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The Effects of Hypoxia on Macrobenthic Production and Function in the lower Rappahannock River, Chesapeake Bay, USA

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Recommended Citation

The Effects of Hypoxia on Macrobenthic Production and Function in the lower

Rappahannock River, Chesapeake Bay, USA.

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A Dissertation

Presented to

The Faculty of the School of Marine Science

The College of William & Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Doctor of Philosophy

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By

S. Kersey Sturdivant
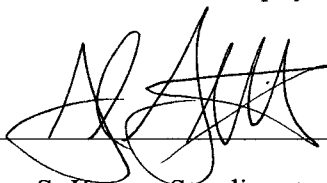
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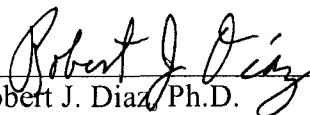
the requirements for the degree of

Doctor of Philosophy

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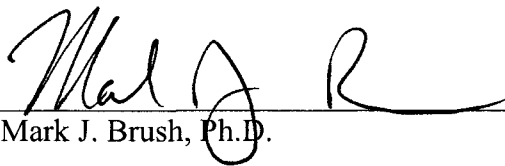
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To my Father and Mother,
who above all else instilled in me the
belief that anything was possible as long
as I applied myself.

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DISSERTATION ABSTRACT

Human development has eroded Chesapeake Bay's health, resulting in an increase in the extent and severity of hypoxia (≤ 2 mg O_2 l^{-1}). The Bay's hypoxic zones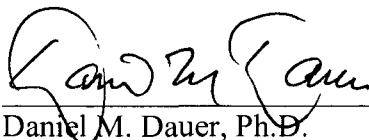 have an adverse affect on community function and secondary production of macrobenthos. The production of macrobenthos is important as these fauna link energy transfer from primary consumers to epibenthic predators and demersal fish, and serve as the foremost pathway that carbon is recycled out of the sediment. Additionally, bioturbation, an essential macrobenthic function that causes the displacement and mixing of sediment particles, increases the quality of marine sediments. In the marine environment bioturbation is primarily mediated by macrofauna which are susceptible to perturbations in their surrounding environment due to their sedentary life history traits.

The effect of hypoxia on macrobenthic production was assessed in Chesapeake Bay and three of its tributaries (Potomac, Rappahannock, and York rivers) from 1996 to 2004. Each year, 25 random samples were collected from each system and macrobenthic production estimated using Edgar's allometric equation. Efforts were then focused on the Rappahannock River, a sub-estuary of Chesapeake Bay known to experience seasonal hypoxia, to assess changes in macrobenthic production and function. During the spring, summer, fall, and following spring of 2007 and 2008, samples were collected each season in each year, and DO concentrations were measured continuously at two sites in 2007 and two in 2008. A benthic observing system (Wormcam) was also deployed in 2009 from early spring to late fall to assess the impact of hypoxia on bioturbation. Wormcam transmitted a time series of *in situ* images and water quality data in near real-time. Results from the previous projects was used to develop a continuous-time, biomass-based model, including phytoplankton, zooplankton, and macrobenthic state variables. The primary focus aimed at predicting the effect of hypoxia on macrobenthic biomass. Z', a sigmoid relationship between macrobenthic biomass and DO concentration, was derived from macrobenthic data collected from the 2007 and 2008 field experiments.

Annual fluctuations in macrobenthic production were significantly correlated with DO. Hypoxia led to a 90% reduction in daily macrobenthic production relative to normoxia, and production at hypoxic sites was composed primarily of smaller, disturbance-related annelids. The reduced production resulted in an annual biomass loss of approximately 7320 to 13,200 metric tons C, which equated to a 6 to 12% annual displacement of the Bay's total macrobenthic productivity due to hypoxia. Macrobenthic production differed across seasons, and sediment reworking rates were significantly higher during normoxia, indicating a change in the functional role of the macrobenthic community. Hypoxia was found to significantly reduce bioturbation through reductions in burrow lengths, burrow rates, and burrowing depth. Although infaunal activity was greatly reduced during hypoxic and near anoxic conditions, some individuals remained active.

The biomass-based model was successfully calibrated and verified, using independent data, to accurately predict B annually. Simulation analysis of the DO formulation showed B strongly linked to DO concentration, with fluctuations in biomass significantly correlated with the duration and severity of hypoxia.

DISSERTATION INTRODUCTION

In the past few decades, a major focus for coastal science has centered on the influence of anthropogenic disturbance in coastal systems. Human activity adversely affects land topography, chemistry of the Earth's atmosphere and water, rates and balance of biogeochemical processes, and biodiversity (Vitousek et al. 1997). The human population in Chesapeake Bay watershed has grown exponentially since colonial times, with a 3-fold increase over the last 100 years (Kemp et al., 2005). Eutrophication, an increase in the supply and accumulation of organic matter to a system (Nixon, 1995; Rabalais, 2004), is pervasive and anthropogenic eutrophication of coastal systems coincides with the introduction of industrially fixed nitrogen in the 1960s (Boesch et al., 2001). Nutrients in fertilizer are designed to enhance terrestrial production, however, when those excess nutrients are leaked to coastal systems, aquatic production is also enhanced and more biomass is produced; when coastal systems become saturated with organic matter, hypoxia develops and biomass is reduced (Boesch 2000; 2001).

This dissertation attempts to elucidate the effects that eutrophication-induced hypoxia has on macrobenthic production and function through historical assessment, seasonal and continuous monitoring, and finally using collected data to construct a predictive ecological model. Our main study site was the upper mesohaline of the lower Rappahannock River, a major tributary in lower Chesapeake Bay, with hydrography that allows for seasonal hypoxia (Kuo and Neilson, 1987; Park et al., 1996). A major accomplishment of this project was quantifying the affect of hypoxia on macrobenthic bioturbation, a key biological function in regulating sediment quality, using an innovative camera system, Wormcam. Wormcam is an *in situ* benthic observing system that is a

combination of a sediment profile camera and water quality datasonde, which can collect a time-lapse series of images and data, transmitting information in near real-time. Results from this dissertation revealed that a significant relationship between hypoxia and macrobenthic production and function exists, with lower production and inhibited function during hypoxia when compared with normoxia.

The dissertation is divided into four chapters, and each chapter is presented in standard manuscript format for the journal of submission. Chapter 1 on the "Relationship between hypoxia and macrobenthic secondary production in Chesapeake Bay" for *Marine Biology*, chapter 2 on the "Effects of seasonal hypoxia on macrobenthic production and community function in the Rappahannock River, VA, USA" for the *Journal of Experimental Marine Biology and Ecology*, chapter 3 on "Bioturbation in a declining oxygen environment, *in situ* observations" for the *Proceedings of the National Academy of Sciences*, and chapter 4 "Modeling the effect of hypoxia on macrobenthic production in the lower Rappahannock River, Chesapeake Bay, USA" for *Marine Ecology Progress Series*. The scientific context and content of each chapter is described next.

1.1 Historical Hypoxia

Hypoxia, dissolved oxygen concentrations ≤ 2.0 mg O_2 l^{-1}, is an emerging threat to coastal marine systems worldwide (Diaz and Rosenberg, 2008) and has been documented to have deleterious effects on marine fauna (Diaz and Rosenberg, 1995; Levin, 2003; Vaquer-Sunyer and Duarte, 2008). Hypoxia can be a natural phenomenon determined primarily by physical factors,

such as water mass movements, temperature, and salinity gradients (Kruse and Rasmussen, 1995; Grantham et al., 2004). There has been a substantial increase in hypoxic/anoxic water in Chesapeake Bay: from approximately 3 km^3 in the 1950s, to approximately 10 km^3 in the 1990s, primarily attributed to anthropogenic eutrophication (Hagy et al., 2004). Areas of low DO adversely affect the inhabitants of the system; the ecological consequences of periodic hypoxia vary and are hypothesized as a mechanism for regulating benthic populations (Dauer et al., 1992; Llansó, 1992).

In chapter 1, we present a historical account of the relationship between hypoxia and macrobenthic production in mainstem Chesapeake Bay and its three major tributaries (Potomac, Rappahannock and York Rivers), from 1996-2004. We address the disparity in macrobenthic production between normoxic and hypoxic sites, as well as the variability in macrobenthic production both spatially and temporally in relation to hypoxic severity. Analyses of production inputs at a species level are taken into account and inferences on impacts for higher trophic levels are made.

1.2 Seasonal Hypoxia

There is a general understanding of hypoxia's effects on community structure, where a series of predictable and graded responses occur, ranging from no obvious change in mild hypoxic regions, to mass mortality of bottom fauna in severe hypoxic areas (Rabalais et al., 2001). There is less of an understanding, at the functional level, of how low DO concentrations interact with macrobenthic secondary production, and the subsequent trophic transfer of production (Baird et

al., 2004). One of the first people to consider the overall flow and balance of matter in an energetic sense was Lindeman (1942). He realized that if all the components of an ecosystem could be expressed in common units of energy, then the functioning of the system could be more easily understood. Thermodynamics is then the common denominator defining the manner of energy transformation and ecological usefulness of varying energy forms (Benke et al., 1988; Wiegert, 1988).

In chapter 2 we assessed macrobenthic production temporally across seasons, sampling the same sites in the spring, summer, fall, and again the following spring to assess recovery. The data were used to determine if a relationship between DO concentration and macrobenthic production existed, and if so, how DO influenced the variation in production between normoxic and hypoxic sites. Taxonomic and functional associations between macrobenthic production and hypoxia were also assessed.

1.3 Bioturbation and Hypoxia

Bioturbation, the biological reworking of sediments by flora, fauna, or microbial activity (Meysman et al. 2006), is a vital function provided to coastal marine systems. Macrofaunal bioturbation is the foremost pathway that carbon is recycled out of the sediment and eventually out of the Chesapeake Bay system (Diaz and Schaffner, 1990), and it plays an important role in regulating the geochemical and physical properties of marine sediments (Aller, 1978; Rhoads and Boyer, 1982). Additionally, bioturbation of macrofauna distributes DO much deeper into the

sediment (Aller, 1982); under normal conditions DO penetrates sediments by physical diffusion to a depth of only a few millimeters (Revsbech et al., 1980).

In chapter 3 we quantify the relationship between bioturbation and DO concentration, *in situ*, through the deployment of Wormcam, a novel adaptation of a sediment profile camera and water quality datasonde. We related burrow depth, burrow rate, and burrow lengths to DO concentration and made inferences on how changes in these structures and processes during hypoxia affected overall macrobenthic bioturbation.

1.4 Modeling Hypoxia

Finally in chapter 4 we used information from the proceeding chapters to develop a continuous-time biomass-based model for the lower Rappahannock River, based on the benthic sub-model in the 2002 Chesapeake Bay Eutrophication Model. The primary focus was aimed at accurately modeling the effect of hypoxia on macrobenthic biomass, and a sigmoid relationship was determined from macrobenthic data collected in the Rappahannock River during earlier field experiments. The equation from the sigmoid curve related macrobenthic biomass to DO concentration and was plugged into our overall model, and inferences about hypoxic duration and severity on the benthic ecosystem were made. Results from this chapter have broad reaching implications on modeling the affect of hypoxia on the benthic environment, confirming that quantitative assessments on the relationship between DO and benthos can and should be derived for application in ecosystem-scale models.

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CHAPTER 1

Hypoxia and macrobenthic secondary

production in Chesapeake Bay

ABSTRACT

Over the years, human development has eroded Chesapeake Bay's health, resulting in an increase in the extent and severity of hypoxia (≤ 2 mg O_2 l^{-1}). The Bay's hypoxic zones have an adverse affect on both community structure and secondary production of macrobenthos. The effect of hypoxia on macrobenthic production was assessed in Chesapeake Bay and three of its tributaries (Potomac, Rappahannock, and York rivers) for the years 1996 to 2004. Each year, 25 random samples were collected from each system and macrobenthic production estimated using Edgar's allometric equation. Annual fluctuations in macrobenthic production were significantly correlated with dissolved oxygen. Hypoxia led to a 90% reduction in daily macrobenthic production relative to normoxia. This resulted in an annual biomass loss of approximately 7320 to 13,200 metric tons C, which equated to a 6 to 12% annual displacement of the Bay's total macrobenthic productivity due to hypoxia. While higher consumers may benefit from easy access to stressed prey in some areas, the large spatial and temporal extent of seasonal hypoxia likely limits higher-trophic-level transfer via the inhibition of macrobenthic production. The loss of macrobenthic production may be detrimental to the overall health of the Bay, as it comes at a time when epibenthic and demersal predators have high energy demands.

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1. INTRODUCTION

Eutrophication, an increase in the supply and accumulation of organic matter to a system (Nixon, 1995; Rabalais, 2004), of estuarine and marine ecosystems is pervasive and has led to a series of counter acting benthic community impacts (Rosenberg, 1985; Nixon, 1995). Reductions in benthic species richness and increases in abundance and biomass are the most obvious and have been documented in many systems (Pearson and Rosenberg, 1978; Rosenberg, 1985). In addition, dissolved oxygen (DO), which is essential in microbial and metazoan metabolism, has declined in many systems experiencing eutrophication and given rise to hypoxia and anoxia (Diaz and Rosenberg, 2008). We define normoxia as DO concentrations >2.8 mg l^{-1}, mild hypoxia 2.8-2.1 mg l^{-1}, and hypoxia as DO concentrations ≤ 2 mg l^{-1} (Tyson and Pearson, 1991).

Seasonal hypoxia occurs throughout Chesapeake Bay and some of its tributaries during the summer months, and was present with the first DO measurements by Newcombe et al. (1939) in the early 1930s for the mainstem of Chesapeake Bay and by Sale and Skinner (1917) in the Potomac in the 1910s. The most severe low oxygen events occur in the mainstem (Officer et al., 1984) creating what was termed an "oxygen desert," and low oxygen conditions in the Bay last approximately 120 days. From the 1950s through the 1990s, there has been a substantial increase in hypoxic/anoxic water in Chesapeake Bay: from approximately 3 km^3 in the 1950s, to approximately 10 km^3 in the 1990s (Hagy et al., 2004). The increase of hypoxia in the Bay is troubling, as hypoxic areas have been well documented to have negative impacts on estuarine benthos (Jørgensen 1980; Rosenberg et al., 1992; Llansó, 1990; Dauer et al., 1992; Rabalais et al., 2001; Tallqvist, 2001). The ecological consequences of periodic and seasonal hypoxia vary

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and are hypothesized as a mechanism for regulating benthic populations (Dauer et al., 1992; Llansó, 1992).

At the community structure level, hypoxic systems exhibit a predictable and graded series of responses to oxygen depletion, ranging from no obvious change, to mass mortality of bottom fauna (Diaz and Rosenberg, 1995). At the initial onset of hypoxia organisms increase respiration (Petersen and Petersen, 1988), and mobile fauna migrate from the area (Pihl et al., 1991). As DO further declines sessile fauna cease feeding and decrease activities not related to respiration (Warren, 1984). Infauna migrate closer to the sediment surface as reduced compounds accumulate, and are observed on or extending above the sediment surface in a moribund condition (Jørgensen, 1980; Tyson and Pearson, 1991). Finally, if the duration of hypoxia is sustained, mass mortality occurs in all but the most tolerant of species (Llansó, 1992; Diaz and Rosenberg, 1995). At the functional level, however, there is less of an understanding of how low DO concentrations interact with macrobenthic secondary production, and subsequent trophic transfer of the production (Baird et al., 2004). Productivity provides an index of community processes proportional to total community respiration and consumption, and integrates the influence of biotic variables and environmental conditions affecting individual growth and population mortality (Edgar and Barrett, 2002; Cusson and Bourget, 2005). Benthic abundance and biomass can supply basic information on potential energy available to higher consumers, but estimates of secondary production provide crucial information on trophic dynamics, and quantitative approximations of energy available to higher trophic levels (Wilber and Clarke, 1998). The derived quantitative production measurements can then be used to make inferences about trophic transfer of energy.

Secondary production, or the heterotrophic production of organic matter, is viewed as an estimate of estuarine health (Diaz and Schaffner, 1990; Dolbeth et al., 2005). The production of benthic invertebrates is important as these fauna serve as a link in the energy transfer from primary consumers to higher trophic levels (Nilsen et al., 2006), and is the foremost pathway that carbon is recycled out of the sediment and eventually out of Chesapeake Bay system (Diaz and Schaffner, 1990). It is estimated that approximately 20-50% of benthic secondary production within the bay is carried over from year to year as standing stock biomass (Baird and Milne, 1981; Holland et al., 1988), and approximately 21,400-27,500 metric tons C (MT C) of benthic organisms are needed to support the Bay's demersal fishery yields (Diaz and Schaffner, 1990). While direct calculations of macrobenthic production are costly and time consuming (Wilbur and Clarke, 1998), methods have been proposed for the indirect calculation of macrobenthic production based on biotic and abiotic variables (Edgar, 1990; Sprung, 1993; Brey et al., 1996).

The increment summation method, the removal summation method, the instantaneous growth method, and a production estimate by the Allen curve are all indirect methods of calculating macrobenthic production that yield similar result (Gillespie and Benke, 1979). However, these methods are based on body weight and cohort abundance sampled at regular time intervals (Sprung, 1993). Our data set has a number of individuals, which cannot be associated with a cohort, making production estimates using these methods non-viable. Further, estimates of production by body size have been related to the quotient of annual production to mean annual biomass, to the body weight at first sexual maturity (Banse and Mosher, 1980) and mean annual body weight (Schwinghamer and Hargrave, 1986). Determining body weight at sexual maturity

would be difficult to obtain for species whose life history is poorly understood, making this estimate of macrobenthic production impractical, and our point method of sampling eliminates production estimates relying on mean annual body weight. For our purposes, we used the Edgar method, which incorporates individual body weight and water temperature (Edgar, 1990). The theoretical bases for Edgar's equation is grounded in the metabolic theory of ecology that shows, among other things, that a constant fraction of metabolism tends to be allocated to production across taxa (Brown et al., 2004).

Using production theory and empirical models developed to quantify macrobenthic production without the requirement of intense sampling, we attempted to relate patterns of macrobenthic production in Chesapeake Bay and its tributaries to DO concentration. Specific objectives of our study were to 1) describe patterns of macrobenthic production spatially (across habitat) and temporally (by year) and assess the relationship with DO concentration; 2) determine taxonomic associations between macrobenthic production and DO concentration; and 3) infer how macrobenthic production losses due to hypoxia impact epibenthic predators and demersal fish.

2. METHODS

2.1. Sampling Design

The Chesapeake Bay Long-Term Benthic Monitoring Program started annual random sampling of Chesapeake Bay and its tributaries in both Maryland and Virginia in 1996 (Fig. 1). The Bay was divided into 10 sampling strata with each having 25 random sampling sites per year. Sites were sampled from late July to early September, with a new set of random sites selected each

year (Dauer and Llansó, 2003). Within the monitoring framework, we included stations from all habitats within the mesohaline and polyhaline Chesapeake Bay Mainstem, Potomac River, Rappahannock River, and York River from 1996 to 2004. These are the main areas within the Chesapeake system that experience hypoxia (Kuo and Neilson, 1987; Hagy et al., 2004). It should be noted that the deep trough (depths greater than 12 km) in the Maryland portion of the mainstem was not sampled. Previous assessments by the Bay program found the 676 km^2 deep trough in Maryland mainstem (roughly 5.8% of the total bay) to be anoxic and azoic during the summer, and it was therefore excluded from the sampling regimen. The Mainstem, Potomac River, and Rappahannock River all experience sustained seasonal hypoxia (Sale and Skinner, 1917; Officer et al., 1984; Kuo and Neilson, 1987), with periodic hypoxia documented in the York River (Kuo and Neilson, 1987).

Samples were collected with a Young grab (440 cm^2 to a depth of 10 cm) and sieved in the field through a 0.5-mm screen. Organisms and detritus retained on the screen were transferred into labeled jars, preserved in a 10% formaldehyde solution and stained with Rose Bengal. Two surface-sediment sub-samples of approximately 120 ml each were collected for silt-clay, organic carbon, and nitrogen analysis from an additional grab sample at each site. At each station, DO, salinity, and temperature were measured approximately 1 m from the bottom using a YSI model 6600 sonde. Samples were processed to identify and enumerate each species present as described in Dauer and Llansó (2003). Ash-free dry-weight biomass was measured for each species by drying to a constant weight at 60°C and ashing in a muffle furnace at 500°C for four hours. Sediment samples were wet-sieve analyzed for percent silt-clay content (Folk, 1966).

2.2. Secondary Production

Prior to estimation of production, data from large-bodied epifaunal and infaunal species known to be over-dispersed and not adequately sampled by the Young grab were removed. These included the bivalves *Crassostrea virginica*, *Mercenaria mercenaria*, and *Geukensia demissa*. Given that our focus was on effects of DO on production, we did not include data from stations in the tidal freshwater and oligohaline zones, as these habitats were subjected to little or no hypoxia.

Edgar (1990) developed a general allometric equation ($P = 0.0049 * B^{0.80}T^{0.89}$) that relates daily macrobenthic production P ($\mu g \cdot C \ day^{-1}$) to ash-free dry weight B (μg) and water temperature T (°C). The only departure from Edgar's method, which uses the mean AFDW of animals retained on a series of sieves of differing mesh size, was the usage of mean AFDW of each species by sample. Biomass measurements at the species level allowed us to examine taxonomic and functional group associations between production and DO. To ensure the quality of our production estimates, *Paraprionospio pinnata* production estimates were compared with direct measurements of *P. pinnata* production from Hinchey (1996) for the Mainstem Bay and York River and found to be approximately comparable. *P. pinnata* was chosen as it is the most numerous of the annelids collected and was ubiquitous across strata and years. These findings provide confidence in the macrobenthic production values reported in this manuscript.

2.3. Analysis Strategy

Given the random selection of stations through time and the possibility that there might be a serial dependence between DO and habitat with time (year), a mixed-effect longitudinal design was used to analyze patterns in the data. Generalized estimating equations (GEE) were applied with the normal distribution, identity link, and cross-year correlations within areas assumed to be equal (Zeger et al., 1988). Analysis of variance (ANOVA) was also used to test for differences between and within areas for quantitative parameters. Normality was checked with the Shapiro-Wilk test and homogeneity of variance with Bartlett's test. If variance was not homogeneous, Welch analysis of variance, which allows standard deviations to be unequal, was used in testing for mean differences (Zar, 1999). Tukey's HSD test was used for multiple mean comparisons. All statistical tests were conducted using SAS® (SAS Institute, Inc. 1989).

3. RESULTS

The total area of Chesapeake Bay and its tributaries is approximately 12,000 km^2. The area of the mesohaline and polyhaline Mainstem, Potomac, Rappahannock, and York Rivers covered by our sampling is approximately 7720 km^2. Therefore, we estimated summer daily macrobenthic production for approximately 65% of Chesapeake Bay. Mean hypoxic volume from the mid-1980s to 2006 was 10.7 x 10^9 m^{-3}; yearly hypoxic volumes for our observation period were compared as either being higher or lower than this mean (Hagy et al., 2004). Estimated summer daily macrobenthic production in Chesapeake Bay from 1996 to 2004 was significantly variable from year to year (Table 1; Fig. 2). Total macrobenthic production was significantly higher from 1999 to 2001, years with below-average hypoxic volume, and lower in 2003 and 2004, years

with above-average hypoxic volume. Production remained relatively constant from 1996-1998

despite a greater than 2-fold increase in hypoxic volume during that time frame.

Daily macrobenthic production was significantly related to DO with higher production at sites

with normoxia as opposed to hypoxia (Table 2, Fig. 3). From 1996 through 2004, normoxic sites

in Chesapeake Bay averaged 39 mg C $m^{-2} d^{-1}$, which was significantly higher than the 4 mg C $m^{-2} d^{-1}$ averaged during hypoxia. The mean daily production of normoxic sites was not significantly

different from the 11 mg C $m^{-2} d^{-1}$ produced by mild hypoxic sites. Overall, hypoxia reduced

daily macrobenthic production by 90% (Fig. 3). Salinity was also found to have a significant

effect on macrobenthic production with higher production at lower salinities. The effect of grain

size on macrobenthic production was marginally significant and depth had no effect (Table 2).

Most of the variability in daily macrobenthic production was associated with DO and salinity.

Production loss due to hypoxia was analyzed in our study area for years 1998 and 2001; these

years represent maximum and minimum volumes of hypoxia for our nine-year study,

respectively (Hagy et al., 2004). In 2001, macrobenthic production averaged approximately 70

mg C $m^{-2} d^{-1}$ within our study area of the Bay, which converts to 0.07 MT C $km^{-2} d^{-1}$. Hypoxic

volume in Chesapeake Bay in 2001 was 6 km^3, covering approximately 960 km^2 (Hagy et al.,

2004). Using the previous values, production for the area affected by hypoxia should have been

67 MT C d^{-1}. Factoring in a 90% reduction for the effect of hypoxia (Fig. 3) approximately 61

MT C d^{-1} of biomass was lost in 2001. A similar calculation was conducted for 1998 when

macrobenthic production averaged approximately 44 mg C $m^{-2} d^{-1}$, and this converts to 0.04 MT

C km^{-2} d^{-1}. Hypoxic volume in 1998 was 18 km^3 covering approximately 3000 km^2, thus

approximately 110 MT C d^{-1} of biomass was lost in 1998. When 61 and 110 MT C d^{-1} are scaled

by 120 days, the average duration of hypoxia in the Bay (Hagy et al., 2004), the annual loss in

biomass in hypoxic areas ranged from 7320 to 13,200 MT C. The habitat-weighted estimate of

macrobenthic production for the entire Chesapeake Bay is 17 g C m^{-2} yr^{-1} (Diaz and Schaffner,

1990), which equates to 114,600 MT C annually. Thus, from 6 to 12% of the Bay's

macrobenthic productivity is either displaced to periods of normoxia or lost to the system due to

hypoxia.

When partitioned by production per unit area, Mainstem Chesapeake Bay was the major

contributor to summer daily macrobenthic production. Macrobenthic production in the

Mainstem Bay was significantly higher (ANOVA, df=3, F=14.23, p < 0.0005) than production in

the Potomac, Rappahannock, and York Rivers. Normoxic sites accounted for the majority of

Mainstem production; a similar pattern was observed in the tributaries (Table 1). Macrobenthic

production trends over time (year) were significantly different for sites that experienced

normoxia and hypoxia in the Mainstem (Paired T-test, df=8, t=4.92, p = 0.002) and Potomac

River (Paired T-test, df=8, t=3.28, p = 0.017). Over the observed period, daily macrobenthic

production in the Rappahannock was not significantly different between normoxia and hypoxia

(Paired T-test, df=8, t=2.78, p > 0.06). This finding is likely influenced by high daily

macrobenthic production in hypoxia for years 1998 and 1999. In the York River, samples were

only collected in hypoxic areas in 2001, 2003, and 2004 (Table 1), due to the random sampling

design and short-term periodic hypoxia in the system (Diaz et al., 1992). Hypoxic production

was compared between the four systems, and the Mainstem and Potomac rivers had significantly

lower (ANOVA, df=3, F=9.67, p = 0.001, Table 1) production during hypoxia than the York

River; the Rappahannock was not significantly different from any system with relatively

intermediate macrobenthic production during hypoxia.

Molluscs, annelids, and arthropods accounted for over 98% of production (Table 3). For all

oxygen levels, daily molluscan production (35.2 mg C m^{-2} d^{-1}) was significantly higher

(ANOVA, df=8, F=83.70, p < 0.0005) than annelid production (8.6 mg C m^{-2} d^{-1}), which was

significantly higher than arthropod production (3.8 mg C m^{-2} d^{-1}). The production was

significantly different between normoxic and hypoxic sites for mollusc, annelids, and arthropods.

Hypoxic sites had 95% lower bivalve and gastropod production; this reduction was only

significant (p = 0.003) for bivalves due to the high variance in gastropod production. Polychaete

(p < 0.0005) and oligochate (p = 0.027) production was also significantly lower at hypoxic sites,

by 70% and 95%, respectively. Amphipods (p = 0.013) and isopods (p < 0.0005) had

significantly lower production at hypoxic sites, by 95% for amphipods and approximately 99%

for isopods.

Over the 9-year observation time, bivalve (df=8, F=2.70 p=0.006), annelid (df=8, F=4.41

p<0.0005), and arthropod (df=8, F=2.59 p=0.008) production were each analyzed separately and

found to be significantly different between years. Tukey's multiple mean comparison was used

to determine significant differences among years, and the maximum and minimum years of

hypoxic volume were assessed for each group. For the maximum hypoxic year of 1998,

bivalves, annelids, and arthropods had 90%, 45%, and 50%, less production, respectively,

compared to 2001, the minimum hypoxia year during our study. These production differences between 1998 and 2001 were significant for molluscs and arthropods and trended in that direction for annelids. Spatially, there were significant differences between study areas in production by major taxon (Table 4).

4. DISCUSSION

We found that daily macrobenthic production in Chesapeake Bay was significantly related to DO, with overall macrobenthic production at hypoxic sites less than 90% of normoxic values. For many major taxonomic groups, production reductions of 95% or greater occurred. Such a drastic reduction in macrobenthic production could have negative consequences for Chesapeake Bay, as benthic invertebrates link energy transfer from primary producers to economically important higher consumers (Möller et al., 1985; Brey, 2001). Additionally, an annual loss in macrobenthic biomass of 7320 to 13,200 MT C was observed during the summer, reducing the yearly productive capacity of the Bay benthos by 6 to 12%; energy demands of epibenthos and demersal fish, predators of benthic organisms, are at their highest during the summer months when these reductions occur.

Daily macrobenthic production in Chesapeake Bay fluctuated from year to year (Fig.2). When production was compared to hypoxic volume for corresponding years (Hagy et al., 2004), there was a noticeable trend of lower macrobenthic production during years of above average hypoxic volume (2003 and 2004), and higher macrobenthic production during years of below average hypoxic volume (1999-2001). Production remained relatively constant from 1996-1998 during a

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greater than 2-fold increase in hypoxic volume. This observation could be best explained by resource compensation in the presence of a deleterious condition. Concentrations of organic matter were not assessed in this study, so the available organic concentrations for macrobenthos were unknown. However, hypoxic volumes in coastal systems are correlated with eutrophication and the subsequent primary productivity generated (Lohrenz et al., 1990); greater primary productivity, greater hypoxic volume to the extent allowable by hydrography (Diaz, 2001). Hypoxic volumes from 1996-1998 were some of the highest observed during our observation period and could have been correlated with above average primary production. The organic content of these blooms would eventually reach the macrobenthos in a relatively shallow system such as Chesapeake Bay, and the organic rich environment fostered by the bloom would be of benefit to macrobenthos adapted to survive in low DO concentrations. While the increased hypoxic volume may have reduced macrobenthic production, the parallel organic rich environment may also have increased macrobenthic production, explaining the relative constant production over time (year). It is also important to note that the sampling design may have affected observed trends. Sediment grabs and DO concentrations were point measurements collected during the daytime, every year in the summer. The limitation of point measurements is the snap-shot view they provide, with little inference as to what occurs between data collection. It is very likely that some sites classified as normoxic when sampled experienced hypoxia at some point or multiple times throughout the season. While the sites may not have experienced sustained hypoxia, periodic hypoxic events stress benthic organisms, causing direct mortality via asphyxiation, indirect mortality through predation, or impede growth (Pihl et al. 1991; Dauer et al., 1992; Llansó, 1992). This hypothesis was substantiated from a field experiment conducted during the summer of 2007 (Sturdivant, unpublished). A site classified as normoxic from the

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random point sample method was monitored throughout the summer of 2007 for water quality, and was found to experience periodic hypoxia (Sturdivant, unpublished). Another explanation for the observed trends in macrobenthic production could be predation pressure. If epibenthic predators and demersal fish are displaced from hypoxic zones, their presence in adjacent normoxic areas could increase the rate of predation and reduce overall macrobenthic production at these sites. This type of hypoxia driven concentration of predators has been documented in Chesapeake Bay (Breitburg, 2002) and the northern Gulf of Mexico (Craig and Crowder, 2005).

While macrobenthic production is linked to DO concentration, the direct role hypoxia plays on the subsequent loss or recovery of macrobenthic production within the ecosystem is not known. The most obvious cause of death from lack of oxygen is asphyxiation (Diaz and Rosenberg, 1995), although H_2S toxicity, which is produced during the reduction of SO_4 during severe hypoxia and anoxia, also contributes to mortality through inhibition of the electron transport chain in aerobic respiration (Torrans and Clemens, 1982). It can be surmised that in Chesapeake Bay regions experiencing hypoxia and anoxia, both processes contribute to the loss of macrobenthic production. Additionally, epibenthic predators and demersal fish can at times capitalize on stressed benthos during mild hypoxic events (Nestlerode and Diaz, 1998; Seitz et al., 2003), although severe hypoxia disrupts the normal energy flow to higher consumers, and instead allows for the microbial community to process macrobenthic secondary production (Baird et al., 2004)

Of the four areas examined (Mainstem, Potomac, Rappahannock, and York), the York River experiences only periodic hypoxia, making this system a likely candidate for hypoxia mediated

macrobenthic production transfer to epibenthic/demersal predation. Strong gravitational circulation in the York River leads to relatively small spatial coverage and short duration hypoxia (Kuo and Neilson, 1987) with no difference between hypoxic and normoxic daily macrobenthic production. Hypoxia in the York may be enough to stress the benthos, but not induce direct mortality or inhibit macrobenthic production. This would allow opportunistic epibenthic invertebrates and demersal fish species to take advantage of stressed benthic infauna that extend their appendages and bodies into the water column, in an attempt to escape dire conditions below the sediment-water interface (Pihl et al. 1992). Areas with periodic hypoxia, such as the York, likely facilitate trophic transfer of energy to epibenthic and demersal predators. However, the area of the York River assessed in this study accounted for only 1% of the area of Chesapeake Bay, and 2% of the observed hypoxic area.

The Rappahannock experiences both periodic and sustained hypoxia (Kuo et al., 1991) with daily macrobenthic production related to the duration and extent of hypoxia. In 1998 and 1999 macrobenthic production during hypoxia was similar to normoxia, but in 1996 and 2004 hypoxia production was significantly less than normoxia (Table 1). In areas of the Rappahannock where periodic hypoxia occurs, it is expected that daily macrobenthic production could be transferred to epibenthic and demersal predators. In the deeper channels of the Rappahannock, where hypoxia is sustained throughout the season, daily macrobenthic production is virtually eliminated. The Potomac and Mainstem both experience severe seasonal hypoxia with >95% reductions in macrobenthic production. Periodic and seasonal hypoxia alters energy flow to epibenthic predators and demersal fish, with the latter shifting energy to the microbial community (Baird et al., 2004). Many epibenthic and demersal predators of macrobenthos already experience

multiple stressors (i.e. HABs, chemical contaminants, disease) given the current health of the Bay (Boesch, 2000; Breitburg et al., 2003). Hypoxia couples these factors with a loss in potential prey energy, loss of habitat, and increased energy expenditure searching for suitable habitat and food.

At the taxonomic level, there were significant reductions in macrobenthic production for the major phyla. Overall, bivalve production dominated during normoxia, particularly in the Mainstem and Potomac River, however, hypoxia significantly reduced bivalve production by 95%. Vaquer-Sonyer and Duarte's (2008) synopsis of species resistant to hypoxia, found that bivalves fared better than any other groups based on LC_{50} (Lethal Concentration to 50% mortality) and LT_{50} (Lethal Time to 50% mortality). The bivalves that overlapped between our study and Vaquer-Sonyer and Duarte's (2008) synopsis, *Macoma balthica* and *Mulinia lateralisi*, had mean LT_{50} of 529 and 159 hours respectively. However, these species accounted for only 15% of our total bivalve production, and were rarely collected at hypoxic sites. Though some bivalves can survive for long periods of hypoxia under laboratory settings, *in situ* there was a trend of less bivalve production during hypoxia. Polychaete production during hypoxia was significantly lower by 65% (Table 3), one of the most minimal observed reductions. Tolerances and behavioral strategies of polychaetes appeared to allow for more efficient survival and less reduction in available production during hypoxia. Capitellids and spionids accounted for 50% of polychaete abundance, and these worms have been previously observed to survive long durations under low DO concentrations, with LT_{50} of 312 (Rosenberg, 1972) and 43 hrs (Llansó, 1991), respectively. Many capitellids and spionids have been observed living in DO conditions around 1 mg l^{-1}, although cessation of feeding and burrowing generally occurs (Warren, 1977; Llansó,

1991). Spionids, such as *Paraprionospio pinnata,* were observed swimming in the water column during low oxygen, and capitellids were seen lying on the sediment surface as strategies to reach more oxygenated water above the sediment-water interface (Diaz et al. 1992). The dominance of polychaete production during hypoxia appears to be a direct result of their morphology and life history strategies, making them more adaptable to changing DO conditions (Vaquer-Sonyer and Duarte, 2008). Arthropod production was also significant reduced by 95% during hypoxia, and they have been noted to be poor in their adaptation to low DO concentrations (Winn and Knott, 1992). Hoback and Barnhart (1996) found that Gammarid amphipods experience LC_{50} at DO concentrations of approximately 2.0 mg l^{-1}. Similar studies have shown amphipods from the same family experience an LT_{50} of 7-15 hours at DO of 2.0 mg l^{-1} (Theede et al., 1969; Agnew and Jones, 1986). Results from our study showed a 95% reduction of available amphipod production at this same DO threshold, indicating that while 50% of the amphipods may still be present at 2.0 mg l^{-1}, their overall available production is drastically diminished, reducing the potential transfer of energy. The amount of uniformity in hypoxia's reduction in production by class (Table 3) was interesting. Previous work has shown that species perform differently in their physiological response to hypoxia (summarized in Vaquer-Sunyer and Duarte, 2008). Despite the differences in hypoxia sensitivity by species, we found large reductions in daily production across taxa; the similar magnitude in daily production reduction for most benthos points to the ubiquity with which hypoxia affects benthic organisms. Reduced daily benthic secondary production across taxa, also translates to reduced trophic transfer potential to higher consumers that prey upon macrobenthic infauna.

5. CONCLUSION

Hypoxia has been a major feature of Chesapeake Bay since at least the 1950s and has had negative effects on ecosystem functions. On average, we found that hypoxia sites had 90% lower daily macrobenthic production; this is based on a comparison between hypoxic and normoxic stations, assuming that hypoxic stations would otherwise be normoxic in a non-hypoxic Bay. Given the extent and duration of hypoxia in Chesapeake Bay during the summer, this amounts to a 6 to 12% reduction in the total annual secondary production. While higher consumers may benefit from easy access to stressed prey in some areas, the large spatial and temporal extent of seasonal hypoxia in the Bay negates higher trophic level transfer via the inhibition of benthic production. The loss of macrobenthic production may be detrimental to the overall health of the Bay, as it occurs when epibenthic and demersal predators (fish and crustaceans) have high energy demands.

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TABLE CAPTIONS

Table 1. Daily macrobenthic production (mg C m^{-2} d^{-1}) averaged by dissolved oxygen category for year and tributary. Periods represent no data.

Table 2. Effect of salinity, percent silt+clay, depth, and DO on daily macrobenthic production. Based on maximum likelihood GEE model with data clustered by year within area (Mainstem, Potomac, Rappahannock, and York).

Table 3. Comparison of oxygen condition and mean daily macrobenthic production by A) phylum, and B) class (±1SE). Letter differences denote significance.

Table 4. Comparison of area and mean daily macrobenthic production by A) phylum and B) class (±1SE). Letter differences denote significance.

FIGURE CAPTIONS

Figure 1. Composite Chesapeake Bay summer DO concentration from 1996 to 2004. Large and small circles represent sample sites. Shading and dot color denotes DO concentration as stated in Figure key.

Figure 2. Comparison of summer daily macrobenthic production by varying oxygen condition (bars) in Chesapeake Bay from 1996 to 2004. Total macrobenthic production significantly different over time (ANOVA, df=8, F=2.43, p=0.013). Hypoxic volume (line) adapted from Hagy et al., 2004. Letter differences denote significance.

Figure 3. Relationship between daily macrobenthic production and dissolved oxygen concentration in Chesapeake Bay. Letter differences represent significance (df=26, F=27.97, p<0.0005). Normoxic areas have significantly higher daily macrobenthic production than hypoxic areas. Error bars represent ±1 SE.

Table 1.

	Normoxia >2.8 mg/l			Mild Hypoxia 2.8-2.0 mg/l			Hypoxia <2.0 mg/l		
	Mean	SD	N	Mean	SD	n	Mean	SD	n
1996	36.3	73.9	80	4.2	.	1	1.3	2.7	21
Mainstem	45.7	91.2	45	.	.	0	1.6	3.0	7
Rappahannock	21.9	25.9	14	.	.	0	1.4	2.3	5
Potomac	61.5	92.0	4	4.2	.	1	1.0	3.0	9
York	17.5	32.0	17	.	.	.	.	.	.
1997	34.5	78.8	83	9.5	18.6	16	3.2	1.6	7
Mainstem	49.1	88.8	33	14.0	14.8	12	5.5	0.2	2
Rappahannock	26.3	73.3	21	4.4	2.7	2	.	.	.
Potomac	58.7	133.2	8	3.5	3.1	5	2.3	0.1	5
York	10.7	3.6	21	.	.	.	.	.	.
1998	34.4	77.8	85	5.3	5.6	6	3.8	5.3	22
Mainstem	47.9	102.4	41	5.8	8.3	3	4.8	4.9	6
Rappahannock	17.3	11.2	15	.	.	0	10.1	6.1	5
Potomac	35.7	82.1	11	4.8	3.2	3	0.5	0.4	11
York	16.9	13.5	18	.	.	.	.	.	.
1999	55.6	167.1	109	13.5	11.7	2	11.0	.	1
Mainstem	107.6	237.3	50	13.5	11.7	2	.	.	.
Rappahannock	14.7	12.1	19	.	.	.	11.0	.	1
Potomac	7.4	12.4	20	.	.	.	.	.	.
York	12.8	8.9	20	.	.	.	.	.	.
2000	56.8	142.5	88	35.6	65.9	7	4.1	5.9	5
Mainstem	101.8	206.6	38	70.6	98.2	3	10.3	3.4	2
Rappahannock	16.6	28.3	18	0.0	0.0	2	.	.	.

Potomac	26.6	43.6	16	18.7	5.0	2	0.0	0.1	3
York	25.5	24.1	16	.	.	.	.	.	.
2001	55.2	144.1	88	12.8	12.0	7	1.6	4.4	10
Mainstem	82.7	184.5	51	11.9	.	1	0.0	.	1
Rappahannock	16.1	7.5	14	9.9	.	1	.	.	.
Potomac	10.5	11.7	6	15.6	20.0	3	0.2	0.4	8
York	20.6	24.4	17	10.4	3.9	2	14.1	.	1
2002	44.4	93.9	106	4.5	4.5	2	5.1	4.2	2
Mainstem	79.3	130.3	45	7.7	.	1	5.1	4.2	2
Rappahannock	16.7	19.6	22	.	.	.	.	.	.
Potomac	20.6	59.2	22	1.3	.	1	.	.	.
York	19.1	10.4	17	.	.	.	.	.	.
2003	15.7	16.4	76	6.9	7.0	10	5.6	7.0	21
Mainstem	19.0	21.5	33	8.5	7.9	6	11.0	3.8	6
Rappahannock	8.4	7.9	19	.	.	0	.	.	.
Potomac	10.1	11.9	4	2.2	3.7	3	0.3	0.8	12
York	19.8	10.1	18	11.0	.	1	16.0	5.4	3
2004	20.3	24.0	60	8.1	6.9	12	2.2	3.0	0
Mainstem	23.6	31.2	27	8.8	8.5	5	1.8	1.9	5
Rappahannock	26.0	14.5	11	11.2	6.5	2	1.8	1.8	3
Potomac	2.3	4.3	10	0.0	0.0	2	0.0	0.0	2
York	22.5	14.8	12	10.4	.	3	9.9	.	1

Table 2.

Parameter	DF	Estimate	SE	Wald 95% CI		Chi-Square	Pr > ChiSq
Intercept	1	29.05	17.95	-6.13	64.22	2.6	0.106
Salinity	1	-3.12	0.71	-4.52	-1.73	19.2	< .001
Silt+Clay	1	0.19	0.10	0.001	0.38	3.9	0.049
Depth	1	-0.004	0.83	-1.64	1.63	0.0	0.996
DO	1	8.92	1.81	5.37	12.47	24.3	< .001

Table 3.

A)

	n	Mollusca		Annelida		Arthropoda	
		p = 0.002		p < 0.0005		p < 0.0005	
normoxia	924	40.8 (4.4)	a	9.6 (0.4)	a	3.2 (0.3)	a
mild hypoxia	64	6.5 (3.1)	a,b	3.7 (0.4)	b	0.7 (0.2)	b
hypoxia	101	3.0 (2.5)	b	2.5 (0.4)	b	0.2 (0.1)	b

B)

	n	Bivalvia		Gastropoda	Polychaetea		Oligochaetea	
		p = 0.003		ns	p < 0.0005		p = 0.027	
normoxia	924	39.9 (4.4)	a	0.8 (0.3)	7.7 (0.3)	a	1.8 (0.3)	a
mild hypoxia	64	6.2 (3.1)	a,b	0.3 (0.1)	3.4 (0.4)	b	0.3 (0.1)	a,b
hypoxia	101	2.9 (2.5)	b	0.1 (0.0)	2.4 (0.4)	b	0.1 (0.0)	b

Amphipoda		Isopoda	
p = 0.013		p < 0.0005	
1.5 (0.2)	a	0.5 (0.1)	a
0.5 (0.2)	a,b	0.1 (0.0)	b
0.1 (0.0)	b	0.0 (0.0)	b

Table 4.

A)

	n	Mollusca		Annelida		Arthropoda	
		p < 0.0005		p < 0.0005		p < 0.0005	
Main Bay	486	52.1 (6.9)	a	9.7 (0.6)	a	2.2 (0.2)	a
Potomac	224	51.8 (9.9)	a	5.7 (0.8)	b	1.8 (0.3)	a
Rappahannock	210	5.1 (1.7)	b	7.5 (0.6)	a,b	4.1 (0.6)	b
York	169	2.1 (0.2)	b	10.3 (0.7)	a	4.4 (1.0)	b

B)

	n	Bivalvia		Gastropoda	Polychaetea		Oligochaetea
		p < 0.0005		Ns	p < 0.0005		ns
Main	486	46.8 (6.8)	a	0.6 (0.2)	8.6 (0.5)	a	2.0 (0.4)
Poto	224	50.1 (9.7)	a	1.7 (1.1)	3.0 (0.4)	b	2.6 (0.7)
Rapp	210	4.8 (1.7)	b	0.3 (0.0)	6.2 (0.6)	c	1.3 (0.2)
York	169	1.9 (0.6)	b	0.2 (0.1)	9.5 (0.7)	a	0.8 (0.1)

Amphipoda		Isopoda	
p < 0.0005		p = 0.008	
0.7 (0.1)	a	0.7 (0.1)	a
0.7 (0.2)	a	0.4 (0.1)	b
2.0 (0.3)	b	0.3 (0.1)	b
3.1 (0.9)	b	0.4 (0.1)	b
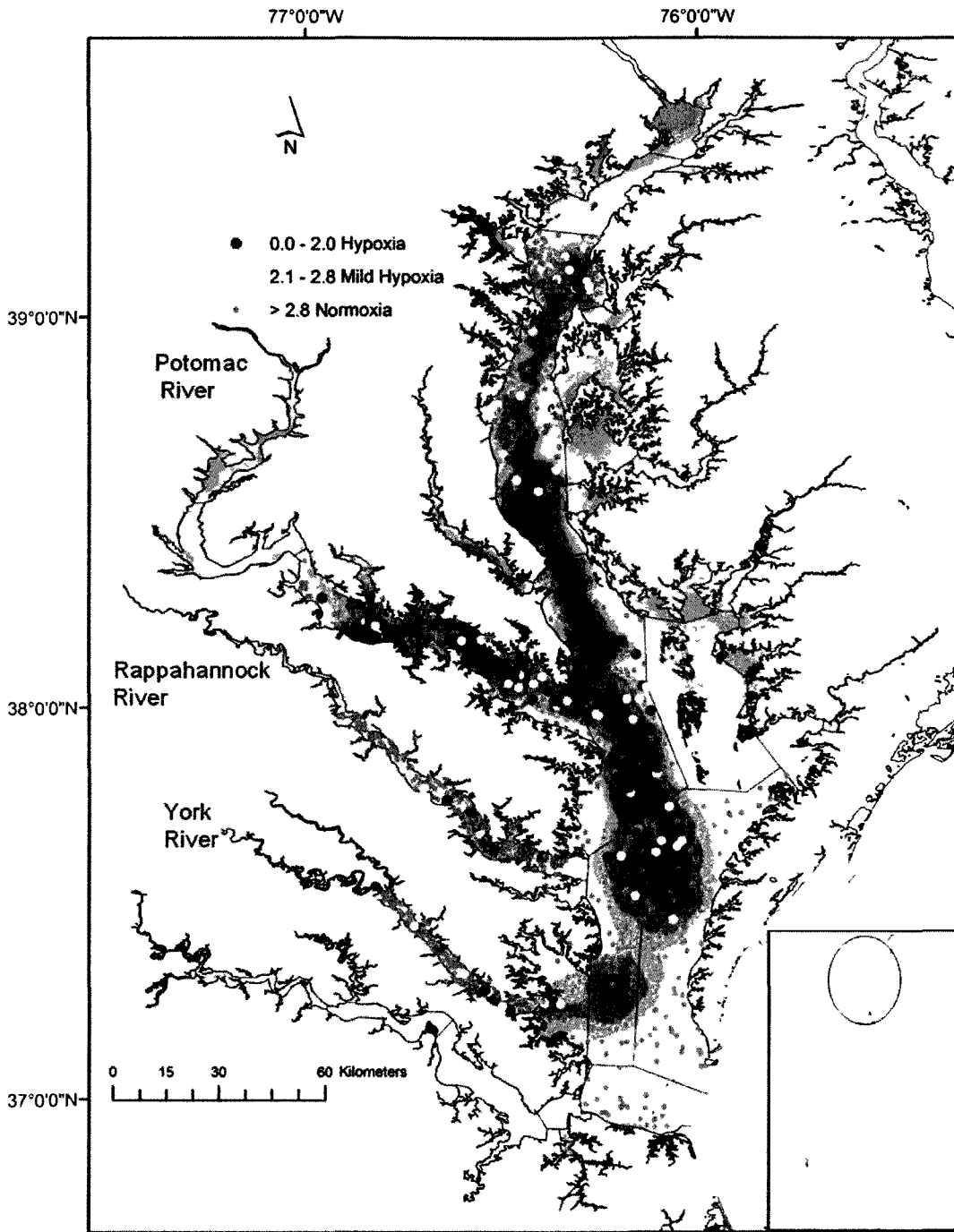
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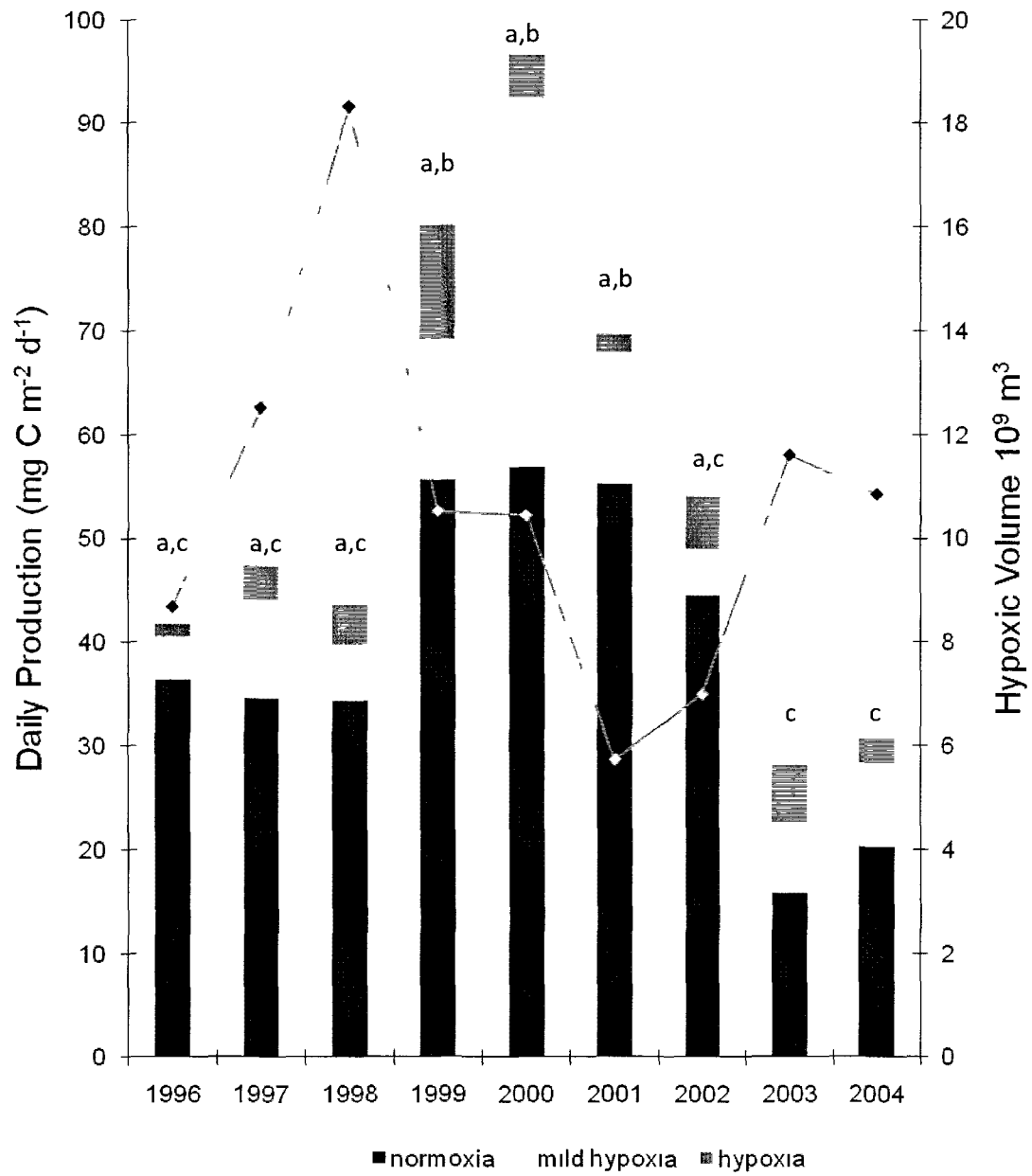
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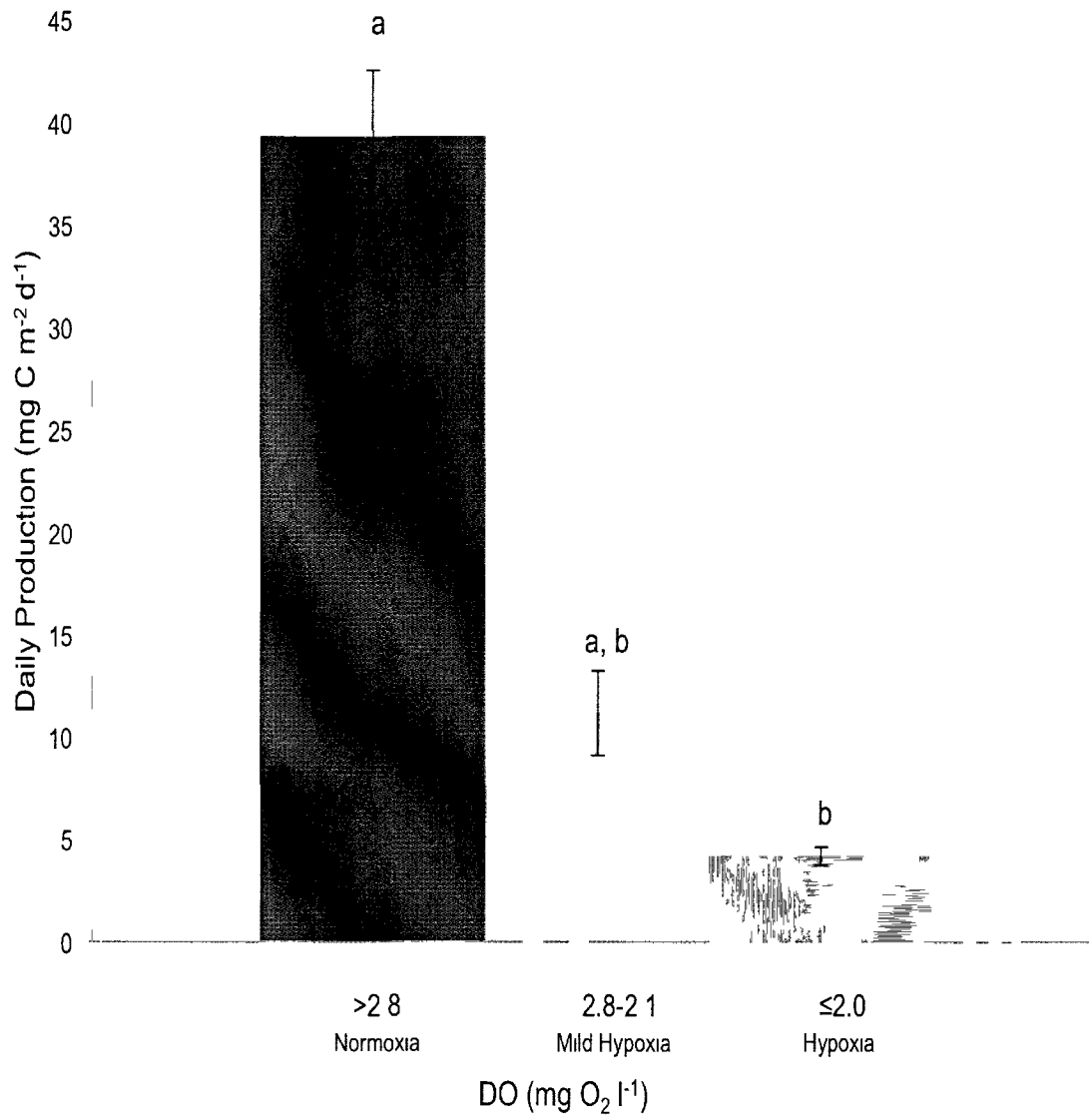
Figure 3.

CHAPTER 2

Effects of seasonal hypoxia on macrobenthic production and

community function in the Rappahannock River estuary, VA, USA

ABSTRACT

Development has eroded Chesapeake Bay's health, resulting in an increase in the extent and severity of hypoxia (≤ 2 mg O_2 l^{-1}), adversely affecting community structure and secondary production of macrobenthos in the Bay and its tributaries. Changes in macrobenthic secondary production were assessed in the lower Rappahannock River, a sub-estuary of Chesapeake Bay in an area known to experience seasonal hypoxia. During the spring, summer, and fall of 2007 and 2008, ten samples were collected each season, and secondary production was estimated using Edgar's allometric equation. From early spring to late fall, dissolved oxygen concentrations were measured continuously at two of the ten sites in 2007 and 2008, and the macrobenthic community was assessed through bi-weekly grab samples. Hypoxic sites had up to 85% lower macrobenthic production, compared to normoxic sites, and macrobenthic production at hypoxic sites was composed primarily of smaller, disturbance-related annelids. Macrobenthic production differed across seasons, and sediment reworking rates were significantly higher during normoxia, indicating that the functional role of the macrobenthic community changed during hypoxia.

1. INTRODUCTION

Oxygen, a key element in the metabolic processes of all metazoan organisms, is found in a dissolved form in aquatic environments as a result of primary production and atmospheric diffusion (Breitburg et al., 2003). Once dissolved into surface waters, the normal condition is for dissolved oxygen (DO) to be mixed down into bottom waters by turbulence. When the supply of DO to the bottom is stymied, typically due to stratification of the water column, and/or the consumption rate exceeds resupply, DO concentrations decline and the system can experience hypoxia (Diaz, 2001). Hypoxia is generally defined by DO concentrations of ≤ 2 mg O_2 l^{-1} (Tyson and Pearson, 1991)

Hypoxia is closely associated with eutrophication arising from altered coastal nutrient budgets that can be linked to increased human population, whether through urbanization in coastal river drainages or through expanded agricultural activities (Diaz, 2001). Since colonial times, the number of humans in Chesapeake Bay watershed has grown exponentially, with a 3-fold increase over the last 100 years (Kemp et al., 2005). Though intermittent hypoxia in the Bay may have been a natural phenomenon, sediment cores indicate the frequency and extent of hypoxia increased with colonization and subsequent land cover changes (Cooper and Brush, 1991; Cooper, 1995). Anthropogenic disturbance has resulted from activities that mobilize the compounds nitrogen and phosphorous through land clearing, application of fertilizer, discharge of human waste, animal production, and combustion of fossil fuels (Cloern, 2001). In Chesapeake Bay, runoff from agricultural practices is the main source of nutrient loading. Non-point sources of nutrient input account for the majority of nutrient loading at approximately 60-

65% (Boyton et al., 1995). Runoff from agriculture account for approximately 40% of the nitrogen and approximately 50% of the phosphorus input into Chesapeake Bay (Magnien et al., 1995). This increased nutrient input promotes a spring phytoplankton bloom, and the particulate organic matter (POM) from this bloom eventually settles to the bottom and is decomposed by microbes. The microbial decomposition process results in the consumption of DO, and depletes DO in bottom waters (Diaz, 2001).

Seasonal hypoxia occurs throughout Chesapeake Bay and some of its tributaries during the summer months. Seasonal hypoxia was present with the first DO measurements in mainstem Chesapeake Bay as observed by Newcombe (1939) in the early 1930s and in the Potomac in the 1910s as observed by Sale and Skinner (1917). The most severe low oxygen events occur in the main stem (Officer et al., 1984; Stow and Scavia, 2008). From the 1950s through the 1990s, there has been a substantial increase in hypoxic/anoxic water in Chesapeake Bay, from approximately 3 km^3 in the 1950s, to approximately 10 km^3 in the 1990s (Hagy et al., 2004). The increase of hypoxia in the Bay is troubling, as hypoxic areas have been well documented to have negative impacts on estuarine benthos (Jørgensen 1980; Llansó, 1990; Dauer et al., 1992; Diaz et al., 1992; Tallqvist, 2001; Rosenberg et al., 2002). Additionally, the outer edge of Chesapeake Bay main stem hypoxic water may be advected into shallow areas, such as the Bay's tributaries, through horizontal transport (Breitburg, 1990). In the Rappahannock River, our area of interest, a combination of tidal mixing and proximity to main stem hypoxic waters controls the seasonal hypoxia, which lasts throughout most of the summer (Kuo and Neilson, 1987; Kuo et al., 1991; Park et al., 1996).

There is a general understanding of hypoxia's effects on community structure, where a series of predictable and graded responses occur (Rabalais et al., 2001). At the initial onset of hypoxia, organisms increase respiration (Petersen and Petersen, 1988), and mobile fauna migrate from the area (Pihl et al., 1991). As DO further declines, sessile fauna cease feeding and decrease activities not related to respiration (Warren, 1984). Infauna migrate closer to the sediment surface as reduced compounds accumulate and have been observed on or extending above the sediment surface in a moribund condition (Jørgensen, 1980; Tyson and Pearson, 1991). Finally, if the duration of hypoxia is sustained, mass mortality occurs in all but the most tolerant of species (Llansó, 1992; Diaz and Rosenberg, 1995). At the functional level, there is less understanding of how hypoxia interacts with macrobenthic secondary production and the subsequent trophic transfer of energy and production (Baird et al., 2004).

Productivity is an intriguing component of the energy budget, as it provides an index of community processes proportional to total community respiration and consumption, and it integrates the influence of numerous biotic and environmental variables affecting individual growth and population mortality (Edgar and Barrett, 2002; Cusson and Bourget, 2005). Production can be defined as the quantity of matter/energy that is available for the next higher trophic level, and a measure or estimate of productivity can be obtained by relating the calculated production to the biomass present (Brey, 2001). Secondary production, or the heterotrophic production of organic matter, is viewed as an estimate of estuarine health (Diaz and Schaffner, 1990; Dolbeth et al., 2005). The production of benthic invertebrates is important, as these fauna serve as a link in the energy transfer from primary consumers to higher trophic levels (Nilsen et al., 2006) and they are the foremost pathway by which carbon is recycled out of the sediment and

eventually out of Chesapeake Bay system (Diaz and Schaffner, 1990). The production of

communities is rarely directly measured due to methodological difficulties (Edgar, 1990). While

direct calculations of macrobenthic production are costly and time consuming (Wilbur and

Clarke, 1998), methods have been proposed for the indirect calculation of macrobenthic

production based on biotic and abiotic variables (Edgar, 1990; Sprung, 1993; Brey et al., 1996).

Using production theory and empirical models developed to quantify macrobenthic production

without the requirement of intense sampling, we attempted to relate patterns of macrobenthic

production in the Rappahannock River estuary to DO concentration. Specific objectives of our

study were to 1) assess the relationship between macrobenthic production and the physical

factors of DO concentration, salinity, and % silt/clay; 2) describe patterns of macrobenthic

production temporally (across weeks and seasons); and 3) determine taxonomic associations

between macrobenthic production and DO concentration.

2. METHODS

2.1 Study Area

Of the three major tributaries of the lower Chesapeake Bay, the Rappahannock is the only sub-

estuary with physical dynamics to allow sustained seasonal hypoxia (Kuo and Neilson, 1987). In

the lower Rappahannock River, a combination of tidal mixing and, to a lesser extent, proximity

to main stem hypoxic waters, control its seasonal hypoxia (Kuo et al., 1991; Park et al., 1996).

The tidal Rappahannock begins at the fall line in Fredericksburg, VA, a distance of

approximately 130 km from its mouth. The 1.0 psu isohaline is normally 75-90 km upriver. The

mean tidal range and surface salinities at the mouth are 0.4 m and 12-18 psu, respectively (Haas, 1977).

2.2 Sampling Design

The Chesapeake Bay Long-Term Benthic Monitoring Program (LTBMP) started annual random sampling of Chesapeake Bay and its tributaries in both Maryland and Virginia in 1996. The LTBMP divided the Bay into 10 sampling strata with each having 25 random sampling sites per year. Sites were sampled by the LTBMP from late July to early September, with a new set of random sites selected each year (Dauer and Llansó, 2003). Within the monitoring framework, we included 10 of the 25 sites within the meso- and polyhaline portions of the Rappahannock River estuary. The meso- and polyhaline regions of the Rappahannock River were selected due to a history of sustained seasonal hypoxia during the summer months (Kuo and Neilson, 1987; Park et al., 1996). Of the ten sites selected, five were chosen in areas that had previously experienced hypoxia and five in areas with a past history of normoxia. DO measurements from the LTBMP were used in site selection. All ten sites were sampled once during the spring, summer, fall, and again in the spring of the following year. Sampling occurred during 2007 and was repeated in 2008.

2.3 Field Methods

At each site, basic water quality parameters of DO concentration, salinity, and temperature were measured at the surface of the water column and approximately 0.5-1 m from the bottom using a YSI model 6600 sonde. Sediment grabs were collected for benthic community analysis using a

Young grab (440 cm^2 to a depth of 10 cm). Sample volume and penetration depth were observed; if the Young grab penetrated less than 7 cm into the sediment, the sample was rejected and the site re-sampled. Samples were sieved *in situ* through a 0.5 mm screen using an elutriative process. Organisms and detritus retained on the screen were transferred into labeled jars and preserved in a 10% formaldehyde solution. They were later stained with Rose Bengal, a vital stain that aids in separating organisms from sediments and detritus. Two surface-sediment sub-samples of approximately 120 ml each were collected for grain-size analysis from an additional grab sample at each site.

Each year, two of the ten sites were selected for continuous DO monitoring; in 2007 sites 18 and 25 were selected, and in 2008 sites 11 and 12. Site selection was based on DO concentration, with one site having a history of normoxia and the other hypoxia; sites 18 and 11 had a history of hypoxia, and 25 and 12 a history of normoxia. Aside from DO, the two sites chosen each year had similar physical parameters. At each of the two locations, a single tripod was deployed with a Hach DS500X water quality datasonde. The sondes were positioned approximately 0.25-0.5 m above the sediment surface. DO concentration, salinity, and temperature measurements were recorded in 20-min increments for a two-week period. Every two weeks, sondes were replaced for maintenance and data retrieval, and new datasondes were deployed. Additional grab samples were collected at each site with a Young grab during the bi-weekly sonde swap, and water quality control measurements were collected approximately 0.5-1 meter from the bottom using a YSI model 6600 sonde. The grab methodology described in the previous paragraph was implemented in the bi-weekly sampling.

2.4 Lab Methods

All macrobenthic samples were processed to identify and enumerate each species present and to measure species-specific ash-free dry weight biomass. Organisms were sorted from detritus under dissecting microscopes, identified to the lowest practical taxonomic unit, and counted. Species identifications were verified when organisms were transferred for biomass measurements. Ash-free dry-weight (AFDW) biomass was measured directly for each species by drying organisms to a constant weight at 60°C and ashing (converting an organic compound into ash, decomposition, by a burner or in a muffle) in a muffle furnace at 500°C for four hours. Sediment samples were wet-sieved for percent silt-clay content (Folk, 1973).

2.5 Macrobenthic Production

Edgar (1990) developed a general allometric equation ($P = 0.0049 * B^{0.80} T^{0.89}$) that relates daily macrobenthic production P ($\mu g \cdot C \, day^{-1}$) to ash-free dry weight B (μg) and water temperature T (°C). Edgar (1990) also developed specific allometric equations for various animal groups (crustaceans, molluscs, and infauna), and these equations were used to estimate production for each respective group; the general equation was used for animals that did not fall into one of the 3 aforementioned groups. Table 1 displays the variation in Edgar's (1990) equations by group. The only departure from Edgar's method, which uses the mean AFDW of animals retained on a series of sieves of differing mesh size, was the usage of mean AFDW of each species by sample. Biomass measurements at the species level allowed us to examine taxonomic and functional group associations between production and DO. The theoretical basis for Edgar's equation is grounded in the metabolic theory of ecology that shows, among other things, that a constant

fraction of metabolism tends to be allocated to production across taxa (Brown et al., 2004). Sturdivant et al. (unpublished) verifies the quality of our production estimate (see Chapter 1).

2.6 Data Analyses

To compare the seasonal replicate data for 2007 and 2008, a repeated-measures analysis of variance (ANOVA) was conducted to determine the variance in production between 4 seasons (spring, summer, fall, and the following spring). A repeated-measures design is one in which multiple measurements or observations are taken on the same replicate data (Zar, 1999). If there is a lot of variability from one replicate to the next, this technique controls for that source of variation. This analysis is needed since the repeated observations on a single replicate are not statistically independent of one another, and therefore, the analysis must reflect this structure of dependence in the data (Gotelli and Ellison, 2004). The model included year as a factor with the randomly selected sites as the repeated measures, and the varying season as the treatment. The physical parameters DO concentration, salinity, and % silt/clay were covariates. Normality was checked with the Shapiro-Wilk test and homogeneity of variance with Bartlett's test. Data found not to be normal was log transformed. Tukey's HSD test was used for multiple mean comparisons.

In an information theoretic approach, general linear models (GLM) were posed, using residual sums of squares (RSS) estimates to determine Akaike's information criterion (AIC) for our seasonal replicate data. AIC is a measure of the explanatory power of a statistical model that accounts for the number of parameters in the model. The RSS, derived from the repeated-measures analysis, of the estimated model parameter (θ) was determined given the data (Gotelli

and Ellison, 2004), and this approach determines the model that best reflects effects on macrobenthic production. When comparing among multiple models for the same phenomenon, the model with the lowest AIC value is considered to be the best model. For this study corrected AIC (AIC_c), a second-order bias correction necessary for small samples (Burnham and Anderson, 2002), was used to determine model strength. AIC_c values were then used to determine AIC differences (Δ_i), relative to the smallest AIC_c value in the set of tested models. Hence, Δ_i rescaled AIC_c values such that the model with the minimum AIC_c value had a $\Delta_i = 0$. Derived Δ_i values were used to determine Akaike weights (w_i). The w_i sum to 1 and were interpreted as the probability that model i is the expected best model for the sampling situation considered. If a "best" model could not be determined, model averaging was conducted. Model averaging takes the β estimates of the parameters and multiplies them by the w_i, and then sums the two for all models, providing model-averaged estimates for the measured variables. Instead of using only those models with a lot of support, all models were used in model averaging to ensure w_i summed to 1. This is an appropriate method of model averaging as models with little or no support essentially get ignored in the calculation, i.e. they are weighted very little (Burnham and Anderson, 2002). Sturdivant et al. (unpublished) determined that DO concentration, salinity, and grain size had significant relationships with macrobenthic production in Chesapeake Bay, thus, the seven models constructed in this paper were based on those findings (Table 2).

For the continuous monitoring data, paired t-tests were used to determine differences in macrobenthic production between sites for each year and to validate differences (or the lack thereof) in the physical parameters at the hypoxic and normoxic sites. Regression and multiple

regression analysis were conducted to determine cause/effect relationships between physical parameters and macrobenthic production for the continuous monitoring data. In a basic sense, regression describes the relationship between a predictor variable and a response variable (Gotelli and Ellison, 2004); multiple regression factors in more than one regression. Macrobenthic data from 2007 and 2008 were regressed against DO concentration. To assess functional group differences between and within sites, ANOVA was run, except for differences in sediment reworking rates (SRR), which were determined using a t-test.

3. RESULTS

The residual sums of squares (RSS) for each of the seven models (Table 2) were used to generate the AIC_c results (Table 3). Based on the calculated Akaike weights (w_i), models g_1, g_2, and g_3 were equally plausible, however, overwhelming evidence for a single superior model, indicated by a $w_i \geq 0.90$ (Burnham and Anderson, 2002), did not exist. To better clarify which variable (DO, salinity, or grain size) was most important to our estimated parameters, the w_i were summed for each model that included a certain variable. Summed w_i were as follows: DO = 0.40, salinity = 0.40, and % silt/clay = 0.49, indicating % silt/clay was the most important of the three measured variables, but strong evidence existed for the importance of each measured variable. Given the strong support for a number of models and parity between each of the 3 measured variables, model averaging was employed for all models. Based on model-averaged results (Table 4), DO concentration and salinity had the most impact on macrobenthic production during 2007 and 2008 in the lower Rappahannock River. Per one mg l^{-1} increase in DO concentration, the rate of macrobenthic production increased by 14.7 mg C m^{-2} d^{-1}, and it

decreased by 12.4 mg C m^{-2} d^{-1} per psu increase of salinity. % Silt/clay had a marginal affect on macrobenthic production, increasing the rate of production by 4.7 mg C m^{-2} d^{-1} per percentage point increase in % silt/clay.

Macrobenthic production differed among seasons (Figure 1), with the highest rate of production in the spring. Summer macrobenthic production was lower than spring production by ~40% and production in the summer was the lowest observed. In the fall, macrobenthic production was higher than summer production but did not equal the magnitude of production observed in the spring. Macrobenthic production in the following spring (represented in Figure 1 as Nxt Spring) was approximately equally to production in the fall and did not parallel macrobenthic production in the initial spring.

DO measurements collected by datasondes at each of the continuously monitored sites from 2007 (Sites 18 and 25) and 2008 (Sites 11 and 12) were compared to corresponding point measurements of DO using a paired t-test to validate the accuracy of sonde readings (Figure 2). There was no significant difference between corresponding sonde readings and point DO measurements for any of the four sites, providing confidence in our DO concentration data. In 2007, 55% of the observed DO measurements at hypoxic site 18 were hypoxic, compared to less than 20% at normoxic site 25. In 2008, 45% of the observed DO measurements at hypoxic site 11 were hypoxic with 15% of the observed DO at anoxic levels. Greater than 80% of the observed DO measurements at normoxic site 12 were higher than 3.0 mg O$_2$ l^{-1}. Note that in 2007, the normoxic site was not a true normoxic site as it experienced hypoxia on several occasions.

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There was no significant difference in salinity, temperature, or % silt/clay between normoxic and hypoxic sites in 2007 or 2008 (Table 5). Depth was significantly different between the normoxic and hypoxic sites in 2007 and 2008, however Sturdivant et al. (unpublished) found that depth had no significant affect on daily macrobenthic production in Chesapeake Bay (see Chapter 1). Therefore, with similar physical factors between the normoxic and hypoxic sites for both years, macrobenthic production was regressed against only DO concentration (Figure 3). A sigmoid relationship was found between daily macrobenthic production and DO concentration; macrobenthic production was low in DO concentrations below ~3.0 mg l^{-1}, rising after ~3.5 mg l^{-1}. Macrobenthic production was also more variable at higher DO concentrations; the standard error (SE) of mean macrobenthic production at DO concentrations > 2.8 mg O_2 l^{-1} was 2.2 mg C m^{-2} d^{-1} compared to a SE of 0.5 mg C m^{-2} d^{-} at DO concentrations ≤ 2.8 mg O_2 l^{-1}.

The bi-weekly macrobenthic production between the normoxic and hypoxic sites in 2007 and 2008 were compared. In 2007, macrobenthic production at the normoxic site was significantly higher than macrobenthic production at the hypoxic site (Figure 4a); the hypoxic site had on average 85% lower production than the normoxic site. Hypoxia-resistant species contributed to half of the macrobenthic production at the normoxic site and approximately 85% of the macrobenthic production at the hypoxic site. The hypoxia-resistant spionid, *Paraprionospio pinnata*, dominated macrobenthic production at the hypoxic site, contributing to 78% of the total macrobenthic production. In 2008, macrobenthic production was not significantly different between sites, but a trend of higher production at the normoxic site existed (Figure 4b). The hypoxic site had on average 36% lower production than the normoxic site, but this assessment

includes early spring, a time period unaffected by hypoxia and when macrobenthic production was approximately equal at both sites. When the difference in production is assessed starting at the development of hypoxia (5/29/08 – 11/12/08) the production between the two sites was found to be significantly different (df = 10, T = 2.25, p = 0.049) with macrobenthic production 50% lower at the hypoxic site compared to the normoxic site. Overall *P. pinnata* contributed to more than half of the observed macrobenthic production at the hypoxic site; by comparison *P. pinnata* contributed to only 30% of macrobenthic production at the normoxic site.

In 2007 and 2008, there were no significant differences in macrobenthic production when tested by functional groups mobility or feeding types (p > 0.05), due to large variances observed in these groups. Using biomass measurements collected in 2007 and 2008, ranges of sediment reworking rates (SRR) were determined at each site using values reported in Diaz and Schaffner (1990). The maximum estimations of SRR and minimum estimations of SRR were compared for normoxic and hypoxic sites each year using a t-test (Figure 5). In 2007, macrobenthos at the normoxic site reworked an average of 18000-21000 mg dry weight sediment individual^{-1} day^{-1}, which was significantly higher than SRR of 1900-2500 mg dry weight sediment individual^{-1} day^{-1} estimated at the hypoxic site. A similar trend was observed in 2008, the normoxic site had significantly higher estimates of SRR at 4300-6100 mg dry weight sediment individual^{-1} day^{-1}, compared to the estimates of 450-1100 mg dry weight sediment individual^{-1} day^{-1} at the hypoxic site.

4. DISCUSSION

In 2007 and 2008, we found a positive correlation and sigmoid relationship between macrobenthic production and DO concentration in the Rappahannock River estuary. In our seasonal study, macrobenthic production increased by 14.7 mg C m^{-2} d^{-1} per unit increase in DO concentration. This positive relationship was expanded in our continuous study, which showed a sigmoid relationship between DO concentration and macrobenthic production. Macrobenthic production was low below ~3 mg l^{-1} rising after 3.5 mg l^{-1}. Seitz et al. (2009) documented similar results, finding sigmoid relationships between macrobenthic biomass and DO concentration in varying salinity regimes of Chesapeake Bay, with a threshold around 3 mg l^{-1} for polyhaline regions. Further, our data indicated hypoxic DO concentrations offered little variability in macrobenthic production, with mean macrobenthic production 3.0 (SE ± 0.5) mg C m^{-2} d^{-1} during hypoxia. The negative impacts of hypoxia on macrobenthic community structure are well documented (Dauer et al., 1992; Llansó, 1992; Diaz and Rosenberg, 1995; Rabalais et al., 2001), and our results indicate hypoxia has equally negative effects on macrobenthic production. It is not known if the observed relationship between DO concentration and macrobenthic production is direct or indirect. A lack of DO in bottom waters can cause direct mortality via asphyxiation (Diaz and Rosenberg, 1995) and inhibit macrobenthic recruitment and growth, hampering production (Nichols, 1977); yet the impact of hypoxia on macrobenthos extends further. At the development of hypoxia, sessile organisms such as macrobenthos decrease feeding and movement (Riedel et al., 2008) in an attempt to depress their metabolism. If the organisms are able to avoid mortality via asphyxiation, such actions during prolonged hypoxic events could lead to starvation. Additionally, during severe hypoxia and anoxia SO$_4$ is

reduced to H_2S, a toxic compound documented to contribute to macrobenthic mortality (Main and Nelson, 1988; Llansó, 1991; Shumway et al., 1993) through inhibition of the electron transport chain in aerobic respiration (Torrans and Clemens, 1982). Predation is another scenario possibly contributing to lowered production during hypoxia. In hypoxic environments macrobenthos have been known to breach and extend their bodies and appendages above the sediment surface, increasing susceptibility to predation (Pihl et al., 1992).

In 2007 and 2008, macrobenthic production differed between seasons, with spring having the highest observed production rate. Macrobenthic production was lower during the summer and there was little recovery of production levels in the fall and following spring. Spring is a productive time of year in coastal estuaries as nutrient input from spring freshets enriches these shallow systems (Boyton et al., 1995; Magnien et al., 1995) and this is also a time when recruitment of many benthic organisms occurs (Simon, 1967; Sandifer, 1972). The shallowness of Chesapeake Bay fosters tight benthic-pelagic coupling, and there exists a high probability that water column productivity reaches the bottom through turbulent mixing and subsequent suspension feeding (Cloern, 2001) or direct sedimentation (Davies and Payne, 1984), thus, fueling benthic production. Hypoxia is pervasive in Chesapeake Bay and its tributaries during the summer months (Kuo et al., 1991; Hagy et al., 2004) and, as was shown in this study, could account for the lower production observed during the summer via the direct or indirect relationships discussed above. That production only partially recovered in the fall is not surprising; it would not be expected that macrobenthic production in the fall would rival spring production. A lack of nutrient input that normally fuels spring production (Hagy et al., 2005), altered hydrography that mixes plankton below the critical depth (Jackson, 2008), and lower

temperatures reducing metabolism (Diaz and Schaffner, 1990) all contribute to less productivity in the colder months. However, the lack of recovery of macrobenthic production in the following spring indicates a possibility of carry-over affects for sites that experienced hypoxia the previous year. This was an interesting result as macrobenthic production from normoxic and hypoxic sites were analyzed in 2007 and 2008. It may be possible that lost production in hypoxic sites impacts overall macrobenthic recruitment. Larval dispersal for macrobenthos occurs through planktotrophy and/or lecithotrophy (Thorson, 1950; Kempf and Hadfield, 1986), and macrobenthic larval settlement is not purely random but selective (Watzin, 1986). We may have observed lower macrobenthic production in the following spring sites due to overall recruitment being limited by the lack of production at hypoxic sites, and/or the changes in the macrobenthic community (discussed below) may have affected larval recruitment and subsequent production.

This study demonstrated that macrobenthic production was up to 85% lower at hypoxic sites, yet the big underlying question is what happened to all the "lost" production? An easy answer is simply that it was never produced. Habitats that are exposed to extensive hypoxia and anoxia have low annual biomass and production (Rainer, 1982; Levin, 2003, Seitz et al., 2009). Macrobenthic production in areas that experience prolonged hypoxia is regulated by the amount of benthic recruitment and growth that occurs during periods of normoxia (Nichols, 1977); the production at these sites is limited by productivity during normoxia. The lower production observed at hypoxic sites would therefore not be a function of its removal, but of the fact that it was never created. Alternatively the "lost" production could have been transferred to higher trophic levels, as hypoxia has been documented to enhance predation as predators capitalize on

stressed prey (Nestlerode and Diaz, 1998; Seitz et al., 2003; Eggleston et al., 2005); although severe hypoxia disrupts the normal energy flow to higher consumers (Baird et al., 2004). Severe prolonged hypoxia instead allows for the microbial community, which can utilize other compounds (NO_3^-, MnO_4, $FeOH$, SO_4^{2-}, and CO_2) as electron acceptors when DO is absent, to process macrobenthic secondary production (Baird et al., 2004).

Functionally there were no significant differences in feeding or mobility groups for macrobenthos at hypoxic vs. normoxic sites. This was driven by large variances in both groups, indicating that hypoxia may affect the production of these functional groups equally. As a whole macrobenthos are sessile in nature, so it is not surprising that the relative mobility of macrobenthic groups was equally impacted by hypoxia. There was a difference in macrobenthic sediment reworking rates (SRR) between normoxic and hypoxic sites in 2007 and 2008; hypoxic sites had on average significantly lower SRR compared to normoxic sites. SRR is analogous with bioturbation, the biological reworking of sediments by flora, fauna, or microbial activity (Meysman et al., 2006), and through this process macrobenthos influence sediment geochemical and physical properties (Lohrer et al., 2004). The consequences of lowered rates of bioturbation include decreases in sediment permeability, remineralization, nutrient flux (Lohrer et al., 2004), and a shallower sediment oxic layer (Sloan and Kennedy, 2002). DO penetrates sediments by physical diffusion only a few millimeters (Revsbech et al., 1980), but bioturbation can distribute DO much deeper in the sediment (Aller, 1982).

The spionid *Paraprionospio pinnata* dominated macrobenthic production at hypoxic sites in 2007 and 2008 contributing to 78% and 50% of the total production, respectively. *P. pinnata* is

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an opportunistic species that is morphologically adapted to deal with a low oxygen environment, having elongated, proliferated and numerous branchia (Dauer, 1985; Lamont and Gage, 2000). In addition to dominating production through its survivability, *P. pinnata* may have also benefited from less competition through reduction of other species during hypoxia (Seitz et al., 2009). In our study, species richness was reduced by 40% at hypoxic sites potentially decreasing competitive pressure and allowing *P. pinnata* to capitalize on the organic-rich environment that generally accompanies eutrophication-induced hypoxic areas. While hypoxic sites were dominated by *P. pinnata*, normoxic sites were characterized by species with high SRR, such as *Loimia medusa*, *Acteocina canaliculata*, and *Heteromastus filiformis*. These species were notably absent at hypoxic sites and contributed to the significant difference in SRR between normoxic and hypoxic sites. Species with life history traits that require high energy demands, such as burrowing to consume food or in search of prey, would be less well adapted to an environment where metabolic depression is important to survival.

In our study, salinity and % silt/clay were documented to affect macrobenthic production to varying degrees. Previous data has indicated a relationship between macrobenthic production and % silt/clay and salinity in Chesapeake Bay, with the effect of salinity significant and % silt/clay only marginally significant (Sturdivant unpublished). Results from our study confirmed these findings; macrobenthic production increased 4.7 mg C m^{-2} d^{-1} per unit increase in % silt/clay, and decreased 12.4 mg C m^{-2} d^{-1} per unit increase in salinity. In estuaries worldwide, salinity is the major governing factor in organism distribution and diversity (Perkins, 1974; Diaz and Schaffner, 1990; Telesh and Khlebovich, 2010), so it comes as no surprise that salinity was observed to have one of the biggest impacts on macrobenthic production in our study. The study

was designed to assess changes in macrobenthic production in a defined upper-mesohaline salinity range of the lower Rappahannock River. Average salinity of all samples over our study period was 14.3 (SD ± 2.6) psu, indicating little variation in salinity. Had our study encompassed a larger salinity range, we suspect impacts on macrobenthic production would have been greater, given the dominance of salinity in regulating macrobenthic populations (Perkins, 1974) and documented impacts of salinity on macrobenthic production (Diaz and Schaffner, 1990). On a total area basis, macrobenthic production is highest in polyhaline habitats and lowest in the euhaline habitats, with the majority of the Bay's macrobenthic production (~70%) occurring in high mesohaline and polyhaline habitats. At moderate to high salinities, or when salinity is constant, patterns of benthic distribution are further correlated with sediment type (Dauer et al., 1984; Cooksey and Hyland, 2007). Percent silt/clay had marginal impacts on macrobenthic production, and this is also likely due to the small spatial extent in which the study took place. Mean % silt/clay across all samples during our study period was 61.2% (SD ± 12.2).

5. CONCLUSION

Macrobenthic production was related to DO concentration with macrobenthic production up to 85% lower at hypoxic sites. The function of macrobenthic communities changed relative to DO concentration, with hypoxia resistant spionids dominant during hypoxia and species with high sediment reworking rates dominant during normoxia. Macrobenthic production differed across seasons, and there were indications that summer hypoxia impacted the recovery of macrobenthic production the following spring. Salinity and grain size were shown to have significant and marginally significant affects on macrobenthic production, respectively; but given the spatial

extent of the study, DO concentration had the biggest impact on macrobenthic production. The observed impacts of hypoxia on macrobenthic production are troublesome, as previous studies have documented negative cascading affects to higher trophic levels as a result of disturbance to macrobenthic communities (Powers et al., 2005).

ACKNOWLEDGEMENTS

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TABLE CITATIONS

Table 1. Equations relating daily production P (μg· C day^{-1}) to faunal ash-free dry-weight B (μg) and water temperature T (°C) for different animal groups, where $P = x*B^y*T^z$. Data derived from Edgar (1990).

Table 2. Parameters for the general linear models (g_x), corresponding to the different hypotheses concerning the effects on macrobenthic production (response = macrobenthic production = θ). k = number of parameters, including σ^2 as a parameter.

Table 3. Constructed AIC table displaying results of each model.

Table 4. Model averaged estimates for the three measured variables of dissolved oxygen concentration (DO), salinity (SAL), % Silt/Clay (% SC). β_j denotes the estimator of β bases on model g_i.

Table 5. Statistical comparison, using a paired t-test, of physical data for A) 2007 sites, hypoxic site 18 and normoxic site 25; and B) 2008 sites, hypoxic site 11 and normoxic site 12. Asterisks denote significant differences.

Figure 1. Comparison of the natural log of mean daily macrobenthic production by season for 2007 and 2008 sites. Samples were collected during the spring, summer, fall, and following spring (represented as Nxt Spring) between spring 2007 – spring 2009. Error bars represent ±1SE.

Figure 2. DO data for each of the four continuously monitored sites (gray line), compared to corresponding point DO measurements (black dots). No significant differences between sonde readings and corresponding point DO measurements for A) the 2007 normoxic Site 25 (df = 6, T = -0.97, p = 0.377), B) the 2007 hypoxic Site 18 (df = 6, T = 0.22, p = 0.834), C) the 2008 normoxic Site 12 (df = 12, T = 0.51, p = 0.62) and D) the 2008 hypoxic Site 11 (df = 12, T = 0.18, p = 0.89).

Figure 3. Relationship between DO concentration and daily macrobenthic production for the continuously monitored hypoxic and normoxic sites in 2007 and 2008. A sigmoid relationship was found between DO and daily macrobenthic production (df = 39, F = 10.31, p = 0.0003). Squares represent 2007 data and triangles 2008. Solid symbols indicate the hypoxic sites, and hollow symbols the normoxic sites.

Figure 4. Display of daily macrobenthic production (gray bars, left y-axis) and corresponding DO concentration (black line, right y-axis) for A) hypoxic site 18 and normoxic site 25 in 2007 and B) hypoxic site 11 and normoxic site 12 in 2008. In 2007 macrobenthic production was

significantly different between sites 18 and 25 (df=6, T=-2.87, p=0.029), and in 2008

macrobenthic production was not significantly different between sites 11 and 12 (df=12, T=-2.11, p=0.056).

Figure 5. Display of sediment reworking rates (mg dry weight individual^{-1} day^{-1}) by year and site. A) In 2007 normoxic site 25 and hypoxic site 18 had significantly different maximum (df=6, T=-3.94, p=0.008) and minimum (df=6, T=-3.70, p=0.010) estimations of sediment reworking rates. B) In 2008 normoxic site 12 and hypoxic site 11 had significantly different maximum (df=12, T=-3.32, p=0.006) and minimum (df=12, T=-3.77, p=0.003) estimations of sediment reworking rates. Error bars represents ±1SE.

Table 1.

	x	y	z	Eqn.
General	0.0049	0.8	0.89	1
Infauna	0.0035	0.79	1.05	2
Crustacea	0.0013	0.81	1.32	3
Mollusca	0.0066	0.87	0.46	4

Table 2.

Model	k	α constant	x_1 DO	x_2 salinity	x_3 % silt/clay
g_1	3	α	β_1		
g_2	3	α		β_2	
g_3	3	α			β_3
g_4	3	α	β_1	β_2	
g_5	4	α	β_1		β_3
g_6	4	α		β_2	β_3
g_7	4	α	β_1	β_2	β_3

Table 3.

Model	k	RSS	AIC_c	Δ_i	w_i	Evidence Ratio
g_1	3	40950168	247.1	0.58	0.22	0.32
g_2	3	40828749	247.0	0.53	0.22	0.31
g_3	3	39611986	246.5	0.00	0.29	0.24
g_4	4	40561010	249.4	2.89	0.07	1.00
g_5	4	39258209	248.8	2.32	0.09	0.75
g_6	4	39349502	248.9	2.36	0.09	0.77
g_7	5	38958470	251.3	4.81	0.03	2.61

Table 4.

Variable	β_j	SE
DO	14.7	23.2
SAL	-12.4	17.6
%SC	4.7	3.1

Table 5.

A)

Parameter	Site 18	Site 25	df	T	p	
Temp (^{o}C)	25.8	26.0	6	-0.6	0.54	
Salinity (psu)	18.6	17.9	6	1.4	0.20	
% Silt/Clay	89.8	90.0	6	-0.4	0.67	
Depth (m)	10.6	9.6	6	11.1	<0.0005	*

B)

Parameter	Site 11	Site 12	df	T	p	
Temp (^{o}C)	21.8	22.2	12	-2.1	0.06	
Salinity (psu)	14.5	14.8	12	-0.7	0.48	
% Silt/Clay	98.4	94.2	12	1.8	0.05	
Depth (m)	9.8	7.7	12	10.6	<0.0005	*

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Sediment reworking rates (mg dry weight sediment individual^{-1} day^{-1})

A) 2007

Site 18
Site 25

B) 2008

Site 11
Site 12

82
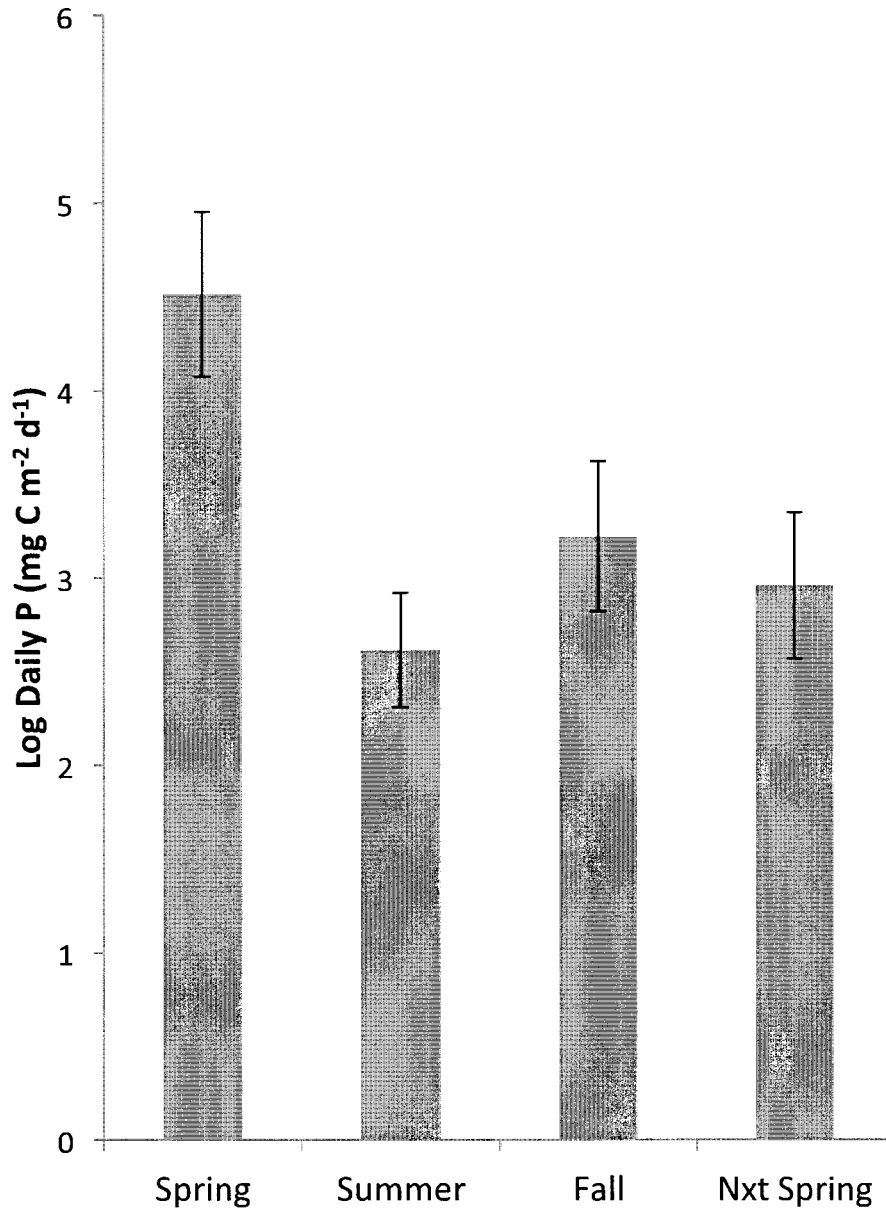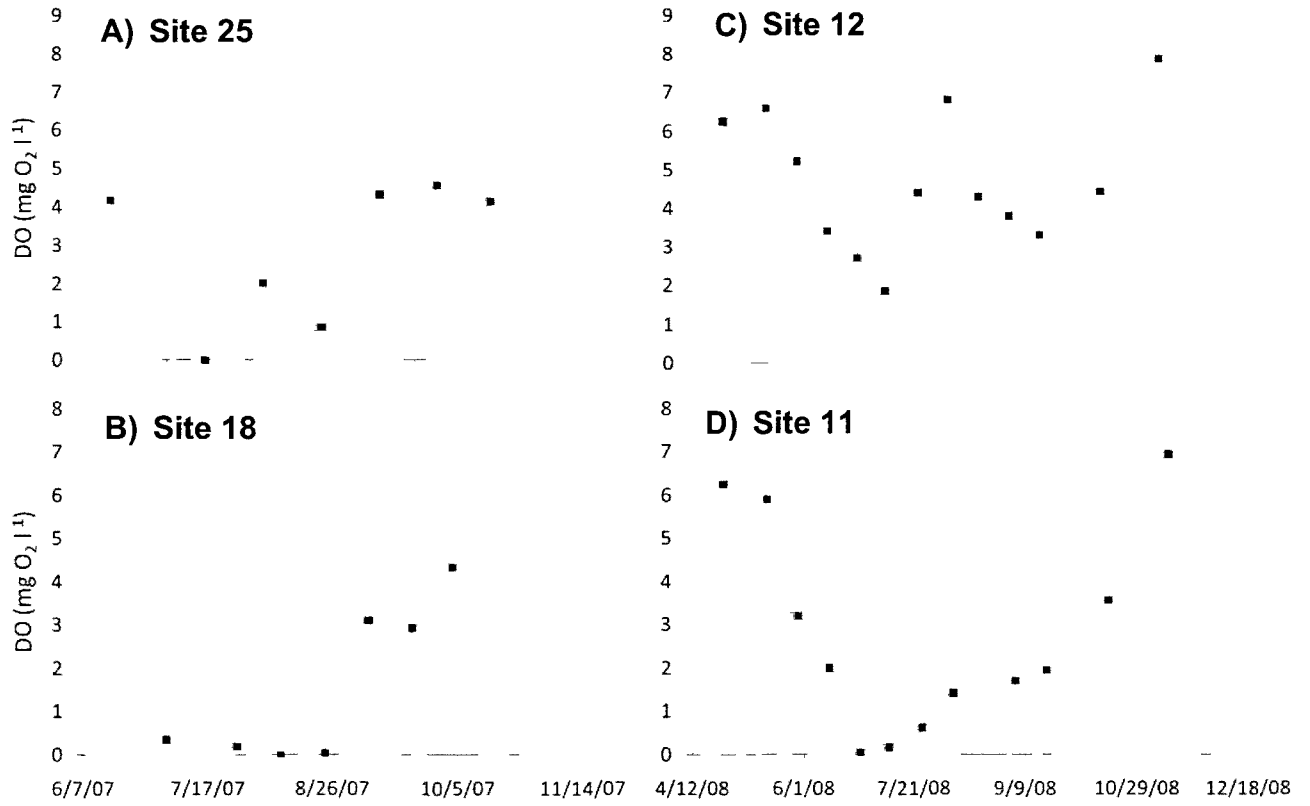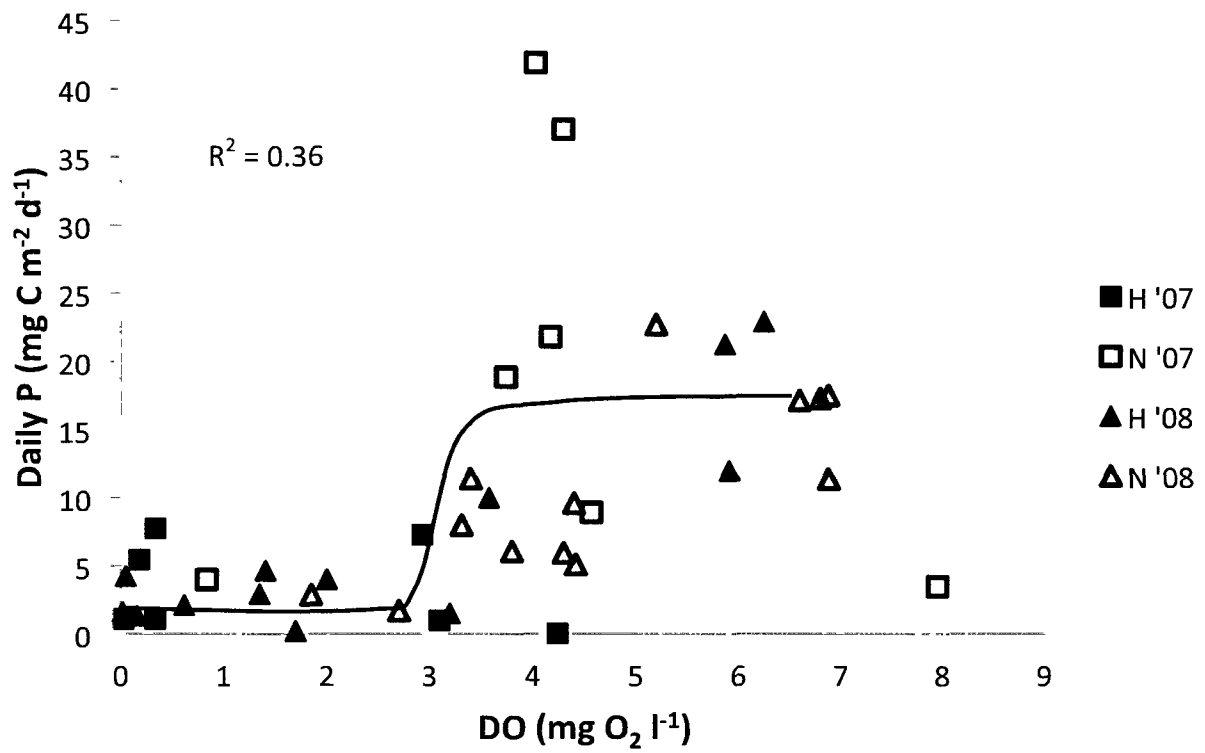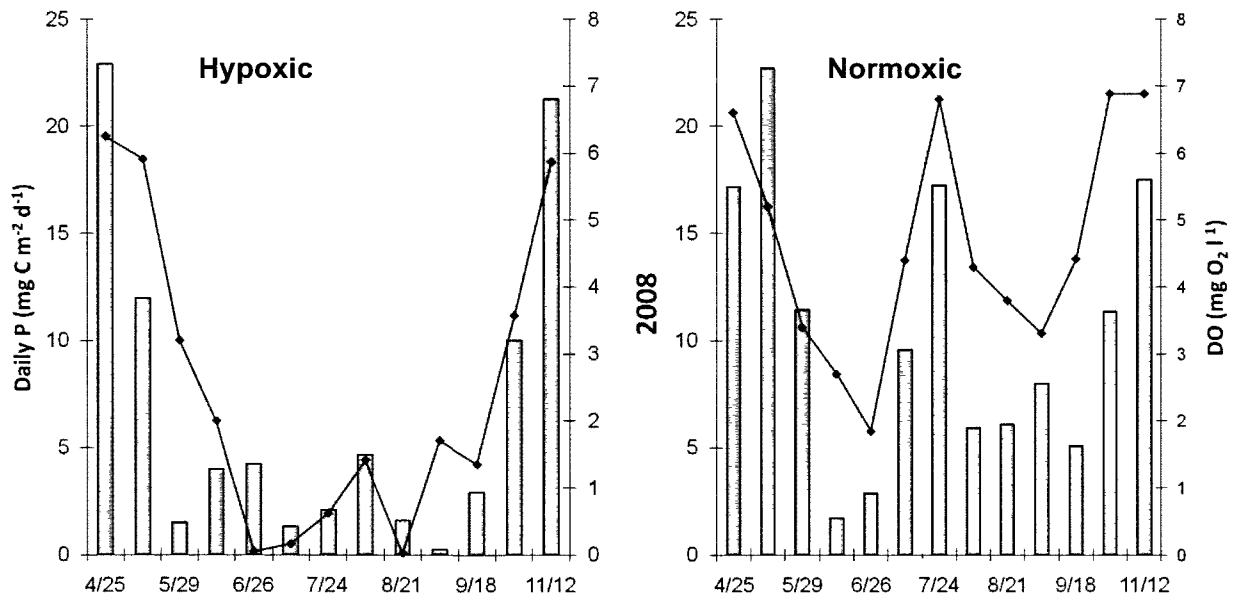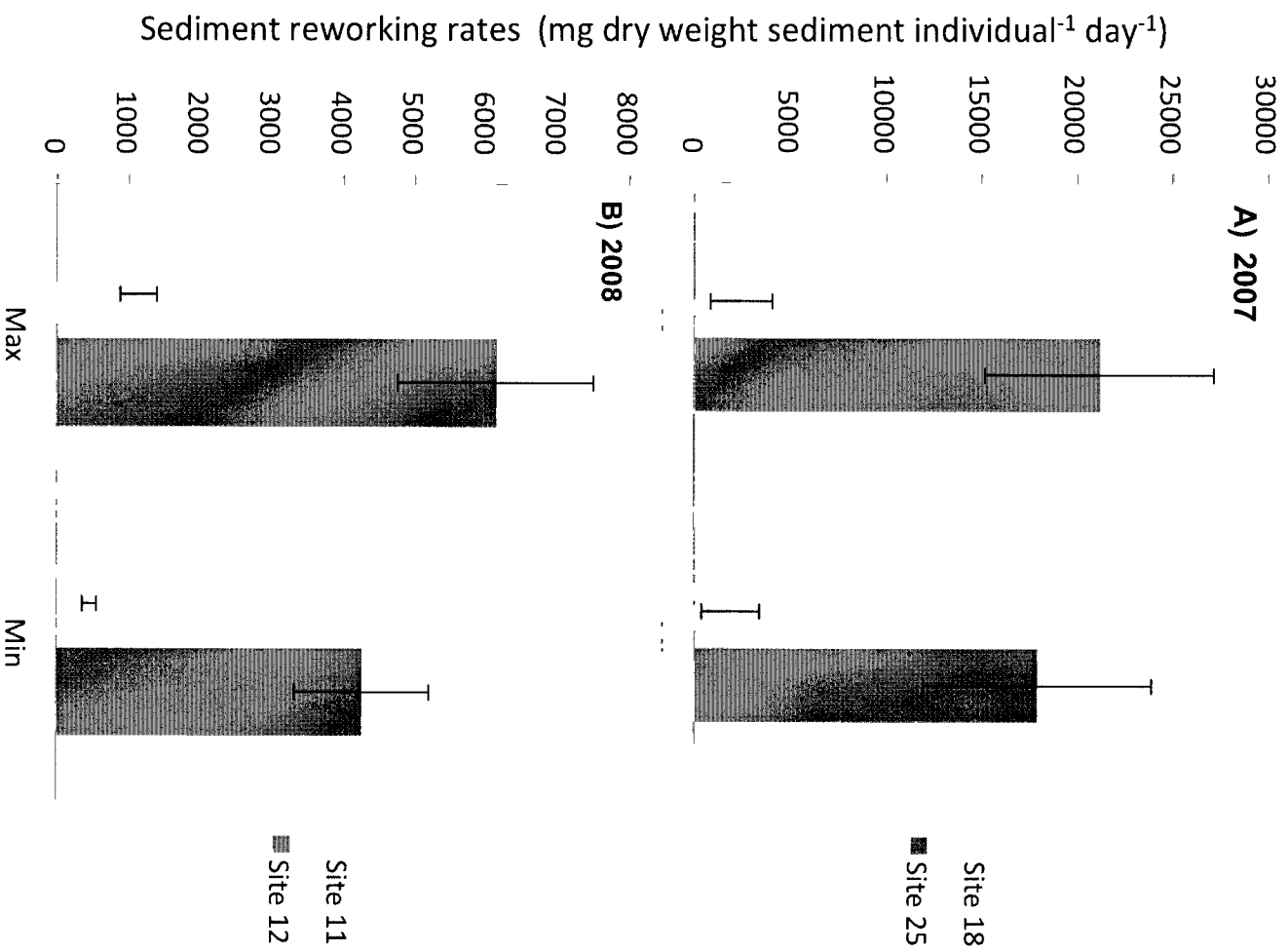
CHAPTER 3

Bioturbation in a declining oxygen environment, *in situ* observations

Manuscript citation: Sturdivant SK, Diaz, RJ, Cutter GR (submitted). Bioturbation in a declining oxygen environment, *in situ* observations. Proc Natl Acad Sci

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ABSTRACT

Bioturbation, the displacement and mixing of sediment particles by fauna or flora, is an essential

process that increases the quality of marine sediments. In the marine environment bioturbation is

primarily mediated by infaunal organisms. Infauna are susceptible to perturbations in their

surrounding environment due to their sedentary life history traits. Hypoxia, dissolved oxygen

(DO) concentrations of ≤ 2 ml l^{-1}, is a prevalent, persistent issue that affects marine life, including

pelagic and bottom fauna, and has been increasing in coastal systems worldwide. A benthic

observing system (Wormcam) consisting of a buoy, telemetering electronics, a sediment profile

camera, and a water quality datasonde was deployed in the Rappahannock River, VA, USA, in

an area known to experience seasonal hypoxia from early spring to late fall. Wormcam

transmitted a time series of *in situ* images and water quality data a shore-based receiver station

via wireless internet for 5 months spanning normoxic and hypoxic periods. Hypoxia was found

to significantly reduce bioturbation through reductions in burrow lengths, burrow rates, and

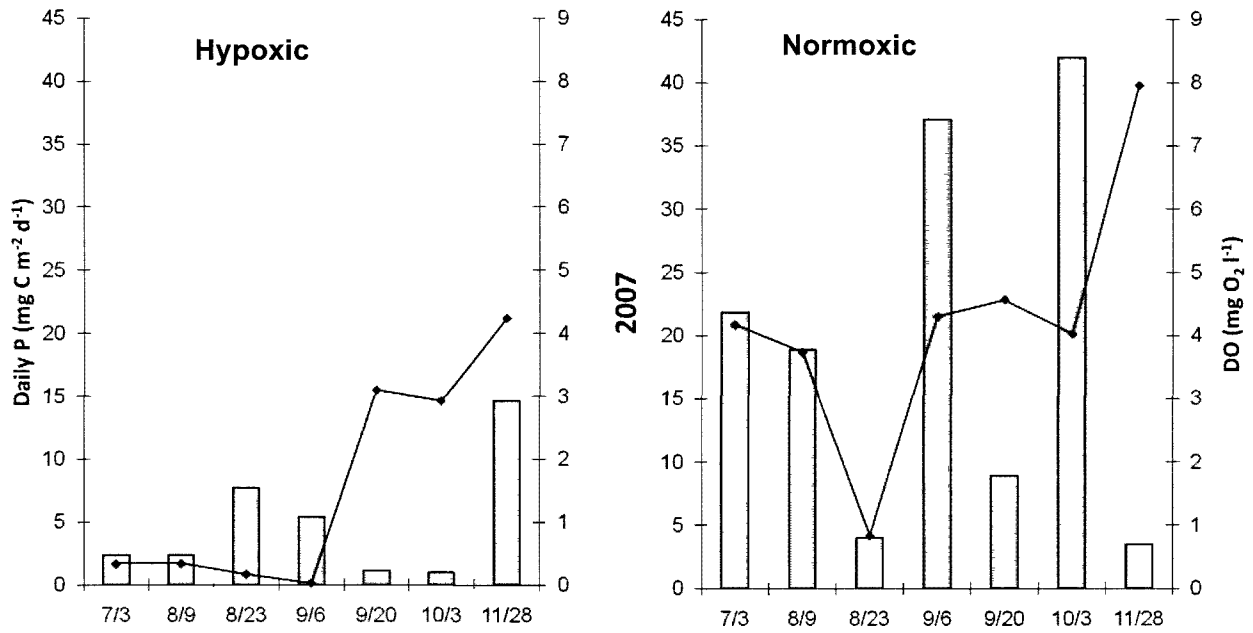burrowing depth. Although infaunal activity was greatly reduced during hypoxic and near

anoxic conditions, some individuals remained active. Low concentrations of DO in the water

column limited bioturbation by infaunal burrowers. This study emphasizes the importance of *in

situ* observations for understanding how components of an ecosystem respond to hypoxia.

1. INTRODUCTION

Bioturbation describes the biological reworking of sediments by flora, fauna, or microbial activity (Meysman et al. 2006). This study focuses on infaunal bioturbation, as it has been shown to play a vital role in regulating marine sediment geochemical and physical properties (Aller, 1978; Rhoads and Boyer, 1982) as well as affecting ecosystem function (Meysman et al. 2006). Sediment permeability, chemical gradients in pore water, remineralization, and inorganic nutrient efflux are a few of the sediment properties and functions regulated by infauna bioturbation (Lohrer et al., 2004).

The sessile nature of the macrobenthos makes them susceptible to changes in the surrounding environment. Consequently, any factors that influence infauna behavior can affect bioturbation. One of the most important is hypoxia, an emergent threat to coastal marine systems worldwide (Diaz and Rosenberg, 2008). Hypoxia, dissolved oxygen (DO) concentrations of ≤ 2 mg l^{-1} (Tyson and Pearson, 1991), has been shown to influence the behavior of infauna (Diaz and Rosenberg, 1995) and eventually lead to death from prolonged exposure (Vaquer-Sonyer and Duarte, 2009). Hypoxia also effects sediment geochemistry resulting in more reduced conditions and a shallowing of the redox-potential discontinuity (RPD) layer (Jørgensen, 1980). Results from laboratory and community field studies suggest that infaunal bioturbation is severely reduced, if not stagnant during periods of hypoxia (Rosenberg et al., 1991; Nilsson and Rosenberg, 2000).

Laboratory studies are effective in providing insight into unknown processes, but can only

attempt to recreate the complexities observed *in situ* (Snelgrove and Butman, 1994). The

development of sediment profile cameras has enabled *in situ* observations of organism-sediment

interactions (Rhoads and Cande, 1971). Diaz and Cutter (2001) and Solan and Kennedy (2002)

used time-lapse profile cameras to document burrowing and formation of other biogenic

structures. We developed Wormcam, an *in situ* benthic observing system that is a combination

of a sediment profile camera and water quality datasonde, to collect a time-lapse series of images

and data. Information collected was transmitted in near real-time, every 30 minutes using a

wireless internet router to our website. The specific objectives of this study were to assess, via *in*

situ observations, the impacts of hypoxia on bioturbation, infaunal behavior, and sediment

geochemistry.

2. METHODS

2.1 Study Area

This study occurred over a five month period from May to mid-September 2009 in the

mesohaline portion of the Rappahannock River (Fig. 1), a sub-estuary of Chesapeake Bay known

to experience seasonal hypoxia (Kuo and Neilsen, 1991; Park et al., 1996). Wormcam was

deployed approximately 2.5 km northeast of La Grange Creek (Middlesex County), Virginia,

based on DO concentrations collected from previous years. Initially Wormcam was deployed in

27 m at Location 1 (37° 41, 24.8' N, 76° 33, 47.9' W), but halfway through the monitoring

period, in mid-July, was moved 0.5 km to the east to 32 m at Location 2 (37° 41, 25.6' N, 76° 33,

37.8' W) to ensure the capture of a prolonged hypoxic event (Fig. 1).

2.2 Wormcam

Wormcam consisted of an IQEye model 705 5-megapixel Ethernet camera, placed in a plastic housing that had a 45 degree angle at the bottom, which formed a wedge to penetrate into the sediments, and a mirror on the back wall in the wedge acted as a prism to image the vertical profile. The field of view was 10 cm wide by 15 cm long. Lighting was provided by a white LED (Lexeon Star model 5C). The camera was set to take a series of 8 to 12 images every half-hour that were stored on the camera's memory card. Wormcam was affixed to a low-profile aluminum frame to minimize flow disturbance and to prevent the camera from fully sinking in the sediment (Fig. 2a). Also, the window extended beyond the edges of the prism to divert water flow and prevent erosion near the corners. A Hach DS500X water-quality datasonde was attached to the frame 20 cm above the sediment and collected DO, salinity, temperature, and depth measurements at 30-min intervals in conjunction with image capture. A few images and water quality data (DO, salinity, temperature, and depth) were transmitted wirelessly to our website via a Sierra Wireless AirLink™ Raven X Ethernet modem for near real-time observation. This allowed us to keep track of water quality and sediment structure conditions. During maintenance trips the memory card was retrieved and images were downloaded for analysis of biogenic structures and sediment oxidation state; sediment grabs were collected using a Young grab (samples an area of 440 cm^2 to a depth of 10 cm) and screened through a 0.5 mm sieve to assess benthic community composition. The entire system was controlled by a Campbell CR1000 microprocessor and solar powered from a surface buoy connected by cable to Wormcam (Fig. 2b). Wormcam was deployed for a period of 5 months, from May 13 - September 15, 2009, and divided into three oxygen regimes: the transition from normoxia to

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hypoxia (May 13 - July 21) , the prolonged exposure of hypoxia and anoxia (July 21 - September

1), and the subsequent rebound to normoxia (September 1 - September 15). Maintenance-

recoveries and redeployments occurred every 3-4 weeks as needed. Point DO measurements

were collected from a surface vessel with a handheld YSI Professional Plus water quality meter

to verify data from the deployed meter. An additional Hach DS500X datasonde was deployed

August 7, 2009 until the end of the project to verify DO results. DO concentrations recorded by

the Wormcam datasonde were not significantly different from the handheld YSI (df=3, T=0.15,

p=0.888) and the additional deployed datasonde (df=1790, T=1.29, p=0.197), based on paired t-

tests.

2.3 Data Analysis

Photoshop (Adobe Systems Inc.) was used to rotate and scale the images, ImageJ (NIH) was

used for digital measurements of sediment oxidation state and biogenic structures, and MatLab

(The Mathworks) code was used to view sequences of images and the corresponding DO

concentration data. While images were captured every half-hour, a 6-h interval was used for

detecting the effects of hypoxia on visual features and infaunal activities. Oxidation state of the

sediment and depth of the apparent-color redox-potential discontinuity (aRPD) was determined

by color: reddish-brown sediment was considered oxidized and grayish-black sediment was

considered reduced (Fenchel, 1969). Centroid and maximum burrow depths were recorded as

estimates of bioturbation activity. Centroid depth was designated as the geometric center of

burrowing activity and max depth was the deepest detectable burrow. The relationships between

DO concentration and aRPD, centroid, and max burrow depths were assessed using linear

regression.

The study record was divided into three periods based on oxygen regime. First was the transition from normoxia to hypoxia (May 13 - July 21), second was the prolonged exposure of hypoxia and anoxia (July 21 - September 1), and third the subsequent rebound to normoxia (September 1 - September 15; Fig. 3). For each of the three oxygen regimes, a random sample of five burrows was analyzed hourly to determine the effect of DO concentration on burrow length and duration. An hour time-frame was used for burrow length and duration quantification to assess finer scale changes in burrow transformation. For a burrow to be measured, it needed a visible connection to the sediment surface, be easily discernible in the images, and extend below the aRPD. Natural log transformation was used to achieve normality for burrow length data. Animating the series of images provided information on burrowing activity and fauna behavior relative to DO concentration.

3. RESULTS

Over the 5-month study, bottom temperature ranged from 18 to 29 °C and salinity from 12 to 19 psu. Burrowing activity was detected a few hours post-deployment of Wormcam; small capitellid-like worms were observed first during normoxic conditions, and small spionid like worms during hypoxia. These were the dominant taxa in corresponding sediment grabs collected less than ~10 m from Wormcam (Table 1). Significant positive relationships were found between DO concentration for both centroid and maximum burrow depths (Fig. 4). As DO concentrations declined the centroid and maximum depth of infauna burrows became shallower, to the point where organisms were seen extending their bodies above the sediment surface during

prolonged periods of low DO (Fig. 5). Over the study period, >90% of the observed maximum burrow depths were <5 cm below the sediment surface.

Burrows were generally well formed within one hour after initial observation in the image sequence. Over the burrows' life span, initial burrow lengths were ≥70% of the maximum length, indicating the majority of burrow formation was completed within an hour, the time interval between images. Initial length of some burrows was >90% of the maximum length. A significant positive relationship was found between burrow length and DO concentration (Fig. 6a), as DO increased, burrow length increased. While non-significant, there was a tendency for increased burrow longevity at higher DO concentrations. Increases in burrow length were primarily attributed to sediment accretion; worms would extend burrows back to the surface within the hour during high accretion events but were rarely observed burrowing deeper during erosion events. Of the observed burrows, the majority were destroyed or abandoned due to erosion or biological disturbance, especially from blue crab (*Callinectes sapidus*) and American eel (*Anguilla rostrata*) foraging during normoxia. Crabs and eels were only present during normoxic conditions and it is presumed that they were searching for prey (Van Engel, 1958; Wenner and Musick, 1975), but neither was observed preying on infauna. During hypoxia burrows remained in place but appeared abandoned.

Over the study period burrow production, defined as the change in total burrow length over time, averaged 3 mm h^{-1} (SD=9). Burrow production during normoxia (>2.8 mg O_2 l^{-1}), 4.3 mm h^{-1} (SD=11.3 mm), was significantly higher (p=0.001, T= -3.29) than during hypoxia, 1.1 mm h^{-1} (SD=3.4 mm), by approximately 75%. Burrows generally had two distinct sections, the portion

of the burrow above and below the aRPD. On average, burrows extended 20 mm (SD=15 mm)

below the aRPD when it was visible. Worms did not appear to favor either side of the aRPD and

were observed readily moving throughout the vertical extent of their burrows during all

conditions. During normoxia, the portion of burrows above the aRPD always appeared oxidized

(reddish-brown in color), and the portion below the RPD became oxidized within an hour to an

average of 1.0 mm (SD=0.3) from the burrow wall. During hypoxia burrows appeared to remain

oxidized above the aRPD, but oxidation was not detectable below. The entire lengths of burrows

appeared completely reduced during periods of anoxia. The affect of hypoxia on sediment

geochemistry was assessed via the depth of the apparent-color RPD (Fig. 6b). As DO

concentration decreased the aRPD depth moved closer to the sediment surface and burrow depth

significantly declined (Fig. 7). When anoxia was reached, the aRPD was not discernible.

During periods of anoxia we observed the dynamic nature of bacterial mat formation. As DO

declined to 0 mg l^{-1} and anoxic conditions spread to the sediment surface, stringy white sulfur

bacteria were observed migrating in mass up through the sediment to the surface (Supplemental

material, Video 1). Although no samples were collected, based on morphology the bacteria

appeared to be *Beggiatoa* spp. Over a 14 day period of anoxia (Aug 1 – 15), bacteria migrated to

the sediment surface at 1.2 mm h^{-1} (SD=), climbed up the face plate of the prism, and produced

copious amounts of organic matter which then settled onto the sediment surface. The original

sediment surface was quickly covered by this unconsolidated mass of bacteria and sediment. As

more was produced the older organic material became consolidated beneath the weight of new

organic material. By the end of the 14 day anoxic period, the sediment surface had risen

approximately 7 cm with 0.5 cm of the new sediment height unconsolidated. As DO

concentration began to rebound, bacteria migrated in mass back down into the sediment (Supplemental material, Video 1), and the 0.5 cm unconsolidated microbial mat left at the sediment surface was eroded by currents within a few hours. A week-long period of normoxia followed and bioturbation was dominated by nereid and capitellid polychaetes. Towards the end of this period, the bacteria started to migrate back to the sediment surface and reformed a bacterial mat over the next hypoxic/anoxic period (Aug 23 - September 1).

During conditions when the water column was anoxic, there was a surprising amount of infaunal activity (Fig. 8). Prior to the onset of anoxia several spionid polychaetes, *Paraprionospio pinnata*, were observed at the sediment surface with their characteristic palps extended into the water column at a DO of 0.1 mg l^{-1} (Fig. 9). As DO concentration declined further to anoxia it appeared that *P. pinnata* continued to burrow throughout the sediment and flocculent bacterial mat. Burrows created during this period remained anaerobic, and worms did not inhabit the burrows for longer than an hour. Sediment grabs collected during anoxia only contained *P. pinnata* (Table 1).

Other behavioral observations from Wormcam were worms retracting into burrows upon the presence of a predator, a nereid worm preying on another worm, a goby searching for food or oxygen during hypoxia, a sea cucumber extending its body and appendages above the sediment-water interface during hypoxia, and apparently a worm using the burrow of another worm (Supplemental material).

4. DISCUSSION

We found hypoxia to interfere with ecosystem function by reducing the rates and depth of bioturbation. Burrow depths and lengths were significantly related to DO concentration with shallower burrow depths and reduced burrow lengths during lower oxygen. Reductions in burrow depths and lengths diminished the area of influence of bioturbators, limiting the amount of sediment reworked. The consequences of inhibiting bioturbation cascade to changes in sediment permeability, remineralization, and nutrient flux. The rate at which sediment was reworked through burrow production was reduced by 75% during hypoxia.

DO concentrations were shown to be significantly positively related to the apparent-color RPD depth, with shallower aRPD depths at lower DO concentrations. In the anaerobic environment below the RPD, reduced conditions dominate and H_2S can be present (Theede, 1973). It is difficult to separate the combined effects of low DO and H_2S toxicity on marine organisms (Vismann, 1990), so to explain the effect these two physical conditions might have on bioturbation, a multiple regression was performed, and a significant positive relationship was found. The interaction of DO concentration and aRPD depth influenced macrobenthos, limiting their bioturbation effectiveness through a reduction in organism activity and burrowing depth. Reductions in bioturbation further reduced DO concentration below the sediment surface affecting sediment geochemistry via reduced oxygen diffusion across burrow walls. During normoxic conditions, oxygen appeared to diffuse an average of 1.0 mm (SD=0.3) from burrow walls below the aRPD. This oxic layer was not discernible around burrow walls below the aRPD during hypoxia. Diaz and Cutter (2001) observed worm activity to correspond with increased

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oxygen diffusion across burrow walls below the aRPD during normoxia. Hypoxia then leads to a reduction in the passive and active diffusion of DO to subsurface sediments.

Analysis of *in situ* Wormcam images quantified the relationship between DO concentration and infaunal bioturbation and revealed the dynamic nature of the benthic environment. Over a 14-day period of anoxia (Aug 1 – 15) filamentous bacteria were observed migrating through the sediment and producing a flocculent mat on the Wormcam faceplate and the sediment surface. Microbial migration and formation of microbial mats has been documented (Jørgensen, 1980; Bagarinao, 1992, Graco et al. 2001), however, the observation of this process and the subsequent burrowing of worms throughout the sediment and bacterial mat are new. We found some portion of the infauna to remain active during hypoxia and even anoxia. Infaunal activity was observed during anoxia and in the presence of sulfur-oxidizing bacteria (Nelson et al., 1986) from the sediment surface to 5 cm below the surface. Other in situ observation of surface fauna behavior during hypoxia also found infauna to surface (Riedel et al., 2008). However, laboratory experimental data would predict mortality and no infaunal activity during anoxia (Vaquer-Sonyer and Duarte, 2008). Although active worm burrowing was observed, the burrows created during this period remained anoxic, indicating that bioturbation could also act as a process to aid the diffusion of anaerobic compounds out of the sediments and into the water column.

The plasticity of *Paraprionospio pinnata*, a worm indentified in images and corresponding sediment grabs, is one hypothesis to explain their activity during prolonged hypoxic/anoxic events. Skipper et al. (2010) defines plasticity as 'the capacity of organisms or cells to alter their phenotype in response to changes in their environment.' Before the onset of anoxia, on multiple

occasions the DO concentrations at our site became hypoxic for a short duration. These low DO events may have pre-conditioned the infauna physiologically for the subsequent anoxia event. Childress and Siebel (1998) discuss 3 methods organisms use to cope with low oxygen: increasing oxygen uptake, decreasing metabolic demands, or utilizing anaerobic metabolism. In response to the infrequent short-duration low oxygen events, worms not killed would have a physiological response to produce more haemoglobin; increasing the capacity of their coelomic fluid to uptake oxygen and subsequently the ability to cope with the next low oxygen event (Mangum, 1970; Bartolomaeus, 1994). *P. pinnata* are also morphologically well adapted to deal with a low oxygen environment having elongated, proliferated and numerous branchia (Dauer, 1985; Lamont and Gage, 2000). We could not determine if *P. pinnata* decreased its metabolic demand by viewing images, but *P. pinnata* observed during anoxia were highly active. Recent work by Gonzalez and Quiñones (2000) showed that *P. pinnata* posses all four subsets of pyruvate oxidoreductases (LDH, ALPDH, OPPDH, and STRDH), which are enzymatic adaptations associated with anaerobic metabolism during low DO. Levin (2003) suggests the high numbers and variety of these enzymes may 'confer metabolic plasticity, and could explain the success of *P. pinnata* in hypoxic settings around the world' as well as at our study site. It is also possible that the organic rich environment created by the bacteria, offset any respiratory deficiencies experienced in a severely oxygen limited environment; even more likely is some combination of the two hypotheses. The scavenging amphipod, *Orchomene obtusus*, has been shown to capitalize on abundant food and lack of predation in anoxic bottom waters, but must reenter oxygenated waters to recover oxygen debt after sometime (De Robertis et al., 2001).

5. CONCLUSION

The results from this study quantify infaunal bioturbation during low DO, and find that hypoxia significantly affects bioturbation. Hypoxia reduces bioturbation through significant reductions in burrow lengths, burrow production, and burrow depth. Although infaunal activity was observed during hypoxic and anoxic conditions, the low concentrations of DO limited diffusion into the sediment. Although some worms were active during hypoxia via plasticity or perhaps capitalizing on the environment enhanced with newly available organic material, the extent to which their ability to process sediment was reduced during anoxic conditions is unknown. Thus a portion of bioturbation may remain unaffected by low DO and some macrobenthic bioturbation may retain their value.

While the results presented in this paper affirm previously held notions about the affect of hypoxia on macrobenthic bioturbation and behavior, observations from Wormcam clearly demonstrate the necessity and importance of *in situ* studies.

ACKNOWLEDGEMENTS

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TABLE CAPTIONS

Table 1. Abundance of species collected in grabs (0.04 m2) at Wormcam site by date.

FIGURE CAPTIONS

Figure 1. Wormcam study site in the lower Rappahannock River. The gray dot is the initial area that Wormcam was deployed (Location 1). Half way through the monitoring period, Wormcam was moved 0.5 km to the east represented by the black dot (Location 2).

Figure 2. Image of the Wormcam apparatus (A) and a cross-sectional diagram of Wormcam (B). Cross-sectional diagram not drawn to scale.

Figure 3. DO data from May to September (gray line); black dotted lines separate the three DO periods. Large black dots represent point DO measurements, and small black dots represent DO measurements from the second datasonde.

Figure 4. Relationship of DO concentrations and centroid (A) and maximum burrow depths (B). Significant positive relationships were found for both centroid ($p<0.0005$, $F=254.48$) and maximum ($p<0.0005$, $F=191.37$) burrow depths.

Figure 5. The holithurm, *Leptosynapta tenuis* (L), observed extending out of the sediment during near anoxic conditions. Scale around image is in cm units, and the blue circle on the graph shows the DO concentration for the image. Light artifacts from reflection in the prism are visible on the edge of the image.

Figure 6. Relationship of DO concentration and burrow length (A) and apparent-color RPD depth (B). Significant positive relationships were found for burrow length (p<0.0005, F=95.32) and RPD depth (p<0.0005, F=399.98).

Figure 7. Relationship of centroid burrow depth with DO concentration and aRPD depth. A significant relationship was found in the interaction between DO and RPD depth (p<0.001, F=432.35) on burrow depth.

Figure 8. Sediment profile image showing *Nereis* spp. worm (W) and worm burrows (Br), during severe hypoxic conditions, and bacteria (Bc) migrating to the sediment surface and producing copious amounts of organic matter. Black tic marks represent 1 cm scale marks, and the blue circle on the graph shows the DO concentration for the image. Light artifacts from reflection in the prism are visible on the edge of the image.

Figure 9. Sediment profile image showing *Paraprionospio pinnata* (P) at the surface during the onset of a near anoxic event. Black tic marks represent 1 cm scale marks, and the blue circle on the graph shows the DO concentration for the image. Light artifacts from reflection in the prism are visible on the edge of the image.

Table 1.

Taxa	05/07/2009	06/02/2009	06/09/2009	07/21/2009	08/03/2009	08/24/2009
Neanthes succinea	1	0	1	0	1	0
Paraprionospio pinnata	7	3	7	1	1	3
Mediomastus ambiseta	5	1	3	0	0	0
Sigambra tentaculata	1	0	1	0	0	0
Tubificoidies spp.	7	0	0	0	0	0
Amphipoda (unknown)	1	0	1	0	0	0
Leitoscoplos robustus	1	0	0	0	0	0
Streblospio benedicti	2	2	0	0	0	0
Eteone heteropoda	0	1	0	0	0	0
Glycinde solataria	0	0	1	0	0	0
Acteocina canaliculata	0	0	1	0	0	0
Sea anemone	0	0	0	0	1	0
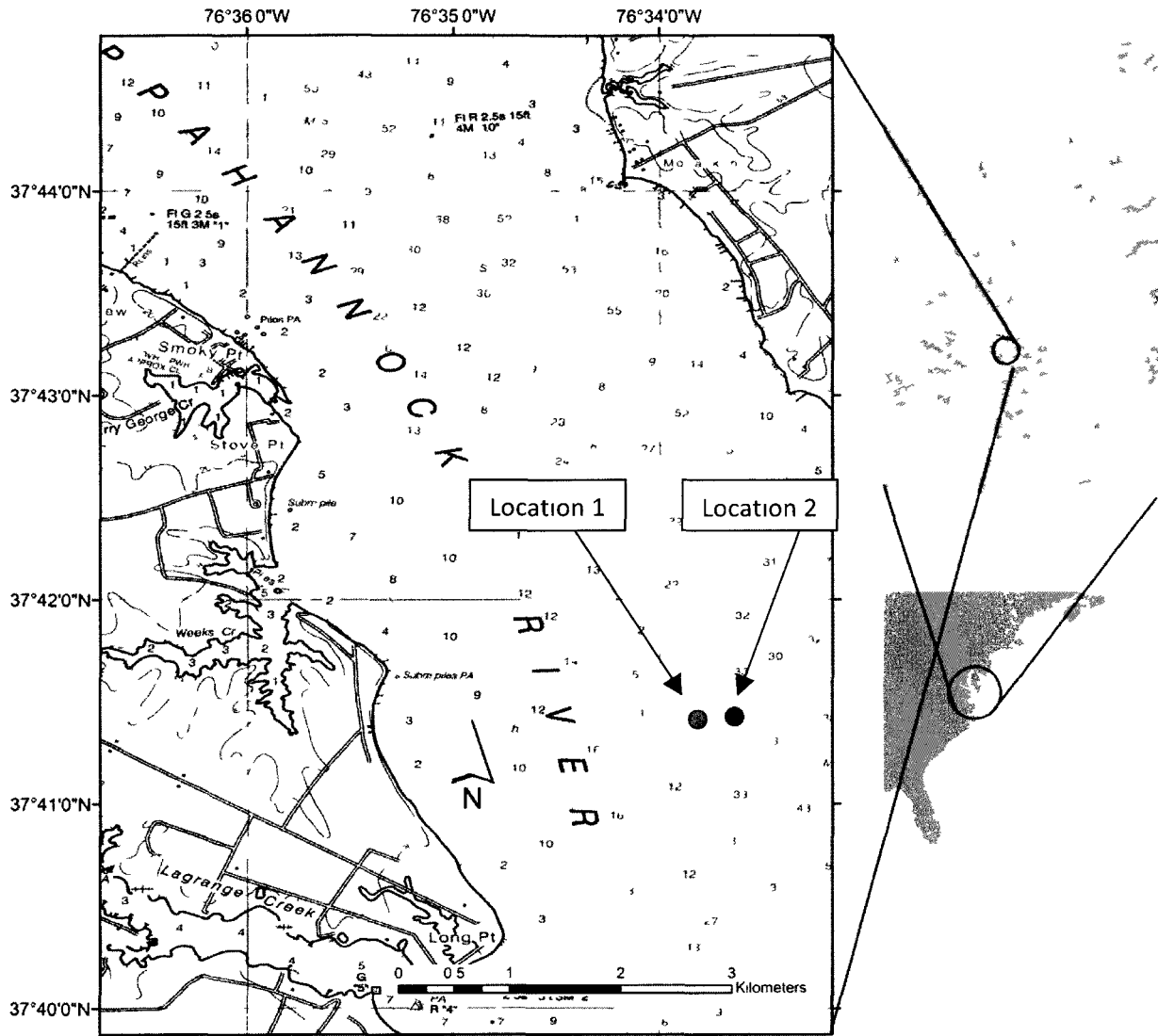
Figure 1.

A)

B)

Figure 2.

Figure 3.

Figure 4.

A)

Centroid Burrow Depth (cm)

$r^2 = 0.52$

B)

Max Burrow Depth (cm)

$r^2 = 0.42$

DO (mg l^{-1})

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Figure 5.

A)

Burrow Length (cm)

r² = 0.19

B)

RPD Depth (cm)

r² = 0.46

DO (mg l⁻¹)

Figure 6.

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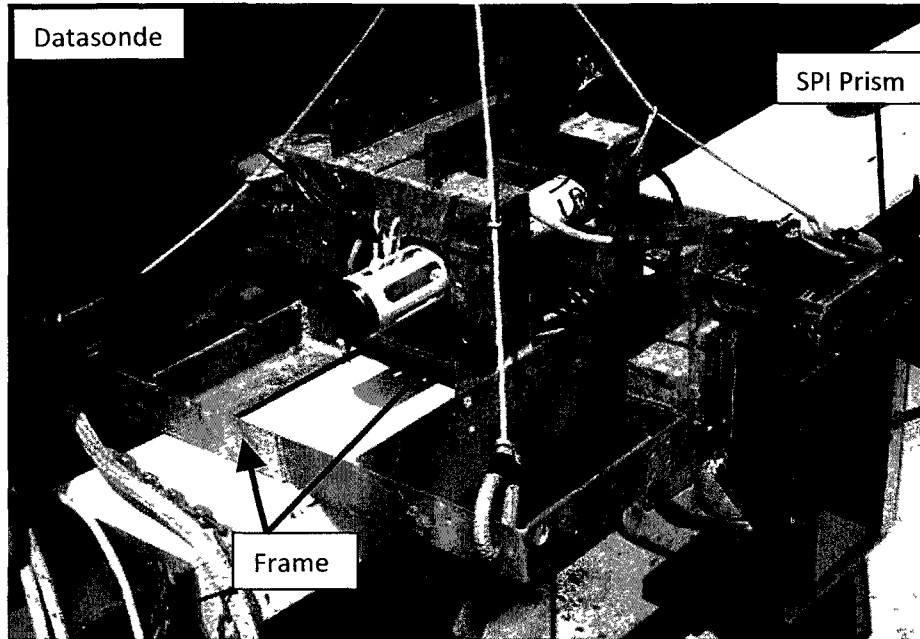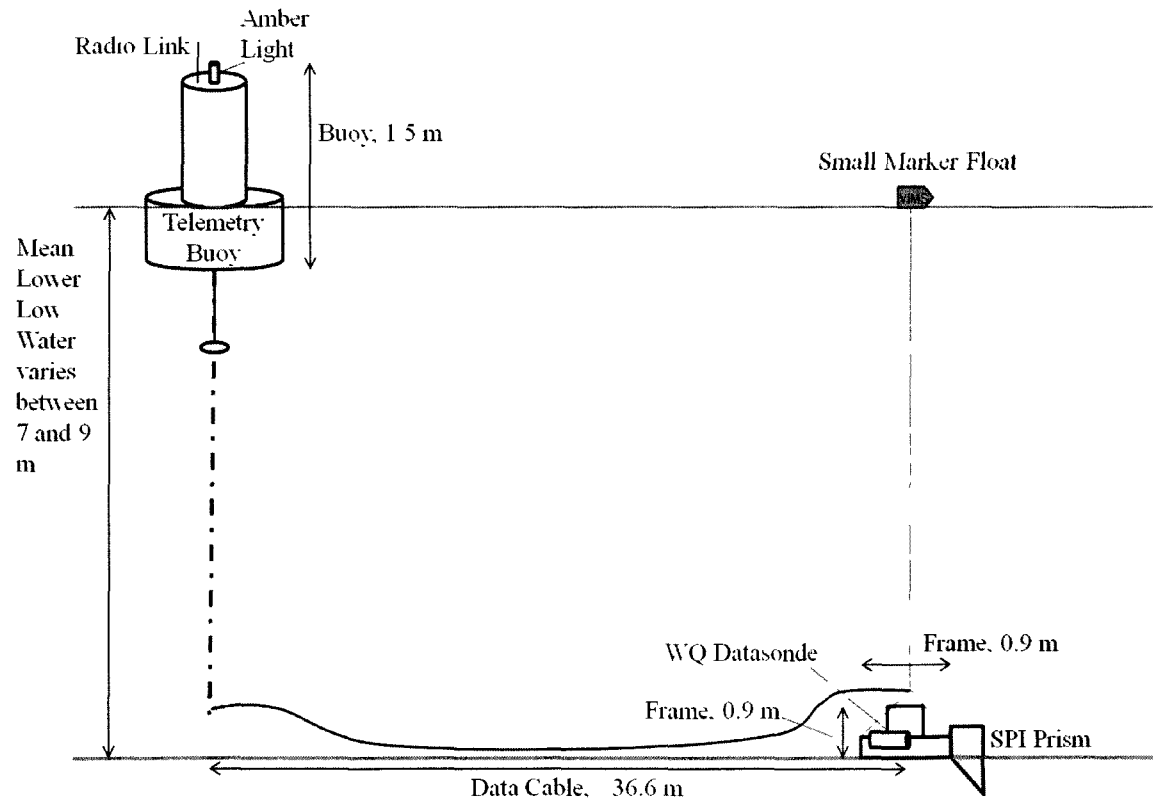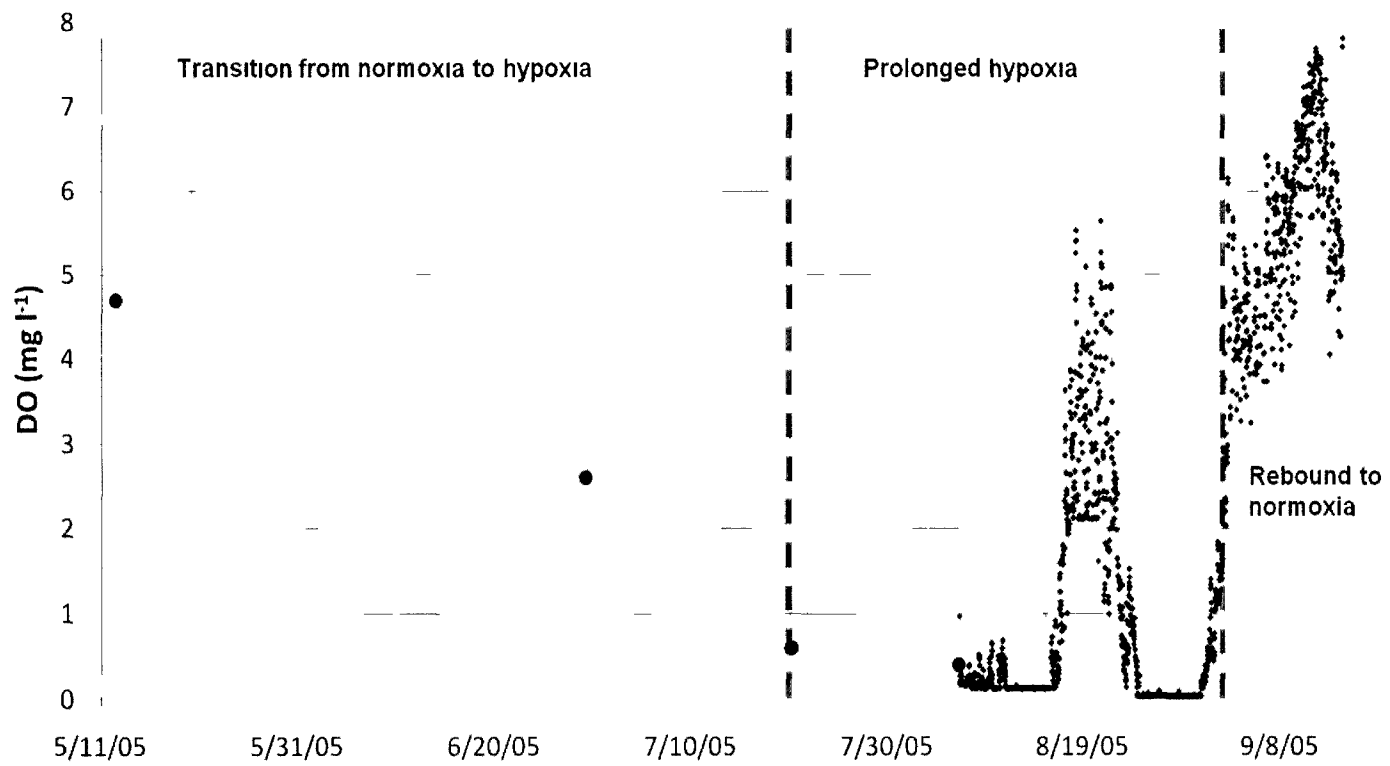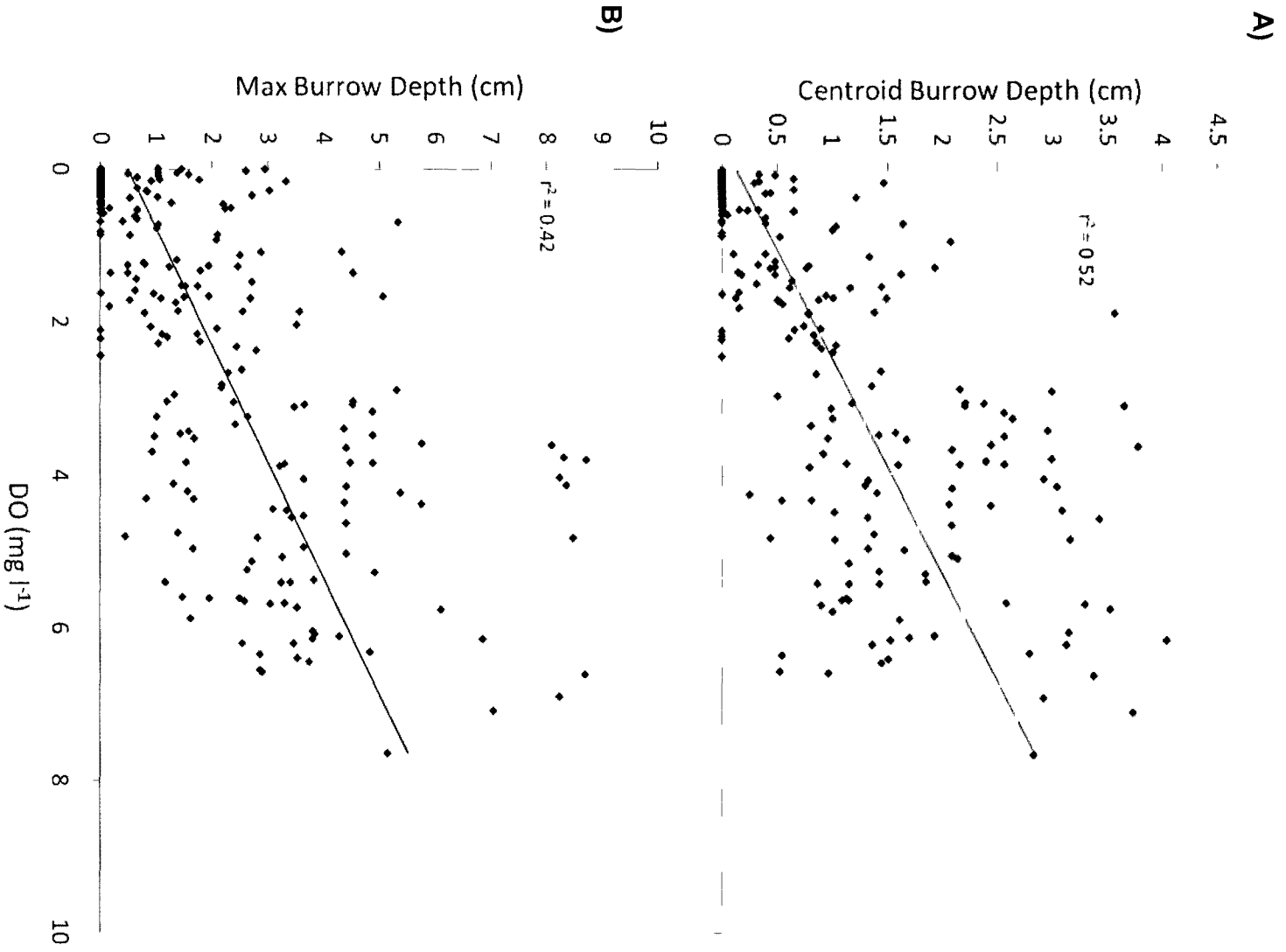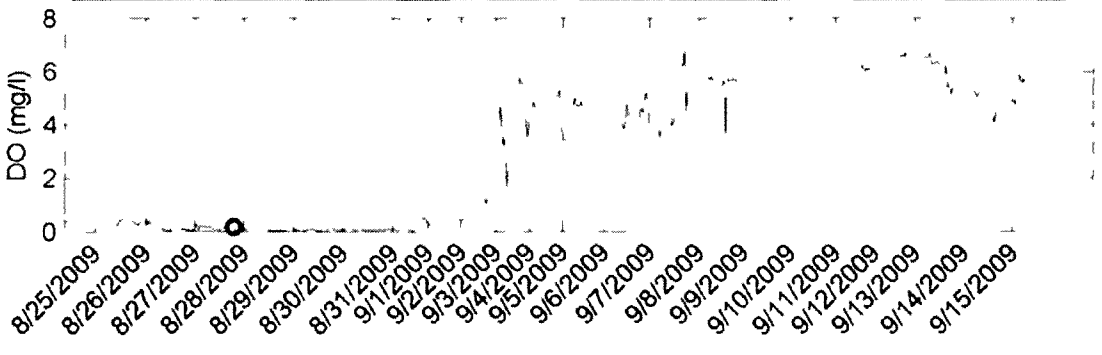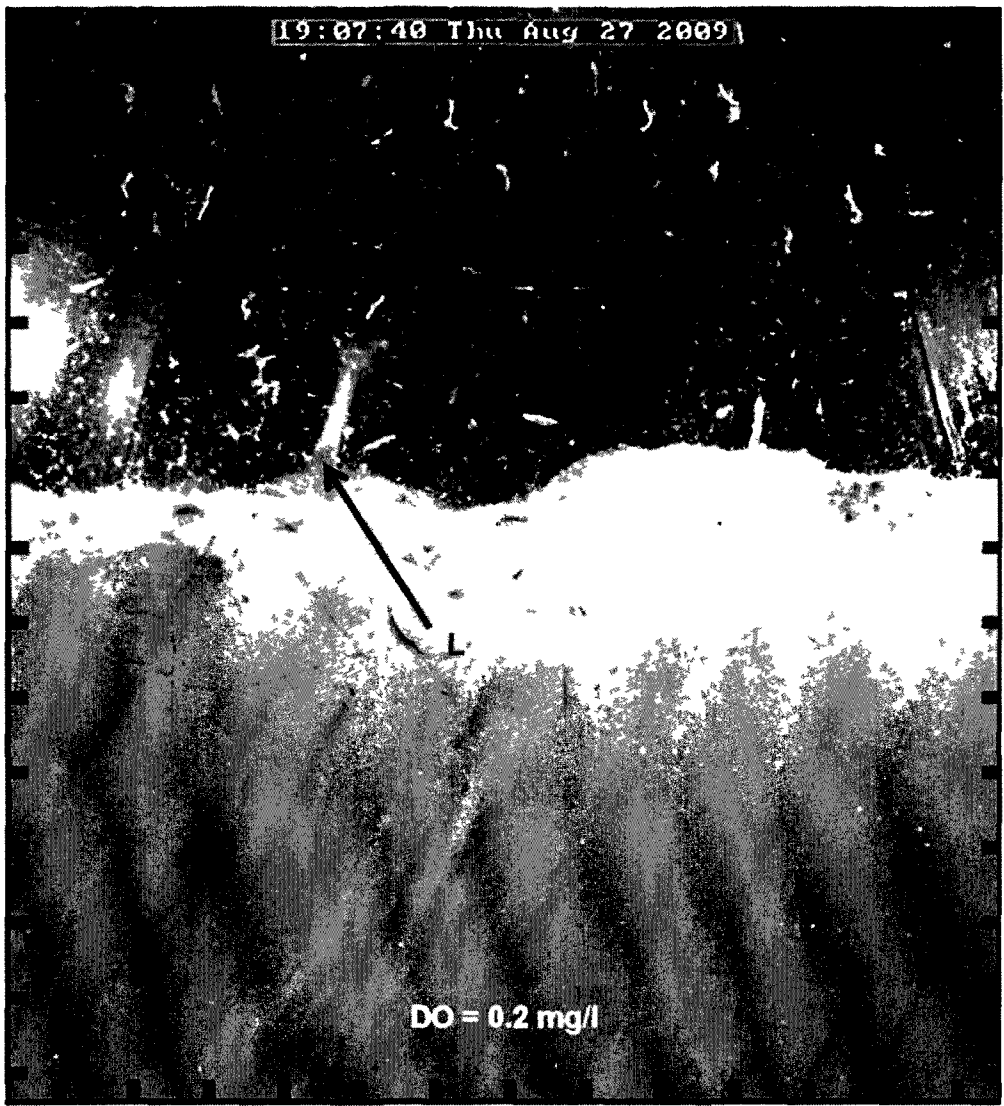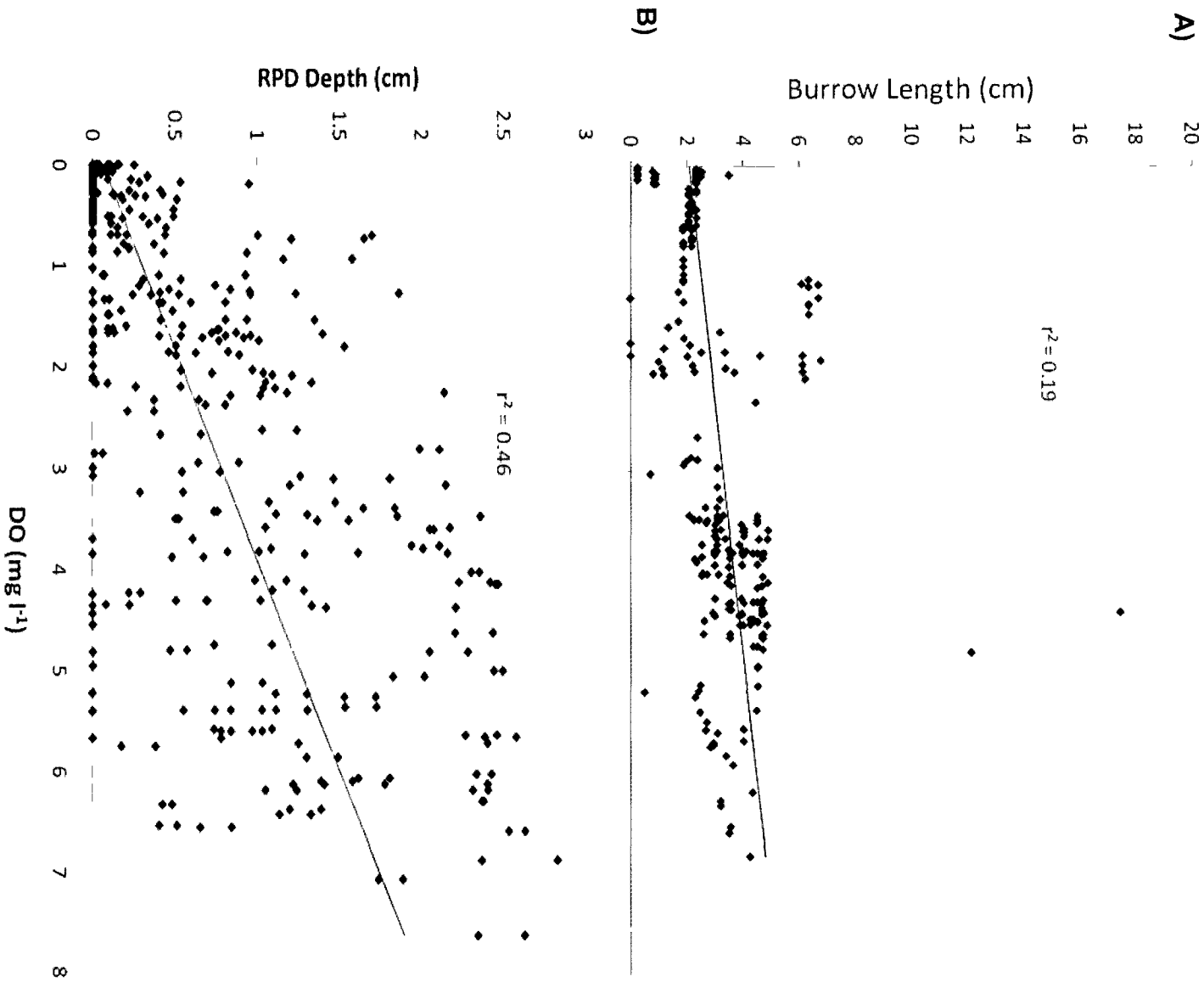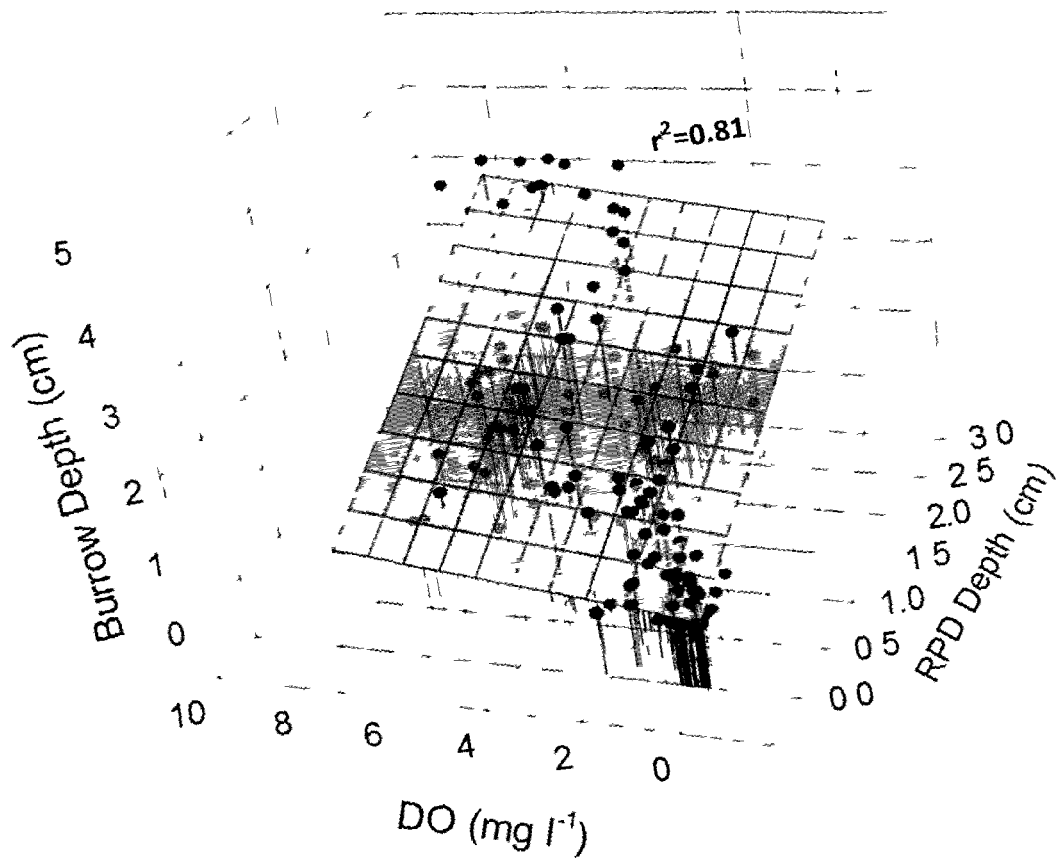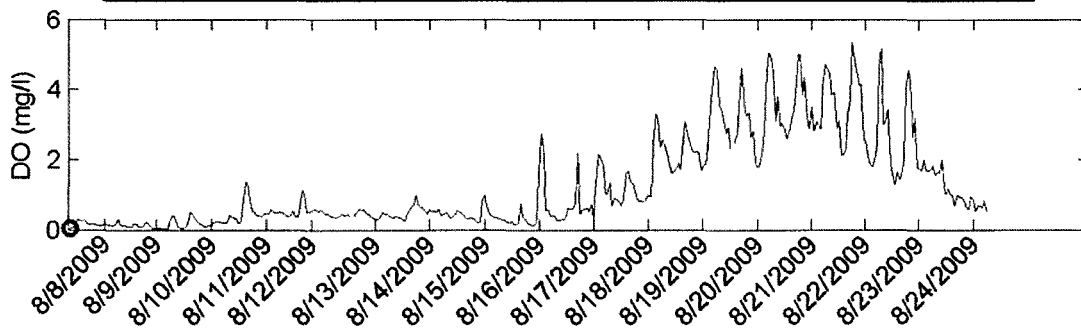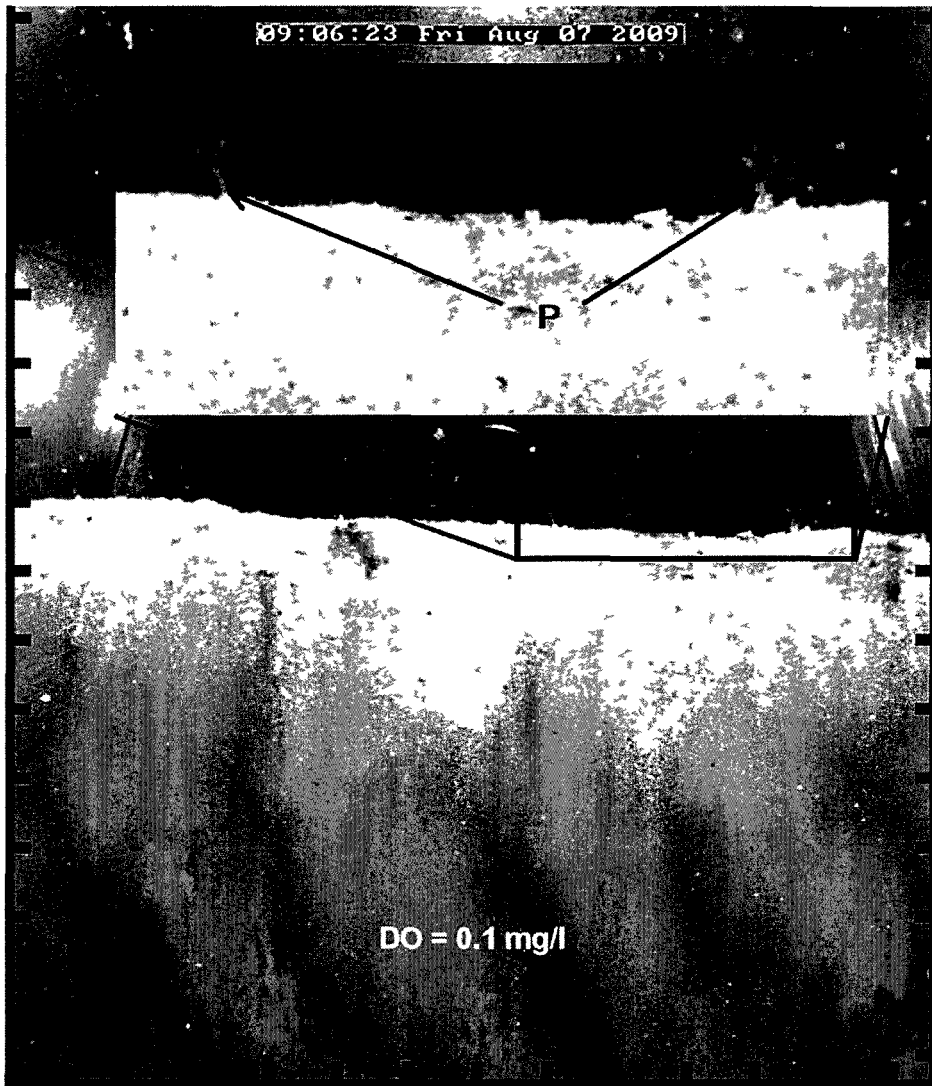
Figure 7.

Figure 8.

Figure 9.

CHAPTER 4

Modeling the effect of hypoxia on macrobenthic production in the

lower Rappahannock River, Chesapeake Bay, USA

ABSTRACT

Hypoxia, DO concentrations of ≤ 2 mg O_2 l^{-1}, in Chesapeake Bay has substantially increased over the past few decades, with detrimental effects on macrobenthic production. The production of benthic invertebrates is important, as these fauna link energy transfer from primary consumers to epibenthic predators and demersal fish. As such, the development of accurate predictive models that determine the impact of hypoxia on macrobenthic production are valuable. A continuous-time, biomass-based model was developed for the lower Rappahannock River, a tributary of Chesapeake Bay prone to seasonal hypoxia, based on the benthic sub-model in the 2002 Chesapeake Bay Eutrophication Model. Phytoplankton, zooplankton, and macrobenthic state variables were modeled, with the primary focus aimed at predicting the effect of hypoxia on macrobenthic biomass (B). Z', a sigmoidal relationship that relates macrobenthic biomass and DO concentration, was derived from macrobenthic data collected in the Rappahannock River during field experiments during the summers of 2007 and 2008, and Z' was appli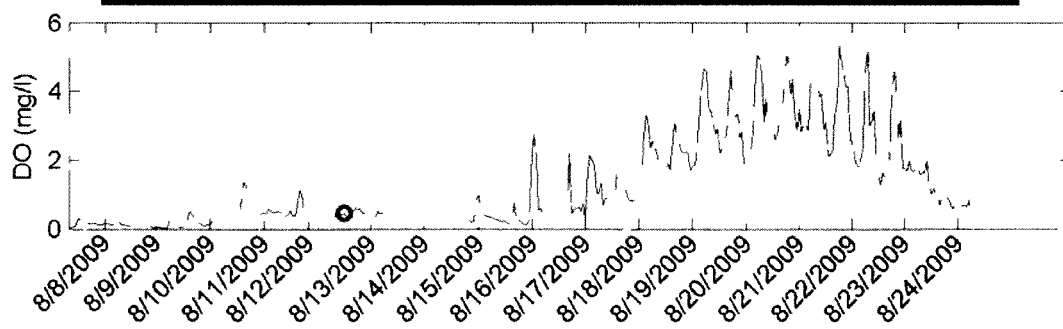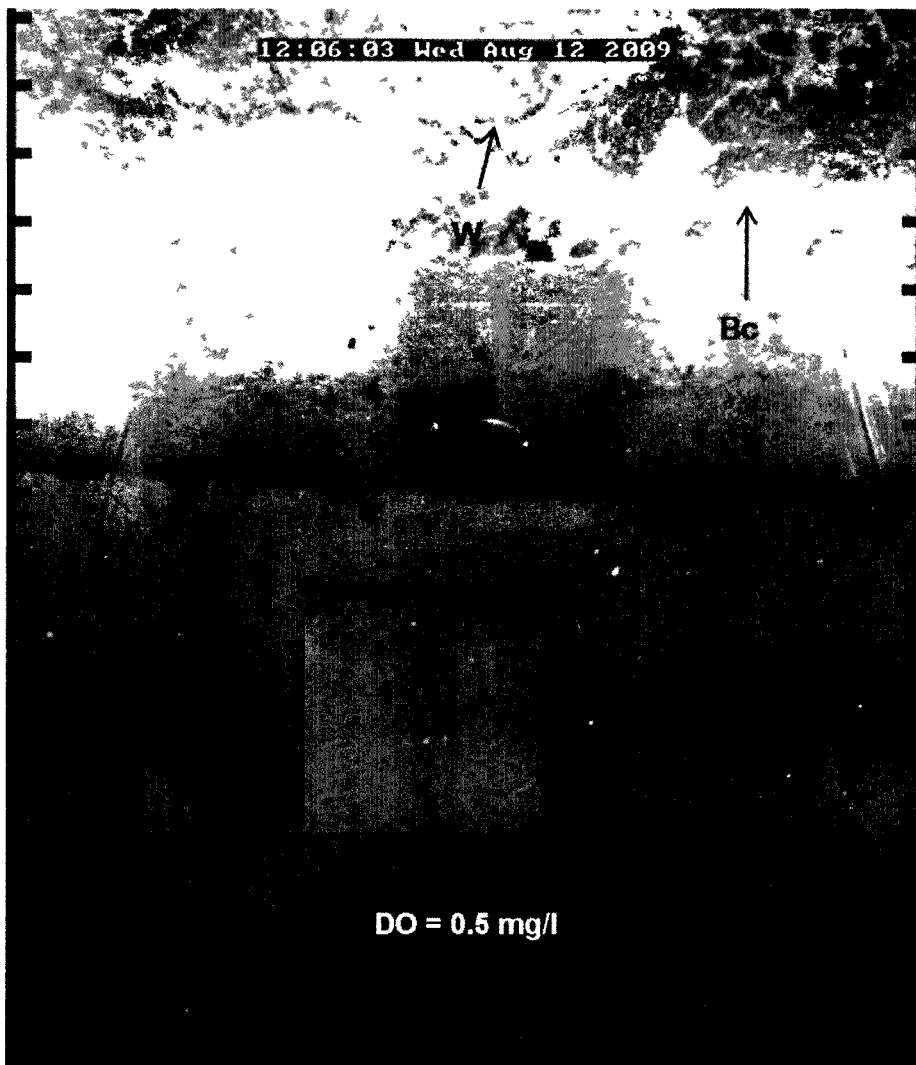ed to the macrobenthic state variable. The biomass-based model was then successfully calibrated and verified, using independent data, to accurately predict B annually. Simulation analysis of the DO formulation showed B strongly linked to DO concentration, with fluctuations in biomass significantly correlated with the duration and severity of hypoxia.

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1. INTRODUCTION

Macrobenthic organisms (> 500 μm) are of great importance to ecological processes in

Chesapeake Bay ecosystem (Fager, 1964; Aller, 1978; Diaz and Schaffner, 1990).

Macrobenthos influence sediment geochemical and physical properties (Rhoads and Boyer,

1982) through bioturbation, the biological reworking of sediments by flora, fauna, or microbial

activity (Meysman et al., 2006). In the estuarine environment, macrobenthos are the foremost

pathway that carbon is recycled out of the sediment and eventually out of Chesapeake Bay

system (Diaz and Schaffner, 1990), and they serve as the energetic link between primary

producers and demersal fish and epibenthic predators (Nilsen, 2006). However, the sessile

nature of macrobenthos makes them susceptible to natural and anthropogenic perturbations (Diaz

and Rosenberg, 1995), a significant concern given the documented importance of macrobenthic

communities in coastal estuaries (Diaz and Schaffner, 1990).

Since colonial times, the number of humans in Chesapeake Bay watershed has grown

exponentially, with a 3-fold increase over the last 100 years (Kemp et al., 2005). Human activity

adversely affects land topography, chemistry of the Earth's atmosphere and water, rates and

balance of biogeochemical processes, and biodiversity (Vitousek et al., 1997); Chesapeake Bay

estuary is no different. Anthropogenic disturbance has resulted from activities that mobilize the

elements nitrogen and phosphorous through land clearing, application of fertilizer, discharge of

human waste, animal production, and combustion of fossil fuels, leading to eutrophication of the

Bay (Cloern, 2001). Hypoxia, dissolved oxygen (DO) concentrations ≤ 2 mg O_2 l^{-1} (Tyson and

Pearson, 1991), is closely associated with eutrophication, an increase in the supply and

accumulation of organic matter to a system (Nixon, 1995), typically arising from altered coastal

nutrient budgets (Diaz, 2001). Low DO concentrations have been documented in mainstem

Chesapeake Bay since the early 1930s (Newcombe et al., 1939) and in the Potomac in the 1910s

(Sale and Skinner, 1917). Presently, seasonal hypoxia forms in the late spring and lasts

approximately 120 days, with the most severe low DO events occurring in mainstem Chesapeake

Bay (Officer et al., 1984). From the 1950s to the present, hypoxic volume has increased

substantially in Chesapeake Bay, from approximately 3 km^3 to 10 km^3 (Hagy et al., 2004). This

increase is of concern given documentation of low DO impairing growth and reproduction and

stressing living resources, increasing faunal susceptibility to disease and other environmental

stresses (Jørgensen 1980; Rosenberg and Loo 1988; Llansó, 1992; Dauer et al., 1992; Diaz et al.,

1992; Tallqvist, 2001). Hypoxic water in the mainstem of the bay may be advected into adjacent

shallow areas, such as Bay tributaries, through horizontal transport (Breitburg, 1990). In the

Rappahannock River, our area of interest, a combination of tidal mixing and proximity to

mainstem hypoxic waters controls the seasonal hypoxia, which lasts throughout most of the

summer (Kuo and Neilson, 1987; Kuo et al., 1991).

The benthic community structure of coastal systems exhibits a series of predictable and graded

responses to hypoxia (Diaz and Rosenberg, 1995). Upon initial decreases in DO concentration,

respiration increases (Petersen and Petersen, 1988) and mobile fauna migrate from the affected

area (Pihl et al., 1991). Fauna incapable of large-scale mobility cease feeding and activities not

related to respiration, in an attempt to depress their metabolism (Warren, 1984). As DO

concentrations continue to decline and reduced compounds accumulate in the sediment, fauna

migrate to the sediment surface, with some extending respiratory appendages above the

sediment-water interface (Jørgensen, 1980; Tyson and Pearson, 1991). With long-lasting and

particularly severe hypoxia, mass mortality will occur in all but the most tolerant of species

(Llansó, 1992; Diaz and Rosenberg, 1995).

As coastal hypoxia continues to increase in the Bay and coastal systems worldwide (Diaz and

Rosenberg, 2008), the development of accurate predictive models that quantify the impact of

hypoxia on macrobenthos are valuable. In this study, we developed a continuous-time biomass-

based model, based on the benthic sub-model in the 2002 Chesapeake Bay Eutrophication Model

(Cerco and Noel, 2004), to model the effect of hypoxia on macrobenthic production in the lower

Rappahannock River. The specific objectives of this study were to 1) utilize independent data to

develop a macrobenthic functional response to DO concentration in the lower Rappahannock

River and to 2) run simulations of varying hypoxic duration and severity to assess and predict

macrobenthic response.

2. METHODS

2.1 Study Area

Estuaries are dynamic transitional-zones where a confluence of inland freshwater is diluted by

salt water from the sea (Schubel and Kennedy, 1984). The tidal Rappahannock begins at the fall

line in Fredericksburg, VA, a distance of approximately 130 km from its mouth. The 1.0 psu

isohaline is normally 75-90 km upriver. The mean tidal range and surface salinities at the mouth

are 0.4 m and 12-18 psu respectively (Haas, 1977). In the Rappahannock River, a combination

of tidal mixing and to a lesser extent, proximity to main stem hypoxic waters, control its seasonal

hypoxia which develops in late May and abates in early September (Kuo et al., 1991). The Rappahannock is also the only major tributary of the lower Chesapeake Bay with the hydrography that allows for sustained seasonal hypoxia (Kuo and Neilson, 1987), making it an ideal location for which to develop our model.

2.2 Field Collection

Macrobenthic data from a previous study were used to calibrate and verify our benthic model (Sturdivant unpublished). From May to October during the summers of 2007 and 2008 two random sites were chosen each year in the lower Rappahannock for continuous monitoring. Each year a normoxic site and a site known to experience seasonal hypoxia were chosen, based on data from the Chesapeake Bay Long-Term Benthic Monitoring Program (www.baybenthos.versar.com). For our model construction, we only used data from all four sites. At each monitored location, a single tripod attached with a Hach DS 5X Hydrolab datasonde was deployed; the datasonde was approximately 0.5 m above the sediment surface. DO concentrations were recorded in 20-min increments for two-week periods. At the end of two weeks, the datasonde was replaced with another Hydrolab datasonde, and a sediment sample was collected with a Young grab (440 cm^2 to a depth of 10 cm) for benthic community analysis. Sediment grabs were sieved in the field through a 0.5 mm screen, and organisms and detritus retained on the screen were transferred into labeled jars, preserved in a 10% formaldehyde solution and stained with Rose Bengal. Samples were processed to identify and enumerate each species present as described in Dauer and Llansó (2003). Ash-free dry weight (AFDW) biomass was measured for each species by drying to a constant weight at 60°C and ashing in a muffle furnace at 500°C for four hours.

2.3 Model Construction

A continuous-time, biomass-based model was constructed using STELLA Modeling and

Simulation Software®. The model was based on the benthic sub-model in the 2002 Chesapeake

Bay Eutrophication Model (Cerco and Noel, 2004), and contained three governing equations.

Phytoplankton biomass was modeled as:

1. $\frac{\delta}{\delta t} P = [(G - R - Wa) * P - PR]$

where:

P = phytoplankton biomass (g C m^{-3})
G = growth rate of phytoplankton (d^{-1})
R = respiration rate of phytoplankton (d^{-1})
Wa = phytoplankton settling velocity (m d^{-1})
PR = predation on phytoplankton (g C m^{-3} d^{-1})

Zooplankton were modeled as the combined biomass of micro- and mesozooplankton for
simplicity as:

2. $\frac{\delta}{\delta t} M = (Gz - BMz - Mz) * M - PRz$

where:

M = zooplankton biomass (g C m^{-3})
Gz = growth rate of zooplankton (d^{-1})
BMz = basal metabolic rate of zooplankton (d^{-1})
Mz = mortality (d^{-1})
PRz = predation on zooplankton (g C m^{-3} d^{-1})

Macrobenthos were modeled as the combined biomass of deposit and suspension feeders, as:

3. $\frac{\delta}{\delta t} B = \left[\alpha * \left(\frac{I_0}{m_2 * 10^9} \right) * (POC + PM) * k_{mn1} * B \right] + Ks - [(r - \beta - m) * B]$

where:

B = macrobenthic biomass (g C m^{-2})
α = assimilation efficiency for carbon
I_0 = ingestion rate of macrobenthos (g C biomass^{-1} d^{-1})
m_2 = sediment solids concentration (kg L^{-1})

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POC = sediment particulate organic carbon concentration (g C m^{-3})
k_{mnl} = Michaelis-Menton growth limitation term for carbon
Ks = recruitment rate of macrobenthos (g C m^{-2})
r = respiration rate of macrobenthos (d^{-1})
β = predation rate (m^2 g C d^{-1})
m = hypoxia mortality rate (d^{-1})
PM = phytoplankton and zooplankton biomass

Phytoplankton and zooplankton groups were included in the model given the tight benthic-pelagic coupling that exist in estuarine and shallow coastal systems (Haven and Morales-Alamo, 1972; Pryor, 1975; Frithsen and Doering, 1986), and importance of both groups as a source of food for macrobenthos (Garber, 1987). Our model excluded the state equation for suspension feeders in the Chesapeake Bay Eutrophication Model, and it combined macrobenthic suspension and deposit feeders into a single state equation. The benthic suspension feeder equation was not included in our model because its construction was based on large bivalve suspension feeders (Cerco and Noel, 2004) that are generally rare in the lower bay, causing the model to over-predict suspension feeder biomass (Schaffner et al., 2002). Further, no oysters or mussels and only a few small clams (primarily *Macoma* spp.) were collected in the field samples used to substantiate the benthic model. Suspension feeders are abundant in the lower bay, but the primary contributor to their biomass is the polychaete *Chaetopterus variopedatus* and a variety of epifaunal species such as tunicates and hydroids (Schaffner et al., 2002). We did not collect any *Chaetopterus* in our Rappahannock samples and the tunicates and hydroids were excluded from our model; these organisms are not macrofauna, and are not adequately sampled with the gear used causing their representation in the data to be overdispersed. Additionally, many of the samples collected in the summer at the hypoxic site were dominated by macrobenthos that both suspension and deposit feed, such as *Leptocherius plumulosus*, *Streblospio benedicti*, *Paraprionospio pinnata*, and *Macoma* spp (Diaz and Schaffner, 1990). Based on the community composition of macrobenthos collected during the summer of 2008, a single governing equation

for macrobenthos was sufficient to model macrobenthic change relative to DO concentration in the lower Rappahannock and maintained our goal of keeping the model as simple as functionally possible.

Water quality data were obtained from daily averages collected by the Chesapeake Bay Water Quality Monitoring Program from 1985 to 2001, with the exception of photosynthetically active radiation (PAR) and temperature. Daily PAR and temperature were forced functions using equations derived by Wetzel and Neckles (1986) for lower Chesapeake Bay.

2.4 Adaptations to Original Model

Some specific changes were made to the original governing equations of the Chesapeake Bay Eutrophication Model. The formulation that represented the response of zooplankton mortality to hypoxia (*Mz*) was altered; in our model, if DO concentration was less than 2 mg DO l^{-1} then:

4. $$Mz = MZEROz * (1 - \frac{DOREF}{DOCRITz})$$

in which:

Mz = mortality of zooplankton group *Z* (d^{-1})
MZEROz = mortality at zero dissolved oxygen concentration (d^{-1})
DOREF = dissolved oxygen concentration when DO < $DOCRIT_z$, otherwise 2 (mg DO l^{-1})
DOCRITz = threshold below which dissolved-oxygen-induced mortality occurs, this value equals 2 (mg DO l^{-1})

In the original equation *DOCRITz* was always 2 mg O_2 l^{-1}, and *DOREF* was the dissolved oxygen concentration when DO < *DOCRITz*, otherwise it was zero. However, this resulted in a linear increase in modeled zooplankton population; therefore, the formulation was amended to equation 8, where the *DOREF* was the dissolved oxygen concentration when DO < *DOCRITz*, otherwise it was 2 mg DO l^{-1}.

The parent Chesapeake Bay Eutrophication model simulates three fractions of sediment organic carbon, a labile, semi-labile, and refractory pool. In the original version of the macrobenthic model, the following portion of Eq. 3, $\left[\alpha * \left(\frac{I_0}{m_2 * 10^9} \right) * POC * k_{mn1} * B \right]$, was computed twice, once for the labile and once for the semi-labile carbon pool. Since our model was not coupled to a larger eutrophication model, in the interest of maintaining simplicity we computed this term in Eq. 3 once using total sediment POC from field measurements. Additionally, the predation rate (β) was originally multiplied by the square of macrobenthic biomass (B^2). However, this formulation caused too great a loss to overall macrobenthic biomass and was replaced with a linear function of B. Finally, during simulations where DO concentrations were less than 2 mg O_2 l^{-1}, the macrobenthic compartment would hit zero due to the hypoxia mortality rate (m):

5. $m = r_d * (1 - Z')$

where:

r_d = intrinsic mortality rate (d^{-1})
Z' = impact of DO concentration on B (mg l^{-1})

The original Eutrophication model had no term to jump-start B once zero was reached, causing B to remain at zero even after hypoxia abated. Therefore, a recruitment parameter (Ks) was created which added a minute amount of B (0.00015 g C m^{-2} d^{-1}) back to the model at each time step. This value was determined by incrementally decreasing the amount of Ks until a value was achieved that did not alter the temporal trend of modeled macrobenthos biomass during normoxia. This was supported by sensitivity analysis results reported later.

2.5 Rappahannock Function Relating Biomass to Hypoxia

In the original Eutrophication model, the impact of DO concentration on macrobenthic respiration (r), ingestion (I_0), and B was represented by the logistic equation Z, where:

$$6. \quad Z = \frac{1}{1 + e^{1.1*(\frac{DO_{gx}-DO}{DO_{gx}-DO_{qx}})}}$$

where:

DO_{gx} = DO at which macrobenthic function is 50% of maximum
DO_{qx} = DO at which macrobenthic function is 25% of maximum

The logistic equation that represents Z was not supported by any data, and our analysis of Chesapeake Bay field data and results from Seitz et al. (2009) indicate a different relationship between DO and macrobenthic biomass than the one represented by the logistic equation Z. Therefore, a sigmoidal function was derived from macrobenthic data collected from the Rappahannock River during the summers of 2007 and 2008 (Figure 1), and a parameter (Z') was created to represent the relationship. The equation Z' was derived from the sigmoidal curve in Figure1 , and used to model the impact of DO concentration on B where:

$$7. \quad Z' = \frac{0.16}{1 + e^{(-\frac{x-3.35}{0.15})}}$$

In our model, Z is still used to model the impact of DO concentration on r and I_0, but Z' is used to more accurately model the impact of DO on B, replacing Z in Eq. 5. Equation 7 was normalized (0 to 1, dimensionless) by replacing the numerator with 1, such that Equation 8 was the equation applied to our model:

$$8. \quad Z' = \frac{1}{1 + e^{(-\frac{DO-3.35}{0.15})}}$$

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where:

DO = dissolved oxygen concentration (mg l⁻¹)

2.6 Model Verification and Simulation Analysis

A single model run encompassed a period of 365 days with a time step of one calculation per day. The three governing equations were verified using Chesapeake Bay Benthic and Water Quality monitoring program data from 1992; this year was chosen at random from years 1985-2001. Stations LE 3.4 and 3.6 in the lower Rappahannock River were compared to model output using a paired t-test. Comparisons found not to be significantly different were generally considered valid. Sensitivity analyses were conducted for the phytoplankton, zooplankton, and macrobenthic state variables by adjusting selected parameters that directly impacted growth or loss (i.e. consumption or predation parameters). Maximum photosynthetic rate ($P^b m$), phytoplankton settling velocity (*Wa*), and predation rate on algae (*Phtl*) were tested for the phytoplankton state variable, predator biomass and clearance rate (*PHTlz*) for the zooplankton state variable, and assimilation efficiency for carbon (α), ingestion limitation (K_I) and recruitment rate (*Ks*) for the macrobenthic state variable. All parameters tested in sensitivity analysis were adjusted at an increment of ±20% and the relative percent difference from the standard run was calculated for each. Parameters with percentage errors greater than 10% were deemed to be sensitive parameters.

A set of simulations analyses were conducted, adjusting DO concentration to model the affect of the severity and duration of hypoxia on the 3 modeled state variables (Table 1). To avoid shock affects in model, the DO concentrations was gradually adjusted to desired DO levels over a period of 3 days before the designated day of hypoxia beginning or ending. The first four

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simulations focused on the sustained duration of hypoxia with the 4th simulation including

intermittent hypoxia; hypoxia occurred every 14 days simulating the development of hypoxia

during neap tidal cycles. Model output verified when DO concentration was normoxic (i.e the

base model results during normoxia after model calibration, verification and sensitivity analyses)

was used as a baseline for comparison between the first four model simulations. A paired t-test

was used to assess differences between the verified model output and each of the 4 hypoxic

simulations for each of the three state variables. Simulations 5-9 modeled the severity of

hypoxia from 0-2 mg O_2 l^{-1}, for a duration of 60 days. Analysis of variance was used to test for

differences between quantitative simulations 5-9 between 1-365 days and 177-238 days.

Normality was checked with the Shapiro-Wilk test and homogeneity of variance with Bartlett's

test (Zar, 1999). Tukey's HSD test was used for multiple mean comparisons. All statistical tests

were conducted using Mini-tab Statistical Software ®, with significant differences at an α-level

of 0.05.

3. RESULTS

Modeled phytoplankton (*P*) and macrobenthic (*B*) biomass were found not to be significantly

different from biomass data collected in the lower Rappahannock River at site LE 3.6 and 3.4,

respectively (Figures 2 and 3). The lack of significant difference provides confidence in the

accuracy of the phytoplankton and macrobenthic state equations. Modeled zooplankton biomass

(*M*) was compared to data collected in the lower Rappahannock River at site LE 3.6 and found to

be significantly different (Figure 4). While zooplankton biomass was significantly different, this

can be attributed to the combination of multiple zooplankton groups (micro- and

mesozooplankton) into a single state variable and calibration difficulties well-documented for zooplankton in the Eutrophication model (Cerco and Cole, 1993). However, the annual pattern of modeled zooplankton biomass was appropriate, and the magnitude accurate, providing us with confidence to use the state variable in our model simulations. Sensitivity analyses were conducted on model constants for each state variable (Table 2). The model was sensitive to a majority of tested parameters, with the phytoplankton state variable sensitive to tested parameters. The zooplankton state variable was found to be insensitive to an increase in predator biomass and clearance rate but sensitive to a decrease. The macrobenthos state variable was sensitive to assimilation efficiency for carbon and insensitive to recruitment rate and ingestion limitation.

Simulations were run assessing the impact of hypoxic duration on phytoplankton, zooplankton, and macrobenthos biomass. Macrobenthos biomass began to decrease as Simulation 1 approached hypoxia. At the start of hypoxia in Simulation 1 the steady decrease acceleratd to an immediate crash of macrobenthos biomass that lasted the duration of the hypoxic event, with a temporal trend in macrobenthos biomass significantly different from one modeled under normoxic conditions (Figure 5A). Macrobenthos biomass began to respond and increase before DO concentrations in the model became normoxic. A few days after hypoxia ended in the model, macrobenthos biomass had increased to above pre-hypoxia biomass. Similar trends were observed in simulations 2 and 3 involving hypoxic durations of 60 and 30 days (Figure 5B and C), with the main difference being the length of the crash of macrobenthos biomass. Simulations with shorter durations of hypoxia resulted in less time with macrobenthos biomass near 0 g C m^{-2}. In the intermittent hypoxia simulation macrobenthos biomass decreased at the onset of

hypoxia and remained near zero for the duration of hypoxia (Figure 6). During the 14-day

intervals when DO concentration was normoxic, macrobenthos biomass began to increase,

however once DO concentration dropped down to hypoxic levels, macrobenthos biomass

decreased back near zero. Macrobenthos biomass fluctuated through this pattern throughout the

entire hypoxic simulation. A few days after hypoxia abated permanently, macrobenthos biomass

began to increase to biomass levels greater than those observed pre-hypoxia.

Modeled phyto- and zooplankton biomass responded oppositely to hypoxic simulations (Figure

7). As DO concentrations began to decline, phytoplankton biomass initially decreased, however,

with the onset of modeled hypoxia, phytoplankton biomass increased. The length of increased

phytoplankton biomass was dependent on the duration of hypoxia, with a longer duration of

hypoxia resulting in higher overall phytoplankton biomass, and to some extent an even greater

magnitude of phytoplankton biomass. Hypoxia had the reverse affect on zooplankton biomass.

As DO concentration decreased to hypoxic levels, zooplankton biomass initially increased and

then declined to near 0 g C m^{-3}. The length of time that zooplankton biomass stayed near 0 g C

m^{-3} was dependent on the duration of hypoxia; lengthy durations of hypoxia coincided with

longer durations of reduced zooplankton biomass. Zooplankton biomass did not initially respond

to an increase in DO concentration, until days to weeks after hypoxia ended in the model.

The effect of hypoxic severity on macrobenthos biomass was tested by adjusting DO

concentration between 2.0 and 0.0 mg O_2 l^{-1} in increments of 0.5 mg O_2 l^{-1} for a series of 5

simulations (Table 3). Macrobenthos biomass was not significantly different between

simulations when compared over 365 days. Simulations 5-9 had similar macrobenthos biomass

during normoxia, as no parameters were changed; macrobenthos biomass did not differ between

simulations until DO concentrations became hypoxic. Therefore, simulations 5-9 were analyzed

starting at the onset of hypoxia on day 177 through the end of hypoxia on day 238. Simulation 5

had significantly higher macrobenthos biomass than simulations 6-9. Mean biomass in

simulation 5, which depicted 60 days of hypoxia at 2.0 mg O_2 l^{-1}, was greater than 3 times higher

than simulations 7-9, which modeled hypoxia at DO concentrations of 0.0-1.0 mg O_2 l^{-1}, and 1.5

times higher than simulation 6. In simulation 6 when the DO was 1.5 mg O_2 l^{-1} macrobenthos

biomass was significantly lower than simulation 5 and greater than 2 times higher than

simulations 7-9. Simulations 7-9 did not significantly differ in biomass.

4. DISCUSSION

The sigmoid relationship (Z') applied to this ecosystem model reflects changes in macrobenthos

biomass (B) over varying hypoxic scenarios. The duration and severity of hypoxia has been

previously shown to impact benthic community assemblages (Vaquer-Sunyer and Duarte, 2008).

In our model, hypoxic duration resulted in prolonged reductions of macrobenthos biomass

relative to the length of hypoxia, with the model suggesting near defaunation (macrobenthos

biomass equal to 0 g C m^{-2}) during the 120, 60, and 30 day hypoxic scenarios (simulations 1, 2,

and 3) at a DO concentration of 0.5 mg O_2 l^{-1}). Scenarios of prolonged hypoxia have been

observed previously in Chesapeake Bay and elsewhere. The deep trough of the mainstem Bay

experiences sustained seasonal hypoxia year after year (Officer et al., 1984), and has been

documented by the Chesapeake Bay Program to be devoid of macrofauna during the summer

months. Further, over extended periods of hypoxic exposure (~40 days) even the most tolerant

of species experienced total mortality (Rosenberg et al., 1991). After DO levels in our model

returned to normoxia, macrobenthos biomass recovered to greater than pre-hypoxic levels. This was unexpected as conditions in Chesapeake Bay that fuel ecological production in the pre-hypoxic spring differ in the post-hypoxic fall (Kemp et al., 2005). Increased nutrient run-off from the spring freshet promotes plankton production; the particulate organic matter from these blooms eventually settles to the bottom promoting benthic growth (Rabalais, 2004). Large plankton blooms seen in the spring are noticeably absent in the fall, and with less primary production one would expect the rate of recovery of macrobenthos biomass to be less in the fall than in the spring. However, data from the continuously monitored sites used to derived Z' indicate macrobenthic production can increase back to pre-hypoxic levels a few weeks post hypoxia, suggesting the macrobenthos biomass increases we observed post-hypoxia may not be inaccurate.

Modeled intermittent hypoxia, simulation 4, depicted hypoxia occurring during neap tides and abating during spring tides. The model output showed macrobenthos biomass being reduced during hypoxia but recovering during normoxia, and cycling in this manner throughout the intermittent series of hypoxic events. Given the severity at which DO concentration was set (0.5 mg O_2 l^{-1}), it is not surprising that macrobenthos biomass decreased to the level that it did. As with previous simulations, the recruitment rate of macrobenthos biomass was very rapid when normoxia did return. Macrobenthos biomass also began recovering ~2-3 days before hypoxia abated, which represents the time frame that DO was increasing from 0.5 to 2.0, indicating a sensitivity to hypoxic severity. Once macrobenthos biomass got to a level where it appeared sustainable, hypoxia returned and macrobenthos biomass was reduced to near zero biomass. This process was then repeated throughout the intermittent hypoxic cycle.

Hypoxic severity had a significant impact on macrobenthos biomass with biomass signficiantly higher at less severy hypoxic simulations. Studies have shown the severity of hypoxia to affect the response of benthic communities; the more severe the hypoxia, the greater the impact on the benthos, directly and indirectly (Diaz and Rosenberg, 1995). Directly, benthic species vary in their tolerances to low DO concentration (Vaquer-Sunyer and Duarte, 2008) as the severity of hypoxia increases towards anoxia, sensitive species die off decreasing the diversity of the affected area and overall biomass. Indirectly, DO concentration can positively and negatively affect benthic predation. Nestlerode and Diaz (1998) showed that benthos may actually have a refuge from predation under mildly hypoxic conditions, and Brante and Hughes (2001) demonstrated that hypoxia reduced the effort of *Carcinus maenas* predation on mussels. During mild hypoxia predators may not effectively prey upon benthos, and hypoxia tolerant benthos would survive and maintain their biomass. However, Seitz et al. (2003) and Long and Seitz (2008) showed that epibenthic predators and demersal fish can at times capitalize on stressed benthos during mild hypoxic events. As oxygen concentrations become lethal, stressed infauna extend their appendages and bodies out of the sediment in an attempt to escape dire conditions below the sediment and sediment-water interface (Phil et al. 1992). Opportunistic mobile predators have been shown to re-enter hypoxic areas and prey on exposed macrofauna during mild hypoxia (Phil et al. 1991). During model simulations, there was no upswing in macrobenthos biomass as DO concentrations declined towards hypoxia. This could indicate that our model does not accurately reflect any macrobenthic predation release due to lowering of DO, or direct hypoxic mortality (Z') has a much greater affect on macrobenthos biomass, dulling any affects of predation release; the latter is likely to be correct. In the macrobenthic state variable, β:

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9. $\quad \beta = \beta' \dfrac{DO}{DO+K_{DO}}$

in which:

β' = the predation rate before considering hypoxic effects
K_{DO} = predation DO half-saturation

accounts for the predation rate on macrobenthos and denotes predation rate as a function of

temperature and DO concentration. Since our predation parameter has factored in the impact of

DO concentration on predators of the macrobenthos, the results observed in the model output

indicate that Z' likely nullifies any affects on β.

Zooplankton biomass (M) was negatively impacted by hypoxia directly, causing zooplankton

biomass to be drastically reduced. Marcus et al. (2004) considered the effect of reduced DO

concentration on the survival and population dynamics of zooplankton, demonstrating the

deleterious affect hypoxia has on zooplankton population and community dynamics. As a result

phytoplankton biomass (P) in our model was indirectly positively influenced by the onset of

hypoxia, due to the release of phytoplankton biomass from grazing pressure by zooplankton.

5. CONCLUSION

Macrobenthic data from the lower Rappahannock River were used to derive Z', a sigmoidal

relationship, to model the effect of DO concentration on macrobenthic biomass (B). Z' was then

applied to an overall biomass-based ecosystem model of the lower Rappahannock and used to

assess the impact of hypoxia on B, while including the important interactions that occur through

benthic-pelagic coupling. Z' is a useful tool in that it can be applied to existing models to

accurately simulate the impact of hypoxia on the macrobenthos, and the methods used to derive

Z' can be applied to other systems to develop site specific Z'.

From our modeling efforts we found that the duration and severity of hypoxia negatively affected

macrobenthos biomass; longer durations and greater hypoxic severity resulted in less biomass.

The ecological and economic importance of macrobenthos to estuarine systems underlies the

significance in understanding processes that positively and negatively impact this group. An

improved understanding of the impact of hypoxia, and the ability to accurately model these

interactions, is a key advancement in benthic ecology.

ACKNOWLEDGEMENTS

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TABLE CAPTIONS

Table 1. Simulations run in the ecosystem model, with varying hypoxic duration and severity. Simulations 1-4 modeled hypoxic duration at a constant concentration of 0.5 mg O_2 l^{-1}; simulation 4 modeled intermittent hypoxia (hypoxia occurring every 14 days on a neap/spring tidal cycle). Simulations 5-9 modeled the affect of hypoxic severity at a constant duration of 60 days.

Table 2. Results of sensitivity analysis for phytoplankton, zooplankton, and macrobenthic state variables. Root mean square deviation (RMS) values over an annual cycle are shown for ± 20% variation for each state variable by parameter. The model was deemed to be sensitive when % difference exceeded 10%.

Table 3. Comparison of macrobenthos biomass to hypoxic severity over (A) a full year, and (B) a partial year, covering the time-frame of simulated hypoxia. Macrobenthic biomass was not significantly different between simulations over a full year (df=364, F=1.62, p=0.17), but significantly different during hypoxia (df=63, F=62.38, p<0.0005). Letter differences denote significance. Mean biomass is shown with ±1 SD in parentheses.

FIGURE CAPTIONS

Figure 1. Comparison of macrobenthic biomass and DO concentration. Data collected from two

sites in 2007 and two sites in 2008 that were monitored bi-weekly throughout the year in the

Rappahannock River. Trendline is a sigmoidal curve, where equation $Z' = \dfrac{1}{1+e^{\left(-\frac{x-3.35}{0.15}\right)}}$.

Figure 2. Verification of the phytoplankton state variable. The black line represents modeled

phytoplankton biomass, and the gray line phytoplankton biomass collected from site LE 3.6 by

the Chesapeake Bay Water Quality Monitoring Program in 1992. Modeled phytoplankton

biomass was not significantly different from observed phytoplankton biomass in 1992 (df=364,

t=-0.06, p=1.93).

Figure 3. Verification of the macrobenthos state variable. The black line represents modeled

macrobenthos biomass, and the gray line macrobenthic biomass collected from site LE 3.4 by the

Chesapeake Bay Benthic Monitoring Program in 1992. Modeled macrobenthic biomass was not

significantly different from observed macrobenthic biomass in 1992 (df=364, t=1.70, p=0.09).

Figure 4. Verification of the zooplankton state variable. The black line represents modeled

zooplankton biomass, and the gray line zooplankton biomass collected from site LE 3.6 by the

Chesapeake Bay Water Quality Monitoring Program in 1992. Modeled zooplankton biomass

was significantly different from observed zooplankton biomass in 1992 (df=364, t=2.17,

p=0.03).

Figure 5. Simulated macrobenthos biomass (*B*) under hypoxic durations of (A) 120, (B) 60, and (C) 30 days. Figure A, B, and C are simulations 1, 2, and 3, respectively, from Table 1. The black line in each graph represents hypoxic simulations, and the gray *B* validated during normoxia. The shaded area indicates the time frame hypoxia occurred during the simulation. *B* during normoxia was significantly different from *B* modeled under 120 (df=364, t=11.81, p<0.0005), 60 (df=364, t=-3.96, p<0.0005), and 30 (df=364, t=-8.23, p<0.0005) days of hypoxia.

Figure 6. Simulated macrobenthic biomass (*B*) under intermittent hypoxia; hypoxia occurred every 14 days simulating the development of hypoxia during neap tidal cycles and its abatement during spring tides; represents simulation 4 from Table 1. The shaded area indicates the time frame hypoxia occurred during the simulation. *B* during normoxia (gray line) was significantly different from *B* modeled during intermittent hypoxia (black line; df=364, t=-5.51, p<0.0005).

Figure 7. Simulated phytoplankton biomass under hypoxic durations of (A) 160, (B) 60, and (C) 30 day, and zooplankton biomass under hypoxic durations of (D) 160, (E) 60, and (F) 30 days. The black line in each graph represents modeled biomass under hypoxia, and the gray line during normoxia. The shaded area indicates the time frame hypoxia occurred during the simulation. The biomass of phytoplankton and zooplankton responded inversely to hypoxia during model simulations.

Table 1.

Simulations	Hypoxia Duration (d^{-1})	Julian Day	Ordinal Date	DO (mg O$_2$ l^{-1})
1	120	148-267	May 28 - Sept 24	0.5
2	60	177-238	Jun 26 - Aug 26	0.5
3	30	192-223	Jul 11 - Aug 11	0.5
4	14 d intervals	162-176, 190-204, 218-232, 246-260	Jun 11-25, Jul 9-23, Aug 6-20, Sept 3-17	0.5
5	60	177-238	Jun 26 - Aug 26	2.0
6	60	177-238	Jun 26 - Aug 26	1.5
7	60	177-238	Jun 26 - Aug 26	1.0
8	60	177-238	Jun 26 - Aug 26	0.5
9	60	177-238	Jun 26 - Aug 26	0.0

Table 2.

State variable	Parameter	-20%	+20%	Average RMS	% Diff -20%	% Diff +20%
Phytoplankton	$P^b m$	0.192	1.267	0.744	74.2*	41.2*
	Wa	0.962	0.383	0.744	22.6*	48.5*
	$Phtl$	1.432	0.237	0.744	48.0*	68.1*
Zooplankton	$PHTlz$	0.110	0.097	0.113	2.7	14.2*
Macrobenthos	α	0.084	0.160	0.121	30.6*	24.4*
	K_l	0.120	0.121	0.121	0.8	0.5
	Ks	0.120	0.122	0.121	0.2	1.0

*Denotes model sensitivity.

Table 3.

A)

Simulation	Hypoxia Duration (d^{-1})	DO (mg O_2 l^{-1})	Julian Day	Mean Biomass (g)	
5	60	2.0	1-365	0.139 (0.08)	A
6	60	1.5	1-365	0.132 (0.09)	A
7	60	1.0	1-365	0.125 (0.09)	A
8	60	0.5	1-365	0.125 (0.09)	A
9	60	0.0	1-365	0.125 (0.09)	A

B)

Simulation	Hypoxia Duration (d^{-1})	DO (mg O_2 l^{-1})	Julian Day	Mean Biomass (g)	
5	60	2.0	177-238	0.117 (0.02)	A
6	60	1.5	177-238	0.077 (0.03)	B
7	60	1.0	177-238	0.033 (0.05)	C
8	60	0.5	177-238	0.027 (0.05)	C
9	60	0.0	177-238	0.027 (0.05)	C
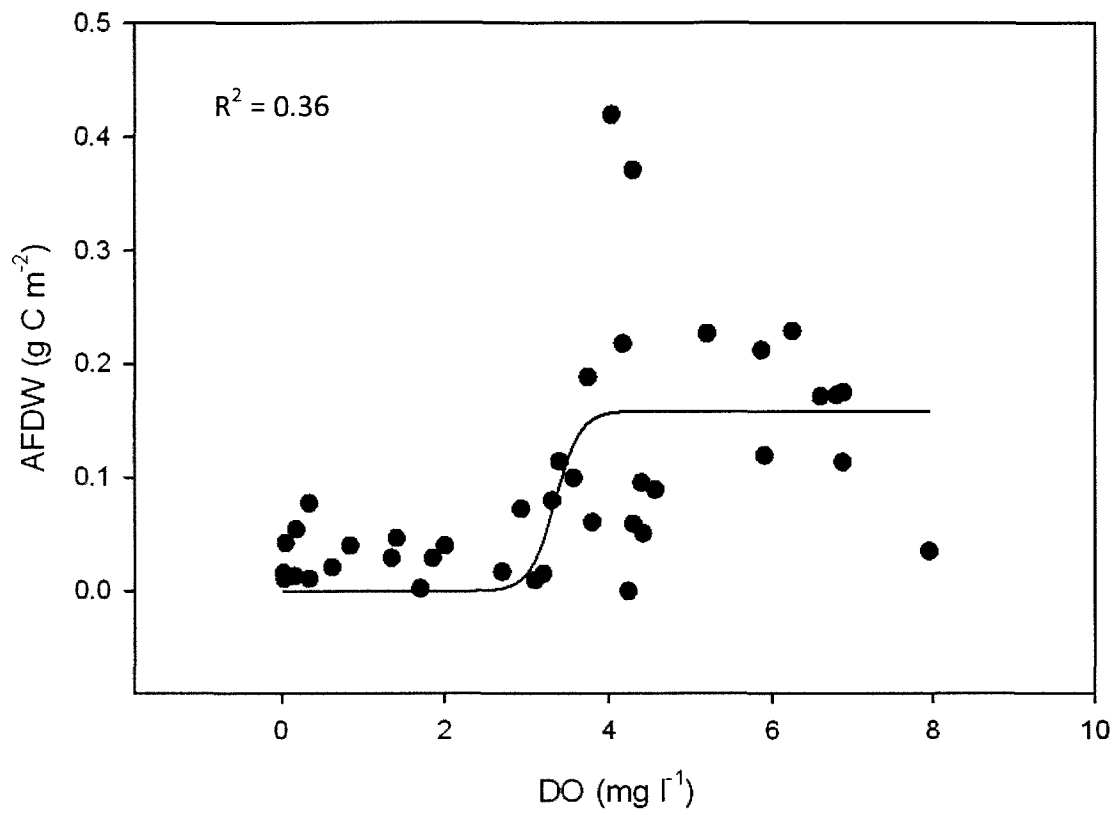
Figure 1.
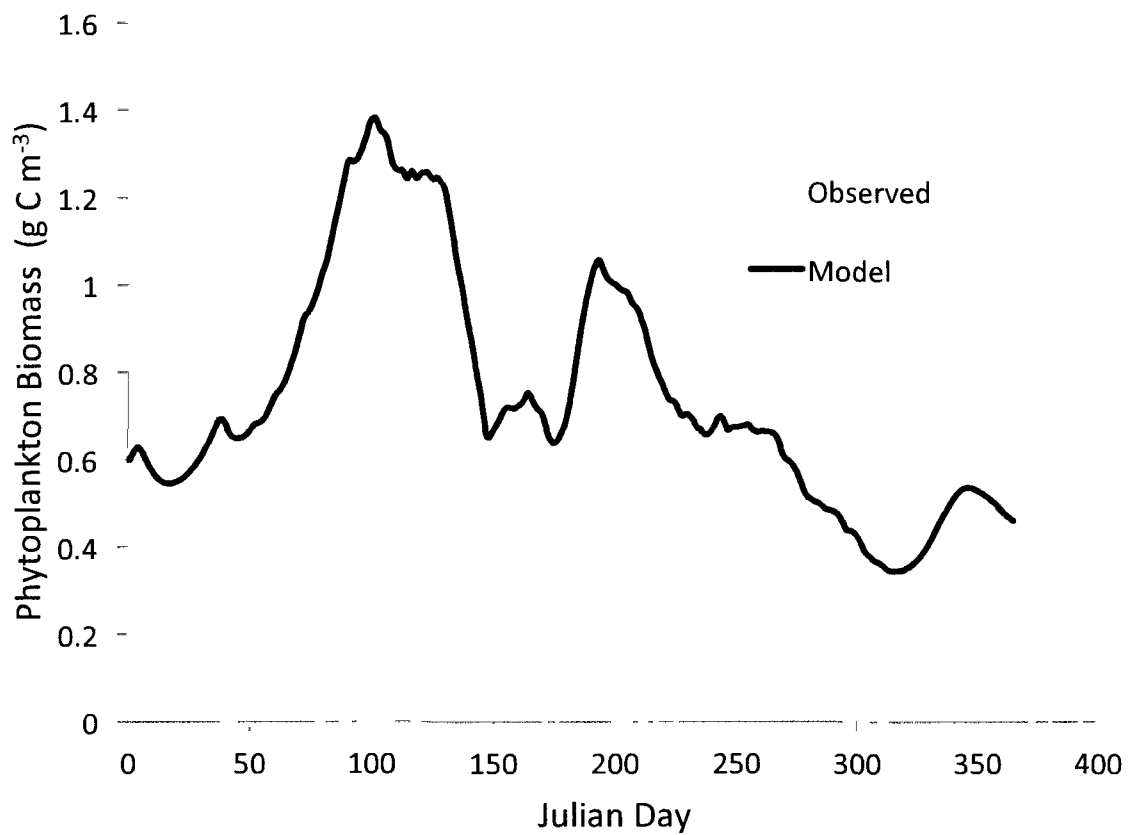
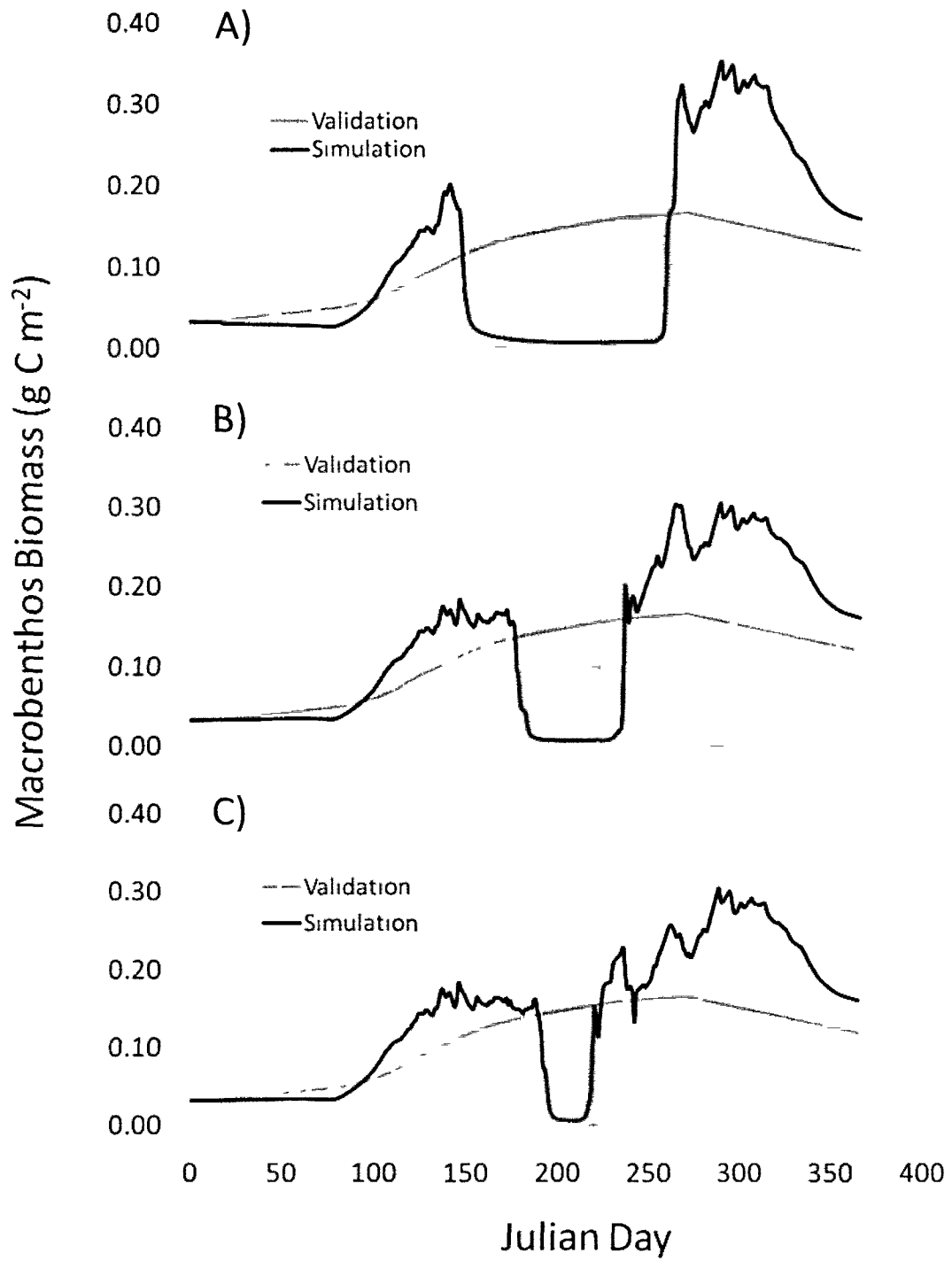**Figure 2.**
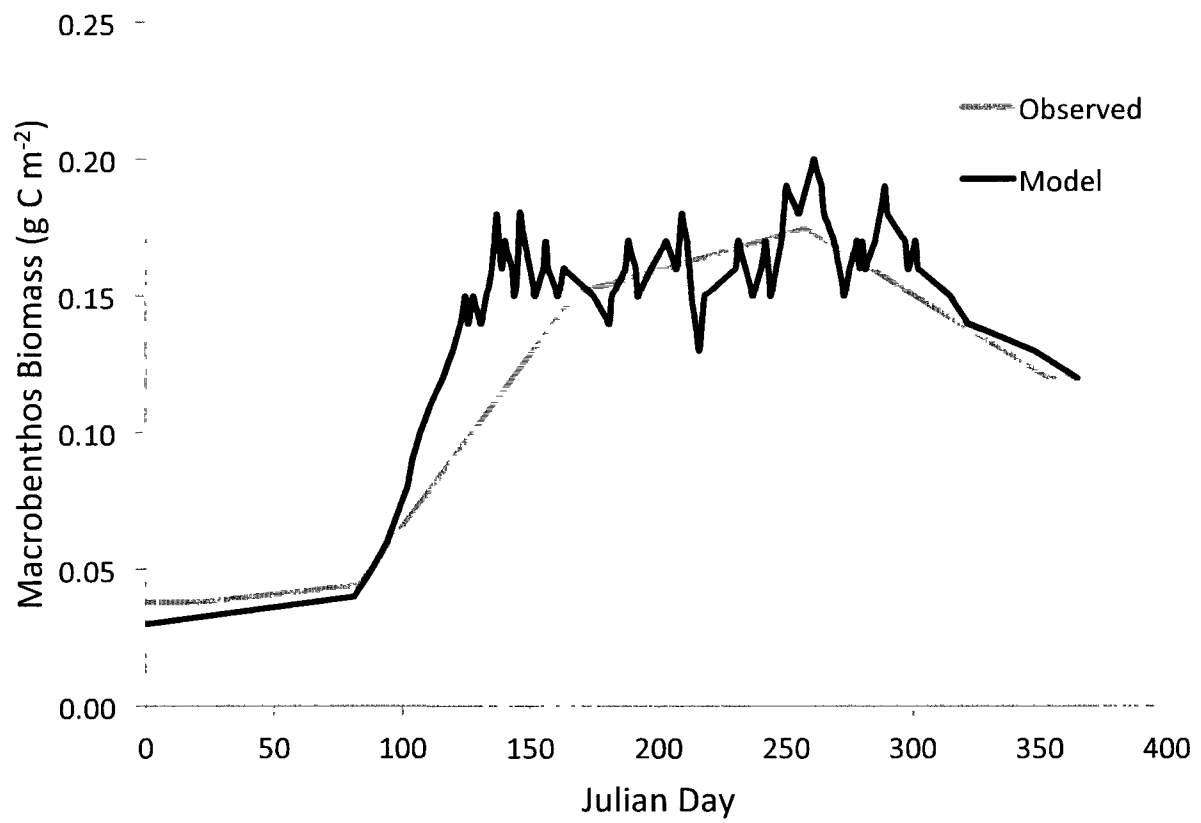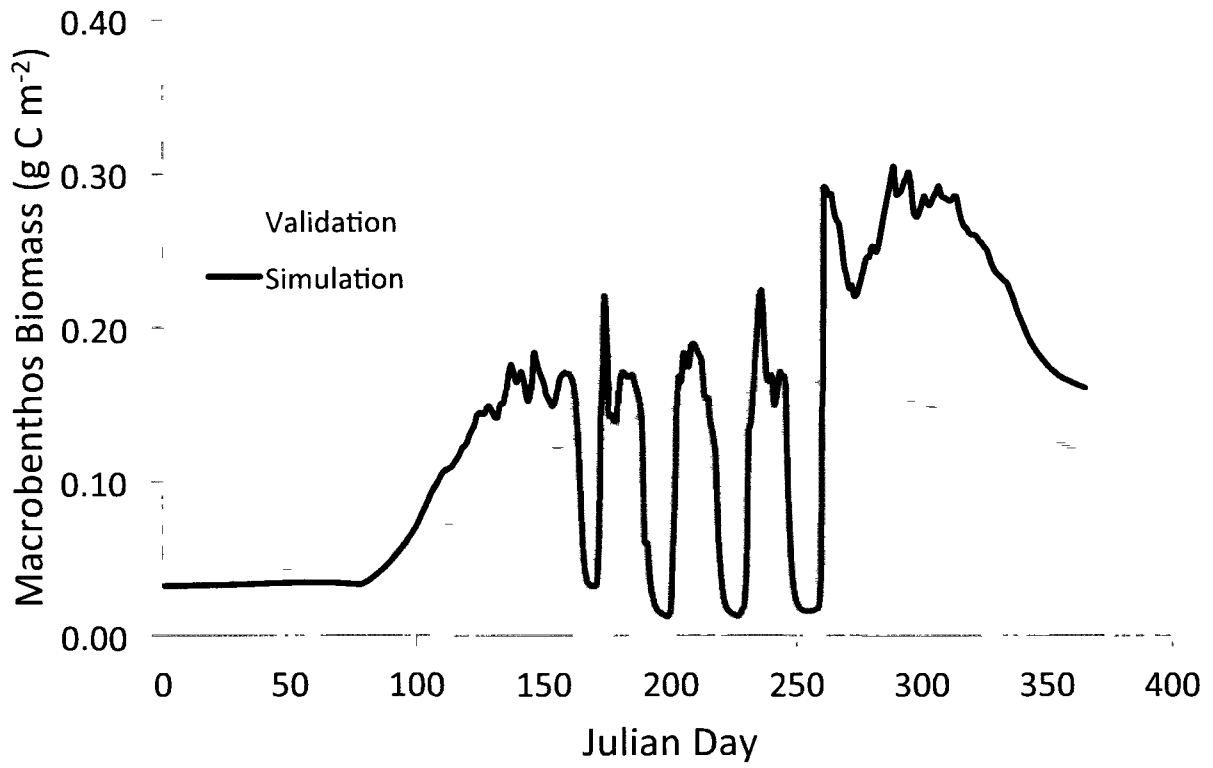
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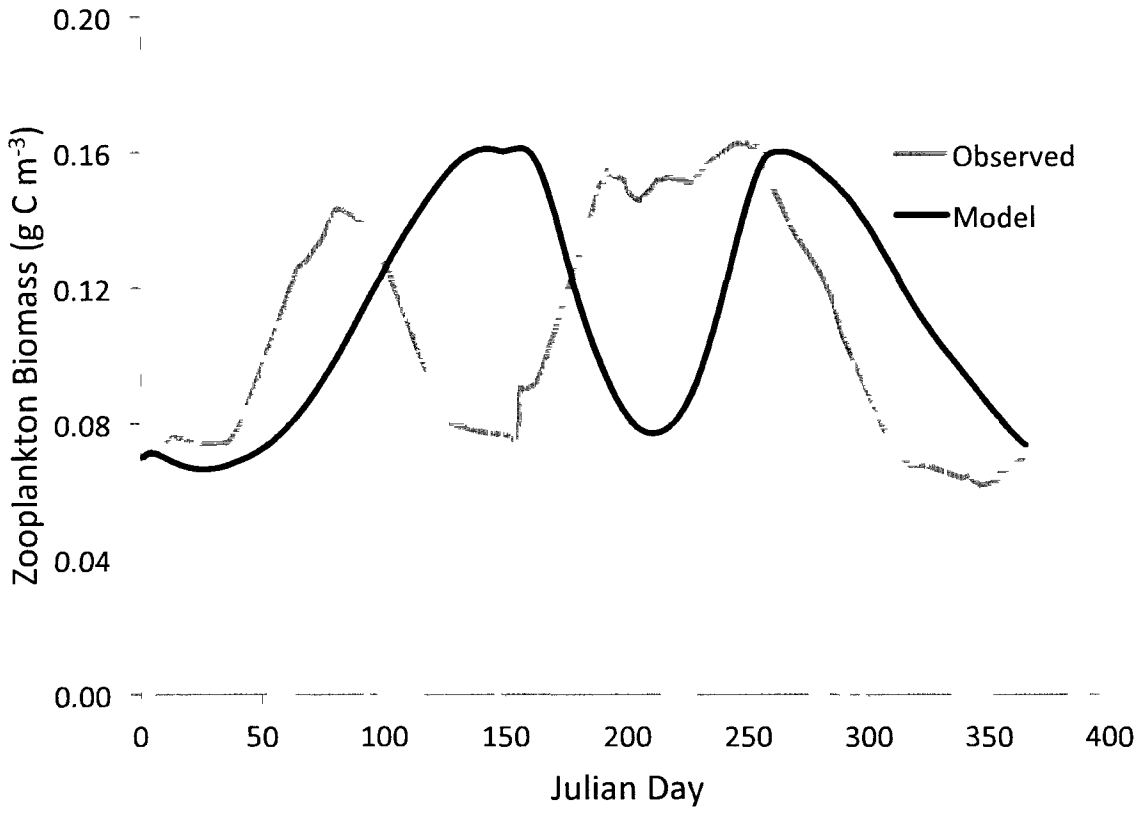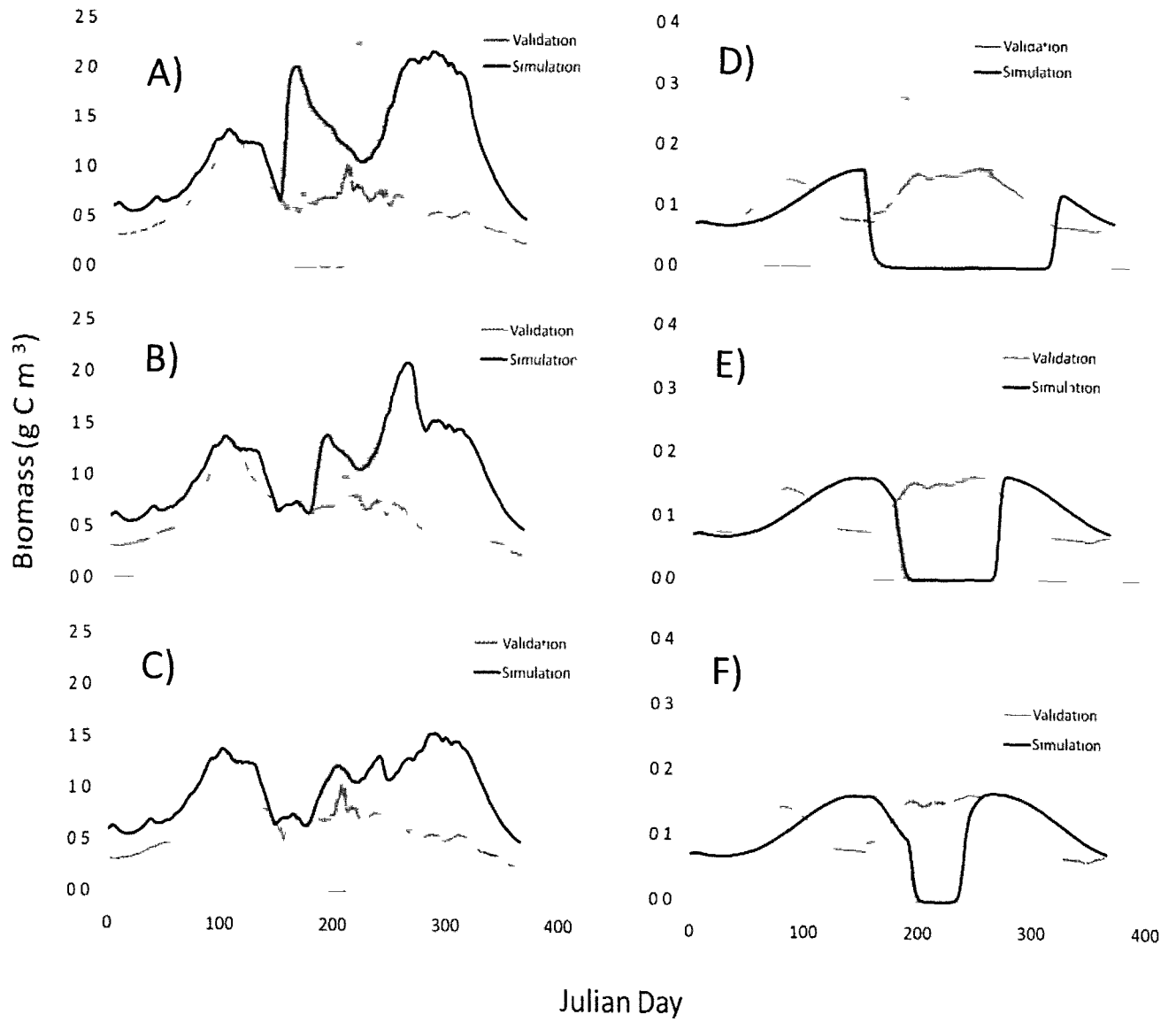
Figure 4.

Figure 5.

Figure 6.

Figure 7.

DISSERTATION CONCLUSION

We assessed the historical temporal trends between hypoxia and macrobenthic production in Chesapeake Bay, and in smaller temporal scales of season and weeks in the lower Rappahannock River. We also used Wormcam to assess the impact of hypoxia on the function of macrobenthos as bioturbators in the lower Rappahannock. Data collected was used to construct a continuous-time biomass-based model of the lower Rappahannock to model the relationship between hypoxia and macrobenthic biomass. Our findings suggest:

- There was a significant relationship between macrobenthic production and the physical parameters DO concentration, salinity, and grain size (represented as % silt/clay), with DO having the biggest impact on macrobenthic production.

- From 1996-2004, on average hypoxic sites a lower daily macrobenthic production by 90%; which amounts to a 6 to 12% loss in the total annual secondary production.

- The function of macrobenthic communities changed relative to DO concentration with hypoxia resistant spionids dominant during hypoxia and species with high sediment reworking rates dominant during normoxia.

- Macrobenthic production differed across seasons and there were indications that summer hypoxia impacted the recovery of macrobenthic production the following spring.

- Hypoxia reduces bioturbation through significant reductions in burrow lengths, burrow production, and burrow depth.

- Although some worms are active during hypoxia via plasticity or perhaps capitalizing on the environment enhanced with newly available organic material, the low concentrations of DO limited diffusion into the sediment.

- Macrobenthic data from the lower Rapphannock River was used to derive Z', a sigmoid relationship to predict the effect of DO concentration on macrobenthic biomass (B). Z' was then applied to an overall biomass-based ecosystem model of the lower Rappahannock and used to assess the impact of hypoxia on B, while including the important interactions that occur through benthic-pelagic coupling.

- Z' is a useful tool in that it can be applied to existing models to accurately simulate the impact of hypoxia on the macrobenthos, and the methods used to derive Z' can be applied to other systems to develop site specific Z'.

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