ABSTRACT

Title of Document: EPIFAUNAL DISTURBANCE BY PERIODIC LOW DISSOLVED OXYGEN: NATIVE VERSUS INVASIVE SPECIES RESPONSE

Elizabeth Bromley Jewett, Doctor of Philosophy, 2005

Directed By: Professor Kenneth Sebens, Department of Biology
Dr. Anson Hines, Smithsonian Environmental Research Center

Invasive species and low dissolved oxygen (DO) threaten the biodiversity and ecosystem health of estuaries worldwide. To test the hypothesis that exposure to low DO reduces resistance of epifaunal community to invasion in the Chesapeake Bay, we conducted experiments using standardized settling panels, including 1) controlled experiments exposing epifaunal communities to low DO; 2) measurement of the short term response of motile and sessile epifauna to low DO; 3) survey of multiple sites in which community structure was correlated with low DO and other environmental variables; and 4) evaluation of the biological and structural effects of an invasive hydroid and a cryptogenic tunicate, both with high tolerance for low DO, on recruitment and development of epifauna.

Periodic hypoxia was correlated with an increased cover of the native serpulid polychaete, *Hydroides dianthus*. Cover of invasive and cryptogenic species increased with exposure to moderate low DO. Cover and incidence of bryozoans, sabellid polychaetes, and cnidarians differed among DO treatments. Nematodes, caprellids, and harpacticoid copepods vacated epifaunal communities in response to low DO.

In the multi-site survey, > 50% cover of invasive and cryptogenic species was associated with exposure to chronic low DO. Six of eight sites in the survey experienced periodic low DO (< 4 mg l^{-1}), but only one experienced chronic low DO (> 40% of days below 4 mg l^{-1} DO). Shifting cover of *Hydroides dianthus*, barnacles, and invasive species was correlated ($\rho > 50\%$) with percent of days experiencing low DO. Epifaunal heterogeneity reflected environmental differences among sites.

Species richness and diversity at local sites declined with increasing abundance of certain taxa in higher salinity, higher diversity areas. Heightened cover of *Molgula manhattensis*, *Hydroides dianthus* or barnacles led to reduced local diversity but regional species diversity was maintained through environmental heterogeneity across sites. Conversely, in lower salinity, lower diversity zone, *Cordylophora caspia*, an invasive hydroid, had a positive effect on some species. Temporal and spatial shifts in cover of dominant species and in species diversity in response to low DO disturbance and other environmental factors may facilitate persistence of less competitive native or invasive species.

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By

Elizabeth Bromley Jewett

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2005

Advisory Committee:
Professor Kenneth Sebens, Co-chair
Dr. Anson Hines, Co-chair
Professor Patrick Kangas
Professor Margaret Palmer
Professor Marjorie Reaka-Kudla
Dr. Gregory Ruiz

Dedication

I dedicate this dissertation to my husband, Stephen John Teach, and to my fabulous children, Owen Sanford Teach and Elizabeth Barris Teach, who supported me, and my work, and believed in me from the beginning. My love of marine science was cultivated at an early age by my generous, enthusiastic and inquisitive parents – Joan and Garry Jewett.

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Dissertation Introduction

Estuaries worldwide are prone to deteriorating water quality conditions because watersheds are densely populated and intensely used for residential and agricultural purposes that promote excessive discharge of nutrients, leading to eutrophication and low dissolved oxygen (DO). Estuaries are sites of industrial ports which receive billions of gallons of ballast water, carrying potentially invasive species propagules transported from other coasts, and focal points for other invasive species vectors such as the live seafood trade and aquaculture. Both invasive species and low DO threaten native ecosystem processes and biodiversity (Diaz & Rosenberg 1995, Stachowicz et al 2002a).

This dissertation analyzed the interaction between these two threats to determine whether low DO reduces resistance to invasion by disturbing competitive dominant sessile and mobile species of shallow estuarine areas of Chesapeake Bay. Other studies have linked environmental variables such as temperature and hypoxia to success of invasive species (Byers 2000, Lawson et al. 2004). Identifying stressors that make systems more vulnerable is an important process to inform predictive models for invasion ecologists. I identified and quantified changes attributable to low DO stress through experimental manipulations of epifaunal communities occurring on replicated, standardized plastic (PVC) settling panels. The findings were used to formulate hypotheses about general epifaunal patterns across sites which might be caused by low DO disturbance. Finally, I tested the effects of two species benefited by low DO to determine the indirect effects of low DO.

Low DO is a deteriorating water quality condition which affects estuarine systems worldwide (Diaz & Rosenberg 1995), most notably the Chesapeake Bay, the Baltic and Black Seas, the Gulf of Mexico, the coasts of Japan, Africa, North and South America (Nixon 1990). Coastal water quality conditions are deteriorating as a result of increased anthropogenic stress including sewage outfall, agricultural run off, shoreline development and global warming. Hypoxia and anoxia have increased substantially in spatial and temporal scale in the Chesapeake Bay in the past 50 years (Officer et al. 1984, Cooper & Brush 1993). Officer et al. (1984) estimated that about .1 billion cubic meters of water in the Chesapeake Bay experienced summer anoxia in 1950 as compared to more than 5 billion cubic meters in the 1980s. Hypoxia has probably existed in the Chesapeake Bay since the 1760s (Cooper & Brush 1993). In contrast, hypoxia in the Gulf of Mexico is a relatively new phenomenon affecting up to 20,000 km^2 since the 1970s (Rabalais et al. 1991, Turner et al. 2005).

An influx of invasive species is also a significant problem which confronts our coastal marine and estuarine environments. Given that non-native species have not co-evolved with other species in the system, the potential for disruption of ecological interactions and ecosystem processes is great. The lack of baseline historical data documenting estuarine biota before the 1800s has made it difficult to track the spread of species beyond their native habitats, but it is generally accepted that the incidence of invasion in our coastal and inland water systems has increased exponentially in the past fifty years primarily as a result of the elevated speed and level of shipping trade among and along continents (Ruiz et al. 2000). Ballast water is recognized as a primary vector of potentially invasive species. Aquaculture, live trade, and hull fouling were major

vectors historically and continue to be important mechanisms of introduction (Ruiz et al. 1997).

Chapter 1 presents results from a combined lab/field experiment which directly tested the effects of hypoxia and low DO on the epifaunal community. The experiment was conducted over two successive summer field seasons in the lower Chesapeake Bay at the Virginia Institute of Marine Science at Gloucester Point, VA. This chapter presents the first manipulative experiments testing the direct effects of periodic hypoxia on natural communities, although other researchers have explored the effects of (1) naturally occurring hypoxic episodes in the field (Holland et al. 1987, Breitburg 1990, Dauer & Ranasinghe 1992, Llanso 1992, Breitburg et al. 2003, Laine 2003) and (2) effects on particular species (Fulton 1962, Diaz & Rosenburg 1995, Sagasti et al. 2000). Hypoxia (DO < 2 mg l^{-1}) did not facilitate invasive or cryptogenic species success, but less intense disturbance by moderately low DO (2 - 4 mg l^{-1}) did.

Short term responses by motile epifauna were also quantified in the manipulative experiments that measured changes in the sessile community. Epifaunal species vacating panels were identified and counted. Chapter 2 details how mtbile (and some sessile) epifauna were affected by low DO conditions as well as potential ramifications for altered predator-prey relationships and for reduction in cover of sessile species.

Chapter 3 presents a season-long field survey that allowed results of the controlled experiments to be related to broader spatial and temporal scales of variation in low DO and other environmental stressors across sites in lower Chesapeake Bay. Dissolved oxygen was monitored at hourly to daily frequency at eight sites where local epifauna was sampled on recruitment panels. Other environmental variables were also

measured and tested for explanatory power. Low DO occurred at six out of the eight sites but was chronic (> 40% of time) at just one site. Both cover of certain dominant indicator species (barnacles and serpulid polychaetes) and relative cover of invasive/cryptogenic species were highly correlated with days exposed to low DO (measured as percent of time daily DO was recorded below 4 mg l^{-1}).

Finally, Chapter 4 examines the biological and structural effects of two epifaunal species (a cryptogenic solitary tunicate and an invasive hydroid) on recruitment and development of the epifaunal community. Both of these species have a high tolerance for low DO conditions. *Cordylophora caspia*, the invasive hydroid, experienced growth during hypoxia in Baltimore Harbor when other native species did not recruit or grow. *Molgula manhattensis*, the cryptogenic tunicate, was not adversely affected by low DO manipulations and had high cover on settling panels at the site in the survey which experienced chronic low DO. Both species caused changes in epifaunal community structure when present.

Both low DO and invasive species threaten the integrity of the world's estuaries and the positive interaction between them may worsen the impact of each individually. Focusing our research on the epifaunal community facilitated quantification of community change in response to disturbance. The approach taken in this dissertation successfully integrated (1) controlled experiments (with replication and rigorous quantification) on whole community units and component species, (2) control of the duration and intensity of disturbance by low DO, (3) sampling across environmental gradients relevant to epifaunal community structure and spatial and temporal scales of low DO disturbance and, (4) evaluation of the impact of introduced species.

Chapter 1: **Epifaunal Disturbance by Periodic Low Dissolved Oxygen: Native versus Invader Response**

Abstract

Hypoxia is increasing in marine and estuarine systems worldwide, primarily due to anthropogenic causes. Periodic hypoxia represents a pulse disturbance with the potential to restructure estuarine biotic communities. We chose the shallow, epifaunal community in the lower Chesapeake Bay to test the hypothesis that low dissolved oxygen (DO) (< 4 mg l^{-1}) affects community dynamics by reducing the cover of spatial dominants, creating space both for less dominant native species and for invasive species. Settling panels were deployed at shallow depths in spring 2000 and 2001 at Gloucester Pt, Virginia and were manipulated biweekly from late June to mid August. Manipulation involved exposing epifaunal communities to varying levels of DO for up to 24 hrs followed by redeployment in the York River. Exposure to low DO affected both species composition (presence or absence) and the abundance of the organisms present. Community dominance shifted away from barnacles as level of hypoxia increased. Barnacles were important spatial dominants which reduced species diversity when locally abundant. The cover of *Hydroides dianthus*, a native serpulid polychaete, doubled when exposed to periodic hypoxia. Increased *H. dianthus* cover may indicate whether a local region has experienced periodic, local DO depletion and thus provide an indicator of poor water quality conditions. In 2001, the combined cover of the invasive and cryptogenic species in this community, *Botryllus schlosseri* (tunicate), *Molgula manhattensis* (tunicate), *Ficopomatus enigmaticus* (polychaete) and *Diadumene lineata* (anemone), was highest

on the plates exposed to moderately low DO (2 mg l^{-1} < DO < 4 mg l^{-1}). All four of these species are now found worldwide and exhibit life histories well adapted for establishment in foreign habitats. Low DO events may enhance success of invasive species, which further stress marine and estuarine ecosystems.

Introduction

Hypoxia in marine and estuarine environments is increasing worldwide as a result of high nutrient run-off from agriculture and urban development (Diaz and Rosenburg 1995). Fifty percent of U.S. estuaries now experience some hypoxia each year (Diaz 2001). Eutrophication, which fuels hypoxia in many systems, is a growing problem in the Baltic, Black and Mediterranean Seas, and along the coastlines of North and South America, Africa, India, Southeast Asia, Australia, China, and Japan (Nixon 1990, Richardson & Jorgensen 1996). Water column stratification by salinity and temperature coupled with weak tidal mixing, a deep central channel and eutrophication make Chesapeake Bay particularly vulnerable to hypoxic and anoxic conditions in the summer (Officer et al. 1984, Cooper & Brush 1991, Harding et al. 1992). However, periodic low oxygen in shallow water habitats may occur when: (1) hypoxic bottom waters move on shore during wind events, (2) as a result of a strong thermocline in near shore waters (Breitburg 1990, Sanford et al. 1990), or (3) due to organic enrichment (Pearson & Rosenberg 1978, Powilleit & Kube 1999, Gray et al. 2002). Shallow embayments with restricted water circulation due to man-made structures are particularly vulnerable to temperature fluctuations that can lead to depleted DO at or near the surface (Breitburg 1990).

The spatial and temporal scale of hypoxia in Chesapeake Bay is well-studied. Large proportions of bottom water in the mainstem and tributaries becomes hypoxic every summer for periods lasting hours to months (Sanford et al. 1990). During summer, 62% of the subpycnocline water volume in the main stem is < 5 mg l^{-1} DO and about 19% is < 2 mg l^{-1} DO (Decker et al. 2004). However, less is understood about the extent to which low DO (< 4 mg l^{-1} DO) occurs in nearshore, shallow water. Breitburg (1990) recorded DO below 4 mg l^{-1} at 4 m depth on 83% of days at a western shore site in 1988. How long these shallow low DO episodes persist or the extent of the geographic area affected is not well understood.

Multiple studies in the Chesapeake Bay, the Baltic Sea and along the coast of Japan have indicated a relationship between low DO and the condition of the benthos (Holland et al.1987, Llanso 1992, Diaz & Rosenberg 1995, Lim & Park 1998, Dauer et al. 2000, Suzuki 2001, Karlson et al. 2002). Dauer et al (2000) found that 42% of the variation in the bottom benthos was explained by relative frequency of low DO events. Few experiments, though, have tried to tease out which changes were directly attributable to low DO versus other correlated physical properties. In addition, more studies have focused on the effects of hypoxia on recruitment (Breitburg 1992, Sagasti et al. 2000, 2003) and on single species tolerance (Sagasti et al. 2001, Gray et al. 2002) without considering the effects on the entire community (Llanso 1992). The question of how single species tolerances scale-up to community change is less clear.

The manner in which episodic disturbance by low DO events structures the estuarine epifaunal community of Chesapeake Bay is complex. Low DO disturbance has

the ability to open up space through killing or stressing of the resident fauna and to change community processes such as predation (Breitburg et al.1999, Brante & Hughes 2001) and competition (Johnston & Keough 2003). A species is benefited if it has a higher tolerance for the disturbance (Schiedek 1997, Byers 2000) or is an aggressive colonizer of open space (Holland et al. 1987, Stachowicz et al. 1999, 2002a). Recruitment in Chesapeake Bay does not seem to be adversely affected by low DO (Huntington & Miller 1988, Mann & Rainer 1988, Sagasti et al. 2000) although some organisms, such as the solitary ascidian, *Molgula manhattensis*, will delay reproduction until after hypoxia has dissipated (Sagasti et al. 2003). Finally, feeding and growth may cease during periods of low DO stress which in turn may alter the relative abundance of species present (Breitburg 1992, Diaz & Rosenburg 1995, Sagasti et al. 2001). In theory, environmental stress can affect assembly dynamics (Belyea & Lancaster 1999) and general ecological processes (Menge & Sutherland 1987).

By causing changes in epifaunal community dynamics, low DO pulse events (24 hrs or less) may enhance success of invasive species**.** Fox and Fox (1986) proposed the idea that invasive species may respond according to an environmental gradient, where invaders occur more frequently in areas of higher stress due to either greater physiological capacity (such as anaerobic respiration, Schiedek 1997) or reduced competition with native species (Brown et al. 2000). Of course, the timing of recruitment with respect to a low DO event (Llanso 1992) is critical for creating an opportunity for invasion. Disturbance may reduce local, native species diversity by eliminating those species that cannot withstand low DO but favor those invasive species with life history

characteristics adapted to the disturbed conditions (Petraitis et al.1989). In this regard, tolerance to low DO may present an advantage for organisms that have low competitive abilities or low predator avoidance capacity (Marcus 2001). Lower diversity communities may also be less resistant to invasion (Law & Morton 1996, Stachowicz et al. 1999, 2002a). On the other hand, low DO pulse events might increase species diversity (Sousa 1979, 1984) through compensatory mortality of competitive dominants (Paine 1966, Connell 1978). By allowing more species to co-exist, the disturbance may create an opportunity for invaders.

Few researchers have explored the relationship between a specific physical variable and the incidence of invasion**.** An exotic snail in California has greater tolerance for hypoxic conditions than the native snail in the same area (Byers 2000). Stachowicz et al. (2002b) discovered a correlation between the percent cover of the invasive tunicate, *Diplosoma listerianum*, and winter temperatures in Long Island Sound. The invasion of a bloom forming dinoflagellate in the Baltic Sea has been associated with increased nitrogen loadings (Pertola et al. 2005). The success of the ctenophore, *Mnemiopsis leidyi*, in the Black Sea may be related to hypoxia because *M. leidyi* has a high tolerance for low DO (Purcell et al. 2001, Breitburg et al. 2003). Eutrophication and higher global temperatures and precipitation are correlated increasingly with invasion success in terrestrial systems (Dukes & Mooney 1999).

To test the effects on community structure and susceptibility to invasion, we conducted experiments manipulating exposure to disturbance by low DO on fouling communities of lower Chesapeake Bay. We conducted experiments over two years with

varying levels and duration of low DO and measured change in species composition and abundance. The epifaunal community served as a good model because of the clear spatial limitation of species abundance and dominance. Unlike hypoxia field studies that evaluated benthic responses to natural low DO events in Chesapeake Bay (Holland et al. 1987, Dauer & Alden 1995, Dauer et al. 2000), our study included both field and lab components to isolate the effects of low DO from other correlated variables such as flow or temperature.

Material and Methods

Study system: epifaunal community dynamics

Since these experiments were conducted over two spring/summer seasons, it is important to place the experimental results in the context of the seasonal sequence of recruitment patterns**.** The shallow water, fouling community of lower Chesapeake Bay is comprised of a broad range of taxonomic groups, including mussels, nudibranchs, barnacles, hydroids, tunicates, amphipods, bryozoans, flatworms, tube worms and errant polychaetes. Barnacles, hydroids and *Botryllus schlosseri* (colonial tunicate) dominate the spring season recruitment, which is followed by *Molgula manhattensis* and spionids in early summer (Otsuka & Dauer 1982), sabellids and serpulids in mid-summer (Dean & Hurd 1980, Otsuka & Dauer 1982), and another round of tunicates and hydroids in the fall (Dean 1981). Mussels, clams and oysters recruit in the fall and winter (Dean & Hurd 1980). In the York River, encrusting byrozoans had highest recruitment in late July and

the anemone, *Diadumene leucolena*, had highest recruitment in late August (Sagasti et al. 2000). How these recruitment periods coincide with experimental DO manipulations helps to explain the final results.

Study system: native v. invader status

Botryllus schlosseri, Ficopomatus enigmaticus and *Diadumene lineata* Verrill (= *Haliplanella luciae*) are recognized non-native (or invasive) sessile species in Chesapeake Bay and *Molgula manhattensis* is considered cryptogenic (http://invasions.si.edu/nemesis). Non-native implies historical evidence demonstrates its introduction to a system, either as a result of range expansion or human mediated vectors. Cryptogenic is applied to a species if, after rigorous examination of the evidence, debate about origin still exists (Carlton 1996). *B. schlosseri* (colonial tunicate) was first described in North American Atlantic waters (in Massachusetts Bay) by Couthouy in 1838 (Couthouy 1838). Greater genetic variability in the *B. schlosseri* populations in Mediterranean suggests that it may have originated there (Rinkevich et al. 1992, 2001, Ben Schlomo et al. 2001, Stoner et al. 2002). *B. schlosseri* also settles preferentially on manmade structures in the North West Atlantic, which suggests an introduced status (Stoner et al. 2002; P. Fofonoff pers. comm.; G. Lambert pers. comm.). *F. enigmaticus* (serpulid polychaete) was first documented in Chesapeake Bay in 1994, but was first described in California in 1921 in Lake Merritt, a lagoon off San Francisco Bay (Fauvel 1923, Carlton 1979a,b). Its likely place of origin is Australia (Allen 1953). In the Mediterranean Sea, *F. enigmaticus* has the capacity to build calcareous reefs up to 3 m

thick (Fornos et al. 1997, Marzano et al. 2003) although bioconstructions of this magnitude are not known in the Chesapeake Bay. *F. enigmaticus* did not recruit to our experimental panels in 2000. *Diadumene lineata* (anemone) was first collected at Cape Charles, VA in 1929 (Richards 1931) having been first described on the Atlantic coast in Long Island Sound in 1892 (Verrill 1898). Debate of geographic origin surrounds *M. manhattensis* (solitary tunicate). Its continuous distribution from Maine to Texas and its colonization of both man-made and natural habitats would suggest a North West Atlantic origin yet it may be conspecific with three described British species (Berrill 1950) which would suggest a North East Atlantic origin.

Although *Hydroides dianthus* (serpulid) is a confirmed native in Chesapeake Bay, it is a recognized opportunist that has invaded other coastal areas around the world. *H. dianthus* has been exported to sites in Western Europe including the British Isles and the Mediterranean Sea where it has been abundant in harbors and lagoons since 1888 (Zibrowius 1991). *H. dianthus* does not inhabit "natural" habitats in the Mediterranean, which is suggestive of its foreign origins (Zibrowius 1991).

Experimental design

To provide standardized surfaces for fouling community analysis, settling plates were deployed in two experiments in the polyhaline zone of lower Chesapeake Bay in the York River subestuary during spring and summer 2000 and 2001. The 14 x 14 x 0.25 cm dark grey plastic (PVC) settling plates were sanded to enhance larval settlement, attached to the horizontal bottom surface of a brick, and suspended from a dock approximately 1

m below mean low water (MLW), a position and orientation that maximizes species richness (Sagasti et al. 2001). Panels were deployed at least 2 m apart. The horizontal, down facing position was chosen to limit sediment load and plant growth on the experimental surface. Following deployment in late April, recruitment proceeded undisturbed until late June when the plates were first manipulated. The timing for experimental manipulation was chosen to coincide with the onset of seasonal low DO episodes in the York River subestuary (Haas 1977, Kuo & Nielson 1987, Kuo et al. 1993). The plates were deployed in shallow water to avoid exposure to York River hypoxia. Hypoxia develops in the York River periodically (every two weeks to a month) every summer (Haas 1977, Kuo & Neilsen 1987, Kuo et al. 1993, Sagasti et al. 2001) but it remains at depths below 8 m. A YSI minisonde hydrolab was deployed from the dock (Ferry Pier at the Virginia Institute of Marine Science) from July 25 to August 8, 2001 at 1 m below MLW. During this period, DO never dropped below 4 mg l^{-1}. Daily monitoring of DO at the same site in 2002, from late June to early September, revealed 0 days of < 4 mg l^{-1} DO (Jewett 2005, Ch. 3).

The experimental protocol in 2000 and 2001 involved three to five discrete manipulations over the course of the summer. Each manipulation entailed removing the plates from the river for exposure to varying levels of DO (and varying exposure periods in 2001) in containers then replacing them in the river for two weeks until the next manipulation. Manipulation (see Table 1-1 for differences between 2000 and 2001 protocols) involved retrieving panels from the York River, photographing them, then placing them in containers which sat in a flow through water table or in bins with

estuarine water that was changed every two hours to maintain a consistent temperature across treatments. Unmanipulated controls were photographed then redeployed within 30 minutes. Over 30 minutes, DO levels in containers with panels were reduced to 1 mg l^{-1} for the hypoxia (H) and 3 mg l^{-1} for the moderately low DO (M) DO treatments with N_2 gas. DO level for normoxia (N) treatments was maintained above 5 mg l^{-1} with constant air bubbling. For the remainder of the exposure periods, all treatments were bubbled with air only to prevent anoxia. Variable flow mini water pumps were deployed in treatments to maintain water movement. All DO levels were checked manually with YSI 85 Temp, Salinity, DO meters every 30 minutes for first two hours then every 3 hours thereafter. Treatments were allowed to vary from 0.5 to 2 mg l^{-1} for H, from 2 – 4 mg l^{-1} for M, and > 5 mg l^{-1} for N (Fig. 1-1). Airflow was adjusted manually to keep the DO level within these ranges. One-third of water (at appropriate DO level) in each container was changed every 6 hours for the 12 and 24 hr treatments to maintain pH above and ammonia levels below stress levels. The pH and ammonia levels were checked randomly before and after water change in 2001. pH never dropped below 7 and ammonia (NH_3) remained below 0.055 mg/l for all treatments which is below stress thresholds determined for fish (Foss et al. 2004, Lemarie et al. 2004). At the completion of the manipulation, the plates were redeployed in the river.

For the 2000 experiment, the Random Incomplete Block Design (RIBD), blocked according to the physical proximity of the deployed panels, dictated that the panels be redeployed to the same location after each manipulation. However, the blocking effect was not significant in statistical analysis so it was dropped in the 2001 experiment which

used a Completely Randomized Design (CRD). The lack of a blocking effect suggests (1) recruitment is random when panels are at least 2 m apart, (2) propagule dispersion may be operating at very small scales and (3) that any other structuring forces were also acting at scales smaller than the block. The CRD design of the 2001 experiment further randomized the effect of larval propagule pressure bias from local, epifaunal sources (from bottom sediments, pier pilings or oyster beds in vicinity) by randomly assigning the panels to location after each manipulation.

At the final retrieval, all plates were photographed, placed in a 0.5 mm mesh bag which was immersed in MgCl (64g per 1 liter seawater) for 15 – 30 minutes to relax organisms, then preserved in 5 % formalin. Biovolume for 2001 panels was measured as the amount of water displaced during immersion (less displacement of the bare panel) when transferring plates from formalin to 70% ethanol within a month of retrieval.

For species percent cover estimates, a 100-point fixed grid was used and the sessile species occupying the substrate was identified at each point. In addition, all mobile and sessile species present were recorded for a measure of species richness. All organisms were identified to genus and/or species where possible. Sponges, due to difficulty in identification, were kept at phylum level. Questionable identifications were reviewed by trained experts. If distinction between genera or species was untenable, the categories were grouped conservatively before analysis.

In general, mobile fauna were counted only in the total species richness lists. However, *Corophium spp*., tube-building amphipods, were counted in the percent cover

when tubes were present and the organism was found in the tubes (Sebens 1985, 1986). Occasionally, *Nereis sp.,* an errant polychaete, was also counted in the percent cover if its mucous tube was present and an individual was protruding from the tube at the designated point.

The multivariate community response was analyzed using Multi-Dimensional Scaling (MDS), Analysis of Similarity (ANOSIM) and Similarity Percent (SIMPER) routines (Plymouth Routines in Multivariate Ecological Research (Primer) software). ANOSIM randomization test (Clarke & Green 1988), which is based on the Bray-Curtis similarity index, does not require normal distribution of data to compute R statistics and significance values. ANOSIM analysis does not test for interaction effects. R statistics, which reflect resolution between communities, were relatively low for all the analyses, although the associated p values (level of significance) were significant. Species incidence, as listed in the ANOSIM tables, refers to sessile species percent cover data transformed to presence/absence data. This changed the data from a measure of relative abundance to relative incidence. Cluster analysis results are visually presented in the MDS figures with circles drawn around treatments which were similar at the 85% level.

SIMPER was performed on both relative percent cover of organisms and on presence/absence data. SIMPER is a multivariate, exploratory routine that uses the Bray-Curtis similarity index to compare treatments to determine which taxonomic groups are driving community differences. SIMPER does not compute pairwise statistical significance for specific species but it does indicate which species contributed most consistently to differences between communities through the use of the ratio of

dissimilarity to standard deviation (Clarke & Warwick 2001). SIMPER analysis is reported for only those treatment comparisons which were determined to be significantly different with the ANOSIM routine.

Analysis of Variance (ANOVA) was used to determine univariate response of dominant sessile species to treatment. For both multivariate and univariate analyses, percent cover data were arcsine square root transformed to meet assumptions of ANOVA (Sokal & Rohlf 1995). Pairwise comparisons based on the least significant difference test (LSD) were reported only when the p value of the main effect F statistic was below 0.05 to reduce experiment-wise error (Fisher's protected F test). The Shannon-Wiener (H') species diversity index, computed on percent cover data (base e), was calculated to compare sessile species diversity between treatments. Species richness was the number of distinct species or genera. Correlation analysis was calculated on untransformed data.

Results

Epifaunal Community Response to Low DO

In both 2000 and 2001, periodic exposure to hypoxia caused change in community composition (Table 1-2). In 2001, exposure to hypoxic conditions for as little as six hours every two weeks led to change in the percent cover of species (Fig. 1-2). Exposure to hypoxic conditions for 12 hours led to change in the composition

(presence/absence) of species (Fig. 1-3). In other words, species replacement did not occur until the community was exposed to hypoxia (< 2 mg l^{-1} DO) for at least 12 hours.

Overall, periodic hypoxia (< 2 mg l^{-1}) led to a significant increase in *Hydroides dianthus* cover relative to the control plates in 2000 and 2001(Fig. 1-4, 1-5, Table 1-3, Appendix I). The difference in cover of *H. dianthus* between the H and N treatments was statistically significant in 2001, but not in 2000. *H. dianthus* experienced highest recruitment in July and August which might explain its success since the DO manipulations occurred throughout both months. Sagasti et al. (2001) showed *H. dianthus* to have a high tolerance for low DO with mortality at 1 mg l^{-1} DO for five days at 0%.

In both years, an inverse relationship between total barnacle cover (*Balanus eburneus* plus *Balanus improvisus*) and *Hydroides dianthus* existed (Fig.1-6). This correlation analysis included all settling panels regardless of treatment. In 2001, *H. dianthus* had the highest percent cover in the hypoxia treated communities at 22% and the lowest in the normoxia communities at 11% (Fig. 1-4).

When barnacles or tube-dwelling serpulids occupied space at high enough densities, they prevented other species from settling. As barnacle cover increased, sessile species diversity (H') increased until approximately 15% barnacle cover was reached, then H' diversity decreased (Fig. 1-7). *Hydroides dianthus* had greater overall cover in 2000, perhaps due to the longer duration of the experiment (Table 1-1), and its effect on settlement of other sessile species was similar to the effect of barnacles in 2001 (Fig. 1-8). H' diversity increased until *H. dianthus* cover reached 11% then H' diversity

decreased with increasing cover of *H. dianthus.* Richness did not vary as cover of either species increased.

Hydroides dianthus, *Balanus eburneus* and *Demonax microphthalma* accounted for 60% of the similarity between treatments. However, the shifting relative percent cover of *B. eburneus* and *H. dianthus* also accounted for the greatest percent dissimilarity between the treatments (Table 1-4). Twelve to 13.4% of the total dissimilarity between any of the paired treatment comparisons was due to *B. eburneus* cover with higher cover always in the relatively higher DO treatments (Table 1-4). *H. dianthus* cover contributed 8 - 9% of the dissimilarity between treatments.

Beyond the five dominant species (*Hydroides dianthus* (serpulid), *Molgula manhattensis* (solitary tunicate), *Botryllus schlosseri* (colonial tunicate), *Balanus eburneus* (barnacle) and *Demonax microphthalma* (sabellid)), differences between communities were due also to small changes in cover of many species. Bryozoans, in general, tended to settle on unoccupied space but also grew over live and dead barnacles and occasionally up the stolons of hydroids. The cover of two encrusting bryozoan species, *Membranipora tenuis* and *Conopeum chesapekensis,* combined accounted for 12.6 to 15% of the differences between the H and the other treatments (Table 1-4). *C. chesapekensis* contributed most consistently to the differences between the H and C multivariate comparison with greater average cover on the H panels. *Halopteris tenella* (hydroid), and *Diadumene leucolena* (anemone) consistently contributed the most to the differences between treatments (H vs N, H vs C respectively) according to species incidence. *H. tenella*, on average, had highest incidence on the N panels and *D.*

leucolena had highest incidence on the H panels (SIMPER does not assign significance to these differences) (Table 1-4).

The total percent cover of combined bare space, dead barnacles and empty sand or sediment tubes was highest for the 24 hr manipulations averaged across DO treatments. All plates exposed for 24 hrs had >15% mean unoccupied space as compared with 11% for the 6 and 12 hr exposure treatments (Table 1-3). The H24 hr treated plates had a mean cover of 24% dead or bare space (Fig. 1-9).

The biovolume of the epifaunal communities was lowest in the hypoxia (H) treated panels and highest for the unmanipulated control (C) in 2001 ($df = 70$, $t = -2.56$, $p = 0.01$). No significant difference existed among the H, M and N treatments (Fig. 1-10). The diversity on the panels without regard to treatment varied inversely with biovolume (Fig. 1-11), which was probably related to the corresponding dominance of barnacles or *Hydroides dianthus* as biovolume increased.

Mobile species were recorded during the general survey of species richness for each panel**.** Composition of mobile taxa did not differ by treatment (ANOSIM, Global $R = -0.06$, $p = 0.90$). Some taxa, such as caprellids and flatworms, were noted as either dead or congregated at the surface of the water in the tanks at the end of the H manipulations.

In the 2001 experiment, diversity as measured by univariate indices (Richness, Shannon-Wiener) did not vary by treatment for either the sessile or the mobile epifauna (Table 1-3). However, the multivariate SIMPER analysis (Table 1-4) and ANOSIM

analysis (Table 1-2) indicate that the composition of species did change according to exposure to low DO. Diversity measures such as H' and S which cannot take into account the changing composition of the communities are less helpful for assessing the impact of environmental conditions (Drake et al. 1999).

Invader Response to Low DO

Although the known invasive and cryptogenic species space occupation was low (< 5%) when compared with the rest of the community, their response clearly differed significantly among treatments in the 2001 experiment (Table 1-3). The combined cover of the four known invader and cryptogenic sessile organisms was highest on the moderately low DO (M) exposed panels (Fig. 1-12). The cover did not differ between the normoxia (N) and hypoxia (H) treatments, but both differed from cover in the M treatments (Table 1-3). *Molgula manhattensis* (cryptogenic tunicate) and *Botryllus schlosseri* (invasive tunicate) were mainly driving this difference. Both *M. manhattensis* and *B. schlosseri* experienced highest cover (4%) on the M exposed panels and lowest on the H exposed panels (< 0.5%). In the 2000 experiment, *B. schlosseri* average cover (0%) in H was significantly lower than its cover (6%) in N. However, in 2000, *M. manhattensis* had increased cover in both the manipulated treatments (H and N) versus the natural control (C) (Fig. 1-5, Table 1-3).

Discussion

Extensive reviews of hypoxia and its effects on mobile and sessile fauna (Gray et al. 2002, Diaz & Rosenberg 1995) frequently overlook the shallow, seasonal DO depletions which occur in localized areas (Sanford et al. 1990). In 1999, Jewett (2005, Ch.4) documented a low DO event in Baltimore Harbor which extended in some sites to the surface with DO levels below 2 mg l^{-1} at 1 m depth at midday. Jewett (2005, Ch. 3) also surveyed DO in eight sites in lower Chesapeake Bay in 2002, six of which experienced periods of moderately low DO (< 4 mg l^{-1}) lasting from hours to weeks. The spatial scale of hypoxic bottom waters has been extensively mapped, both in the Chesapeake Bay and estuaries worldwide, but the spatial and temporal scale of moderately low DO in shallow areas is not well understood.

Moderately low DO may represent a refuge for some species as exhibited by the relative increase in cover of invasive and cryptogenic species (Fig. 1-12). Exposure to hypoxia did not facilitate invasion success. All four known invasive and cryptogenic species had lowest percent cover on the hypoxia (H) treated plates in 2000 and 2001 (Fig. 1-5, 1-12). However, periodic exposure to moderately low DO (2 mg l^{-1} < DO < 4 mg l^{-1}) led to an increase in invasive and cryptogenic species cover (Fig. 1-12). If the intermediate disturbance effect were responsible (Connell 1978, Petraitis et al. 1989), one would expect species diversity to be higher on the M treatments; but neither H' diversity nor richness were significantly different according to treatment (Table 1-3). Laine (2003) concluded that declining richness due to low DO stress in the Baltic Sea may make that

system more vulnerable to invasion but richness alone did not correlate with invasion success in this experiment.

Hypoxia has the ability to cause local mortality, to shift dominance thus changing species diversity, and/or to serve as a refuge for less dominant members of the community. Species specific local mortality could occur if DO levels drop below 2 mg l^{-1} for a minimum of 6 hours, given that the composition of species changed across DO treatments (Table 1-2) and that the percent cover of at least two dominant species, *Demonax microphthalma* and *Botryllus schlosseri,* was either reduced or nonexistent on the hypoxia exposed panels (Fig. 1-5). Hypoxia also caused shifts in the dominant species, as was shown in the reduction of *D. microphthalma* (Fig. 1-5) and *Balanus spp.* cover and the increase in *Hydroides dianthus* cover (Fig. 1-4) which in turn led to changes in species diversity (Fig. 1-7, 1-8) depending on the extent of the *H. dianthus* or barnacle cover.

The dynamics of recovery after hypoxic or anoxic events has been described by the Pearson-Rosenberg (1978) organic enrichment model (Heip 1995; Powilleit & Kube 1999). The model is based on changes in species number, abundance and biomass (SAB) as the effect of organic enrichment diminishes spatially from a central source. The authors and others (Heip 1995) suggest that the model could also be applied to time elapsed since an hypoxic disturbance with small bodied, opportunistic species dominating recruitment following a low DO event. In our experiment, communities exposed to hypoxia for 24 hrs (the highest level of disturbance in this study) exhibited a reduction in biovolume (Fig. 1-9) and the opportunistic polychaete, *Hydroides dianthus*, increased in

the H treatments (Fig. 1-4, 1-5). Other studies in Chesapeake Bay (Dauer & Alden 1995), in Kattegat (Hagerman et al 1996), in Norwegian fjords (Mirza & Gray 1981, Gray et al. 1988) and in the Gulf of Mexico (Rabalais et al. 1991) have documented the increasing dominance of opportunistic species in response to degraded conditions. From 1985 to 1991, the percent of total biomass in the central Chesapeake Bay composed of opportunistic species increased as DO conditions worsened (Dauer & Alden 1995).

Timing of exposure to disturbance can influence community response (Anderson 1998, Nandakumar 1996). The timing of the low DO manipulations, which happened from mid-June through August (when low DO disturbance occurs in the Bay), probably affected which species benefited. Since peak recruitment of *Hydroides dianthus* happens in July and August (Dean & Hurd 1980, Otsuka & Dauer 1982) and the adults have a tolerance for low DO (Sagasti et al. 2001), the increase in *H. dianthus* cover was not unexpected. *H. dianthus* may take advantage of space clearing disturbance, because its recruitment is inhibited by most other sessile species (Dean & Hurd 1980) and it preferentially settles on bare space (Dean 1981). In addition, *H. dianthus* larvae tend to settle near other conspecific adults (Toonen & Pawlik 2001), so its response to cleared space is amplified. Finally, *H. dianthus* larvae are planktonic for up to two weeks (Toonen & Pawlik 2001), as compared to < 1 day for most tunicates, enhancing the likelihood of finding bare space created by low DO disturbance due to greater dispersal. Serpulids, in general, are considered good colonizers and poor competitors (Keough 1984, Dunstan & Johnson 2004). Research after a severe hypoxic event in the Baltic Sea

also concluded that timing of the event affected the pool of larval recruits and thus community composition (Powilleit & Kube 1999).

In 2000 and 2001, *Balanus spp.* experienced heightened mortality mid-summer in the low DO treatments after strong recruitment in the spring**.** *Balanus* larvae were available throughout the summer. Cory (1967) and Otsuka & Dauer (1982) attributed barnacle mortality to predation by the flatworm, *Stylochus ellipticus*. However, *S. ellipticus* has a low tolerance for DO stress. In lab experiments, *S. ellipticus* experienced 59% mortality when exposed to hypoxia (1 mg l^{-1} DO) for five days (Sagasti et al. 2001). It is possible that, in the estuary, these flatworms experience high mortality during hypoxic periods so predation on barnacles during hypoxia would decrease. However, predation on stressed barnacles after redeployment may account for increased vulnerability. Fish and crabs in the York River prey on stressed organisms, such as barnacles, upon redeployment (Breitberg 1997, Nestlerode & Diaz 1998, Seitz et al. 2003). Mud crabs, *S. ellipticus*, and blue crabs all eat barnacles. It would take some time for mud crabs and *S. ellipticus* to recruit to the stressed settling panels, but very mobile fish and blue crabs might have had an immediate impact. In natural conditions, predators have been reported to return to affected areas before prey has recovered (Pihl et al. 1992, Nestlerode & Diaz 1998).

Other species had positive responses to low DO including the anemone, *Diadumene leucolena,* and two encrusting bryozoans. *D. leucolena* experienced a recruitment pulse in late July which may account for its, on average, higher incidence on the H panels (Table 1-4). It may also have a high tolerance for low DO given that

cnidarians are found in low DO conditions worldwide (Purcell et al. 2001). Reduced predation may account for the higher cover and incidence of *Membranipora tenuis* and *Conopeum chesapekensis* on the H panels (Table 1-4). Predation on encrusting bryozoans in the disturbed panels may have been reduced, since the nudibranch, *Doridella obscura*, which grazes on bryozoans has a low tolerance for hypoxia (Sagasti et al. 2001).

The mechanism facilitating increased cover of invasive and cryptogenic species in the moderately low DO treatments was probably multi-factorial**.** These invasive and cryptogenic species were clearly sensitive to hypoxic conditions, yet they also must have had some tolerance to lower DO conditions. They may have had the capacity to capitalize on open space by increasing sexual and/or asexual reproduction during stressful conditions when predation by barnacles and other filter feeders was reduced. The stress of the low DO manipulation may have induced spawning in *Molgula manhattensis* (Osman, pers. comm.). Because *M. manhattensis* has a short larval stage (< 1 day) (Costello et al. 1957) and its tadpole larvae have been shown to settle near spawning adults (Graham & Sebens 1996), it might capitalize on local patch clearings near resident adults. However, in lab experiments (Sagasti et al. 2001), *M. manhattensis* adults delayed reproduction and the larvae delayed settlement until after the hypoxia passed. *Botryllus schlosseri* adult tolerance for periodic hypoxia was low (Fig. 1-5, 1-12), and this species was not found in deep waters where periodic hypoxia occurred (Sagasti et al. 2000). Tunicates, in general, are better competitors than colonizers (Keough 1984, Dunstan & Johnson 2004), so the increased percent cover of these tunicates in moderately

low DO may also have resulted from overgrowing of other less competitive, less low DO tolerant sessile species such as sponges and barnacles.

Ficopomatus enigmaticus, serpulid worm, a recent arrival to the Chesapeake Bay, also had higher cover in communities subjected to moderately low DO. However, tunicates and serpulids probably succeed for different reasons. This invasive serpulid was observed settled on and around *Hydroides dianthus*. It may cue settlement on *H. dianthus* adults or at least prefer similar conditions. *F. enigmaticus* has a high tolerance for low DO and tolerates a broad salinity range (Fornos et al. 1997).

How the geographic area affected by a low DO disturbance compares to the colonizing capacity of the resident species will determine changes in local species diversity (Sousa 1984). The scale of a disturbance event can determine the speed of recovery in soft sediment communities (Gamenick et al. 1996). However, if the frequency of low DO episodes is low, then regional species richness will be maintained because competitive species, such as some colonial tunicates and sponges, will not be eliminated (Miller 1982). Temporary low DO in shallow areas differs fundamentally from hypoxia in deep zones of the Bay which can last for months. The patchiness of disturbance by low DO in shallow waters may lead to a mosaic of environments; some dominated by *Hydroides dianthus*, others by *Balanus eburneus* and still others by a more heterogeneous community depending on the severity, frequency and geographic extent of the low DO episodes.

Conclusion

Our experiments illustrated how composition of species in local areas may change with increased stress from low DO. Certain species, such as *Hydroides dianthus*, dominated the stressed communities, leading to local aggregations that, in turn, reduced the incidence and cover of other species. The DO stress directly reduced the cover of non-tolerant species, such as *Demonax microphthalma*. Invasive and cryptogenic species benefited from moderately low DO, a condition prevalent in near shore areas. The opportunity for recruitment and spread of invasive species may be facilitated by the low DO disturbance. Low DO pockets may enhance overall survival of invasive and cryptogenic species by providing source populations for the region. High temperatures are correlated with low DO conditions worldwide which may make marine and estuarine systems more vulnerable to invasion as global temperatures rise due to climate change.

It may be possible to use a sample of the epifaunal community to gauge the health of the local waters. Many studies have documented a predominance of opportunistic species and a decline in species richness in areas exposed to hypoxia (Heip 1995, Hagerman et al 1996, Laine 2003). Dauer et al. (2003) proposed that over abundance of certain taxa may reflect degraded environmental conditions. In the Chesapeake Bay, the existence of extensive *Hydroides dianthus* aggregations probably is an indicator of stressful local conditions. In addition, greater percent cover of invasive and cryptogenic species may indicate that stressful but not lethal episodes of hypoxia have occurred.

Acknowledgements

Dr. Anson Hines and Dr. Gregory Ruiz of the Smithsonian Environmental Research Center supported this research both financially and with scientific guidance. The following people deserve special mention for advice on experimental design, loan of laboratory space or assistance in doing the experiments: K. Sebens, R. Osman, A. Sagasti, J. Crooks, L. McCann, L. Schaffner, M. Seebo, J. Blum, J. Kurtin, J. van Montfrans, A. Key, T. Gurley, G. Smith, J. Lawshe and M. Kramer. This work was made possible through the generous funding of the Smithsonian Institution and a University of Maryland Chesapeake Bay Summer Grant. I especially would like to thank Virginia Institute of Marine Science for allowing me to do these experiments at their facility.

Table 1-1 Experimental protocol. H = hypoxia (< 2 mg l^{-1}), M = moderately low DO (2 mg l^{-1} < DO < 4 mg l^{-1}), N = normoxia (< 5 mg l^{-1}), C = unmanipulated control. # = total number of manipulations. RIBD = Randomized Incomplete Block Design. CRD = Completely Randomized Design.

Year	n / total panels	Design	Manipulation period : #	DO levels	Exposure time	Set-up	Water Change	Redeployment	Analysis	Biovolume measured
2000	7 / 20	RIBD	June 12 – August 23: 5	H, N, C	24 hrs	Plate + brick in 20 L container in sea table	NO	Same location each time	Live	No
2001	8 / 80	CRD	June 23 – August 9: 3	H, M, N, C	6, 12, 24 hrs	Plate only in 5 L container in sea table	Every 6 hrs	Random placement	Preserved	Yes

Table 1-2. ANOSIM (Analysis of Similarity) results comparing assemblages on panels for DO and exposure time treatment levels for 2001 and 2000 data. DO treatment abbreviations include H = hypoxia, M = moderately low DO, N = normoxia, C = unmanipulated control. Significant p values in bold.

	Main Effect : DO Treatment n = 18				Main Effect : Exposure Time (hrs) n = 18			
2001	Global R	Pairwise comparisons			Global R	Pairwise comparisons		
		H v M	H v N	M v N		6 v 12	6 v 24	12 v 24
Primary cover								
R	0.15	0.21	0.23	0.04	0.06			
p	**< 0.002**	**< 0.004**	**< 0.004**	> 0.260	0.110			
Species Incidence								
R	0.10	0.17	0.12	0.02	0.08	0.15	0.03	0.07
p	**< 0.02**	**< 0.006**	**< 0.040**	0.320	**0.040**	**< 0.020**	0.370	0.150

2000	Global R n = 7	H v N	H v C	N v C
Primary cover				
R	0.36	0.33	0.61	0.06
p	**0.001**	**0.003**	**0.001**	0.240
Species Incidence				
R	0.13	0.27	0.09	0.04
p	**0.052**	**0.010**	0.180	0.310

Table 1-3 ANOVA analysis (2-way) of treatment effects on primary cover of 5 dominant taxa plus other indicators. DO treatments: H = hypoxia, M = moderately low DO, N = normoxia, C = unmanipulated control. Invaders / Cryptogenic = *Molgula manhattensis* , *Botryllus schlosseri* , *Ficopomatus enigmaticus* and *Diadumene lineata* (combined cover). Empty space = bare space, empty tubes or dead barnacles. H' = Shannon-Wiener diversity index (base e). Total Richness = all distinct taxonomic groups present on a panel including mobile and sessile fauna. Pairwise comparison t values are in parentheses. P values significant at the .05 level are in bold.

	DO treatments		Pairwise Comparisons			Exposure Time		Pairwise Comparisons			DO x Exp	
	n = 18; df = 2, 51		H v M	H v N	M v N	n = 18; df = 2, 51		6 v 12	6 v 24	12 v 24	n = 6; df = 4,51	
2001	**F value**	**p**	**p**	**p**	**p**	**F value**	**p**	**p**	**p**	**p**	**F value**	**p**
*Balanus (*two species combined)	1.1	0.3412				0.01	0.9882				0.28	0.8867
Botryllus schlosseri	7.61	**0.0013**	**0.0003**	**0.037**	0.089	0.56	0.5755				0.88	0.386
			(3.90)	(2.14)	(-1.74)							
Demonax microphthalmus	1.33	0.272				9.22	**0.0004**	0.252	**0.004**	**0.0001**	2.29	0.073
								(-1.16)	(2.98)	(-4.17)		
Hydroides dianthus	5.17	**0.009**	0.0763	**0.0023**	0.1564	0.23	0.814				0.27	0.911
			(-1.81)	(-3.21)	(-1.44)							
Molgula manhattensis	3.88	**0.027**	**0.0079**	0.2669	0.1076	1.38	0.26				3.04	**0.0253**
			(2.77)	(1.12)	(-1.64)							
Invasive/Cryptogenic Species	5.86	**0.0051**	**0.0013**	0.162	**0.054**	1.07	0.35				0.92	0.46
			(-3.41)	(1.42)	(-1.97)							
Empty space	1.81	0.174				4.34	**0.018**	0.832	**0.01**	**0.02**	1.23	0.262
								(-0.21)	(-2.64)	(2.46)		
Total Richness	0.45	0.643				0.04	0.957				0.32	0.863
H' diversity	0.34	0.713				0.01	0.985				0.61	0.655

	F	p	Pairwise p values		
2000	**n = 7; df = 2, 17**		**H v N**	**H v C**	**N v C**
Balanus (two species combined)	4.46	**0.030**	0.376	**0.026**	0.096
			(-0.93)	(-2.73)	(-1.84)
Botryllus schlosseri	9.95	**0.003**	**0.001**	**0.007**	0.281
			(-4.24)	(3.25)	(-1.13)
Demonax microphthalmus	31.82	**<.0001**	**<.0001**	**<.0001**	0.545
			(-6.37)	(7.27)	(0.62)
Hydroides dianthus	3.81	**0.043**	0.083	**0.016**	0.469
			(1.84)	(-2.69)	(-0.74)
Molgula manhattensis	3.70	**0.053**	0.612	0.055	**0.025**
			(-0.52)	(-2.10)	(-2.52)
Total Richness	1.84	0.189			
H' diversity	3.10	0.071			

Table 1-4 SIMPER results for DO treatment comparison for arcsin squareroot and for presence absence transformed percent cover 2001 data. % δ = percent of dissimilarity (calculated by the average contribution from this species to the overall dissimilarity between groups) contributed by this species. δ/ SD = average dissimilarity / standard deviation which is a measured of how consistently a species contributes to δ between two groups (the larger this ratio the more discriminating the species). H = hypoxia, M = moderately low DO, N = normoxia, C = unmanipulated control. Invasive or cryptogenic species in bold. The species contributing most consistently to the pairwise dissimilarity is marked with an asterisk. Tmt > cover indicates in which treatment that species had greater average percent cover. Tmt > Inc indicates in which treatment the species incidence was higher.

Percent Cover Data

H,M	% δ	δ/SD	Tmt > cover	H, N	% δ	δ/SD	Tmt >cover	H,C	% δ	δ/SD	Tmt >cover
B. eburneus	13.4	1.3	M+	*B. eburneus*	12.3	1.4	N+	*B. eburneus*	12.2	1.2	C+
H. dianthus	9.2	1.2	H+	*H. dianthus* *	9.6	1.5	H+	*H. dianthus*	8.0	1.5	H+
M. tenuis	6.4	1.3	H+	*M. tenuis*	7.6	1.4	H+	*C. chesapekensis**	7.9	1.8	H+
C. chesapekensis	6.2	1.3	H+	*C. chesapekensis*	7.2	1.4	N+	*M. tenuis*	7.1	1.3	H+
***B. schlosseri** *	5.9	1.8	M+	*D. microphthalma*	5.9	1.3	H+	*D. microphthalma*	6.7	1.3	C+
D. microphthalma	5.9	1.4	M+	*O. dichotoma*	5.6	1.2	N+	*Corophium spp.*	6.3	1.3	C+
B. improvisus	5.5	1.3	H+	*Corophium sp.*	5.2	1.4	N+	*H. tenella*	5.9	1.2	C+
Corophium sp.	5.4	1.4	M+	*Polydora spp.*	5.1	1.3	N+	*Polydora spp.*	5.5	1.4	H+
O. dichotoma	5.0	1.1	H+	*Nereis spp.*	4.2	1.3	N+	*B. improvisus*	5.0	1.2	H+
M. manhattensis	4.9	1.3	M+	***B. schlosseri***	4.2	1.1	N+	*B. schlosseri*	4.5	1.3	C+
Polydora spp.	4.7	1.2	H+	***M. manhattensis***	3.7	1.1	N+	*D. leucolena*	4.4	1.5	H+

Presence Absence Transformed

H,M	% δ	δ/SD	Tmt > Inc	H,N	% δ	δ/SD	Tmt > Inc	H,C	% δ	δ/SD	Tmt > Inc
***M. manhattensis** *	7.1	1.2	M+	***M. manhattensis***	6.6	1.1	N+	*D. leucolena* *	8.7	1.6	H+
B. improvisus	6.8	1.1	H+	*Metafolliculina sp.*	6.6	1.1	H+	Porifera	7.8	1.3	C+
B. schlosseri	6.3	1.0	M+	*H. tenella* *	6.6	1.1	N+	*H. tenella*	7.4	1.2	C+
Metafolliculina sp.	6.2	1.0	H+	Porifera	6.4	1.0	N+	*O. dichotoma*	7.3	1.2	H+
H. tenella	5.9	1.0	M+	***B. schlosseri***	6.2	1.0	N+	*Metafolliculina spp.*	7.2	1.2	H+
D. leucolena	5.9	0.9	H+	*D. leucolena*	6.2	1.0	H+	*C. tenuissemum*	6.6	1.1	H+
C. tenuissemum	5.7	0.9	M+	*C. tenuissemum*	6.2	1.0	H+	*Polydora spp.*	6.3	1.0	H+
F. enigmaticus	5.6	0.9	M+	*B. improvisus*	5.5	0.9	H+	***B. schlosseri***	6.2	1.0	C+
A. palmata	4.8	0.8	H+	***F. enigmaticus***	5.3	0.9	H+	***M. manhattensis***	6.1	1.0	C+
Corophium sp.	4.6	0.7	M+	*Corophium sp.*	5.2	0.8	N+	*B. improvisus*	5.0	0.8	H+
O. dichotoma	4.4	0.7	M+	Other ciliates	5.2	0.9	N+	*Corophium* spp.	4.9	0.8	C+
Polydora spp.	4.2	0.7	H+								

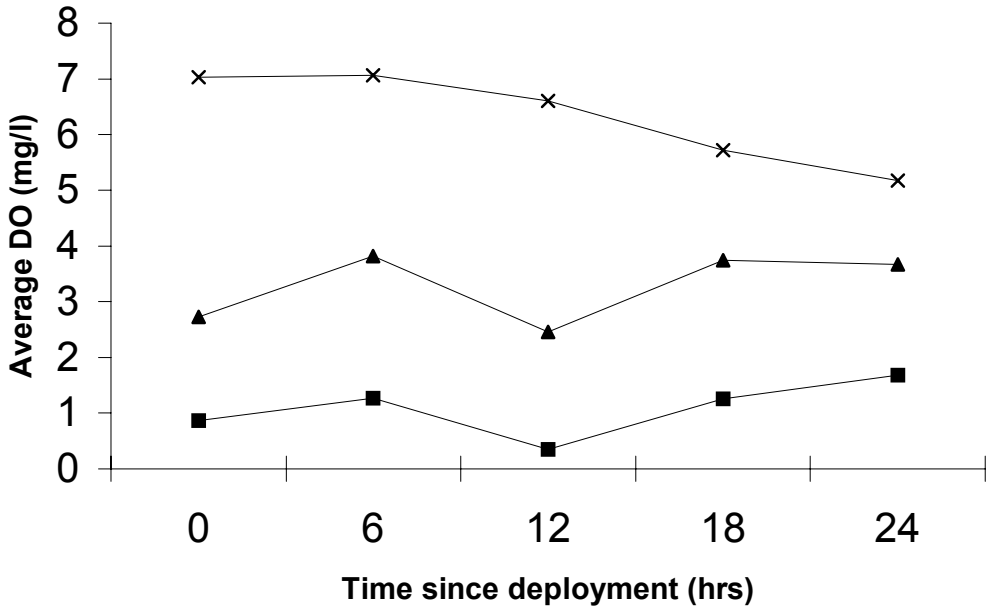


Fig. 1-1 Average DO levels experienced by communities during a 24 hour period. H = hypoxia (squares), M = moderately low DO (triangles), N = normoxia (crosses). (n=8).

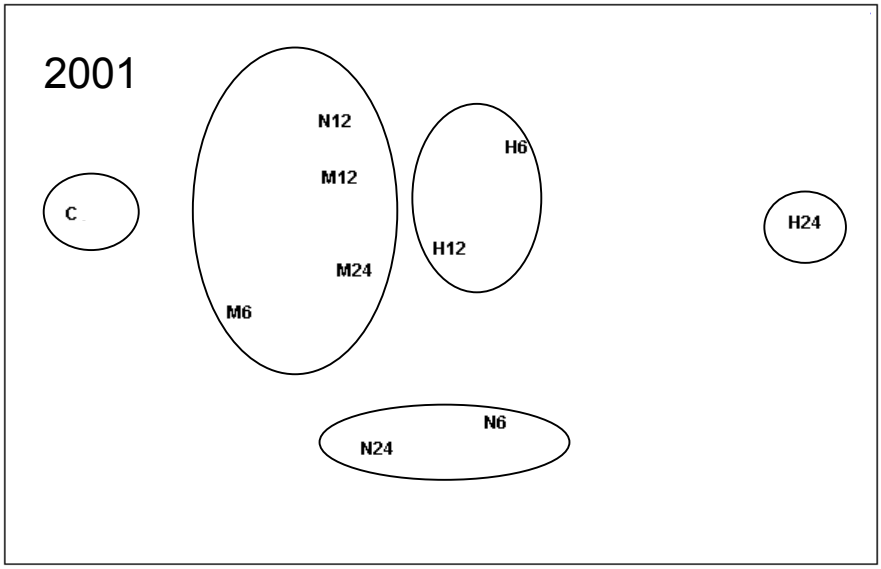


Fig. 1-2 Results of Multi Dimensional Scaling (MDS) analysis performed on transformed primary percent cover data averaged by treatment for 2001 data. Circles represent 83% similarity according to cluster analysis. Letters refer to DO treatment and numbers refer to exposure time for each treatment. Stress = 0.07.

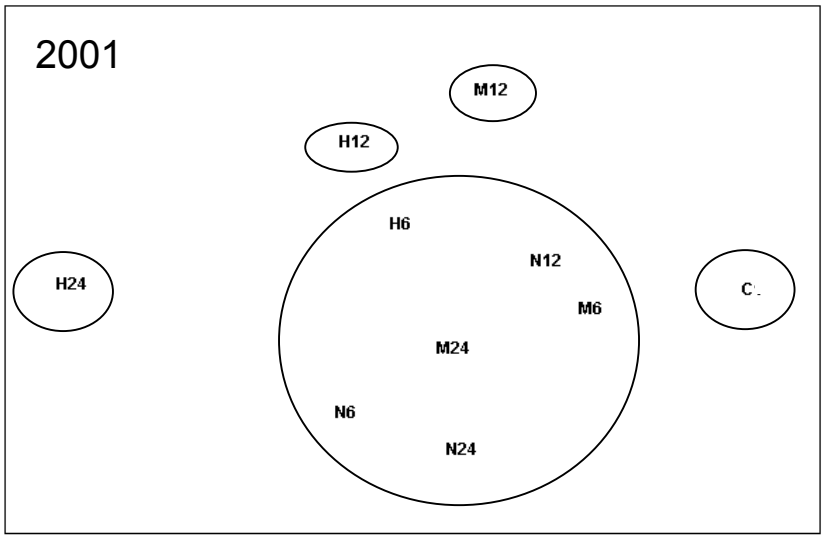


Fig. 1-3 Results of Multi Dimensional Scaling (MDS) analysis performed on presence absence transformed primary and secondary percent cover data averaged by treatment for 2001 data. Circles represent 85% similarity according to cluster analysis. Stress = 0.08.

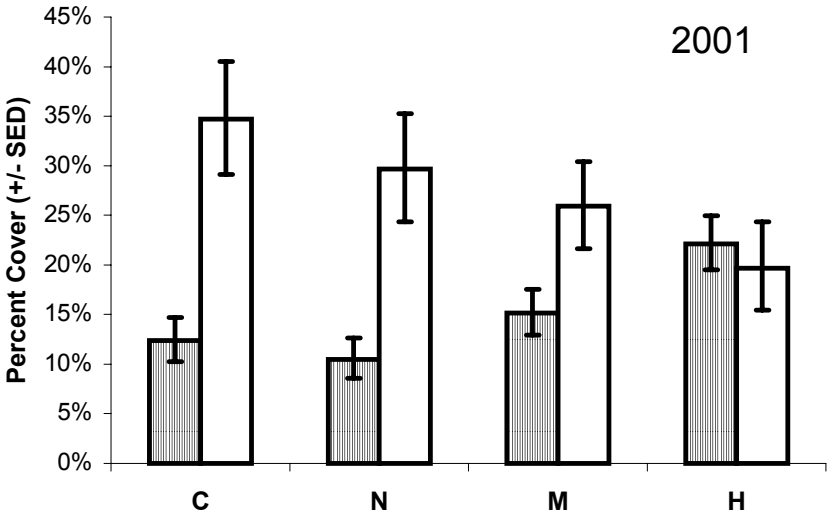


Fig. 1-4 Percent cover of *Balanus* spp. (*Balanus eburneus* plus *Balanus improvisus*) and *Hydroides dianthus* by treatment in 2001 experiment. C = Unmanipulated control, N = normoxia , M = moderately low DO, H = hypoxia. See Table 3 for statistical differences among treatments. n = 18. *H. dianthus* = striped bars, *Balanus* spp. = open bars.

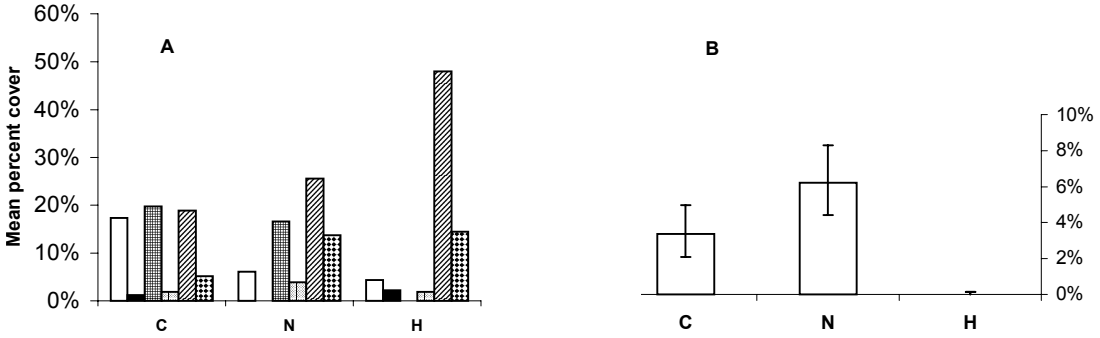


Fig. 1-5 Species cover according to treatment for the 2000 experiment. C = unmanipulated control, N = normoxia, H = hypoxia. See Table 1-3 for ANOVA analysis. **A**: Barnacles = open bars, encrusting bryozoans = solid bars, *Demonax microphthalma* = cross hatched bars, *Diadumene leucolena* = stippled bars, *Hydroides dianthus* = striped bars, *Molgula manhattensis* = diamond bars. **B**: *Botryllus schlosseri* cover according to treatment. Bars represent standard error of the difference (SED).

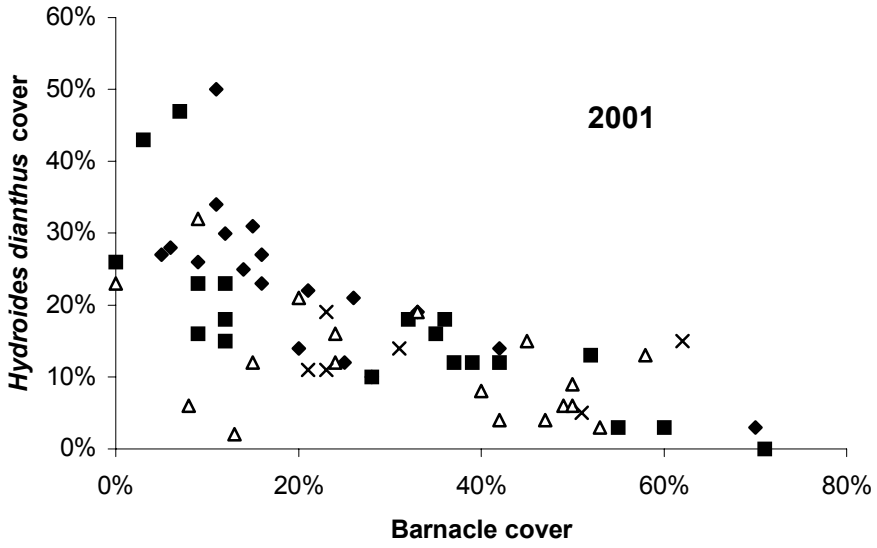


Fig. 1-6 *Hydroides dianthus* percent cover versus barnacle cover (*B. eburneus* and *B. improvisus* combined percent cover) for 2001 data. Correlation analysis: $r = -0.72$, $p < 0.0001$ ($n = 60$). Correlation analysis was computed on all data regardless of treatment. H = diamond, M = square, N = triangle and C = cross.

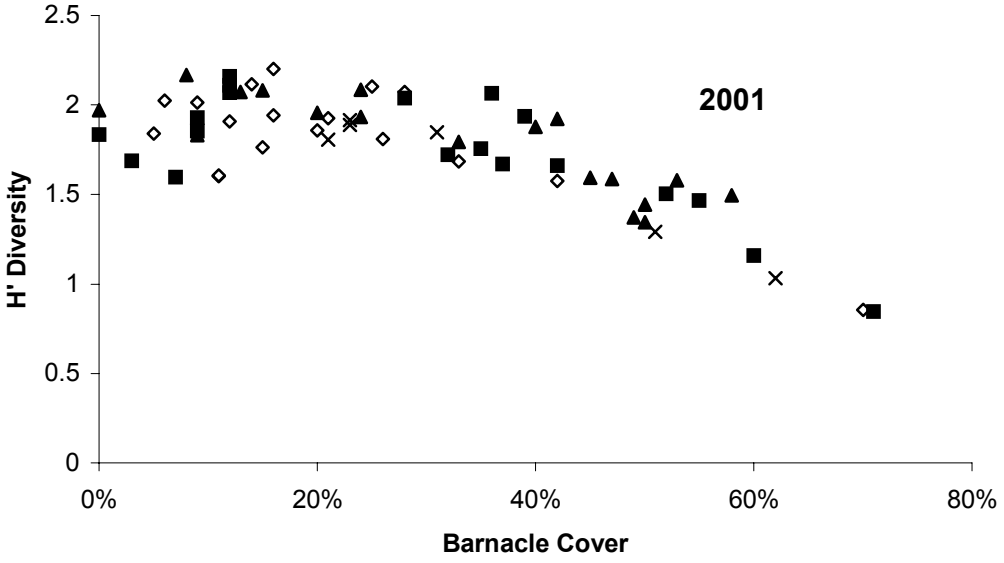


Fig. 1-7 H' diversity (Shannon-Wiener index) versus barnacle cover (*B. eburneus* and *B. improvisus* combined) for 2001 data. Correlation analysis: $r = -0.78$, $p < 0.0001$. H = diamond, M = square, N = triangle and C = cross. Correlation computed on all data regardless of treatment (n = 60).

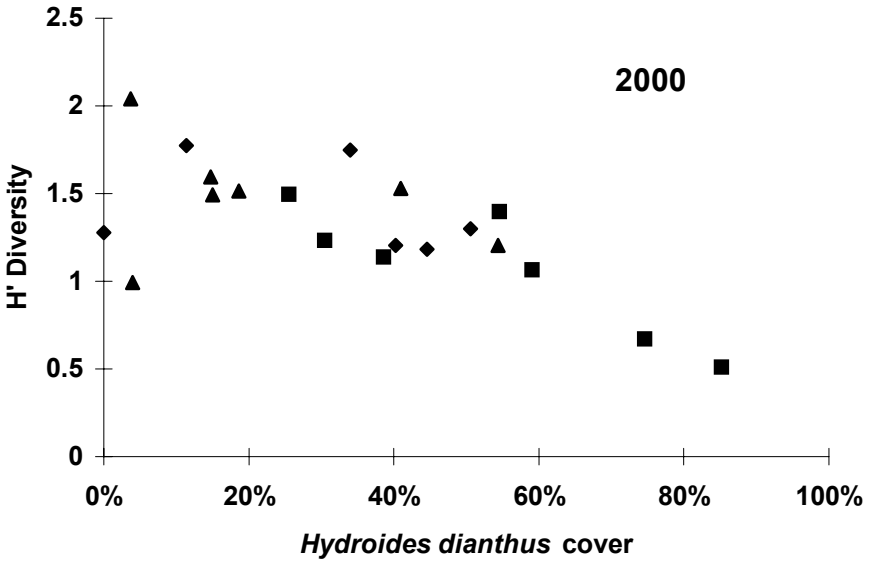


Fig. 1-8 H' diversity (Shannon-Wiener Index) vs. *Hydroides dianthus* percent cover for 2000 data. Correlation analysis : $r = -0.67$, $p < 0.01$. Correlation computed on all data regardless of treatment (n = 20). H = square, N = diamond, C = triangle.

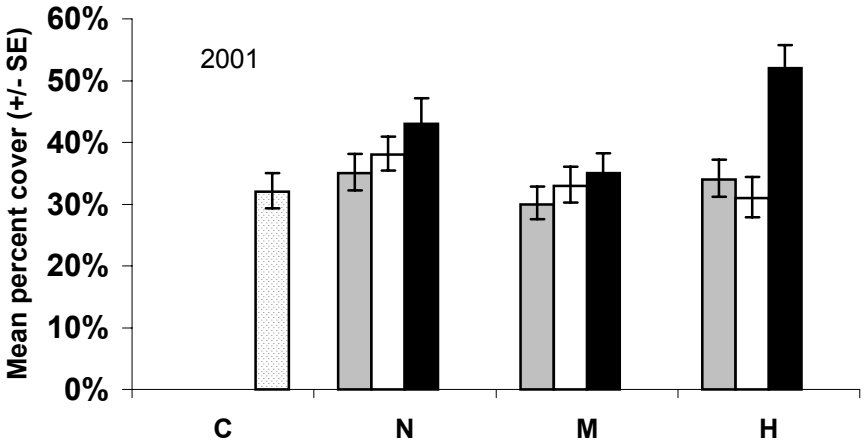


Fig. 1-9 Mean empty space (empty = bare space, vacated tubes or dead barnacles) for 2001 data. C = unmanipulated control (stippled), N = normoxia , M = moderately low DO, H = hypoxia. Exposure time within DO treatment: 6 hrs (gray), 12 hrs (open), 24 hrs (solid). See Table 1-3 for ANOVA analysis. n = 6.

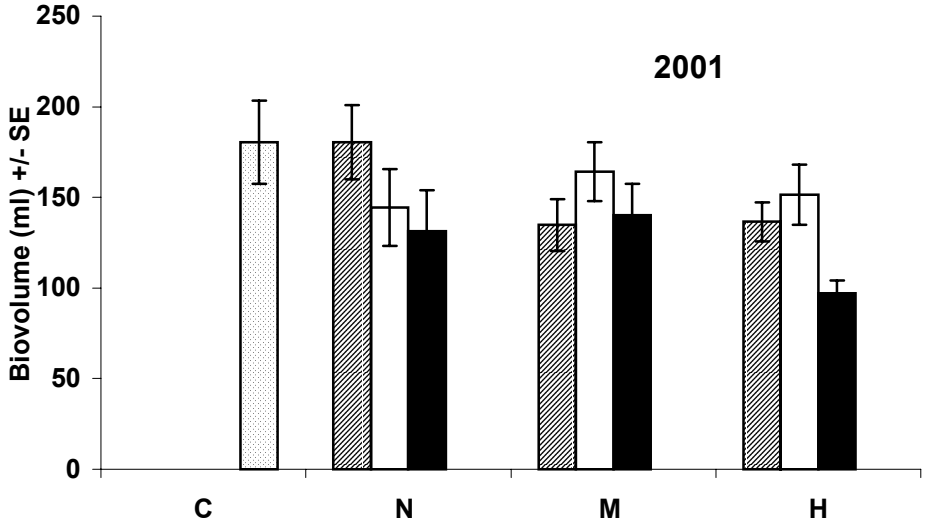


Fig. 1-10. Mean biovolume according to treatment for 2001 data. Biovolume represents amount of water displaced by community assemblage on a panel. DO treatment: C = unmanipulated control (stippled), N = normoxia, M = moderately low DO, H = hypoxia. Exposure time within DO treatment: 6 hrs (striped), 12 hrs (open), 24 hrs (solid). n = 8.

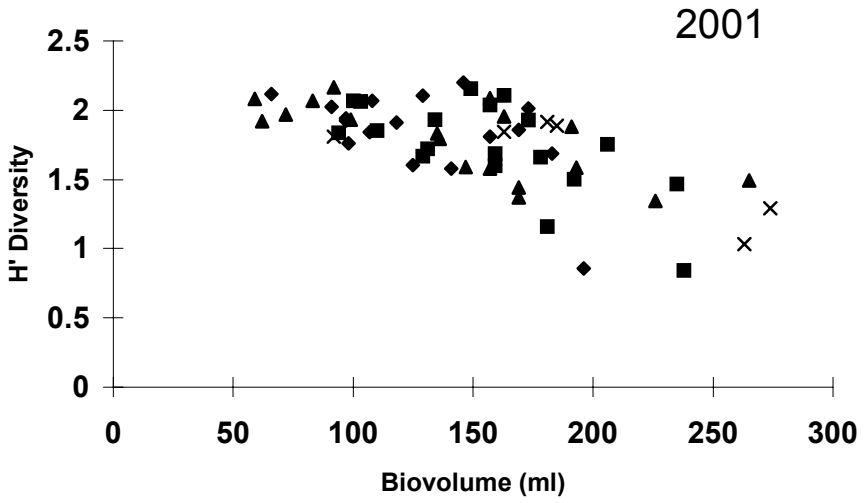


Fig. 1- 11 H' diversity (Shannon - Wiener index) versus biovolume for 2001 data. Correlation analysis: r = - 0.63, p < 0.001. Correlation computed on all data regardless of treatment (n = 60). H = diamonds, M = squares, N = triangles, C = crosses.

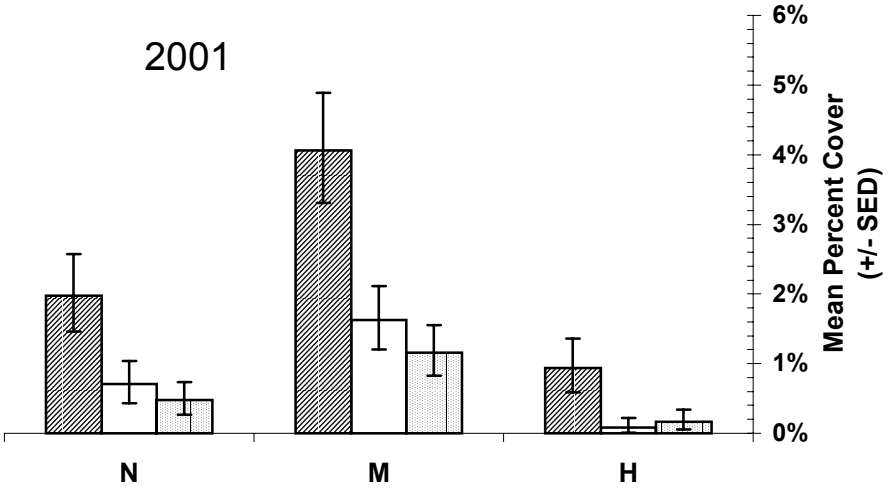


Fig. 1-12 Invader / cryptogenic species cover according to treatment for 2001 experiment. H = hypoxia, M = moderately low DO, N = normoxia. Total invader/cryptogenic (striped bar) includes *Botryllus schlosseri* (open bar), *Molgula manhattensis* (stippled bar), *Diadumene lineata and Ficopomatus enigmaticus*. See Table 1-3 for ANOVA analysis. n = 18.

Chapter 2: Short Term Response of Motile and Sessile Epifauna to Low Dissolved Oxygen Disturbance

Abstract

Low dissolved oxygen (DO) events in estuaries periodically affect shallow water habitats. These events can restructure sessile epifaunal communities (Jewett 2005, Ch. 1), but the effects on motile epifauna and interactions among motile and sessile organisms is another critical component of the disturbance. We conducted an experiment to test how motile epifaunal species (flatworms, harpacticoid copepods, gammarid amphipods, nematodes, nereid polychaetes and nudibranchs) responded to varying DO levels (hypoxia (< 2 mg l^{-1}), moderately low DO (2 - 4 mg l^{-1} DO), normoxia (> 5 mg l^{-1}) and exposure periods (6, 12, 24 hrs) during summer 2001 at Gloucester Point, VA. Caprellids, harpacticoids, nematodes, flatworms and sabellid polychaetes vacated the epifaunal community when exposed to low dissolved oxygen (< 4 mg l^{-1}). Harpacticoids and flatworms were equally sensitive to hypoxia and moderately low DO conditions. Low DO events in shallow water estuarine systems may cause a net energy transfer away from the disturbed sites. These events might also render motile epifauna more susceptible to predation by sessile epifaunal predators (hydroids, anemones) or create a refuge from predation for sessile prey (bryozoans, hydroids) if motile predators (such as flatworms) vacate the community. Although low DO disturbance did temporarily decrease abundances of some organisms, motile epifaunal richness did not differ by treatment a month later. However, the recovery process following an actual low DO event in the bay is less certain.

Introduction

Periodic low dissolved oxygen (DO) is a pulse disturbance which can cause changes in the epifaunal communities of estuaries (Jewett 2005, Ch. 1). Some of these changes may be due to the ecological interaction of small motile organisms living within the epifaunal habitat. For example, *Stylochus ellipticus* and *Doridella obscura* experienced relatively high mortality compared to other members of the community when exposed to severe hypoxia (Sagasti et al. 2001). Both are known predators on sessile epifauna, the former on barnacles and the latter on encrusting bryozoans. If these predators are killed or forced to migrate out of an area, the relaxation in predation pressure could lead to changes in the sessile community (Osman & Whitlatch 2004).

Low DO stress may increase the chances of prey capture by inhibiting predator avoidance behavior or by increasing predator – prey encounter rates (Breitburg et al. 1999, Decker et al. 2004). Calanoid copepods, which are prey for several taxa (Turner 2004) are captured at a higher rate by ctenophores when DO drops below 3 mg l^{-1}. Copepod jumping rates decreased during hypoxia (Decker et al. 2004). Harpacticoids disappeared from sediments in the laboratory after intermittent exposure to hypoxia in a Baltic Sea experiment (Modig & Olafsson 1998). In a modeling experiment, fish larvae encountered higher predation rates when bottom water became hypoxic (Breitburg et al. 1999). Hydroids and anemones, which consume harpacticoids and caprellids, may benefit when prey defenses are compromised by hypoxia. *Diadumene leucolena*, the prevalent anemone in sessile communities of lower Chesapeake Bay, has a high tolerance for low DO (Sagasti et al. 2001).

Other effects of low DO include slowed growth and feeding in motile epifaunal species. The number of barnacles eaten by *Stylochus ellipticus* (flatworm) and *Neopanopeus sayi* (mud crab) dropped significantly when exposed to hypoxia (1 mg l^{-1}) (Sagasti et al. 2001). Low DO delayed molting in some crustaceans (Clark 1986, Mugnier & Soyez 2005). *Doridella obscura* reduced its feeding rate on encrusting bryozoans during hypoxia (Sagasti et al. 2001).

We conducted an experiment on motile epifauna as part of a larger experiment analyzing the effect of low DO on sessile epifauna of a temperate estuary. Motile organisms were collected from the treated water after epifaunal communities were exposed to varying levels of DO for varying exposure periods. To explain changes in the sessile community structure, we needed to understand how low DO affected the motile epifauna which can serve as predators (Sagasti et al 2001, Osman & Whitlatch 2004), prey (Holohan et al 1998) and facilitators (Bruno et al. 2003) of sessile organisms.

Methods

We collected motile organisms (including one predominantly sessile worm) as part of a summer long experiment examining the effects of low DO on the sessile communities growing on fouling panels described in Jewett (2005, Ch. 1). The samples analyzed in this study were collected during the second manipulation of a longer term experiment. The 14 x 14 cm^2 dark grey, plastic PVC panels were deployed in the York River subestuary of the Chesapeake Bay from the Virginia Institute of Marine Science Ferry and Oyster Piers in early May 2001 (see Jewett 2005, Ch. 1). In order to analyze

the effects of low DO on shallow water epifauna, panels were exposed to three levels of dissolved oxygen (hypoxia (H) < 2 mg l^{-1} DO, moderately low DO (M) 2 mg l^{-1} < DO < 4 mg l^{-1}, normoxia (N) > 5 mg l^{-1} DO) for three discrete time periods (6, 12, 24 hrs) every two weeks from late June until early August. For treatment manipulations, panels were retrieved from the York River and placed face-up in seawater in 5 l containers, which sat either in a sea table or in bins with estuarine water that was changed every two hours to maintain a consistent temperature across treatments. Panels were deployed from a dock about 2 m above the water surface so some motile species were probably lost during retrieval when panels were hoisted to the dock. For instance, mud crabs and fish did not remain on the panels. All hypoxic (H) and moderately low DO (M) treatment containers were fitted with both N_2 and air hoses for manipulating DO level. Variable flow water pumps were deployed in these treatments to maintain water movement.

Over 30 minutes, DO levels were lowered to 1 mg l^{-1} for the hypoxic and 3 mg l^{-1} for the moderately low DO treatments. As soon as these levels were attained, N_2 gas was terminated and all treatments were bubbled with air only for the duration of the manipulation. Respiration of settling plate organisms naturally reduced DO levels so air was necessary to keep water in containers from going anoxic. All DO levels were checked manually with YSI 85 Temp, Salinity, DO meters every 30 minutes for the first two hours then every 3 hours thereafter. Treatments were allowed to vary within the target range: 0.5 to 2 mg l^{-1} for the hypoxia (H) DO level, 2 – 4 mg l^{-1} for the moderately low DO (M) and above 5 mg l^{-1} for normoxia. Airflow was adjusted manually for each container to keep the DO level within the target range. Gas delivery and water pumps

were never directed toward the experimental surface of the plate to prevent dislodging organisms. At the completion of the exposure periods (6, 12 or 24 hrs), the panels were removed for redeployment to the York River and the water in the containers was sieved with a 500 μm sieve. Collected organisms were preserved in 10% formalin then transferred to 70% ethanol for later analysis. It was not determined whether organisms were dead before preservation so existence in the sample was only evidence that the organisms were no longer living within the epifaunal habitat on the panels. We assumed that an epifaunal organism would not leave the sessile community differentially in response to treatment unless it was experiencing some stress.

One-third of the water (at appropriate DO level) in each treatment container was changed every 6 hours for the 12 and 24 hr treatments to maintain pH above and ammonia levels below stress levels and to prevent build-up of toxic ammonia wastes. The pH and ammonia levels were checked randomly before and after water changes. pH never dropped below 7 and ammonia (NH_3) remained below 0.055 mg/l for all treatments which is below stress thresholds determined for fish (Foss et al. 2004, Lemarie et al. 2004). The removed water in the 12 and 24 hr treatments was not sieved but considerable care was taken not to remove any visible organisms with the water. Therefore, the 12 and 24 hour samples may not include all individuals which vacated the panels. As a result, the data were analyzed separately for each exposure period, given that the treatments were different.

One way Analysis of Variance (ANOVA) was performed on log (x + 1) transformed abundance data using SAS software. All taxa collected were analyzed.

Transformation was used so that data would meet ANOVA assumptions of equal variance and normal distribution of data. Correlation analysis was used to check that means were not correlated with variances. We used Fisher's Protected F test, which prevented analysis of pairwise treatment comparisons unless the overall F value was significant, to control experimentwise error. ANOVA mixed model was used to determine whether *Demonax microphthalma* percent cover was different according to treatment as of July 10, 2001. Photos, which were taken of all panels prior to low DO exposure, were used for percent cover estimates of sessile organisms. A 50 point fixed grid was superimposed over each photo and species cover was estimated according to the number of points occupied by that species. Percent cover data was arcsine squareroot transformed to meet ANOVA assumptions.

In addition to the univariate analysis, the entire suite of species which vacated the panels was analyzed using Analysis of Similarity (ANOSIM), Multi Dimensional Scaling (MDS) and Similarity Percent (SIMPER) routines by Plymouth Routines in Marine Ecological Research software (PRIMER v. 5). All abundance data were log (x +1) transformed before analysis to prevent overweighting of species with high abundances. SIMPER was used to determine species driving multivariate differences but the findings agreed with univariate analysis so it was not reported.

Results

The community composition of motile organisms found in the water at the end of the hypoxia (H) and moderately low DO (M) treatments was different than that found in

the water after normoxia exposure when averaged across exposure periods (Fig. 2-1) (ANOSIM: DO Global R = 0.231, p = 0.001; H vs M - R = 0.05 p = 0.20; H vs N - R = 0.56 p = 0.001; M vs N - R = 0.15 p = 0.029). There was no significant difference in motile epifaunal species composition between the hypoxia and moderately low DO treatments which indicates that the species driving the differences were as sensitive to moderately low DO as to hypoxia. The MDS graph represents the multivariate data of all species and their abundances per experimental unit.

Caprellids (Fig. 2-2), nematodes (Fig. 2-3), harpacticoids (Fig. 2-4) and the tube dwelling polychaete (Fig. 2-5), *Demonax microphthalma,* all left the panels in significant numbers in response to hypoxia and moderately low DO conditions (Table 2-1). Caprellid abundance was only significantly different for the 6 hour exposure period, perhaps because the water changes during the other two treatments (12, 24 hrs) removed dead caprellids. Caprellids were observed floating on the surface of the water after exposure to hypoxia, so impacts of low DO may have occurred primarily in the initial hours of exposure.

Although analysis of flatworm abundance did not differ if each exposure period was considered separately (Table 2-1), significantly more flatworms were found in the hypoxia and moderately low DO treatments when differences by DO level were averaged across exposure periods ($F = 5.04$, $df = 2, 49$, $p = 0.01$). However, the known barnacle predator, *Stylochus ellipticus*, abundance did not differ by treatment when analyzed separately (Table 2-1).

Another dominant member of the sessile community, *Demonax microphthalma*, responded to low DO stress only after 12 hours of exposure (Fig. 2-5). Sabellid

polychaetes have been observed leaving their tubes and free swimming during microscope analysis of live communities. In Jewett (2005, Ch. 1), cover of *D. microphthalma* virtually disappeared on the panels exposed to hypoxia. Sabellid percent cover did not differ among the treated panels at the time of the motile species collection, so relative abundance of *D. microphthalma* by treatment was not a factor (Fig. 2-6).

Several taxonomic groups did not respond to low DO treatments including nereid polychaetes, *Polydora* spp., *Corophium* spp., gammarid and ampithoid amphipods. Mud crabs, ostracods, snails and isopods were not abundant enough in treatments to determine a univariate response although they were represented in the MDS and ANOSIM analyses.

Discussion

Periodic low DO affects estuarine systems worldwide (Diaz & Rosenberg 1995, Modig & Olaffson 1998, Suzuki 2001, Gray et al 2002) and can cause changes in sessile (Jewett 2005, Ch. 1) and motile (Breitburg et al. 2003) community structure. Changes in the sessile community were readily evident in our experiments (Jewett 2005, Ch. 1) but analysis here indicates that low DO conditions also affect small, motile organisms which form an integral part of the hard substrate epifaunal community. Some of the organisms responded as predicted but others did not. Given the strong response of some motile epifaunal species, predator-prey dynamics both between sessile and motile organisms and within the motile community would be temporarily altered.

Caprellids, harpacticoids, nematodes and flatworms all vacated the sessile epifauna in significant numbers. Caprellids, harpacticoids and nematodes responded

quickly (< 6 hrs). Flatworm response increased with exposure time, but was only close to significant after 24 hours. The lack of low DO tolerance by flatworms and copepods has been reported by other researchers (Roman et al. 1993, Modig & Olafsson 1998, Qureshi & Rabalais 2001, Sagasti et al. 2001, Decker et al. 2004). Nematode tolerance to low DO is species-specific, with some species exhibiting tolerance and others not (Hendelberg & Jensen 1993, Modig & Olafsson 1998). All nematodes were grouped for this analysis, so species specific analysis was not possible. Despite crustaceans being labeled "sensitive" to low DO conditions (Gray et al. 2002), neither *Corophium* spp. nor the Gammarid amphipods responded to low DO stress (Table 2-1). Caprellids were sensitive to hypoxia and experienced high mortality. Unlike other motile epifauna whose status at the end of the manipulations was difficult to assess due to microscopic size, caprellids were visibly dead after exposure to low DO.

Three important predators did not vacate the panels in response to low DO stress. Nudibranchs, in general, and *Doridella obscura*, specifically, which is a known grazer of encrusting bryozoans, were not found in our collection of the organisms that dropped out of the epifaunal community. Sagasti et al. (2001) noted decreased feeding and higher mortality for *D. obscura* when exposed to 1 mg l^{-1} DO in lab experiments. The lack of significant response in our experiment appears to refute the explanation that mortality of *D. obscura* caused the increase of the encrusting bryozoan, *Conopeum chesapekensis*, observed by Jewett (2005, Ch.1) on panels exposed to hypoxia. An important barnacle predator, *Stylochus ellipticus*, also did not respond as predicted given its lower tolerance in other studies (Sagasti et al 2001). Finally, *Nereis* spp. larvae, which have been found in

high numbers in low DO water (Powers et al 2001) may reflect the higher tolerance of this group and its lack of response despite its presence in all treatments.

Space is a limiting resource in most epifaunal communities. Low DO caused *Demonax microphthalma* to leave its sand tubes. The relatively lower cover of the sabellid in hypoxia treated panels may have been caused by adults leaving the panels (Jewett 2005, Ch. 1). The cover of *D. microphthalma* was, on average, lowest on the panels repeatedly exposed to hypoxia for up to 24 hours (Jewett 2005, Ch. 1). However, the ability of *D. microphthalma* to recolonize may be enhanced if it can disperse as an adult (Breitburg 1992). It is unclear whether this sabellid polychaete can recruit and build a new tube as an adult.

Implications for changes in predator-prey dynamics within the epifaunal community are evident. The increased vulnerability of stressed prey has been documented in pelagic systems (Purcell et al. 2001, Decker et al 2004) and for benthic macrofauna (Breitburg 1992, Pihl et al. 1992, Nestlerode & Diaz 1998, Breitburg et al. 1999). Harpacticoids are prey to anemones (Holohan et al. 1998), grass shrimp (Gregg & Fleeger 1998), nematodes (Kennedy 1994), flatworms (Kennedy 1994), caprellids (Caine 1980), larval and demersal fish (Gee 1987, Marcus 2001, Turner 2004) and perhaps hydroids. If harpacticoids are flushed out of their epifaunal habitat by low DO, their encounter rates with predators may increase although this depends on the effect of low DO on their predators. If they are able to escape the initial layer of sessile predators, which include anemones and hydroids, their exodus represents a net energy transfer away from the disturbed area. The same holds for caprellids, flatworms and nematodes. Caprellids are prey to fish (Caine 1980) and amenones (personal observation), nematodes

are prey to other nematodes (Kennedy 1994), and flatworms are prey to fish and crabs. Of course, fish and crabs are also sensitive to low DO but have been reported to move into areas immediately post disturbance before prey have recovered (Pihl et al 1992, Nestlerode & Diaz 1998). Energy may be transferred away from the impacted sites until recolonization, the speed of which may depend on the geographic scale of the disturbance.

It is important to recognize that the motile epifaunal response was not limited to hypoxia but included responses to less severe low DO. The harpacticoid response was not limited to hypoxia. By 12 hours, these benthic copepods responded to moderately low DO (< 4 mg l) levels with an increase in movement (Fig.2-4). The delayed response to moderately low DO may benefit harpacticoids during diel low DO events which generally do not last for more than two hours in the pre dawn period (Jewett 2005). Caprellids and nematodes only responded in significant numbers to hypoxic conditions.

Although the geographic scale of hypoxic disturbance in estuaries is relatively well understood, the scale of moderately low DO conditions is not (Jewett 2005, Ch.1). Recolonization of hard substrates by motile epifauna can happen quickly and may be independent of the scale of the disturbance given the high mobility of the organisms (Cristoni et al. 2003). Breitburg (1992) posits that motile, benthic organisms have an advantage over sessile ones because adults and juveniles can capitalize on resources more quickly than the larvae of sessile organisms. In the end, the effects on the sessile organisms, such as sabellids, may be longer lasting than the motile residents because they must rely on larval supply to recover.

Acknowledgements

Dr. Anson Hines and Dr. Gregory Ruiz of the Smithsonian Environmental Research Center supported this research both financially and with scientific guidance. I would like to acknowledge the valuable assistance given by J Blum in conducting the experiment, collecting the organisms and in identifying and counting them. This work would not have been possible without K Sebens' advice and use of his laboratory space. I gratefully acknowledge financial support from the Smithsonian Environmental Research Center and the Smithsonian Institution Predoctoral Fellowship program.

Table 2-1 One way ANOVA results comparing species abundances according to DO treatments within each exposure period. All abundance data were log (x + 1) transformed before analysis.

Species	Exposure Time	6 hrs F	6 hrs p	12 hrs F	12 hrs p	24 hrs F	24 hrs p
Arthropods	**Caprellids**	8.43	**0.004**	2.05	0.154	0.37	0.696
	Harpacticoids	8.91	**0.003**	9.10	**0.002**	11.01	**0.001**
	***Corophium* spp.**	1.76	0.206	0.65	0.531	0.56	0.583
	Gammarids	1.15	0.343	0.90	0.422	0.74	0.495
Nudibranchs	***Doridella obscura***	2.61	0.106	0.42	0.667	1.91	0.184
	All Nudibranchs	1.90	0.185	0.07	0.933	1.14	0.349
Polychaetes	***Demonax microphthalma***	0.58	0.560	3.04	0.071	4.53	**0.030**
	***Polydora* sp.**	1.92	0.181	2.66	0.095	0.96	0.407
	Nereids	0.27	0.771	0.67	0.522	1.14	0.347
Nematodes	**Nematodes**	4.11	**0.038**	4.30	**0.028**	1.76	0.209
Flatworms	***Stylochus ellipticus***	0.59	0.567	1.60	0.226	0.16	0.855
	All Flatworms	1.41	0.275	2.19	0.139	2.86	0.091

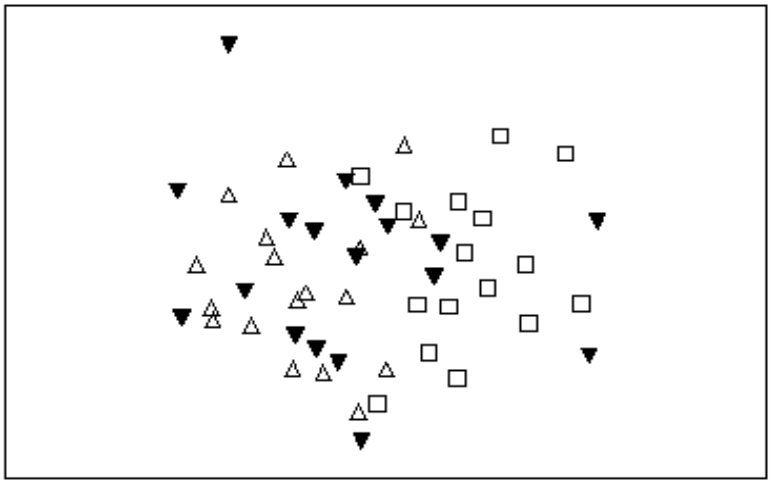


Fig. 2-1 Multi Dimensional Scaling (MDS) graph based on mobile epifaunal species abundances (log(x+1) transformed) collected from water at end of manipulations. N (square) = normoxia, M (downward triangle) = moderately low DO, H (upward triangle) = hypoxia. Stress = 0.21.

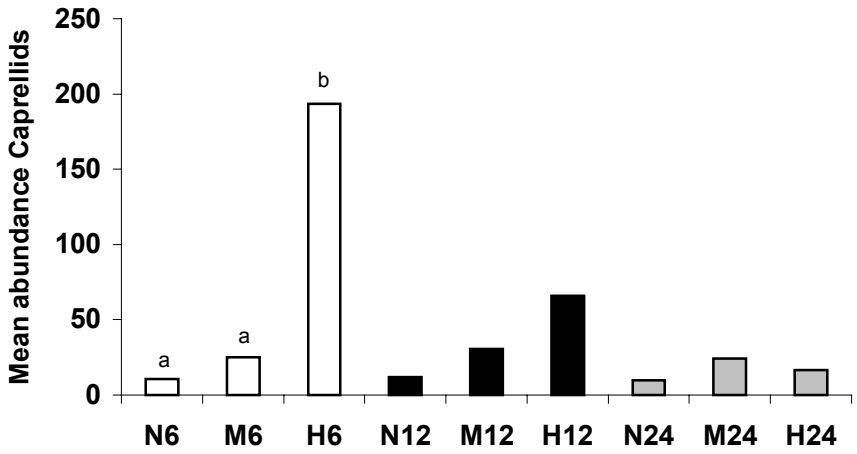


Fig 2-2. Mean caprellid abundance sieved from water per container at end of designated treatment (1 panel per container). H = hypoxia, M = moderately low DO, N = normoxia. Treatment numbers refer to exposure time (hrs). Pairwise comparisons were only made within each exposure time and differences were only significant for the panels exposed for 6 hours. Treatments with same letter were not statistically different. Data were log (x + 1) transformed for analysis then detransformed for graph. See Table 2-1 for ANOVA analysis. n = 6.

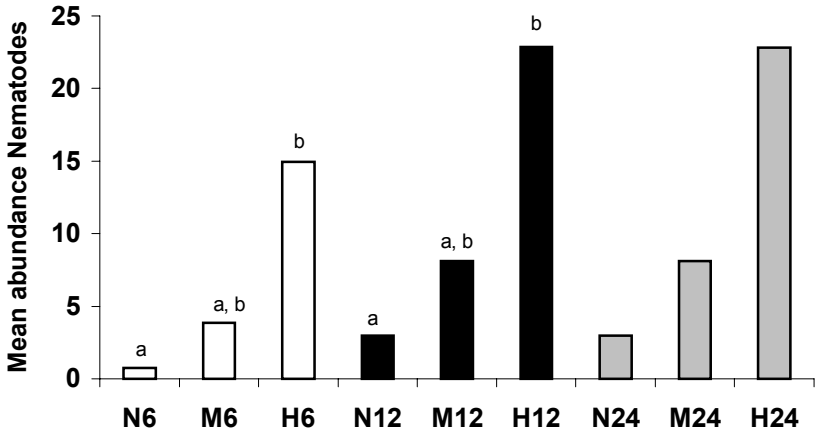


Fig 2-3. Mean nematode abundance sieved from water per container at end of designated treatment (1 panel per container). H = hypoxia, M = moderately low DO, N = normoxia. Treatment numbers refer to exposure time (hrs). Pairwise comparisons were only made within each exposure time (see methods) and were significant only for the panels exposed for 6 and 12 hours. Treatments with same letter were not statistically different at .05 level. Data were log transformed for analysis then detransformed for graph. See Table 2-1 for ANOVA analysis. n = 6.

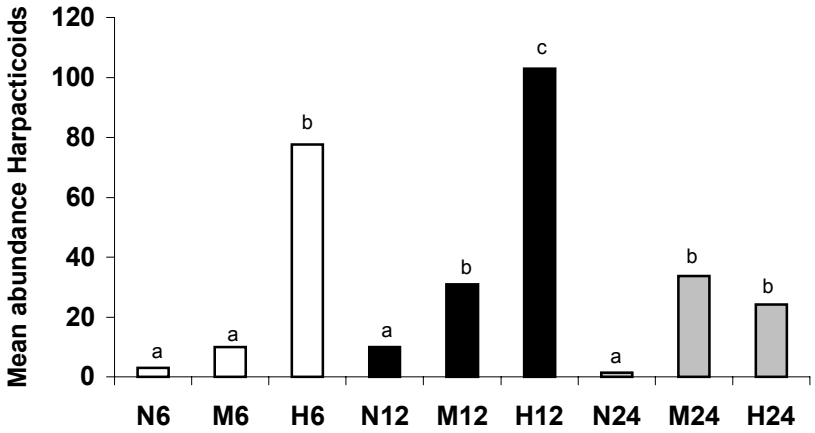


Fig 2-4. Mean harpacticoid abundance sieved from water per container at end of designated treatment (1 panel per container). H = hypoxia, M = moderately low DO, N = normoxia. Treatment numbers refer to exposure time (hrs). Pairwise comparisons were only made within each exposure time (see methods). Treatments with same letter were not statistically different at .05 level. Data were log transformed for analysis then detransformed for graph. See Table 2-1 for ANOVA analysis. n = 6.

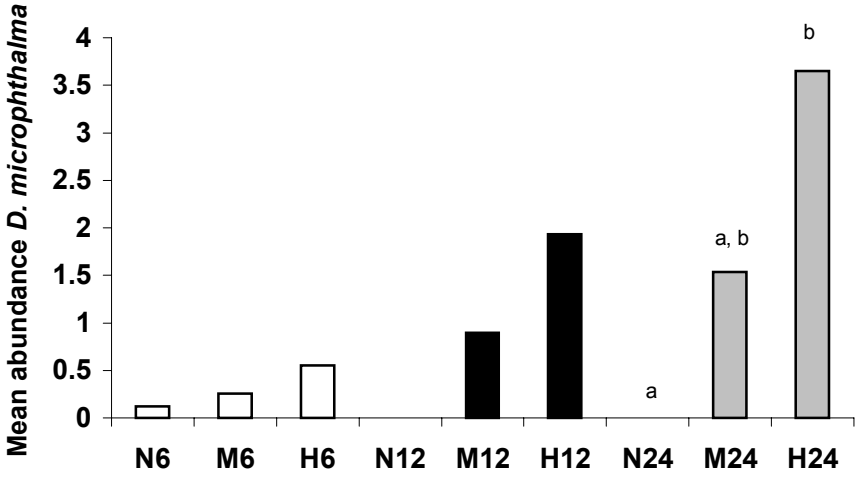


Fig 2-5. *Demonax microphthalma* abundance sieved from water per container at end of designated treatment (1 panel per container). H = hypoxia, M = moderately low DO, N = normoxia. Treatment numbers refer to exposure time (hrs). Pairwise comparisons were only made within each exposure time (see methods) and were significant only for the panels exposed for 24 hours. Treatments with same letter were not statistically different at .05 level. Data were log transformed for analysis then detransformed for graph. See Table 2-1 for ANOVA analysis. n = 6.

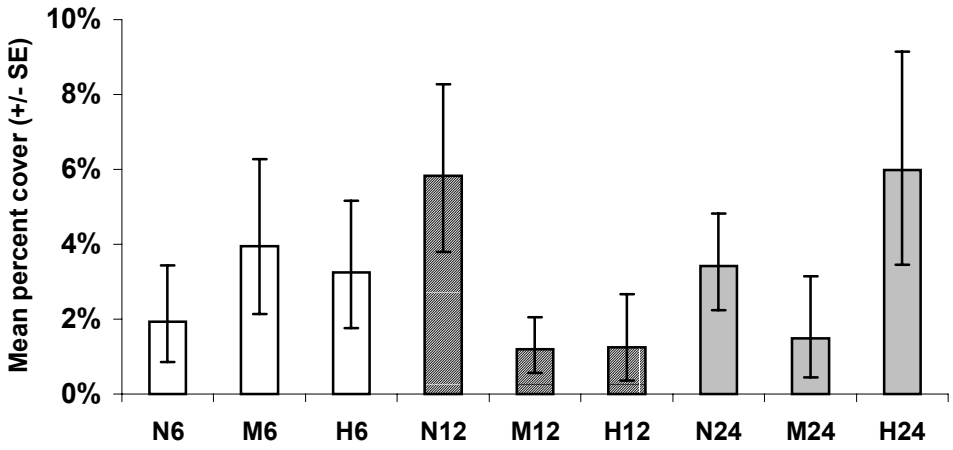


Fig 2-6. *Demonax microphthalma* percent cover on July 10, 2001 according to treatment. H = hypoxia, M = moderately low DO, N = normoxia. Treatment numbers refer to exposure time (hrs). One way ANOVA analysis among treatments was not significant (df = 8, 59; F = 1.22, p = 0.31). n = 8.

Chapter 3: Effects of Near Shore Low Dissolved Oxygen on Epifaunal Community Composition

Abstract

We investigated how shifts in the hard substrate, epifaunal community of lower Chesapeake Bay may be correlated with duration of exposure to low dissolved oxygen (< 4 mg l^{-1}) at shallow depths. Prior research indicated a shift in dominance from barnacles to a serpulid polychaete (*Hydroides dianthus*) with exposure to low dissolved oxygen (DO). We hypothesized that such shifts may change benthic processes and create opportunities for non-indigenous species. At eight shallow sites, we measured physical and biological environmental variables including dissolved oxygen, temperature, salinity, chlorophyll a, turbidity, water circulation, bacteria level and macro-predator abundance to determine the power of low DO events versus other variables to explain the variation in community composition and specifically the cover of invasive and cryptogenic species. Settling panels were deployed in May 2002 at each site to measure seasonal composition of the fouling community. Upon retrieval in late summer, percent cover of sessile species and species richness for sessile and motile species were measured for each panel. Percent of total days exposed to low dissolved oxygen (%LDO), water circulation, and salinity together explained 80% of the variability in the cover of sessile native species. The cover of invasive or cryptogenic species including three tunicates (*Diplosoma listerianum, Botryllus schlosseri,* and *Molgula manhattensis*), one serpulid (*Ficopomatus enigmaticus*) and one hydroid (*Garveia franciscana)* was also correlated with %LDO ($\rho = 0.51$). Barnacle cover decreased and *H. dianthus* cover increased as exposure to moderately low DO (2 mg $l^{-1} < DO < 4$ mg l^{-1}) increased. A high biomass, low diversity community

dominated by invasive/cryptogenic species was associated with chronic low DO (< 4 mg l^{-1} DO for > 40% days). Species distribution overall was patchy, and biotic and abiotic environmental conditions explained 24 to 80% of the distribution of distinct taxa.

Introduction

Physical and biological aspects of estuaries have been studied to determine how they covary with the composition and biomass of marine and estuarine epifauna and infauna. Community composition or certain species abundances have been correlated with organic (eutrophication) and inorganic (metals) pollution (Pearson & Rosenberg 1978, Warwick 1986, Kocak et al. 1999), dissolved oxygen (Sagasti et al. 2000, Mistri et al. 2002, Laine 2003), ammonia (Mistri et al. 2002), sediment type (Sherk 1971, Ysebaert & Herman 2002, Laine 2003), chlorophyll a (Ysebaert & Herman 2002), flow (Bingham & Young 1995, Watson & Barnes 2004), turbidity (Bourget et al. 2003), salinity (Giangrande & Fraschetti 1996, Powilleit & Kube 1999, Ysaebert & Herman 2002, Laine 2003), and temperature (Hutchins 1947, Santos et al 1996, Roy et al. 1998, Bourget et al. 2003). Most studies have related these environmental variables to infaunal distributions (Cory 1967, Mannino & Montagna 1997, Ritter & Montagna 1999, Ysebaert & Herman 2002) but correlations with epifauna have also been found (Sagasti et al. 2000, Broitman et al 2001, Bourget et al. 2003).

Dissolved oxygen (DO) is one of many environmental variables used to assess water quality. Low DO represents a serious threat to estuarine biota (Diaz & Rosenberg 1995). Estuaries are particularly vulnerable to periods of DO deficit due to anthropogenic

stresses including eutrophication, shoreline modification, dredging for shipping lanes, shipping traffic, pollution and sewage outfall, and global warming. Twenty to fifty percent of mid-Atlantic waters experience eutrophic conditions and depleted DO and, as a result, 37 % of all mid-Atlantic estuaries (Kiddon et al 2003) and 47% of the Chesapeake Bay (Dauer & Llanso 2001) are considered to have degraded benthic communities. Eutrophication is recognized as a growing problem in many systems including the Baltic, Black and Mediterranean Seas, and along the coastlines of North and South America, Africa, India, Southeast Asia, Australia, China and Japan (Nixon 1990, Richardson & Jorgensen 1996). Hypoxia and anoxia occur seasonally throughout the mainstem of the Chesapeake Bay in deep waters below 6 m, but moderately low DO (2 - 4 mg l^{-1}) can also occur periodically in shallow waters due to organic enrichment, high surface temperatures and reduction of natural water circulation (Pearson & Rosenberg 1978, Breitburg 1990, Diaz & Rosenberg 1995). Global warming may worsen the conditions, especially in shallow water environments.

Abiotic environmental conditions were once credited with controlling the distribution of organisms (Hutchins 1947, Kinne 1971), until researchers showed that life history and ecological relationships (Connell 1961, Paine 1966, Woodin 1976) shaped distribution of some marine epifauna. Now focus has shifted back to understanding how environmental conditions affect ecological relationships and population distributions (Lubchenco & Menge 1978, Menge & Sutherland 1987, Borcard et al. 1992, Ysaebert et al. 2003). Computer intensive, multivariate analysis techniques have facilitated matching complex sets of biological data with environmental data to determine correlation between environmental and biological variation (Clarke &Warwick 2001).

Most studies have focused on how hypoxia (DO < 2 mg l^{-1}) and anoxia affect individual species (Sagasti et al. 2001) and communities (Ritter & Montagna 1999), but little attention has been given to how moderately low DO (2 - 4 mg l^{-1}) conditions in shallow waters may structure biotic communities. We conducted this field survey to assess how DO varies in shallow water environments and to determine whether exposure to low DO affected epifaunal community development. Unlike other studies which relied on DO data collected infrequently (e.g. twice a month) (Dauer & Alden 1995, Powillet & Kube 1999, Laine 2003) to study its effects on biota, this study collected DO data 5 –7 (or more) times per week at the eight sites. Daily DO readings monitored closely the fluctuating DO conditions and made it possible to estimate low DO exposure at time scales (days) that can have significant effects on epifaunal community structure for the designated shallow water environments (Jewett 2005, Ch.1 & 2). Frequency, intensity, duration and patch size are important aspects of a disturbance and may determine how species are affected (Miller 1982, Sousa 1984). The data collected in this study contributed to an understanding of the dynamics of shallow water low DO events including: how often DO drops to stressful levels, how low it drops, and how long it stays there.

The goals of this study were: 1) to measure spatial variation in biotic community structure (cover and composition of sessile and motile species) in fouling community among sites; 2) to characterize spatial and temporal variation among microhabitats for DO and six other environmental variables; and 3) to test for correlation of local epifaunal community structure with microhabitat variation in environmental conditions. We were particularly interested in whether invasive and cryptogenic species as a group were

benefited by stressful DO conditions in the field as indicated by controlled experiments (Jewett et al. 2005). Additional environmental variables were measured because they have been shown to regulate structure of biotic communities in other systems.

Concern about non-native species in marine systems and effects they may have on biodiversity has compelled scientists to examine why certain marine systems are more vulnerable to invasion than others (Elton 1958, Tilman 1997, Lonsdale 1999, Levine 2000). Non-native species may respond to certain environmental conditions with an increase in abundance (Fox & Fox 1986) depending on: level of anthropogenic degradation; propagule supply; timing of the arrival of recruits; diversity of habitats; match between the donor and receiving environment; and level of competition and predation by native species (Carlton 1996a, Ruiz et al. 2000). Disturbance experienced by the receiving environment is also a factor that may facilitate invasion by freeing up otherwise limited resources. Opportunist species, such as some non-native species, may benefit from disturbance, especially if local populations increase rapidly in response to cleared space (Grassle & Grassle 1974).

Methods

Site Description

Eight sites with previously surveyed fouling communities were selected in the polyhaline zone of the Chesapeake Bay (Fig. 3-1). Sites were chosen with varying epifaunal communities to facilitate the matching of the environmental variability with the biotic community differences. These sites were all located along the lower western shore

of the Chesapeake Bay and had salinity regimes within 2-3 ppt of each other as measured during three summers prior to this study. The polyhaline zone was chosen because species diversity increases with salinity, which maximized our ability to detect effects of varying DO levels on benthic diversity. Six of the sites were marinas (BI, BP, HYC, NYC, WB and YC) and of the other two sites, one (NT) was a docking facility for navy and cruise ships and the site of the USS Wisconsin battleship museum and the other (VS) was the "ferry pier" (research facility) maintained by the Virginia Institute of Marine Science. Five of the sites had fixed docks (BI, NT, NYC, VS, YC) and three had floating docks (BP, HYC, WB) for attachment of the epifaunal sampling panels. Regions around sites were categorized by NOAA (1997) as highly to moderately polluted due to effluent from industrial and military facilities and to other human activities. Excessive chlorophyll a levels have been reported in the James and York Rivers (NOAA 1997, Kiddon et al. 2003). Most, except VS and YC, were located in and around the highly populated Norfolk city.

Epifaunal Sampling

Epifaunal community structure was characterized by analysis of species on settling panels, which consisted of 14 cm x 14 cm x 0.5 cm sanded, dark gray PolyVinylChloride (PVC) plates. Two sets of five panels each were attached to the underside of a brick and hung settling surface down with line from docks or piers between 1 and 2 m below mean low water (MLW) 1 – 2 m apart. The first set was deployed from mid-May until mid-August and the second set from mid-May until mid-

September Depth of sites varied, but the distance from the bottom substrate to the panel was never greater than 3 m at high tide or < 0.5 m at low tide.

Upon retrieval, all settling plates were preserved by first soaking an entire panel in MgCl solution (64 g MgCl per 1 liter seawater) for 15 minutes to relax organisms before placing them in 10% formalin. The September settling panels were analyzed with dissecting microscope with 100 point fixed grid to determine percent cover of organisms. Fixed grid was preferred over random sampling because open grid (nylon wire attached to metal frame) allowed exploration of species attached to the panel without removing the grid. Species richness for motile and sessile fauna and abundances of invasive species were recorded for each plate. Species identification and percent cover analysis for the August settling panels for four of the sites (BP, NT, WB, YC) were conducted by the Smithsonian Environmental Research Center (SERC) Invasions Lab but all SERC panels were also reviewed by Jewett to ensure agreement on species identification. A 50 point (dictated by the SERC invasion laboratory protocol) fixed grid was used to estimate species percent cover analysis for the August settling panels. Wet biomass or biovolume of the epifaunal community was estimated by measuring the differences in water displacement of the clean panel and panel with fouling organisms. Biovolume measurements were conducted when transferring plates from formalin to 70% ethanol within a month of retrieval.

Environmental Assessment

Environmental variables were measured at each site throughout the summer. Beginning at the end of June until mid-September, dissolved oxygen (DO), salinity and temperature were measured at least five times per week with YSI DO meters by trained, on-site volunteers. At all of the sites, except VS and NYC, salinity was measured with either a refractometer (NT and YC) or a hand-held, specific gravity salinity gauge. All DO meters were calibrated before taking each reading and compensated for salinity and temperature. Each meter was checked and adjusted biweekly. At VS and NYC, YSI 85 meters were used to measure DO, salinity and temperature. YSI hydrolabs were deployed intermittently at WB, VS and HYC to measure DO, salinity and temperature when volunteers were not available. Only the noon readings from the hydrolabs were used to calculate the average DO and %LDO (percent of total time with DO below 4 mg/l) (Fig. 2). By including both %LDO and average DO in the model, we tested for the different effects of total time spent below critical DO thresholds (below 4 mg/l) (Ritter & Montagna 1999) versus the general DO conditions.

Chlorophyll a and turbidity were measured on a biweekly basis. A Niskin grab sampling device was used to collect two 200 ml water samples from 1m depth. Each sample was filtered, acetone processed then frozen until analyzed with 10-AU digital fluorometer. Secchi depth was measured with 70 cm diameter secchi disk (Preisendorfer 1986).

Water movement and bacterial counts at each site were measured once during the summer. Because flow (water movement) measurement was integrated over three days, one deployment was considered sufficient to distinguish sites. Water movement (or flow)

was measured over three days (August 11- 14, 2002) using plaster spheres (n=2) attached to bottom surface of bricks that were deployed at 1 m below MLW. A control sphere was also deployed inside a 20 L bucket with holes (to prevent plaster saturation) on site in calm waters (usually sitting on the bottom) to measure plaster dissolution in seawater of the same salinity and temperature as the spheres exposed to flow. Spheres were dried at 65 ^{o}C for $\geq$ 24 hours then weighed both before and after deployment to calculate total mass transfer. The diffusion factor is the ratio of weight of material dissolved from an experimental sphere to that of the mass lost from the control sphere maintained simultaneously in calm water of the same temperature and salinity (Doty 1971). The relationship between current velocity and weight loss of plaster spheres has been found to be linear unless the sphere loses more than 70% of its original mass (Jokiel & Morrissey 1993). The relationship is not linear in turbulent flow (Porter et al. 2000). At two of the sites, NYC and VS, the dissolution spheres were completely gone at the end of three days so distinguishing between these two sites was not possible although they could be characterized conservatively as having "high water movement". In addition, the two spheres deployed at NT lost slightly more than 70% of original mass (down to 26%).

Two bacterial samples were collected from each site on July 23, 2002 according to the following protocol. Scintillation vials were preloaded with 270 microliters of filtered, buffered 10% formalin. At sample retrieval, two 10 ml water samples collected separately at 1 m depth with Niskin grab sampler was added to each preloaded scintillation vial. Samples were analyzed for total bacterial count using flow cytometry. Given that the bacteria measurements were limited to one collection, we did not use this

for the biotic environmental (BioENV) correlation analysis, but we included the findings for descriptive purposes.

To gauge the potential level of predation on the sessile community by large motile predators, the September panels were retrieved using a box net with 5 mm mesh mounted on a PVC frame to trap motile organisms residing on and around the sessile community. The box net was submerged carefully below the panel then the panel and net were retrieved together to trap organisms swimming around and clinging to the panel. These organisms were identified, counted and released at the time of plate retrieval.

Data Analysis

All percent cover data were arcsine squareroot transformed before applying statistical analysis to meet assumptions of normal distribution and equal variance by data. One way Analysis of Variance (ANOVA) was used to determine differences in species cover, biovolume and species diversity (H') (base e) according to site (treatment). Most of the biotic – environmental matching analyses were performed on the percent cover data from the September retrieval. An exception to this was an analysis of the August cover of *Hydroides dianthus,* since the cover of live *H. dianthus* diminished between August and September. Species incidence for Multidimensional Scaling graphing (MDS) and Analysis of Similarity (ANOSIM) analyses was calculated on motile and sessile species presence/absence data from August and September retrievals combined.

The correlation between environmental variables and biotic composition was calculated with Biotic-Environmental Matching (BioENV) on PRIMER software version

5 (Clarke & Warwick 2001) or, for the sole case of analyzing the correlation between environmental variables and entire suite of native species, BioENV and Biotic Variable Step (or BEST) in PRIMER version 6 (Clarke et al. 2005). RELATE was used to test the a priori hypothesis that %LDO affected percent cover of barnacles, invasive/cryptogenic species and *Hydroides dianthus* as suggested by prior experiments (Jewett 2005, Ch. 1). Significance values for correlation coefficients were only reported for the BEST and RELATE analyses. The BioENV routine was used only to explore potential relationships between measured environmental variables and biological data so no significance values were generated (R. Clarke, pers. comm.)

Ysaebert & Herman (2002) concluded that long term averages in environmental variables were more important for explaining differences in the benthic community by station than short term temporal variation in those variables. Therefore we used the seasonal averages for measured environmental variables in the BioENV analysis. Differences in percent cover and incidence of individual species and groups of species between sites were measured against differences in environmental parameters at these same sites to generate a measurement of correlation (ρ). The correlation statistic indicates the percent of variation in the biotic community explained by the differences in environmental variables. The normalized Euclidian distance was used when generating a similarity matrix for the environmental data, which was then compared to a Bray-Curtis similarity index of the multivariate biotic data.

Both the average DO per site (calculated from midday DO reading) and a %LDO variable calculated as the percent of the summer days which experienced DO below 4 mg l^{-1} were used in the BioENV analysis. The BioENV routine expects correlation between

environmental variables so using two parameters based on the same data was allowed (R. Clarke pers. comm.).

Results

Environmental Conditions Across Sites

One site, NT, was distinguishable from the others according to four of the environmental variables. It experienced the most days of moderately low DO (Fig.3-2), had the lowest average chlorophyll a level and bacteria count, and had the clearest water (Fig. 3-3). It was more similar to the other sites in terms of salinity, flow and temperature (Fig. 3-3). Both NYC and NT were distinguished by the MDS graph of the multivariate environmental data from each site. %LDO, low chlorophyll a and clear water (deep secchi depth) strongly distinguished NT while high chlorophyll a and high flow distinguished NYC (Fig. 3-4).

DO at the sites never dropped below 2 mg l^{-1} and it ranged daily across sites from just above 2 to 10 mg l^{-1} with most of the readings between 4 and 6 mg l^{-1} (Fig. 3-5). All sites experienced some days with DO below 5 mg l^{-1}, a level which has been shown to cause stress in estuarine biota (Fig. 3-2) (www.EPA.org, Diaz & Rosenberg 1995). NT and YC were notable in that the readings at these two sites dropped below 3 mg l^{-1} (Fig. 3-2). For NT, 40% of the days had readings below 4 while at YC this happened for only a few days in late July and early August and comprised < 10% of days.

The hydrolab data (readings up to six times per day) reflected the diel fluctuations in DO at three sites. YSI hydrolabs were intermittently used at WB, VS and HYC during periods when volunteers were not available. One 3-day period in late August was isolated when both HYC and WB experienced moderately low DO (< 4 mg l^{-1}). HYC had two DO minima during each 24 hr period, one around 10:00 hrs and another at 24:00 hrs. However, at WB, DO dropped below 4 mg l^{-1} DO on the second morning and remained consistently low for almost two days (Fig. 3-6). This is evidence that daily readings can only approximate the underlying fluctuations and that sites which had DO readings above 4 mg l^{-1} at midday were probably experiencing DO below that threshold at other times of day (or night). The difference in DO fluctuations between these two sites reflects the difference between a site experiencing unnaturally long low DO (WB) versus a site experiencing expected diel, short term low DO (HYC). The low DO event at WB was registered in the %LDO calculation (Fig. 3-2) unlike the HYC event.

Secchi depth was greatest both on average and over time for NT, BP and WB (Fig. 3-3). Secchi depth is a measurement of light attenuation caused by sediment, dissolved organic matter (DOM), algae and other plankton in the water.

Average water movement (or flow) as measured by mass diffusion was greatest at NYC and VS (Fig. 3-3). Given the loss of the spheres it is impossible to distinguish flow between these two sites. NYC is located at the mouth of the Lafayette River as it meets the Elizabeth River and VS is located 8 km up from the mouth of the York River. Both are strongly influenced by tidal currents. BP, a yacht club off the Lynnhaven River, also experienced relatively high flow although a strong current was not noticeable. At the other end of the spectrum, BI, located where tidal marsh meets the Back River, had the

lowest average flow (Fig. 3-3). Flow and %LDO were not correlated with each other although the highest flow sites, VS and NYC, experienced very few to no days of low DO.

BP, the site located closest to the mouth of the bay, experienced the highest average salinity of all the sites at > 26 ppt (Fig. 3-3). Although the average salinity for YC was similar to BI and NT around 23 ppt, the daily salinity readings were more frequently lower at YC, closer to 20 ppt. BI, on the other hand, had readings closer to 25 ppt for most of the summer but then, in early September, experienced a dramatic drop (see bars on salinity graph (Fig. 3-3) which indicated maximum and minimum salinity for the season) which accounts for its lower overall average. The other four sites' average salinity ranged between 24.1 ppt at NYC and 25.4 at WB (Fig. 3-3).

Average chlorophyll a levels were generally similar across sites although temporal variability differed. All sites experienced one period of high primary production, in late June or early July, except for NYC which experienced two dramatic peaks (Fig. 3-7). NYC and NT experienced the highest (19.6 µg/l) and lowest (5.7 µg/l) average primary productivity respectively (Fig. 3-3). These two sites are located only a few miles apart on the Elizabeth River (Fig. 3-1). At NYC, the two chlorophyll a peaks exceeded 30 µg /l. The James River into which the Elizabeth River flows has been cited for increasing chlorophyll a levels (NOAA 1997). All sites experienced medium to high chlorophyll a levels over the sampling period as defined by NOAA (1997). For the first two sampling periods, primary productivity at NT was not different than six of the sites, but then it dropped and remained significantly below the others starting in mid July until late August. The decline in DO at NT coincided with this reduction in primary

productivity (Fig. 3-5, 3-7). Given that phytoplankton is a primary supplier of DO to surface waters, this correlation may explain the drop in DO. Reasons for the reduction in primary productivity are less clear. Toxic pollutants such as heavy metals which may cause mortality in phytoplankton have been recorded at dangerously high levels in the Elizabeth River where both NT and NYC are located (Chesapeake Bay Program 1999).

Average water temperatures varied between 26.5^{o} C and 28.0 ^{o} C across the eight sites. BP and WB, the two sites closest to the mouth, had the lowest average temperatures (Fig. 3-3). The fluctuations over time were remarkably consistent across all sites although only four sites were graphed (Fig. 3-8). The cyclical temperature swings correlated roughly with the tides, such that neap tides corresponded with higher temperatures in shallow waters due to low mixing of the water column, while spring tides had the opposite effect. BI experienced the greatest temperature swings, from 22.4^{o} C to 30.8 ^{o} C. NT had the highest temperatures of all the sites in late summer (Fig. 3-8). Both water temperature (Fig. 3-8) and DO levels (Fig. 3-5) followed tidal cycles. Higher water temperatures and lower DO generally corresponded to neap tides which did not generate sufficient water column mixing. Lower temperatures and higher DO corresponded to spring tides which mixed deeper, cooler water with warmer surface waters.

Bacteria levels were measured at each site once at mid summer (July 10, 2002). The average counts (two independent samples were taken at each site) ranged from 6.5 to 10 million cells/ml for seven of the sites (Fig. 3-3). NT, the exception, had 3.2 million cells/ml. If bacterial consumption of DO were fueling the low DO conditions at NT (Officer et al. 1984), then bacteria levels should have been higher or at least comparable

to the other sites. Low bacteria level did correspond with the drop in chlorophyll a levels at NT (Fig. 3-6).

General Characterization of the Hard Substrate Epifauna

Each site had a different suite of dominant species including bryozoans, barnacles, tunicates, serpulids, sabellids, sponges, hydroids and bivalves. All are sessile filter feeders and primary space occupiers in the lower Chesapeake Bay fouling community. Although this community was sampled with artificial panels, these same species are also found on oyster shells in the lower bay (pers. obs.). Thirty eight sessile species were identified across the eight sites (Table 3-1). In addition, 33 micro-motile species (vs macro-motile species which were identified and counted at retrieval) were identified (Table 3-2). The micro-motile species, those found living integrated within the sessile community, included errant polychaetes, nudibranchs, flatworms, amphipods, crabs, snails, pycnogonids and nematodes.

Seven sessile and three motile invasive or cryptogenic (sensu Carlton 1996b) species were identified among the eight sites including two invasive, colonial tunicates (*Diplosoma listerianum, Botryllus schlosseri)* and a cryptogenic solitary tunicate (*Molgula manhattensis*), an invasive serpulid (*Ficopomatus enigmaticus*), an invasive hydroid (*Garveia franciscana)*, an invasive anemone (*Diadumene lineata*), an invasive barnacle (*Balanus amphitrite*), an invasive nudibranch (*Cuthona perca*) and a cryptogenic nudibranch (*Tenellia adspersa*) and a recently identified invasive isopod (*Synidotea laevidorsalis?*) (invasions.si.edu/nemesis). See Jewett (2005, Ch. 1) for

invasion history for *B. schlosseri*, *M. manhattensis, D. lineata* and *F. enigmaticus*. When invasion history is not strongly documented by baseline data, species are labeled cryptogenic.

Diplosoma listerianum most likely originated from the North East Atlantic. It was first identified along North East coast of U.S. around 1880 but was not positively identified in Chesapeake Bay until 2001 (invasions.si.edu/nemesis). *Garveia franciscana* was identified by Frey (1946) in Chesapeake Bay and has an Indo Pacific origin (Carlton 1979a,b). *Balanus amphitrite* also has an Indo-Pacific origin (Cohen & Carlton 1995) and was first identified in Lynnhaven Bay at the mouth of the Chesapeake Bay in 1967. *Cuthona perca*, of tropical Western Atlantic origin, was first positively identified in the Chesapeake Bay in 1994 by Ruiz (invasions.si.edu/nemesis). *Tenellia adspersa* is a cryptogenic nudibranch which was first identified in the Chesapeake Bay in 1967 (Cory 1967). Finally, *Synidotea laevidorsalis?* was positively identified on fouling panels at NT from this study (Marilyn Schotte, personal communication) and was originally described in California (Poore 1996).

Total species richness including sessile and motile species ranged from 38 to 48 species per site over the two retrieval periods (Table 3-1, 3-2). The difference in the number of sessile species accounted for the largest variation by site (Table 3-1). Six of the sites had between 22 – 24 sessile species while two of the sites, HYC and YC, had 29 and 28 sessile species respectively. Although many of the sites averaged the same species richness when both motile and sessile species were combined, the identity and incidence of those species was not the same across those sites (ANOSIM Global R =

0.518, p = 0.001) (Fig. 3-9). Species composition was indistinguishable only between WB and BP. The rest of the pairwise comparisons were significant at p = 0.001.

Species diversity as measured by the Shannon-Wiener index (H') differed according to site with species diversity at NT significantly lower than all other sites except YC (Fig. 3-10). Species diversity was calculated for the September retrieval percent cover data. The sites can be divided into three groups according to diversity: highest species diversity was found at HYC, NYC, WB, VS, mid level at BI and BP and lowest at NT and YC.

These epifaunal biotic communities were generally less space limited in 2002. Average cover of empty space, either bare or vacated by tube worms or barnacles, varied between 12% at YC to 36% at VS in August and between 16% at BP and 47% at YC in September (Fig. 3-11). This compares with < 10% bare space at VS in August 2001 (Jewett 2005, Ch.1). Biovolume was greatest at NT, and lowest at BI and HYC (Fig. 3-12). The NT panels were dominated by the solitary cryptogenic tunicate, *Molgula manhattensis*, which accounts for the greater volume displacement at this site. Biovolume overall was low across all sites. In 2001, biovolume measurements of undisturbed panels at VS averaged 180 ml (Jewett 2005, Ch. 1) as compared with 79.6 ml at VS in 2002.

Epifaunal Differences by Site

Five general types of communities developed across the eight geographic sites based on species occupying the primary space: (1) at HYC and BP, panels were > 50%

covered by barnacles and bryozoans, (2) at BI and NT, panels were 23 to 50% covered by tunicates, *Perophora viridis*, a colonial native, at the former and *Molgula manhattensis*, a solitary cryptogenic form, at the latter, (3) at YC and WB, panels were between 40 – 60% covered by *Hydroides dianthus* and sponge, (4) at NYC, *Demonax microphthalma*, a tube dwelling sabellid, dominated the panels and (5) at VS, hydroids and bivalves had higher abundances than at any of the other sites (Fig.3-11). The cover of *H. dianthus* at YC and WB declined between August and September (Fig. 3-11). The panels at these two sites were still covered by calcareous tubes in September but the worms were not present.

Abundances of invasive or cryptogenic species differed significantly among sites and increased between August and September for five of the sites (Fig. 3-13). Two other sessile invasive species were identified: *Diadumene lineata*, an anemone, and *Balanus amphitrite*, a barnacle, but their incidences were so low they never occupied as much as 1% of the primary substrate. *Botryllus schlosseri* was only found at WB and NT and the abundance did not differ between them. *Molgula manhattensis* and *Ficopomatus enigmaticus* had highest abundances at NT. VS, which was one of four sites with the highest diversity of sessile species (Fig. 3-10), also had the lowest abundance of invasive/cryptogenic species (Fig. 3-13). The panels at NT had the highest cover of invasive/cryptogenic species of all the sites and the lowest cover of native species (Fig. 3-11). Regression analysis reveals a negative relationship between panel diversity and cover of invasive/cryptogenic species if NT site panel data were included ($y = -0.2675x + 0.8178$, $R^2 = 0.11$, $F = 4.80$, $p = 0.03$). Data were arcsine squareroot transformed before regression analysis. However, if the NT data were excluded (as outliers), then there was a

positive relationship ($y = 0.1761x - 0.1623$; $R^2 = 0.13$, $F = 4.85$, $p = 0.02$). As H' increased, the cover of invasive/cryptogenic species increased (Fig. 3-14). Sessile species richness did not vary significantly with invasive/cryptogenic species cover. If *Molgula manhattensis*, the cryptogenic tunicate with high cover at NT (Fig 3-11), was removed from the analysis, the relationship between H' diversity and invasive cover was no longer significant.

The number of distinct species of micro-motile organisms was comparable among sites although the identity of these species changed from site to site (Fig. 3-9, Table 3-2). Eight of the micro-motile species were present on at least one panel at every site (two amphipods, mud crabs, a nudibranch, two flatworms including *Stylochus ellipticus*, *Nereis* sp. and nematodes) while 9 – 12 other motile species were only present at some of the sites (Table 3-2). During the September retrieval, the macro-motile community included fish, mud crabs, grass shrimp, isopods and spider crabs. This community was dominated at most sites by mud crabs and fish with VS having a significantly high abundance of both. HYC also had a high abundance of fish. NT's macro-motile community was dominated by the newly invasive isopod *Synidotea laevidorsalis?* (identified for the first time in the Chesapeake Bay) which ranged in size from 1 – 2 cm long (Fig. 3-15). Isopods were completely absent at the rest of the sites except for one individual at NYC, which was not identified to species.

Correlation between Environmental Variables and Epifaunal Composition

Species composition of epifaunal communities was highly correlated with variation in DO among sites. %LDO, flow and salinity explained 80% of the native

community variation by site (Table 3-3). The native suite included all sessile species besides the recognized invasive or cryptogenic species. %LDO explained 51% of combined cover of the five prevalent invasive or cryptogenic species ($p = 0.07$) (Table 3-3). %LDO explained 78% of *Hydroides dianthus* cover ($p = 0.004$) in the August retrieval (but only 4% for the September retrieval) and 68% of barnacle cover ($p = 0.02$). Finally %LDO and salinity explained 64% of differences in average species diversity (H') by site (Table 3-3).

As predicted, the primary cover of barnacles decreased and the cover of *Hydroides dianthus* increased with increasing exposure to low DO across sites (Figs. 3-2, 3-11). The exception to this was at NT, which was chronically exposed to low DO for most of the summer. NT had negligible cover of both species but was dominated by *Molgula manhattensis* (Fig. 3-11).

The cover by the suite of invasive and cryptogenic species (*Botryllus schlosseri, Ficopomatus enigmaticus, Diplosoma listerianum, Molgula manhattensis and Garveia franciscana*) was best explained with two parameters, %LDO and secchi depth however %LDO alone did explain a significant amount of variation in this group of species (Table 3-3). The cover of invasive/cryptogenic species was highest at NT (Fig. 3-14), the site with chronic low DO (Fig. 3-5). In addition, the macro-motile community at NT, which was not included in the BioENV analysis, was dominated by an invasive isopod (Fig. 3-15).

These panel communities had significant bare or empty space. In some cases, such as at YC, the unoccupied space on the September panels was covered with vacated serpulid tubes. Average salinity explained 59% of the variability in empty space across

sites (Table 3-3) with the lower salinity sites exhibiting relatively higher open space (Fig. 3-3, 3-11).

The relative cover of bryozoans and sponges correlated well with measured environmental factors, which was not true for *Demonax microphthalma* or hydroids. Seventy three percent of the variability in bryozoan cover was explained by average DO and chlorophyll a levels, and sponge cover was correlated (57%) with differences in flow, temperature, secchi depth and chlorophyll a levels (Table 3-3). Other studies have shown a relationship between abundance of sabellid polychaetes and sediment type which was not analyzed in this study.

Water movement (or flow) was a factor to which several of the taxonomic groups responded. When analyzed with multivariate approach, native species as a group were correlated with flow, although when the analysis was partitioned, not all species or groups within the native species data set responded in the same way. Hydroids had highest cover at the two sites with the highest flow, VS and NYC (Fig. 3-3), where bryozoans and sponges had lower relative cover (Fig. 3-11).

Sponges, bryozoans and macro motile species had lower abundance at sites where chlorophyll a levels were high. NYC had the highest chlorophyll a levels (Fig. 3-3) and high hydroid cover but lower bryozoan cover as compared to other sites (Fig. 3-11). Abundances of macro-motile species were low at NYC (Fig. 3-16) and dominated by mudcrabs. The site with the lowest chlorophyll a levels, NT, was dominated by the invasive isopod, *Synidotea laevidorsalis?,* which may prey on hydroids (P. Foffonoff, personal communication).

Sponge and invasive species (mostly tunicates) cover was correlated with water clarity (Table 3-3). At NT, where water clarity was highest (Fig. 3-3), sponges had negligible cover, while tunicate cover was higher than at any other site (Fig. 3-11).

Finally, species diversity and native species cover was correlated with salinity. The lower salinity sites (NT, YC and BI) exhibited on average lower H' diversity (Fig. 3-10) and lower native species cover.

Discussion

Periodic low DO was associated with shifts in spatial dominance from barnacles to serpulid polychaetes across sites, but with only marginal increases in cover of invasive/cryptogenic species. However, invasive or cryptogenic species had high cover at the site with chronic low DO. The timing of low DO events was important for determining the relative dominance of species. Low DO not only stresses resident adults opening space for newly arriving recruits (Giangrande & Fraschetti 1995, Jewett et al. 2005), but also affects larval supply available for recruitment (Powilleit & Kube 1999). A dominance of polychaete larvae in low oxygen waters reflects a colonization advantage for this taxon because they are available to settle in open space created by low DO disturbance (Powers et al. 2001).

Polychaetes have widely dispersing larvae and the ability to settle quickly to exploit local resources, such as recently cleared space (Grassle & Grassle 1974). The Pearson-Rosenberg model (1978) described species distributions in relation to organic enrichment or the related hypoxia with the areas closest spatially or temporally to the

disturbance dominated by opportunist species (Powilleit & Kube 1999, Dauer 2001). Sites with periodic low DO conditions (YC, WB, BI) had relatively higher *Hydroides dianthus* cover than the sites with no incidence of DO below 4 mg/l (Fig. 3-11). The lack of *H. dianthus* at the site with chronic low DO (NT) may also be due to food limitation, since both chlorophyll a and bacteria were lower than at any other site (Fig. 3-3). *Hydroides* is a bacterivore (Gosselin & Qian 2000).

Low DO functions as a potential space-clearing disturbance, which may benefit less competitive species such as bryozoans and sponges. Bryozoans need space clearing events to thrive (Keough 1984). The sites with higher chlorophyll a, such as NYC, experienced only minor low DO in shallow waters. The lowest sponge and bryozoan cover occurred at NYC. Competition for space in areas with high food availability may account for lower cover of less competitive species.

At the site with the most stressful low DO conditions (NT), invasive and cryptogenic tunicates (*Molgula manhattensis and Botryllus schlosseri*) dominated (Fig. 3-11). Other studies have found correlation between high cover of invasive species and stressful environmental conditions (Stachowicz et al. 2002b, Lawson et al. 2004). Tunicates have the capacity to filter significant water volume (Flood et al. 1992) which may explain partly why they had higher cover in clearer, nutrient limited water. NT had the deepest secchi depth and the lowest chlorophyll a levels of all sites. Chronic low DO may have forced fish and crabs out of the area perhaps reducing predation pressure on the tunicates. In a caging experiment in the lower bay, *Molgula manhattensis* attained high cover in cages which may be evidence of predation on the tunicate (Jewett 2005, Ch. 4).

Although not tested directly, we did explore the relationship between diversity and invasion through regression analysis. The low diversity at the site with chronic low DO (NT) may have created an opportunity for invasive/cryptogenic species (Fig 3-14), perhaps due to reduced competition from other sessile species (Brown et al. 2000). Stachowicz et al. (1999, 2002a) concluded that simpler (lower diversity) communities were more prone to invasion as a result of increased vulnerability to space-clearing events. In other words, a disturbance affecting a dominant species in a low diversity assemblage will open more space, simply because that dominant species occupies more space than in a comparable high diversity community. Across all sites and panels, as species diversity increased, invasive/cryptogenic species cover decreased, which agrees with other research in terrestrial (Elton 1958, Fargione & Tilman 2005) and marine systems (Stachowicz et al 1999). However, the relationship between species diversity and cover of invasive and cryptogenic species depended on whether the most disturbed site (NT) was included. The relationship reversed if the data from the most disturbed site was removed. As H' increased, total invasive/cryptogenic species cover increased in the second analysis as found for terrestrial plants (Lonsdale 1999, Levine 2000). Therefore, for most sites with only periodic low DO exposure, an increase in species diversity may reflect an increase in available resources, such as space, at the local scale (Shea & Chesson 2002). The cryptogenic species, *Molgula manhattensis*, was driving the effect on species diversity, given that the relationship was not significant when its contribution to invasive/cryptogenic cover was removed. *M. manhattensis* had a negative effect on species richness when tested (Jewett 2005, Ch. 4).

Epifaunal spatial dominants in the lower Chesapeake Bay which included tunicates, bryozoans, serpulid and sabellid polychaetes, hydroids and sponges, were present at all eight sites but their relative dominance shifted according to the site-specific environmental conditions (Fig. 3-11, Table 3-1). The persistence of these primary species across sites is evidence of estuarine resilience (Boesch 1974). These species are well adapted to the disturbed conditions of the estuary which produce a mosaic of micro-environments (Picket & White 1985). This shifting dominance also ensures persistence of regional species richness across the sites with some sites acting as sources of propagules and others as sinks (Underwood & Keough 2000). Even sites that were in relatively close geographic proximity (NT and NYC) lacked spatial and temporal environmental homogeneity which was reflected in differences in community biovolume, species diversity (H') and species composition between sites. NT panels had higher biomass and lower H' than the less disturbed site, NYC. The biovolume – H' diversity tradeoff follows models which have related species diversity and biovolume in response to disturbance (Pearson & Rosenberg 1978).

The effects of shifts in species dominance across sites on benthic-pelagic processes, ecosystem function and energy flow are not well understood (Heip 1995). Functions performed by the benthos include detrital processing, bacteria and DO consumption (Heip 1995), DOM production, phytoplankton consumption, habitat and protection for micro-motile fauna and infauna (Dean 1981) and food source for motile fauna including fish and blue crabs and infauna (Baird & Ulanowicz 1989). *Hydroides* and some bryozoans consume bacteria but barnacles do not (Gosselin & Qian 2000). A shift in dominance from barnacles to *Hydroides* may increase the capacity for bacterial

processing but how the shift in dominance affects energy flow would require more analysis of epifaunal predation on these two species.

Conclusion

This study found a strong correlation between variability in benthic community structure and fluctuation in shallow water dissolved oxygen. Shallow water DO deficits may be caused by artificial modifications to flow, localized enrichment and high temperatures. Low DO pulse disturbance was correlated with a shift from a barnacle to serpulid dominated community. Serpulids have been associated with more disturbed, polluted conditions worldwide (Kocak et al. 1999, Gambi & Giangrande 1986). Chronic low DO led to dominance by invasive and cryptogenic species, probably as a result of reduced competition by native species and the capacity of some invasive species to survive in stressful conditions. Reduced predation pressure may also have contributed to high cover of invasive and cryptogenic species. Given the relatively low diversity of the epifaunal community in the Chesapeake Bay, it is necessary to study other estuarine systems which experience low DO before generalizing an invasive species response to deteriorating environmental conditions. Shifts in the average cover of other spatial dominants, such as bryozoans and sponges, was correlated with variability in other environmental factors including water clarity and chlorophyll a levels.

Acknowledgements

Dr. Anson Hines and Dr. Gregory Ruiz of the Smithsonian Environmental Research Center supported this research both financially and with scientific guidance. This research would not have been possible without the generous contribution of time and support from trained, on-site volunteers: Odean Vant Hul, Paul Richardson, Melanie Spring, Maritza Montegross, Linda Crewe, Gwen McCowin, Kevin Fonda, Cliff Hardy, and Dino Kunic. Robert C Clarke and Larry Douglass gave important advice on statistical analysis. Marilyn Schotte, Dale Calder, Gretchen Lambert and Paul Fofonoff assisted in taxonomic identifications of tricky species. Kenneth Sebens, Monaca Noble, Marty Bell, Midge Kramer, John Sherman, Tami Huber, George Smith, Sharon Hendrick, Dennis Whigham, William Jackson, and Denise Breitburg contributed in various ways either through the loan of equipment or space, help in troubleshooting collection techniques or general mentoring. The following institutions allowed panels to be deployed at their docks: Virginia Institute of Marine Science, Nauticus Museum, York Yacht Haven, Hampton Yacht Club, Norfolk Yacht Club, Belle Isle Marina, Willoughby Harbor Marina, Bay Point Marina. Funding for this study was provided by the Smithsonian Institution and a University of Maryland Research and Training Grant.

Table 3-1 Sessile species presence by site. Bold font indicates species present at all eight sites. I = invasive species, C = cryptogenic species.

SITE		BI	BP	HYC	NT	NYC	VS	WB	YC
	Amphipoda								
Corophium sp.		+	+	+	+	+	+	+	+
	Bivalvia								
Unidentified clam				+		+	+		+
Crassostrea virginica		+	+	+	+	+	+	+	+
Ischadium recurvem			+	+	+	+	+	+	+
	Bryozoa								
Alcyonidium verrilli							+		
Anguinella palmata		+		+				+	+
Conopeum seurati		+	+	+		+		+	+
Conopeum tenuissemum		+	+	+	+	+	+	+	+
Electra monostachys			+	+	+	+			
Membranipora chesapekensis		+	+	+	+	+	+	+	+
Membranipora tenuis			+	+	+	+	+	+	+
Upright bryozoan		+	+	+	+	+	+	+	+
	Chordata								
Botryllus schlosseri [I]			+		+		+	+	+
Perophora viridis		+		+					+
Molgula manhattensis [C]		+	+	+	+	+	+	+	+
Diplosoma listerianum [I]			+						
	Cirripedia								
Balanus amphitrite [I]				+					
Balanus eburneus		+	+	+	+	+	+	+	+
Balanus improvisus		+	+	+	+	+	+	+	+
	Cnidaria								
Bougainvillea rugosa						+	+		
Scyphozoan polyp								+	+
Clytia sp.									+
Diadumene lineata [I]			+	+	+				
Diadumene leucolena		+	+	+	+	+	+	+	+
Garveia franciscana [I]		+			+		+		
Halopteris tenella			+	+		+			+
Obelia bidentata		+	+	+	+	+	+	+	+
Obelia dichotoma		+		+	+	+	+		
Proboscydactila ornata			+		+	+			
	Entoprocta								
Barentsia benedeni		+							
	Polychaeta								
Demonax microphthalma		+	+	+	+	+	+	+	+
Ficopomatus enigmaticus [I]		+	+	+	+	+		+	+
Hydroides dianthus		+	+	+	+	+	+	+	+
Polydora sp.		+		+	+	+	+	+	+
	Porifera								
Other Porifera		+	+	+	+		+	+	+
Microciona sp.				+					+
	Protista								
Foraminifera sp.		+		+			+		+
Metafolliculina sp.		+	+	+		+	+	+	+
Sessile Richness		23	24	29	23	24	24	22	28
Total Richness		41	40	48	39	38	40	39	46
H' Sessile Diversity		1.86	1.92	2.17	1.54	2.17	2.14	2.31	1.88

Table 3-2 Epifaunal mobile species presence by site. Bold font indicates species present at all eight sites. I = invasive species, C = cryptogenic species.

SITE		BI	BP	HYC	NT	NYC	VS	WB	YC
	Amphipoda								
Ampithoids			+	+	+	+	+	+	+
Caprellids		+	+	+	+	+	+	+	+
***Gammarus* sp.**		+	+	+	+	+	+	+	+
Red amphipod		+		+	+			+	+
Jassa falcata		+	+	+	+			+	+
	Decapoda								
Callinectes sapidus				+	+				
Neopanopeus sayi				+			+		
Palaemonetes spp.			+					+	
Rithropanopeus harissi		+	+	+	+	+	+	+	+
	Flatworms								
Euplana gracilis		+	+		+	+			+
Leptoplana spp.		+	+	+	+	+	+	+	+
Other flatworms						+		+	+
Stylochus ellipticus		+	+	+	+	+	+	+	+
	Isopoda								
Synidotea laevidorsalis?	[I]				+				
Other isopod				+					
	Nematoda								
Nematodes		+	+	+	+	+	+	+	+
	Nudibranchs								
Cratena pilata				+			+	+	+
Cuthona perca	[I]	+							
Doridella obscura		+	+	+	+	+	+	+	+
Other nudibranch		+					+		+
Stiliger fuscatus				+					
Tenellia adspersa	[C]	+				+			
	Polychaeta								
***Nereis* sp.**		+	+	+	+	+	+	+	+
Ophelia sp.									+
Other worm (green)		+	+	+		+	+	+	+
Family *Phyllodocidae*							+		
Lepidonatus spp.							+		
Family *Syllidae*		+	+	+	+		+	+	+
	Pycnogonida								
Pycnogonid			+	+					
	Snail								
Libinia sp.		+							
Other snail					+	+		+	
Mobile Richness		18	16	19	16	14	16	17	18
Total Richness		41	40	48	39	38	40	39	46

Table 3-3 Correlation between environmental variables and biotic community as measured by **A:** RELATE or BioEnv (Biotic Environmental matching) and BVStep or **B:** Bio-Env routine only (see methods). ρ (rho), the correlation coefficient, reflects the percent of variability in biotic data described by environmental variation. EV = environmental variables (Avg DO = average DO, C = average chl a, F = flow, %LDO = percent of total days when DO below 4 mg/l, S = average secchi depth, and Sal = salinity). Invasive species include *Botryllus schlosseri, Diplosoma listerianum, Ficopomatus enigmaticus, and Garveia franciscana. Molgula manhattensis* is cryptogenic. September data used unless noted.

A

Biotic variables	EV	ρ	p value
Barnacles	%LDO	0.68	**0.020**
***Hydroides dianthus* (Aug)**	%LDO	0.78	**0.004**
***Hydroides dianthus* (Sept)**	%LDO	0.04	0.270
Invasive/cryptogenic Species	%LDO	0.51	0.071
Native Species	%LDO, F, Sal	0.80	**0.003**

B

Biotic variables	EV	ρ
Biovolume	C, S	0.50
Bryozoans	Avg DO, C	0.73
Demonax microphthalma	F	0.24
Empty Space	Sal	0.59
***Hydroides dianthus* (Sept)**	Sal, S	0.52
H'	%LDO, Sal	0.64
Hydroids	S	0.37
Invasive/cryptogenic Species	%LDO, S	0.65
Mobile Species	%LDO, C	0.52
Porifera	C, F, S, T	0.57

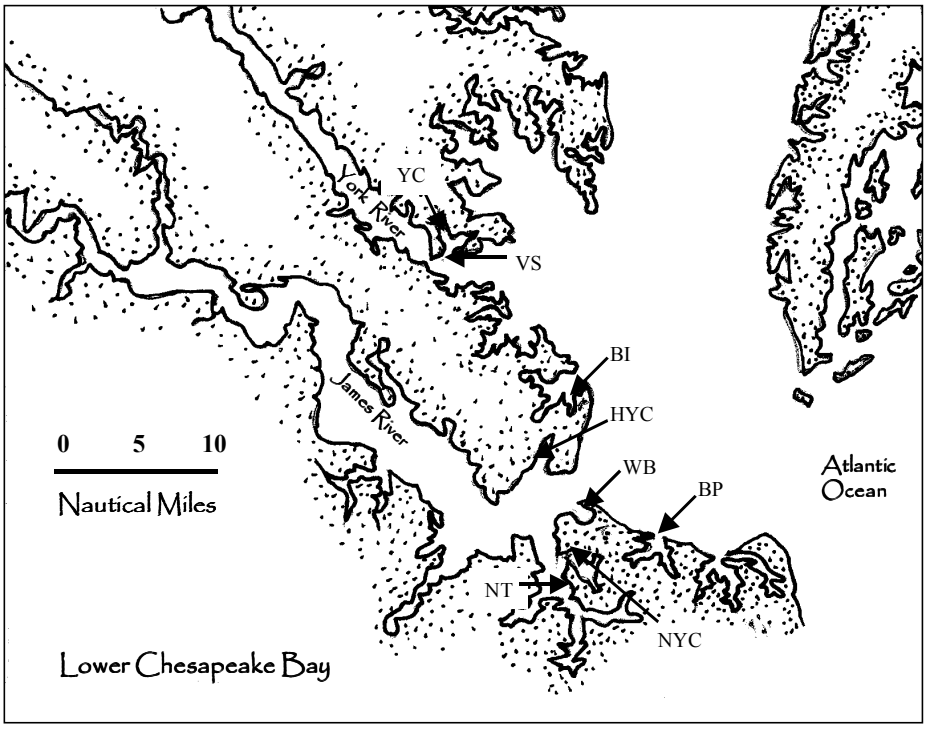


Fig. 3-1 Map of lower Chesapeake Bay showing sampling sites. BI = Belle Isle Marina, BP = Bay Point Marina, HYC = Hampton Yacht Club, NT = Nauticus Museum, NYC = Norfolk Yacht Club, VS = Virginia Institute of Marine Science Ferry Pier, WB = Willoughby Bay Marina, YC = Yorktown Yacht Club

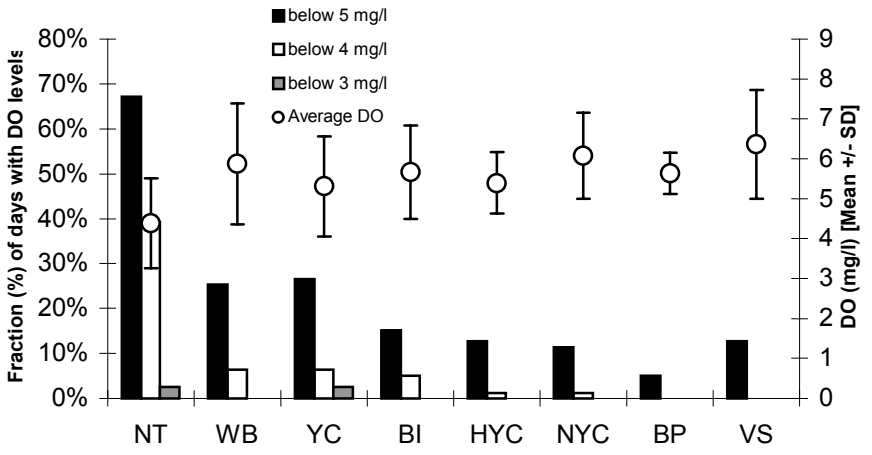


Fig. 3-2 Variation in dissolved oxygen (DO) among eight study sites in lower Chesapeake Bay recorded from late June until mid September 2002 and based on one reading per day at 1M depth at midday. Sites are ordered from site with highest %LDO (< 4 mg/l) days to least. Total days does not include early recruitment period (from mid May to late June). Histograms indicate fraction (%) of days with DO levels at < 5 mg/l, < 4 mg/l, and < 3 mg/l. Open circles indicate mean (+/- SD) DO level. See Fig 3-1 for site abbreviations.

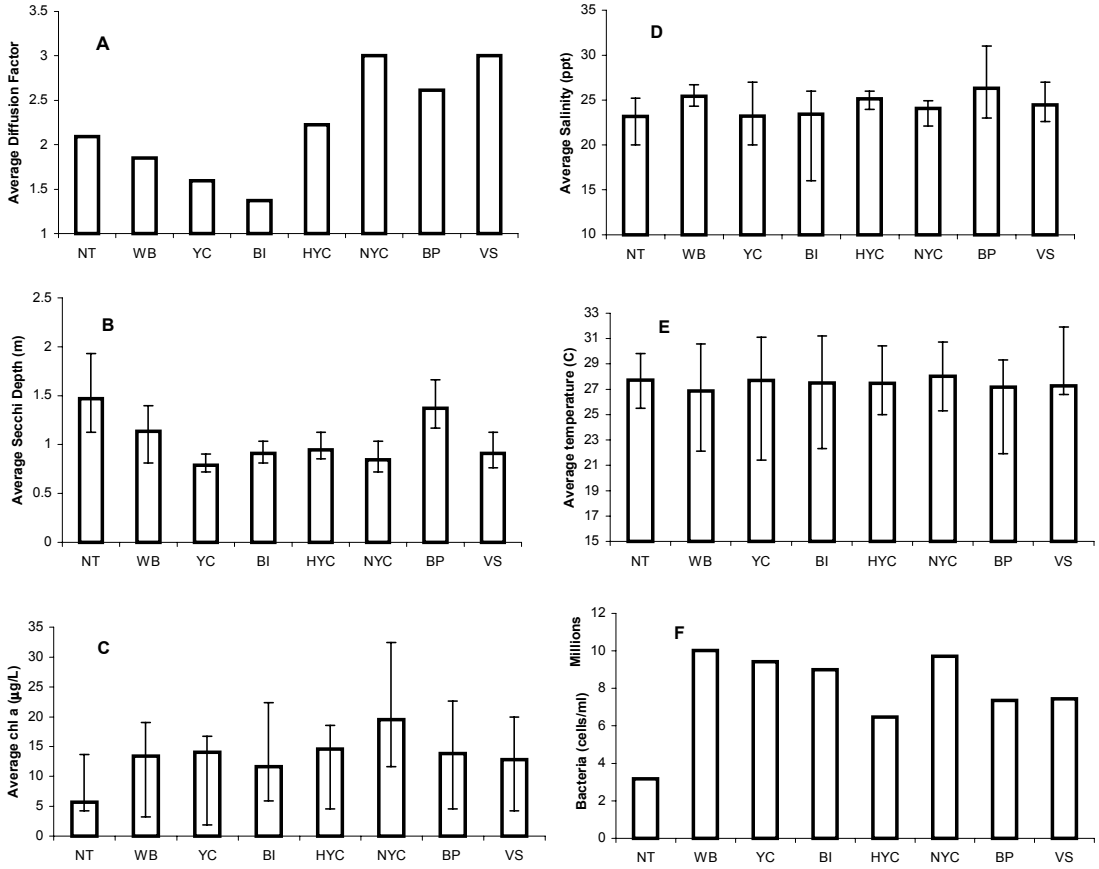


Fig. 3-3 Environmental variables measured at each site. Sites are ordered from site with highest %LDO (< 4 mg/l) days to least. **A** The **diffusion factor** is the ratio of weight of material dissolved from an experimental block to that of the control block maintained simultaneously in calm water (Doty 1971). The flow spheres at VS and NYC were completely dissolved at retrieval which made distinguishing flow between the sites impossible. The NT flow sphere had lost more than 70% of its mass at retrieval which requires caution in interpreting this result. **B, C** Secchi depth and chl a readings were taken biweekly (see methods). **D** The **salinity** data was taken daily with either refractometers, meters or specific gravity gauges.The salinity at NT was estimated from biweekly readings. **E** The **temperature** data except for NYC is from HOBO temp loggers which logged temperatures at 1M depth every 6 hours from late June to retrieval in mid September. The NYC temperature data is from handheld YSI 85 DO meter readings taken 1 - 2 times per day which may account for the discrepancy. **F Bacteria** counts were only taken once in mid summer (see methods). Bars indicate minimum and maximum of each variable as measured during entire experimental period.

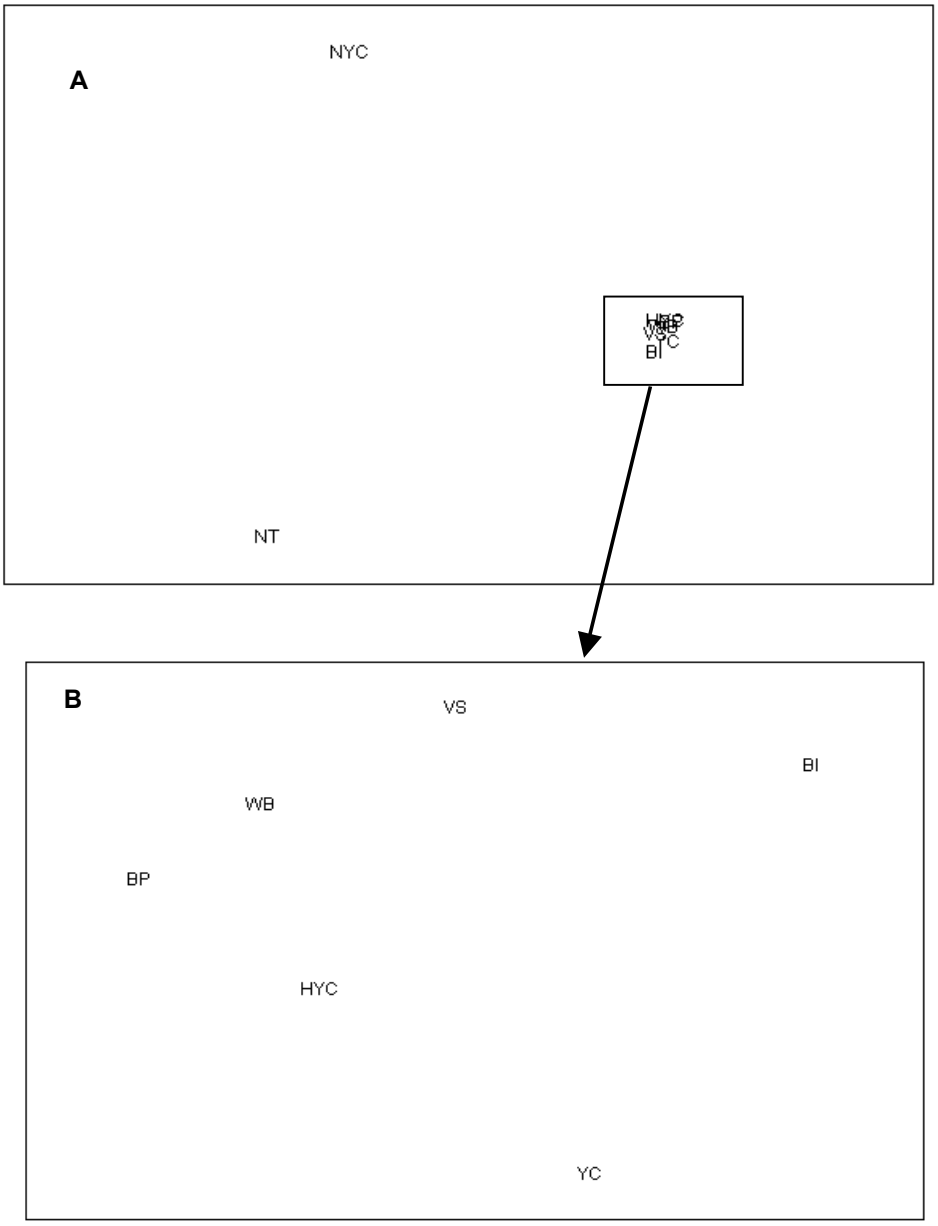


Fig. 3-4 Multidimensional Scaling (MDS) graphs of environmental variables by site including average dissolved oxygen, %LDO, chlorophyll a, bacteria, temperature, salinity, secchi depth and flow. **A** includes all sites. In **B**, NT and NYC were removed. Stress = 0.01 for both graphs.

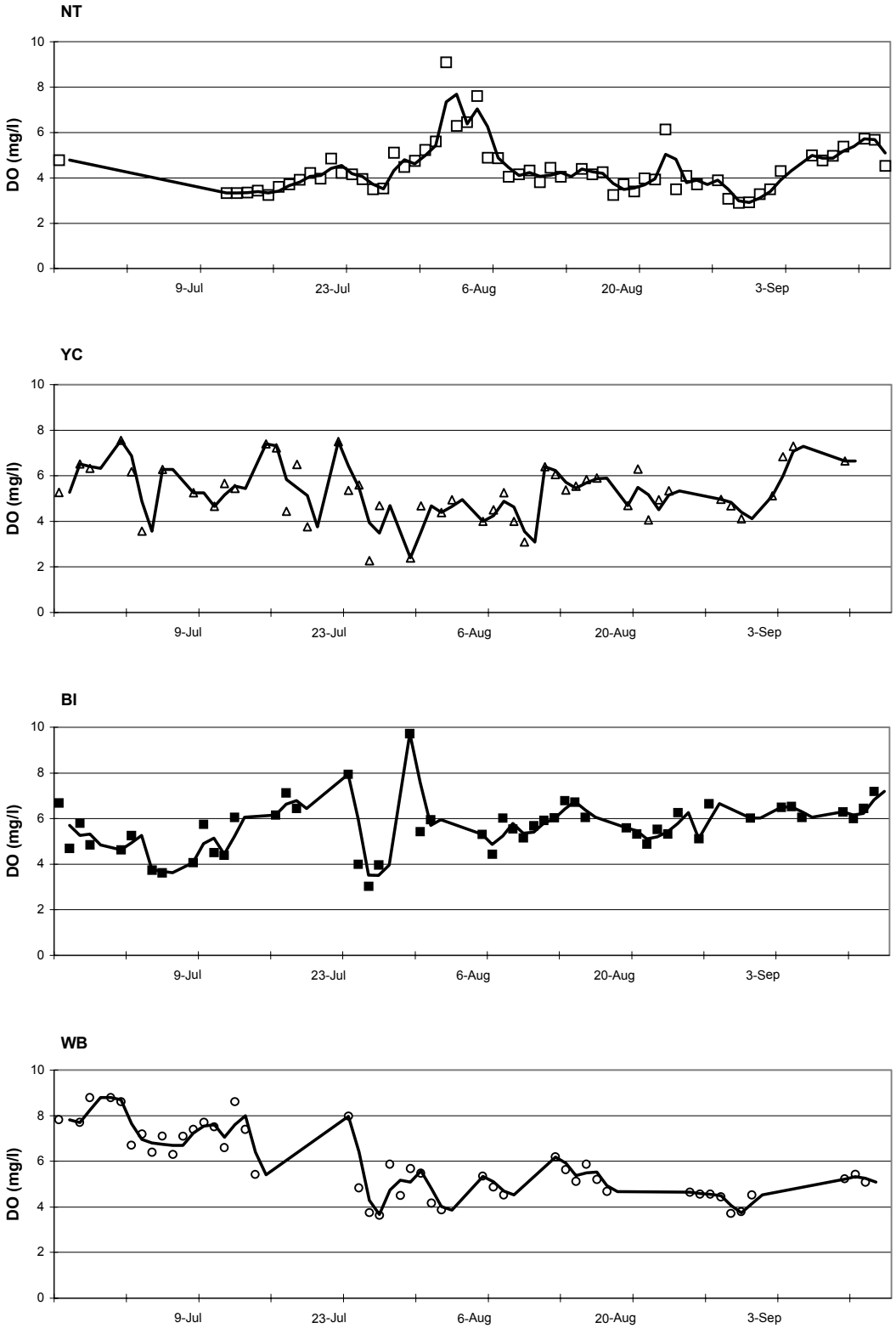


Fig. 3-5 Daily dissolved oxygen readings at four sites in lower Chesapeake Bay which experienced moderate low DO (2 mg/l < DO< 4 mg/l). Line represents two recorded data point moving average. All readings were taken around midday. See Fig. 3-5 for hydrolab readings for WB which indicates diell DO fluctuations relative to these daily readings.

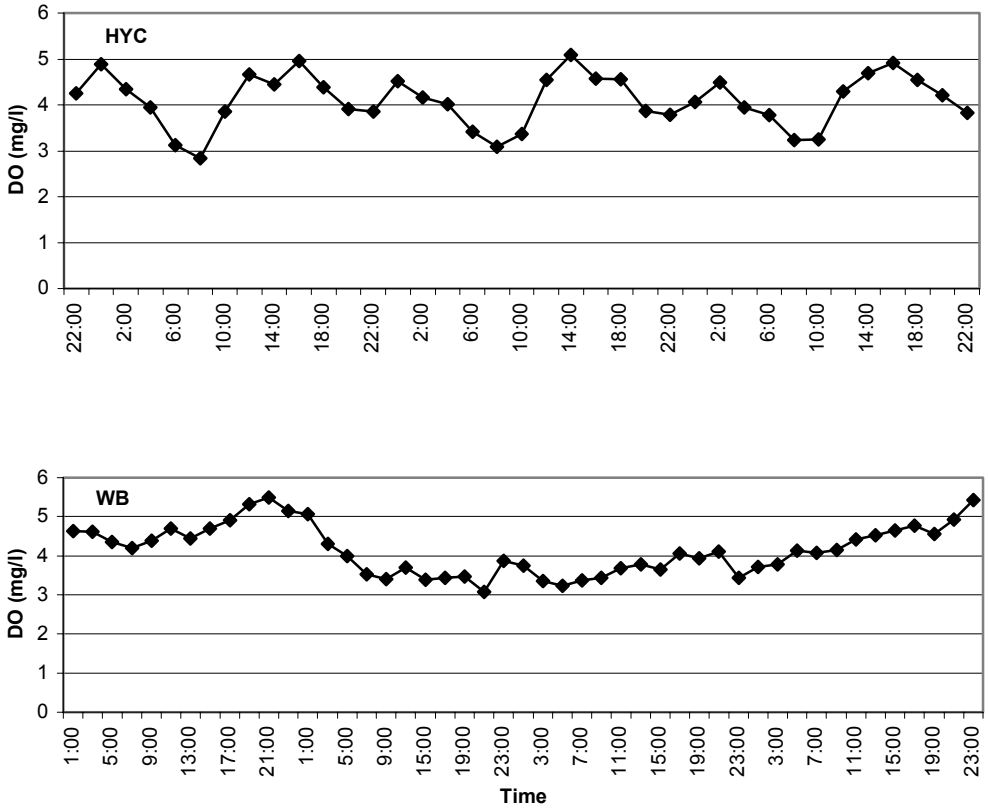


Fig. 3-6 Hydrolab readings from HYC and WB for the same time period from August 28 - 31, 2002. Both hydrolabs were suspended 1 M below a floating dock.

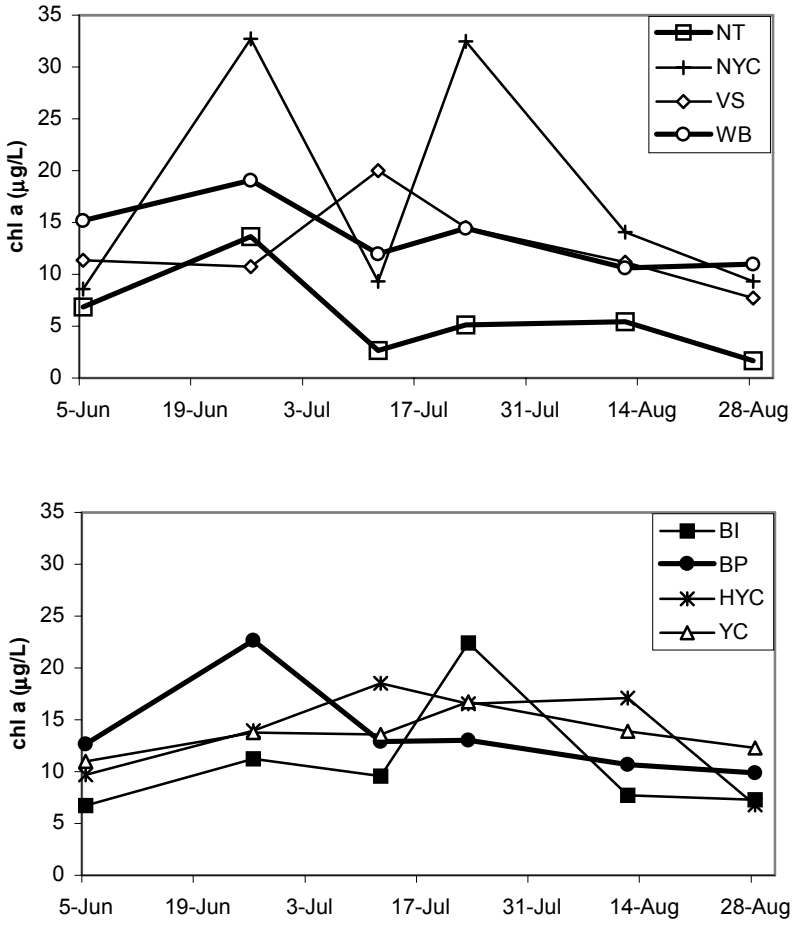


Fig. 3-7 Chlorophyll a levels at biweekly intervals throughout the experimental period. Each point represents the average of two replicate samples. Sites were arbitrarily divided into two groups for graphing so that individual readings could be discerned.

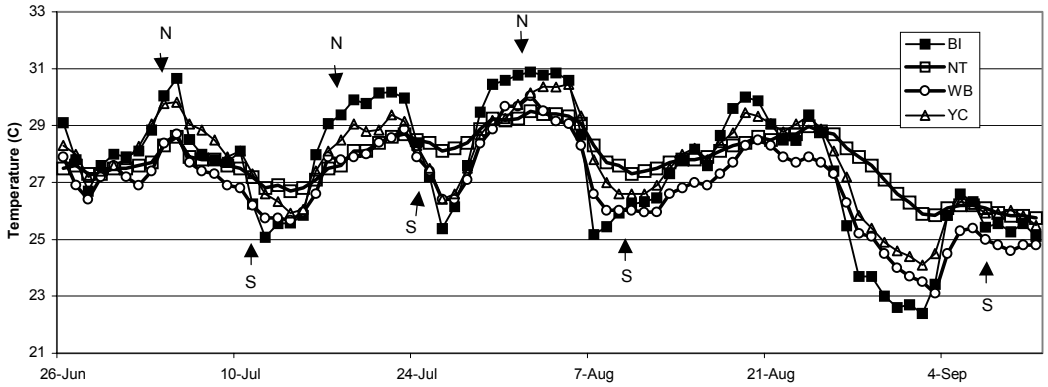


Fig. 3-8 Average daily temperature readings measured at four of the eight sites monitored in the lower Chesapeake Bay. Hobo temp loggers recorded temperature every four hours. Six readings per day were averaged for this graph. The other sites were removed to make it easier to view the individual readings. Temperature readings at all the sites followed the same cycle as those in the figure. Temperatures at BI were consistently higher and lower depending on the tidal cycle than all the other sites. N indicates neap tide and S spring tide.

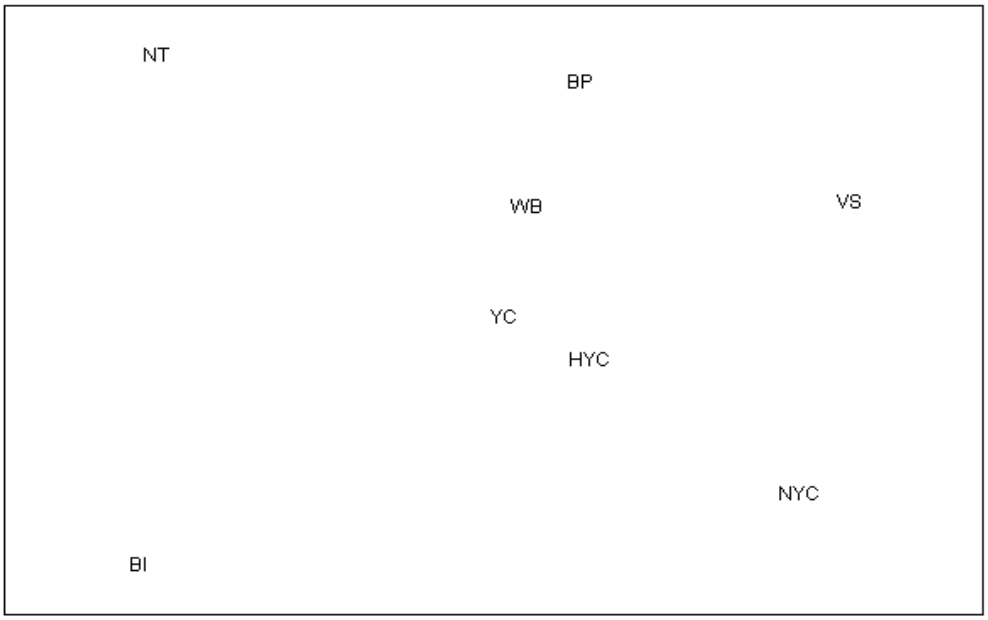


Fig. 3-9 Multi Dimensional Scaling (MDS) graph of mobile and sessile fauna, presence (absence) transformed, for August and September retrievals combined. Stress = 0.06.

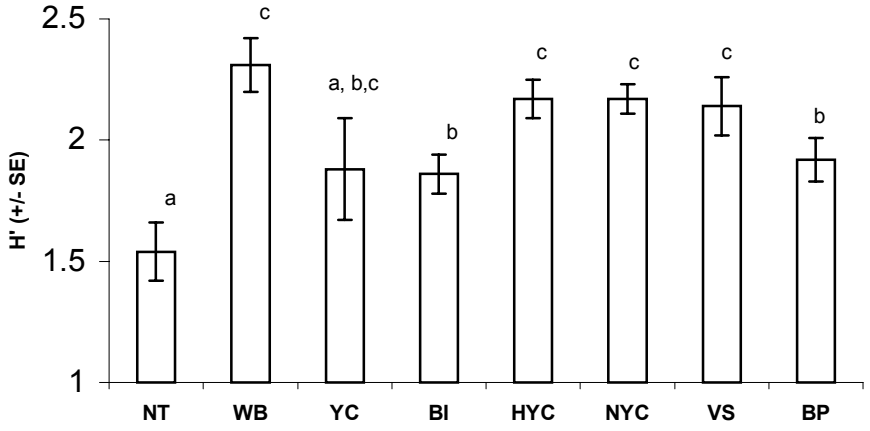


Fig 3-10 Shannon-Wiener diversity index (base e) according to site for sessile species cover only. Sites are ordered from site with highest %LDO (< 4 mg/l) days to least. ANOVA F = 4.311, p = 0.002. Same letters indicate no significant difference between sites. Only September sessile species data included. n = 5.

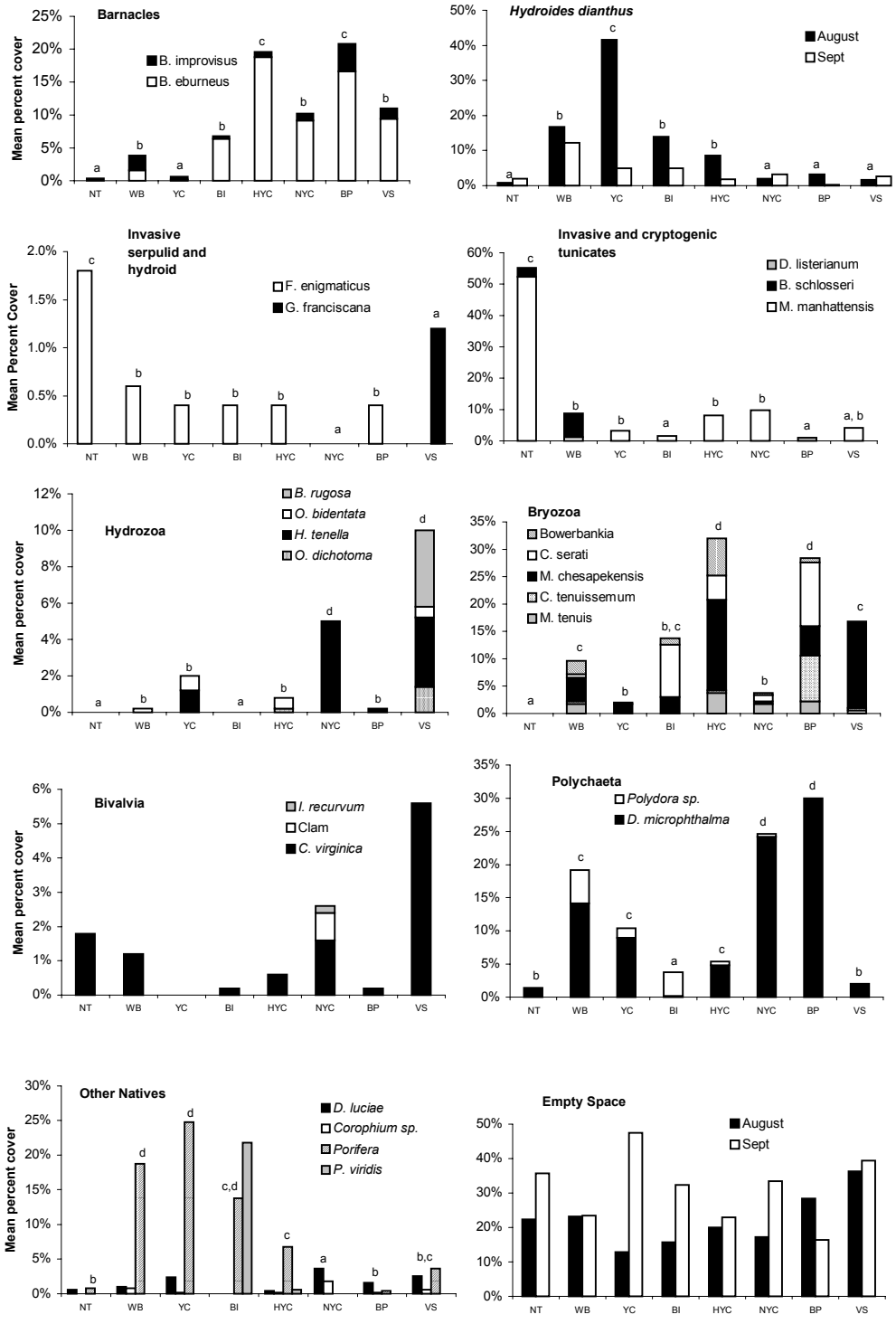


Fig. 3-11 Mean percent cover by site for groups of species. All data are from September retrieval unless otherwise noted. Sites are ordered from site with highest %LDO (< 4 mg/l) days to least. Letters indicate significant difference at $p < .01$ for one way ANOVA analysis using panels as replicates. See Fig 1 for site abbreviations. Letters in invasive serpulid and hydroid graph refer to serpulid (*Ficopomatus engimaticus*) only. Letters in Polychaeta graph refer to *Demonax microphthalma* only. Letters in Other Natives graph refers to sponge cover only. n = 5.

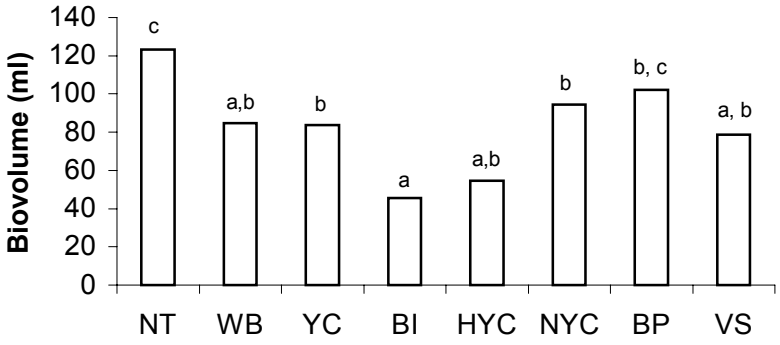


Fig 3-12. Biovolume of settling panel communities retrieved in September as measured through water displacement (ml). Sites are ordered from site with highest %LDO (< 4 mg/l) days to least. Data was log transformed for statistical analysis then backtransformed. ANOVA ($F = 3.97$, $df = 7, 32$, $p = 0.003$). $N = 5$.

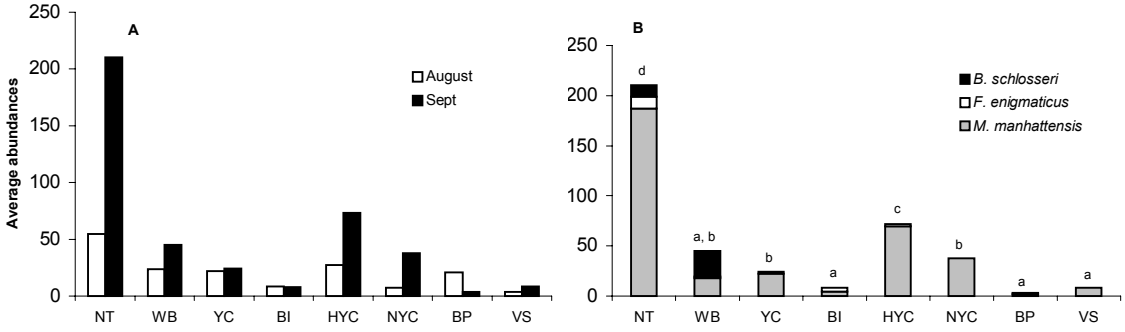


Fig. 3-13 Average abundances per plate of all sessile invasive species except *Garveia franciscana* . Abundance for *Botryllus schlosseri* is number of distinct colonies. **A** includes three invasive species: *B. schlosseri, Diplosoma listerianum, Ficopomatus enigmaticus,* and one cryptogenic species: *Molgula manhattensis* . **B** is a break out of the September data only. *D. listerianum* was not included in B due to the small number of individuals. Different letters in **B** represent significant difference for *M. manhattensis* log transformed abundance. Abundance of *B. schlosseri* not different between NT and WB. In **B**, BP bar includes both *M. manhattensis* (1.6) and *F. enigmaticus* (1.8), YC dark section of bar represents *F. enigmaticus* (2), HYC dark section of bar represents *F. engimaticus* (2.2). Sites are ordered from site with highest %LDO (< 4 mg/l) days to least. n = 5.

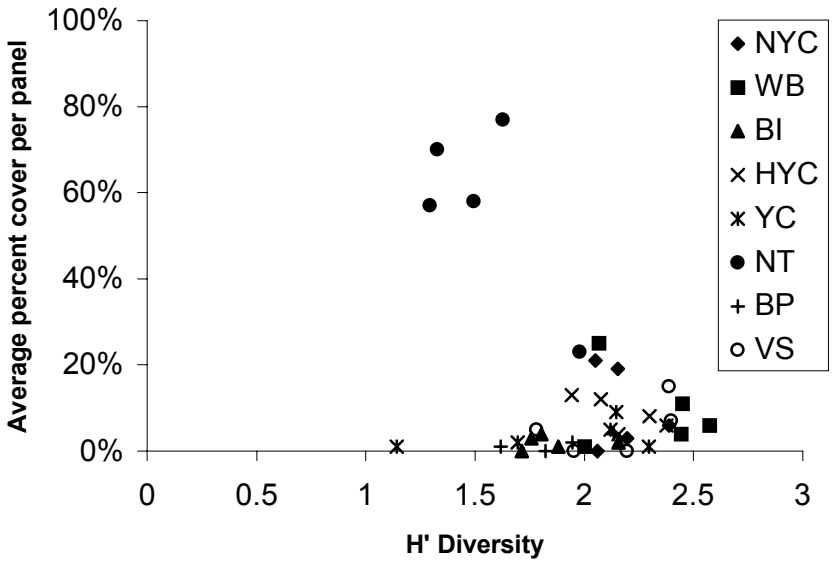


Fig. 3-14 Invasive and cryptogenic species cover as a function of H' (Shannon - Wiener diversity index base e). September data only.

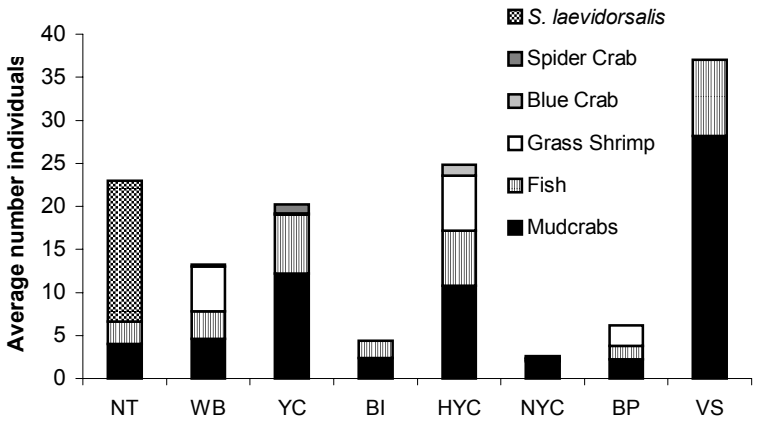


Fig. 3-15 Average abundances of larger mobile organisms living in and clinging to each settling panel retrieved during September retrieval only. Sites are ordered from site with highest %LDO (< 4 mg/l) days to least. N = 5.

Chapter 4: Assessing Impact of an Invasive Hydroid and a Cryptogenic Tunicate on Epifaunal Community Structure

Abstract

To determine the biological and structural effects of two sessile species on recruitment and early community development of the hard substrate epifauna, two settling panel experiments were conducted: one in the lower diversity, oligohaline zone of the Chesapeake Bay with the invasive hydroid, *Cordylophora caspia*; and one in the higher diversity, polyhaline zone with the cryptogenic tunicate, *Molgula manhattensis*. The *M. manhattensis* experiment included a predator exclusion treatment factor which had high (> 50% substrate) cover of the tunicate by retrieval. A decrease in species richness especially among organisms that settle secondarily such as some bryozoans and sponges was associated with an increased abundance of *M. manhattensis*. Biological and structural effects of the hydroid were significant with ciliates and motile species responding positively and *Victorella pavida*, a cryptogenic bryozoan, responding negatively. Predation by motile predators may exert a top down control on both the tunicate and the hydroid, reducing the magnitude of their impact.

Introduction

Marine species are moving around the globe at an unprecedented rate via anthropogenic vectors (Carlton 1985, 1987, Ruiz et al. 2000, Mack et al. 2000). Species introductions are considered the second largest threat to biodiversity after habitat

destruction by some (Simberloff 2000) and not as threatening by others (Gurevitch & Padilla 2004). All agree that it is necessary to evaluate introductions and potential introductions on a case by case basis. Unfortunately, field experiments testing the effects of non-indigenous marine and estuarine species in their new habitats are limited (Berman et al. 1992, Crooks 1996, 1998, Crooks & Kim 1999, Ruiz et al. 1999, Holloway & Keough 2002, Byrnes & Witman 2003, Branch & Steffani 2004). Introduced species, if successful, can have a range of impacts including: reducing or causing extinction of native species through direct competition or predation (Leppakoski et al. 2002, Byrnes & Witman 2003, Walton et al. 2002) or re-engineering the habitat (Crooks 2002, Schwindt et al. 2001); compromising native species through hybridization (Huxel 1999); indirectly increasing native species or other invasive species by providing novel prey or facilitation (Crooks 1998, Simberloff & Von Holle 1999, Branch & Steffani 2004); displacing or narrowing range of native species (Branch & Steffani 2004); or modifying ecosystem processes such as redirecting energy from pelagic to benthic systems or increasing nutrient cycling (Leppakoski et al 2002). Some studies have failed to detect a significant effect despite high abundances of a new species (Holloway & Keough 2002, Lawson et al. 2004).

We conducted two settling panel experiments to test the effects of two sessile, benthic species on estuarine epifaunal communities in the Chesapeake Bay. *Molgula manhattensis* is a cryptogenic (sensu Carlton 1996), solitary tunicate prevalent in the polyhaline zone (http://invasion.si.edu/nemesis), while *Cordylophora caspia* is an invasive hydroid which was first identified in the oligohaline zone of the upper bay in 1877 (Clarke 1878). Debate of geographic origin surrounds *M. manhattensis*. Its

continuous distribution from Maine to Texas, and its colonization of both man-made and natural habitats would suggest a North West Atlantic origin. However, it may be conspecific with three described British species (Berrill 1950), which would suggest a North East Atlantic origin. Species are defined as cryptogenic if, after evidence is weighed, invasion status is unclear (Carlton 1996).

Both experiments used settling panels treated with live organisms, artificial species mimics and blank panels to separate the structural from the biological impact of these species. The mimics were used to control for the physical versus the biological impact (e.g. consuming incoming prey, secreting toxin that prevent secondary settlement in its vicinity or changing small scale flow through suspension feeding activities (Andre & Rosenberg 1991, Ahn et al. 1993)). Physical structure has been implicated in changing benthic community recruitment through alteration of small scale flow (Russ 1980, Dean 1981) or increase in sedimentation rates, both of which can affect larval recruitment (McDougall 1943). Ecosystem engineering, even though its primary impact is physical versus biological, is considered part of the entire impact of an invasive species (Vitousek 1986) with ecosystem level effects.

Assessing the impact of an introduced species in an estuarine system with spatial and temporal variability in physical conditions (Jewett 2005, Ch 3) is difficult. The oligohaline zone, where *Cordylophora caspia* is found, experiences dramatic changes in salinity and temperature especially in early spring when freshwater runoff dominates (Officer et al 1984). As a result of these conditions, many benthic species (bryozoans, hydroids, copepods) of the oligohaline community possess the ability to revert to a resting stage until the return of a more suitable environment (Roos 1979, Jormalainen et

al. 1994). During these periods, effects of resting species on other species are minimized. However, when amenable conditions return, the species may experience explosive growth (Stachowicz 2002a). A sessile species' effect on the benthic community can be temporary; although if widespread and persistent enough, it may compromise the ability of affected species to maintain viable populations.

Short term effects may not reflect the average impact of the species. Holloway & Keough (2002) caution that effects which may seem evident after a relatively short deployment period may disappear if the experiment is allowed to run for more than 6 months. Their experiments with an introduced sabellid polychaete were conducted in a geographic area (Adelaid, South Australia) that does not undergo regular seasonal defaunation.

Predator-prey relationships with the potential to affect the structure of the benthic community include: larger motile predators (e.g. fish and crabs) consuming sessile prey (Osman & Whitlatch 2004); small motile micropredators (e.g. nudibranchs or small snails) consuming sessile prey (Osman et al. 1992, Osman & Whitlatch 2004, Blezard 1992); and sessile filter feeders (e.g. ascidians, serpulids, sabellids, hydroids and barnacles) consuming incoming larvae, phytoplankton or zooplankton (Bingham & Walters 1989, Sutherland & Karlson 1977). Motile predators (fish in the case of *Molgula manhattensis* and nudibranchs in the case of *Cordylophora caspia*) may control local populations of these two species.

Methods

Cordylophora caspia Experiment

The *Cordylophora caspia* experiment, deployed in the vicinity of Baltimore Harbor in the oligohaline zone of the Chesapeake Bay, used a 3 x 3 factorial, randomized complete block design (RCBD). The experimental design included 9 treatment combinations of two factors with three levels each: depth (1 m, 3 m and 7 m) and panel treatment (seeded with *Cordylophora caspia*, a mesh mimic and blank). Site (five sites were used) was a random, blocking factor. To control for the structural complexity of *C. caspia*, a mesh mimic was used to distinguish the structural from the biological effect of the hydroid (Appendix I). The mesh hydroid mimic consisted of 1 x 1 cm^2 black plastic mesh which was cut into 3 x .5 cm^2 pieces to resemble the branching stolon of a hydroid. Approximately nine pieces were attached with glue to the panel surface in a uniform pattern.

To seed blank panels with live hydroid, *Cordylophora caspia* was harvested from Baltimore Harbor in early June. Individual hydranths were attached with rubber bands to 14 x 14 cm^2 sanded, PVC settling panels that were suspended in 40 L aquaria. *C. caspia* zooids were fed newly hatched *Artemia* every other day in lab aquaria (18^{o}C, 12 ppt) and allowed to attach and spread across the settling panels (Fulton 1960, Appendix I). Hydroids were raised in dark to avoid algae growth. All estuarine water for culturing was filtered through 10 micron sieve to remove live organisms. Half of water in each aquarium was changed each week. Blank panels and panels with mesh mimic were seeded with bacterial film through placement in aquaria with *C. caspia* plates for two

weeks prior to facilitate larval settlement. Before deployment, percent cover of *C. caspia* was measured through use of 25 point fixed grid.

Five sites (blocks) with ample depth (> 10 m) and geographic spread (> 1 km apart) (Fig. 4-1) were chosen in the vicinity of Baltimore Harbor for deployment. All nine treatments were deployed at each site, arrayed in random order. PVC panels were deployed experimental side down, attached to the underside of bricks and hung from line attached at the surface. Only one panel was attached to each brick. Dissolved oxygen, temperature and salinity were recorded at each depth at each site at deployment, at mid deployment and at retrieval. DO was measured with a YSI DO meter Model 57. Temperature and salinity were measured with a thermometer and a refractometer in water retrieved from depth with Niskin bottle. All panels were deployed from July 12 to August 6, 1999.

At retrieval, all panels were collected and transferred to the laboratory for live analysis under a dissecting scope. Percent cover of individual taxa and total species richness were assessed for each panel. A 25 point fixed grid was placed over the live community on each panel and the identity of the species at each point was recorded to create an estimate of the percent cover of each species. In addition, the identification of all sessile and motile species was determined to the extent possible. A 4 cm^2 scrape sample was also removed from the center of each panel and preserved for species abundance estimates.

Both multivariate and univariate statistical techniques were used to assess the impact of *Cordylophora caspia* on community development. All percent cover data was arcsine squareroot transformed and abundance data was log (x + 1) transformed for

statistical analysis so data would meet the assumptions of ANOVA (Sokol & Rohlf 1995). All means and standard errors were back transformed for graphs. Fisher's protected F test, used to reduce experimentwise error, did not allow pairwise comparisons unless the main effect F statistic and p value were significant. Analysis of Similarity (ANOSIM), Similarity Percent (SIMPER), and Multi Dimensional Scaling (MDS) routines available through PRIMER v.5 were used to analyze the overall community differences according to treatment. The cover and abundance of *C. caspia* were not included in the multivariate analysis because we were interested in its effect on the community not in the presence absence or abundance of the hydroid itself. *C. caspia* was taken into account as a fixed factor in the experiment.

Molgula manhattensis **Experiment**

A 3 x 2 factorial, completely randomized design (CRD) was used to assess the impact of the solitary tunicate, *Molgula manhattensis*, on the early fouling community development in the polyhaline zone of the Chesapeake Bay. The entire experiment was deployed from the Ferry Pier at the Virginia Institute of Marine Science (VIMS) near the mouth of the York River (Fig. 4-1). Two fixed factors used were panel treatments with three levels (panels with live *Molgula*, *Molgula* mimics and blank panels) and predation control with two levels (caged versus not caged) with four replicates of each individual treatment combination (n = 4). The cage consisted of 1 cm^2 black plastic mesh sitting 10 cm from the experimental surface. The cage was devised to prevent larger, motile predators such as fish and crabs from grazing on the panels although small,

micropredators such as nereids and flatworms would not be excluded. Little fouling of cage was evident at retrieval.

As in the hydroid experiment, both live *Molgula* and *Molgula* mimic panel treatments were created to separate the structural from the biological effects of the organism. To seed panels with live *Molgula*, individual *Molgula* zooids were harvested from collecting panels at VIMS and maintained in lab aquaria for several weeks before deployment. Tunicates were attached to panels with superglue. After attachment, the panels were maintained in the lab for several days to ensure viability of the tunicates. Any dead *Molgula* were removed and replaced before deployment. Artificial *Molgula* mimics (sanded plastic grapes) were arrayed in similar patterns to live *Molgula* treatments (Appendix II). Prior to deployment, all panels were allowed to develop a bacterial film by placing in lab aquaria with estuarine water for several weeks. All panels were deployed in the field for four weeks in July 2001.

Upon retrieval, percent cover was estimated for all panels using a 50 point fixed grid. Total species richness and identification of motile and sessile species were assessed from the preserved panels. In addition, abundances of all non-indigenous and cryptogenic species were counted, which included *Ficopomatus enigmaticus* tubes, *Molgula manhattensis* zooids and *Botryllus schlosseri* colonies. Statistical analysis of panels was the same as for the *Cordylophora caspia* experiment including both multivariate and univariate techniques. Linear regression was used to test how species richness varied with cover of *Molgula manhattensis*.

Results

Cordylophora caspia Experiment

The main species recruiting in the upper bay were barnacles, ciliates, the upright bryozoan, *Victorella pavida*, serpulids (*Hydroides dianthus* and *Ficopomatus enigmaticus*), the tube forming spionid, *Polydora* sp., the cryptogenic entoproct, *Loxosomotoides laevis* (Wasson et al. 2000), and a variety of motile species including nudibranchs, mud crabs, and flatworms. In general, the benthic community of the oligohaline zone is low growing and spatially sporadic.

Cordylophora caspia did not recruit naturally during the experimental period. Only the experimental panels that were originally seeded with the hydroid had *C. caspia* cover at retrieval. *C. caspia* cover on the experimental panels at the end of the deployment period ranged from < 4 – 58% depending on depth and site (Fig. 4-2). The hydroid's persistence was site specific, with the greatest growth occurring at 7 m depth at site 5 (Fig. 4-3). Abundance of viable *C. caspia* zooids in scrape samples did not differ significantly by depth (Fig. 4-4), although the mean number of viable polyps was greatest at the mid-depth (3 m) primarily due to the high cover at site 3 (Fig. 4-2). Change in *C. caspia* cover by depth varied across the five sites (Fig. 4-3). At all sites except site 4, *C. caspia* cover decreased on the shallow panels, and increased on the deep panels. If site 4 is removed from the analysis, the mean change across depths was significantly different (ANOVA $F = 5.36$, $p = 0.03$), with the deep sites experiencing the most growth (Fig. 4-5).

The physical conditions in Baltimore Harbor varied predictably by depth and time. The mid-Atlantic region of the East Coast experienced a prolonged drought throughout the summer of 1999. Salinity, which normally does not exceed 10 ppt in the oligohaline zone of Chesapeake Bay, exceeded 16 ppt in early August (Fig. 4-6). Dissolved oxygen (DO) dropped to stressful levels (< 4 mg/l) at all depths in late July (mid deployment). Sites 3 and 4 experienced hypoxic conditions (< 2 mg/l), even at the shallow depth (1 m) (Table 4-1). *C. caspia* final percent cover was greatest at site 3 (Fig. 4-2). In addition, despite all the sites experiencing hypoxia at 7 m depth, *C. caspia*, on average, grew most on the panels deployed at this depth (Fig. 4-3).

Data analysis of effects on individual species and groups of species revealed, in general, a greater effect of depth than hydroid treatment. *Victorella pavida*, an upright bryozoan of cryptogenic origin, may be a competitor for space with *Cordylophora caspia*. In general, *V. pavida* recruited to all panels at all sites but it had very low cover at 7 m depth (Fig. 4-7). The plates seeded with the hydroid had the lowest and the blank panels had the highest *V. pavida* cover (Fig. 4-7). The bryozoan cover on the mimic control did not differ from the other two treatments. This may be evidence that the lower cover of the bryozoan on the seeded panels was not just a negative response to the structure of the hydroid.

Ciliate abundance on the hydroid seeded panels did not differ significantly from either the blank or the mimic panels (Fig. 4-8), although ciliate abundance was significantly greater on the mimic than the blank panels (ANOVA depth $F = 6.36$, $p = 0.005$; panel treatment $F = 4.81$, $p = 0.02$). The sessile ciliates grouped for this analysis included *Metafolliculina* sp., *Stentor* sp., *Zoothamnium* sp., *Vorticella* sp., and a

suctorian. These ciliates colonize the stolons of other upright species including hydroids and bryozoans and recruit to open space although they are not good competitors so they have higher abundances as secondary settlers on other species. Ciliate abundance also decreased by depth (Fig. 4-8).

Loxosomotoides laevis, cryptogenic entoproct, also recruited to panels but its cover and abundance did not differ according to hydroid treatment.

Total abundance of motile species, counted in the scrape samples, was highest on the hydroid mimic treatments with no significant difference between the live hydroid seeded and the blank panels (Fig. 4-9). Abundance of motile organisms also decreased with depth with no difference in abundance between the 1m and 3m depths. Abundances of *Tenellia adspersa*, invasive nudibranch, did not differ by panel treatment (ANOVA $F = .49$, $p = 0.6$).

Motile and sessile species incidence (presence/absence), abundance (from scrape samples) and sessile species percent cover did not differ according to panel treatment but did differ according to depth (multivariate analysis, Table 4-2). In other words, *Cordylophora caspia* presence on the panels, in the live hydroid treatment, did not affect the overall composition, percent cover or abundances of species when analyzed as an entire community.

Molgula manhattensis **Experiment**

The dominant species recruiting in the lower Bay at midsummer were *Hydroides dianthus* (serpulid polychaete), *Demonax microphthalma* (sabellid polychaete), *Molgula*

manhattensis and *Balanus eburneus* (barnacle). The species list also included a variety of upright and encrusting bryozoans, *Corophium* spp. (amphipod), *Diadumene leucolena* (anemone) and *Polydora* sp. (spionid polychaete). *Botryllus schlosseri* (colonial tunicate) and *Ficopomatus enigmaticus* (serpulid polychaete) were two invasive (http: invasions.si.edu/nemesis) species that also recruited.

Although the experiment was designed to test for both the effects of *Molgula manhattensis* and the effects of predation on early community assembly, the caged treatments allowed a dramatic increase in the cover and abundance of *M. manhattensis* (Fig. 4-10). In contrast, the three uncaged panel treatments (tunicate seeded, mimic and blank panels) did not have significantly different cover of *M. manhattensis* by the end of the experimental period (one month) ($F = 2.44$, $p = 0.12$). Therefore, to analyze the effects of the tunicate on community composition we considered both the differences between the caged and uncaged treatment and the response to the panel treatments (averaged across caging factor). Of course, the caged versus uncaged comparisons cannot fully distinguish the effect of the presence of the tunicate from the effect of the caging. Given the dramatic difference in *M. manhattensis* cover between the caged and uncaged panels, it is possible that a predator removed biomass, particularly tunicates, from the uncaged panels.

As the number of *Molgula manhattensis* individuals increased on a panel (regardless of treatment), the sessile species richness decreased (at the rate of .05 species per *M. manhattensis* individual) (Fig. 4-11). Cover of *M. manhattensis* explained 38% of the species richness per panel ($R^2 = .38$, $p = 0.002$). All sessile species were included

regardless of whether they occupied primary or secondary space but observations showed fouling on *M. manhattensis* individuals was uncommon.

Community composition of species as measured by percent cover differed by both the panel treatment and the caging factor, although the resolution between treatments was greater for the caging factor (as indicated by the much larger R value, Table 4-2). The dissimilarity among panel treatment communities was greater for the uncaged treatments (Fig. 4-12). Barnacles which dominated the uncaged panels (Fig. 4-13) accounted for 68% of the dissimilarity between caging treatments (SIMPER analysis) for the percent cover data. Of the three invasive or cryptogenic species, only *Molgula manhattensis* had significantly higher abundances on the caged panels (Fig. 4-14).

Identity of settling species differed depending on *Molgula* dominance of panel. Species incidence (species data transformed to presence absence on each panel) differed between caging factors and among *Molgula* treatments (Table 4-2). The incidence of *Molgula* itself was not included in the analysis. Similarity percent routine analysis (SIMPER) was used to highlight the species which accounted for the community level differences (Table 4-3). Both *Demonax microphthalma* and *Hydroides dianthus* were prevalent on all panels. The higher incidence of *Balanus eburneus* and *Ficopomatus enigmaticus* on the uncaged panels contributed significantly to the similarity of the uncaged panels. No one species contributed to more than 10% of the dissimilarity in species incidence among panels.

Overall, the community composition as measured by percent cover of general sessile taxonomic groups (barnacles, tunicates, serpulids, sabellids, encrusting bryozoan) was not different between the *Molgula* and mimic treatments but both were different

from the blank panels when averaged across caging treatments (Table 4-2). Barnacles again contributed most to this difference but, in this analysis, barnacles had higher cover on panels with more structure (Table 4-4). *Hydroides dianthus* ranked second for distinguishing between panel treatments with higher cover on blank panels. Species incidence (including motile and sessile species) was only different between *Molgula* and blank panels (Table 4-2). Varying recruitment of barnacles, bryozoans, *Botryllus schlosseri* and nereids accounted for some of the differences between *Molgula* and blank panels (Table 4-4). In addition, the cover of *Molgula manhattensis* on the caged panels did not differ between the panels seeded with *Molgula* and the structural mimic but both were significantly less than the tunicate cover on the caged blank panels ($t = 3.31$, $df = 18$, Tukey adj $p = 0.04$).

Discussion

Decline in Species Richness

The decline in species richness with the increase in *M. manhattensis* abundance (Fig. 4-11) may reflect the effectiveness with which the tunicate preempted space and/or prevented secondary settlement. The uncaged panels which had low cover of *M. manhattensis* (Fig. 4-10) had higher incidence of *Balanus eburneus, Ficopomatus enigmaticus*, upright and encrusting bryozoans (except for *Membranipora tenuis*), *Corophium* sp., *Polydora* sp. and sponges relative to the caged panels (Table 4-3). Both bryozoans and sponge frequently settle secondarily on other organisms such as barnacles

or serpulid tubes. The decline in their incidence on *Molgula* dominated panels (caged treatments) may reflect an anti-fouling property of *Molgula* tunics (Osman & Whitlatch 1995a,b). Contrary to our finding, Otsuka & Dauer (1982) recorded *Molgula* fouled by *Polydora, Corophium* and *Membranipora*. It is possible that the age of the *Molgula* determines their susceptibility to fouling. Although tunicates are efficient filter feeders (filtering between 2 and 18 l water/hr), in one experiment they did not have an appreciable effect on larval availability (Bingham & Walters 1989). The change in the number and identity of species on the caged panels may not reflect direct predation by the tunicate on settling larvae.

Localized aggregations of *Molgula manhattensis* in the lower bay could increase vulnerability to species invasion because spatial dominance by one species may increase the probability of open space (Stachowicz et al. 1999, 2002a). Through removal of tunicates, fish and crabs may have a beneficial, top down effect (Paine 1966) of maintaining the benthic community more resistant to invasion by preventing dominance.

Invasion Meltdown?

Neither experiment provides evidence that these species are facilitating other introduced species. Simberloff & Von Holle (1999) warn that, once introduced, invasive species may facilitate new invasions through habitat modifications which they termed "invasion meltdown". In the lower bay, *Ficopomatus enigmaticus* had higher incidence on the uncaged (lower *Molgula* cover) panels (Table 4-3). *Botryllus schlosseri* incidence contributed to differences between the *Molgula* seeded, mimic and blank panel

communities with higher incidence on the blank panels (Table 4-4). *Cordylophora caspia* may inhibit recruitment and growth of *Victorella pavida*, a cryptogenic bryozoan. *Tenellia adspera*, invasive nudibranch, preys on *Cordylophora caspia*, so its populations should be benefited by the hydroid. However, abundances of the nudibranch were not higher on the hydroid seeded panels. The nudibranch controls the hydroid population (Chester et al. 2000) and the relationship is moderated by the different tolerances each species has for salinity (Blezard 1992). *F. enigmaticus* did not recruit in sufficient numbers in Baltimore Harbor to detect a response to the hydroid. Finally, the cover and abundance of *Loxosomotoides laevis*, a cryptogenic entoproct, was not correlated with the cover of *C. caspia*.

Biological Interactions

Cordylophora caspia and *Molgula manhattensis* consume live prey and might have an impact on larval recruitment. Standing (1975) suggested that hydroids might consume barnacle larvae, but in this study with *Cordylophora caspia*, barnacle cover was not lower on the seeded panels. It may be that *C. caspia* has a greater predation effect on the abundances of zooplankton (Arndt 1984) that were not measured in this study. The abundances of benthic motile organisms such as harpacticoid copepods or insect larvae, considered hydroid prey (Roos 1979), did not differ between the seeded panels and the controls. *Molgula* does not consume barnacle larvae (Bingham & Walters 1989), so the decreased barnacle cover on the caged panels is more likely the result of space preemption and not direct larval predation.

Competition is difficult to prove (Connell 1961, 1980), and our study was limited in its ability to do so. The major difference between the caged and uncaged panels in the lower bay experiment may be due to competition for space between *Molgula manhattensis* and *Balanus eburneus.* However, newly settled barnacles are not considered good at maintaining the space against further settlement (Otsuka & Dauer 1982, Dean & Hurd 1981, Dayton 1971, Lubchenco & Menge 1978, Peterson 1979a). Colonial species (including tunicates and bryozoans) may avoid barnacles (Young & Gotelli 1988). Bullard et al (2004) experimented with larval settlement and recruitment on panels seeded with various tunicates (*Molgula manhattensis, Diplosoma listerianum* and *Botryllus schlosseri*) and concluded that invertebrate larvae of species considered competitively inferior to tunicates, such as bryozoans and barnacles, do not avoid settling near the tunicate adults. Bryozoans, serpulids and barnacles, which are considered inferior competitors, had higher incidence on the uncaged panels which had lower percent cover of *Molgula* (Table 4-3).

Cordylophora caspia had a negative effect on the cover of *Victorella pavida* and ciliates. The cover of *V. pavida* was even lower on the seeded than the mimic panels (Fig. 4-7), which may indicate that *C. caspia* is having a biotic rather than a structural effect on the upright bryozoan. However, upright bryozoans have been negatively correlated with structural complexity (Russ 1980). In other years with higher *C. caspia* abundance (Von Holle & Ruiz unpublished), effects on this species might have been greater.

Structural Effects

Structural complexity can have various effects on community structure, including: providing surface area for epibionts (Dean & Hurd 1981, Roos 1979), providing refuge from fish predation (Sutherland 1974, Russ 1980, Caine 1987), changing the flow to benefit sessile filter feeders (Pequegnat 1974), attracting motile species (Dean 1981, Caine 1987) and increasing sedimentation rates which repels some larvae (McDougall 1943). Invasive ecosystem engineers, those organisms that can change the habitat to suit their needs, may outcompete resident species (Cuddington & Hastings 2004). *Musculista senhousia*, a non indigenous mussel in San Diego bay, creates extensive mats on tidal flats, which bind the sediment and increase species richness and infaunal density (Crooks 1999). Through the use of structural mimics, Crooks (1999) demonstrated that the structural effect of the mussels and their mats outweighed the biological effects of the mussel alone.

Abundance of attached ciliates was facilitated by *Cordylophora caspia* in one study in the Netherlands (Roos 1979) and inhibited in another study in the Chesapeake Bay (Von Holle & Ruiz unpub.). However, ciliate abundance did not differ among the panels seeded with the hydroid and the blank or mimic panels (Fig. 4-8). Even so, a significant difference in ciliate abundance between the blank and mimic panels (Fig. 4-8) may indicate the ciliate preference for structural complexity. In Roos (1979), ciliates and suctorians were observed settled on the stolon of *C. caspia*. The hydroid may serve as a preferred, structural substrate for the ciliates, which improves their capacity to feed.

Hydroids may also represent a refuge for other motile organisms (Caine 1987), such as nereid worms and caprellids that either prey on sessile species or consume algae

and bacteria (Caine 1980, Jensen & Andre 1993). Small motile species responded to structural complexity (panels with hydroid mimics) in the *C. caspia* experiment with significantly higher abundances on the mimic panels than the seeded or the blank panels (Fig. 4-9). The mimic panels represented the seeded hydroid structure closely at the beginning of the experiment, but the adverse environmental conditions caused some of the hydroid to die back (Fig. 4-2). This may account for the difference in response by the ciliates and the motile organisms to the mimics versus the live hydroid, both of which have been shown to prefer structure (Roos 1979, Dean 1981). Filamentous structure of hydroids might also facilitate recruitment of mussels in early fall in some habitats (Bayne 1964, Seed 1969, Dean & Hurd 1980, Okamura 1986).

Cordylophora caspia is one of a few hydroids in the upper Chesapeake Bay and one of two (the other is also non-indigenous – *Garveia franciscana*) with economic costs, given its propensity to foul ship motors, hulls, and water intake pipes for power plants (Leppakosi et al 2002). This hydroid may represent a substantial substrate change in the bay if ciliates, mussels and encrusting bryozoans comprised the benthic community before their arrival. *Victorella pavida* (s.l.) is also a potential newcomer to the Chesapeake Bay (http://invasions.si.edu/nemesis).

Interpretation of the structural effects as opposed to the biotic effect of the tunicate was difficult since the high recruitment and growth of *Molgula* on the caged panels overwhelmed the structural mimic. In addition, the live *Molgula* were removed by motile predators from the uncaged panels. However, despite this, the percent cover analysis revealed less difference between the panels seeded with *Molgula* and the *Molgula* mimic than between either of them and the blank panels (Table 4-2). The

primary effect of the tunicate may therefore be structural given the similar response of species to the live tunicate and the mimic (Table 4-4).

Cordylophora caspia and Low DO

Other studies have linked higher abundances of non-indigenous species in response to specific environmental variables including higher temperatures, salinities or low DO (Byers 2000, Stachowicz et al. 2002b, Lawson et al. 2004). Competition between species may be mediated by stressful conditions (Johnston & Keough 2003). *Cordylophora caspia* has a high tolerance for low DO conditions (Fulton 1962). Its capacity to recruit and grow in low DO waters may provide a competitive advantage with a potential spatial or temporal refuge from competition from resident species. *Cordylophora caspia* grew most on the panels deployed at 7 m despite the stressful low DO conditions (Fig. 4-5).

Caging Effect

Predation may have had a strong impact on the abundance of *Molgula manhattensis,* given that the tunicate's cover was significantly higher for the caged panels (Fig. 4-10). Gregarious settlement is probably not the cause of the high cover (Durante 1991). Some argue that predation exclusion is not necessarily distinguishable from small scale flow change or larval entrapment by organisms fouling the cage (Virnstein 1978, Peterson 1979b). Cages were fouled only minimally by *Polydora* sp. and *Corophium* spp.. However, predation on ascidians by fish is well documented in other systems

(Sutherland 1974, Ojeda & Dearborn 1991, Osman & Whitlatch 2004). Tunicates may also be eaten by blue crabs (Otsuka & Dauer 1982). Of course, these motile predators may be removing other species besides ascidians and this experiment cannot separate out the more general predation effect. Small predators, such as snails and perhaps flatworms, have also been found to prey on newly settled asidians (Osman & Whitlatch 2004) and these micropredators would not have been excluded from the caged panels. Otsuka & Dauer (1982) also found a caging effect in the Chesapeake Bay with increased *Molgula*, sabellids and *Botryllus schlosseri* on the caged panels and increased cover of *Hydroides dianthus* on the uncaged panels. In retrospect, a caged control treatment should also have been used to separate out the predation effect from the other caging artifacts (Peterson 1979b).

Conclusion

When either *Cordylophora caspia* or *Molgula manhattensis* is locally abundant, they can have structural and biological effects on the benthic systems. *Molgula manhattensis* preempts space when locally abundant which may prevent other organisms from settling. *Molgula* does overwinter (Cory 1967) so its effect on other species might continue over several seasons. Low DO may provide a temporal or spatial refuge for *C. caspia* given its ability to survive and grow during hypoxia episodes. *C. caspia* provides structural substrate for some sessile species and a potential refuge for motile species. Both *C. caspia* and *M. manhattensis* are prey to motile organisms. Predation on these two species and competition from other sessile species may prevent these species from increasing the vulnerability of the benthic community to further invasion.

Acknowledgements

First and foremost, I must recognize the first class research done by Julia Blum in conducting the *Molgula manhattensis* experiment as a summer intern under my guidance at the Smithsonian Environmental Research Center. Dr. Anson Hines and Dr. Gregory Ruiz of the Smithsonian Environmental Research Center supported this research both financially and with scientific guidance. I gratefully acknowledge the invaluable assistance and support received in developing and trouble shooting these experiments from Jeff Crooks, Greg Ruiz, Tuck Hines, Linda McCann, Kenneth Sebens, and Midge Kramer. Financial support came from a University of Maryland Chesapeake Bay Summer Research Grant and the Invasions Program at the Smithsonian Environmental Research Center.

Table 4-1 Dissolved oxygen as measured during experimental period at each site at each depth. All readings below 4 mg/l are in bold font.

Site	Depth	12-Jul	26-Jul	6-Aug	29-Sep
1	S	8.4	8.8	15.8	9.25
	M	5.5	7.5	7.4	7.9
	D	4.4	**2.4**	**2.6**	7.38
2	S	9.2	**2.3**	6.7	8.15
	M	7.2	**2**	5.2	7.33
	D	**3.3**	**1.2**	0.52	6.08
3	S	4.5	**2**	7.5	8.24
	M	**2.4**	**0.8**	5.2	7.7
	D	**1**	**0.45**	**1.25**	6.7
4	S	10.2	**1.4**	7.3	10.5
	M	**3.2**	**0.8**	6.2	10.09
	D	**2.8**	**0.6**	**0.22**	7
5	S	8.6	9.2	8.7	9.58
	M	8.2	4.1	7.05	8.7
	D	5.1	**2.4**	**3**	8.24

Table 4-2. Analysis of Similarity (ANOSIM) Global R and p values for treatment comparisons for each experiment. Number in parenthesis is p value for the Global R statistic above. E = experimental panel seeded with live organism, M = panel with structural mimic, B = bare control panel.

Cordylophora caspia	**Factor 1 Panel treatment**	**Factor 2 Depth**
Percent cover	-0.03	0.12
sessile species	(0.640)	**(0.035)**
Abundances	-0.07	0.23
all species	(0.880)	**(0.003)**
Incidence	-0.06	0.11
all species	(0.850)	**(0.013)**

Molgula manhattensis	**Factor 1 Panel treatment**	**E vs. M**	**E vs. B**	**M vs. B**	**Factor 2 Caging**
Percent cover	0.30	0.19	0.42	0.28	0.75
	(0.004)	(0.059)	**(0.001)**	**(0.033)**	**(0.002)**
Incidence					
sessile species	0.08				0.30
	(0.110)				**(0.004)**
all species	0.15	0.14	0.21	0.12	0.22
	(0.024)	(0.137)	(0.059)	(0.111)	**(0.006)**

Table 4-3. Sessile species richness from *Molgula* experiment. Similarity percent (SIMPER) routine analysis comparing sessile species richness on caged versus uncaged treatments. Av. Sim = average similiarity within treatment, Av. Diss = average dissimilarity between treatments, SD = standard deviation. The larger the Sim/SD ratio the more that species consistently contributes to differences (or similarities) between panels within or between treatments. % = percent of the total similarity or dissimilarity contributed by the designated species. H.I. = which treatment had the higher incidence of the indicated species. *Molgula manhattensis* was not included in the analysis since we were analyzing its effects on the community.

Caged	**Av. Sim**	**Sim/SD**	**%**
Demonax microphthalma	10.72	8.28	14.38
Hydroides dianthus	10.72	8.28	14.38
Obelia bidentata	8.9	2.12	11.94
Balanus eburneus	8.81	2.14	11.81
Metafolliculina sp.	8.47	2.18	11.36
Diadumene leucolena	7.01	1.44	9.4
Ficopomatus enigmaticus	6.79	1.45	9.1
Membranipora tenuis	4.69	0.84	6.29
Anguinella palmata	3.12	0.67	4.18
No Cage	**Av. Sim**	**Sim/SD**	**%**
Balanus eburneus	7.44	2.12	11.59
Demonax microphthalma	7.44	2.12	11.59
Hydroides dianthus	7.44	2.12	11.59
Ficopomatus enigmaticus	7.44	2.12	11.59
Obelia bidentata	5.82	1.44	9.04
Upright bryozoan	3.64	0.85	5.61
Metafolliculina sp.	3.7	0.85	5.47
Polydora sp.	3.47	0.85	5.42
Membranipora tenuis	2.95	0.67	4.62
Anguinella palmata	2.85	0.67	4.79
Conopeum tenuissemum	2	0.53	3.1

Caged vs No Cage	**Av Diss**	**Diss/SD**	**%**	**H.I.**
Diadumene leucolena	3.07	0.99	8.58	Cage
Upright bryozoan	3.1	1.29	8.4	No Cage
Polydora sp.	2.95	1.18	8.01	No Cage
Balanus improvisus	2.78	0.97	7.53	Cage
Membranipora tenuis	2.64	0.8	7.15	Cage
Conopeum tenuissemum	2.46	0.97	6.66	No Cage
Metafolliculina	2.34	0.69	6.33	Cage
Conopeum chesapekensis	2.32	0.87	6.3	No Cage
Corophium sp.	1.92	0.76	5.21	No Cage
Obelia bidentata	1.65	0.48	4.47	Cage
Ficopomatus enigmaticus	1.57	0.5	4.25	No Cage
Porifera	1.35	0.63	3.67	No Cage

Table 4-4. Similarity percent (SIMPER) routine analysis comparing **A** sessile species percent cover and **B** mobile and sessile species incidence for panel treatments. Av. Diss = average dissimilarity between treatments, Diss/SD (standard deviation) is a measure of how consistently a species contributes to average dissimilarity across all such pairwise comparisons. The larger the Diss/SD ratio the more that species consistently contributes to differences between panels within or between treatments. % = percent of the total dissimilarity contributed by the designated species. H.C. = which treatment had higher percent cover and H.I. = which treatment had the higher incidence of the indicated species. *Molgula manhattensis* was not included in the analysis since we were analyzing its effects on the rest of the species.

A Sessile Species Cover

Molgula vs Mimic	**Av. Diss**	**Diss/SD**	**%**	**H.C.**
Barnacles	28.03	1.41	60.06	Molgula
Hydroides dianthus	13.35	1.01	28.59	Molgula
Demonax microphthalma	4.01	0.95	8.6	Molgula
Molgula vs Blank	**Av. Diss**	**Diss/SD**	**%**	**H.C.**
Barnacles	30.04	1.33	60.22	Molgula
Hydroides dianthus	24.28	1.11	24.28	Blank
Demonax microphthalma	8.76	0.91	8.76	Molgula
Mimic vs Blank	**Av. Diss**	**Diss/SD**	**%**	**H.C.**
Barnacles	26.56	1.44	54.42	Mimic
Hydroides dianthus	14.38	1.24	29.57	Blank
Demonax microphthalma	3.41	1	6.98	Blank

B Species Incidence (Mobile + Sessile species)

Molgula vs Mimic	**Av. Diss**	**Diss/SD**	**%**	**H.I.**
Rhithropanopeus sp.	2.73	1.64	7.92	Mimic
Anguinella palmata	2.17	1.09	6.3	Mimic
Upright bryozoan	2.14	1.08	6.2	Molgula
Conopeum tenuissemum	2.13	1.09	6.19	Mimic
Balanus improvisus	1.97	0.95	5.73	Molgula
Molgula vs Blank	**Av. Diss**	**Diss/SD**	**%**	**H.I.**
Balanus improvisus	2.47	0.99	6.32	Molgula
Upright bryozoans	2.36	1.01	6.04	Molgula
Nereis sp.	2.27	0.89	5.81	Molgula
Botryllus schlosseri	2.19	1	5.59	Blank
Anguinella palmata	2.18	0.99	5.57	Blank
Mimic vs Blank	**Av. Diss**	**Diss/SD**	**%**	**H.I.**
Conopeum tenuissemum	2.57	1.1	6.51	Mimic
Nereis sp.	2.29	0.8	5.8	Mimic
Botryllus schlosseri	2.28	1.08	5.77	Blank
Caprellids	2.26	1.07	5.71	Blank
Rhithropanopeus sp.	2.23	0.88	5.63	Mimic

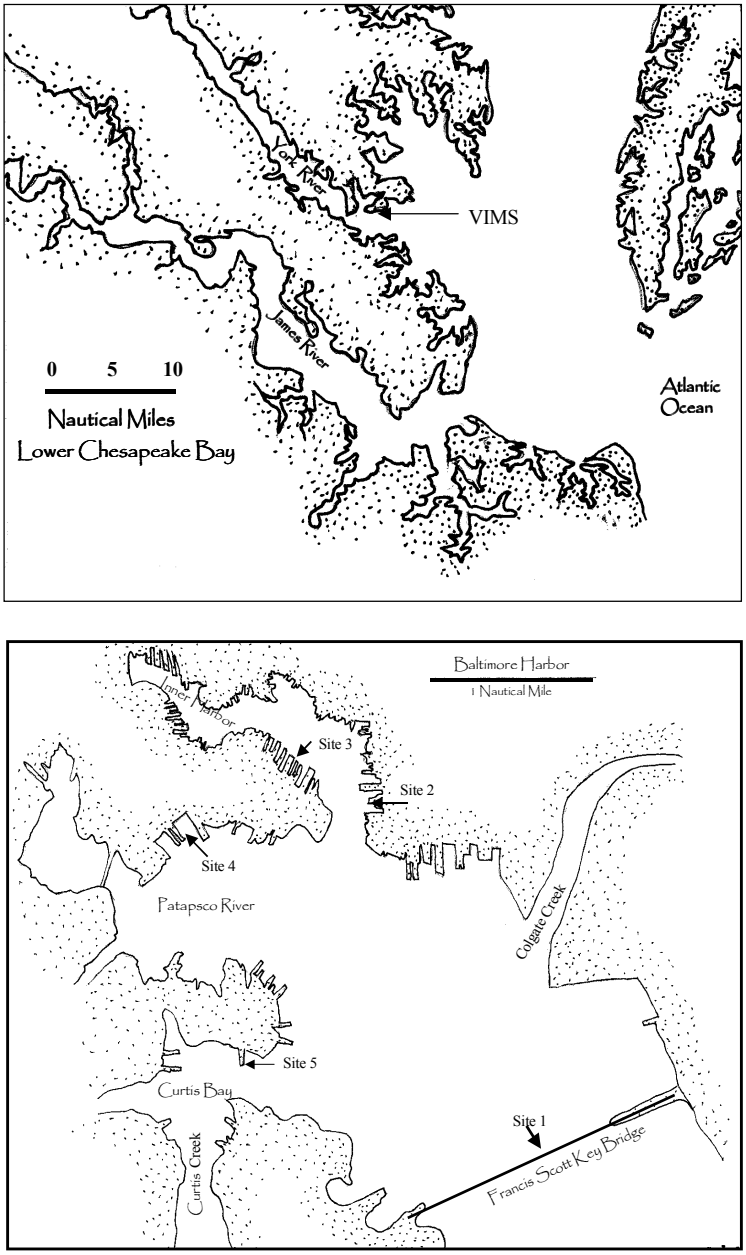


Fig. 4-1 Map of lower Chesapeake Bay with Virginia Institute of Marine Science (VIMS) and map of Baltimore Harbor with experimental sites.

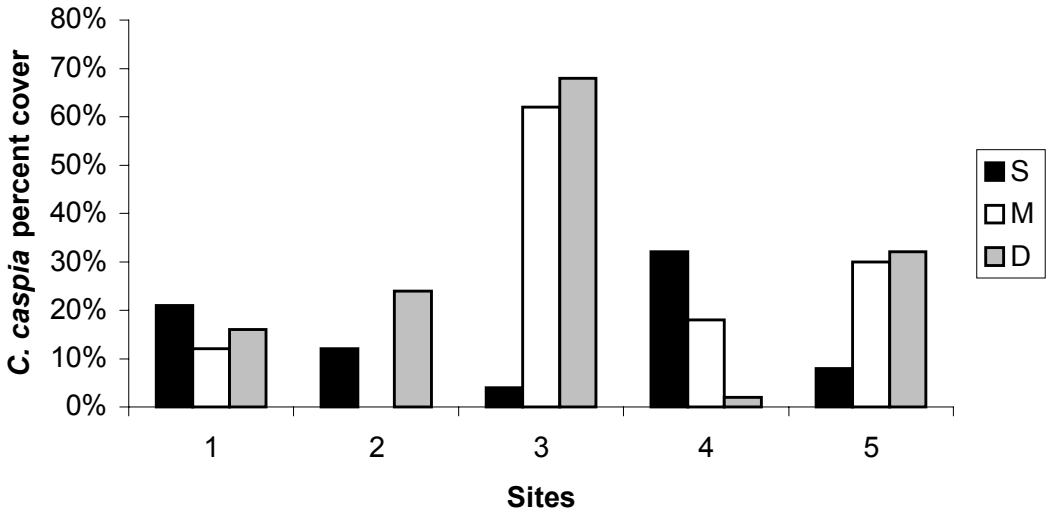


Fig. 4-2 Final percent cover of *C. caspia* on seeded experimental panels according to depth and site. Both healthy and stressed hydroid (without feeding zooids) was included in the analysis since lack of feeding hydranths does not indicate mortality (Roos 1979). All panels had some remaining hydroid although at the site 2 mid depth panel, the hydroid had less than 4% cover (minimal detectable with fixed grid method). S = 1 M, M = 3M, D = 7M deep. See Fig 4-1 for map of sites.

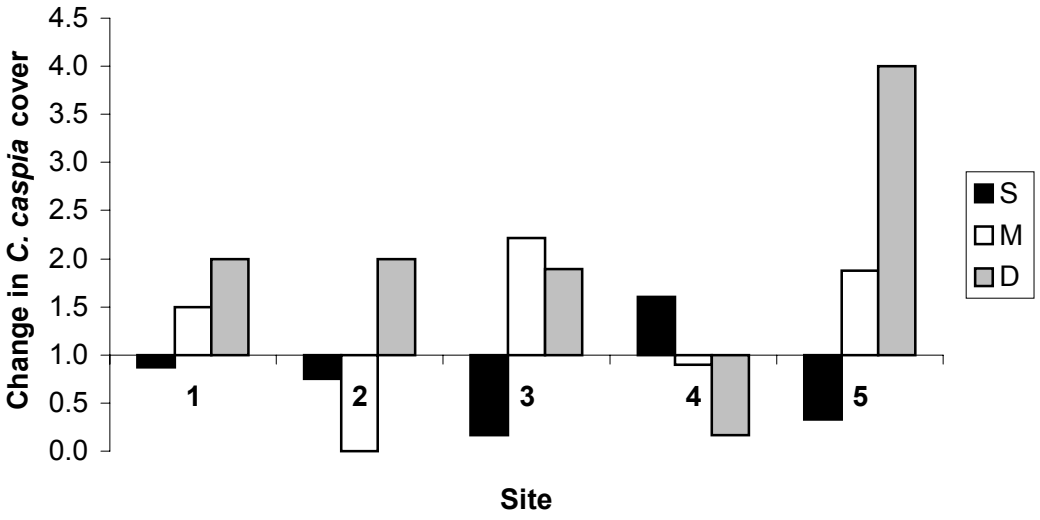


Fig. 4-3 Change (after/before) in cover of *Cordylophora caspia* at each depth at each site (n = 1). Note that cover decreased at the shallow depth and increased at the deep depth at four of the sites. The pattern was reversed only at site 4. Change in cover at the mid depths (3 M) did not follow the same pattern. S = 1 M, M = 3 M and D = 7 M below mean low water (MLW). See Fig 4-1 for Site map.

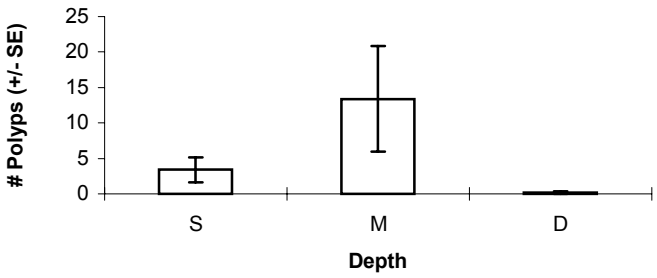


Fig. 4-4 Viable *Cordylophora caspia* polyps counted in 2 x 2 cm scrape samples taken from panels after percent cover analysis. Viable polyps were those with distinguishable hydranth and feeding tentacles. ANOVA F = 2.41, p = 0.13

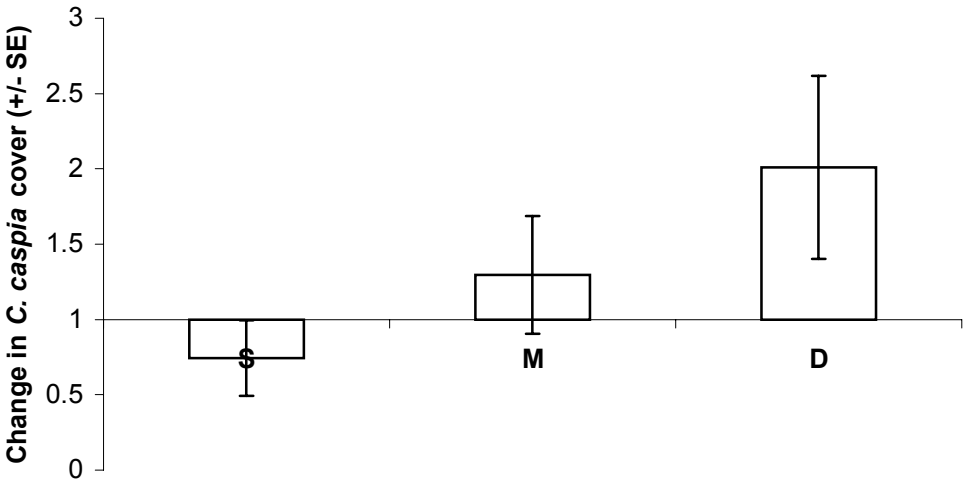
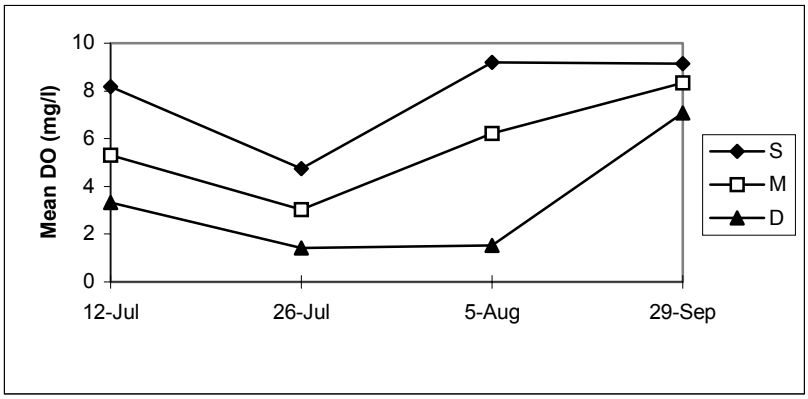
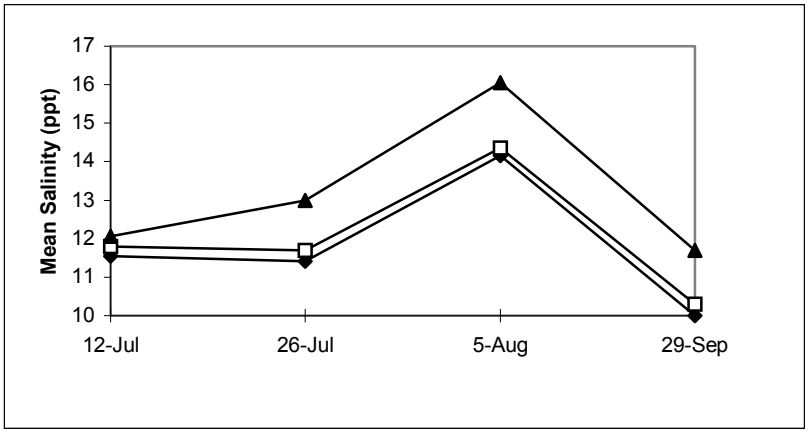


Fig. 4-5 Change in percent cover of *Cordylophora caspia* over the experimental period. 1 = no change in cover. If all sites are included in the analysis, the overall difference in cover change by depth is not significant: ANOVA F = 2.06, p = 0.17. However, if you remove Site 4 from the analysis (see Fig 4-2), the difference in change by depth is significant. ANOVA F = 5.39, p = 0.03.





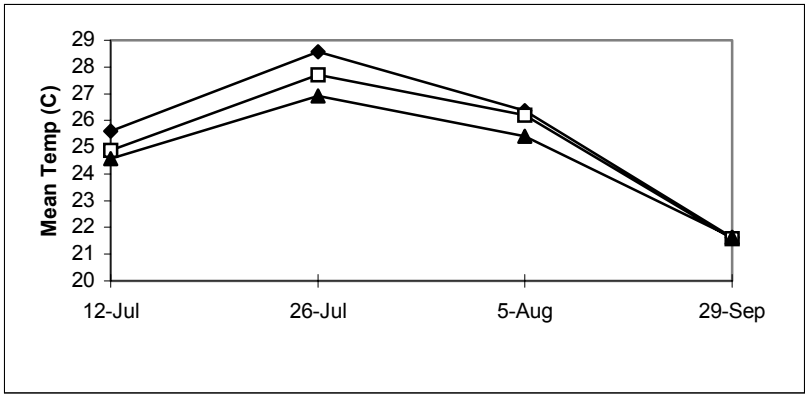


Fig. 4-6 Mean dissolved oxygen (DO), salinity and temperature as measured over the experimental period and in late September 1999. See Table 4-1 for DO measurements at each site and depth.

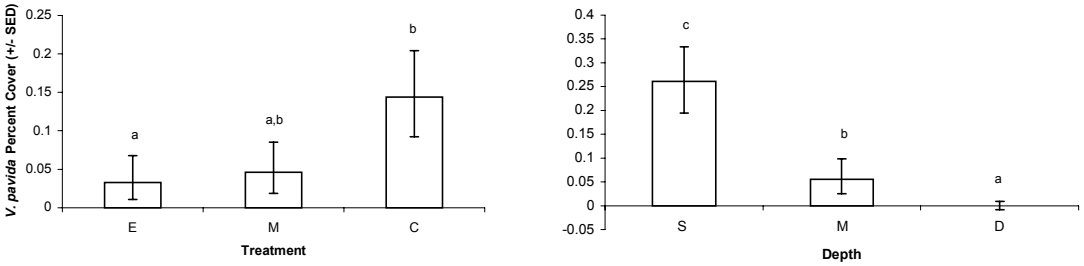


Fig. 4-7 *Victorella pavida* , bryozoan, mean percent cover as measured on settling panels in *Cordylophora caspia* experiment. ANOVA significant for both main effects but not for the interaction. All percent cover data was asin squrt transformed for statistical analysis then back transformed for graphs. ANOVA (ddf = 31) treatment $F = 3.58$, $p = 0.04$, depth $F = 19.41$, $p < .0001$. Treatments with same letter were not significantly different at the $p < 0.05$. E = all panels seeded with C. caspia, C = bare control panels, M = panels with structural mimic, S = all panels deployed at 1 M, M = 3 M and D = 7M

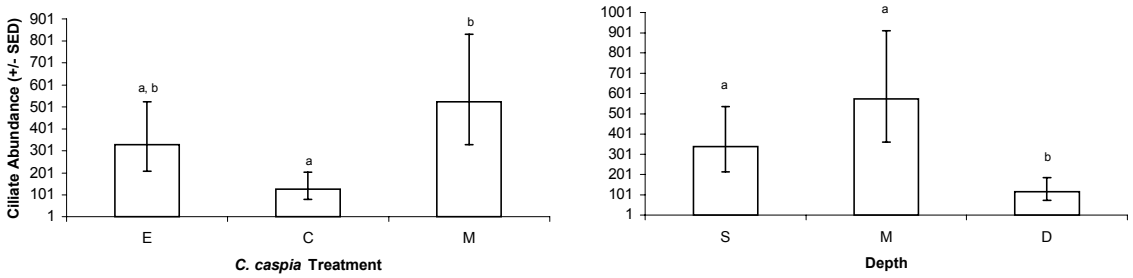


Fig. 4-8 Mean ciliate abundance (log +1 transformed) according to the main effects (*C. caspia* treatment and depth). ANOVA depth F = 6.36, p = 0.005; ANOVA treatment F = 4.81, p = 0.02. Same letters indicate no significant difference at the $p < 0.05$. See Fig. 4-7 for abbreviations.

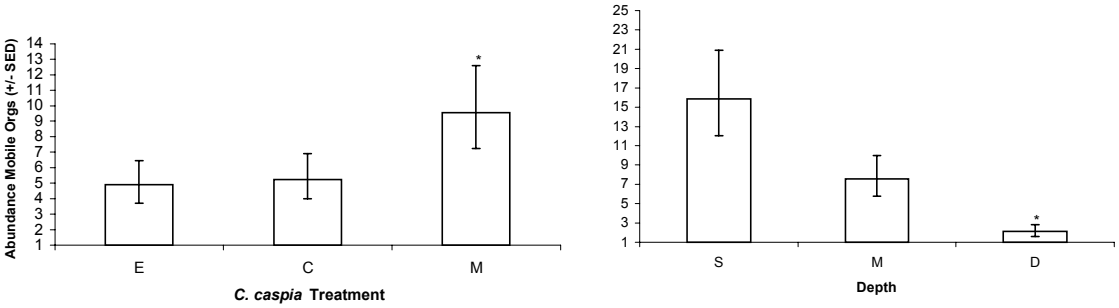


Fig. 4-9 Total abundance of all mobile organisms found in 2 x 2 cm scrape sample from the experimental panels. Mobile organisms included Nereis sp., nematodes, copepods, flatworms, and nudibranchs. Data was log transformed for statistical analysis then back transformed for graphs. ANOVA ddf = 31, depth F = 26.64, p = 0.0001; C. caspia treatment F = 3.53, p = 0.04. Asterisk indicates significant difference at $p < 0.05$. No significant interaction found. See Fig. 4-7 for abbreviations.

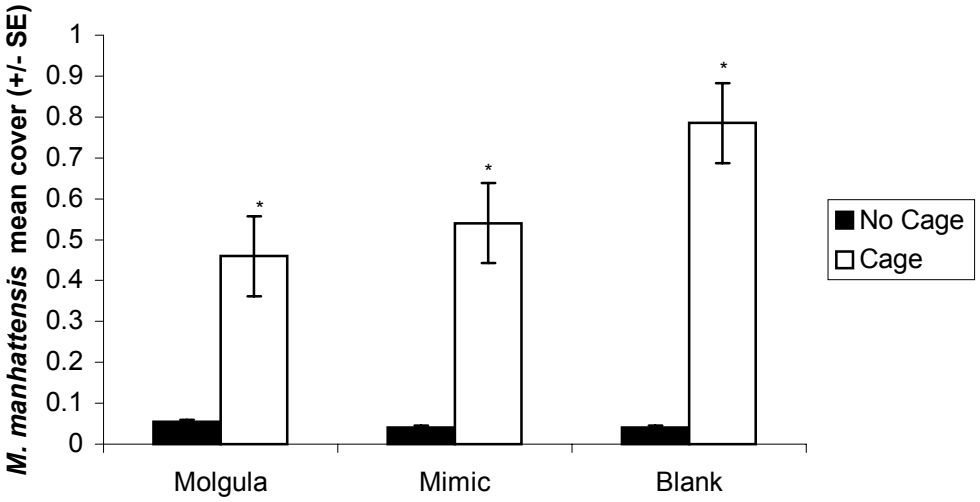


Fig. 4-10 *Molgula manhattensis* percent cover according to treatment as measured on live panels at the end of experiment. Factorial, mixed model ANOVA results: *Molgula* treatment F = 2.44, p = 0.11; Caging treatment F = 132.21, p < 0.0001; Molgula x Caging F = 3.56, p = 0.05. Asterisk indicates signficant difference at 0.05 level between caged and uncaged treatments for each type of panel treatment.

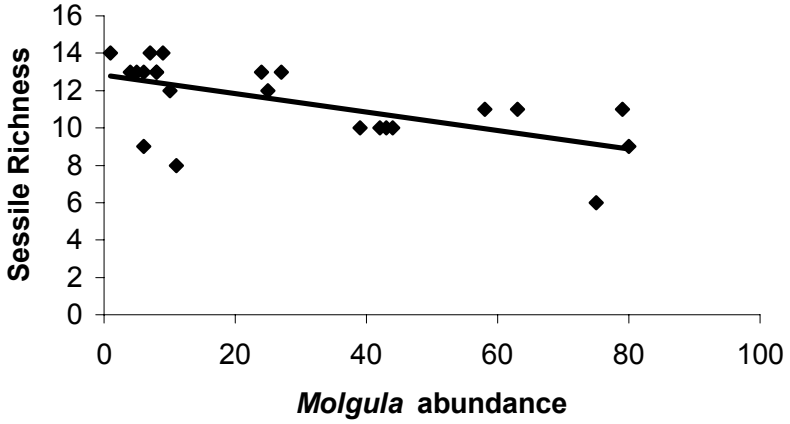


Fig. 4-11 Sessile species richness (# species) as a function of *Molgula manhattensis* abundance across per panel all treatments. Slope of linear regression line y = -0.0493x + 12.837, R^2 = 0.38, p = 0.002.

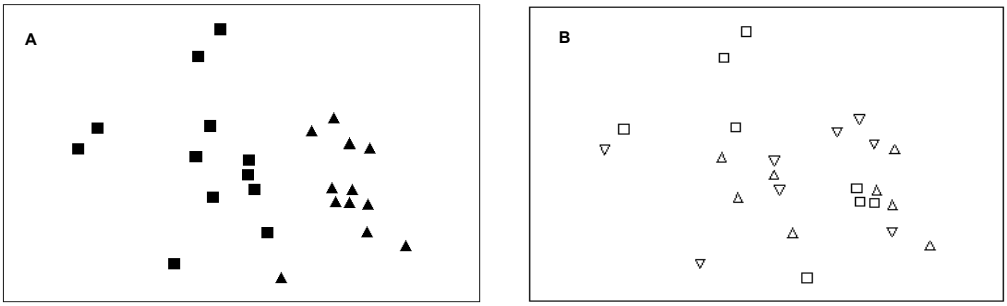


Fig. 4-12 Multi Dimensional Scaling graph (MDS) of asin squrt transformed primary percent cover for cage vs no cage factor. **A** Comparison of caged versus uncaged (square = cage, triangle = no cage) and **B** panel treatments (upright triangle = live Molgula, flipped triangle = Mimic, square = blank) for sessile species percent cover data. See Table 4-2 for ANOSIM analysis. Stress = 0.15.

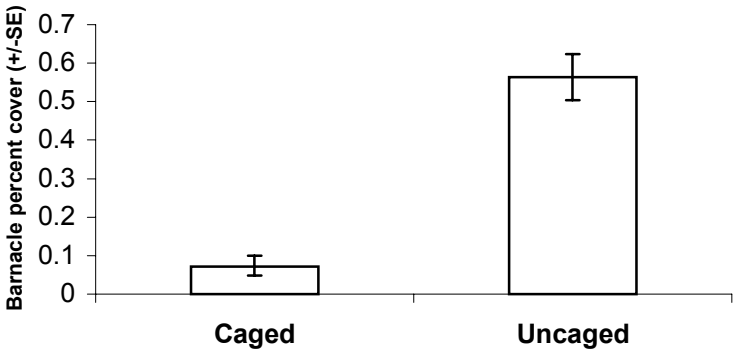


Fig. 4-13 Barnacle percent cover according to cage treatment in the *Molgula* effects experiment. ANOVA $F = 49.44$, $p < 0.0001$

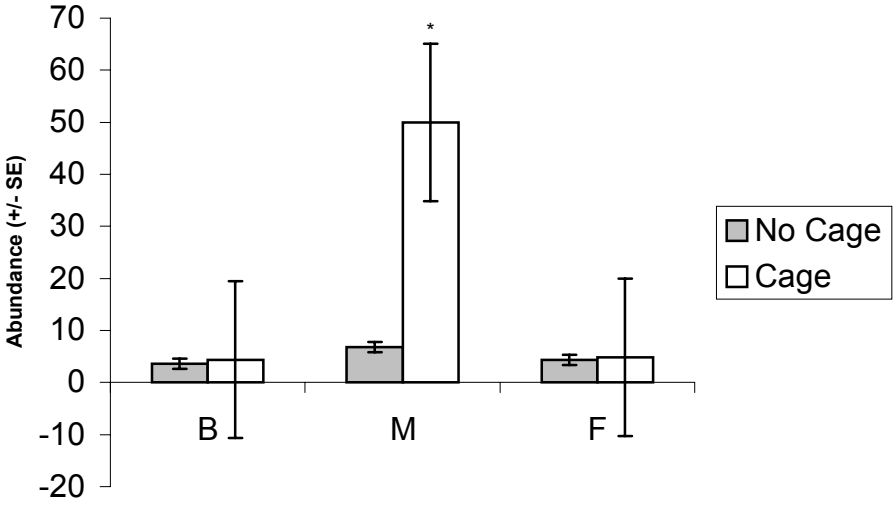


Fig. 4-14 Abundances of invasive and cryptogenic species according to caged treatment. B = *Botryllus schlosseri*, M = *Molgula manhattensis*, F = *Ficopomatus enigmaticus*. ANOVA on *Molgula* abundance (log transformed) for the cage treatment $F = 46.82$, $p < 0.00001$.

Dissertation Conclusion

Although hypoxia clearly represents a serious disturbance for epifaunal communities leading to changes in species incidence and cover, the significant effect of moderately low DO (2 - 4 mg l^{-1}) on the cover of invasive and cryptogenic species was not predicted. The hydrodynamics that cause this lesser form of hypoxia are not well understood but may be widespread in shallow areas of estuaries (www.cbp.org/wquality). The multi-site survey established the importance of length of exposure to low DO (< 4 mg l^{-1}) to structure of the epifaunal community.

The changes in relative dominance of sessile species in these experiments, from barnacles to *Hydroides dianthus*, may have created opportunities for less common native species (such as bryozoans, anemones and sponges) and invasive/cryptogenic species (especially *Ficopomatus enigmaticus*, *Botryllus schlosseri* and *Molgula manhattensis*). Sabellid polychaetes also left their tubes in response to low DO conditions opening space for other species. The changes in dominance were partly attributable to life history and larval availability during and after periods of low DO that disturbed dominant species and opened up space for settlement on primary substrate (Powers et al. 2001). The species with higher abundances of larvae in low DO waters were more likely to be available to capitalize on opened space.

The limitations of the controlled experiment emphasize the importance of the multi-site field survey. The lab/field experiment could not replicate all aspects of a low DO event. For instance, epifaunal communities on panels, stressed by low DO exposure, were returned to the York River and exposed to predation and larval recruitment. In a natural low DO episode, it might take days for larvae, fish and crabs to return to the

affected area. The field survey, however, documented similar results to those from the manipulative experiment: 1) increased cover of serpulid polychaetes and reduced cover of barnacles with increasing exposure to low DO, 2) shifting incidences and cover of bryozoans, sponges and sabellid polychaetes and 3) an increase in the cover of invasive/cryptogenic species.

Through several investigations, H' (Shannon-Wiener) diversity or richness (S) declined when a single species monopolized space. Barnacles achieved high cover in high DO conditions and when predation removed more competitive species. *Molgula manhattensis* attained high cover when predation was removed or when chronic low DO occurred (which may also have excluded predators from the area). *Hydroides dianthus* achieved high cover when the epifaunal community was exposed to periodic low DO. In all three cases, species richness declined with increasing cover or abundance of the dominant species. Loss of diversity at the local level is significant whether it is caused by high cover of an invasive species or a native species. Therefore, temporal and spatial environmental heterogeneity of the system may be important for maintaining regional species diversity to compensate for loss of diversity at the local level.

Deteriorating water quality conditions in the Chesapeake Bay may be facilitating the establishment of two newly arrived species. We must monitor the invasive serpulid, *Ficopomatus enigmaticus*, and isopod, *Synidotea laevidorsalis?,* given their positive association with low DO conditions (demonstrated in this dissertation). *F. enigmaticus* has created massive reefs on the West Coast of the U.S. and in the Mediterranean Sea with negative ecosystem effects (Schwindt et al. 2001). *Synidotea laevidorsalis?*, the invasive isopod first reported in the Chesapeake Bay in this dissertation, may also pose a

significant threat to epifaunal communities given its possible predation on sessile species such as hydroids (P. Fofonoff, personal communication).

Although introduced (invasive or cryptogenic) and native species were distinguished in this dissertation, identity and life history characteristics of particular species may have been more important to the results (R. Osman, personal communication). Only through further exploration and replication of experiments both in the Chesapeake Bay and other systems can an introduced species response to deteriorating environmental conditions be generalized. However, through rigorous, controlled experimental manipulation combined with systematic sampling of epifaunal communities and environmental conditions within naturally occurring low DO events in shallow waters of lower Chesapeake Bay, we established that shifts in epifaunal community structure, species dominance and diversity are happening in response to low DO. These changes may be compromising the resistance of the community to invasion in the Chesapeake Bay.

Appendix I

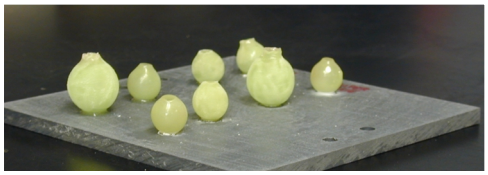
2000 Experiment. Community on panel exposed to normoxia only.

2000 Experiment. Community on panel exposed to hypoxia only.

2000 Experiment. Non-manipulated Control.

Appendix II

Cordylophora caspia mimic attached to PVC settling panel



Molgula manhattensis mimics attached to PVC settling panel

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