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ABSTRACT

Eight studies were conducted within the Hudson River Estuary under the auspices of the Tibor T. Polgar Fellowship Program during 2012. Major objectives of these studies included: (1) assessing the effects of sea level rise and increased salinity on decomposition dynamics in Hudson River tidal marshes, (2) determining the abundance and persistence of fecal indicator bacteria in Hudson River sediments, (3) documenting oyster recruitment at six Hudson River sites, (4) evaluating the role of mud crabs in post-settlement mortality of juvenile Eastern oysters and its possible effects on oyster restoration efforts, (5) assessing the role of temperature in the shift in size structure of zebra mussels in the Hudson River, (6) studying the effect of zebra mussel introduction on larval and juvenile striped bass prey diversity, (7) quantifying parasite diversity in banded killifish as a preliminary indicator of ecosystem health in the comparison of three littoral habitat types in the upper Hudson, and (8) refining Bioimpedance Assessment for determining lipid content in American eel and its use as a tool for quantifying eel health.

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PREFACE

The Hudson River estuary stretches from its tidal limit at the Federal Dam at Troy, New York, to its merger with the New York Bight, south of New York City. Within that reach, the estuary displays a broad transition from tidal freshwater to marine conditions that are reflected in its physical composition and the biota its supports. As such, it presents a major opportunity and challenge to researchers to describe the makeup and workings of a complex and dynamic ecosystem. The Tibor T. Polgar Fellowship Program provides funds for students to study selected aspects of the physical, chemical, biological, and public policy realms of the estuary.

The Polgar Fellowship Program was established in 1985 in memory of Dr. Tibor T. Polgar, former Chairman of the Hudson River Foundation Science Panel. The 2012 program was jointly conducted by the Hudson River Foundation for Science and Environmental Research and the New York State Department of Environmental Conservation and underwritten by the Hudson River Foundation. The fellowship program provides stipends and research funds for research projects within the Hudson drainage basin and is open to graduate and undergraduate students.

Prior to 1988, Polgar studies were conducted only within the four sites that comprise the Hudson River National Estuarine Research Reserve, a part of the National Estuarine Research Reserve System. The four Hudson River sites, Piermont Marsh, Iona Island, Tivoli Bays, and Stockport Flats exceed 4,000 acres and include a wide variety of habitats spaced over 100 miles of the Hudson estuary. Since 1988, the Polgar Program has supported research carried out at any location within the Hudson estuary.

The work reported in this volume represents the eight research projects conducted by Polgar Fellows during 2012. These studies meet the goals of the Tibor T. Polgar Fellowship Program to generate new information on the nature of the Hudson estuary and to train students in estuarine science.

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FIELD AND LABORATORY INVESTIGATIONS ON THE EFFECTS OF SALINITY ON DECOMPOSITION DYNAMICS AMONG THE HUDSON RIVER'S FRESHWATER TIDAL WETLANDS

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

Sea level rise due to climate change will expose Hudson River tidal marshes to chronic shifts in salinity, thus altering habitat conditions and biogeochemical processes. Increased salt intrusion may affect macroinvertebrates and microbial colonies important in the decomposition of the invasive plant species, *Phragmites australis.* It was hypothesized that litter breakdown varies along the Hudson River salinity gradient, and salinity intrusion will negatively affect macroinvertebrate and micro-organisms. To study the role of salinity in dictating decomposition dynamics, leaf packets were deployed along the Hudson River for measurements of microbial respiration, fungal biomass, and mass loss. The tolerance of a freshwater isopod (*Ligidium sp.*) and microbial colonies to varying salt concentrations found along the Hudson River was examined. Salinity negatively affected isopod survivorship and microbial activity in controlled laboratory treatments. However, the effect of a varying salinity regime on field measurements is unclear. This study provides a model of a river undergoing continuous sea level rise and changing decomposition dynamics.

Key words: decomposition, salinity, respiration, litter, fungi, microbial colonies, Hudson River, *Phragmites australis*

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INTRODUCTION

Rising temperatures causing thermal expansion of oceans and melting of continental sheet ice have made global sea level rise a current and future concern (Larsen et al. 2010). Global sea level rise has increased through the 20th century and is projected to accelerate, adding 190 cm by 2100 (Vermeer and Rahmstorf 2009). Immediate impacts include increased coastal flooding and salt intrusion of surface waters (Nicholls and Cazenave 2010). Wetlands are highly valued ecosystems (Costanza et al. 1998), with services linked to plant biomass, sediment and nutrient retention, and high rates of above- and below- ground net production (Findlay et al. 1990; Findlay et al. 2002B). Tidal wetlands are detritus-based systems with large quantities of organic matter available for decomposition (Mendelssohn et al. 1999; Quintino et al. 2009). As high impact areas, sea level rise threatens the long-term persistence and functioning of coastal wetlands (Titus 1988; Morris et al. 2002; Nicholls and Cazenave 2010; Larsen et al. 2010). Tidal wetlands show spatial patterns of salinity variations from the daily scale of tides to the annual scale of climate patterns, making them prone to increasing salt intrusion (Quintino et al. 2009). Decomposition of plant material supplies organic matter for the metabolism of rivers and wetlands, nutrient cycling for sustaining food chains and primary production, and supports a rich biodiversity of macroinvertebrates, fish, and wildlife (Jordan et al. 1989; Gessner and Chauvet 1994; Baldy et al. 1995; Mendelssohn et al. 1999; Komínková et al. 2000; Quintino et al. 2009). Litter breakdown involves tissue softening and consumption by fungi, microbial colonies, and detritus feeding macroinvertebrates (Webster and Benfield 1986; Gessner and Chauvet 1994; Baldy et al.

1995; Komínková et al. 2000; Graça 2001; Hieber and Gessner 2002; Van Ryckegem et al. 2007).

Phragmites australis (common reed) is an invasive species in tidal wetlands. *Phragmites* stands are productive systems that enhance wetland biomass, modify community structure, and promote resource availability (Gessner 2001; Morris et al. 2002; Findlay et al. 2002B; Quintino et al. 2009). *Phragmites* is tolerant of brackish conditions and is expected to spread as the brackish zone of the Hudson River Estuary moves north (Baldwin and Mendelssohn 1998; Chambers et al. 2003).

Several studies have examined how salinity and macro-and micro-organisms control rates of decomposition of *Phragmites* along a salinity gradient (Reice and Herbst 1982; Hemminga et al. 1991; Mendelssohn et al. 1999; Piscart et al. 2005; Quintino et al. 2009). In response to salinization and the extensive invasion of *Phragmites*, it is important to identify factors controlling organic matter decomposition in tidal freshwater wetlands for future mitigation and wetland restoration projects (Mendelssohn et al. 1999).

This study examined the effect of sea level rise on decomposition within a major river with a salinity gradient. Literature has indicated that mass loss from field measurements (Jordan et al. 1989; Findlay et al. 1990; Hemminga et al. 1991; Windham 2001; Findlay et al. 2002B; Hieber and Gessner 2002; Quintino et al. 2009), respiration by microbial colonies (Findlay et al. 1990; Komínková et al. 2000), ergosterol content as an indicator for fungal biomass (Baldy et al. 1995; Komínková et al. 2000; Gessner 2001; Gessner and Newell 2002; Findlay et al. 2002A; Hieber and Gessner 2002), and salt treatments to macroinvertebrates and microbial colonies (Blasius and Merritt 2002; Baumann and Marschner 2011) are appropriate measures to predict the contribution of

consumer organisms to decomposition, and the effect of salinity on macroinvertebrate and microbial activity. This study presents a novel approach to use well established field and laboratory techniques to measure decomposition within emergent, tidal wetlands along a river spanning a full estuarine salinity gradient.

METHODS

Site Description

The Hudson River Estuary extends 250 km (154 miles) from the Federal Lock and Dam at Troy, New York to The Battery, at the southern tip of Manhattan Island (Yozzo et al. 2005). Mean tidal amplitude ranges from 0.8 m (West Point) to 1.4 m (Troy). The estuary is tidal freshwater (< 0.1 ppt) from Troy south to Poughkeepsie (River Mile "RM" 75) (Yozzo et al. 2005). Depending on freshwater discharge rates, the salt front migrates between the Tappan Zee Bridge (RM 30) and Newburgh (RM 60) throughout the year (Limburg et al. 1986; Yozzo et al. 2005). Intertidal wetlands occur throughout the estuary and tidal freshwater wetlands are found from Albany south to Manitou Marsh (Yozzo et al. 2005). Oligohaline and mesohaline marshes occur along an increasing salinity gradient from Manitou Marsh south to New York City (Yozzo et al. 2005). This study was conducted at the Piermont, Iona, Manitou, Con Hook, and Constitution Marshes from early June to late August 2012 (Fig. 1a, b). Piermont Marsh is a tidal mesohaline marsh (5-15 ppt) located along the west shoreline of the Hudson River about 26 km south of Iona Island (Yozzo and Osgood 2012). Piermont Marsh is covered predominately by *Phragmites* (65% in 1991); native vegetation is restricted to the interior of the marsh (Yozzo and Osgood 2012). Iona Marsh is an oligohaline intertidal marsh and upland habitat, also predominately covered by *Phragmites* (70%

Phragmites; 30% narrowleaf cattail, *Typha angustifolia*) as well as other minor vegetation: broadleaf emergent plants [e.g., pickerelweed (*Pontedaria cordata*) and arrow-arum (*Peltandra virginica*)] (Yozzo and Osgood 2012).

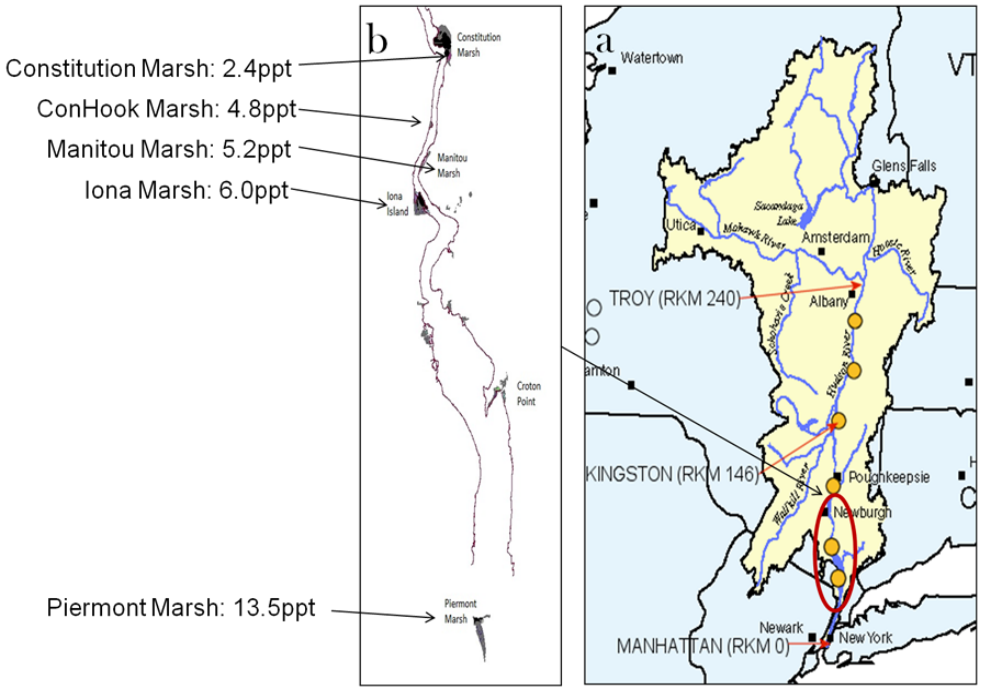

Figure 1. A map of the Hudson River Watershed (a) and Estuary (b). Salinity weekly average for Piermont, Iona, Manitou, Con Hook, and Constitution Marshes were measured during mid-August.

Manitou Marsh is a freshwater to slightly brackish enclosed tidal marsh (0-7 ppt) located on the eastern shore of the Hudson River (Limburg et al. 1986). Manitou Marsh is partially isolated from the Hudson River by a railroad and natural ledge and contains *Phragmites*, narrowleaf cattail, purple loosestrife (*Lythrum salicaria*) and spotted touch-me-not (*Impatiens capensis*). Con Hook Marsh is ~1 km distance north of Manitou Marsh and likely has similar salinity ranges. Constitution Marsh is a freshwater to slightly brackish, enclosed tidal marsh (0-5 ppt) located on the eastern shore of the Hudson River (Limburg et al. 1986). Constitution Marsh is dominated by narrowleaf cattail, but arrow-

arum, pickerelweed, broadleaf arrowhead (*Sagittaria latifolia*), rice cutgrass (*Leersia oryzoides)*, and wild rice (*Zizania sp.*) are also common, while *Phragmites* stands are minor and controlled.

Salinity Range

Salinity data for Piermont, NY and Hastings, NY were analyzed using long-term USGS records (archived conductivity data provided by Gary Wall, USGS, Troy, NY). Daily averages were calculated from October 2005-November 2010 for Hastings and November 2010-September 2011 for Piermont due to differing lengths of records. A histogram of the frequency of salinities (ppt) containing bins: 0, 5, 10, 15, 20 was used to assess the range of salinities from October 2005-September 2011. The frequency of daily averages clearly reached 15 ppt (13% days from 2005-2010, Hastings; 1.8% days from 2010-2011, Piermont) for experimental purposes. During the experimental period, salinity data were collected with YSI Sondes logging at 15 min intervals at all five sites, and averages were calculated during 9 -14 days from 11 July-3 August. Additional salinity measurements were made from grab samples collected during field work at each site in mid-July and early-August.

Macroinvertebrate and Microbial Tolerance to Salinity

A laboratory experiment was conducted to examine macroinvertebrate tolerance to salinity. A simple feeding experiment was performed, giving a common freshwater isopod (*Ligidium sp.*) 1 cm leaf discs of *Phragmites.* Mass loss measurements of leaf discs were made 16 days later. In addition, *Ligidium sp.* were collected at Constitution Marsh and were exposed to salt concentrations similar to conditions spanning the Hudson River Estuary (Blasius and Merritt 2002; Yozzo and Osgood 2012). Daily salinity

averages calculated from October 2005 – September 2011 for Hastings and Piermont were used to select salinity concentrations (ppt): 0, 5, 10, 15, and 20. Salt solutions were made using Instant Ocean and freshwater from the Hudson River and monitored using a YSI salinity meter. Isopods were held in aerated tanks until exposed to salt treatments. Each experimental unit was replicated 4x per treatment and contained four isopods, 20 ml of water varying in salinity, and some detritus. Isopods were exposed to salt treatments for 96 hrs with observations of behavior and mortality recorded every 24 hrs. A similar controlled, laboratory experiment was conducted to assess the effect of salinity on microbial respiration. *Phragmites* leaves were incubated at Constitution Marsh for four weeks. Upon retrieval, 1 cm diameter leaf discs were placed into cups with water from Constitution Marsh and salt amendments (ppt) to comprise: 0, 5, 10, 15, and 20. Leaf discs sat in salt treated water for 96 hrs, while water was changed every 24-48 hrs. After 96 hrs, 10 leaf discs were placed into 60 ml BOD bottles to measure respiration (mg DO ml^{-1} hr^{-1}) over a 12-13 hr interval.

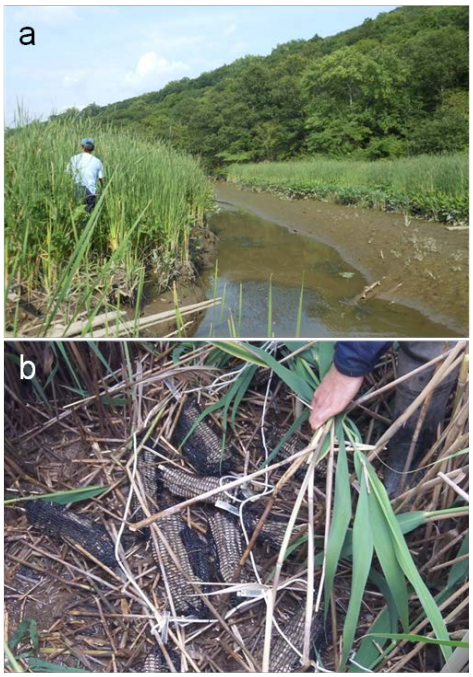

Figure 2. Research approach (a) Typical *Phragmites australis* stand at Manitou Marsh, (b) leaf packet design.

In situ Mass Loss

To study the effect of a salinity gradient on mass loss, leaf litter packets containing 5 g dry weight of *Phragmites* leaves were deployed for approximately four and eight weeks at the five marsh sites (Fig. 2 a, b). Leaf packets were deployed 4-7 June. The first round of packets

was collected 11-19 July, and the second round was collected 31 July- 3 August. Leaf packets consisted of a 1 cm x 1 cm mesh size to allow macroinvertebrate colonization. Twenty-three leaf packets were deployed at each site and were held together by a nylon rope and PVC piping staked into the marsh sediment. Leaf packets were transported to the field individually in paper bags in order to collect mass loss during handling. Three leaf packets at each site were removed immediately to estimate mass loss during field transfer. Leaf packets were placed in varying densities of *Phragmites* and narrowleaf cattail during low tide and in small tributaries off the main channel of the Hudson River, where disturbance would be minimal. Attempts were made to place packets at the same elevation so they were inundated equally.

Upon retrieval, leaf litter was cleaned of sediment and a portion of each leaf packet was removed for leaf discs and small segments for respiration and ergosterol measurements. Leaf litter was dried at 70^{o}C for 24 hrs and combusted in a muffle furnace at 450^{o}C for four hours for ash free dry mass (AFDM) determination. Mass loss during handling and field transfer, and mass removed for respiration discs and ergosterol segments were converted to an AFDM corresponding to the percent loss AFDM of original leaf matter from packets. These values were added back to the AFDM of litter from packets to obtain a final percent AFDM after four and eight weeks. The average mass lost due to handling and field transfer, and mass removed for respiration and ergosterol measurements were 2.7% and 12.7% respectively of the average mass of leaf litter from packets.

Microbial Respiration

To estimate rates of respiration (mg DO ml^{-1} hr^{-1}), dissolved oxygen remaining in

BOD bottles containing leaf litter associated microbes over a 12-13 hr interval was measured. Respiration was measured on leaf litter collected after four and eight weeks. Prior to measurements, leaf litter was cleaned using Hudson River water from the corresponding site to minimize variation between laboratory and field conditions and prevent shock to microbial colonies. Leaf discs were cut using a core (1 cm diameter) immediately following retrieval of packets and placed in BOD bottles filled with corresponding Hudson River water. Hudson River water was used at room temperature to prevent supersaturation of DO. BOD bottles were measured for initial DO concentration and a final DO concentration after a 12-13 hr interval.

Fungal Biomass

To estimate fungal biomass, ergosterol content (μg erg/mg DM) was measured (Gessner and Newell 2002; Findlay et al. 2002B). Upon retrieval, sediment was cleaned with Hudson River water from corresponding sites and 10 leaf segments (2 cm long) were made from random leaves. Leaf segments were stored in 20 ml methanol and placed in a freezer (-20^{o}C). For the first round of leaf packets, an additional 10 leaf segments per leaf packet were made for AFDM determination. Samples were extracted for two hours at 65^{o}C and then cooled in an ice bath. A 5 ml saponification solution containing 4% KOH was used, followed by additional warming and cooling. A series of 10 ml and 5 ml pentane solutions were added and mixed. Pentane containing ergosterol was extracted and evaporated to dryness. Dissolved ergosterol residue was put in 1 ml methanol, sonicated, and then filtered using acrodisks in 2 ml HPLC vials. Samples were stored in the freezer until HPLC analysis. The UV detector was set to 282 nm, while methanol as a mobile phase was set to 1.0-1.5 ml/min. Ergosterol standards were

included during the HPLC analysis. Retention time of ergosterol was dependent on flow rate, temperature, and column properties, and ranged from 4-5 min. A 20 µl injection of samples was used for analysis of amount of ergosterol.

Statistical Analysis

Statistica software was used for statistical analysis. ANOVA was used to compare cumulative mortality of *Ligidium sp.* and microbial respiration across salinity treatments. ANOVA was also used to compare field microbial respiration, ergosterol content, and decomposition across sites. Model significance required p-value ≤ 0.05. Tukey's post-hoc test was used to delineate significance among treatments and report data as mean ±SE. The data do not meet the ANOVA assumption of normality when log transformed due to small sample sizes.

RESULTS

Average salinities (ppt) for Piermont, Iona, Manitou, Con Hook, and Constitution Marshes were 13.5, 6.0, 5.2, 4.8, and 2.4, respectively. Spot measurements for these marshes after four and eight weeks were in the range of average salinities (ppt) calculated from 11 July- 3 August: 7.5 and 9.7, 3.8 and 3.0, 2.6 and 2.4, 2.9 and 2.1, and 1.9 and 1.6, respectively. Results show *Ligidium sp.* fed on leaf litter causing roughly a 2%/day reduction in mass of leaf discs. Cumulative mortality over the 96 hr salinity exposure showed significant differences among treatments (Fig. 3, ANOVA $p< 0.001$). No mortality occurred in 0 ppt, indicating that mortality was due to salt treatments and lab conditions provided suitable conditions. Immediate negative salinity effects were found with significant differences from exposure to 15 ppt and 20 ppt (2.75± 0.71, $p <0.05$, LSD test; 4.0± 0, $p < 0.05$, LSD test). Observations of isopod behavior exhibited less

responsiveness to prodding and lethargy when exposed to salinity compared to the control. This behavior intensified in 10 ppt, 15 ppt, and 20 ppt treatments with increased exposure. Microbial respiration rates (mg DO ml^{-1} hr^{-1}) significantly differed among treatments (Fig. 4, ANOVA $p < 0.001$). Freshwater-colonized microbes had decreased respiration at all salinities greater than 2 ppt (25%, $p < 0.05$, LSD test).

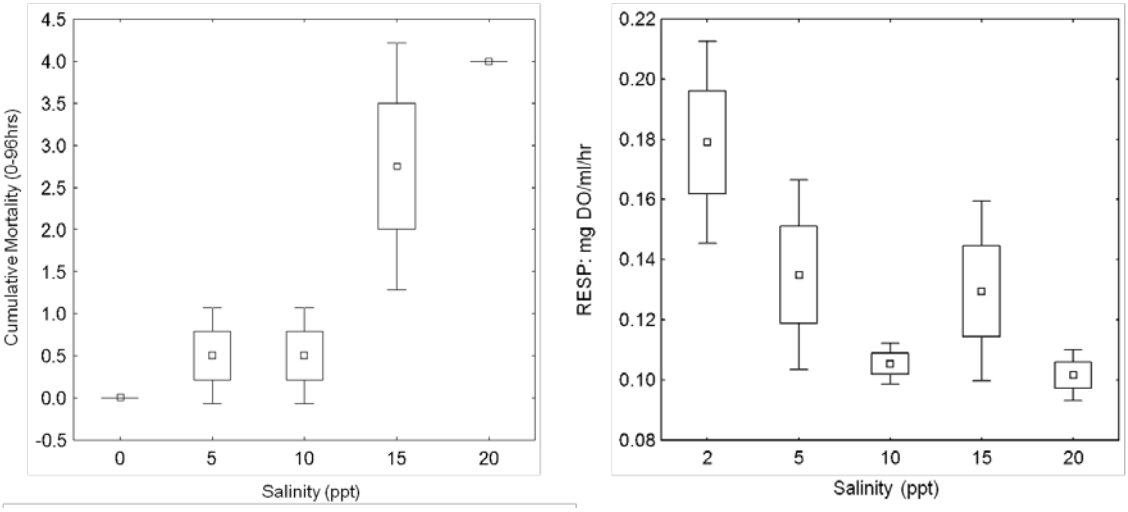

Figure 4. Microbial respiration across salinity treatments after 96hrs.

In situ mass loss experiments showed field measurements of percent mass loss AFDM (g) after eight weeks were significantly different among sites (Fig. 5, ANOVA $p<0.0001$); however, there was no clear relationship between salinity and percent mass loss. Percent mass loss was highest at Iona Marsh (62.95± 1.46%) and lowest at Con Hook Marsh (37.61± 0.92%). Field respiration rates (mg DO $ml^{-1}hr^{-1}$) after four and eight weeks were combined since there was no effect of time in the field. Respiration was significantly different among sites (Fig. 6, ANOVA $p<0.001$), but there was no clear relationship between salinity and *in situ* microbial respiration. Microbial respiration was highest at Piermont Marsh (0.40± 0.02 mg DO $ml^{-1}hr^{-1}$) and lowest at Con Hook Marsh

(0.23± 0.01 mg DO $ml^{-1}hr^{-1}$). Fungal biomass (reported as ergosterol) was significantly different among sites (Fig. 7, ANOVA *p<0.001*), but there was no clear relationship between salinity and ergosterol content. Ergosterol content was highest at Con Hook Marsh (0.17 ± 0.02 μg erg/mg DM) and lowest at Iona Marsh (0.06± 0.005 μg erg/mg DM).

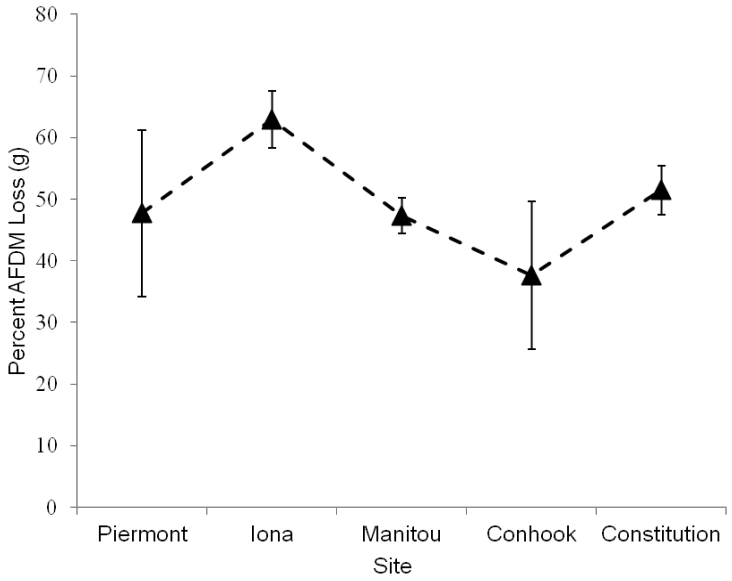

Figure 5. Percent ash free dry mass (AFDM) loss after 8 weeks across the Hudson River salinity gradient.

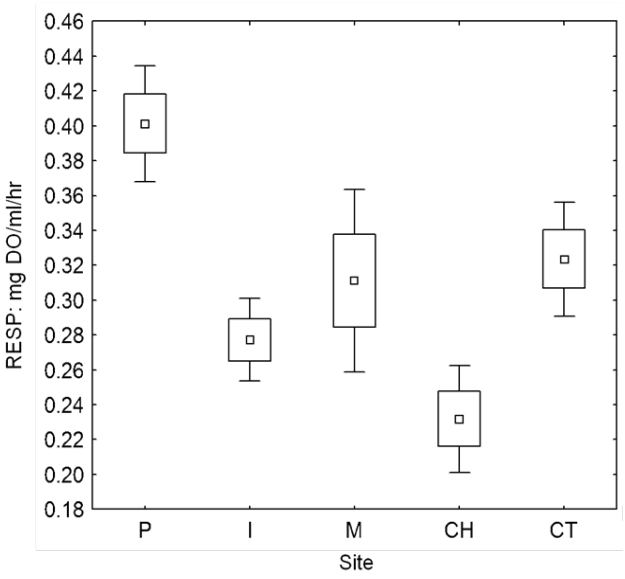

Figure 6. Field microbial respiration across the Hudson River salinity gradient combined 4 and 8 week measurements: Piermont (P), Iona (I), Manitou (M), Con Hook (CH), and Constitution (CT).

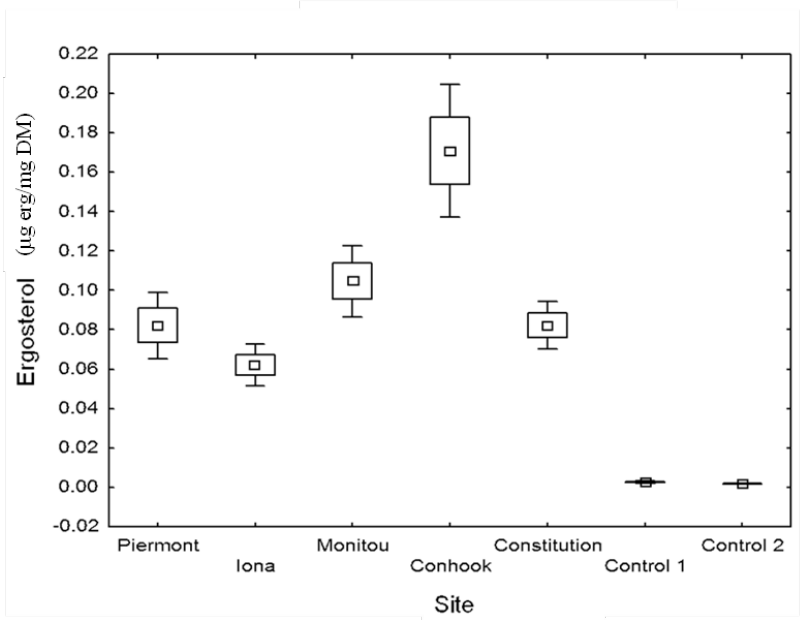

Figure 7. Field ergosterol content across the Hudson River salinity gradient 4 and 8 week measurements combined.

DISCUSSION

The impacts of sea level rise from climate change are a current and future concern for the structural integrity and community composition of wetlands (Titus 1988; Costanza et al. 1998; Morris et al. 2002; Nicholls and Cazenave 2010; Larsen et al. 2010). Litter decomposition has been widely studied in inland wetlands, providing ecosystem services that drive nutrient cycling, and primary and secondary productivity (Webster and Benfield 1986; Jordan et al. 1989; Hemminga et al. 1991; Gessner 2001; Windham 2001; Findlay et al. 2002B). The dominant wetland macrophyte, *Phragmites australis* (common reed) has received considerable attention due to its extensive invasiveness and ability to drive wetland detrital processes (Findlay et al. 2002B). Several studies have examined the role of salinity in dictating decomposition dynamics of *Phragmites* associated with litter mass loss, microbial respiration, and fungal abundances (Reice and Herbst 1982; Hemminga et al. 1991; Mendelssohn et al. 1999; Blasius and Merritt 2002; Piscart et al. 2005; Roache et al. 2006; Quintino et al. 2009; Baumann and Marschner 2011). Only

one study to date has considered the effect of sea level rise and salt intrusion on decomposition dynamics of *Phragmites* across a full salinity gradient (Quintino et al. 2009).

Salinity changes at 15 -20 ppt negatively affected the freshwater isopod, *Ligidium sp.*. Isopods held at 5 -10 ppt exhibited less responsiveness to prodding and a gradual mortality compared to freshwater conditions. This behavior intensified at 15 -20 ppt and with increasing time. Isopods exposed to 10 -20 ppt exhibited immediate mortality, indicating intolerance to rapid, large changes in salinity. Macroinvertebrates contribute to shredding and breakdown of leaf litter (Brinson et al. 1981; Webster and Benfield 1986; Graça 2001; Blasius and Merritt 2002; Collins et al. 2007). *Ligidium sp.* is a common macroinvertebrate at Constitution Marsh that shreds and decomposes *Phragmites*. Increasing salinity at sites near freshwater conditions would be expected to decrease decomposition of *Phragmites* by *Ligidium sp.*. Shifts in salinity might change the biodiversity of common shredding macroinvertebrates of the Hudson River, leading to further decreases in decomposition rates (Yozzo and Osgood 2012). Similar patterns were seen of increasing mortality of *Gammarus* (Amphipoda) from exposure to high road salt concentrations in a laboratory study on the effect of road salts (NaCl) on macroinvertebrate communities in Lake Michigan (Blasius and Merritt 2002).

Exposing microbial colonies on *Phragmites* to acute salinity increases showed a strong negative effect on microbial respiration. Respiration decreased from changes as low as 5 ppt. This trend intensified at 10 -15 ppt, indicating intolerance to chronic changes in salinity. Microbial assimilation of detritus is critical for softening of leaf tissue and contributes to the decomposition of leaf matter (Brinson et al. 1981; Webster and

Benfield 1986; Gessner and Chauvet 1994; Baldy et al. 1995; Hieber and Gessner 2002). Thus, increasing salinity would be expected to decrease the decomposition of *Phragmites* by microbial colonies adapted to freshwater conditions. Similar decreases in microbial respiration with increasing soil salinity were observed in a study of drying and rewetting (Baumann and Marschner, 2011). Evidence from laboratory experiments also showed reduced microbial activity on a gradient of increasing salinity in a freshwater wetland near Gippsland Lakes, eastern Victoria, Australia (Roache et al. 2006).

Natural variation in salinity among Hudson River marshes did not reveal clear patterns in field respiration, fungal biomass, and mass loss from decomposition. Recent studies on *Phragmites* decomposition along a full salinity gradient (34.6 ppt at the mouth-0 ppt, at the head) of the Mira Channel, Ria de Averio in Western Portugal, showed clearer relationships (Quintino et al. 2009). It was estimated that a 51%, 71%, 70%, and 71% mass loss occurred in 5 mm litter bags containing 3 g *Phragmites* after 60 days in salinities (ppt) of approximately 34.6, 16.2, 2.4, and 0.0, respectively.

Studies show an inverse relationship between water or soil salinity and microbial activity. Decreasing micro-organism activity with increasing salinity was seen in a terrestrial ecosystem from shifts in species diversity (van Bruggen and Semenov 2000). Other studies in terrestrial ecosystems also indicated decreasing microbial biomass (Muhammad et al. 2006; Wichern et al. 2006), and activity (Rietz and Haynes 2003; Sardinha et al. 2003) with increasing salinity. Rising salinity along a gradient has been shown to reduce denitrification activity and the diversity of nitrogen cycling communities, suggesting nitrogen removal capacity will reduce as freshwater marshes become more saline (Larsen et al. 2010). Evidence from laboratory studies showed a

decrease in leaf mass decay for three plant species along an increasing salinity gradient (Roache et al. 2006). Discrepancy between laboratory and field results could be due to varying conditions at field sites compared to a controlled lab setting. These conditions include tides of changing salinity, and sediment on leaf matter which alters respiration rates and the AFDM. A "shock" from instant exposure to salt treatments may contribute to differences between field and laboratory results. The effect that salinity has on fungal communities is poorly understood, but could contribute to variations in fungal dynamics.

In years and decades to come, this pressing issue will become increasingly important as chronic changes in salinity will continue to alter and drive wetland processes. Consequences include adaptation, changes in productivity and community diversity, and diminishing of buffering and restorative services of wetlands. Future studies examining decomposition of *Phragmites* across a salinity regime are required to enhance the knowledge of wetland responses to salt intrusion from sea level rise. Increasing salinization in tidal wetlands is a current issue for preventing the expansion of *Phragmites australis,* and for establishing restoration goals. Climate change projections indicate continuous sea level rise; hence, this study provides a useful approach and initial benchmark for future studies examining projected salinity increases in tidal wetlands along the Hudson River and other freshwater-tidal rivers.

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REFERENCES

Baldy, V., M.O. Gessner, and E. Chauvet. 1995. Bacteria, fungi and the breakdown of leaf litter in a large river. Oikos 74: 93-102.

Baldwin, A.H., and I.A. Mendelssohn. 1998. Effects of salinity and water level on coastal marshes: an experimental test of disturbance as a catalyst for vegetation change. Aquatic Botany 61: 255-268.

Baumann, K., and P. Marschner. 2011. Effects of salinity on microbial tolerance to drying and rewetting. Biogeochemistry. Doi: 10.1007/s10533-011-9672-1.

Brinson, M.M., A.E. Lugo, and S. Brown. 1981. Primary productivity, decomposition and consumer activity in freshwater wetlands. Annual Review of Ecology and Systematics 12: 123-161.

Blasius, B.J., and R.W. Merritt. 2002. Field and laboratory investigations of the effects of road salt (NaCl) on stream macroinvertebrate communities. Environmental Pollution 120: 219-231.

Chambers, R.M., D.T. Osgood, D.J. Bart, and F. Montalto. 2003. *Phragmites australis* invasion and expansion in tidal wetlands: interactions among salinity, sulfide, and hydrology. Estuaries 26: 398-406.

Collins, B.M., W.V. Sobczak, and E.A. Colburn. 2007. Subsurface flowpaths in a forested headwater stream harbor a diverse macroinvertebrate community. Wetlands 27: 319-325.

Costanza, R., R. d'Arge, R. de Groot, S. Farber, M. Grasso, B. Hannon, K. Limburg, S. Naeem, R.V. O'Neill, J. Paruelo, R.G. Raskin, P. Sutton, and M. van den Belt. 1998. The value of ecosystem services: putting the issues into perspective. Ecological Economics 25: 67-72.

Findlay, S.E.G., K. Howe, and H.K. Austin. 1990. Comparison of detritus dynamics in two tidal freshwater wetlands. Ecology 71: 288-295.

Findlay, S.E.G., J. Tank, H.M. Valett, P.J. Mulholland, W.H. McDowell, S.L. Johnson, S.K. Hamilton, J. Edmonds, W.K. Dodds, and W.B. Bowden. 2002. A cross-system comparison of bacterial and fungal biomass in detritus pools of headwater streams. Microbial Ecology 43: 55-66.

Findlay, S.E.G., S. Dye, K.A. Kuehn. 2002. Microbial growth and nitrogen retention in litter of *Phragmites australis* and *Typha angustifolia*. Wetlands 22: 616-625.

Graça, M.A.S. 2001. The role of invertebrates on leaf litter decomposition in streams- a review. International Review of Hydrobiology 86: 383-39.

Gessner, M.O., and E. Chauvet. 1994. Importance of stream microfungi in controlling breakdown rates of leaf litter. Ecology 75: 1807-1817.

Gessner, M.O. 2001. Mass loss, fungal colonization and nutrient dynamics of *Phragmites australis* leaves during senescence and early aerial decay. Aquatic Botany 69: 325-339.

Gessner, M.O., and S.Y. Newell. 2002. Biomass, growth rate, and production of filamentous fungi in plant litter. Manual of Environmental Microbiology 2: 390-408.

Hemminga, M.A., J. de Leeuw, W. de Munck, and B.P. Koutstaal BP. 1991. Decomposition in estuarine salt marshes: the effect of soil salinity and soil water content. Vegetatio 94: 25-33.

Hieber, M., and M.O. Gessner. 2002. Contribution of stream detrivores, fungi, and bacteria to leaf breakdown based on biomass estimates. Ecology 83: 1026-1038.

Jordan, T.E., D.F. Whigham, and D.L. Correll. 1989. The role of litter in nutrient cycling in a brackish tidal marsh. Ecology 70: 1906-1915.

Komínková, D., K.A. Kuehn, N. Büsing, D. Steiner, and M.O. Gessner. 2000. Microbial biomass, growth, and respiration associated with submerged litter of *Phragmites australis* decomposing in a littoral reed stand of a large lake. Aquatic Microbial Ecology 22: 271-282.

Larsen, L., S. Moseman, A.E. Santoro, K. Hopfensperger, and A. Burgin. 2010. A complex-systems approach to predicting effects of sea level rise and nitrogen loading on nitrogen cycling in coastal wetland ecosystems. American Society of Limnology and Oceanography Eco-DAS VIII Symposium Proceedings Chapter 5: 67-92.

Limburg, K.E., M.A. Moran, and W.H. McDowell. 1986. The Hudson River ecosystem. Springer-Verlag, New York pp 6-39.

Mendelssohn, I.A., B.K. Sorrell, H. Brix, H.H Schierup, B. Lorenzen, and E. Maltby. 1999. Controls on soil cellulose decomposition along a salinity gradient in a *Phragmites australis* wetland in Denmark. Aquatic Botany 64: 381-398.

Morris, J.T., P.V. Sundareshwar, C.T. Nietch, B. Kjerfve, and D.R. Cahoon. 2002. Responses of coastal wetlands to rising sea level. Ecology 83: 2869-2877.

Muhammad, S., T. Müller, and R.G. Joergensen. 2006. Decomposition of pea and maize straw in Pakistani soils along a gradient in salinity. Biology and Fertility of Soils 43: 93-101.

Nicholls, R.J., and A. Cazenave. 2010. Sea-level rise and its impact on coastal zones. Science 328: 1517-1520.

Piscart, C., J.C. Moreteau, and J.N. Beisel. 2005. Biodiversity and structure of macroinvertebrate communities along a small permanent salinity gradient (Meurthe River, France). Hydrobiologia 551: 227-236.

Quintino, V., F. Sangiorgio, F. Ricardo, R. Mamede, A. Pires, R. Freitas, A.M. Rodrigues, and A. Basset. 2009. *In situ* experimental study of reed leaf decomposition along a full salinity gradient. Estuarine, Coastal, and Shelf Science 85:497-506.

Reice, S.R., and G. Herbst. 1982. The role of salinity in decomposition of leaves of *Phragmites australis* in desert streams. Journal of Arid Environments 5: 361-368.

Rietz, D.N., and R.J. Haynes. 2003. Effects of irrigation-induced salinity and sodicity on soil microbial activity. Soil Biology and Biochemistry 35: 845-854.

Roache, M.C., P.C. Bailey, and P.I. Boon. 2006. Effects of salinity on the decay of the freshwater macrophyte, *Triglochin procerum*. Aquatic Botany 84: 45-52.

Sardinha, M., T. Müller, H. Schmeisky, and R.G. Joergensen. 2003. Microbial performance in soils along a salinity gradient under acidic conditions. Applied Soil Ecology 23: 237-244.

Titus, J.G. 1988. Sea level rise and wetland loss: an overview. Office of Policy Analysis U.S. Environmental Protection Agency 1: 1-35.

van Bruggen, A.H.C., and A.M. Semenov. 2000. In search of biological indicators for soil health and disease suppression. Applied Soil Ecology 15: 13-24.

Van Ryckegem, G., M.O. Gessner, and A. Verbeken. 2007. Fungi on leaf blades of *Phragmites australis* in a brackish tidal marsh: diversity, succession, and leaf decomposition. Microbial Ecology 54: 600-61.

Vermeer, M., and S. Rahmstorf. 2009. Global sea level linked to global temperature. Proceedings of the National Academy of Sciences 106: 21527-21532.

Webster, J.R., and E.F. Benfield. 1986. Vascular plant breakdown in freshwater ecosystems. Annual Review of Ecology and Systematics 17: 567-594.

Wichern, J., F. Wichern, and R.G. Joergensen. 2006. Impact of salinity on soil microbial communities and the decomposition of maize in acidic soils. Geoderma 137: 100-108.

Windham, L. 2001. Comparison of biomass production and decomposition between *Phragmites australis* (common reed) and *Spartina patens* (salt hay grass) in brackish tidal marshes of New Jersey, USA. Wetlands 21: 179-188.

Yozzo, D.J., J.L. Andersen, M.M. Cianciola, W.C. Nieder, D.E. Miller, S. Ciparis, and J.V. McAvoy. 2005. Ecological Profile of the Hudson River National Estuarine Research Reserve. New York State Department of Environmental Conservation.

Yozzo, D.J., and D.T. Osgood. 2012. Invertebrate communities of low-salinity wetlands: overview and comparison between *Phragmites* and *Typha* marshes within the Hudson River Estuary. Estuaries and Coasts. Doi: 10.1007/s12237-012-9543-6.

INVESTIGATION OF ESTUARINE SEDIMENT AS A RESERVOIR FOR SEWAGE ASSOCIATED BACTERIA

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

The Fecal Indicator Bacteria (FIB), *Escherichia. coli* and *Enterococci*, are commonly used by Hudson River monitoring programs to quantify the level of sewage pollution in estuarine water and to provide information about the health risk to recreators from sewage associated bacteria. It is generally assumed that these FIB do not persist in the water for extended periods of time and that their presence represents a recent release of sewage into the environment. However, much less is known about the abundance and environmental persistence of FIB in sediments, as opposed to the water column, of the Hudson River Estuary. In this study, FIB were quantified, using cultivation-based techniques, in water and sediment samples collected from six locations in the estuary, and the persistence of FIB in sediment was investigated in laboratory incubation experiments. FIB were found to be widely distributed in both sediment and water from the estuary. *E. coli* and *Enterococci* displayed correlated abundances in sediment, consistent with sewage pollution as a shared source for both FIB in the environment. However, the levels of FIB were not correlated in paired water and sediment samples collected simultaneously from the same sites, suggesting that environmental persistence of these FIB differs in water versus sediment. *Enterococci* concentrations were found to decrease over time in laboratory incubations of estuarine sediment but remained at detectable levels for weeks after collection. In order to confirm the presence of *Enterococci,* and rule out the possibility of false positives from the cultivation-based assay, isolated bacterial colonies were characterized using molecular genetic techniques and the vast majority (96%) were confirmed as *Enterococci*.

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INTRODUCTION

Fifty years ago, the lower Hudson River and connected waterways surrounding New York City were commonly considered to be inappropriate for any recreational contact due to extensive pollution, with the river often acting as an open sewer for the surrounding population. Enforcement of the Clean Water Act and major investment in wastewater treatment facilities since the 1970s initiated a trend of improving water quality (Steinberg et al. 2004; NYC DEP 2009). Long-term environmental monitoring data from New York Harbor clearly document the prior history of poor water quality and the resulting improvements in mean seasonal water quality in recent decades (Brosnan and O'Shea 1996; Hetling et al. 2003; Brosnan et al. 2006; NYC DEP 2009). Along with improvements in water quality, there has been a widespread increase in recreational use of the Hudson at official and "unofficial" swimming beaches (Lawler, Matusky, and Skelley Engineers 2005), and management action aimed at re-claiming the waterfront and increasing public access to the river (New York-New Jersey Harbor and Estuary Program 2013; City of New York 2013).

Despite improvement in wastewater treatment infrastructure and in seasonal water quality, raw and partially treated sewage continues to enter the river and continues to be a management challenge. It is estimated that approximately 27 billion gallons of stormwater, mixed with raw sewage, are still released each year into New York Harbor through Combined Sewer Overflow (CSO) events (NYC DEP 2010). Numerous studies from other aquatic systems have demonstrated that waterways contaminated with sewage contain potentially pathogenic strains of microbes such as *Salmonella*, *Campylobacter*, and *Escherichia coli* 0157:H7 (Obiri–Danso and Jones 2000; Walters et al. 2007).

Similarly, a recent study in the Hudson River Estuary found that the abundance of antibiotic resistant microbes was correlated to the concentration of Fecal Indicator Bacteria (FIB) and that levels of these bacteria increased following rainfall (Young et al. 2013), presumably due to sewage discharge from CSOs.

Increased public access to the waterfront and improved mean seasonal water quality has led to a widespread demand from the public for more detailed water quality data. *Enterococcus* is an Environmental Protection Agency (EPA) approved FIB used for recreational water quality management whose presence in water has been shown to correlate with the occurrence of gastrointestinal illness in recreators (US-EPA 2004). Multiple monitoring programs in the lower Hudson River (NYC DEP 2013; New York Water Trails Association 2013; Riverkeeper 2013) now collect data on the concentration of FIB, and distribute these data to the public. *Enterococci* are commonly detected in the Hudson River Estuary (HRE) at elevated levels, with 21% of water samples (from 75 locations in the lower HRE) tested from 2006 through 2010 deemed unacceptable by EPA standards for primary contact recreation (Riverkeeper 2011).

Microorganisms released into the coastal environment are subjected to numerous stressors such as temperature change (Davies et al. 1995; Thomas et al. 1998), salinity (Mezrioui et al. 1995), nutrient deficiencies (Ozkanca and Flint 1997), and sunlight (Sinton et al. 1999). As a result, FIB and other sewage-associated bacteria are generally not thought to live for very long in the water column. In fact, short environmental persistence is a desired characteristic of FIB, because their detection is intended to reflect the recent input of sewage to the environment. However, in the HRE water column a high percentage of *Enterococci* are attached to particles, (Suter et al. 2011) and settle out

of the water column to the underlying sediment more quickly than "free-living" bacteria. Environmental conditions in the sediment are quite different than in the water column, including reduced sunlight, protect against predators, increased nutrient and organic carbon availability, and increased colonizable surfaces (Brettar and Holfe 1992; Davies et al. 1995; Blumenroth and Wagner-Dobler 1998; Sinton et al. 1999). In combination, sediment conditions may lead to increased environmental persistence for FIB, as compared to the water column (Lee et al. 2006).

For the past two years, data on the persistence of sediment associated *Enterococci* and *E. coli* in the Hudson River has been collected and analyzed in the O'Mullan laboratory at Queens College and the Juhl laboratory at Columbia University. Sediment samples collected from the environment in areas impacted by sewage pollution and incubated under a range of temperatures in the laboratory suggest that cultured FIB can remain at detectable levels for weeks to months in Hudson sediment (O'Mullan and Juhl, unpublished data).

Although local monitoring programs typically only measure FIB in water, recreators may also come into contact with contaminated sediment. In addition, the turbulence from large storm events and disturbance from boats or recreators may reintroduce sediment FIB, and associated pathogens, back into the water column creating a connection between water quality and sediment quality. Therefore, studying the persistence patterns of FIB in the all compartments of the environment, including sediment, is imperative to understanding the ecology of sewage associated bacteria in the environment and to interpreting water quality patterns from local monitoring programs.

The goals of this study were to: 1) quantify FIB abundance in sediment and water from six locations in the estuary, using cultivation-based approaches; 2) to determine if FIB abundance would be correlated in paired water and sediment samples; and 3) to use DNA-based assays to confirm that FIB enumerated, with cultivation-based methods, from sediment in laboratory persistence experiments were correctly identified as *Enterococci*. The hypothesis was tested that FIB would be widely distributed in sediment samples from the estuary, but that their abundance would not be well correlated in paired water and sediment samples due to longer FIB persistence in sediment relative to water. In addition, the hypothesis was tested that the majority of isolated colonies characterized using DNA based assays would be confirmed as *Enterococci* and that FIB cultivation-based methods applied to sediment would not be prone to false positives.

METHODS

Paired Water and Sediment Field Sampling

Water and sediment samples for FIB analyses were collected from six field locations in Flushing Bay, Sparkill Creek, and the lower Hudson River (Figure 1) between late May and mid-July in 2012. Samples were collected four to six times from each of the six field sites. Approximately 40 ml of surface water was collected from along the shoreline (depth of less than 0.3 m), just above the paired sediment sample that was also collected (see below), into sterile 50ml plastic tubes that had been triple rinsed with water from the environment. A surface sediment core (approximately 20 ml from the top 3 cm of sediment) was collected using a modified sterile 60ml syringe barrel as a coring device, along with a metal putty knife to help retain the core in the syringe barrel

during collection. The sediment was then extruded into a sterile 50ml tube for storage and transport to the laboratory. Both water and sediment sampling tubes were placed into a cooler, away from light, and transported to the laboratory for processing within six hours of collection.

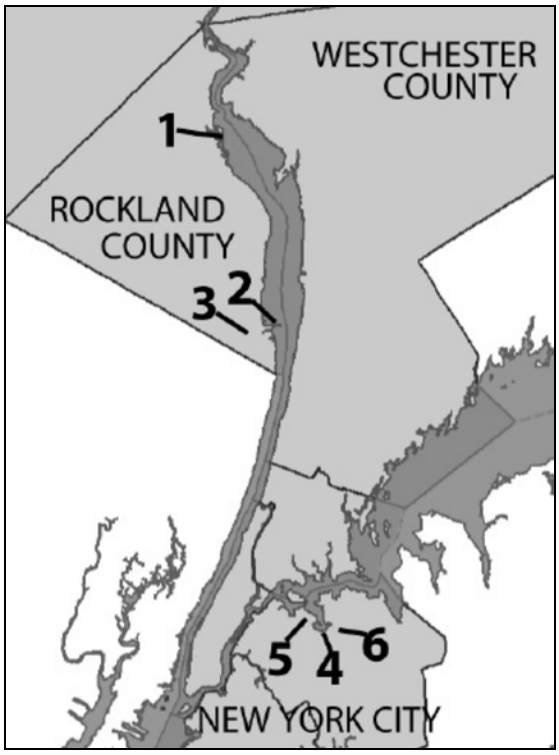

Figure 1. Map of sampling sites. Tappan Zee region stations: 1- Stony Point, 2- Piermont Pier, 3- Sparkill Creek; Flushing Bay region stations: 4- Flushing Bay Boat Launch, 5- Flushing Bay Marina, 6- Flushing River-Corona Park.

Laboratory Persistence Experiments with Sediment

Bulk samples of sediment, scraped from the top 3 cm using a cleaned trowel, were removed from the estuary and transferred to clean plastic incubation chambers (approximately 20cm long x 10cm wide x 10cm tall). Two samples were collected in late May 2012; one from a muddy, organic rich, site near the public boat launch in Flushing Bay, and one from a sandy site also in Flushing Bay but closer to Flushing Bay Marina

(Figure 1). A third sample was collected, also from the sandy Flushing Bay Marina site but in late June 2012. All samples were immediately transported to the laboratory and incubated at 4^{o}C in the dark for approximately five weeks. This incubation temperature was selected based upon prior research (O'Mullan and Juhl, unpublished) to allow for weeks of FIB persistence in order to test for false positives after an extended incubation.

Sediment samples were collected from each incubation chamber periodically over the five weeks to quantify the persistence of FIB in the sediment sample, using the same procedures for *Enterococcus*, as described below, for field sediment samples. It is worth noting that other samples from the O'Mullan and Juhl labs have been used to more completely characterize persistence rates of *Enterococci* in laboratory incubations using a variety of conditions (e.g., variable temperature). The purpose of the persistence incubations was to obtain cultured isolates of *Enterococci* that could be processed for DNA based identification using the 16S rRNA genes, to confirm that cultivation based approaches were actually quantifying *Enterococci* and were not prone to false positive results. The rates of decay are not reported quantitatively in this report, as this was not a goal of the study and is best estimated with a more complete data set spanning more persistence experiments.

FIB Enumeration Procedures

Microbes were extracted from sediment samples, using a method modified from Van Elsas et al. (2002), by mixing 10ml of sediment with 100ml of extraction buffer containing 0.1% sodium pyrophosphate and 0.1 mM EDTA in a sterile, sealed 500ml container shaken at 200 rpm for 30 minutes. Mass of replicate sediment samples was

recorded before and after drying at 60^{o}C for two days to determine wet and dry sediment mass and to allow normalization of microbial counts per gram dry weight of sediment.

After extracting sediment samples, microbial processing of sediment and water samples for FIB was identical, although only *Enterococci* was measured from water, while both *Enterococci* and *E. coli* were measured from sediment. *Enterococci* and *E. coli* were enumerated using the IDEXX Enterolert and Colilert methodology (www.Idexx.com). A 10% dilution of sample water in sterile water and growth media was sealed into a quanti-tray 2000 (IDEXX) vessel and incubating at 41^{o}C (*Enterococcus*) and 37^{o}C (*E. coli*) for 24 hours. After incubation, samples were exposed to UV light and the Most Probably Number (MPN) of *Enterococci* or *E. coli* cells was calculated per 100 ml (for water samples) or per gram of dry sediment weight (for sediment). In addition, a subset of samples were also processed using the EPA approved membrane filtration technique (US-EPA 2007) so that the isolated colonies could be used for DNA based taxonomic identification (described below) to confirm that the cultivation based technique was not prone to false positive results.

DNA Characterization of Sediment FIB and Statistical Analyses

Isolated *Enterococci* colonies from membrane filtration based enumeration of laboratory persistence samples were picked off petri dishes using sterile pipette tips and transferred into tubes with 40 µl of sterile water for molecular analysis. Colonies were then heated to 95^{o}C for 5 minutes using an Eppendorf mastercycler to lysis cells and the 16S rRNA gene was amplified from the released DNA using universal primers 8F and 1492R, followed by gene sequencing by SeqWright Inc. using the conditions described

by Young et al. (2013). The resulting gene sequences were taxonomically classified using the Ribosomal Database Project (RDP; http://rdp.cme.msu.edu/) and searched against the Genbank database (www.ncbi.nlm.nih.gov/genbank/) to confirm species identification. Non-parametric tests, including the Spearman's coefficient, were preformed using the GraphPad Prism (Version 4C, May 2005) statistical analysis software.

RESULTS

Paired Water and Sediment Field Sampling

Enterococci were detected in both water and sediment at all of the six sampling sites (Table 1). Only one water sample, out of 30 total samples enumerated, was found to be below detection for *Enterococci* (Stony Point, MPN <10/100ml). All 30 sediment samples were found to have detectable levels of FIB. Stony Point, in the Tappan Zee, had by far the lowest water and sediment FIB concentrations compared to all other sites (Table 1, Figure 2) with a maximum *Enterococci* MPN of only 113/100ml in the water, more than four times lower than any other site; and a maximum *Enterococci* MPN of only 19.7/g in sediment, more than an order of magnitude lower than any other site.

Levels of *Enterococci* and *E. coli*, the two common FIB used in water quality monitoring programs, were positively correlated (Spearman $r = 0.622$; $p < 0.001$) when sediment samples were compared among all sites (Figure 3). In contrast, levels of *Enterococci* in paired water and sediment samples, collected at the same site and at the same time, were not correlated (Spearman $r = 0.124$; $p = 0.515$) (Figure 4).

Site	# of samples	*Enterococci* water MPN/100ml		*Enterococci* sediment MPN/gram dry wt.		*E. coli* sediment MPN/gram dry wt	
		Minimum	maximum	minimum	maximum	minimum	maximum
1) Stony Point	4	<10	113	5	20	2	12
2) Piermont Pier	4	63	471	46	3788	274	3170
3) Sparkill Creek	4	121	>24196	12	2377	188	2378
4) Flushing Bay Boat Launch, muddy	6	10	>24196	134	4327	484	4327
5) Flushing Bay Marina, sandy	6	20	>24196	97	2866	143	2296
6) Flushing River-Corona Park	6	20	>24196	76	2818	112	2818

Table 1. FIB samples processed from the Hudson River Estuary.

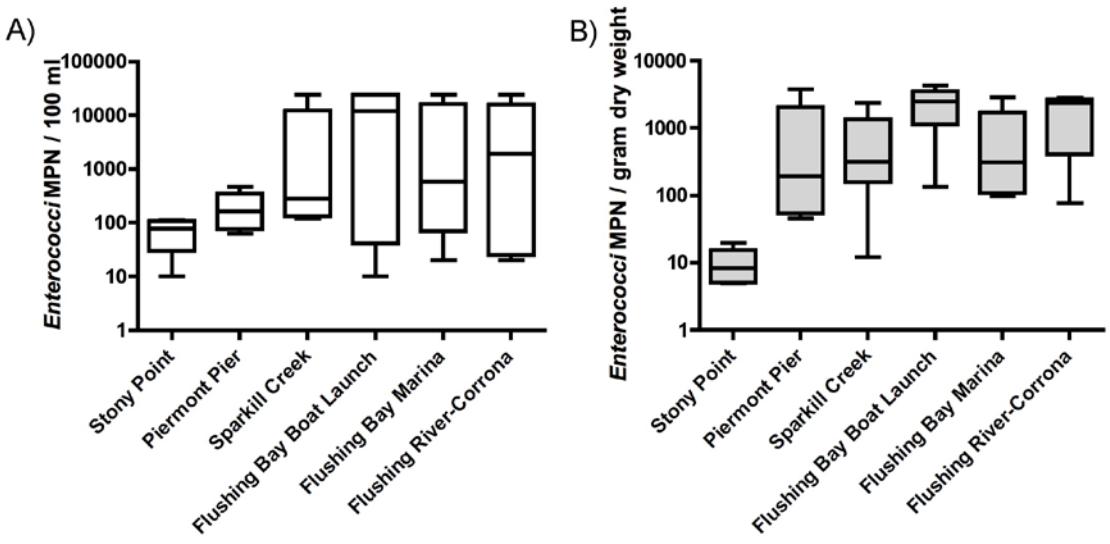

Figure 2. *Enterococci* Concentrations in Water and Sediment. A) water concentration and B) sediment concentration from the six spatial sampling sites. Stony Point, in the Tappan Zee, had the lowest concentrations of *Enterococci* for both water and sediment.

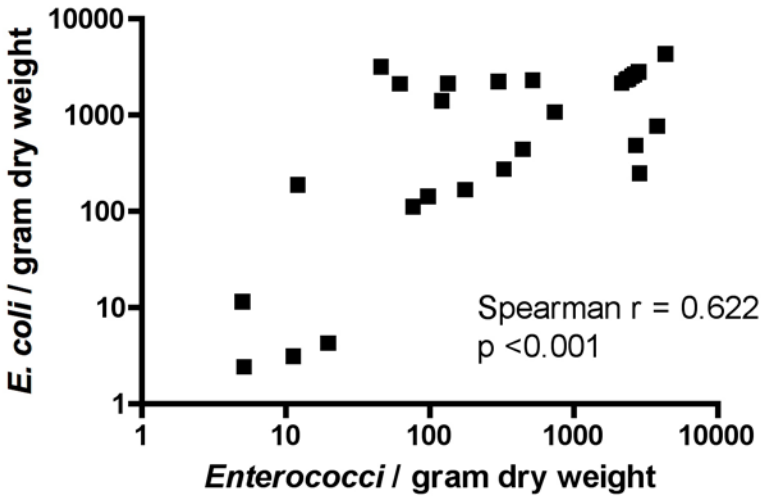

Figure 3. ***Enterococci*** **and** ***E. coli*** **Correlation in Sediment. Concentrations of** ***Enterococci*** **and** ***E. coli*** **measured from the same sediment samples were found to have a significant positive correlation.**

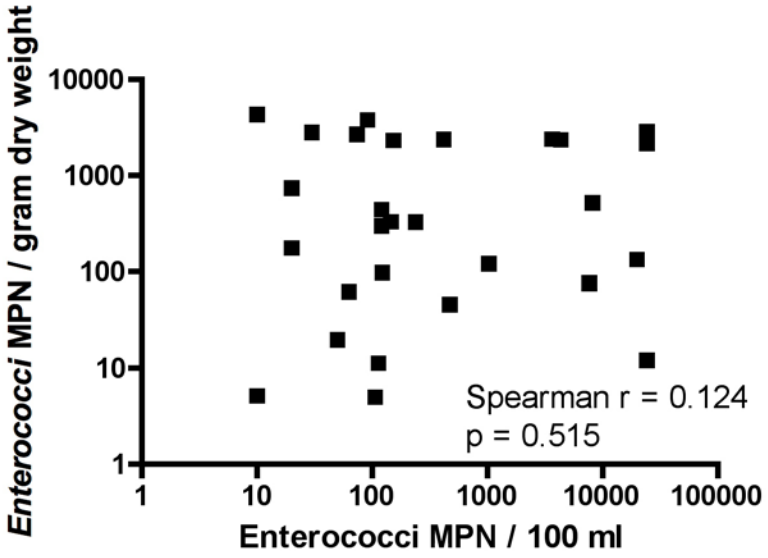

Figure 4. ***Enterococci*** **in Paired Water and Sediment.** ***Enterococci*** **concentrations in paired water and sediment samples were not significantly correlated.**

Sediment Persistence Experiments and Molecular Identification of *Enterococci*

Enterococci concentrations were found to decrease over time in laboratory incubations of estuarine sediment, based on cultivation-dependent techniques, but all three samples still had detectable FIB levels even five weeks after collection (Figure 5). In order to confirm the presence of *Enterococci,* and rule out the possibility of false positives from the cultivation-based assay, isolated bacterial colonies from the sediment persistence experiment were characterized using 16S rRNA gene sequencing. Of 113 colonies identified, the vast majority (96%) were classified as belonging to the genus *Enterococcus*, with most (72 sequences) classified as *Enterococcus faecium,* a bacterium commonly found in the intestine of humans. Four percent of sequences obtained from cultured isolates were false positives and classified as either *Lactobacillales*, *Desemzia* or *Klebsiella,* closely related enteric organisms.

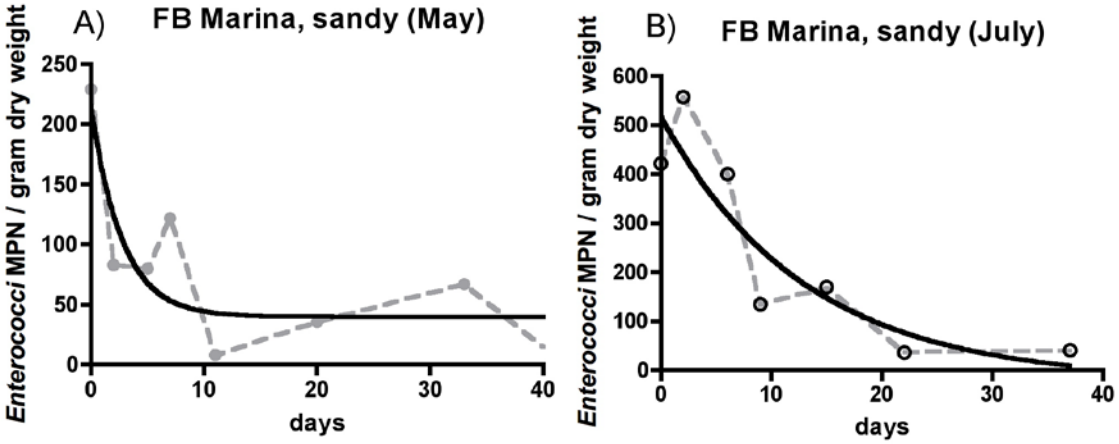

Figure 5. Sediment FIB Decay Curves. FIB decay curves from laboratory incubation experiments conducted at 4°C with Flushing Bay sediment. Dotted lines connect observed data and solid line represents a calculated exponential decay curve.

DISCUSSION

Paired Water and Sediment Field Sampling

Despite the improving water quality of Hudson River, FIB were widely distributed, detected in both water and sediment at every sampling site and within every individual sediment sample analyzed (Table 1, and Figure 2). These data suggest that sediment in the HRE act as a significant reservoir for FIB, as has been found in other similar systems (Anderson et al. 2005; Boehm et al. 2005; Bonilla et al. 2007). The correlated abundances of *Enterococci* and *E. coli* in sediment samples provides added confidence in the use of each indicator and would indicate that both FIB share a common delivery mechanism (Nobel et al. 2003), consistent with sewage as a source for both FIB to the Hudson sediment. Given the correlations of FIB to known pathogens (e.g. Walters et al. 2007), recreator illness (e.g. Haile et al. 1999), and antibiotic resistant bacteria (Young et al. 2013) from prior studies, the current FIB results are also strong evidence that other microbial agents of concern, beyond the FIB themselves, are likely to be widely distributed in Hudson sediment.

Recreators could be exposed to sewage-associated pathogens residing in sediment through activities along the shoreline, for example wading (Phillip et al. 2009), when sediment is directly contacted, but also through other activities when sediment becomes resuspended into the water column. Boat traffic (Pettibone et al. 1996), wave-shore interactions (LeFevre and Lewis 2003), high flow conditions in rivers and estuaries (Jamieson et al. 2005; Wilkinson et al. 2006), and even high winds (Roslev et al. 2008) can cause sediment re-suspension events that may negatively impact water quality. Some

water quality models are now attempting to incorporate FIB resuspension as a central factor controlling water quality (Liu et al. 2006).

Sediment Persistence Experiments and Molecular Identification of *Enterococci*

FIB in Hudson sediments from this study were found to persist for more than five weeks at detectable levels (Figure 5), a similar time scale found in some other aquatic systems (e.g. Haller et al. 2009). Extended persistence is not only expected in sediments, but there is also some evidence that particle-associated microbes in the water column may persist for longer periods than free-living FIB (Fries et al. 2008). This may be significant in the Hudson, where a high percentage of FIB are found to be particle associated (Suter et al. 2011).

A recent review (Jamieson et al. 2005) of water quality modeling studies identified gaps in the understanding of FIB and enteric ecological behavior within the environment as a significant obstacle to the generation of improved prediction systems. Variable persistence in high versus low nutrient environments, the significance of particle attachment for transport, and interactions between sediment and water column associated microbes are all important areas of continued research toward the goal of next generation water quality models (US-EPA 2007; Surbeck 2009; Kim et al. 2010).

Finally, the molecular genetic characterization of FIB isolates suggest that the vast majority (96%) of isolates obtained using cultivation based approaches were *Enterococci*. This finding is significant because it supports the use of cultivation-based approaches to quantify FIB in estuarine sediments and it confirms the long persistence of FIB in estuarine sediment, as suggested by cultivation based approaches.

CONCLUSION

The results from this study demonstrated that FIB are widely distributed in Hudson River sediment and appear to act as a reservoir for sewage associated pathogens. The microbes within this reservoir can persist for weeks, complicating the interpretation of FIB monitoring data. The high FIB content in sediments and attached to particles suggestions that additional research is required to understand the ecology of FIB in the Hudson and to allow improved approaches to water quality monitoring and modeling. Finally, the molecular genetic results from this study confirm that cultivation based approaches can be confidently used to enumerate FIB from sediments, supporting this method for studying FIB ecology in the environment.

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REFERENCES

Anderson, K.L., J.E. Whitlock, and V.J. Harwood. 2005. Persistence and differential survival of fecal indicator bacteria in subtropical waters and sediments. Applied and Environmental Microbiology 71:3041-3048.

Blumenroth, P., and I. Wagner-Dobler. 1998. Survival of inoculants in polluted sediments: effect of strain origin and carbon source competition. Microbial Ecology 35:279-288.

Boehm, A.B., D.P. Keymer, and G.G. Shellenbarger. 2005. An analytical model of enterococci inactivation, grazing, and transport in the surf zone of a marine beach. Water Research 39:3565-3578.

Bonilla, T.D., K. Nowolsielski, M. Cuvelier, A. Hartz, M. Green, N. Esiobu, D.S. McCorquodale, J.M. Fleisher, and A. Rogerson. 2007. Prevalence and distribution of fecal indicator organisms in South Florida beach sand and preliminary assessment of health effects associated with beach sand exposure. Marine Pollution Bulletin 54:1472-1482.

Brettar, I., and M.G. Holfe. 1992. Influence of ecosystematic factors on survival of Escherichia coli after large-scale release into lake water mesocosms. Applied and Environmental Microbiology 58:2201-2210.

Brosnan, T.M., and M.L. O'Shea. 1996. Long-term improvements in water quality due to sewage abatement in the lower Hudson River. Estuaries 19:890-900.

Brosnan, T.M., A. Stoddard, and L.J. Hetling. 2006. Hudson River sewage inputs and impacts: past and present. pp. 335-348 in Levinton, J. S., and J.R. Waldman (Eds.), The Hudson River Estuary. Cambridge University Press, New York.

City of New York. 2013. PLANYC 2030 www.nyc.gov/planyc

Davies, C.M., J.A. Long, M. Donald, and N.J. Ashbolt. 1995. Survival of fecal microorganisms in marine and freshwater sediments. Applied and Environmental Microbiology 61:1888-1896.

Fries J.S., G.W. Characklis, and R.T. Noble. 2008. Sediment-water exchange of *Vibrio* sp. and fecal indicator bacteria: Implications for persistence and transport in the Neuse River Estuary, North Carolina, USA. Water Research 42:941-950.

Haile, R.W., J.S. Witte, M. Gold, R. Cressey, C. McGee, R.C. Millikan, A. Glasser, N. Harawa, C. Ervin, P. Harmon, J. Harper, J. Dermand, J. Alamillo, K. Barrett, M. Nides, and G. Wang. 1999. The health effects of swimming in ocean water contaminated by storm drain runoff. Epidemiology 10:355–363.

Haller, L., E. Amedegnato, J. Pote, and W. Wildi. 2009. Influence of freshwater sediment characteristics on persistence of fecal indicator bacteria. Water, Air and Soil Pollution 203:217-227.

Hetling, L.J., A. Stoddard, T.M. Brosnan, D.A. Hammerman, and T.M. Norris. 2003. Effect of water quality management efforts on wastewater loadings during the past century. Water Environment Research 75:30-38.

Jamieson R.C., D.M. Joy, H. Lee, R. Kostaschuk, and R.J. Gordon. 2005. Resuspension of sediment-associated *Escherichia coli* in a natural stream. Journal of Environmental Quality 34:581-589.

Kim J.-W., Y.A. Pachepsky, D.R. Shelton, and C. Coppock. 2010. Effect of streambed bacteria release on *E. coli* concentrations: Monitoring and modeling with the modified SWAT. Ecological Modeling 221:1592-1604.

Lawler, Matusky, and Skelley Engineers. 2005. Swimming in the Hudson River, Feasibility Report on Potential Sites. www.dec.ny.gov/lands/5452.htm

Lee, C.M., T.Y. Lin, C.C. Lin, G.A. Kohbodi, A. Bhatt, R. Lee, and J.A. Jay. 2006. Persistence of fecal indicator bacteria in Santa Monica Bay beach sediments. Water Research 40:2593-2602.

LeFevre, N.M., and G.D. Lewis. 2003. The role of resuspension in *Enterococci* distribution in water at an urban beach. Water Science and Technology 47:205-210.

Liu, L., M.S. Phanikumar, S.L. Molloy, R.L. Whitman, D.A. Shivley, M.B. Nevers, D.J. Schwab, and J.B. Rose. 2006. Modeling the transport and inactivation of *E. coli* and *Enterococci* in the near-shore region of Lake Michigan. Environmental Science and Technology 40:5022-5028.

Mezrioui, N., B. Baleux, and M. Troussellier. 1995. A microcosm study of the survival of *Escherichia coli* and *Salmonella typhimurium* in brackish water. Water Research 29: 459-465.

Nobel, R.T., D.F. Moore, M.K. Leecaster, C.D. McGee, and S.B. Weisberg. 2003. Comparison of total coliform, fecal coliform, and *Enterococcus* bacterial indicator response for ocean recreational water quality testing. Water Research 37:1637 – 1643.

New York-New Jersey Harbor and Estuary Program. 2013. www.harborestuary.org/publicaccess.htm

New York Water Trails Association. 2013. 2013 Citizens' Water Quality Testing Program. http://www.nycwatertrail.org/water_quality.html

NYC DEP. 2009. New York Harbor Survey Program, celebrating one hundred years, 1909-2009. www.nyc.gov/html/dep/pdf/hwqs_centennial.pdf

NYC DEP. 2010. NYC Green Infrastructure Plan: A Sustainable Strategy For Clean Waterways." New York City Department of Environmental Protection, New York, NY, 2010 p. 8.

NYC DEP. 2013. New York City Department of Environmental Protection Harbor Water Sampling Data. http://www.nyc.gov/html/dep/html/harborwater/harbor_water_sampling_results.shtml

Obiri-Danso, K., and K. Jones. 2000. Intertidal sediments as reservoirs for hippurate negative campylobacters, *Salmonellae* and fecal indicators in three EU recognised bathing waters in north west England. Water Research 34:519-527.

Ozkanca, R., and K.P. Flint. 1997. Relationship between respiratory enzymes and survival of *Escherichia coli* under starvation stress in lake water. Journal of Applied Microbiology. 82:301-309.

Pettibone, G.W., K.N. Irvine, and K.M. Monahan. 1996. Impact of ship passage on bacteria levels and suspended sediment characteristics in the Buffalo River, New York. Water Research 30:2517-2521.

Phillip, D.A., P. Antoine, V. Cooper, L. Francis, E. Mangal, N. Seepersad, R. Ragoo, S. Ramsaran, I. Singh, and A. Ramsubhag. 2009. Impact of recreation on recreational water quality of a small tropical stream. Journal of Environmental Monitoring 11: 1192-1198

Riverkeeper. 2011. How is the Water? 2012. Sewage Contamination in the Hudson River Estuary. http://www.riverkeeper.org/wp-content/uploads/2012/12/RvK_How-Is-the-Water-2012.pdf

Riverkeeper. 2013. Hudson River Water Quality. http://www.riverkeeper.org/water-quality/

Roslev, P., S. Bastholm, and N. Iversen. 2008. Relationship between fecal indicators in sediment and recreational waters in a Danish estuary. Water, Air, and Soil Pollution 194:13-21.

Sinton, L.W., R.K. Finaly, and P.A. Lynch. 1999. Sunlight inactivation of fecal bacteriophages and bacteria in sewage-polluted seawater. Applied and Environmental Microbiology. 65:3605-3613.

Steinberg N., D.J. Suszkowski, L. Clark, and J. Way. 2004. Health of the Harbor: The first comprehensive look at the state of the NY/NJ Harbor Estuary: A report to the NY/NJ Harbor Estuary Program. Hudson River Foundation, New York, NY. 82 pp.

Surbeck, C.Q. 2009. Factors influencing the challenges of modeling and treating fecal indicator bacteria in surface waters. Ecohydrology 2:399-403.

Suter, E., A.R. Juhl, and G.D. O'Mullan. 2011. Particle association of *Enterococcus* and total bacteria in the lower Hudson River Estuary, USA. Journal of Water Resource and Protection. 3:715-725.

Thomas, C., H. Gibson, D.J. Hill, and M. Mabey. 1998. *Campylobacter* epidemiology: an aquatic perspective. Journal of Applied Microbiology 85:168S-177S.

U.S. Environmental Protection Agency (U.S. EPA). 2004. Method 1600: *Enterococci* in water by membrane filtration using membrane-*Enterococcus* Indoxyl-B-D Glucoside Agar (mEI). EPA-821-R-06-009.

U.S. Environmental Protection Agency (U.S. EPA). 2007. Report of the experts scientific workshop on critical research needs for the development of new or revised recreational water quality criteria. EPA 823-R-07-006.

Van Elsas J.D., K. Smalla, A.K. Lilley, and M.J. Bailey. 2002. Methods for sampling soil microbes. pp. 505-515 in Hurst, C.J., R.L. Crawford, G.R. Knudsen, M.J. McInerney, and L.D. Stetzenbach (Eds), Manual of Environmental Microbiology 2nd Ed. ASM Press, Washington, D.C.

Walters S.P., V.P. Gannon, and K.G. Field. 2007. Detection of Bacteroidales fecal indicators and the zoonotic pathogens *E. coli* 0157:H7, *Salmonella*, and *Campylobacter* in river water. Environmental Science and Technology 41:1856-1862.

Wilkinson J., D. Kay, M. Wyer, and A. Jenkins. 2006. Processes driving the episodic flux of faecal indicator organisms in streams impacting on recreational and shellfish harvesting waters. Water Research 40:153-161.

Young, S., A. Juhl, and G.D. O'Mullan. 2013. Antibiotic-resistant bacteria in the Hudson River Estuary linked to wet weather sewage contamination. Journal of Water and Health 11:297-310.

RECRUITMENT OF OYSTERS WITHIN THE HUDSON RIVER ESTUARY

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

The eastern oyster, *Crassostrea virginica*, was once a major ecological element in the Lower Hudson, but was nearly driven to extinction by the early 20th century owing mainly to sewage-related pollution, but also affected by overexploitation and habitat alteration. Today, the eastern oyster is generally rare from the Tappan Zee to New York – New Jersey Harbor, and no longer is a major resource species or a foundation species for marine communities. A healthy larval supply is essential for oyster restoration. To examine variation in oyster recruitment, oyster-shell bags were placed from the Tappan Zee to Governors Island to examine spatial differences in oyster recruitment. Three sites were established within the Tappan Zee at Ossining, Philips Manor and Irvington and three more were established at lower Manhattan: one at Pier 40 and two at Governers Island. Water temperature, salinity, and dissolved oxygen were monitored biweekly. Observations of invertebrate recruitment were made with emphasis on barnacles and bivalves. Only zebra mussels recruited to Ossining, which suggested a salinity limitation to oyster survival at this site. Strong recruitment of the barnacle *Balanus improvisus* occurred in July within the Tappan Zee; this species was present at Pier 40 in lower Manhattan, but absent from two sites at Governors Island. A pattern of larval retention within the lower Hudson estuary explains this. Oyster recruitment was strongest at the southern end of the Tappan Zee at Irvington but also occurred in the mid-Tappan Zee at Philips Manor. No oysters recruited to the Manhattan sites. It seems likely that the Tappan Zee recruitment came from a larval source within the Tappan Zee.

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INTRODUCTION

The eastern oyster *Crassostrea virginica* is a bivalve mollusk that can provide many ecosystem services when abundant in estuarine and coastal environments on the east and Gulf coasts of North America. They filter algae, bacteria, fine sediments and occasionally toxins from the water (Officer et al. 1982, Newell 1988). Oyster reefs provide habitat for marine organisms such as shrimp, crabs, clams, snails and worms, as well as many species of fish such as snook, grouper, redfish, black drum and more (Coen et al. 2007). They also can strongly influence nitrogen cycling in estuaries (Newell et al. 2005). Oysters are also an indicator of regional watershed properties due to their differential sensitivity to changes in water quality and climate (Levinton et al. 2011).

Unfortunately the ecosystem services provided by oysters are not being fully exploited because of destruction of oyster reefs due to pollution, habitat alteration, overexploitation and disease (Coleman and Williams 2002), especially within the Hudson River Estuary (Franz 1982, Kurlansky 2006). The lack of adults in the current lower Hudson results in too few larvae to sustain oyster populations, especially reefs, and there is not enough shell to attract larval settlement and promote sustainability of oyster mounds (Mann and Powell 2007).

The Hudson River was formerly known to contain oysters in high abundance (Franz 1982). This study examined the possible availability of recruiting larvae of these oysters in the Hudson River, as well as the distribution of larval recruitment in the Lower Hudson, and settlement of other species, including predators of oyster larvae as they settle. Larval settlement can be monitored by placing bags of shell that can be used to collect recruits with the assumption that recruitment is an estimate of larval abundance.

The main objectives of this study were to estimate recruitment and measure of oyster recruit growth rates while monitoring important physical variables for oysters including temperature, salinity, and oxygen.

The hypothesis that settlement rates will vary between sites was tested. It was expected that settlement in the Tappan Zee would be observed because a preliminary study showed settlement in 2008 (J. Levinton, unpublished data) and settlement has been observed at a nearby Oyster Restoration Research Program (ORRP) experimental reef at Hastings, New York, (B. Peterson, verbal communication). If oysters settle in New York Harbor, it is expected that growth rate of recruits will be greater in these higher salinity waters (Pier 40, Governors Is.) in comparison to the growth rates in lower salinity environments within the Tappan Zee-Haverstraw Bay area. Finally, two sites have been established on Governors Island, one close to an existing ORRP experimental reef and one approximately 1 km away. It was hypothesized that if oyster recruitment occurs, it will be greater closer to the experimental reef and that this reef will cause higher recruitment than other sites. This would be explained by a source of larvae from the experimental reef.

METHODS

Site Descriptions

Six sites were chosen for study, selected for access to the Hudson River in two general geographic areas (Figure 1). These sites were divided between lower Manhattan-Governors Island and the Tappan Zee. The structure of sampling allowed for overall comparison of the Tappan Zee versus New York Harbor recruitment, and also allowed an

investigation of the salinity gradient within the Tappan Zee (salinity declines toward the north), and the possible effect of the ORRP experimental reef at Governors Island.

Lower Manhattan / Governors Island Sites:

1. Pier 25, north side (P25): Shell bags were suspended from the vessel *Lilac* on the north side of the pier.

2. Yankee Pier, Governors Island (Gov-Y). This pier is ca 200 m from the Hudson River Foundation experimental oyster reef.

3. Pier 1, Governors Island (Gov-M). This pier is on the north side of Governors Island, near the oyster study area of the Harbor School.

Up river sites – Tappan Zee and Lower Haverstraw Bay:

4. Ossining (Oss). This is a bulkhead located in back of Westerly Marina, directly on the Hudson River.

5. Phillips Manor, Sleepy Hollow, NY (PM). This is a floating dock located in the Phillips Manor Boat and Beach Club, immediately west of the Phillips Manor train station.

6. Irvington, Irvington Boat Club (Irv). This is a floating dock located in a small bay of the Hudson River.

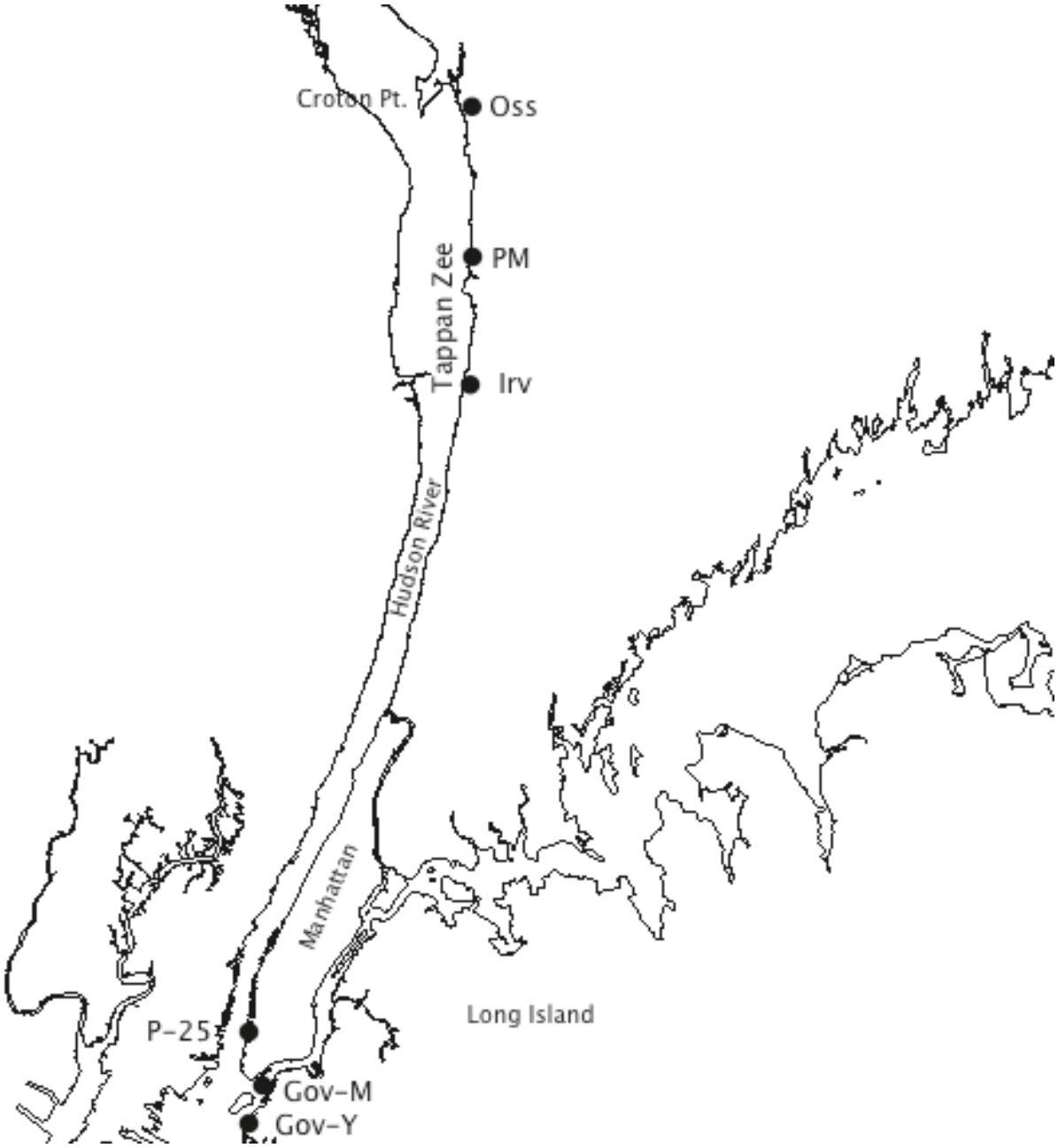

Figure 1. Sampling locations

Figure 2. Shell bag such as those used in recruitment studies

Field Methods

At each site, five bags (Figure 2) of oyster shell in plastic mesh were suspended about 1.0-1.5 m below water level. The shell bags used were constructed of 5 mm mesh with the dimensions 42.3 cm X 29 cm with a 30.5 cm long lead weight along the bottom. The bags were cleaned and filled with two horizontal rows of five shells so in total there were ten shells in each bag. All the shells were in the same orientation from back-side to front so that they could be identified individually within the bags without numbering them. Plastic ties were used to secure the mesh bags, and small pieces of rope were tied to the left and right top corners of the mesh bags. All of the bags were suspended about 1.5 meters below low tide (fixed sites) or below the water (floating docks) at each site by the rope. Bags were put in place on June 20, 2012, checked on July 5 2012 and

approximately every two weeks thereafter until August 31, 2012. One last sampling was done on October 12.

Biweekly Sampling Methods

Fouling organisms were cleaned off of mesh bag surface along with any mud or debris that could interfere with the settlement of oyster larvae. Water Quality was measured using a YSI Model 85 temperature-salinity-oxygen meter. Temperature (^{o}C), salinity (ppt), percent saturation and absolute oxygen concentration (mg L^{-1}) were recorded at each site. An example of data collection is shown in Table 1.

Table 1. Oyster water quality monitoring data for 6/20/12.

Locality	Temperature (^{o}C)	Salinity (ppt)	% Saturation	Dissolved Oxygen
Gov-Y	21.9	21.2	61.0	5.0 mg L^{-1}
Gov-M	22.4	20.7	65.3	5.17 mg L^{-1}
P25	21.4	19.8	62.0	5.0 mg L^{-1}
Irv	23.7	6.0	78.1	6.19 mg L^{-1}
PM	25.7	4.8	78.3	7.90 mg L^{-1}

RESULTS

Water Quality

Temperature variation was uniform (Figure 3) among the three New York Harbor sites, throughout the two months the study took place with a slight variation of two degrees Celsius. In the Tappan Zee, there was a slightly larger variation of temperature of about four degrees Celsius. On the second sampling date (6/18-20/2012), a slight dip in temperature of three degrees Celsius was recorded in both regions. Water temperature was consistently higher within the Tappan Zee – Haverstraw Bay sites relative to the New York Harbor sites, which were closer to the open ocean. The most southerly Tappan Zee site, Irvington, had the lowest temperature, which might indicate mixing with the cooler southerly part of the Hudson.

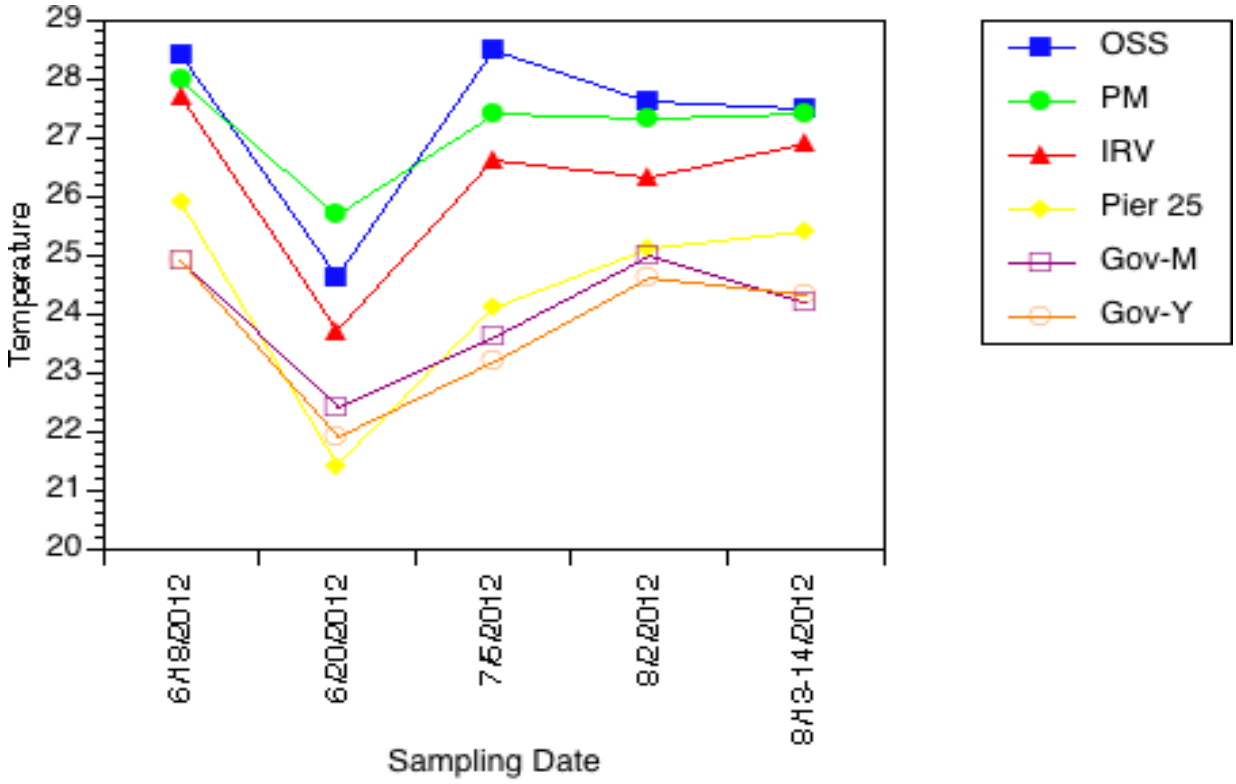

Figure 3. Temperature (°C) variation among the sampling sites.

Salinity (Figure 4) in the New York Harbor sites was distinctly higher than the Tappan Zee-Haverstraw sites. Salinity variation throughout the sites located in the Tappan Zee area was fairly uniform with an average salinity of approximately 4-10 ppt. The lower Hudson also exhibited a fairly uniform salinity of approximately 19-25 ppt. All of the sites exhibited a small decrease of about 2 ppt on the second sampling date (6/20/2012), corresponding to the temperature drop. For most of the sampling period, Ossining had the lowest salinity whereas Irvington had the highest.

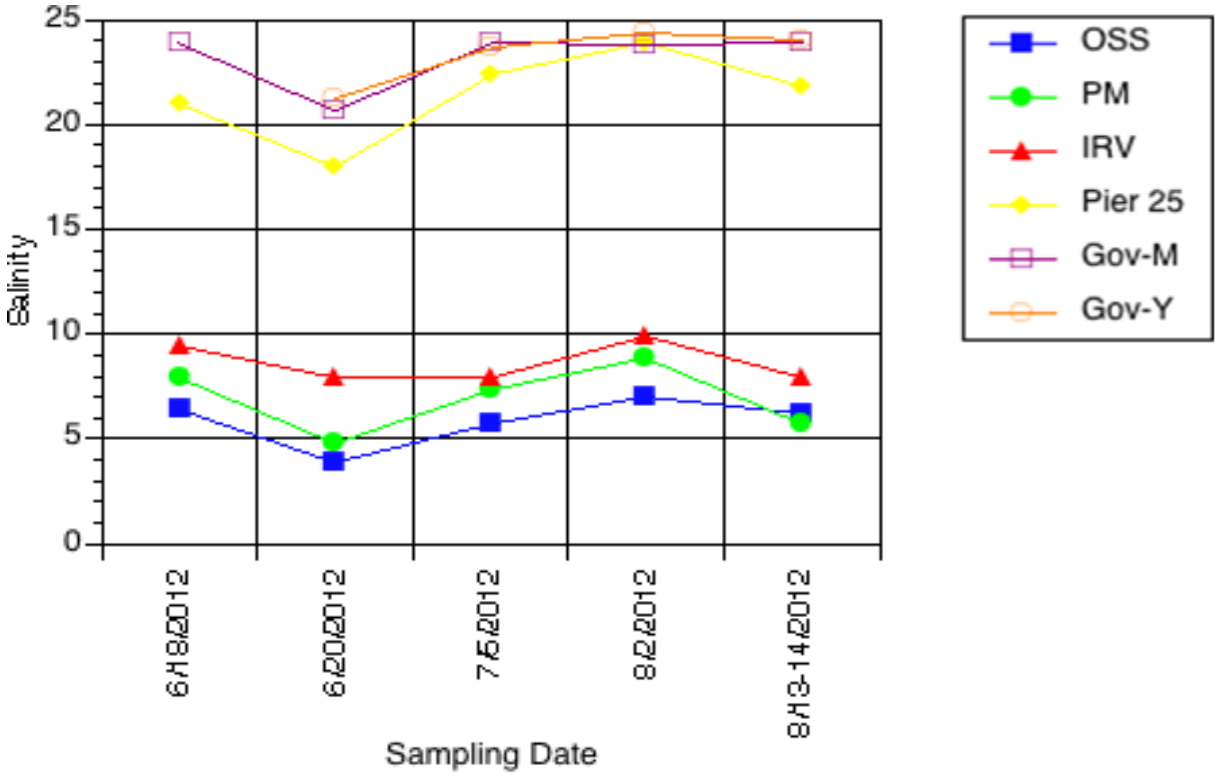

Figure 4. Salinity (ppt) over time at the sampling sites.

Oxygen concentration throughout these sites was fairly scattered but declined generally after June 20 (Figure 5). The variation had a range of about 3 mg/l throughout the sampling period with an average concentration of around 5 mg/l with some outliers noted at Ossining, Philips Manor, and the Governors Island site Gov-M. Oxygen reached a high of about 8 mg/l at Ossining on 7/5/2012 and at Philips Manor on 6/20/2012. Oxygen reached a high of about 8 mg/l and the Governors Island site along with Philips Manor Reached a low of 2 mg/l at Governors Island and Philips Manor on 6/18/2012. All in all, however, dissolved oxygen was usually above 4 mg/l and likely not a source of stress to oysters.

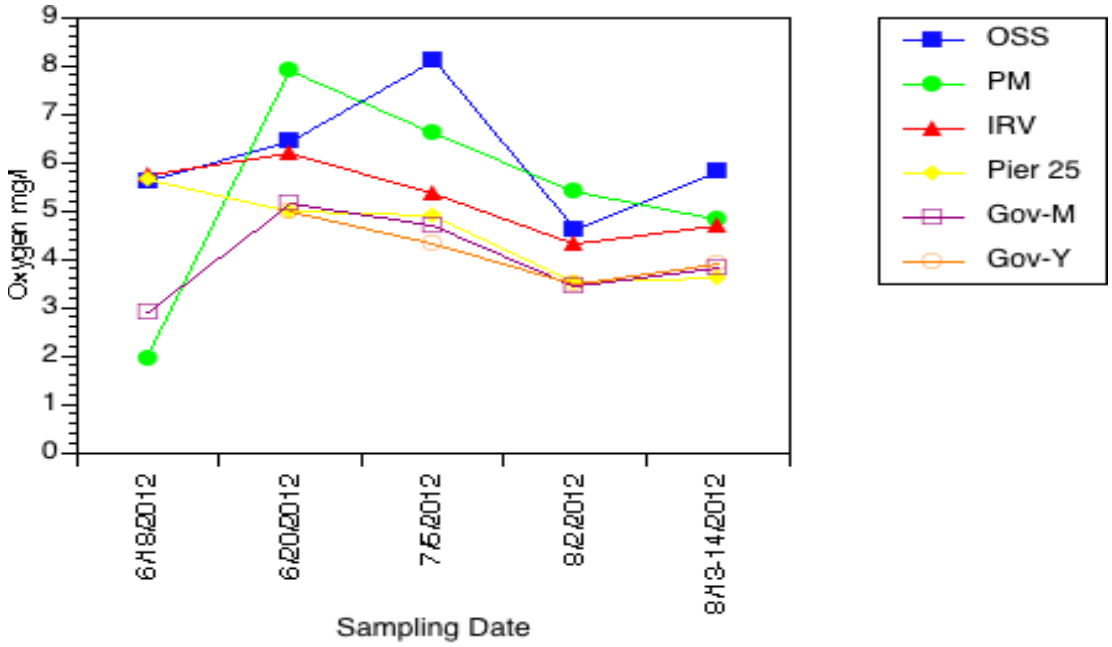

Figure 5. Dissolved oxygen concentration (mg L^{-1}) **at the sampling sites.**

Water quality data collected for this study were compared to data from the Piermont Pier Hudson River Environmental Conditions Observing System (HRECOS) station (www.hrecos.org). This continuously operating station is located between the Philips Manor and Irvington study sites, but on the opposite (western) shore. Average Daily values from the Piermont Pier HRECOS station were in the same range as the data collected at the Philips Manor and Irvington study sites (Table 2).

Table 2. Water quality data from the Piermont Pier HRECOS station.

Date	Average Daily Salinity (ppt)	Dissolved Oxygen (mg/L)	Water Temp (C)
6/18/12	4.9	8.1	22.6
6/20/12	5.5	8.0	23.5
7/5/12	8.2	6.2	27.0
8/2/12	ND	ND	ND
8/14/12	7.8	5.7	27.3

Recruitment

Oyster recruitment

The only sites where oyster settlement was observed throughout the study were Irvington (Figure 6) and Philips Manor, both within the Tappan Zee; these were last sampled on 10/12/2012. Oyster recruitment density declined from Irvington (mean = 36.8) to Philips Manor (mean = 6), with no recruitment observed at Ossining (Figure 7). Recruitment was significantly greater at Irvington than Phillips Manor (ANOVA, $F = 127.5$, $p < 0.0001$). Mean shell length was greater at Phillips Manor (18.3 mm) than Irvington (13.3, ANOVA, $F = 36.4$, $p < 0.0001$). A few zebra mussels were found at Ossining, even when the salinity was 4-6 ppt. No oyster recruitment was found in any of the New York Harbor sites.

Figure 6. Oyster shell taken from shell bag at Irvington on October 12, 2012, showing settled oyster recruits and barnacles.

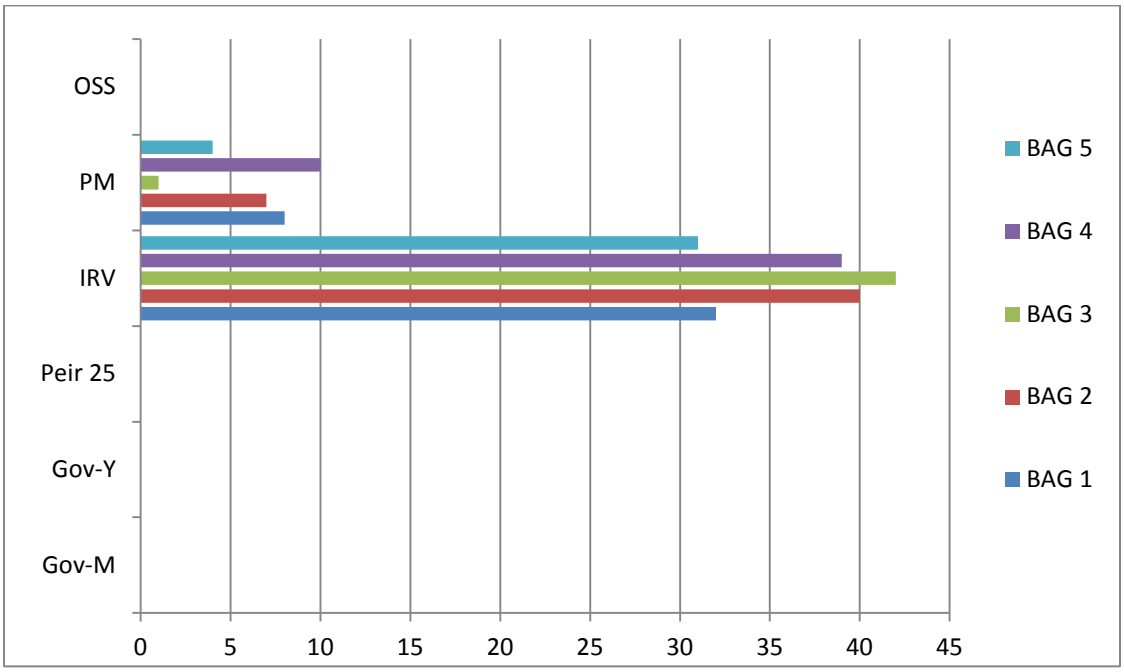

Figure 7. Oyster recruitment numbers, by bag, at the six sites.

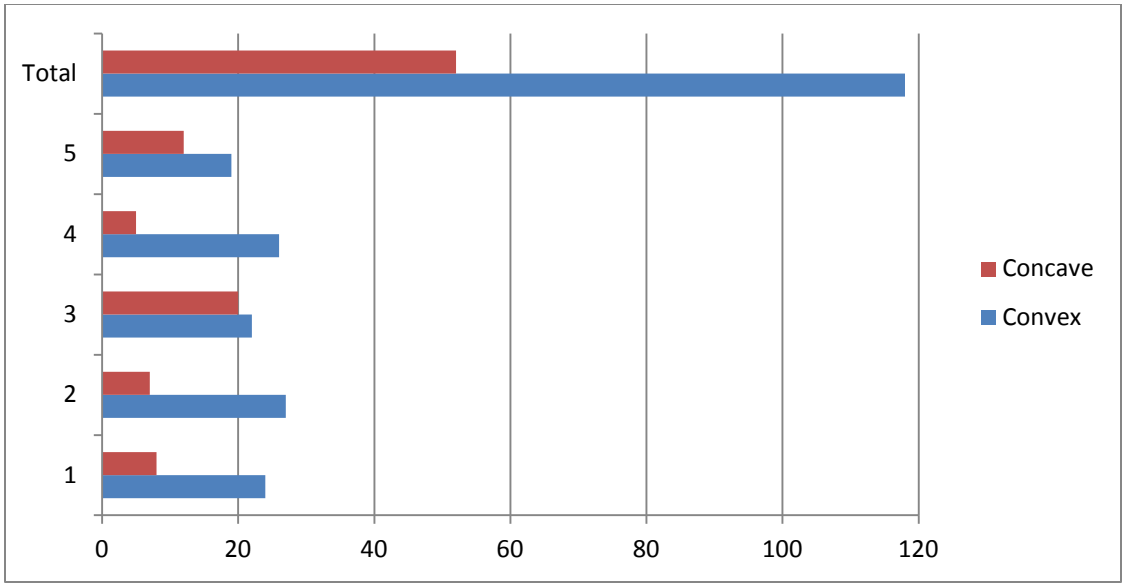

Figure 8. Number of oysters settled on interior and outside of valves.

The numbers of settled oysters were counted on the exterior (convex side) and interior (concave side) of the oyster valves. The numbers are substantial only for the Irvington site, which are reported in Figure 8. As can be seen, oysters settled more frequently on the exterior of the oyster valves, although there was variation among bags. The total numbers were tested for departure from a random distribution, using a Wilcoxon Signed Rank test, which tested the null hypothesis that settlement on the inside and outside of shells was the same frequency. This test suggests that there was a non-random concentration of oyster settlement on the exterior of the oyster shells, since the test was statistically significant (X^2 approximation = 5.8, $p \sim 0.02$).

Barnacle recruitment

In the first sampling period, a strong settlement of the barnacle *Balanus improvisus* was observed (Figure 7*)*. Because one shell bag had to be replaced at Phillips Manor, it was apparent that settlement ended in July. There was an abundance of barnacles that settled on the oyster shells that were placed in the Tappan Zee area. The Ossining, Philips Manor and Irvington sites contained barnacles on all 10 shells in each bag. There were also barnacles that settled on almost all the shells at Pier 25, but the density was conspicuously lower than within the Tappan Zee. No settlement was found at the other two sites at Governors Island (Figure 9).

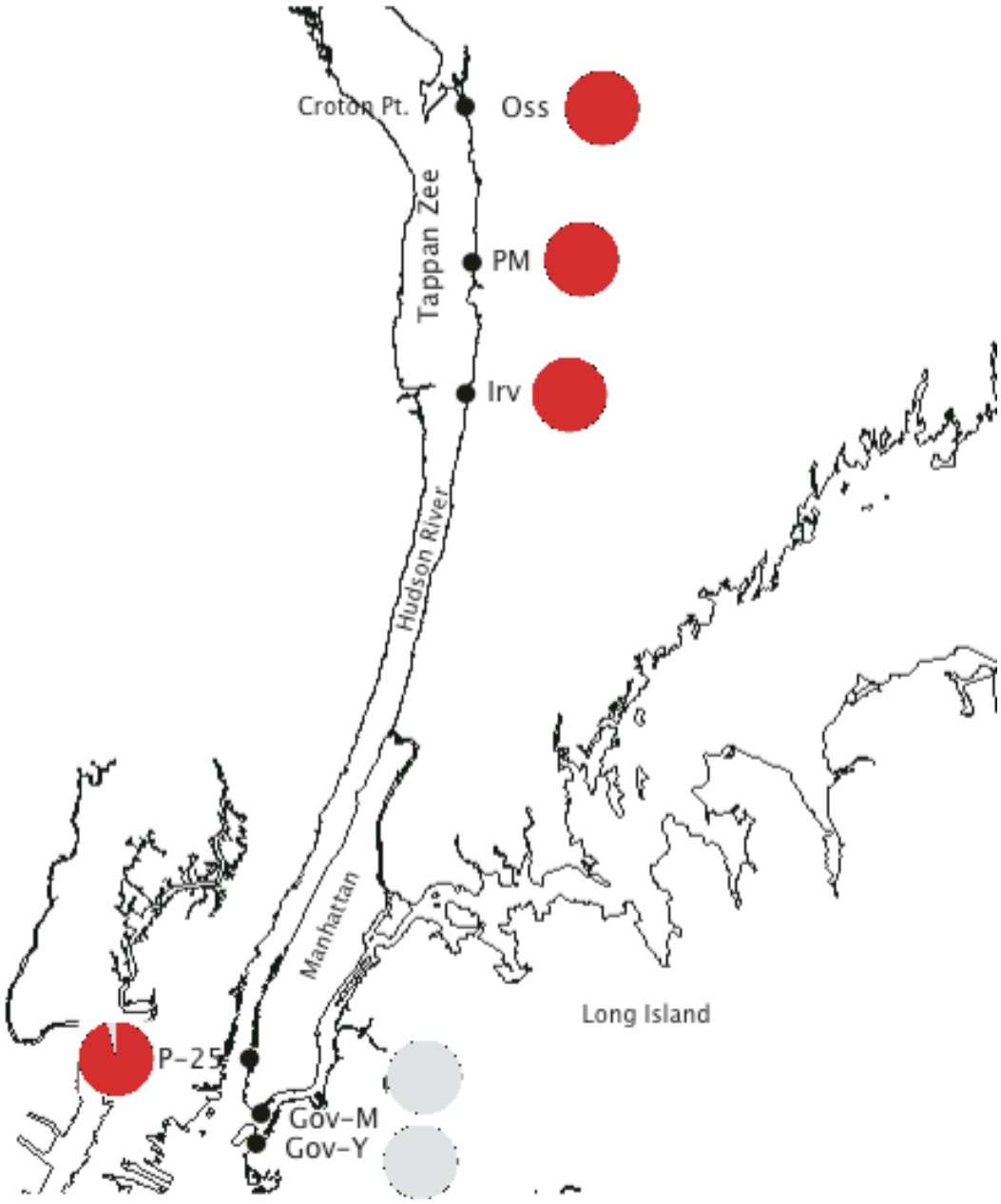

Figure 9. Proportion of shells from all five sample bags per locality, colonized by the barnacle *Balanus improvisus* (red, gray indicates absence).

Other species

A number of other species recruited to the oyster shell bags. The most conspicuous was the sea squirt *Molgula* sp., which nearly covered shell bags at the two Governors Island sites (Figure 10, 11). Also common was the orange colonial sea squirt *Botrylloides violaceus*. The mud crab *Rhithropanopeus harrisii* was common in shell bags within the Tappan Zee. A number of polychaete annelids, amphipods and other species were found within the Tappan Zee shell bags. The snails *Crepidula fornicata* and *C. plana* recruited to shells in the bags at Pier 25 (Figure 12), as did large numbers of the isopod *Idotea sp* (Figure 13).

Figure 10. Colonization of a shell bag at Pier 1, Governors Island by the sea squirt *Molgula sp*.

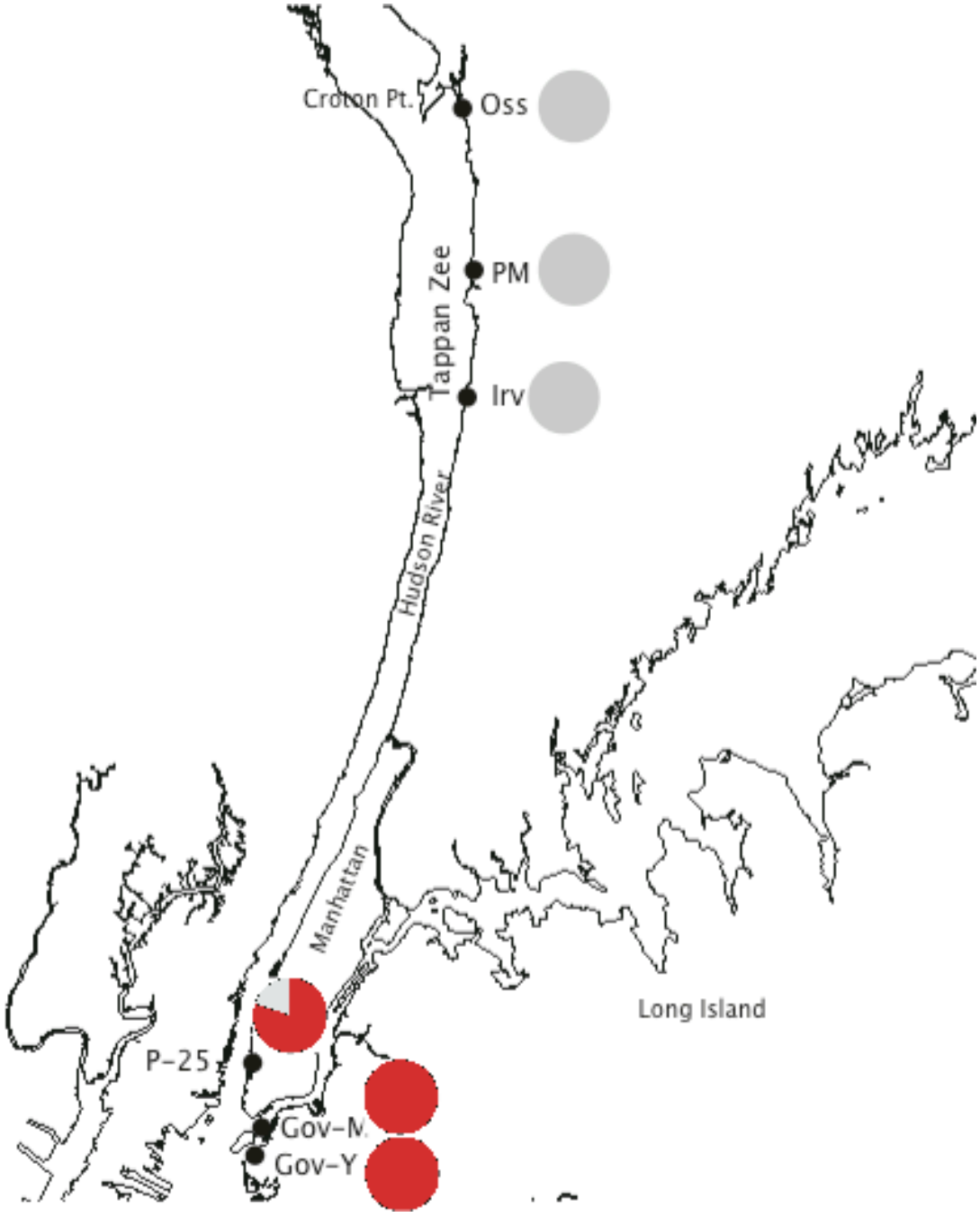

Figure 11. Proportion of shell bags with *Molgula sp*. at the sites (red, light gray indicates absence).

Figure 12. Settlement by the slipper shell (*Crepidula fornicata*) at Pier 25.

Figure 13. Settlement by the isopod *Idotea sp*. at Pier 25.

Diversity

Table 3 shows the total number of identified taxa in the Tappan Zee and New York Harbor samples. As can be seen, a greater number of taxa were found in the New York Harbor set. Much of the difference was explained by colonial ascidians found in New York Harbor.

Table 3. Taxa identified from the two major regions.

New York Harbor sites	Tappan Zee sites
Balanus improvisus, bay barnacle	*Balanus improvisus*, bay barnacle
Molgula sp.	polychaete
Botrylloides violaceus, orange sheath tunicate	*Rhithropanopeus harrisii*, mud crab
Idotea metallica	polychaete
Crepidula fornicata	amphipod
Crepidula plana	*Mytilus edulis*, blue mussel
polychaete	*Dreissena polymorpha*, zebra mussel
Rhithropanopeus harrisii, mud crab	*Idotea metallica*
hydroid	
amphipod	
Mytilus edulis, blue mussel	
Botryllus schlosseri, star tunicate	
Tube worm	
encrusting bryozoan	

DISCUSSION

In this study, the hypothesis that oyster recruitment would be greater closer to the experimental reef was shown to be false. Oyster settlement was not greater in the higher salinity waters of New York Harbor, as represented by the Governors Island and Pier 25 sites. Possible reasons for this are: 1) settlement of other species (e.g., ascidians) may have taken up too much surface area for the larvae to attach to the oyster shells, or 2) they may have been consumed by other organisms that settled before the oyster larvae. Some species that could have prevented and/or disrupted oyster larvae settlement include *Botrylloides*, *Molgula sp.*, and *Balanus improvisus*. The barnacles likely had no effect since the Tappan Zee oyster shells were covered with living barnacles when the oysters recruited in September. A 3rd possibility is that the currents in the vicinity of the constructed reefs are not successfully transporting larvae to the sampling sites. Finally it is possible that there were no larvae at all in the water column of the lower Hudson River/New York – New Jersey Harbor Estuary.

Strong settlement of the barnacle *Balanus improvisus* was observed within the Hudson Estuary, but low settlement was observed in waters off lower Manhattan, and no settlement was observed at Governors Island. This may be due to larval behavior, which promoted retention within the lower Hudson River. Many crustacea in the Hudson River estuary have rhythmic behavior, coincident with rising and falling tides (Morgan 2006).

In conclusion, oyster settlement failed in New York Harbor, at least at the sites established in this study. However, oyster settlement was successful in the lower portion of the Tappan Zee. It is suspected that the larvae in the Tappan Zee originated from within this water body. Settled oysters have been preserved, and they will be analyzed

using neutral genetic markers (microsatellite loci) in the laboratory of Matthew Hare, Cornell University. The markers may demonstrate that Tappan Zee includes an isolated and perhaps locally adapted population of oysters. The data on barnacle settlement suggest that larval behavior may also contribute to localized settlement within the Hudson River estuary, perhaps isolating many species from the oceanic coastal environment.

ACKNOWLEDGEMENTS

I thank the staff of the Irvington Boat Club, the Phillips Manor Beach Club, and the Westerly Marina at Ossining for assistance. Special thanks go to Pete Malinowski of The Harbor School for arranging for localities on Governors Island and to Cathy Drew and especially Nina Zain of The River Project for help on the Lilac at Pier 25. Thanks also to Helena Andreyko, David Yozzo and Sarah Fernald for numerous recommendations for revisions of this manuscript.

LITERATURE CITED

Coen, L. D., R. D. Brumbaugh, D. Bushek, R. Grizzle, M. W. Luckenbach, M. H. Posey, S. P. Powers, and S. G. Tolley. 2007. Ecosystem services related to oyster restoration. Marine Ecology Progress Series 341:303-307.

Coleman, F. C. and S. L. Williams. 2002. Overexploiting marine ecosystem engineers: Potential consequences for biodiversity. Trends in Ecology and Evolution 17:40-43.

Franz, D. R. 1982. An historical perspective on mollusks in Lower New York Harbor, with emphasis on oysters. Pages 181-197 *in* G. F. Meyer, editor. Ecological Stress and the New York Bight: Science and Management. Estuarine Research Federation, Columbia SC.

Kurlansky, M. 2006. The Big Oyster: History on the Half Shell. Ballantine Books, New York.

Levinton, J. S., M. Doall, D. Ralston, A. Starke, and B. Allam. 2011. Climate change, precipitation and impacts on an estuarine refuge from disease. PLoS one 6:e18849. doi:10.13.1371/journal.pone.0018849.

Mann, R. and E. N. Powell. 2007. Why oyster restoration goals in the Chesapeake Bay are not and probably cannot be achieved. Journal of Shellfish Research 26:905-917.

Morgan, S. G. 2006. Larval migrations between the Hudson River Estuary and New York Bight. pp. 157-170, *in* J. S. Levinton and J. R. Waldman, editors. The Hudson River Estuary. Cambridge University Press, New York.

Newell, R. I. E. 1988. Ecological changes in Chesapeake Bay: Are they the result of overharvesting the American oyster, *Crassostrea virginica*? Pages 536-546 Understanding the estuary: Advances in Chesapeake Bay Research. Proceedings of a Conference, March, 1988, Chesapeake Bay Research Consortium, Solomons MD.

Newell, R. I. E., T. R. Fisher, R. R. Holyoke, and J. C. Cornwell. 2005. Influence of eastern oysters on nitrogen and phosphorus regeneration in Chesapeake Bay, USA. pp. 93-120, *in* R. Dame and S. Olenin, editors. The Comparative Roles of Suspension Feeders in Ecosystems. Springer, Netherlands.

Officer, C. B., T. J. Smayda, and R. Mann. 1982. Benthic filter feeding: A natural eutrophication control. Marine Ecology Progress Series 9:203-210.

WHO CONTROLS WHOM? LINKING PREDATOR-PREY DYNAMICS BETWEEN MUD CRABS AND JUVENILE EASTERN OYSTERS TO RESTORATION EFFORTS IN THE NEW YORK METROPOLITAN REGION

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

Predation of newly settled juvenile Eastern oysters (*Crassostrea virginica*; spat) often dominates post-settlement mortality. Mesopredators such as the flat mud crab (*Eurypanopeus depressus*), Say mud crab (*Dyspanopeus sayi*) and white-fingered mud crab (*Rhithropanopeus harrisii*) are abundant on the newly constructed oyster reefs in the New York Metropolitan Region at both Hastings and Soundview Park, and potentially control spat post-settlement mortality. Predator-exclusion studies were conducted at both sites over the summer using glued hatchery-reared oyster singles and naturally recruited oysters. The study not only separated the effect of spat predators by size classes (all sizes, <25 mm, and <5 mm), but also examined the role oyster reefs have in enhancing or decreasing predation pressure. While there was a site and cage treatment interaction ($P<0.001$), there was not a reef structure effect. The naturally recruited tiles showed no difference between 25 mm and exposed cage treatments, regardless of settlement time period and site ($P<0.05$). Results indicate that mesopredators are not important in spat post-settlement mortality at Hastings or Soundview Park.

Since the cage field study could not measure mud crab predation directly, an additional pilot study was performed to test the plausibility of using stable isotope signatures for species-specific interactions. $\delta^{13}C$ and $\delta^{15}N$ signatures were compared between oyster spat, *D. sayi* fed an all-spat diet, and control *D. sayi* not fed oyster spat. While control *D. sayi* had significantly enriched $\delta^{13}C$ signatures compared to laboratory *D. sayi* ($P<0.001$), the $\delta^{15}N$ values did not differ ($P>0.05$). Further research is needed to evaluate whether filter feeders are an important food resource for *D. sayi*, as results suggest *D. sayi* may depend on benthic and not pelagic carbon fixation.

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INTRODUCTION

Oyster reef restoration efforts have been increasing in recent years with the goal of enhancing both oyster fisheries and the ecosystem benefits provided by oyster reefs. The Hudson River Foundation (HRF) has been developing oyster reefs throughout the New York Metropolitan Region. Constructing oyster reefs in the New York Metropolitan Region has the potential to restore ecosystem services, ranging from improving water quality (Nelson et al. 2004; Grizzle et al. 2008) to habitat provision (Wells 1961; Tolley and Volety 2005).

Understanding the community's response to the restoration process is not only important in evaluating restoration success, but also in understanding food-web dynamics. Juvenile Eastern oysters (spat) are an important food resource for a multitude of predators, including the Xanthid mud crab mesopredators. Xanthid mud crabs are abundantly found throughout the Western Atlantic in a wide variety of structured intertidal and subtidal habitats ranging from seagrass meadows to oyster reefs. The four most common species in the mid-Western Atlantic (common mud crab, *Panopeus herbstii*; flat mud crab, *Eurypanopeus depressus*; Say mud crab, *Dyspanopeus sayi*; and white-fingered mud crab, *Rhithropanopeus harrisii*) are found in different salinity regimes and are thus restricted to specific portions of estuaries (Ryan 1956).

There are important species-specific ecological differences between these mud crab species. For example, Kulp et al. (2011) found that *E. depressus* consumed four times more oyster spat than *R. harrisii* in a laboratory experiment. There may be important site-specific differences in the ecological roles for mud crabs, as each species prefers different environmental conditions. Most laboratory studies have examined *P.*

herbstii, which has the largest carapace width of the mud crabs (Ryan 1956), the highest per capita juvenile oyster predation rate (Bisker and Castagna 1987; Kulp et al. 2011) and a salinity preference (35 psu) coincident with the highest oyster growth rates (e.g. Paynter and Burreson 1991). Yet, many oyster reefs and restoration efforts are being conducted in estuaries where *P. herbstii* are not the most abundant mesopredator. Preliminary data suggest that there are low to non-existent abundances of *P. herbstii* on the newly constructed Hudson River oyster reefs (Peterson, *unpublished results*). Additionally, *E. depressus*, *D. sayi* and *R. harrisii* abundances have been estimated to lie between 50 and 150 m^{-2}. Therefore, further research is needed to understand whether *E. depressus*, *D. sayi* and *R. harrisii* play roles similar to *P. herbstii* in the Hudson River.

Examining *E. depressus*, *D. sayi*, and *R. harrisii* are not only important for conservation efforts, but also for evaluating their ecological roles in benthic food webs. Mesopredators transfer energy to higher trophic levels (e.g. Harley and Lopez 2005), thereby serving as an important food source for commercially important organisms such as blue crabs (*Callinectes sapidus*). With the current decline in apex predators worldwide (Heithaus et al. 2008), mesopredators may serve a more critical role in developing food web communities. O'Connor et al. (2008) found that *P. herbstii* could fulfill the functional roles served by blue crabs and stone crabs (*Menippe mercenaria*) if they were of equivalent biomass. The understudied *E. depressus*, *D. sayi*, and *R. harrisii* species may similarly play important roles as adult blue crabs and spider crabs (*Libinia* spp.).

Studying species-specific predation on a resource, such as oyster spat, becomes challenging in the field when there is high predator species diversity (e.g. Eggleston 1990; Newell et al. 2000; O'Connor et al. 2008). Underwater photography/videography

can be costly and challenging to deploy in turbid, urban settings. Therefore, molecular approaches such as stable isotope analysis could be useful in evaluating predator-prey interactions. Stable isotope techniques evaluate long-term trends in diet composition, help determine major nitrogen and carbon sources fueling a food web, and provide a more precise method of examining energy transfers between trophic levels. As such, stable isotopes have been used widely in the study of food web structure and function (e.g. Darnaude 2005; Parker et al. 2008). Yet, a major limitation of this technique is that each prey resource needs to have distinctive $\delta^{13}C$ and $\delta^{15}N$ signatures. Organisms from the same functional feeding group, such as the Eastern oyster and barnacles (*Balanus* spp.), have the potential to consume the same food source and thus could have similar $\delta^{13}C$ and $\delta^{15}N$ values. Since mud crabs are omnivores, capable of consuming multiple filter-feeder species, the $\delta^{13}C$ and $\delta^{15}N$ signatures fractionated from oyster spat may be masked. Additionally, since stable isotope analysis can be costly, pilot studies need to be conducted before performing a large-scale food-web study.

The goals of this study were to determine the role oyster spat and reef structures have in enhancing mud crab populations, and to quantify how these mud crab populations influence spat abundance. The reef structure was expected to increase mesopredator abundances and coincide with increased predation rates. To partition the spat mortality from mesopredators, two predator-exclusion cages were used. The largest sized cage (25 mm lobster wire) was used to determine mesopredators contribution to overall spat mortality at the population level. Conversely, the smallest size cage (5 mm plastic mesh) was used to prevent all oyster spat predators from accessing the oyster spat and measure the natural mortality of oyster spat due to other environmental factors. The

mesopredators were expected to contribute at least 25% of the total spat mortality. Additionally, a laboratory stable isotope study was conducted to determine whether $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures in mud crab cheliped tissues could be distinguished between individuals on an all-oyster diet or no-oyster diet. The stable isotope approach was expected to show significantly different isotopic signatures between treatments, providing evidence for conducting a larger-scale stable isotope experiment.

Mud crabs are abundant mesopredators, whose populations have the potential to regulate oyster post-settlement mortality and impact the trophic transfer and community development of oyster reefs. The completed research worked to clarify the roles of mud crabs on restored oyster reefs, examine the biotic control of post-settlement mortality of oyster spat, and predict the effect of predation by mud crabs on restoration efforts currently underway in the Hudson River.

METHODS

Site selection. Experiments were conducted at two newly constructed preliminary oyster reefs in the New York Metropolitan Region: Hastings and Soundview Park (Figure 1). In 2010, the HRF and partners built the oyster reefs by laying shell veneer on top of a transplanted bedrock base. The footprints of the Hastings and Soundview Park sites are approximately 69 m^2 and 40 m^2, respectively. The two sites have different salinity regimes (Soundview Park: *ca.* 20-25 psu; Hastings: *ca.* 5-10 psu), which influenced the dominant mesopredator mud crab species. *E. depressus* and *D. sayi* both were found only at Soundview Park, whereas *R. harrisii* was found only at Hastings. While oysters naturally settle at both sites, hatchery-reared juvenile oysters settled on shell (spat-on-

shell) were planted at both sites in two installments, October/November 2010 and June 2011.

After visiting the sites in May, there was evidence of tidal erosion, as a large proportion of the spat-on-shell and veneer shell layer were gone. The Hastings site additionally had high turbidity, such that the west side of the reef was often covered in mud after a heavy storm. Regardless, both sites allow for the effects of a reef structure and presence of different mesopredator species to be tested.

In each site, there was an on and off-reef experimental location. The off-reef site had the same footprint as the reef site, approximately 25 m east and north of the reef sites at Soundview Park and Hastings, respectively. While the off-reef site was characterized by no structure, there was mixture of gravel and sandy substrate at Soundview Park. Conversely, the substrate at Hastings consisted of unconsolidated mud with no additional structure.

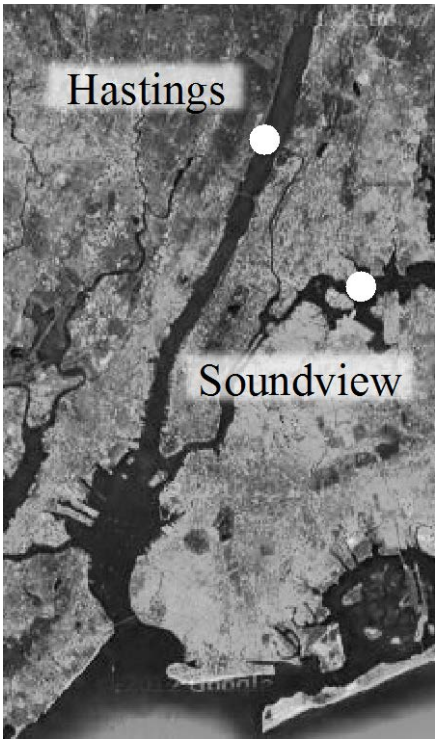

Figure 1. **Study site locations within the New York Metropolitan Region. Hastings was at a lower salinity site than Soundview Park (Soundview Park: *ca.* 20-25 psu; Hastings: *ca.* 5-10 psu). The footprint of Hastings and Soundview Park were 69 m^2 and 40 m^2, respectively. Each site had an off-reef study location with an equal footprint. Map was generated from Google Maps, ©2012Google.**

Predator-exclusion experiment. A randomized 2-factorial design comparing cage treatment by within site location was conducted at both Hastings and Soundview Park to test the effect different size classed predators have on post-settlement spat mortality, as well as how the reef structure influences predation patterns. The same design was conducted using two alternative strategies for exposing spat (<20 mm shell height [SH]) to predation. The first strategy was to standardize the number, size and arrangement of oysters by gluing oyster singles onto 10 x 10 cm ceramic tiles. The second strategy was to measure natural oyster recruitment onto the top and bottom of 10 x 10 cm ceramic tiles. Recruitment was defined as the number of settled spat present at the time of retrieval.

For the glued tiles, hatchery-reared oyster singles (5-8 mm SH) were provided by the Cornell Cooperative Extension's hatchery in Southold, NY and the East Hampton Town Shellfish hatchery in East Hampton, NY. Artificially adhered oysters were given at least one week to grow in the sea tables at Stony Brook University's Marine Station, Southampton, NY. All sea tables were supplied continuously with ambient seawater. Individuals that died from the gluing process were replaced and given at least 1 day to recover before deployment. As glued oysters were exposed to ambient seawater, the growth rate in the sea table changed during the course of the experiment. At the time of deployment, oysters were all within a size range mud crabs were capable of consuming (8-20 mm; *e.g*. Kulp et al. 2011).

To measure the potential effects of artificially attaching oysters onto tiles, a laboratory experiment was conducted to compare the number of glued oysters, naturally settled and spat-on-shell oysters consumed by *D. sayi*. Glued and naturally recruited

oysters were adhered to 10 x 10 cm ceramic tiles. Larval oysters were allowed to naturally settle on tiles and grow at the hatchery of the Urban Assembly New York Harbor School two months prior to the experiment. The spat-on-shell were similarly obtained from the New York Harbor School. Of the 30 tiles provided to the hatchery, 13 had successful settlement and could be used for the mesocosm study. Spat are known to be aggregate settlers (Tamburri et al. 2007), explaining the variable oyster densities observed on the spat-on-shell and recruited tiles. If there were more than twenty-five spat on the spat-on-shell and recruited tiles, then the extra spat were randomly removed. To standardize the glued tile treatments, 25 oysters were adhered with super glue and given 1 week to grow in the sea table. All oyster sizes were within the same range (5 to 15 mm shell height [SH]).

The experiment was conducted in 95 L flow-through mesocosms for 72 hours. *D. sayi* (15-23 mm carapace width [CW]) were collected from Shinnecock Bay and starved for 24 hours in the mesocosms before oyster treatments were added. There were four replicate controls of each oyster treatment without a mud crab present to measure natural oyster spat mortality. All treatment replicates were performed in one experimental run. Any molted or dead crabs were not included in the analysis. Since not all oyster treatments had 25 oysters initially present, percentages of oysters consumed were calculated and compared between treatments.

In the field predator-exclusion experiment, each tile was assigned to one of three predator-exclusion treatment cages (exposed, 25 mm aperture, and 5 mm aperture) that offered varying levels of protection from predation. The exposed treatment offered no protection from predation. The 25 mm cage (10 x 10 x 35 cm) was made of lobster wire

with 25 mm aperture, so that predators >25 mm could not access the prey. The 5 mm cage also had a 25 mm lobster wire frame, but with 5 mm polyvinyl plastic lining the interior to prevent predators > 5 mm entry. To test for potential cage artifact effects (Steele 1996), a cage control cage was used. The cage control was identical to the 5 mm cage treatment, except that 2 of the 6 sides were missing. Tiles were zip-tied within each cage treatment and a brick was attached onto the cage bottom for stability. The brick was then inserted into the substrate, making the cage relatively level with the substrate. There were four replicates of each treatment randomized and lined into two rows at the on and off-reef sites (Figure 2).

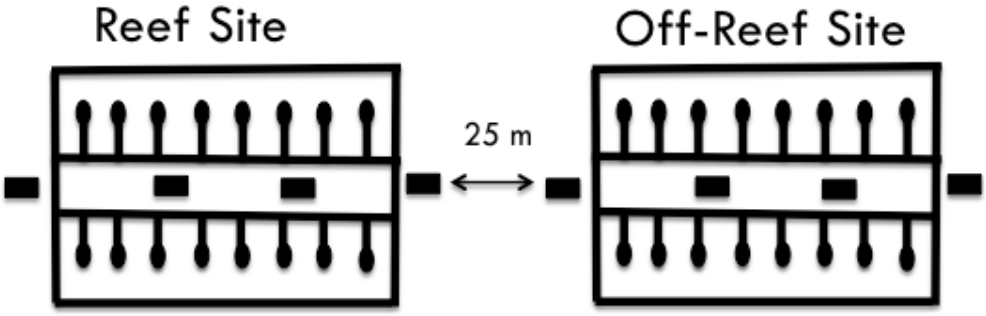

Figure 2. Diagram of on and off-reef study locations. The two study sites had the same footprint and were approximately 25 m apart. Cage treatments were distributed in two rows across the site and recruitment trays were inserted in a row in the middle of the study sites.

A picture was taken before and after deployment for the glued tiles to determine the number of oysters consumed. Any oysters missing at retrieval were assumed consumed. Four deployments were made between May and July 2012. Methods were slightly altered between deployments due to different logistical constraints. For the May deployment, 49 oysters were glued on a tile using super glue and deployed for 48 hours. Since less than 40% of oysters were consumed, 30 oysters were glued per tile for the next

three deployments to decrease gluing effort in the laboratory. Additionally, approximately half of the tiles lost at least one oyster during transport to the field. As such, an alternative adhesive, Z-Spar epoxy, was used in the June deployment. A maximum of 4 oysters $tile^{-1}$ were consumed during the June deployment. Unlike the super glue, oysters were pushed into the Z-Spar epoxy, smoothing the edges around the oyster and potentially increasing the handling time required for consumption. Therefore, super glue was used for the last two deployments in July to remove any artificial edge protection created by the Z-Spar. The deployment periods for the last two deployments in July were increased from 48 hours to one week to increase predator exposure. However, due to logistical constraints, the second July deployment at Hastings was retrieved after five days. Due to the changes in methodology throughout the summer, the June deployment was not included for trend analysis and the May and July deployments were analyzed separately.

Between glued tile deployments, 10 x 10 cm ceramic tiles were placed in the predator-exclusion cages to test predator effects on oyster recruitment. In June and July, tiles were retrieved after three weeks, and in August, the tiles were retrieved after six weeks. The number and size of oysters on the top and bottom of each tile was recorded after retrieval. Only oyster size ranges were recorded in the July retrieval at Soundview Park.

To estimate resident mesopredator abundances, four replicate trays (45 x 30 x 10 cm) lined with fiberglass window screen were inserted into the on and off-reef sites for three, five and six weeks before retrieval in June, July and August, respectively. The trays placed at the on-reef site were filled with veneer shell, whereas trays at the off-reef

site were filled with the excavated sediment. After retrieval, the number of mesopredators was quantified. Additionally, two mud crab traps were placed at the on and off-reef sites during the glued tile deployment to obtain a mesopredator catch per unit effort measurement. The mud crab traps had a 5 mm polyvinyl mesh lining a lobster wire frame. The square traps had a 25 mm door, thus targeting mesopredators capable of entering the 25 mm predator-exclusion cage treatment. One baited fish trap (*c.a.* 0.25 m diameter doors) was deployed at an on and off-reef site for 48 hours when the recruitment tiles were deployed. The off-reef site was not in the same location the cage and trays were deployed, but were at least 25 m from the reef.

Stable Isotope Analysis. A pilot stable isotope laboratory experiment was conducted to explore whether a large-scale stable isotope experiment could be performed at the oyster reefs. Thirty *D. sayi* collected from Heady Creek, Shinnecock Bay, NY were fed oyster spat (10-20 mm SH) for 28 days to allow sufficient time for tissue turnover. All spat were reared at the East Hampton hatchery and kept in the Marine Station's sea table for at least two weeks prior to consumption. A subsample of 15 oyster individuals (14-20 mm SH) were selected and frozen for determining baseline isotopic signatures prior to fractionation by crab individuals. At the conclusion of the experiment, thirty additional *D. sayi* were collected from Heady Creek. The Heady Creek site was characterized by having high densities of the common slipper shell (*Crepidula fornicata*) that lived on top of a *Crepidula* shell hash base. As such, Heady Creek offered a low relief, three-dimensional habitat that were abundantly inhabited by *D. sayi* (*ca.* 80-150 m^2). There have been no recorded oyster recruitment in Shinnecock Bay in the past two summers

(Peterson, *unpublished*), thus the control *D. sayi* samples represent individuals without an oyster spat diet. All samples were frozen before processing.

Fifteen crabs (15-22 mm CW) were randomly selected from individuals fed an all-oyster diet and those collected from Heady Creek. Cheliped muscle was extracted from the crab samples to standardize the tissue type, as tissues can have different turnover rates (Llewellyn and Peyre 2011). To have enough material for the analysis, the entire oyster body tissue was used. Samples were dried, ground, and 1 ± 0.1 mg of tissue sample was sent to the Stable Isotope Laboratory at Boston University for $\delta^{13}C$ and $\delta^{15}N$ analysis. Accuracy and precision of analysis was verified through the use of standards and duplicate samples.

Data Analysis. For the glued and naturally recruited oyster tile experiment, the data could not be normalized. As such, glued and naturally recruited oyster tile experiment data were rank-transformed and the parametric analysis of variance on ranks (ANOVA on ranks; Potvin and Roff 1993) used for statistical comparisons. For the glued oyster tile experiment, a three-way ANOVA on ranks was performed, comparing the site, treatment location and cage treatment effects. For the naturally recruited oyster tile experiment, a three-way ANOVA on ranks was performed, comparing recruitment month, treatment location and cage treatment effects between the July and September at Soundview Park. Additionally, a three-way ANOVA on ranks was performed, comparing site, treatment location and cage treatment effects in August. Due to low replication the abundances from the fish and crab traps were not statistically compared. Catch per unit effort was summarized into tables.

A one-way ANOVA was performed on the laboratory trial performed in the mesocosms between oyster treatment and percentage of oysters consumed. Data was arcsine transformed to meet necessary parametric assumptions. Student's t-test was used to compare $\delta^{13}C$ and $\delta^{15}N$ signatures from control and experimental *D. sayi*. To normalize data, $\delta^{15}N$ signatures were raised to the 10^{th} power before analysis was performed.

Tukey's HSD post-hoc test was performed when significant differences existed between treatment means. All statistical analyses were performed in R statistical software, version 2.15.1. Statistical significance α was set at 0.05.

RESULTS

Predator-exclusion experiment. The May and July glued tile deployments showed similar trends; therefore only July deployments will be summarized. Soundview Park had more than 50% oyster consumption compared to Hastings in all the cage treatments except the 5 mm cage. There was a significant interaction between oyster consumption differences in cage treatment types observed between sites ($P<0.001$; three-way ANOVA on ranks; Figure 3). At Soundview Park, significant differences were observed between the exposed and 5 mm treatment, as well as the exposed and 25 mm treatments ($P<0.05$; Tukey's HSD). The exposed treatment had 64.6% ± 40.2% oysters consumed $tile^{-1}$ (mean ± 1 SD) with a maximum of 30 and minimum of zero oysters consumed $tile^{-1}$. Conversely, there were no differences between the exposed and cage control or the 5 and 25 mm treatments. Unlike Soundview Park, no significant differences were observed between cage treatments at Hastings ($P>0.05$; Tukey's HSD). Furthermore, no

significant differences were observed between on and off-reef oyster consumption (P >0.05; three-way ANOVA on ranks).

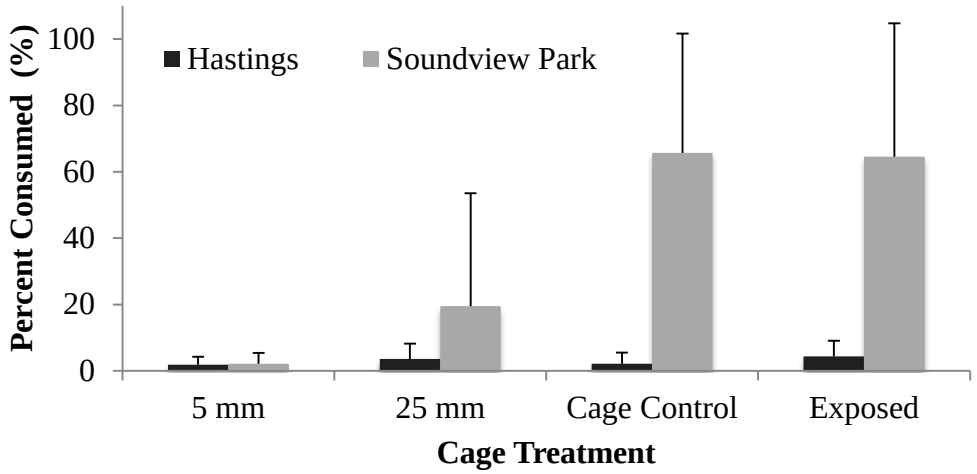

Figure 3. **Percent oyster consumed by site and cage treatment in July from the on-reef location. At Hastings and Soundview Park, there were three different cage exclusion treatments: a cage with 5 mm mesh, 25 mm lobster wire and an exposed treatment. There was also a cage control that had four 5 mm mesh sides. The average percent consumed was calculated for two deployments in July, left out for one week each. The error bars are +1 SD. A significant interaction was observed between site and cage treatment (P<0.001; three-way ANOVA on ranks).**

Oysters naturally recruited to bare tiles at Soundview Park in July and August and at Hastings in August. At Soundview Park, recruitment in July and August was low with 4.6 ± 3.3 oysters tile^{-1} and 1.5 ± 1.3 oysters tile^{-1} averages, respectively. Even though oyster densities decreased in August, the oyster sizes were greater, ranging from 3-15 mm SH in comparison to 1-5 mm in July. There was an interaction between recruitment month and cage treatment ($p<0.05$; three-way ANOVA on ranks; Figure 4). There was additionally a significant treatment location effect ($p<0.01$; three-way ANOVA on ranks), where the off-reef location had twice as many oysters recruited than the reef location.

When comparing the August oyster recruitment period between Hastings and Soundview Park, there was a significant site and treatment location interaction ($P<0.001$; three-way ANOVA on ranks; Figure 5). Hastings had a higher number of oysters recruited than Soundview Park, averaging between 25 and 36 oyster tile^{-1} in each cage treatment type, and a larger size range, ranging between 2-21 mm SH. Furthermore, the on and off-reef site location significantly influenced predation at Soundview Park, but not at Hastings ($P<0.05$; Tukey's HSD). The cage treatments had a significant effect on percentage of oysters recruited ($P<0.05$; three way ANOVA on ranks), where the 5 mm cage treatment had fewer oysters recruited than the cage control ($P<0.05$; Tukey's HSD).

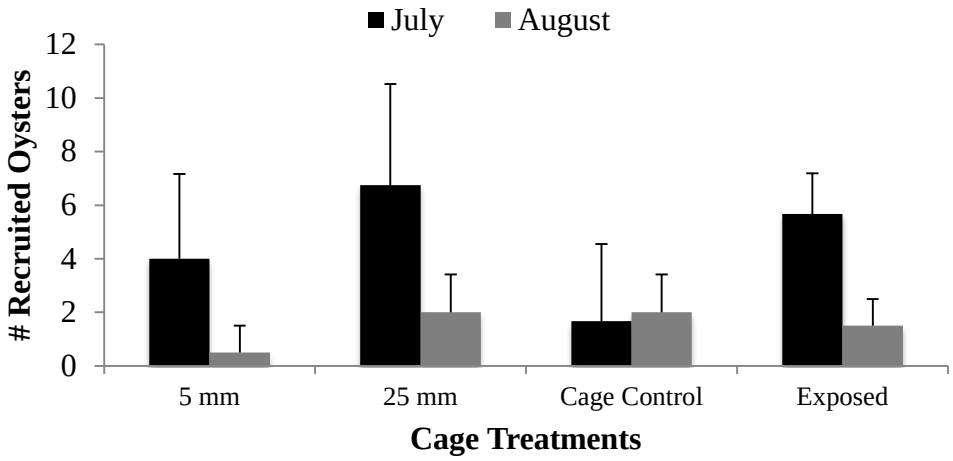

Figure 4. **Number of oysters recruited at Soundview Park in July and August at the on-reef location. Tiles were deployed for 3 weeks in July and 6 weeks in August. Averages include the number of oysters recruited on the top and bottom of tile. There were significant differences in number of oysters recruited between July and August, as well as the on and off-reef site location ($P<0.05$; three-way ANOVA on ranks). There were no cage treatment effects observed ($P>0.05$; three-way ANOVA on ranks). Error bars are +1 SD.**

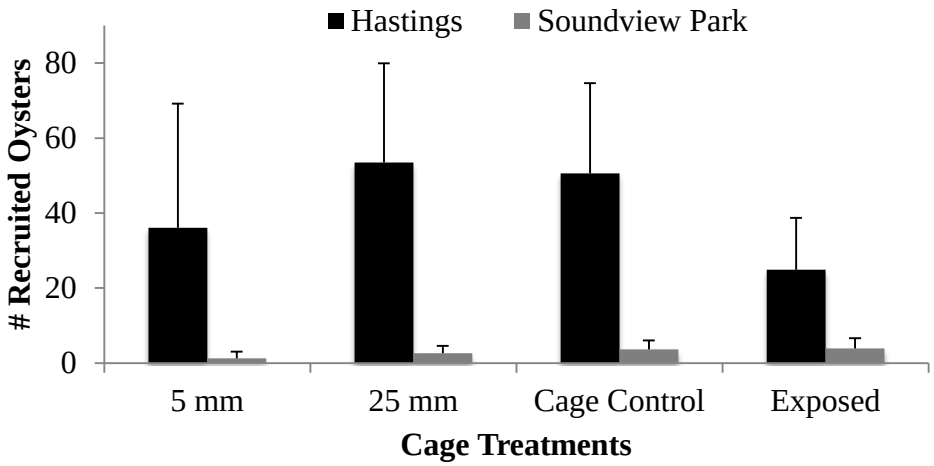

Figure 5. **Number of oysters recruited onto a tile in August at Hastings and Soundview Park. Averages include the number of oysters recruited on the top and bottom of tile retrieved from the on and off-reef site. There were significant differences between site and treatment location ($P<0.05$; three-way ANOVA on ranks). The cage treatments also had a significant effect on percentage of oysters recruited ($P<0.05$; three-way ANOVA on ranks). Error bars are +1 SD.**

A maximum of four mud crabs were collected from the mud crab traps with averages ranging from zero to one mud crab collected on and off-reef at both sites over the summer. There was one outlier of three crabs collected during the July retrieval at Hasting's off-reef location. The low crab collections may be a result of trap malfunction. To test this hypothesis, three traps were deployed in separate mesocosms with 13 mud crabs. Crab abundances in the trap reached four crabs within two hours, but then subsequently decreased, indicating that the cage design may not have effectively prevented escape. Therefore, the cage trap counts were not estimating catch per unit effort, but served to confirm the presence or absence of predators. No differences were

found between predator species caught on and off the reef. At Soundview Park, mud crab traps caught not only *D. sayi*, but also juvenile blue crabs. Conversely, *R. harrisii* were the only predator species caught at Hastings. A small number of juvenile blue crabs (2 individuals) were also collected at Hastings inside the 5 and 25 mm cage treatments during tile retrieval in September, but not within the crab traps.

Fish traps were additionally used to measure catch per unit effort over 48 hours (Table 1). Blue crabs and spider crabs were collected at Soundview Park both on and off-reef over the summer, whereas blue crabs were collected at Hastings only during the June deployment. White perch (*Morone americana*) were additionally collected at Hastings throughout the summer, while no fish were collected at Soundview Park. Unlike fish traps, the trays inserted into the on-reef location recorded higher abundances of resident crustacean predator species (Table 2). The on-reef trays at Hastings showed the highest mud crab abundances throughout the summer, reaching average densities of 61 ± 18.4 *R. harrisii* individuals in September. While Hastings had a gradual increase in mud crab abundances over the course of the summer, Soundview Park showed a peak in July with 23.5 ± 9.3 *D. sayi* individuals and 8.3 ± 2 *E. depressus* individuals. As expected, the off-reef location showed lower mud crab abundance. At both sites, averages ranged from zero to one mud crab over the summer. Hastings had the maximum number of mud crabs recorded with two mud crab individuals tray^{-1} in September.

	On-Reef		Off-Reef	
	Callinectes sapidus	*Libinia* spp.	*Callinectes sapidus*	*Libinia* spp.
May	1	1	0	2
June	8	3	3	2
July	0	0	3	0
September	4	0	1	0

Table 1. Crustacean catch per unit effort from fish traps in on and off-reef locations at Soundview Park. One baited fish trap was deployed for 48 hours in May, June, July and September at an on and off-reef location. The fish trap off-reef site was not in the same location as the off-reef tray abundance location.

	Hastings		Soundview Park			
	Rhithropanopeus harrisii		*Dyspanopeus sayi*		*Eurypanopeus depressus*	
	On-reef	Off-reef	On-reef	Off-reef	On-reef	Off-reef
June	23.3±12.9	0.3±0.5	9.5±4.2	0.3±0.5	0.5±0.6	0
July	43.3±26.4	1±1	23.5±9.3	0.3±0.5	8.3±2.2	0
Sept	61±18.1	0.3±0.6	19±10.5	0	2±2	0

Table 2. Number of mud crabs collected at on and off-reef locations. Numbers were averaged across four recruitment trays that were deployed for 3, 5 and 6 weeks before retrieval in June, July and August, respectively. Values represented as the average ± 1 SD.

In the mesocosm predator experiment, glued tiles had the highest consumption with an average of 45.8% ± 33.5% oysters consumed, while the spat-on-shell and naturally recruited oyster treatments had 18% ± 18.3% and 23% ± 24.7%, respectively. The statistical test resulted in a P value of 0.0502 (one-way ANOVA; n = 13 for glued, n = 14 for spat-on-shell, and n = 9 for naturally recruited oysters; Figure 6), indicating there were differences in the percentage of oyster spat consumed among the three forms of oyster prey: glued oysters, naturally recruited oysters, and spat settled on oyster shell. The glued tiles were the only treatment that had 100 percent consumption of the 25 oysters; however, there was high variability in oyster consumption, with values ranging from zero to 25 oysters.

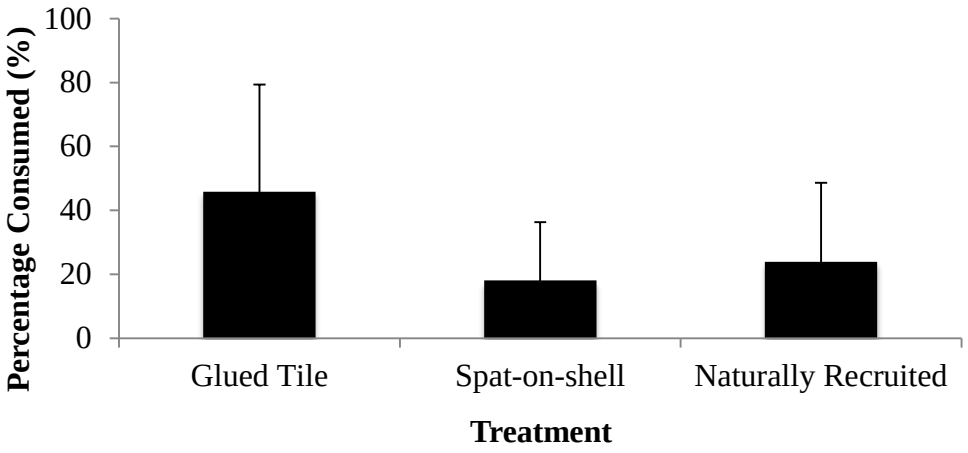

Figure 6. **Percentage of oysters consumed in the mesocosm predator experiment. The three oyster treatments were glued oyster singles (GT), spat-on-shell (SOS), and naturally recruited oysters (NRT). Experiments were conducted within flow-through mesocosms over a 72 experimental period with *Dyspanopeus sayi* individuals. The test statistic had a p value of 0.0502 (one-way ANOVA; n = 13 for GT; n = 14 for SOS; n = 9 for NRT), indicating oyster treatments were different. Error bars are + 1 SD.**

Stable isotope analysis. The $\delta^{13}C$ signatures from the oyster-fed laboratory *D. sayi* were significantly different from control non-oyster fed *D. sayi* $\delta^{13}C$ signatures collected from Shinnecock Bay, NY ($P<0.001$; Student's t-test; n = 15; Figure 7). Control *D. sayi* were enriched in $\delta^{13}C$ with values of -14.9 $^{0}/_{00}$ ± 0.6 $^{0}/_{00}$ compared to -16.6 $^{0}/_{00}$ ± 0.5 $^{0}/_{00}$ from laboratory *D. sayi* that were fed oysters exclusively. Conversely, $\delta^{15}N$ signatures were not statistically different between the laboratory and control *D. sayi* individuals ($P>0.05$; Student's t-test; n=15). Sampled oyster spat $\delta^{13}C$ signature was -19.0 $^{0}/_{00}$ ± 1.4 $^{0}/_{00}$ and $\delta^{15}N$ signature was 8.8 $^{0}/_{00}$ ± 0.2 $^{0}/_{00}$. Laboratory *D. sayi* fractionated spat $\delta^{13}C$ and $\delta^{15}N$ signatures by 2.5 $^{0}/_{00}$ and 2.3 $^{0}/_{00}$, respectively.

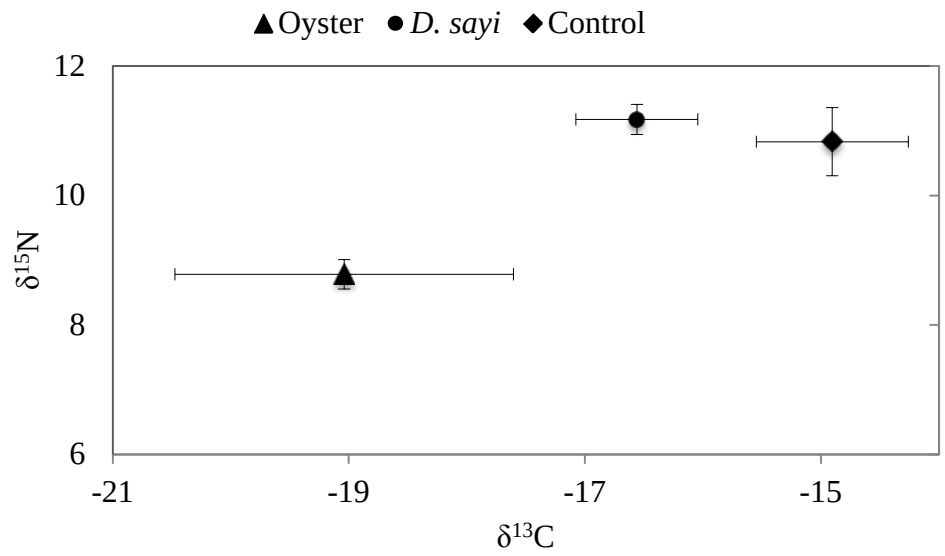

Figure 7. **Comparing $\delta^{13}C$ and $\delta^{15}N$ signatures between *Dyspanopeus sayi* with or without an all-oyster diet. Control *D. sayi* individuals were collected at Heady Creek, Shinnecock Bay, NY, where no oyster spat were present. Experimental *D. sayi* were fed an all-oyster diet in the laboratory for 28 days. The oyster spat $\delta^{13}C$ and $\delta^{15}N$ signatures represent baseline values before fractionation by *D. sayi*. There were no significant differences between laboratory and control *D. sayi* in $\delta^{15}N$ signatures ($P>0.05$; Student's t-test; n=15); however, there were significant differences in $\delta^{13}C$ signatures ($P<0.001$; Student's t-test; n=15). Error bars are ± 1 SD.**

DISCUSSION

The conducted experiments were aimed at evaluating the role of mesopredators in post-settlement mortality of juvenile Eastern oysters. The hypothesis that mud crabs contribute to 25% of overall oyster spat consumption was not supported, as the percentage of oysters consumed did not differ between the 25 mm and 5 mm cage treatments. Since mud crabs at Hastings and Soundview Park have rarely been observed to be larger than 25 mm CW (Peterson, *unpublished observations*), the 25 mm treatment was intended to record the contribution of mud crab predation. The results were

unexpected, as previous laboratory research by Kulp et al. (2011) estimated that these mud crab populations have the potential of consuming half the seeded spat planted on Maryland oyster reefs. The results provide evidence that laboratory predation rates may not be representative of population-level predation rates. Under laboratory conditions, crustacean predation studies typically starve individuals for 24 to 72 hours, separate predators into individual containers, and provide one prey resource (Eggleston 1990; Kulp et al. 2011). Even though these laboratory procedures standardize predation and decrease variability, biological and physical interactions occurring in the field may override results found in the laboratory. While recent laboratory experiments have increased design complexity by testing for prey preference (Mascaró and Seed 2001) and effects of conspecific and interspecific biological interactions (Griffen 2006; Bèlair and Miron 2009), there are still limitations to extrapolating results to field conditions. Perhaps spat are not the preferred prey resource of these mud crab species in the field, or the field consumption rates are less than those observed in the laboratory.

Prey preference may not only be related to the prey species, but also the level of risk associated with consuming a resource. Predator driven behavioral changes that do not involve direct consumption are known as trait-mediated indirect interactions (TMIIs; Werner and Peacor 2003). Such non-consumptive interactions of predators on mesoconsumers can lead to decreased control of lower trophic levels. *Panopeus herbstii,* another mud crab species dominant in southern coastal oyster reef systems, has been used as the model mesoconsumer in studying TMIIs. Grabowski (2004) found that the presence of a mud crab predator, the oyster toadfish (*Opsanus tau*), decreased mud crab foraging rates on oyster spat. Recently, Griffen et al. (2012) examined how TMIIs vary

depending on the habitat and size of *P. herbstii*. Results indicate that small individuals alter their behavior more than large individuals. Griffen et al. (2012) had used *P. herbstii* between 30 and 40 mm, which are larger than the mud crab species found at Hastings and Soundview Park. As such, predators of mud crabs, including blue crabs and oyster toadfish, that were found in the fish traps at both Hudson oyster reef sites may have a stronger effect on *D. sayi*, *E. depressus*, and *R. harrisii* individuals than *P. herbstii* due to their smaller sizes. The observed low oyster consumption in the predator-exclusion experiment may have been due to TMIIs exhibited by mud crab species. On the other hand, the experimental design may have created an artificial risk by using a flat ceramic tile opposed to the concave oyster cultch. In other mud crab predation studies (Grabowski 2004), oyster cultch was used instead of ceramic tiles, which could provide increased structural complexity and decrease risk associated with oyster consumption. Future experiments could incorporate assessments of the potential risk associated with exposing prey to predators in the field.

Another potential explanation for decreased mesopredator oyster spat consumption involves the size of oysters used in the experiment. Bivalve size has consistently been shown to impact the predation rate of crustacean predators, as predators usually will prefer smaller sized bivalves (Seed 1980; Eggleston 1990; Kulp et al. 2011). While the sizes used in the glued oyster tile experiment were within the size range consumed by mud crabs (Bisker and Castagna 1987; Kulp et al. 2011), they were not at the size that showed the highest consumption rates (Kulp et al. 2011). As predation rate declines with increasing size, the glued oysters may not have been within a desirable size range to overcome the risk associated with accessing the oyster prey.

The naturally recruited oysters, which were at smaller sizes than the glued tile experiment, also did not show a 25 mm treatment effect. The naturally recruited oysters could have been more challenging to remove from the tile than the glued oysters. While predation rates between treatments in the mesocosm study were not statistically different (P=0.0502), there could have been an important biological difference. Glued tiles were the only treatment type that had 100% consumption and had the highest mean consumption rate. As such, the glued oyster singles could have been easier to remove from the substrate than the settled oyster spat that conformed to the substrate. Additionally, perhaps the density of naturally recruited oysters was not high enough at Soundview Park to attract mud crab predators. Densities were no greater than 12 oysters on the tile bottom, which was almost completely protected from macroalgal growth and likely represents potential spat settlement on the top of the tile. Conversely, Hastings had high oyster densities, up to 72 oysters on the tile bottom and 69 oysters on the tile top. *Rhithropanopeus harrisii*, the mud crab living in high densities at Hastings, was shown to eat a minimal amount of oyster spat in previous studies (Kulp et al. 2011). As such, *R. harrisii* was not expected to be a dominant oyster predator. Low predation at Hastings may have been related to low abundances of other oyster predators; only one blue crab was collected in the fish trap during the study period.

In addition to investigating the role of mud crab predation, the experiment also examined the role oyster reef structure has on predation rates. The field experiments did not support our hypothesis that the oyster reef would enhance predation rate. One potential reason could involve predator mobility. Since the exposed treatments showed the highest oyster consumption at Soundview Park, the largest size classes of predators,

such as blue and green crabs, were the most important contributors to oyster spat mortality. These larger-sized predators are transient species and have the ability to migrate large distances. For example, male blue crabs have been recorded to travel 85 m day^{-1} (Wrona 2004); therefore, the off-reef site (25 m off the reef) could have been easily accessible by these highly mobile larger crustacean predators. There was no cage artifact effect observed in the study, as there was no difference in oyster consumption between the cage control and exposed treatment. As such, the lack of a reef effect on predation rates was most likely due to the mobility of the predators and not from a cage artifact effect. Blue crabs have been well recorded as an important oyster spat predator (e.g. Egglestone 1990; O'Connor et al. 2008), so the results from the predation study confirm their important predation role in post-settlement mortality of oyster spat.

A limitation of the field experiment was the inability to confirm the identity of the predators responsible for the consumption on the tiles. Therefore, the final component of the mud crab experiment was to determine whether stable isotopes would be useful in evaluating the species-specific interactions between mud crabs and oyster prey. While *D. sayi* fractionated oyster tissue by an expected 2.3 $^{0}/_{00}$ for $\delta^{15}N$ signature, *D. sayi* fractionated the $\delta^{13}C$ signature by an unexpected 2.5 $^{0}/_{00}$. Standardized fractionation values have been estimated to be approximately 1.0 $^{0}/_{00}$ for $\delta^{13}C$ and 2.5 $^{0}/_{00}$ (Vanderklift and Ponsard 2003; Zanden and Rasmussen 2001). As such, the $\delta^{13}C$ signature value was more enriched than expected. Llewellyn and Peyre (2011) performed a 20-day laboratory feeding study with blue crabs and found that the muscle tissue was more enriched than expected while the hepatopancreatic tissue was not. The cheliped muscle similarly may need more than 28 days to turnover tissue $\delta^{13}C$ values. Regardless of the unexpected

enriched $\delta^{13}C$ signatures in the laboratory *D. sayi*, the *D. sayi* individuals collected directly from Shinnecock Bay had significantly different $\delta^{13}C$ signatures from the laboratory *D. sayi* ($P<0.001$; Student's t-test; $n=15$). Pelagic and benthic primary production fractionate solubilized carbon differently, thus having different $\delta^{13}C$ values (France 1995). Thus, the more enriched $\delta^{13}C$ signatures found in the control *D. sayi* individuals suggests that the mud crabs at Heady Creek in Shinnecock Bay consume prey that feed on the benthos or benthic detritus directly and not those that filter-feed. Perhaps one reason why filter feeders are not consumed at Heady Creek involves increased risk associated with consuming filter-feeding prey. Therefore, stable isotope results suggest that TMIIs could also be an important biological interaction in *Crepidula* benthic environments. Additional studies need to be performed before conclusions can be drawn about whether filter feeders are an important resource for *D. sayi* in benthic habitats, as well as whether oyster tissue $\delta^{13}C$ and $\delta^{15}N$ signals differ from other filter feeders.

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LITERATURE CITIED

Bélair, M. and G. Miron. 2009. Predation behavior of *Cancer irroratus* and *Carcinus maenas* during conspecific and heterospecific challenges. Aquatic Biology 6: 41-49.

Bisker, R. and M. Castagna. 1987. Predation on single spat oysters *Crassostrea virginica* (Gmelin) by blue crabs *Callinectes sapidus* Rathbun and mud crabs *Panopeus herbstii* Milne -Edwards. Journal of Shellfish Research 6: 37-40.

Darnaude, A.M. 2005. Fish ecology and terrestrial carbon use in coastal areas: implications for marine fish production. Journal of Animal Ecology 74: 864-876.

Eggleston, D.B. 1990. Foraging behavior of the blue crab, *Callinectes sapidus*, on juvenile oysters, *Crassostrea virginica*: effects of prey density and size. Bulletin of Marine Science 46: 62-82.

France, R.L. 1995. Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. Marine Ecology Progress Series 124: 307-312.

Grabowski, J.H. 2004. Habitat complexity disrupts predator-prey interactions but not the trophic cascade on oyster reefs. Ecology 85: 995-1004.

Griffen, B.D. 2006. Detecting emergent effects of multiple predator species. Community Ecology 148: 702-709.

Griffen, B.D., Toscano, B.J., and J. Gatto. 2012. The role of individual behavior type in mediating indirect interactions. Ecology 93: 1935-1943.

Grizzle, R.E., Greene, J.K., and L.D. Coen. 2008. Seston removal by natural and constructed intertidal eastern oyster (*Crassostrea virginica*) reefs: a comparison with previous laboratory studies, and the value of in situ methods. Estuaries and Coasts. 31: 1208-1220.

Harley, C. D. G. and L.P. Lopez. 2005. The natural history, thermal physiology, and ecological impacts of intertidal mesopredators, *Oedoparen*a spp. (Diptera: Dryomyzidae). Invertebrate Biology 122: 61-73.

Heithaus, M . R., Frid, A., Wirsing, A. J., and B. Worm. 2008. Predicting ecological consequences of marine top predator declines. Trends in Ecology and Evolution 23: 202-210.

Llewellyn, C. and M.L Peyre. 2011. Evaluating ecological equivalence of created marshes: comparing structural indicators with stable isotope indicators of blue crab trophic support. Estuaries and Coasts 34:172-184.

Kulp, R., Politano, V., Lane, H. A., Lombarde, S. A., and K.T. Paynter. 2011. Predation of juvenile *Crassostrea virginica* by mud crabs in the Chesapeake Bay. Journal of Shellfish Research 30: 261-266.

Mascarò, M. and R. Seed. 2001. Foraging behavior of juvenile *Carcinus maenas* (L.) and *Cancer pagurus* (L.). Marine Biology 139: 1135-1145.

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

Newell, R.I.E., Alspach, G.S., Kennedy, V.S., and D. Jacobs. 2000. Mortality of a newly metamorphosed eastern oysters (*Crassostrea virginia*) in mesohaline Chesapeake Bay. Marine Biology 136: 665-676.

O'Connor, N.E., Grabowski, J.H., Ladwig, L.M., and J.F. Bruno. 2008. Simulated predator extinctions: predator identity affects survival and recruitment of oysters. Ecology 89: 428-438.

Parker, J.D., Montoya, J.P., and M.E. Hay. 2008. A specialist detritivore links *Spartina alterniflora* to salt mash food webs. Marine Ecology Progress Series 364: 87-95.

Paynter, K.T. and E.M. Burreson. 1991. Effects of *Perkinsus marinus* infection in the Eastern oyster, *Crassostrea virginica*: II. Disease development and impact on growth rate at different salinities. Journal of Shellfish Research 10: 425-431.

Potvin, C. and D.A. Roff. 1993. Distribution-free and robust statistical methods: viablealternatives to parametric statistics. Ecology 74: 1617-1628.

Ryan, E.P. 1956. Observations on the life histories and the distribution of the Xanthidae (mud crabs) of Chesapeake Bay. American Midland Naturalist 56: 138-162.

Seed, R. 1980. Predator-prey relationships between the mud crab *Panopeus herbstii*, the blue crab, *Callinectes sapidus* and the Atlantic ribbed mussel *Geukensi*a (=*Modiolus*) *demissa*. Estuarine and Coastal Marine Science 20: 445-458.

Steele, M.A. 1996. Effects of predators on reef fishes: separating cage artifacts from effects of predation. Journal of Experimental Marine Biology and Ecology 198: 249-267.

Tamburri, M.N., Zimmer, R.K., and C.A. Zimmer. 2007. Mechanisms reconciling gregarious larval settlement with adult cannibalism. Ecological Monographs 77: 255-68.

Tolley, S.G. and A.K. Volety. 2005. The role of oysters in habitat use of oyster reefs by resident fishes and decapods crustaceans. Journal of Shellfish Research 24: 100-1012.

Vanderklift, M.A. and Ponsard, S. 2003. Sources of variation in consumer-diet δ^{15} N enrichment: a meta-analysis. Oecologia 136: 169-182.

Wells, H.W. 1961. The fauna of oyster beds, with species reference to the salinity factor. Ecological Monographs 31: 239-266.

Werner, E. E. and S. D. Peacor. 2003. A review of trait-mediated indirect interactions in ecological communities. Ecology 84: 1083-1100

Wrona, A.B. 2004. Determining movement patterns and habitat use of blue crabs (*Callinectes sapidus* Rathbun) in a Georgia saltmarsh estuary with the use of ultrasonic telemetry and a geographic information system (GIS). Doctoral dissertation. University of Georgia, Athens, Georgia.

Zanden, M.J. and J.B. Rasmussen. 2001. Variation in δ^{15} N and δ^{13} C trophic fractionation: Implications for aquatic food web studies. Limnology and Oceanography 46: 2061-2066.

TEMPERATURE AS A DRIVER OF A SIZE-STRUCTURE SHIFT IN ZEBRA MUSSELS (*DREISSENA POLYMORPHA*) IN THE HUDSON RIVER

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

The introduction of zebra mussels to the Hudson River in 1991 caused strong impacts throughout the ecosystem. Since 2005, these ecosystem impacts have changed, likely as a result of a shift toward smaller-bodied mussels. Since this shift in size-structure has had a dramatic influence on the ecosystem, the cause of the size-structure is of interest in this project. Specifically, the role of temperature as a driver of the size-structure shift was studied using a combination of parameter estimations for a stage-structured matrix model based on long-term data, respiration differences among size classes at different temperatures (acute thermal effect), and the effect of increasing temperature on mortality (chronic thermal effect). The goal of this study was to test the following alternative hypotheses related to temperature: i) mortality increased in large mussels, favoring small-bodied mussels; ii) mortality increased in all size classes such that few mussels survive to a large size class; iii) high temperatures reduced growth rates, leading to smaller mussels. While the results from the analyses of the mechanism by which temperature affects size structure are not conclusive, they suggest that, based on the matrix model results and the acute thermal tolerance results, large mussels are not less thermally tolerant. Further, the experiments on chronic exposure to high temperatures indicated that there is a strong temperature effect on mussel survival, beginning at temperatures that frequently occur in the Hudson River during the summer. This means that high temperatures could be a significant source of mortality for zebra mussels in the Hudson River.

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INTRODUCTION

Zebra mussels (*Dreissena polymorpha*) first became abundant in the Hudson River in 1991, and their introduction led to dramatic changes to nearly every aspect of the ecosystem. The changes included an 80–90% reduction in phytoplankton (Caraco et al. 2006), a 70% reduction in zooplankton (Pace et al. 2010), a decrease in dissolved oxygen (Caraco et al. 2000), and increases in the deposition of organic matter (Roditi et al. 1997) and water transparency (Newell 2004). However, these impacts have not been constant over time. Zebra mussels exhibit strong cohort dynamics, which results in an oscillation between small- and large-bodied dominance in the population (Figure 1). When survivorship of adult mussels fell ~100 fold in 2005, there was a near elimination of large zebra mussels (Strayer et al. 2011) and zooplankton biomass recovered to pre-invasion levels by 2010, but phytoplankton biomass remained low (Pace et al. 2010). This suggests that the size-structure is a critical factor in the zebra mussels' impacts. This is expected because body size is important in many areas of ecology since it is closely related to physiological rates, interactions among organisms, and organisms' interactions with the environment. As a result, shifts in body size distributions often lead to shifts in ecological function

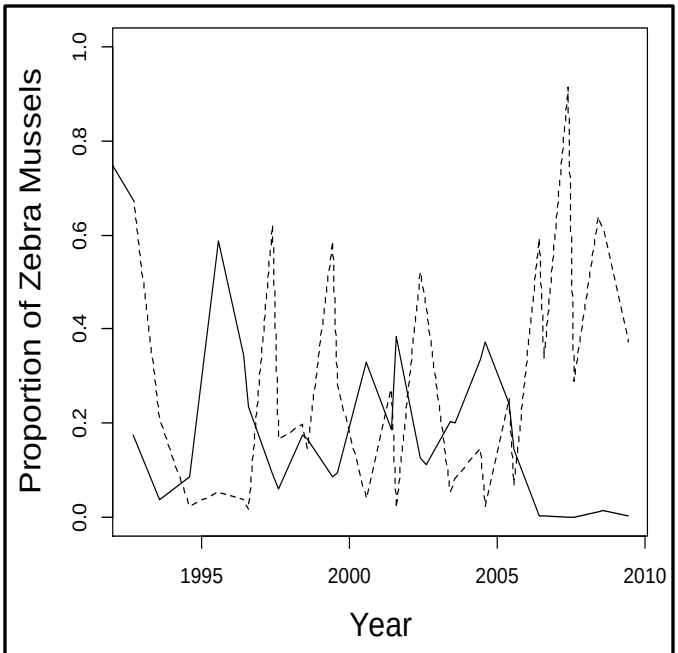

Figure 1: Proportion of zebra mussels belonging to the small (4–6 mm) size class (dashed line), and large (20–30 mm) size class (solid line) based on zebra mussel densities.

(Young 1996). Since changes in size-structure can dramatically alter ecosystem impacts, the cause of the shift toward small-bodied mussels is of interest.

The size-structure shift likely resulted from increased mortality, with an observed drop in survivorship of approximately 100-fold (Strayer et al. 2011). This increase in mortality has partially been attributed to blue crab (*Callinectes sapidus*) predation (Carlsson et al. 2011). Blue crabs migrate into the freshwater portion of the Hudson River during the summer, and Carlsson et al. (2011) tested whether blue crabs were the source of the increased mortality in the Hudson River using exclosure experiments. Higher mortality was observed where blue crabs were not excluded, indicating that blue crabs are a source of mortality for the zebra mussels. However, this did not entirely explain the change because mortality rates in both the control and experimental sites were higher than those previously observed from 1993 to 2008 (Carlsson et al. 2011). Further, higher mortality rates were observed in both the control and the exclosure before blue crabs arrived at the site. Another potential source of mortality is high temperature. Simulation and experimental evidence suggest that zebra mussels may be adversely impacted by warming temperatures and the Hudson is warming (Seekell and Pace 2011). Experiments have also provided evidence for decreased growth rates and increased mortality in large zebra mussels at high temperatures (Allen et al. 1999).

The effect of water temperature in well-mixed riverine systems like the Hudson, is likely more dramatic than in lake or reservoir systems where bottom-dwelling mussels experience lower temperatures. In the Hudson, when water temperatures exceed 25ºC, as they do in summer, even mussels at depth are exposed to this high temperature because of uniform temperatures over depth (Limburg et al. 1986). Further, the highest number of

days observed above threshold temperatures over the range 25 to 28 degrees all occurred in 2005, the same year as the size-structure shift in the zebra mussel population (Figure 2, data from USGS).

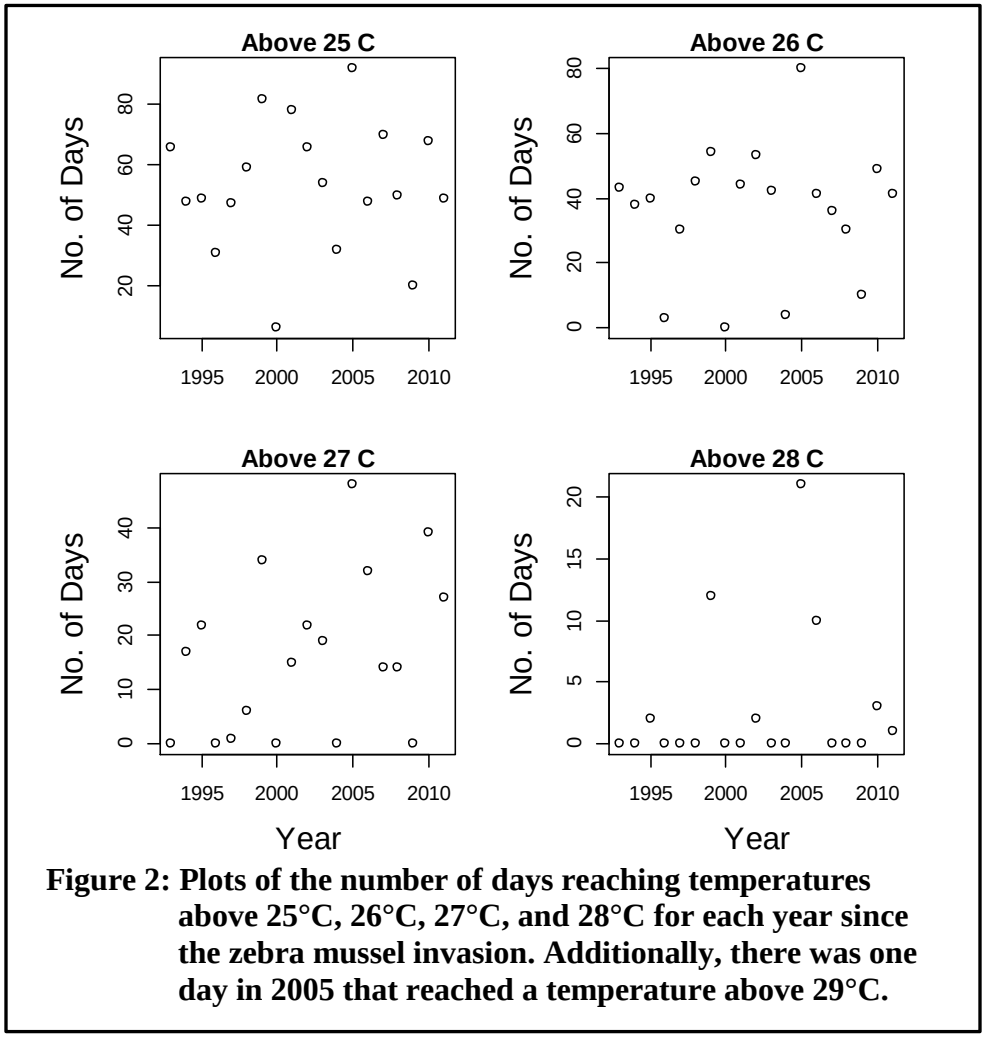

Figure 2: Plots of the number of days reaching temperatures above 25°C, 26°C, 27°C, and 28°C for each year since the zebra mussel invasion. Additionally, there was one day in 2005 that reached a temperature above 29°C.

This study provides a stage-structured matrix model to consider the parameters which most likely led to the observed change in dynamics based on long-term population data by testing the following alternative hypotheses related to temperature: i) mortality increased in large mussels, favoring small-bodied mussels; ii) mortality increased in all

size classes such that few mussels survive to a large size class; iii) high temperatures reduced growth rates, leading to smaller mussels. To experimentally test the first hypothesis, acute thermal stress differences among zebra mussel size classes were studied by comparing respiration rates of individual mussels at temperatures between 18 and 24°C. To test the temperature at which chronic effects on mortality would occur, the number of dead mussels were counted daily in tanks held at 18 (control), 25, 27, and 29°C.

METHODS

STUDY SITE AND ZEBRA MUSSEL SAMPLING

Zebra mussel population data was collected in the freshwater, tidal zone of the Hudson River, extending from Troy at river kilometer 248 (measured from the Battery in Manhattan) to Newburgh at river kilometer 100 (Figure 3). The Hudson's freshwater tidal reach is 900m wide and 8.3 m deep on average (Strayer and Malcolm 2006). The water is turbid, moderately hard, and nutrient rich (Strayer and Malcolm 2006). Water temperatures reach 25–28°C during the summer (based on USGS data). Since the Hudson River is well-mixed, temperature is generally uniform with depth (Limburg et al. 1986).

From 1993 to 2012, demographic data was collected on the zebra mussel populations by sampling 6–7 rocky sites throughout the Hudson River. These rocky sediments were sampled by collecting 10 rocks (15–40 cm in dimension) using a diver. In the laboratory, all mussels >2 mm long were removed from the rocks and counted. The area of each rock was estimated by tracing the outline of the rock. Subsamples of zebra mussels were saved for measurement of shell length (approximately 300 mussels

per site, when possible) to determine the size-structure of the population. Only rocky areas were used for demographic information because these samples contained a large enough number of mussels to study population demographics, and >75% of the population in the middle estuary is represented by rocky areas (Strayer and Malcolm 2006). Sampling was conducted and the resulting data was provided by David Strayer's laboratory at the Cary Institute of Ecosystems Studies. Data was provided in the format of estimates of the density of small (4–6 mm), medium (12–18 mm), and large (20–30 mm) zebra mussels each year for late June to early July, and mid-August to early September.

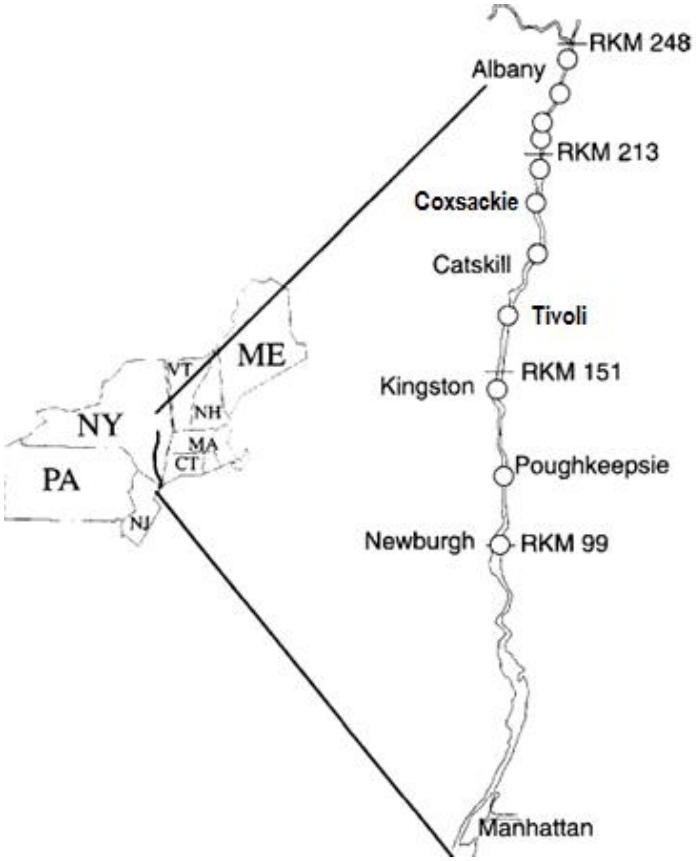

Figure 3: A map of sampling locations used to determine zebra mussel population size and size-structure (circles), and the two locations where experimental organisms were collected. Map is modified from Strayer and Smith 1996.

Zebra mussels used in laboratory studies were collected by divers in mid-June near Coxsackie, NY, and in mid-July near Tivoli, NY (Figure 3). Rocks with zebra

mussels attached were brought back to the laboratory, where the mussels were removed by cutting the byssal threads with a razor blade. The shells were cleaned using a toothbrush, and the mussels were placed in 10 gallon tanks with untreated well water, held at 18–19°C in a climate controlled room. The water was oxygenated using air stones and pumps in each tank. Mussels were fed 0.32g of green algae (*Chlorella* sp.) per 100 animals each day. The water in the holding tanks was changed one to two times per week. In temperature treatment tanks, 2L of water was removed from each tank daily and replaced with new untreated well water so as not to substantially alter the temperature.

POPULATION MATRIX MODEL

A stage-structured population matrix was developed to study which parameters most likely led to the observed change in dynamics. Parameters for the period before the size-structure shift (1993–2005) were compared to those after the size-structure shift (2006–2010). Long-term population size data from the Hudson River was used to fit the following three stage matrix model:

$$\begin{bmatrix} Xsm,\ t+1 \\ Xmd,\ t+1 \\ Xlg,\ t+1 \end{bmatrix} = \begin{bmatrix} (Fsm + Ssm - Csm) & (Fmd - Cmd) & (Flg - Clg) \\ (Gsm) & (Smd) & 0 \\ 0 & (Gmd) & (Slg) \end{bmatrix} \begin{bmatrix} Xsm,\ t \\ Xmd,\ t \\ Xlg,\ t \end{bmatrix},$$

where F is the fecundity (per mussel), S is the proportion of mussels remaining in same size class, C is the cannibalism of veligers (per mussel), G is the proportion of mussels growing to next size class, and X is the abundance of mussels in small (sm), medium (md), and large (lg) size classes. The fecundity minus cannibalism term will be referred to as "net fecundity." Parameter estimations were calculated in MATLAB (R2012a)

using a least squares method described in Caswell (2000). Since the changes in parameters could have been caused by factors other than temperature, temperature as the cause of the shifts in parameters was then explored experimentally.

OXYGEN CONSUMPTION AMONG SIZE CLASSES

To measure acute thermal stress differences among the zebra mussel size classes, oxygen consumption rates were measured on three individual mussels from each size class, and an empty control at 18, 22, 26, 30, and 34°C. Untreated tap water, which was filtered through a 0.2 μm filter and kept oxygenated with an air stone, was used during the experiments. Each mussel's shell was cleaned with a toothbrush, before it was placed in a 60 ml biological oxygen demand (BOD) bottle. An optical dissolved oxygen probe (YSI ProODO) was inserted into the BOD bottle, and the bottle was then placed into a water bath. The water in the bottle was allowed 15 minutes to reach the experimental temperature before data was collected. Oxygen consumption rates were determined either over a 2 hour period or until the animal expired, with the level of dissolved oxygen (mg O_2/L) recorded every 5 minutes. The slope of the least squares regression line for the level of dissolved oxygen over time was used to determine the oxygen consumption rate (mg O_2/L/hr). Oxygen consumption rates were averaged to create a plot for the three size classes at the five experimental temperatures. Metabolism speeds up with increasing temperature to a point, and then the animal begins shutting down its metabolic processes. The temperature at which the decline in oxygen consumption begins after the initial increase was taken as a measure of the thermal stress point. This thermal stress point was then compared among size classes.

ZEBRA MUSSEL SURVIVAL AT INCREASED TEMPERATURES

To study the effect of chronic exposure to high temperatures, mussel survival was monitored in tanks with experimental temperatures of 18 (control), 25, 27, and 29°C. Zebra mussels were brought to the experimental temperatures by raising the water temperature by 1°C per day using an aquarium heater. Tanks were observed daily and dead mussels were removed. This experiment was conducted twice, first using 20 medium mussels per tank, and then using 40 medium mussels per tank.

RESULTS

The parameter estimations based on long-term zebra mussel size data showed a decrease in survival and growth parameters for medium- and large-bodied mussels (Table 1). Medium mussels went from an estimate of 74% surviving without growing into the next size class, to 0%, and large mussels went from 51% surviving to 30% surviving. Growth from the medium size class to the large size class also decreased from 6% to 0%. The results also showed a decrease in net fecundity for medium mussels by 0.13 veligers/mussel, but an increase for large mussels by 10.44 veligers/mussel. For small-bodied mussels, net fecundity plus survival, and growth parameters both increased. Related to the hypotheses, these results show a decrease in growth and survival for medium and large mussels, but an increase in both for small mussels.

Parameter	1993-2005	2006-2010	Change
$F_{sm}+ S_{sm}- C_{sm}$	0.00	0.06	0.06
G_{sm}	0.00	0.49	0.49
$F_{md} - C_{md}$	0.13	0.00	-0.13
S_{md}	0.74	0.00	-0.74
G_{md}	0.06	0.00	-0.06
$F_{lg} - C_{lg}$	0.33	10.77	10.44
S_{lg}	0.51	0.30	-0.21

Table 1: Parameter estimations for the 3x3 stage-structured population matrix, where F is the fecundity (per mussel), S is the proportion of mussels remaining in same size class, C is the cannibalism of veligers (per mussel), and G is the proportion of mussels growing to next size class.

The oxygen consumption rate comparison among size classes at 18, 22, 26, 30, and 34°C did not provide support for the hypothesis that large mussels are more adversely affected by high temperatures than smaller mussels. In fact, it appears that small mussels are less thermally tolerant, with the thermal stress point where oxygen consumption begins to decline after initial increase occurring at 22°C in small mussels, and at 26° in large mussels (Figure 4).

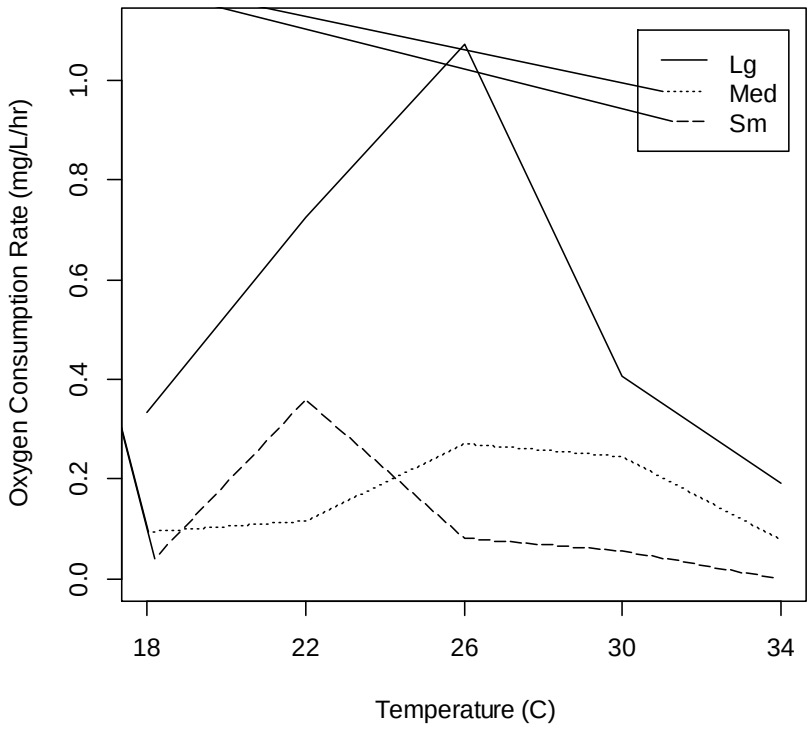

Figure 4: Average oxygen consumption rate at five experimental temperatures for each size class.

Mortality rates for medium mussels at 18, 25, 27, and 29°C could not be compared because too few mussels remained by the time the tanks reached their experimental temperatures. For example, only 3 of the 60 individuals survived to 29°C. The proportion of mussels surviving as the temperature was raised by 1°C per day shows a rapid decrease in survival between 23 and 25°C, while the reference tank (held at 18°C) experienced a very high survival rate (Figure 5).

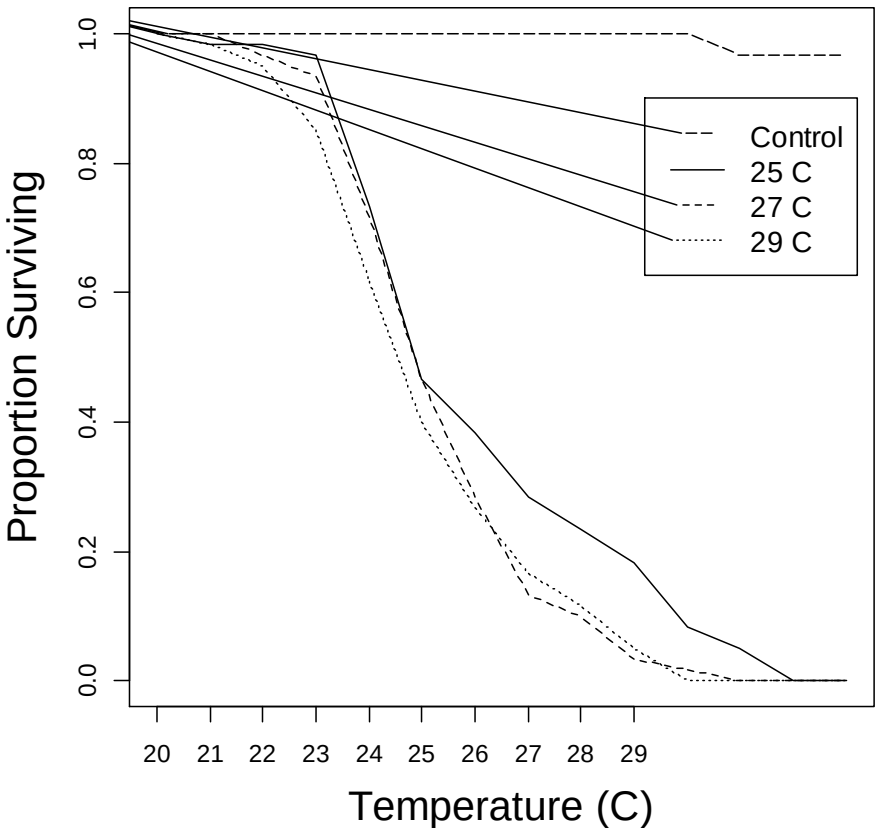

Figure 5: A plot of the proportion of mussels surviving as the temperature was raised 1°C per day to the experimental temperature. After the experimental temperature was reached, the tank was kept at that temperature for each day as the other tanks continued to be raised to their experimental temperatures. The control tanks were kept at 18°C on all days.

DISCUSSION

The results from these analyses were not conclusive in determining the most likely mechanism by which temperature leads to a shift in size-structure. The results do suggest that it is unlikely that large mussels are less thermally tolerant based on the matrix model results and the acute thermal tolerance results. However, the changes in parameter estimates from before the size-structure shift to after do not all seem reasonable. For example, none of the parameters are likely to be zero in either time period, and it seems unlikely that net fecundity would decrease for medium mussels (by

0.13 veligers/mussel), but increase in large mussels (by 10.44 veligers/mussel). The parameter estimation may be improved in the future by including biologically relevant constraints, and using maximum likelihood methods instead of a least squares approach. Studying the nonlinear dynamics of the model would also provide insight into how shifts in parameters could lead to different size-structures.

The acute thermal tolerance results suggest that small mussels are less thermally tolerant than large mussels, but since the experiment only focused on respiration, conclusions are limited because there are other physiological parameters relevant to thermal stress. These results do motivate further experiments using a scope for growth (SFG) approach. SFG provides a measure of an organism's stress by measuring the energy acquisition (feeding and digestion) against energy expenditure (metabolism and excretion) (Widdows et al. 1995). Further work using this approach would be valuable.

The experiments on chronic exposure to high temperatures indicated that there is a strong temperature effect on mussel survival, beginning at temperatures much lower than expected. The effect of temperature on mortality became clear between 23 and 25°C, temperatures that the Hudson River frequently reaches during the summer. Even mussels at depth are exposed to these temperatures because the Hudson River is well-mixed with uniform temperatures over depth. Therefore, high temperatures could be a significant source of mortality for zebra mussels in the Hudson River.

ACKNOWLEDGEMENTS

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REFERENCES

Allen, Y.C., B.A. Thompson and C.W. Ramcharan. 1999. Growth and mortality rates of the zebra mussel, Dreissena polymorpha, in the Lower Mississippi River. Canadian Journal of Fisheries and Aquatic Sciences 56:748–749.

Caraco, N.F., J.J. Cole, S.E.G. Findlay, D.T. Fischer, G.G. Lampman, M.L. Pace and D.L. Strayer. 2000. Dissolved oxygen declines in the Hudson River associated with the invasion of the zebra mussel (*Dreissena polymorpha*). Environmental Science and Technology 34: 1204-1210.

Caraco, N.F., J.J. Cole and D.L. Strayer. 2006. Top down control from the bottom: Regulation of eutrophication in a large river by benthic grazing. Limnology and Oceanography 51: 664-670.

Carlsson, N.O.L., H. Bustamante, D.L. Strayer and M.L. Pace. 2011. Biotic resistance on the increase: native predators structure invasive zebra mussel populations. Freshwater Biology 56:1630–1637.

Caswell, H. 2000. Matrix population models: Construction, analysis, and interpretation. Sinauer Associates, Inc., Sunderland, MA. Second edition.

Limburg, K. E., M. A. Moran, and W. H. McDowell. 1986. The Hudson River ecosystem. Springer Verlag, New York

New York, USAMATLAB version 7.14.0.739. 2012. (computer software), The MathWorks Inc., Natick, Massachusetts.

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

Pace, M.L. D.L. Strayer, D. Fischer and H.M. Malcom. 2010. Recovery of native zooplankton associated with increased mortality of an invasive mussel. Ecosphere 1(1):art3. doi:10.1890/ES10-00002.1.

Roditi, H.A., D.L. Strayer and S.E.G. Findlay. 1997. Characteristics of zebra mussel (*Dreissena polymorpha*) biodeposits in a tidal freshwater estuary. Archiv für Hydrobiologie 140: 207-219.

Seekell, D.A. and M.L. Pace. 2011. Climate change drives warming in the Hudson River Estuary, New York (USA). Journal of Environmental Monitoring 13: 2321–2327.

Strayer, D.L., N. Cid and H.M. Malcom. 2011. Long-term changes in a population of an invasive bivalve and its effects. Oecologia 165: 1063-1072.

Strayer, D.L. and H.M. Malcom. 2006. Long-term demography of a zebra mussel (*Dreissena polymorpha*) population. Freshwater Biology 51: 117-130.

Widdows, J., P. Donkin, M.D. Brinsley, S.V. Evans, P.N. Salkeld, A. Franklin, R.J. Law and M.J. Waldock. 1995. Scope for growth and contaminant levels in North Sea mussels *Mytilus edulis*. Marine Ecology Progress Series, 127: 131–148

Young, B.L. 1996. The importance of size-frequency relationship for predicting ecological impact of zebra mussel populations. Hydrobiologia 332: 151.

THE IMPACTS OF THE ZEBRA MUSSEL (*DREISSENA POLYMORPHA*) ON THE FEEDING ECOLOGY OF EARLY LIFE STAGE STRIPED BASS (*MORONE SAXATILIS*)

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

Despite numerous studies of the ecological effects of the zebra mussel (*Dreissena polymorpha*) invasion in the Hudson River Estuary, the impacts on larval and juvenile fishes have been poorly characterized. In this study, changes in early life stage fish diets upon invasion of the zebra mussel were analyzed, focusing on the striped bass (*Morone saxatilis*). Changes in prey diversity, frequency of prevalent prey items, and a prey habitat index from 1988 (before the mussels arrived), to 2008 were quantified. Sample years bracketed a period of increasing mussel impacts, followed by a period of apparent ecosystem recovery. For the striped bass, prey diversity increased during peak invasion years and then declined in 2008. A similar trend was seen with the frequency of prevalent prey. After they arrived, zebra mussels became one of the main components of the diet. Over time, bass fed increasingly on benthic prey rather than pelagic prey. Overall, the zebra mussel has changed many aspects of the striped bass diet, some in surprising ways, and although some of those aspects are returning to their pre-invasion condition, others are remaining the same as they were during peak invasion years.

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INTRODUCTION

Invasive species have a remarkable ability to alter the environment into which they are introduced. The zebra mussel (*Dreissena polymorpha*) is an invasive bivalve from Eurasia that has thrived outside of its native range. Zebra mussels first arrived in the Great Lakes region of the United States in the mid 1980s. The mussels have since spread rapidly throughout the freshwater systems of the eastern half of the country. By 1991, zebra mussels had reached the northernmost point of the Hudson River Estuary, the Federal Lock and Dam at Troy, and have since become well established throughout the freshwater tidal length of the river (Strayer and Malcom 2006).

After arriving in the Hudson, zebra mussels began to change both the abiotic and biotic components of the ecosystem. The benthic substrate of the freshwater tidal Hudson was once dominated by mud and sand, but as the mussels have spread the substrate is now dominated by the mussels' hard shells (Strayer 2009). Zebra mussels are highly efficient filter feeders that feed on a variety of freshwater organisms depending upon their size. Small mussels feed primarily on phytoplankton, while larger mussels can consume both phytoplankton and small zooplankton (Pace et al. 2010). By 1992, zebra mussels had caused an 80-90% decline in phytoplankton biomass (Pace et al. 1998). Declines in phytoplankton led to increases in water clarity and nutrient levels, including nitrogen and phosphorous (Strayer 2009). All microzooplankton groups in the freshwater tidal portion of the Hudson River declined after the zebra mussel invasion, and an overall 70% decline in zooplankton biomass was seen by 1995 (Pace et al. 1998). These declines in primary consumers were likely caused by both bottom-up food web effects from the

phytoplankton decline as well as the direct consumption of zooplankton by larger zebra mussels (Pace et al. 1998; Strayer et al. 2011).

Despite the huge changes that have occurred in the Hudson River Estuary since the zebra mussels' arrival, the ecosystem has shown signs of recovery. In recent years, the Hudson has seen declines in zebra mussel population density, filtration rate, body size, and annual survivorship in the river (Strayer et al. 2011). It is thought that these declines could be driven by natural predators, such as blue crabs, in the river utilizing zebra mussels as a food source (Carlsson et al. 2011), leading to a recovery in primary consumer abundance (Pace et al. 2010).

Many of the impacts of the zebra mussel are poorly understood due to the difficulties associated with studies covering large spatial scales and because many of the variables are difficult to measure and analyze (Strayer 2009). One of these poorly understood areas is the impact that zebra mussels have had on the secondary consumers of the Hudson, particularly early life stage fish species. The Hudson River estuary serves as an important nursery habitat for a variety of larval and juvenile fish species, and a decline in phytoplankton could continue to translate up the food web to the fish that live in the river. An extensive study of both pelagic and littoral larval and juvenile fish in the Hudson River Estuary found that pelagic species have declined in population size and exhibited slower growth rates since the arrival of the zebra mussel, while littoral species were relatively unaffected (Strayer et al. 2004). Early life stage pelagic fish are dependent upon their food source of pelagic primary consumers to grow and thrive. Presumably, the decline in pelagic food sources caused these fish to begin foraging in the benthos, where the populations of primary consumers were less affected. Being less

successful at obtaining food in these new foraging habitats could cause declines in populations and growth rates. Although it is assumed that the declines in early life stage fish were caused by the declines in their pelagic food source, no studies have been done to observe how the diet and feeding ecology of these fish has changed. This study focuses on changes in the diet of the early life stages of one Hudson River Estuary pelagic fish species, the striped bass (*Morone saxatilis*), over a twenty year time period that spans from before the zebra mussels arrived in the river until the present.

The ability of striped bass populations to maintain stable population abundances, distributions, and apparent growth rates, may indicate that they were able to successfully change their diets in response to changes made by zebra mussels. Striped bass are known to be generalist predators and may have been better able to adapt to changes in prey availability when compared to the other pelagic fish species in the study by Strayer et al. 2004.

It is predicted that as striped bass forage for unfamiliar prey items during the transition from pelagic to benthic feeding the diversity of their prey will increase over time. The most abundant prey items should also change over time due to the presumed pelagic to benthic feeding shift. In the most recent study year, results could be similar to those found before the zebra mussel invasion due to the observed recovery of zooplankton in the Hudson River.

METHODS

Year and Fish Selection:

All of the fish used in this study were provided by Hudson River Utilities annual survey of fish populations in the Hudson River, and methods for the survey can be found in the annual Year Class Report for the Hudson River Estuary Monitoring Program (ASA Analysis and Communication 2001). Fish were caught in the river during a 20 year period spanning across the zebra mussel invasion. 1988 was selected as the initial year of the study to show what fish diets were like before the zebra mussels arrived in the river. The years 1995, 1999, and 2008 were selected to represent years during peak zebra mussel invasion and the present day. Fish were preserved in formalin and were identified to species by Normandeau Associates in Bedford, New Hampshire. The fish that were caught in 1988 were transported to the ichthyology collection at the New York State Museum in Albany, New York where they were transferred from formalin to 70% ethanol and their species identifications were confirmed. The fish from all other years remained in the storage facilities of Normandeau Associates. Fish from 1995, 1999, and 2008 were obtained from Normandeau's facilities in Bedford. The fish from 1988 were obtained from the New York State Museum in Albany.

Fish were selected for dissection based on a variety of factors including their size, the condition of their preservation, confirmation of their species identification, and where they were caught in the river. Fish were excluded if they were dried out due to evaporation of the formalin they were preserved in, or if their bodies had been otherwise damaged. Fish needed to be large enough to dissect with 2 mm cutting surface spring

dissecting scissors, and most fish were less than 50 mm long. Once a fish was selected to be dissected, it was confirmed that it was a striped bass by counting the number of anal fin rays it possessed. A striped bass should possess 13-14 anal fin rays as opposed to the white perch, which has 12 anal fin rays (Waldman et al. 1999). Using this external character to distinguish between striped bass and white perch was found to be 96% accurate when the fish were 8.0 mm and larger (Waldman et al. 1999). All of the bass selected came from the freshwater tidal length of the river between river kilometer 100 and river kilometer 248.

Dissection and Identification:

Once a striped bass was selected, it was prepared for dissection and then carefully dissected to mitigate specimen damage. Each bass was assigned a unique sample number, its standard length was measured, and it was weighed in a sealed container filled with water. The fish was dissected under a dissecting scope using 2 mm or 4 mm spring dissecting scissors. The fish was first cut from the vent up towards the lateral line and then across to the operculum. A second cut was then made through the pectoral girdle and the operculum angled up towards the eye. Finally, a cut was made through the operculum connecting the first and second cuts. This allowed the esophagus, stomach, and intestines to be easily removed from the body cavity with forceps. Once removed from the body cavity, the intestines and any part of the gill basket that may have been extracted with the stomach were snipped off with the dissecting scissors. Any fat bodies that were still attached to the stomach were removed with forceps.

After the stomach was removed and cleared of any fat, the gut contents were removed. To do this, a cut was made from the esophagus to the end of the stomach. The stomach was then spread open and its contents were removed with a pipette. The contents of the stomach were preserved in 70% ethanol in microcentrifuge tubes marked with the fish's sample number. This procedure was followed for thirty striped bass from each year for a total of 120 fish. Stomach contents were identified on a Sedgewick-Rafter gridded counting slide under a compound light microscope. Each prey item was counted and identified to the lowest taxonomic level possible using Peckarsky et al.'s Freshwater Macroinvertebrates of Northeastern North America (1990) and the University of New Hampshire's Image-Based Key to the Zooplankton of the Northeast (USA) (Haney et al. 2010). Some prey items that could be identified to taxon were noted for presence but not counted individually because it was likely that they were ingested in conjunction with another prey item (i.e. copepod eggs and spermatophores).

Data analysis:

Changes in prey diversity over time were determined using the Shannon-Wiener Diversity Index:

$$\mathrm{H} = (-\sum_{i=1}^{n} p_i \log p_i)/\mathrm{S}$$

where p_i is the number of individuals for species number i divided by the total number of individuals, and S is the species richness of the sample (Shannon 1948). The length of each fish was log transformed and an analysis of covariance (ANCOVA) was conducted [using SAS version 9.3] for the two main effects of length and year as well as the

interaction effect of length-by-year. If the interaction effect of length by year was found to be not significant, it was dropped and only the two main effects were run. A least-squares-means estimation was used to correct for the effect of variation in fish size on the diversity of prey present in each year. The length-corrected least squares mean of diversity was graphed for each year to show how prey diversity changed over time. A bar graph was generated to demonstrate the overall diversity present in the striped bass diet throughout the entire study. The abundance of each prey item was log transformed to enhance the presence of prey items present in low abundance and diminish the presence of prey items present in high abundance so that prey diversity could be better visualized.

To determine the changes in predominant prey items, the four prey items that were present in the highest numbers in the fish diet were determined. The decision to look at the four most abundant prey items was arbitrary. The total number of each prey item present in an individual fish and the length of the fish were then log transformed. An ANCOVA was conducted on the incidence of each one of the predominant prey items. The two main effects of length and year were tested as well as the interaction effect of length by year. If length by year was not significant, it was dropped and only the two main effects were run. A least squares means estimation was run in SAS to correct for the effect of fish size. The logarithm of frequency of prey items for each year was then plotted with the error for the least squares means.

To determine the degree to which the fish for each year were feeding pelagically or benthically, a weighted average was used. For each fish, the total number of each prey item was multiplied by two if the prey item lived in the benthos or by four if the prey

item lived in the pelagic zone. These values were then totaled and divided by the total number of prey items found within that fish. The resulting value was termed the fish's prey habitat index (PHI). This method is a modified version of the trophic level equation used by Pauly and Palomeres (2000) and Stergiou and Karpouzi (2002). Each fish's PHI within a specific year was then averaged together to achieve an overall PHI for that year. The changes in the yearly PHIs can then be compared to determine how the feeding habitats of the fish have changed over time. PHIs closer to four indicate that the fish are feeding mostly pelagically, while PHIs closer to two indicate that the fish are feeding mostly benthically. The PHIs obtained for each year were plotted with standard errors.

RESULTS

Prey Diversity:

Copepods were the most dominant prey item, which is best demonstrated by the non-log-transformed frequencies in Figure 1. A log-transformation reveals that the most prevalent prey items in the diet were copepods, amphipods, zebra mussels and *Leptodora sp.* (Figure 2). Items classified as "Other" included items that could not be attributed to a specific organism such as eggs, dismembered arthropod legs, and small worms that could not be identified to a taxon. These items occurred rarely and often only within a single fish. Although some amphipods, isopods, and copepods could be identified to more specific levels of classification, many of the finer details of the organisms were damaged or lost due to ingestion by the bass and the preservation process. In order to better demonstrate the portion of the diet contributed by each of these groups, organisms that

could be identified to higher taxonomic levels were combined with the less specific group.

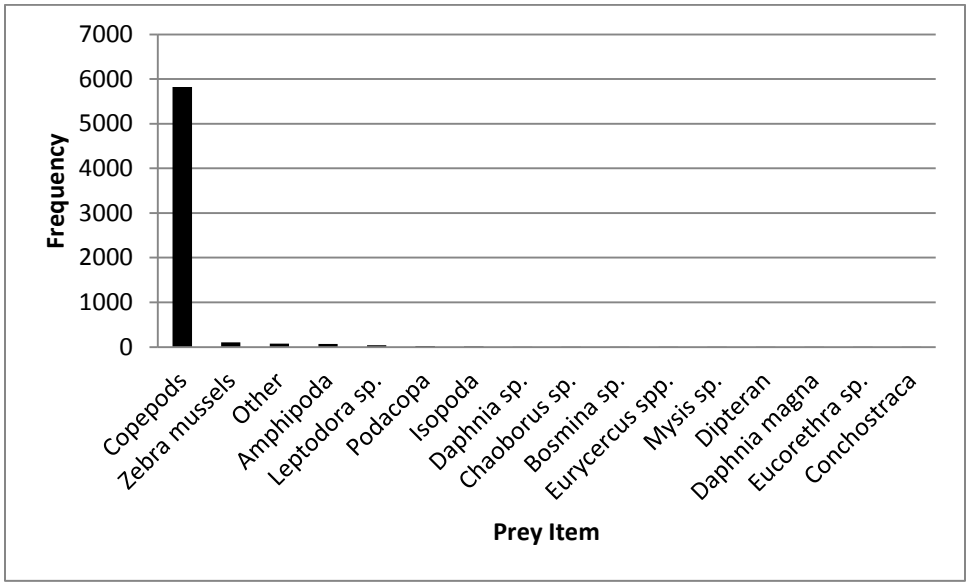

Figure 1. The frequency of prey items found in the striped bass diet across all four years (1988, 1995, 1999, and 2008) without log transformation illustrates the dominance of copepods in the diet.

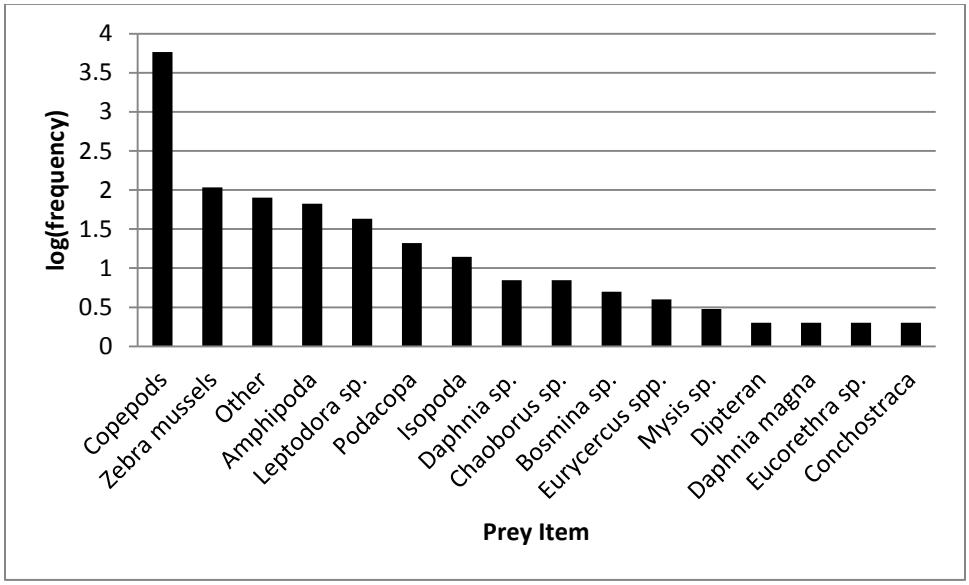

Figure 2. After a log transformation, the contribution of each prey item to the makeup of the striped bass diet across all years four becomes clearer.

The Shannon-Wiener diversity index showed that diversity was higher during the years of peak mussel invasion, 1995 and 1999, and then declined in 2008. In the first ANCOVA that was run, the effect of length by year was found to be not significant with a p-value of 0.3038. The reduced model showed that the year effect had a p-value of less than .0001 and the length effect had a p-value of 0.0690 (Table 1). Diversity in the diet significantly increased between 1988 and 1995, remained the same in 1999, and then declined significantly in 2008 to levels lower than in 1988 (Figure 3). These changes in diversity appear to have been strongly driven by changes in species number rather than changes species evenness.

Table 1. ANCOVA for changes in the Shannon-Wiener diversity index by year and length.

Source	DF	Type III SS	Mean Square	F	Pr > F
Year	3	0.758	0.253	7.84	<.0001
Length	1	0.109	0.109	3.37	0.0690
Error	115	3.71	0.0322		

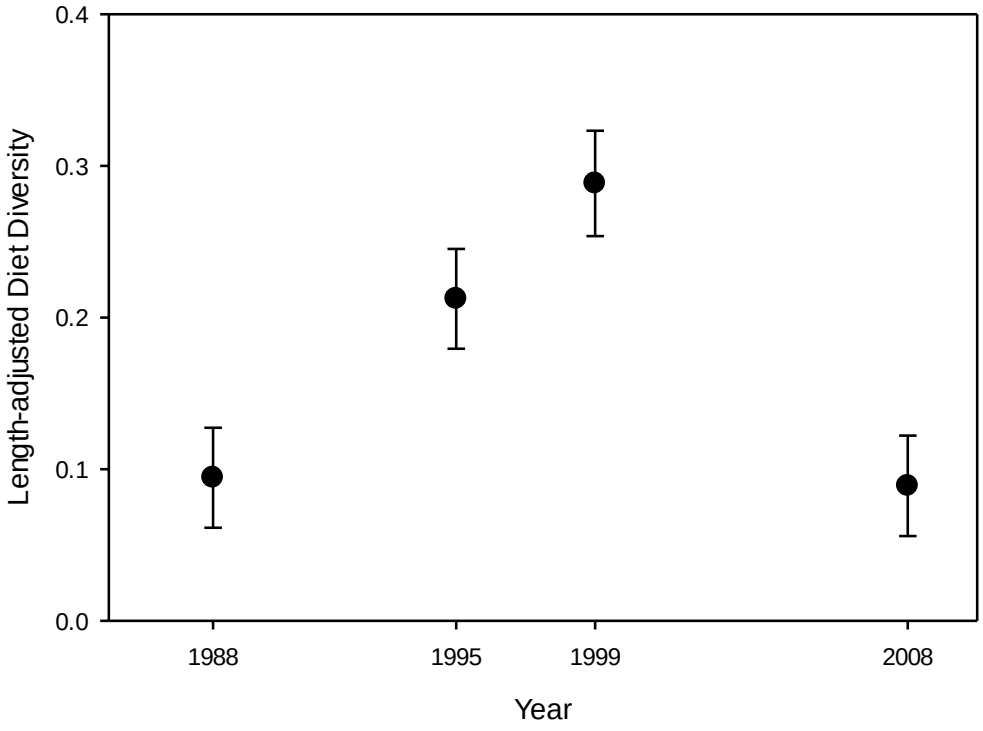

Figure 3. The changes in the Shannon-Wiener diversity index over time show that there was a significant increase in prey diversity between 1988 and 1995. A significant decrease in prey diversity between 1999 and 2008 was also seen.

Prevalent Prey Items:

An analysis of the prey diversity showed that the most prevalent prey items were copepods, amphipods, zebra mussels, and *Leptodora sp*. The abundance of each prey item varied from year to year (Table 2 a-d). Figure 4 shows that for each prey item the size adjusted log transformed frequency in the diet increases from 1988 to 1995 and then declines from 1999 to 2008. This trend is most pronounced in the copepods. Zebra mussels were not present in the diet in 1988, but appeared in striped bass stomachs in 1995 and remained present in the diet through 2008. Generally, as fish length increased the number of prey items within each fish also increased (Figure 5). This length effect

was significant for each prey item except copepods (Table 2a). The length effect varied among years for only one prey species, *Leptodora* (Table 2d). In larger fish, the number of prey items seems to decrease with length. This could be due to the low sample size of larger fish or because the fish are able to eat larger prey items and thus consume fewer individuals. *Leptodora sp.* was the only prey item in which there was a significant interaction effect of length by year.

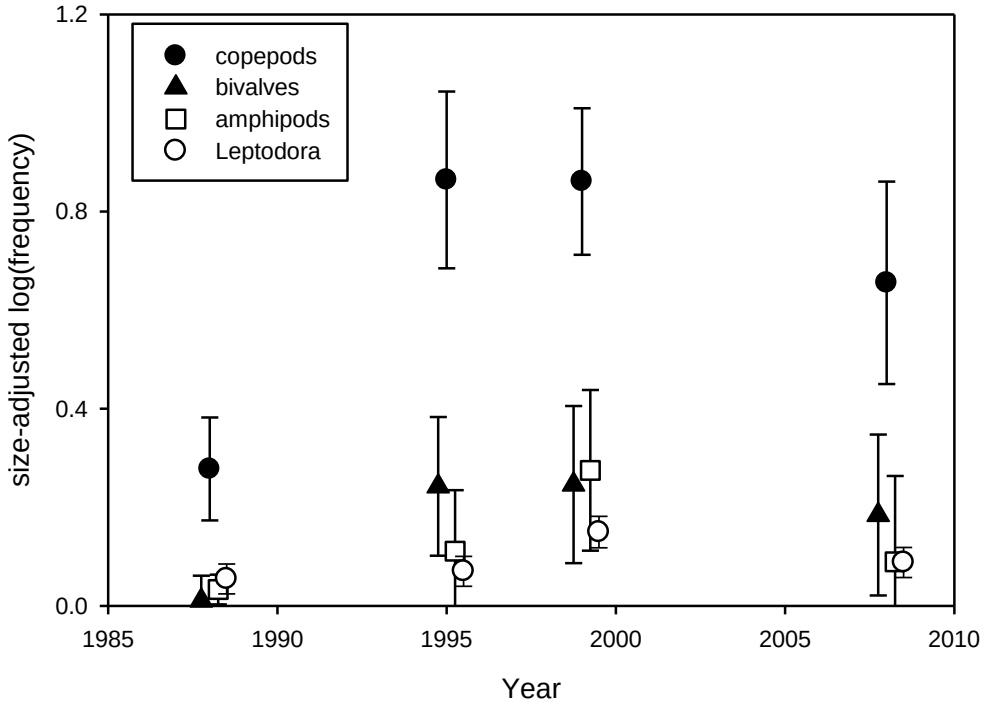

Figure 4. Changes in the size adjusted log transformed frequency of the most prevalent prey items (copepods, zebra mussels, amphipods, and *Leptodora sp.*) in the diet over time.

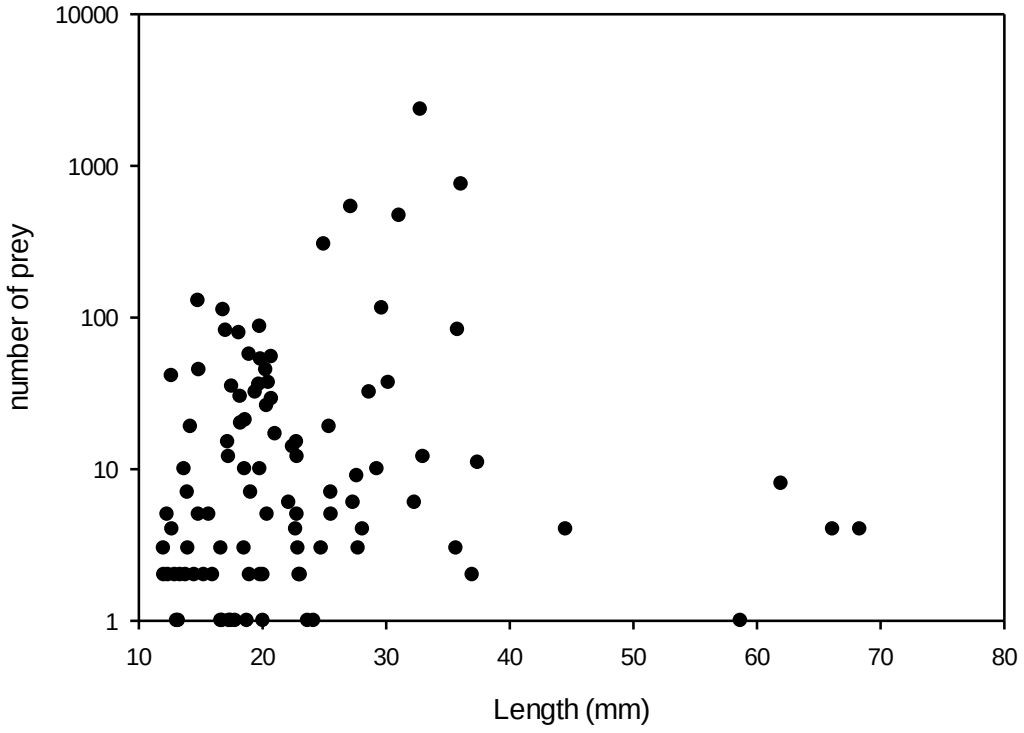

Figure 5. The number of prey items in a fish generally increased until the fish was around 40 mm in length and then decreased.

Table 2. ANCOVA tables for each prevalent prey item: a) copepods b) amphipods c) zebra mussels e) *Leptodora sp*.

a) Copepods

Source	DF	Type III SS	Mean Square	F	Pr > F
Year	3	6.62	2.21	3.78	0.0125
Length	1	0.236	0.236	0.4	0.526
Error	115	67.2	0.584		

b) Amphipods

Source	DF	Type III SS	Mean Square	F	Pr > F
Year	3	0.846	0.282	9.62	<.0001
Length	1	0.425	0.425	14.5	0.0002
Error	115	3.37	0.0293		

c) Zebra Mussels

Source	DF	Type III SS	Mean Square	F	Pr > F
Year	3	1.07	0.356	6.01	0.0008
Length	1	0.276	0.276	4.66	0.0330
Error	115	6.82	0.0593		

d) *Leptodora sp.*

Source	DF	Type III SS	Mean Square	F	Pr > F
Year	3	0.345	0.115	4.65	0.0042
Length	1	0.0711	0.0711	2.87	0.0928
Length by Year	3	0.369	0.123	4.97	0.0028
Error	112	2.77	0.0247		

Prey Habitat Index:

The PHI was used to determine the degree to which bass were feeding pelagically or benthically. The PHI in 1988 was nearly four, meaning that striped bass were feeding almost entirely pelagically. In subsequent years, the PHI steadily declined towards two,

but never fell below three. This indicates that the fish never fed entirely benthically, but the proportion of their diet that came from the benthos significantly increased as indicated in Figure 6. An ANOVA showed that the effect of year on PHI was significant (Table 3). The benthic and pelagic scoring of each prey item can be found in Table 4.

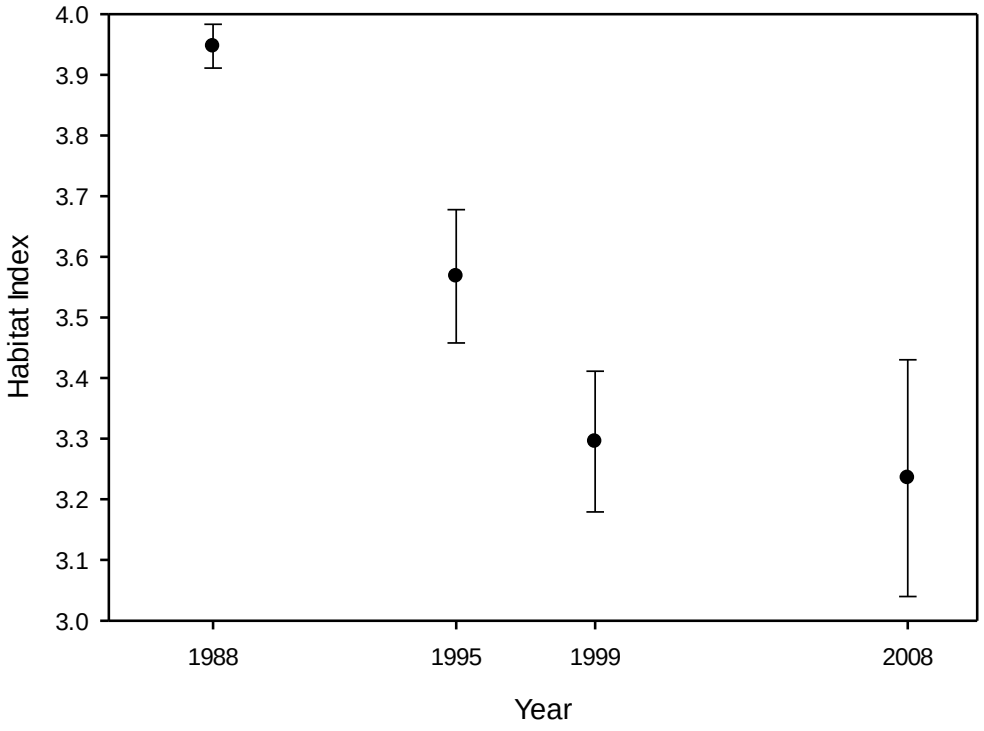

Figure 6. The PHI over time shows a steady decline in value from 4 (pelagic feeding) towards 2 (benthic feeding) as striped bass diets adjusted to zebra mussel induced ecosystem changes. PHI was measured on a scale from two to four where two represented feeding entirely on prey items from the benthos and four represented feeding entirely on prey items from the pelagic zone.

Table 3. ANOVA for changes in PHI by year.

Source	DF	Type III SS	Mean Square	F	Pr > F
year	3	6.58	2.19	5.59	0.0014
error	93	36.4	0.392		

Table 4. List of every identifiable prey item in the striped bass diet with their habitat type (benthic or pelagic) and value that was assigned to them for the PHI calculation.

Prey Item	Habitat	Value
Daphnia sp.	pelagic	4
Copepod - Cyclopoida	pelagic	4
Crustacea - Podacopa	pelagic	4
Crustacea - Conchostraca	pelagic	4
Copepod	pelagic	4
Chaoboridae - *Chaoborus*	pelagic	4
Amphipoda	benthic	2
Amphipoda - *Gammarus*	benthic	2
Amphipoda - *Pontoporeia affinis*	benthic	2
Amphipoda - *Hyalella*	benthic	2
Leptodora kindtii	pelagic	4
Dipteran	pelagic	4
Copepod - Calanoida	pelagic	4
Bosmina sp.	pelagic	4
Copepod nauplii	pelagic	4
Daphnia magna	pelagic	4
Zebra Mussel	benthic	2
Isopoda - *Lirceus*	benthic	2
Isopoda - *Caecidotea*	benthic	2
Mysid - *Mysis sp.*	benthic	2
Chaoboridae - *Eucorethra*	pelagic	4
Eurycercus spp.	pelagic	4
Isopoda	benthic	2

DISCUSSION

In the years following the zebra mussel invasion, prey diversity significantly increased, the abundance of prevalent prey items significantly increased, and prey habitat index declined indicating a transition from pelagic feeding to benthic feeding in early life stage striped bass. Prey diversity then declined in 2008 to a level similar to what was seen in 1988. In 2008, the abundance of each prevalent prey item also declined. No

recovery was seen in the 2008 prey habitat index, indicating that although there were some signs of diet recovery, striped bass were still feeding more benthically than pelagically. The changes in prey diversity and PHI during peak invasion years were consistent with how the diet was expected to change, while changes in the abundance of prevalent prey items as well as the lack of PHI recovery in later years were not expected.

In accordance with the hypothesis, prey diversity in the diet increased after the arrival of zebra mussels in the river. This was most likely because the documented decline in pelagic prey items in the river (Pace et al. 1998; Pace et al. 2010; Strayer et al. 2011) forced striped bass to search in new places for food and resulted in the ingestion of new prey items. In 2008, the Shannon-Wiener diversity index returned to a value similar to what was seen in 1988, consistent with the ecosystem recovery reported by Pace et al. (2010). Overall, the results for diversity changes in the striped bass diet corresponded with the predicted response for a generalist predator.

Abundance of prevalent pelagic prey items actually increased during peak zebra mussel invasion years, rather than decreasing as was hypothesized. Pelagic copepods were the most abundant of all of the most prevalent prey items and were primarily from the order Calanoida. The dramatic increase in the number of copepods that were present in the diet between 1988 and 1995 could be explained by Pace et al.'s (1998) study of zooplankton in the Hudson River. Copepods were one of the groups that were least affected by the zebra mussel invasion and they maintained pre-invasion population abundances and dynamics through 1995. This could mean that striped bass consumed more copepods during peak invasion years because they were one of the only food sources left in the pelagic zone. Most amphipods were too damaged to classify more

specifically, but of those that were, many were *Gammarus sp*. As benthic invertebrates, the increased presence of amphipods in the striped bass diets during peak invasion years supports the hypothesis that the bass would be feeding more heavily on benthic prey items.

There were no obvious changes in the patterns of prey use after the arrival of zebra mussels, contrary to what was expected. Copepods, amphipods, and *Leptodora sp.* were all present in the diet of the 1988 striped bass. The most interesting addition to the bass diet after the zebra mussels arrived in the river was the zebra mussels themselves. It has not yet been definitively confirmed that the bivalves present in the striped bass stomachs are zebra mussels; however, there are several factors that indicate that this is a safe assumption. The mussels did not appear in the striped bass diet in the 1988 fish, but were present frequently and abundantly in fish from 1995, 1999, and 2008. In addition, zebra mussels have been found in the stomachs of larval white perch (*Morone americana*), American shad (*Alosa sapidissima*), and alewife (*Alosa pseudoharengus*) from the Hudson River (K. Limburg SUNY ESF pers. comm. 2012). It would be logical to then conclude that striped bass could also utilize zebra mussels as a food source. A positive identification has been precluded thus far due to the absence of the characteristic zebra stripes on the mussel, possibly due to discoloration during the preservation process or because the mussels themselves were not mature enough to possess their stripes. Many of the mussels that were observed had byssal threads, meaning that they had already settled to the bottom of the river where the bass then consumed them. Some of the natural predators in the Hudson River Estuary, particularly blue crabs and larval white perch, have begun to utilize zebra mussels as a food source, which may explain a decline

in zebra mussel size and population density in recent years (Carlsson et al. 2011; Strayer et al. 2011; K. Limburg SUNY ESF pers. comm. 2012). The ability of striped bass to utilize zebra mussels as a food source, and have them contribute to such a large proportion of the diet, may explain why the bass were able to maintain their population sizes and growth rates despite the drastic changes that were occurring in the Hudson River.

As expected, the striped bass were feeding pelagically in 1988, before the zebra mussels arrived in the Hudson River, and then began to feed increasingly in benthic habitats after zebra mussels had spread throughout the length of the river. Although the amount of benthic organisms present in the diet did increase in 1995, 1999, and 2008, causing the PHI to decline from four towards two, the PHI never fell below three, meaning that for all years the striped bass were never feeding more benthically than pelagically. In 2008, the PHI remained similar to that seen in 1999 instead of increasing to indicate a return towards pelagic feeding as was expected based on the evidence for ecosystem recovery.

This study is part of a larger proof-of-concept study which includes studying the changes in diet in early life stage alewife, blueback herring (*Alosa aestivalis*), and American shad. In the future it will be expanded to include many more years as well as an increase in the sample size in order to more accurately discern changes in prey diversity, prevalent prey items, and PHI.

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REFERENCES

ASA Analysis and Communication. 2001. 1998 year class report for the Hudson River Estuary Monitoring Program. Washingtonville, NY

Carlsson, N. O. L., H. Bustamante, D. L. Strayer, and M. L. Pace. 2011. Biotic resistance on the increase: native predators structure invasive zebra mussel populations. Freshwater Biology 56:1630-1637.

Haney, J.F., M. A. Aliberti, E. Allan, S. Allard, D. J. Bauer, W. Beagen, S. R. Bradt, B. Carlson, S. C. Carlson, U. M. Doan, J. Dufresne, W. T. Godkin, S. Greene, A. Kaplan, E. Maroni, S. Melillo, A. L. Murby, J. L. Smith, B. Ortman, J. E. Quist, S. Reed, T. Rowin, M. Schmuck, R. S. Stemberger. 2010. "An-Image-based Key to the Zooplankton of the Northeast, USA" version 4.0 released 2010. University of New Hampshire Center for Freshwater Biology <cfb.unh.edu>

Pace, M. L., S. E. G. Findlay, and D. Fischer. 1998. Effects of an invasive bivalve on the zooplankton community of the Hudson River. Freshwater Biology 39:103-116

Pace, M. L., D. L. Strayer, D. Fischer, and H. M. Malcom. 2010. Recovery of native zooplankton associated with increased mortality of an invasive mussel. Ecosphere 1:1-10.

Pauly, D. and M. L. Palomares. 2000. Approaches for dealing with three sources of bias when studying the fishing down marine food web phenomenon. Fishing Down the Mediterranean Food Webs? Kerkyra, 26-30 July 2000.

Peckarsky, B. L., P. R. Fraissinet, M. A. Penton, and D. J. Conklin, Jr. 1990. Freshwater Macroinvertebrates of Northeastern North America. Cornell University Press. Ithaca and London.

Shannon, C. E. 1948. A mathematical theory of communication. The Bell System Technical Journal 27:379-423.

Strayer, D. L., K. A. Hattala, and A. W. Kahnle. 2004. Effects of an invasive bivalve (*Dreissena polymorpha*) on fish in the Hudson River estuary. Canadian Journal of Fisheries and Aquatic Sciences. 61:924-941.

Strayer, D. L. and H. M. Malcom. 2006. Long-term demography of a zebra mussel (*Dreissena polymorpha*) population. Freshwater Biology. 51:117-130

Strayer, D. L. 2009. Twenty years of zebra mussels: lessons from the mollusk that made headlines. Frontiers in Ecology and the Environment 7:135-141.

Strayer, D. L., N. Cid, and H. M. Malcom. 2011. Long-term changes in a population of an invasive bivalve and its effects. *Oecologia*. 165:1063-1072.

Stergiou, K. I. and V. S. Karpouzi. 2002. Feeding habits and trophic levels of Mediterranean fish. *Reviews in Fish Biology and Fisheries*. 11:217-254

Waldman, J. R., J. R. Young, B. P. Lindsay, R. E. Schmidt, H. Andreyko. 1999. A comparison of alternative approaches to discriminate larvae of striped bass and white perch. North American Journal of Fisheries Management 19:470-480.

FISH PARASITES IN THE HUDSON RIVER ESTUARY'S LITTORAL HABITATS: A PRELUDE TO RESTORATION

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

Banded killifish (*Fundulus diaphanus*) parasite communities were examined from three different littoral habitat types (main channel fringe, secondary channel, and contiguous backwater) in New York's upper Hudson River Estuary at four different sites. High parasite species richness and diversity at a site should indicate a similar amount of free living species richness and diversity at the location. Species diversity is one portion of ecosystem "health." The sites were different in terms of the environmental variables measured, with the two secondary channel sites being the most similar. Parasite species abundance, prevalence, diversity, and community similarity were considered. Twenty eight different parasite species were found. Statistically, the composition of parasite species and their abundances were significantly different between sites according to the Multi-response Permutation Procedures (MRPP) and Non-metric Multidimensional Scaling (NMS). The abundance of *Posthodiplostomum minimum, Proteocephalidae* metacestodes, and nematode sp.1 cysts were the main influences on the differences in the sites' parasite community composition. *Posthodiplostomum minimum* and nematode sp. 1 showed the highest mean abundance in the secondary channel habitat. Many fish had heavy infections of *Myxobolus funduli* at all sites. The sites shared some species in common as indicated by high Sørensen's similarity coefficients. Parasite diversity, as expressed using the Shannon-Wiener Index, was high at all sites and highest in the two secondary channel sites, indicating a related level of abundance and diversity of free-living host organisms present in the habitat. This initial investigation of killifish parasites begins to build knowledge of fish parasite community composition in the Hudson River.

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INTRODUCTION

Parasitism is the most common consumer lifestyle on earth, and it has been estimated that half of the animals living in the world are parasites (and this would be a cautious approximation) (Price 1980). These numerous, influential organisms are present in all natural aquatic ecosystems. Ecological studies of parasites often focus on measuring the abundance, diversity, and interactions of communities by studying disturbance, competition, and predation in the ecosystem (Bernot and Lamberti 2008; Wellborn et al. 1996). Despite their large contribution to the species diversity of habitats and ecological dynamics, parasites have often been overlooked in habitat research on community composition and diversity, food webs, and overall ecology (Landsberg et al.1998; Lafferty and Kuris 1999; Marcogliese 2004). The parasite communities of the Hudson River Estuary are poorly represented in the parasitological literature, providing an opportunity to describe these communities and include them in a broader ecological context.

The first objective of this study was to provide information on the parasite species composition, richness, abundance, and prevalence in banded killifish (*Fundulus diaphanus*), beginning a record of the parasite communities in the Hudson River. The second objective was use this information of the parasite communities at different littoral sites in the Hudson River Estuary to determine if there are differences in parasite community composition between sites and habitat types. The comparison of parasite community composition between sites can suggest the presence of certain trophic interactions and can indicate that there are different conditions present in each habitat.

Banded killifish are small fish ranging from 5 to 10 cm in length. Banded killifish spawn in very shallow, vegetated littoral habitats in still water (Werner 2004). These fish are abundant in the Hudson River (Kraft et al. 2006), and play an important role as intermediate links in littoral food webs. Killifish are known to eat ostracods, cladocerans, copepods, chironomid larvae, amphipods, trichopterans, turbellarians, small flying insects, young odonate nymphs, small mollusks, algae, and plant seeds (Becker 1983; Werner 2004). They are important prey for larger fish and water birds (Chippett 2003; Johnson and Dropkin 1993).

Because of their abundance and intermediary role in these food webs, banded killifish are an excellent study organism for examining parasites as a "mirror" on ecosystem complexity. As free-living host organisms increase in richness, parasites increase in richness (Lafferty 2012). Parasites can be used as indicators of the ecosystem's host species richness. A large diversity of parasites indicates a functioning, complicated, and interacting group of free-living organisms (Lafferty 2012). Fish or snails that are abundant, easy to sample, and contain a diversity of parasites with complex life-cycles are well-suited as host species in studies of parasites as biological indicators.

Banded killifish were determined to be an appropriate host to examine to gain information on the parasites of tidal, littoral habitats in the Hudson River Estuary. Many of these habitats are important nursery areas for juvenile fishes and are areas of high biotic diversity in the Hudson River (Levinton and Waldman 2006). They are significant habitats that could be either models for restoration, or potential sites for future restoration. Specifically, the Rogers Island area was included in the study. This site is one of the few secondary channel habitats (braided channel areas that branch off the main

channel) that remains intact on the Hudson River and could be used as a model for restoration of secondary channel habitats, giving this area particular significance. Insight into the ecology of littoral habitats in the Hudson River Estuary gained from studying the parasite communities at ecological reference sites could inform restoration and management decisions.

Parasitological studies have asserted that an assessment of aquatic parasite communities can serve as a good indicator of water quality and of overall habitat health and could also be used in conjunction with other biological indices to provide a more in-depth understanding of the quality of an area (Crafford and Avenant-Oldewage 2009; Huspeni and Lafferty 2004; Marcogliese and Cone 1996; Minguez et al. 2011; Schludermann et al. 2003). In the past, most fish health indices have simplified parasite dynamics to the extreme point of saying that presence of parasites (that is any parasite of any kind) signifies poor health and poor habitat quality (e.g., Karr 1981). Fortunately, more comprehensive and detailed investigations of parasitology in the context of environmental health, anthropogenic change, and water quality began to appear in the scientific literature over the last 30 years. Scientists began to overcome the over-simplified view of parasites as "bad" and began to examine this large category of organisms in a more detailed way in the context of aquatic ecology. Numerous studies have proposed the use of parasites as biological indicators. Shea et al. (2012) determined that some parasites are more sensitive to and better indicators of heavy metal pollution than macroinvertebrate diversity indices. Groups of parasites vary in their responses to pollution and anthropogenic disturbance. However, most instances of habitat pollution and degradation negatively affect parasites and most species of parasites will decrease

(Blanar et al. 2009). The parasites often are killed directly by water contaminants or their numbers decline due to a decrease in their hosts' abundance (Blanar et al. 2009; Lafferty 2012).

Many parasites have complex life cycles that depend on a number of different hosts. Several parasite species are host-specific; thus, a diversity of parasites would suggest a diversity and abundance of specific host animals sufficient enough to support the parasites. Parasite species richness declines when free-living host species richness declines (Lafferty 2012). This means that ecosystems with high free-living species diversity should be likewise characterized by a richness and abundance of diverse parasites (Hechinger et al. 2007; Hudson et al. 2006; Huspeni and Lafferty 2004; Huspeni et al. 2005; Marcogliese 2005). A specific example of this was demonstrated by Hechinger and Lafferty (2005) through a field study which found that with an increase in diversity of final avian hosts, there was an increase in the richness of digenean parasite communities found in snails (the intermediate hosts of these parasites). Research has shown that knowledge of the parasite species present in an area can reveal specific trophic connections and, therefore, provide information about the entire habitat (Huspeni et al. 2005). Lafferty (2012) states that an increase in free-living organism diversity and an increase in parasite diversity should result from the proper restoration of native biodiversity. Huspeni and Lafferty (2004) showed that larval digeneans parasitizing snails increased as a result of habitat restoration in a salt marsh. The consistent rate of increase in larval digeneans occurred for over six years after a saltmarsh restoration project was completed (Huspeni and Lafferty 2004). Digeneans increased because the project effectively restored a proper, functioning habitat which was able to support a

diversity of birds, fishes, and invertebrates which served as hosts for the parasites. These studies illustrate that examination of parasite communities can help scientists determine if restoration efforts have been more than superficially successful by demonstrating that proper ecological connections have been restored along with the physical improvements in the structure of the habitat.

Given the widespread effects of humans on aquatic ecosystems, it is important to develop useful and practical ways of evaluating human impact and to assess restoration efforts in inland waters. The Hudson River Estuary has a history of anthropogenic pollution and disturbance (Levinton and Waldman 2006). Contaminants such as PCBs, chlorinated hydrocarbon pesticides, dioxins, and trace metals are still present in the sediment of the Hudson (Baker et al. 2006). However, due to the Clean Water Act, other important legislation, and the work of scientists, managers, foundations, and citizen action groups, many harmful inputs to the river have been eliminated. Improvements in sanitation have reduced the amount of untreated sewage entering the Hudson River Estuary, contributing to better water quality (Brosnan et al. 2006). Progress made in the realm of water quality has allowed managers and researchers to concentrate on the important efforts of habitat restoration (Levinton and Waldman 2006).

The physical restoration of habitat types in the Hudson River Estuary is a topic of interest to managers in the region. The river channel around river kilometer 185 (measured as distance from the Battery in New York City) was altered greatly from its natural meandering and shallow state in order to make this portion navigable for large barges traveling intentionally (Miller et al. 2006). Many littoral habitats in the upper reaches of the river were dredged and transformed into deep channels, and the dredged

material was moved to nearby littoral habitats. Therefore, many of the shallow, intertidal habitats were dramatically changed into a deep main channel habitat or changed into a vegetated upland habitat. Miller et al. (2006) states that "Restoration of Hudson River Estuary shallow-water and intertidal main-channel habitats for fish and other organisms faces many challenges. Identifying the need for and goals of restoration efforts depends on understanding the historic structure and function of the Hudson River Estuary prior to navigation channel dredging (p. 5)." Therefore, research investigating the ecology of the remaining secondary channel habitats that were not drastically altered by dredging activities, could inform efforts to restore these missing habitats in ways that reflect their original ecology and function.

Objectives

- The first objective of this study was to provide information on the parasite species composition, diversity, abundance, and prevalence in banded killifish (*Fundulus diaphanus*)—to begin a record of the parasite communities in the Hudson River. By beginning to investigate this large group of organisms that have been understudied, researchers will begin to have a fuller picture of the ecology of the habitats examined.
- The second objective was to determine if there are differences in parasite community composition between sites and habitat types in the Hudson River Estuary. This information might be used in the future after restoration efforts have been conducted to determine if similar parasite communities emerge at the restored sites, showing that ecological interactions and species diversity have been restored.

METHODS

Field collection

Banded killifish were collected in three different littoral habitat categories on July 14th and 15th, 2012. The habitat categories included: main channel fringe habitats (sites in the littoral areas of the major channel of the river), secondary channel habitats (shallow water sites located in a smaller channel that "branched off" the large, main channel), and contiguous backwater habitats (littoral sites that are more isolated but still remain connected to the surface flow of the main channel). Historically, braided, secondary channels (shallow water habitat located in a smaller channel that "branched off" the large, main channel) were an abundant habitat feature in the upper Hudson River (Collins and Miller 2012). Navigational dredging activities undertaken resulted in the destruction of these once-numerous secondary channel areas. The sites are located between Schodack Island and Kingston, NY in the upper section of the Hudson River Estuary. The study sites included: 1) a secondary channel site at Rogers Island (RI); 2) a secondary channel site at Stockport (SP) for replication; 3) a main channel fringe site at Schodack Bay (SB); and 4) a contiguous backwater site at North Schodack (NS).

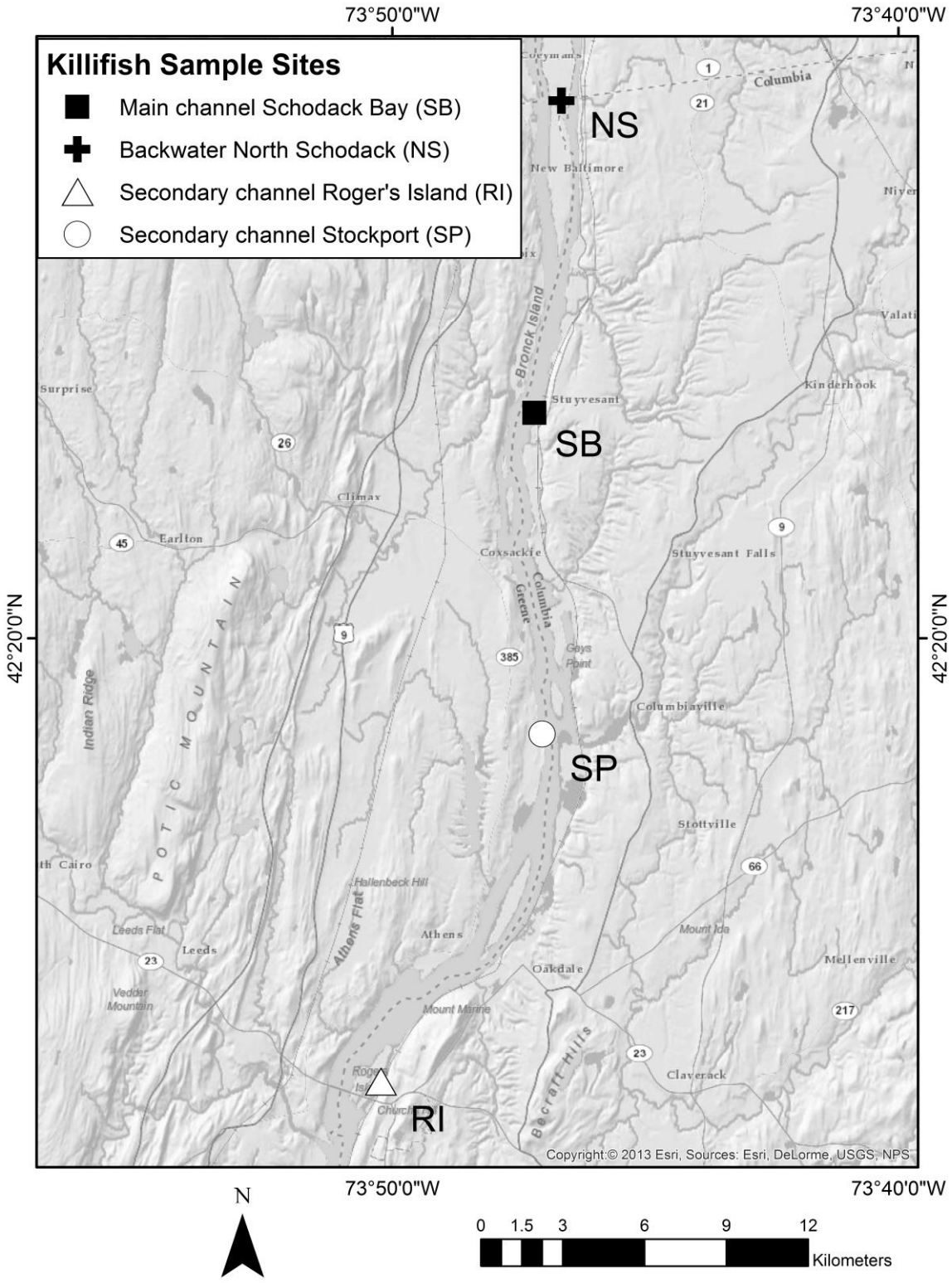

Figure 1. Map of four sites in the upper Hudson River Estuary.

A beach seine was used to collect fish at each site and euthanized fish were put on dry ice as quickly as possible after being caught. Environmental data [temperature (°C), conductivity (µS/cm), dissolved oxygen (mg/L), pH, and velocity (m/S)] were collected using a YSI Model 556 multi-parameter meter, and a Marsh-McBirney Model 201d flow meter.

Sample processing

Banded killifish were necropsied and the following tissues examined for parasites: the external surface including fins and operculum; esophagus, all gill arches, heart, liver, spleen, kidney, swim bladder, gonads, urinary bladder, intestine, mesenteries, eyes, and gall bladder (when it was intact). Typically, a census count of all individuals of particular species is done, and for most of the digeneans, acanthocephalans, and nematodes this was possible; however, for different parasite groups, it was a matter of necessity to quantify or enumerate them differently. They were preserved using the methods listed by Upton (2005). Nematode sp.1 occurred in cysts of varying sizes that were filled with small nematodes, and usually a multitude of nematodes were present in each cyst (exceeding hundreds inside many cysts). Therefore, the number of cysts full of these nematodes was counted to quantify the abundance of this parasite. Monogeneans found in many of the fish's gills were recorded as present or absent, and were preserved but not counted. *Myxosporea* were frozen for preservation for later identification using molecular techniques, recorded as present for presence/absence data, and photographed and measured using Spot102 Basic software (Diagnostic Instruments, Inc. Spot RT Software 4.6 Sterling Heights, Michigan). An exception was with *Myxobolus funduli*, which was

quantified by examining 30 gill filaments and counting the number of filaments out of 30 that were infected with at least one *M. funduli* cyst. Many of the cysts were quite large in size and many of the fish had several cysts on each filament. Therefore, a gill arch from each fish was preserved for histological sectioning so that a more detailed quantification of the infection can be gained in further research. The parasite identifications were made primarily using Hoffman (1999). *Creptotrema funduli* (Mueller 1934) was identified to species with the use of a type specimen obtained from the Justus F. Mueller Parasite Collection at SUNY-ESF.

Analysis

The environmental data were compared between sites using Principal Component Analysis (PCA) and Multi-Response Permutation Procedures (MRPP) with an $\alpha = 0.05$ in PC-ORD version 5.33 (MjM 118 Software, Gleneden Beach, Oregon, U.S.A). MRPP is a statistical test to determine if there is a significant difference between sampling units (in this case the sampling units are fish and are grouped by site). Mann-Whitney *U* tests were performed in MiniTab for site pair-wise comparisons of each fish's parasite abundance for each species of tallied parasite with a *p*-value of less than 0.05 considered significant (Minitab 16.2.2). Sørensen's similarity coefficients (SSC) were calculated for each site comparison to determine parasite community similarity. These values serve as an index number to evaluate the community similarity between the sites compared. The range of this index is 0 to 1 with 0 indicating the sites share no species in common and 1 indicating they share all the same species. Using PC-ORD version 5.33, an MRPP with an $\alpha = 0.05$ was performed on the parasite abundance data for each fish by site to

determine if there was a significant difference in the parasite communities between each site. Non-metric Multidimensional Scaling (NMS) was used as an ordination method to illustrate the results of the MRPP in two-dimensional space. Shannon-Wiener Index (H') numbers were calculated for the four sites to determine species diversity. Percent prevalence of infection was calculated using the presence/absence data for all the parasite species by counting the number of individuals of a certain parasite species present within a given site and dividing that count by the number of hosts examined at the given site. This value was then multiplied by 100 to determine a percentage.

RESULTS

The environmental data [(velocity (m/s), dissolved oxygen (mg/L), pH, depth (m), Secchi depth (m), conductivity (µS/cm), and temperature (°C)] were analyzed to determine if there was a significant difference between these variables for each site. Table 1 shows the environmental data taken during the fish collection at each site. The scatterplot of the Principal Component Analysis (PCA) illustrates the differences in sites among environmental variables (Fig. 2). Multi-Response Permutation Procedures (MRPP) yielded p-values $< 10^{-5}$ for all of the pair-wise comparisons of environmental data by site, showing that there is a statistically significant difference between the environmental data at all the sites (including RI and SP).

Table 1. Environmental parameter data at the four sites.

Site	D.O. (mg/L)	pH	Conductivity (µS/cm)	Velocity (m/s)	Secchi Depth (cm)	Depth (cm)	Temperature (°C)
RI	7.72	7.96	267	0.09	40	80	27.57
SP	8.55	7.76	271	0.01	30	90	27.14
SB	10.31	7.94	297	0.15	30	70	28.17
NS	9.94	7.92	350	0.01	30	80	26.9

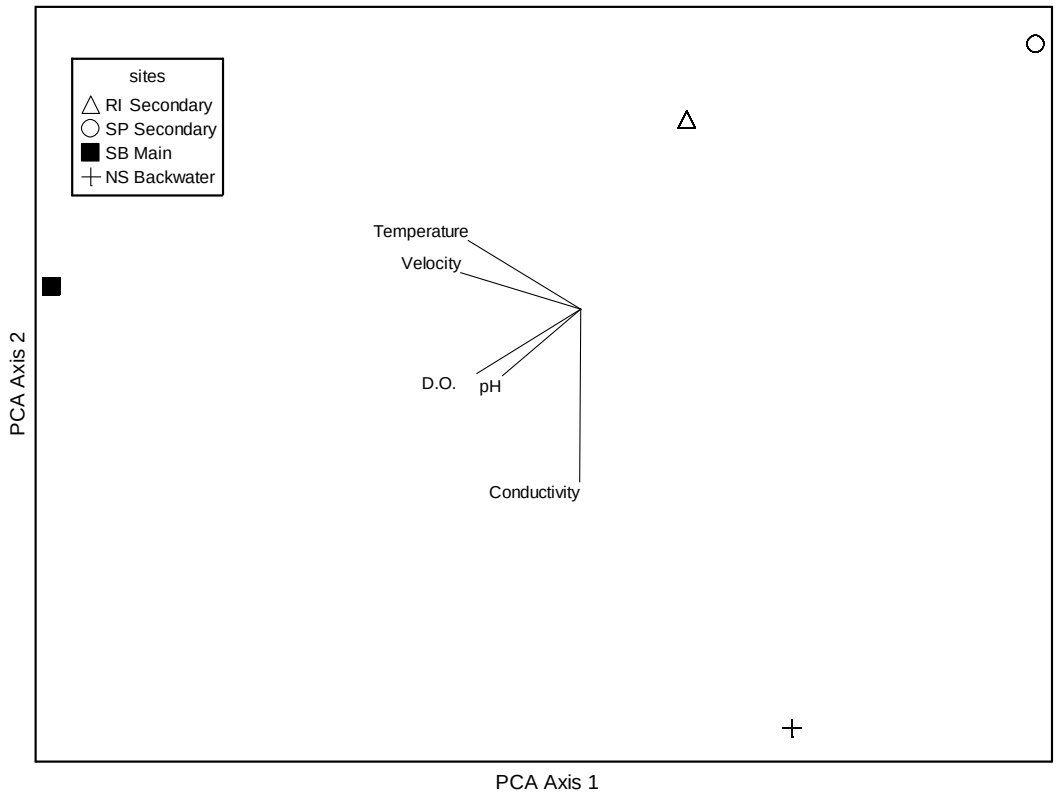

Figure 2. Scatterplot of PCA ordination of environmental variables. Arrangement of four sites—Rogers Island (RI), Stockport (SP), Schodack Bay (SB), and North Schodack (NS)—in two-dimensional space by environmental data variables. Vectors are labeled and illustrated with lines and labeled with the environmental variable.

Six different small, microscopic parasite species were found in the four sites: two different *Myxidium* species, two different *Myxobilatus* species, two different *Myxobolus* species (including *Myxobolus funduli),* and one species of Trichidinids. Twenty-two different larger parasite species were found (Table 7).

The mean abundance of each of the 23 quantifiable parasite species is shown in Table 2. The mean abundance is equal to the total of the parasites found within all the fish at a particular site divided by the number of fish examined from the site.

Table 2. Mean abundance with standard error (SE) of parasite species.

	Rogers Island (RI)	Stockport (SP)	Schodack Bay (SB)	North Schodack (NS)
	Secondary Channel	Secondary Channel	Main Channel Fringe	Contiguous Backwater
Acanthocephala				
acanthocephalan sp. 1	**0.83** (0.36)	**0.29** (0.17)	**0.45** (0.18)	**0.15** (0.07)
cystacanth spp. 1	**0.87** (0.20)	**3.65** (1.35)	**0.39** (0.10)	**0.77** (0.20)
Cestoda				
cestode sp. 1	**0.43** (0.17)	**1.29** (0.44)	**0.23** (0.12)	**0.35** (0.14)
Proteocephalidae adult	**1.37** (0.36)	**0.47** (0.17)	**0.61** (0.19)	**0.35** (0.16)
Proteocephalidae metacestode	**26.33** (3.76)	**13.29** (2.33)	**31.45** (7.01)	**26.10** (12.70)
Digenea				
Creptotrema funduli	**0**	**0**	**0**	**0.692** (0.65)
digenean sp. 1	**1.43** (0.89)	**1.47** (1.01)	**0**	**0**
digenean sp. 2	**0**	**0**	**1.52** (0.90)	**0**
digenean sp. 3	**0**	**0**	**0**	**0.08** (0.08)
digenean sp. 4	**0**	**0**	**0**	**0.04** (0.04)
digenean sp. 5	**0**	**0**	**0.03** (0.03)	**0**
Neascus metacercaria	**2.57** (1.03)	**8.47** (3.22)	**2.03** (0.69)	**6.38** (2.38)
Phyllodiplostimum sp.	**0**	**0**	**0.13** (0.06)	**0**
P. minimum	**13.30** (2.69)	**41.76** (8.29)	**10.39** (2.00)	**21.69** (8.03)
Nematoda				
nematode sp. 1	**38.70** (3.60)	**22.59** (3.96)	**10.19** (1.60)	**17.46** (2.81)
nematode sp. 2	**1.63** (0.39)	**2.24** (0.56)	**0.61** (0.17)	**1.96** (0.47)
nematode sp. 3	**0.17** (0.12)	**0.77** (0.46)	**0.61** (0.35)	**0.73** (0.26)
nematode sp. 4	**0.13** (0.10)	**0**	**0**	**0**
nematode sp. 6	**0**	**0.12** (0.08)	**0**	**0**
Crustacea				
copepod sp.1	**0.03** (0.03)	**0**	**0**	**0**
Myxosporea				
Myxobolus funduli	**23.25** (4.02)	**21.24** (3.02)	**19.59** (1.96)	**19.42** (2.19)

The abundances of *Proteocephalidae* metacestodes were significantly different between the two secondary channel sites (RI and SP), between the RI secondary channel site and the NS backwater site, between the SP secondary channel site and the SB main channel site, and between the NS backwater site and the SB main channel site. The RI secondary channel site had a higher mean abundance of this parasite (26.33) than SP secondary channel site (13.29), the SB main channel fringe site had the highest mean abundance (31.45), and the NS backwater site had a mean abundance (26.10) similar to RI.

The abundance of *Posthodiplostomum minimum* was significantly different between the fish examined in the two secondary channel sites (RI and SP), between the SP secondary channel site and the SB main channel site, between the SP secondary channel site and the NS backwater site, and between the NS backwater and SB main channel sites. The mean abundance of *P. minimum* was the highest at the SP secondary channel site (41.76) and the lowest at the SB main channel site (10.39). The RI secondary channel site had a mean abundance of 13.30 and the backwater site had a mean abundance of 21.69, the second highest.

The cysts filled with small nematodes were labeled as nematode sp.1 and the number of cysts was significantly different between all sites except for one comparison. The SP secondary site was not statistically different than the NS backwater site in terms of number of cysts full of nematode sp. 1.

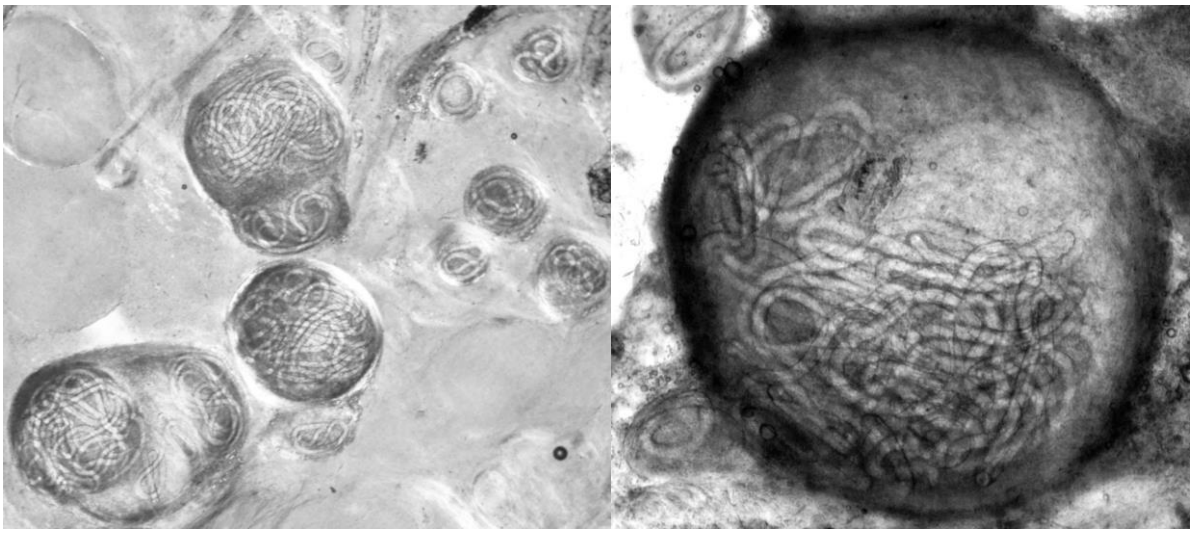

Figure 3. Cysts filled with nematode sp. 1 (Left: cysts in mesenteries at 4x magnification Right: A cyst at 10x magnification)

There were seven adult digenean parasites, and each was found exclusively in one habitat type (Table 4). *Cretotrema funduli,* digenean sp. 4, and digenean sp. 5 were found only in the contiguous backwater site (NS). *Phyllodiplostimum* sp., digenean sp. 3, and digenean sp. 6 were all only found in fish from the main channel fringe site (SB). Digenean sp.1 was only found in the secondary channel sites (RI and SP) and there was no statistically significant difference between the abundance of this parasite at these two sites.

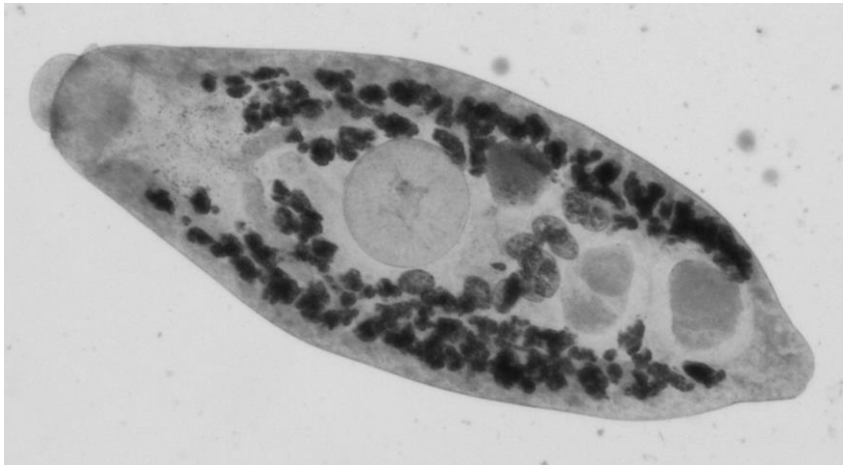

Figure 4. Photograph of *Creptotrema funduli.*

Table 3. **The *p*-values produced by Mann-Whitney *U* tests for site pair-wise comparisons. The sites are: Rogers Island (RI), Stockport (SP), Schodack Bay (SB), and North Schodack (NS) and the tests that yielded p-values that were not statistically significant are shown as >0.05. The categories of habitat type are listed for each comparison.**

	RI & SP	RI & SB	RI & NS	SP & SB	SP & NS	SB & NS
	2ndary & 2ndary	2ndary & Main	2ndary & Backwater	2ndary & Main	2ndary & Backwater	Main & Backwater
Acanthocephala						
Acanthocephalan sp. 1	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Cystacanth spp. 1	0.0035	>0.05	>0.05	0.0001	0.0085	>0.05
Cestoda						
Cestode sp. 1	0.0318	>0.05	>0.05	0.0029	0.0323	>0.05
Proteocephalidae adult	>0.05	>0.05	0.0094	>0.05	>0.05	>0.05
Proteocephalidae metacestode	0.026	>0.05	0.0315	0.009	>0.05	0.015
Digenea						
digenean sp. 1	>0.05					
digenean sp. 2						
digenean sp. 3						
digenean sp. 4						
digenean sp. 5						
Neascus metacercaria	>0.05	>0.05	0.0431	0.0447	>0.05	0.0334
Posthodiplostomum	0.0004	NS	NS	0.0001	0.0011	>0.05
Nematoda						
Nematode sp. 1	0.0041	0	0	0.004	>0.05	0.0257
Nematode sp. 2	>0.05	0.0494	>0.05	0.0065	>0.05	0.0032
Nematode sp. 3	>0.05	>0.05	0.012	>0.05	>0.05	>0.05
Myxosporea						
Myxobolus funduli	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Table 4. **Parasites present only at one site. A list of the banded killifish parasite species present only within one site.**

Parasite species	Site
digenean sp. 2	SB
digenean sp. 3	NS
digenean sp. 4	NS
digenean sp. 5	SB
Cretotrema funduli	NS
Phyllodiplostimum sp	SB
Nematode sp. 4	RI
Nematode sp. 6	SP
Copepod sp.1	RI

Table 5. Sørensen's similarity coefficients (SSC) for site comparisons. The number of species the two compared-sites shared. RI and SP belong to the same habitat category.

Site Category comparisons	Site comparisons	Shared Species	SSC
2ndary vs. 2ndary	RI vs SP	16	0.865
2ndary vs. Main	RI vs NS	15	0.811
2ndary vs. Backwater	SP vs SB	14	0.800
2ndary vs. Main	SP vs SB	14	0.778
2ndary vs. Backwater	SP vs NS	14	0.778
Main vs. Backwater	SB vs NS	13	0.743

Non-metric Multidimensional Scaling (NMS) was used as an ordination method to place the parasite species abundances into two-dimensional space. A scatterplot of this ordination shows how the parasite abundance data are positioned on two axes. The fish are marked as data points for each site and the position of these points was determined by the ordination of each fish's parasite abundance. All four sites overlap on the NMS scatterplot, showing that there is similarity between the communities of parasite species at each site, as the relatively high Sørensen's similarity coefficients also showed. The two secondary channel sites (RI, represented by open triangles and SP, represented by open circles in Fig. 5) overlap the most in their ordination groupings.

The vectors driving the differences in sites' arrangement and the length and angle of the vector lines (shown as dashed lines) show the strength and direction of the relationship between certain parasites and the ordination scores for the entire parasite abundance matrix for the sites. Proteocephalidae metacestode, *Posthodiplostomum minimum*, and the number of cysts of nematode sp.1 are the main "drivers" of the sites' plot points on the NMS axis 1 and axis 3.

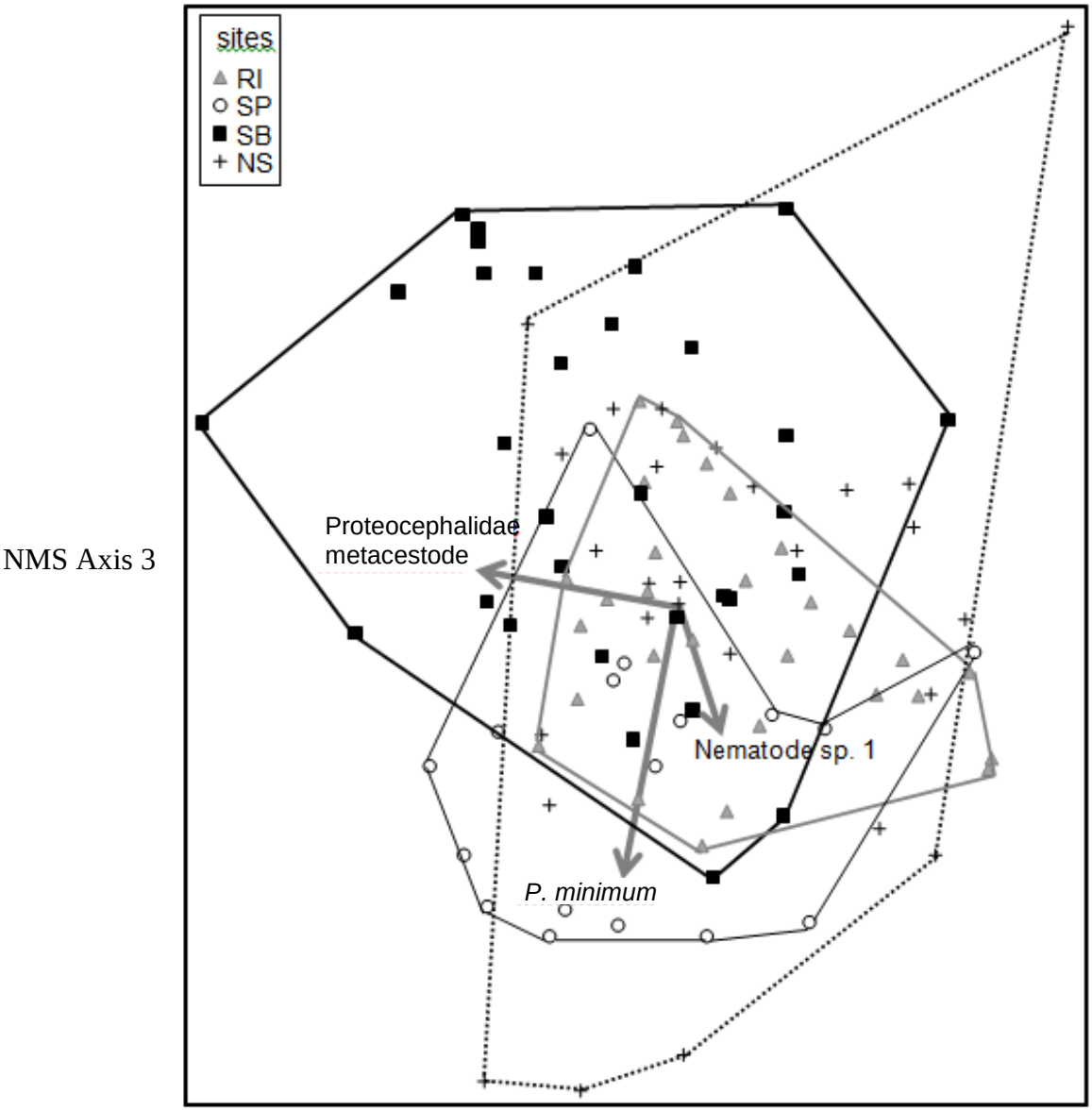

Figure 5. **Scatterplot of the NMS ordination of parasite abundance at the four sites. Rogers Island (RI) is pictured as a gray triangle, Stockport (SP) is an open circle, Schodack Bay (SB) is a closed square, and North Schodack is a plus sign. The vectors are labeled with their parasite name and signified by gray arrows.**

The Multi-Response Permutation Procedures (MRPP), like the pair-wise comparisons of the parasite abundance data variables, were statistically significant at $\alpha = 0.05$ and the sites were different in terms of the recorded environmental variables. Table

5 shows the *p*-values related to the NMS scatterplot (Fig. 5) for each pair-wise comparison of sites.

Table 6. Multi-Response Permutation Procedures (MRPP) p-values for sites' parasite abundance comparisons.

Site Category comparisons	Site comparisons	p-value
Secondary vs. Secondary	RI vs. SP	$<10^{-4}$
Secondary vs. Main	RI vs. SB	$<10^{-4}$
Secondary vs. Backwater	RI vs. NS	$<10^{-4}$
Secondary vs. Main	SP vs. SB	$<10^{-4}$
Secondary vs. Backwater	SP vs. NS	0.0012
Main vs. Backwater	SB vs. NS	0.0047

The Shannon-Wiener Index (H') was calculated to measure species diversity at each of the four sites (Table 7). The Shannon-Wiener Index (H') uses species richness and abundance to determine an index number ranging from 1 to 5 (1 signifying low species diversity and 5 signifying high species diversity).

Table 7. Shannon-Wiener Index (H') for the four sites. Rogers Island (RI), Stockport (SP), Schodack Bay (SB), and North Schodack (NS). H' ranges from 1-5.

Habitat Categories	Sites	H'
Secondary	SP	4.838
Secondary	RI	4.485
Main	SB	4.247
Backwater	NS	4.154

The percent prevalence numbers were calculated for all the parasites found in the fish examined from the four sites (Table 8). The abundance of *Myxobolus funduli* (pictured in Fig. 6) found on the 30 gill filaments examined on each fish was not

significantly different between any of the sites. The prevalence of infection of *M. funduli* is also extremely high for all of the sites, ranging from 100% prevalence to 96% prevalence.

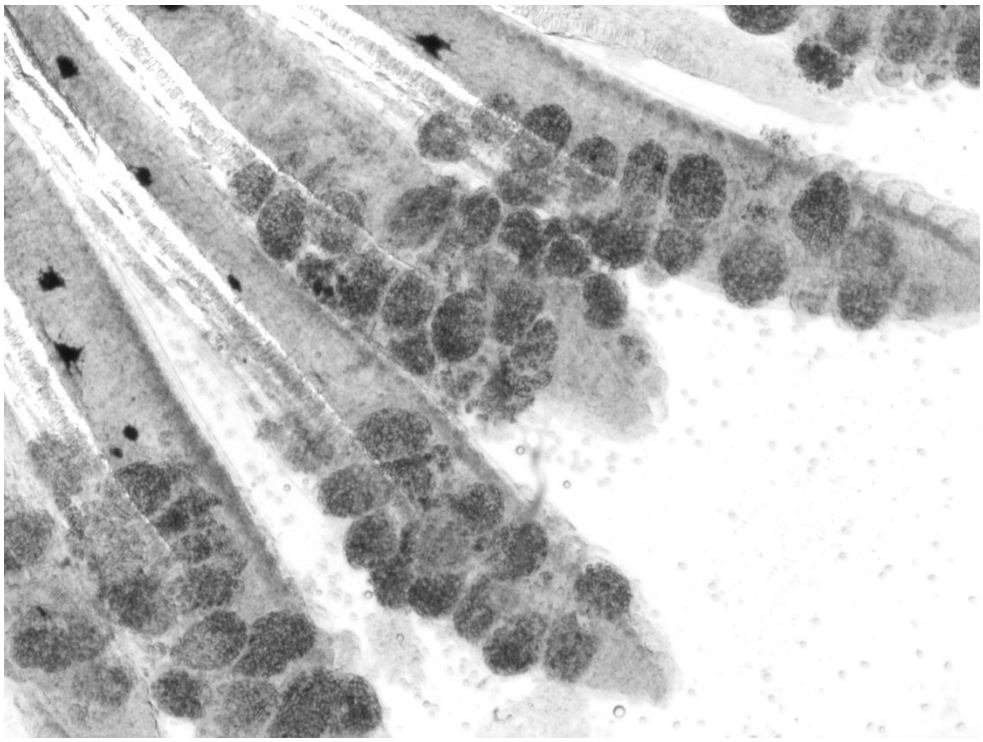

Figure 6. Photograph of a gill infection of *Myxobolus funduli*. The gill filaments are pictured and the darker circular objects are the *Myxobolus funduli* cysts. This was an average, moderate infection for the killifish examined.

Table 8. Percent prevalence of all killifish parasites found.

	Rogers Island (RI)	Stockport (SP)	Schodack Bay (SB)	North Schodack (NS)
Acanthocephala				
Acanthocephalan sp. 1	33	18	23	15
Cystacanth spp. 1	40	82	35	42
Cestoda				
Cestode sp. 1	20	53	13	23
Proteocephalidae adult	53	35	35	19
Proteocephalidae metacestode	100	94	100	96
Digenea				
Cretotrema funduli	0	0	0	8
Digenean sp. 1	20	12	0	0
Digenean sp. 2	0	0	23	0
Digenean sp. 3	0	0	23	0
Digenean sp. 4	0	0	0	4
Digenean sp. 5	0	0	3	0
Neascus metacercaria	47	65	42	69
Phyllodiplostimum sp.	0	0	13	0
Posthodiplostomum minimum	90	100	77	88
Nematoda				
Nematode sp. 1	100	100	87	96
Nematode sp. 2	57	71	42	73
Nematode sp. 3	7	18	16	35
Nematode sp. 4	7	0	0	0
Nematode sp. 6	0	12	0	0
Crustacea				
Copepod sp.1	3	0	0	0
Myxosporea				
Myxidium sp. 1	73	6	3	12
Myxobilatus sp.1	0	6	0	0
Myxobilatus sp. 2	0	0	0	4
Myxobolus funduli	100	100	97	96
Myxobolus sp.1	73	100	0	77
Monogenea				
Monogenean sp. 1	63	88	77	15
Litostomatea				
Trichodina	60	6	13	0

DISCUSSION

This study was conducted to begin to provide information on the parasite communities of the upper Hudson River Estuary through an examination of banded killifish hosts. The research investigated four different sites in three different littoral habitats in order to determine if a difference in parasite community composition existed between habitats with different environmental variables.

The environmental variables separated the sites distinctly in the PCA and these differences were statistically significant (Fig. 2). The two secondary channel sites were different in terms of the environmental variables measured but were much more similar to each other (and closer in two dimensional space in the PCA) compared to the other sites. SP had a higher concentration of dissolved oxygen and lower pH than RI.

The NMS scatterplot (Fig. 5) and the associated MRPP p-values (Table 5) show that there is a significant difference between each site in terms of the abundances and community of parasites. The sites share some species in common, as shown by the overlap of sites in Figure 6 and as demonstrated by the SSCs. It was shown that the parasite communities of the two secondary channel sites, RI and SP, are somewhat different but were the most similar sites to each other.

The vectors shown in the NMS scatterplot show that the abundance of *Proteocephalidae metacestodes*, *Posthodiplostomum minimum* metacercaria, and cysts of nematode sp. 1 are the main factors contributing to the fish plot points (the visual representation of where each fish's parasite community is positioned related to other individuals' parasite communities in the same and different sites). *Posthodiplostomum minimum* had the highest mean abundance at SP, a secondary channel site. RI, the other

secondary channel site, had the highest mean abundance of nematode sp. 1 cysts. *Proteocephalidae* metacestodes were most abundant at the main channel site (SB). Because this study examined parasites found within one fish species in one river, it was expected that there would be some similarity between parasite communities; however, the different habitat category types (and to a lesser extent all the different sites) supported distinct parasite communities.

Posthodiplostomum minimum has a complex life cycle and needs different host species to complete different stages of its life cycle. Great blue heron (*Ardea herodias*) is the common natural definitive host (hosts that support the adult, reproductive stage of the parasite) (Campbell 1972). *Physa* spp. snails are the common natural first intermediate host (Hoffman 1958) and Turner and Beasley (1982) found *Ferrissia fraglis*, *Hebetancylus extricus*, and *Laevapex fuscus* to be intermediate ancylid snail hosts. Therefore, this type of snail is probably present in great enough abundance at the Hudson River sites to support this parasite. The four sites in the study had significantly different abundances of *P. minimum*, yet all sites had at least a mean abundance of 10.39. SP, a secondary channel site, supported the highest mean abundance of *P. minimum* suggesting that the snail first intermediate hosts and the avian definite hosts are present in sufficient abundance at the sites where these parasites were found, to not only provide linkages for the parasite's life cycle, but also support a great abundance of this parasite. Thus, this is a case where the presence of a relatively benign parasite is a positive indication that several ecological linkages are in place.

Bernot and Lamberti (2008) found that *Physa* spp. snails infected with *P. minimum* grazed more heavily than uninfected snails and that this parasite-induced

behavioral change reduced algae blooms. When comparing the relative abundance of periphytic taxa on ceramic tiles, snails with high infection rates (50% of snails infected) left more *Cladophora glomerata*, (a filamentous green algae) present in the environment and grazed more heavily on cyanobacteria and on diatoms, compared to the snails with no infection. This difference in feeding behavior due to parasite infection adds another aspect to the complicated interactions involved with the presence of this particular parasite in these habitats. It supports the theory that parasites are responsible for a greater influence on ecosystem dynamics than their small, individual body size might suggest.

Trematodes of the genus *Creptotrema* use various vertebrates as definitive hosts. Curran et al. (2012) determined that adult *Creptotrema funduli* had been reported 10 times (including their study and the first discovery of the parasite) and in all reported cases the definitive host was a fish. The intermediate hosts have not been reported, and thus the life cycle is unknown.

A high prevalence (100%, 100%, 97%, and 96% as listed in Table 6) of the myxozoan parasite *Myxobolus funduli* characterized the parasite infracommunities at all Hudson River sites. Cone et al. (2006) reported prevalences of 42% and 20% or less in banded killifish. Barse (1998) reported mummichog (*Fundulus heteroclitus*) with *Myxobolus funduli* prevalences as high as 94% during one spring (with other sampling efforts yielding prevalences of 58%, 58%, 65%, 67%, 79%, 81%, and 89%).

Not only was there a high prevalence of *Myxobolus funduli* in the Hudson River banded killifish examined, but in many of the fish the cysts of this myxozoan completely covered the gills. This is significant because this type of gill infection could easily have a direct impact on respiration, and therefore health and survival, whereas some of the

intestinal parasites encountered in this study are generally considered benign. The U-tests found that there was no significant difference in the abundance of this parasite between the sites. Further investigation of this parasite infection will be done to more precisely quantify the infections (histological sections of the gills will be done to carefully quantify the severity between sites). This preliminary investigation of the July killifish simply counted the number of infected gill filaments out of 30 that were infected with at least one cyst.

Another interesting finding of this study was the habitat specificity of the adult digenean species. *Creptotrema funduli*, *Phyllodiplostimum* sp., and digenean sp. 1-5 were found within one habitat category each. Digenean sp. 1 was the only adult digenean found at two sites and those two sites were both secondary channel sites. Because the sites were within the same river, habitat characteristics might be a determining factor for the presence and range of these different parasites, and adult digeneans may remain within certain habitat types.

The Shannon-Wiener Index values showed that species diversity of parasites was different at all the sites and highest at the secondary channel sites. This indicates that species richness of free-living organisms at these sites may also be high, since many of these parasites have complex life cycles which depend on free-living organisms. The parasite species diversity was lowest at NS, a backwater site, as shown in Table 6; however, this lower H' value of 4.15 is still a very high number for this diversity index, which in this case has a range of 1 (lowest diversity) to 5 (highest diversity).

While parasite communities are often overlooked in research on community ecology, parasitological studies can provide insight into differences between habitat types

and can suggest the complex interactions between different free-living organisms and parasites within those habitats. This study found that the abundance of some parasite species was significantly different among three different habitat categories that each exhibited significantly different environmental variables. The parasite communities showed some level of community similarity between the sites (especially between sites within the same habitat category) but were significantly different at each site. A simple index of species diversity suggested that the parasite communities had a high diversity at all sites and that diversity was highest at the secondary channel sites. Since many studies have found that parasite richness and diversity often decreases as a result of habitat degradation and pollution, the high parasite species diversity in these sites could reflect positively on the condition of these habitats. Richness and diversity of parasites with complex life cycles is dependent on the richness of free-living host species, so higher parasite diversity suggests higher free-living organism abundance. In addition, the presence of particular parasites whose complex life cycles are known shows that the necessary host species are present in sufficient abundance in the habitats which support the parasite. This study suggests that parasite communities differ from sites within backwater habitat, within main channel fringe habitat, and within secondary channel habitat. This is a preliminary portion of ongoing work and further information will add to this examination of banded killifish parasites at these sites.

This information could be used if restoration projects are conducted as a part of the assessment of sites post-restoration. Killifish from a restored site could be examined for parasites at various times after restoration efforts. A basic examination could be conducted if time and parasitological expertise are limited. Larger parasites or intestinal

parasites could be the main focus and parasites could be placed in large grouping instead of identified to species. This post-restoration parasitological assessment could show if trophic linkages and species diversity have been successfully restored via comparison of parasites communities of the new, restored sites relative to the model sites. If there are no or very few parasites found, then this could suggest that the restoration efforts have not fully formed all the functioning ecological dynamics yet, and that more time is needed for organisms to establish and for complete restoration to be accomplished (Huspeni and Lafferty 2004). If the normal functioning of the habitat is fully restored and the habitat is able to support a diversity of free living organisms, it would follow that parasite species diversity would also be supported (Hechinger and Lafferty 2005; Hechinger et al. 2007; Huspeni and Lafferty 2004). Digenean diversity and abundance has been shown to directly reflect the diversity and abundance of final host organisms (Huspeni et al. 2005. This could be part of a valuable assessment of the success of a restoration project, along with other assessment criteria (Huspeni et al. 2005). Further research into parasite communities will illuminate more about the complex interactions between parasites and the ecosystem in which they reside and provide more insight into the influences of these understudied components of the ecology of the Hudson River Estuary.

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LITERATURE CITED

Baker, J.E., W. F. Bohlen, R.F. Bopp, B. Brownawell, T.K. Collier, K.J. Farley, W. R. Geyer, R. Nairn, and L. Rosman. 2006. PCBs in the upper and tidal freshwater Hudson River estuary: the science behind the dredging controversy. pp. 349-367 in Levinton, J.S. and J.R. Waldman (eds.), The Hudson River Estuary. Cambridge University Press.

Barse, A. M. 1998. Gill parasites of mummichogs, *Fundulus heteroclitus* (Teleostei : Cyprinodontidae): Effects of season, locality, and host sex and size. Journal of Parasitology 84: 236-244.

Becker, G. C. 1983. Fishes of Wisconsin. University of Wisconsin Press.

Bernot, R. J., and G. A. Lamberti. 2008. Indirect effects of a parasite on a benthic community: an experiment with trematodes, snails and periphyton. Freshwater Biology 53: 322-329.

Blanar, C. A., K. R. Munkittrick, J. Houlahan, D. L. MacLatchy, and D. J. Marcogliese. 2009. Pollution and parasitism in aquatic animals: A meta-analysis of effect size. Aquatic Toxicology 93**:** 18-28.

Brosnan, T.M., A. Stoddard, and L.J. Hetling. 2006. Hudson River sewage inputs and impacts. pp. 335-348 in Levinton, J.S. and J.R. Waldman (eds.), The Hudson River Estuary. Cambridge University Press.

Campbell, R. A., 1972, New experimental hosts for *Posthodiplostomum minimum* (Trematoda Diplostomatidae. Journal of Parasitology 58: 1051-1051.

Chippett, J. D. 2003. Update COSEWIC status report on the banded killifish *Fundulus diaphanus*, Newfoundland population in Canada, in COSEWIC assessment and update status report on the banded killifish *Fundulus diaphanus* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. 1-21.

Collins, M. J., and D. Miller. 2012. Upper Hudson River Estuary (USA) floodplain change over the 20th century. River Research and Applications 28: 1246-1253.

Cone, D. K., D. J. Marcogliese, A. M. Barse and M. D. B. Burt. 2006. The myxozoan fauna of *Fundulus diaphanus* (Cyprinodontidae) from freshwater localities in eastern North America: Prevalence, community structure, and geographic distribution. Journal of Parasitology 92: 52-57.

Curran, S. S., E. E. Pulis, D. O. Hugg, J. P. Brown, L. C. Manuel and R. M. Overstreet. 2012. Phylogenetic position of *Creptotrema funduli* in the Allocreadiidae based on partial 28S rDNA sequences. Journal of Parasitology 98: 873-875.

Crafford, D., and A. Avenant-Oldewage. 2009. Application of a fish health assessment index and associated parasite index to *Clarias gariepinus* (Teleostei: Clariidae) in the Vaal River system, South Africa. African Journal of Aquatic Science 34**:** 261-272.

Hechinger, R. F., and K. D. Lafferty. 2005. Host diversity begets parasite diversity: bird final hosts and trematodes in snail intermediate hosts. Proceedings of the Royal Society Biological Sciences 272: 1059-1066.

Hechinger, R. F., K. D. Lafferty, T. C. Huspeni, A. J. Brooks, and A. M. Kuris. 2007. Can parasites be indicators of free-living diversity? Relationships between species richness and the abundance of larval trematodes and of local benthos and fishes. Oecologia 151**:** 82-92.

Hoffman, G. L. 1999. Parasites of North American Freshwater Fishes. 2nd ed. Cornell University Press.

Hoffman, G. L. 1958. Experimental studies on the cercaria and metacercaria of a strigeoid Trematode, *Posthodiplostomum minimum*. Experimental Parasitology 7**:** 23-50.

Hudson, P. J., A. P. Dobson, and K. D. Lafferty. 2006. Is a healthy ecosystem one that is rich in parasites? Trends in Ecology and Evolution 21**:** 381-385.

Huspeni, T. C., and K. D. Lafferty. 2004. Using larval trematodes that parasitize snails to evaluate a saltmarsh restoration project. Ecological Applications 14**:** 795-804.

Huspeni, T.C., R.F. Hechinger, and K.D. Lafferty. 2005. Trematode parasites as estuarine indicators: opportunities, applications and comparisons with conventional community approaches. pp. 297-314 in Bortone, S.A. (ed.), Estuarine Indicators. CRC Press, Boca Raton, FL.

Johnson, J. H., and D.S. Dropkin. 1993. Diel variation in diet composition of a riverine fish community. Hydrobiologia 271: 149-158.

Karr, J.R. 1981. Assessment of biotic integrity using fish communities. Fisheries 6**:** 21-27.

Kraft, C.E., D.M. Carlson, and M. Carlson. 2006. Inland Fishes of New York (online), Version 4.0. Department of Natural Resources, Cornell University, and the New York State Department of Environmental Conservation.

Lafferty, K. D., and A. M. Kuris. 1999. How environmental stress affects the impacts of parasites. Limnology and Oceanography. 44: 925-931.

Lafferty, K. D. 2012. Biodiversity loss decreases parasite diversity: theory and patterns. Philosophical Transactions of the Royal Society Biological Sciences 367: 2814-2827.

Landsberg, J. H., B. A. Blakesley, R. O. Reese, G. McRae, and P. R. Forstchen. 1998. Parasites of fish as indicators of environmental stress. Environmental Monitoring and Assessment 51**:** 211-232.

Levinton, J.S., and J.R. Waldman. 2006. The Hudson River Estuary: executive summary. pp. 1-10. in Levinton, J.S. and J.R. Waldman (eds.), The Hudson River Estuary. Cambridge University Press, New York.

Marcogliese, D. J., and D. K. Cone. 1996. On the distribution and abundance of eel parasites in Nova Scotia: Influence of pH. Journal of Parasitology 82**:** 389-399.

Marcogliese, D. J. 2004. Parasites: Small players with crucial roles in the ecological theater. EcoHealth 1: 151-164.

Marcogliese, D. J. 2005. Parasites of the superorganism: Are they indicators of ecosystem health? International Journal for Parasitology 35**:** 705-716.

Miller D., J. Ladd, and W. Nieder. 2006. Channel morphology in the Hudson River Estuary: historical changes and opportunities for restoration. pp. 29–38 in Waldman J.R., Limburg K.E., Strayer D.L. (eds.), Hudson River Fishes and Their Environment, American Fisheries Society, Symposium 51: Bethesda, MD.

Minguez, L., D. P. Molloy, F. Guerold, and L. Giamberini. 2011. Zebra mussel *(Dreissena polymorpha*) parasites: Potentially useful bioindicators of freshwater quality? Water Research 45: 665-673.

Mueller, J. F. 1934. Two New Trematodes from Oneida Lake Fishes. Transactions of the American Microscopical Society 53: 231-236.

Price, P. W. 1980. May, R.M. (ed.), Evolutionary Biology of Parasites. Princeton University Press, Princeton.

Shea, J., G.J. Kersten, C.M. Puccia, A.T. Stanton, S.N. Stiso, E.S. Helgeson, and E.J. Back. 2012. The use of parasites as indicators of ecosystem health as compared to insects in freshwater lakes of the Inland Northwest. Ecological Indicators, 13, 184-188.

Schludermann, C., R. Konecny, S. Laimgruber, J. W. Lewis, F. Schiemer, A. Chovanec, and B. Sures. 2003. Fish macroparasites as indicators of heavy metal pollution in river sites in Austria. Parasitology 126: 61-69.

Turner, H. M., and S.M. Beasley. 1982. Ancylid Snails as Hosts for *Posthodiplostomum minimum* (MacCallum, 1921) (Digenea: Diplostomatidae). Proceedings of The Helmintological Society of Washington 49: 143.

Upton, S. J. 2005. Animal Parasitology Biology 625: Laboratory Manual. Kansas State University, Fall Semester. Manhattan, Kansas.

Wellborn, G. A., D. K. Skelly and E. E. Werner. 1996. Mechanisms creating community structure across a freshwater habitat gradient. Annual Review of Ecology and Systematics 27: 337-363.

Werner, R.G. 2004. Freshwater Fishes of the Northeastern United States. A Field Guide. Syracuse University Press.

DEVELOPING A NONLETHAL FIELD METHOD FOR DETERMINING LIPID CONTENT OF AMERICAN EELS (*ANGUILLA ROSTRATA*)

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

American eels (*Anguilla rostrata*) were collected from Hudson River tributary streams, and resistance was measured with a tetrapolar Bioimpedance Assessment device. Lipid content of 20 eels was then determined in the laboratory using the Folch method. Correlation between resistance corrected for distance between the electrodes and total lipid content was substantial ($R^2 = 0.84$). However, measures of lipid content were not well correlated with Fulton's K (0.25 – 0.33).

A field study showed that American eels in one Hudson River tributary (Hannacrois Creek) were significantly higher in lipid content than eels in a second tributary (Black Creek). A test of the hypothesis that eels would have a higher lipid content in upstream locations compared to eels near the tributary mouth confirmed that this was the case.

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INTRODUCTION

Research concerning the health of freshwater eels has become increasingly important as eel populations decline across the globe. In the case of the American eel (*Anguilla rostrata*), concern over eel populations has resulted in the species being brought under review for protection via the Endangered Species Act (Federal Register 2011). The task of protecting the American eel is made more difficult by the unique life-cycle of freshwater eels wherein they migrate to the Sargasso Sea in order to reproduce. There is still much to be learned about their migratory behavior, as well the physiological and environmental requirements of this migration. Any research into effective management strategies for eel populations will be enhanced by an accurate field method for quantifying eel health.

While many fisheries studies utilize weight/length equations to quantify fish health, this methodology makes cross population comparisons difficult if not impossible. When using a condition index, an individual with a greater than the average mass for a given length receives a higher condition rating. Determining an "average" mass for a population can become quite complicated, especially when a study is being conducted over time or between populations. Length to weight ratios are greatly influenced by a myriad of environmental factors that determine fish size and shape (Froese 2006).

Traditionally, a more quantitative approach to fish health is to look at lipid content. Proximate analysis of tissue was the only way to gain insight in to the actual composition of individuals. By quantifying body metrics, researchers could make judgments about an individual's energy consumption and storage. In particular, data concerning non-polar lipid content can communicate much about an organism's energy

storage and consumption (Tocher 2003). Since lipids have the highest energy to weight ratio, they allow organisms to store energy while limiting body mass and whole-fish lipid content has been positively correlated with whole-fish caloric content (Peters et al. 2007). This is especially important for *A. rostrata* given its catadromous life cycle in which they migrate from river tributaries to the Sargasso Sea. This migration requires large stores of energy and larger eels are known to have very high lipid content. The physiological aspects of the migration are still poorly understood due to the reluctance of the scientific community to sacrifice breeding eels.

More recently, however, Bioimpedance Assessment (BIA) has used non-lethal electric current to derive information about an animal's chemical composition. BIA thus offers the accuracy of proximate analysis while also providing a non-lethal, efficient, and economical field methodology. Currently, the biggest limitation to using BIA to study eels is its limited application to fish in general and eel shaped fish in particular. Although previous studies have found strong correlations between impedance measurements and lipid content, the relationship differs between organisms (Duncan 2008; Cox and Hartman 2005). Body shape plays a large role in determining how current from the BIA device is distributed through the organism. Up to this point, no studies have been published on eel shaped fish, thus BIA remains an unproven method.

The purpose of this study was examine lipid content in eels using proximate analysis and BIA to determine if the there was a correlation between BIA and lipid content for the American eel. If an appropriately significant relationship was obtained, the methodology could then be applied to investigate differences in total lipid content between upstream and downstream eel populations. Machut (2006) had investigated this

question previously using condition indices and found no significant difference in condition between the upstream and downstream populations. The hypothesis was that eels in upstream habitats, where they live at relatively low densities (Machut et al. 2007), have access to more food and thus would have a higher lipid content.

METHODS

Initial Field Collection and Chemical Analysis

The first part of this study focused on determining whether or not there was a correlation between lipid content and bioelectrical impedance. For this purpose, 20 eels of varying size were caught in the Vlockie Kill, Castleton, New York, a small tributary of the Hudson River, using a Smith-Root LR 24 backpack electroshocker. The eels were brought back to the laboratory where they were over-anesthetized with clove oil.

Impedance measurements were taken using a Quantum X tetrapolar BIA device (RJL systems) fitted with 2 cm sub-dermal needles. Prior to sampling, the device was calibrated using an included 500 ohm resistor. Individuals were placed on a non-conductive surface, which consisted of a lab bench covered in burlap, and were dried with paper towels. The electrodes were inserted laterally directly behind the gill opening and at a point near the tail that was sufficiently fleshy to allow current to pass between electrodes. Each electrode consists of a positive and negative needle and each was placed horizontally with the positive needles closest to one another. The distance between the positive poles was measured with a ruler.

Figure 1. Electrode placement and measurement of the distance between innermost electrodes during BIA.

Outputs from the BIA device, which consisted of resistance and reactance measurements, were then recorded. After BIA analysis, each individual was weighed with a triple-beam balance and total length (TL) was taken with a measuring board. The eels were then stored in Ziploc freezer bags at -79° C for later chemical analysis.

Chemical analysis of lipid content was done to determine the correlation between lipid content and impedance measurements. Each eel was first homogenized using an industrial blender. Eel skin is extremely resistant to blending and it was necessary to score it using a razor blade prior to blending. Additionally, smaller eels (<25 g) often required further homogenization using a smaller handheld homogenization device. Larger eels (>100 g) had to be cut into smaller sections prior to blending. Once homogenized, three one-gram samples were taken from each individual and used for lipid analysis. Lipid content from the three samples was averaged for each eel.

Eel lipid content was measured using the Folch method for determining whole body lipid content of fish (Folch et al. 1957). Each sample was placed in 20 ml of 2:1

chloroform/methanol solution. The solution containing the homogenate was then manually shaken every 10 min over the course of an hour. The chloroform/methanol solution was then transferred into a separatory funnel while the homogenate was filtered out using #1 qualitative filter paper. The remaining solution was shaken with 4 ml of 0.1 M NaCl, which caused the more polar methanol layer, containing polar lipids and proteins, to separate from the less polar chloroform, which contained the non-polar lipids. The chloroform layer was then transferred into a 50 ml round bottom flask and evaporated using a rotary evaporator with a 30 °C water bath. Round-bottom flasks were then allowed to further dry under a fume hood until a stable mass was achieved. The lipid mass was then recorded and used to calculate total body lipid for each eel.

Analysis consisted of determining regressions between total body lipid and resistance and reactance measurements from the BIA device. Resistance measurements were corrected following Duncan (2008): Whole body resistance = (Distance between positive electrodes)^2/Resistance. A curve was fit to the whole body resistance (ohms) to total lipid (g) relationship. Additionally, both total lipid and percent lipid were regressed against Fulton's Condition Factor (K) using both linear and power functions.

Applied Field Study

The second part of this study consisted of field observations comparing BIA measurements between different eel populations. Eels were sampled at an upstream and downstream location in two tributaries of the Hudson River, Black Creek (Esopus, NY) and Hannacrois Creek (Coeymans, NY).

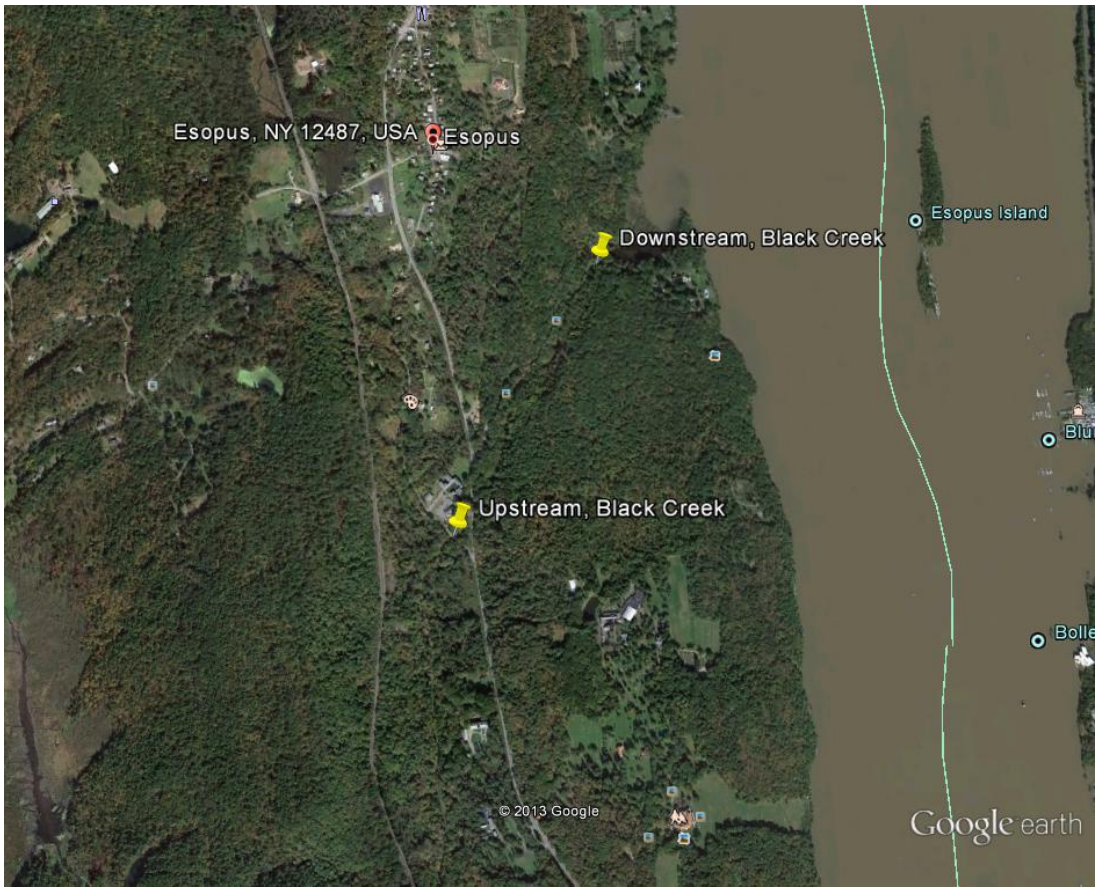

Figure 2. Map of eel sampling sites on Black Creek, Ulster County, New York. Distance between sites was 0.87 km.

In Black Creek, the downstream site was just upstream of the bridge on Winding Brook Rd., approximately 50 m upstream of the tidal Hudson River. The upstream site on Black Creek was upstream of the western bridge on Black Creek Road.

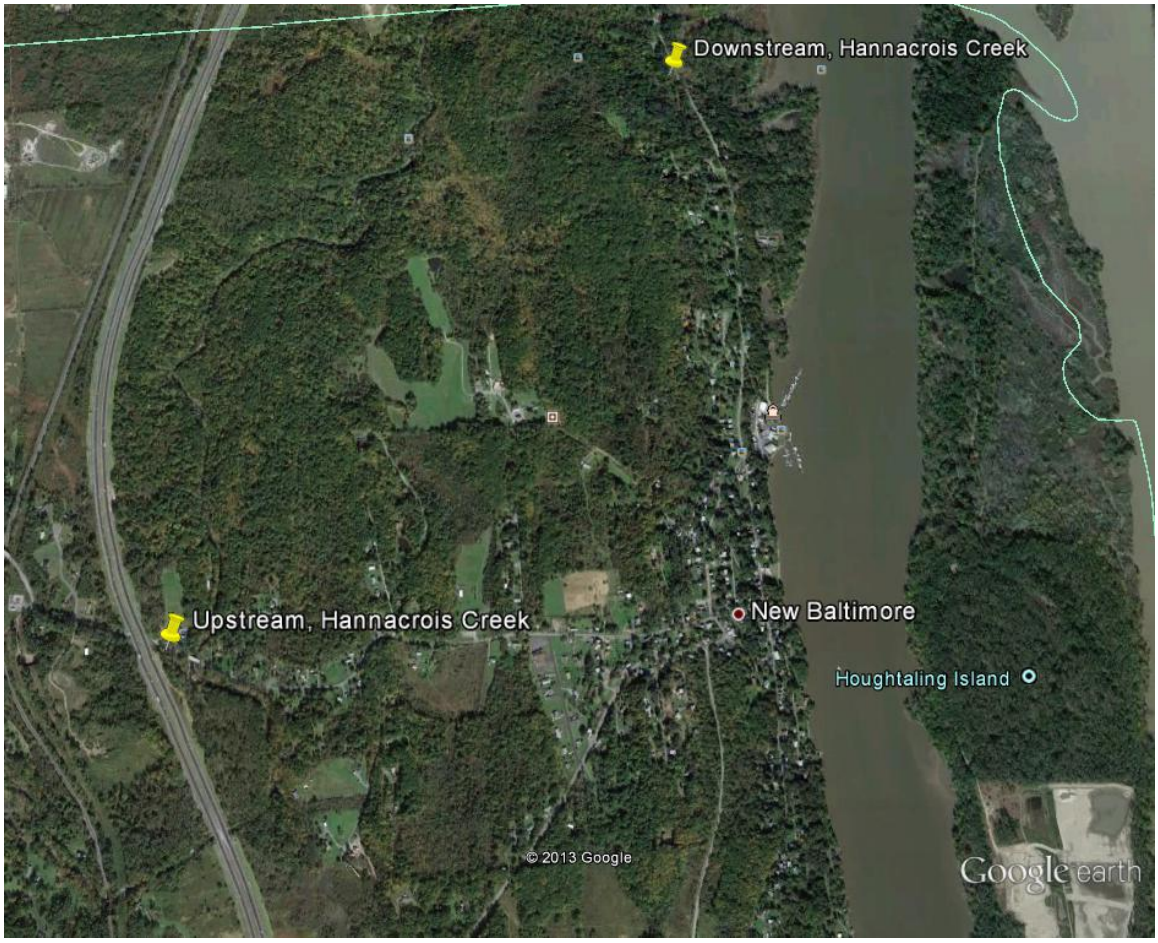

Figure 3. Map of eel sampling sites on Hannacrois Creek, Greene/Albany County, New York. Distance between sampling sites was 2.62 km.

The downstream site on Hannacrois Creek was upstream of the Rt. 144 bridge, in tidal water. The upstream site on Hannacrois Creek was downstream of the Rt. 9W overpass.

At each of the sites, 25 eels were caught for analysis after approximately an hour of electrofishing, except for the upstream Hannacrois site at which only six eels were caught over the course of an hour. Eels were anesthetized with clove oil and measured, weighed, and impedance measured according to the same procedures described above. Eels analyzed in the field were placed on a portable, non-conductive surface constructed

using two 1x4 inch pieces of wood and a cotton towel to measure impedance. Prior to using the BIA device at each site, it was calibrated using the 500 ohm resistor. The device showed very little variance when connected to the resistor and no corrections were required for the impedance data.

After measurements were taken, eels were placed in a recovery bucket filled with fresh water from the creek. Once the eels recovered from the clove oil, they were released back into the creek. Eels that did not recover from the clove oil were taken back to the lab and frozen for future analysis. Using the relationship determined in the first part of the study, total lipid content was calculated for each eel. Total lipid content was then compared between upstream and downstream populations in the respective creeks using a two-tailed T-test. Additionally, a two-tailed T-test was used to determine if there was a significant difference in lipid content between the creeks using combined data from upstream and downstream populations.

RESULTS

Initial Field Collection and Chemical Analysis

A total of 21 American eels were collected in the Vlockie Kill. The average total length was 34.4 cm (20.0-61.0) and the average weight was 99.8 g (17.5-436.5). When corrected for electrode width, resistance measurements showed a strong correlation (R^2 = 0.84) with total lipid content (Figure 1). The best fit regression was a power relationship with the equation:

$$Y = 31.536\ X^{1.4262}$$

where Y is total lipid content in grams and X is the corrected whole body resistance.

Fulton's condition factor did not correlate well with either percent lipid or total lipid of the collected eels (Figures 2 & 3). Percent lipid ranged from 3.1-15.6, within the range of percent lipid reported for eels from the tidal Hudson River (Steinbacher 2001; Steinbacher and Baker 2002).

Applied Field Study

Percent lipid content for eels in upstream and downstream populations in Hannacrois Creek was found to be significantly different ($P = 0.0493$). In Hannacrois Creek, the average percent lipid content per eel was 21.4% downstream and 26.7% upstream (Figure 4). In Black Creek where the difference between upstream and downstream lipid content was highly significant ($P = 0.000192$) the average percent lipid content per eel downstream was 11.6% while upstream it was 16.8%. Difference in percent lipid content for eels between the two creeks was found to be highly significant ($P < 0.0001$).

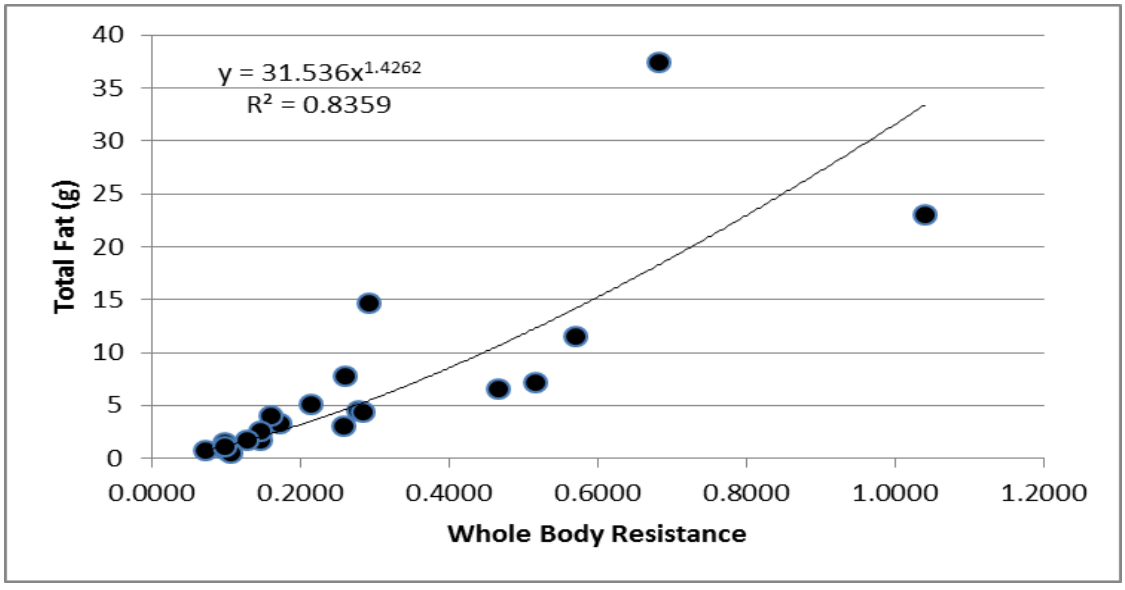

Figure 4. Regression showing correlation between total body lipid and corrected resistance.

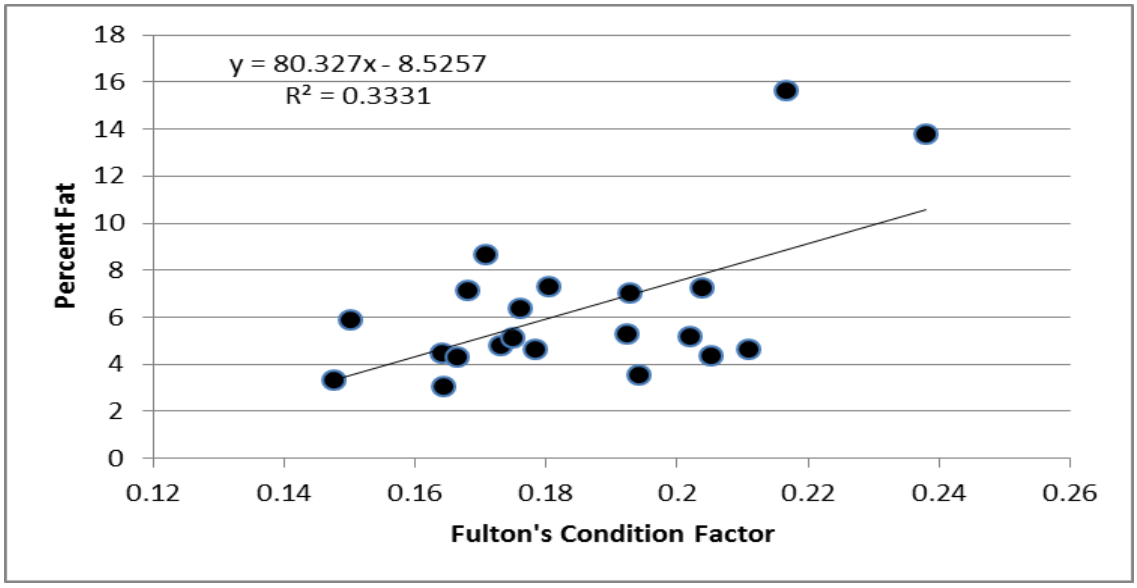

Figure 5. **Linear regression showing lack of correlation between Fulton's Condition Factor (K) and percent lipid in eels.**

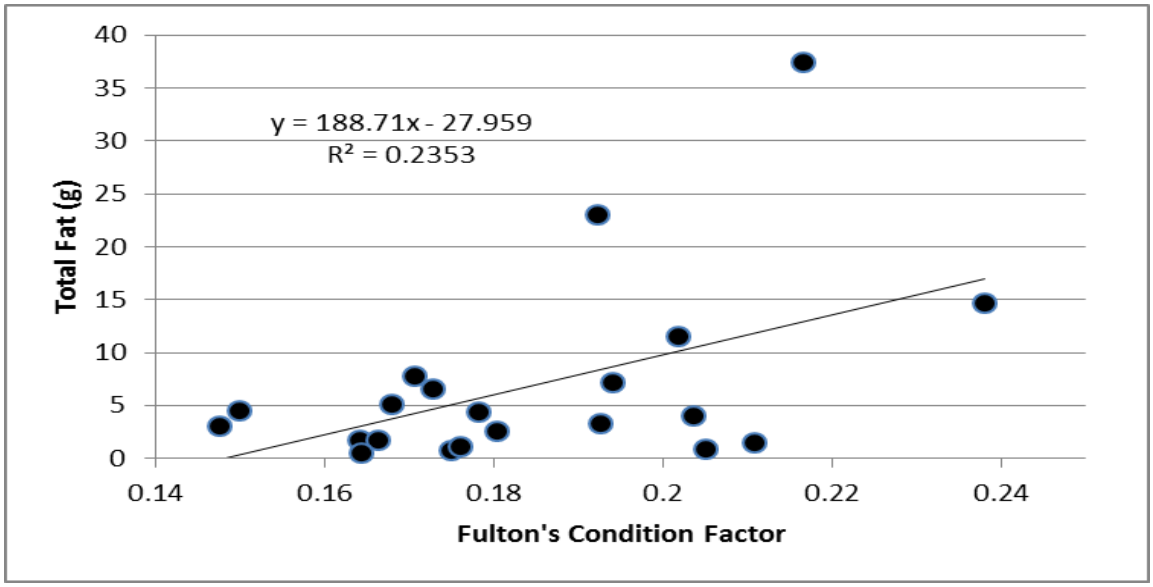

Figure 6. **Linear regression showing lack of correlation between total Fulton's K and total lipid in collected eels.**

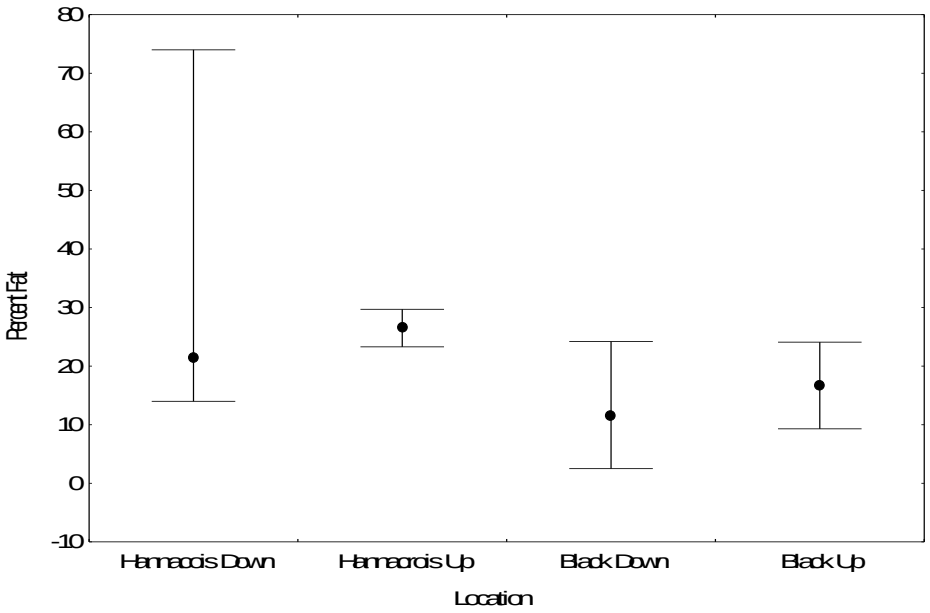

Figure 7. Mean (solid circle) and range (capped line) of percent lipid content of American eel upstream and downstream in Hannacrois Creek and upstream and downstream in Black Creek, Hudson River, NY.

DISCUSSION

Based on the results of this study, Bioimpedance Analysis appears to be a viable field method for determining lipid content in American eels. The strong statistical correlation between total lipid content and corrected resistance ($R^2 = 0.84$) indicates that lipid content could be successfully predicted using the regression developed in this study. Duncan (2008) performed a similar study on four different species of fish, and found R^2 values ranging from 0.94 to 0.75 when relating total body lipid to corrected resistance. Both extracted lipid levels from the initial sample group and predicted lipid levels were within the known range for American eels. It is worth noting that the majority of previous BIA studies on fish have used the Bligh and Dyer (1959) method for lipid extraction, which is a modified version of the Folch method (Folch et al. 1957), to determine total body lipid. According to Iverson et al. (2001), the Bligh and Dyer

method should only be used for the analysis of muscle tissue on fish with low lipid content. The decision to use the Folch method was therefore warranted, but might complicate lipid content comparisons with previous BIA studies.

Fulton's Condition Index predicted lipid content poorly, as expected. Findings of this study confirm observations that both Fulton's condition factor and the Heptosomatic Index (HSI) were a poor indication of lipid content in fish (Peters et al. 2007). Although more population specific condition indices can be derived (Froese 2006), the relative ease and effectiveness of BIA makes it a more appealing alternative. Additionally, once the eels are anesthetized for BIA, taking length and weight measurements is quite simple. Condition indices need not be sacrificed in order to perform BIA and a combination of the two methods could result in a broader understanding of the population(s) being studied.

Due to its quantitative and non-lethal nature, Bioimpedance Analysis constitutes a significant step forward when it comes to performing larger scale studies of eel health between populations. Although the method is relatively simple to perform, it should be noted that uniform electrode placement is difficult to achieve with eels due to their lack of anatomical landmarks. Though it was desirable to place the electrodes as far away from one another as possible, so the data would best represent the whole eel, the meter would read maximum when the electrode was placed too near the tail. This was solved by moving the electrodes closer to one another. Another difficulty of BIA is that it requires the eels to be completely immobile. Though this is achievable with clove oil, it requires careful application to avoid overdose. In the end the benefits of BIA clearly outweigh the few difficulties posed in its application.

The results of the field study confirmed the initial hypothesis that upstream eel populations (mean TL = 41.8 cm) would have a higher lipid content than downstream populations (mean TL = 33.1 cm) in Hannacrois Creek and in Black Creek (mean TL upstream = 28.7 cm; downstream = 31.3 cm). The second finding was that eels in Hannacrois Creek were had a higher lipid content than those in Black Creek. These data, however, are confounded by differences in average size of the eels collected. Larger eels are expected to have higher lipid content. Given the number of variables at play, it is difficult to hypothesize what other factors may be responsible for this pattern, but it certainly warrants further investigation. The qualitative observations at the two streams indicated that eel populations were much lower upstream and that crayfish, primary prey of large eels (Machut 2006), were more abundant. It has been suggested that the higher density of eels downstream, where there is greater competition between individuals, is directly responsible for reduced condition (Machut 2006) or in this case reduced lipid content. It has also been suggested that urbanization plays a broader role in eel health including direct effects such as pollution and barrier construction (Machut et al. 2007). Since these observations have thus far relied on condition indices, it would certainly be worth reinvestigating them using Bioimpedance Analysis.

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LITERATURE CITED

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

Cox, M. K. and K J Hartman. 2005. Nonlethal estimation of proximate composition in fish. Canadian Journal of Fisheries and Aquatic Sciences, 62: 269-275.

Duncan, M. B. 2008. The use of bioelectrical impedance analysis for estimating the body composition of various fish species. MS Thesis, Virginia Polytechnic Institute and State University.

Folch, J., M. Lees, and G. H. S. Stanley. 1957. Journal of Biological Chemistry, 226: 497-509.

Froese, R. 2006. Cube Law, Condition Factor and Weight-length Relationships: History, Meta- analysis and Recommendations. *Journal of Applied Ichthyology* 22.4: 241-53.

Iverson, S. J., L. Shelley, C. Lang, and M. H. Cooper. 2001. Comparison of the Bligh and Dyer and Folch methods for total lipid determination in a broad range of marine tissue. Lipids 36: 1283-287.

Machut, L. S. 2006. Population dynamics, *Anguillicola crassus* infection, and feeding selectivity of American eel (*Anguilla rostrata*) in tributaries of the Hudson River, New York. MS Thesis, State University of New York College of Environmental Science and Forestry.

Machut, L. S., K .E. Limburg, R. E. Schmidt, and D. Dittman. 2007. Anthropogenic impacts on American eel demographics in Hudson River tributaries, New York. Transactions of the American Fisheries Society 136: 1699-1713.

Peters, A. K., M. L. Jones, D. C. Honeyfield, and J. R. Bence. 2007. Monitoring energetic status of Lake Michigan Chinook salmon using water content as a predictor of whole-fish lipid content. Journal of Great Lakes Research 33: 253-263.

Steinbacher, J. C. 2001. The American eel, Anguilla rostrata, as a sentinel species of PCB contamination in the Hudson River. M.S. thesis, University of Maryland, College Park, Maryland.

Steinbacher, J. C. and J. E. Baker. 2002. A spatial examination of Hudson River American eel (Anguilla rostrata) and surficial sediment PCB contamination. Pp. 106-125. In. D. H. Secor and J. E. Baker. Ecology and Contamination of the Hudson River American Eel. Final Report to the Hudson River Foundation.

Tocher, D. R. 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Reviews in Fisheries Science 12: 107-184.

Pictured (left to right): Craig T. Connolly, Ryan Carthan, Sean O.I. Swift, Erin Schneider, Grace A. Casselberry, Jessica A. Gephart, Emily Ogburn, and Rebecca E. Kulp

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