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Quantifying the nutrient bioextraction capacity of restored eastern oyster populations in two coastal bays on Long Island, New York

A Thesis Presented

by

Daria Sebastiano

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Abstract of the Thesis

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Degradation of coastal areas due to eutrophication is becoming evident on a global scale. Like many estuaries, Jamaica Bay and Great South Bay, NY have been impacted by eutrophication at varying degrees. The eastern oyster, *Crassostrea virginica*, has been suggested as a way to remediate eutrophication through nutrient bioextraction. Eastern oyster populations were once abundant in both Jamaica Bay and Great South Bay. Overfishing, habitat destruction, and pollution have caused ecological extinction of populations in these regions. The abundance of oysters that once existed in these regions may drive the desire for restoration in these areas. Although appealing, invested individuals must be cautious to start restoration based on historical populations as indicators of potential for restoration. In order to determine the potential of eastern oysters to act as bioextraction tools in these areas a two year aquaculture-based assessment in Jamaica Bay, and a five month aquaculture-based assessment in Great Bay were implemented. Our assessments revealed fast growth rates in shell height and tissue growth in these regions. Mean values of shell and tissue growth in the first-year season of growth (mid-June to mid-October) in Jamaica Bay 2010 were approximately 50.40mm and 1.54g, respectively. In 2011 in Great South Bay mean values over the same time period were approximately 33.64mm and 0.84g. Results also showed high cumulative survivorship by the end of the first growing season. Average cumulative survivorship was 95.5% in Jamaica Bay and 75.5% in Great South Bay. Information from these physiological assessments was combined with a quantification of nutrient assimilation by oyster tissue and shell. Total nitrogen content of aquacultured oysters was of particular interest and was quantified by measuring total nitrogen content in the tissue and shell of oysters after the first growing season. Results reveal the average sized oyster to be approximately .19gN in Jamaica Bay and .11gN in Great South Bay.

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Chapter 1

A physiological assessment of *Crassostrea virginica* in two coastal bays on Long Island, NY

Introduction

The eastern oyster, *Crassostrea virginica* (Gmelin 1791) is found in the western Atlantic and has a northernmost limit in the St. Lawrence River estuary in eastern Canada. Populations exist south of this limit along the east coast of North America and through the Gulf of Mexico and the Caribbean. The southernmost limit of this species is found in Brazil (Carriker & Gaffney 1996). Oysters are often referred to as ecosystem engineers—organisms capable of biologically, chemically or physically altering the surrounding environment in a way that has potential to affect other organisms (Jones 1994). Ecosystem services provided by the eastern oyster are reviewed by Grabowski (2007) and Coen (2000) and include increased habitat complexity, carbon sequestration, stabilization of adjacent habitats and shorelines, increased fishery yields, water filtration, benthic pelagic coupling, and denitrification.

Jamaica Bay, New York once produced up to 700,000 bushels of oysters per year (Franz 1982). The thriving oyster industry in this region collapsed following the closure of oyster beds in response to public health concerns. Oyster beds soon became degraded due to sewage, industrial pollution, and harbor dredging. The rapid growth in human population and development has further reduced water quality and has rendered oyster populations in this region inconsequential. Temperature and salinity regimes within the bay remain favorable. Favorable environmental conditions in conjunction with New York City's current efforts to improve water quality may present potential for oyster restoration in the region.

Great South Bay, New York is found east of Jamaica Bay and once supported an oyster industry that began in the 1800s and prospered for over a century. Multiple events led to the demise of oyster populations in the mid-1900s. A coastal storm induced the opening of Moriches Inlet in 1930. This event increased the salinity of the Bay, allowing oyster predators to thrive and reduce the natural production of oysters (Nature Conservancy 2011). By the 1940s and 1950s blooms of small-form algae were linked to the input of organic wastes from duck farming (Ryther 1954). Natural oyster populations have not been able to recover in the Bay since these events. Suitable environmental factors such as salinity, and temperature still persist. Efforts to reduce organic waste input to improve water quality may present an opportunity for successful restoration in an area that once supported considerable oyster production.

Recent restoration efforts in multiple fields appear to have been moving towards ecosystem-based restoration that aims to restore functionality rather than individual species. The

many ecosystem services provided by the oyster suggest that this species may have a disproportionate effect on ecosystems in relation to its abundance. In addition to being a keystone species and ecosystem engineer, the oyster displays great resiliency. It is capable of surviving a wide range of salinities, temperatures and dissolved oxygen concentrations (Shumway 1996). Other life history traits that contribute to its resiliency include fast growth, early age of maturity, high fecundity, and multiple spawns per season. The significant effects this organism can have on the functionality of an ecosystem in addition to its resiliency make it a potentially suitable organism for restoration efforts.

The Hudson River Estuary (HRE) environmental restoration program was authorized by Congress in 1999. It exemplifies restoration efforts that attempt to restore specific ecosystem properties or features, referred to as target ecosystem characteristics in the HRE plan (Bain et al. 2007). One goal specified by the plan is the restoration of 500 acres of oyster reefs by 2015 and 5000 acres by 2050 in the Hudson River Estuary. In order to ensure the success of this and other restoration projects in the New York region it will be necessary to understand the physiological responses of oysters to the surrounding environment. Although historical populations are an indicator that this environment was once suitable, changes in water quality and habitat may have altered the species' ability to persist and grow in the two bays studied in this assessment. The objective of these studies is to make a physiological assessment of the eastern oyster in aquaculture based experiments in Jamaica Bay and Great South Bay.

Methods & Materials

Study Areas

Jamaica Bay, NY

Jamaica Bay is a shallow, highly eutrophic estuary located within an extensively urbanized portion of southwestern Long Island, New York (Figure 1). The Bay serves as contrast to the highly developed and densely populated region of New York City that surrounds it. Jamaica Bay drains parts of Brooklyn, Queens and Nassau County and discharges to the Atlantic Ocean via Rockaway Inlet. It has a residence time of approximately 35 days (National Academy of Sciences 1971). Four waste water treatment plants encircle the bay and are principal contributors to the degradation of water quality by means of high nitrogen input (Kinney & Valiela 2011).

Three sites within Jamaica Bay were selected and established along a longitudinal gradient (Figure 1, Table 1). The sites span from the high energy western portion of the bay that undergoes extensive mixing and oceanic influence to the low energy eastern portion of the bay that experiences poor mixing and small oceanic influence. Water quality and phytoplankton biomass varies along the east-west gradient.

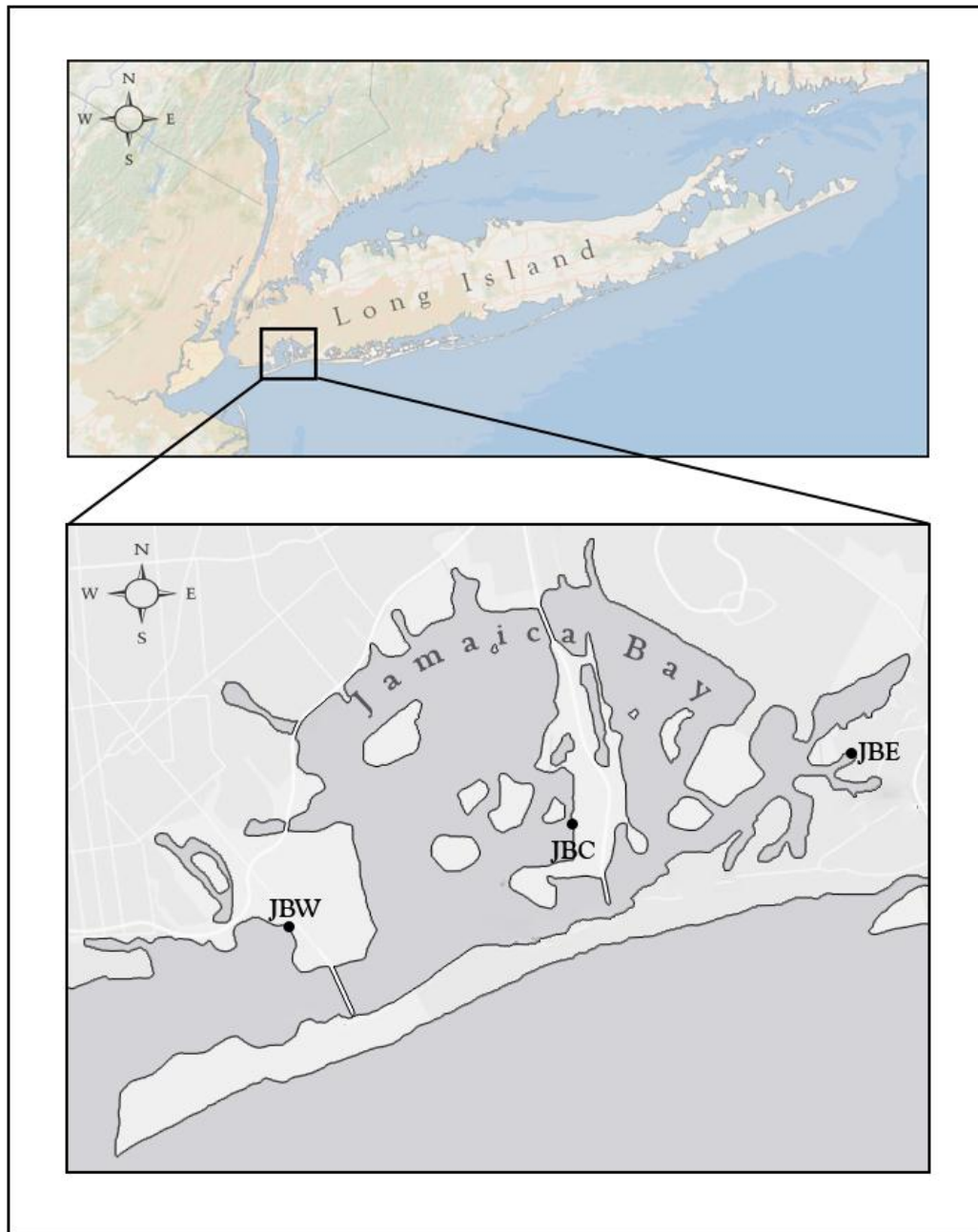


Figure 1 (Top) Southwestern Long Island; the location of the Jamaica Bay study area. (Bottom) Study site locations within Jamaica Bay, NY.

Table 1 Site names, IDs, descriptions, and coordinates for study sites in Jamaica Bay, NY.

Site Name	Site ID	Site Description	Latitude	Longitude
Jamaica Bay West	JBW	Gateway marina; Brooklyn, NY Floating dock	40°34'59.78" N	73°54'00.82" W
Jamaica Bay Central	JBC	Personal residence; Broad Channel, NY Floating dock	40°36'16.24" N	73°49'03.11" W
Jamaica Bay East	JBE	Inwood marina; Inwood, NY Floating dock	40°37'02.56" N	73°45'28.44" W

Oyster seed was obtained from Fishers Island Oyster Farm. Oysters spawned and larvae settled in the summer of 2009. Seed was then overwintered in waters adjacent to the oyster farm and transferred to 10mm semi-rigid polyethylene mesh grow-out bags in late June of 2010. The dimensions of these grow-out bags are 94x43x7.6cm. At the time of deployment the shell height of these oysters averaged approximately 29mm. Each bag contained 300 oysters—a density comparable to that of a restored population. Two bags were placed within each two-tiered wire cage. The lower tier is several centimeters below the upper tier. The cage has feet approximately 20cm. Feet ensure that bags are never directly resting in the sediment. Two cages were placed at each of the three sites, yielding a total of 1,200 oysters per site. These cages were suspended at approximately 1-2m depth from docks at each location. Cages remained submerged at average low tide level.

This study took place from June 2010 until October 2011. Sites were monitored bi-weekly from April to November—when oysters experience greatest growth. Monitoring was carried out monthly for the remainder of the year. Monitoring at each site took place within the same week to minimize temporal variation in the data. During site visits, temperature, salinity, dissolved oxygen concentration, chlorophyll *a* concentration, mortality, and shell growth data were collected. Dead oysters were not replaced. Fouling was controlled during each visit by cleaning cages and bags with wire brushes.

Great South Bay, New York

Great South Bay is a shallow barrier beach estuary on the south shore of Long Island, New York. The Bay is located approximately halfway between New York City and Montauk Point (Figure 2). Water exchanges with the Atlantic Ocean primarily through the narrow Fire Island Inlet, and residence time of water in the Bay is approximately 96 days (Conley 2000). Although the surrounding watershed is not as developed and densely populated as Jamaica Bay, increasing urbanization may elicit concern for degradation of water quality in the near future. Current estimates classify the bay as being low to moderately eutrophic (Kinney & Valiela 2011).

Three sites were chosen along a longitudinal gradient. Two of the sites were located on the northern shore of the Bay, and one on the southern shore (Figure 2, Table 2). Higher salinities can be found at sites closer to the Fire Island inlet. A reference site (Figure 2) was

selected in the highly eutrophic estuary of Jamaica Bay in order to observe how varying levels of eutrophication may affect oyster physiology and assimilation of nutrients.

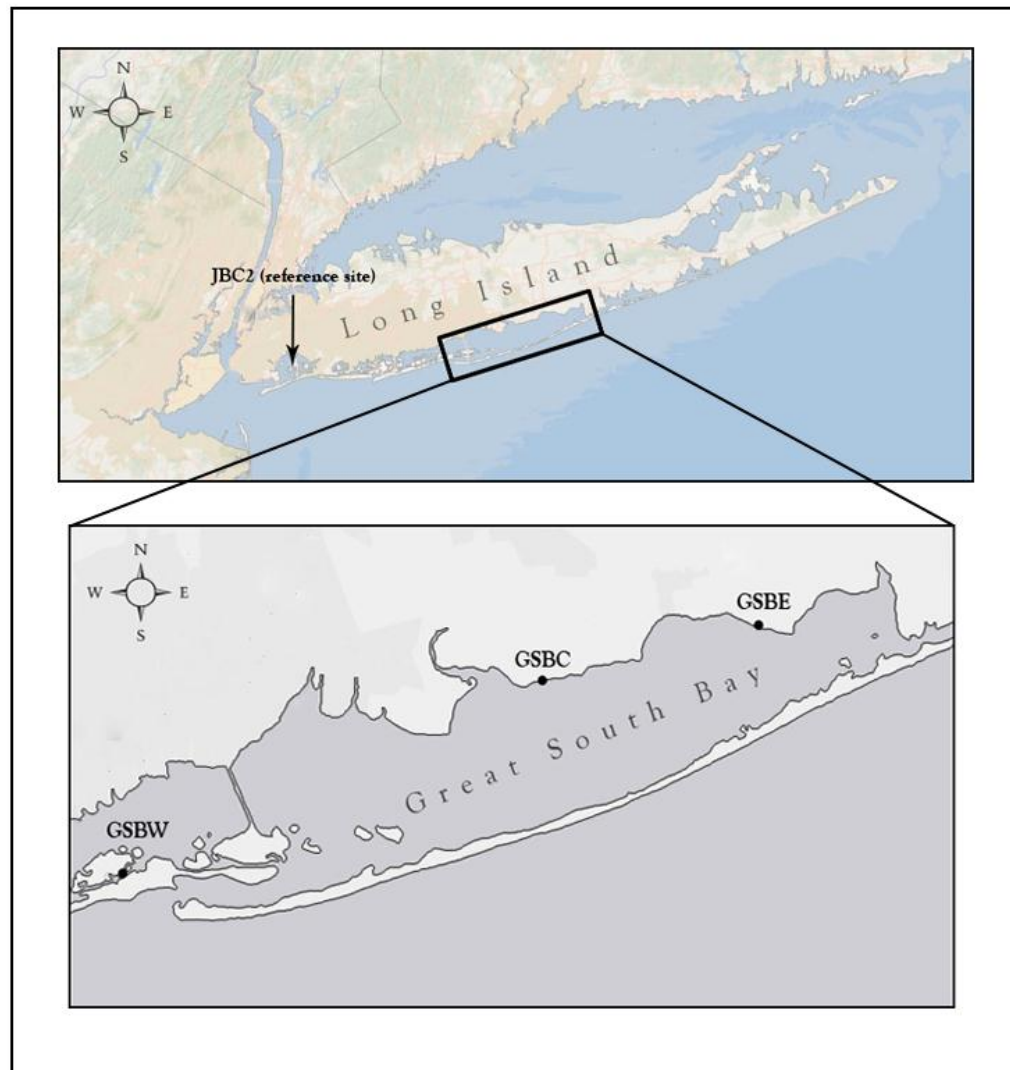


Figure 2 (Top) Southern Long Island; the location of the Great South Bay study area and a reference site located in central Jamaica Bay. (Bottom) Study site locations within Great South Bay, NY.

Table 2 Site names, IDs, descriptions, and coordinates for study sites in Great South Bay, NY and Jamaica Bay reference site.

Site Name	Site ID	Site Description	Latitude	Longitude
Great South Bay West	GSBW	Cedar Beach Marina; Babylon, NY Floating dock	40°38'08.36" N	73°20'32.30" W
Great South Bay Central	GSBC	West Sayville Boat Basin; West Sayville, NY Floating dock	40°43'15.73" N	73°05'29.99" W
Great South Bay East	GSBE	Shores Yacht Basin; Patchogue, NY Floating dock	40°36'16.24" N	73°49'03.11" W
Jamaica Bay Central (reference)	JBC2	Personal Residence; Broad Channel, NY Floating dock	40°44'58.08" N	72°58'34.80" W

Oyster seed were obtained from Fishers Island Oyster Farm. Oysters spawned and seed settled in the summer of 2010. Oysters were then overwintered in waters adjacent to the farm and transferred to 10mm semi-rigid polyethylene mesh grow-out bags in late June of 2011. The dimensions of these bags were 94x43x7.6cm. At the time of deployment oyster shell height averaged approximately 31mm. Each bag contained 300 oysters—a density comparable to that of a restored population. Two bags were placed within each two-tiered wire cage. Two cages were placed at each of the three sites, yielding a total of 1,200 oysters per site. These cages were suspended at approximately 1-2m depth from docks at each location. Cages remained submerged at average low tide level.

This study took place from June to October 2011. Sites were monitored bi-weekly throughout the project. All sites were visited within the same week to minimize temporal variation. During site visits, temperature, salinity, dissolved oxygen concentration, chlorophyll *a* concentration, mortality, and shell growth data were collected. Dead oysters were not replaced. Fouling was controlled during each visit by cleaning cages and bags with wire brushes.

Environmental Parameters

Four environmental parameters were measured and recorded during biweekly monitoring visits. Temperature, salinity and dissolved oxygen concentration were recorded at surface, cage depth, and bottom using a handheld YSI model 85 environmental TSO meter. Temperature was also logged using a TidbiT v2 water temperature data logger that was attached to the outside top of one cage at each locality. The logger recorded temperature once every fifteen minutes.

Water samples for chlorophyll *a* were collected several centimeters below the water surface and vacuum filtered through Whatman GF/F filters. Water was filtered on site when possible. Filters were placed into conical screw cap tubes and placed in a -80°C freezer until they were analyzed. Chlorophyll *a* concentration was determined for four size fractions: whole sea water, 2µm, 5µm, and 20µm. In order to obtain these size fractions water was filtered through filters with four different pore sizes. Each size treatment had three replicates. These measurements were made using standard fluorometric techniques described by Strickland and

Parsons (1972) and a Turner Designs Trilogy fluorometer. Concentration of chlorophyll *a* >5µm was used as a proxy for food availability since particles larger than 6µm in diameter have uniformly high retention efficiencies by the gills (Haven and Morales-Alamo 1970).

Physiological Measurements

Survivorship

The number of dead and live oysters was counted for each bag in order to calculate a cumulative survivorship between biweekly visits for each site. Gaping and boxed oysters were considered dead; solid, closed oysters were considered alive. Once a month ten oysters per bag were haphazardly collected without replacement to be used for condition index analysis. In making survivorship calculations it was assumed that these individuals had the same probability of surviving as the remaining oyster population. Predation was not accounted for in this study and it was considered to be negligible due to the containment of oysters in small-mesh bags. No evidence of crushed or drilled shells was observed.

Growth & Condition

Shell growth

In this study oyster growth and condition was analyzed using three metrics: shell height, condition index and dry tissue weight. During each visit oysters (n=20) from each bag were haphazardly sampled, and measurements of shell height (anterior to posterior), length (dorsal to ventral) and width (across two valves) were made using digital calipers that are accurate to 0.01mm. These oysters were replaced to the grow-out bag they were sampled from. Measuring valve size is a simple and non-destructive way to measure growth in oysters. During each visit shell growth measurements were averaged for all oysters on a per site basis. This allowed for comparisons of seasonal growth between sites.

Condition Index

Condition index (CI) is a ratio of dry meat weight to the internal capacity of the shell. It is used to indicate how well the organism has utilized the total volume that is available for tissue growth (Higgins 1938). The method for gravimetrically determined condition index was used in this study because it is shown to have the least chance for measuring errors, a lower coefficient of variation, and is the easiest and fastest method (Crosby & Gale 1990). Samples for condition index were taken once a month in order to address concerns of depleting the population due to destructive sampling.

Oysters (n=10) were haphazardly selected from each bag and brought back to the lab for analysis of condition index. Before measuring, all oysters were cleaned of any fouling organisms and sediment. The whole oyster live weight (g), shucked dry shell weight (g), and dry soft tissue weight (g) were recorded for each oyster using a balance that is accurate to 0.001 g.

Soft tissue was dried at 70°C for a minimum of seven days before weighing. The formula used to calculate condition index is given by A.E. Hopkins and is described by Higgins (1938) as follows:

$$\text{Condition Index} = (\text{dry meat weight (g)}) (100) / (\text{internal capacity volume in cm}^3)$$

The internal capacity volume is determined by subtracting the weight in air of the oyster's valves from the weight in air of the oyster live weight. This is a valid method under the assumption that the effective density of the cavity contents is approximately 1 g cm⁻³ (Lawrence 1982). The dry tissue samples were stored in an oven after being weighed and were later used for analysis of carbon and nitrogen content. In addition to these measurements, gonadal tissue smears were conducted on all of the sampled oysters in order to determine the sex and ripeness of each individual. Gonadal smears were made by extracting small samples of the gonad. Smears were then examined microscopically in order to determine the sex and ripeness of the oyster.

Dry Tissue Weight

Tissue samples used for the analysis of condition index were dried at 70°C for at least seven days, which allowed for the complete dehydration of tissues. Dry tissue was weighed using a balance that is accurate to 0.001 g. The weight (g) of these samples was averaged for each condition index sampling date on a per site basis.

Analysis of data

Growth in shell and dry tissue weight were used as a measure of overall physiological response between sites during the first growing season (initial planting to October). A significance level of $\alpha = 0.05$ was used for all comparisons between sites. All non-transformed data for shell height passed tests for normality (Shapiro-Wilk) and equal variance (Levene's test). A one-way ANOVA was used to compare mean shell height in the Jamaica Bay and Great South Bay studies. The Holm-Sidak method was used as a post-hoc test for multiple pairwise comparisons between sites.

Data for dry tissue weight for the Jamaica Bay and Great South Bay study areas passed tests for equal variance but failed tests for normality. The non-parametric Kruskal-Wallis test was used for between site comparisons of dry tissue weight. Post-hoc analysis was carried out to make multiple pairwise comparisons between sites. A Tukey test was used for Jamaica Bay data and Dunn's method was used for Great South Bay data to account for varying sample sizes due to a lost bag at the GSBE site. All statistical analyses were obtained using SigmaPlot[®] 11.0.

Results

Jamaica Bay

Environmental Parameters

Temperature

The three study sites in Jamaica Bay followed similar monthly and seasonal trends in temperature at cage depth (Figure 3). JBW had more moderate summer and winter temperatures in comparison to the other two sites. This was expected due to its proximity to the inlet. Temperatures during this two year study fell within the typical temperature range of tolerance for adult oysters. This range is reported as -2°C to 36°C (Galtsoff 1964). Maximum temperatures were observed to be approximately 32°C during mid-July; minimum temperatures were approximately -1.5°C during mid-January. Temperatures were $<5^{\circ}\text{C}$ from early January to early March. Galtsoff (1928) reported that under these conditions no feeding or current was produced and oysters were in a state of hibernation. According to observed temperatures in Jamaica Bay, the potential growth season for oysters is approximately nine months out of the entire year. Missing data are due to broken or malfunctioning temperature loggers.

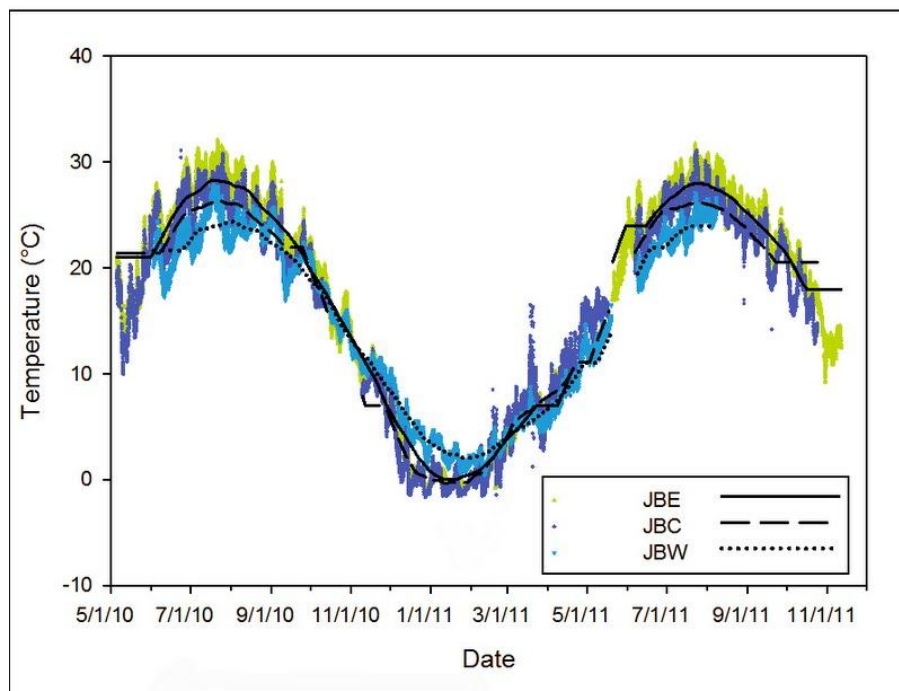


Figure 3 Seasonal water temperature ($^{\circ}\text{C}$) in the Jamaica Bay study area. Black lines represent running averages that were calculated using a smoothing algorithm (0.1 sampling proportion) in Sigmaplot[®] 11.0.

Salinity

The salinities at cage depth observed in Jamaica Bay during this study period ranged from 21 to 29ppt (Figure 4). Galtsoff (1964) suggested that the eastern oyster has an optimal salinity range of 14 to 28ppt. With the exception of several dates that lie slightly outside of this range, all sites were within the optimal salinity range for the duration of the study. Highest salinities were generally observed during summer due to high rates of evaporation. JBW consistently experienced the highest salinities throughout the study due to oceanic proximity. The greatest variation in salinity was observed at JBE.

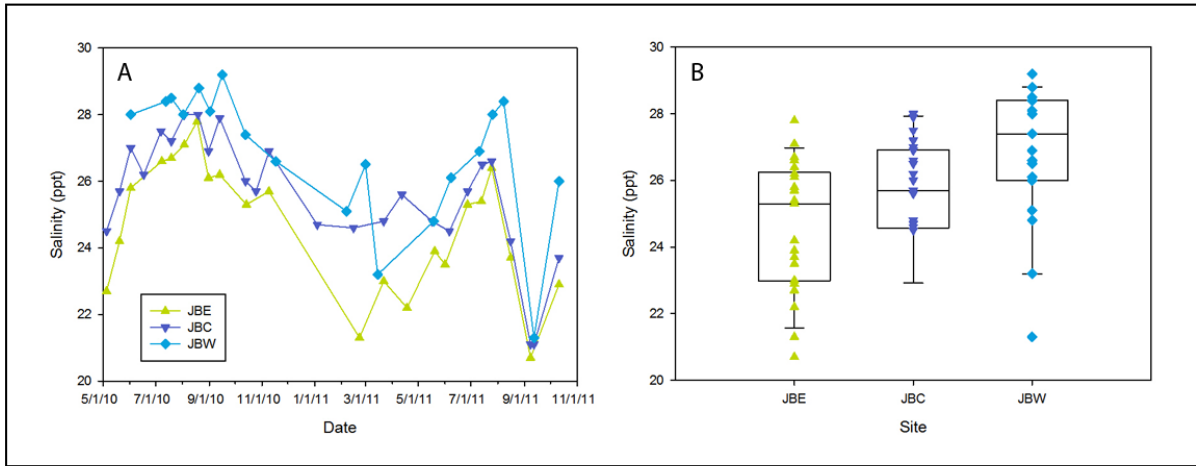


Figure 4 Salinity (ppt) at cage depth in Jamaica Bay. (A) Seasonal trends in salinity. (B) Comparison of salinity between sites. Boxplots are representative of median, and upper and lower quartiles.

Dissolved oxygen

Seasonal trends in oxygen at cage depth showed a range of 2.32 to 18.31mg/L with the exception of one hypoxic event in which dissolved oxygen was 0.07mg/L at JBC in July 2011 (Figure 5). It is unknown how long this hypoxic event lasted, but fish kills and ‘crab jubilees’ were evident during the monitoring visit. This event occurred after successive, unusually high temperatures. Dissolved oxygen concentrations were generally lowest during times of elevated temperature. Median dissolved oxygen levels were similar between sites.

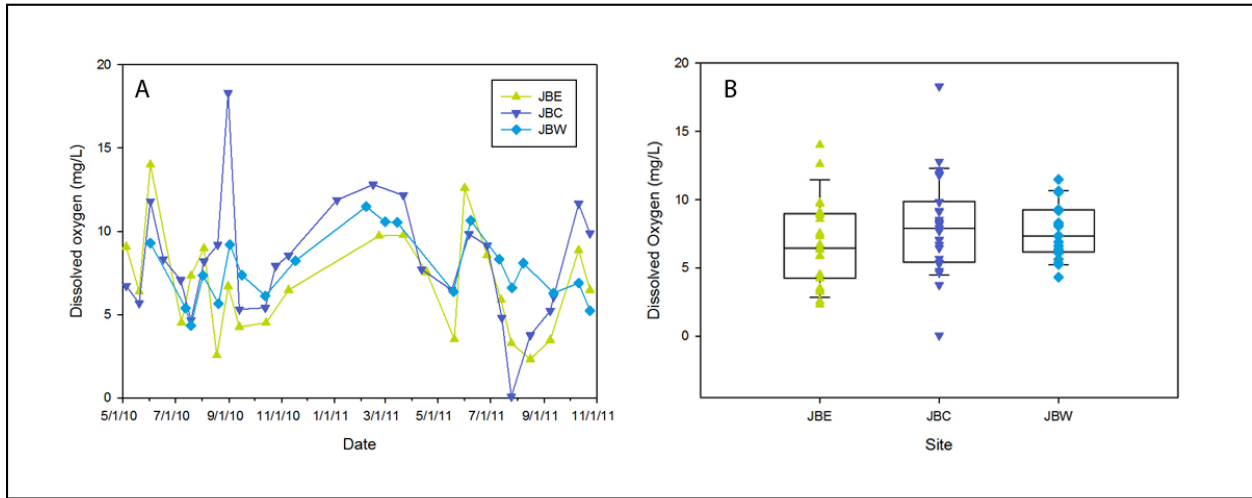


Figure 5 Dissolved oxygen concentrations (mg/L) at cage depth in Jamaica Bay. (A) Seasonal trends in dissolved oxygen concentration. (B) Trends in dissolved oxygen concentration per site. Boxplots are representative of median, and upper and lower quartiles.

Chlorophyll *a*

Two phytoplankton blooms were evident in Jamaica Bay (Figure 6). The first bloom occurred in winter/spring and was characterized by greater production than the second bloom in summer. In both years, the summer bloom started in June, peaked in August, and diminished by September. The winter bloom began in January, peaked in March and diminished by April. The summer bloom in 2010 was two times greater in magnitude than the 2011 bloom. The proportion of available food $> 5\mu\text{m}$ ranged from 99.83 to 25.93% and followed similar trends as total chlorophyll *a* concentration. Median values of chlorophyll *a* concentrations are similar for the east and central Jamaica Bay sites (Figure 7); they are both consistently higher than the west site, with the exception of the $>20\mu\text{m}$ size fraction. Across all size fractions JBC experienced the greatest variation in chlorophyll *a* concentrations. The least amount of variance was observed at the JBW site for all size fractions.

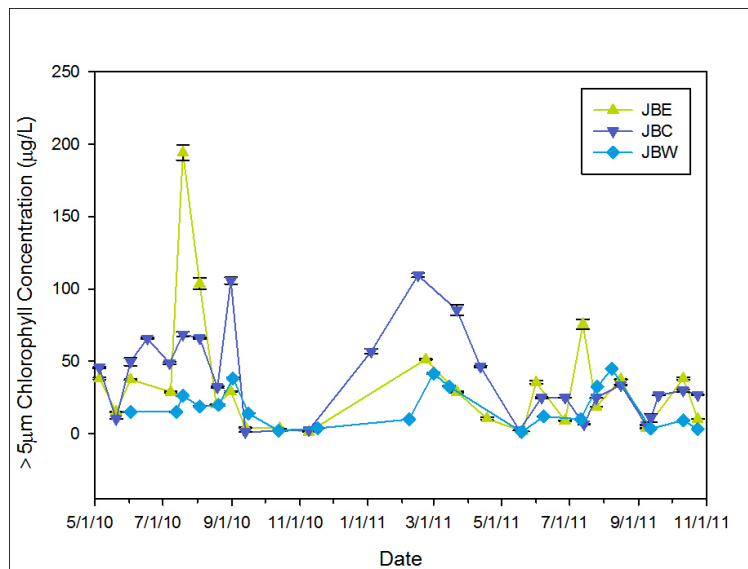


Figure 6 Seasonal trends in chlorophyll *a* $>5\mu\text{m}$ concentration ($\mu\text{g/L}$) at the Jamaica Bay study sites. Error bars represent $\pm\text{SE}$.

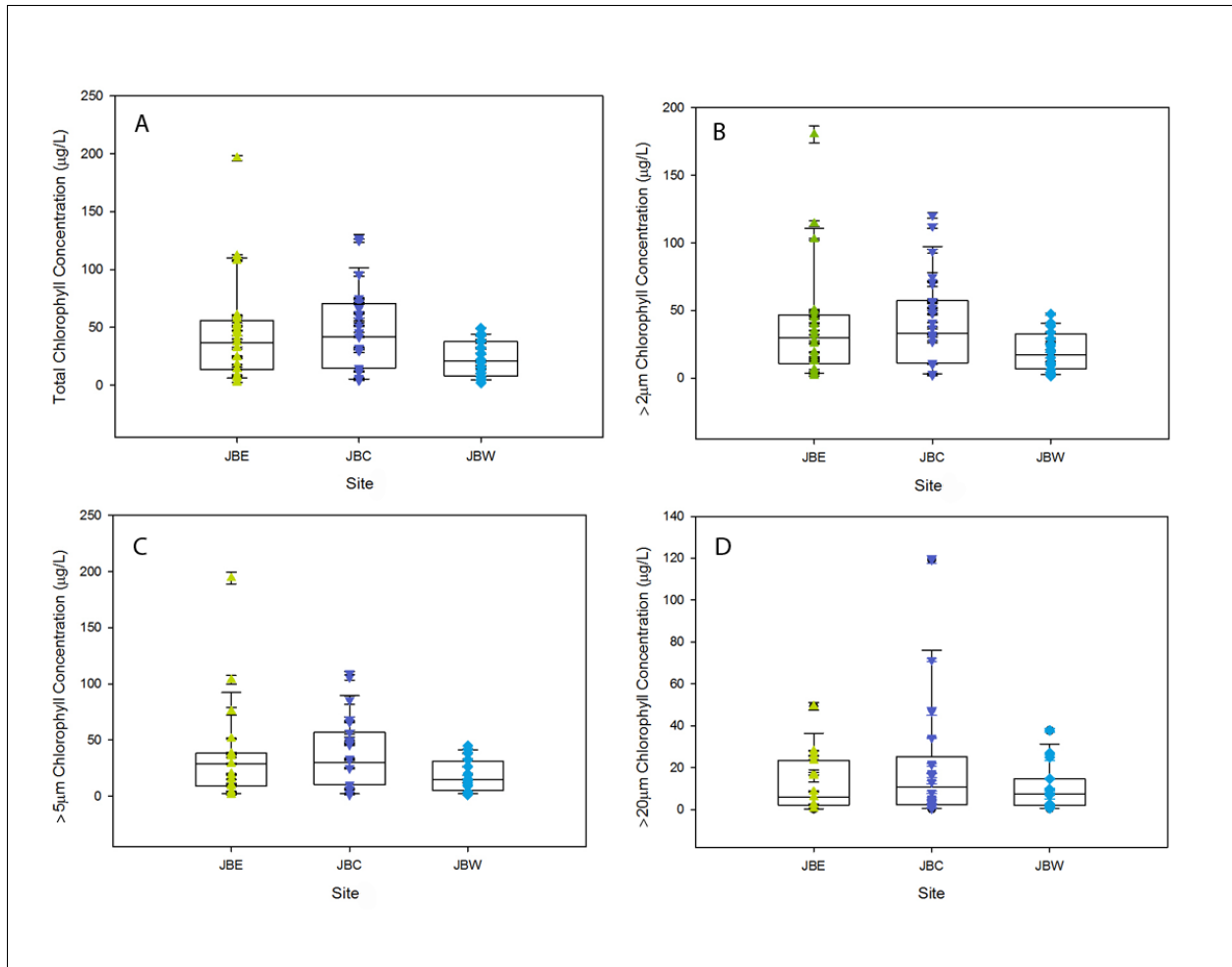


Figure 7 Site comparisons of chlorophyll *a* concentrations (µg/L) of varying size fractions at the Jamaica Bay study sites. (A) Total chlorophyll *a* concentration (µg/L). (B) >2µm chlorophyll *a* concentration (µg/L). (C) >5µm chlorophyll *a* concentration (µg/L). (D) >20µm chlorophyll *a* concentration (µg/L). Boxplots are representative of median, and upper and lower quartiles. Error bars are ±SE.

Physiological measurements

Survivorship

Cumulative survival of oysters in Jamaica Bay ranged from 93.5 to 98.3 % after the first growing season (June to October) (Figure 8). Greatest survival was observed at JBW and lowest observed at JBC. Survival rates decreased only slightly as the season progressed in 2010 and temperature increased. Survival continued to be stabilized at the onset of cooler water temperatures and minimal mortality occurred during the winter. As temperature began to increase in the second year survival rates began to drastically decrease. The greatest decrease in survival during the second year was observed at JBC and it occurred at the same time as the recorded anoxic event that occurred in early August. By the end of the study the survivorship range in Jamaica Bay was 17.79 to 47.34%.

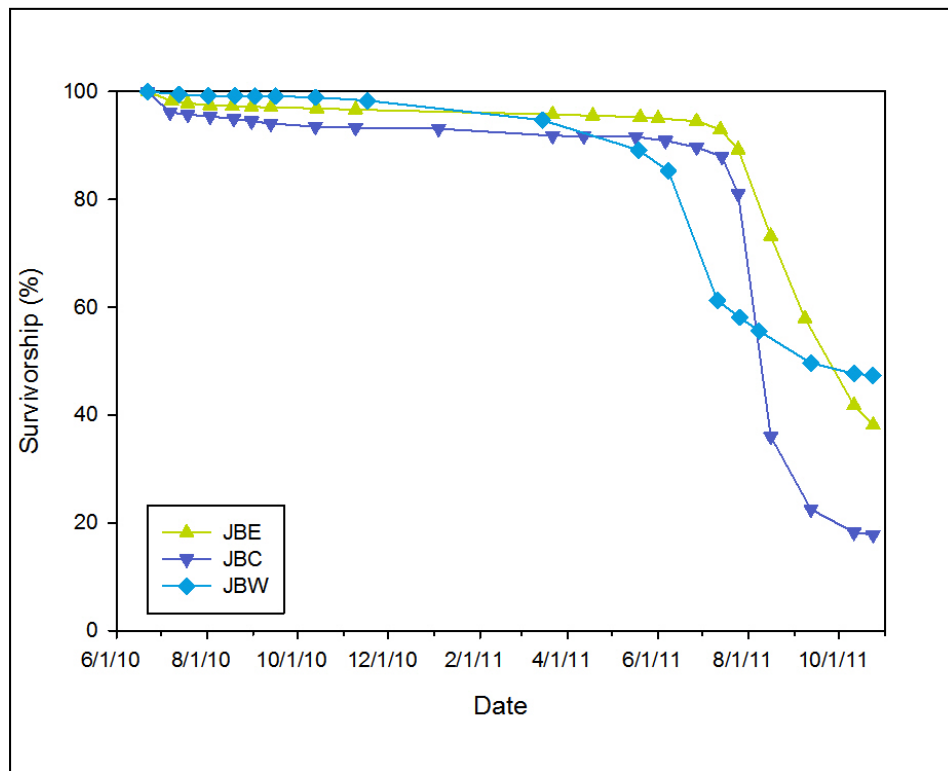


Figure 8 Seasonal trends of survivorship (%) at each site in Jamaica Bay.

Shell and tissue growth

Shell height is used as a proxy for overall trends in shell growth (Figure 9a). Shell height was strongly correlated with shell length ($r = 0.863$, $p < 0.001$) and shell width ($r = 0.857$, $p < 0.001$). Increases in shell height were evident from mid-May until early November. Oysters were observed to grow approximately seven months out of the entire year. No growth was evident from November to May. The greatest growth was observed at JBW. A one-way ANOVA reveals significant differences between sites ($p < 0.001$) after the first growing season. A post-hoc pairwise analysis using the Holm-Sidak method revealed significant differences between all pairwise comparisons except that there was no significant difference in shell height between JBC and JBE ($p = 0.117$).

Growth in tissue as determined by dry tissue weight follows similar seasonal patterns as shell growth (Figure 9b). However, there is a decrease in tissue weight after the overwintering period, and growth does not recommence until June-August, depending on the site. As with shell growth, the greatest growth was experienced at JBW. A Kruskal-Wallis one-way analysis of variance revealed significant differences in mean dry tissue weight between sites ($p = 0.010$) after the first season of growth (initial planting to mid-October). A post-hoc analysis using a Tukey test revealed significant differences ($p < 0.001$) between all pairs of sites except for the comparison between JBW and JBC.

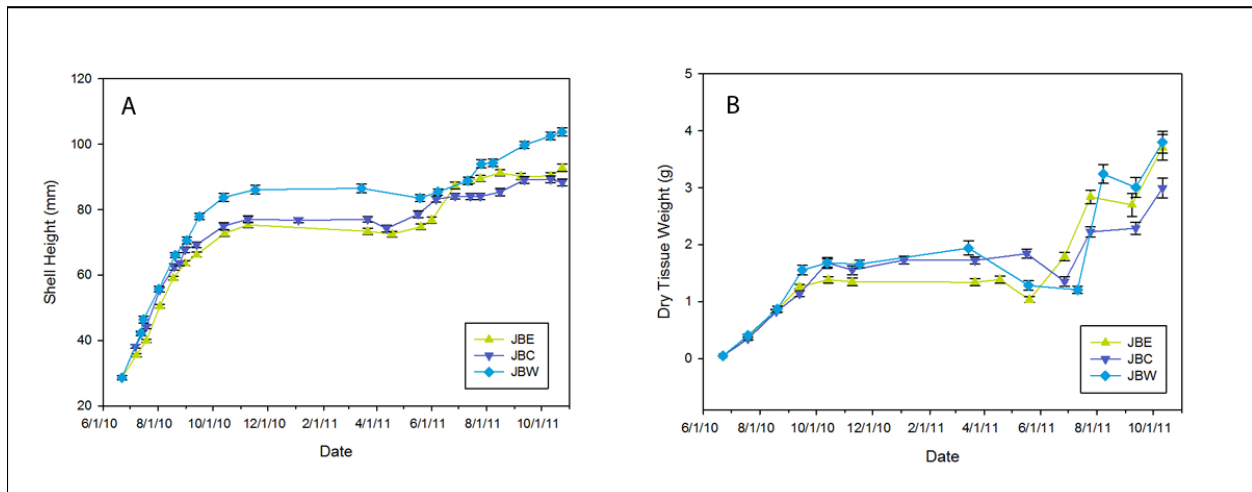


Figure 9 (A) Seasonal trends in mean shell height (mm) from Jamaica Bay study sites. (B) Mean dry tissue weight (g) from Jamaica Bay study sites. Error bars are \pm SE.

Condition index

Condition index increased through July but then decreased, coinciding with spawning and then high summer temperatures and possibly low fall food concentrations (Figure 10). After planting in the first year, almost all oysters were sexually differentiated by mid-July. The sex of almost all oysters were unknown by mid-September, possibly indicating that the sampled oysters were spawned out. These estimates of spawning period are similar to those suggested by (Loosanoff 1965) in a study of gonad development of the eastern oyster in Long Island Sound, Connecticut. Similar patterns were evident in the second year of the study, but condition index values are of lesser magnitude. There was also a decrease in condition after the overwintering period between May and July. Gonadal smears that were conducted suggested that oysters may have begun spawning in mid-July and stopped spawning in early October. These changes occur around the same time as decreases in condition index. Increases in condition index in early August coincide with increases in food availability that arises from the summer phytoplankton bloom. Condition index remains fairly stable over winter.

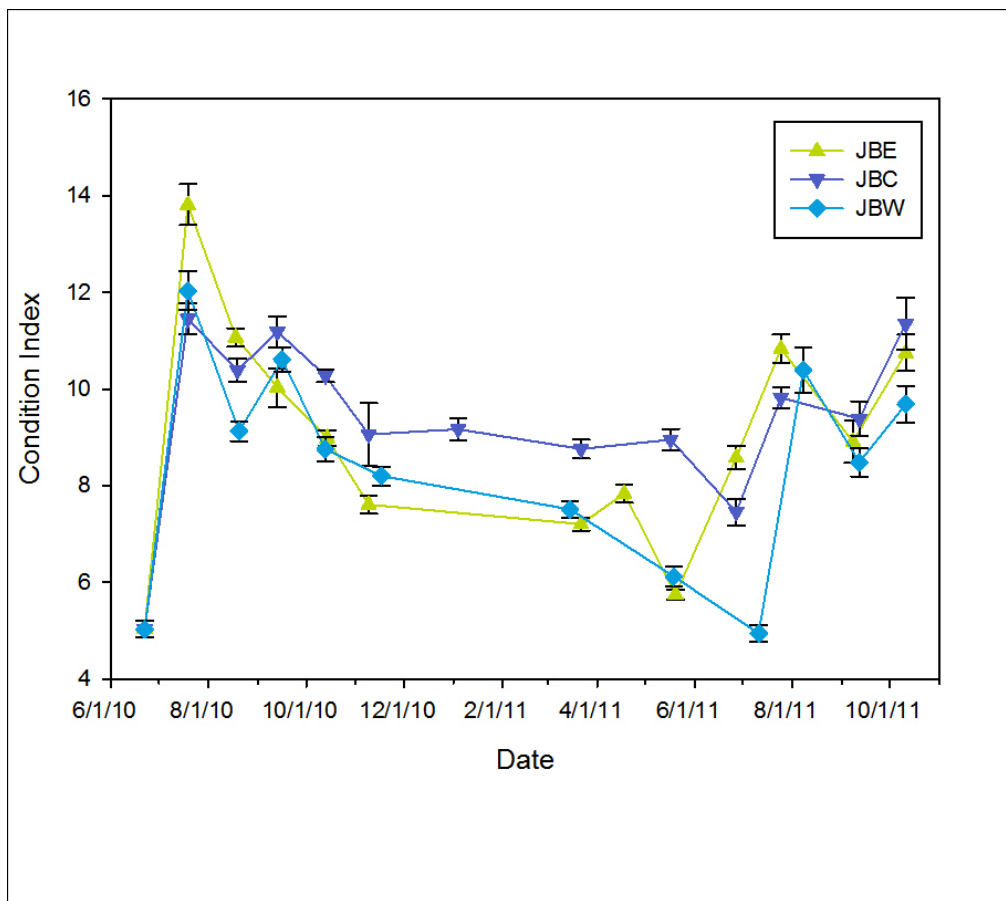


Figure 10 Seasonal trends of mean condition index at each site in Jamaica Bay. Error bars represent \pm SE.

Great South Bay

Environmental parameters

Temperature

Monthly and seasonal trends in temperature at cage depth were similar at all three Great South Bay study sites (Figure 11). The maximum temperature found across all sites was 30.7°C in mid-July; the minimum temperature was 15.3°C and was observed in late October. Stanley (1986) reported optimal temperatures for growth, reproduction, and survival of the American oyster to be approximately 20°C to 30°C. Temperatures in Great South Bay during this five month study period were within this range for approximately four months. Missing data is attributed to broken or malfunctioning data loggers.

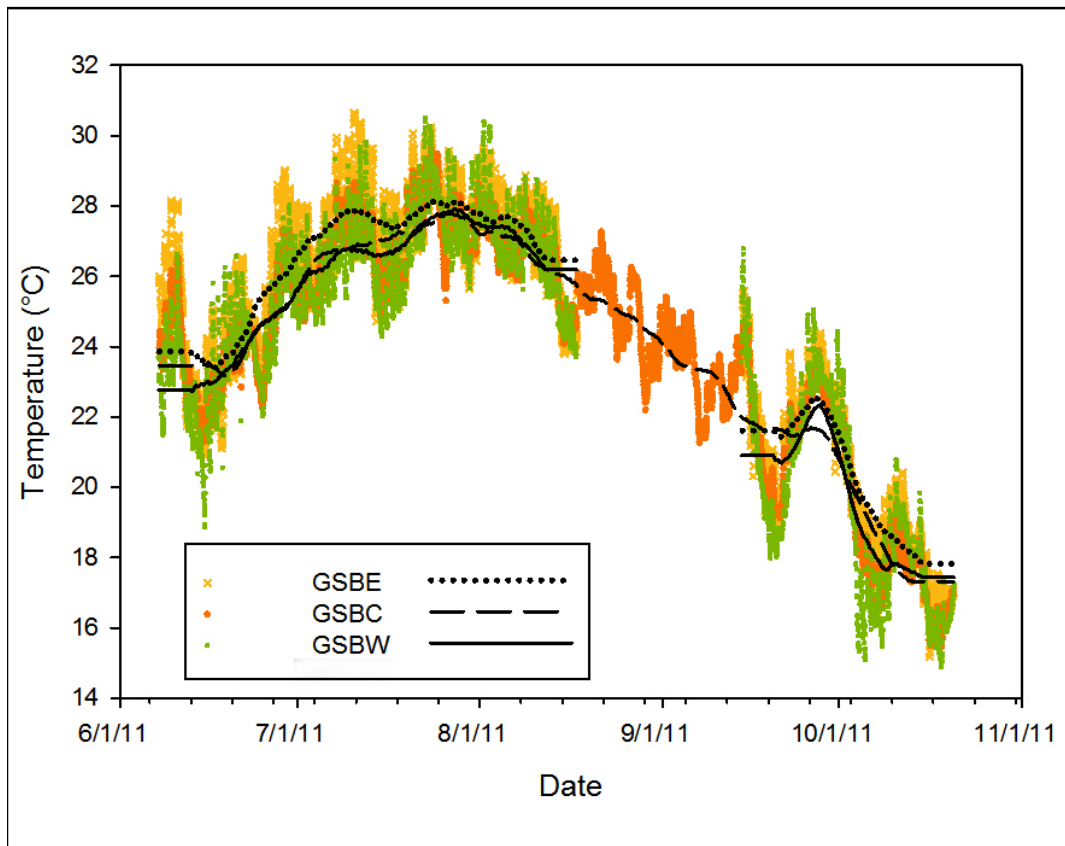


Figure 11 Seasonal water temperature (°C) trends in the Great South Bay study area. Black lines represent running averages that were calculated using a smoothing algorithm (0.1 sampling proportion) in Sigmaplot® 11.0.

Salinity

Salinity in Great South Bay ranged from 16 to 30ppt during the study (Figure 12). With the exception of several points from the GSBW site, the majority of salinity measurements at all sites remained within the optimal salinity range of 14 to 28ppt that was suggested by Galtsoff (1964). A considerable drop in salinity was observed in late June and early July at the GSBE site. Salinity dropped from approximately 23 to 16ppt. As distance from the Fire Island inlet decreased, median salinities were observed to increase. Salinity at the Jamaica Bay reference site (JBC2) was most similar to the central site in Great South Bay; however, it was slightly more variable.

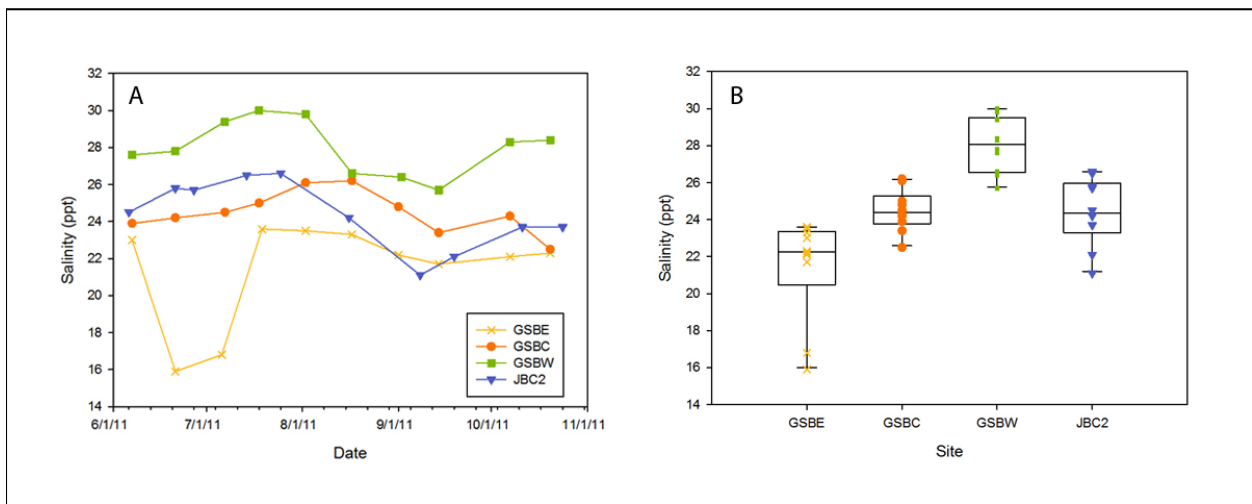


Figure 12 Salinity (ppt) at cage depth in Great South Bay sites and the Jamaica Bay reference site in 2011. (A) Seasonal trends in salinity. (B) Comparison of salinity between sites. Boxplots are representative of median, and upper and lower quartiles.

Dissolved oxygen

Dissolved oxygen levels at cage depth in Great South Bay ranged from 2.70 mg/L to 7.96 mg/L (Figure 13). The reference site (JBC2) ranged from 0.07 mg/L to 11.66 mg/L and showed greater variability than the GSB sites. All sites in GSB remained above hypoxic conditions for the duration of the study; JBC2 remained above levels of hypoxia with the exception of one hypoxic event that followed occurred at elevated temperatures. It is unknown how long this event persisted.

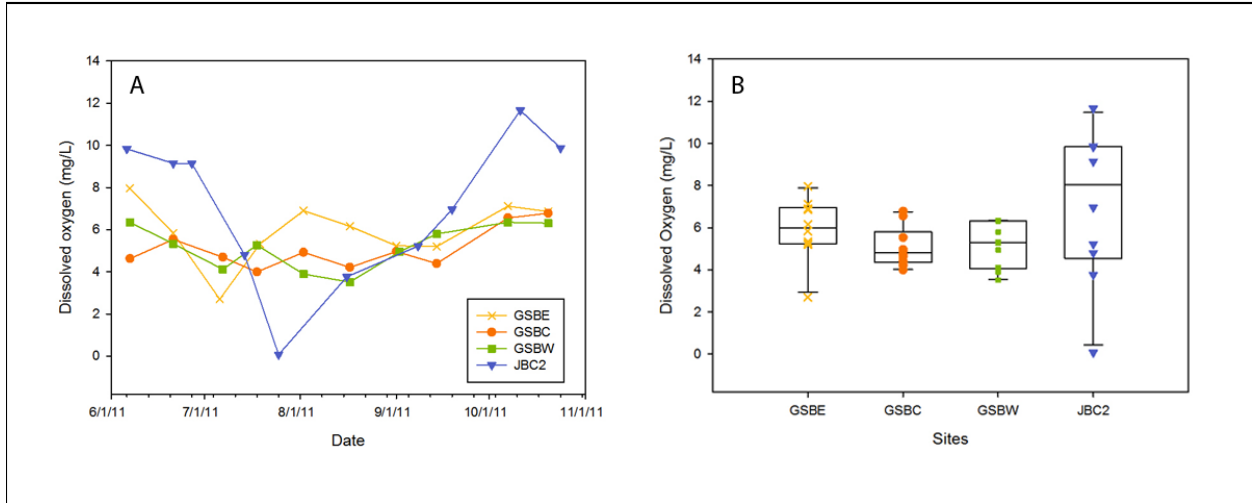


Figure 13 Dissolved oxygen concentrations (mg/L) at cage depth in Great South Bay and the Jamaica Bay reference site in 2011. (A) Seasonal trends in dissolved oxygen concentration. (B) Trends in dissolved oxygen concentration per site. Boxplots are representative of median, and upper and lower quartiles

Chlorophyll *a*

The presence of a summer bloom is not as well defined as the summer bloom observed in Jamaica Bay. Seasonal trends of $>5\mu\text{m}$ chlorophyll *a* concentration generally do not follow similar patterns among sites (Figure 14). All sites except GSBW experienced a peak in chlorophyll *a* in mid-October. Comparisons between sites show the greatest median value of chlorophyll *a* concentration to be JBC2 for all size fractions (Figure 15). Of the three Great South Bay sites, the east site generally had the greatest median value and greatest variation in chlorophyll *a* concentration for all size fractions. This trend is especially apparent in the $>5\mu\text{m}$ size fraction. The GSBW site consistently experienced the least amount of variation and lowest median concentration of chlorophyll *a*.

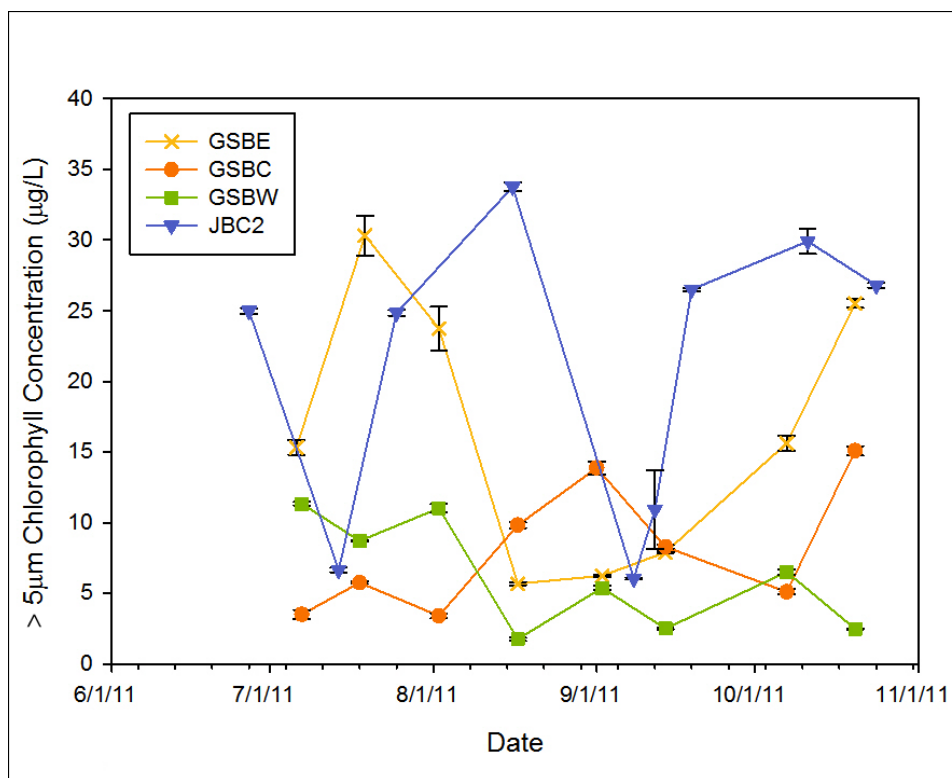


Figure 14 Seasonal trends in chlorophyll *a* $>5\mu\text{m}$ concentration ($\mu\text{g/L}$) at the Great South Bay study sites and Jamaica Bay reference site in 2011. Error bars are $\pm\text{SE}$.

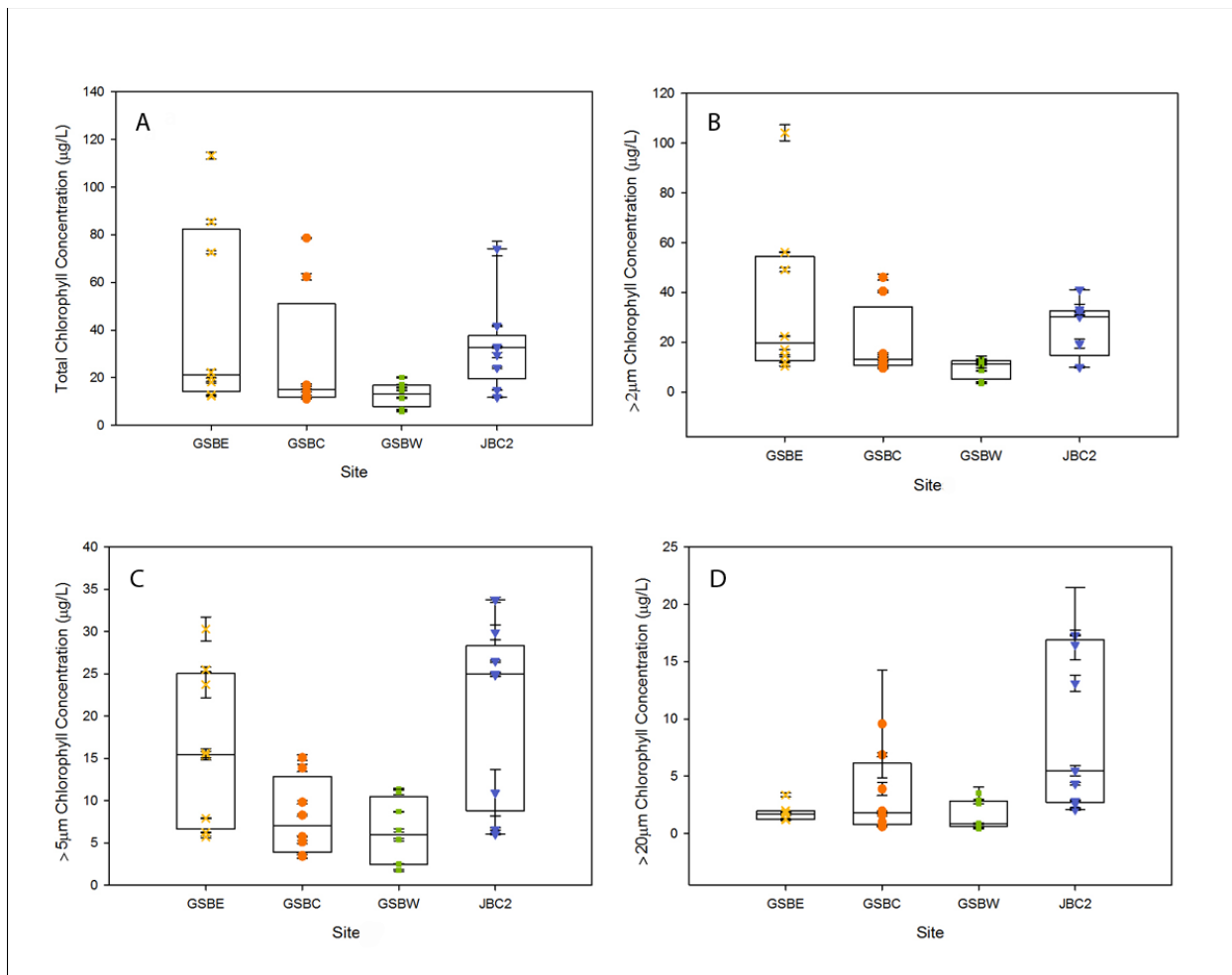


Figure 15 Site comparisons of chlorophyll *a* concentrations (µg/L) of varying size fractions at the Great South Bay study sites and Jamaica Bay reference site in 2011. (A) Total chlorophyll *a* concentration (µg/L). (B) >2µm chlorophyll *a* concentration (µg/L). (C) >5µm chlorophyll *a* concentration (µg/L). (D) >20µm chlorophyll *a* concentration (µg/L). Boxplots are representative of median, and upper and lower quartiles. Error bars are ±SE.

Physiological measurements

Survivorship

Considerable decreases in survivorship were observed at all sites during the first monitoring visit after the initial planting (Figure 16). These initial decreases in survivability were not evident in the 2010 Jamaica Bay study. After an initial acclimation, survival rates appear to stabilize in mid-August and there was minimal mortality after this point. Survivorship ranged from 70.8 to 79.9% at the end of the growing season (initial planting to October) in Great South Bay. JBC2 had the overall highest survivorship at 84.0%.

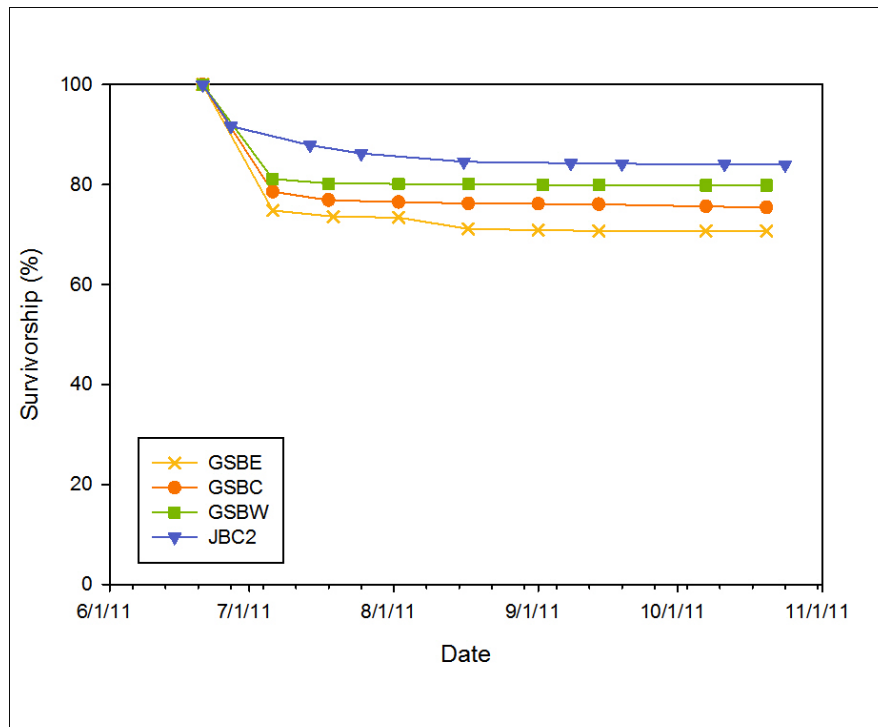


Figure 16 Seasonal trends of mean cumulative survivorship at each site in Great South Bay and the Jamaica Bay reference site in 2011.

Shell and tissue growth

Shell height was strongly correlated with shell length ($r = 0.911$, $p < 0.001$) and shell width ($r = 0.857$, $p < 0.001$). The greatest shell growth was observed at the reference site, JBC2 (Figure 17a). A one-way ANOVA of mean shell height after one growing season revealed significant differences among sites ($p < 0.001$). Of the Great South Bay sites, GSBW has the greatest mean shell height growth by the end of the season. A post-hoc analysis of multiple pairwise comparisons reveals significant differences ($p < 0.001$) between all pairs of sites except for GSBE and GSBW ($p = 0.386$)

Shell growth appears to experience rates of growth that are greatest at beginning of the season and decrease over the course of the season. Tissue growth rates appear slow at first, increase mid-season, and then slows again toward the end of the season. As with shell growth, the greatest growth in tissue was observed at the reference site. A Kruskal-Wallis one-way analysis of variance revealed significant differences ($p < 0.001$) in the means of dry tissue weight between sites after one growing season. Post-hoc analysis using Dunn's method revealed significant differences between all pairwise comparisons ($p < 0.001$) except for the comparison between GSBE and GSBW. GSBE had the highest mean tissue growth of the Great South Bay sites by the end of the growing season. However, this difference was not statistically significant. Shell growth observed at the Great South Bay sites was greatest at the GSBW site, whereas tissue growth was greatest at the GSBE site. Shell and tissue growth at the GSBE and GSBW were however not significantly different.

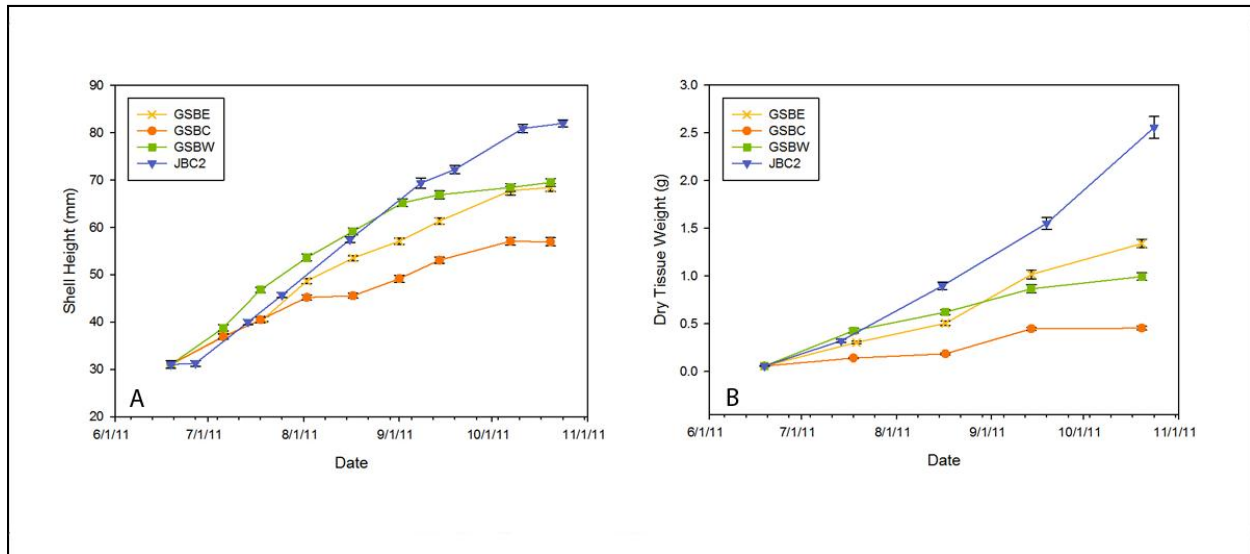


Figure 17 (A) Seasonal trends in mean shell height from Great South Bay sites and Jamaica Bay reference site in 2011. (B) Mean dry tissue weight from Great South Bay study sites and Jamaica Bay reference site in 2011. Error bars are \pm SE.

Condition index

The three Great South Bay sites generally had similar trends in condition index over the course of the growing season (Figure 18). Results from gonadal smears indicate that almost all oysters at each site were sexually differentiated by mid-July and spawned out by mid-October. Initial decreases in condition index appear to coincide with periods of spawning. Increases in condition index occur during the same time as increases in food availability due to a phytoplankton blooms in early September. This increase in condition index was not evident at the GSBW site. This site was the only site that did not experience increased food availability in mid-August. JBC2 followed a slightly different trend. At this site, spawning was observed to begin in mid-July and end in late October. Initial decreases in condition index were not evident in mid-July, but this site experienced a pulse in food availability at this time that did not occur at the Great South Bay sites (Figure 14). JBC2 had slight decreases in condition index in mid-August while Great South Bay sites experienced increases in condition index. This decline in condition index at JBC2 coincides with low food availability at JBC2 from August to September. Another increase in food availability at JBC2 occurred in early September, similarly to the Great South Bay sites.

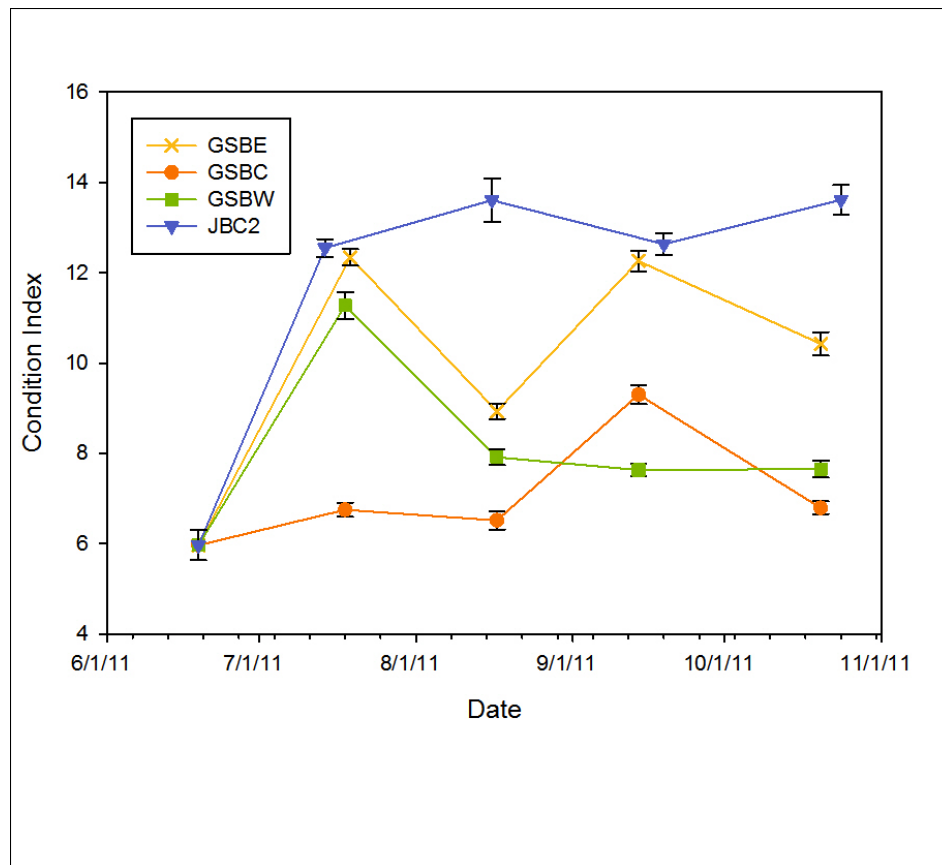


Figure 18 Seasonal trends of mean condition index at each site in Great South Bay and the Jamaica Bay reference site in 2011. Error bars represent \pm SE.

Discussion

From our observations, it is immediately evident that the eastern oyster is quite euryplastic. Not only is the eastern oyster capable of surviving a large range of environmental conditions, but it also capable of substantial growth. Our assessments reveal that the eastern oyster can maintain high rates of survivorship and growth in the first growing season (initial planting to October) at all of the study sites. Although growth continues at a slightly slower rate in the second year, significant mortality in this year may be a limiting factor for restoration efforts in areas such as Jamaica Bay. Longer assessments than the one provided in this study need to be carried out in Great South Bay in order to determine second year mortality.

Substantial second-year mortality in Jamaica Bay may be the result of the interaction of environmental stressors and disease pressure from *Perkinsus marinus* (Dermo). Extensive mortality is observed at the onset of high annual temperatures from July to August. This pattern is similar to observations of Paynter (1996) that observed infection in the first summer, and large-scale mortality in the second summer associated with high water temperatures from August to September. Results from a Ray's fluid thioglycollate medium tissue assay indicated increased prevalence of Dermo when moving along a west to east gradient in Jamaica Bay in 2011 (B. Allam, unpublished data). Mortality of oysters in the second year was lowest at the western site and highest at the eastern site. Despite lower disease rates, the central site experienced significantly greater mortality than the eastern site. This may be due to the combined pressure of disease and hypoxia. Prolonged exposure to hypoxia may reduce the capacity of oysters to suppress the proliferation of *Perkinsus marinus*, which intensifies infection and leads to increased mortality (Lennihan 1999). The steepest decline in survivorship at the central site was associated with the hypoxic event that occurred in August 2011. The oysters from the reference site (JBC2) for the Great South Bay study in their first year of growth did not experience any mortality associated with this hypoxic event. This suggests that the interaction of environmental stressors and disease may be more important than the effects of these stressors independently.

In comparison to the 2010 Jamaica Bay study, the seed planted at the JBC2 reference site for the Great South Bay study in 2011 experienced a 10% higher mortality rate by the first monitoring visit. Considerably higher initial rates of mortality in the Great South Bay study in comparison to the 2010 Jamaica Bay study were observed at all sites. This may be due to the quality of the seed received for this project. Bags of seed contained large amounts of dead seed in 2011; this was not observed in the seed used for the project in the previous year. It is unknown if the quality of the seed or environmental stressors were the cause of initial high mortality rates. Regardless of cause, unpredictable mortality due to disease, environmental stressors, and seed quality are hard to account for when planning and may hinder restoration efforts.

Other limiting factors for successful restoration may include recruitment. Recruitment to oysters in aquaculture bags was observed at all sites in Great South Bay, but none of the sites in

Jamaica Bay. Despite this, a resident living nearby at the central Jamaica Bay site remarked that an oyster had recruited to the inside of a crab pot. The source of this oyster is unknown, but the oyster had the same markings as the hatchery oysters used for this study. Observed recruitment in Great South Bay was minimal. Spat was never found to settle in density greater than one spat per oyster, and the most settlement observed was about three settled oyster spat per site. This does not suggest that oyster larvae are not retained in the Bay, only that they were not observed to settle in this very localized area. A formal evaluation of recruitment was not a part of this assessment, but it is significant parameter that should be studied in future work to determine if these populations can be self-sustaining.

It is important to measure a multitude of environmental variables affecting organisms when making physiological assessments. As suggested by Alderdice (1972), the synergistic effect of variables can have greater biological significances than any variable can have independently. In an attempt to account for this, multiple indicators of water quality were monitored during this assessment. They include temperature, salinity, dissolved oxygen concentration, and food availability as determined by chlorophyll *a* concentration $>5\mu\text{m}$. Although these variables encompass temperature and salinity, which are suggested by Shumway (1996) to possibly have the most profound synergistic effects on the biology of the eastern oyster, more comprehensive assessments may consider including variables such as pH, availability of hard substrate, water flow, turbidity, parasites, disease prevalence, predators, and competitors.

While water temperature in Jamaica Bay and Great South Bay falls within the annual range of -2°C to 36°C (Galtsoff 1964), temperature in these regions are likely the primary limiting factor of growth for eastern oyster populations. Galtsoff (1928) found no feeding currents produced below 5°C . Loosanoff (1958) later modified this statement to reflect empirical results that showed that most oysters were closed or not pumping at temperatures below 10°C . This limitation fits with oyster filtration data obtained for Jamaica Bay oysters in 2011 (J. Levinton, unpublished data). Temperatures in Jamaica Bay are $\leq 5^{\circ}\text{C}$ from January to March, which would limit growth to at least 9 months of the year. No shell growth is evident from December to May despite the presence of the winter/spring bloom during this period. Growth was found to be limited in these regions to about 6 or 7 months of the year. Periods of growth are in agreement with the findings of shell growth for oysters in Long Island Sound (Loosanoff and Nomejko 1949). A temporal mismatch in food availability and suitable temperature for feeding may limit growth of these populations. Despite short growing seasons, oysters Jamaica Bay and Great South Bay are capable of sufficient growth when temperatures are suitable. In Great South Bay, oyster shell growth ranged from 25-39mm during a period of 4 months. In the same amount of time, shell growth in Jamaica Bay ranged from 44-55mm among sites.

Westernmost sites in both study areas have longer growing seasons due to moderated temperatures from oceanic influence. This may partially explain better shell growth at these study sites in both locations. Variability in mean salinity between sites may explain additional

differences in shell growth. Westernmost sites had the highest salinity and experienced the greatest shell growth within each study area; however, in Great South Bay this growth was not significantly different from GSBE, which had the lowest salinity of all sites. The reference site JBC2, displayed greater shell growth despite having lower salinity than GSBW. Levinton et al. (2011) described a strong positive correlation between salinity and shell height, while Shaw (1966) found no significant difference in growth between oysters grown at 12ppt and 30ppt in Maryland bays. These observations suggest that other environmental variables may alter shell growth trends associated with salinity. Of the measured environmental parameters from this study it is likely that food availability is the limiting growth factor at high salinity sites in Great South Bay.

Tissue growth among sites in Jamaica Bay, as determined by dry tissue weight followed the same trend as shell growth. However, tissue growth at JBW was not significantly greater than JBE. Among the Great South Bay sites, GSBE tissue grew more than GSBW, unlike observations of shell growth. This may suggest food limitation at the western sites in both studies. Western sites generally have the smallest concentration of food available across all size fractions, while eastern sites generally have the greatest food availability. Using the $>5\mu\text{m}$ size fraction of chlorophyll *a* concentrations is a good approximation of food availability as particles larger than $6\mu\text{m}$ are retained by the gills at a uniformly high rate (Haven and Morales 1970); particles less than $1\mu\text{m}$ are poorly retained (Langdon and Newell 1990). The Great South Bay sites displayed clear differences in available food $>5\mu\text{m}$ in diameter. Food availability at this size fraction decreased when moving from the east to west site in Great South Bay. The median concentration of $>5\mu\text{m}$ particles at JBC2 is higher than GSBE, and is likely attributed to the more excessive nutrient input in Jamaica Bay they may relieve nutrient limitations for phytoplankton growth. Less food availability at western sites may be due to the dilution of limiting nutrients for phytoplankton growth by oceanic mixing. Despite observations of total food availability based on chlorophyll *a* concentration, the nutritive properties of food sources at each site are unknown but are important indicators of how efficiently oysters may be feeding and growing in these areas. Declines in tissue weight throughout the year may be attributed to changes that are associated with condition index.

Trends in condition index on a per site basis in both study areas show different trends than both shell and tissue growth. This highlights the importance of using multiple metrics for growth and condition when making physiological assessments. Butler (1953) suggested that differences in shell growth do not necessarily reflect differences in tissue or meat yield. The measurement of shell volume provides a more critical evaluation of growth than does shell height. Condition index in both regions revealed that all sites appear conducive to healthy and productive oyster populations. Variables that were most likely to affect condition index in this study were food availability and temperature. General trends of synthesis, storage, and use of biochemical reserves follow those observed in the literature. Declines in condition index are associated with the use of energy reserves used to initiate gametogenesis (Thompson 1996).

Similar to observations of Thompson (1996), reserves in our study appear to be sequestered at times of high food availability in the late summer and fall. This is evident in the Great South Bay study. Almost all sites experienced increases in condition index associated with a bloom in the late summer/fall. The GSBW site did not experience this increase in condition index, but also was the only site that did not experience a phytoplankton bloom during this time. All Jamaica Bay sites experienced substantial declines in condition index after winter. This may be attributed to the net loss of glycogen during winter as it may be catabolized to meet maintenance requirements (Deslous-Paoli and Heral 1988). High values of condition index however resumed as temperatures increased and oysters were able to feed and restore glycogen concentrations.

Despite seemingly suitable conditions at the GSBC site, oysters experienced significantly low values for all four parameters of physiological health. While analyzing these oysters for condition index, the tissue of almost all oysters appeared green in color. This observation may be explained by the accumulation of copper (Galtsoff 1964). Wood preserved with chromated copper arsenate, the most common type of pressure treated wood, has been found to be associated with green colored oysters (Weis 1993). The Weis (1993) study found negative correlation ($r = 0.506$) between oyster soft tissue weight and copper concentration. The docks at this location appear to be made of pressure treated wood and may contribute to the green color of most oysters at this site. Other sources of copper may be from antifouling paint that is used on boats found in the marina. Oysters exposed to copper have not been shown to experience significantly decreased shell growth (Shuster and Pringle 1969) or increased mortality (Abbe 2000). It is unknown if copper contributed to less than expected growth and survivorship at the GSBC site. Future studies may consider including measurements of metal concentrations as an additional indicator of water quality.

These observations provide preliminary data for off-bottom aquaculture based studies. Future studies should involve on-bottom aquaculture to determine the effect of sedimentation in these areas. Furthermore, once sufficient data are collected from these preliminary assessments, it will be necessary to begin small-scale reef projects in these regions in order to encompass the entire range of variables that affect oyster populations. The Hudson River Fund is currently undertaking an oyster research restoration project in which they placed six experimental reefs throughout the Hudson River Estuary. These reefs were built to mimic natural reefs and are 50m² and 20-50cm high (USACE & PANYNJ 2009). Data are not yet available, but these projects will be important and necessary indicators of the potential for large-scale restoration in these regions.

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Chapter 2

A quantification of the bioextraction capacity of *Crassostrea virginica* in two coastal bays on Long Island, NY

Introduction

The expansion of human populations has caused rapid alterations in the surrounding environment. As human populations become denser in coastal areas the export of nitrogen to surrounding bodies of water will increase, leading to enhanced primary productivity (Nixon 1992; Nixon 1995). Increased primary productivity will cause greater turbidity and reduced levels of dissolved oxygen in bottom waters as it decomposes in oxygen consuming processes (Johannessen and Dahl 1996). Additional impacts of eutrophication may include the loss of submerged aquatic vegetation and marsh habitat, proliferation of nuisance and harmful algal blooms, and a reduction in biodiversity (reviewed by Cloern 2001; Hinga 1991).

This study focuses on two estuaries that vary in the degree that they are affected by eutrophication. Jamaica Bay, NY is a shallow estuary that receives approximately 5.8×10^6 kg N y^{-1} and is classified as being highly eutrophic (Benotti et al. 2005). The other site of interest is Great South Bay, NY; it is a shallow estuary receiving about 8.5×10^5 kg N y^{-1} and is classified as being low to moderately eutrophic (Kinney & Valiela 2011). Wastewater-derived nitrogen is the dominant source in both estuaries, accounting for 89% of nitrogen input to Jamaica Bay (Benotti et al. 2005) and 55% of nitrogen input to Great South Bay (Kinney & Valiela 2011). As instances of eutrophication are becoming more common on a global scale, the necessity for research and management of anthropogenic nutrient input and control has developed.

Both Jamaica Bay and Great South Bay historically supported populations of the eastern oyster. Populations in these regions collapsed due to a combination of fisheries exploitation, disease, habitat change and degradation, and additional stresses. Although it is difficult to currently find wild oysters in these areas, our physiological assessment suggests that aquacultured oyster populations can exist with minor mortality and fast growth. The eastern oyster has recently been suggested as a supplemental means to reduce the effects of eutrophication. It is an active filter feeder that is capable of maintaining high clearance rates of approximately $1-10 \text{ L h}^{-1} \text{ g}^{-1}$ dry weight tissue (Jordan 1987) and efficiently removing particles $>6\mu\text{m}$ in diameter (Haven and Morales 1970). At high abundances eastern oysters can exert top-down control of phytoplankton biomass through grazing pressure (Cerco 2007; Fulford 2007). Local ecosystem services associated with biofiltration of oysters may have been lost in these regions as populations diminished. Some of these services include increased water clarity,

reduced local concentrations of suspended solids, carbon, and chlorophyll *a*, increased benthic-pelagic coupling, enhanced nutrient burial, and a removal of nitrogen from the local system through microbially mediated denitrification of biodeposits (Ruesink 2005).

The diet of oysters is composed primarily of phytoplankton. Oysters can also feed on detritus and bacteria. At seston concentrations of 5 to 20 mg L⁻¹ the eastern oyster assimilates approximately 50% of the particulate organic nitrogen that is cleared from the water during feeding (Newell and Jordan 1983). Oysters assimilate this nitrogen into tissue and shell biomass. Newell (2005) found that the nitrogen content of Chesapeake Bay wild oyster tissue and shell are on average 7% and .3% per g dry weight, respectively. At harvest, sequestered nutrients are permanently removed from the local system. Higgins (2011) estimated that the removal of 1 t N in Chesapeake Bay would require the harvest of 7.7 million 76mm-TL cultivated oysters. This suggests that in addition to local top-down control on phytoplankton biomass, the eastern oyster can act as a sink for nutrients that enter a eutrophic water body. Restored oyster populations and expansions of oyster aquaculture can act as *in situ* sites for nutrient removal. In the case of aquaculture, harvest of oysters constitutes regular removal of nitrogen from a eutrophic bay system. While sites such as Jamaica Bay are closed to human consumption, culture and removal is still possible. Closed sites are also candidates for relay to cleaner areas. Other areas such as Great South Bay can be used directly for combined aquaculture, harvest, and nitrogen removal.

Local nutrient removal through bioassimilation has been proposed for various locations using several species of bivalves (Lindahl 2005; Gifford 2005; Jones 2002). The objective of this study is to quantify the nitrogen sequestered by the eastern oyster during one growing season. It is also of interest to determine the scale, feasibility and limits of using this organism as a supplementary means to remediate nitrogen loading. Although it may not be possible to return eutrophic estuaries back to baseline conditions, restoration of foundation species such as the eastern oyster may help to restore the functional integrity of these ecosystems. Our preliminary physiological assessment revealed that both Jamaica Bay and Great South Bay have potential to be suitable sites for oyster restoration projects. From the initial planting in mid-June until mid-October, average tissue growth, shell growth, and survivorship is summarized in Table 3. Caution must be taken in comparing the 2010 Jamaica Bay study and 2011 Great South Bay as environmental conditions may have varied between years. However, these observations in combination with those from the 2011 Jamaica Bay reference site indicate that Jamaica Bay may be more conducive to the growth and survival of oysters. Increased growth may be due to substantially higher levels of available food in Jamaica Bay that were observed during this study. Elevated food availability may be due to the higher level of eutrophication that is present in Jamaica Bay. More excessive nutrient inputs in Jamaica Bay may act to relieve nutrient limitation on phytoplankton growth and thus relieve food limitation on oyster growth as long as there is no shift to small phytoplankton forms upon which oysters are unable to efficiently retain. These findings in conjunction with the findings of this chapter will help in determining the

ability of oyster populations to act as bioremediation tools in regions that are becoming progressively eutrophic.

Table 3 Summary of mean shell growth, tissue growth, and survivorship from the first-year season of growth (initial planting in mid-June to mid-October). Mean values are \pm SE.

Study Area	Shell growth(mm)	Shell growth(g)	Tissue growth(g)	Survivorship(%)
Jamaica Bay 2010	50.40 \pm 0.74	28.43 \pm 0.70	1.54 \pm 0.05	95.5
Great South Bay 2011	33.64 \pm 0.63	15.86 \pm 0.62	0.84 \pm 0.08	75.5
Jamaica Bay (reference) 2011	50.99 \pm 0.75	30.00 \pm 1.01	2.50 \pm 0.11	84.0

Methods & Materials

Study Areas

Jamaica Bay, NY

Jamaica Bay, NY is a shallow, highly eutrophic estuary located in southwestern Long Island (Figure 19). Queens, Brooklyn, New York City, and Nassau County are highly urbanized boroughs that surround the Bay. To keep up with wastewater demands from these regions, four wastewater treatment plants are situated around Jamaica Bay. Benotti et al. (2005) estimated that 92% of nitrogen loading to the Bay was from point sources such as wastewater-treatment plants, combined sewer overflow, and subway dewatering. The remainder of nitrogen loading was due to nonpoint sources that include landfill leachate, groundwater flow, and atmospheric deposition. Prior to the 1900s nitrogen loading to the Bay was estimated at 35.6kg/day; nitrogen loading as of 2005 was estimated to be 15,800kg/day (Benotti et al. 2005). This recent estimate is about 450 times the predevelopment estimate. Methods for reducing nitrogen within wastewater plants in Jamaica Bay are now being tested, so oyster growth and harvest can supplement these activities

Study sites were located along a longitudinal gradient from eastern to western Jamaica Bay (Figure 19). Water quality in this region varied along the gradient (Chapter 1). The western site experienced substantial oceanic influence, high salinity, and low primary productivity in relation to the eastern site that had relatively poor mixing, low salinity, and high primary production. At the western site water residence time is about 0-5 days and at the eastern site it is about 35-40 days (Benotti et al. 2005). Concentrations of nitrogen are generally highest in the eastern parts of the Bay where they are retained to a greater extent due to slow flushing.

This study was the continuation of a 1.5 year physiological assessment of oysters. Two wire cages were suspended from the docks at each study site and remained below average low tide level at all times. Each wire cage contained two semi-rigid polyethylene 10mm-mesh grow-

out bags with dimensions of 94x43x7.6cm. Each bag contained 300 oysters that had an average shell height of approximately 29mm at the initial planting. Oyster seed was obtained from Fishers Island Oyster Farm. Oysters spawned and seed settled in the summer of 2009. Oysters were then overwintered in waters adjacent to the oyster farm and transferred to grow-out bags in late June of 2010.

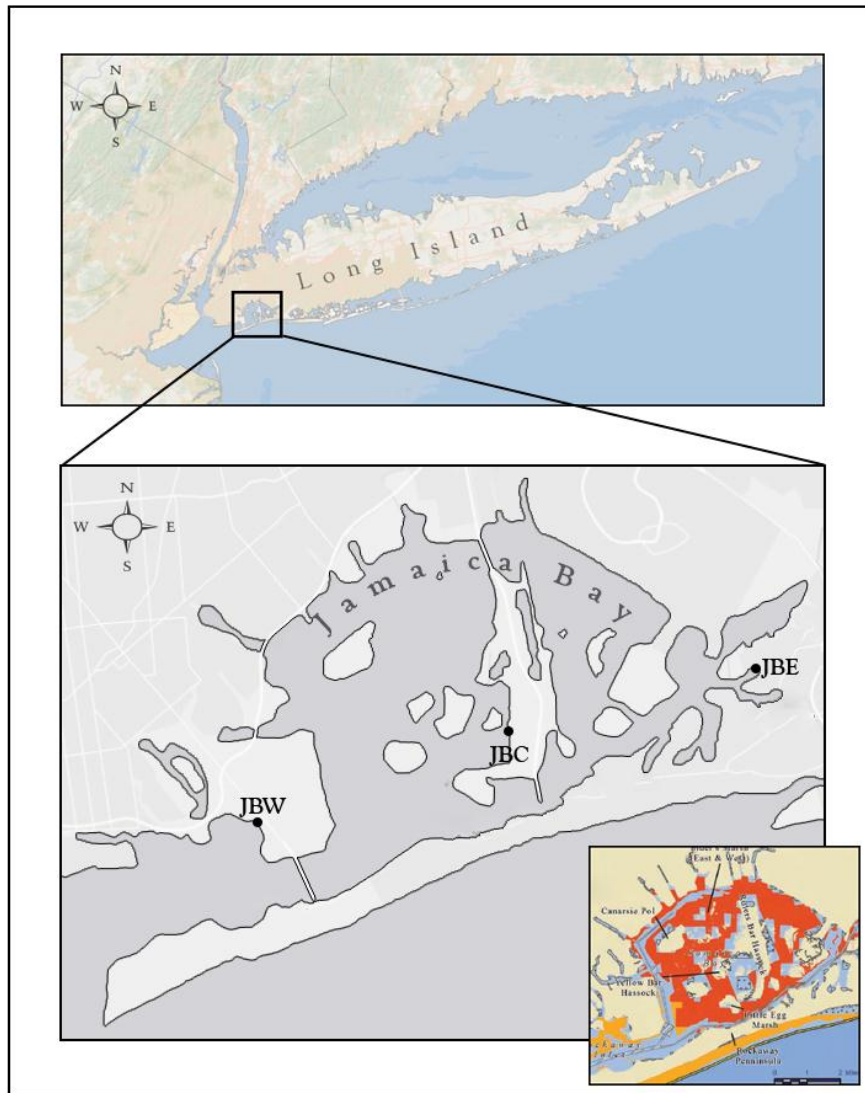


Figure 19 (Top) Southwestern Long Island; the location of the Jamaica Bay study area. (Bottom) Study site locations within Jamaica Bay, NY. Inlay is from the Hudson-Raritan Estuary Comprehensive Plan; the red area represents the area that meets four suitability criteria (bathymetry, salinity, dissolved oxygen, and total suspended solids) for oyster restoration in this region (USACE and PANYNJ 2009). Suitable area was calculated using ArcGIS® and estimated to be approximately 6,387 acres.

Great South Bay, NY

Great South Bay is a shallow barrier beach estuary that is on the low end of the range of eutrophic estuaries (Kinney & Valiela 2011). It is located approximately halfway between New York City and Montauk Point (Figure 20). Wastewater-derived nitrogen accounts for 55% of the total nitrogen loading to the Bay. Atmospheric deposition accounts for approximately 31% and fertilizer 15% of the remaining nitrogen load (Kinney & Valiela 2011). The watershed has high retention capacity and retains approximately 77% of the nitrogen that enters the region (Kinney & Valiela 2011). Although the watershed currently displays high retention capacity for nitrogen, land-use changes due to urbanization will reduce retention efficiency (Walbridge 2007). Despite Great South Bay's current status of relatively low eutrophication, the expansion of human populations will increase eutrophication if plans for remediation and reduction of nitrogen are not developed.

Three sites were chosen along a longitudinal gradient (Figure 20). Two of the sites were on the north shore, and one on the south shore of the Bay. A reference site was chosen in Jamaica Bay to compare physiological responses and nutrient assimilation in two estuaries that differ in their degree of nutrient input. Water quality varied along the east to west gradient (Chapter 1). The western site experienced the greatest mixing and oceanic influence due to its proximity to the inlet. Salinity decreased and primary productivity generally increased from west to east.

Two wire cages were suspended from docks and remained below mean low tide level at each locality. Each cage contained two 10mm-mesh polyethylene grow-out bags with dimensions of 94x43x7.6cm. Bags contained 300 oysters each, for a total of 1,200 oysters per site. At the initial planting oysters averaged approximately 31mm in shell height. Oyster seed was obtained from Fishers Island Oyster Farm. Oysters spawned and seed settled in the summer of 2010. Oysters were then overwintered in waters adjacent to the farm and transferred to grow-out bags in late June of 2011. This study was the continuation of a five month physiological assessment that lasted from mid-June until mid-October 2011.

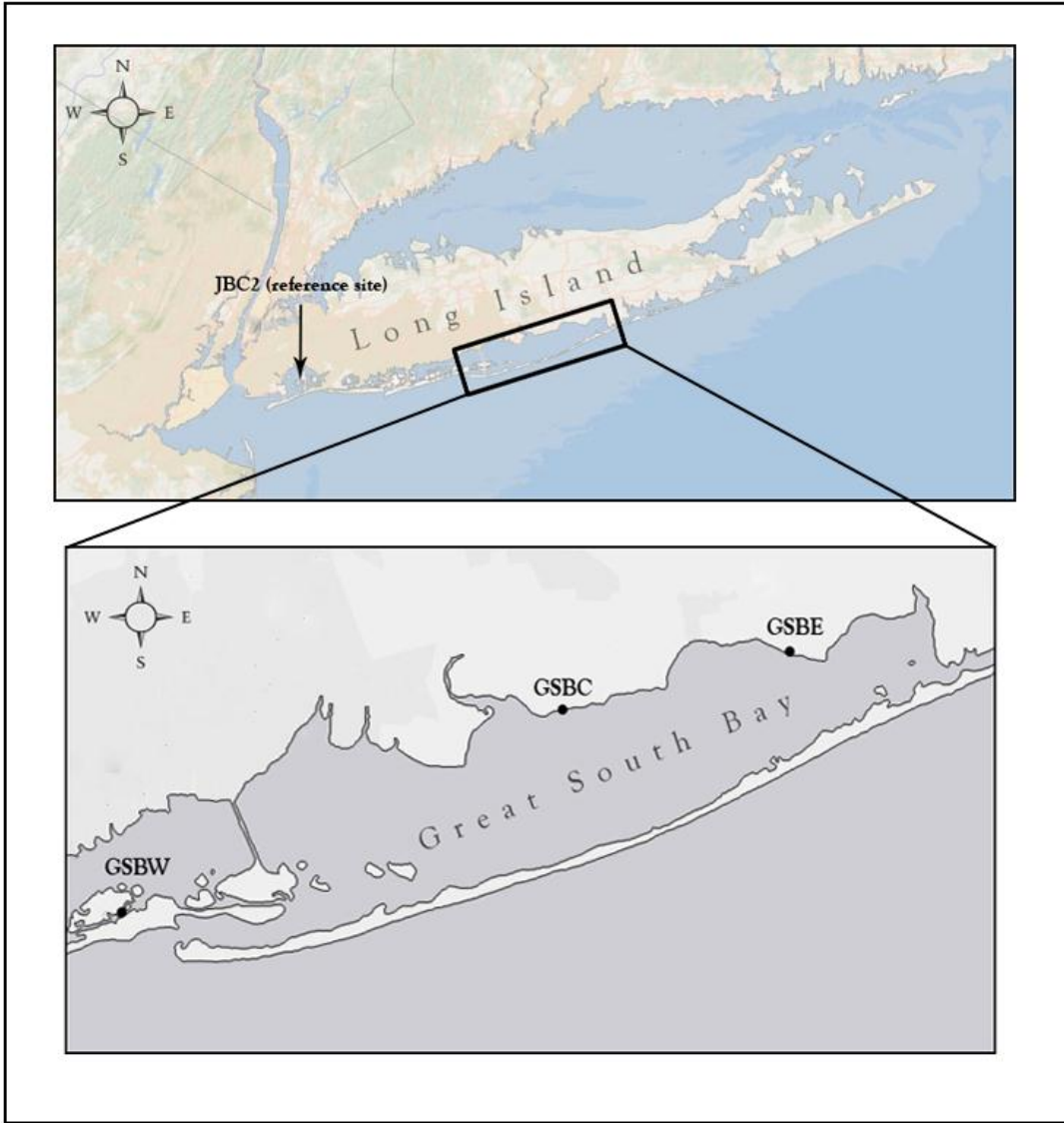


Figure 20 Southern Long Island; the location of the Great South Bay study area and a reference site located in central Jamaica Bay. (Bottom) Study site locations within Great South Bay, NY.

Carbon and nitrogen analysis

Once a month ten oysters were haphazardly collected from each grow-out bag. Oysters were then analyzed to determine values of condition index. Oyster tissue was dried for at least 7 days at 70°C for the analysis of condition index and was saved for carbon and nitrogen analysis that took place at a later date. Drying time allowed for complete dehydration of the tissue. In Great South Bay three dates were chosen and all tissue samples from these dates were processed for carbon and nitrogen content (n=120 per site). These dates were July 20th, August 18th, and October 20th 2011. In Jamaica Bay two dates were chosen. These dates were July 19th and October 13th 2010 (n=120 per site). Dry tissue weight (g) had previously been recorded during condition index analysis. Tissue was ground using a porcelain mortar and pestle. Samples were ground to a fine, homogenized powder before being prepared by standard procedures for carbon and nitrogen analysis. Carbon and nitrogen were determined by combustion using a CE Elantech[®] Flash Elemental Analyzer 1112. Prior to analysis a standard curve was produced using aspartic acid samples. Carbon and nitrogen were expressed as %C and %N of the sample. Twenty replicates of one sample of tissue from a single oyster from Great South Bay were analyzed for %C and %N in order to quantify differences in %N and %C that may be due to sample preparation or measurement error by the carbon nitrogen analyzer. The average %N was 8.09±0.21 SD and %C was 45.05±0.19 SD.

Shell from Great South Bay was saved from the final condition index analysis that took place on October 20th 2011 (n=10 per site). Shell height (mm) and shell weight (g) were recorded previously during analysis of condition index. As the result of an oversight, shell was not saved during the course of the 2010 monitoring period for the Jamaica Bay study. However, shell was collected in November 2011 from each site (n=10 per site) and shell height (mm) and weight (g) were measured. In preparation for carbon and nitrogen analysis all shell was scrubbed under running water to remove sediment and epibionts. Dry shell was then ground using an agate mortar and pestle to make a fine, homogenized powder. Additional procedures were the same as those used for tissue analysis, but atropine was used as a standard to create a standard curve. Six replicates of one sample of shell from Great South Bay were analyzed for carbon and nitrogen to quantify differences in %N and %C they may arise from sample preparation or measurement error by the carbon nitrogen analyzer. The average %N was 0.15±0.01 SD and %C was 11.07±1.40 SD.

Analysis of data

Differences in carbon and nitrogen content of dry tissue between sites were of interest in this study. A significance level of $\alpha = 0.05$ was used for all between site comparisons. Jamaica Bay data for %C of tissue passed a test for equal variance, but failed a test for normality (Shapiro-Wilk). Data for %N of tissue passed a test for normality but failed a test for equal variance. Values of tissue %C and %N of tissue in Great South Bay failed tests of normality and equal variance. A Kruskal-Wallis one way analysis of variance was therefore used to analyze differences in median values of %C and %N of dry tissue in Jamaica Bay and Great South Bay. A post-hoc analysis was carried out for Great South Bay data using Dunn's method to make multiple pairwise comparisons between treatment groups with unequal sample size.

Regression analysis was used to analyze the relationship between dry tissue weight and %N. This analysis was primarily used to determine an equation from which tissue %N could be predicated from a given dry tissue weight. Of most interest was the %N of the average dry tissue weight after one season of growth. In Jamaica Bay and Great South Bay \log_{10} -transformed data for dry tissue weight (g) versus \log_{10} -transformed data for %N passed tests of normality (Shapiro-Wilk) and constant variance. Data for the Jamaica Bay reference site failed a test for normality and passed a test for constant variance. A normal probability plot (Figure 26a) for this data shows fairly normally distributed data, and a scatter plot of the residuals (Figure 26b) appears to exhibit homoscedastic data.

Regression analysis was also used to determine an equation to predict %N from a given shell height in Great South Bay. \log_{10} -transformed data for shell height and %N passed tests of constant variance and normality. In order to calculate values of total nitrogen by weight of shell, a regression was needed for shell height versus shell weight. A regression model for \log_{10} -transformed shell height versus \log_{10} -transformed shell weight was developed for each study area (Jamaica Bay, Great South Bay, and the Jamaica Bay 2011 reference site).

Results

Jamaica Bay

Tissue carbon and nitrogen content

Mean values of carbon content in tissue ranged from 45.39 to 45.77% among sites (Figure 21). A Kruskal-Wallis one way analysis of variance detected no significant differences ($p=0.611$) between median values of %C content of tissues between sites. Mean values of nitrogen content in tissue ranged from 9.18 to 9.50%. A Kruskal-Wallis one way analysis of variance detected no significant differences among sites ($p < 0.056$) in median values of %N of dry tissue.

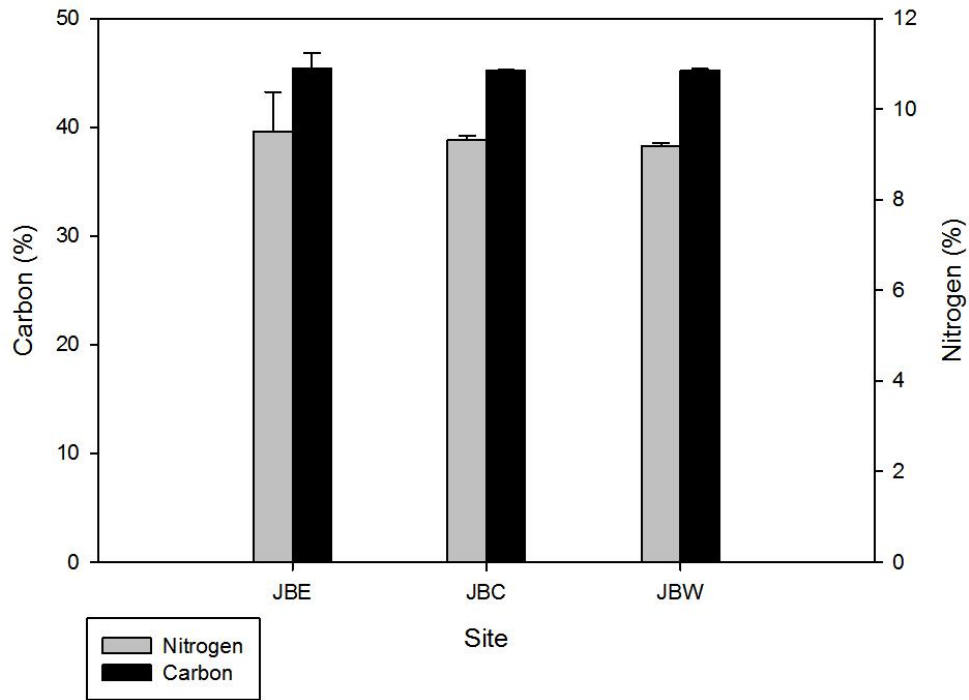


Figure 21 Mean %C and %N at each site in Jamaica Bay. Samples are from two dates during the first growing season in 2010. Error bars represent \pm SE.

Linear regression analysis yielded a negative relationship (Figure 22) between \log_{10} -transformed dry tissue weight and \log_{10} -transformed %N. Despite this apparent trend, there is a fair amount of variability about the regression line for a given weight of dry tissue. The equation developed from this regression model was used to predict %N for the average weight of dry tissue after the first year season of growth. The average weight of dry tissue for Jamaica Bay was 1.58g and was composed of 8.93% nitrogen. The regression is significant at a level of $p < 0.001$.

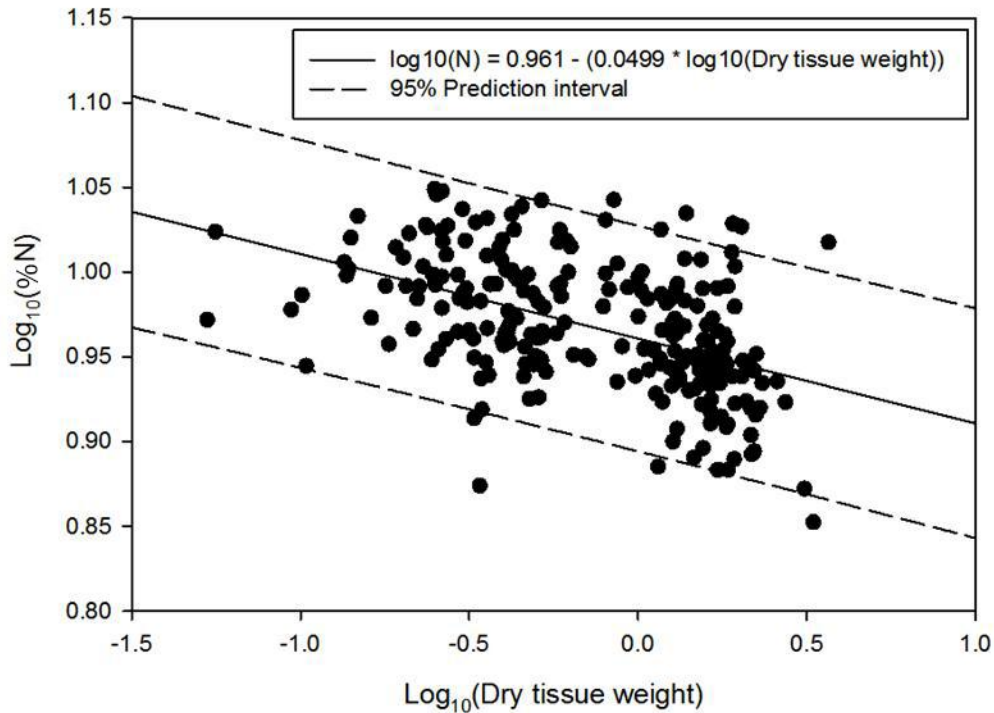


Figure 22 Linear regression of \log_{10} -transformed %N vs. \log_{10} -transformed dry tissue weight (g) for the three Jamaica Bay sites. $R^2 = 0.237$.

Great South Bay

Tissue carbon and nitrogen content

Mean values of carbon content in tissue ranged from 41.79 to 44.46% among Great South Bay sites (Figure 23). The Jamaica Bay reference site had a mean carbon tissue content of 44.71%. A Kruskal-Wallis one way analysis of variance detected significant differences ($p < 0.001$) in median values of tissue carbon content. A post-hoc analysis revealed significant differences between all pairwise comparisons except for between GSBE and JBC2. Mean values of nitrogen tissue content in Great South Bay ranged from 9.10 to 10.04%. The Jamaica Bay reference site had a mean value of 8.49%. A Kruskal-Wallis one way analysis of variance revealed significant differences ($p < 0.001$) in median values of tissue nitrogen content. A post-hoc analysis using Dunn's method for multiple pairwise comparisons found significant differences for nitrogen content in tissue in all possible pairs except for the GSBW and GSBE comparison. The overall differences in percent nitrogen, however, do not affect to any great degree my conclusions below concerning the total amount of nitrogen that can be removed by oysters.

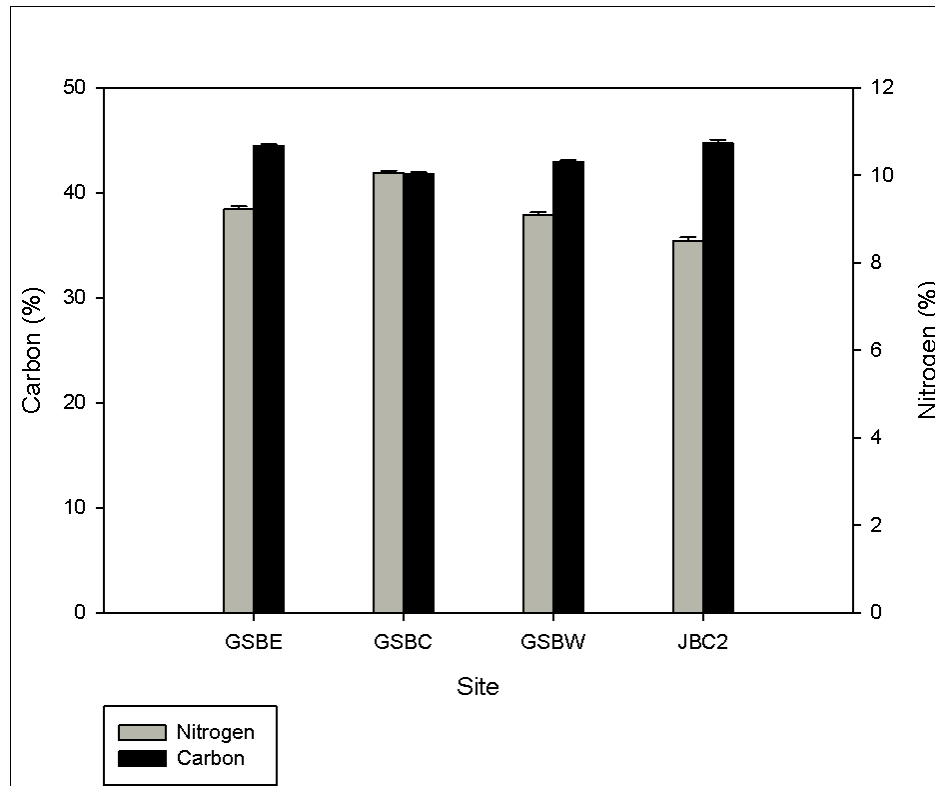


Figure 23 Mean %C and %N at each site in Great South Bay and the Jamaica Bay 2011 reference site. Samples are from three dates during the first growing season. Error bars represent \pm SE.

Linear regression analysis yielded a negative relationship (Figure 24) between the independent variable (\log_{10} -transformed dry tissue weight) and the dependent variable (\log_{10} -transformed %N). Although this trend is apparent, there is a fair amount of variability around the regression line for a given weight of dry tissue. The equation developed from this regression model was used to predict %N for the average weight of dry tissue after the first year season of growth. The average weight of dry tissue for Great South Bay was 0.89g and was composed of 8.94% nitrogen. The regression is significant at a level of $p < 0.001$.

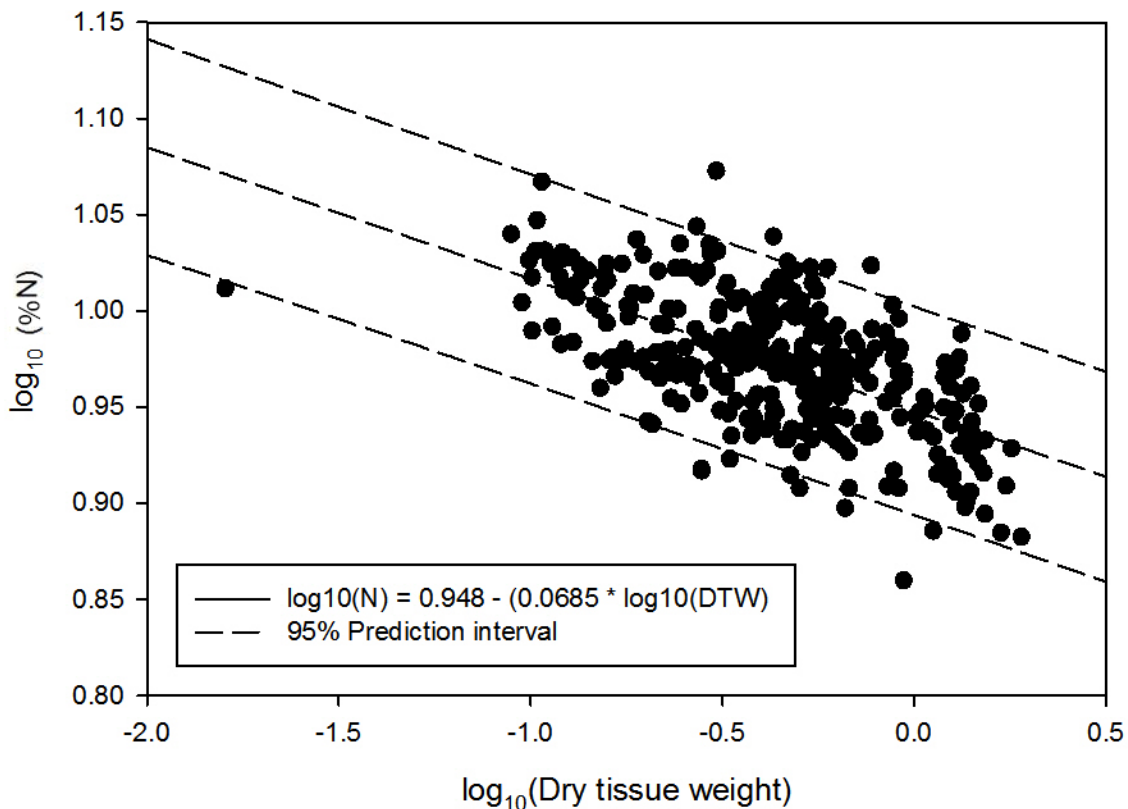


Figure 24 Linear regression of \log_{10} -transformed %N vs. \log_{10} -transformed dry tissue weight (g) for the three Great South Bay sites. $R^2 = 0.384$.

Linear regression analysis showed a negative relationship between \log_{10} -transformed dry tissue weight and \log_{10} -transformed nitrogen content of dry tissue (Figure 25). There is a fair amount of variability around the regression line for a given value of dry tissue weight. Linear regression was primarily used to predict values of %N for a given dry tissue weight. The average dry tissue weight after the first year season of growth at the Jamaica Bay 2011 reference site was 2.56g and was composed of 7.65% nitrogen. The regression is significant at a level of $p < 0.001$.

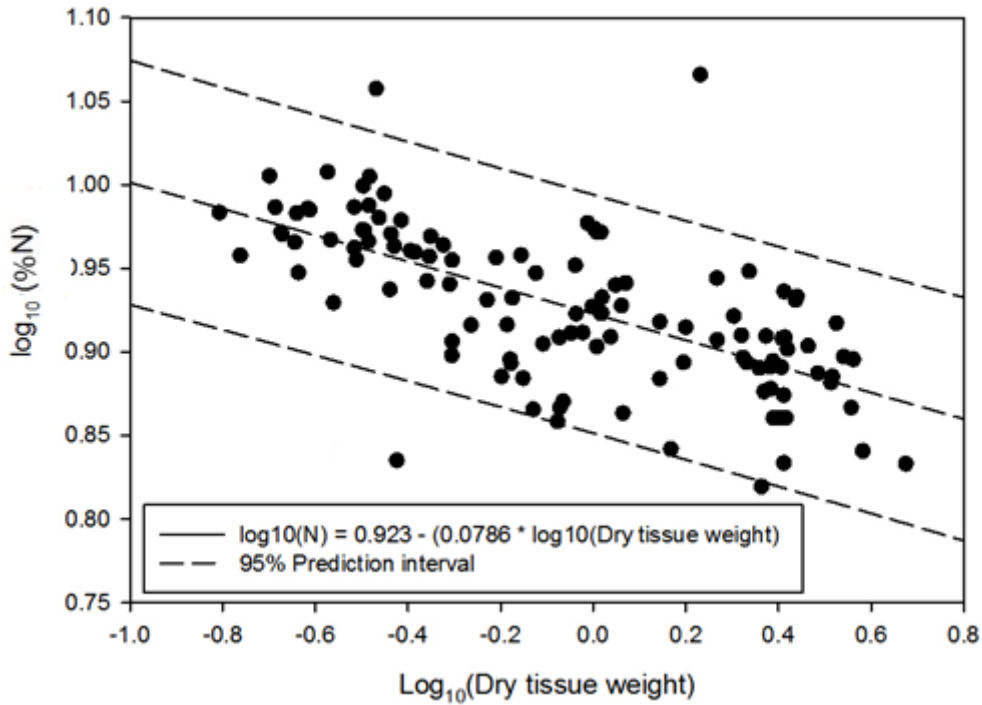


Figure 25 Linear regression of \log_{10} -transformed %N vs. \log_{10} -transformed dry tissue weight (g) for the Jamaica Bay 2011 reference site. $R^2 = 0.578$.

Data for the Jamaica Bay reference site for \log_{10} -transformed dry tissue weight and \log_{10} -transformed %N (Figure 25) failed tests for normality constant variance. A normal probability plot (Figure 26a) for this data shows fairly normally distributed data, and a scatter plot of the residuals (Figure 26b) appears to exhibit homoscedastic data. A large F-value (161.747) and small p-value (<0.001) give confidence in the use of this regression model, despite failed tests of normality and constant variance.

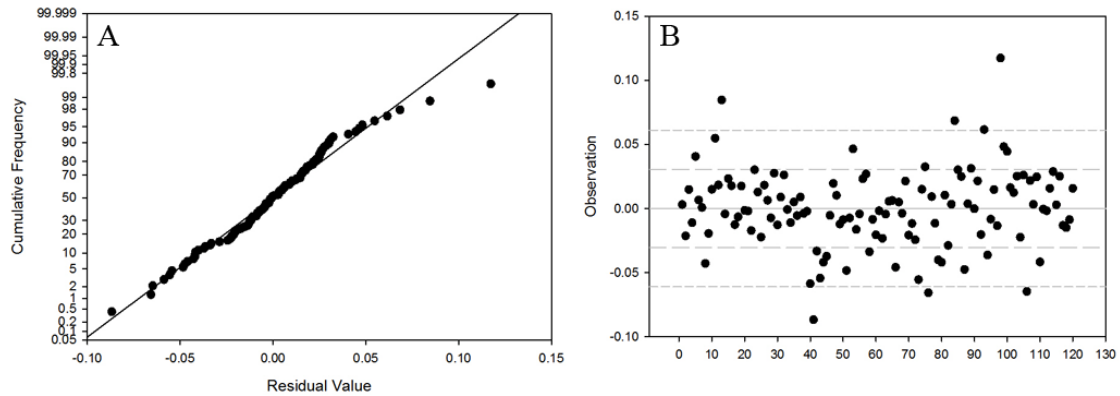


Figure 26 (A) Normal probability plot. Plot of the normalized cumulative sums of the residuals of \log_{10} -transformed data for %N and dry tissue weight (g) for the Jamaica Bay reference site in 2011. (B) Scatter plot of the residuals of \log_{10} -transformed data for %N and dry tissue weight (g) for the Jamaica Bay reference site in 2011.

Shell nitrogen content

Linear regression of \log_{10} -transformed data for shell height and \log_{10} -transformed nitrogen content of shell displays a positive relationship (Figure 27). Although this trend is apparent, there is considerable variance in %N for a given shell height. Three groupings are evident upon inspection of the linear regression. All three sites in Great South Bay were plotted separately and these groupings are not site or date specific. The order that samples were analyzed was also plotted separately; there is no apparent relationships between the order samples were run and the groupings evident in the regression. The regression is significant at a level of $p < 0.001$.

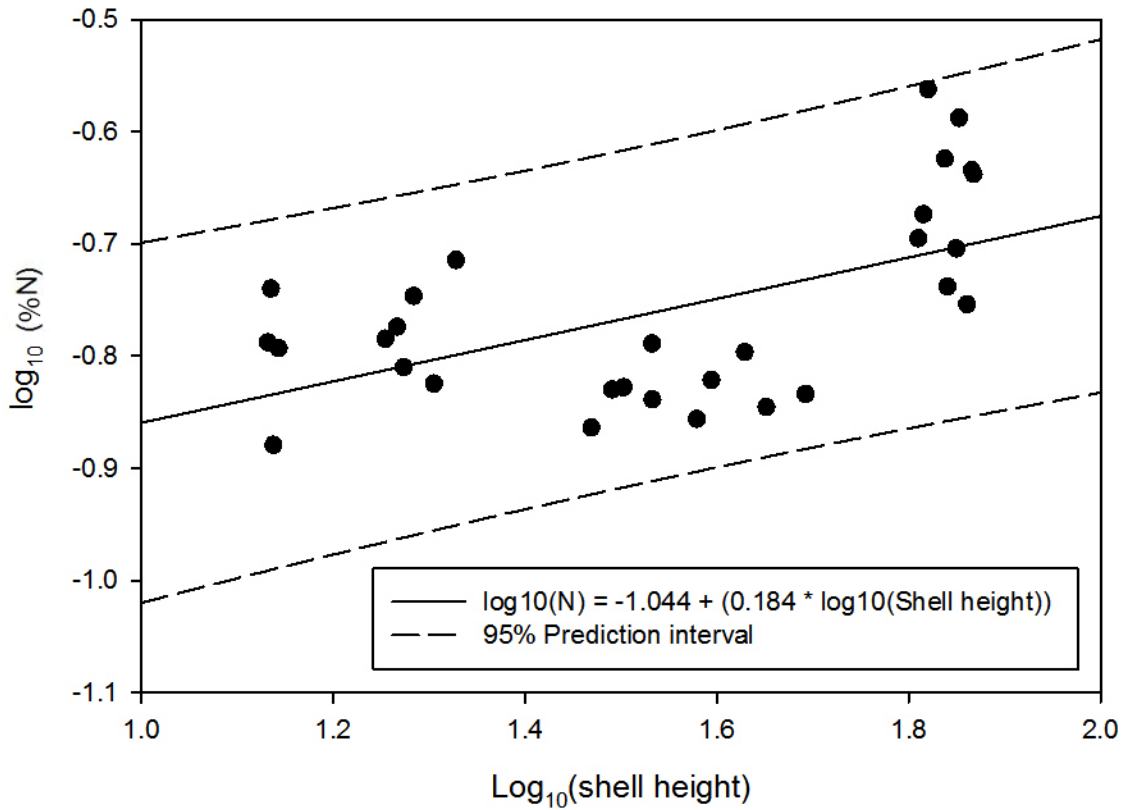


Figure 27 Linear regression of \log_{10} -transformed %N vs. \log_{10} -transformed shell height (mm) for the Jamaica Bay 2011 reference site. $R^2 = 0.319$.

Nitrogen Sequestration

Predicted values of %N for a given dry tissue weight (Table 4) are similar to those estimated by Higgins (2011). A similar trend of decreasing nitrogen content with increasing dry tissue weight appears in their estimates. This trend is not apparent in estimates provided by Grizzle (2011), but that may be due to small sample size (n=10). Observed dry tissue weight was higher than that observed in native oyster populations by Newell (2005); an oyster 76mm in height was observed to have a dry tissue weight of 1g.

Table 4 Summary of final estimates for dry tissue weight (g), nitrogen (%), and nitrogen (g) after one season of growth for each study area. Dry tissue weight is the mean observed dry tissue weight \pm SE. Nitrogen is derived from a linear equation \pm the standard error of the estimate.

Tissue	Dry tissue weight(g)	Nitrogen (%)	Nitrogen(g)
Jamaica Bay 2010	1.58 \pm 0.05	8.93 \pm 0.03	0.14 \pm .004
Great South Bay 2011	0.89 \pm 0.04	8.94 \pm 0.03	0.08 \pm .004
Jamaica Bay (reference) 2011	2.56 \pm 0.11	7.65 \pm 0.03	0.20 \pm .008

The Great South Bay regression analysis was used to predict %N for a given shell height for all study regions. Due to an oversight, shells from the Jamaica Bay sites were not collected during the first year of growth. The regression shows a positive relationship between shell height and %N. Therefore, using shells collected during the second year may overestimate the predicted value of %N for first year shells. Using the Great South Bay regression yielded a more conservative estimate of shell nitrogen content in Jamaica Bay. The estimates for %N shown in Table 5 are similar to those observed by Higgins (2011) for similar sized oyster shell. Newell (2005) observed that native oyster shell 76mm in height weighs approximately 150g. This observed shell weight is substantially higher than the weights observed in this study.

Table 5 Summary of the final estimates for shell height (mm), shell weight (g), nitrogen (%), nitrogen (g) after one season of growth for each study area. Shell height is the mean observed shell height \pm SE. Shell weight, and %N were derived from linear equations \pm the standard error of the estimate.

Shell	Shell height(mm)	Shell Weight(g)	Nitrogen(%)	Nitrogen(g)
Jamaica Bay 2010	77.19 \pm 0.71	25.86 \pm 0.10	0.20 \pm 0.07	0.05 \pm 0.02
Great South Bay 2011	64.66 \pm 0.63	15.06 \pm 0.11	0.19 \pm 0.07	0.03 \pm 0.01
Jamaica Bay (reference) 2011	82.01 \pm 0.75	29.28 \pm 0.11	0.20 \pm 0.07	0.06 \pm 0.02

Estimates for total nitrogen sequestered per oyster, and per cage for Great South Bay over the same approximate growth season is about half that sequestered by oysters in Jamaica Bay (Table 6). This is largely attributed to greater shell and tissue growth observed in Jamaica Bay; nitrogen content for tissue and shell was similar in the Jamaica Bay 2010 and Great South Bay 2011 studies. The %N for tissue in the Jamaica Bay 2011 reference was approximately 1% lower than that observed in Great South Bay. In this study total oyster growth appears to have the most profound effect on nitrogen assimilation. Cumulative mortality rates in Great South Bay were about 10% higher than the Jamaica Bay 2011 reference site and contribute to the lower estimated values of total nitrogen sequestration per cage.

Table 6 Estimates for total N per oyster (gN) are representative of mean oyster dry tissue weight(g) and mean shell height(mm) after one season of growth in each respective study region. Total N assimilated per cage (gN) accounts for site specific mortality.

Study Area	Total N/oyster(gN)	Total N/cage(gN)
Jamaica Bay 2010	0.19±0.02	108.3±2.17
Great South Bay 2011	0.11±0.01	49.83±0.50
Jamaica Bay reference 2011	0.26±0.02	131.04±2.62

Estimates of total nitrogen sequestration (Table 7) by oyster populations suggest that oysters can remove a substantial amount of nitrogen upon harvest. It is important to note that in order to remove a large amount of nitrogen from the system, restoration efforts must take place and be successful on very large scales that cover the majority of the Bay. Even at smaller, more practical scales of restoration a significant amount of seed is needed to produce a relatively small effect on the total yearly nitrogen load.

Table 7 Jamaica Bay nitrogen sequestration potential. Estimates are based on proposed restoration areas from the Hudson-Raritan Comprehensive Restoration Plan (USACE & PANYNJ 2009). Estimates of the total number of oysters that can cover these proposed areas assume 50% coverage of the total area defined as suitable for oysters to allow for sufficient water flow in an aquaculture-based design similar to that presented in this study. Site specific mortality is accounted for in all estimates. The percent of yearly nitrogen load assimilated by oysters is based on the estimate of 5.8x10⁶kg N yr⁻¹ for annual N load (Benotti et al. 2005).

Proposed restoration area (acres)	Total # of oysters	Jamaica Bay 2010	
		Total N sequestered(kgN)	% of yearly N load
6,387	1.8x10 ¹⁰	3.4x10 ⁶ ±3.6x10 ⁵	61±6
5,000	1.4x10 ¹⁰	2.7x10 ⁶ ±2.8x10 ⁵	47±5
500	1.4x10 ⁹	2.7x10 ⁵ ±2.8x10 ⁴	5±1

No proposed restoration efforts exist for Great South Bay, but these estimates were provided for comparison to Jamaica Bay (Table 8). Although estimates in Great South Bay for total nitrogen removal for a given unit area is about half of Jamaica Bay, oysters in Great South Bay have a relatively larger effect on the total nitrogen load to the Bay due to lower yearly nitrogen input. Smaller, more practical restoration efforts in areas of relatively low nitrogen input may be more justifiable as means of supplementary nitrogen removal.

Table 8 Great South Bay nitrogen sequestration potential. Estimates are based on proposed restoration areas from the Hudson-Raritan Comprehensive Restoration Plan (USACE & PANYNJ 2009). Estimates of the total number of oysters that can cover these proposed areas assume 50% coverage of the area to allow for sufficient water flow in an aquaculture-based design similar to that presented in this study. Site specific mortality is accounted for in all estimates. The percent of yearly nitrogen load assimilated by oysters is based on the estimate of 8.5×10^5 kg N yr⁻¹ for annual N load (Kinney and Valiela 2011).

Proposed restoration area (acres)	Great South Bay 2011		
	Total # of oysters	Total N sequestered(kgN)	% of yearly N load
6,387	1.5×10^{10}	$1.6 \times 10^6 \pm 1.6 \times 10^5$	190±19
5,000	1.1×10^{10}	$1.3 \times 10^6 \pm 1.3 \times 10^5$	148±15
5,00	1.1×10^9	$1.3 \times 10^5 \pm 1.3 \times 10^4$	15±2

Discussion

The inherent variability of nitrogen content for individual oysters is evident in our regression analysis for both tissue and shell. This displays the importance of analyzing sufficient sample sizes to develop regression models that accurately predict nitrogen content. There was no significant difference in median values of nitrogen content of dry tissue across Jamaica Bay study sites. However, there were significant differences observed in the 2011 Great South Bay study. This observation may be the result of different growth rates of tissue between sites. Differences in tissue growth among sites may be explained by differences in food availability or composition of diet. Laboratory growth experiments have shown growth of juvenile *C. virginica* to be positively correlated with C:N ratios of cultured diet (Flaak & Epifanio 1978). In support of this finding, Utting (1986) found a negative correlation between the protein content of algal diets and growth of *C. gigas*. Larger oysters may have a greater proportion of storage products that are carbon rich and nitrogen poor. This may partially explain observations of our linear regression analysis that show a negative trend between dry tissue weight and nitrogen content. At sites that experienced greater tissue growth it may be expected that nitrogen content at these sites would be lower. This pattern is observed in our data and may partially explain site to site

significant differences in nitrogen content. Predicted values of %N for a given dry tissue weight (Table 4) are similar to those estimated by Higgins (2011). A similar trend of decreasing nitrogen content with increasing dry tissue weight appears in their estimates. This trend is not apparent in estimates provided by Grizzle (2011), but that may be due to small sample size (n=10) used in their study.

Newell (2005) observed that native oyster shell 76mm in height weighs approximately 150g. This shell weight is substantially more than the weights observed in this study (Table 5). Shell weight is dependent on growth rate and age of the organism. Lower shell weight may be the result of using younger individuals. Additionally, lower shell weight in aquacultured oysters may be environmentally determined. Oysters in aquaculture bags and cages do not undergo extensive disturbance by predators and other secondary factors, and therefore allocate less energy to making thick shells. This pattern was described and noticed by Paynter and DiMichele (1990) when comparing native and tray-cultured oysters. As a result of this, more energy may be available to allocate towards reproduction and tissue growth, and may explain dry tissue weights (Table 4) that are larger than the 1g dry tissue weight of a 76mm native oyster described by (Newell 2005). Newell (2005) also estimated nitrogen sequestration by an individual 76mm oyster to be approximately 0.52g. The difference between this value and those described in this study can be attributed to the substantially smaller shell weights in this study. Although nitrogen sequestration values for native oyster populations by Newell (2005) are more than double those described by our study (Table 6), oysters under aquaculture conditions grow relatively rapidly, so on a rate basis this may compensate for lower shell growth in terms of nitrogen sequestration.

Physiological assessments are necessary and helpful indicators in determining the potential for organisms to restore ecosystem services to a region. Our aquaculture based assessment shows potential for the eastern oyster to act as a bioextraction tool. In this assessment the oyster displayed substantial growth and minimal mortality across a sizable range of environmental variables. Despite the unsuitable conditions that are often associated with eutrophic estuaries, environmental conditions in both regions appeared congenial for oyster growth and survival. Aquacultured oysters with an initial planting size of approximately 30mm in these environmental conditions grow rapidly and reach market size within about 6 months. The aquaculture based design of this study avoids mortality due to predation since oysters are contained in small mesh bags. Therefore, the results summarized in Table 6, 7, and 8 can only be directly applied to aquaculture-based restoration efforts. Further research must be done to quantify mortality due to predation and sedimentation if reef restoration is the ultimate goal.

In our assessment significantly greater growth was evident in Jamaica Bay. Greater observed growth is likely explained by the greater primary productivity present in Jamaica Bay. Greater primary productivity may be explained by enhanced nutrient input that relieves nutrient limitation on phytoplankton growth, combined with a phytoplankton response that includes a large proportion of the phytoplankton with retainable cell sizes over 5 μm . Kirby (2005) showed that in relation to periods prior to eutrophication, the eastern oyster experienced greater growth

as eutrophication increased. However, there was an upper-bound to the benefits of increased eutrophication. During exceptionally high periods of eutrophication other factors such as hypoxia, increased blooms of harmful algae, and increased incidence of parasitic disease may counteract the benefits of increased food supply. Therefore, it is important to note that the eastern oyster should only be used as a bioremediation tool in conjunction with other efforts to reduce nitrogen loading. If eutrophication continues to increase oysters may no longer be a viable option to help remediate eutrophication in the region.

Observed environmental variables in Jamaica Bay and Great South Bay such as salinity, and dissolved oxygen concentrations remained favorable throughout most of the year. The main constraint on growth, and consequently bioextraction capacity in these regions is temperature. Our assessment shows that oysters are only capable of growth at temperatures above approximately 10°C, limiting growth to only 6 or 7 months of the year. During the remaining months no growth is observed, and therefore no nitrogen will be assimilated into tissue or shell biomass. Importantly, oysters cannot reduce phytoplankton density in Jamaica Bay during the winter phytoplankton bloom, which is a major part of annual production (M. Doall and others, unpublished data). Seeing as there is no nitrogen sequestration during this period, our assessment for bioextraction capacity of the oyster focused on the first year growing season. Oysters grow fastest during their first 3 months of life (Bahr 1976) and therefore will assimilate the greatest relative amount of nitrogen during this time.

Using first year growth does not account for substantial second year mortality observed in Jamaica Bay that is likely due to a combination of disease and environmental stress. Further research should be done to quantify recruitment in order to determine if these populations are self-sustaining. Restocking large-scale projects may not be economically feasible. Estimates for nitrogen removed per oyster and per cage of oysters at each study region are summarized in Table 6. Cage estimates account for region specific first-year mortality observed during the physiological assessment. Estimates do not include a subtraction of the nitrogen sequestered during hatchery growth. At a large-scale it is assumed that the supply of a large amount of seed will need to be grown on-site to keep up with substantial numbers of seed needed for these projects. The number of oysters needed for varying restoration scales is summarized for Jamaica Bay (Table 7) and Great South Bay (Table 8). At large-scales obtaining and growing seed at such large quantities may still be problematic. These estimates fail to account for nitrogen returned to the system due to mortality. The nitrogen sequestered in tissue will be expected to remineralize quickly, while nitrogen sequestered in shell will remain sequestered for a longer period of time.

Three oyster restoration scales were proposed by the Hudson-Raritan Estuary comprehensive restoration plan (USACE & PANYNJ 2009). The 6,387 acres restoration size refers to the estimated suitable habitat in Jamaica Bay that meets four suitability criteria (i.e. salinity, bathymetry, dissolved oxygen concentration, and total suspended solids). A visual representation of this area can be seen in the inlay of Figure 19. This suitable area is likely an

overestimate, but the total nitrogen sequestered by a restoration effort of this size is calculated in Table 7 for Jamaica Bay. The other two scales are proposed areas for restoration efforts by the HRE plan. This plan hopes to restore 500 acres of oysters to the Hudson-Raritan Estuary by 2015, and 5,000 acres by 2050. Areas for oyster restoration in Great South Bay have not been proposed, but are calculated in Table 8 for comparison.

The percent of the yearly load of nitrogen to the estuary that can be potentially sequestered by oysters is calculated in Tables 7 and 8. In Jamaica Bay the yearly nitrogen load is 5.8×10^6 kg N yr⁻¹ (Benotti et al. 2005); in Great South Bay the yearly nitrogen load is 8.5×10^5 kg N yr⁻¹ (Kinney and Valiela 2011). It is evident that oysters in Jamaica Bay are capable of removing more nitrogen on a per acre basis. This larger amount is attributed to greater growth seeing as %N of tissue and shell were fairly similar. Smaller values of cumulative mortality in Jamaica Bay also contribute to higher overall nitrogen sequestration in relation to nitrogen sequestration in Great South Bay. Due to the lower input of nitrogen to Great South Bay, restored oyster populations in this region have a greater effect on remediating nitrogen input to the Bay. Therefore, the scale of restored oyster populations for a desired reduction in nitrogen in a less eutrophic bay may be more practical and feasible in relation to a highly eutrophic bay.

Estimates for nitrogen sequestration by oysters per unit of area assume 50% coverage of the area by aquaculture cages. This coverage is assumed to allow for sufficient water flow. Galtsoff (1964) stated that the free exchange of water is necessary to maintain the growth, fattening, and reproduction of oysters. Sufficient water flow provides food and oxygen while taking away metabolites and feces. The stocking density of oysters should be considered in more detail when planning restoration efforts on large-scales. The ability of oysters to remediate water conditions in eutrophic estuaries is likely not a linear function of bivalve density; positive effects may exist at low or moderate densities and lost at extremely high densities (Newell 2004). Extensive aquaculture may cause overenrichment of the sediment by feces and pseudofeces. This may result in intense microbial activity that can cause localized anoxia and the accumulation of hydrogen sulfides.

The provided estimates of nitrogen sequestration do not attempt to quantify other sources that of nitrogen sequestration and removal that may be the byproduct of oyster cultivation. One notable mechanism that may remove additional quantities of nitrogen from the localized system is the denitrification and burial of nitrogen. Newell (2005) estimated that the nutrient removal for oysters at a density of 1g DW m⁻² over 4,290 acres is approximately 13,080kg N. Based on the estimate of .52g N per individual oyster (Newell 2005), this population would annually sequester approximately 9,000kg N. Although removal of nitrogen via denitrification is substantial, removal is highly dependent on certain environmental conditions. In order for nitrogen to be lost from the system as N₂, an aerobic layer of sediment must exist above an anaerobic layer (Newell 2004). If these conditions do not occur, coupled nitrification-denitrification will cease. Suitable conditions dependent on dynamics of the region may be highly variable. The quantification of nitrogen sequestration and removal through harvest of

bivalves is a more direct and less variable calculation. The relative ease in these estimates may facilitate programs and regulatory plans that promote aquaculture and bioextraction through assimilation credits.

Fouling organisms associated with oyster aquaculture may add to values of nitrogen sequestration. No fouling was evident in Great South Bay, but extensive fouling was observed in Jamaica Bay during spring to fall. The most commonly observed fouling organisms were ascidians sponges, barnacles, tunicates, and mussels. Although these organisms may be an additional source of nitrogen sequestration, it is unknown how fouling affected the growth and survival of oysters. If these effects are substantial, the net sequestered nitrogen may be less than a simple summation of nitrogen sequestered by oysters and fouling organisms. For large-scale operations it would be extremely labor-intensive to clean cages of fouling, it may be necessary to use antifouling coating on cages and bags. Additional nitrogen may be sequestered as the result of fisheries augmentation, which is often viewed as an ecosystem service provided by oyster populations (Coen et al. 2007). The structural complexity introduced to a habitat by oyster populations has been found to be associated with increased abundance, biomass, and species richness (Coen and Luckenbach 2000). Additional biomass attributed to oyster restoration can be viewed as additional nitrogen sequestration in the biomass of a variety of species.

Although the eastern oyster displays noteworthy potential for restoration efforts and use as a bioextraction tool, other considerations of practicality must be considered. According to the Department of Environmental Conservation all lands within Jamaica Bay are closed to shellfishing. Although these sites are closed to shellfishing for human consumption, the culture and removal of oysters in these areas are still possible. These oysters cannot be sold as food, fertilizer, or chicken feed due to contamination, and so no monetary gain will result from their harvest. However, the monetary gain that results from ecosystem services provided by oysters may be greater than their sale for a variety of uses. In Great South Bay a fair portion of the bay is closed to shellfishing. These closed areas mainly consist of tributaries, canals, and creeks. Closed areas forbid the taking and use of shellfish for food but may be removed and transported to state approved relay programs (see <http://www.dec.ny.gov/regs/4014.html#12835>) for regulations and closures). Oysters raised in closed areas for the purpose of enhancing water quality may be seen as an attractive nuisance. The problems associated with poaching and consumption of oysters in polluted regions may add additional costs to enforce and regulate associated legal and health concerns.

The eastern oyster shows great potential to act as a supplementary means of remediation for eutrophication if restoration is successful on large scales. Although calculations show that eastern oyster populations are capable of sequestering and removing nitrogen from estuaries upon harvest, the practicality of such large-scale restoration efforts remains an important question. The startup cost of a 5,000 acre off-bottom oyster restoration project in Jamaica Bay was calculated based on cost estimates from Wieland (2008). Maintenance costs are not considered, but the startup cost of a project this size would be approximately \$827 million. In

addition to this point, the survivorship and growth of organisms is often rather variable on a year-to-year basis. The success of restoration efforts, and therefore success of the eastern oyster as a bioremediation tool is inherently variable. Upgrading wastewater treatment plants are costly, but are not variable in nature. Upgrades will ensure nitrogen reductions; while efficiency of oyster populations in removing nitrogen will vary. The use of oysters as bioextraction tools may be most useful in removing nitrogen from nonpoint sources and used in conjunction with wastewater treatment plant upgrades.

Conclusion

These studies reveal that both of these regions are still conducive to the growth and survival of eastern oyster populations despite habitat degradation and eutrophication. Although fast growth rates and high cumulative survivorship may suggest promise for using the eastern oyster as a nutrient bioextraction tool in these areas, caution must be taken to realize the inherent variability in restoring oyster populations. This variability will translate to the use of eastern oyster populations as a bioremediator. Additionally, the size of restoration projects needed to act as substantial sinks for nitrogen is important to note. Aside from economic concerns, it is unknown if restoration at such a large-scale is possible from an ecological perspective. Oyster populations may be best used as supplements to nutrient reduction programs that aim to reduce nutrient loading through wastewater treatment plant upgrades. The use of the oyster as a nutrient bioextraction tool may be best suited for the remediation of nonpoint sources of nutrients. These aquaculture-based assessments may act as preliminary results for larger scale projects in these regions. Further research must be done to quantify the differences in growth between aquaculture-based and on-bottom or reef restoration. It is likely that these results will be more variable as aquaculture restoration efforts are not affected by variables such as predation and sedimentation.

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