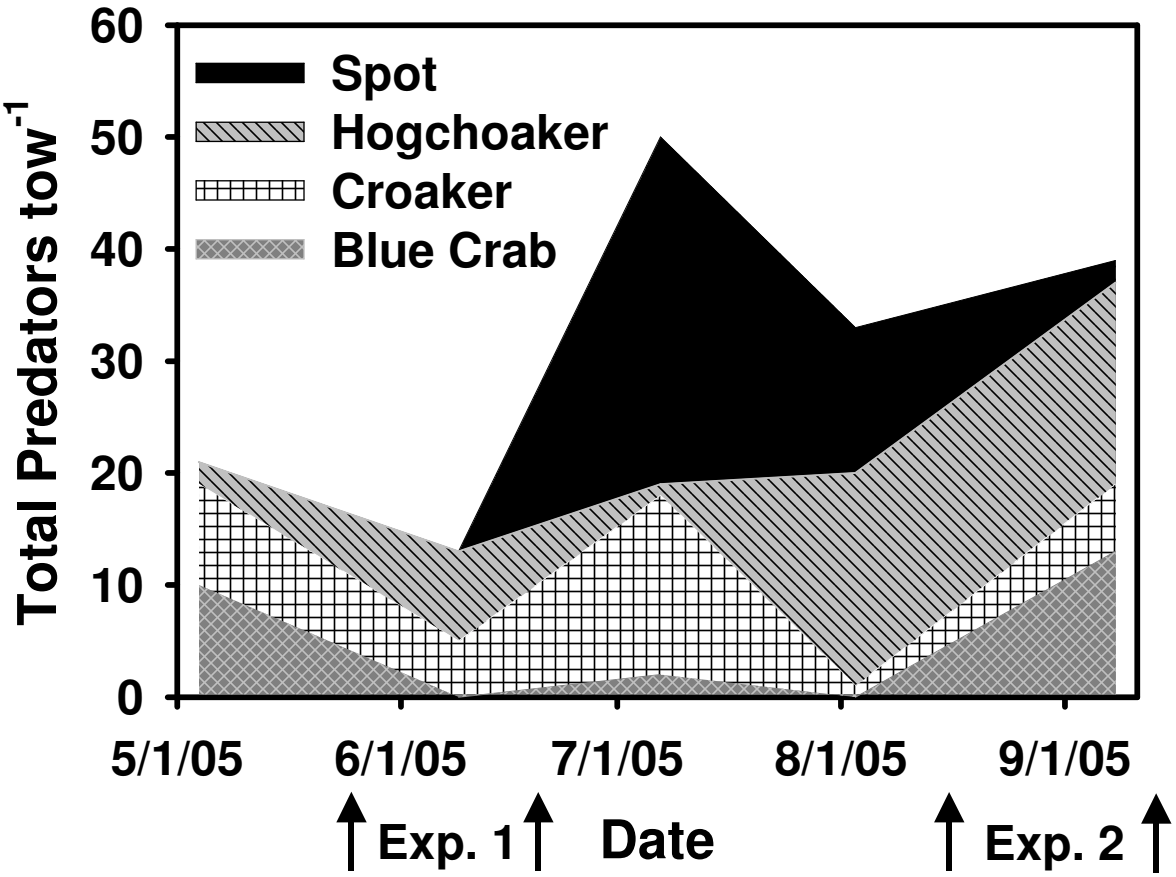

Predation rate (day-1)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Shallow
Deep
A
A
A
B
a
Pre
Post
Predation rate (day-1)
Partial Cage
Uncaged
A
A
A
B
b
Pre
Post
Ambient Clam Density (m-2)
120
100
80
60
40
20
0
Shallow
Deep
*
c
Pre
— * — Post
Onset of Hypoxia

Figure 4



Cumulative predator density from the VIMS Juvenile Finfish and Blue Crab Trawl Survey. Only epibenthic predator species are shown. Arrows indicate the beginning and end of Experiment 1 (June, prior to hypoxia), and Experiment 2 (August, during hypoxia).

Figure 5

Illustrations of predator and prey behavior (a) before the onset of hypoxia and (b) after onset of hypoxia (includes episodes of hypoxia). Stippling indicates sediment, light gray shading is normoxic water column, and dark gray shading is extent of hypoxic water. Infaunal clams and polychaetes are pictured with relative position in relation to the sediment-water interface. (a) Before hypoxia predators feed in shallow areas where prey are more abundant. (b) During hypoxia prey species migrate vertically, and become more vulnerable to epibenthic predators. Predators move in to feed either during a hypoxic episode of after relaxation before the prey rebury.

Figure 5a

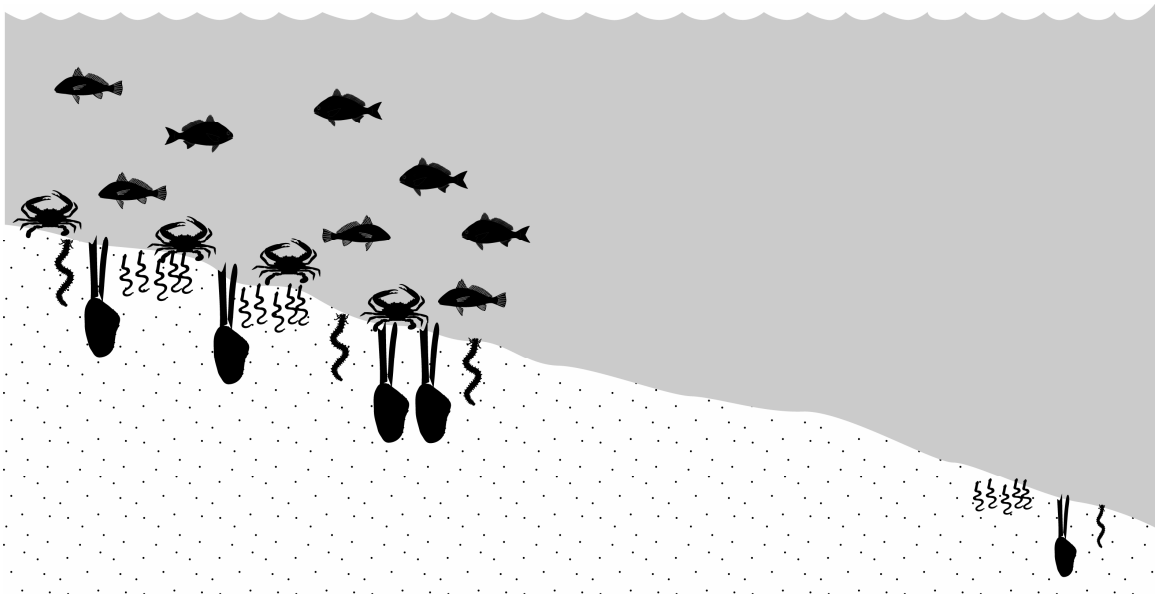
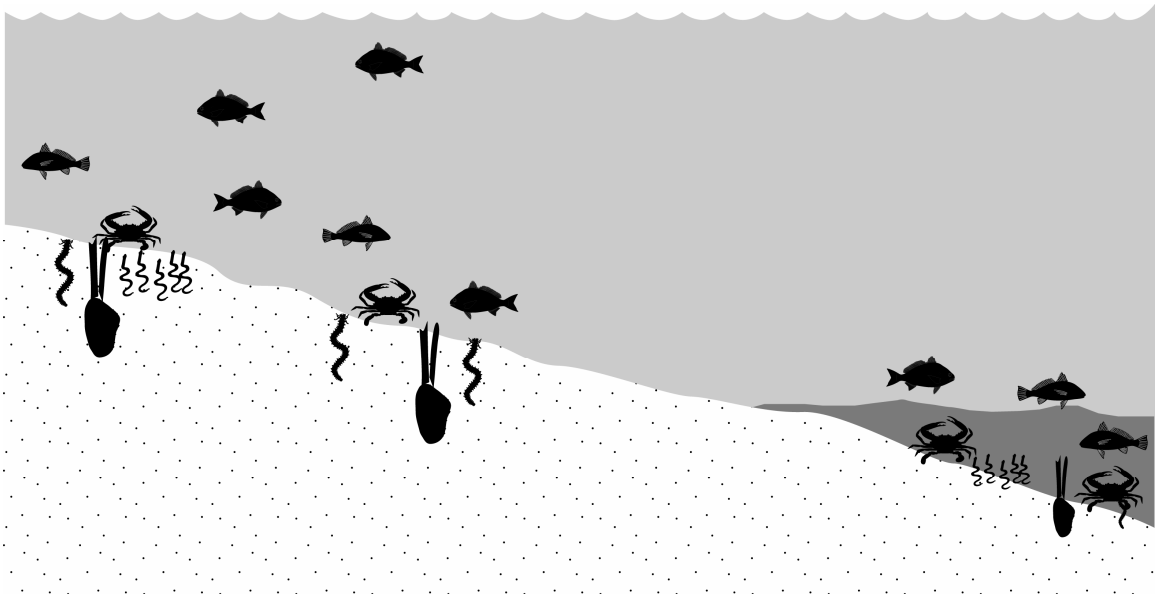


Figure 5b



CHAPTER 5:

INDIVIDUAL, POPULATION, AND ECOSYSTEM EFFECTS OF HYPOXIA: *MACOMA BALTHICA* IN CHESAPEAKE BAY

ABSTRACT

Hypoxia, or low dissolved oxygen, is a serious environmental stressor that can substantially reduce the abundance, biomass, and diversity of benthic communities and subsequently affect the whole ecosystem. In this study, we examined the effects of hypoxia on the biomass-dominant clam, Macoma balthica, in the York and Rappahannock Rivers (Chesapeake Bay, USA). We performed a survey of the population of M. balthica in both rivers in 2003 and 2004, a laboratory experiment, in which we held M. balthica under a range of DO conditions to examine effects on fecundity, a predator-exclusion field experiment to establish the effects of hypoxia and prey density on predation of M. balthica. We used this empirical data to parameterize a density-independent matrix model which we used to analyze the effect of hypoxia on the population. In both rivers we surveyed, hypoxia resulted in local extinction of the population, and may have decreased individual clam growth. In the laboratory, hypoxia caused female M. balthica to produce fewer eggs but to invest more protein in each. In the predator-exclusion field experiment, hypoxia increased predation rates three-fold and may change the functional response of predators to M. balthica from a stabilizing type III functional response to a destabilizing type I or II. In the matrix model, hypoxia resulted in coupled source-sink metapopulation dynamics, with hypoxic areas acting a black hole sink. Moreover, increasing the spatial and temporal extent of hypoxia was expected to cause the population to decline toward extinction. A second model, which incorporated density-dependent predation, suggested that under mild hypoxic conditions, trophic

transfer from M. balthica to epibenthic predators could increase, but that increasing the spatial and temporal extent of hypoxia decreased trophic transfer. Increasing the intensity of hypoxia resulted in a major decline in trophic transfer to predators, as the majority of the M. balthica biomass entered the microbial loop. Our model further predicted that there are alternative stable states for the M. balthica population with the switching threshold increasing with the spatial extent of hypoxia. We underscore how effects of a stressor at the individual level can combine and have substantial ecosystem-level effects.

INTRODUCTION

The development of hypoxia and its ecological consequences

Anthropogenic eutrophication of estuarine systems can have substantial negative effects on costal ecosystems (Kemp et al. 2005). One important consequence of eutrophication is the development of hypoxia, or low dissolved oxygen (DO), in bottom waters (Diaz and Rosenberg 1995). Hypoxia usually develops when excessive algal growth, fueled by nutrient inputs, results in the flux of large amounts of labile organic material to bottom waters. If respiration depletes the oxygen faster than it can be replenished, this leads to the development of hypoxia, DO < 2.0 mg/L, or anoxia, DO = 0.0 mg/L (Seliger et al. 1984, Officer et al. 1984).

Hypoxia is an important stressor in aquatic systems, and is especially detrimental to benthos. Severe hypoxia can directly cause the death of benthic species and a subsequent decrease in abundance, biomass, and diversity of the benthic assemblage (e.g., Dauer et al. 1992, Powers et al. 2005, Motagna and Ritter 2006). Also of interest are its sub-lethal effects on the individual level. Hypoxia can reduce the feeding rate (Sagasti et al. 2001), growth (e.g. Nilsson 1999, Wiklund and Sundelin 2001), and reproduction (e.g., Condon et al. 2001, Wu and Or 2005) of individuals. In addition, behavioral responses, such as decreased burial depth, and extension of siphons or palps are common in benthic infauna (Taylor and Eggleston 2001, Seitz et al. 2003b, Montagna and Ritter 2006, Long et al. unpublished manuscript a).

Although much work has been conducted on the effects of hypoxia at the individual level, few studies explicitly consider how these effects interact over multiple scales to affect the population or ecosystem, though this is an important step in understanding large-scale patterns (Diaz and Rosenberg 1995, Altieri and Witman 2006). In this study, we consider the effects of hypoxia on the growth, reproduction, and survival of the Baltic macoma, Macoma balthica, in the York and Rappahannock Rivers. We use these results to explore population-level effects by constructing a matrix model that examines how hypoxia-induced source-sink population dynamics affect population stability. We consider the effects that this will have on higher and lower trophic levels by performing a caging experiment to determine effects of hypoxia and prey density on the rate of predation. We incorporate this into a density-dependent model and explore the net results on the ecosystem and ecosystem services.

The study area and organisms

Our studies were performed in the mesohaline regions of the York and Rappahannock Rivers (Chesapeake Bay, VA, USA) in unstructured soft-bottom habitats (Fig. 1). Both rivers experience episodic hypoxia during the summer (Diaz and Rosenberg 1995). Typically, hypoxia in the Rappahannock is more severe, with lower DO levels, a greater areal extent of hypoxic waters, and a longer duration (Kuo and Neilson 1987).

M. balthica, a small (shell length < 40 mm), thin-shelled clam, is the biomass-dominant species in these habitats, comprising over 85% of the biomass in some areas (Holland et al. 1977). With an LT_{50} (Lethal time for 50% of the population) of 4.5-15

days under near-anoxic conditions, it is considered tolerant of hypoxia (Henriksson 1969, de Zwaan et al. 2001). Under hypoxic conditions it is known to migrate vertically in the sediment and to extend its siphons (Bradfield 1969, Seitz et al. 2003b, Long et al. unpublished manuscript b), which can make it more vulnerable to predation (De Goeij et al. 2001), because its primary defense against predation is deep burial (Hines and Comtois 1985, Seitz et al. 2001). It is an important part of the food web, contributing to the flow of biomass from primary producers to upper trophic levels, such as the commercially important Callinectes sapidus (Baird and Ulanowicz 1989). In Chesapeake Bay M. balthica comprises approximately 50% of the adult C. sapidus diet (Hines et al. 1990). Piscine predators, such as the Atlantic croaker (Micropogonias undulatus), spot (Leiostomus xanthurus), and hogchoker (Trinectes maculatus), are generally unable to reach whole clams due to deep burial, but will feed extensively on M. balthica siphons (Pihl et al. 1992, Derrick and Kennedy 1997, Powers et al. 2005), which can induce M. balthica to migrate vertically to a shallower burial depth (De Goeij et al. 2001).

Source-sink population dynamics

Source-sink theory recognizes that species occupy a variety of interconnected habitats that differ in quality and carrying capacity within a heterogeneous landscape. These habitats can be separated into sources and sinks. In sources, births and emigration exceed deaths and immigration; there is positive population growth, with emigration of excess individuals. In sink habitats, the death rate exceeds the birth rate, and the populations are only sustained by immigration from source habitats (Pulliam 1988). In

this way, a species can extend its range into areas where, without immigration, the population would suffer local extinction.

It may seem counterintuitive that an organism would live in a sub-optimal (i.e., sink) habitat, but this is common (Pulliam and Danielson 1991). Sink habitats can be populated due to competition forcing individuals out of the source habitat, or through passive dispersal leading to recruitment in the sink (Dias 1996). When the latter occurs, sink habitats can have a negative impact on the source populations (Pulliam and Danielson 1991). Many sedentary marine invertebrates fall within this second category. Even though M. balthica larvae are capable of discerning the quality of habitats to some degree (Beukema and de Vlas 1989, Armonies 1992, Seitz et al. 2001), settlers can only detect current conditions and not future adverse conditions. As M. balthica recruits in the fall and spring, and hypoxia occurs in the summer, settlers are unlikely to avoid hypoxic areas. Thus, we hypothesize that hypoxic areas of Chesapeake Bay tributaries may act as population sinks that may negatively influence normoxic populations.

Predation and density dependence

The functional response (FR) of a predator relates the rate of predation to the density of prey. Three general types of functional response are type I, or linear and density independent; type II, or hyperbolic, which is inversely density dependent; and type III, or sigmoid, which is density dependent (Holling 1959, Hassell et al 1977). Type II FRs are common where the prey species uses armor to increase handling time and reduce the rate of predation. As the density of prey increases, the proportional mortality decreases, resulting in a high-density refuge from predation. When prey avoid predators

by hiding or using camouflage, thus increasing the searching time and predation rate, Type III FRs are common. As the densities of these prey decrease, the proportional mortality decreases, so the prey have a low-density refuge from predation (Taylor and Eggleston 2000, Seitz et al. 2001). Blue crabs have a type III FR towards M. balthica (Eggleston et al. 1992), but as hypoxia should decrease predator search time for M. balthica, we hypothesize that it may change the FR from a type III to a type I or II. This could have negative implications for the population, since both type I and type II FRs can lead to the local extinction of prey populations.

MATERIALS AND METHODS

Survey of populations in the York and Rappahannock Rivers

Sampling:

The M. balthica populations in the York and Rappahannock Rivers were surveyed by box-coring before and after summer hypoxic events in 2003 and 2004. We randomly selected 10 sites within each of three depth strata: shallow (depth < 3m), mid (6 m < depth < 3 m), and deep (depth > 6 m). In 2003 in the Rappahannock, only 5 sites were sampled in each strata because of logistic constraints. We stratified by depth to ensure that we sampled enough hypoxic sites; hypoxia is strongly correlated with depth in both rivers (Kuo and Neilson 1987). We sampled the York River in the spring on June 19, 2003, and May 25, 2004, for the before hypoxia samples, and in the fall on October 14, 2003, and November 11, 2004, for the after hypoxia samples. The Rappahannock was sampled before hypoxia in the spring on June 24, 2003, and June 7, 2004, and after hypoxia in the fall-winter on February 10, 2004, and November 19, 2004. We revisited the sites during hypoxia in July of 2003, and in June, July, and August of 2004 to measure bottom temperature, salinity, and dissolved oxygen with a DO probe (YSI Model 85, Yellow Springs Instruments, Dayton, Ohio, USA).

We used a 25 cm x 25 cm Gray O'Hara box-core to take benthic samples on each date. In 2003, one 10 cm diameter core down to 15 cm was taken from the center of each box core and sieved on a 0.5 mm screen. A second 2.5 cm diameter core was taken down to 5 cm for grain size analysis. The remainder of the core was sieved on a 2 mm screen.

Everything retained on the sieves was frozen and taken back to the lab for processing. Bottom temperature, salinity, and dissolved oxygen were measured, as were water depth and box-core penetration. Cores with penetration < 15 cm were not used for 2 mm analyses and cores with penetrations < 5 cm were not used for 0.5 mm analyses (Hines and Comtois 1985). In 2004, the sampling was the same, except that we took three replicate box-cores at each site and only sub-cored the first.

The 2 mm and 0.5 mm samples were sorted in the lab and all organisms were identified to the lowest taxonomic level possible (usually species). The 0.5 mm samples were stained with Rose Bengal before sorting. We measured the shell length of all bivalves. In 2004, the dry and ash-free dry masses (AFDM) were determined for all bivalves. Grain-size analysis was conducted using a modified phi pipet technique (Folk 1974).

Data analysis:

We used maximum likelihood techniques to examine the effect of dissolved oxygen on the survival of M. balthica over the summer in 2004. We calculated the survival at each site where M. balthica were found as the average density after hypoxia divided by the average density before hypoxia. We modeled survival using the following base equation:

$$S = \frac{Max_S}{1 + \left(\frac{DO}{DO_{50}} \right)^b}$$

where S is survival, and DO is the average summer dissolved oxygen. The parameters are Max_S, the maximum survival, DO_{50}, the concentration of dissolved oxygen at which survival is 50%, and b, the slope parameter. We assumed a normal distribution of errors

with a standard deviation that increased with the cube of the DO. We examined this model and a second that allowed the survival to change with River. The Alkaike's Information Criterion, corrected for sample size (AIC_c) was calculated for each model and the 95% confidence intervals calculated for the best-fit model (Matlab, Version 7.2.0.232). In this and all similar analyses, we considered models with a $\Delta AIC_c < 2$ to explain the data equally well (Burnham and Anderson 2002).

M. balthica shell length frequencies from 2004 were analyzed using maximum likelihood techniques. We hypothesized a priori that the shell lengths could be modeled as a bimodal normal distribution, with two means and two standard deviations (representing the adult and the juvenile cohorts) and a mixing parameter. We considered three factors: River, time (before or after hypoxia), and dissolved oxygen. As we were interested in sub-lethal effects of dissolved oxygen, we used a DO cutoff above hypoxia, dividing the sites into low DO sites, average summer DO < 3.5 mg/L, and high DO sites, average summer DO > 3.5 mg/L. This cutoff point both provided an adequate sample size for the low DO sites and represents the point where survival is reduced by 15% of the maximum (see results of previous analysis). Thus, sites above 3.5 mg/L are unlikely to be substantially affected by low DO, whereas sites below may be expected to be affected. We considered nine models where the means of each cohort, as well as the mixing parameter, were linear functions of these factors (Table 1). Time was considered an important factor since the means were expected to increase over the summer with the growth of the clams. In addition, it was likely that time would have an effect on the mixing parameter, as the smallest clams would have higher mortality than the larger ones. We hypothesized that low DO was likely to decrease the average size of the clams,

because stressed animals would have less energy to devote to growth. We also considered that sites with low DO would have populations of clams that were dominated by juveniles, as mortality from low DO the previous year would reduce adult populations; thus, we allowed DO levels to vary the mixing parameter. The potential effects of River were unknown, but we considered each river may have a different effect on the means. We calculated the AIC_c for each model and used them to select the best fit model. We then calculated the 95% confidence intervals for each of the parameters for the best fit model (Table 2).

We used maximum likelihood techniques to look for effects of Time, River, and DO on the length-mass relationship in M. balthica. We used a standard exponential growth curve as the base model: $Mass = a*Length^b$. We assumed the errors had a normal distribution, with the standard deviation increasing with the cube of the Length. We analyzed 10 *a priori* models wherein 'b' was a linear function of Time, River, and DO (Table 3). Once again, Time was considered important as in the fall the clams may have lost mass to spawning, low DO was investigated to see if the stress would lead to a decrease in mass, and River was included to control for any potential special effects. We also considered an interactive term between Time and DO, as the effects of low DO were likely only to be noticeable after summer hypoxia. The AIC_c was calculated as before for each model and the 95% confidence intervals calculated for the parameters in the best fit model (Table 4).

Effects of hypoxia on growth and reproduction

We performed a laboratory experiment to test the effects of sub-lethal hypoxia on the growth and reproduction of M. balthica. We used sixteen 40 L plastic tanks which were filled with 20 cm of mud, collected from shallow coves in the York River where M. balthica are abundant, and that was passed through a 2 mm sieve to remove most bivalves. M. balthica with shell lengths > 10 mm were collected from representative muddy sites in the York River and brought back to the lab. Only healthy clams with whole shells and a quick siphon-withdrawal reflex were used. Twenty clams were randomly selected for each tank. Each clam was dried, measured, and marked with a letter using permanent ink. Clams were allowed to bury for at least 24 hrs before the experiment was started to allow acclimation to normal burial depth (Seitz et al. 2003). Any clams that failed to bury in 24 hrs were removed and replaced.

We produced hypoxic water by flowing unfiltered seawater through a fluidized mud-bed reactor (Long et al. unpublished manuscript b). Hypoxic water was stored in a 400 L distribution tank which we bubbled with nitrogen to maintain hypoxia. A second distribution tank was filled with unfiltered seawater that was kept normoxic by vigorously bubbling air through it. Each tank was randomly assigned to one of four treatments (1) Hypoxic (DO < 2.0 mg/L), (2) Moderately hypoxic (DO 2.5 ± 0.5 mg/L), (3) Nearly normoxic (DO 3.5 ± 0.5 mg/L), and (4) Normoxic (DO > 4.0 mg/L). To enable the measurement of dissolved oxygen, temperature, and salinity without opening the tank and letting oxygen in, each tank was covered with a lid that had a hole drilled in it; a cork was inserted into the hole when measurements were not being taken.

The DO in the tanks was brought down to the nominal DO range by mixing differing amounts of hypoxic and normoxic waters from the distribution tanks and

allowing the mixed water to flow through the tanks. The DO, temperature, and salinity were monitored approximately twice a day, and the flow of water to each tank was adjusted when necessary to keep each tank within its nominal range. The experiment ran for 28 days, from August 21 to September 18, 2006, after which all the tanks were brought to normoxic conditions. The tanks were monitored and a high flow rate of normoxic water was maintained until November 10, when the clams were harvested by passing the mud through a 5 mm screen. Mortality rates were calculated for each tank using the equation $S = Ne^{-mt}$ where S is the number of survivors, N the initial number, t the time in days, and m the mortality rate. The final shell length was measured and growth of each clam determined as the change in shell length. All clams were opened and sexed (male, female, or juvenile), and the AFDM of each clam was determined.

For fecundity measurements, all female clams were shucked and homogenized in 15 ml of phosphate-buffered saline (PBS; 10 mM Na_2HPO_4, 150 mM NaCl, pH 7.2) and then centrifuged at 1700 g for one hour. An enzyme-linked immunosorbent assay (ELISA) was performed on each sample to determine the relative concentration of M. balthica heat-shock protein 70 (mb-HSP70), a protein which is specific to egg tissue that can be used to quantify egg production in M. balthica (Long et al. unpublished manuscript c). Clams from both an hypoxic tank and a normoxic tank were used to establish the relationship between the concentration of mb-HSP70 and egg concentration (Long et al. unpublished manuscript c). Samples of eggs were removed from the ovaries, suspended in PBS, and counted with a hemocytometer. The samples were centrifuged at 15,000 g for one hour at 4 ºC, homogenized and centrifuged again. The mb-HSP70 concentration in the supernatant was determined by ELISA, and the protein concentration

was determined with a bicinchoninic acid protein assay kit (Sigma) using bovine serum albumin for the standards. We calculated the amount of mb-HSP70 in each egg, as well as the amount of protein in each egg. We used the mb-HSP70:egg ratio to calculate the number of eggs produced by each clam.

Temperature measurements were analyzed with a nested Analysis of Variance (ANOVA with tank nested within DO treatment; Minitab, Version 14.1). Growth and fecundity were also analyzed with a nested ANOVA (tank nested within DO treatment). The mortality rates were analyzed with a one-way ANOVA with DO treatment as the factor. The mb-HSP70:egg and the protein:egg ratios were analyzed with a nested ANOVA (tank nested within DO treatment). We used maximum likelihood techniques, as above, to examine the effect of Treatment on the length-mass relationship in M. balthica: $Mass = a*Length^b$. We analyzed 2 a priori models, wherein 'b' was a constant, or allowed to vary with the four Treatments. A posteriori, it was noted that treatments 1-3 were similar, so we tested a modified version of the second model with one parameter for Treatments 1-3 and one for Treatment 4. The AIC_c was calculated for each model and the 95% confidence intervals calculated for the parameters in the best fit model.

Hypoxia, density dependence, and predation

In the summer of 2006 we performed a caging experiment to determine the effects of hypoxia on predation on M. balthica. We haphazardly selected four sites at each of two depths: shallow (~3-4 m) and deep (~10-12 m) in the York River near Gloucester Point (Fig. 1). Sites were selected to keep environmental factors, especially sediment type, temperature and salinity, as consistent as possible. The experiment was performed

twice, once from June 6-29, and once from August 3-31. During the June experiment, both the deep and shallow sites remained normoxic, while, during August, the deep sites experienced episodic hypoxia and the shallow sites remained normoxic. At each site, four 50 cm x 50 cm plots were established by SCUBA and marked with a frame made of PVC pipes. Each plot was a combination of a caging treatment--caged, partially caged, or uncaged--and a clam density treatment: high (40 clams/plot or 160 /m^2) or low (10 clams/plot or 40 /m^2). These densities are within the normal range found in the York River (Seitz et al. 2005). We used these two densities because it is possible to distinguish between a type II and type III FR using only two prey densities; a type II response will lead to higher predation rate at low densities, whereas a type III will lead to the opposite (Taylor and Eggleston 2000). The plots were as follows: (1) Caged, high density; (2) Partially caged, high density; (3) Uncaged, high density; (4) Uncaged, low density. Live M. balthica, shell lengths > 10 mm, were collected from the York River and brought back to the lab, where they were marked with permanent ink and allowed to recover for 24 hrs. Only healthy clams with whole, un-chipped shells, and a quick siphon withdrawal were used. They were transplanted at each plot at the nominal density and covered with a full cage for at least 24 hrs to allow burial and recovery (Seitz et al. 2001). After acclimation, the full cage was removed from the uncaged plots, replaced with a partial cage in the partially caged plots, and left in place in the caged plots. The cages were made from galvanized steel hardwire cloth (mesh size 1 cm) and were 50 cm x 50 cm on the top and 14 cm high. Partial cages had a 25 cm x 25 cm square cut in the center of the top, and a 25 cm wide by 7 cm tall hole cut in the center of each side to allow access by predators.

All cages were inserted 7 cm into the sediment so that the bottoms of the holes in the sides of the partial cages were flush with the sediment surface.

The experiments were run for approximately 28 days before all plots were re-sampled with a suction sampler down to a depth of 40 cm (Eggleston et al. 1992). Samples were brought back to the lab and frozen before processing. Each sample was sorted, marked M. balthica were counted, and percent recovery was calculated for each plot. Marked, whole M. balthica shells were counted as recovered when we calculated predation since they represented non-predatory mortality. Unmarked bivalves were identified and measured.

A continuous water-quality recorder (model Hydrolab DS5X with a luminescent dissolved oxygen probe, Hach Environmental, Loveland, CO) was deployed at one of the deep sites during both experiments. This instrument recorded temperature, salinity, and dissolved oxygen every five minutes. It was downloaded and serviced once every 7 – 10 days. Unfortunately, during the first deployment in June, the probe temporarily failed. A five point running average was applied to the data to smooth it. Spot measurements were made at each of the sites using a DO probe throughout the experiment (YSI Model 85, Yellow Springs Instruments, Dayton, Ohio, USA).

Predators were sampled by the Virginia Institute of Marine Science's Juvenile Finfish and Blue Crab Survey. The Survey samples sites throughout the Virginia portion of Chesapeake Bay and its tributaries. Monthly, five-minute trawls are performed using a 9 m semi-balloon otter trawl with a 38.1 mm stretch mesh body and a 6.35 mm mesh cod liner. All animals caught in the trawl are identified down to species and length is

measured. We present average benthic predator abundance from two of the survey sites near Gloucester Point.

We calculated predation as a rate using the equation $N = N_0 e^{-pt}$ where N is the number of clams surviving after time t, N_0 is the initial number, and p is the rate of predation. The rate of predation may include a caging effect (from the partial cages) and a density-dependent effect. We calculated the predation rate for each of the uncaged high-density, uncaged low-density, and partially caged plots using the number of clams recovered in the caged plot at that site as N to account for recovery efficiency (i.e., non-recovered clams). For the low-density plots, we assumed that recovery efficiency was non-density-dependent, and used the proportional recovery x10 as N. In some instances we had cage failure; either the cage was broken or missing when we returned to the site, most likely caused by fisheries activity. In these cases, we used the average recovery in the caged plots within that time and depth as N instead. We also estimated non-predatory mortality in the uncaged and partially caged plots with the same equation using the number of dead, whole M. balthica recovered as '1 – S' and the number recovered in the caged plot as N. We analyzed the predation rates with an ANOVA with Depth (Deep or Shallow), Time (June or August), Plot (uncaged high density, uncaged low density, or partially caged) as main factors and Site (nested within Depth and Time) as a blocking factor. Where an interaction term was significant, a Student-Newman-Keuls post-hoc multiple comparison test (SNK test) was performed.

Modeling the M. balthica population

Density-independent model:

We used the results from the previous experiments to parameterize and analyze a matrix model for the M. balthica population in the York River (Caswell 2001). We used two age classes, adults (A), and juveniles (J), and divided the metapopulation into two populations: individuals in areas that remain normoxic (subscript N), and those in areas that go hypoxic (subscript H), giving the following population vector:

$$\begin{bmatrix} J_N \\ J_H \\ A_N \\ A_H \end{bmatrix}$$

The population census took place after recruitment in the spring before predators entered the river. We assume that predation only occurred from the late spring through early fall when predators are common in the system and actively foraging (Hines et al. 1990), which we estimated as May 1 to September 30, or 153 days. For the density independent model, we assumed that there was no density dependent predation, as the results of our predation experiment suggested. Survival over the summer in normoxic areas is calculated as: $S_N = e^{-(p_N + m_N)t}$, where S is the proportion surviving, p is the predation rate, and m is the non-predatory mortality rate. To calculate survival in hypoxic areas, t_H is the time in days that the river experiences episodic periodic hypoxia, which we estimated as ~90 days (Kuo and Neilson 1987). We assumed that during normoxia predatory and non-predatory mortality are the same in all areas of the river. Thus, survival in hypoxic areas is given by: $S_H = e^{-(p_H + m_H)t_H} e^{-(p_N + m_N)(t - t_H)}$. We estimated p from our predation experiment and m from our laboratory experiments for both hypoxic and normoxic areas. Our initial estimate of p_N resulted in summertime survival rates that were much lower than those observed, so we lowered it slightly (while

still remaining within the 95% CI) until the S_N predicted was close to that observed in the field. Our estimate of p_N may have been high because our predation experiments were performed during periods of higher predator abundance and did not account for lower predation rates in the early spring or late fall. The S_H predicated was very close to the observed survival, so we did not need to adjust the values of p_H. Fecundity was estimated from our laboratory experiment for clams in both hypoxic and normoxic areas. We assumed that recruitment R was equal to the number of eggs produced times a constant survival term. We calculated R as the average density of recruits in the spring of 2004 divided by average density of adult clams in the Fall of 2003. Previous work shows that juvenile clams that attain a size of 18 mm shell length or greater will reproduce in their first year and produce about 1/5 the number of eggs as an adult (Long et al. unpublished manuscript c). We estimated the proportion of juveniles that attain this shell length (M) in hypoxic and normoxic areas by the predicted shell length frequencies calculated from the population survey. We assumed that recruitment was equal across all areas, and that d is the proportion of the river that remains normoxic and 1-d is the proportion that goes hypoxic. We further assumed that over-winter survival is 100%, that all juveniles graduate to the adult size class after their first year, and that clams do not move from one area to another after recruitment. From this we constructed the following population projection matrix:

$$\begin{bmatrix} S_N 0.2 M_N R_N d & S_H 0.2 M_H R_H d & S_N R_N d & S_H R_H d \\ S_N 0.2 M_N R_N (1-d) & S_H 0.2 M_H R_H (1-d) & S_N R_N (1-d) & S_H R_H (1-d) \\ S_N & 0 & S_N & 0 \\ 0 & S_H & 0 & S_H \end{bmatrix} \begin{bmatrix} J_N \\ J_H \\ A_N \\ A_H \end{bmatrix}$$

This model contains three parameters that allowed us to explicitly examine effects of a changing hypoxic regime on the population. The duration of hypoxia, t_H, and the areal extent of hypoxia, '1-d', are explicit in the model. We further examined the intensity of hypoxia, i.e. the duration of hypoxic episodes, and the proximity of dissolved oxygen levels to anoxia, with the m_H parameter, which should increase with increasing hypoxic intensity.

We calculated the dominant eigenvalue and eigenvectors and performed sensitivity and elasticity analyses on the matrix. We further examined the effect of various parameters on the long-term stability of the population and on the population's stable age structure.

Incorporating density dependence:

The predator functional response to M. balthica in Chesapeake Bay is a type III, or sigmoid, in both the laboratory (Eggleston et al. 1992) and the field (Seitz et al. 2001). We used this to create a hybrid model that contains both a continuous part (mortality and growth over the summer) and a discrete part (reproduction and graduation of size classes). We used the same population vector as above, changing the units to the density (clams/m^2). This gave rise to the following population projection matrix, similar to the one above but lacking survival terms:

$$\begin{bmatrix} 0.2M_N R_N d & 0.2M_H R_H (1-d) & R_N d & R_H (1-d) \\ 0.2M_N R_N d & 0.2M_H R_H (1-d) & R_N d & R_H (1-d) \\ 1 & 0 & 1 & 0 \\ 0 & 1 & 0 & 1 \end{bmatrix} \begin{bmatrix} J_N \\ J_H \\ A_N \\ A_H \end{bmatrix}$$

The following differential equation describes summer mortality: $\frac{dN}{dt} = -mN - g(N)P$, where N is the density of *M. balthica*, m is non-predatory mortality (as above), g(N) is the predator functional response, and P is the density of predators. We assumed a type three functional response (Hassell 1987): $g(N) = \frac{bN^2}{1 + cN + bT_{hand}N^2}$, where b and c are parameters of the instantaneous attack rate (Hassell 1987) and T_{hand} is the handling time. We used parameters estimated by Eggleston et al. (1992) for Callinectes sapidus feeding on M. balthica in the mud: b = 0.006, c = 0.001, T_{hand} = 4.06 hrs. Predator densities in the York River are strongly correlated with prey densities (Seitz et al. 2003a; Seitz et al. 2007). We used the relationship between blue crab density and clam density in the York River described by Seitz et al. (2003a) to estimate the predator density at each clam density: $Crabs/m^2 = \frac{5.99 * e^{0.006 * Clam/m^2}}{66}$. The rate of predation during hypoxia was increased by a factor of 3.0, the observed increase in the predation rate observed in our predation experiment. We calculated the number and biomass of clams consumed by predators and lost to non-predatory mortality by integrating these coupled differential equations:

$$\frac{dN}{dt} = -mN - g(N)P$$

$$\frac{dN_P}{dt} = g(N)P$$

$$\frac{dN_m}{dt} = mN$$

$$\frac{dB_P}{dt} = g(N)B_c(L(t))P$$

$$\frac{dB_m}{dt} = mNB_c(L(t))$$

N_p and N_m are the number of clams/m^2 that suffer predatory and non-predatory mortality; B_p and B_m are the biomass of those clams; B_c is the biomass of an individual clam and is a function of the length, L. We use the length-mass relationship determined above for clams in the York River in the spring of 2004 for B_c. L(t) is the von Bertalanffy (1938) growth function: $L(t) = L_\infty - (L_\infty - L_0)e^{-kt}$, where L_∞ is the maximum size, L_0 is the initial size, and k is the growth rate. We estimate these parameters using preliminary unpublished results from an ongoing caged marked-recapture experiment in the York River (L_∞ = 32.7 mm; k = 0.85 $years^{-1}$; sample size = 441; Brylawski unpublished data). L_0 is the observed average length of clams in each age class and area from the York River in the spring of 2004.

The census was taken immediately after recruitment in the spring. We then ran the continuous portion for five months (153 days) after which the population vector was multiplied by the population project matrix. We assumed that hypoxia took place in the middle of the summer. We performed sensitivity analysis on the model and examined how changing the hypoxic regime affected the equilibrium populations of M. balthica and how it affected the number and biomass of clams consumed by predators and lost to non-predatory mortality.

RESULTS

Survey of populations in the York and Rappahannock Rivers

Hypoxia was more extensive in the Rappahannock than in the York, and more extensive in 2004 than 2003 (Fig. 1). In the Rappahannock, 44% of the sites went hypoxic in 2003, and 47% in 2004. In the York, 16% of the sites went hypoxic in 2003, and 27% in 2004. In 2003, hypoxia was more intense in the Rappahannock River, where the average DO concentration observed at hypoxic sites was 0.5 mg/L vs. 1.3 mg/L in the York. However, in 2004 the trend was reversed: the average DO in hypoxic areas of the York was 0.4 mg/L vs. 0.7 mg/L in the Rappahannock.

The M. balthica populations in both rivers followed the general pattern expected; there were recruitment pulses in the fall and spring, followed by a decline in population over the summer (Fig. 2). Densities decline in all areas over the summer, but in hypoxic areas there was virtually complete local extinction (Fig. 2). Recruitment was patchy, but no clear trends differentiating recruitment in hypoxic and normoxic sites were found. In February, 2004, recruitment tended to be higher in hypoxic sites than in normoxic sites in the Rappahannock (Fig. 3), but in May 2004, the normoxic sites in the York had higher densities than the hypoxic sites (Fig. 2).

Survival was correlated with the average summer DO observed (Fig 4). The average maximum survival was 0.42 (95% CI 0.26 -- 0.60) and the average summer DO that resulted in a 50% reduction in survival was 2.6 mg/L (95% CI 2.3 -- 3.1). The CI for the River parameter included zero (-0.009 -- 0.009) and reduced the AICc by 2.5,

indicating that there is no evidence that River influences survival (Burnham and Anderson 2002).

In best-fit model for shell-length frequencies, the means of both distributions were functions of Time, River, and Dissolved Oxygen, and the mixing parameter was a function of Time and Dissolved Oxygen (Table 1). Under low DO the mean shell length of the juveniles (μ_1) was increased, the shell length of the adults (μ_2) was decreased, and population was shifted towards the juveniles by 20% (Table 2, Figs. 5 and 6). The juveniles grew by about 14 mm over the course of the summer and the adults remained essentially unchanged, while the populations shifted towards the adults by 40% (Table 2, Figs. 5 and 6). River had a mixed effect; in the York, the average shell length of the juveniles was slightly larger, but that of adults was slightly smaller (Table 2, Figs. 5 and 6).

There were two models of the relationship between shell length and AFDM that had substantial weight (Table 3). Both of these indicated an effect of Time and River; clams in the fall (after hypoxia) had, on average, lower AFDW at a given shell length and the clams in the York had a higher AFDM (Figure 7). There was some evidence for a small effect of Low DO (Table 3 and 4), but it was weak; the parameter represented a biologically insignificant change of ~0.25% in '$\underline{b}$'. Thus, for clarity, results presented in figure 7 do not include this term.

Effects of hypoxia on growth and reproduction:

The DO for each tank within each treatment generally stayed within its nominal range (Figs 8 and 9). The only exception to this was Hypoxic tank 3, which was about

halfway between Hypoxic and Moderately Hypoxic treatments and overlapped each. There was substantial variation in DO over time in each of the tanks (Fig. 9, we show only the first tank in each treatment type), which is characteristic of DO in natural systems such as the York River, which we were trying to mimic. The average temperature during the experiment was 24.2 ± 1.1 (SD) ºC and it did not differ among Treatments (ANOVA, $F_{3,687} = 1.36$, $p = 0.25$) or Tanks (ANOVA, $F_{12,687} = 0.67$, $p = 0.79$).

There was no detectable growth in any of the treatments; average growth over the experimental period was 0.02 ± 0.38 (SD) mm. There was no detectable difference in mortality among treatments during the 28 day experiment (ANOVA; $F_{3,12} = 1.12$; $p = 0.38$); the average mortality rate was 0.0014 day^{-1} ± 0.00025 (SE). The best fit model for the length-mass relationship indicated that there was a difference between the Normoxic treatments and the other three (Fig. 10). The next best fit model had a ΔAIC of 3.8 and increased the number of parameters by 2, indicating there was no substantial evidence for it (Burnham and Anderson 2002).

Hypoxia decreased the number of eggs produced by female M. balthica, but increased the protein investment in each. The ratio of mb-HSP70:eggs was influenced by Treatment (ANOVA; $F_{1,12} = 7.28$; $p = 0.019$) and Clam (ANOVA; $F_{3,12} = 11.33$; $p = 0.001$), with the hypoxic tanks having a higher ratio than the normoxic (Fig. 11a; Tukey's test). The same pattern was observed in the ratio of protein:egg; both Treatment (ANOVA; $F_{1,12} = 9.40$; $p = 0.01$) and Clam (ANOVA; $F_{3,12} = 7.45$; $p = 0.004$) had an effect, and the hypoxic tanks had a higher ratio than the normoxic (Fig. 11b; Tukey's test). As the mb-HSP70:egg ratio differed between the hypoxic and normoxic treatments,

we were able to calculated egg production for clams from hypoxic and normoxic treatments, but not from the two intermediate treatments. We therefore performed two analyses. We ran a nested ANOVA on the concentrations of mb-HSP70 in each clam (a measure of maternal investment), with Treatment and Tank nested within treatment as factors, using all female clams from all treatments. We then calculated the number of eggs produce by each clam in the normoxic and hypoxic tanks and performed a second identical nested ANOVA. There was neither an effect of Treatment (Fig 11c; ANOVA; $F_{3,56} = 0.31$; $p = 0.818$) nor Tank (ANOVA; $F_{11,56} = 1.05$; $p = 0.417$) on the concentration of mb-HSP70. There was an effect of Treatment (ANOVA; $F_{1,31} = 4.72$; $p = 0.038$), but not Tank (ANOVA; $F_{6,31} = 1.31$; $p = 0.28$) on egg production. Clams in hypoxic tanks produced 40% fewer eggs than those in normoxic tanks (Fig 11d; Tukey's test). In making the standard curve for the ELISA results, it was noticed that one of the females from the normoxic group had an empty gonad, indicating that it had already spawned and it was not used in analyses.

Hypoxia, density dependence, and predation in the field

During the June field experiment, dissolved oxygen measurements remained well above 2.0 mg/L in both the deep and shallow areas as expected (Fig. 12a). Although we lost some data from the water-quality recorder at the beginning of the experiment, we have data from during the neap tides when hypoxia is most likely (Kuo and Nelson 1987). In August, the shallow sites remained normoxic during the whole experiment but the deep sites suffered one strong hypoxic episode that lasted ~5 days, beginning August 3, and also had some short excursions into hypoxia starting August 28 (Fig 12b). During

both experiments, temperatures in the deep area were ~1 ºC cooler than shallow areas and ~1 ppt more saline.

Hypoxia increased the rate of predation, but not of non-predatory mortality. Average recovery of clams in caged plots was 92% in June and 85% in August. We lost one deep site during June, the caged, uncaged high, and partially caged plots from one deep site, and the uncaged low plot from a second deep site in August. This left us with at least three replicates of each plot type in each depth and time period, which was adequate for the analysis. There was an significant interaction effect between Time and Depth (ANOVA; $F_{1,19} = 14.9$; $p = 0.001$). Predation rate in the deep, hypoxic sites during August was significantly higher than all other depths and time periods (SNK; $p < 0.01$; Fig. 13a). There was also a significant effect of Site on the predation rate (ANOVA; $F_{11,19} = 2.49$; $p = 0.02$); however, the only difference was between the 2 deep sites in August that had lost one or more plots and therefore had sample sizes of 1 and 2, making the comparison unreliable (Tukey's test). There was a significant interactive effect of Time and Depth on non-predatory mortality (ANOVA; $F_{1,19} = 4.7$; $p = 0.043$). The rate of non-predatory mortality was higher in the deep sites in August than in the deep sites in June (SNK test), but there was no difference between the deep and shallow sites in August.

Density did not have a significant effect on predation rate, but the trends are interesting (Fig. 13b). One of the major difficulties in the interpretation of the results was the presence of ambient unmarked clams that increased the actual density of M. balthica above the desired density and blurred the distinction between nominally high- and low-density plots. This problem was especially prominent the shallow areas. In the deep sites

during hypoxia, predation rates were so high that we recovered no live clams in one of our partially caged, high-density plots. With such high losses in all density plots, differences in proportional mortality were slight. In August, however, during hypoxia, there was a trend toward higher proportional mortalities in the high-density plots in the shallow sites, whereas in the deep sites there was trend for higher proportional mortalities in low-density plots.

The overall density of predators was low in June-July, and was dominated by hogchoaker and croaker (Fig. 14). In August-September, the abundance of predators increased 10 fold and the relative number of blue crabs increased, though the population was still dominated by piscine predators (Fig. 14).

Model Simulation:

Density-independent model:

Our population projection matrix, based on the estimate parameters (Table 5), was:

$$\begin{bmatrix} 0.02 & 0.00 & 2.06 & 0.02 \\ 0.02 & 0.00 & 1.37 & 0.02 \\ 0.34 & 0 & 0.34 & 0 \\ 0 & 0.01 & 0 & 0.01 \end{bmatrix}$$

The dominant eigenvalue of this matrix (λ) is 1.04, indicating an increasing population ($\lambda > 1$ implies that the population is increasing, and $\lambda < 1$ that population is decreasing; Caswell 2001). Sensitivity analysis indicated that the dominant eigenvalue was sensitive to the reproduction and survival of clams in normoxic areas as well as recruits produced by maturing juveniles in hypoxic areas that recruit into normoxic area (Fig 15a).

Elasticity analysis showed that the three matrix elements that contribute substantially to λ are survival of clams in normoxic areas, and recruitment into normoxic areas from adults in normoxic areas (sum of these elements: 0.98). Clams in hypoxic areas contributed almost nothing to λ (sum of elements including hypoxic clams: 0.006; Fig. 15b).

The dominant eigenvalue, λ, is very sensitive to changes in the extent of normoxia, d, along its full range and is relatively insensitive to changes in the duration of hypoxia, T_H, between about 153 and 40 days but increases rapidly between 40 and 0 days (Fig 16). As predicted from the elasticity analysis, λ is almost completely insensitive to reproduction from clams in hypoxic areas (Fig. 17). λ is fairly sensitive to changes in predation in hypoxic areas, p_H, from 0 to 0.15 day^{-1} and insensitive at larger values (Fig. 17).

The stable age structure associated with our matrix shows that ~80% of the population is juveniles and ~20% is adults in normoxic areas; adults in hypoxic areas are a negligible part of the population (Figure 18). The stable age structure was not much affected by changes in t_H or p_H. At small values of t_H and p_H, A_H approached A_N; this is expected, since when conditions in hypoxic areas approach those in normoxic areas, the age structure in hypoxic areas should resembles those in normoxic (Fig. 18 a&d). As d decreases, the population becomes progressively dominated by J_H, while, as R_N decreases, the populations becomes dominated by A_N (Fig. 18 c&d).

Density-dependent model:

The model predicts cyclical population dynamics with recruitment in the spring and fall followed by mortality over the summer (Fig 19), which is typical of M. balthica

(Holland et al. 1987). The population reaches equilibrium rapidly, after about 10-25 generations. In normoxic areas in the early spring, the density of adult population is about 200 clams/m^2 and the density of juveniles is around 400 clams/m^2, which is a moderate density of clams in shallow areas in the York (Seitz et al. 2007). Summer mortality is much higher in hypoxic areas, and the total population drops to less that 12 clams/m^2, compared with 190 clams/m^2 in normoxic areas (Fig. 19). Numerical analysis suggest that under certain conditions there are two stable equilibria, one high and one low density, one unstable equilibrium at zero; which equilibrium the population approaches depends on the initial population vector. The population can also switch from the high to low density equilibrium if the population densities dropped below a critical density (which represents a second unstable equilibrium), as might be caused by some catastrophic event. Under other conditions, the lower equilibrium becomes the only stable one regardless of the initial population.

The model predicts that the metapopulation of M. balthica is sensitive to the extent (1-d), and duration (t_H), of hypoxia, but not to its intensity (m_H, Fig. 20). The density of clams decreases with increasing extent of hypoxia, exhibiting a threshold response at d = 0.49 (when just over half the river is hypoxic), below which the metapopulation is functionally extinct (Figure 20a). Additionally, as the extent of hypoxia increases, the threshold density, i.e. the density at which the population will switch between the high to the low equilibrium densities, increases indicating that the resilience of the population to perturbation decreases. The population of adult clams in hypoxic areas decreases with increasing hypoxic duration until it becomes functionally extinct when the duration is greater than 100 days (Fig. 20b). Varying the intensity of

hypoxia did not change the equilibrium densities of the population even when the rate of non-predatory mortality was increased to those observed under anoxia (Fig. 20c). Hypoxia also affects predation and trophic transfer (Fig. 21). Increasing the extent of hypoxia decreases the number and the biomass of clams eaten in both hypoxic and normoxic areas (Fig 21a&b). Although the number of clams consumed in hypoxic areas is generally predicted to be higher than that in normoxic areas, the biomass is generally much lower because a greater proportion of those clams is juveniles. The exception for this trend occurs when the duration of hypoxia is less than 40 days, in which case it is predicted that the biomass consumed in hypoxic areas will be greater than that in normoxic areas (Fig 21c). The amount of biomass lost to non-predatory mortality is very low and almost always much lower than that consumed by predators. However, increasing the intensity of hypoxia, i.e. increasing the rate of non-predatory mortality in hypoxic areas, changes this ratio. When the rate of predation and the rate of non-predatory mortality are approximately equal, the biomass lost to each is equal, but as the non-predatory mortality rates approach those observed under anoxic conditions, the biomass lost to non-predatory mortality greatly exceeds that lost to predation (Fig. 21d).

DISCUSSION

Effects of hypoxia on the individual level

Low DO has a multitude of effects on individual M. balthica. Periodic hypoxia, such as was experienced in our lowest DO treatment in our tank experiment and in August in the deep area during our predation experiment in the field, did not increase the non-predatory mortality rate, as seen in other studies (Modig and Ólafsson, 1998). An increase in non-predatory mortality has been observed in laboratory experiments (e.g. de Zwaan et al. 2001, Seitz et al. 2003b), but only after long periods of continuous hypoxia. In our studies, sub-lethal hypoxia decreased egg production in M. balthica, similar to what is observed in other species (eg. Chrysaora quinquecerrha, Condon et al. 2001; Mnemiopsis leidyi and Chrysaora quinquecirrha, Grove and Breitburg, 2005, Acartia tonsa, Richmond et al. 2006). Interestingly, in our experiment, as the number of eggs decreased their protein content increased. This is consistent with predictions based on evolutionary and life-history theory that individuals in stressful environments will maximize their fitness by producing fewer offspring but investing more in each (McGingley et al. 1987, Sibly et al. 1988). M. balthica also have behavioral responses to hypoxia that include increasing siphon extension (Seitz et al. 2003b), and reducing burial depth (Bradfield 1963, Long et al. unpublished manuscript b) to gain access to higher DO levels above the benthic boundary layer. Both of these responses take place within 24-48 hrs of the beginning of hypoxia.

Our analysis of shell-length frequencies supports the theory that hypoxia might affect the growth of M. balthica, as is commonly observed in other species (e.g. McNatt and Rice 2004, Grove and Breitburg 2005, Richmond et al. 2006). In May, the juveniles in areas that experienced low DO had shell lengths that were ~1mm longer than those in high DO areas; however, the adult populations in the same area had shell lengths that ~1mm shorter. This suggests that the negative effects of low DO on growth are subtle and only detectable after a year, explaining why we did not detect find an effect in our laboratory experiment; the duration was not long enough for differences in growth to be noticeable. Juveniles may be larger in hypoxic sites because lower ambient clams densities in these areas (from reduced abundances due to hypoxic episodes the previous year) lead to reduced competition (Rhodes and Young 1970, Woodin 1974); M. balthica growth, though not mortality, is affected by density (Ólafsson 1986). Low DO did not have a substantial effect on the relationship between clam length and mass, indicating that the clams use energy for the maintenance of optimal mass rather than for growth. In our lab experiment, clams in normoxic tanks had a slightly lower mass at each length compared to clams in other treatments; however, this may be because some of the clams in these tanks had spawned earlier, reducing their mass. Other studies have shown that hypoxia can result in delayed reproduction (Richmond et al. 2006, Brouwer et al. 2007).

Effects of hypoxia on the population level

Combined, the multiple effects of sub-lethal levels of hypoxic stress on M. balthica can lead to local extinction of populations, a substantial decrease in production and transfer of biomass up the food web, and possibly the collapse of the metapopulation.

In the York and Rappahannock Rivers, this combination of effects leads to source-sink dynamics along a dissolved oxygen gradient. In our field study mortality was complete in hypoxic areas leading to local extinction. Thus, areas where the dissolved oxygen drops below ~1.5 mg/L represent a black-hole sink (*sensu* Gomulkiewicz et al. 1999) for M. balthica and areas where the dissolved oxygen averages between ~1.5 and 2.5 mg/L represent a traditional sink habitat. In a sink habitat the rate of reproduction is not sufficient to maintain the population in that area (Pulliam 1988, Dias 1996, Holt 1997). In a black-hole sink the organism recruits, but it dies before it can reproduce (Dias 1996, Gomulkiewicz et al. 1999). Thus, M. balthica that recruit into hypoxic areas will not be able to maintain the population, as they produce fewer eggs, or will die before reproduction. Though source-sink dynamics have been documented in marine invertebrate species (e.g., Lipcius et al. 1997, Peterson et al. 2001), ours is the first empirical evidence for source-sink dynamics in an infaunal bivalve.

The populations of M. balthica in the York and Rappahannock Rivers do not appear to suffer from recruitment limitation; recruitment is strong both in areas that remain normoxic as well as in areas that experience hypoxia. Indeed, recruitment in the Rappahannock River in the spring of 2004 tended to be higher in the areas that typically go hypoxic, possibly because of reduced competition (Rhodes and Young 1970, Woodin 1974) due to death of the benthos during the previous year's hypoxia. This pattern of strong recruitment of M. balthica in areas that go hypoxic is typical in both the Chesapeake (Holland et al. 1987), and elsewhere (Powers et al. 2005). Summer survival of M. balthica was zero at all but one site in 2 years where the dissolved oxygen averaged below 1.5 mg/L DO, representing black hole sinks in hypoxic zones. This combination

of high recruitment and low survival in hypoxic areas gives rise to a population dominated by juveniles, which is both what we observed, and what was predicted by both our models. Areas between 1.5 and 2.5 mg/L DO represent a traditional sink habitat, where low survival of M. balthica, combined with reduced growth and fecundity, result in a level of reproduction insufficient for maintenance of the population (e.g., Kreuzer and Huntly 2003). The populations in these areas are replenished each year by recruits from shallow, normoxic habitats that act as sources.

Our models of the population dynamics of M. balthica predict what may happen to the population under changing hypoxic conditions. For example, source-sink population dynamics can lead to destabilization of the population as a whole. Our model predicts that when the extent and duration of hypoxia reach a threshold, the population of M. balthica will decline towards extinction, as more general models predict (e.g. Delibes et al. 2001b). Increasing the intensity of hypoxia, or decreasing reproduction in clams in hypoxic areas, has little effect on population stability, because mortality in hypoxic areas is already near 100%. This is why the dominant eigenvalue of the model is sensitive only to changes in survival and reproduction in normoxic areas. Our model unrealistically assumes that the system is closed, and that M. balthica larvae do not immigrate from areas outside the coupled source-sink pair; such immigration could help stabilize a population. However, if the population in the River required external inputs of larvae, that would mean that the population in the River as a whole was functioning as a sink. Increasing the scale in this way does not affect the general prediction that, once the threshold conditions are reached on the largest effective scale, the population will decline.

For species that have a more active role in choosing their habitat, a sink habitat is often occupied by individuals forced out of source habitats by competition (Pulliam 1988). In this case, sinks are only populated after the source habitats have reached carrying capacity, and thus the total population size with the sink habitat is greater than it would be with the source habitat alone (Dias 1996). However, for species that disperse passively, or that are unable to distinguish between source and sink habitats, a sink habitat can reduce the population in the source (Pulliam and Danielson 1991, Delibes et al. 2001 a&b). This latter case is what our density dependent model predicts, and what our observations support. Increasing the extent and duration of hypoxia in the model can cause the equilibrium population density in the normoxic areas to drop by more than 40%, and the population density of M. balthica in the Rappahannock, which generally has a greater extent and duration of hypoxia, is lower than in the York, as our model predicts. This suggests that hypoxia may contribute to the reduced M. balthica population densities in the Rappahannock.

Our model further predicts that there are alternative stable states for the M. balthica population, and that increasing hypoxic stress can decrease the resilience of the population by increasing the threshold density at which a switch between the high and low density states occurs. A switch in states is predicted to cause the population to decline by two orders of magnitude or more. A decline in the density of a species of this magnitude has already been observed for Crassostrea virginica, the eastern oyster, in both Chesapeake Bay and the Neuse River, where declining water quality combined with high fishing mortality and disease has resulted in a collapse of the species (Rothschild et al. 1994, Lenihan and Peterson 1998).

Effects of hypoxia on trophic interactions

Our predation study demonstrated that hypoxia can have a substantial effect on trophic transfer. The rate of predation in the deep areas in August was increased three fold compared with the predation rates under normoxic conditions. During the experimental period there was a substantial hypoxic episode at the beginning of the experiment, and a slight one at the end. The duration of the first episode was five days, which is long enough for M. balthica to exhibit a behavioral response but not long enough for non-predatory mortality to occur (Seitz et al. 2003b, Long et al. unpublished manuscript b). These results corroborate those obtained in a similar experiment the previous year (Long et al. unpublished manuscript d) and provide strong evidence that hypoxic episodes of sub-lethal duration substantially increase vulnerability of M. balthica to predation, and that predators in the system take advantage of this.

Although motile predators typically avoid hypoxic areas (Phil et al. 1991, Eby and Crowder 2004, Bell and Eggleston 2005), there are examples of predators entering hypoxic water to forage both in estuarine (Nestlerode and Diaz 1998, Robertis et al. 2001) and freshwater systems (Rahel and Nutzman 1994), indicating that predation can occur during hypoxic events. In addition, predators do not avoid episodic hypoxia to the same extent as they do chronic hypoxia (Bell et al. 2003, Bell and Eggleston 2005). Finally, M. balthica will exhibit a behavioral response to hypoxia at DO concentrations higher than the concentrations which piscine and crustacean predators avoid (Bell and Eggleston 2005, Long et al. unpublished manuscript). Epibenthic predators were present in abundance in the general area of our field experiments and may have been able to take advantage of stressed infaunal prey during or just after hypoxic events. Taken together,

these responses offer a large window of opportunity for increased predation during and after hypoxia. Since in previous studies in the York River, the predation rate was lower during hypoxia (Nestlerode and Diaz 1998), the more likely mechanism for the increased predation in hypoxic areas in our study is reinvasion by predators immediately after a hypoxic event (Phil et al. 1991), before the prey species have a chance to recover (Phil et al. 1992). If this is the case, then short-lived hypoxic episodes, with duration of about 1 week, are important for predation to be enhanced.

Although we did not detect a significant effect of density on the predation rates, our results suggest that hypoxia changes the functional response of predators to M. balthica. In shallow areas, ambient clams increased the density in our plots above our desired density, making detection of a density-dependent response difficult. However, the trend in shallow plots of higher rates of predation in higher density plots is consistent with a type III functional response. This response has been demonstrated for M. balthica in multiple field and laboratory experiments (Eggleston et al. 1992, Mansour and Lipcius 1991, Seitz et al. 2001). In our deep sites, the predation rate was so high that we observed complete local extinction in one of our high density plots, which is why there was no statistical difference between high- and low-density plots, though the trend was for higher predation rates in low-density plots. This trend is consistent with a type I or type II functional response and in opposition to a type III (Taylor and Eggleston 2000). Finally, a type III functional response implies the existence of a low-density refuge at which the prey are safe from predation (Eggleston et al.1992). For M. balthica this refuge exists between 12 and 50 clams m^{-2} in the field in the York River (Seitz et al. 2001). Our observation of extinction in a high-density plot, and the fact that our low-

density hypoxic plots were reduced from an average of 40 clam/m^2 to 8 clams/m^2, shows that the clams no longer have a low density refuge from predation, providing further evidence against type III and providing support for either a type I or II functional response.

Effects of hypoxia at the ecosystem level

Although on the individual and population levels the distinction between predatory and non-predatory mortality is somewhat irrelevant, at an ecosystem level it is of central importance (Altieri and Witman 2006). Clams that die from non-predatory mortality enter the microbial loop and are lost to higher trophic levels (Baird et al. 2004), but clams that are consumed by predators are passed up the food chain (Phil et al. 1992). If the majority of the biomass that is lost during hypoxia goes to predators, then the overall effect on the ecosystem will be far less than if this biomass is metabolized by microbes.

In the York, we observed an increase in predation rate under hypoxic conditions, but no commensurate increase in non-predatory mortality, compared to normoxic sites. This has the effect of increasing the both the rate of trophic transfer and the ratio of biomass consumed to biomass lost to microbes; predators consume so many clams that there are few left to die from other causes. Our model predicts that a greater number of clams are consumed in hypoxic areas than in normoxic. However, because the population of clams in hypoxic areas is dominated by juveniles, the biomass consumed is generally substantially less in hypoxic areas.

The magnitude of hypoxia is very important in determining the rate of trophic transfer. As the areal extent of hypoxia increases, the equilibrium population levels in both the hypoxic and normoxic areas decrease. This lowers the number of clams available and subsequently the number and biomass of the clams consumed by predators in all areas. The duration of hypoxia has a interesting range of effects on trophic transfer. When the duration is low (less than 40 days) the biomass consumed in the hypoxic areas is higher than in normoxic areas. A low duration of hypoxia allows predators a short window of opportunity for higher consumption rates, accounting for the high amount of biomass consumed. However, the level of predation is not high enough to cause local extinction of the population, so benthic production remains high, and some of the juveniles survive to become adults, increasing the biomass available in the areas. As the duration of hypoxia increases, predators consume greater proportions of the prey, production is negatively affected, and the total biomass consumed decreases when the system reaches equilibrium. The situation is analogous to human over-harvesting of natural resources leading to a decrease in total harvest; maximum yields occur at a moderate rate of exploitation (e.g. Rieman and Beamesderfer 1990). Also, intermediate disturbance leads to highest density and diversity of benthic species (Connell 1978, Sommer 1995).

Increasing the intensity of hypoxia, that is, moving from episodic to chronic hypoxia and decreasing the DO concentrations towards anoxia, increases the rate of non-predatory mortality. This substantially decreases the biomass of clams consumed by predators and commensurately increases the biomass lost to the microbial loop. At rates of mortality equal to those observed for M. balthica under anoxic conditions, the majority

of biomass in hypoxic sites enters the microbial loop. This is what is predicted by other models (Baird et al. 2004), and what is observed in systems with more intense hypoxia (Powers et al. 2005, Altieri and Witman 2006).

An important ecosystem-level effect of hypoxia is the decrease in filtration by the benthic assemblage. Although M. balthica is a facultative deposit feeder and thus may not be a major contributor to overall benthic filtering capacity (Ólafsson 1986), when M. balthica densities decrease in association with hypoxia, the overall density and biomass of the benthic assemblage, which includes deposit, filter, and suspension feeders, also decline. Since the population filtration rate is proportional to the biomass, the filtration rate will decline along with the biomass. Filtration by bivalves and other benthic fauna has the potential to reduce the effects of eutrophication in the system by removing labile organic material from the system (Officer et al. 1982, Newell et al. 2007), so reduction in bivalve biomass by hypoxia could, through a feedback mechanism, exacerbate the problems caused by eutrophication (Altieri and Witman 2006), as has been proposed for oysters in Chesapeake Bay (Newell et al. 2007) .

Another ecosystem-level effect of hypoxia that is observed in other systems is an increased density of predators in normoxic areas due to flight from hypoxic waters (Eby and Crowder 2002). This can result in an increase in predation in the shallow habitat (Lenihan et al. 2001) and potentially increased antagonism and cannibalism within predator populations (Eggleston et al. 2005, Aumann et al. 2007). We did not observe an increase in predation in the shallow areas during hypoxic episodes, suggesting that habitat compression is not an important factor in this system (Phil et al. 1992). This may be either because the areal extent of hypoxia is not great enough to produce an observable

effect, or because predators are inefficient at avoiding episodic hypoxia such as we observe in this system (Bell et al. 2003, Bell and Eggleston 2005).

These ecosystem-level effects are sobering, especially if one considers that the effects of hypoxia on benthic species are the same as for pelagic, and particularly planktonic species, where hypoxic similarly affects growth (Breitburg 1992, Grove and Breitburg 2005), reproduction (Condon et al. 2001, McNatt and Rice 2004, Richmond et al. 2006), mortality (Breitburg, 1992), and predation (Breitburg et al. 1994, Breitburg et al. 1997). Adding the effects of hypoxia on benthos and the water column further decreases the overall services the ecosystem is able to provide, and decreases its stability and resilience (Baird et al. 2004).

CONCLUSIONS

In this study, we have explored the effects of hypoxia on the biomass dominant species in an estuarine system and, through modeling, integrated those effects over time, space, and the ecosystem. The effects cascade up through the various scales. Individual effects on growth, reproduction, and mortality decrease benthic abundance, biomass, and production in local populations. Further, behavioral changes in individual M. balthica result in a higher rate of predation, increasing trophic transfer but decreasing benthic biomass and secondary production. Integration over space by considering linkages between local sub-populations, gives rise to source-sink population dynamics and results in the population in hypoxic areas reducing the equilibrium abundances in normoxic areas. We predict that a short duration of hypoxia may result in a slight increase in ecosystem services (e.g., increased trophic transfer and production in commercially important species), but over the long term, increasing the extent and duration of hypoxia could lead to a population crash and an elimination of these important ecosystem services. Out of these dynamics emerge ecosystem-level effects, where short-term increases in predation may lead to long-term decreases in energy transfer from primary producers to predators, and a feedback mechanism between the loss benthic filtration services and the development of hypoxia could further decrease the stability and potential resilience of the system to eutrophication.

ACKNOWLEDGMENTS

I would like to thank K Delano, K Knick, K Erickson, R Burke, J Falls, D Lambert, A Lawless, A Smith, and M Seebo for help with the field sampling, K Knick and J Long for help with the laboratory work, and D Wolcott, and J Long for help with the revision of this manuscript. WC Long was funded by an EPA GRO fellowship. Additional funding was provided by NOAA National Sea Grant, Essential Fish Habitat Program, by the Commonweath of Virginia, by a grant to the Blue Crab Advanced Research Consortium from the NOAA Chesapeake Bay Program, by a Student Research Grant by the School of Marine Science, College of William and Mary, and by a grant from the VIMS Graduate Student Association.

Tables:

Table 1: Ranking of models of shell length frequencies for M. balthica in 2004 based on Alkaike's Information Criterion with small sample size correction (AIC_c). The base model is a bimodal normal distribution with means of μ_1 and μ_2, standard deviations of σ_1 and σ_2 and a mixing parameter, ω. Parentheses indicate that the parameter is a linear function of the parenthetical factors. Factors include 'O', Low Dissolved Oxygen, 'R', River (York), and 'T', Time (Fall).

Model	Parameters	AIC_c	ΔAIC_c	Likelihood	AIC_c Weights
μ_1(ORT),σ_1,μ_2(ORT),σ_2,ω(OT)	13	10434.30	0.00	1.00	1.00
μ_1(ORT),σ_1,μ_2(ORT),σ_2,ω(T)	12	10481.79	47.49	0.00	0.00
μ_1(RT),σ_1,μ_2(RT),σ_2,ω(T)	10	10518.83	84.53	0.00	0.00
μ_1(OT),σ_1,μ_2(OT),σ_2,ω(T)	10	10615.61	181.31	0.00	0.00
μ_1(T),σ_1,μ_2(T),σ_2,ω(T)	8	10633.37	199.06	0.00	0.00
μ_1(OR),σ_1,μ_2(OR),σ_2,ω	9	11182.41	748.11	0.00	0.00
μ_1(R),σ_1,μ_2(R),σ_2,ω	7	11212.37	778.07	0.00	0.00
μ_1(O),σ_1,μ_2(O),σ_2,ω	7	11322.34	888.04	0.00	0.00
μ_1,σ_1,μ_2,σ_2,ω	5	11334.90	900.60	0.00	0.00

Table 2: Maximum likelihood estimates (MLE) and 95% confidence intervals (CI) for the parameters of the best fit model of shell length frequencies of juveniles (μ_1) and adults (μ_2) in the York and Rappahannock Rivers in 2004. Factors include 'O', Low Dissolved Oxygen, 'R', River (York), and 'T', Time (Fall).

Parameter	MLE	95% CI	
μ_1	6.63	6.78	6.48
μ_1(O)	0.96	1.16	0.75
μ_1(R)	1.58	1.74	1.41
μ_1(T)	14.48	15.15	13.81
σ_1	2.23	2.35	2.12
μ_2	22.35	22.71	21.99
μ_2(O)	-0.81	-0.12	-1.50
μ_2(R)	-3.82	-3.40	-4.25
μ_2(T)	-0.29	0.20	-0.79
σ_2	4.52	4.78	4.27
ω	0.60	0.63	0.58
ω(O)	0.20	0.24	0.17
ω(T)	-0.47	-0.40	-0.55

Table 3

Ranking of models of relationship between shell length and AFDM in M. balthica in 2004 using Alkaike's Information Criterion with small sample size correction (AIC_c). The base model is Mass = a*Length^b, assumes a normal distribution of errors with a standard deviation = s*length^3. Parentheses indicate that the parameter is a linear function of the parenthetical factors. Factors include 'O', Low Dissolved Oxygen, 'R', River (York), and 'T', Time (Fall).

Model	Parameters	AIC_c	ΔAIC_c	Likelihood	AIC_c Weights
a,b(ORT),s	6	5873.71	0.00	1.00	0.56
a,b(RT),s	5	5874.20	0.49	0.78	0.44
a,b(OT),s	5	6186.67	312.96	0.00	0.00
a,b(T),s	4	6193.81	320.10	0.00	0.00
a,b(T,O*T),s	5	6194.99	321.28	0.00	0.00
a,b(O*T),s	4	6786.86	913.15	0.00	0.00
a,b(R),s	4	6857.66	983.95	0.00	0.00
a,b(OR),s	5	6858.61	984.90	0.00	0.00
a,b(H),s	4	6916.42	1042.71	0.00	0.00
a,b,s	3	6922.34	1048.63	0.00	0.00

Table 4

Maximum likelihood estimates (MLE) and 95% confidence intervals (CI) for the parameters of the best fit models of AFDMs of M. balthica in 2004. Factors include 'O', Low Dissolved Oxygen, 'R', River (York), and 'T', Time (Fall).

	a,b(ORT),s			a,b(RT),s		
Parameter	MLE	95% CI		MLE	95% CI	
a	0.00072	0.00073	0.00071	0.00075	0.00076	0.00074
b	3.72	3.73	3.72	3.71	3.72	3.71
b(H)	0.010	0.019	0.000			
b(R)	0.14	0.15	0.13	0.14	0.14	0.13
b(T)	-0.28	-0.27	-0.30	-0.28	-0.27	-0.30
S	0.0015	0.0016	0.0015	0.0016	0.0016	0.0015

Table 5

Estimates of parameters used in population projection matrix.

Parameter	Estimate
p_N (days^{-1})	0.006
p_H (days^{-1})	0.05
m_N (days^{-1})	0.001
m_H (days^{-1})	0.001
T (days)	153
T_h (days)	90
M_N	0.06
M_H	0.34
R_N (recruits clam^{-1})	10
R_H (recruits clam^{-1})	6
d	0.6

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Figure 1

Map of Chesapeake Bay and study areas. Inset a) Boxcoring sites in the Rappahannock River in 2004. Contours represent interpolated dissolved oxygen concentrations on August 5, 2004 (ArcGIS, Version 9.1). Inset b) Boxcoring sites in the York River in 2004. Contours represent interpolated dissolved oxygen concentrations on July 30, 2004. Inset c) Map of the predation experiment in the York River 2006. Circles and triangles represent the sites where the caging experiments were performed, and stars represent sites where predator trawls were performed.

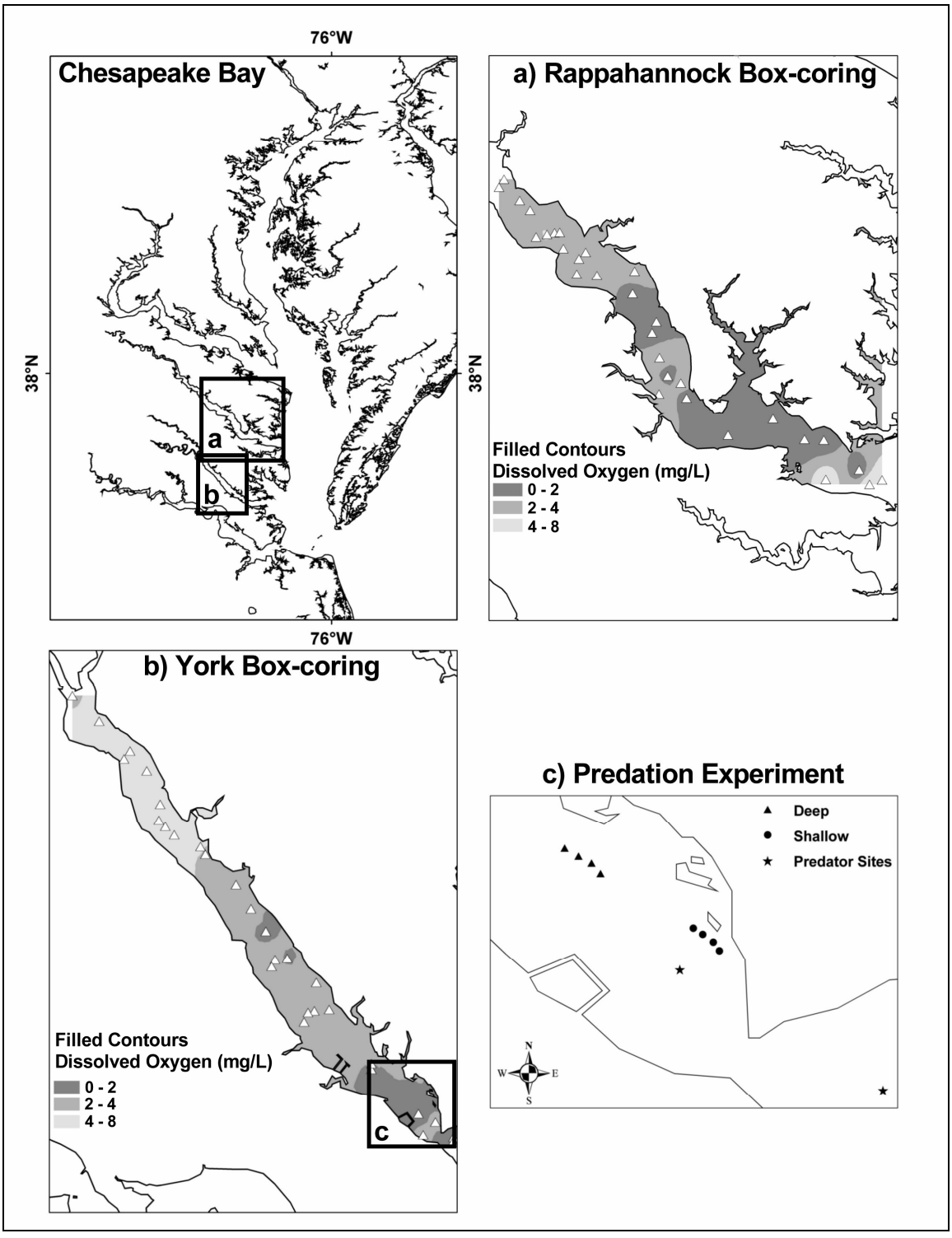
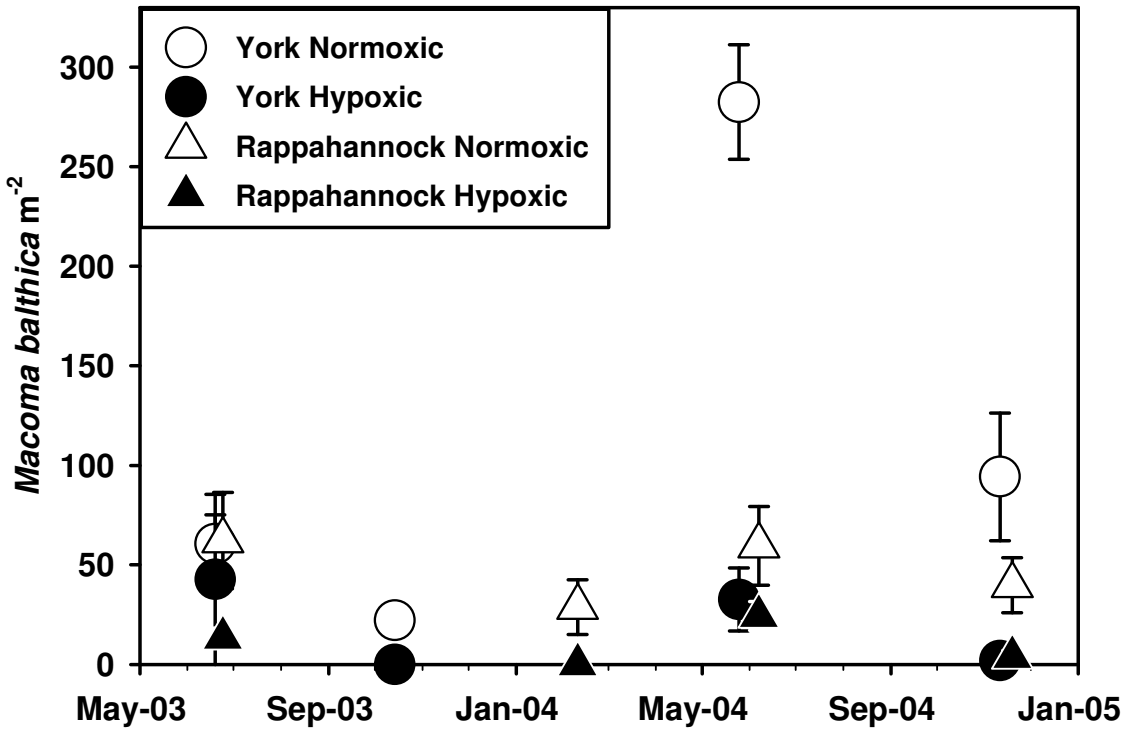
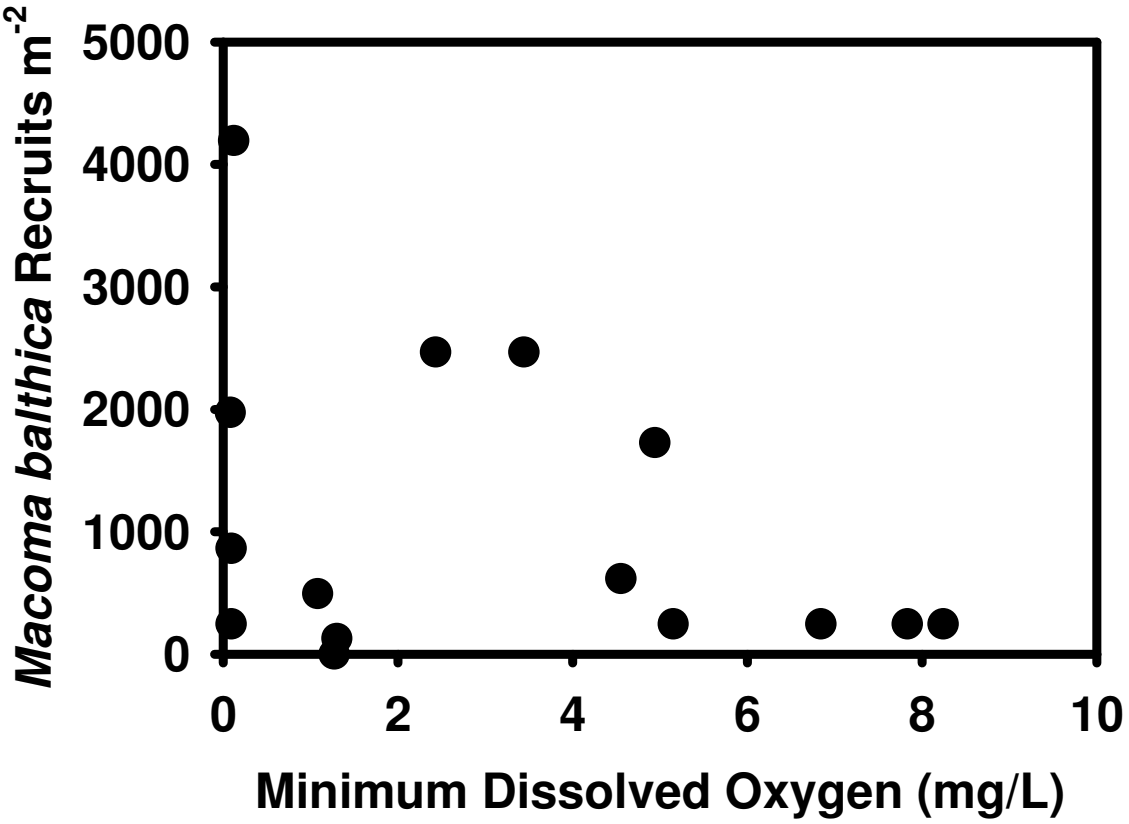

76°W
Chesapeake Bay
38°N
a
b
a) Rappahannock Box-coring
Filled Contours
Dissolved Oxygen (mg/L)
0 - 2
2 - 4
4 - 8
76°W
b) York Box-coring
Filled Contours
Dissolved Oxygen (mg/L)
0 - 2
2 - 4
4 - 8
c
c) Predation Experiment
Deep
Shallow
Predator Sites
N
W
E
S

Figure 2



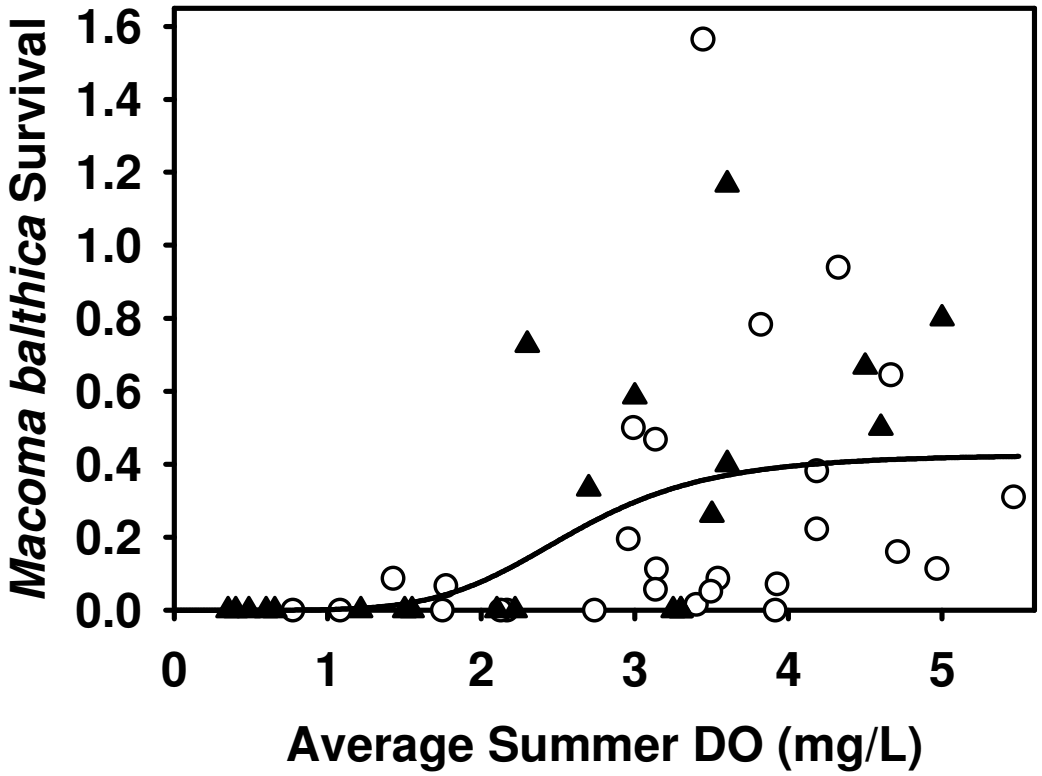
Density of M. balthica in the York (circles) and Rappahannock (triangles) Rivers in 2003 and 2004. Points are average density ± 1 SE. Normoxic sites are hollow and hypoxic sites solid.

Figure 3



Densities of M. balthica recruits in the Rappahannock River in February 2004 plotted against minimum observed dissolved oxygen from the previous year.

Figure 4



Proportional survival of M. balthica from sites in the York (circles) and Rappahannock (triangles) Rivers in 2004. Predicted line represents the maximum likelihood estimates for the best-fit model.

Figure 5

Shell length frequency histogram for the M. balthica in the York River (a) before and (b) after hypoxia in 2004. White bars represent the High DO site (DO > 3.5 mg/L) and gray bars the low DO sites (DO < 3.5 mg/L). Lines are the predicted probability distributions functions (PDFs) estimated by maximum likelihood for high DO (dashed line) and low DO (solid line) sites.

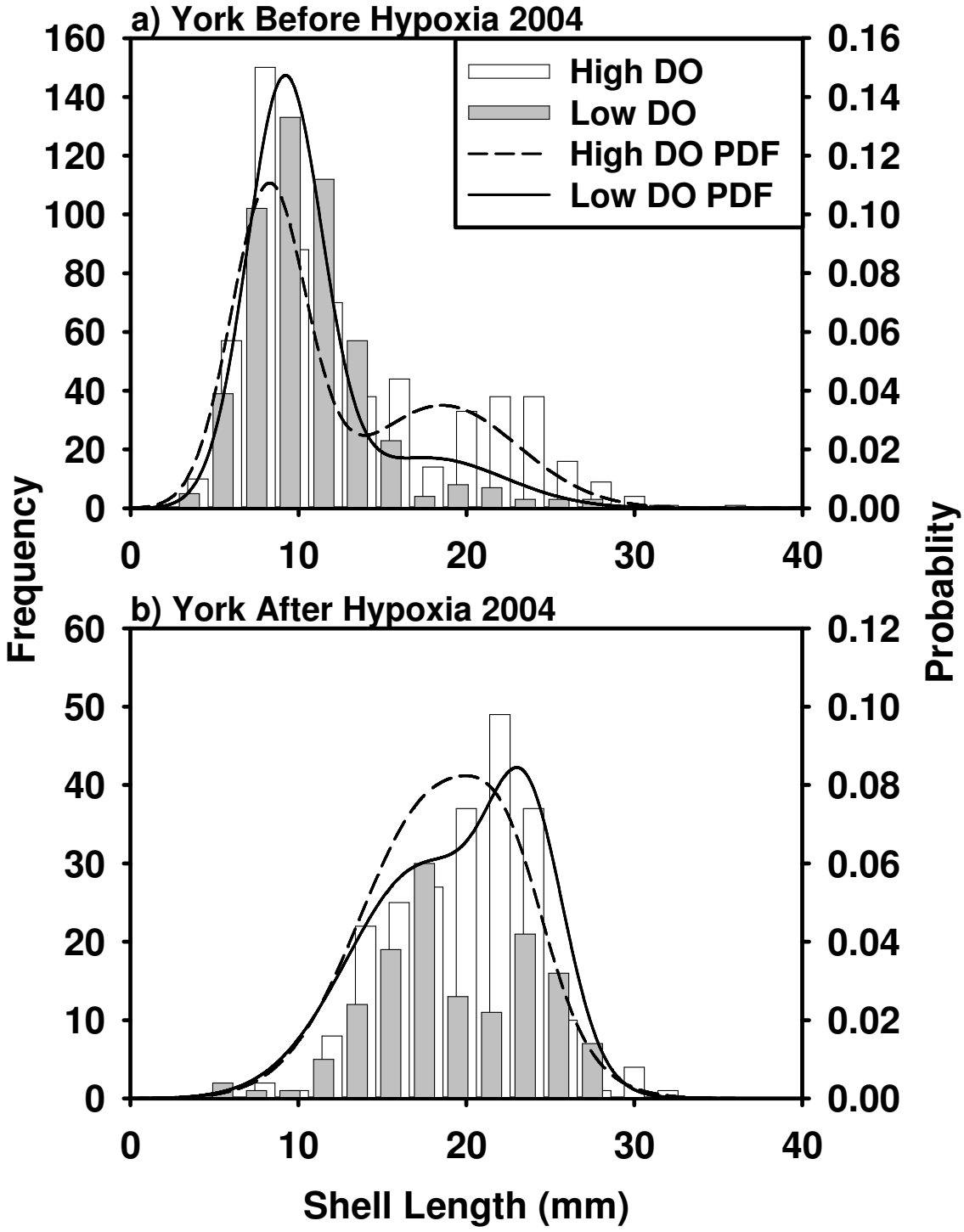

a) York Before Hypoxia 2004
High DO
Low DO
High DO PDF
Low DO PDF
160
140
120
100
80
60
40
20
0
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
10
20
30
40
b) York After Hypoxia 2004
60
50
40
30
20
10
0
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
10
20
30
40
Frequency
Probablity
Shell Length (mm)

Figure 6

Shell length frequency histogram for the M. balthica in the Rappahannock River before (a) and after (b) hypoxia in 2004. White bars represent the High DO site (DO > 3.5 mg/L) and gray bars the low DO sites (DO < 3.5 mg/L). Low DO bars are offset by -0.5 mm. Bins are 2mm in size. Lines are the predicted probability distributions functions (PDFs) estimated by maximum likelihood for high DO (dashed line) and low DO (solid line) sites.

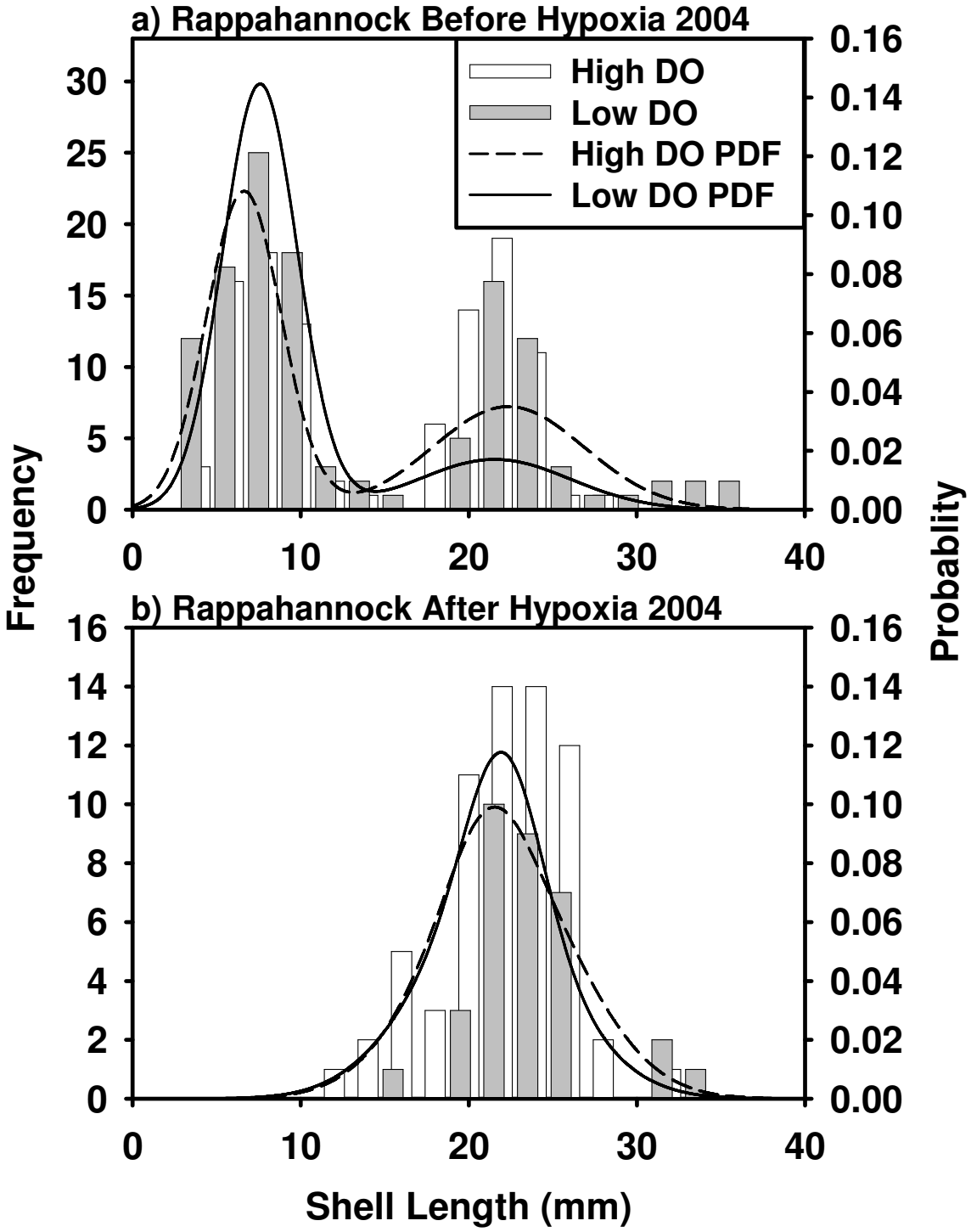

a) Rappahannock Before Hypoxia 2004
High DO
Low DO
High DO PDF
Low DO PDF
b) Rappahannock After Hypoxia 2004
Frequency
Probablity
Shell Length (mm)

Figure 7

Observed (points) and predicted (lines) relationship between shell length and AFDM for M. balthica in the York (a), and Rappahannock (b) Rivers in 2004.

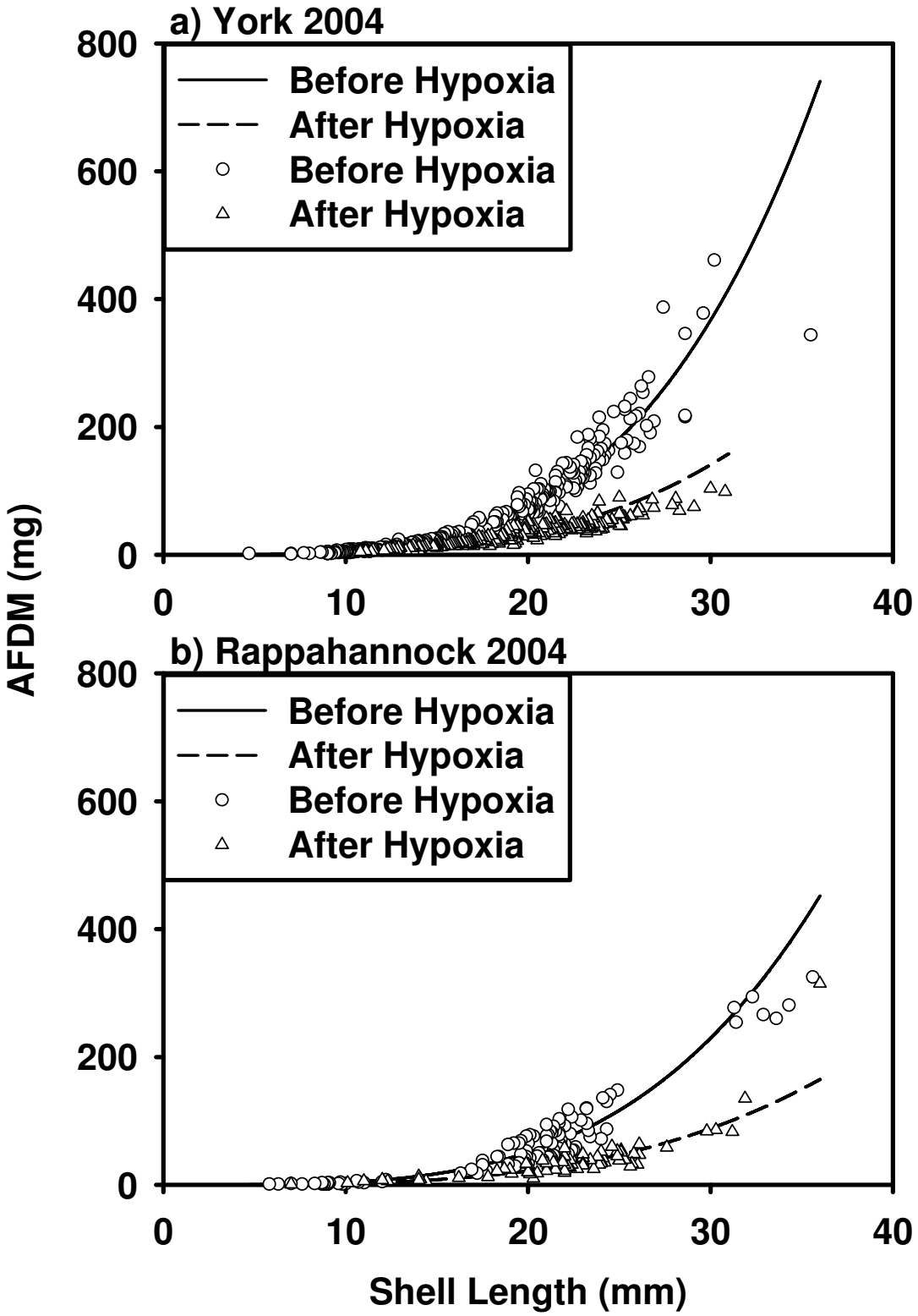
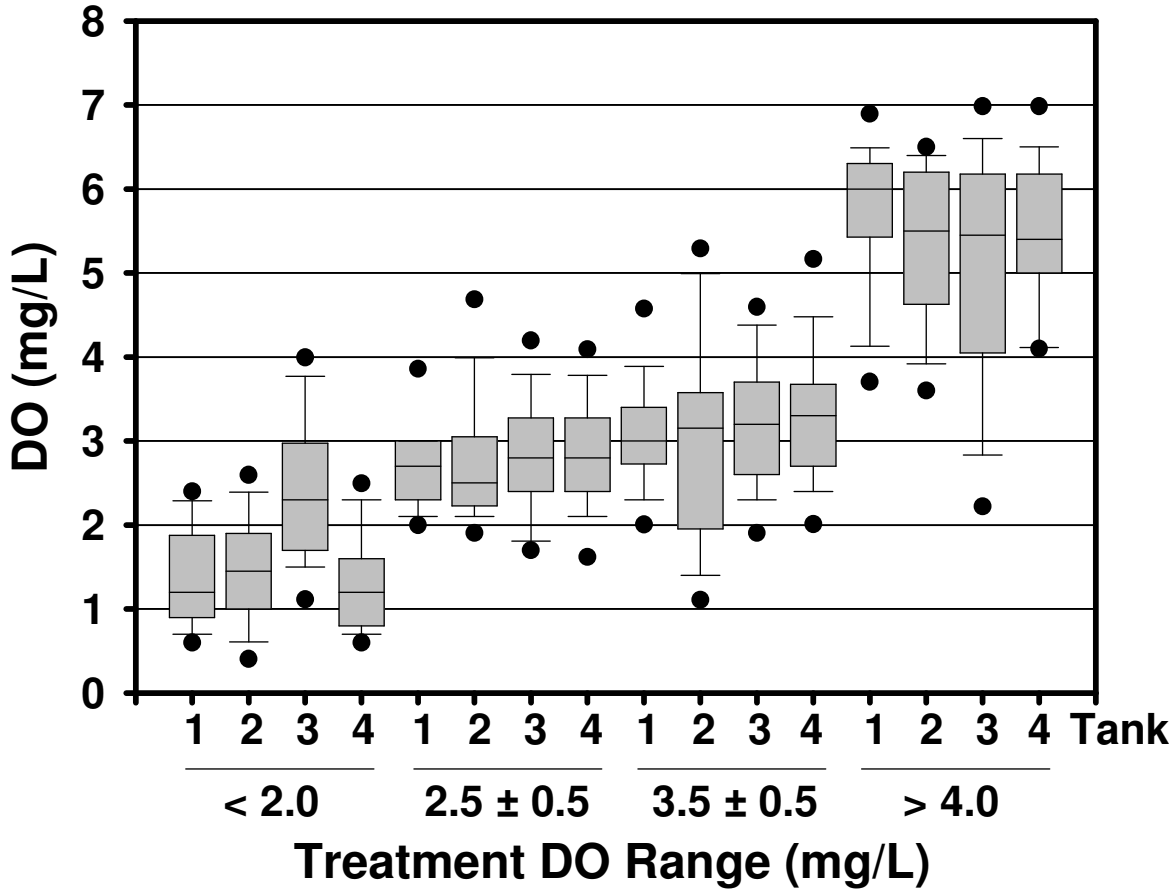
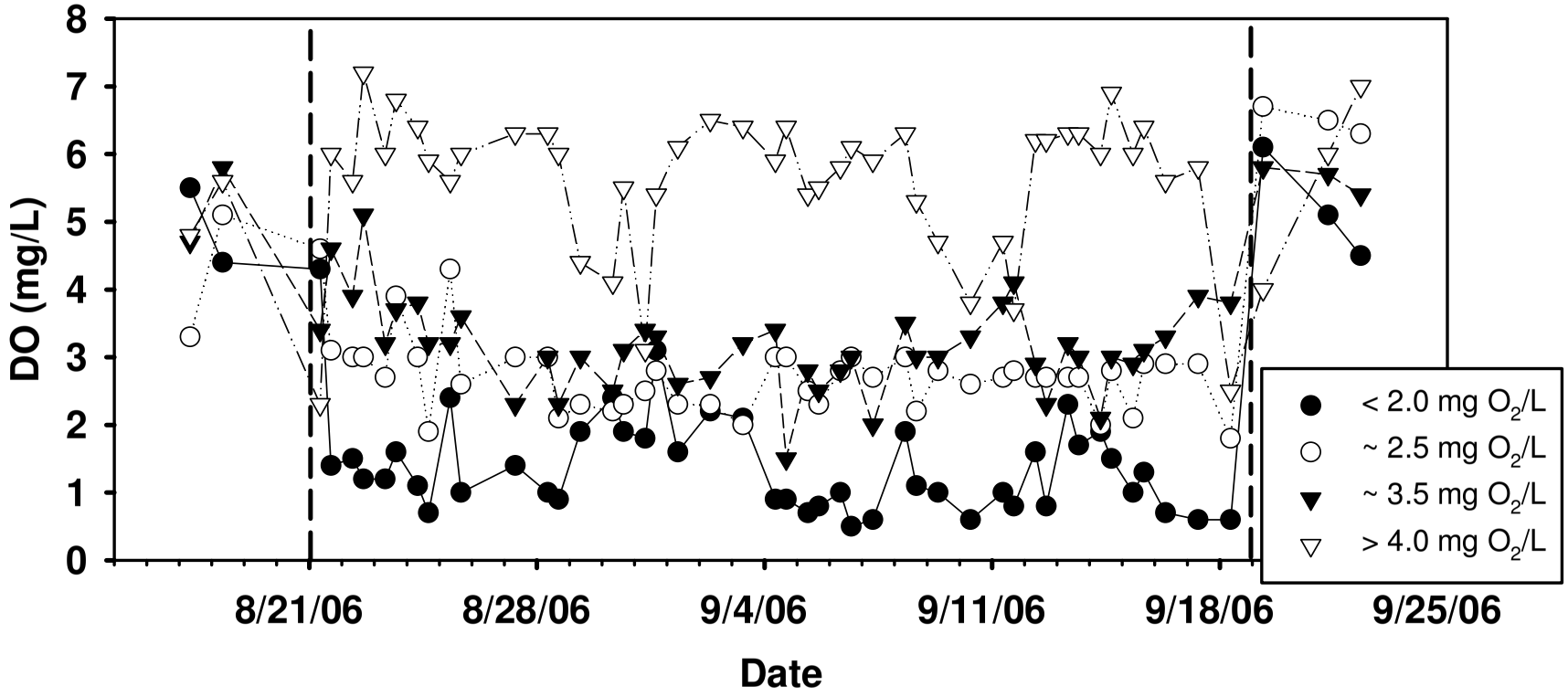

a) York 2004
Before Hypoxia
After Hypoxia
Before Hypoxia
After Hypoxia
800
600
400
200
0
0
10
20
30
40
b) Rappahannock 2004
Before Hypoxia
After Hypoxia
Before Hypoxia
After Hypoxia
800
600
400
200
0
0
10
20
30
40
AFDM (mg)
Shell Length (mm)

Figure 8



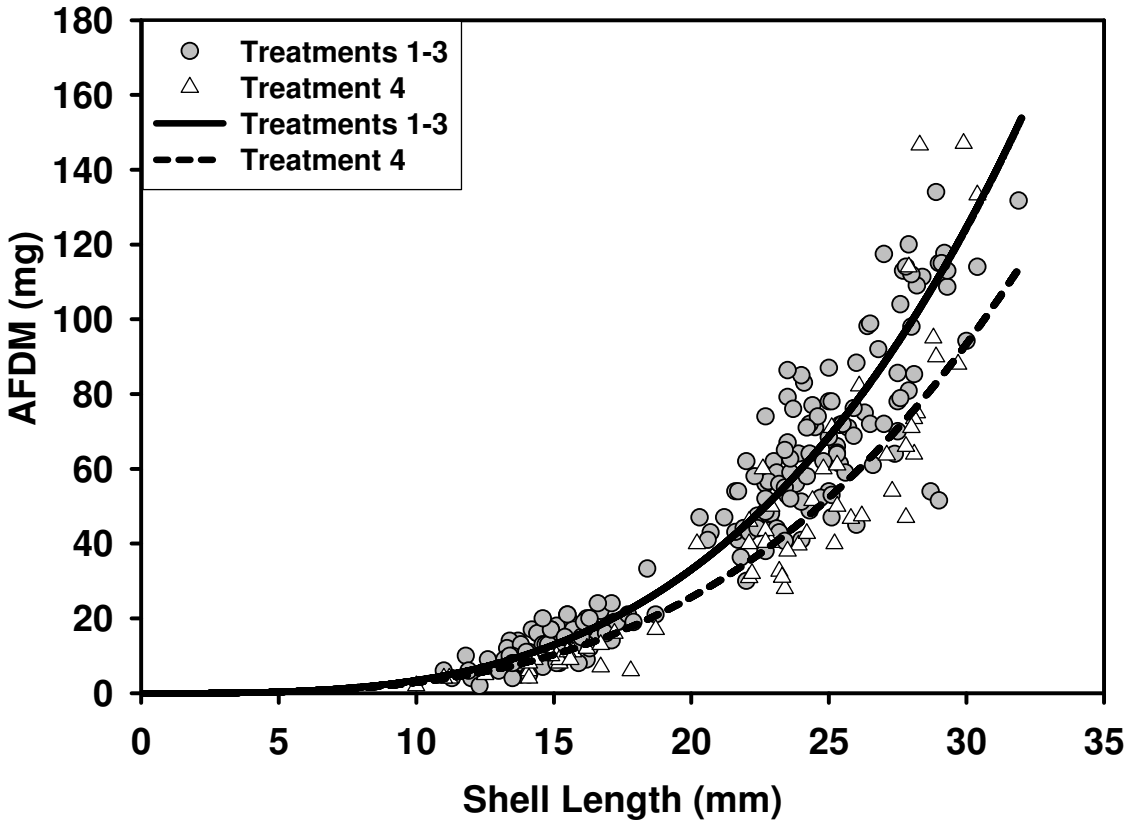
Box plots of dissolved oxygen measurement in each tank and treatment level. Horizontal lines represent the median DO, boxes the 25th and 75th percentiles, whiskers the 10th and 90th percentiles, and dots the 5th and 95th percentiles.

Figure 9



Time series of DO measurements in one representative tank of each Treatment type. Vertical dashed lines indicate dates the DO treatments began and ended.

Figure 10



Length-mass observations and modeled relationship for M. balthica from laboratory experiment. Points are observations and lines represent the maximum likelihood fits.

Figure 11

Effect of dissolved oxygen on fecundity in M. balthica. Bars with different letters above them differ at the 0.05 level (ANOVA; Tukey's test). a) Average mb-HSP70:Egg ratio for all samples from Hypoxic and Normoxic tanks + 1 SE. b) Average Total Protein:Egg ratio for all samples from Hypoxic and Normoxic tanks + 1 SE. c) Average mb-HSP70 concentrations for each treatment + 1 SE. d) Average fecundity of female clams from Hypoxic and Normoxic tanks + 1 SE.

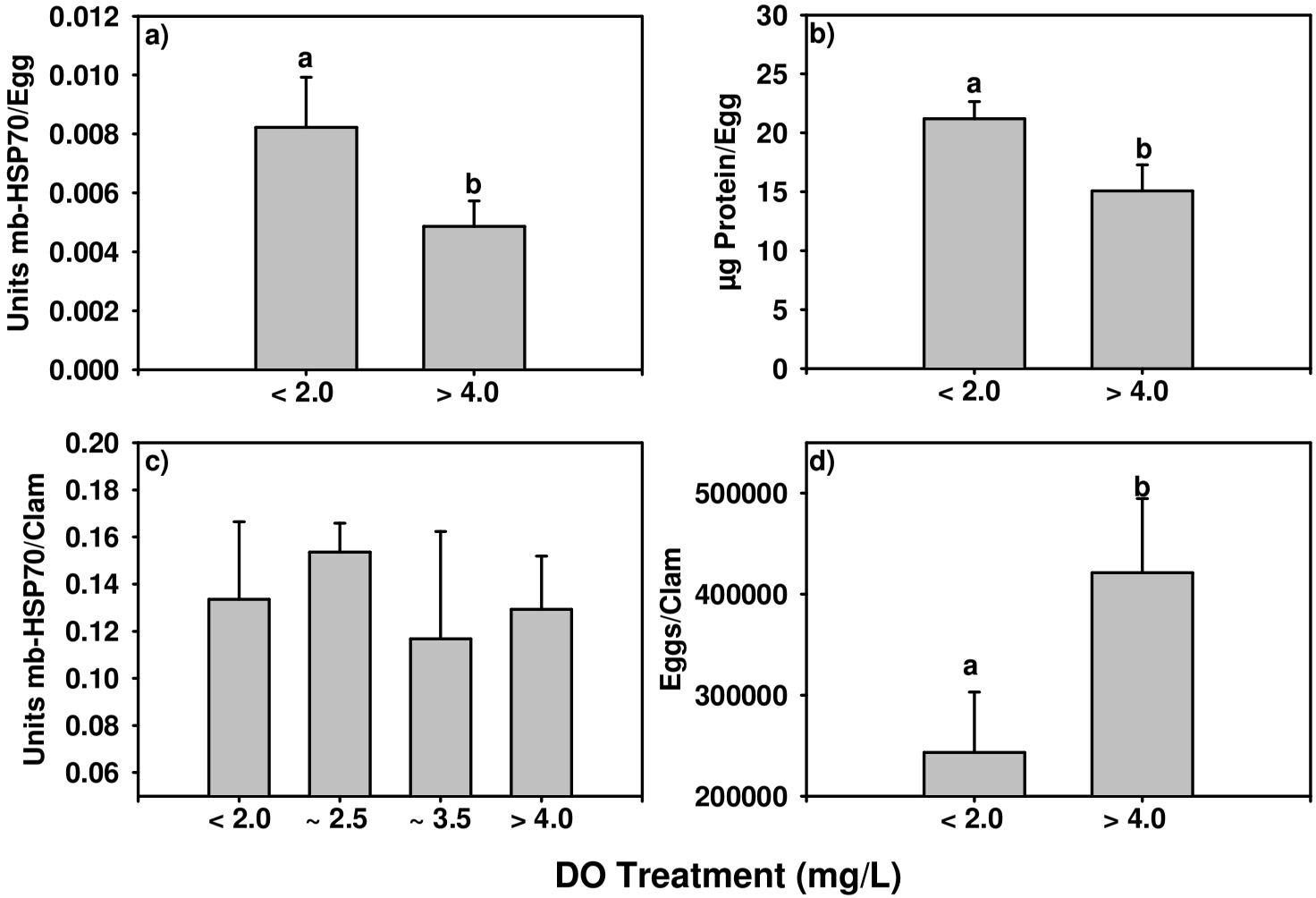

a)
Units mb-HSP70/Egg
0.012
0.010
0.008
0.006
0.004
0.002
0.000
a
b
< 2.0
> 4.0
b)
µg Protein/Egg
30
25
20
15
10
5
0
a
b
< 2.0
> 4.0
c)
Units mb-HSP70/Clam
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
< 2.0
~ 2.5
~ 3.5
> 4.0
d)
Eggs/Clam
500000
400000
300000
200000
a
b
< 2.0
> 4.0
DO Treatment (mg/L)

Figure 12

Dissolved oxygen measurements made during the (a) June and (b) August predation experiments. Black lines represent the smoothed readings from DO recorder in the deep sites. Grey triangles represent spot DO measurements taken in the shallow area. Moon diagrams indicate new (unfilled), quarter, and full (filled) moons. Arrows indicate the beginning of hypoxic observations in the deep sites. Readings below dashed horizontal line are considered hypoxic.

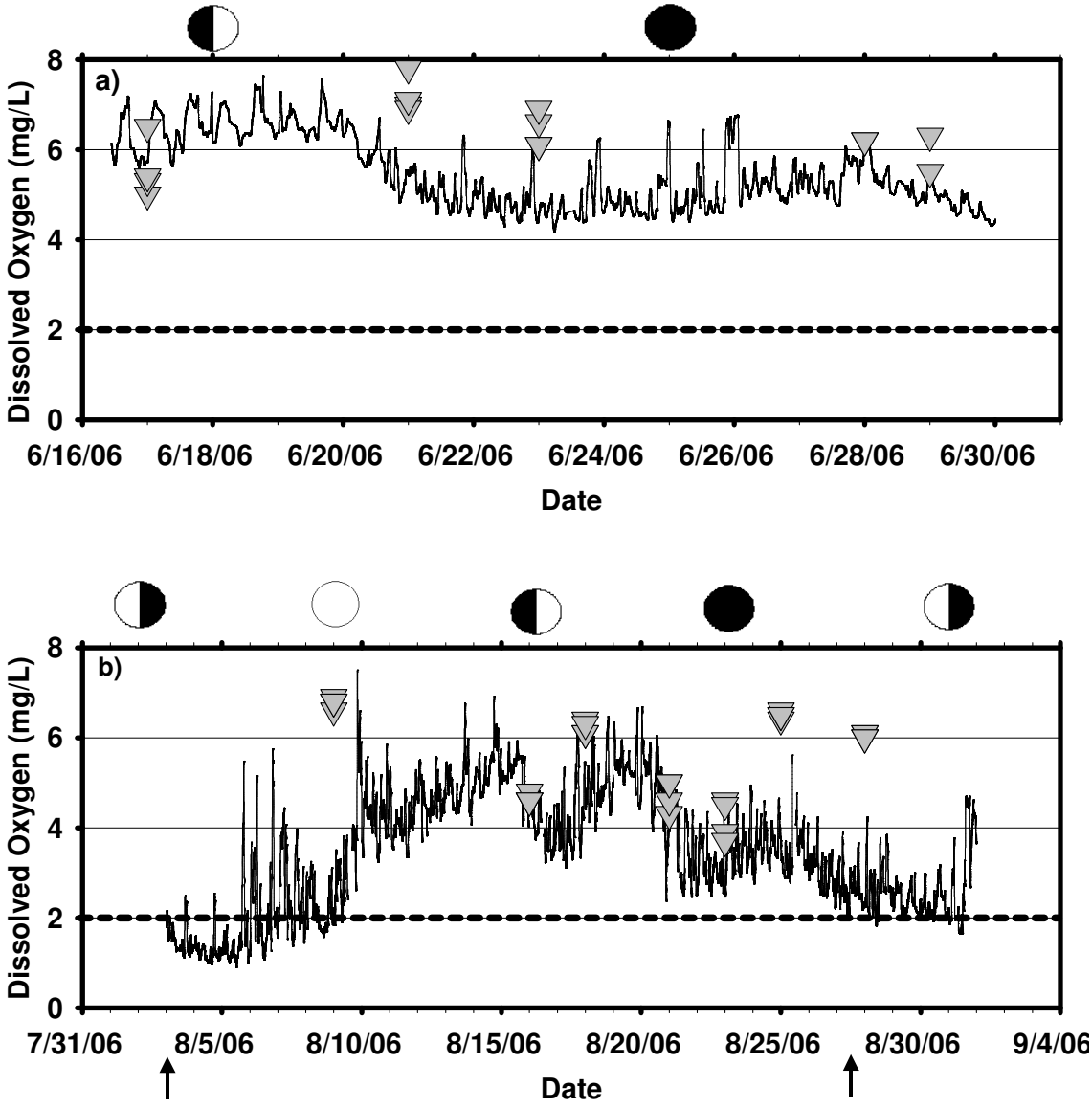
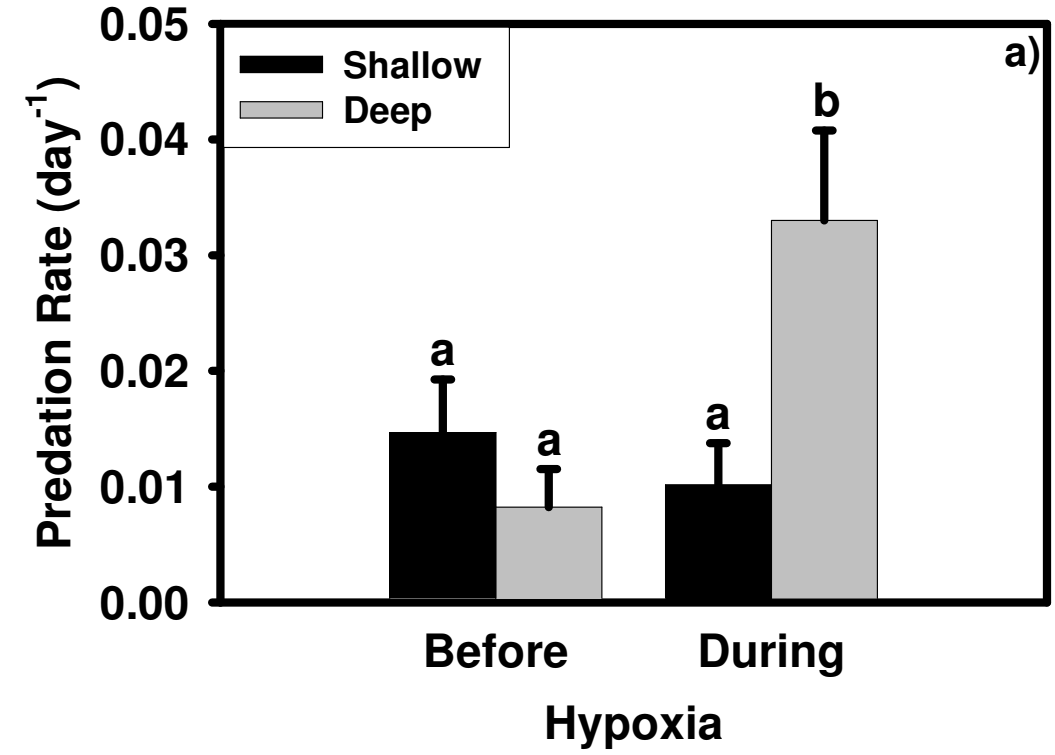

a)
Dissolved Oxygen (mg/L)
0
2
4
6
8
6/16/06
6/18/06
6/20/06
6/22/06
6/24/06
6/26/06
6/28/06
6/30/06
Date
b)
Dissolved Oxygen (mg/L)
0
2
4
6
8
7/31/06
8/5/06
8/10/06
8/15/06
8/20/06
8/25/06
8/30/06
9/4/06
Date

Figure 13

(a) Predation rates on transplanted M. balthica in the York observed in the caging experiment in both deep and shallow plots before and during hypoxia. Bars are mean + 1 SE. Bars with different letters above them differ at the 0.05 level (SNK test). (b) Trends in the rates of density-dependent daily proportional predation in the Deep and Shallow plots during hypoxia in August. Bars are mean + 1 SE.


a)
Shallow
Deep
Predation Rate (day-1)
0.05
0.04
0.03
0.02
0.01
0.00
a
a
a
b
Before
During
Hypoxia

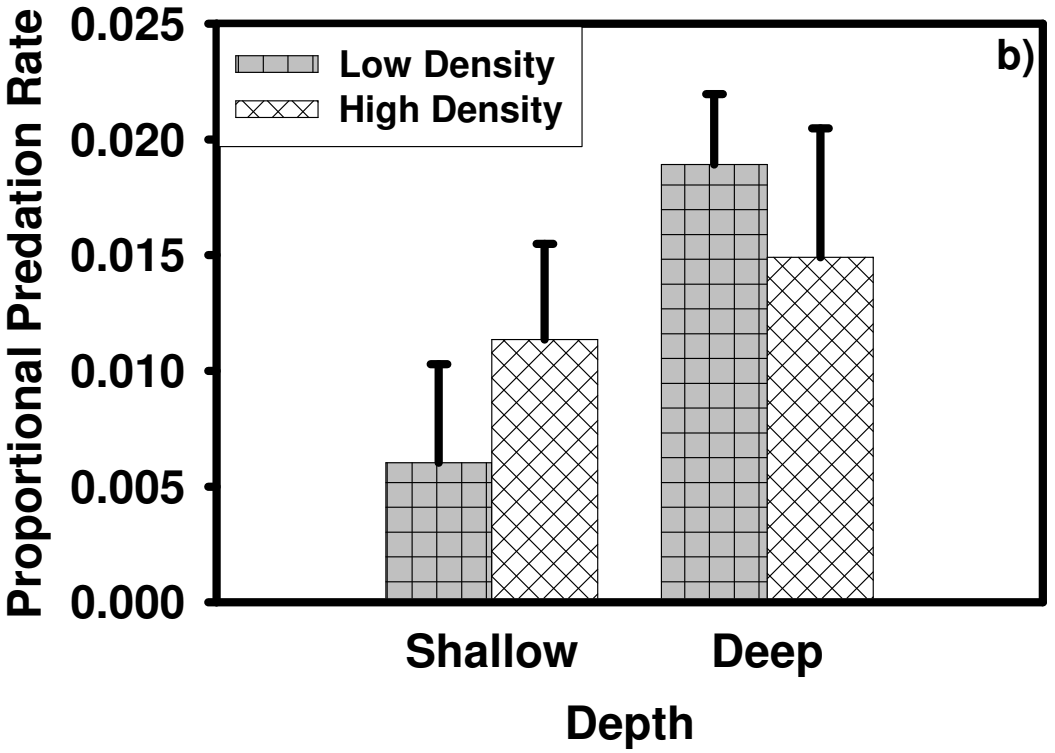
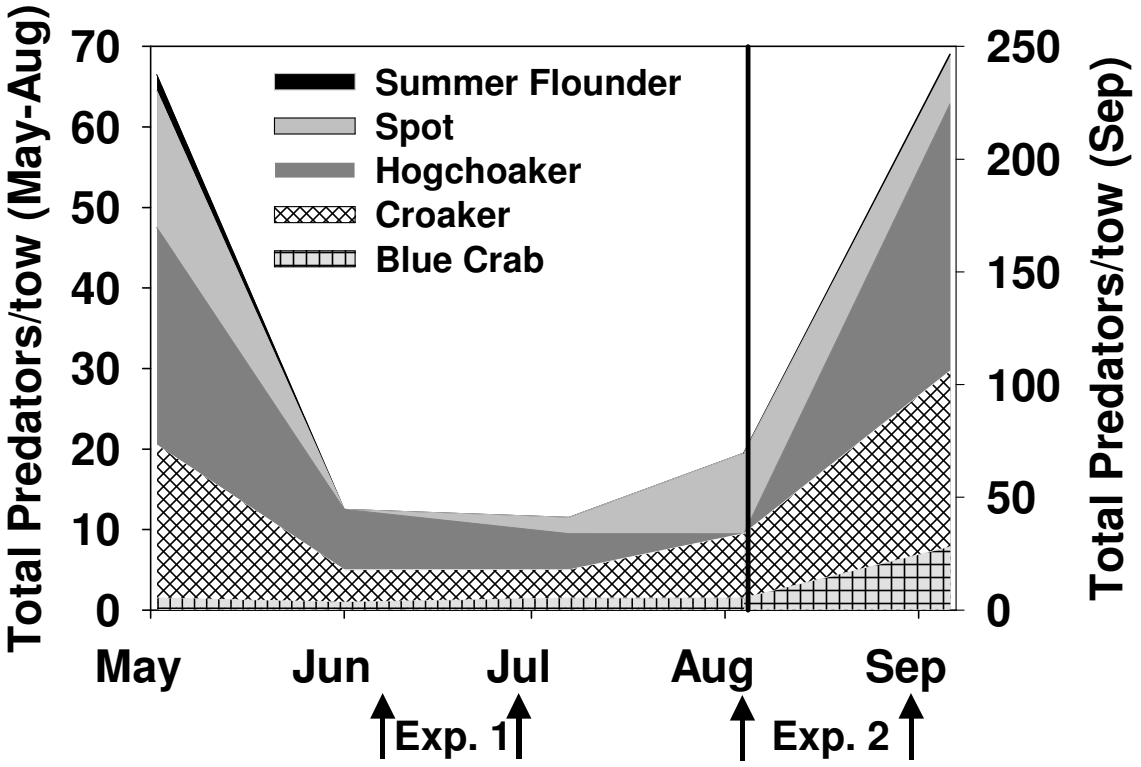

b)
Low Density
High Density
Proportional Predation Rate
0.025
0.020
0.015
0.010
0.005
0.000
Shallow
Deep
Depth

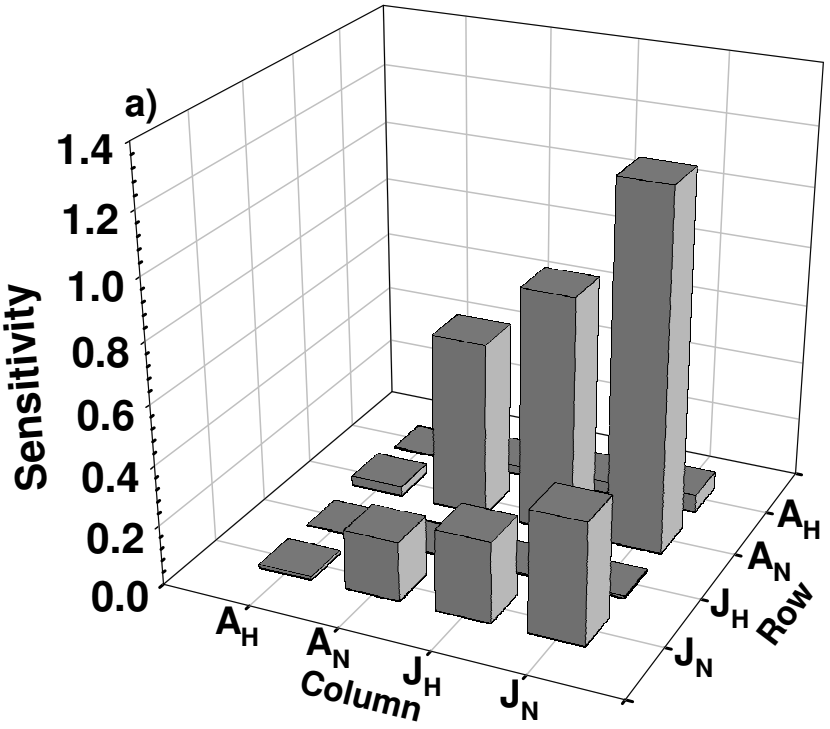
Figure 14



Total abundance of benthic predators in the York River during the summer of 2006. Points to the left of the horizontal line are plotted against the left y-axis and those to the right against the right y-axis. A change of scale was necessary to show the details of the predator guild during the middle of the summer. Arrows indicate time period during which the first and second predation experiments were performed.

Figure 15

Sensitivity (a) and elasticity (b) of λ to each element of M. balthica population projection matrix.


a)
Sensitivity
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
A_H
A_N
J_H
J_N
Column
Row

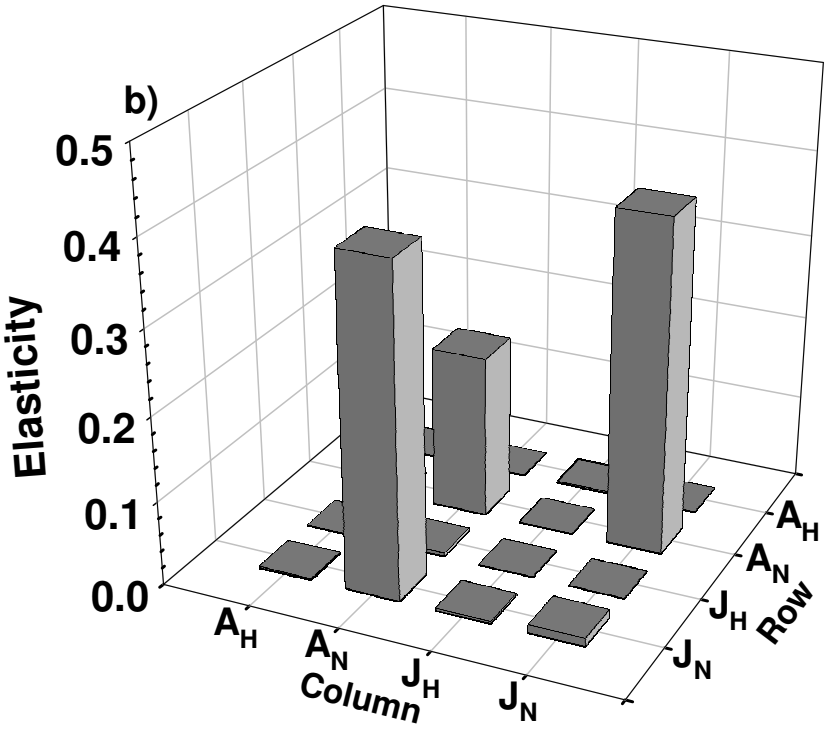
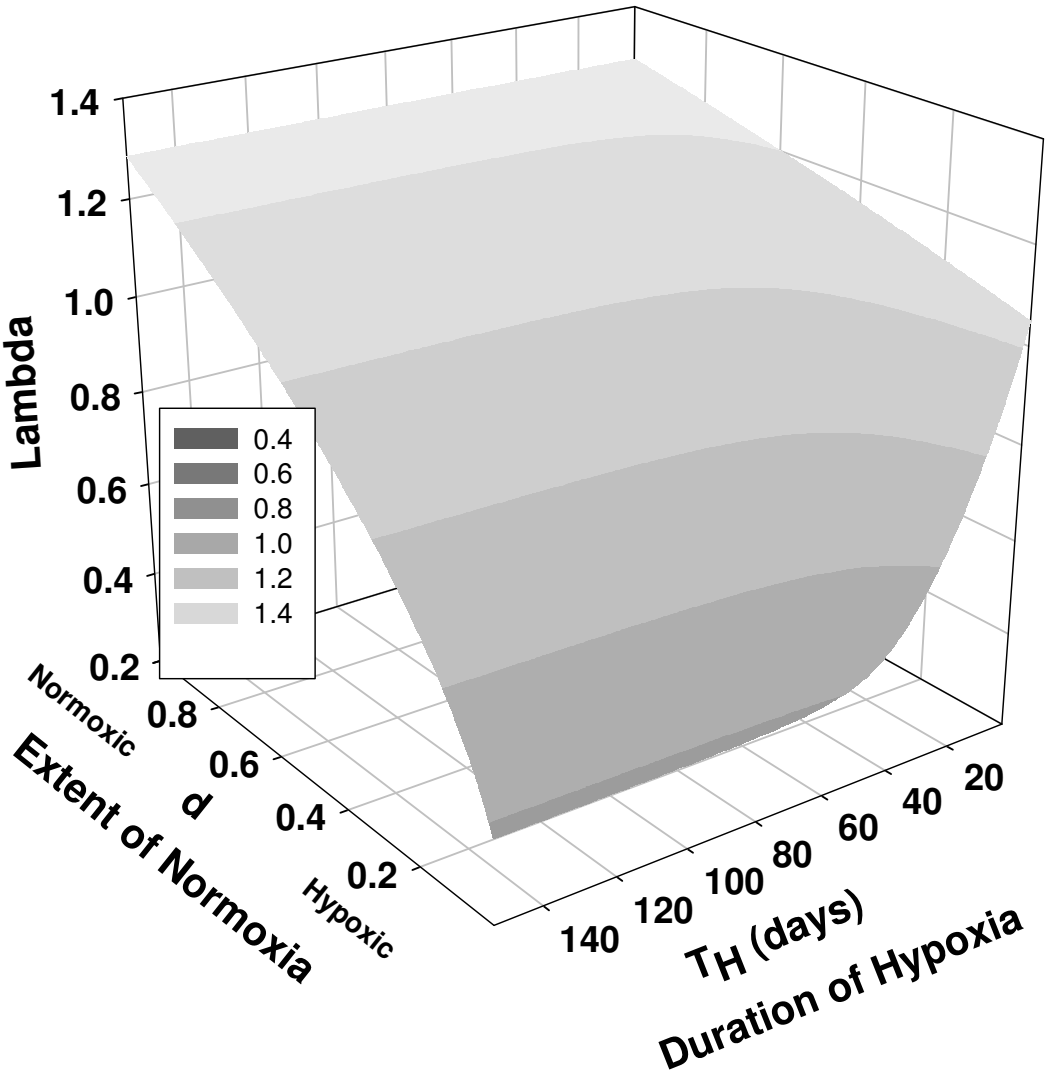
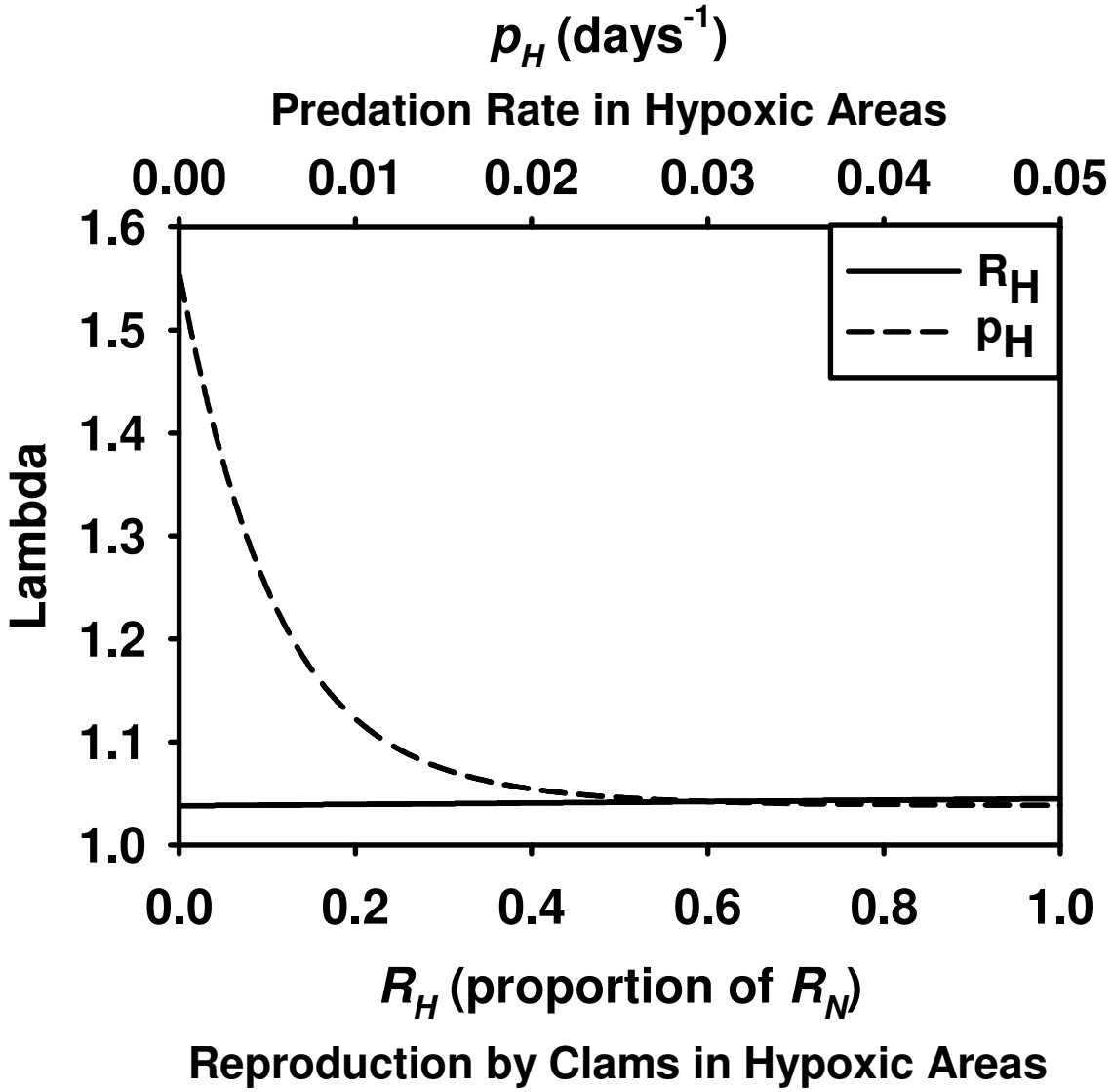

b)
Elasticity
0.5
0.4
0.3
0.2
0.1
0.0
A_H
A_N
J_H
J_N
Column
Row

Figure 16



Sensitivity of λ to the extent of normoxic water (d) and duration of hypoxia (T_H). The population is stable or increasing when $\lambda \geq 1.0$, and decreasing when $\lambda < 1.0$ (Caswell 2001).

Figure 17



Sensitivity of λ to the rate of predation mortality ($\underline{p_H}$; dashed line, upper x-axis) and reproduction by clams in hypoxic areas ($\underline{R_H}$; solid line, lower x-axis). $\underline{R_H}$ is expressed and a proportion of the reproduction of clams in normoxic areas so that when $\underline{R_H} = 1$ they reproduce at the same rate. Note that because mortality is density independent, the effect of varying p_H is identical to varying m_H.

Figure 18

Effects of the a) duration of hypoxia (T_H), b) the extent of normoxic water (d), c) reproduction of clams in normoxic areas (R_N), and d) predation rate in hypoxic areas (P_H) on the stable age structure predicted by the M. balthica population projection matrix. The population is comprised of juveniles (J) and adults (A) in normoxic (subscript N) and hypoxic (subscript H) areas.

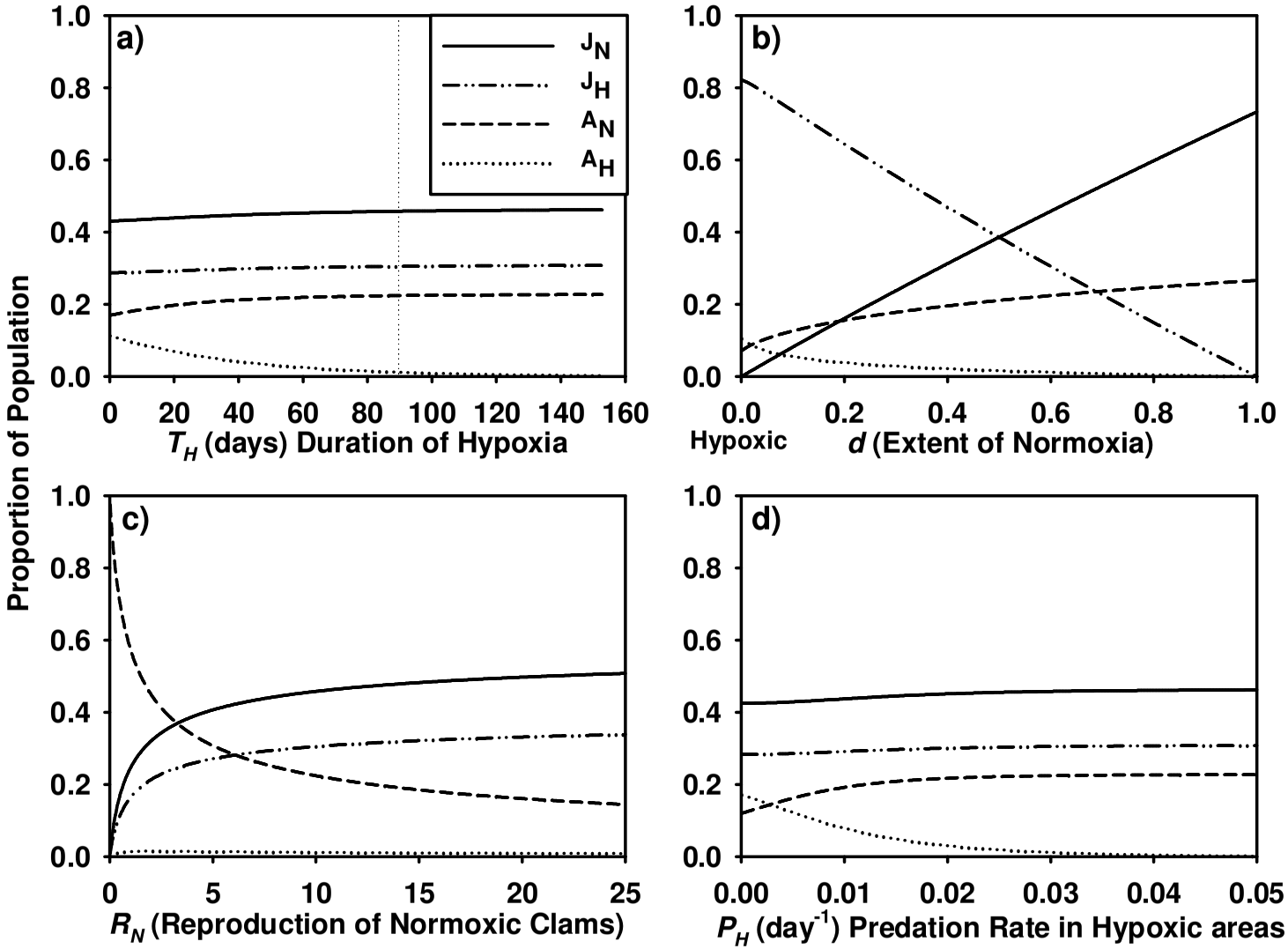

a)
b)
c)
d)
J_N
J_H
A_N
A_H
Proportion of Population
1.0
0.8
0.6
0.4
0.2
0.0
0 20 40 60 80 100 120 140 160
T_H (days) Duration of Hypoxia
0.0 0.2 0.4 0.6 0.8 1.0
Hypoxic
d (Extent of Normoxia)
0 5 10 15 20 25
R_N (Reproduction of Normoxic Clams)
0.00 0.01 0.02 0.03 0.04 0.05
P_H (day^{-1}) Predation Rate in Hypoxic areas

Figure 19

The population densities of juvenile (J) and adult (A) M. balthica in normoxic (subscript N) and hypoxic (subscript H) areas over eight years predicted by the base density-dependent model. Starting population was 170 clams/m^2 at all ages and areas. Parameters used are those calculated in this paper, or gleaned from others (see methods).

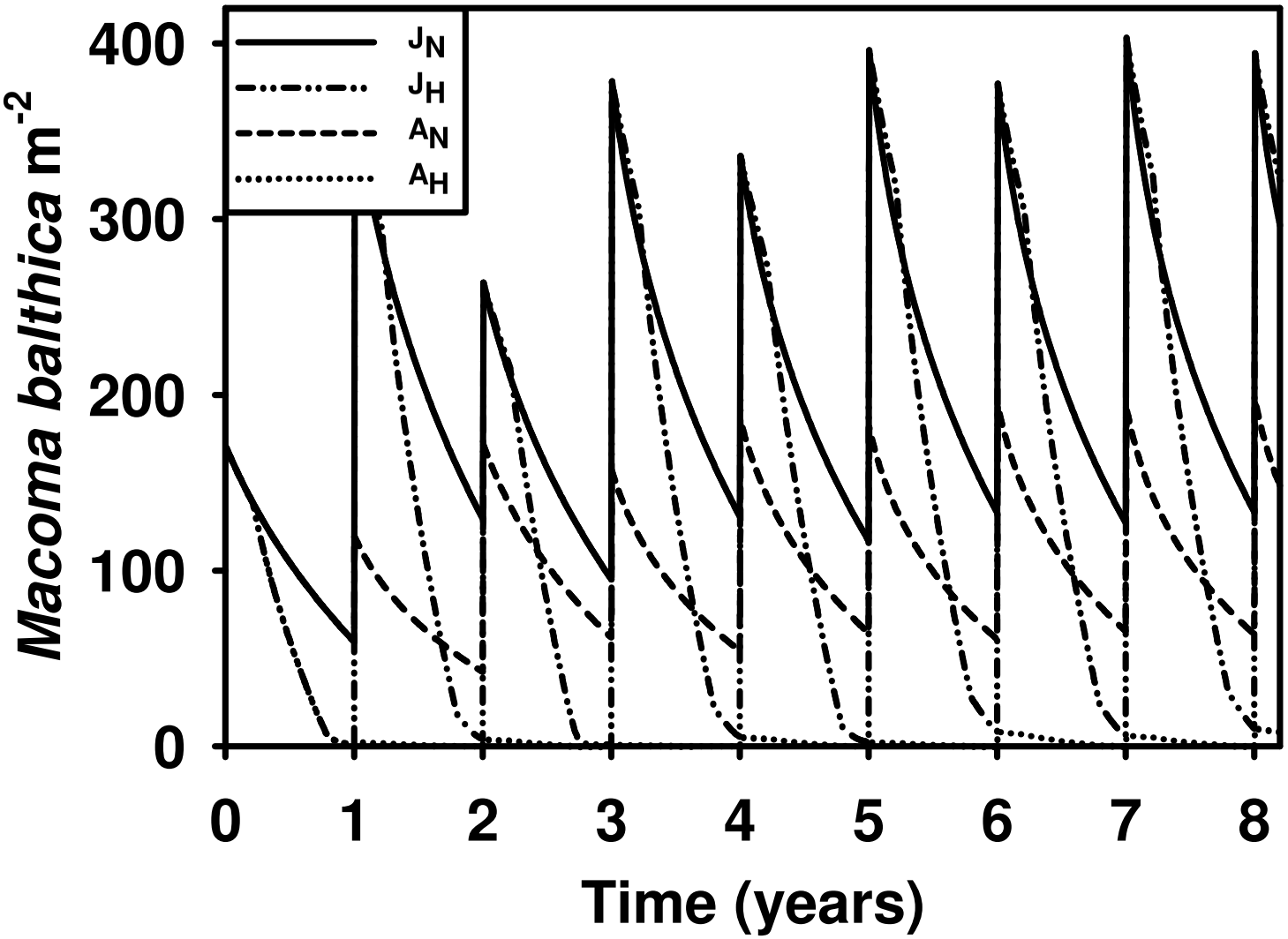

400
300
200
100
0
Macoma balthica m-2
JN
JH
AN
AH
0
1
2
3
4
5
6
7
8
Time (years)

Figure 20

The effects of a) the extent of hypoxia (1-d), b) the duration of hypoxia (T_H), and c) the intensity of hypoxia as measured by the rate of non-predatory mortality ((m_H) on the equilibrium density of M. balthica in the York, predicted by the density-dependent model. Each point represents the predicted density after 50 generations immediately after spring recruitment. Note that, because we assume recruits settle equally in all areas, the density of juveniles (J) in normoxic (subscript N) and hypoxic (subscript H) areas is the same, whereas the density of adults (A) vary between areas. Effect of the extent of hypoxia (1-d) on the Threshold density (the density of A_N at which the population will switch between the high and low equilibria, given a starting density of 170 clams/m^2 in all other ages and areas) is shown in plot (a).

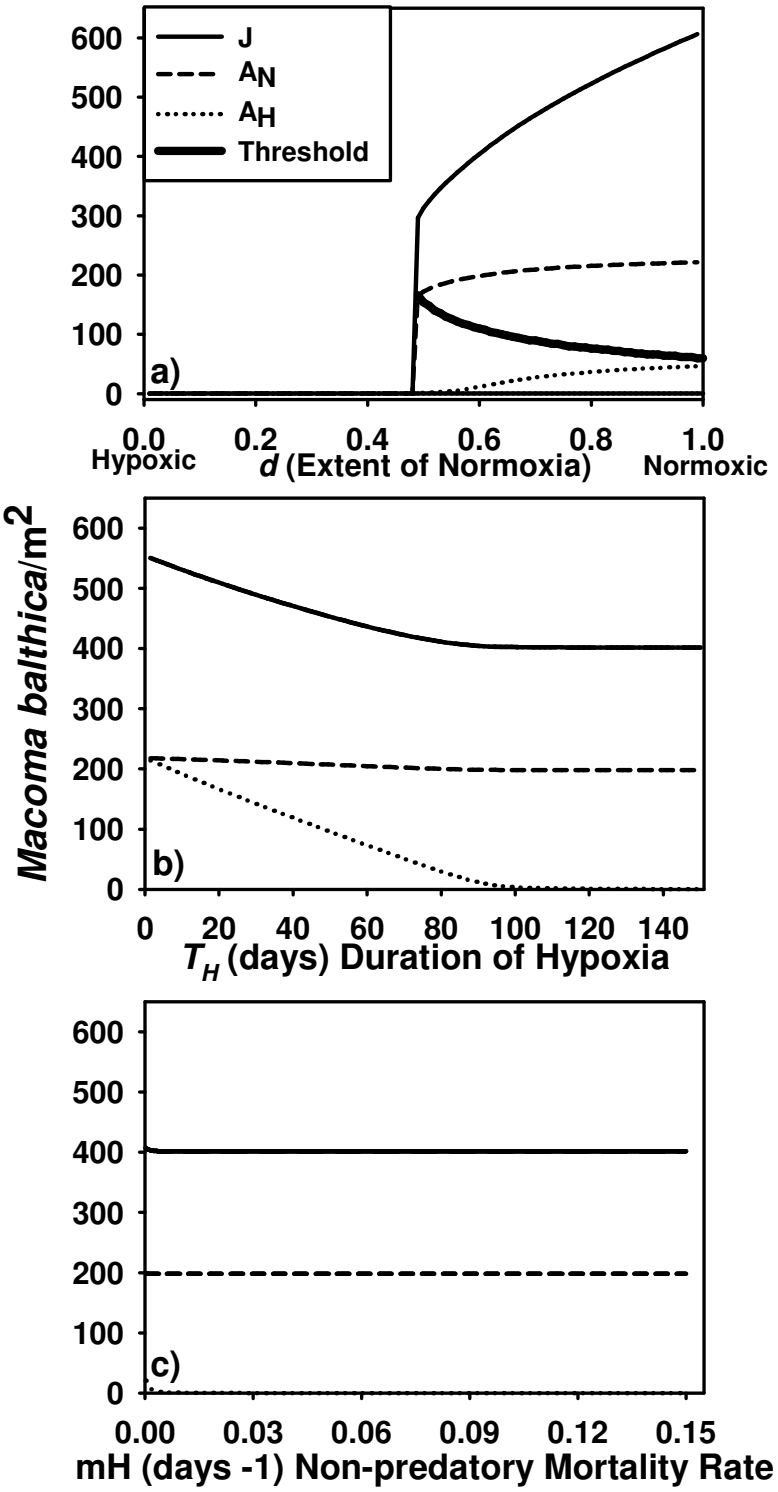
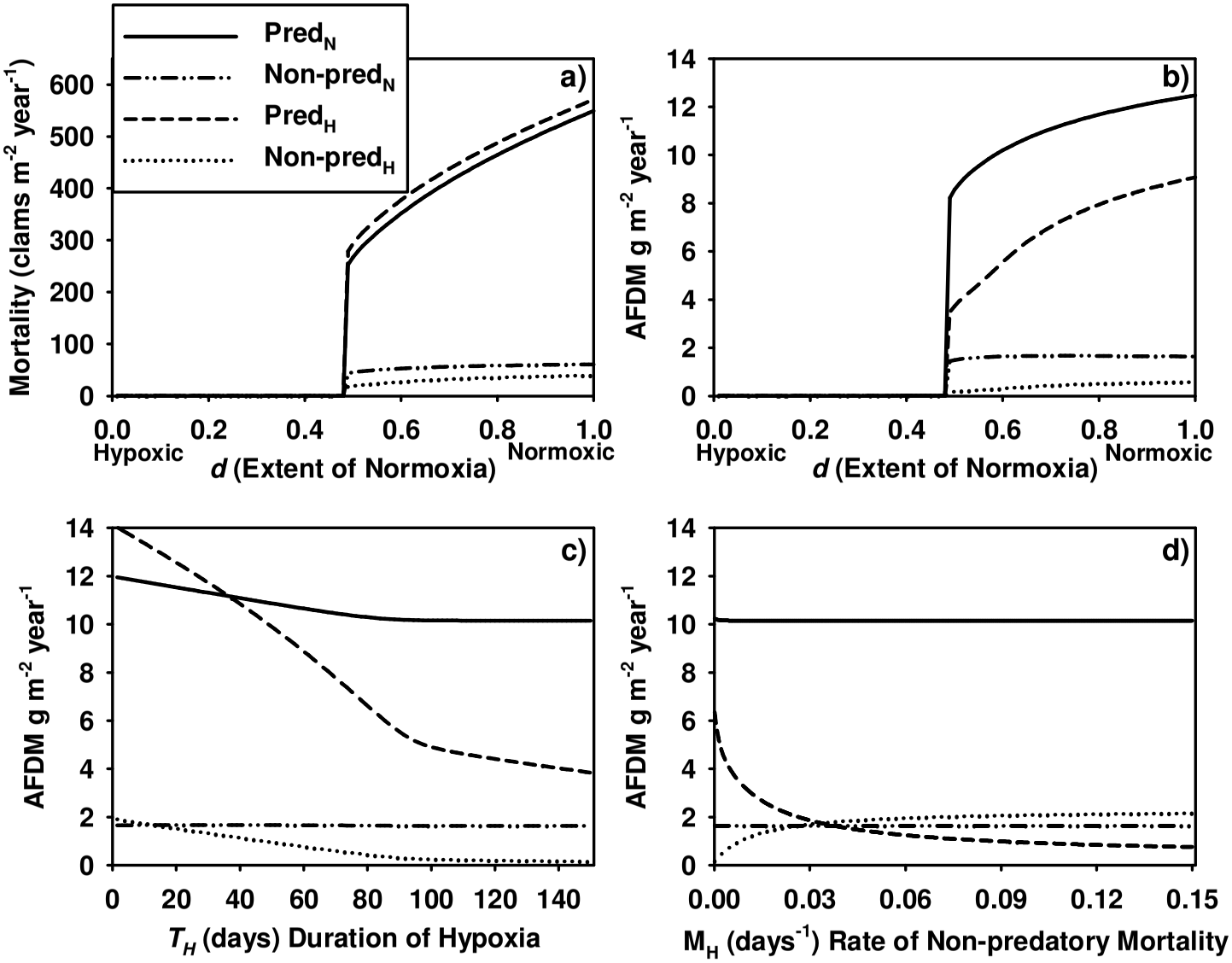

J
A_N
A_H
Threshold
a)
600
500
400
300
200
100
0
0.0
0.2
0.4
0.6
0.8
1.0
Hypoxic
d (Extent of Normoxia)
Normoxic
Macoma balthica/m2
b)
0
20
40
60
80
100
120
140
T_H (days) Duration of Hypoxia
c)
0.00
0.03
0.06
0.09
0.12
0.15
mH (days -1) Non-predatory Mortality Rate

Figure 21

The effects of (a & b) the extent of hypoxia (1-d), c) the duration of hypoxia (T_H), and d) the intensity of hypoxia as measured by the rate of non-predatory mortality (m_H) on number and biomass (N) of M. balthica in the York lost to predatory (Pred) and non-predatory (Non-pred) mortality in normoxic (subscript N) and hypoxic (subscript H) at equilibrium, as predicted by the density dependent model. Each point represents the prediction after 50 generations. The maximum value of m_H, 0.15 day^{-1}, represents the highest rate of mortality observed in laboratory experiments with M. balthica held under anoxic conditions (de Zwaan et al. 2001).


Pred$_N$
Non-pred$_N$
Pred$_H$
Non-pred$_H$
a)
Mortality (clams m^{-2} year^{-1})
0
100
200
300
400
500
600
0.0
0.2
0.4
0.6
0.8
1.0
Hypoxic
Normoxic
d (Extent of Normoxia)
b)
AFDM g m^{-2} year^{-1}
0
2
4
6
8
10
12
14
0.0
0.2
0.4
0.6
0.8
1.0
Hypoxic
Normoxic
d (Extent of Normoxia)
c)
AFDM g m^{-2} year^{-1}
0
2
4
6
8
10
12
14
0
20
40
60
80
100
120
140
T_H (days) Duration of Hypoxia
d)
AFDM g m^{-2} year^{-1}
0
2
4
6
8
10
12
14
0.00
0.03
0.06
0.09
0.12
0.15
M_H (days^{-1}) Rate of Non-predatory Mortality

VITA

William Christopher Long (on right) was born in Garrett County, Maryland. However, shortly after, his parents moved his family to live in the Kalahari Desert in Botswana, Africa. Chris graduated from Rift Valley Academy in Kenya in June of 1997. He received a BS in Biology at Wheaton College, from whence he graduated with honors in 2000. After working on recombinant protein expression and analysis at Argonne National Laboratory for a year and a half, he started at the Virginia Institute of Marine Science in the fall of 2002. He received an EPA GRO fellowship and finished his PhD program in September 2007. He begins work at the Smithsonian Environmental Research Laboratory in October 2007 as a Marine Science Network Postdoctoral Fellow. Chris enjoys teaching Sunday School at his church, making his son loop the loop in his baby carrier, writing fiction, jumping off piers with his daughter, cooking, and his wonderful wife.