THE EFFECTS OF AN URBANIZED ESTUARY ON THE PHYSIOLOGY AND METAL STORAGE OF THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT:

Prior to the urbanization of the Hudson River Estuary (HRE), New York City was the center of a flourishing oyster industry. The Eastern oyster (*Crassostrea virginica*) was found in large reefs and was a significant contributor to the ecological and economic health of the region. However, due to overharvesting, pollution, and declining water quality, this ecosystem engineer has become 'functionally extinct' in the lower HRE. Recent efforts to restore the important bivalve have yielded mixed results, possibly due to the unique suite of contaminants seen in the HRE. Exposure to contaminants, particularly metals, can lead to alterations in the physiology of the oyster. Disruptions in mitochondrial energetics, changes in the relative percentages of energy stores (proteins, carbohydrates), and binding of metals to specific fractions within the cell can all lead to changes in the overall health of the oyster, and may prevent growth, reproduction, and survival. A field-based study examining the condition, biochemistry, and metal storage of juvenile *C. virginica* at a variety of sites within the urbanized HRE was conducted in the summer of 2010. Juvenile oysters were placed in the subtidal areas of four sites along a contamination gradient, and subsampled every two weeks to determine the changes in physiology and metal storage over time. Site-specific differences in condition index, biochemistry, and Cd accumulation were seen over time (Factorial ANOVA; $p < 0.05$). These results indicate that restoration of *C. virginica* to the urbanized HRE must take into account the differences in energy budgets and condition of juveniles. Future studies on adult physiology and biochemistry will complement this research.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

INTRODUCTION

Urbanization of estuaries along the Atlantic coast has lead to the destruction

of ecological niches and the loss of many important species. In the Hudson River

Estuary (HRE), sewage pollution (leading to lowered dissolved oxygen content in

the water column) and overharvesting of the population were most likely the primary

causes for the loss of the Eastern oyster, *Crassostrea virginica.* The input of various

contaminants (e.g., metals, pesticides, PCBs and other organics) and the destruction

of estuarine habitat (e.g., marsh loss, bulkheading of canals, dredging of the harbor)

have also contributed to the continued loss of this ecological engineer (Yozzo et al.

2004; Wakeman & Themelis 2001; Franz 1982)

Oysters are able to filter large amounts of water through their gills, removing

suspended particulates from the water column and depositing feces or pseudofeces

onto the benthos, thus acting as benthic-pelagic couplers within the ecosystem

(Newell 2004; Brumbaugh et al. 2000; Gerritsen et al. 1994). This action allows for

increasing amounts of light and oxygen to penetrate the water column and become

available to other organisms, affecting the entire estuarine community (Nelson et al.

2004; Coen & Lukenbach 2000). Dense populations of oysters, such as those that

historically occurred in the HRE, therefore have the ability to control phytoplankton

assemblages, nutrients, and suspended matter within an estuary (Nelson et al. 2004;

Coen & Lukenbach 2000; Gerritsen et al. 1994).

Alteration of the HRE over the past centuries has lead to an urbanized

environment, which makes it very different from the one present when oysters were

abundant (Yozzo et al. 2004; Wakeman & Themelis 2001; Franz 1982). Increased

pollution (including sewage run-off, organic, and inorganic pollutants), dredging of the harbor, removal of fringing marshes, and other impacts of urbanization have led to lowered dissolved oxygen levels, increased nutrients, and increased sediment loads within the HRE (Yozzo et al. 2004; Crawford et al. 1994). PCBs and PAHs have also been found at levels exceeding NOAA's ER-M (Effects Range Median) guidelines (Dimou et al. 2006; Feng et al. 1998). The HRE ranks second behind the San Francisco Bay Estuary in terms of metal contaminant levels within an estuary (Levinton & Waldman 2006). Cd, Cu, Hg, and Zn are found in elevated quantities throughout the HRE (Yozzo et al. 2004; Feng et al. 1998; Crawford et al. 1994). All of these contaminants have been shown to effect metabolism, causing mortality, reproductive failure, and decreased growth rates, as well as alterations in cellular responses (i.e., lysosomal destabilization) in oysters and other bivalves (Cherkasov et al. 2007; Ringwood et al. 1998; Roesijadi 1996).

Contaminants, such as metals (i.e., Cd, Cu, and Hg), may have profound effects on the physiology of oysters, and may have played a part in their demise in urbanized estuaries. Oysters are able to bioconcentrate metals up to several orders of magnitude higher than ambient concentrations before experiencing toxicity (O'Connor 2002; Mouneyrac et al. 1998). Accumulated metals can be separated within a variety of subcellular compartments, including heat denatured proteins (HDP), heat stable proteins (HSP), insoluble metal-rich granules (INS), organelles (ORG), and cell debris (CD) (Wallace et al. 2003), after which the metal can be sequestered, eliminated, or transferred along the food chain. At low concentrations, metals may be stored in subcellular compartments or eliminated with no ill-effects to

the oyster (Geffard et al. 2002). Metals may be bound to metallothionein proteins or granular hemocytes in large quantities, which presumably render the metal biologically inert (Amiard et al. 2006; Wallace et al. 2003; Roesijadi 1996).

The exposure to, and storage of, metals within oysters can have drastic impacts on energy stores (Pridmore et al. 1990). Increased exposure to non-essential metals (i.e., Cd) has negative effects on growth and reproduction of oysters (Volety 2008; Ringwood et al. 2004). The amount of energy reserves within the oyster, reproductive condition, and environmental parameters (e.g., food, temperature, salinity) can affect the amount of metallothioneins present within the oyster, and thus the amount of metal that can be successfully sequestered (Erk et al. 2008; Amiard et al. 2006). If excess metal is present in the environment, accumulations from the environment can outpace detoxification mechanisms, leading to toxicity and mortality (Amiard et al. 2006).

Understanding the relationship between environmental contaminants and oyster health is a critical aspect of restoration efforts within the HRE. Linking the effects of the environment (including contaminants) to changes in oyster physiology (i.e., energy stores, reproduction) will allow for a greater understanding of how oysters can be restored in urbanized estuaries. In many cases, a suite of contaminants as well as various environmental stresses are typically responsible for adverse effects on organisms and it is often difficult to discern which variables are the primary causes for stress. Oysters that sequester and eliminate toxins may deal with physiological trade-offs including lowered reproductive output, respiration and metabolic rates, and smaller size (Lanning et al. 2008; Hartwell et al. 1991).

Crassostrea gigas living in a polluted estuary with high Cu and Zn concentrations were found to have lower glycogen content and reduced overall condition than those living in a cleaner area (Pridmore et al. 1990). Additionally, the binding of metals onto different subcellular fractions may adversely impact oyster metabolism and energy budgets, leading to mortality. For example, cadmium (Cd) has been found to bind to mitochondria in oysters even at low concentrations, which can lead to disruption of the electron transport system and alterations of the oysters' metabolism (Cherkasov et al. 2009). Mitochondrial bioenergetics may be affected at low levels of Cd exposure, including those that are environmentally relevant within the HRE (Sokolova et al. 2005). If metals are able to preferentially bind with the HDP fraction (which contains enzymes), energy storage and reproduction may be impaired (Blanchard et al. 2009; Wallace et al. 2003). Considering the many different environmental variables (i.e., pollutants, dissolved oxygen, temperature and salinity) occurring within the HRE, a field study examining linkages between important environmental variables and metal toxins would allow for a more thorough comparison of metal uptake and biochemistry of transplanted oysters. Field studies would allow for the examination of multiple variables, at environmentally relevant concentrations.

The objectives of this study are 3-fold: (1) to determine the effects of a highly urbanized environment (the HRE) on the physiology of the Eastern oyster, *Crassostrea virginica*; (2) to determine the body burden of accumulated metals, and the internal storage of these metals to different subcellular fractions, incurred by *C. virginica* while living in an urbanized estuary; and (3) to determine the relationships

among physiology, as measured by overall condition and biochemistry (carbohydrates, proteins, and lipids), the storage of metals within the oyster tissue (both total body burdens, and in different metal fractionations), and the environmental variables present at each site. It is hypothesized that oysters placed at impacted sites will exhibit a lower overall condition, lower energy reserves, and higher total body burdens of metals (Cd, Cu, Hg) than oysters placed at a reference site. Furthermore, oysters placed at impacted sites are expected to accumulate metals in different subcellular compartments. Linkages between energy reserves and the partitioning of metals to different subcellular compartments should differ among oysters from contaminated sites and oysters from a clean reference site.

METHODS

Field Deployment of Oysters:

Oysters were placed at the field sites from July 12-15, 2010. Three chronically contaminated sites within the Hudson River Estuary were chosen based on (1) high levels of contaminants, (2) potential or current usage in oyster restoration projects within New York Harbor, and (3) probability that native oysters were historically found at the site (Waldman 1999; Kurlansky 1996; Franz 1982). The sites chosen were (1) Spring Creek, a small dead-end creek located at the Northern end of Jamaica Bay, Queens, NY (**JB**); (2) Floyd Bennett Field, along the Rockaway Inlet (between Jamaica Bay and New York Bay, Brooklyn, NY; **FBF**); and (3) Soundview Park, located at the confluence of the Bronx River and the East

V-9

River, Bronx, NY (**SVP**). A clean reference site, located outside the urbanized HRE, was also chosen at Rutgers Marine Field Station, Tuckerton, NJ (**TK**) (Figure 1). The three impacted sites have been shown to have elevated concentrations of pollutants, including essential and non-essential metals (Bopp et al. 2006; Wirgin et al. 2006; Feng et al. 1998; Adams et al. 1996; Seidemann 1991). Sediment samples from 2009 concur with previous data showing that the three impacted sites have higher burdens of important metals (Cd, Cu, Hg, and Zn) (Mass, unpubl. data). Tuckerton has been used as a local reference site in several other studies concerning metal uptake by invertebrates (Khoury et al. 2009).

Juvenile oysters (18-25mm) were obtained from a local hatchery (Aeros Cultured Oyster Co., Southold, NY) on July 13, 2010, and transported on ice to the various sites. At each location there were two polyethylene mesh bags (Aquatic Ecosystems, FL) of juvenile oysters, each with approximately 350 oysters, for a total of 700 oysters per site. At the three impacted sites, the bags were secured to a cinderblock and placed in the subtidal zone; at TK the bags were suspended off a dock in the subtidal area. A subsample of the oysters was taken prior to being placed in the field (July 12-14, 2010), and then on bi-weekly intervals until October 18-22, 2010 (7 sampling events). The bags were retrieved during a low tide, and a random subsample of 35 juvenile oysters was removed from each bag. All oysters were placed on ice, and immediately transported back to the laboratory. Of the oysters subsampled, 25 were used to determine overall condition and biochemistry (carbohydrates, lipids, and proteins), 5 were used to estimate ICP metal burdens and subcellular fractionation (Cd, Cu), and another 5 to estimate total Hg. Additionally,

salinity, temperature, and dissolved oxygen readings were taken in the field with a

hand-held YSI Pro-20 water quality probe during each field sampling. Water

samples were collected, then filtered back in the laboratory for total suspended

particulates, and total and size-fractionated (5-28 µm, <5 µm) chlorophyll *a* analysis.
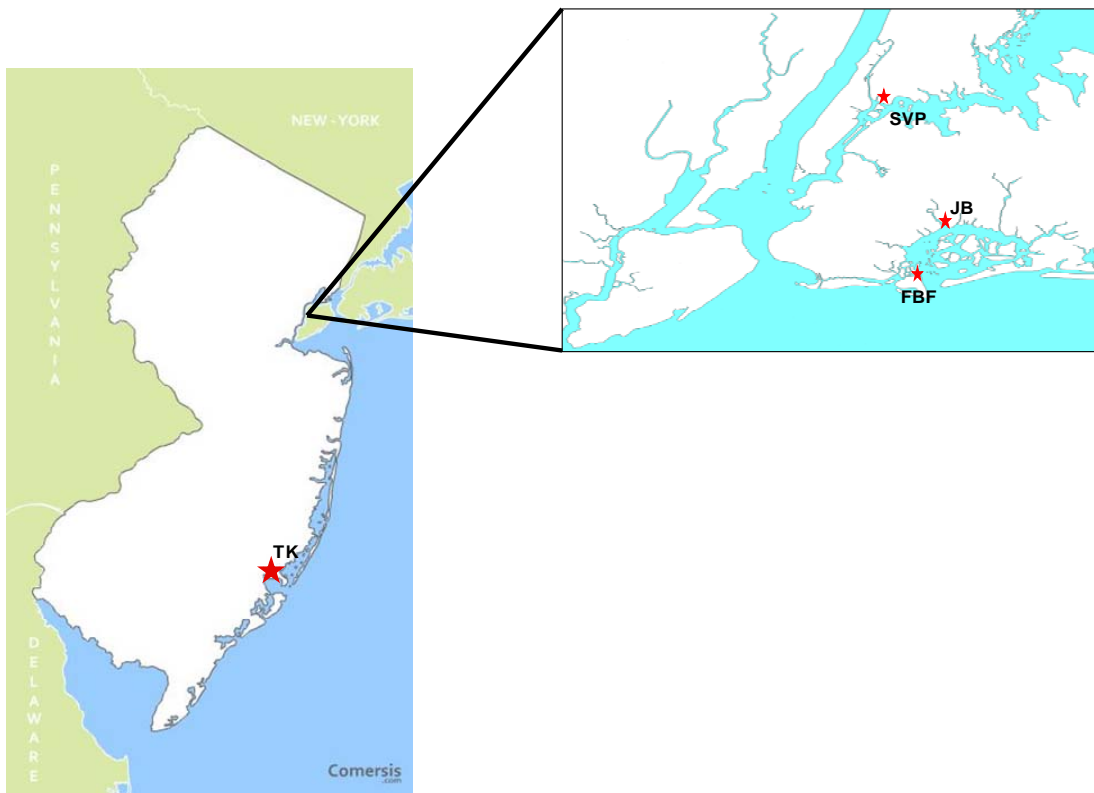
Figure 1: Map of study sites. TK= Tuckerton, NJ (Rutgers University Marine Field Station), FBF= Floyd Bennett Field, Brooklyn, NY (Gateway National Recreation Area, National Parks Service), JB= Spring Creek, Jamaica Bay, Queens, NY (Gateway National Recreation Area, National Parks Service), SVP= Soundview Park, Bronx River, Bronx, NY (NYC Department of Parks and Recreation).

Laboratory Assays

All oysters were brought back from the field sites and stored at -80°C until the assays were performed. Prior to flash-freezing on dry ice, oysters collected for metal body burden and subcellular fractionation were depurated in clean water (DI water mixed with Reef crystals; salinity 25ppt, 23°C) for 24-48 hours, to rid the gut of any foreign particles, and then stored in the –80°C freezer. At each site, 25 oysters were measured for total length (mm), shucked, and dried to determine the condition index (Crosby & Gale 1990). Dried tissue was then pooled into groups (*n*=25), and used to determine carbohydrate, lipid, and protein levels of the oysters. Lipid levels were determined using chloroform-methanol extraction technique (Bligh & Dyer 1959). Total protein content was determined using a CHN elemental analyzer (Perkin Elmer 2400 Series II CHNS/O Analyzer), and adjusted using stoichiometric ratios to determine the protein content (Gnaiger & Bitterlich 1984). Total carbohydrates were determined using the phenol-sulfuric acid method (Dubois et al. 1956).

To determine total body burdens and metal allocation within different subcellular fractions, four juvenile oysters were weighed for wet weight (g), and homogenized with TRIS buffer (pH 7.6). A 0.8 ml subsample was then removed to determine total body burden (TOT). Then, sequential centrifugation and heat treatment steps were used to separate the oyster tissue into operationally-defined fractions (ORG, CD, HDP, HSP, and INS) (Wallace et al. 2003). All fractions (including TOT) were placed in a drying oven for 2-3 days, digested with trace-metal grade nitric acid, resuspended in 2% nitric acid, and analyzed on an Atomic

Absorption Spectrophotometer (Perkin Elmer 3100) to estimate the amount of Cd present (Brown & Luoma 1995). Analysis is pending on an additional five oysters, which will be used to determine total Hg (THg) body burdens (Klajovic-Gaspic et al. 2006; Hatch and Ott 1968). Tissue samples will be homogenized and digested (HNO_3), and THg concentrations will be determined by Cold-Vapor Atomic Absorption Spectrophotomy (Perkin Elmer FIMS-100 Hg analyzer) using standard techniques ($SnCl_2$ will be added to digested tissue prior to analysis).

Water samples (1-3 L) from each site were filtered through a 1.8 µm GF/F glass fiber filter to collect total suspended matter. The filter was then dried in a 60^oC oven to determine the total particulate matter (TPM), and subsequently ashed in a muffle furnace at 450^oC to determine the ash-free dry weight (total organic matter, TOC/POM) (Bayne 2002). Additional water samples at each site (100-500 ml) were filtered through a 0.45 µm nitrocellulose filter, digested in 90% acetone, and run on a UV-spectrophotometer (GENESYS 10) to determine total and size-fractionated chlorophyll *a* (Parsons et al. 1984).

Statistics:

Data was analyzed to compare biochemistry, metal accumulation and subcellular distribution, and environmental variables at each site and to identify relationships between these variables. Regressions were used to determine linkages between variables. Two-way ANOVAs were performed to compare the effects of site and time on the different variables. Post-Hoc tests (Tukey or Unequal N HSD) were performed to identify all significant differences (Zar 1999).

RESULTS

Condition Index:

Juvenile oysters grow quite rapidly in size and body mass. The condition index (ratio of wet flesh weight to total body weight) is a measure of the overall health of the oyster (Crosby & Gale 1990). The condition index values differed significantly among sites over time. While each site showed significant changes in condition index over the summer (ANOVA, $p<0.05$), amongst site differences give us more information as to the site-specific trends in overall oyster health. As the summer began, SVP and TK were significantly different than JB and FBF; however, as time at each site increased, condition index values at SVP became significantly lower than the other three sites, while TK began to display similar condition index values as FBF and JB (Factorial ANOVA, $p< 0.05$) (Figure 2).

Condition Index
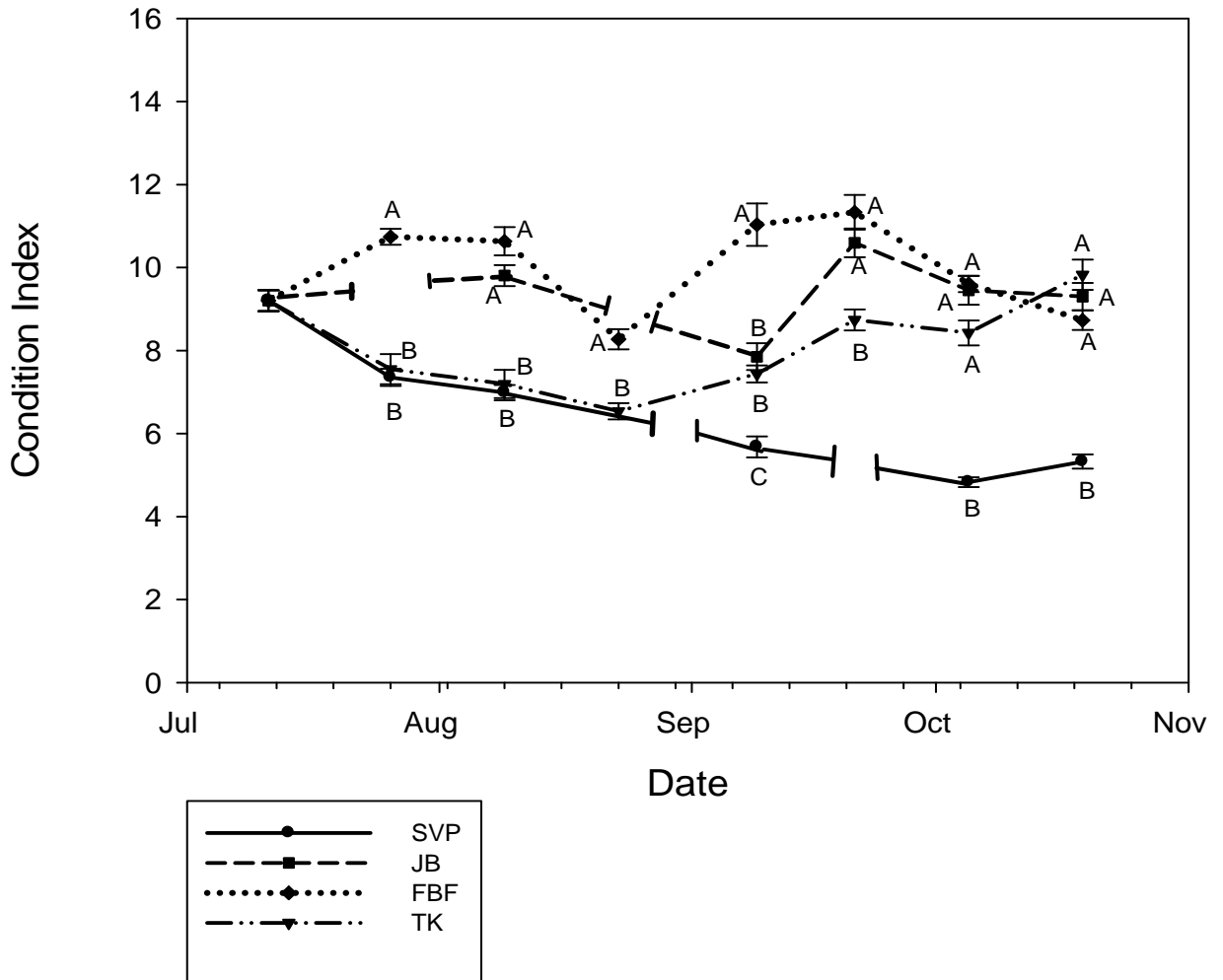
Figure 2: Condition Index of juvenile *Crassostrea virginica* at four field sites over time. Condition index values expressed as means (*n*=25) with standard error bars. At SVP, data was not collected at the 4th and 6th sampling events (8/23/10 and 9/21/10) due to inclement weather (represented with broken lines). At JB, data was not collected at the 2nd and 4th sampling events (7/26/10 and 8/23/10) due to tidal height restriction (represented with broken lines). Letters (A, B, C) represent statistically significant (Factorial ANOVA, $p < 0.05$) differences between sites at each sampling event.

Biochemistry:

The proportion of carbohydrates, lipids, and proteins within oyster tissue is an indicator of the health of the juvenile. Juveniles will put more energy into growth while they are small, meaning more of the energy gained from feeding will end up as carbohydrates within the body, versus protein and lipid.

Within sites, significant changes in carbohydrate storage over time were seen at all four sites (ANOVA, $p < 0.05$, arc-sin transformation of data). SVP showed a steady decline in carbohydrate storage from 8/9/10 through 10/7/10, while TK showed a steady increase of carbohydrate storage from 8/23/10 through 10/22/10. FBF and JB oysters had more variable carbohydrate storage, with fluctuating levels from 7/13/10 through the end (although with an upward trend). Significant differences between sites were seen over all dates (Factorial ANOVA, $p < 0.05$, arc-sin transformation of data) (Figure 3).

Protein storage also showed significant changes in stored proteins within each site over time (ANOVA, $p < 0.05$, arc-sin transformation of data). The percent of protein per gram dry weight was much higher than that of carbohydrate or lipids (40-60% of tissue). All sites showed a decline of protein storage over time, with FBF declining to the lowest levels of protein storage by the end of the summer. JB maintained the highest percentage of stored protein over the summer, while SVP and TK fluctuated as well. Significant differences between sites were found on 7/26/10, 8/9/10, 9/9/10, and 10/22/10 (Factorial ANOVA, $p > 0.05$, arc-sin transformation of data).

The amount of lipids found in tissue was significantly different over time within SVP; however, within the other three sites, no significant change was seen between 7/13/10 and 10/22/10 (ANOVA, p>0.05, arc-sin transformed data). SVP oysters steadily decreased the percentage of lipids within tissues as the summer went on, as TK oysters steadily increased lipid stores from 8/22/10 to 10/22/10. FBF oysters displayed fluctuating lipid stores, with sharp drops on 8/22/10 and 9/22/10 (with recovery periods after). JB oysters displayed a similar pattern as FBF oysters, with a sharp decline in lipid storage on 10/7/10. Across sites, significant differences in lipid storage were seen over the summer (except 7/26/10 and 8/22/10; Factorial ANOVA, p> 0.05, arc-sin transformed data) (Figure 5).
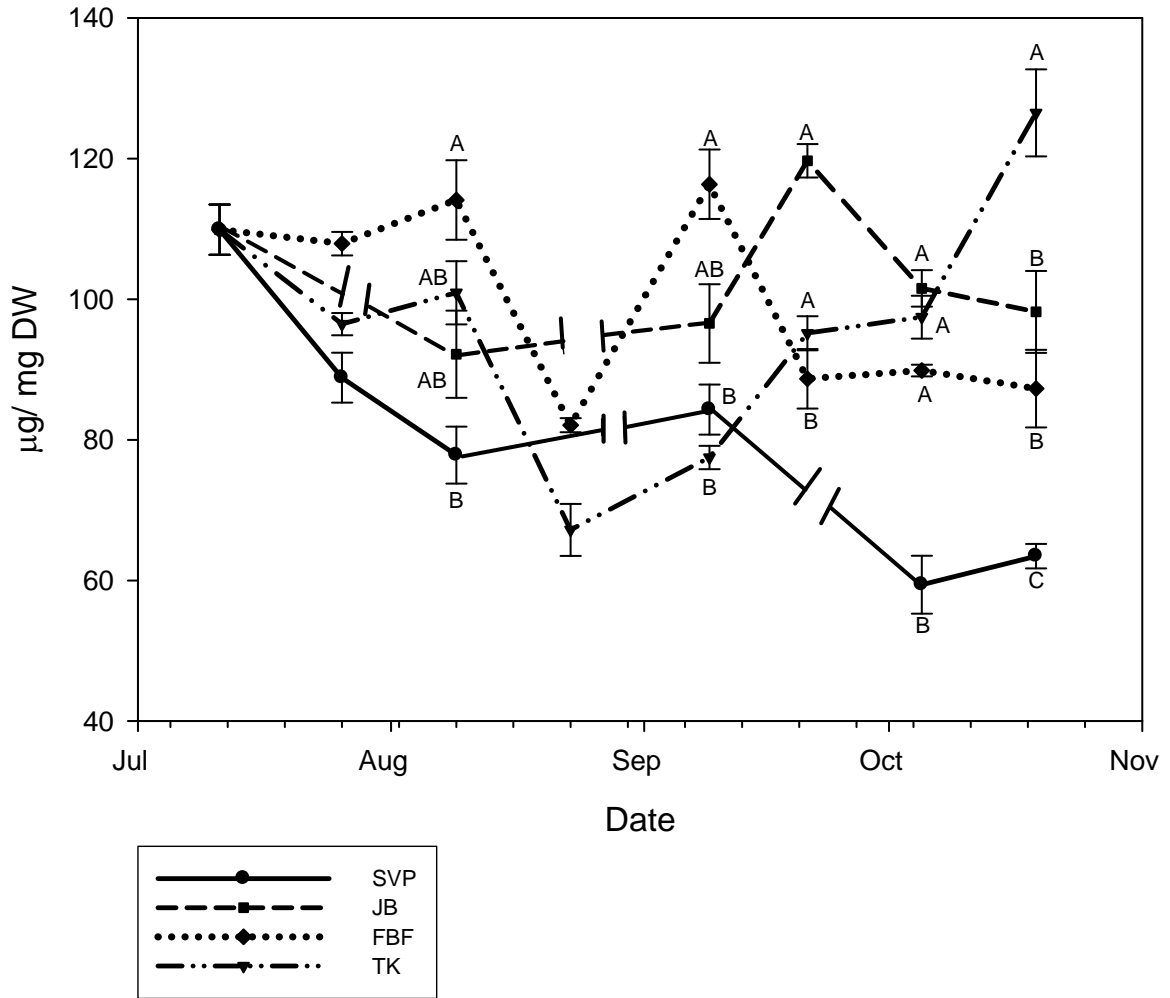
Carbohydrates
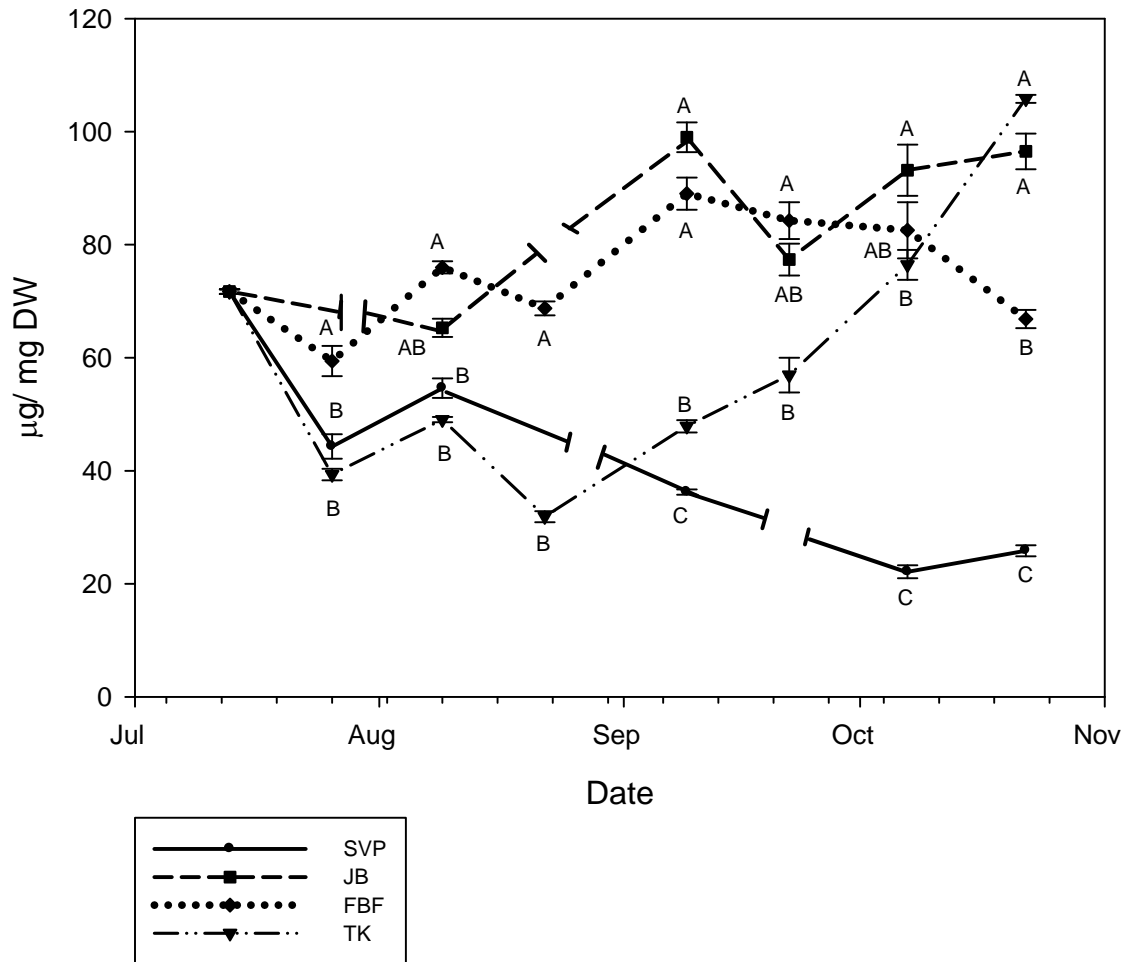
Figure 3: Carbohydrates (microgram per gram dry weight) of juvenile oysters, *Crassostrea virginica*, at four field sites during the summer of 2010. Carbohydrate values are expressed as means (*n*=3) with standard error bars. At SVP, data was not collected at the 4th and 6th sampling events (8/23/10 and 9/21/10) due to inclement weather (represented with broken lines). At JB, data was not collected at the 2nd and 4th sampling events (7/26/10 and 8/23/10) due to tidal height restriction (represented with broken lines). Letters (A, B, C) represent statistically significant (Factorial ANOVA, $p<0.05$) differences between sites at each sampling event.

Proteins
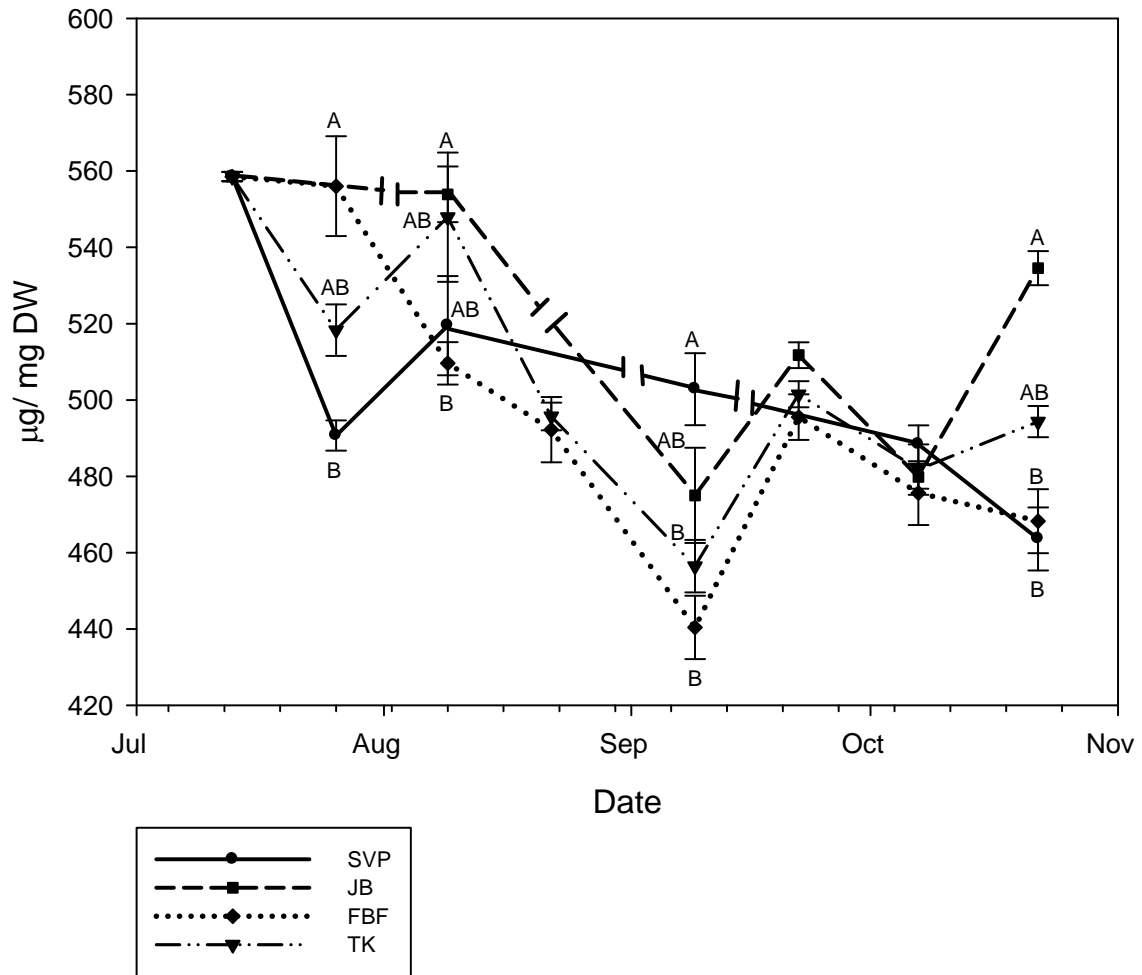
Figure 4: Protein (microgram per gram dry weight) of juvenile oysters, *Crassostrea virginica*, at four field sites during the summer of 2010. Protein values are expressed as means (*n*=3) with standard error bars. At SVP, data was not collected at the 4th and 6th sampling events (8/23/10 and 9/21/10) due to inclement weather (represented with broken lines). At JB, data was not collected at the 2nd and 4th sampling events (7/26/10 and 8/23/10) due to tidal height restriction (represented with broken lines). Letters (A, B) represent statistically significant (Factorial ANOVA, p<0.05) differences between sites at each sampling event.

Figure 5: Lipids (microgram per gram dry weight) of juvenile oysters, *Crassostrea virginica*, at four field sites during the summer of 2010. Lipid values are expressed as means (*n*=3) with standard error bars. At SVP, data was not collected at the 4th and 6th sampling events (8/23/10 and 9/21/10) due to inclement weather (represented with broken lines). At JB, data was not collected at the 2nd and 4th sampling events (7/26/10 and 8/23/10) due to tidal height restriction (represented with broken lines). Letters (A, B, C) represent statistically significant (Factorial ANOVA, p<0.05) differences between sites at each sampling event.

Water Quality:

Environmental data was taken during each sampling event. Water collected for chlorophyll *a* was filtered and processed for both total and size-fractionated chlorophyll. Total chlorophyll *a* values reached a wide range at each site, due to the annual summer bloom of algae that occurs in the HRE (Taylor et al., 2003; Sambrotto 2001). JB recorded the highest chlorophyll *a* values during the peak on 7/26/10 (120.32 mg/L), while the other sites recorded much lower values (SVP-60.075 mg/L, FBF- 63.792 mg/L, TK- 19.693 mg/L) during this date. Significant differences among sites with regards to total chlorophyll *a* were found (Factorial ANOVA, $p>0.05$) on all sampling dates except 9/9/10 and 10/7/10 (Figure 6). At each site, the chlorophyll *a* measurements for the 5-28 μm fraction of phytoplankton follow the same pattern as the total chlorophyll *a* measurements. The 5-28 μm fraction is 40% or higher of the total chlorophyll a at each site (Figure 6).

Water was filtered through a preweighed GF/F filter and dried at 60°C to determine the total particulate matter (TPM) at each site. Significant differences among sites with respect to TPM were seen (Factorial ANOVA, $p>0.05$, log10 transformed data). SVP water had the highest amounts of TPM throughout the entire sampling season (Figure 8). After being weighed for dry weight, filters were then put into a muffle oven and ashed, and the particulate organic matter (POM) calculated from the difference between the ash weight and the dry weight. Again, statistically significant differences were seen in the POM among sites (Factorial ANOVA, $p>0.05$, log10 transformed data) (Figure 9).

MacDonald & Ward (1994) quantified seston quality as the percent of particulates in the water column that are photosynthetic (algae) particles. The amount of chlorophyll *a* (µg) per gram of suspended particulate (TPM) can act as a proxy for the amount of nutritional particles being ingested by the oyster. A measure above 1 µg chl *a* g TPM^{-1} quantifies as "good" seston quality; below 1 µg chl *a* g TPM^{-1} is considered "poor" seston quality (Figure 10). SVP had consistently poor seston quality, only reaching above 1 µg chl *a* g TPM^{-1} on 7/26/10. FBF and JB had high seston quality most of the sampling season, only falling below 1 µg chl *a* g TPM^{-1} after 10/7/10.

Water was filtered through GF/F filters, and a sample run on the CHNS/O elemental analyzer to determine the ratio of carbon to nitrogen in the water, which can indicate seston quality as well. SVP had a significantly higher C:N ratio throughout the sampling season than all other sites (Factorial ANOVA, p>0.05, arc-sin transformed data) (Figure 11).

At each sampling event, environmental parameters (temperature, salinity, and dissolved oxygen) were taken with a handheld YSI probe (Table 1). Environmental parameters are within ranges typically seen at these sites (Mass, unpubl. data).

Total Chlorophyll *a*
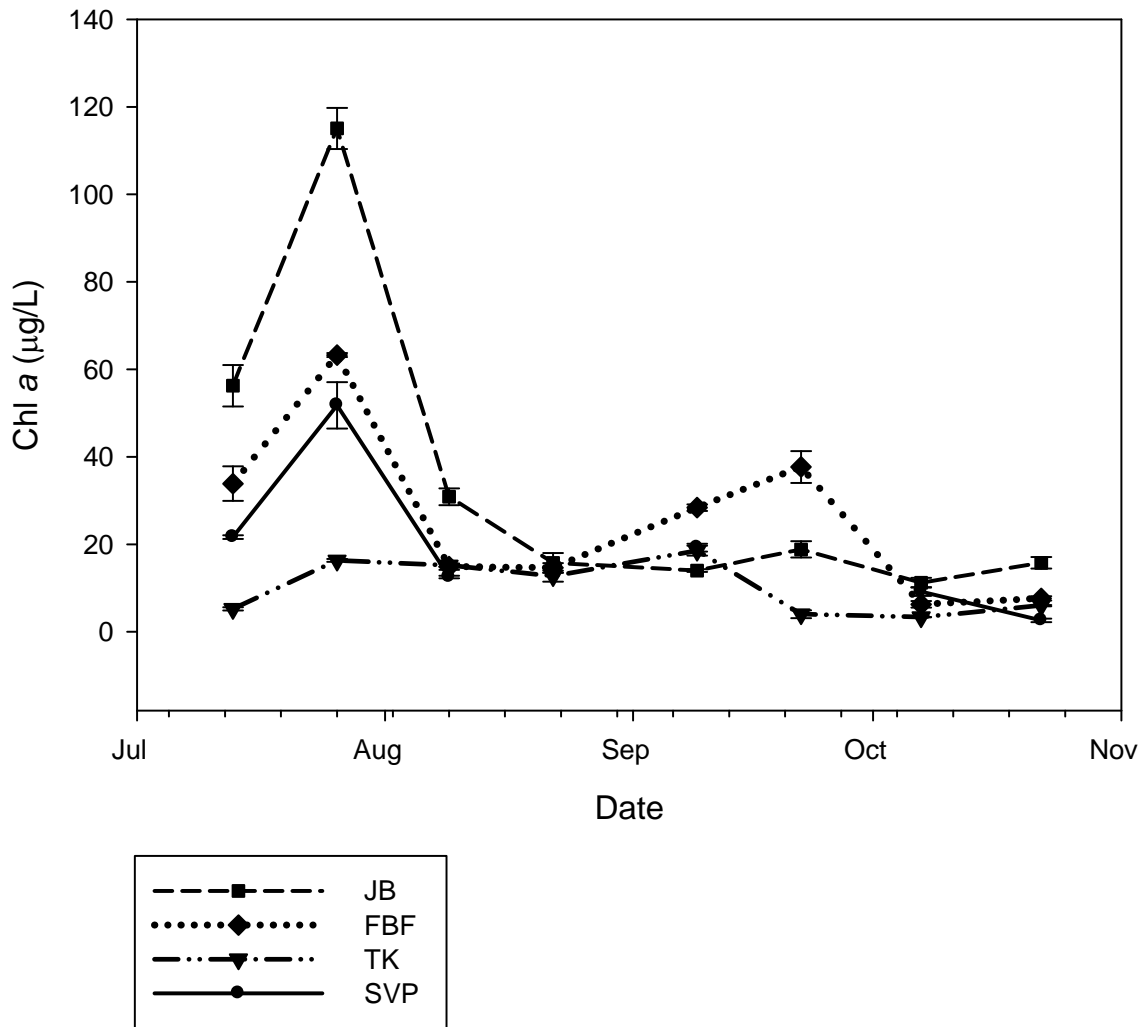
Figure 6: Total chlorophyll *a* (µg/L) at four field sites during the summer of 2010. Data is expressed as mean (*n*=3) values with standard error. At SVP, data was not collected at the 4th and 6th sampling events (8/22/10 and 9/22/10) due to inclement weather.

Size-Fractionated Chlorophyll a
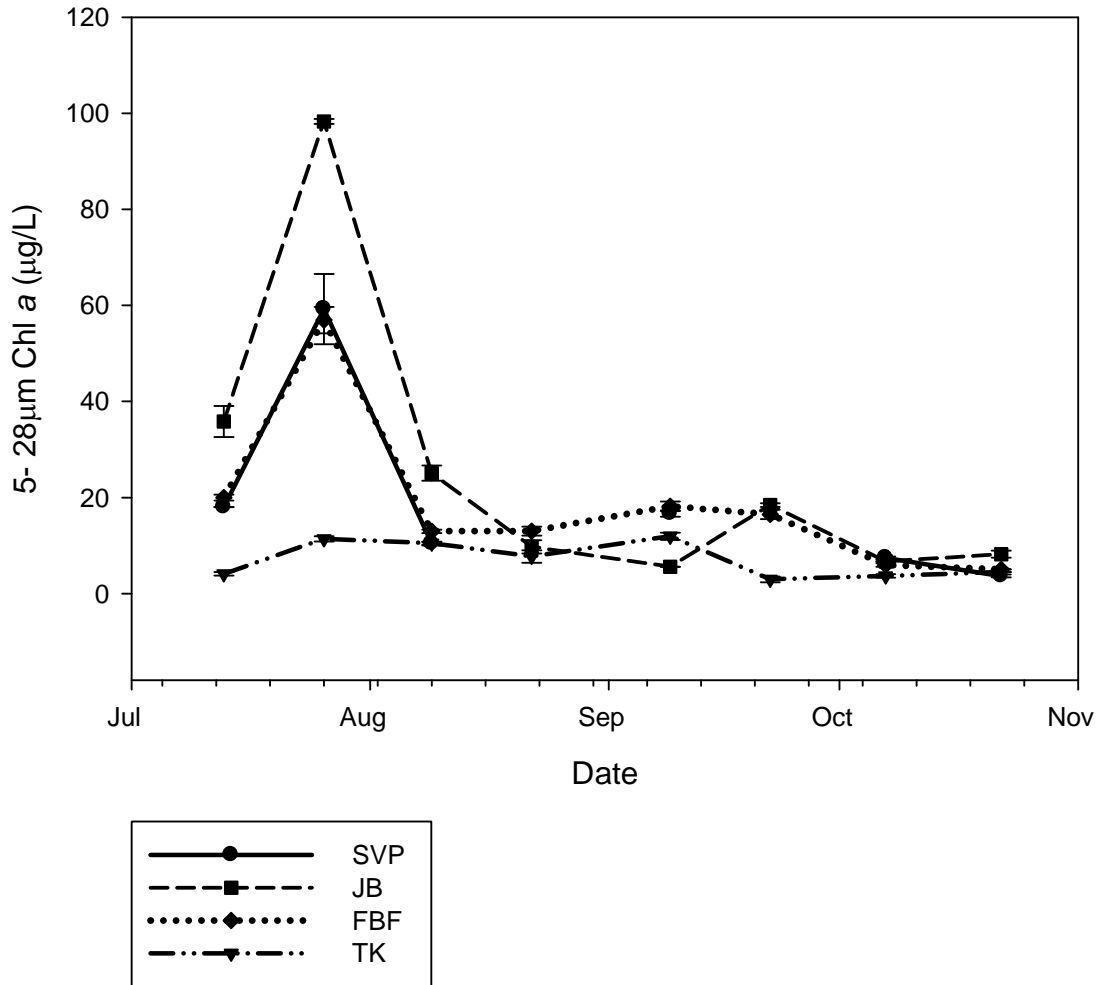
Figure 7: Size-fractionated (5-28μm) chlorophyll *a* (μg/L) at four field sites during the summer of 2010. Data is expressed as mean (*n*=3) values with standard error. At SVP, data was not collected at the 4th and 6th sampling events (8/22/10 and 9/22/10) due to inclement weather.

Total Particulate Matter

Figure 8: Total particulate matter (mg/L) at four field sites during the summer of 2010. Data is expressed as mean (*n*=3) values with standard error. At SVP, data was not collected at the 4th and 6th sampling events (8/22/10 and 9/22/10) due to inclement weather.

Particulate Organic Matter

Figure 9: Particulate organic matter (mg/L) at four field sites during the summer of 2010. Data is expressed as mean (*n*=3) values with standard error. At SVP, data was not collected at the 4th and 6th sam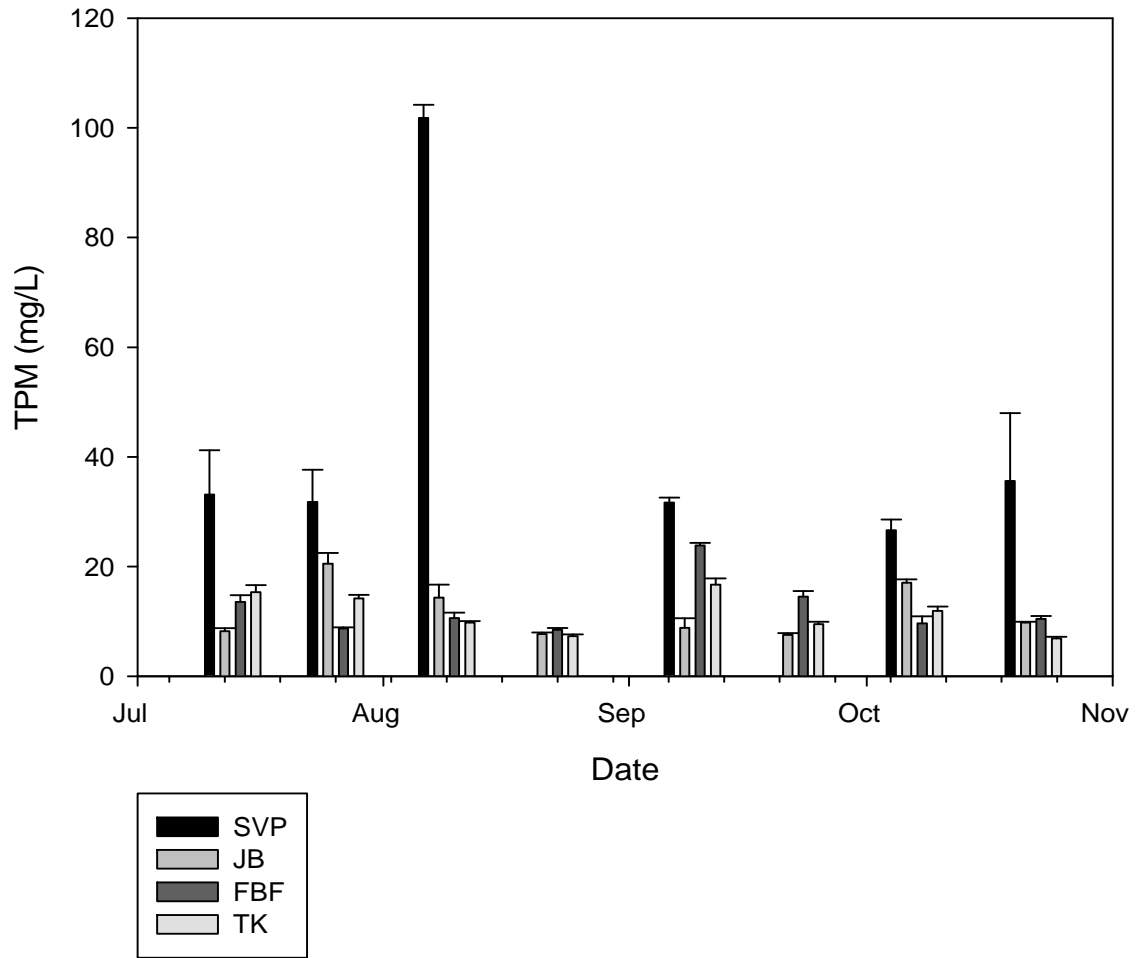pling events (8/22/10 and 9/22/10) due to inclement weather. The scale was kept the same as TPM to highlight relative percentage of TPM that is POM.

Seston Quality

Figure 10: Seston quality (μg Chl *a*/ g TPM) at four field sites during the summer of 2010. Data is expressed as mean (*n*=3) values with standard error. At SVP, data was not collected at the 4th and 6th sam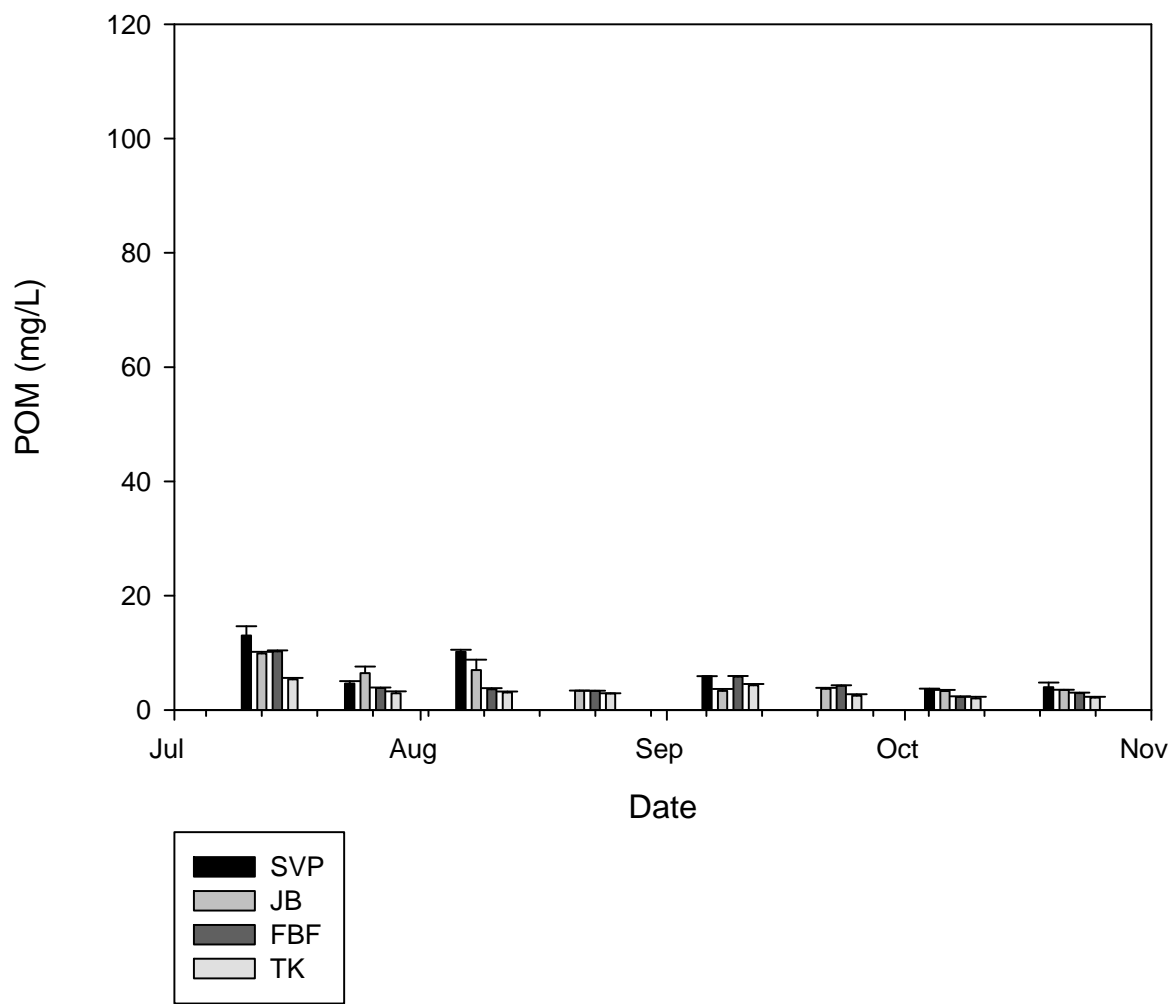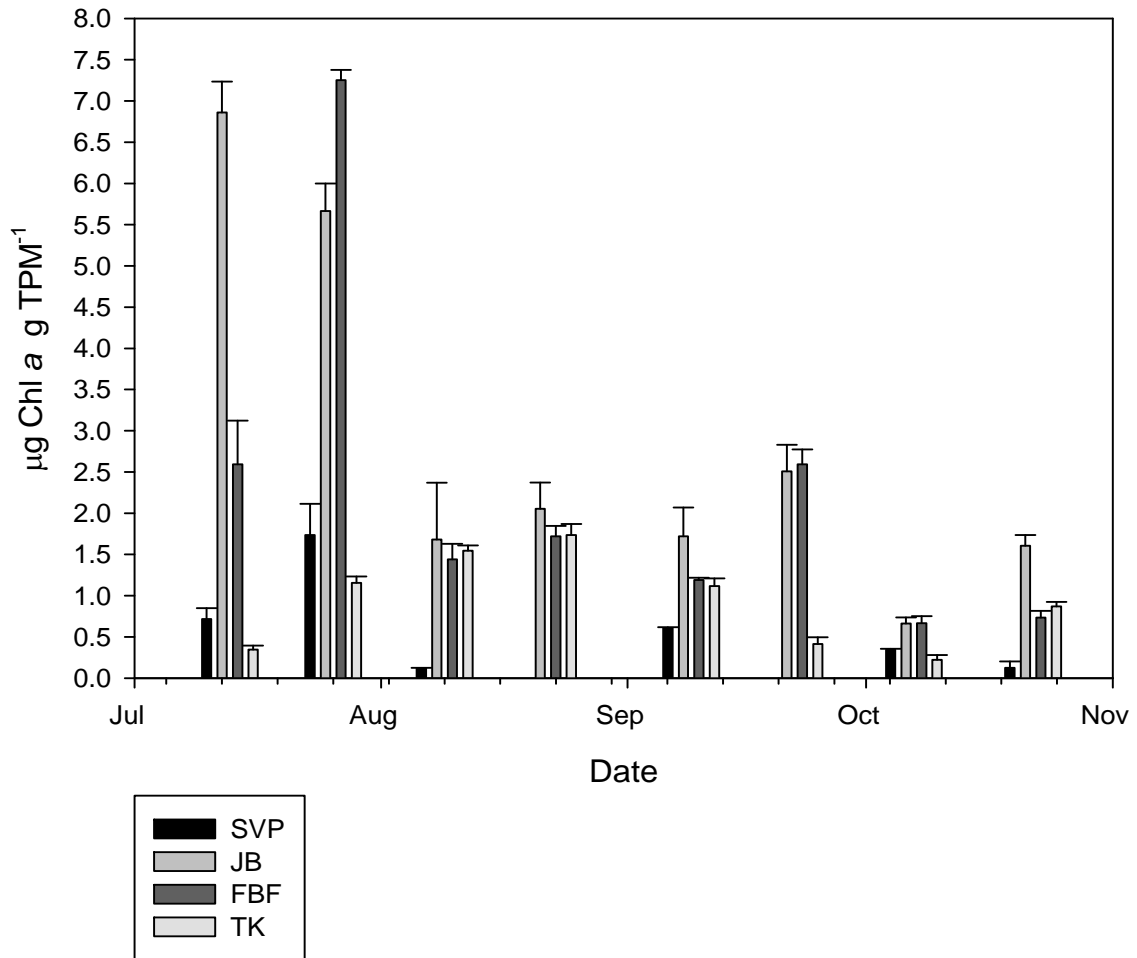pling events (8/22/10 and 9/22/10) due to inclement weather. Values above 1.0 μg Chl *a*/ g TPM indicate high quality seston.

C:N Ratio (Water Quality)

Figure 11: Ratio of elemental carbon to elemental nitrogen in water samples from four field sites during the summer of 2010. Data is expressed as mean (*n*=3) values with standard error. At SVP, data was not collected at the 4th and 6th sampling events (8/22/10 and 9/22/10) due to inclement weather.

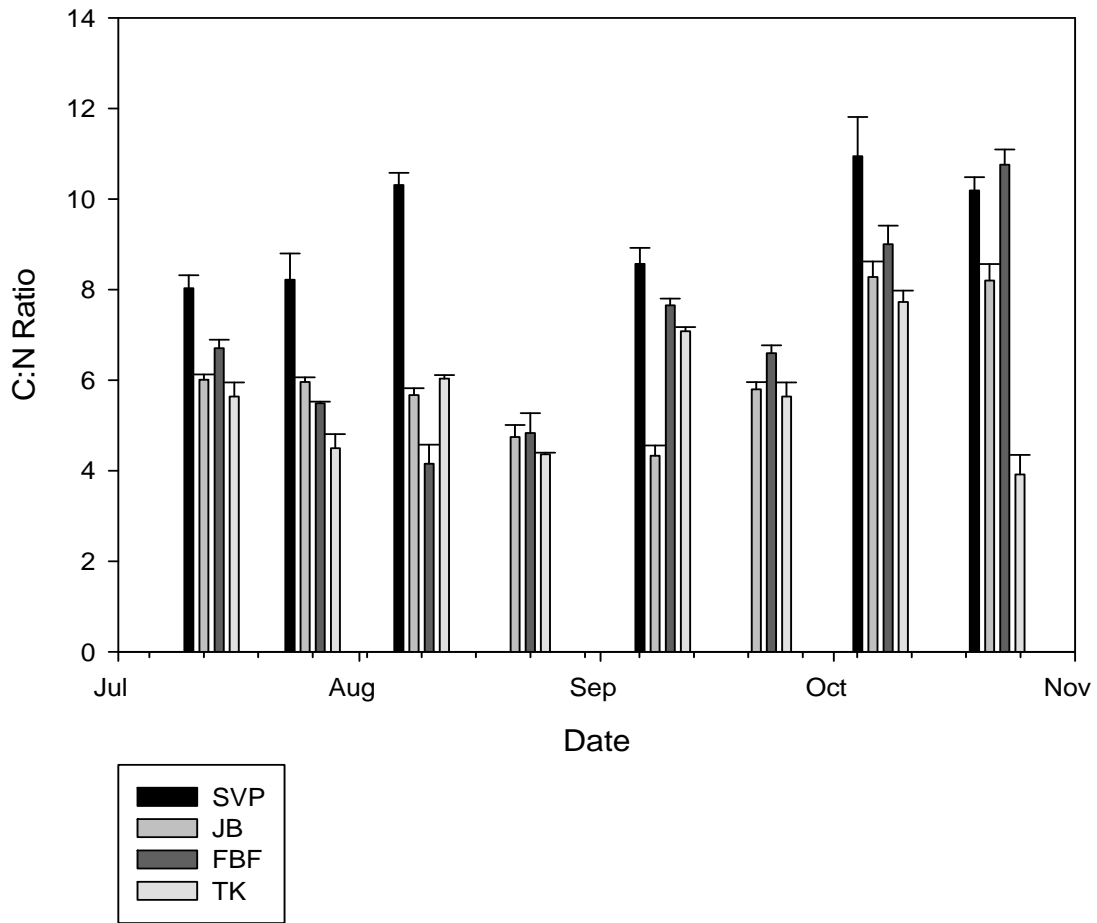|	DATE	7/13/2010	7/26/2010	8/9/2010	8/22/2010	9/9/2010	9/22/2010	10/7/2010	10/23/2010
SVP	TEMPERATURE (^{o}C)	22.6	23.5	23.6	--	22.4	--	18	16.5
	SALINITY (ppt)	25	25	25	--	23	--	30	18
	D.O. (mg/L)	3.78	1.75	3.76	--	6.5	--	6.12	9.63
JB	TEMPERATURE (^{o}C)	25.4	24.9	29.3	21.9	21.1	23.2	19.2	12.3
	SALINITY (ppt)	28	26	26	28	29	27	29	29
	D.O. (mg/L)	2.95	8.63	12.01	6.11	7.07	12.03	8.4	10.19
FBF	TEMPERATURE (^{o}C)	25.9	25.6	24.9	23	20.1	23.1	18.3	14.2
	SALINITY (ppt)	28	28	30	31	32	30	30	31
	D.O. (mg/L)	6.98	9.61	7.21	6.09	8.01	10.61	7.05	8.66
TK	TEMPERATURE (^{o}C)	28.7	27.6	27.6	24.7	25.5	21.1	16	15.1
	SALINITY (ppt)	30	30	30	29	30	33	33	32
	D.O. (mg/L)	5.91	7.36	7.36	8.72	7.32	7.78	7.69	7.66

Table 1: Environmental parameters (temperature, salinity, dissolved oxygen) at four field sites from 7/13/2010- 10/23/10.

Metals:

Metal body burdens were determined using Atomic Absorption Spectrophotometry. Whole body burdens of Cd were highest in SVP oysters, peaking on 10/7/10 at 55.05 μg Cd g dry tissue^{-1} (Figure 12). Statistical differences between sites were seen on 10/7/10 (Factorial ANOVA, p< 0.05), with SVP having significantly more Cd within tissues than TK. JB accumulated the least amount of Cd throughout, with only 10.25 μg Cd g dry tissue^{-1} on 10/23/10 (Figure 12).

At SVP, sequential centrifugation steps to separate different subcellular cytosolic partitions yielded differences in Cd burdens. More Cd was seen in the INS fraction than ORG, HDP, or HSP. The least amount of Cd accumulation was found

in the HSP fraction, which has metallothioneins. Statistically significant differences in Cd burdens within fractions were seen on 7/15/10, 9/9/10, and 10/7/10 (Figure 13).

Total body burdens of Hg were analyzed using Cold-Vapor Atomic Absorption Spectrophotometry. Body burdens of juvenile *C. virginica* were elevated at the initial sample, obtained from the hatchery on Long Island (0.756 µg/ g dry weight). At SVP, body burdens decreased as the season progressed, but detectable levels of Hg remained in tissues until the last sampling event (0.101 µg/ g dry weight on 10/23/10). Oysters in JB and FBF had increases in Hg body burdens in September 2010, but then quickly decreased to undetectable levels in October 2010. Oysters at TK remained at low, but detectable, body burdens through September, before becoming undetectable (Figure 14).

Correlations:

Correlations were calculated between variables to determine any linkages. At SVP, condition index was positively correlated with carbohydrate and lipid percentages in tissue, but not with the amount of protein stored in tissue (Pearson correlation, $p < 0.05$). At JB, environmental parameters were correlated with carbohydrate storage. Salinity was positively correlated with the amount of carbohydrates present, while temperature showed a negative correlation with carbohydrate storage (Pearson correlation, $p < 0.05$). FBF oysters had protein storage positively correlated to both temperature and seston quality, but negatively correlated with salinity (Pearson correlation, $p < 0.05$). At TK, condition index was positively

correlated with carbohydrate and lipid storage in tissues, but not protein (Pearson correlation, p< 0.05).   Condition index was not significantly correlated with either total chlorophyll *a* or seston quality at any of the sites. Cd body burdens were not correlated with any physiological (condition index, carbohydrates, lipids, proteins) or environmental (chlorophyll *a*, seston quality, temperature, salinity, dissolved oxygen) at any of the sites. Hg body burdens were positively correlated with condition index, carbohydrates, and lipid levels at SVP, but not at any of the other sites (Pearson correlation, p<0.05).

TOT Cd Burdens
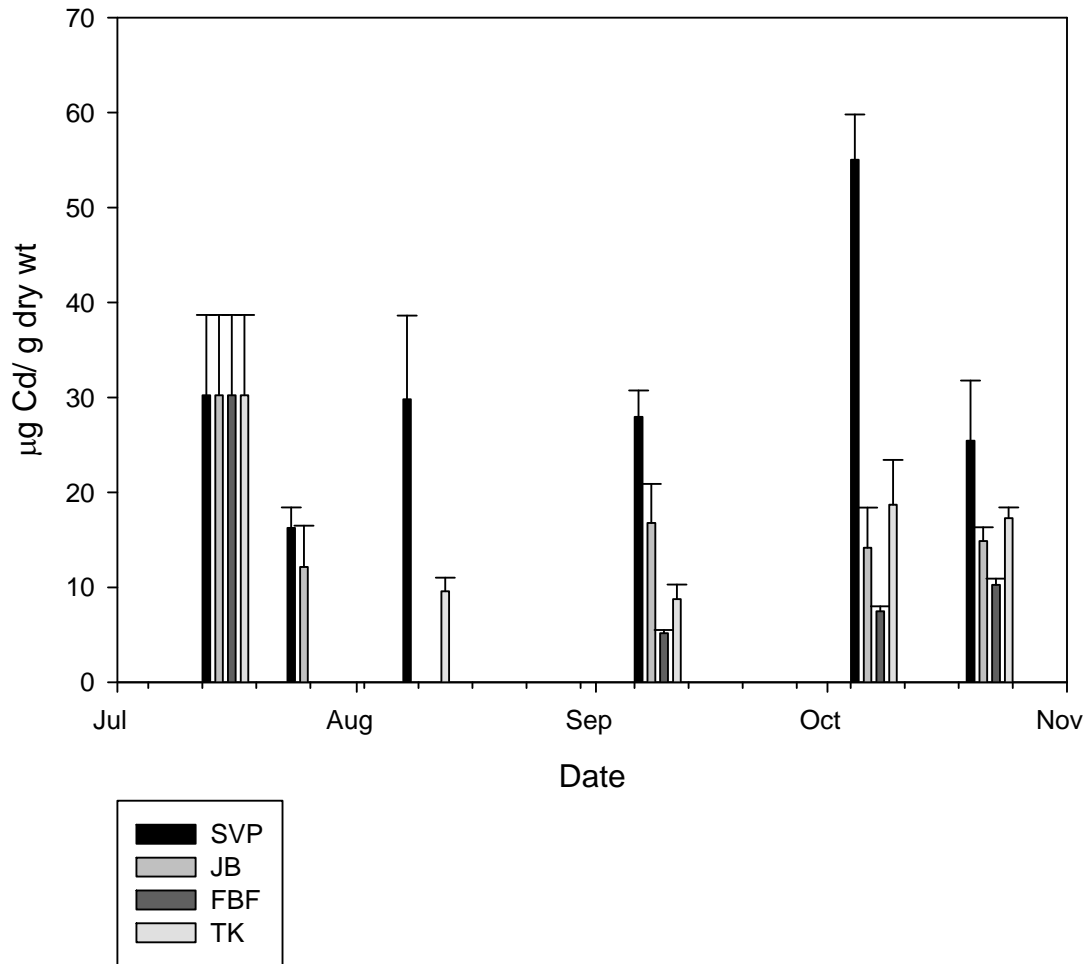
Figure 12: Total body burdens of Cd (μg) per unit dry weight of juvenile oysters, *Crassostrea virginica*, at four field sites during the summer of 2010. Cd burdens are expressed as means (*n*=4) with standard error. At SVP, data was not collected at the 4th and 6th sampling events (8/23/10 and 9/21/10) due to inclement weather. At JB, data was not collected at the 2nd and 4th sampling events (7/26/10 and 8/23/10) due to tidal height restriction. At TK, oysters from the 2nd sampling event (7/26/10) were preserved incorrectly and not useable for analysis. Body burdens from missing FBF (8/10/10, 8/22/10, 9/22/10), JB (8/10/10, 9/22/10), and TK (8/22/10, 9/22/10) dates were not analyzed yet due to AA machine problems.

SVP: Subcellular Cd Burdens
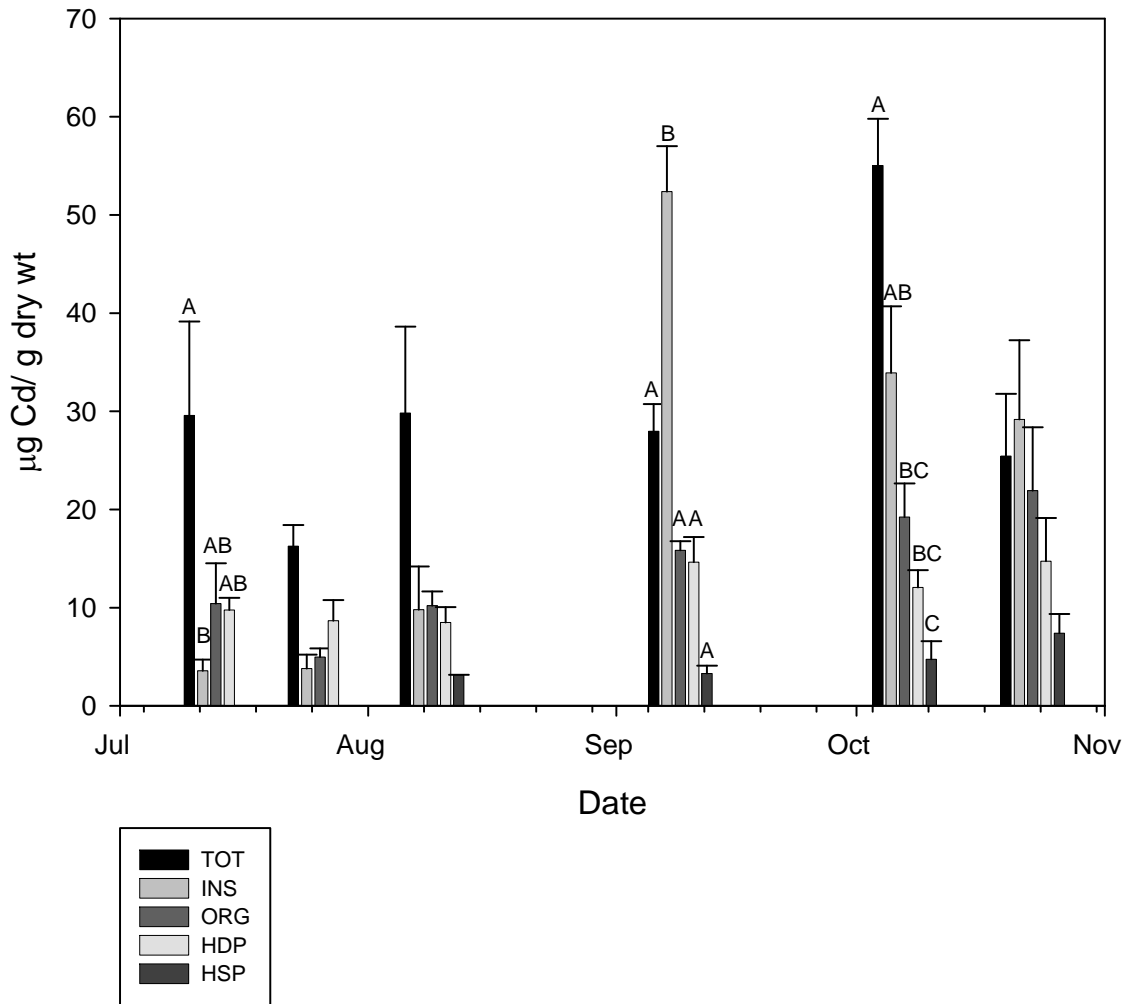
Figure 13: Subcellular Cd burdens found in different cytosolic fractions at SVP during 2010. Cd burdens are expressed as means (*n*=4) with standard error. Data was not collected at the 4th and 6th sampling events (8/23/10 and 9/21/10) due to inclement weather. TOT= total body burden, INS= insoluble granules, ORG= organelles, HDP= heat-denatured proteins (i.e., enzymes), HSP= heat-stable proteins (i.e., metallothioneins). No detectable Cd burdens were found in the HSP fraction on 7/15/10 and 7/26/10. Letters (A,B,C) represent significant differences between fractions on that date (Factorial ANOVA, $p < 0.05$).

Total Body Burdens- Hg
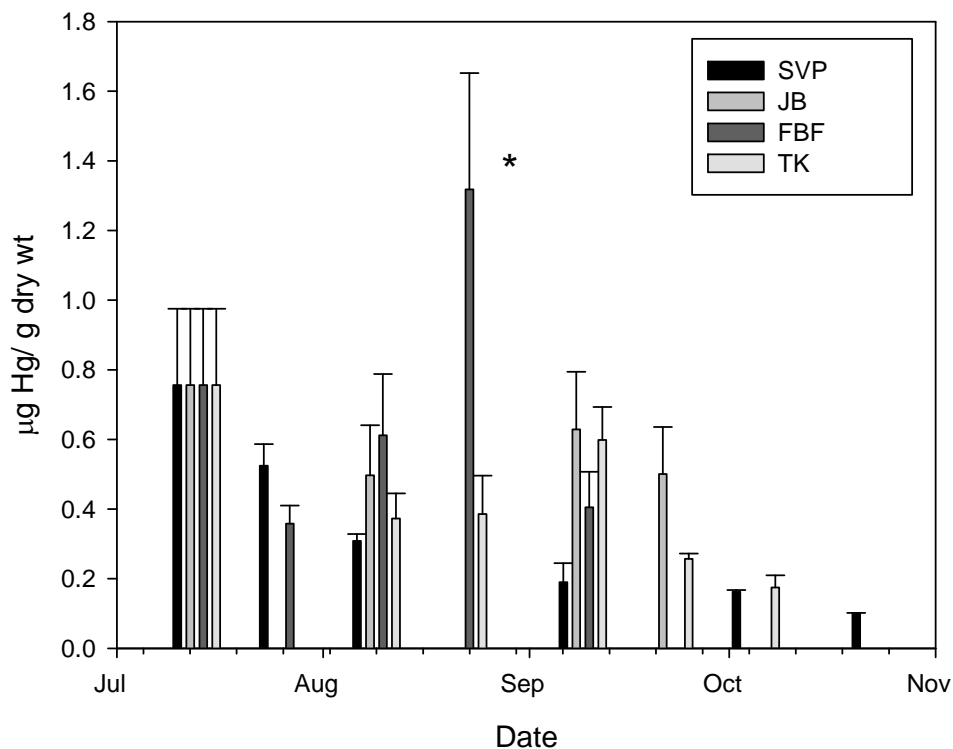
Figure 14: Total body burdens of Hg in juvenile *C.virginica* from field sites during 2010. Hg burdens are expressed as means (*n*=4) with standard error. At SVP, data was not collected at the 4th and 6th sampling events (8/23/10 and 9/21/10) due to inclement weather. At JB, data was not collected at the 2nd and 4th sampling events (7/26/10 and 8/23/10) due to tidal height restriction. At TK, oysters from the 2nd sampling event (7/26/10) were preserved incorrectly and not useable for analysis. Other missing values indicate undetectable levels of Hg in tissues. A * indicates a significant difference between sites (Factorial ANOVA, $p < 0.05$).

DISCUSSION

Previous findings have shown elevated concentrations of pollutants (including Cd, Cu, and Hg) within the HRE (Mass, unpubl. data; Bopp et al. 2006; Wirgin et al. 2006; Feng et al. 1998; Adams et al. 1996; Seidemann 1991). Analysis of sediments from SVP and JB revealed similar concentrations of Cd (0.170 µg/L Cd at SVP; 0.167 µg/L Cd at JB; Mass, unpubl. data). These concentrations have been shown to cause deleterious physiological effects in oysters in lab exposures (Ivanina et al. 2009). Exposure to 0.05 µg/L of Cd led to significant accumulation of Cd within tissues, and also increased expression of heat-sensitive proteins and metallothioneins (Ivanina et al. 2009). After an initial loss phase, oysters at SVP were shown to accumulate Cd within tissues throughout the summer, significantly more than the other three sites (Factorial ANOVA, $p > 0.05$). Oysters had significantly higher TOT Cd burdens on 10/7/10 and 10/23/10 than the initial condition (ANOVA, $p < 0.05$). As the summer progressed, and oysters continued to filter water and particulates through their gills, exposure to Cd ions and increased accumulation became more likely. Cadmium burdens increased through October, coinciding with a decrease in condition index (Figure 2 & 12). Abbe et al (2000) found that increases in Cd burdens occurred in Chesapeake Bay oysters as the condition index was decreasing, which could be due to loss of tissue mass or retention of metals in insoluble granules or metallothioneins. SVP oysters also showed increased Cd burdens in both the INS and HSP fractions as the summer progressed (Figure 13). More Cd was accumulated in the INS fraction, where granular hemocytes are found (Roesijadi 1996). These insoluble granules may help

to detoxify oysters by binding the free Cd within tissues and sequestering it, thus helping to detoxify the organism (Roesijadi 1996). Among the fractions, oysters accumulated more Cd into the ORG and HDP fractions than HSP (Figure 13). Organelles (such as mitochondria; ORG) and enzymes (HDP) necessary for metabolism, defense, and growth may be impacted by the accumulated Cd. Metals binding to mitochondria and important enzymes may denature the enzymes, and alter the metabolic regime of the oyster. Differences in energy budgets can lead to large-scale effects (i.e., lowered reproductive output, slower filtration rate) that will affect potential restoration of healthy oyster reefs (Sokolova et al. 2005). Preliminary data (Mass, unpubl.) on filtration rate of juvenile oysters at the four field sites (from 2010) has shown a significant decrease in filtration rate as Cd accumulates within SVP oysters. This may be due to the binding of metals to sensitive intracellular targets within ORG and HDP that control filtration activity for *C. virginica*. More investigation into the alteration of physiological variables such as filtration and assimilation is necessary to determine if changes are due solely to Cd accumulation.

No significant correlations were seen between TOT Cd burdens and any physiological or environmental parameters, which was surprising. Cd complexes easily with free chlorine ions to form $CdCl_2$, a form that is not bioavailable for oysters to uptake (Blackmore & Wang 2003); therefore, salinity was predicted to affect the accumulation of Cd by oysters at the various sites. JB is located furthest from the mouth of Jamaica Bay in an enclosed creek, where salinities may become higher due to low flushing and dry summers (as seen in 2010). On the other hand, TK was located on a well-flushed inlet, yet had higher salinity due to its proximity to

the Atlantic Ocean. Additionally, a correlation between TOT Cd and condition

index was predicted, since the loss of tissue mass may affect the µg Cd per unit dry

mass (if the Cd is not being offloaded, due to spawning activities or sequestration).

Future analysis is needed to determine if metal accumulation is the root cause for the

differences seen in physiology at these sites, including a laboratory exposure and

further metal analysis.

Total body burdens of Hg were not significantly different between sites over

time except for 8/22/10 when elevated concentrations of Hg in tissues at FBF were

seen (Factorial ANOVA, $p<0.05$; Figure 14). After this date, there was a decline in

condition index that indicates possible spawning by the oysters (Figure 2).

Formation of gonad tissue was found in FBF oysters at this date and prior sampling

as well (pers. obsv.). A spawning event may have provided the oysters with a way to

detoxify; Hg will easily bind with gonad tissue, allowing for depuration as the oyster

spawns (Kehrig et al. 2006; Gagne et al. 2002).   Gagne et al (2002) found high

levels of class IIb metals (which include Hg and Cd) in gonads of female *Mya*

arenaria along with elevated condition index values which indicate the clam is

getting ready to spawn. A positive correlation between condition index,

carbohydrate and lipid content, and Hg concentrations found at SVP support this

statement; as the oysters were building up gonad tissue, Hg was accumulating

(Figures 2, 3, 5, & 14).

Condition index values at FBF and JB increased over time from 7/13/10-

8/3/10, followed by a sharp decrease from 8/22/10- 9/9/10, and again a significant

increase until 10/7/10. This fluctuation in condition index levels may indicate a

spawning event, where the tissue mass of the oyster would decrease quickly over time due to the release of gametes (Li et al. 2009).   The decreasing condition index values correspond with decreasing lipid levels (although not significantly correlated) at FBF as well. This decrease in lipid storage may also indicate spawning because oysters store large quantities of lipid in gametes (Pazos et al. 1996). Post- spawning, recovery occurs with increasing quantities of lipids. Both SVP and TK showed condition indices correlated with carbohydrate and lipid levels; however, no correlation between condition index and lipid or carbohydrate levels was seen at FBF or JB, and no correlation with protein levels was seen at any of the sites (Pearson correlation; $p< 0.05$). Oysters use carbohydrate stores first during somatic and gametic growth phases, followed by lipid stores. Protein percentages within tissue were at the lowest levels on 9/9/10, as condition index values were increasing after sharp decreases the previous weeks (Figures 2 & 4). This may be due to large quantities of carbohydrates and lipids being depleted, and oysters depending on proteins for somatic growth as food supplies diminish. The decrease in condition index occurs along with the decline in food quantity (total chlorophyll *a*) (Figure 2 and 6). Juvenile oysters rapidly feed on algae when it is available, putting ingested energy towards shell growth and tissue growth. Oysters are able to particle-sort on the velum, and are able to reject particles that are less nutritious into their pseudofeces (Baldwin & Newell 1995), which may be why at lower concentrations of algae, the oysters had lower condition indices. As the condition index declined, the amount of stored carbohydrates increased. Storing carbohydrates when food availability is lower is a way to ensure energy during low-food times (such as

winter), when oysters will use energy for basic metabolic demands rather than for growth and reproduction.

It was observed that the shells at SVP and TK were much thinner than those at FBF and JB, which may be due to the diversion of energy more towards general metabolism and less towards shell growth at these two sites, both of which had the lowest condition index values throughout the season (Figure 2). Both sites also saw a decrease in stored biochemical compounds (carbohydrates, proteins, and lipids) until 9/9/10; however, carbohydrate and lipid levels remained low in SVP while TK oysters were able to recover and increase beyond what was seen in the initial sample (Figures 3-5). This change in percentages of biochemical storage compounds indicates that the oysters were transferring energy from growth and reproduction into stored compounds for metabolic demands. JB and FBF oysters did not see a sharp decline in carbohydrates, nor as much lost from protein and lipid storage as seen at other sites. This may be due to higher chlorophyll *a* amounts available, or better seston quality (Figures 6 & 10). No statistically significant correlations between food availability or quality and overall condition index were found at JB or FBF; however, these correlations do not take into account the food availability seen between bi-monthly sampling events. A more consistent chlorophyll/ seston quality monitoring program would show more temporal trends in food availability, and help to explain why thinner shells were seen at the two sites outside of Jamaica Bay.

Environmental parameters (i.e., seston quantity and quality, temperature, salinity, oxygen levels) can have a profound effect on oyster physiology (Paterson et al. 2003; Pridmore et al. 1990; Ivanina et al. 2009). Salinity can influence the

V-39

binding state of metal ions and thus the amount of metal that is accumulated in the tissue. Changes in temperature, especially elevated temperatures, can increase metabolic demands and increase the expression of heat-shock proteins and metallothionein proteins, which can affect the accumulation and detoxification of metals (Cherkasov et al. 2007; Ivanina et al. 2009). Between June-August, 2010, the HRE experienced a very hot and dry summer, with temperatures reaching a peak of $25.5^{o}C$ and salinity between 23-32 ppt (Table 1). Along with an increase in temperature comes a decrease in dissolved oxygen levels; sites experienced values between 1.75- 12.03 mg/L. Values below 2.5 mg/L indicate hypoxic conditions, which may affect oyster metabolism and lead to higher metal body burdens (Baker & Mann 1994).

Evaluation of oyster physiology when placed at various field sites along a contamination gradient will allow for a greater understanding of bivalve responses to urbanization. While numerous lab-based studies have examined a single variable and the oyster's response, this situation allows us to determine the interactions of multiple variables on oyster condition, energy budgets, and storage of harmful contaminants. A suite of contaminants exists in the HRE, including organic pollutants (PAHs, PCBs) which can alter oyster physiology, and this study will provide a starting point for discussions on restoration. Various institutions and organizations in New York City and New Jersey have begun oyster restoration projects within the HRE, and knowledge of how our unique system affects the growth, reproduction, and larval dynamics of *Crassostrea virginica* is imperative for the success of such projects.

ACKNOWLEDGEMENTS

LITERATURE CITED

Abbe, G.R., G.F Riedel, and J.G. Sanders. 2000. Factors that influence the accumulation of copper and cadmium by transplanted eastern oysters (*Crassostrea virginica*) in the Patuxent River, Maryland. Marine Environmental Research 49: 377-396.

Adams, D.A., J.S. O'Connor, and S.B. Weisberg. 1996. Sediment quality of the NY/NJ Harbor system- an investigation under the Regional Environmental Monitoring and Assessment Program. Final report to the US EPA.

Amiard, J.-C., C. Amiard-Triquet, S. Barka, J. Pellerin, and P.S. Rainbow. 2006. Review: Metallothioneins in aquatic invertebrates: Their role in metal detoxification and their use as biomarkers. Aquatic Toxicology 76: 160-202.

Baker, S.M, and R. Mann. 1994. Feeding ability during settlement and metamorphosis in the oyster *Crassostrea virginica* (Gmelin, 1791) and the effects of hypoxia on post-settlement ingestion rates. Journal of Experimental Marine Biology and Ecology 181: 239-253

Baldwin, B.S., and R.I.E Newell. 1995. Feeding rate responses of oyster larvae (*Crassostrea virginica*) to seston quantity and composition. Journal of Experimental Marine Biology and Ecology 189: 77-91.

Bayne, B.L., 2002. A physiological comparison between Pacific oysters *Crassostrea gigas* and Sydney rock oysters *Saccostrea glomerata*: food, feeding and growth in a shared estuarine habitat. Marine Ecology Progress Series 232, 163–178.

Blackmore, G., and W.-X. Wang. 2003. Inter-population differences in Cd, Cr, Se, and Zn accumulation by the green mussel *Perna viridis* acclimated at different salinities. Aquatic Toxicology 62: 205-218.

Blanchard, J., K. Brix, and M. Grosell. 2009. Subcellular fractionation of Cu exposed oysters, *Crassostrea virginica,* and Cu accumulation from a biologically incorporated Cu rich oyster diet in *Fundulus heteroclitus* in fresh and sea water. Comparative Biochemistry and Physiology, Part C. 149: 531-537.

Bligh, E.G., and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37: 911-917.

Bopp, R.F., S.N. Chillrud, E.L. Shuster, and H.J. Simpson. 2006. Contaminant chronologies from the Hudson River sedimentary records. pp. 383-397 *in*: Levinton, J.S. and J.R. Waldman (Eds). The Hudson River Estuary. Cambridge University Press, New York.

Brown, C.L., and S.N. Luoma. 1995. Use of the euryhaline bivalve *Potamocorbula amurensis* as a biosentinel species to assess trace metal contamination in San Francisco Bay. Marine Ecology Progress Series 124: 129-142.

Brumbaugh, R.D., L.A. Sorabella, C.O. Garcia, W.J. Goldsborough, and J.A. Wesson.   2000. Making a case for community-based oyster restoration: an example from Hampton Roads, Virginia, USA.   Journal of Shellfish Research 19: 397– 400.

Cherkasov, A.S., S. Grewal, and I.M. Sokolova. 2007. Combined effects of temperature and cadmium exposure on haemocyte apoptosis and cadmium accumulation in the eastern oyster *Crassostrea virginica*.   Journal of Thermal Biology   32: 162-170

Cherkasov, A.S., C. Taylor, and I.M. Sokolova. 2009. Seasonal variation in mitochondrial responses to cadmium and temperature in eastern oysters *Crassostrea virginica* (Gmelin) from different latitudes. Aquatic Toxicology 97: 68-78.

Coen, L.D., and M.W. Lukenbach. 2000. Developing success criteria and goals for evaluating oyster reef restoration: Ecological function or resource exploitation? Ecological Engineering   15: 323-343.

Crawford, D.W., N.L Bonnevie, C.A. Gillis, and R.J. Wenning. 1994. Historical changes in the ecological health of the Newark Bay Estuary, New Jersey. Ecotoxicology and Environmental Safety 29: 276-303.

Crosby, M.P., and L.D. Gale. 1990. A review and evaluation of bivalve condition index methodologies with a suggested standard method. Journal of Shellfish Research   9: 233–237.

Dimou, K.N., T.-L. Su, R.I. Hires, and R. Miskewitz. 2006. Distribution of polychlorinated biphenyls in the Newark Bay Estuary. Journal of Hazardous Materials   136: 103-110.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28: 350-356.

Erk, M., B.T.A. Muyssen, A. Ghekiere, and C. Janssen. 2008. Metallothionein and cellular energy allocation in the estuarine mysid shrimp *Neomysis integer* exposed to cadmium at different salinities. Journal of Experimental Marine Biology and Ecology 357: 172-180

Feng, H., J.K. Cochran, H. Lwiza, B.J. Bronawell, and D.J. Hirschberg. 1998. Distribution of heavy metal and PCB contaminants in the sediments of an urban estuary: The Hudson River. Marine Environmental Research 45: 69-88.

Franz, D.R. 1982. *A historical perspective on mollusks in lower New York Harbor, with emphasis on oysters. In*: G.F. Mayer. (Ed.) Ecological Stress and the New York Bight: Science and Management. Estuarine Research Foundation, Columbia, S.C.

Gagne, F., C. Blaise, J. Pellerin, and S. Gauthier- Clerc. 2002. Alteration of the biochemical properties of female gonads and vitellins in the clam *Mya arenaria* at contaminated sites in the Saguenay Fjord. Marine Environmental Research 53: 295-310.

Geffard, A., J.C. Amiard, and C. Amiard-Triquet. 2002. Kinetics of metal elimination in oysters from a contaminated estuary. Comparitive Biochemistry and Physiology, Part C. 131: 281-293.

Gerritsen, J., A.F. Holland, and D.E. Irvine. 1994. Suspension-feeding bivalves and the fate of primary production: an estuarine model applied to Chesapeake Bay. Estuaries 17: 403–416.

Gnaiger, E., and G. Bitterlich. 1984. Proximate biochemical composition and caloric content calculated from elemental CHN analysis: a stoichiometric concept. Oecologica 62: 289-298.

Hartwell, S.I., D.A. Wright, R. Takacs, and C.H. Hocutt. 1991. Relative respiration and feeding rates of oyster and brackish water clam in variously contaminated waters. Marine Pollution Bulletin 22: 191-197.

Hatch, W.R., and W.L. Ott, 1968. Determination of sub-microgram quantities of mercury by atomic absorption spectrophotometry. Analytical Chemistry 40: 2085-2087.

Ivanina, A.V., C. Taylor, and I.M. Sokolova. 2009. Effects of elevated temperature and cadmium exposure on stress protein response in eastern oysters *Crassostrea virginica* (Gmelin). Aquatic Toxicology 91: 245-254.

Kehrig, H.A., M. Costa, I. Moreirac and O. Malma. 2006. Total and Methyl
Mercury in Different Species of Molluscs from Two Estuaries in Rio de
Janeiro State.   Journal of the Brazilian Chemical Society   17: 1409-1418.

Khoury, J.N., E. Powers, P. Patnaik, and W.G. Wallace. 2009. Relating disparity in
competitive foraging behavior between two populations of fiddler crabs to
subcellular partitioning of metals. Archives of Environmental Contamination
and Toxicology   56: 489-499.

Klajovic-Gaspic, Z., N. Odzak, I. Ujevic, T. Zvonaric, M. Horvat, and A. Baric.
2006. Biomonitoring of mercury in polluted coastal areas using transplanted
mussels. Science of the Total Environment 368: 199-209.

Kurlansky, M. 2006. The Big Oyster: History on the Half Shell. Random House,
New York, NY.

Lanning, G., A.S. Cherkasov, H.O. Portner, C. Bock, and I.M. Sokolova. 2008.
Cadmium-dependent oxygen limitation affects temperature tolerance in
eastern oysters (*Crassostrea virginica* Gmelin). American Journal of
Physiology: Regulated, Integrative, and Comparative Physiology 294: 1338-
1346.

Levinton, J.S. and J. R. Waldman. 2006. The Hudson River Estuary: Executive
Summary. pp. 1-13 *in*: Levinton, J.S. and J.R. Waldman (Eds). The Hudson
River Estuary. Cambridge University Press, New York.

Li, Y., J.G. Qin, X. Li, and K. Benkendorff. 2009. Monthly variation of condition
index, energy reserves, and antibacterial activity in Pacific oysters,
Crassostrea gigas, in Stansbury (South Australia). Aquaculture   286: 64-71.

MacDonald, B.A., and J.E. Ward. 1994. Variation in food quality and particle
selectivity in the sea scallop *Placopecten magellanicus* (Mollusca: Bivalvia).
Marine Ecology Progress Series   108: 251-264.

Mouneyrac, C., J.C. Amiard, and C. Amiard-Triquet. 1998. Effects of natural
factors (salinity and body weight) on cadmium, copper, zinc, and
metallothionein-like protein levels in resident populations of oysters
Crassostrea gigas from a polluted estuary. Marine Ecology Progress Series
162: 125-135.

Nelson, K.A., L.A. Leonard, M.H. Posey, T.D. Alphin, and M.A. Mallin. 2004.
Using transplanted oyster (*Crassostrea virginica*) beds to improve water
quality in small tidal creeks: a pilot study. Journal of Experimental Marine
Biology and Ecology   298: 347-368.

Newell, R.I.E. 2004. Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: A review. Journal of Shellfish Research 23: 51-61.

O'Connor, T.P. 2002. National distribution of chemical concentrations in mussels and oysters in the USA. Marine Environmental Research 53: 117-143.

Parsons, T.R., Y. Maita, and C.M. Lalli. 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Oxford.

Paterson, K.J., M.J. Schreider, and K.D. Zimmerman. 2003. Anthropogenic effects on seston quality and quantity and the growth and survival of Sydney rock oyster (*Saccostrea glomerata*) in two estuaries in NSW, Australia. Aquaculture 221: 407-426.

Pazos, A.J., C. Ruiz, O. Garcia- Martin, M. Abad, and J.L. Sanchez. 1996. Seasonal variations of the lipid content and fatty acid composition of *Crassostrea gigas* cultured in El Grove, Galicia, N.W. Spain. Comparative Biochemistry and Physiology 114B: 171-179.

Pridmore, R.D., D.S. Roper, and J.E. Hewitt. 1990. Variation in composition and condition of the Pacific oyster, *Crassostrea gigas*, along a pollution gradient in Manukau Harbour, New Zealand. Marine Environmental Research 30: 163-177.

Ringwood, A.H., D.E. Conners, and A. DiNovo. 1998. The effects of copper exposures on cellular responses of oysters. Marine Environmental Research 46: 591-595.

Ringwood, A.H., J. Hoguet, C. Keppler, and M. Gielazyn. 2004. Linkages between cellular biomarker responses and reproductive success in oysters- *Crassostrea virginica.* Marine Environmental Research 58: 151-155.

Roesijadi, G. 1996. Environmental Factors: Response to Metals. pp. 515-538 *in*: Kennedy, V.S., R.I.E. Newell, and A.F. Eble (Eds.) The Eastern Oyster, *Crassostrea virginica*. Maryland Sea Grant College. College Park, MD.

Sambrotto, R. 2001. Nitrogenous Nutrients and Plankton Production in Jamaica Bay. *in*: Integrated Reconnaissance of the Physical and Biogeochemical Characteristics of Jamaica Bay: Initial Activity Phase (A Coordinated Program of the Gateway National Recreational Area and the Columbia Earth Institute). Final Report.

Seidemann, D.E. 1991. Metal pollution in sediments of Jamaica Bay, New York, USA- An urban estuary. Environmental Management 15: 73-81.

Sokolova, I.M., E. P. Sokolov, and K. M. Ponnappa. 2005. Cadmium exposure affects mitochondrial bioenergetics and gene expression of key mitochondrial proteins in the eastern oyster *Crassostrea virginica* Gmelin (Bivalvia: Ostreidae). Aquatic Toxicology 73: 242-255.

Taylor, G.T, J. Way, and M. I. Scranton. 2003. Planktonic carbon cycling and transport in surface waters of the highly urbanized Hudson River estuary. Limnology and Oceanography 48: 1779-1795.

Volety, A.K. 2008. Effects of salinity, heavy metals, and pesticides on health and physiology of oysters in the Caloosahatchee Estuary, Florida. Ecotoxicology 17: 579-590.

Wakeman, T.H., and N.J. Themelis. 2001. A basin-wide approach to dredged material management in New York/ New Jersey Harbor. Journal of Hazardous Materials 85: 1-13.

Waldman, J. 1999. Heartbeats in the Muck. Lyons Press, New York, NY.

Wallace, W.G., B.G. Lee, and S.N. Luoma. 2003. Subcellular compartmentalization of Cd and Zn in two bivalves. 1: Significance of metal-sensitive fractions (MSF) and biologically detoxified metal (BDM). Marine Ecology Progress Series 249: 183-197.

Wirgin, I., J.S. Weis, and A.E. McElroy. 2006. Physiological and Genetic Aspects of Toxicity in Hudson River Species. pp. 441-460 in: Levinton, J.S. and J.R. Waldman (Eds). The Hudson River Estuary. Cambridge University Press, New York.

Yozzo, D.J., P. Wilber, and R.J. Will. 2004. Beneficial use of dredged material for habitat creation, enhancement, and restoration in New York- New Jersey Harbor. Journal of Environmental Management 73: 39-52.

Zar, J.H. 1999. Biostatistical Analysis. Upper Saddle River, NJ. Fourth edition.