REPORTS OF THE TIBOR T. POLGAR

FELLOWSHIP PROGRAM, 2008

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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 2008. Major objectives of these studies included: (1) discovering evidence for a hypervelocity bolide impact event around 2300 years ago that produced a tsunami, and detailing the extent of that tsunami's effects, (2) monitoring the nutrient overenrichment-eutrophication-hypoxia (NOEH) cycle on Newtown Creek to test the hypotheses that oxygen levels are microbially-mediated and related to the balance between autotrophy and heterotrophy, (3) investigating the effects of surface roughness and exposure to wave intensity on the accumulation of organic matter, growth of chlorophyll *a* and the colonization of macroinvertebrates, (4) analyzing the effects of shoreline habitat complexity, substrate diversity, and disturbance exposure on the abundance and diversity of snails, (5) applying the equilibrium temperature concept to test the hypothesis that changes in water temperature are a result of the zebra mussel invasion, (6) determining the ecological, morphological, and genetic relationships among populations of clam shrimp from three localities, (7) examining pre-winter energy reserves and otolith microstructure to determine hatch-dates, and cohort structure, and compare cohort-specific growth rates of juvenile bluefish, and (8) assessing the Double-crested Cormorant diet to determine if fish species with difficult-to-digest spiny fins would show up in pellets in higher proportions than those species with soft-rayed fins, relative to their proportions in the bolus samples.

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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 2008 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 estuary 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 2008. 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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EVIDENCE FOR A TSUNAMI GENERATED BY AN IMPACT EVENT IN THE NEW YORK METROPOLITAN AREA APPROXIMATELY 2300 YEARS AGO

A Final Report of the Tibor T. Polgar Fellowship Program

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Cagen, K. and D. Abbott. 2009. Evidence for a tsunami generated by an impact event in the New York metropolitan area approximately 2300 years ago. Section I: 23 pp. *In* S.H. Fernald, D. Yozzo and H. Andreyko (eds.), Final Reports of the Tibor T. Polgar Fellowship Program, 2008. Hudson River Foundation.

ABSTRACT

Oceanic impacts such as the one discussed in this paper, which we propose occurred approximately 2300 years ago in the Atlantic Ocean, are a poorly understood phenomenon. Though the Earth is seventy percent covered with water, scientific investigations have focused on continental events. This is in part due to the difficulties inherent in examining submarine impact structures. Oceanic impacts lack many of the known features of continental events. However, oceanic impacts, unlike their continental counterparts, produce catastrophic tsunami events that may be used to identify them. Recent discoveries point to a tsunami event that affected the New York metropolitan area approximately 2300 years ago (Goodbred et al. 2006). Here it is shown that impact ejecta found in the tsunami deposit layer underneath the Hudson River indicate an oceanic impact as the source of the tsunami. The sharp resolution of the stratigraphic study of the cores suggests that the sediment containing impact ejecta was deposited in a tsunami-like event, rather than being reworked from an older event. Individual ejecta grains were identified through an examination of samples from the tsunami layer with optical and electron microscopy, as well as compositional analysis via energy dispersive X-ray spectroscopy. Carbon and aluminum silicate impact spherules were found in the samples. Also present in the samples were shock-metamorphosed phases of feldspar, ilmenite, and olivine exhibiting planar deformation features and Brazil twinning consistent with studies of known impact ejecta. TEM studies of the spherules revealed the presence of associated hexagonal nanodiamonds, also known as lonsdaleite, which are uniquely related to shock formation. In addition, the New York area lacks the extreme seismic and volcanic activity that might produce similar results, leaving a hypervelocity bolide impact as the most likely source for the tsunami event and associated impact ejecta. As oceanic impacts pose a serious threat to coastal communities around the world, it is necessary to understand both their frequency and effects. It is hoped that this method of identifying an oceanic impact via the ejecta found in tsunami deposits will improve our understanding of submarine impact events.

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INTRODUCTION

We propose that the source of the tsunami event recently discovered to have occurred in the New York area approximately 2300 years ago was the submarine impact of a hypervelocity bolide, a type of event that is poorly understood by the scientific community. Though the Earth is approximately seventy percent covered by water, less than twenty percent of known impact craters are located underwater (Gersonde et al. 2002). This discrepancy is the combined result of a lack of investigation into submarine impacts and the difficulty inherent in identifying them. The geological structures produced by submarine impacts are difficult to find, and are often changed by erosion and blanketed by new sediment. They also differ greatly from their continental counterparts, with which scientists are more familiar (Strelitz 1979). In the search for oceanic impacts, it is necessary to create more reliable methods of identification, understanding that the characteristics of submarine impacts can vary greatly from those of continental events.

One of the most unique features of a submarine impact is the tsunami. A submarine impact can produce a megatsunami wave with a run-up in the hundreds of meters, depositing oceanic sediment and impact ejecta (terrestrial material recognizably altered by the pressure and heat of an impact; impact debris) kilometers from the impact site (Bryant 2001; Crawford and Mader 1998). It is therefore possible to identify an impact through the traces that the ensuing tsunami left in the geological record. The deposition of sediment from tsunami waves of the magnitude produced by bolide impacts occurs over an incredibly short interval, from minutes to a few hours, rarely even days (Dypvik and Jansa 2003). This sudden deposition produces a well-defined, visible contact between the tsunami deposit and the underlying sediment.

Goodbred et al. (2006) provide compelling evidence for a tsunami event in the New York metropolitan area approximately 2300 years ago. They record horizontal runups

of hundreds of meters inland from Long Island's Great South Bay, which correspond to an abnormally large wave. We hope to expand on Goodbred et al.'s work on samples from the Great South Bay by further detailing the extent of the tsunami's effects up the Hudson River and establishing the origin of the tsunami to be a hypervelocity bolide impact by discovering evidence for an impact in material deposited by the tsunami further up the Hudson River. Sampling of the tsunami layer in the Hudson has revealed multiple pieces of impact ejecta (impact debris), including shocked minerals and impact spherules. These ejecta strongly suggest that an impact event around 2300 years ago produced a tsunami that hit the New York metropolitan area.

METHODS

Sediment samples of the tsunami layer are from core samples of the bottom of the Hudson River near Piermont, New York. Samples were taken from the layer in sediment cores CD01-01, CD01-02, SD30, and VM 32-2 from the Hudson River (see Figure 1). The thickness of the layer ranged from approximately half a meter in CD01-02 to four centimeters in VM32-2. Both the macroscopic descriptions of the cores and photographs of the cores were used to identify potential ejecta layers, specifically descriptions that indicated the presence of glass or large coarse fractions and core photographs that showed sharp contacts between layers of sediment, indicative of a sudden deposit of material like that from a tsunami.

Individual samples generally span two to three centimeters within the core. Control samples were taken from above and below prospective tsunami deposit layers. Samples were sieved with de-ionized water, separated into >150 micrometer, >63 micrometer, and >38 micrometer fractions, and then dried in an oven at 60° Celsius. The >150 micrometer fraction (coarse fraction) was then examined with a Nikon SMZ2800 optical microscope. Grains that seemed to have an impact origin, including spherules, broken or strangely-

shaped grains, quartz with shock lamellae, and magnetic fragments were selected for further examination. Selected grains were placed on mounts, which were then examined with scanning electron microscopy. After the samples were coated in platinum with a Cressington SputterCoater 208, high-magnification photographs were taken with a Philips ESEM (Environmental Scanning Electron Microscope). Elemental analysis was also performed with an attached EDAX (Energy dispersive X-Ray analysis system). These analyses provide important insight about the nature of individual grains of ejecta, and therefore accompany the SEM photomicrographs. Final analysis was based on both the photographs and elemental analyses.

RESULTS

The impact ejecta found in the samples can be organized into four categories: impact spherules, shock-metamorphosed minerals, impact generated textures, and impact generated rocks.

Impact Spherules

Spherules are millimeter-sized glassy spheres composed of minerals that were melted in the impact and re-solidified in the near-vacuum left in the wake of the impactor (Melosh and Vickery 1991). Spherules of varying chemical compositions were found throughout the sampled tsunami layers.

Many of the spherules are composed primarily of carbon, though they vary in texture, appearing both vesicular (Figures 2 and 3) and glassy (Figures 4 and 5). Some of the carbon spherules contain mixtures of other elements, or have splash (once-melted minerals, visible in Figure 6) on their surfaces (Figures 4 through 7). We also found a spherule with many partially formed spherules (microspherules) on its surface (Figures 8 through 13). Some spherules, such as the spherule in Figures 12 and 13, exhibit quench texture, a surface of randomly oriented crystals that is produced by the rapid cooling of the

melted spherule as it lands in the water and further solidifies the association of spherules with an impact event. The other spherules are composed primarily of silicate, SiO_2. Some of these, such as the spherule in Figures 14 and 15, have extraordinarily smooth surfaces. Others, such as the spherules in Figures 16 through 21, also exhibit quench texture or other surface features, such as the ilmenite needles in Figures 22 and 23.

Shock-metamorphosed minerals

Pressure on the host rock during an impact event can exceed over one hundred gigapascals at the point of impact. This immense pressure affects minerals in ways unique to hypervelocity bolide impacts, called shock-metamorphic effects. These changes are often visible as planar deformation features (PDF's) in quartz, feldspar, and other minerals. These features are visible in the feldspar in Figures 24 though 27 and the olivine in Figures 28 through 31. (Chao 1967, French 1998). Impact breccias are the result of several angular pieces of rock smashed together to form a single grain under immense pressure (French 1998). Though breccias are produced by several geological processes, the ilmenite in Figures 32 and 33 also exhibits classic shock-metamorphism, with offsets of curved lamellae similar to those of known shocked ilmenite specimens (Harris et al. 2005). Brazil twinning is a particular zig-zag pattern of shock lamellae produced under high pressure (Stöffler and Langenhorst 1994). It is clearly visible in Figures 26 and 27 that the lamellae are generally continuous and less than one micrometer apart. The olivine grain in Figure 28 also exhibits very closely spaced planar fractures, visible in the close-up (Figures 30 and 31).

Impact Generated Rocks

Ilmenite, or iron titanium oxide ($FeTiO_3$), has been found in two significant forms in the tsunami deposits. Figure 32 shows a shocked ilmenite breccia, while Figures 22 and

23 show nanoscale needle-like forms of ilmenite, both of which imply an impact origin for the tsunami.

Nanodiamonds

Nanodiamonds are formed under the intense pressure of impact, are incredibly rare and almost exclusively related to impact events. In particular, the form of diamond called lonsdaleite, which has a specific hexagonal structure, has been found exclusively associated with meteorites and impact events (Lipschutz and Anders 1961). Hexagonal-form nanodiamonds (Figures 34 through 37) were found associated with some of the carbon spherules from the tsunami deposit layers, and are visible as Transmission Electron Microscope images in Figures 34 and 36.

These nanodiamonds were identified as lonsdaleite through their electron diffraction patterns, as visible in Figures 35 and 37. These patterns are produced by observing the scattering of a beam of electrons due to the internal lattice structures of a grain. These patterns are therefore uniquely related to the internal structure of a grain, and in this case the hexagonal pattern allowed us to conclusively identify the nanodiamonds found with the associate spherules as lonsdaleite.

DISCUSSION

Spherules

The presence of spherules strongly suggests an impact origin; spherules are associated with numerous impact events, including events in Bosumtwi (Ghana), South Africa (Koeberl 1994, Lowe and Byerly 1986), and Western Austraila (Lowe and Byerly 1986). Spherules have also been found in the Barringer Meteorite Cater (Arizona) (Masaitis 2006) the Wabar crater (Saudi Arabia) (Mittlefehldt et al. 1992,) and the Lonar crater (India) (Murali et al. 1987). Spherules have been found in abundance in distal ejecta deposits from the Chicxulub impact, at the Cretaceous-Tertiary boundary (Powell 1998).

Spherules are particularly common in lunar materials, where their abundance may reflect the high frequency of small impacts due to the lack of a lunar atmosphere (Symes et al. 1998). In particular, carbon spherules similar to those found in this investigation were found at the site of a proposed impact that contributed to the Younger Dryas cooling (Firestone et al. 2007).

Shock-metamorphism

The shock-metamorphism discovered in the tsunami layer is a clear indicator of an impact event; impacts are the only natural source for such effects. In particular, the shock-metamorphosed ilmenite breccia (Figure 32) and feldspar with Brazil twinning (Figures 24 through 27) are undeniably the results of an impact. True shock metamorphism is unique to impact events, and therefore the presence of both shock metamorphism and brecciation is strongly indicative of an impact origin. Although widely spaced Brazil twinning can be produced by geological processes other than impacts, the spacing of the twin planes in Figures 26 and 27 indicates an impact origin. While a spacing of less than one micrometer is not uncommon for impact events, planes from other processes tend to be at least 10 micrometers apart and vary in continuity (Alexopoulos 1988; Koeberl 2002). Thus, the shock-metamorphosed feldspar in Figure 24, which exhibits Brazil twinning, clearly has an impact origin. The occurrence of planar fractures in olivine is also characteristic of shock metamorphism (Stoeffler 1972).

Impact Generated Rocks

The ilmenite breccia is a clear indicator of an impact event. Similarly indicative of an impact origin are the ilmenite needles exhibited in Figures 22 and 23. These ilmenite needles are very similar to ilmenite needles found in lunar craters (Heiken and Vaniman 1990) and have been identified in ejecta from impact structures on Earth

(Glikson and Allen 2004). Like spherules, the presence of nanoscale ilmenite needles in the tsunami deposits is indicative of an impact origin.

Nanodiamonds

Lonsdaleite is a particular hexagonal form of carbon that is exclusively related to impacts (Frondel and Marvin 1967; Lipschutz and Anders 1961). Nanodiamonds such as the ones found in this event have been found associated with a number of well-known impact structures, including the KT Boundary and the Younger Dryas impact event (Powell 1998; Firestone et al. 2007; Masaitis 1998; Koeberl et al. 1997; Heymann et al. 1966). Electron diffraction analysis has confirmed that the nanodiamonds found in the tsunami deposits are lonsdaleite, which is an unequivocal link between the tsunami and a hypervelocity bolide impact event.

Alternative Explanations

All of the evidence gathered so far supports an impact event. There is simply no other explanation for the tsunami layer, the shocked feldspar, the impact breccia, and the spherules. Though explosive volcanism and high-magnitude seismic activity can produce similar results, the lack of extreme volcanic and seismic activity in the New York area leaves an impact event as the only reasonable cause. Also, the grains show no signs of long-term erosion or transport by water, suggesting that they were produced near their depositional sites. Although the eastern United States has experienced several tsunami-like events during historical times, none in the New York metropolitan area have been of a magnitude comparable to the event described here (Lockridge et al. 2002). The Atlantic, unlike the Pacific, is not bordered by subduction zones, where earthquakes with large vertical offsets would displace enough water to generate a megatsunami (Lockridge et al. 2002). The lack of any source of explosive volcanism in the area further rules out volcanic activity as the source of the tsunami.

Location and Date

Though the proposed impact is submarine, the presence of quartz and K-feldspar, abundant on the continents but not on the seafloor, suggests an impact on the continental shelf. The tsunami layers vary in thickness from 10 to 200 centimeters, indicating proximity to the impact site. The presence of impact ejecta upriver indicates a serious local event, though we have not yet determined the upriver extent of the tsunami.

The date for the impact, approximately 2300 years ago, is given from carbon dating of material in the tsunami layer performed by Goodbred et al. (2006) This date is imprecise due to the lack of a reservoir correction for carbon dating in the Hudson. Future investigations will include more rigorous dating of the event.

CONCLUSION

Our examination of the Hudson River sediments strongly supports our supposition that the tsunami event was generated by an impact. We were able to establish widespread presence of impact ejecta, including spherules and shock-metamorphosed minerals. The presence of ejecta in multiple cores at 2300 B.P. indicates that there was a local impact event at that time. Our discovery of exclusively impact-related nanodiamonds (lonsdalite) at the same age further cements our impact hypothesis. We have conclusively determined that there was an impact event approximately 2300 years ago in the New York area. We hope in future studies to better document the extent of the resulting tsunami, its potential effects on the Hudson River area, and locate the impact site.

FIGURES

Figure 1. Location of core samples in the Hudson River

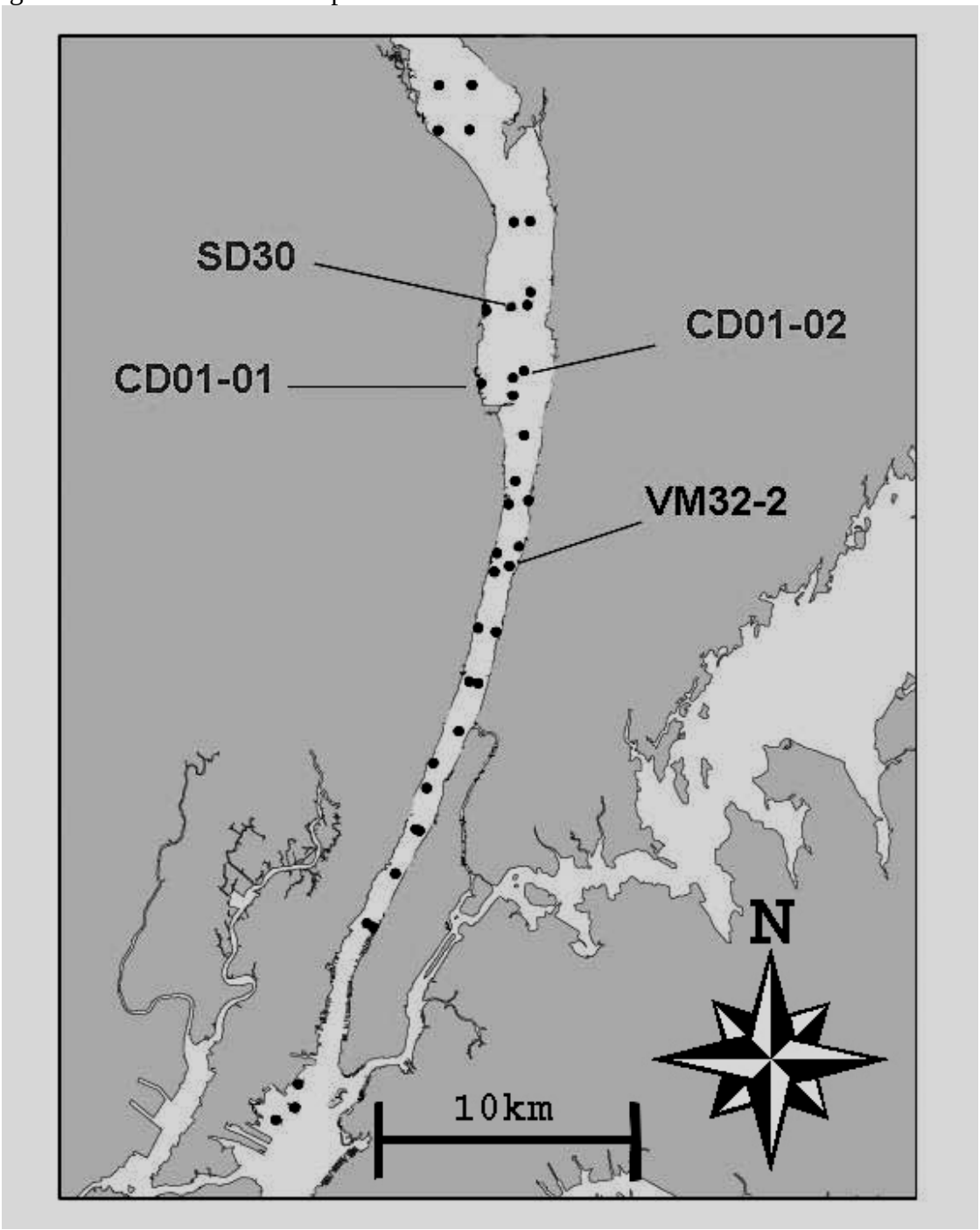


Figure 2. Vesicular carbon spherule

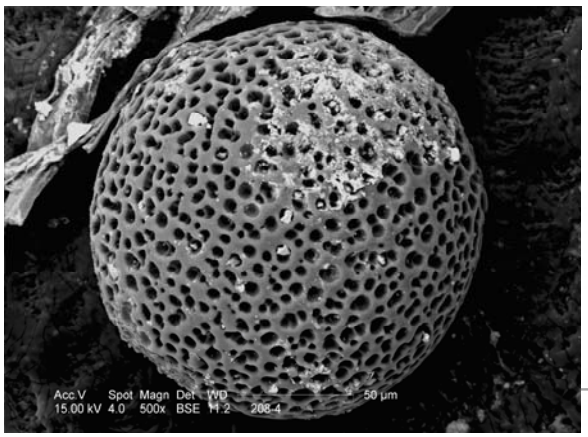


Figure 3. Chemical composition of spherule in Figure 2

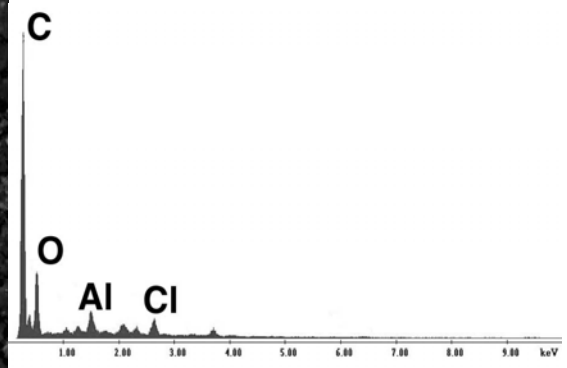


Figure 4. Smooth carbon spherule with pyrite splash

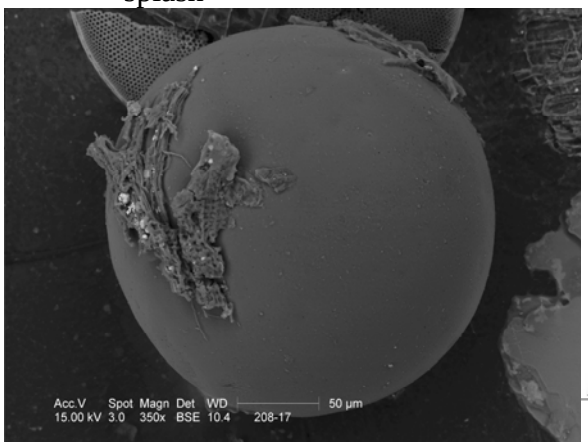


Figure 5. Chemical composition of spherule in Figure 4

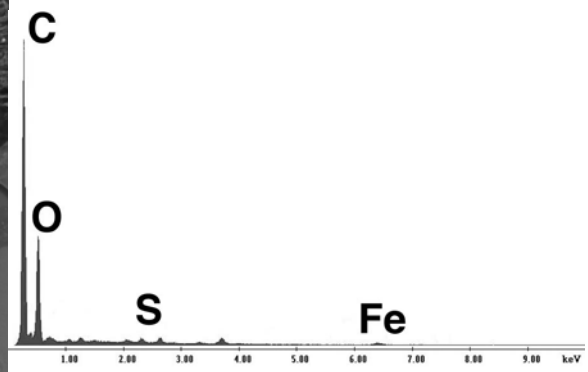


Figure 6. Close-up of pyrite splash on Figure 4

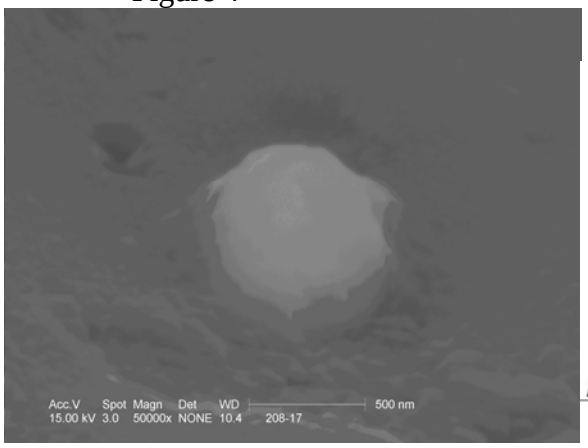


Figure 7. Chemical composition of pyrite in Figure 6

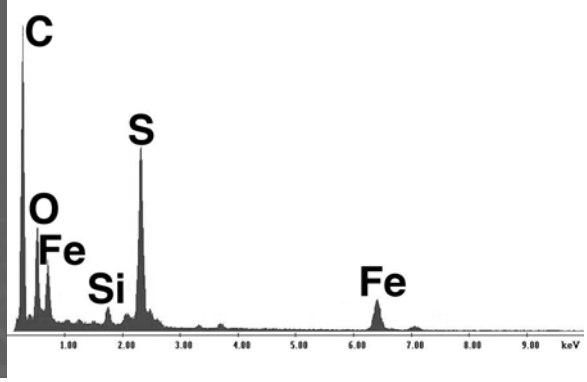


Figure 8. Carbon spherule with pyrite microspherules

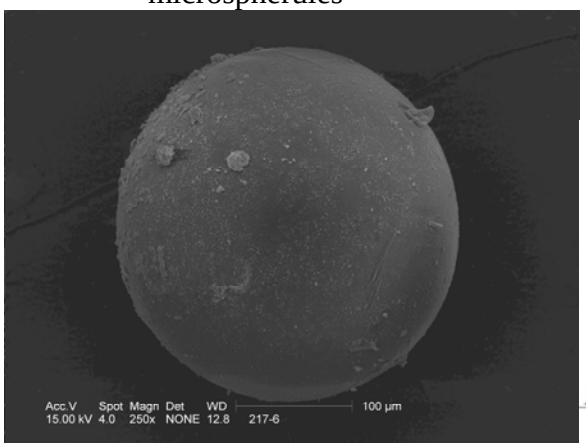


Figure 9. Chemical composition of spherule in Figure 8

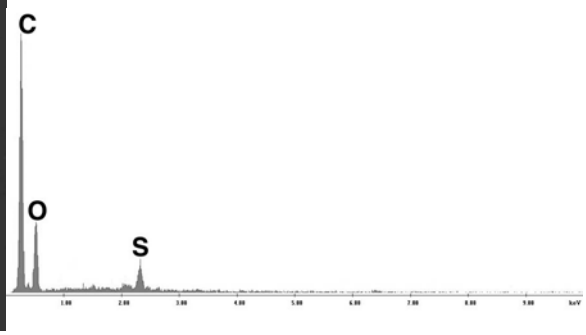


Figure 10. Close-up of microspherules on Figure 8

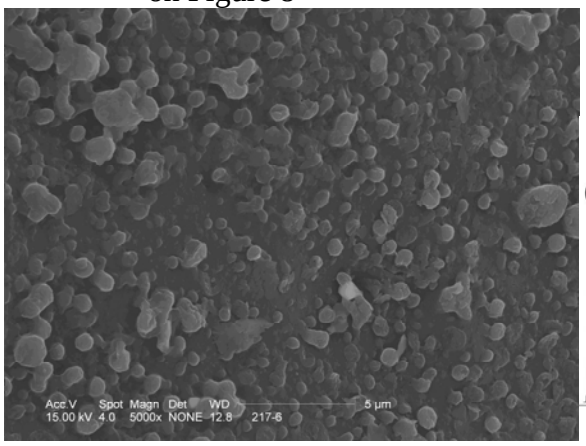


Figure 11. Chemical composition of microspherules in Figure 10

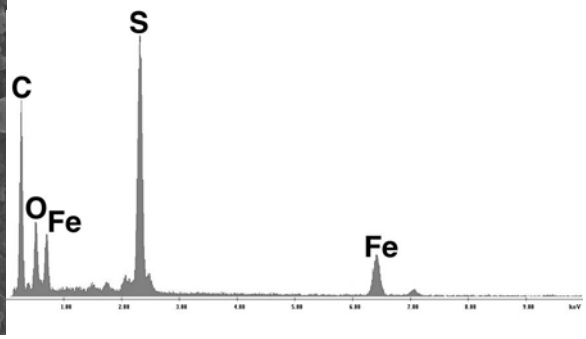


Figure 12. Iron oxide spherule with quench texture

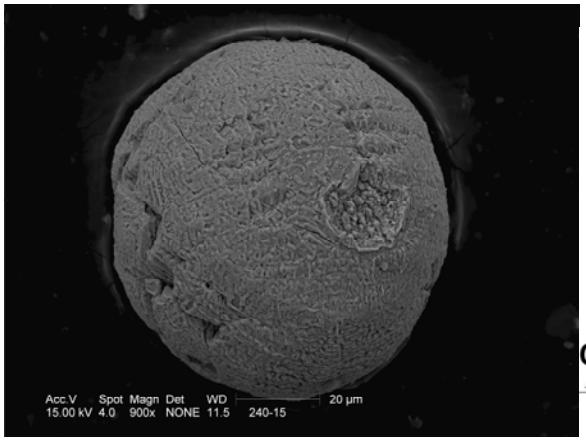


Figure 13. Chemical composition of spherule in Figure 12

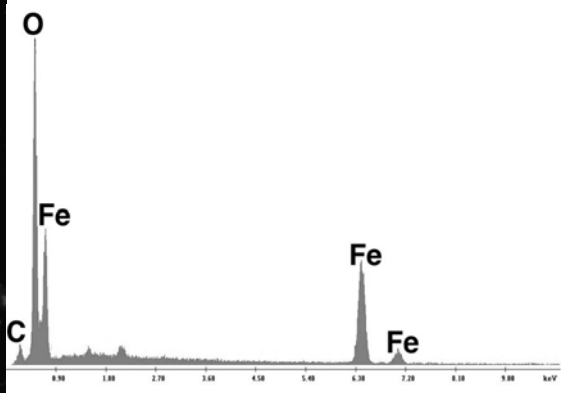


Figure 14. Smooth aluminum silicate spherule

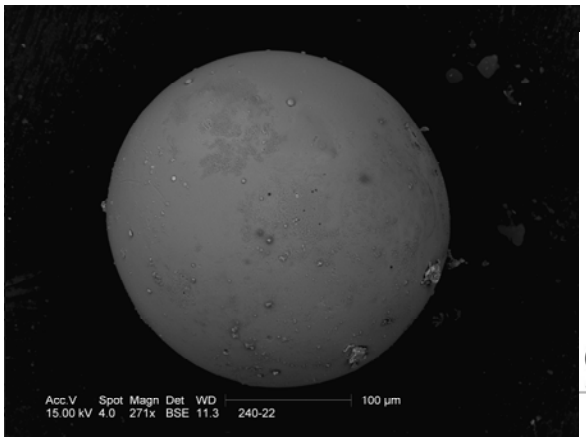


Figure 15. Chemical composition of spherule in Figure 14

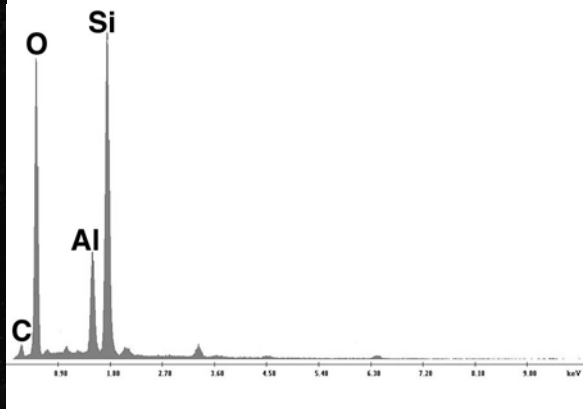


Figure 16. Aluminum silicate spherule with quench texture

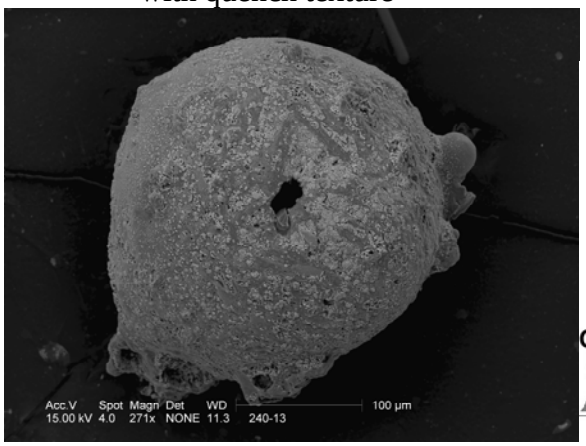


Figure 17. Chemical composition of spherule in Figure 16

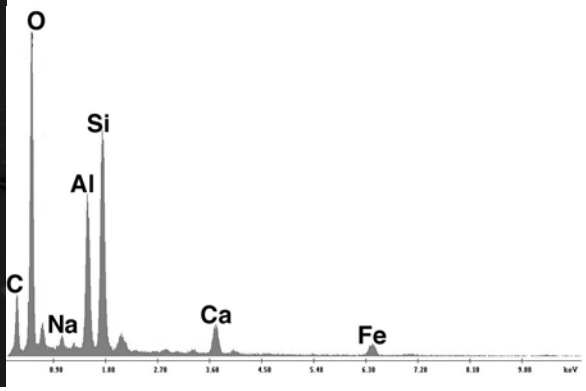


Figure 18. Close-up of quench texture on Figure 16

Figure 19. Chemical composition of surface in Figure 18

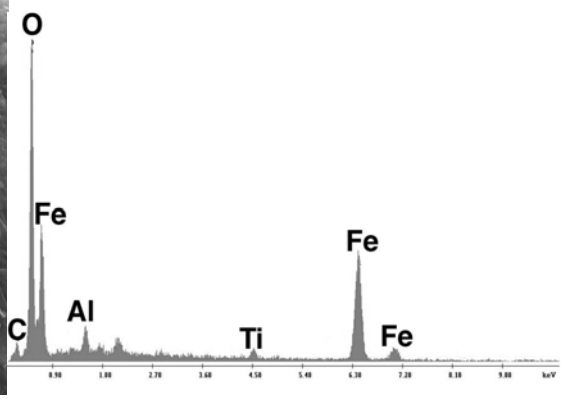


Figure 20. Joined aluminum silicate spherules with ilmenite needles

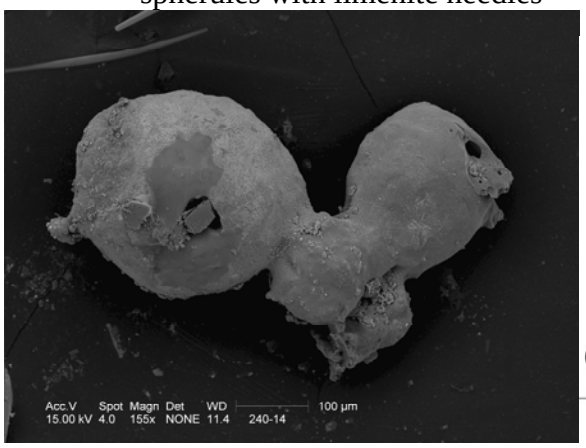


Figure 21. Chemical composition of spherules in Figure 20

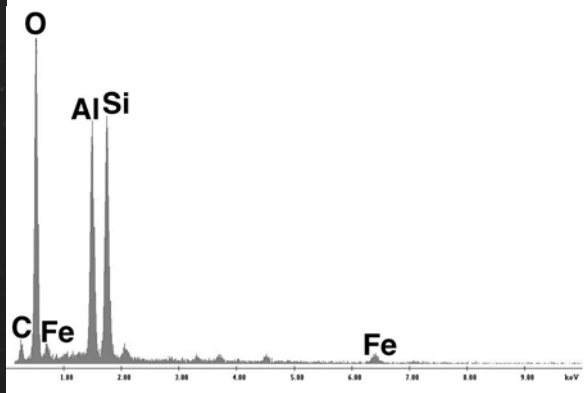


Figure 22. Close-up of ilmenite needles on Figure 20

Figure 23. Chemical composition of needles and substrate in Figure 22

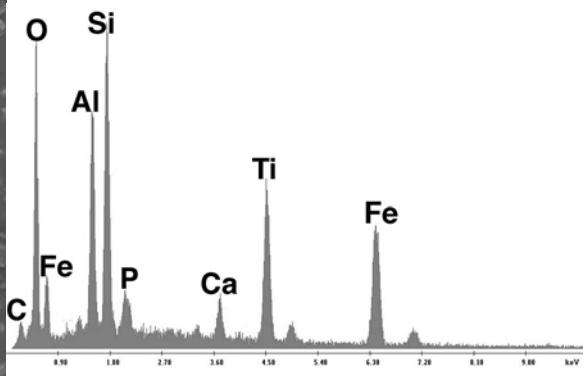


Figure 24. Shocked feldspar with Brazil twins

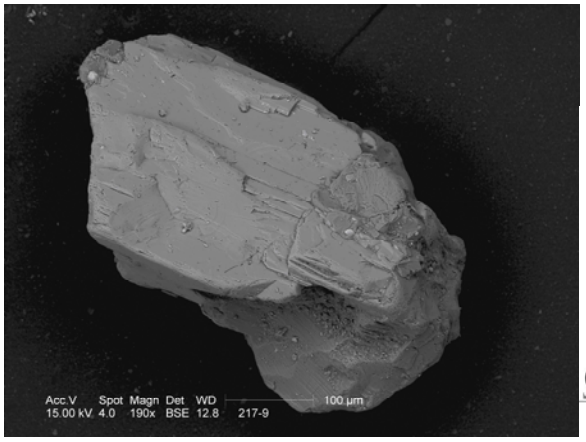


Figure 25. Chemical composition of feldspar in Figure 24

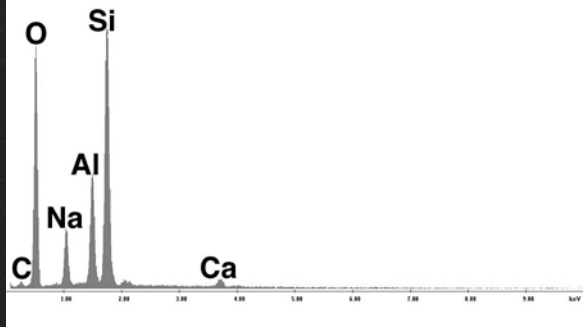


Figure 26. Close-up of Brazil twins on Figure 24 #1

Figure 27. Close-up of Brazil twins on Figure 24 #2

Figure 28. Olivine with planar features

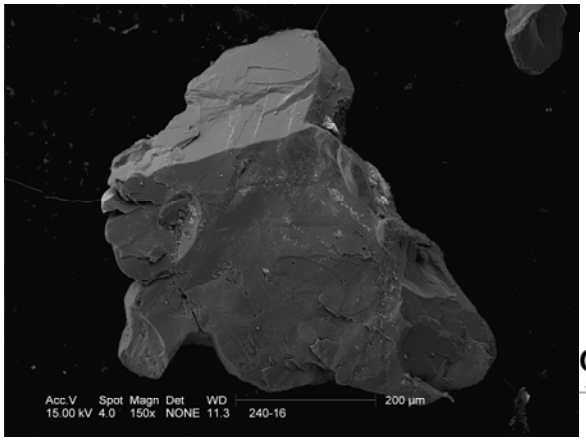


Figure 29. Chemical composition of olivine in Figure 28

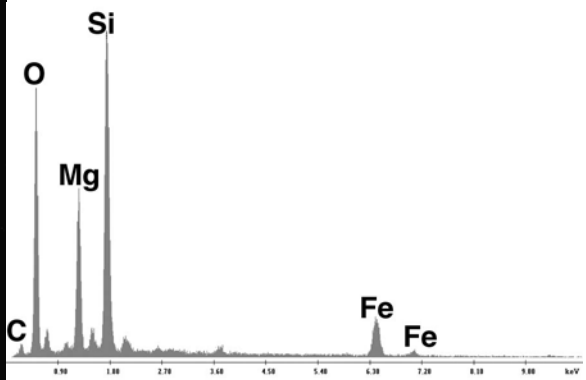


Figure 30. Close-up of planar features on Figure 28

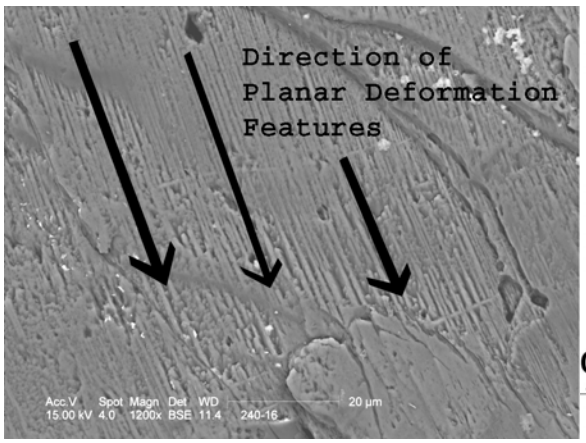


Figure 31. Chemical composition of planar features in Figure 30

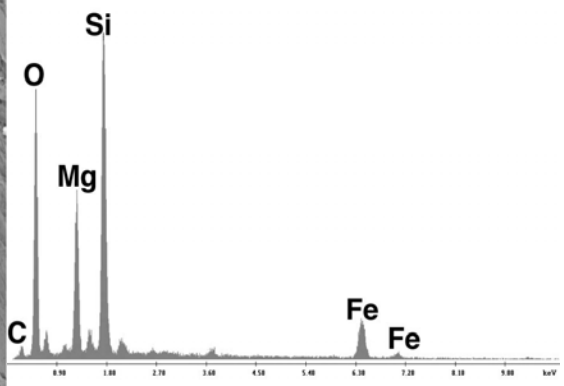


Figure 32. Shocked ilmenite breccia

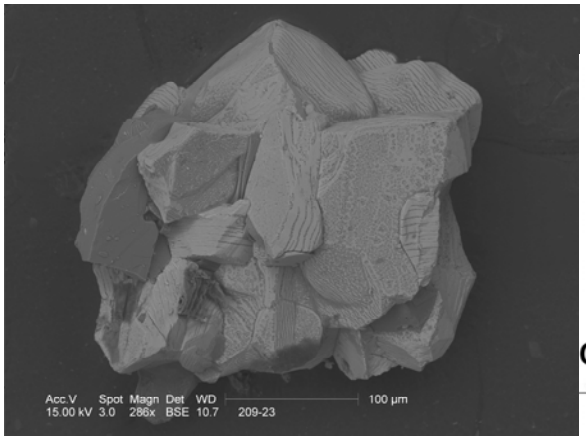


Figure 33. Chemical composition of breccia in Figure 32

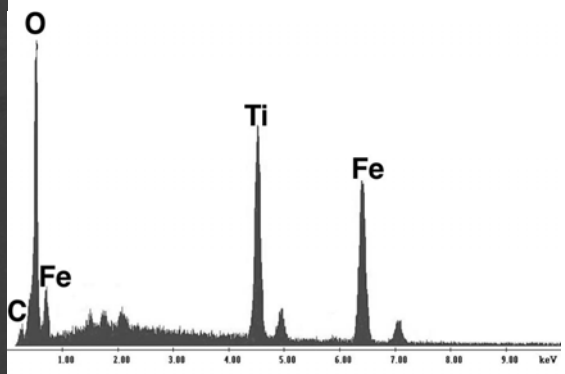


Figure 34. Nanodiamond #1

Figure 35. Electron diffraction pattern of nanodiamond in Figure 38

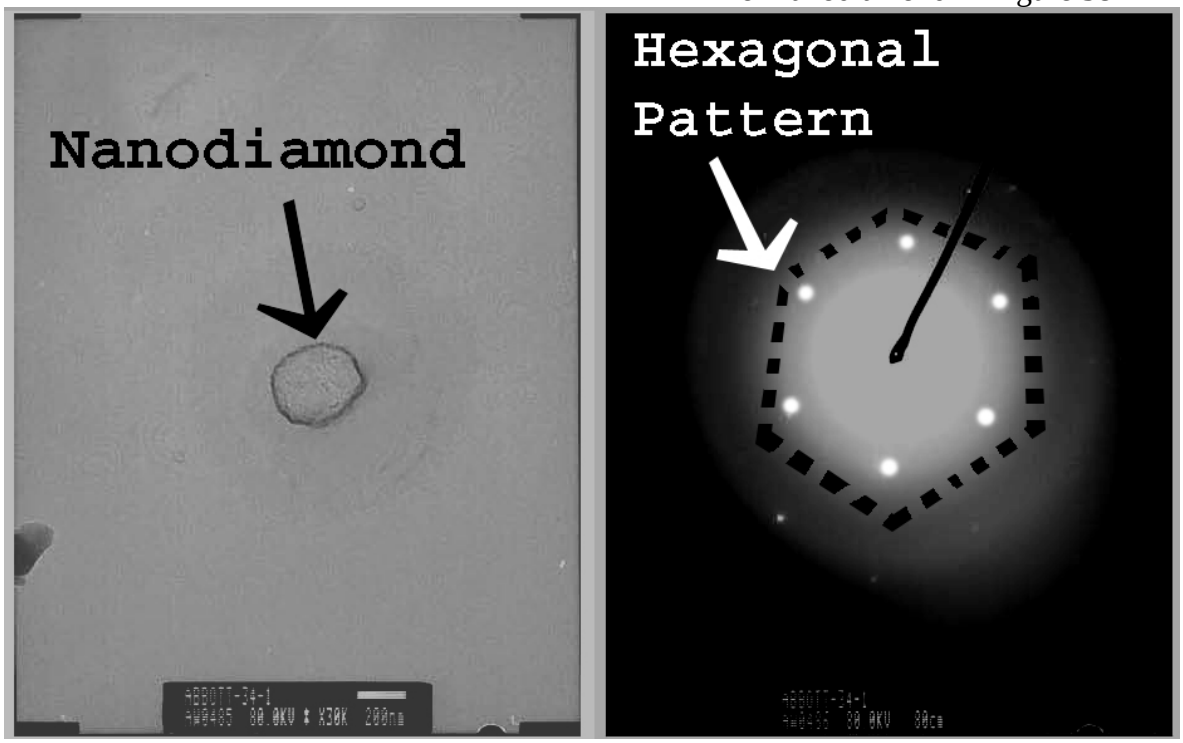


Figure 36. Nanodiamond #2

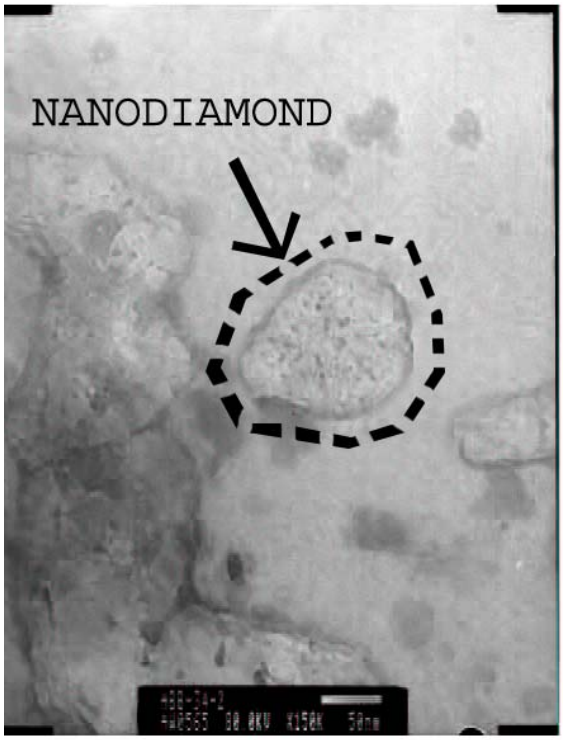
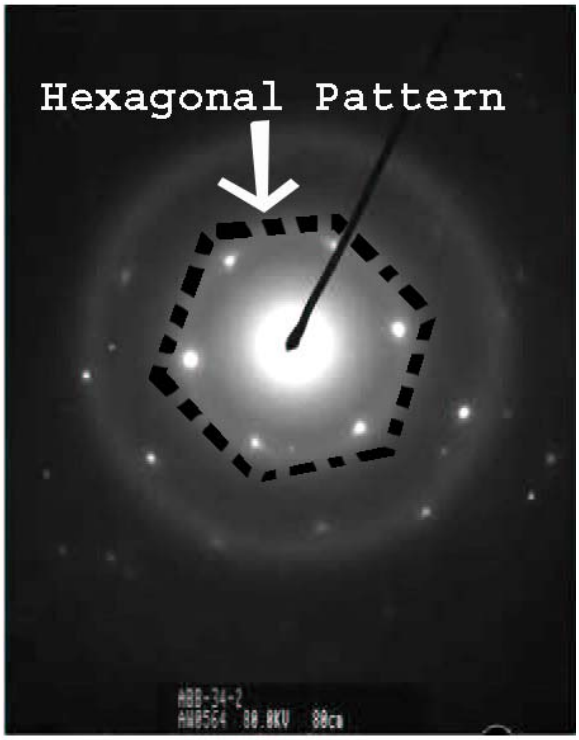


Figure 37. Electron diffraction pattern of nanodiamond in Figure 36



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CAPTURING THE NUTRIENT OVERENRICHMENT-EUTROPHICATION-HYPOXIA CYCLE IN NEWTOWN CREEK

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

Nutrient loading in estuarine waters leads to alterations to biogeochemical cycling, decreased oxygen levels and massive fish and shellfish kills. In highly-urbanized areas, nutrient loads can be delivered by rain events containing raw sewage and allochthonous bacteria including pathogens. Past transect sampling of Newtown Creek, an urbanized tributary of the Lower Hudson River Estuary, has revealed that this tributary experiences a nutrient overenrichment-eutrophication-hypoxia (NOEH) cycle, throughout the summer, displaying high variability in chlorophyll and oxygen levels in surface waters. In order to assess the drivers behind this extreme variability, a major summer rain event was captured during a week of daily sampling on the creek. This sampling revealed that the tributary fluctuates between physical and biological extremes on a daily and even hourly basis in terms of variability in chlorophyll *a* concentrations, bacterial abundance, oxygen saturation and nutrient concentrations. A comparison of environmental conditions at surface and depth in the waters of this creek also underscores the importance of surface water processes in this body of water, where surface waters alternate between hypoxia and oxygen supersaturation within days. The combination of the establishment of a strong thermo/halocline and the persistent oil slick observed on the creek's surface waters in summer may result in surface waters disconnected from the physical processes of mixing and diffusion at the sea-air interface. A comparison of the ratio of autotrophic:heterotrophic microbial concentrations in surface waters to dissolved oxygen saturation during the week of sampling reveals a significant ($p=0.00164$) relationship.

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INTRODUCTION

One of the major environmental factors influencing estuarine ecosystems in urbanized regions is the influx of nutrients from wastewater and other sources, which can result in dramatically increased levels of dissolved inorganic and organic material in the water column (Roman et al. 2000). Tributaries of the Hudson River Estuary have been implicated as major contributors to estuarine nutrient loading and sewage inputs in particular (Howarth et al. 2003; O'Mullan 2008). The overenrichment of estuarine nutrient cycles has a variety of effects, and perhaps the most acute from an ecological perspective is the decrease in oxygen levels due to eutrophication. Hypoxia (defined here as less than 50% oxygen saturation) in estuarine waters results from elevated microbial respiration during decomposition of algal blooms, and can result in fish and shell fish kills (National Research Council 2000).

Newtown Creek, a tributary that runs along the border between Brooklyn and Queens and empties into the East River (Fig. 1), sustains high levels of sewage input through both the largest Water Pollution Control Plant (WPCP) in New York City and Combined Sewer Outfalls (CSO's) located along its shores. Transect sampling in Summer 2007 revealed a system caught in a cycle of nutrient overenrichment-eutrophication-hypoxia (NOEH) and suffering wide fluctuations in surface oxygen levels (Fig. 2) (Dueker et al. 2008). Newtown Creek surface waters were routinely either oxygen supersaturated by massive phytoplankton blooms (saturation levels reaching as high as 270%) or oxygen-depleted by microbial processes (saturation levels reaching as low as 5%).

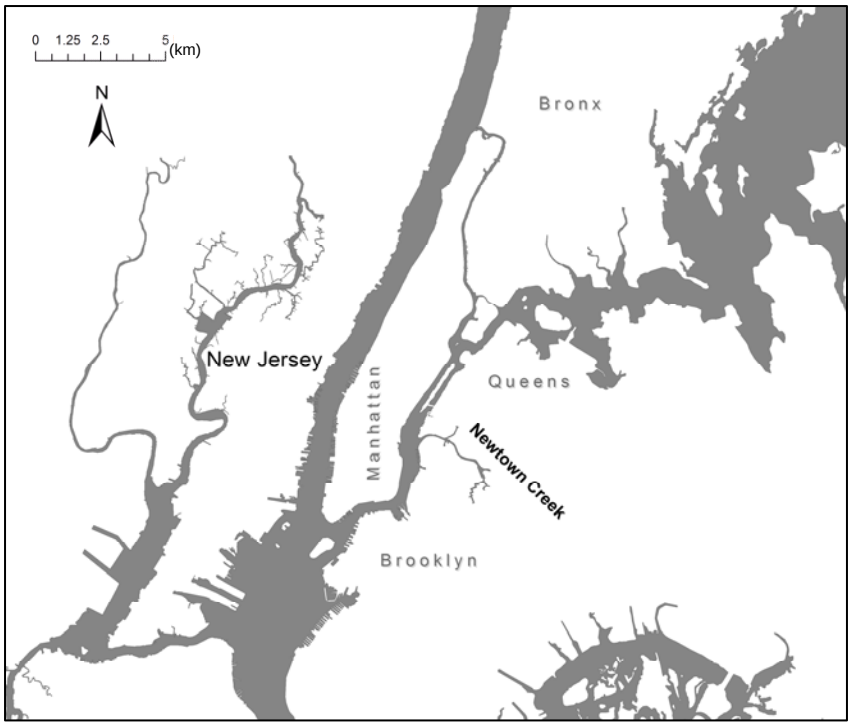

Figure 1. Location of Newtown Creek, running along the border between Brooklyn and Queens and emptying into the East River.

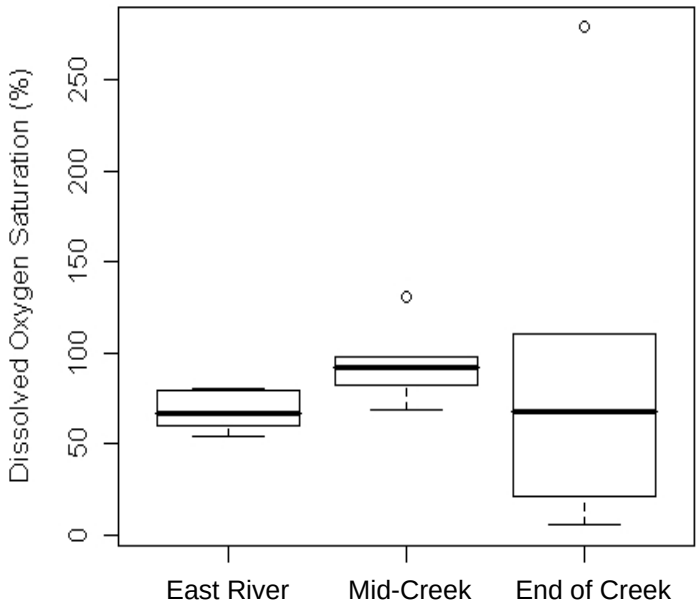

Figure 2. Boxplot of Summer 2007 surface dissolved oxygen saturation (%) along Newtown Creek transect.

The combination of summer heat, urban runoff, combined sewer overflows during rain events, and a persistent oil slick creates a unique microbial habitat in the surface waters of Newtown Creek. Surface waters are routinely out of equilibrium with the atmosphere in terms of oxygen content, with extreme high and low levels of oxygen sustained longer than expected through physical processes of diffusion (Dueker et al. 2008). This diffusion disconnect may indicate that the oil slick often covering these waters impedes diffusion and creates a gas exchange cap on surface waters.

This study tests the hypothesis that, much like biogeochemical cycling of nutrient pools, the oxygen levels in the surface waters of the creek are microbially-mediated (Fig. 3). As a heavily-nutrient enriched marine system, Newtown Creek microbial

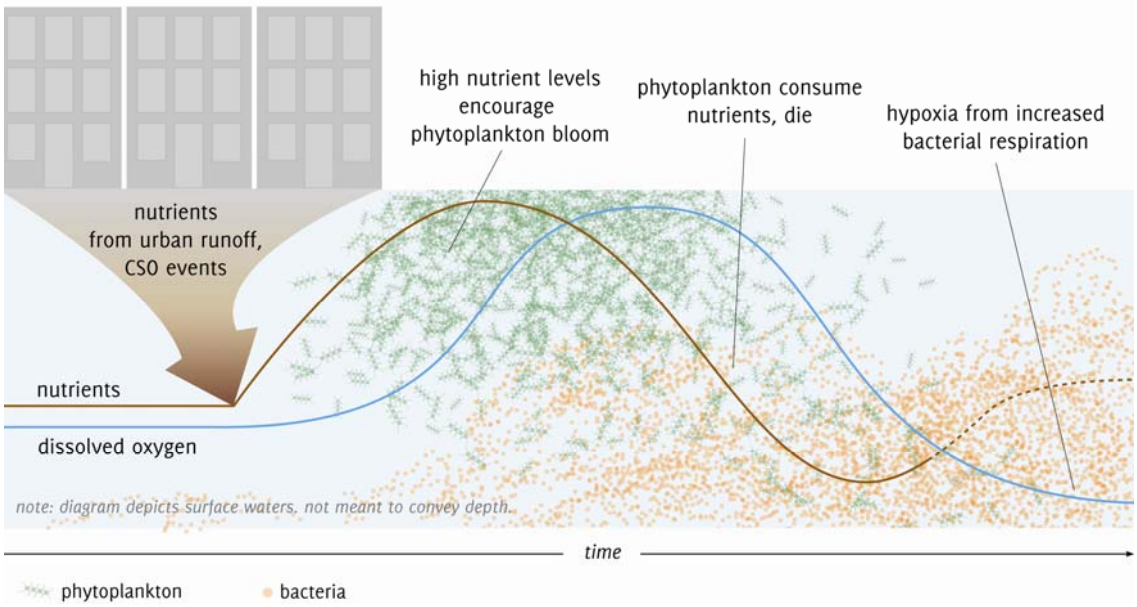

Figure 3. Diagram of proposed mechanisms creating surface water hypoxia in Newton Creek. (Diagram designed for this study by Jamie Stafford-Hill.)

communities are dominated by phytoplankton and bacteria, therefore, levels of oxygen in the water column should relate to the balance between autotrophy (creation of oxygen) and heterotrophy (respiration of oxygen) in surface waters.

Furthermore, biological control of oxygen levels in the surface waters of this tributary is predicted to result in additional environmental instability, with major implications for the maintenance of microbial community structure and diversity in this challenged tributary. To test these hypotheses, the NOEH cycle on the creek was monitored during a daily sampling regime conducted in the summer of 2008.

METHODS

Sample sites and timeframe. The transect for this study spans Newtown Creek (Fig. 4), with sampling sites at its mouth (ER1), mid-point (NT1), and toward its farthest inland reach (NT2). Sampling was conducted at slack tide daily at each site from July

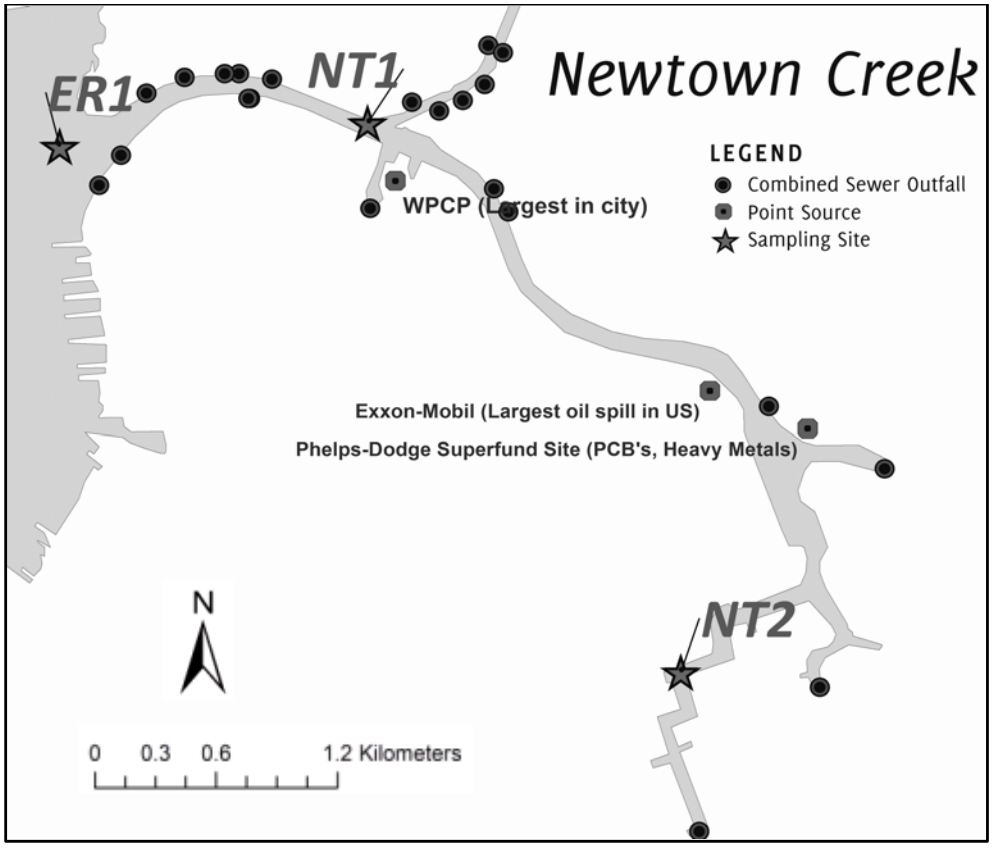

Figure 4. Newtown Creek with sampling sites, CSO locations and other pollution point sources identified.

20-23, 2008. An intensive daylight-hours observation of NT2 was conducted on July 24 to monitor the microbial activity in near-anoxic surface waters.

Sample Collection. Environmental transect samples were collected from shore using a Niskin bottle and a two-meter extension pole to reach into the Creek channel. At each site, samples were taken both at the surface and 0.2 meters above the Creek bottom. Oxygen levels were determined using a handheld Hach oxygen meter. Transparency was determined using a secchi disk. Samples for microbial community assessment were collected from the Niskin bottle and preserved. Ciliate, phytoplankton and dinoflagellate samples were preserved immediately with Acid Lugol's solution and stored in coolers for return to the lab. Samples were preserved for bacterial abundance counts using 1% formalin (final concentration). Samples for nutrient analyses (dissolved inorganic nitrogen (DIN), dissolved inorganic phosphorus (DIP)) were filtered through a 0.22 um Sterivex filter and frozen pending analysis. Nutrient analyses were performed at Horn Point Laboratory using colorimetric analysis protocols (Parsons et al. 1984). 300 ml of surface water from each site were filtered through 0.45 μm pre-combusted glass fiber filters (GFF). These filters were immediately frozen for later analyses of chlorophyll *a* concentrations using an ethanol extraction and calibrated fluorometer.

Ciliate, Phytoplankton and Dinoflagellate Enumeration. Preserved samples (100 ml) treated with Acid Lugol's solution were counted within 3 months of collection. After initial settling, samples were transferred to 15 ml centrifuge tubes and gently centrifuged. The supernatant was removed to leave a pellet of 1 ml. 5-10 sub-samples (20-50 μl drops) were then scanned and enumerated for ciliate, phytoplankton and dinoflagellate cells. All identifications were made to the lowest taxonomic level possible

using descriptions in Lee et al. (2000) and Jahn et al. (1949). Size categories were recorded for organisms that could not be identified taxonomically.

Bacterial Abundance. Bacteria were counted using an epifluorescent enumeration method according to Patel et al. (2007). Briefly, within 2 months of collection, 250-750 µl aliquots (volume depending on cell density) of formalin-preserved sample were filtered onto 0.22 µm black polycarbonate filters (Nucleopore, Pleasanton, Calif.). 10 µl of SYBR Green stain (causes bacterial DNA to fluoresce bright green) was applied to the filter for 15 minutes and then filtered through with a sterile phosphate-buffered saline wash. Filters were then mounted on a glass microscope slide, and a coverslip was applied over a 10µl drop of 50% glycerol, which acted as a cryoprotectant. The slides were immediately frozen until counting. At least 200 cells per filter were counted using epifluorescence microscopy.

Statistical Analyses. To test for significant relationships between physical parameters and microbial abundances I used linear models fitted using least squares regression in R statistical software (http://www.r-project.org).

RESULTS

Precipitation, Temperature and Salinity. The July sampling week began during the driest and hottest period of the NYC summer. A small rain event (0.2 in.) occurred early on the morning of July 23 to break this heat-wave, followed by an even larger rain event (over 1.5 inches) the evening of July 23, continuing into the morning of July 24 (Fig.5).

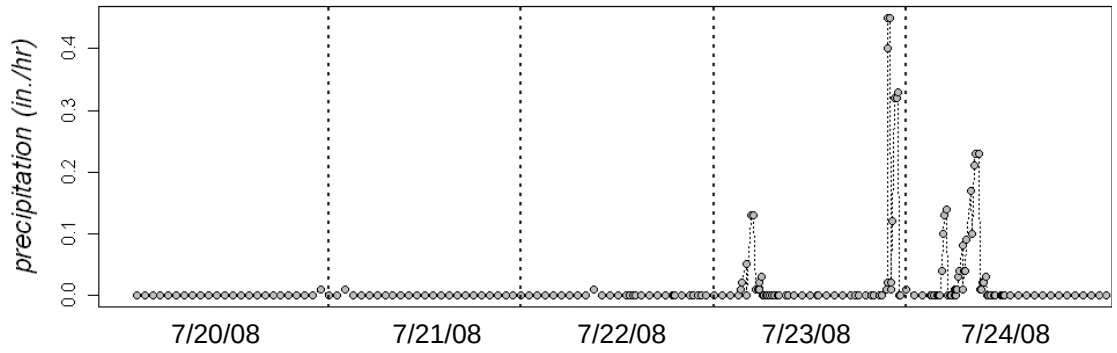

Figure 5. Precipitation during study (in./hr). Data from Central Park weather station.

ER1 consistently had a well-mixed water column, with salinity and water temperature (Fig. 6) similar both at surface and depth, ranging from 23-24ºC. NT1 had warmer (24-28ºC) and fresher surface waters than bottom waters (24-26ºC), indicating the establishment of a thermocline/halocline that could create a stable barrier between surface and bottom water processes (Fig. 6). NT2 was generally cooler than NT1

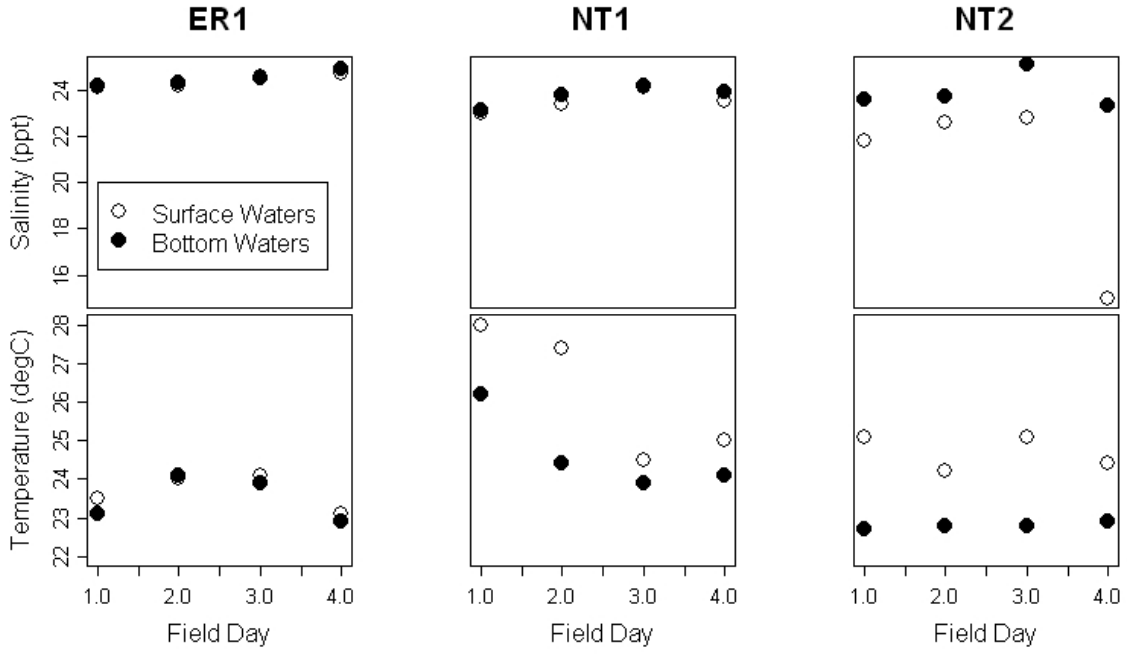

Figure 6. Salinity and temperature during sampling period, at depth (filled circles) and at surface (open circles). Field Day 1 = July 20, 2008.

(surface temperatures ranging from 24-25ºC) and displayed the largest difference between surface and bottom water temperature and salinity (Fig. 6), indicating the establishment of a strong thermo/halocline in these waters over the time period studied, even through the rain event.

Transparency. The ER1 water column was consistently more transparent than creek waters (Fig. 7). NT2 had the lowest transparency throughout the week, decreasing along with NT1 during the rain event on day 4 (Fig. 7).

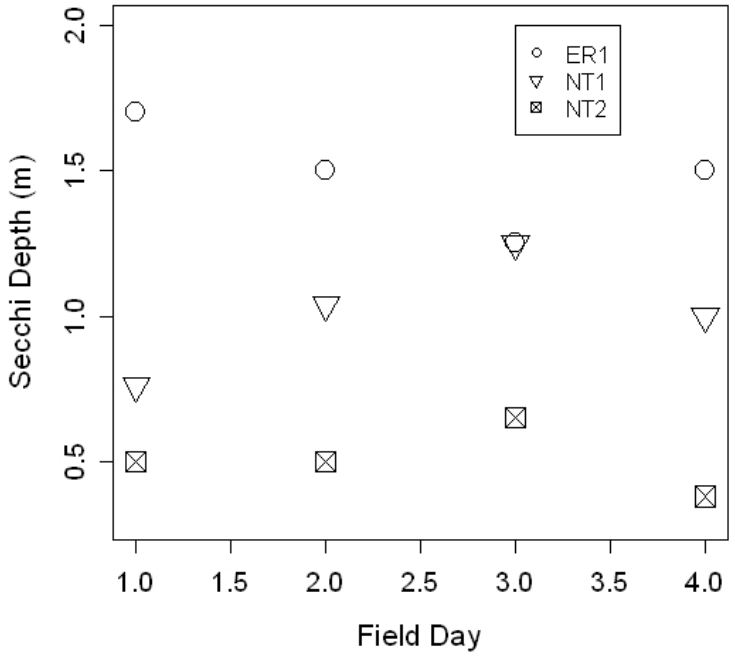

Figure 7. Water column transparency as determined by secchi disk measurements along the transect over the course of the study period.

Bacterial Abundance. Bacterial abundances were highest at NT2 and lowest at ER1, both in surface and bottom waters (Fig. 8). Whereas the bottom and surface bacterial abundances were similar each day for ER1 and NT1, NT2 surface waters had almost an order of magnitude greater bacterial concentration than its bottom waters.

Bacterial abundance along the transect was significantly correlated with secchi depth (R^2=0.821, p<0.0001), but not with chlorophyll (R^2=-0.03204, p=0.5778).

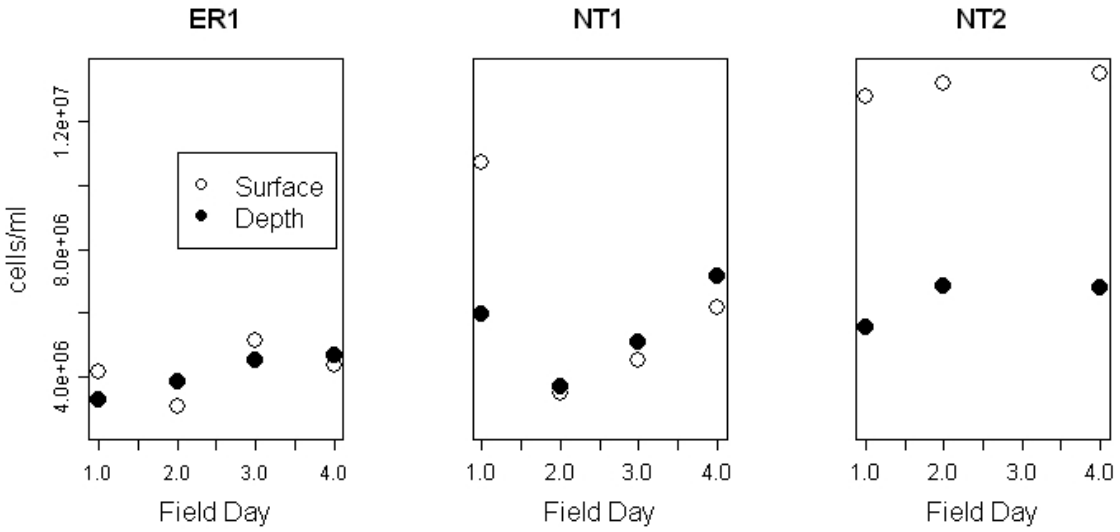

Figure 8. Bacterial abundances along transect during study.

Oxygen levels. Oxygen levels at ER1 hovered at about 55% both at the surface and at depth throughout the week, with a slight decrease after the major rain event (Fig. 9). The mid-creek station (NT1) initially showed oxygen supersaturation at the surface

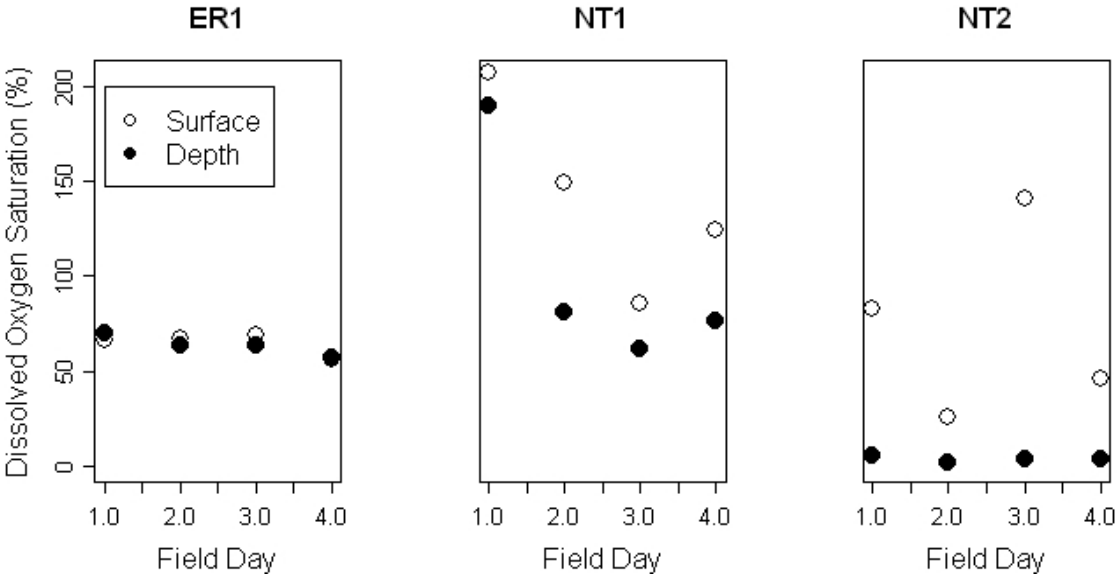

Figure 9. Dissolved oxygen saturation along transect during study.

and bottom waters that declined steadily until the July 23 (Field Day 4) rain event (Fig. 9). The inland station (NT2) had fluctuating surface oxygen levels during the week, oscillating daily from well-oxygenated to hypoxic conditions. The largest shifts occurred on the days with rain (3 and 4). Notably, the bottom waters of NT2 were consistently anoxic and, unlike the other stations, did not correspond with surface water conditions.

Nutrient concentrations. Nutrient concentrations in ER1 surface waters were relatively stable, in contrast to bottom water concentrations of NH_4 and NO_3, which had high variability (Fig. 10). In general, ER1 surface waters maintained elevated NO_3

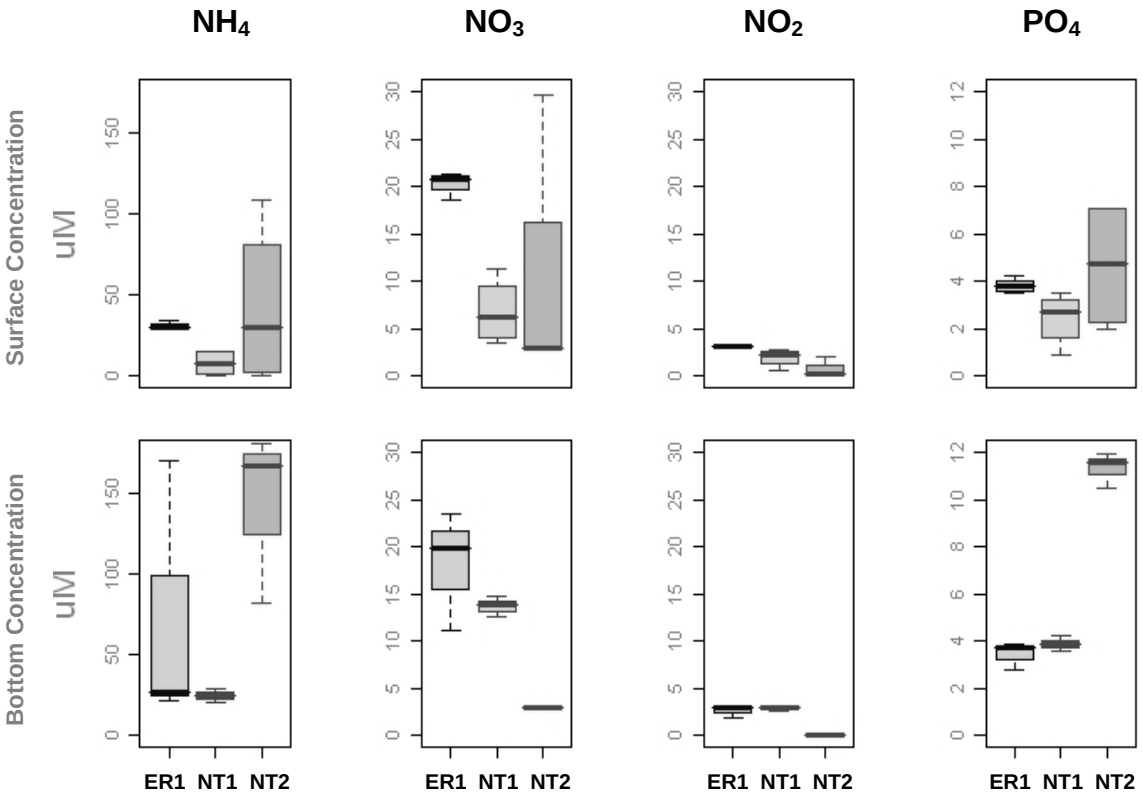

Figure 10. Boxplots of nutrient concentrations during study period by site. The top row shows surface water concentrations, the bottom row shows concentrations at depth.

concentrations (at about 20 μM) when compared to the inner creek sites. Nutrient concentrations in surface waters were more variable at inner creek sites, with NT2

displaying highest variability, particularly in the NH_4, NO_3, and PO_4 pools. NO_2 levels were low both in surface waters and at depth at all sites. NT2 had significantly elevated NH_4 and PO_4 concentrations at depth. At NT2, bottom waters were notably depleted in NO_3 and NO_2.

All sites maintained surface water N:P ratios below the 16:1 Redfield ratio (solid line, Fig. 11) except after the major rain event on the evening of the third field day. ER1 maintained an N:P ratio just below 15:1 in its surface waters throughout the week (Fig. 11). The inner creek sites maintained low N:P levels, closer to the average N:P of

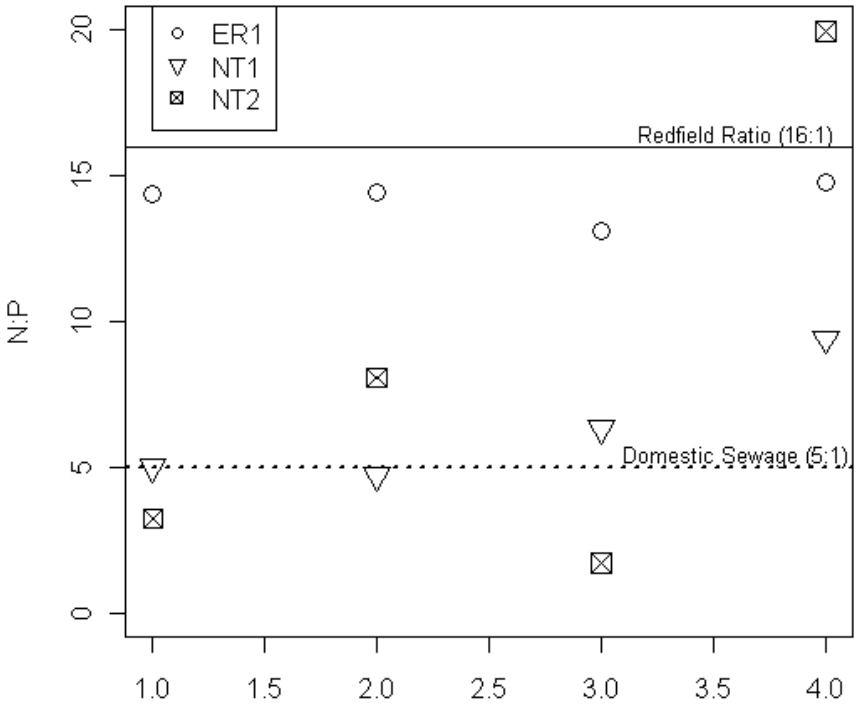

Figure 11. Nitrogen:Phosphorus ratios of surface waters along transect during study. Solid line denotes Redfield Ratio of 16:1, dotted line denotes domestic sewage ratio of 5:1).

domestic sewage, which is about 5 (Dunstan and Menzel 1971). NT1's N:P was 5 at week's start, but increased after the rain event. NT2 fluctuated wildly, with the biggest

variation occurring the day of the first small rain event, when it jumped from 1.75 to 20 (Fig. 11).

Chlorophyll *a*. ER1 had consistently lower Chl*a* concentrations than the mid and end-creek sites (Fig. 12). NT1 chlorophyll concentrations increased over the week, but levels fluctuated widely by day at NT2. NT2 chlorophyll concentrations peaked a day after NT1 concentrations peaked, suggesting possible advection of blooming phytoplankton from NT1 to NT2 surface waters.

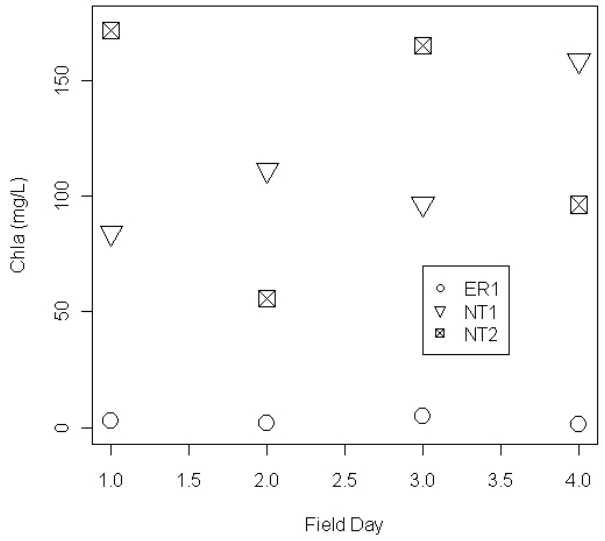

Figure 12. Chl*a* concentrations in transect surface waters during study period.

Comparing the chlorophyll *a* concentrations:bacterial abundance ratio to the oxygen saturation in these surface waters revealed a significant relationship across the transect (Fig. 13). This excludes an outlier from NT1 on July 20.

Surface water microplankton communities. The microplankton communities (cells in the size range of 10 μm-100 μm) in surface waters at NT2 varied greatly both in number and composition. In the five days NT2 was observed, total microplankton fluctuated greatly (Fig. 14). The phytoplankton communities were dominated by

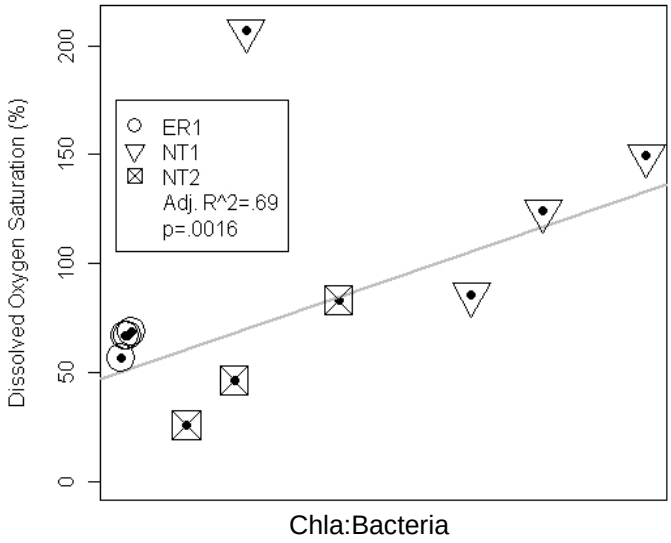

Figure 13. Dissolved Oxygen Saturation in transect surface waters regressed against the ratio of Chl*a* concentrations:Bacterial concentrations. Grey line denotes linear model fitted using least squares regression.

euglenoids, dinoflagellates and centric diatoms. Small (30 - 40 µm) aloricate ciliates dominated the heterotrophic microplankton communities (Fig 14). Loricate ciliates were present only on days when phytoplankton were abundant in the microplankton communities (July 20 and July 23). The aloricate ciliate bloom community on July 23 was dominated by 30 µm *Mesodinium rubrum*, a mixotroph which "enslaves" chloroplasts to make a living in eutrophic waters.

DISCUSSION

Newtown Creek experienced considerable shifts in both physical and biological parameters during the week of sampling, presenting a complicated case study of the NOEH cycle. Low transparency in marine systems can often be attributed to enhanced mixing of particulate organic materials into the water column from depth, but the strong thermocline and halocline (which would reduce turbulent mixing) clearly developed at

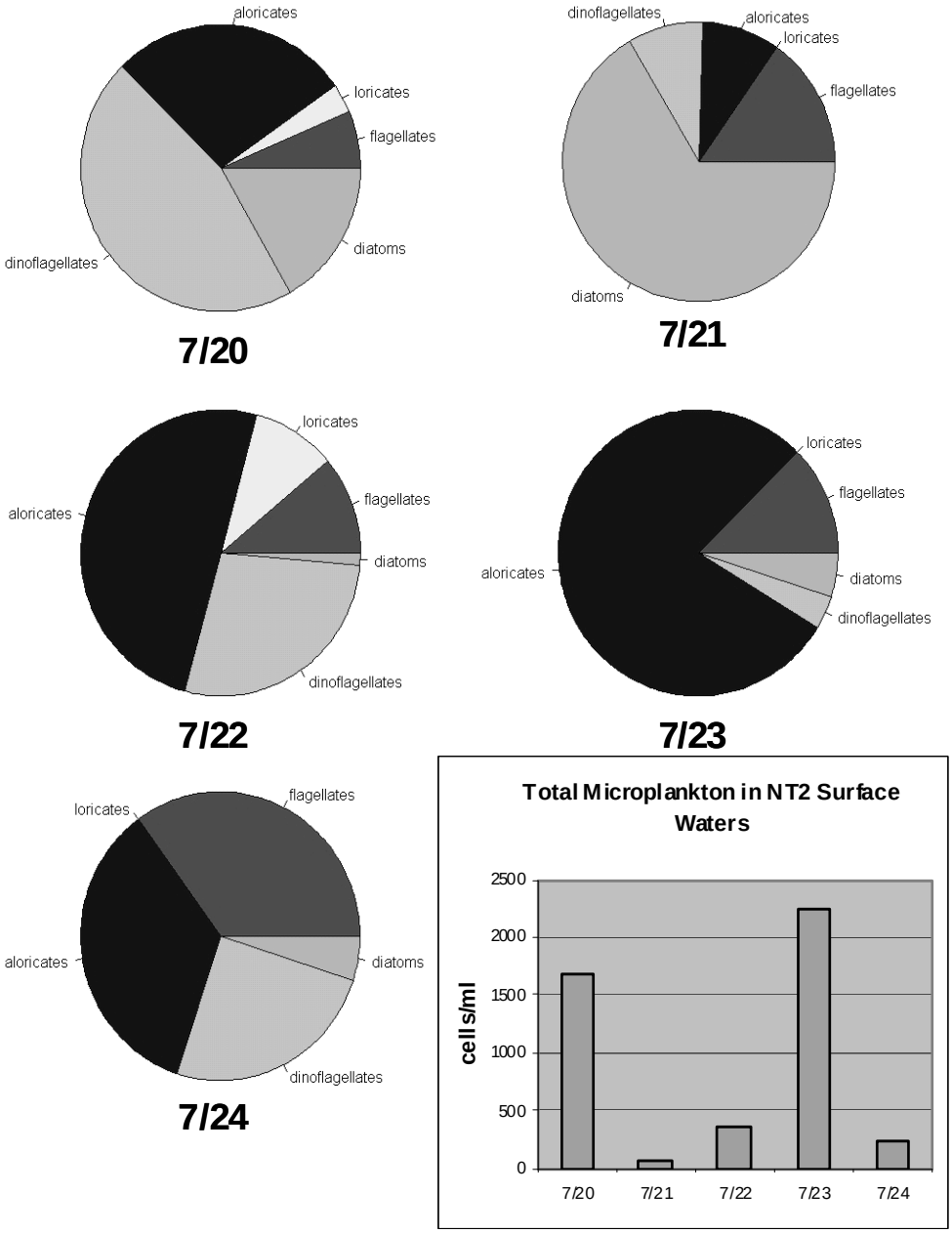

Figure 14. Microplankton community structure and abundances in the surface waters of NT2 during the sampling period.

NT2 suggests that the addition of particulate matter to the water column is allochthonous (National Research Council 2000). While sorting out environmental drivers to microbial community change in this scenario is further complicated by unknown sewage-release practices and uncharacterized point sources, a few possible suspects do arise.

Nutrient levels in the creek were consistently below the Redfield ratio, closer to the average levels observed in domestic raw sewage (Fig. 11) (Dunstan and Menzel 1971). The consistently low N:P ratios measured underscore the immense human influence on this tidal creek through inputs of high-P sewage effluent. Studies have demonstrated a connection between low N:P ratios and phytoflagellate dominance in marine eutrophication scenarios (Hodgkiss and Ho 1997; Hodgkiss and Lu 2004). Dinoflagellates and euglenoid flagellates do play a major part in bloom communities in the creek (Fig. 14). When oxygen levels were low at NT2 (Fig. 9), bacterial abundances remained elevated (Fig. 8), but the grazer populations were fluctuating wildly (Fig. 14). This suggests a possible "disconnect" of functional relationships between grazer microplankton and bacterial populations during the NOEH cycle, resulting in the complete removal of top-down control of bacterial populations (Kirchman 2000).

High nutrient inputs translate to the potential for increased microbial activity, as demonstrated both by the high chlorophyll *a* concentrations and bacterial concentrations measured at NT1 and NT2 during the sampling period (Figs. 8, 12). NT1 maintained high phytoplankton activity throughout, as evidenced by oxygen supersaturation in the entire water column (Fig. 9). NT2, however, fluctuated wildly between high chlorophyll-high oxygen levels and low chlorophyll-low oxygen levels associated with the NOEH cycle resulting in hypoxia. Bacterial levels were consistently elevated at NT2 (almost an order of magnitude higher than ER1 counts) (Fig. 8), suggesting that variability in phytoplankton abundance, balanced against a consistently high bacterial oxygen demand, may regulate the large variability in oxygen levels observed there.

Increased microbial activity in surface waters, and in particular, blooms of certain species, creates a context where nutrient pools can shift quickly. The microbial mediation of these nutrient pools requires oxygen, as most processes in surface waters are assumed to be aerobic (Stumm 1996). Because Newtown Creek surface waters appear to be inhibited in physical processes of diffusion, oxygen levels become a competition between photosynthesis and respiration, an unusual situation for a surface water system.

I used the chlorophyll *a* concentrations:bacterial abundance ratio to represent an assessment of the autotrophic vs. heterotrophic dominance in surface waters of the transect. A significant correlation was detected between chlorophyll:bacteria and oxygen levels during this week-long observation (Fig. 13), which suggests that microbes mediate not only the nutrient cycling occurring in these waters, but also the oxygen saturation of the water column. The NT1 outlier from the first day's sampling occurred during conditions of oxygen supersaturation but relatively low chlorophyll *a* concentrations. This outlier may be a result of oils at the surface inhibiting diffusion, indicating that excess oxygen produced by phytoplankton is not able to escape to the atmosphere as predicted by diffusion processes.

CONCLUSIONS

This study showed that Newtown Creek fluctuates between physical and biological extremes on a daily and even hourly basis in terms of variability in chlorophyll *a* concentrations, bacterial abundance, oxygen saturation and nutrient concentrations in its water column. Nutrient levels along the creek transect were consistently below the Redfield ratio, closer to the average levels observed in domestic raw sewage, highlighting

the immense human influence on this tidal creek through inputs of high-P sewage effluent.

A comparison of environmental conditions at surface and depth in the waters of this creek also underscores the importance of surface water processes in this body of water, where surface waters alternate between hypoxia and oxygen supersaturation within days. The combination of the establishment of a strong thermo/halocline and the persistent oil slick observed on the creek's surface waters in summer results in surface waters disconnected from the physical processes of mixing and diffusion at the sea-air interface. Surface microplankton community structure and abundance fluctuated wildly along with bacterial abundances in response to nutrient additions at NT2. This suggests that functional predator-prey relationships between grazers and bacteria are disrupted during hypoxic events. The control of surface dissolved oxygen levels by the relative abundances of phytoplankton and bacteria in these surface waters reveals an additional stressor to a system already challenged by extreme nutrient and bacterial loading from anthropogenic sources.

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I would like to thank Riverkeeper, Greg O'Mullan, Andy Juhl, O. Roger Anderson, Sarah McGrath, Liz Suter, Jamie Stafford-Hill, LDEO's Office of the Director, the Earth Microbiology Initiative, and the Hudson River Foundation for support for this project.

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EFFECTS OF SURFACE ROUGHNESS ON ECOLOGICAL FUNCTION: IMPLICATIONS FOR ENGINEERED STRUCTURES WITHIN THE HUDSON RIVER SHORE ZONE

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

Shorelines are a critical and changing aspect of the Hudson River ecosystem; however, relationships between shoreline structure and ecological function of the Hudson River are not well understood. Surface complexity may affect algal accumulation, organic matter, and macroinvertebrate abundance, but no study has examined all of these components of ecological function simultaneously. In this experiment, the relationships between ecological function, surface roughness and exposure to wave energy were tested. The objective was to determine if the manipulation of surface roughness on artificial structures alters ecological function within the shore zone. Tiles with different surface roughness were deployed at four sites in the freshwater tidal Hudson River (two high-energy sites, two low-energy), and the accumulation of algae, organic matter and macroinvertebrates was measured. The macroinvertebrate community varied with surface roughness, and significantly greater macroinvertebrate density (other than zebra mussels) was found on rougher tiles. This experiment showed that surface roughness can alter ecological function, but that the effects depend at least partially on exposure to wave energy, the pre-existing food web structure, and other site-specific factors.

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INTRODUCTION

Shorelines are a critical and changing aspect of the Hudson River ecosystem. At the interface between the aquatic and terrestrial world, shorelines provide a buffer from storm-induced erosion, and serve as habitats where many aquatic and terrestrial organisms forage, find refuge and reproduce (Wei et al. 2004; Brauns et al. 2007). Shorelines may accumulate organic matter from either the terrestrial or aquatic system and there may be significant biogeochemical transformations of materials as they pass through this interface (Lambert and Sommer 2007). All of these ecological functions are likely to vary dramatically with shoreline characteristics such as slope and roughness but it is not presently know how particular functions are related to particular shorelines.

Shorelines provide people with access to water, as well as recreational and aesthetic opportunities. Waterfronts are extremely attractive areas for residential development, as can be seen with the recent increase in development in the Hudson Valley. Natural shoreline is rapidly disappearing along the Hudson River; about half of the natural Hudson River shoreline has already been replaced with engineered structures (Miller et al. 2006). Much of the modified length was altered decades ago for purposes of stabilization and as these structures have degraded there are opportunities for novel design. Based on a long history of development combined with recent improvements in water quality and the public's perception of the Hudson River, shoreline development is likely to intensify, and old engineered structures will need repair or replacement.

Relationships between shoreline structure and ecological function of the Hudson River are not well understood. Literature suggests that rougher substrates are usually linked to greater macroinvertebrate abundance and richness, as a result of a variable surface that provides microhabitats and refuges from physical stresses (Clifford et al. 1992; Way et al. 1995; Schmude et al. 1998; Strayer and Smith 2000). Experiments conducted using concrete blocks with variable surface roughness in the Mississippi River revealed more than twice as many macroinvertebrates on concrete blocks with grooves than the original smooth concrete block. The drastic difference was attributed to the microhabitat refuge from high velocity flow. Even blocks with more shallow surface irregularities were found to harbor significantly more macroinvertebrates, but taxa

richness was not reported (Way et al. 1995). Texture or surface complexity can also affect algal accumulation on hard substrates with surface irregularities promoting greater accumulation of algae (Clifford et al. 1992) and organic matter (Scealy et al. 2007). Complex woody debris added to a lowland river in Australia increased macroinvertebrate richness and abundance (Scealy et al. 2007), with more organic material trapped in complex woody debris in riffle sites (i.e. higher energy) than in pools. The relationships between substrate complexity, organic matter, and macroinvertebrate richness and abundance suggest that potential modifications in shoreline construction or materials could increase ecological function within the shore zone.

Algae, organic matter and macroinvertebrates provide the ecological foundation (i.e. food resources) for higher trophic levels, including fish and bird populations. In this study, the hypothesis that rough tiles retain greater organic matter, algae and more macroinvertebrates than smooth tiles was tested. The specific objective was to investigate the effects of surface roughness and exposure to wave intensity on the accumulation of organic matter, growth of chlorophyll *a* and the colonization of macroinvertebrates within the shore zone of the tidal freshwater Hudson River.

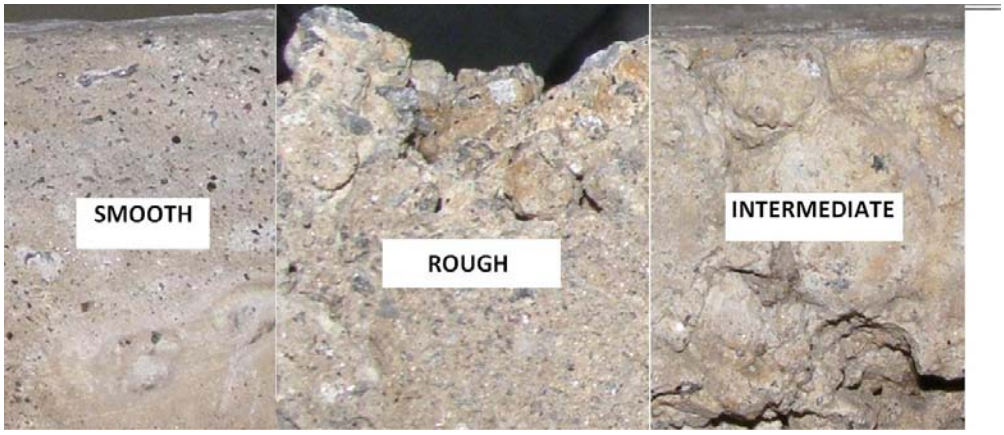
METHODS

During the first week of May 2008, 120 experimental concrete tiles were created using 15.24 in^2 square, commercially available plastic and hand-made wood molds. Forty tiles of each surface roughness were crafted to be smooth, intermediately rough and rough (Figure 1). Surface roughness was manipulated by varying the amount of water added to the concrete mix. Each tile was about 1.5 inches thick. The smooth tiles were smoothed to prevent cracks and crevices. Rough tiles were manipulated to include crevices and peaks, while intermediate tiles were smoothed, but crevices were enhanced.

Four sites (Figure 2) were selected within the Mid-Hudson region: two high-energy sites (exposed) and two low-energy sites (sheltered). Rough tiles in high energy environments were expected to act as a refuge from the stressful outer environment, thus having a stronger effect on organic matter accumulation, algal growth and macroinvertebrate abundance than smoother tiles. Tiles were placed vertically to mimic

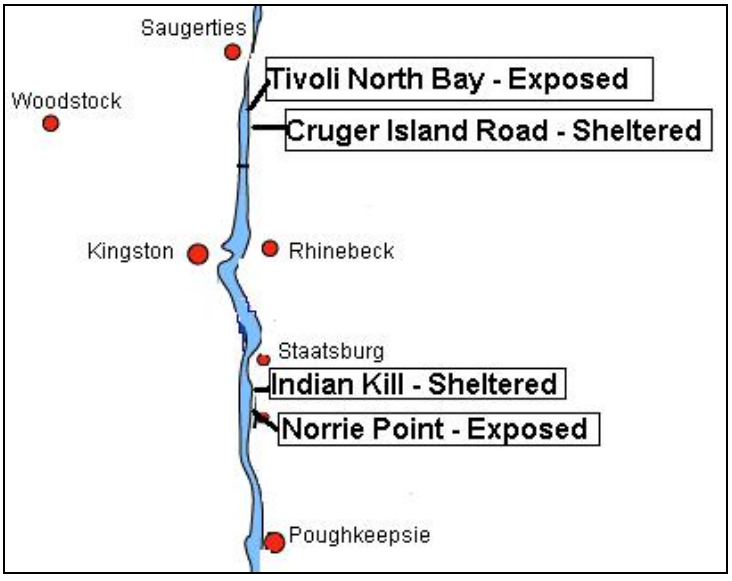
the walls of artificial structures and to avoid deposition of detritus and sediment from the water column (Vollenweider 1969).

Figure 1: Cross-section of tiles showing surface roughness.



Sheltered sites were established in tidal creeks less than 5 m wide with densely vegetated stream banks adjacent to the Hudson River. Both sheltered sites experienced minimal human disturbance during the study. Exposed sites shared a rocky bottom substrate. Submerged vegetation increased in sheltered sites during the study. Water chestnut became prevalent at the Norrie Point exposed sites, but was not as much of a factor at the Tivoli North Bay exposed site.

Figure 2: Site Locations



Tiles were placed within the sheltered sites on May 13 and 14, 2008, and were grouped in ten clusters of three, each consisting of a smooth, intermediate and rough tile. The soft stream bottom within sheltered areas allowed us to anchor tiles directly to the bottom using heavy-duty wire braces (Figure 3, left). Tiles were placed approximately 0.5 m below the low water mark.

Figure 3: Tile stabilization structures. Wire anchors for sheltered sites (left) and concrete composites for exposed sites (right).

The exposed tile composites (Figure 3, right) were placed into the river at ten locations within the site reach on May 20 and 21, 2008. A single tile of each substrate type was glued to a concrete patio block 18”x 6”x 2.5” using a hybrid waterproof adhesive and sealant. The composites were required to maintain vertical alignment in the high energy sites where rocky substrate did not allow for wire braces.

Tiles were removed after ten weeks during the week of July 21-28, 2008. The increase in submerged vegetation and sediment made it difficult to find tiles. Tiles were placed in individually sealed bags and transported to the lab at the Cary Institute of Ecosystem Studies. In the lab, tiles were scrubbed into a bucket of water to create a 500 ml slurry solution. A toothbrush, hairbrush and barbeque brush were used to scrub the tiles. Tiles were scrubbed within 12 hours of removal from the river. A 40 ml subsample for organic matter analysis and a 30 ml subsample for chlorophyll *a* analysis were collected from the slurry. The remaining 430 ml solution was filtered through a 0.5 mm mesh for macroinvertebrate identification and counting.

Organic matter analysis

Organic matter samples were placed in small tin collection pans and placed in the drying oven (18-24hours), re-weighed, and combusted at 450 degrees C for 4 hours. When cooled the tins were reweighed to obtain the ash-free dry mass (organic matter).

Chlorophyll a analysis

The chlorophyll *a* samples were centrifuged to collect material in a pellet and water was then gently decanted. The tube with remaining pellet was stored in a freezer until the day before extraction when tubes were frozen to -20 degrees C. Then 20 ml of methanol was added to each sample tube and heated in a water bath at 65 degrees C for 5 minutes. Samples were removed and stored in the dark at room temperature for 24 hrs. This methodology was adapted from (Sartory and Grobbelaar 1984) to facilitate the chlorophyll *a* extraction from benthic samples.

The following day, samples were diluted (1 ml extract + 5 ml MeOH) into fluorometer tubes. Wavelength absorption was measured at 665 nm before acidification (0.1 ml N HCl), and 750 nm after acidification to account for chlorophyll *a* and pheophytin (Steinman et al. 2006). If the fluorescence response exceeded 800 units before acidification the samples were diluted again by combining 1 ml of the prepared solution to another 5 ml of MeOH. The final chlorophyll *a* content of samples was calculated using the equations provided in Parsons et al. (1984).

Macroinvertebrates

Macroinvertebrates were filtered from the slurry and stored in 70% ethanol until they could be counted and identified under a dissecting scope. All organisms found in each sample were counted. Where zebra mussels exceeded 20 individuals in the first subsection of the collection plate, all non-zebra mussel taxa were counted, then subsampled to count zebra mussels. Organisms were identified to class, and where possible order. A list of all taxa observed and their functional feeding groups is provided in Table 1. Functional feeding groups are broad categories based on body structure and behavioral mechanisms used for feeding (Voshell 2002).

Table 1: Taxa observed in Hudson River artificial substrate study May-July 2008.

Class	Order	Function Group
Gastropoda	-	Scrapers
Bivalvia	Veneroida (predominantly Dreissenidae)	Collector-Filterer
Hirudinea	-	Predator
Oligochaeta	-	Collector-Gatherer
Turbellaria	-	Predators, Collector-Gatherer
Insecta	Ephemeroptera	Collector-Gatherer, Scraper
Insecta	Plecoptera	Shredder, Predator
Insecta	Coleoptera (predominantly Elmidae)	All (scraper, collector-gatherer)
Insecta	Diptera	Shredders, Collectors
Insecta	Trichoptera	All
Insecta	Odonata	Collector-Gatherer
Arachnida	Hydracarina	Predator
Crustacea	Isopoda	Mostly Collector-Gatherer
Crustacea	Amphipoda	Multiple functional feeding groups
Coelenterata	-	Predator
Crustacea	Ostracoda	Collector-Filterer
Crustacea	Cladocera	Collector-Filterer

Statistical analysis

The null hypotheses for organic matter and chlorophyll *a* were tested using a mixed model analysis of variance where site was a random effect and exposure and surface roughness as fixed (treatment) effects. The chlorophyll *a* and organic matter data was log-transformed to adjust for a non-normal distribution. A similar approach was used to test the null hypothesis for macroinvertebrate abundance, and non-metric multidimensional scaling (NMDS) was used to analyze the macroinvertebrate community

structure among substrate types in exposed and sheltered sites. NMDS calculates a Sorenson distance matrix and creates an ordination that illustrates this matrix in a low-dimensional space (2 or 3 dimensions) (Zuur and Ieno 2007). NMDS is based on the ranking or order of distances between subjects.

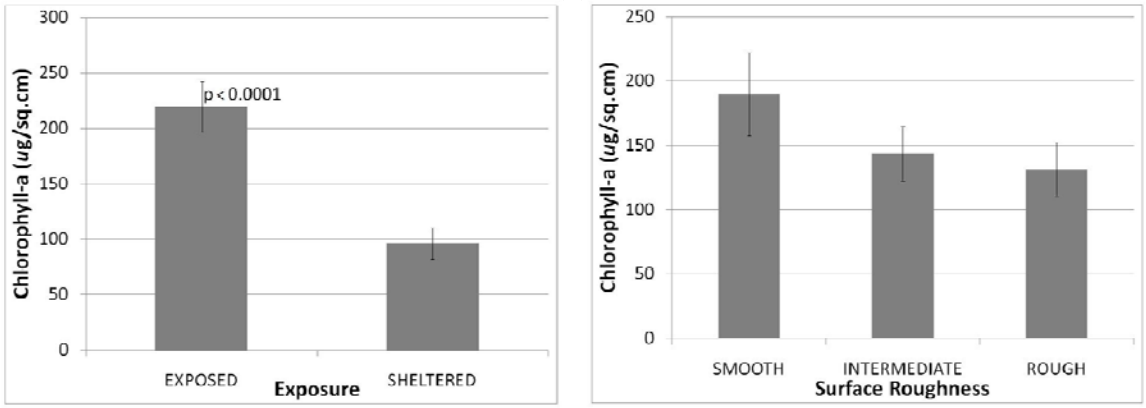
The relationship between organic matter and macroinvertebrate abundance, and chlorophyll *a* concentrations and macroinvertebrate abundance was tested statistically by linear regression. Only tiles for which both sets of data existed (i.e. organic matter or chlorophyll *a* and macroinvertebrate abundance) were included in this regression analysis.

RESULTS

Chlorophyll *a* and Organic Matter

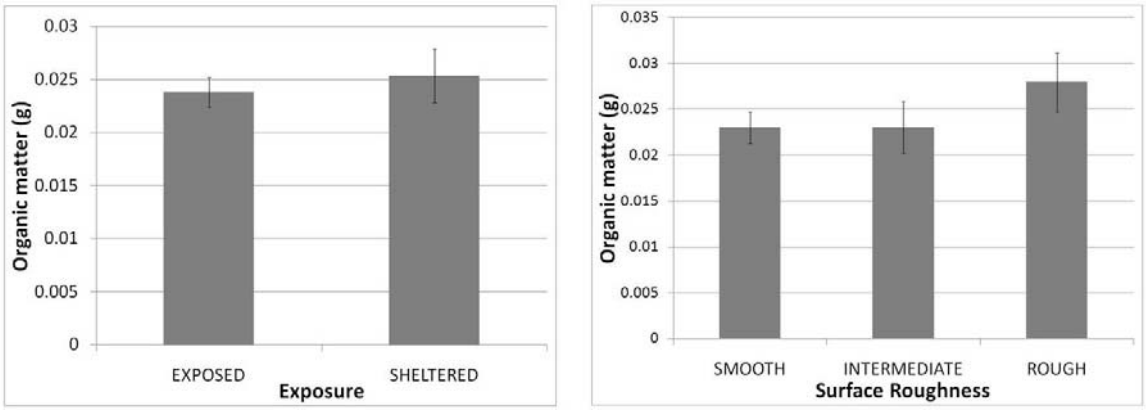
Surface roughness was not a significant determinant of chlorophyll *a* accumulation; exposure to wave energy was the only statistically significant factor ($p < 0.0001$) explaining the observed results (Figure 4).

Figure 4: The effects of exposure (left) and surface roughness (right) on chlorophyll *a*. Exposure was a significant factor ($p<0.001$), however surface roughness was not significant ($p = 0.7371$). Bars represent standard error.



OM accumulation did not differ significantly between exposure or among roughness ($p = 0.47$ and $p = 0.81$, respectively); therefore, the null hypothesis could not be rejected (Figure 5).

Figure 5: The effects of exposure (left) and surface roughness (right) on organic matter accumulation. There was no significant difference in organic matter accumulation between exposed and sheltered sites (p = 0.47) or among roughness tiles (p = 0.81). Bars represent standard error.

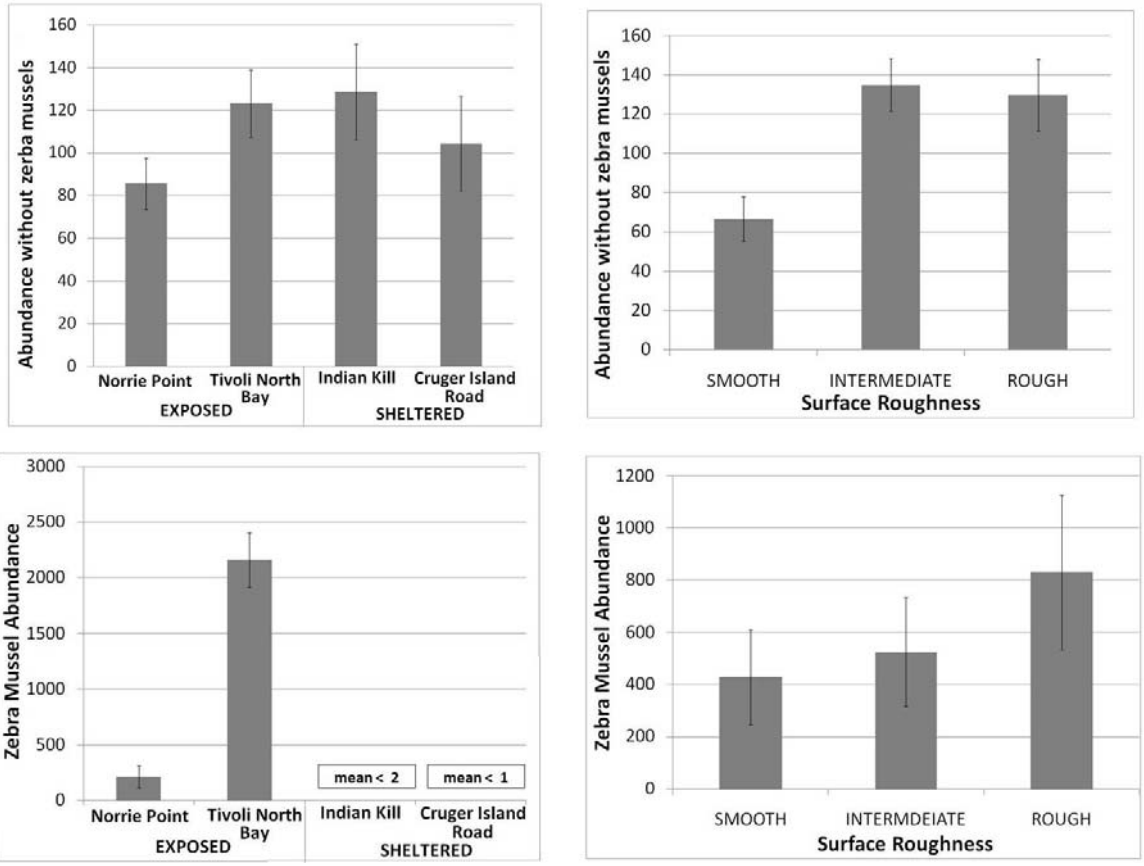


Macroinvertebrates

Abundance

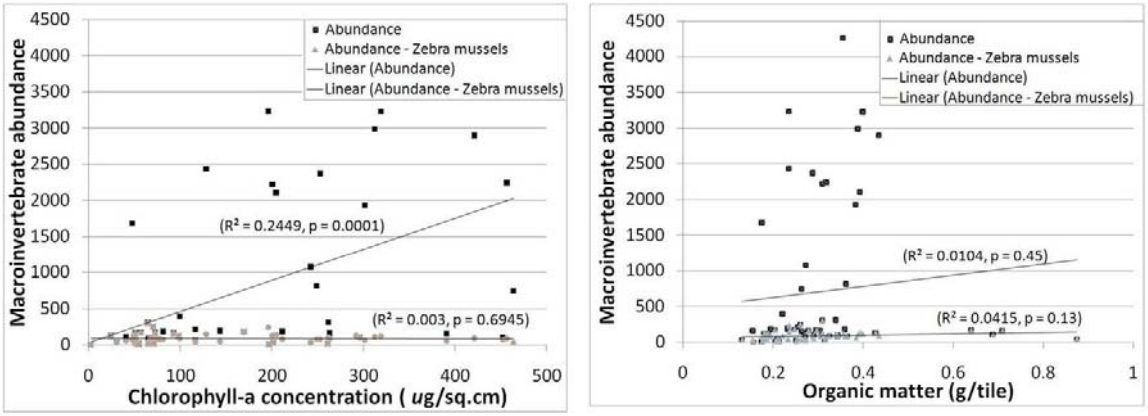
Exposure was a statistically significant factor ($p < 0.0001$) explaining the variability in density observed in this experiment. Tiles in exposed sites supported more macroinvertebrates than in sheltered sites. This disproved the original expectation of more macroinvertebrates within sheltered sites where wave energy was less intense. Neither surface roughness ($p = 0.14$) or the interaction of both factors ($p = 0.22$) was a significant factor explaining macroinvertebrate abundance. The effect of exposure was attributed to the dominant presence of zebra mussels within exposed sites, particularly the Tivoli North Bay site (Figure 6; bottom left). When tile density was recalculated to exclude zebra mussels, exposure was no longer a significant factor ($p = 0.48$), but surface roughness became a significant factor ($p = 0.0028$) explaining observed variability (Figure 6, top). In both scenarios, intermediate and rough tiles supported more macroinvertebrates overall, and more zebra mussels, than smooth tiles.

Figure 6: The effects of exposure (top left) and surface roughness (top right) on macroinvertebrate abundance without zebra mussels and the effects of exposure (bottom left) and surface roughness (bottom right) on zebra mussel abundance Bars represent standard error. Exposure was a significant factor ($p < 0.0001$) in the observed abundance of macroinvertebrates (minus zebra mussels), but surface roughness was not ($p = 0.14$). Likewise, exposure was a significant factor explaining observed zebra mussel abundance ($p < 0.001$), while surface roughness was not ($p = 0.1675$).



There was not a strong relationship between organic matter accumulation and macroinvertebrate density on individual tiles; however, there was a significant ($p<0.0001$) positive relationship between chlorophyll *a* concentration and total macroinvertebrate abundance (Figure 7).

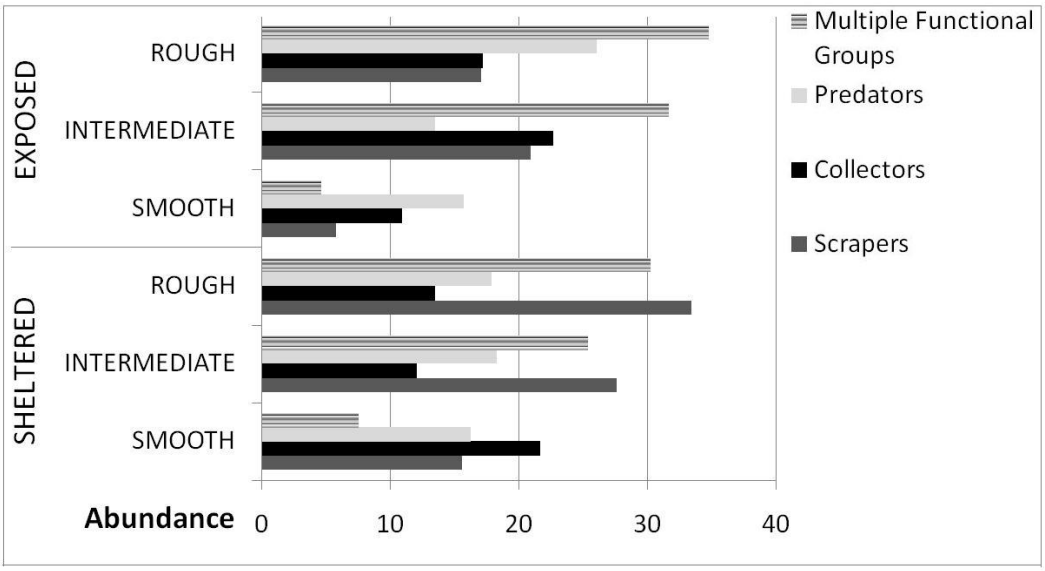
Figure 7: The relationship between macroinvertebrate density and organic matter and chlorophyll *a* accumulation.



Functional Feeding Groups

Figure 8 illustrates functional feeding group (FFG) density on all tiles at exposed and sheltered sites. Each functional group includes organisms that are considered members of only one function group. Those that play several functional roles are aggregated in the "Multiple Functional Groups" class. The predator group included Turbellaria, Hirudinea, and Hydracarina; the scraper group included Gastropoda and

Figure 8: The effects of exposure and surface roughness on different functional feeding groups. Predators = Turbellaria, Hirudinea, and Hydracarina; Collectors = Oligochaeta, Bivalvia, and Diptera; Scrapers = Gastropoda and Coleoptera larvae; Multiple Functional Groups = Isopoda, Amphipoda, and Trichoptera. Zebra mussels were excluded from collectors group (denoted with *) in this graph to facilitate comparisons.



Coleoptera larvae; the collector group included Oligochaeta, Bivalvia, and Diptera; and the "multiple functional groups" included Isopoda, Amphipoda, and Trichoptera (Voshell 2002). Zebra mussels are excluded from the exposed sites in Figure 8 to enhance the view for comparison. The density of scrapers found on tiles in sheltered areas was less than found in the exposed sites, but the density of predators was higher. Macroinvertebrate community structure was expressed by NMS ordinations illustrated in Figures 9-11. The distance between points (individual tiles) in an ordination is inversely proportional to their taxonomic similarity. Table 2 reports the dimensionality of the ordination results and the stress level. Stress measures the fit of the ordination created. According to Clarke's rules of thumb, stress value less than 10 is considered good with a small chance of misinterpretation, between ten and twenty is considered fair, but stress greater than 15 is considered unreliable (Clarke 1993).

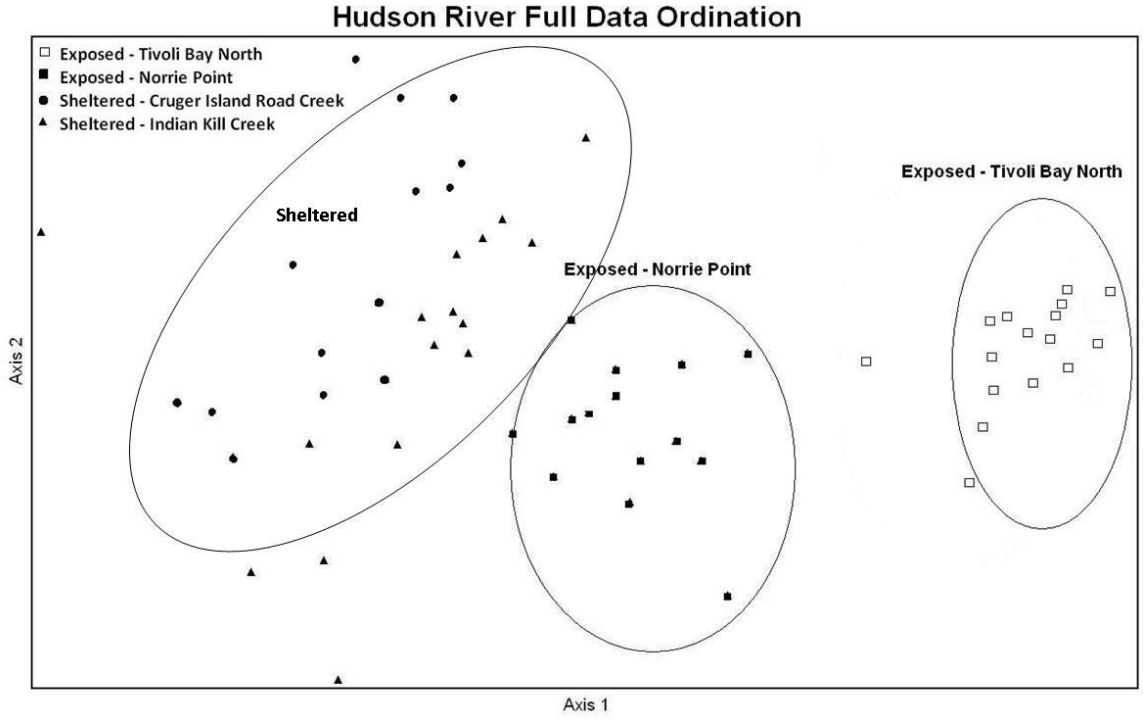
Table 2: Dimensionality and stress results from the NMS ordination.

Site	Number of axes	Stress
Full Hudson River Study Data	2	11 (Fairly good ordination)
Indian Kill (S)	2	14 (Fair ordination)
Cruger Island Road Tidal Creek (S)	2	9 (Good ordination)
Tivoli North Bay (E)	2	No ordination possible
Tivoli North Bay (E), no zebra mussels	2	11 (Fairly good ordination)
Norrie Point Environmental Center (E)	2	7 (Good ordination)

The full data set ordination indicated a fairly clear grouping by site. The ordination suggested that the macroinvertebrate communities within exposed sites had stronger intra-site similarity than the sheltered sites (Figure 9). There is greater distance between the two exposed sites than there is between the sheltered sites, suggesting greater similarity between sheltered sites than between exposed sites.

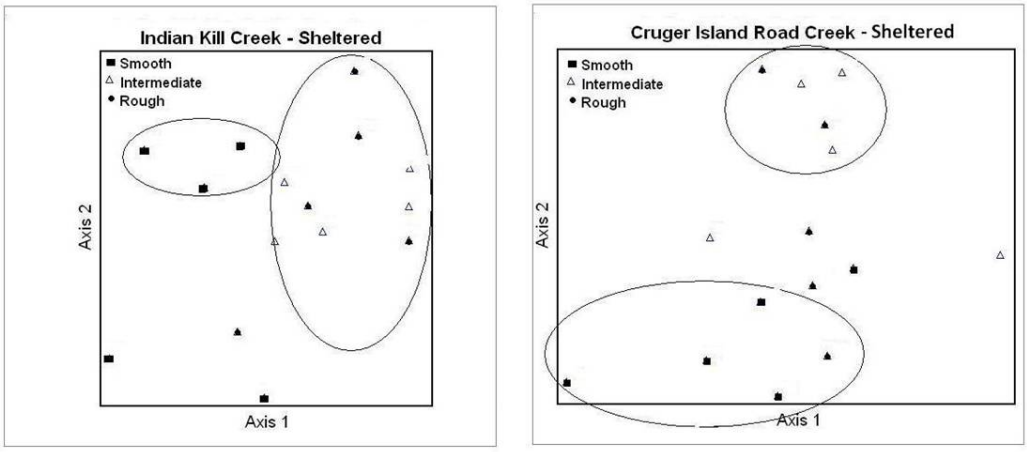
Site-specific ordinations were run to facilitate the visual analysis of trends with respect to surface roughness. The Indian Kill sheltered site shows a grouping of intermediate and rough tiles being more similar to each other than with the community of smooth tiles (Figure 10). Community structure among smooth tile replicates varied based on this graphical depiction of Bray-Curtis distance, with only three of five tiles being

Figure 9: Macroinvertebrate community structure at all sites. (n = 120).



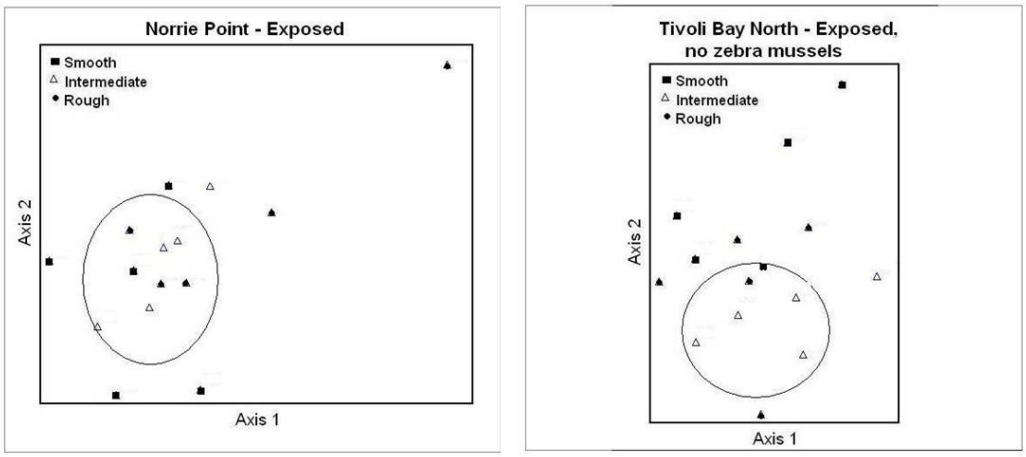
placed adjacent to each other. The Cruger Island Road Tidal Creek site demonstrated stronger clustering between rough and intermediate tile communities than the other sites (Figure 10). The variability among smooth tile replicates was also less than other tiles, with 4 of 5 tiles placed adjacent to each other.

Figure 10: Macroinvertebrate community structure at sheltered sites: Indian Kill Creek (n = 30) and Cruger Island Road Creek (n = 30).



Community structure on smooth tiles was especially variable at the Norrie Point exposed site as is illustrated with the scattered depiction in Figure 11. Intermediate and rough tile communities appear more similar to each other than with smooth tile communities. A relatively tight clustering of rough and intermediate tiles (8 of 10) is evident from this ordination. A useful ordination was not found for the exposed site at Tivoli North. This is either because the structure of the community was too weak or a single variable has too much weight. The latter is most probable due to the prevalence of zebra mussels at this site (mean density = 2160 mussels). When the ordination was rerun on community data excluding zebra mussels a fairly good ordination was found. In general, clustering is not as strong in this ordination. Only the intermediate tiles are somewhat closely related to each other. Overall, there is a relatively weak pattern of intra-site similarity among tile types.

Figure 11: Macroinvertebrate community structure at exposed sites: Norrie Point (n = 30) and Tivoli North Bay (n = 30). The ordination presented for Tivoli North Bay excluded zebra mussels.



DISCUSSION

Overall, surface roughness did not strongly affect chlorophyll *a*, organic matter, or macroinvertebrate abundance. These results differed from other results cited in the literature (Way et al. 1995) and from expectations at the onset of this experiment. A greater accumulation of both organic matter and chlorophyll *a* in sheltered areas was expected where water movement was slower and calmer than at the exposed sites.

Instead, there was greater chlorophyll *a* growth in exposed sites and on smooth tiles. While organic matter accumulation was somewhat greater within sheltered sites and on rough tiles, the differences were not statistically significant. Organic matter accumulation was expected to follow a similar pattern as chlorophyll *a*, but this was not observed in this experiment. Moreover, prior studies suggest zebra mussels increase macroinvertebrate density through the provision of increased substrate for colonization (Hovarth et al. 1999), but there were not significantly more macroinvertebrates on tiles with zebra mussels than tiles without zebra mussels. In fact, the highest macroinvertebrate tile density (excluding zebra mussels) was found at Indian Kill Creek sheltered site where mean zebra mussel density was less than 2 individuals (Figure 6).

Table 3: A summary of experiment results. Statistical significance ($p < 0.05$) is denoted by (*).

Variables	Exposure	Roughness
Organic matter	Exposed < Sheltered	$S = I \leq R$
Chlorophyll *a*	Exposed > Sheltered*	$S > I \geq R$
Macroinvertebrate abundance	Exposed > Sheltered	$S = I = R$
Macroinvertebrate abundance, without zebra mussels	Exposed = Sheltered	$S < I > R$*
Macroinvertebrate community structure	Exposed: strong within-site similarity. Sheltered: weak within-site similarity	Weak clusters
Chlorophyll *a* and Macroinvertebrate abundance	Highly significant, but fairly weak correlation (0.2449, p = 0.0001)	
Organic matter and Macroinvertebrate abundance	No significant correlation	

Surface roughness did appear to play a role in determining the structure of macroinvertebrate communities. A consistent similarity was found between the macroinvertebrate community structure of rough and intermediate tiles from our within-site analysis. Although these tiles were created to be different, intermediate tiles were more similar to rough tiles than to smooth tiles. The similarity detected among

intermediate and rough tiles to microhabitat heterogeneity was attributed to the provision of refuge from predators, and the actual design of the tiles.

Implications for shore zone development

Surface roughness of artificial structures may influence ecological function within the shore zone, but the effect seemed to vary with exposure and with location. Despite the conflicting results of the organic matter and chlorophyll *a* analyses, macroinvertebrate abundance was higher on intermediate and rough tiles than on smoother tiles. An overall positive correlation between chlorophyll *a* and macroinvertebrate abundance was found, but it is difficult to know what is determining the observed chlorophyll *a* results. For instance, low chlorophyll *a* could represent low photosynthetic production or high grazing pressure. Experiments that specifically test production and predation would strengthen the understanding of the relationship between chlorophyll *a* and surface roughness. Similarly, the direct cause of observed macroinvertebrate abundance could be higher chlorophyll *a* production or greater refuge from predators. Again, experiments that isolate these processes are needed to better explain our results.

Even though the results of this study support the idea that macroinvertebrate abundance increases with increased surface roughness, the question remains whether a numerical increase in macroinvertebrates is equivalent to enhancing ecological function. There were more zebra mussels at exposed sites. In these cases, an increase in macroinvertebrates may not be considered ecological enhancement. Perhaps a better measure would be taxonomic richness or diversity. The functional feeding group approach may also provide a helpful snapshot of the community-level response to surface roughness. In both exposed and sheltered areas, the abundance of predators and multiple functional group taxa increased with increasing surface roughness. This could suggest rougher surfaces promote a more diversified community of prey resources that is attractive to predators. However, a study that identifies macroinvertebrates to genus or species could avoid the ambiguity (i.e. the “multiple feeding groups” class) associated with the functional feeding group analysis presented here.

This experiment attempted to minimize variability within exposed and sheltered sites, but differences between sites of a given exposure were detected for chlorophyll *a*

and the macroinvertebrate community. In the future it would be helpful to quantify more site characteristics (e.g. wave energy, water clarity, benthic substrate, predation pressure, etc) in efforts to minimize variability among treatment sites and increase consistency within the results.

The results of this experiment suggest that surface roughness of artificial structures has the potential to affect ecological function, but that the magnitude of the effects depends on exposure to wave energy, the presence of ecosystem-altering species (i.e. zebra mussels), and perhaps other site-specific characteristics. Nevertheless, there was not a negative relationship between surface roughness and chlorophyll *a*, organic matter, or macroinvertebrate abundance. Therefore, where possible, short-term experiments should be conducted at specific sites targeted for waterfront development to determine the potential ecological enhancements associated with surface roughness. Ecological function can be compared to the pre-existing ecological function to determine which roughness is most appropriate to maintain or enhance ecological function. When experimentation is not possible, this study supports the use of rougher substrates in waterfront development projects, providing the cost difference is small.

ACKNOWLEDGEMENTS

We would like to thank the Hudson River Foundation and the Tibor T. Polgar Fellowship selection committee for choosing and funding this research. We would also like to thank Sarah Fernald of the Hudson River National Estuarine Research Reserve for logistical support and comments, Heather Malcom for amazing lab support, David Fischer for assistance with the chlorophyll *a* extraction and analysis, as well as David Yozzo and Helena Andreyko for comments on all drafts.

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GASTROPODS OF THE HUDSON RIVER SHORELINE: SUBTIDAL, INTERTIDAL, AND UPLAND COMMUNITIES

A Final Report of the Tibor T. Polgar Fellowship

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ABSTRACT

The tidal Hudson River shore zone is a unique and dynamic ecotone, supporting a great abundance of wildlife and fulfilling numerous ecological roles, but simultaneously subject to intense human activity. A survey was completed for gastropods inhabiting six dominant shore zone types along the Hudson River, including natural and altered shoreline structures: sand, bedrock, unconsolidated rock, riprap, sea wall and timber cribbing. Each of these shore zone types was surveyed at three river sections, lower, mid and upper, from Poughkeepsie to Albany. Three elevations were sampled at each site: sub-tidal, inter-tidal and upland. Eighteen sites between Poughkeepsie and Albany were sampled in June during two intensive trips, resulting in a total of 23 taxa of gastropods. Of these, three were exotic, including *Bithynia tentaculata*, which was represented by only one specimen, indicating a significant decline for this species since the mid-1980s. Three aquatic species were new records for the Hudson River, including *Floridobia winkleyi*, which was present in significant numbers, as well as the recently recorded *Littoridinops tenuipes*, also present in large numbers. Another new record was the presence of two specimens of the New York state listed snail *Valvata lewisi*. Two landsnails, *Pomatiopsis lapidaria* and *Vallonia costata* were also found below high tide. ANOVAs examining abundance and diversity of gastropods by shore type, elevation and river section, indicate that mid-river sites, in combination with riprap and unconsolidated rock, at inter-tidal elevations, contain significantly higher abundances and diversity of gastropods. Regression analysis indicated that fine-scale environmental variables such as site slope, rugosity and complexity do not explain the abundance and diversity of gastropods at this level of analysis. Additional analysis included NMS ordination and qualitative analysis.

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INTRODUCTION

The Hudson River is tidal for 248 kilometers from New York City to Troy. Its shoreline is complex and dynamic, and like many ecotones, supports a very diverse fauna. At the same time, the tidal shoreline is not well understood ecologically (Strayer and Smith 2000) and the human impact on it is of great significance (Limburg and Schmidt 1990). Large-river shorelines like the Hudson have sustained significant damage to their original ecological functions by virtue of the destruction of wetlands, the replacement of natural shoreline habitats with artificial and erosion-resistant riprap and bulkheads, development and pollution. Moreover, they continue to support a great deal of human activity through recreation, fishing, transportation and water withdrawals for commercial, agricultural and urban needs (Daniels et. al. 2005). Shorelines at once serve as the point of impact for many of these human activities, while simultaneously providing significant ecological services as a dynamic interface between the aquatic and upland communities.

One class of organisms common along shorelines is the gastropods (Jokinen 1992). There are at least 50 species of freshwater snails in the Hudson River Valley, of which at least four are exotic (non-indigenous to North America), and another 4-6 may be invasive (introduced from other North American regions) (Strayer 1987). In addition, there are at least 85 species of land snails in New England (Nekola 2005), most of which can be found in the Hudson Valley, though not necessarily on shore lines. Gastropods are well known as being sensitive to ecological change, particularly sensitive to pollution, and a substantial resource base for fish, crayfish, waterfowl and small mammals (Dillon

2000, 2005). However, the understanding of the role of these invertebrates within the multitude of habitats in which they exist is minimal.

Efforts to understand and conserve invertebrates was not a pressing issue for most states until the mid 1980's, and the understanding of their ecological role remains weak (McCollough 1997). Currently there are 23 snail species on New York State's rare animal list (NYSDEC 2008), and the status, trends and ecological needs of most of these species are poorly known.

While invertebrates make up nearly 99% of all animal diversity, they have received significantly less attention than vertebrates, with mollusks receiving even less attention despite their being considered one of the most threatened groups of animals (Lydeard et al. 2004). Between 1500-2008, 257 gastropods became extinct (37% of all animal extinctions during that time) and the number of gastropods on the International Union for Conservation of Nature Red List of Threatened Species in 2008 was 883 (IUCN 2008). Despite these numbers, less than 2% of known mollusk species have been properly assessed (Lydeard et al. 2004). Given the general decline of snail species in the United States, as well as the loss of habitat associated with that decline (Burch 1989; Lydeard et al. 2004), the ecological importance of snails (Kabat and Hershler 1993), the contribution of rare invertebrates to species richness in aquatic systems (Cao et al. 1998), and the relative lack of knowledge of gastropods, there is a need to develop a greater understanding of the gastropod communities in the Hudson River shore zone. This report provides baseline data on shoreline gastropods, and contributes to the understanding of gastropod communities along the Hudson River.

This project is part of a larger study, the *Ecological Functions of Hudson River Shorelines*, being conducted by David Strayer and Stuart Findlay of the Cary Institute of Ecosystem Studies in collaboration with the Hudson River National Estuarine Research Reserve (HRNERR). One key objective of this broader study is to provide basic information required for maintenance and restoration of the shore zone, and to articulate the different functions of natural and engineered structures and ecological communities. Operating within this framework, gastropods were examined across six shoreline types: sand, unconsolidated rock, bedrock, riprap, timber cribbing and sea wall (revetment and sheet pile). Two main questions were addressed: 1) is the abundance and diversity of snails greater on complex habitats and diverse substrates, and 2) is the abundance and diversity of snails associated with exposure to disturbance? Under the first hypothesis habitats with greater diversity of plant cover, complexity of substrate and higher levels of organic material would contain greater densities and higher diversity of snails (Lodge et. al 1987, Thorp et. al 1997, Strayer and Smith 2000). Under the second hypothesis increased exposure to wind, waves and human disturbance would result in lower numbers of snails (Strayer and Smith 2000).

In addition to contributing to the understanding of the ecological importance of gastropod communities in the shore zone, this study provides critical information for understanding the impact of current and future alien species (Loo et. al. 2007), notably the established *Bithinia tentaculata,* the anticipated New Zealand Mud Snail (*Potamopyrgus antipodarum*), and the recently discovered molluscivore, the Chinese mitten crab (*Eriocheir sinensis*) (Schmidt 2008, pers. comm.). The full impact of invasives remains unknown but continues as a significant concern (Mills et al. 1996).

METHODS

Gastropod communities from three sections of the Hudson River (lower, mid, upper), from Poughkeepsie to Albany (Fig. 1), were sampled across six shoreline types: sand, unconsolidated rock, bedrock, riprap, timber cribbing and sea wall. For each of these shoreline types data were collected on three elevation zones: subtidal, intertidal and upland. Sampling took place during two overnight field trips in the early summer of 2008, the first taking place June 1st through the 3rd, and the second June 29th through July 1st. Each site was identified using GPS coordinates provided by Dr. Strayer and HRNERR.

Because of the variety of habitat structures found among the shoreline types and elevations, a variety of sampling techniques were employed, using a 3x3 quadrat design at each site (Fig. 2). The sub-tidal zone is that portion of the river bank that lies just below low-tide level, the intertidal zone is that portion of the river bank that is cyclically inundated with water as the tide rises and falls, and the upland zone is the shore bank immediately above the high-tide mark. These three zones mark the dynamic interface between the river and the surrounding landscape, with each providing different species composition, habitat structure and function.

Site Protocols

Three different collection protocols were used to cover the three elevations at each of the sites. These protocols combine quantitative plot sampling with full-site visual

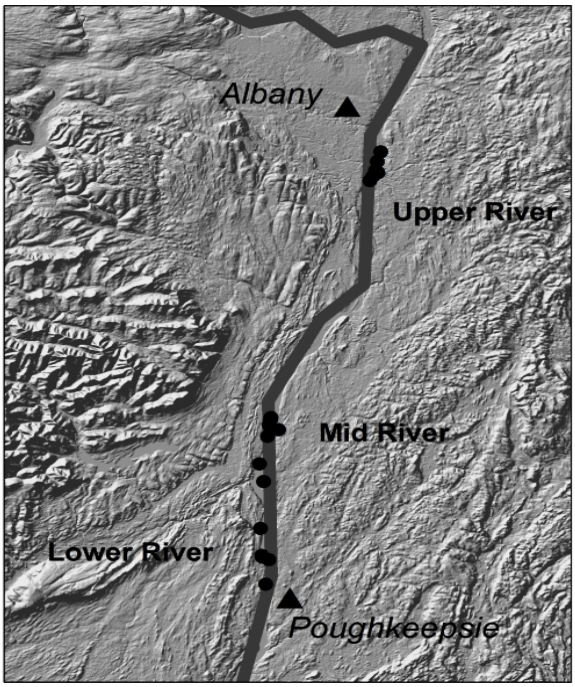

Figure 1. Map of the study area showing sample sites at each river section.

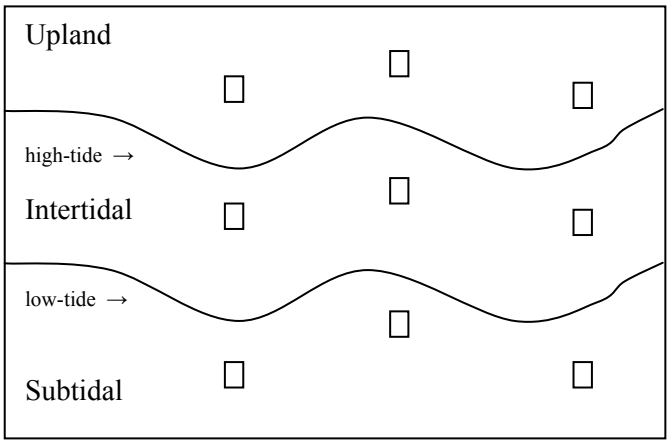

Figure 2. Illustration of site with sample quadrats and their relative position.

inspections to capture unusual habitats or species. For each of the 18 sites, three quadrats along 100 meter transects were laid parallel to the shoreline for each elevation. Figure 2 shows an idealized quadrat arrangement, but it does not indicate the complexity of habitat structures for a given site; thus, actual layout in the field was more complex. For

example, sea wall sites were typically in deep water and therefore the idealized shore profile did not exist. Regardless, in all cases this basic design was employed, sampling parallel to the shoreline with each quadrat at least 10 meters apart.

In the sub-tidal zone, three 30-second sweeps of substrate were completed with a 1,200 μm mesh D-net. Each sweep covered one-meter square, at one-meter depth below mean low tide. The selection of sample plots was selected in-situ based on appropriateness of habitat (e.g., inclusion of vegetation, exclusion of sharp rock substrate, etc.). Due to low visibility, in most cases a priori determination of vegetation was not possible, but vegetation was included in the sample whenever possible.

In the intertidal zone, three one-meter square plots were sampled at mid slope (during low tide), using a combination of handpicking and a 1.4 mm sorting sieve. Depending on substrate type, picking frequently included turning over of rocks, pulling of submerged vegetation and the washing of sediments. In a few cases, snails were exceptionally abundant in the inter-tidal zones. In these cases quadrats were divided into either half or quarters and total numbers determined accordingly.

For the upland zone, sampling took place within 10 meters of the high tide mark. This habitat was by far the most complicated, including multiple scaled structures and micro-habitats. Selection of quadrats attempted to include as broad a range of habitats as possible. Handpicking and a 6.6 mm and 1.4 mm sieve were used for sorting the detritus, soil, and rocks. Sampling included the examination of large rocks, plants, and trees.

A number of abiotic response variables have been identified including: mean slope, shoreline complexity, rugosity, sediment grain size, organic content, vegetation structure, coarse woody debris, wrack, turbidity, peak wave energy and exposure (D.

Strayer and S. Findlay, pers. comm.). Mean slope, complexity and rugosity are included in analysis here. Slope was determined using two methods, first using a line level from 1 meter below low tide to 1 meter above high tide, and second, using a depth finder to determine slope from the 1 meter point below low tide, to 5 meters perpendicular off shore. Complexity was measured using 1 meter calipers and measuring the shoreline as it ran parallel to the 100 meter straight measure, using the ratio of the length measured by the calipers to the taut 100 meter line. Complexity equals 1 for a shoreline that runs exactly parallel to the 100 meter line, or higher for shorelines that deviate from the 100 meter line. Rugosity is the vertical roughness of the shoreline substrate, and was measured using a 1 meter chain lain as closely as possible to the substrate contours. The covered distance is then measured by a taut tape, and a ratio determined for chain length (1 meter) to the tape length.

Sample Processing

While all sub-tidal samples were collected with a dip net, the inter-tidal and upland samples were typically handpicked or run through sieves to collect all snails greater than 2 mm. All snails collected were preserved in full strength, 95% analytic grade ethanol for later identification and possible genetic analysis (Dillon 2005). Snails were identified in the lab to genus or species using standard references (Pilsbry 1939-1948; Harman and Berg 1971; Burch 1962, 1989; Jokinen 1992). Voucher specimens will be deposited at the National Museum of Natural History, Washington, D.C.

Analysis

Because of the distinctive elevation community types (subtidal, intertidal, and upland) and the different sampling techniques and conditions, statistical comparisons across elevations should be viewed cautiously. Statistical analysis includes ANOVAs for density (abundance) and diversity on elevation, shore type and river section, as well as regression analysis for diversity and density against exposure, rugosity and slope. For diversity, both species richness as well as Shannon's diversity index (H′) were used. Non-metric multidimensional scaling (NMS) (McCune and Grace 2002) was also used to assess differences in gastropod community composition across different types of shorelines. Statistical analysis includes only the data collected as part of the primary 3x3 sampling design for each site as described above, and does not include data collected as part of each site survey, or from additional sampling. These additional data are included in qualitative analysis.

RESULTS

Qualitative Analysis

The diversity of aquatic gastropods collected in this project is consistent with previous studies (Strayer 1987), representing 14 of the 30 taxa known from the river (Table 1). In addition, three newly reported aquatic species in the Hudson were documented in this study. Figure 3 includes pictures of the selected species discussed below. Table 1 lists all reported historical records and covers dozens of separate studies (Strayer 1987). Three of the species in Table 1 are known exotics, and four are suspected invasives via the Erie Canal, of which one of each was collected in this study. *Bithynia*

tentaculata is an exotic snail and is known to be an intermediate host for a number of lethal waterfowl trematodes (Sauer et al. 2007). The suspected invasive is represented by a single recently empty shell of *Pleurocera acuta*. No detrimental effects of this invasive have been reported.

Live specimens of three previously unreported species for this section of the river were also collected during this study. Several *Littoridinops tenuipes* were found throughout the lower and mid river study sites, and, although this species was reported for the first time in the lower river in 2001 (Strayer & Smith 2001), this is their first documented finding north of Poughkeepsie. Two individuals on the New York state list of species of special concern, *Valvata lewisi*, and several *Floridobia winkleyi,* were also found. It is important to note that the original identification of *F. winkleyi* in this study was *Marstonia lustrica*, a species previously reported from the river by a number of researchers, and morphologically almost identical to *F. winkleyi*. After the original identification, genetic analysis was completed on several specimens for the mitochondrial gene COI (Liu pers. comm.). These results unequivocally identified these snails to be *F. winkleyi*. No specimens of *M. lustrica* were identified in this study. This first record of *F. winkleyi* in the river, and in the state of New York, represents a significant shift in its known range, with the only other records occurring in tidal coastal fresh and brackish waters extending from Connecticut to Maine (Davis & Mazurkiewicz 1985; Smith 1994).

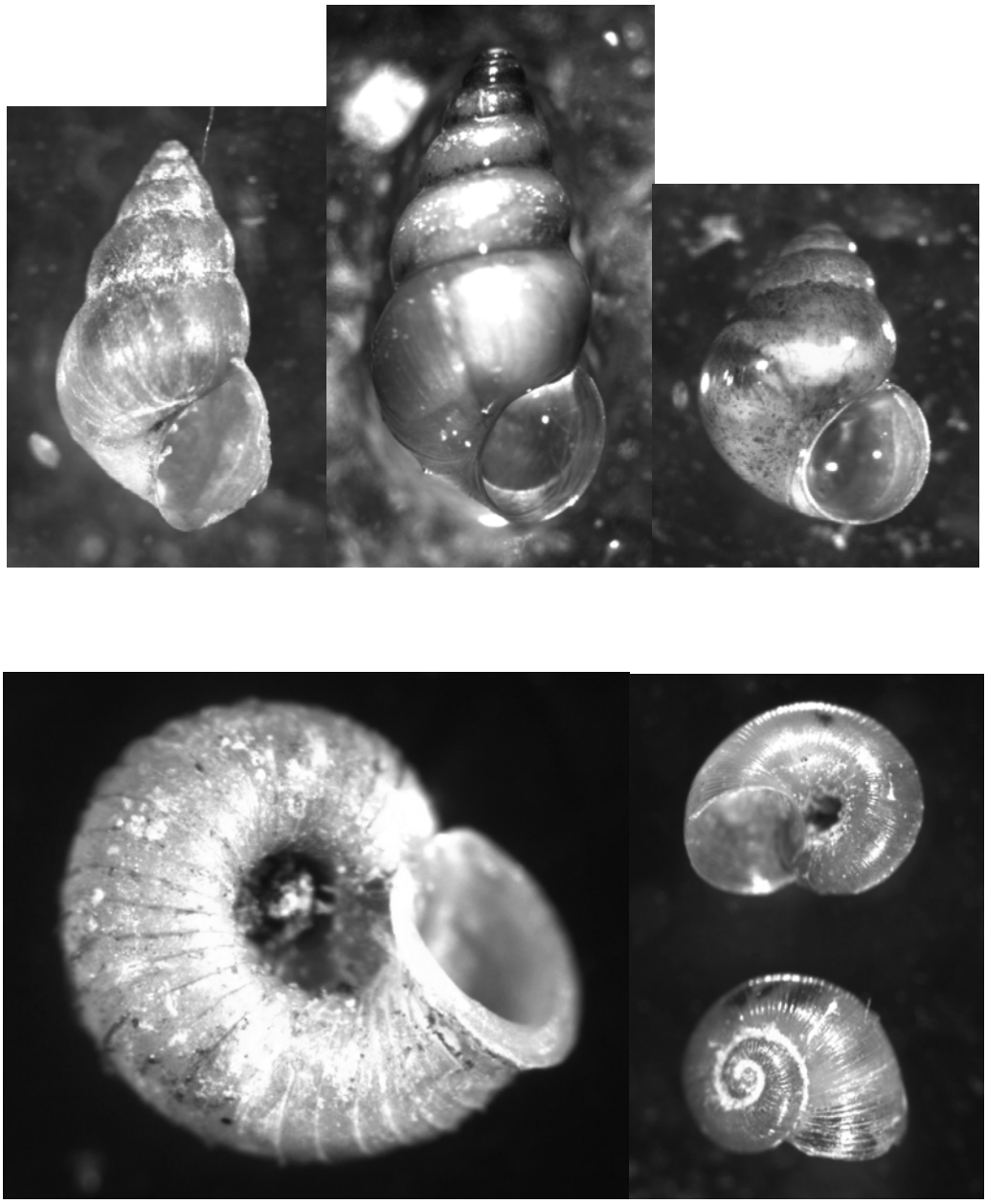

Figure 3. Selected photographs of some snails found during this study. Clockwise starting from the top: *Littoridinops tenuipes*, *Pomatiopsis lapidaria*, *Floridobia winkleyi*, *Vallonia costata*, and *Valvata lewisi*. Scale bar is approximately 1.0 mm: ----------

Two upland species not previously reported in Hudson River surveys were collected below the high-tide mark. *Vallonia costata* was represented by one specimen, and was found on bricks in timber cribbing in the inter-tidal elevation. *Pomatiopsis*

lapidaria, known to be amphibious, was found at several sites in the mid and upper river sections, was represented by several specimens, and was found both in the upland and inter-tidal elevations. Due to its amphibious nature, ***P. lapidaria*** has rarely been reported in New York (Strayer, pers. comm.), with only a handful of records. No specimens were reported by Strayer (1987) or Harman and Berg (1971), and Jokinen only reported one live population in her survey of New York (Jokinen 1992).

The results for land snails overall are disappointing. The upland elevations yielded fewer species and lower numbers than what was expected (Table 2). The two genera of slugs found, Arionidae and *Deroceras*, are invasives, and are common throughout the northeast United States. While sampling in the upland elevations at each of the sites was extensive, it appears that relatively dry weather resulted in low incidence during the primary upland sampling trip in early June. This is evident when comparing the fifteen upland sites surveyed in early June to an additional three sites sampled in late June (Figure 4), which followed rain events throughout the month. Due to logistical constraints, additional sampling of the upland sites was not feasible.

Prosobranch snails	1867-1900	1936	1972-1985	2008
Valvata lewisi				x
Valvata piscinalis[1]			x	
Valvata sincera			x	
Valvata tricarinata	x	x	x	
Viviparus georgianus[1]		x	x	
Campeloma decisum	x	x		
Lioplax subcarinata	x	x		
Bithynia tentaculata[1]	x	x	x	x
Probythinella lacustris	x	x	x	x
Gillia altilis	x	x		
Birgella subglobosa[2]		x		
Littoridinops tenuipes[3]				x
Marstonia lustrica	x			
Amnicola limosa	x		x	x
Amnicola pupoidea			x	
Goniobasis (=Elimia) livescens[2]	x	x	x	
Goniobasis (=Elimia) virginica	x	x	x	x
Pleurocera acuta[2]			x	E
Floridobia winkleyi				x
Pulmonate snails				
Pseudosuccinea columella			x	
Lymnaeidae	x	x	x	x
Physidae *(Physella)*	x	x	x	x
Gyraulus deflectus	x		x	
Gyraulus parvus	x	x	x	x
Helisoma anceps	x	x	x	
Micromenetus (Menetus) dilatetus			x	E
Planorbella trivolvis	x	x	x	
Promenetus exacuous		x	x	x
Ferrissia rivularis			x	E
Laevapex fuscus		x		
Land snails found below high tide in 2008				
Pomatiopsis lapidaria				x
Vallonia costata				x

Table 1. Historical survey results of gastropods in the Hudson Valley (Strayer 1987) combined with this study's findings. (E=recent empty shells, 1=Exotic, 2=Suspected Invasive, 3=First reported in 2001 (Strayer & Smith 2001))

Upland Gastropods	Abundance
Stenotrema hirsutum	1
Euchemotrema fraternum	1
Deroceras sp. [1]	7
Arionidae *sp.* [1]	6
Novisuccinea ovalis	21
Helicodiscus parallelus	1
Retinella rhoadsi	1
Vallonia costata [2]	2
Pomatiopsis lapidaria [2]	13

Table 2. List of land snails found in this study. (1. exotic 2. inter-tidal)

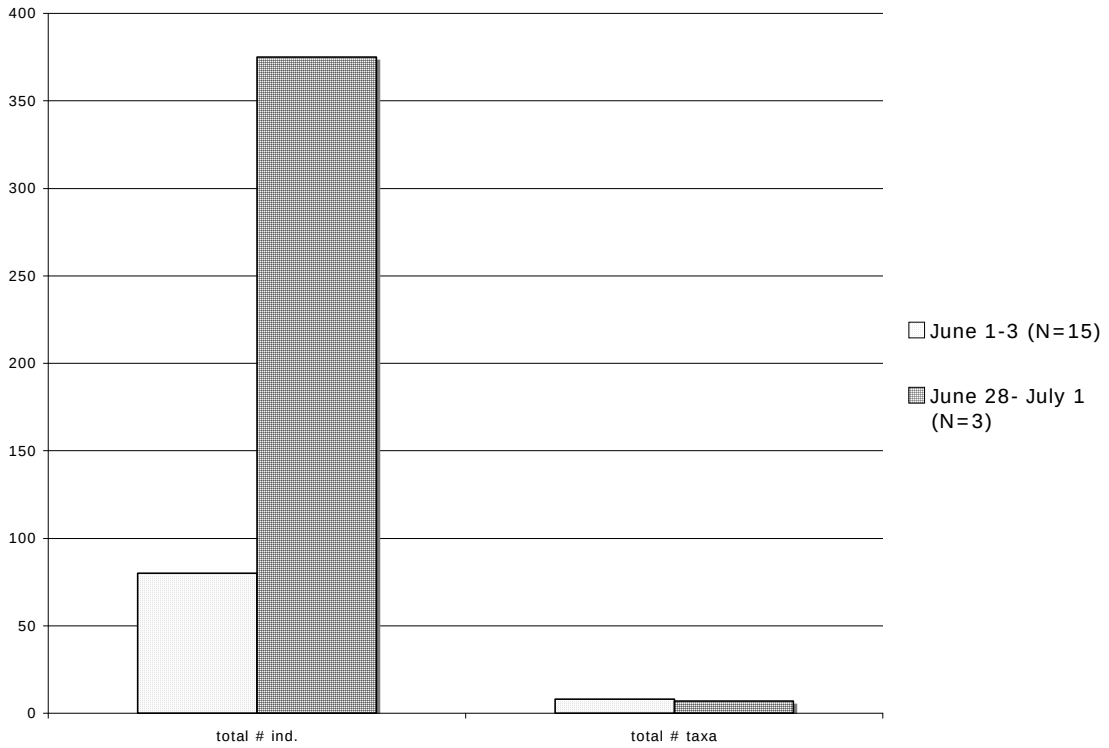

Figure. 4. Incidence of land snails in the beginning of June and the end of June. 15 upland sites were surveyed from June 1-3 and 3 sites were sampled from June 28-July 1.

Quantitative Analysis

The distribution of snail counts in the samples deviated significantly from normal (*Shapiro-Wilk* = 0.29) and so the data were subjected to a $\log_{10}$ transformation. Following this transformation, the distribution of the data still deviated from normal, but less so (*Shapiro-Wilk* = 0.78). Species richness distributed more closely to normal (*Shapiro-Wilk* = 0.78) and $\log_{10}$ transformation did not result in any improvement, so these data were not transformed for analyses. The transformed snail count data were subjected to a 3-way ANOVA, examining the effects of the three independent variables: elevation, shore type and river section.

For abundance, the ANOVA revealed significant main effects of elevation, shore type and river section (Table 3). Tukey post-hoc comparisons indicated that across shore types, sand and sea wall contained significantly lower numbers of snails, while unconsolidated and riprap contain significantly higher numbers. The mean snail abundances are also higher in the lower and mid river sections than at the upper river section. Post-hoc tests also indicated the highest snail count at the inter-tidal elevation, as expected due to sampling efficiency.

All of these main effects were qualified, however, by a significant three-way interaction (Table 3). Examination of the means across the 54 cells revealed higher snail counts in the riprap and unconsolidated rock river sections, and highest at the intertidal elevation (Table 4).

ANOVA

total_snails_log10		Experimental Method				
		Sum of Squares	df	Mean Square	F	Sig.
Main Effects	(Combined)	40.805	9	4.534	23.893	.000
	elevation	24.843	2	12.421	65.459	.000
	shore type	13.811	5	2.762	14.557	.000
	river section	2.151	2	1.076	5.668	.005
2-Way Interactions	(Combined)	24.730	24	1.030	5.430	.000
	elevation * shore type	13.948	10	1.395	7.351	.000
	elevation * river section	2.569	4	.642	3.389	.012
	shore type * river section	8.212	10	.821	4.328	.000
3-Way Interactions	elevation * shore type * river section	14.239	20	.712	3.752	.000
Model		79.774	53	1.505	7.932	.000
Residual		20.494	108	.190		
Total		100.268	161	.623		

Table 3. Three-way ANOVA summary table for $\log_{10}$ transformed snail abundance.

Elevation	upland			intertidal			subtidal		
Riversection	lower	mid	upper	lower	mid	upper	lower	mid	upper
bedrock	0.33	0.00	0.00	8.67	89.33	16.33	7.33	3.33	0.33
cribbing	2.00	0.00	0.33	23.67	184.00	23.00	0.00	1.33	2.33
unconsolidated	18.67	83.33	10.67	**267.33**	**224.00**	0.00	5.00	5.00	0.00
riprap	0.00	0.00	0.00	12.33	**582.67**	**154.00**	30.67	7.00	1.67
sand	1.33	5.00	2.00	0.00	0.00	3.67	0.00	4.00	2.33
seawall	0.00	0.00	0.00	0.00	0.00	98.00	0.00	0.67	0.00

Table 4. Raw mean snail counts by cell (cell n's = 3). Bold indicates significantly (p=.05) higher counts.

The ANOVA on the number of species (richness) indicated significant main effects of elevation, shore type and river section (Table 5). Tukey post-hoc analysis indicates that across shore types, sea wall contains significantly lower numbers than riprap, unconsolidated rock and bedrock; across elevation, species richness was significantly lower at the upland sites.

These main effects were again qualified, however, by a significant three-way interaction (Table 5). Examination of the mean total species counts across the 54 cells again revealed higher counts in riprap and unconsolidated rock, and again at the intertidal, but also sub-tidal, elevations (Table 6).

To test for possible effects of upland and subtidal elevations on the inter-tidal elevation, an additional ANOVA was run on the inter-tidal elevation only. There was a highly significant effect for shore type on total species richness, $F(5,36) = 5.48$, $p<.001$, and a marginal effect of river section, $F(2,36) = 2.38$, $p=.109$. Post-hoc analysis indicates there was no effect for shore type in the lower or upper river sections, but species richness was significantly greater for riprap in the middle river section versus sand and seawall.

ANOVA

total_species		Experimental Method				
		Sum of Squares	df	Mean Square	F	Sig.
Main Effects	(Combined)	62.352	9	6.928	7.687	.000
	elevation	26.012	2	13.006	14.432	.000
	shore type	32.994	5	6.599	7.322	.000
	river section	6.272	2	3.136	3.429	.034
2-Way Interactions	(Combined)	76.926	24	3.205	3.557	.000
	elevation * shore type	45.395	10	4.540	5.037	.000
	elevation * river section	7.284	4	1.821	2.021	.097
	shore type * river section	24.297	10	2.425	2.690	.006
3-Way Interactions	elevation * shore type * river section	37.309	20	1.865	2.070	.009
	Model	179.512	53	3.387	3.758	.000
	Residual	97.333	108	.901		
	Total	276.846	161	1.720		

Table 5. Three-way ANOVA summary table for species totals.

Elevation	upland			intertidal			subtidal		
River section	lower	mid	Upper	lower	mid	Upper	lower	mid	upper
Bedrock	0.33	0.00	0.00	2.67	**3.33**	1.00	2.33	1.33	0.33
Cribbing	1.33	0.00	0.33	0.67	2.67	1.33	0.00	1.00	1.00
Unconsolidated	1.00	1.00	1.67	**3.33**	**3.00**	0.00	1.00	2.33	0.00
Riprap	0.00	0.00	0.00	1.67	**3.00**	2.33	**3.00**	2.67	1.00
Sand	1.00	1.33	1.67	0.00	0.00	0.67	0.00	1.00	1.33
Seawall	0.00	0.00	0.00	0.00	0.00	1.67	0.00	0.33	0.00

Table 6. Mean species counts (cell n's = 3). Bold indicates significantly (p=.05) higher species counts.

There was also a significant effect for shore type on $\log_{10}$ abundance, $F(5,36)= 14.53$, $p<0.001$, and for river section, $F(2,36)= 6.17$, $p=0.005$. Post-hoc analysis indicates that there was a significant difference between sand (low abundance) and unconsolidated rock (high abundance) in the lower river section, a significant difference for sand and seawall (low) versus unconsolidated rock, riprap, bedrock and cribbing (high) in the middle river section, and a significant difference for unconsolidated rock (low) versus riprap (high) in the upper river section.

Regressions of $\log_{10}$ slope, complexity and rugosity on the transformed snail abundance data and H′ (Shannon Index) revealed no significant relationship between slope, complexity or rugosity on snail count or H′ (Table 7).

NMS ordination was attempted on all samples ($\log_{10}$) but was unsuccessful. Ordinations were then completed without species that occurred in fewer than three samples, resulting in no significant ordination (stress=29.76). Additional analysis was completed without species that occurred in fewer than 3 samples, as well as all samples with fewer than 2 species. This resulted in a reasonable (stress =12.72), 3-axis ordination for both samples and species (Figures 5 & 6).

Correlation analysis for the three ordination axis for samples, along with slope, rugosity, complexity, elevation, river section and shore type were completed (Figure 5). The results indicate that 17.6% (r^2) of axis one can be explained by slope, 20.1% can be explained by rugosity, and 34.5% by elevation. For axis 2, only elevation has any explanatory power accounting for 21.2% of the results, and for axis 3, elevation and river section explain 19.8% and 14.8% of the data respectively. The clusters of species in Figure 6 coincide with the clustering of samples in Figure 6, showing that the ordination has grouped the data according to the dominant species for each sample. Despite low numbers (less than 10 individuals), the upland species are clearly clustered together where no other species occurred. Samples that were dominated by Physidae and Lymnaeidae are clustered at the top of axis 3. *F. winkleyi* and *P. lapidaria* are clustered with Physidae and Lymnaeidae because they are present in those same samples at relatively high numbers. Of the 22 samples represented by the cluster of Physidae, Lymnaeidae, *F. winkleyi* and *P. lapidaria*, 19 are inter-tidal, two are upland and three are sub-tidal. Samples that were dominated by *A. limosa* and *L. tenuipes*, and had only one or two individuals from other species, are clustered at the bottom of axis 1 and 2. Three of these samples are inter-tidal and 16 are sub-tidal. Overall, the patterns of snail occurrences are relatively weak, but do indicate that there is some effect of environmental variables on the distribution of gastropods, particularly elevation.

		slope	rugosity	abundance	complexity	H
slope	Pearson Correlation	1	.477	-.238	-.368	.179
	Sig. (2-tailed)		.045	.341	.133	.476
	N	18	18	18	18	18
rugosity	Pearson Correlation		1	-.461	-.098	.163
	Sig. (2-tailed)			.054	.698	.518
	N		18	18	18	18
abundance	Pearson Correlation			1	.435	.232
	Sig. (2-tailed)				.071	.354
	N			18	18	18
complexity	Pearson Correlation				1	.117
	Sig. (2-tailed)					.642
	N				18	18
H	Pearson Correlation					1
	Sig. (2-tailed)					
	N					18

Table 7. Correlations between slope, complexity, and rugosity on H' and snail abundances (N). Slope and abundances were non-normal and subjected to $\log_{10}$ transformation.

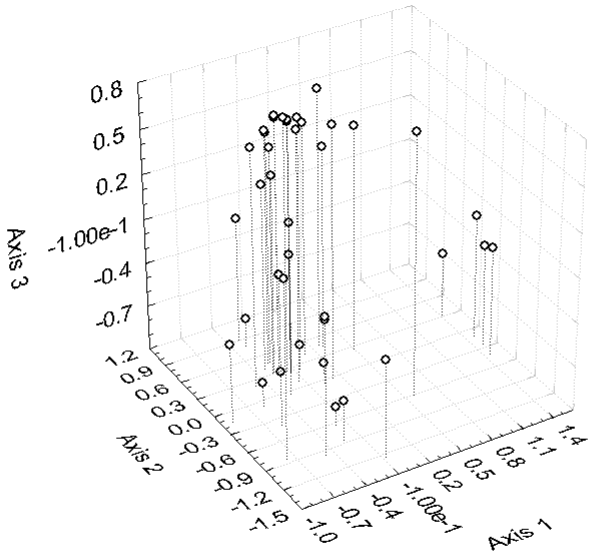

Figure 5. NMS ordination of samples.

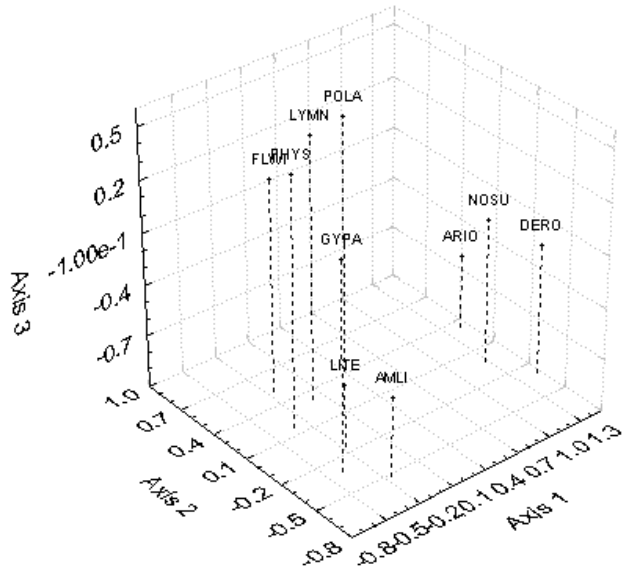

Figure 6. NMS ordination for species.

DISCUSSION

The goal of this project was to survey gastropods across the six different shore types, and to draw some conclusions on how the various assemblages correlated with the various ecological and physical structures. It is clear from the analysis that the inter-tidal elevations supported the majority of snails, although the data on the upland and sub-tidal elevations are relatively weak. At the inter-tidal elevation, the unconsolidated rock and riprap habitats supported the most snails and the most species, although bedrock also supported a high number of species. Sand beaches and seawall structures were the most depauperate.

Generally speaking this study suggests that stable, medium scale structure (brick, small rock, timber cribbing) may be beneficial to gastropod communities, whereas unstable, fine scale (sand and unconsolidated rock), or stable large smooth structures (seawall and large exposed basalt surfaces) are not as supportive. An issue for management of shorelines then may be not so much a question of natural versus man-made, but rather which type of structure is best suited to protect the shoreline while simultaneously promoting aquatic life.

The environmental variables tested in this paper did not provide explanatory power for the gastropod communities. This agrees with field observations, specifically that there are a plethora of microhabitats in each of the major shoreline types, precluding any distinct larger-scale pattern from emerging. For example, within the timber cribbing sites, the variability in habitat structure ranged from large smooth faced basalt, to small

rough bricks, to the wood support structure, with each type of habitat supporting different abundances and different species. This type of variability existed at most of the sites. However, NMS ordination does support elevation as a potential selecting factor, distinguishing samples dominated by *L. tenuipes* and *A. limosa* as occurring primarily in sub-tidal elevations, as opposed to Physidae, Lymnaeidae, *F. winkleyi*, and *P. lapidaria* as dominating inter-tidal elevations.

Table 1 highlights several interesting results. First is the incidence of the relatively rare and state-listed snail *Valvata lewisi*, which has no previous record in the Hudson Valley. In this study, two live individuals were collected from mid-river cribbing habitat, in the sub-tidal elevation. There are only two records for this species in New York, one from a ditch in Onondaga County, in the St. Lawrence River watershed, and one from Oneida Lake in central New York (Jokinen 1992).

Floridobia winkleyi is one of the most surprising finds. This species is new to New York State, and represents a substantial increase in its known range. It is of particular interest because of its close similarity morphologically to *Marstonia lustrica*, which has been recorded occasionally in the Hudson River, but was not found during this survey. The identification of *F. winkleyi* came about after initial identification as *M. lustrica* based on morphology. Several specimens were then submitted for genetic analysis as part of another project on *M. lustrica*, with the surprising result that they were *F. winkleyi.* Further work needs to be completed to check historical lots and records to determine if *F. winkleyi* is indeed new to the river, or if it has been mistakenly identified as *M. lustrica*, or if both species are present and *M. lustrica* was simply not found during this survey.

Another species only recently reported for the upper Hudson River is *Littoridinops tenuipes*. It is generally considered an Atlantic seaboard species, extending from Florida to the northeastern shore of Massachusetts, with records only for the lower Hudson River (below Poughkeepsie) (Smith 1987; Strayer pers. comm). This species was a common occurrence throughout the lower and mid-river sections in this study (both above Poughkeepsie), but was not represented in the upper river section. It is possible that this species is a relatively recent introduction to the river and is moving upstream.

Pomatiopsis lapidaria is a species that is also considered rare in New York, yet it occurred at four locations, in all three river sections. The land snail *Vallonia costata* was found attached to bricks in timber cribbing in the inter-tidal elevation, raising the possibility that it is amphibious. Only one specimen of the exotic *Bithynia tentaculata* was found, which is in stark contrast to Strayer's (1987) findings in 1985 when they covered the rocks of the inter-tidal zone of the shoreline. While this study is not conclusive, it does appear that this species has declined significantly.

The results of this project highlight the great variety of habitats and community structures, and variety of gastropods that inhabit those environments, the multiple scales of interest, and the dynamic nature of the Hudson River shoreline. It also highlights how little is understood about gastropods and their relationship to the environment, while highlighting that such understanding is not beyond grasp, and that this information is critical for appropriate management. Recommendations for further study include repeating the work attempted here in the upland elevations, as well as looking closer at the potential differentiation of species occurrence in the inter-tidal and sub-tidal elevations.

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DID THE ZEBRA MUSSEL (*DREISSENA POLYMORPHA*) ALTER THE THERMAL BALANCE OF THE HUDSON RIVER?

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

The ecological effects of the zebra mussel (*Dreissena polymorpha*) invasion on the Hudson River have been well documented. Higher bivalve filtration rates after the invasion increased water clarity. This should have decreased water surface albedo and may have lead to changes in water temperature. This study used the equilibrium temperature concept to explore potential changes in water temperature due to change in albedo. Equilibrium temperature is the water temperature at which the sum of energy fluxes across the water surface equals zero. Equilibrium temperature is a function of meteorological and hydrological conditions including albedo. We calculated equilibrium temperature for the Hudson River for observed conditions and for modeled conditions where the influence of zebra mussel filtration was removed. Changes in albedo due to zebra mussel filtration were small and the resulting changes in equilibrium and water temperature were negligible. The equilibrium temperature concept may be useful for further exploration of drivers of a known warming trend.

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INTRODUCTION

Climate change due to global warming is likely to profoundly alter estuaries (Scavia et al. 2002). Critical physical factors such as the timing and volume of freshwater inflows will change and lead to shifts in water residence time, salinity, and mixing that determine or influence many properties of estuaries (Knowles and Cayan 2002; Scavia et al. 2002). Warming may also increase nitrogen fluxes resulting in eutrophication (Howarth et al. 2006). Warming will alter biological communities and ecological interactions within estuaries leading to likely changes in resource species (Oviatt 2004; Meynecke et al. 2006; Mackenzie et al. 2007). In addition to climate induced warming, many systems are also being invaded by exotic species. In 1992 the zebra mussel (*Dreissena polymorpha*) invaded the Hudson River. Impacts on macrozoobenthos (Strayer et al. 1998), native bivalves (Strayer and Malcom 2007), phytoplankton (Roditi et al. 1996; Caraco et al. 1997), zooplankton (Pace et al. 1998), fish assemblages (Strayer et al. 2004), dissolved oxygen (Caraco et al. 2000) cyanobacteria (Fernald et al. 2007) and sediments (Strayer and Malcom 2006) have been documented. Strayer et al. (1999) and Caraco et al. (2000) suggested that zebra mussels have likely increased water clarity in the Hudson. This is because the zebra mussel population has a higher filtration rate than exerted previously by native bivalves and as a consequence achieve greater removal of particles from the water column. The water surface albedo of clear water is less than that of turbid water (Rosenberg et al. 1983). Increasing water clarity should alter the thermal balance of the river, warming it by allowing more incoming solar radiation to enter the water. This could contribute to the known warming trend in the Hudson that

has accelerated in years since the zebra mussel invasion (Pace and Seekell 2009). Changing temperature during spring and early summer months could change reproductive timing and success of fish species. It also may impact the development of some species and their ecological interactions as they undergo ontogenetic shifts in diet and predator susceptibility (Mehner et al. 1996; Tetzlaff et al. 2005). Warmer summer months could lead to further constraints on thermally suitable habitat for species during what are already the warmest months of the year (Mohseni et al. 2003).

Equilibrium temperature is the water temperature at which the sum of all energy fluxes across the water surface equals zero (Mohseni and Stefan 1999; Bogan et al. 2003; Caissie et al. 2005). It is a function of meteorological and hydrological conditions including albedo (Mohseni and Stefan 1999; Bogan et al. 2003; Caissie et al. 2005). At coarse temporal scales river water temperatures closely track equilibrium temperature (Bogan et al. 2003). This study uses the equilibrium temperature concept to test the hypothesis that changes in water temperature are a result of the zebra mussel invasion. The equilibrium temperature approach better accounts for the major drivers of water temperature than simple regression models but has more manageable data requirements than full energy budgets. Albedo is an important component of equilibrium temperature and thus modeling albedo is an important part of this exercise. Albedo was calculated based on observed conditions. Albedo without zebra mussel filtration was then estimated. These values were used to calculate observed and potential (without zebra mussel filtration) equilibrium temperature time series to test for an effect of mussels on temperature.

METHODS

Data

Meteorological data from 1989-2007 were obtained from a long term environmental monitoring site in Millbrook NY (Long W 073.741447°, Lat N 41.785823°, elevation 128m) (Cary Institute of Ecosystem Studies 2008). This is the closest weather station to the Hudson River sampling sites that records all of the necessary meteorological parameters over the period of time examined. Photosynthetically active radiation (μmol s^{-1} m^{-2}) was measured with a Licor Model LI-190SB sensor 2 m (1989-2002) and 2.5 m (2002-2007) above the ground. Incoming shortwave radiation (W m^{-2}) was measured with an Eppley Model 8-48 sensor 2 m (1989-2002) and 2.5 m (2002-2007) above the ground. Relative humidity was measured with a Phys Chem Corp. PCRC-11 or PCRC-55 (1989-1997) and a Campbell Scientific HMP45C (1997-2007) sensor 1.6 m above the ground inside a motor aspirated shield. Air temperature (°C) was measured with a Campbell Scientific Model 107 or 207 (1989-1998) and HMP45C (1998-2007) sensor 1.6 m above the ground inside a motor aspirated shield. Wind speed (km h^{-1}) was measured with a Campbell Scientific Model 014A sensor (1989-2002), Met One Instruments Model 50.5 solid state sensor, and Climatronic Corp sonimometer on a 10 m tower. All measurements were taken over a flat, mowed grass surface. Solar angle was calculated using the standard equations (see Allen et al. 1998).

Hudson River data from 1989-2007 were collected off of Kingston NY (Long W 74.004088° Lat 41.928877°) bi-weekly during the ice free season (typically April to

November). This portion of the river is well mixed and turbid with suitable substrate and salinity for zebra mussels (Caraco et al. 1997). Photosynthetically active radiation (PAR)(µmol s^{-1} m^{-2})(L_z) was measured with a Licor LI-193SA spherical quantum sensor. Zebra mussel filtration was estimated from a geographically stratified (to include multiple bottom surface types) sample and regression model based on abundance and size (Strayer et al. 2006). Water temperature was measured at a USGS station just south of Poughkeepsie. Water flow was measured as freshwater inputs into the lower Hudson over the gauging station at the Federal dam in Troy. Inorganic seston was calculated the product of total seston and 1 - organic seston mg L^{-1} / total seston mg L^{-1}. Seston was collected and measured from water samples with glass fiber filters. The organic composition was determined by combustion.

Light Extinction Coefficient

The light extinction coefficient k (m^{-1}) was calculated based on irradiance profiles obtained with the quantum sensor. Light extinction is calculated as the regression coefficient of the natural log of L_z at 0.5m, 1m, and 2m relative to irradiance at 0.5m. This can be used as a proxy for water clarity (Caraco et al. 1997). A multiple regression model of k as a function of zebra mussel filtration, inorganic seston, calendar day, and water flow was used (Table 1). To estimate k in a zebra mussel free scenario filtration is set to zero.

Table 1. Parameter estimates for a multiple regression model of the light extinction coefficient.

Parameter	Coefficient	Std. Error	Std. Coef.	P
Constant	-0.834	0.084	0	-
Inorganic Seston	-0.05	0.003	-0.624	< 0.001
Julian Day	$-8.18*10^{-4}$	$3*10^{-4}$	-0.089	0.005
Zebra Mussel Filtration	0.019	0.008	0.068	0.021
Water Flow	$-1.37*10^{-4}$	$1.4*10^{-5}$	-0.371	< 0.001

Cloud Cover

Cloud cover is an important component of equilibrium temperature and surface albedo. Clouds non-selectively refract incoming solar radiation. Consequently, diffuse radiation increases with cloud cover. This decreases water temperatures and increases albedo. Cloud cover is difficult to measure and is rarely recorded (Lhomme et al. 2007). Cloud cover was estimated using the proxy given by Lhomme et al. (2007) and Lenters et al. (2005) to estimate fractional cloud cover (f_c). Here f_c is the ratio of the of incoming solar radiation (H_s) for each daylight hour to potential incoming solar radiation on a cloud free day ($H_{s,0}$)(equation 1). To eliminate time of day effects the sum of H_s and $H_{s,0}$ for each daylight hour were used to find daily cloud cover.

[1] $$f_c = \frac{H_s}{H_{s,0}}$$

Albedo

There are several components of surface water albedo (α) (Nunez et al. 1972). Incident radiation can be reflected at the water surface, backscattered by particulates in

the water or reflected off of the river bed. All components are rarely measured (Nunez et al. 1972). Albedo was calculated after Caraco et al. (1997) as a ratio of irradiance in the air (L_{air})(µmol s^{-1} m^{-2}) and irradiance at the surface (L_0)(µmol s^{-1} m^{-2})(equation 2).

[2] $$\alpha = 1 - \frac{L_{air}}{L_0}$$

The sensor only measures in the 400-700 nm range; thus, it is necessary to assume that all wavelengths reflect equally on the water surface. Because L_0 was only measured between 1989 and 1993 albedo was calculated for the rest of the series with an empirical model of L_0. L_0 cannot be estimated from the light extinction coefficient and irradiance measurements at a known depth (L_z) because turbulence in the upper 0.5 m of the water column alters the light extinction relationship (Wetzel 2001). This approach generally over estimates surface irradiance values. In clear sky conditions (no diffuse radiation) on a flat surface albedo is a function of the solar angle (Wetzel 2001). However this condition rarely occurs at the study site. An ordinary least squares regression with solar angle, L_{air}, k, and inorganic seston (mg L^{-1}) as predictors explained over 97% of variance in L_0 ($\log_e$ transformed) (Table 2). Solar angle and L_{air} were $\log_e$ transformed.

Table 2. Parameter estimates for an empirical model of L_0.

Parameter	Coefficient	Std. Error	Std. Coef.	P	VIF
Intercept	-0.15	0.37	0.00	---	---
Solar Angle	-0.35	0.07	-0.11	<0.001	1.16
L_{air} (Log_e)	1.11	0.03	0.94	<0.001	1.19
Inorganic Seston	-0.01	0.005	-0.08	0.056	3.90
K	-0.11	0.05	-0.08	0.046	3.92

Albedo varies with time of day because of changing solar angle. On a typical summer day albedo has a maximum value near dawn, decreases to a minimum at noon and then increases again in the afternoon (Figure 1). To eliminate the time of day effect from the analysis the sum of L_0 and sum of L_{air} for each daylight hour were used to calculate daily albedo.

Equilibrium Temperature

Equilibrium temperature is the water temperature at which the sum of energy fluxes across the water surface equals zero (Mohseni and Stefan 1999; Bogan et al. 2003). At the coarse time scales (one day or greater) this is expressed as

[3] $H_s + H_l - H_e = 0$

where H_l is net long wave radiation (MJ m^{-2} day^{-1}) and H_e is evaporative heat flux (MJ m^{-2} day^{-1})(Caissie et al. 2005; Bogan et al. 2003). Convective heat flux is not accounted for because it has been found to be insignificant in water temperature modeling (Caissie et al. 2005). The equilibrium temperature can be expressed in linear form as a function of meteorological conditions (Mohseni and Stefan 1999). A simple approximation is presented by Caissie et al. (2005) as

[4] $T_e = B_1 H_s + B_2 T_a + B_3 T_d + B_4$

where T_e is equilibrium temperature (°C), T_a is air temperature (°C), and T_d is the dew point temperature (°C). The coefficient B_1 adjusts incoming solar radiation (H_s) for shading (assumed to be zero) and albedo. The product of B_1 and H_s represents the

radiation that enters the water body. Coefficient B_2 is atmospheric long wave radiation which is a linear function of T_a. Coefficient B_3 represents the amount of energy able exit the water body through evaporative heat flux at the dew point T_d. The coefficient B_4 is long wave radiation from the water degraded by atmospheric emissivity (a function of air water vapor pressure and cloud cover) and water surface emissivity (see Mohseni and Stefan 1999 and Caissie et al. 2005 for derivation of the coefficients).

Modeling Approach

Equilibrium temperature was first calculated using observed meteorological and light extinction data. Equilibrium temperature was then recalculated with observed meteorological data but with modeled light extinction data where the influence of zebra mussel filtration was removed. This alters albedo, changing the amount of solar energy entering the water and thus the equilibrium temperature. The impact of zebra mussel filtration on Hudson River equilibrium temperature was assessed by comparing the results of the two calculations.

RESULTS

The observed light extinction in the Hudson varies with time of year (Cole et al. 1992). The median light extinction coefficient was -1.79 m^{-1} with a minimum of -7.94 m^{-1} and a maximum of -0.84 m^{-1}. The modeled light extinction coefficients were similar to the observed. Fit was evaluated by root mean square error (RMSE). RMSE is the standard deviation of difference between observed and estimated values. The RMSE of modeled light extinction coefficients was 0.33 m^{-1}. The mean difference between the observed light extinction coefficient and the light extinction coefficient with zebra mussel filtration

set to zero is 0.066 m^{-1} (standard deviation 0.045). The portion of the light extinction coefficient attributed to zebra mussel filtration varies cyclically over time (Figure 2).

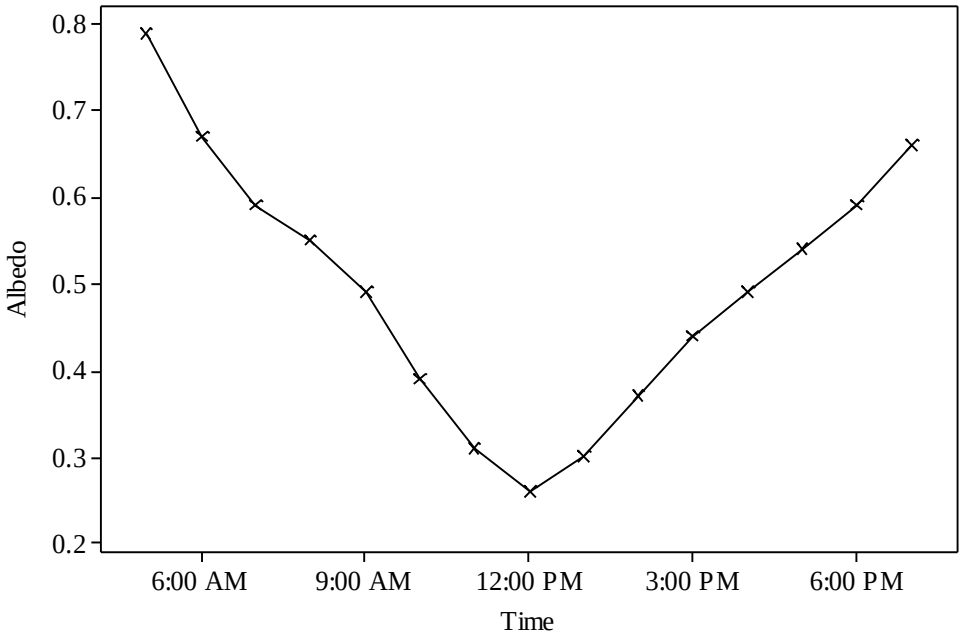

Figure 1. Hudson River albedo over the course of a typical summer day. Solar angle is largely responsible for the time of day effect (Rosenberg et al. 1983)

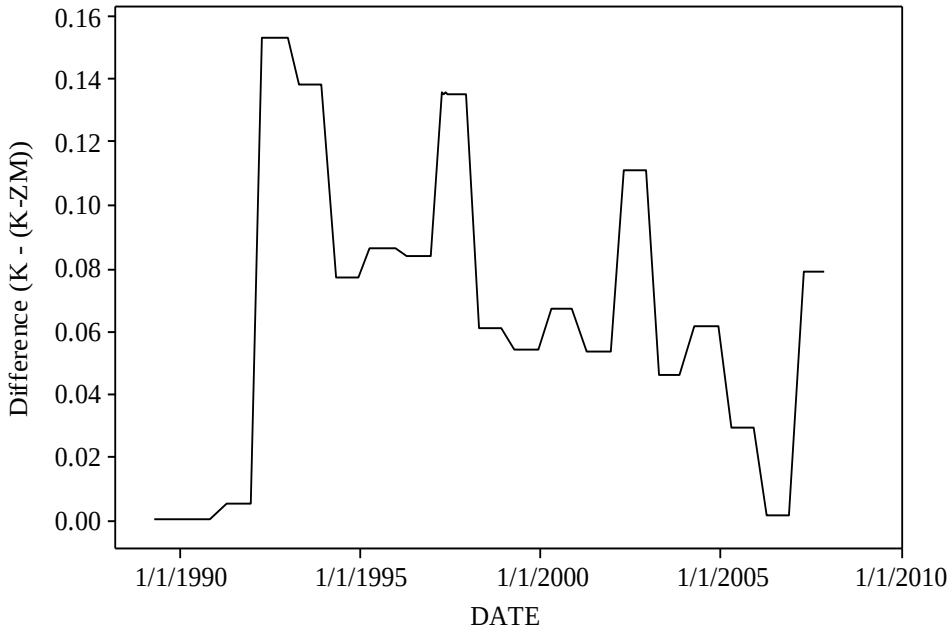

Figure 2. The difference in light extinction coefficient between observed and minus zebra mussel calculations. This difference is attributed to zebra mussel filtration. This portion of the light extinction coefficient follows the same 2-4 year cycle as the Hudson River zebra mussel population (Strayer and Malcom 2006).

Albedo varied greatly over the study period due to time of year, cloud cover and humidity. The median albedo was 0.455. Observed albedo ranged from 0.325 to 0.668. The modeled albedo closely tracked the observed albedo with a RMSE of 0.02161. The mean difference between modeled albedo and albedo minus the influence of zebra mussel filtration is 0.004234 with a standard deviation of 0.003. The change in albedo attributed to zebra mussel filtration follows the same two to four year cycle as the portion of the light extinction coefficient attributed to zebra mussel filtration (Figure 3). However, the difference between observed albedo and albedo minus zebra mussel filtration is

negligible. Consequently it is not necessary to calculate equilibrium temperature for the scenario without zebra mussels because any difference would be well within measurement error and routine environmental variability.

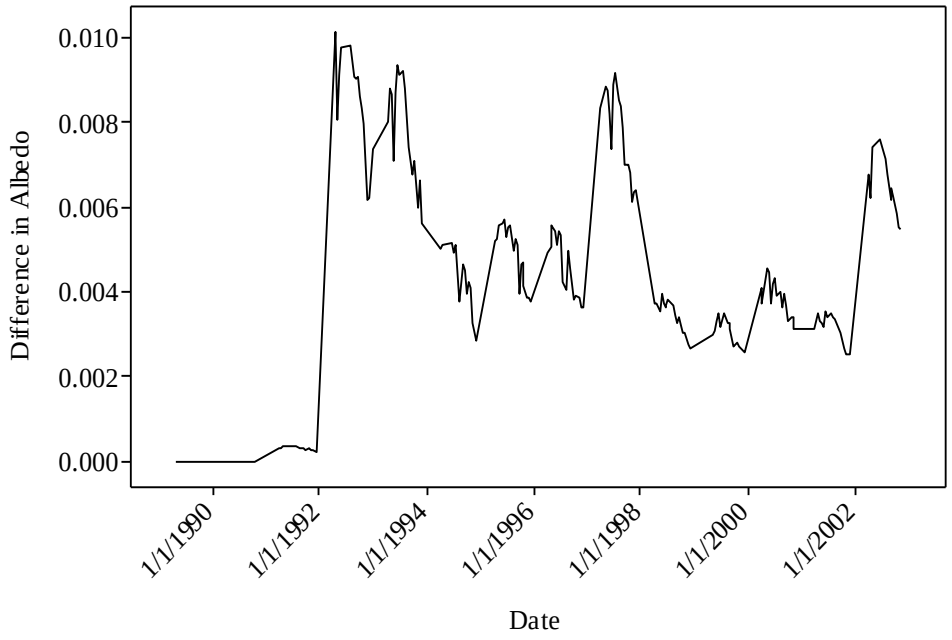

Figure 3. The difference in albedo between the observed data and a zebra mussel free scenario. The value is the change in albedo which is attributed to zebra mussel filtration. It follows the same cycle as the light extinction coefficient (figure 2) and the zebra mussel population cycle (Strayer and Malcom 2006).

The average air temperature over the period was 13.89°C (SD 6.86°C). Air temperature was roughly normally distributed with a minimum of -2.02°C and the maximum was 26.73°C. The mean equilibrium temperature was 18.74°C (standard deviation 13.73°C). Equilibrium temperature also roughly follows a normal distribution with a minimum of -10.76°C and maximum of 51.19°C. The upper extreme seems unreasonable. This could be due to the use of a relatively short time interval. At weekly

or monthly time intervals the equilibrium temperature is expected to more reasonable. Water temperature roughly followed a heavy tailed normal distribution with a mean of 16.74°C, a minimum of 1.00°C and a maximum of 28.00°C. It should be noted that these values are based off of a biweekly sampling scheme during the ice free season and do not represent annual mean temperatures. Temperature trends were not tested for because it data were not collected at the equally spaced intervals generally necessary for trend analysis (Hirsch et al. 1991).

There was a linear relationship between equilibrium and water temperature. The ordinary least squares regression slope of the relationship is 0.35 (standard error 0.026). Equilibrium temperature explains 48.8% of variance in water temperature. Air temperature and equilibrium temperature also have a linear relationship with a least squares regression slope of 1.67 (standard error 0.079). Air temperature explains 69.7% of the variance in equilibrium temperature. Air temperature explained 68.4% of variance in water temperature. The slope of the relationship between air and water temperature was 0.83 (standard error 0.04). However, the air temperature/water temperature relationship begins to depart from linearity above approximately 20°C and below approximately 5°C.

DISCUSSION

The light extinction coefficient was difficult to model using the available data. While both light extinction and albedo changes attributed to zebra mussel filtration follow the two to four year cycle of the zebra mussel abundance (and hence filtration

rates), the models did not resolve the change in light extinction found by Caraco et al. (1997. Standardized coefficients showed that zebra mussel filtration was unimportant relative to other parameters in the light extinction model. Light extinction, in turn, had relatively little impact in the albedo model. The small change in light extinction coefficient due to zebra mussel filtration results in a trivial changes in albedo and equilibrium temperature. The simple models used in this study are unable to show any zebra mussel induced changes in water temperature.

An alternative approach for assessing the influence of zebra mussel filtration may be to de-trend the influence of flow on water temperature and regress the residuals on zebra mussel filtration. Light extinction is jointly controlled by grazing and water flow (Strayer et al. 2008). Removing the influence of flow may make any zebra mussel dependent signal more apparent. This approach could be hampered by the relatively small number of zebra mussel samples.

While it does not find temperature changes due to increased filtration, this exercise does demonstrate the utility of the equilibrium temperature concept. The Hudson River is known to be warming (Ashizawa and Cole 1994). The relatively simple equilibrium temperature calculations may give more insight into the processes controlling water temperature. The impacts of changing cloud cover, thermal pollution, and relative humidity may be evaluated in ways that more simplistic air/water temperature regressions cannot.

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NOTATION

f_c	fractional cloud cover, dimensionless
H_s	incoming solar radiation, W m^{-2}
$H_{s,0}$	potential incoming solar radiation with a cloudless sky, W m^{-2}
H_l	net long wave radiation, MJ m^{-2} day^{-1}
H_e	evaporative heat flux, MJ m^{-2} day^{-1}
H_c	convective heat transfer, MJ m^{-2} day^{-1}
α	water surface albedo, dimensionless
L_{air}	irradiance in the air, µmol s^{-1} m^{-2}
L_0	irradiance at null depth, µmol s^{-1} m^{-2}
T_e	equilibrium temperature, °C
T_a	air temperature, °C
T_d	dew point temperature, °C

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GENETIC, MORPHOLOGICAL AND ECOLOGICAL RELATIONSHIPS AMONG HUDSON VALLEY POPULATIONS OF THE CLAM SHRIMP, *Caenestheriella gynecia*

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

This project was designed to better understand the clam shrimp species, *Caenestheriella gyneca*, by comparing its ecological, morphological, and genetic characteristics from pools in three localities: the Hackensack Meadowlands of New Jersey; Saugerties, New York; and Pittsfield, Western Massachusetts (the first two occurring in the Hudson River Watershed). Little is known about the ecology of *C. gynecia. Caenestheriella gynecia* was first discovered in 1939 in a single pool in Oxford, Ohio. Schmidt and Kiviat reported four new localities of *C. gynecia* in New York and New Jersey, three within the Hudson Valley of New York and one in northeastern New Jersey. *Caenestheriella gynecia* may have originated from a very small founder population due to unusual dispersal vectors from its natural range to the west. Egg samples and hatched individuals were obtained from study sites. Specimens were raised in the lab to estimate several growth and survivorship traits. In the field, puddle habitats were observed between the months of May and August where water quality parameters (i.e. dissolved oxygen, temperature, conductivity and pH, and nutrient composition) were recorded. Genetic comparisons across the study sites were made using nuclear DNA sequencing and random amplified polymorphic DNA (RAPD). The investigations outlined in this proposal should provide a substantial extension of fundamental knowledge of this species. There is a wide variation among water quality values within and among sampling sites. Puddles found in the Meadowlands are warmer, deeper and have a higher salinity than the other two sites. Morphologically, all populations possess meristics within the range of those discovered by Mattox in 1950.

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INTRODUCTION

Clam shrimp are small freshwater organisms belonging to the Branchiopoda (Crustacea). There are about 800 species of Branchiopoda found worldwide, mostly inhabiting fresh or brackish temporary pools (Follo and Fautin 2001). Clam shrimp belong to the order Conchostraca. Conchostracans are frequently referred to as clam shrimp because of their similarities to bivalved mollusks. All conchostracans possess a

bivalved carapace with a dorsal hinge controlled by a strong adductor muscle. Besides being filter feeders, these organisms can tear apart their food and will scavenge organisms in their environment (Martin and Boyce 2004). Clam shrimp are eaten by amphibians and other predators including notonectid hemipterans (backswimmers), Mallards and other ducks; shore birds like Killdeer, and Great Blue Heron, Great Egret and other wading birds. The protein from clam shrimp can provide important nutrition for migrating birds which visit vernal pools to gather nutrients they need to grow new feathers, migrate and lay their eggs. In Michoacan, Mexico, clam shrimp have been collected and used commercially as dry pet food (Martinez – Pantoja et al. 2002).

This project focused on *Caenestheriella gynecia* (suborder Spinicaudata; Fig. 1). *Caenestheriella gynecia* Mattox 1950 is a poorly known representative of the clam shrimp family Cyzicidae (Smith and Gola 2001). Little is known about the ecology of *C. gynecia*. *Caenestheriella gynecia* was first discovered in 1939 in a single pool in Oxford, Ohio. Schmidt and Kiviat (in press) reported four new localities of *C. gynecia* in New York and New Jersey, three within the Hudson Valley of New York and one in northeastern New Jersey.

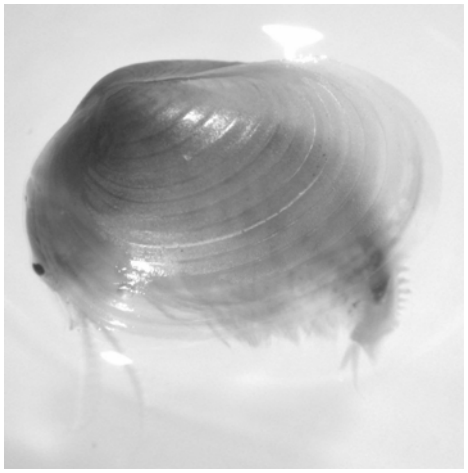

Fig. 1 Study organism – *Caenestheriella gynecia.* Lateral view of living organism, head to left. Specimen collected from Meadowlands, NJ.

The majority of clam shrimp are sexually dimorphic. The first two pairs of thoracic appendages in males have differentiated into claw-like claspers which they use to grab the shells of females and then hang on at right angles to the female's long axis (Martin and Boyce 2004). There are studies that show that "females" are actually functional hermaphrodites possessing both well-developed ovarian and testicular tissues (Sassaman and Weeks 1993). Contrary to the norm for clam shrimp, *Caenestheriella gynecia* is a parthenogenetic (asexual) species with no record of males. It is possible that males are uncommon and have yet to be discovered. Eggs are present in females between June and October and can remain viable without hatching for nearly 8 years. Individual specimens can live up to 6 months (Mattox and Velardo 1950). Egg viability is affected by temperature and time required for development and hatching varies with changes in temperature as was shown by Mattox and Velardo in 1950. Water persistence is necessary in a pool for a given amount of time in order for the animals to survive.

As a parthenogenetic species, the genetic variation of *C. gynecia* should be low. Parthenogenesis is a form of asexual reproduction in which females produce eggs that develop without fertilization. Another factor which would contribute to low variation is that the eastern populations of *C. gynecia* may have originated from a very small founder population due to a dispersal event from its natural range to the west (Schmidt and Kiviat, in press).

Caenestheriella gynecia is so little known that only four scientific papers have been published about it, but it appears to have ecological significance as a food source for migratory birds. It also seems to be expanding its distribution, most likely through the unusual vector of all terrain vehicles (ATVs). Its habitat is found in open, muddy puddles

on manmade dirt roads. Although this environment is odd for a clam shrimp, it is a common habitat in the U.S. and it seems likely that it will continue to expand its range to other similar locations. Wherever it occurs, either as a native or non-native, it contributes a unique set of life history characteristics that are intriguing, but still poorly defined. The investigations outlined in this report should provide a substantial extension of fundamental knowledge of this species. Moreover, as we gain a better understanding of *C. gynecia* habitat, behavior and distribution, this information can be used to create pools that support the species in the face of pool loss and insure the persistence of *C. gynecia.*

The goal of this project was to better understand the biology of *C. gynecia* by comparing its ecological, morphological, and genetic characteristics from pools in three localities: the Hackensack Meadowlands of New Jersey; Saugerties, New York; and Western Massachusetts (the first two occurring in the Hudson River Watershed). The first objective of the project was to determine the morphological and ecological relationships among populations of *C. gynecia*. The second objective was to determine the genetic relationships between *C. gynecia* found in vernal pools in the Meadowlands and the forms found in New York and Massachusetts. The third objective of the project was to assess the differences among pools, found in the Meadowlands, which render them habitable or inhabitable to *C. gynecia.*

METHODS

Study sites

Hackensack Meadowlands

The Hackensack Meadowlands are composed of approximately 8,300 hectares of wetlands, uplands, and developed areas in the Hackensack River watershed of

northeastern New Jersey (Kiviat and MacDonald 2004). *Caenestheriella gynecia* appears to occur only in puddles on the gas pipeline road in 1.07 km section of the Empire Tract and this is its only known locality in New Jersey. The actual study site was 10 rain puddles on the dirt surface of this road (Fig. 2 and 3). This road is regularly used by all terrain vehicles (ATVs) and other sport vehicles and these activities may help create and maintain the puddle habitats. The road is elevated around 1.5 m above the surrounding tidal marsh.

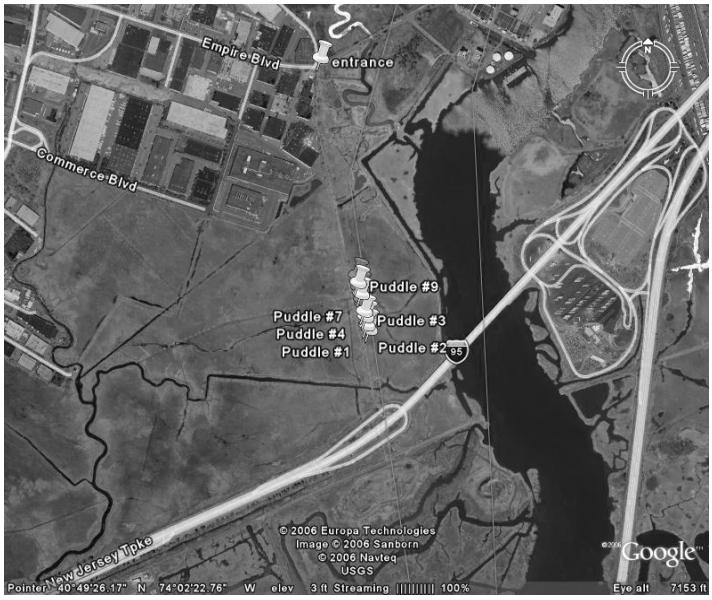

Fig. 2 Aerial map showing puddle sites located between Empire Blvd and I-95, Meadowlands, Bergen County, NJ. *Google maps.*

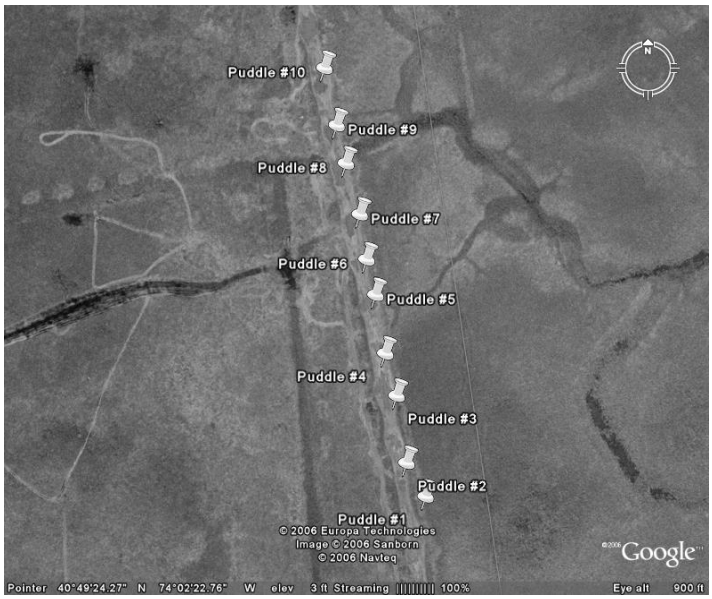

Fig. 3 Aerial map of puddle distribution on the gas pipeline road, Empire Tract, Bergen County, NJ. *Google Maps.*

Saugerties, New York

In July 2007, populations of *C. gynecia* were discovered by Erik Kiviat (Hudsonia Limited) in puddles on a dirt road in Bristol Beach State Park, Saugerties, New York (Fig. 4). The park is one of the 10 sub-units of the northern Ulster Scenic Area of State Significance found in the Hudson River Valley. In 1997, 58 acres of Hudson River shorefront was added to the 53 acres of undeveloped park. The park consists of riverfront, meadows, woodland, marsh and tidal flat habitats. In addition, the park is interspersed with several ATV trails.

Fig 4. Topographic map showing location of Bristol Beach State Park. Saugerties, NY. www.topozone.com

Lenox and Pittsfield, Massachusetts

Puddles located in Lenox, MA occur at the edge of a large wetland in the Housatonic River floodplain along an abandoned dirt road (Fig. 5). The areas surrounding the puddles provide a dense canopy of pine, maple, elm and other trees, which shades them. Like the Meadowlands site, the dirt road is visited and the puddles are probably maintained by off-road vehicles. Puddles located in Pittsfield, MA contain soils that are a mixture of tunbridge (loamy, well-drained soils that formed in Wisconsin-age glacial till) and muck and are slightly acidic to slightly alkaline (Fig. 6). Compared to the Lenox site, the pH of the water ranges from moderately to very slightly acidic. Direct sunlight is largely prohibited by a canopy of pine, hemlock, birch and oak trees (Smith and Gola 2001).

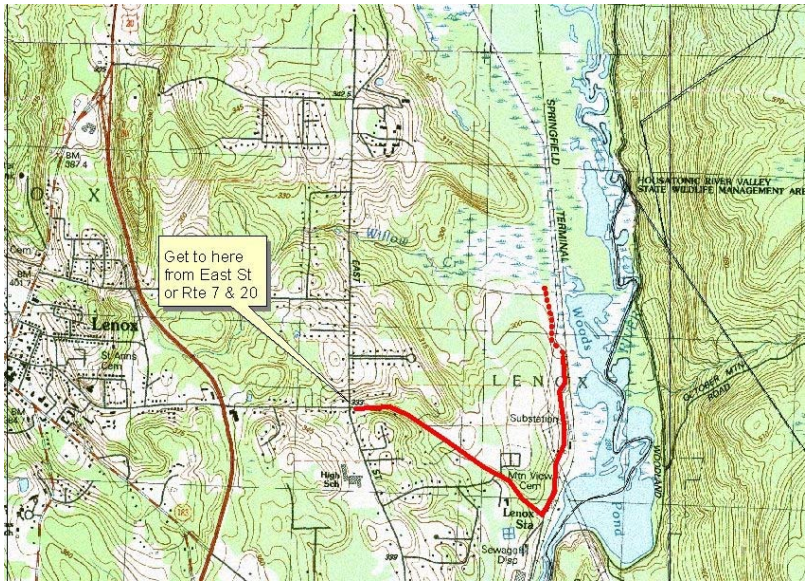

Fig 5. Topographic map of Lenox, MA site.
www.topozone.com

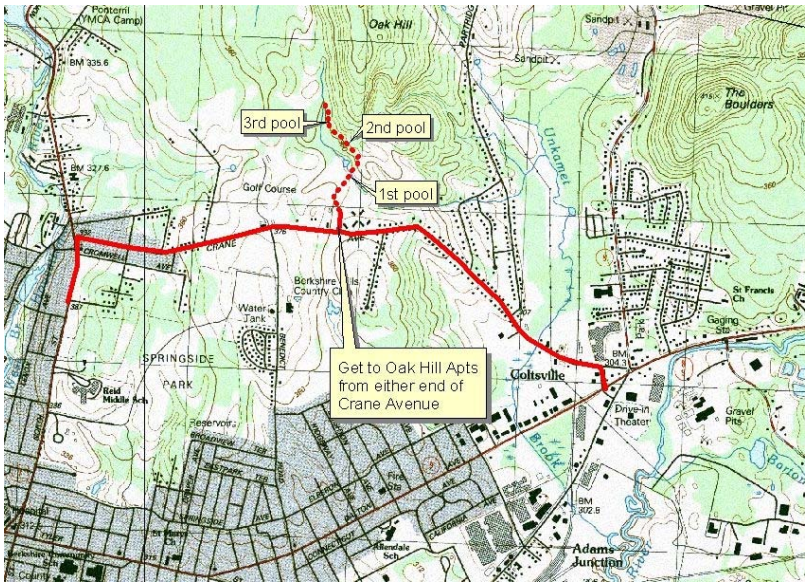

Fig. 6. Topographic map of Pittsfield, MA site.
www.topozone.com

Ecology

In the field, at the Meadowlands site, puddles were selected every 35m from a transect through the study site and marked for observation between the months of May and October 2008. Water quality parameters (dissolved oxygen, temperature, salinity and pH) were recorded weekly using an YSI 5562 Multiprobe Meter. Puddle depth was measured using a metric ruler. Nutrient composition (calcium, chloride and silica) of the

puddle was determined using a LaMotte 5917-01 water pollution kit. Three puddles were tested in July 2008 at the Pittsfield site and one at the Saugerties site.

Egg samples were obtained from dormant cysts in soil collected from these study sites (Zucker et al. 1997; Weeks and Zucker 1999; Marcus and Weeks 1997). Hatched individuals were collected via dipnetting. Water from the puddles was placed in plastic vials in which the specimens were stored. Specimens were raised in the lab to estimate growth and fecundity and to determine whether there is a correlation between age banding in their shells. Aquaria were kept at a temperature of 29 ± 2°C and under constant lighting using sunlight-simulating fluorescent bulbs. Crushed Tetramin© algal pellets were the only food source directly provided (algae and bacteria that colonize the tanks may have been consumed). Hatching time was recorded from the date/time of hydration while age was recorded as the time since hatching.

Soil samples were removed from the site, air dried and rehydrated in a 10L tank to collect larvae for observation. Daily size measurements were made using a Mitutoyo dial caliper. Daily growth increments were calculated for each shrimp by subtracting its size at day X from its size at day X + 1. Two measurements were attained, a dorsal measurement (measured between the two ends of the elliptical shell) and a lateral measurement (measured from the umbone of the carapace to the end of the shell, see Figure 7). Age at maturity was determined as the day at which eggs in the brood chamber were first noted. Longevity was determined by the length of time (in days) each individual survived and the carapace was preserved for ring counts at death. Molts were counted and collected/removed daily.

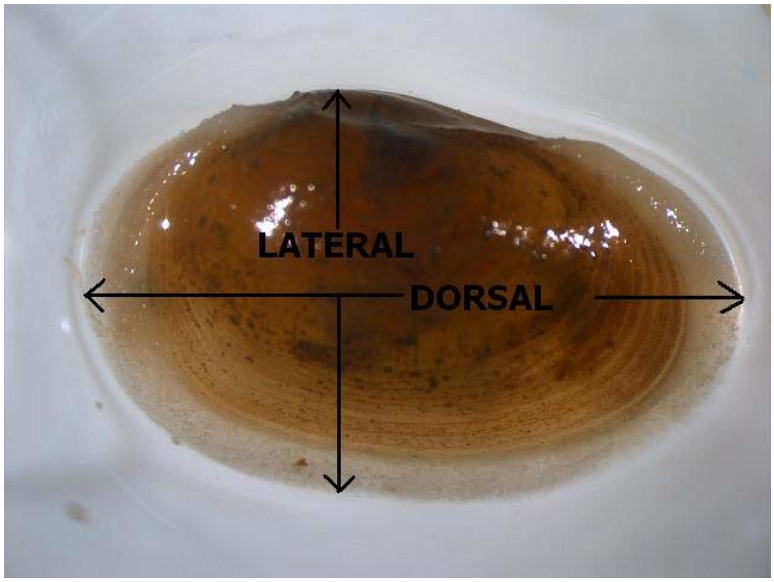

Fig. 7
Diagram of growth measurements

Population-Level Relationships

Populations examined included those in the Meadowlands, populations in the Hudson Valley, and Pittsfield and Lenox, Massachusetts. Population-level relationships were achieved by combining two sensitive molecular approaches: the random amplified polymorphic DNA (RAPD) and mitochondrial DNA sequencing. RAPD is a PCR-based method that can be performed without previous knowledge of DNA sequences of the species under study (Kautenburger 2006; Williams et al. 1990; Richardson et al. 1995). It uses arbitrary primers to detect changes in the DNA sequence at sites in the genome which anneal to the primer.

DNA extraction

Individuals were dissected and its digestive tract was removed. Genomic DNA was extracted from the remaining animal section using 250 µL aliquots of proteinase K extraction buffer. DNA was extracted with phenol chloroform followed by ethanol precipitation (Martinez et al. 2006).

Nuclear DNA sequencing

COI, 12S, 16S and 18S was examined in *Caenestheriella gynecia* and other species belonging to order Spinicaudata using the primers developed by deWaard et al. (2006), Carvalho et al. (2004), and Duff et al. (2004).

PCR conditions

The 22 µL PCR reactions contained 1µL of DNA template, 5 µL of 10x PCR buffer, 5 µL of each primer at 5µM (0.5 µM final) , 3 mM of $MgCl_2$, 2 mM of each dNTP, and 1 unit of *taq* DNA polymerase (Table 1).

Table 1. Primer sequences and PCR conditions

Primers	COI	12S	16S	18S
Forward	5'-CTGGTATAGT GGGAACTGCT – 3'	5'- TCCCTTTATTA GGGAGAGCG-3'	5'-TGAACGGCTA AACGAGAAAA-3'	5'-TTAAGCCAT GCATGTCTA AG-3'
Reverse	5'- AGGGTCAAAA AAAGAGGTGT-3'	5'- GTTAGACGAA GGACCCAAAT -3' or 5'-GTTAGAGAAG GACCCAAATA-3'	5'-AGGTCGAACA GACCTTTTGT-3'	5'-CAACTACG AG CTTTTTAAC C-3'
PCR cycling	1.5 min at 94°C, followed by 35 cycles of 45s at 93°C, 1 min at 50° C and 1 min at 72° C, followed by 1 cycle of 5 min at 72° C.	40 cycles of 94°C, followed by 30s denaturing, 40° C/45s annealing, and 72°C/45s extensions	1.5 min at 94°C, followed by 35 cycles of 45s at 93° C, 1 min at 50 C and 1 min at 72° C, followed by 1 cycle of 5 min at 72° C.	1 cycle at 94° C, 35 cycles of 30s at 93° C, 30s at 50° C, and 3 min at 72° C, followed by 1 cycle of 5 min at 72° C

PCR purification

One unit of Shrimp Alkalane Phosphotase (SAP) and 3 units of Exonuclease I was added to the PCR product. PCR products were diluted to 100 µl with Tris/EDTA and another100 µl of phenol was added. After vortexing, it was placed in a centrifuge (Eppendorf Centrifuge 5417R) for 10 minutes at 10,000 rpm at 22˚C. The aqueous top layer was placed in a new tube with 100 µl of phenol chloroform/isoamyl mix. It was again placed in the centrifuge for 10 min. One µl of glycogen was added to the aqueous top layer. Ten to twenty µl of sodium acetate was then added, depending on the final volume retrieved. Three hundred µl of 100% ethanol was added and the mixture was kept at -40˚C for 5 minutes. After incubation, the mixture was spun at 10,000 rpm for 15 minutes at 4˚C. The ethanol was poured off and 300 µl of 70% ethanol was added. The mixture was spun in the centrifuge again for 5 minutes. Thirty µl of distilled water was added and PCR fragments will be sent to Microgen in Korea to be sequenced or the solution will be stored at 37˚C until ready to be used.

RAPD analysis

PCR reactions were performed in a final volume of 25 µl, containing 25 pmol RAPD primer (see Table 2), 50 – 100 ng template DNA and a standard quantity of Ready To Go RAPD Analysis mixture using the kit from Amersham Pharmacia Biotech, Inc. #27-9502-01. The mixture was denatured for 5 minutes at 95°C followed by 45 cycles of 1 minute denaturation at 95°C, 1 minute annealing at 36°C and 2 minutes extension at 72°C. The amplified product was resolved by electrophoresis on 1.5% agarose gel in 1x

TAE buffer for 3 hours at 150 volts. The gel was stained with ethidium bromide and immediately photographed under UV light. Sizes were estimated by comparison with a 100 bp ladder.

RAPD bands were scored as present/absent and only well-resolved bands were considered. Selection of primers were based on reproducibility of the RAPD profiles and its consistency of producing polymorphic bands for DNA concentration ($1 - 5$ ng μl^{-1}) Williams et al. 1990).

Table 2. RAPD analysis primers

RAPD analysis primers	Primer sequence
Primer 1	5' – d[GGTGCGGGAA] – 3'
Primer 2	5' – d[GTTTCGCTCC] – 3'
Primer 3	5' – d[GTAGACCCGT] – 3'
Primer 4	5' – d[AAGAGCCCGT] – 3'
Primer 5	5' – d[AACGCGCAAC] – 3'
Primer 6	5' – d[CCCGTCAGCA] – 3'

RESULTS

Ecology

Table 3. Water chemistry of pools containing *Caenestheriella gynecia* in three sites in Pittsfield, MA, one site in Saugerties, NY and the main study site in the New Jersey Meadowlands. New Jersey values are the average of the 10 puddles at this site. All measurements were taken around midday. (DO: Dissolved oxygen saturation.)

Location	Temperature ºC	DO %	DO Mg/L	Salinity ppm	pH
Pittsfield 1	23.01	26.0	2.22	0.02	7.25
Pittsfield 2	24.32	6.8	0.53	0.01	5.58
Pittsfield 3	21.39	17.5	1.4	0.04	5.95
Saugerties	26.36	91.9	7.4	0.08	6.99
New Jersey	28.91	86.8	6.85	0.28	6.88

Water temperature in Pittsfield averaged 23˚C (Table 3). The water temperatures were higher in both Saugerties (26.4°C) and New Jersey (28.9°C). Dissolved oxygen saturation ranged from 6.8 – 26% in Pittsfield. However, the other sites had higher dissolved oxygen values – Saugerties at 91.9% and New Jersey at 86.8%.

The Meadowlands has higher temperatures than Saugerties and Pittsfield sites with an average minimum temperature of 70˚F. Figure 8 shows the minimum, average and maximum temperatures among the three sites for the month of July.

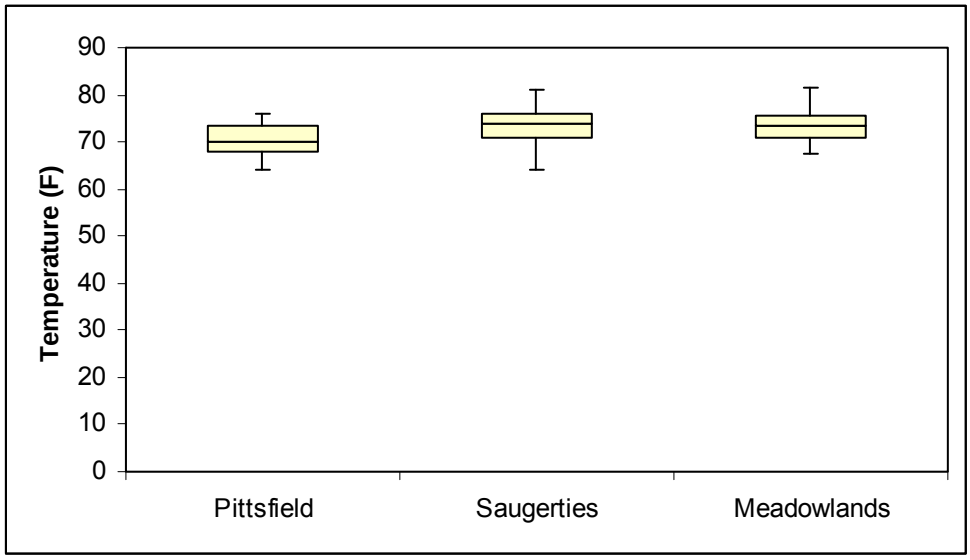

Figure 8. Temperature comparisons for the month of July among the three sampling sites. Data retrieved from Teterboro Airport at www.wunderground.com

Pittsfield, MA received almost five times (4.96x) the amount of precipitation (4.81 in.) than the Meadowlands site (0.97 in.) and almost three times (2.75x) the amount of precipitation than the Saugerties site (1.75 in.) Data was collected from Teterboro Airport at www.wunderground.com in July 2008.

Puddles (n=10) found in the New Jersey Meadowlands are 2.26x deeper (10.4 cm) than puddles found in Pittsfield (4.6 cm) and 1.79x deeper than puddles found in Saugerties, NY (5.83 cm) (see Fig. 9).

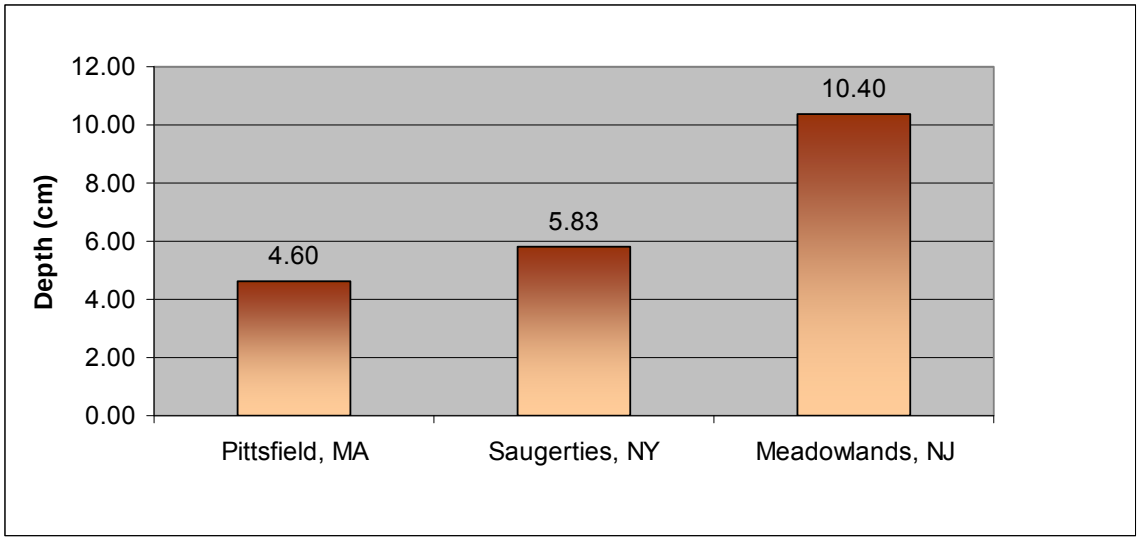

Fig. 9 Depth comparisons among the different sampling sites, Pittsfield, Saugerties and the Meadowlands.

Life history

Table 4. Life history comparisons among New Jersey and Pittsfield, MA populations of *Caenestheriella gynecia*.(D = dorsal length; L = lateral length)

	Daily Growth (D) (mm/day)	Daily Growth (L) (mm/day)	Molts (days/molt)	Age at maturity (days)	Daily egg count (eggs/day)	Long-evity (days)	Ring count (rings/day)
NJ (n=267)	0.037	0.040	2.0	14.2	11.30	25.43	0.396
MA (n=31)	-0.004	-0.007	1.3	0	0.00	35.67	0.180

New Jersey populations of *C. gynecia* had an average daily dorsal growth of 0.037 mm/day, an average lateral growth of 0.040 mm/day, and produced 0.40 rings/day. From the day of collection, individuals lived on average 25.43 days within the laboratory setting. Individuals molted every two days, developed eggs around 14.2 days and produced 11.30 eggs/day.

Massachusetts populations of *C. gynecia* had an average daily dorsal growth of -0.004 mm/day, an average lateral growth of -0.007 mm/day, and produced 0.18 carapace

rings/day. From the day of collection, individuals lived an average 35.67 days within the lab. Individuals molted every 1.3 days. None of the MA individuals reached maturity.

Morphology

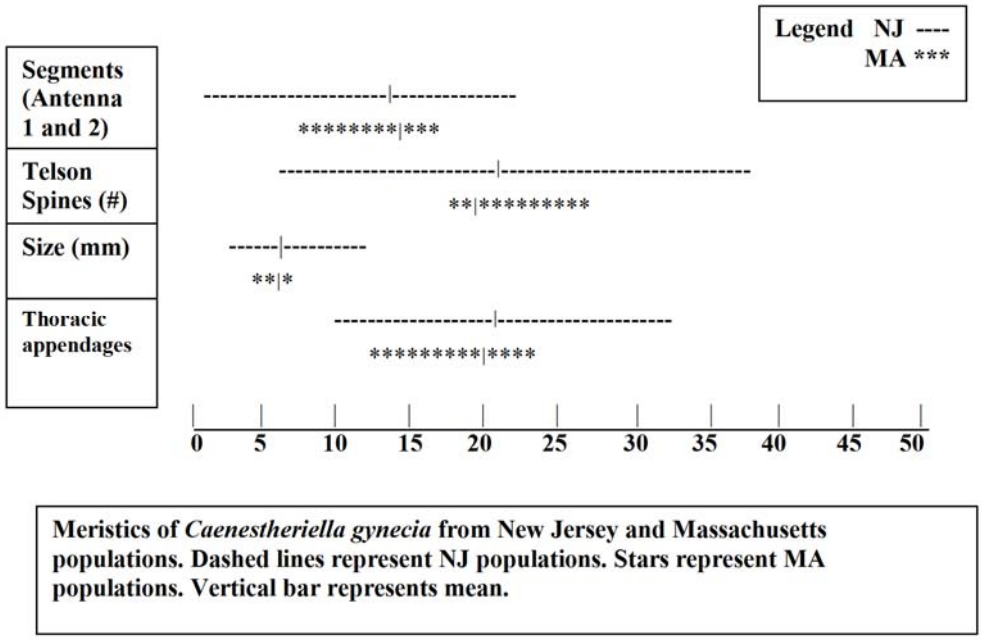

Fig. 10 Summary of meristics among *Caenestheriella gynecia* populations. New Jersey (n = 267); Pittsfield, MA (n=31)

Morphological measurements varied for populations of *C. gynecia* found in New Jersey and Massachusetts (Fig. 10). Individuals found in New Jersey had an antennal segment range between 1 and 23 ($\bar{x}$ = 13), while individuals from Massachusetts had an antennal segment range between 7 and 18 ($\bar{x}$ = 14). Individuals found in New Jersey possessed 6 to 38 ($\bar{x}$ = 19) spinal pairs (telson), while individuals from Massachusetts possessed 17 to 26 ($\bar{x}$ = 20) spinal pairs. Individuals from New Jersey ranged in size from 2.12 mm to 11.54 mm ($\bar{x}$ = 6.6 mm) total carapace length. Individuals from Massachusetts ranged in size from 4.86 mm to 8.12 mm ($\bar{x}$ = 6.6 mm). Number of thoracic appendages ranged from 13 to 24 pairs ($\bar{x}$ = 20) in the New Jersey population,

while it varied from 10 to 33 pairs ($\bar{x}$ = 20) in the Massachusetts population (Figure 11). All individuals examined were females as no specimens possessed any male claspers.

Figure. 11
Thoracic appendages of *Caenestheriella gynecia*.

We were unable to find live individuals to dissect for morphological comparisons on the sampling trip to Saugerties, NY. However, we successfully collected larvae from the rehydrated soil and are in the process of monitoring the life history traits and use them for genetic analysis.

Genetics

This portion of the project is still underway due to the unsuccessful collection of organisms from the Lenox and Saugerties site. A total of 100 individuals were collected from NJ and 15 from Pittsfield, MA. Soil was collected from all sites and taken back to the lab to dry and rehydrate to obtain larvae usable for genetic comparisons. To date, we have successfully collected 13 larvae from New York and 15 from Lenox, MA. These were the two sites that were dried out and no live individuals were found.

DISCUSSION

Ecological trends

There is a wide variation among values for the water quality parameters within and among sampling sites (Table 1). When looking at the water chemistry data for the three pools in Pittsfield, MA, it is evident that there is a range of values even though the pools are in close proximity to each other (within 2 - 4 minute walking distance). Variations in water temperature may arise from the amount of shade provided by the surrounding canopy. Some pools were completely shaded while others were exposed to pockets of sunlight. This also holds true for pools monitored at the main sampling site in the Meadowlands of New Jersey. Difference in water temperature among puddles can be as large as 20°C in a day. Pools here also share the same characteristics of the Pittsfield site in that some puddles receive direct sunlight at all times of the day and others are shaded by cottonwood, silver maple or honey locust trees. Variations in dissolved oxygen values may also result from the presence of dense algal mats that were found at both of these sites.

Salinity

Freshwater invertebrates inhabit discrete sites that are typically surrounded by inhospitable terrestrial landscape (Bilton et al. 2001). Freshwater is defined as water with less than 0.5 parts per thousand dissolved salts. Salinity values are consistent with freshwater aquatic systems in the Pittsfield and Saugerties (0.03 ppm and 0.08 ppm respectively). However, salinity is significantly higher in the Meadowlands (0.28 ppm) where salinity has reached to brackish levels (0.95 ppm). This is due to the fact that the dirt road in which these pools are located is surrounded by tidal marsh. After heavy

rainfall and extremely high tides, water from the marsh overflows onto the road and mixes with the water in the pools.

pH

When Smith and Gola (2001) sampled the Pittsfield site in 2001, they found that pools located there had a pH ranging from moderately to slightly acidic (6.2 – 7.6) with an average pH of 6.65. During my 2008 sampling of the Pittsfield site, pH values ranged from moderately acidic to nearly neutral (5.58 – 7.25). Pools found in the New Jersey Meadowlands, on average had a pH of 6.88 although values have dipped to as low as 4.34 and as high as 9.66. This is in part due to the increased truck presence within the area. They have recently started to repair the levees on the Hackensack River and are repeatedly transporting and dumping tidal soil back and forth between puddles. It has been shown that soil that is more saline is known to have a lower pH and puddles with high amounts of plant and animal detritus have a high pH (Al-Busaidi and Cookson 2003, Yee and Juliano 2006).

Depth

Comparing the maximum depths of all sampling sites, the New Jersey Meadowlands were 2.26x deeper than puddles found in Pittsfield and 1.79x deeper than puddles found in Saugerties, NY. All sites are frequented by recreational traffic of ATVs and SUVS. The major difference is that the Meadowlands site are subjected to not only the 650 lb. all terrain vehicles, but also 30,000 lb dump trucks and plow trucks whose immense tires kick up a lot of dirt and mud while traveling along the road.

Air temperature and precipitation

The Meadowlands are considerably warmer than the other two sites, as shown in Figures 8 and 9. However, the Meadowlands is also the site which received the least amount precipitation. This might correlate with the fact that we were only able to find 1 clam shrimp among the 10 puddles in the Meadowlands for the month of July. Higher temperatures combined with little rainfall does not leave enough water necessary for *C. gynecia* to complete its life cycle. Factors that affect pond duration constrain the length of life and time available for reproduction. Clam shrimp that live in smaller ponds, with low average rainfall, experience a shorter total time available for development than those found in larger ponds, with higher rainfall (Marcus and Weeks 1997).

Conversely, the Pittsfield site received the most rainfall and "cooler" temperatures out of the three sites. Of the three puddles sampled, all contained clam shrimp. Although cooler, pools in Pittsfield are inundated longer, which increases the time period in which branchiopods can complete their reproductive life cycle (Pike 2005).

These results indicate that even though pools are in close range, they can serve as micro-habitats that may result in differences in clam shrimp densities among pools. Modest changes in climate affects small pools which provide marginal reproductively suitable habitats with the potential to shift from favorable to unfavorable conditions in short periods of time (Pike 2005).

Life History

The life history measures reported herein are as a result from collection of individuals at an unknown stage in life. Exact ages at collection were undetermined and longevity and age at maturity are denoted as days since collection in the field.

Nonetheless, we feel that these data can be used for uncovering the relationship between certain life history traits in these organisms.

The last published study to look at life history traits for *C. gynecia* was by Emberton (1980). However, this study only looked at the absence/presence of eggs within the carapace and the maximum carapace length of individuals. Mattox and Velardo (1950) found ovigerous C. *gynecia* as small as 7 mm and laboratory cultures of *C. gynecia* to have a life cycle as brief as 23 days, growing from <1 to 11 mm in that time. Within my study, an individual completed its life cycle (egg to egg bearing) in 24 days which is consistent with their results. However, this individual grew to a maximum length of 5.91 mm. It must be noted that only one of 20 larva reached maturity under laboratory conditions.

When the project first began, it was observed that when daily dorsal measurements were taken alone, clam shrimp would increase and then decrease in size. This puzzlement led to the recording of and distinction between the two carapace measurements as dorsal and lateral. It was observed that at some instances, if the dorsal length decreased, the lateral length increased leading to the negative values in Table 4. More studies on the development of this species, from egg to maturity, must be done to establish its typical colonist life history traits in determining best conservation practices of the species. *Eulimnadia texana*, for instance, is optimized for life in short-lived water bodies displaying high initial growth, early reproduction, and then early senescence and death (Weeks et al. 1997).

Morphology

A total of 304 individuals from New Jersey and 31 individuals from Massachusetts were dissected. As shown in Figure 10, there is a wide range in variation of the different meristic measurements between the two populations of *C. gynecia.* These measurements included number of antennal segments, number of dorsal telson spines, carapace size, and the number of thoracic appendages.

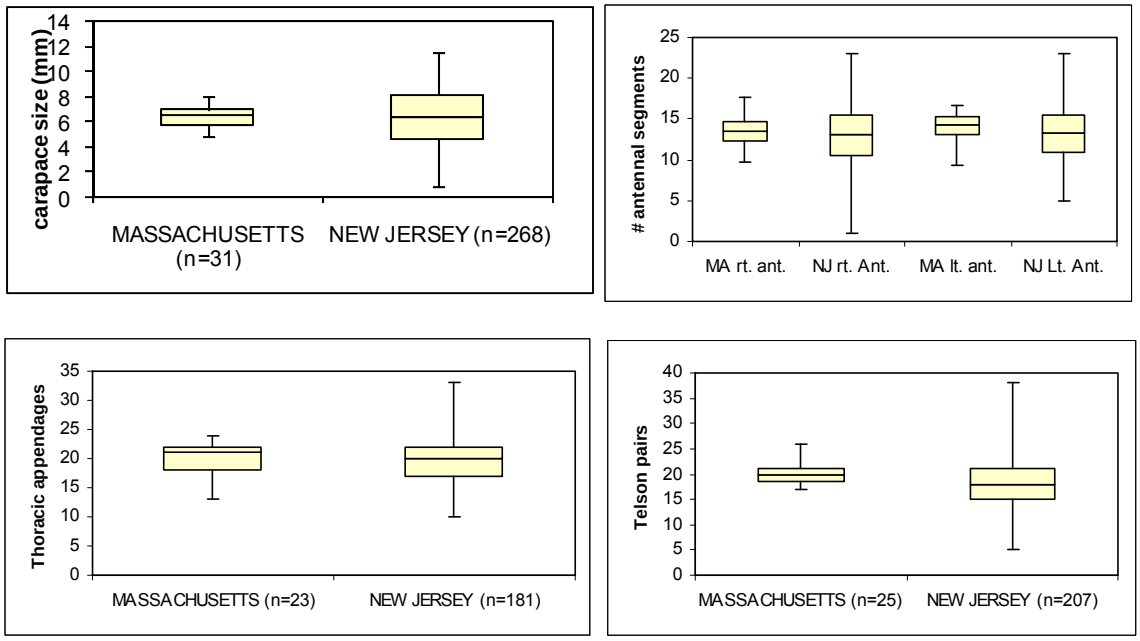

Fig. 12 Comparison of meristics among Massachusetts and New Jersey populations of *Caenestheriella gynecia.* rt. ant: Right antenna segment. lt. ant: Left antenna segment.

There has been speculation as to whether *C. gynecia* has been wrongly placed within *Caenestheriella* and perhaps should be included within *Cyzicus*. This hypothesis was based solely on morphological comparisons and the fact that there have been no males recorded for *C. gynecia* (Smith and Gola 2001). Although wide in range, the mean values of each meristic feature are actually close if not the same between the NJ and MA populations (Fig. 12). All values are within those described by Smith and Gola of the original population discovered by Mattox in 1950 and also their sample population (n

=186) in Massachusetts (2001), see Figure 13. Individuals (n = 11) collected by Schmidt and Kiviat also had measurements which fell into the same range as those that I examined (2005).

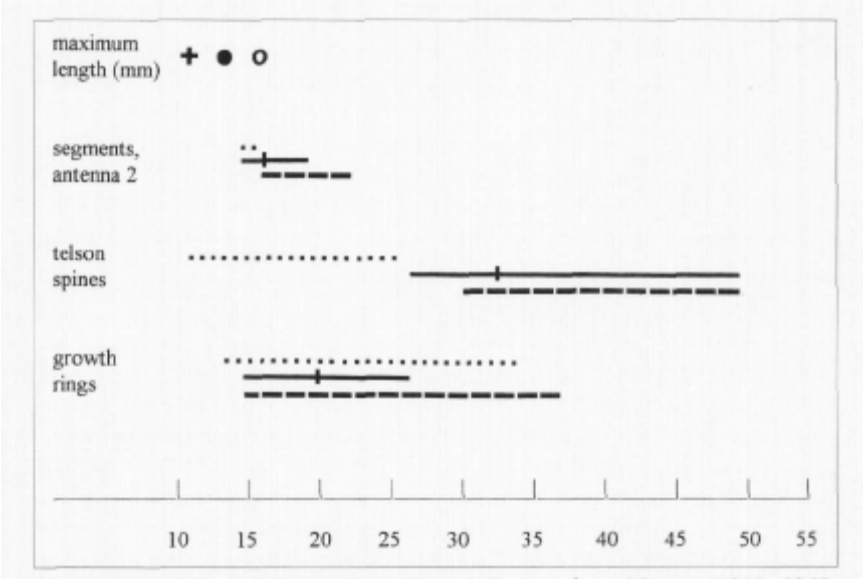

Fig. 13 - Figure 6. excerpted from Smith and Gola, (2001). Dotted lines, Ohio *C. gynecia* population; dashed lines, *Cyzicus species*; solid lines, Massachusetts populations.

Genetics

Although we were unsuccessful in collecting individuals from New York during this study, preliminary RAPD analysis of the New Jersey and the Pittsfield populations of *C. gynecia* were performed in 2007, (Figure 14). The DNA of the individual from Massachusetts (lane 6) shows a marked difference from individuals collected in NJ. It also shows that there are intra- and inter-puddle differences in DNA as well and there is a strong possibility that *C. gynecia* is not a true clonal species. The next step is to sequence the DNA fragments to see if there are more than one clone per puddle and if the New Jersey population is geographically centered.

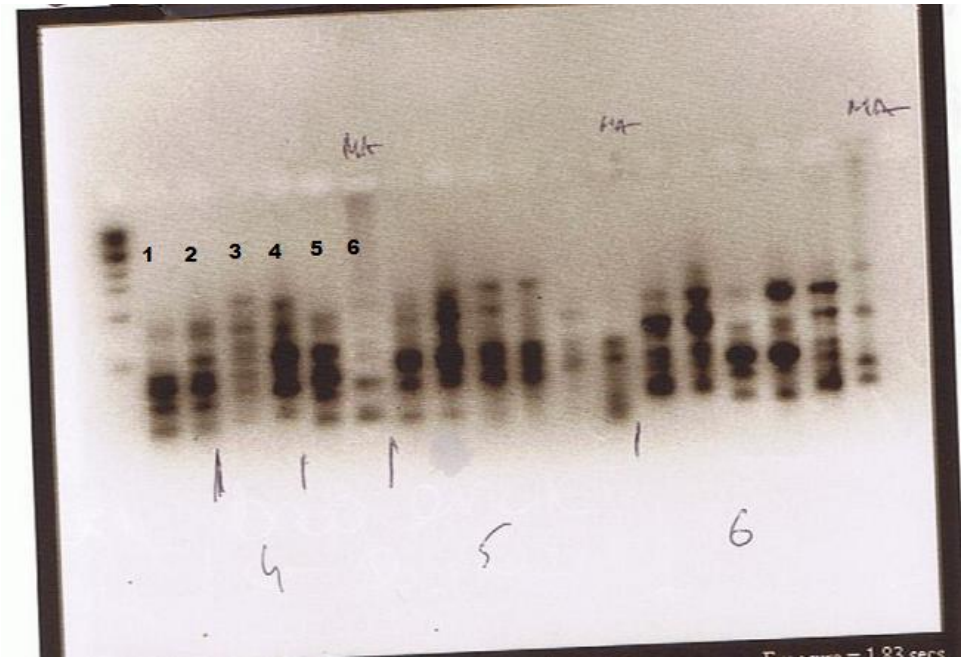

Figure 14. Image of RAPD analysis of New Jersey individuals (lanes 1-5) and an individual from Pittsfield, MA (lane 6). Lanes 1 and 2 are individuals from puddle #2; lanes 3 and 4 are individuals from puddle #5; Lane 5 is an individual from puddle #7.

Hopefully, as we gain a better understanding of *C. gynecia* biology and ecology, the information gathered from this project will assist in preserving the vernal pool habitat of *C. gynecia* as well. Clam shrimp are designated as an "At Risk" invertebrate species. Vernal pools serve as habitat and food sources to a variety of wildlife such as birds, amphibians and invertebrates, many of which are state-listed rare species. Invertebrates are vital to the vernal pool ecosystem as they function as both predator and prey.

Vernal pools formed in the Meadowlands may support *C. gynecia* because of the reduced predation or competition that would have been present in larger vernal pools. From what is known, *C. gynecia* occurs only in the pools formed on the gas pipeline road in a 1.07 km section and is the only known locality for this species in NJ.

Despite having a passive dispersal, migration among puddles is possible during periods of increased precipitation. However, during periods of drought, connectivity is

decreased. Populations will be lost if inter-puddle movement is prevented and there is a reduced likelihood of re-colonization events following population extinction. Hopefully, the genetic comparisons of the different populations will also lend a hand in determining the dispersal of the species from its first known location in Ohio by showing divergence times between populations.

There are many proposals to construct mega-malls, housing, and hiking trails to "renovate" the Empire Tract area. Providing awareness of the inhabitants of the area should help preserve and/or create and maintain the vernal pools that support *C. gynecia* in its new localities.

ACKNOWLEDGEMENTS

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COHORT STRUCTURE, GROWTH, AND ENERGY DYNAMICS OF JUVENILE BLUEFISH IN THE HUDSON RIVER ESTUARY

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

The mechanisms of recruitment in marine fishes have been topics of much interest for several decades. Estuaries serve as nursery areas for their inhabitants by providing resources for fast growth, energy and protection against predation, ultimately increasing recruitment potential. Bluefish, *Pomatomus saltatrix*, is a species that uses the Hudson River estuary as a nursery area through summer and early fall.

In this study, the cohort structure of juvenile bluefish was investigated during summer residency in the Hudson River estuary by determining individual hatch-dates and examining length-frequency distributions. Growth rates and lipid content of white muscle were compared between the spring- and summer-spawned cohorts of juvenile bluefish through the summer of 2008.

Peak hatch-dates for the spring and summer cohorts occurred in mid-April and late June respectively. Spring-spawned juvenile bluefish comprised the entire catch until late July when the first summer-spawned fish was captured in the estuary. The summer cohort recruited to the estuary by mid-August, making up approximately half of the juvenile abundance (61% by the end August). The spring and summer cohorts exhibited similar growth rates. Lipid content of both cohorts was also similar when all time periods were combined. However, spring-spawned fish entered summer with higher lipid content and depleted energy reserves through the summer, whereas the summer cohort accumulated lipid content over time.

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INTRODUCTION

In fishes, small changes in growth rates of juveniles can have large impacts on the number surviving to the adult population (Taylor et al. 2007). Estuaries vary greatly in their biotic and abiotic characteristics with direct implications to fish populations. Variability in temperature, salinity and productivity has been associated with small-scale changes in growth for many species that reside in estuaries during early life stages (Castro and Cowen 1991; McBride et al. 1995; Cooper et al. 1998).

During estuarine residency, temperate and tropical fishes also accumulate energy reserves in the form of lipids and this energy acquisition is often essential for survival (Schultz and Conover 1999). For migratory fishes, foraging is greatly reduced or ceases during migration, and energy reserves are rapidly depleted. As a result, migrating species must rely on the stored energy acquired during estuarine residency to survive (Morley et al. 2007). For juveniles that spend the summer and early fall in estuaries, it is critical that adequate energy reserves are accumulated and available for the migration and over-winter survival.

One such species that uses estuaries extensively during early life-history stages is bluefish, *Pomatomus saltatrix.* Bluefish is a highly migratory species found worldwide in subtropical and temperate waters, except for the eastern Pacific (Juanes et al. 1996). In the United States, bluefish occur seasonally along the eastern coastline from Maine to Florida (Kendall and Walford 1979).

Bluefish reproduce multiple times along the eastern coast of the U.S. during annual spawning migrations. Although the exact temporal and spatial patterns of bluefish spawning remains uncertain, at least two cohorts (spring and summer) are evident as a

result of spawning over the continental shelf (Hare and Cowen 1996). Eggs and larvae develop offshore, and juveniles recruit to nearshore and estuarine waters (Nyman and Conover 1988). Multiple cohorts are rare in many species but may contribute to dampened recruitment variability in bluefish.

From the early 1970s to the mid-1990s, spring-spawned bluefish have dominated juvenile abundances, corresponding to a healthy adult population (Conover et al. 2003). However, for reasons unknown, a shift in recruitment dynamics from the mid-1990s through the turn of the century has appeared to favor the summer-spawned cohort, which has been implicated in the decline of the adult population (Conover et al. 2003). The coast-wide cohort structure from 2000 to the current time is unresolved.

Increased size-dependent mortality of summer-spawned bluefish could be contributing to decreased recruitment potential and subsequent declining adult stock size. Mortality during periods of cold stress is often related to size, with smaller individuals suffering higher mortality rates (Sogard 1997). Conover et al. (2003) observed greater abundances of age-0 summer-spawned bluefish than spring-spawned fish along the southern and mid-Atlantic coast, but found little evidence of the summer cohort in the adult population. Size-related over-winter starvation of small summer-spawned juvenile bluefish, and subsequent cohort-specific recruitment failure, were implicated in the recent decline of adult stock abundance (Conover et al. 2003). Differences in body size and energy acquisition between the spring and summer cohorts during estuarine residency coupled with an apparent shift in cohort structure may be associated with variable juvenile survival and recruitment potential (Slater et al. 2007). One explanation for this

disparity in relative abundance could be that many summer-spawned bluefish do not have the energy reserves to survive their first winter migration.

Summer-spawned bluefish are considerably smaller than the spring-spawned group when they first appear in the Hudson River estuary (Juanes et al. 1993), but there can be considerable overlap in the length distributions between the two cohorts. Juanes et al. (1993) suggested that cohort structure of juvenile bluefish may display considerable variation, and recommended a thorough examination of the cohort structure to better understand bluefish recruitment dynamics.

Length-frequency analysis has typically been the method used in evaluating cohort-specific recruitment success of juvenile bluefish. However, the examination of otolith microstructure may provide better resolution in cohort identification by counting daily growth rings to elucidate hatch-dates. Additionally, otoliths can provide an estimate of daily growth rate by back-calculating daily growth increments. Otolith microstructure analysis has recently been validated for aging juvenile bluefish (Roemer and Oliveira 2007), and has been used to examine cohort structure and estimate growth rates for juvenile bluefish inhabiting oceanic and estuarine environments (Taylor and Able 2006; Taylor et al. 2007; Roemer and Oliveira 2007). This aging technique can provide the same information as long-term mark-recapture studies or costly laboratory experiments in age determination and growth rate estimation (Roemer and Olivera 2007). However, otolith microstructure analysis has not been used to examine cohort structure or estimate growth rates for juvenile bluefish residing in the Hudson River estuary.

Examination of lipid content has proved a powerful approach in assessing the energy dynamics of freshwater and marine fishes (Miranda and Hubbard 1994; Griffiths

and Kirkwood 1995; Eckmann 2004; Abdulkadir and Tsuchiya 2008). Temporal dynamics, cohort structure, and environmental characteristics have been implicated in the differences in energy acquisition and depletion observed for juvenile bluefish during the over-wintering period (Morley et al. 2007; Slater et al. 2007). Slater et al. (2007) examined over-winter survival of the spring- and summer-spawned juvenile bluefish in mesocosm, but suggested a thorough investigation of lipid content in wild fish to estimate energy levels prior to the fall migration.

In this study, the hypothesis was tested that smaller individuals of the summer cohort accumulate lipids more rapidly or efficiently than larger spring-spawned bluefish at the time of estuarine egress. The specific objectives were to examine otolith microstructure to determine hatch-dates and cohort structure, and to compare cohort-specific growth rates of juvenile bluefish inhabiting the Hudson River estuary. Also, pre-winter energy reserves were assessed for juvenile bluefish during summer residency in the Hudson River estuary.

METHODS

Fish sampling was conducted biweekly from early July to late August 2008 at 30 stations over a 65 kilometer (km) section of the lower Hudson River estuary (Figure 1). Bluefish were collected with a 61 meter (m) x 3 m beach seine with 13 millimeter (mm) stretched mesh wings and a 6 mm stretched mesh bag. Seine hauls were set from a boat and parallel to shore. Catches were processed on shore, with juvenile bluefish preserved frozen for subsequent age and lipid analysis. Hydrographic (temperature, salinity, and

dissolved oxygen), and habitat (depth, tidal stage and substrate type) measurements were recorded for each seine haul.

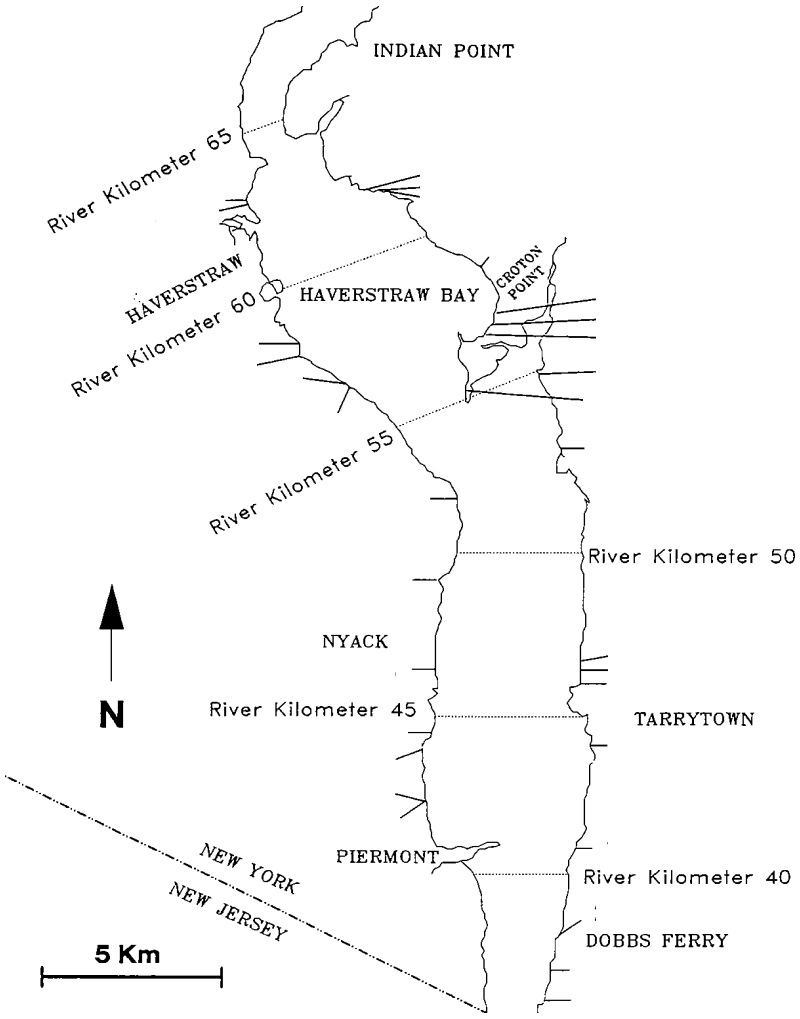

Figure 1. Area of the Hudson River estuary selected for sampling juvenile bluefish. Straight lines terminating at river margin indicate sampling locations.

Cohort structure, and growth

Juvenile bluefish were aged by enumerating growth increments on sagittal otoliths. Whole otoliths were fixed, concave side down, to glass microscope slides and embedded in Crystalbond glass adhesive (SPI supplies). To facilitate interpretation,

embedded otoliths were ground by hand on 1,200-grit silicon carbide sandpaper and polished with alumina micropolish until growth increments were visible. Hatch-dates and daily increments were enumerated by viewing photographic images of sectioned otoliths produced by an Olympus compound microscope (Olympus America, Inc.) at a magnification of 400x. The images were transferred to image analysis software (Image J) for augmentation of growth increments. Hatch-dates were calculated by subtracting the number of otolith increments (age in days) from the date on which juvenile bluefish were collected in the field.

In the laboratory, individual juvenile bluefish were measured to ± 1.0 mm fork length (mm FL; measured from the tip of the jaw or tip of the snout with closed mouth to the center of the fork in the tail) and weighed (± 0.1 grams wet weight). Juvenile bluefish growth was derived from regression slopes of age-length relationships. Differences in the slopes of the linear regressions between body size and otolith increment counts (hatch-date or age) were analyzed with analysis of covariance (ANCOVA) models, with age in days (d) as the covariate and cohort (spring and summer) as the explanatory variables.

Lipid content

Approximately 2-4 grams (g) of white muscle were removed from individual bluefish representing each cohort and time of residency. White muscle was selected for lipid content analysis because this tissue is an appropriate proxy for overall energy content of juvenile bluefish (Slater et al. 2007). Tissue samples were weighed (± 0.001 g), dried at 60 ºC for at least 72 hours, and reweighed to determine dry weight and percent water weight per sample. Dry tissue was transferred to pre-weighed porous

Alundum (fused alumina) thimbles for lipid extraction. An automated soxhlet extractor with di-ethyl ether was used to dissolve neutral lipids with a method similar to Shahidi (2001). Soxhlet extraction was used to remove the soluble components (lipids) from a solid sample (dried muscle tissue). The soxhlet extractor consisted of a flask which contained the solvent. As the flask was heated the solvent vapor rose (passing through the extraction thimble), and entered a water-cooled condensor, and liquified. When the liquid level in the extractor reached the top a siphon tube returned it as an extract-enriched solvent to the flask and this process was repeated until the entire lipid was dissolved. The extraction time was between 2-5 hours depending on the sample aggregation distillation rate. After extraction, the thimbles were dried again at 60 ºC for 24 hours to ensure evaporation of remaining solvent prior to final weighing. Weights of post-extracted dry tissue and pre-extracted dry tissue were used to determine lipid content. Lipid levels were expressed as a proportion of the sample dry weight. Analysis of variance (ANOVA) and ANCOVA were used to compare lipid content between the spring and summer cohorts and among 4 time periods. Data were natural log (ln) transformed to meet assumptions of normality and homogeneity of variances. A significance level of ≤ 0.05 was considered statistically significant.

RESULTS

Cohort structure, and growth

A total of 269 juvenile bluefish were collected from the Hudson River estuary from 15 July 2008 to 27 August 2008, ranging in size between 43 – 192 mm FL (mean

FL = 125 mm; # of seine hauls = 156). Otolith microstructure was used to determine ages (d) of juvenile bluefish and ranged between 33 and 165 d (mean = 76 ± 33 d; n = 47). Back-calculated hatch-date distributions were bimodal, indicating the occurrence of spring and summer cohorts (Figure 2). Juvenile bluefish with hatch-dates on or before 15 May 2008 were assigned to the spring cohort and individuals with hatch-dates after 15 May 2008 were classified as summer-spawned. Peak hatch-dates for the spring and summer cohorts occurred in mid-April and late June respectively (Figure 2).

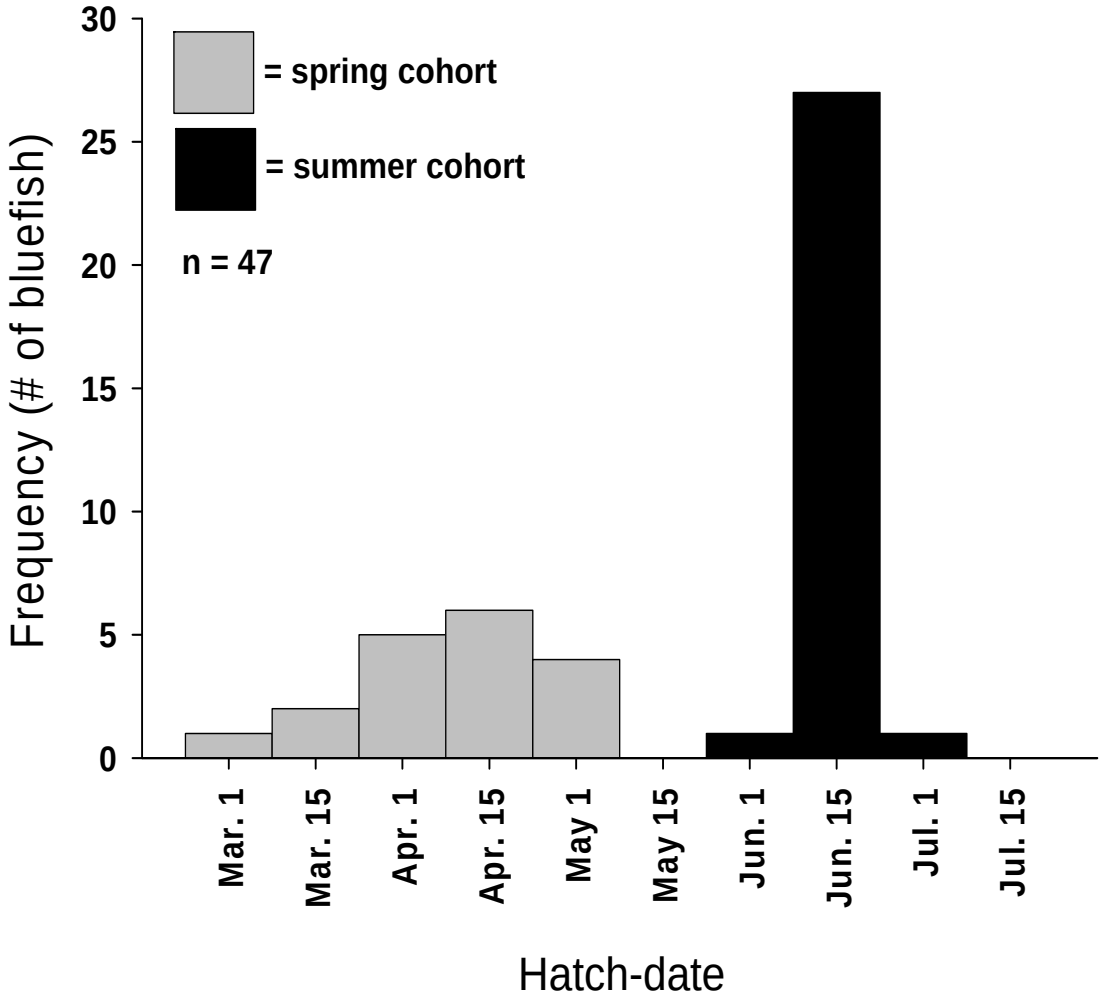

Figure 2. Hatch-date distributions of juvenile bluefish collected from the Hudson River estuary during summer 2008, aged by otolith microstructure analysis.

Hatch-date to length relationships of 47 juvenile bluefish were used to construct age-length keys for the remaining bluefish collected (n = 222) during each sampling event (15 July = T1; 29 July = T2; 13 August = T3; 27 August = T4; Figure 3). These data

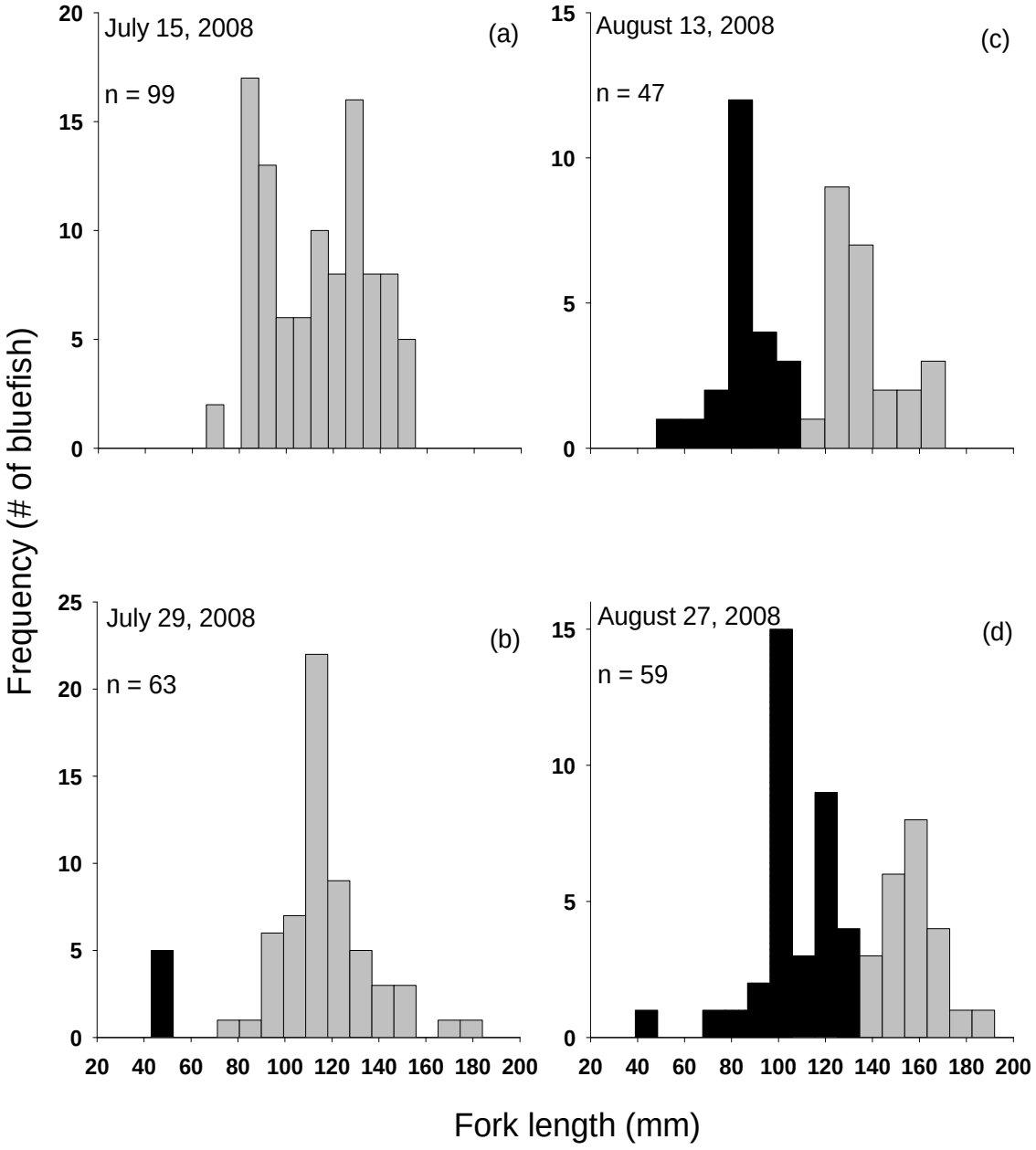

Figure 3. Length-frequency distributions of spring-spawned (gray bars) and summer-spawned (black bars) juvenile bluefish collected from the Hudson River estuary on: (a) T1: 15 July 2008, (b) T2: 29 July 2008, (c) T3: 13 August 2008, and (d) T4: 27 August 2008.

corroborated the presence of two major spawning periods (spring and summer). In early summer (T1), all juvenile bluefish collected resulted from spring spawning (Figure 3a). Summer-spawned bluefish first appeared in the Hudson River estuary in late July (T2), and constituted approximately 50% of the bluefish catch by mid-August (T3). In late summer (T4), multiple pulses of summer-spawned bluefish were evident as the length frequency distribution of this cohort resulted in 2 modes (mean = 101 mm FL, mode 1; mean = 120 mm FL, mode 2; Figure 3d).

Growth was estimated from the slope of the linear regression between juvenile bluefish size and age for the spring and summer cohorts. Covariate analysis showed that growth was similar between the cohorts (ANCOVA; age x cohort: $F = 1.24, df = 1, P = 0.27$; Figure 4).

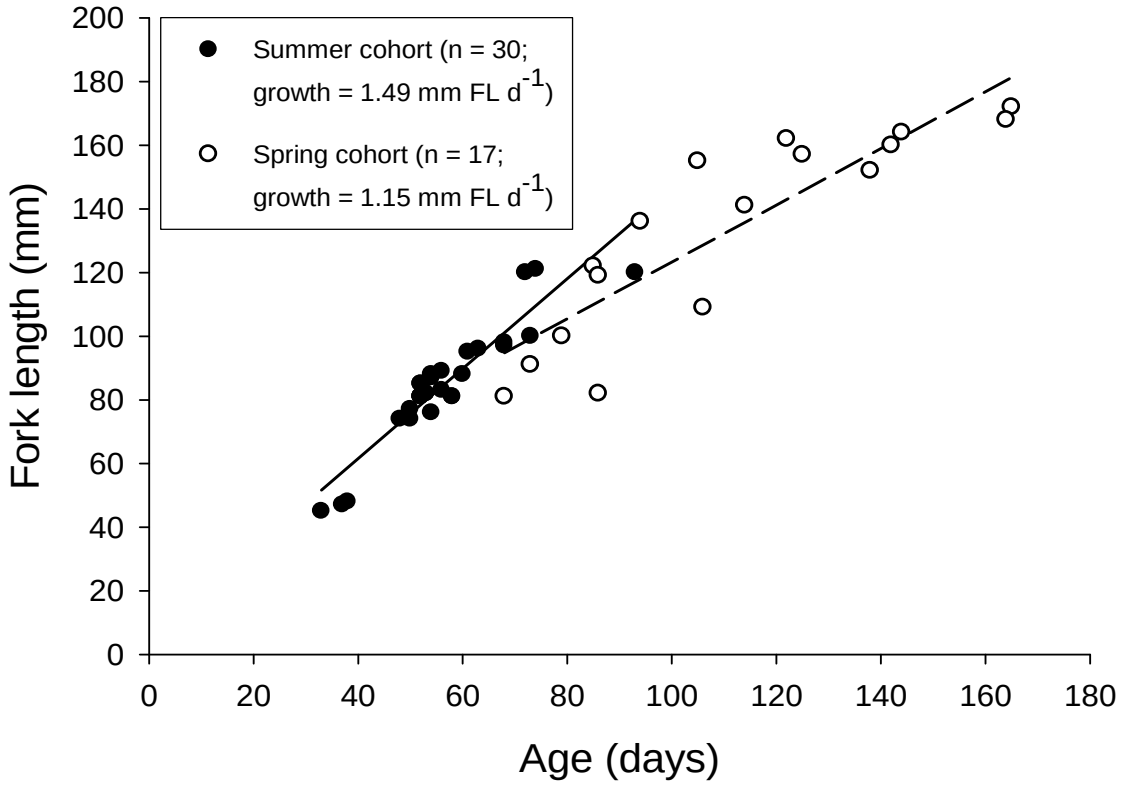

Figure 4. Body size (mm FL) to age (# of otolith increments) relationships of juvenile bluefish as a function of cohort (spring and summer).

Lipid content

Total neutral lipids constituted between 2.0 and 8.2 % (mean ± standard error = 5.42 ± 0.19 %, n = 46) of dried muscle for the spring and summer cohorts combined. Lipid content ranged from 2.0 – 8.2 % of dried muscle for the spring cohort (mean ± standard error = 5.43 ± 0.24; n = 31) and from 3.0 – 7.2 % (mean ± standard error = 5.34 ± 0.32; n = 15) for the summer cohort. The lipid percentage in dried muscle did not differ between the spring and summer cohorts of juvenile bluefish inhabiting the Hudson River estuary after pooling time periods (F = 0.04, df = 1, P = 0.85; Figure 5).

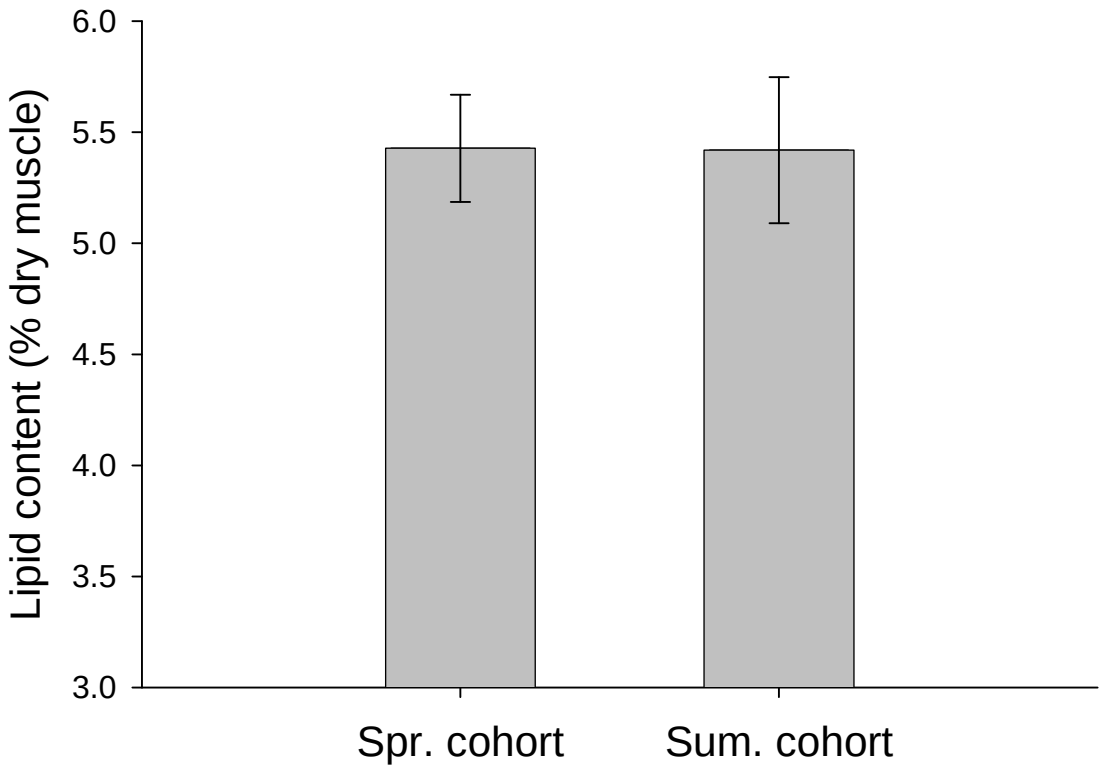

Figure 5. Mean (±SE) lipid content of white muscle for spring- and summer-spawned juvenile bluefish cohorts over all time periods combined.

To examine the effect of time period on lipid content of the spring and summer cohorts combined, T1 was omitted because no summer-spawned individuals were collected.

Lipid content was similar among times 2 – 4 (F = 0.33, df = 2, P = 0.72) for the spring and summer cohorts of juvenile bluefish combined. Lipid content declined modestly with increasing bluefish length when cohorts were combined (r^2 = 0.10, P = 0.04; Figure 6).

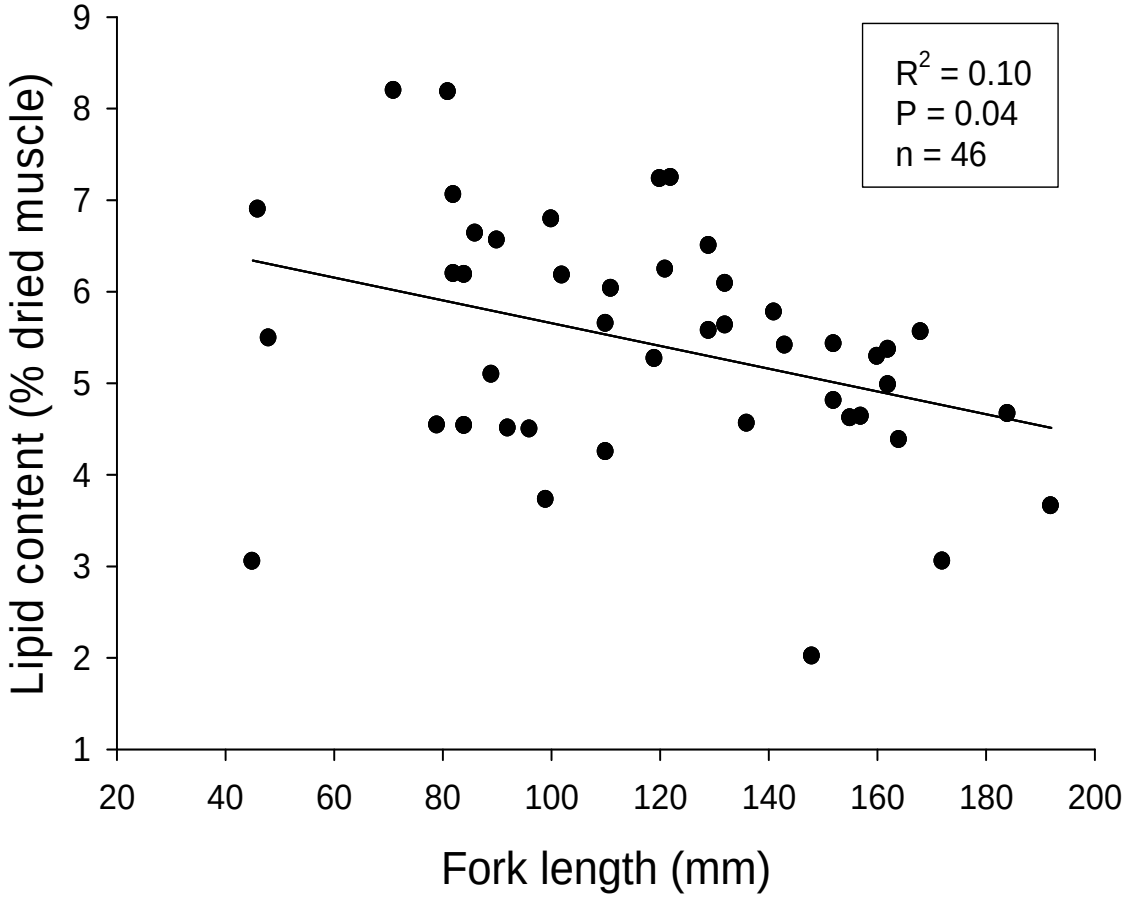

Figure 6. Lipid content as a function of length (mm FL) for the spring and summer cohorts of juvenile bluefish inhabiting the Hudson River estuary during summer 2008 combined (n = 46).

Cohort-specific lipid content of juvenile bluefish over time was examined using ANCOVA. The slope coefficient of the lipid content to time period relationship differed between the cohorts (t = 2.33, P = 0.02). Lipid content of spring-spawned juvenile bluefish was highest during the first time period and decreased through the summer. The

summer-spawned cohort exhibited the lowest lipid content levels upon arrival into the Hudson River estuary and increased over time (Figure 7).

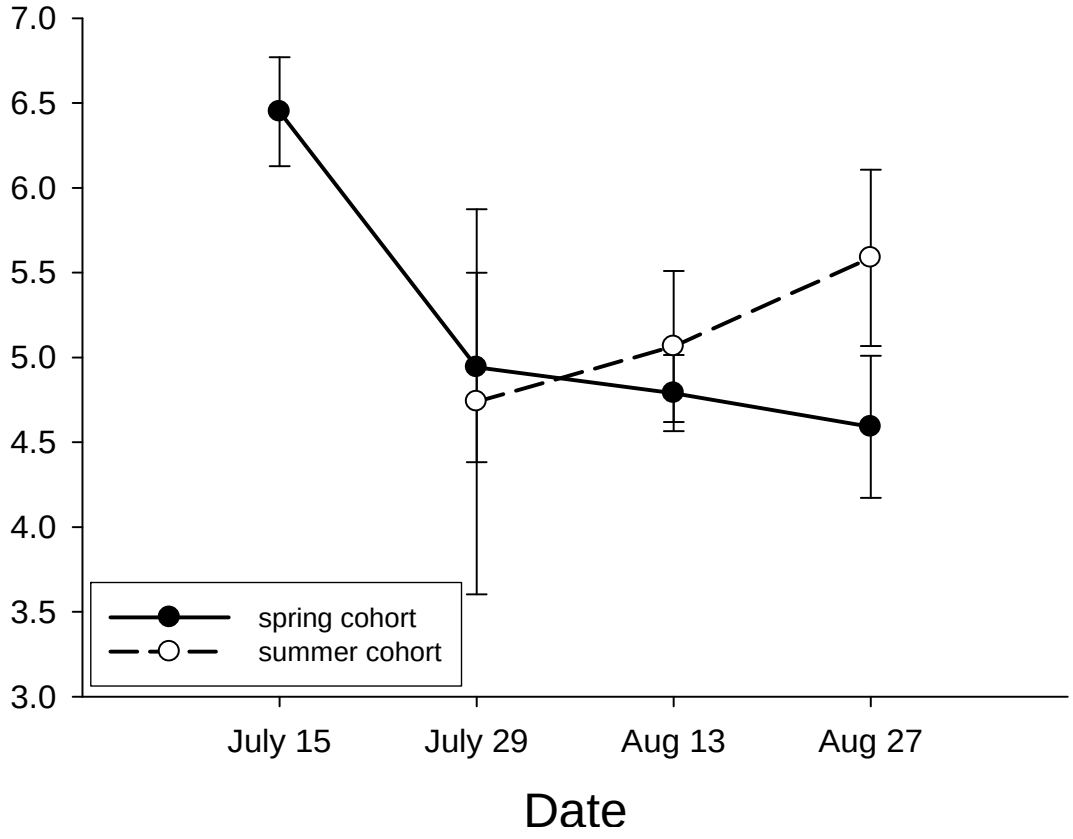

Figure 7. Mean (± SE) white muscle lipid content for the spring and summer cohorts of juvenile bluefish residing in the Hudson River estuary as a function of time period.

DISCUSSION

Cohort structure, and growth

This study supports the function of the Hudson River estuary as an important juvenile bluefish nursery area, as the estuary is used by the spring and summer cohorts of juvenile bluefish throughout the summer. Juvenile bluefish have previously been classified as estuarine dependent (Kendall and Walford 1979); however, the summer cohort may preferentially inhabit the coastal ocean zone during late summer and early fall

(Able et al 2003). The results of this study indicate estuarine use by the 2 cohorts of juvenile bluefish throughout the summer. Ocean sampling was not included in this study, but would be necessary to determine cohort-specific habitat selection. Further, movement between the coastal ocean and estuary by juvenile bluefish during summer and fall remains largely unresolved. Nevertheless, it was evident by consistent abundances of the spring and summer cohorts that the Hudson River estuary provided adequate resources for summer-long residency of juvenile bluefish.

In early summer, the entire catch of juvenile bluefish in the Hudson River estuary was comprised of spring-spawned individuals, while the summer cohort did not ingress until late July. These findings were consistent with previous observations of bluefish recruitment to the Mid-Atlantic Bight (MAB) (Wilber et al. 2003; Taylor and Able 2006). By late summer, catches of summer-spawned bluefish were greater than the spring cohort providing evidence for summer-spawned cohort dominance of the age-0 year class as suggested by Conover et al (2003). Estimates of cohort-specific abundance in the Hudson River estuary and associated coastal ocean zone through fall are required to further elucidate the pre-migration cohort structure. Examination of otolith microstructure allowed for hatch-date distributions and corresponding age-length keys to be constructed, which corroborated the identification of two principal bluefish spawning events (Figure 2). Mean hatch-dates of mid-April and late June indicated the two spawning events occurred in spring and summer respectively. The timing of spawning periods was consistent with previous studies of juvenile bluefish recruitment dynamics in other Atlantic coast estuaries (Taylor et al. 2007; Callihan et al. 2008).

Length-frequency distributions (Figure 3) were consistent with a previous study that indicated the presence of at least 2 cohorts with the possibility of multiple intra-summer-spawned cohorts of juvenile bluefish inhabiting the Hudson River estuary (Taylor and Able 2006). Concomitant with earlier hatch-dates of the spring cohort, and similar cohort-specific growth rates, members of the spring cohort (mean = 147 mm FL) were larger in absolute size than summer-spawned bluefish (mean = 94 mm FL) by the end of August. The disproportionately low contribution of summer-spawned individuals to the adult bluefish population observed by Conover et al. (2003) could result from size-selective mortality of summer-spawned individuals as they emigrate from inshore nurseries to over-wintering waters of the South Atlantic Bight.

Discrete estimates of cohort-specific growth (spring cohort = 1.15; summer cohort = 1.49 $mmFLd^{-1}$, Figure 4) were comparable with juvenile bluefish growth rates observed elsewhere in the MAB (McBride and Conover 1991; Able et al. 2003). However, the range of growth rates estimated in this study was lower than reported for juvenile bluefish in the Chesapeake Bay, Maryland (Callihan 2005). This discrepancy may be explained by spatial and inter-annual variation in water temperature between the Hudson River estuary and the Chesapeake Bay. Bluefish growth is optimized at a water temperature of approximately 24 °C (Hartman and Brandt 1995), and it is likely (Callihan 2008) that the Chesapeake Bay area maintains sea surface temperatures closer to this optimum for a longer period through the summer than the Hudson River estuary, thus maximizing growth potential. All the same, this study reaffirms that bluefish growth rates are among the fastest of any temperate fish species (Able and Fahay 1998).

Summer-spawned bluefish may be expected to exhibit faster growth than spring-spawned juveniles to compensate for the size advantage incurred by earlier hatch-dates and dietary shift to piscine prey of the spring cohort (Juanes and Conover 1995). Taylor et al. (2007) reported faster growth of summer-spawned juvenile bluefish than the spring cohort in New Jersey waters, but the observed growth rate differences corresponded to habitat whereby growth rates were higher in the coastal ocean than the estuary irrespective of cohort classification. In this study, growth rates of the spring and summer cohorts of juvenile bluefish residing in the Hudson River estuary during summer were similar.

Lipid dynamics

For many fishes, accumulation of lipids in muscle has been associated with increasing body length (Hutchings et al. 1999). Juvenile bluefish do not seem to follow these patterns as lipid content declined with increasing length when individuals of each cohort were examined together (Figure 6). However, when examined separately, larger body size of the spring cohort conferred higher energy content at the beginning of the summer, and subsequently declined with length, while the summer cohort exhibited increasing lipid reserves with length (Figure 7). The observed cohort-specific lipid content to length relationships corresponded to the growth rate estimations and length-frequency analyses. The summer cohort did not exhibit an increased rate of size-specific compensatory growth, indicating considerable resource intake to energy storage in the form of lipids.

The lipid content in dried muscle of the spring and summer juvenile bluefish cohorts inhabiting the Hudson River estuary were similar after pooling time periods

(Figure 5). However, a significant time effect was evident, revealing a decline in energy content of the spring cohort, and an accumulation of lipids in summer-spawned juvenile bluefish through the summer of 2008 (Figure 7). Morley et al. (2007) reported higher energy content in the spring cohort than summer-spawned bluefish at the beginning of summer from the nearshore waters of North Carolina. However, over-summer lipid dynamics were not examined, and June was the final time period analyzed by the investigators (Morley et al. 2007). In this study, the summer cohort did not appear in the Hudson River estuary until late July, and approximately 1 month after spring-spawned bluefish. It is possible that an earlier dietary shift by the spring cohort from low energy prey to a more lipid rich fish diet (Juanes et al. 1994) resulted in higher initial energy content of spring-spawned fish, while the summer cohort suffered from an early summer lipid accumulation deficit.

CONCLUSION

A risk tradeoff between migration (or over-winter starvation) and predation may explain the observed cohort-specific growth rates and differences in lipid allocation versus mobilization strategies (Post and Parkinson 2001). If adequate energy storage is not obtained prior to the fall migration, energy depletion and starvation may increase natural mortality. Conversely, smaller individuals are at greater risk to predation than larger conspecifics, and could suffer higher predation mortality by allocating a greater proportion of energy intake to lipid storage over somatic growth (Sogard 1997). Results of this study indicated that the spring and summer cohorts of juvenile bluefish inhabiting the Hudson River estuary grew at similar rates and that summer-spawned fish

accumulated lipids while the spring cohort depleted energy reserves over time. Perhaps, energy acquisition was selected over growth in the summer cohort in preparation of the fall migration. Consequently, size-specific predation mortality rather than starvation during migration may explain the recruitment failure exhibited by the summer cohort of juvenile bluefish to the adult population.

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FEEDING HABITS AND THE EFFECTS OF PREY MORPHOLOGY ON PELLET PRODUCTION IN DOUBLE-CRESTED CORMORANTS, *PHALACROCORAX AURITUS*

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

The contents of pellets and boli collected from the New York Harbor population of Double-crested cormorants, *Phalacrocorax auritus*, were analyzed for species composition to investigate possible biases associated with use of cormorant pellets for diet studies. During the breeding and chick-rearing seasons, boli and pellet samples were collected from three island colonies, Hoffman, Swinburne and South Brother. Comparison between the two largest colonies, Swinburne and South Brother, generated a Schoener Index value of 0.337, indicating a medium level of dietary overlap . The most common species found in the boli were black seabass, *Centropristis striata* (14.4%), and scup, *Stenotomus chrysops*, (12.9%). Neither of two local species with conservation concerns (striped bass, *Morone saxatilis*, and winter flounder *Pseudopleuronectes americanus*) made up a significant portion of the diet. The samples were also analyzed to examine a possible bias in pellet production associated with the spininess of prey species. Ninety-five percent of species found in pellets were spiny compared to 63% in the boli. This and other evidence suggest that spininess of prey species is a factor affecting their representation in pellets. Other morphological factors such as prey size and otolith morphology were shown to be unlikely to account for the observed differences in species makeup; however, boniness is one morphological factor which could not be eliminated. Even so, the evidence for the effect of prey spininess on pellet composition remains strong. Future research including is recommended to further investigate the issue.

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INTRODUCTION

The Double-crested Cormorant, *Phalacrocorax auritus*, is a large, colonial waterbird that catches its prey through foot-pedaled pursuit diving (Carss 1997). Most of their hunting is restricted to shallow waters less than 8 m deep and within 30 km of their roosts (Hatch and Weseloh 1999). This research was conducted on the Double-crested Cormorant population of New York Harbor, which has been growing and breeding since its appearance in 1984 (Parsons 1987). Their numbers have flourished locally during the last three decades with the 2008 breeding population consisting of over 1300 pairs spread out among seven colonies (S. Elbin, NYC Audubon, personal communication). The subspecies found in New York Harbor area is *Phalacrocorax auritus auritus* (Hatch and Weseloh 1999).

Cormorants primarily eat schooling and bottom-dwelling fish, and invertebrates (Hatch and Weseloh 1999), a diet which often leads to conflict with humans, particularly commercial and recreational fishermen. The only way to determine whether a local population is exploiting the same resources as humans is through studies of their diet. Diet studies on cormorants have been numerous, with results varying by area and approach. While some studies have found that the local cormorant population depleted fish populations sufficiently to warrant cormorant population control (Collis et al. 2002; Johnson et al. 2005; Rudstam et al. 2004), research in other locations found that the proposed threat posed by the local colony was exaggerated (Glahn et al. 1998; Somers et al. 2003; Withers and Brooks 2004).

There are three primary methods used to assess the diet of cormorants – direct stomach content analysis, identification of bolus contents, and identification of pellet

contents. Stomach content analysis involves dissection of the gizzard and esophagus of dead birds (Derby and Lovvorn 1997). Bolus and pellet analyses both rely on studying regurgitated material. A bolus is a partially digested food item, usually regurgitated by young cormorants in response to disturbance, such as a perceived predator near their nest. Pellets are gelatinous sacs containing otoliths and other bones that can be used to identify the species of fish eaten. Otoliths – small, white structures found in the heads of all fishes other than sharks , rays and lampreys -- are used for hearing and balance, and are highly species-specific in morphology, making them useful to researchers for species identifications (Campana 2004). The most commonly used method is the dissection of pellets, which are popular for diet analysis because they greatly increase the number of food items identified, compared to boli, which often represent one food item in each bolus (Derby and Lovvorn 1997).

Sources of error associated with the use of pellets are well known. They can be damaged in the digestive processes, rendering them unidentifiable; smaller otoliths are especially susceptible to this which could cause some species to be underrepresented (Carss 1997). Damage from digestion can also lead to errors in estimating fish size (Carss 1997; Johnson et al. 2001). Secondary consumption, in which otoliths from the latest meals eaten by the cormorants' prey show up in the pellets, has also been shown to be problematic (Carss 1997). Despite these findings, little research has been performed on the process of pellet production and its effects on diet analysis. One such study found that European shags, *Phalacrocorax aristotelis*, a closely related species, produced pellets over the course of 1 to 7 days, with an average of 3.5 days (Russell et al. 1995),

but many researchers still go by the older assumption of one pellet being produced per day (Derby and Lovvorn 1997; Johnson et al. 2001).

The hypothesis was tested that fish species with difficult-to-digest spiny fins (and other bony protrusions and large hard parts, such as the head plates of searobins, *Prionotus* spp.) would show up in pellets in higher proportions than those species with soft-rayed fins, relative to their proportions in the bolus samples. A possible cause of this hypothetical bias could be a protective response to the sharp nature of the spines themselves, prompting the birds' digestive tracts to encase the spines and other hard parts in pellets and eject them sooner than might otherwise occur. The specific goals of our research were to assess the dietary composition of the New York Harbor Double-crested Cormorant population, and to examine a possible artifact affecting the accuracy of pellets in such work. The first aspect of the study was an analysis of the local population's diet through the study of boli and pellets. Major questions were: (1) which species of fish the cormorants eat, and (2) in what proportions they eat these species. Also of concern was identifying to what extent, if any, they preyed on local species valuable to humans – striped bass, *Morone saxatilis* and winter flounder, *Pseudopleuronectes americanus*. The second aspect of the research was an investigation into the role of prey morphology on pellet production.

METHODS

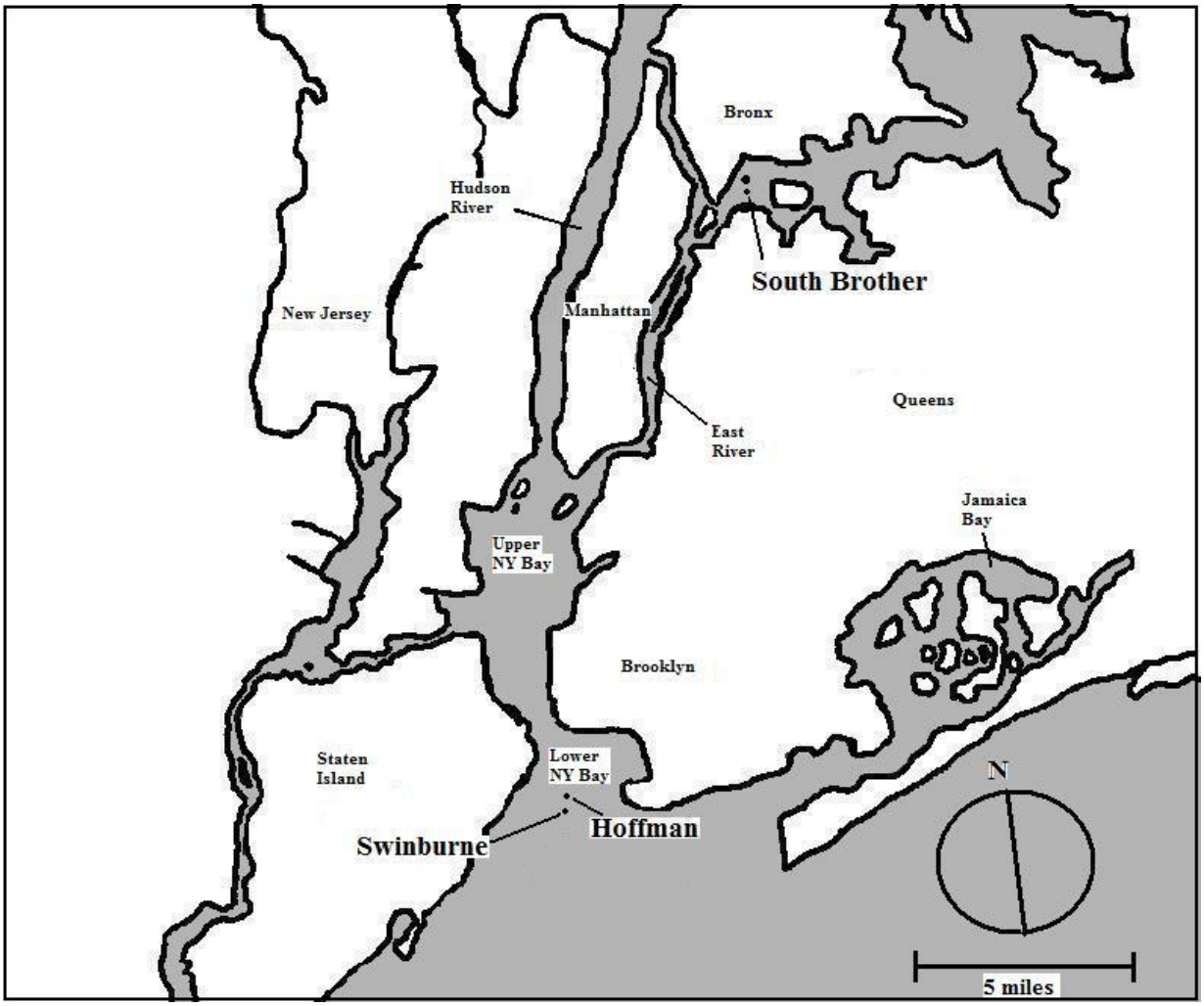
Samples consisting of boli and pellets were collected between May and July 2008. Three islands were visited: Hoffman and Swinburne, located off the east coast of Staten Island, and South Brother, located in the East River between Queens and the Bronx

(Figure 1). Hoffman and Swinburne are man-made islands located in Lower New York Bay between Staten Island Brooklyn; both were completed in the early 1870's (Figure 1). Hoffman's four hectares are now devoid of the buildings that once stood on it, instead the island is covered in vegetation comprising 34 identified species (Seitz and Miller 1996, Bernick 2007). Cormorants were first recorded nesting on the island in 2002; today the colony is primarily located on the southern end of the island in a stand of black locust trees, *Robinia pseudo-accacia* (Bernick 2007). Construction of Swinburne Island was completed in 1870. There are still three buildings standing on the island, one without a roof, and the foundations of other buildings are also present. The cormorants have probably been nesting on the island since the early 1990's (Kerlinger 1998). Nests are found on the buildings as well as a group of black locusts and several other trees (Bernick 2007). South Brother Island is a naturally occurring 5 ha island located in the East River between the Bronx and Queens (Figure 1) (Seitz and Miller 1996, Parsons 1987). Covered in a dense canopy of trees, the islands flora are diverse with 27 species recorded in a survey conducted in 2007 (Bernick 2007). There is a large wading bird colony on the island, nesting, for the most part separate from the cormorant colony. The cormorants primarily inhabit the central portion of the island, nesting in a stand of black locust (Bernick 2007). Boat troubles throughout the season limited our access to the islands, particularly South Brother, which was only sampled twice. Swinburne was visited five times, and Hoffman was visited once.

The collected boli and pellets were stored in freezers for further analysis. Boli were weighed, measured and identified to the lowest taxonomic level possible. Pellets were dissolved in a solution of detergent and water before being dissected and examined

for otoliths and other parts, which could be used to identify food items. When multiple otoliths from a species were found, the total number was divided in half to get the estimated number of fish present. Otoliths which showed a high degree of wear or which could not be identified with a high degree of confidence were listed as unknowns and excluded from further analyses.

Figure 1: Map of New York Harbor area, Hoffman, South Brother and Swinburne colonies indicated.



The composition of species between the two main colonies surveyed – Swinburne and South Brother – was examined using the Schoener index. This index can be used to quantify the dietary overlap of two species, and to compare the diets of neighboring communities of the same species (Schoener 1968). The diet of the South Brother colony was compared with the combined diets of the Swinburne and Hoffman colonies. The two samples collected from Hoffman were combined with those from Swinburne due to their low number (n=2) (Table 1), and the islands' close proximity of 1.1 km (Figure 1).

The Schoener Index is:

$$PSIxy = 1 - 0.5\ (\Sigma|Pxi - Pyi|)$$

where Pxi is the proportion of species i in the diet of population x and
Pyi is the proportion of species I in the diet of population y.

Values can range from 0, indicating no overlap, to 1 indicating complete overlap.

The Shannon-Weaver Index was used to estimate the diet diversity as well as to quantify the difference in the diversity of food items as identified in boli and pellets. The formula is:

$$H' = -\Sigma\ P_i(\log P_i),$$

where P_i is the proportion of items of species i in the sample (Cortes et al. 2002). Diet diversity increases as the index increases.

Due to the similarities among the otoliths of the three herring species identified, the counts for those species were combined to avoid errors caused by misidentifications. It was likewise useful to group other closely related species together for the analysis. Due to decomposition of the boli due to digestion, several searobins could not be identified beyond the level of their genus, *Prionotus*; however, the morphologies of the two occurring species are similar enough that, for this analysis, identification down to the species level was deemed unnecessary. The same was also true of the hakes, genus *Urophycis*, and sculpins, genus *Myoxocephalus*. Cunner (*Tautogolabrus adspersus)*, and tautog (*Tautoga onitis)*, though not congeneric, were too similar in appearance to be identified down to species and were combined as the family Labridae. The final such grouping was for eel specimens that could not be identified as either American eels, *Anguilla rostrata*, or conger eels, *Conger oceanatus*; these species, though not congeneric, are nearly identical in morphology.

RESULTS

Over the field season, a total of 434 boli and 88 pellets were collected. Four of these were combinations of boli and pellets, each containing one bolus along with the normal pellet contents, and were removed from the comparison analysis. Of the boli, 402 were identified and included in the analysis. There were 32 species of fish and two species of crustacean identified in the boli (Table 1). In the pellets, all 88 samples were

analyzed, with 249 food items were identified, comprising 17 fish and three crustacean species (Table 1). Species found included those associated with marine, freshwater, and estuarine environments. The most common species were black seabass (*Cetropristis striata)*, which made up 14.4% of the items identified in the boli and scup (*Stenotomus chrysops)*, which made up 12.9%.

Table 1: Species found in samples, numbers in parentheses indicate species identified in pellets, numbers outside parentheses indicate species identified in boli.

Species	Number		
Fish	South Brother	Swinburne	Hoffman
american eel, *Anguilla rostrata*	1	3	-
atlantic thread herring, *Opisthonema oglinum*	-	9	-
bay anchovy, *Anchoa mitchilli*	12	-	-
black seabass, *Centropristis striata*	2	55 (71)	-
blueback herring, *Alosa aestivalis*	-	1	-
bluefish, *Pomatomus saltatrix*	-	1	-
bluegill, *Lepomis macrochirus*	1	3 (2)	-
brown bullhead, *Amerius nebulosus*	1	-	-
conger eel, *Conger oceanitus*	-	5	-
cunner, *Tautogolabrus adspersus*	7 (6)	10 (20)	-
goldfish, *Carassius auratus*	1	-	-
grubby sculpin, *Myoxocephalus aenaeus*	1	15 (3)	-
gulf stream flounder, *Citharichthys arctifrons*	-	2	-
hogchoker, *Trinectes maculatus*	3	-	-
lined seahorse, *Hippocampus erectus*	-	1	-
menhaden, *Brevoortia tyrannus*	2	20 (2)	-
mummichog, *Fundlus heteroclitus*	22	24	-
northern pipefish, *Syngnathus fuscus*	4	-	-
northern searobin, *Prionotus carolinus*	-	16 (28)	-
oyster toadfish, *Opsanus tau*	3	12 (1)	-
pumpkinseed, *Lepomis gibbosus*	18 (10)	-	-
red hake, *Urophycis chuss*	-	1	-
rock gunnel, *Pholis faciata*	-	2	-
silverside, *Menidia sp.*	1	0	-

Table 1 (continued):

Species	Number		
Fish	South Brother	Swinburne	Hoffman
spotted hake, *Urophycis regis*	3	4	-
striped searobin, *Prionotus evolans*	-	17 (24)	1
summer flounder, *Paralichthus dentatus*	-	2	-
tautog, *Tautoga onitis*	-	9 (4)	-
weakfish, *Cynoscion regalis*	0 (2)	-	1
white perch, *Morone americana*	20 (17)	3 (1)	-
winter flounder, *Pseudopleuronectes americanus*	-	7	-
yellow perch, *Perca flavescens*	-	1	-
unidentified *Anguillaformes*	-	2	-
unidentified *Clupeid sp.*	-	1 (8)	-
unidentified *Labridae sp.*	1	4	-
unidentified *Myoxocephalus sp.*	-	2	-
unidentified *Prionotus sp.*	-	6 (4)	-
unidentified *Urophicys sp.*	-	5	-
Crustaceans			
blue crab, *Callinectes sapidus*	-	0 (2)	-
lady crab, *Ovalipes ocellatus*	0 (1)	0 (1)	-
sand shrimp, *Crangon septemspinosa*	2	0 (2)	-
crustacean sp.	-	0 (2)	-
Combined species			
Anguilliform sp. (american eel, conger eel, unidentified *Anguilliform sp.*)	1	10	-
Clupeid sp. (atlantic thread herring, blueback herring, menhaden, unidentified *Clupeid sp.*)	2	30 (10)	-
Labridae sp. (cunner, tautog, unidentified *Labridae sp.*)	7 (6)	19 (24)	-
Myoxocephalus sp. (grubby sculpin, unidentified *Myoxocephalus sp.*)	1	17 (3)	-
Prionotus sp. (nothern searobin, spotted searobin, unidentified *Prionotus sp.*)	-	39 (56)	-
Urophycis sp. (red hake, spotted hake, unidentified *Urophycis sp.*)	3	10	-

The Schoener Index value for the overlap between South Brother and the Swinburne and Hoffman colonies was 0.108, indicating a low amount of overlap. Slightly different results were obtained by analyzing boli and pellets separately. Looking at pellets only, the score remained low at 0.106, but the score derived from the bolus samples was higher, 0.337, indicating a medium level of overlap.

Bolus samples, comprising 33 species, showed greater diversity than pellets, which had 18 species (Table 2). Every species of fish identified in the pellets was also identified in the boli. There were 62 otoliths which could not be identified due to erosion. It could not be determined if some of these belonged to species not found in the boli. All of the otoliths in good condition were identified to species. The reverse trend was true of crustaceans – several were found in the pellets compared to one in the bolus samples.

Table 2: Proportions of spiny vs. non-spiny fish species in pellets and boli. Spininess column indicates whether each species is spiny or not; y = yes, n = no.

Species	Spiny?	P pellets	P boli
american eel, Anguilla rostrata	n	-	1.01%
bay anchovy, *Anchoa mitchilli*	n	-	3.02%
black seabass, *Centropristis striata*	y	30.09%	14.36%
bluefish, *Pomatomus saltatrix*	y	-	0.25%
bluegill, Lepomis macrochirus	y	0.88%	1.01%
brown bullhead, *Amerius nebulosus*	y	-	0.25%
conger eel, *Conger oceanicus*	n	-	1.26%
cunner, *Tautogolabrus adspersus*	y	8.85%	4.28%
goldfish, *Carassius auratus*	n	-	0.25%
grubby sculpin, *Myoxocephalus aenaeus*	y	1.33%	4.03%
gulf stream flounder, *Citharichthys arctifrons*	n	-	0.50%
hake sp.	n	-	1.26%
hogchoker, *Trinectes maculatus*	y	-	0.76%
lined seahorse, *Hippocampus erectus*	n	-	0.25%

Table 2 (continued):

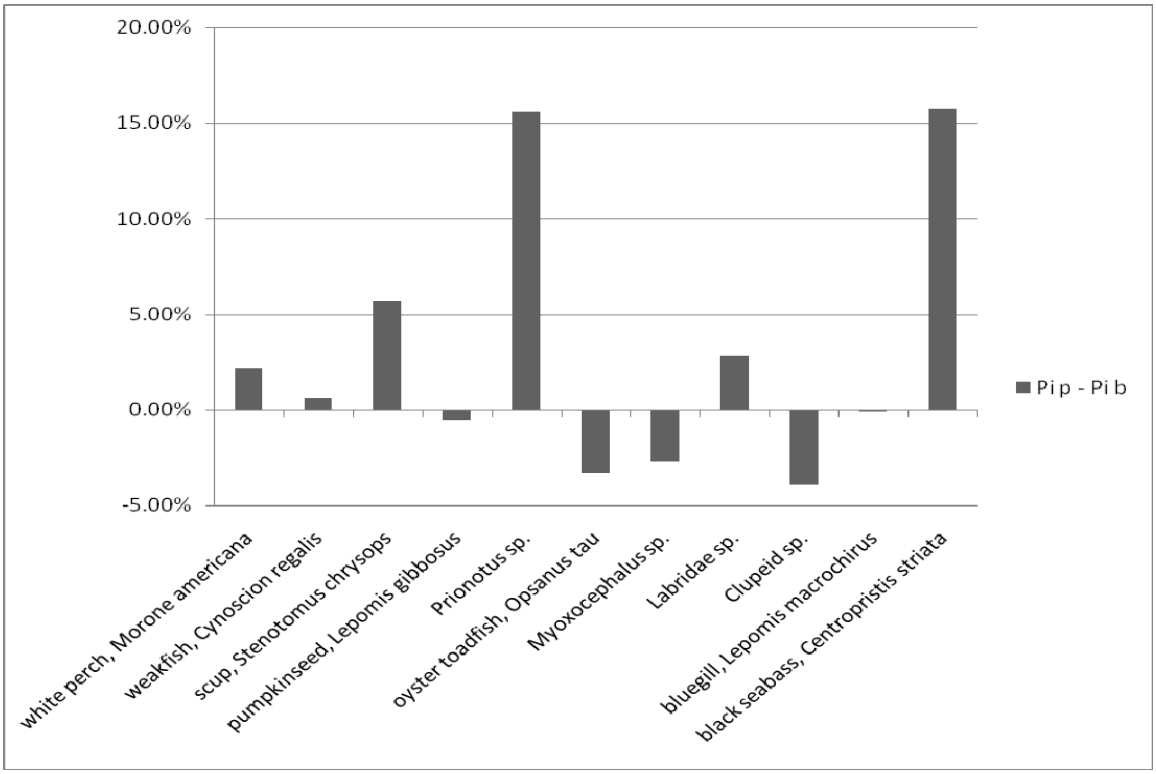
Species	Spiny?	P pellets	P boli
mummichog, *Fundlus heteroclitus*	n	-	11.08%
northern pipefish, *Syngnathus fuscus*	n	-	0.76%
oyster toadfish, *Opsanus tau*	n	0.44%	3.78%
pumpkinseed, *Lepomis gibbosus*	y	3.98%	4.53%
hake, red, *Urophycis chuss*	n	-	0.25%
rock gunnel, *Pholis faciata*	y	-	0.50%
sculpin sp.	y	-	0.50%
scup, *Stenotomus chrysops*	y	18.58%	12.85%
searobin sp.	y	1.77%	1.51%
searobin, northern, *Prionotus carolinus*	y	11.95%	4.03%
searobin, striped, *Prionotus evolans*	y	10.62%	4.53%
hake, spotted, *Urophycis regis*	n	-	1.76%
summer flounder, *Paralichthus dentatus*	n	-	0.50%
tautog, *Tautoga onitis*	y	1.77%	2.27%
weakfish, *Cynoscion regalis*	y	0.88%	0.25%
white perch, *Morone americana*	y	7.96%	5.79%
winter flounder, *Pseudopleuronectes americanus*	n	-	1.51%
yellow perch, *Perca flavescens*	y	-	0.25%
Combined species			
Anguilliform sp.	n	-	2.27%
Clupeid sp.	n	4.42%	8.31%
Labridae sp.	y	10.62%	7.81%
Myoxocephalus sp.	y	1.33%	4.53%
Prionotus sp.	y	25.66%	10.08%
Urophycis sp.	n	-	3.27%

Comparing the proportions of spiny and non-spiny food items in pellets, several important observations were made: 1) 11 of the 13 species found in pellets had spiny fins compared to the ratio of 14 out of 31 species found in boli (Table 2); 2) spiny fish species made up 63% of items identified in the bolus samples versus 95% of items identified in pellets; 3) every fish species which was found in the pellets with frequency of greater than 5% was spiny; and 4) when non-spiny species did occur in pellets, their relative

frequencies were always less than their relative frequencies in the bolus samples. This trend was reversed for 5 of the 8 spiny species found in the pellets (Figure 2).

The biggest differences could be seen in black seabass which collectively increased 15.7% in relative frequency between boli and pellets, and the searobin species which collectively increased 15.6% in frequency in the same measure. The largest decrease could be seen in the clupeids which decreased 3.9%.

Figure 2: Differences in species proportions between pellets and boli. Columns indicate the difference in relative frequencies of fish species found in pellets (Pi p) versus their relative frequencies in the boli (Pi b). The non-spiny species listed are oyster toadfish and the clupeid spp.



DISCUSSION

Comparison of Boli and Pellets

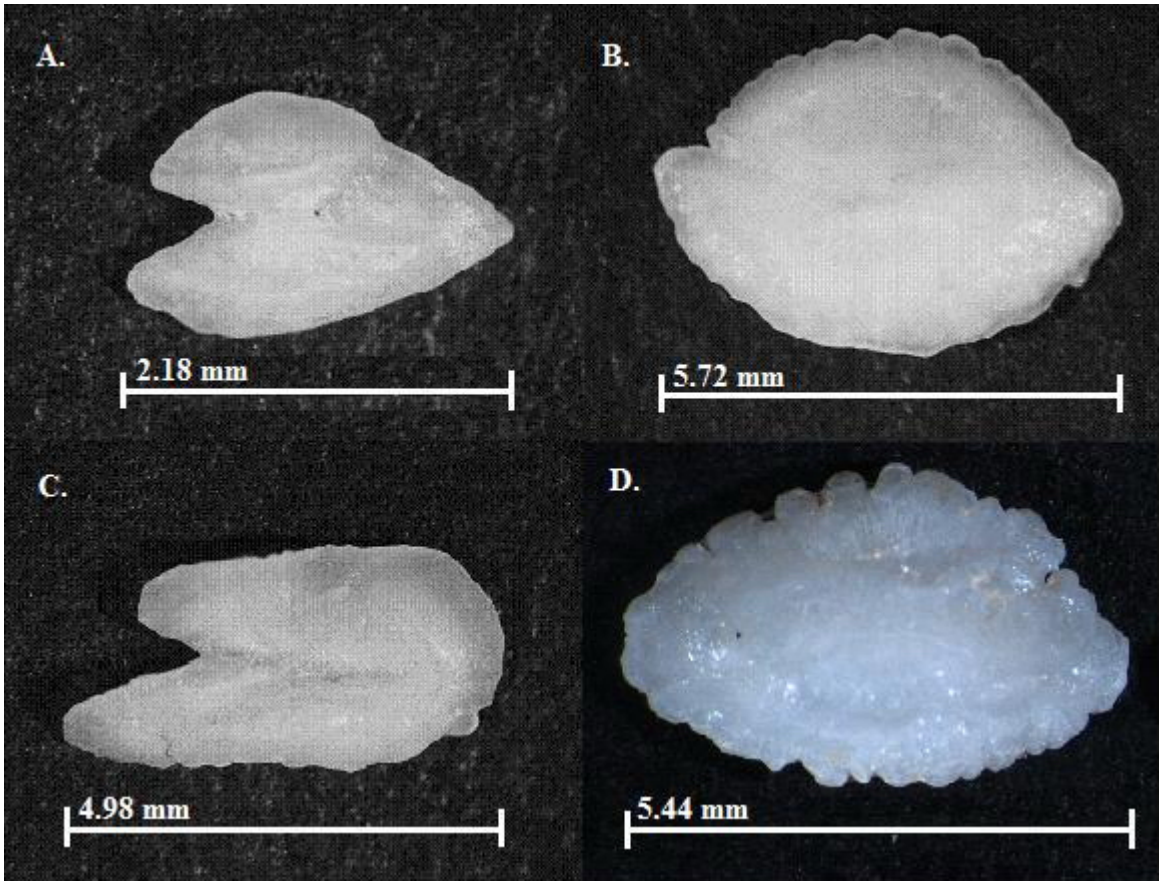
The species assemblages of the boli and pellets were very different, with boli being more diverse than pellets (Table 2). All fish species found in pellet samples were also found in the bolus samples while less than half of the species found in the boli were also found in the pellets. An important difference between the species which were common in pellets and those which were not, is that the former are spiny and the latter are not (Table 2). Previous studies have also shown that pellets do not represent all species equally, and that the time in which pellets are produced can vary greatly, (Casaux et al. 1995; Russell et al. 1995). Russell et al. (1995) showed that for shags, the age and sex of the birds did not affect pellet production, the only exceptions being the nestlings, which were not found to produce pellets. It was hypothesized that this was due to their having a more acidic gastric environment (Russell et al. 1995). The possibility that prey morphology could play a part in pellet production has been raised before but not well studied (Carss 1997, Caseaux et al. 1995). Three possibilities are fish size, otolith morphology and bone content (Carss 1997, Caseaux et al. 1995); of these, only otolith morphology has been previously studied (Johnstone et al. 1990, Caseaux et al. 1995).

Fish size is often mentioned as a factor that could affect the production of pellets (Carss 1997). The digestion processes render accurate estimation of fish size difficult for both pellets and boli (Carss 1997). The effects of fish size on pellet production are hard to quantify. Most boli are partially digested, and using them to estimate the sizes of the fish before digestion is problematic. Some soft-fleshed species are rarely found as intact

boli. For example, all the hakes, genus *Urophycis*, found in 2008 were unrecognizable balls of flesh and could only be identified through the discovery of identifiable otoliths, skin or bones. Although accurate estimation of fish size is difficult, by using fairly complete bolus samples, it was shown not to be the major morphological aspect acting on pellet production. There were a few examples of this. Both herring species and oyster toadfish, *Opsanus tau* tended to be sizeable but rarely showed in the pellets, while cunner showed more frequently in pellets than boli, despite generally being smaller. Both mummichogs and grubby sculpins, *Myoxocephalus aenaeus*, are very small species, their average weights in the bolus samples being 4.5 g and 10.6 g, respectively, and yet sculpins showed up in the pellets three times while mummichogs never did (Table 1). This shows that observed differences cannot be easily explained by prey size, however additional research is needed.

Otolith morphology is another possible factor in species representation in pellets. Casaux et al. (1995) conducted a feeding trial involving the Imperial Cormorant, *Phalacrocorax atriceps*. Seven species of fish were fed to a captive bird, which was monitored for its rate of pellet production and how well the fish species were represented in the pellets. The researchers were able to show a large variation in the number of otoliths lost by different fish species. They hypothesized that the differences observed were due to differences in the morphologies of the otoliths but, because this was a preliminary study and their primary interest was in providing estimates in the correction of pellet analysis data for the local cormorant population, they did not actually provide much evidence to support their hypothesis. All seven species used in the trial were spiny, so it is not possible to draw conclusions on the importance of spininess from their data.

Figure 3: Otolith morphologies. Otoliths of different species have different shapes. Otoliths with thin projections might be more vulnerable to digestion than those compact in shape. Depicted are the otoliths of A) cunner, B) Atlantic thread herring, C) striped searobin, and D) oyster toadfish. Pictures are not to scale.



The feeding trial conducted by Caseaux et al. (1995) involved only one bird and was not comprehensive enough to truly answer the question. The study by Caseaux et al. (1995) involved only one bird and was not comprehensive enough to truly answer the question of how otolith morphology affects species representation in the pellets but it did lend support for the idea, and so it was considered here as well. The sizes and shapes of

otoliths in the observed species varied greatly. Labrids have very small otoliths compared to the sizes of the fish and yet cunner otoliths often survive to be regurgitated in pellets. In addition, their shape is roughly similar to that of clupeid otoliths (Figure 3), which in this study were found in pellets less frequently than would be expected given the proportion of herring in the boli. In contrast, the large otoliths of striped searobins, *Prionotus evolans*, and oyster toadfish (Figure 3) are similar in size and shape and yet while striped searobins increased in frequency between boli and pellets by 4.5%, oyster toadfish decreased in frequency by 3.8%. This indicates that while otolith morphology can affect their survival in pellets, this is not enough to discount the affects of spininess.

The total bone content, and the size and shape of large bones of prey species is another possible morphological factor in pellet production. Data on the relative boniness on these species is not available, and will be explored in future research. The presence of soft-shelled crustaceans in the pellets is possible evidence of this, however. None of the five pellets containing soft-shelled crustaceans contained evidence of any other species. This could indicate that the pellets were ejected immediately due to the high load of indigestible material contained in these food items. More evidence of this was the presence of searobin skulls in the pellets. Searobins were the only species for which whole skulls could be found in the pellets. Their thick skulls along with the bony plates covering their heads could prove too much for the cormorant digestive processes, requiring them to be ejected in pellets faster than spininess alone would account for. Finally, the importance of crustaceans in the diet is probably overstated by the biases associated with pellets. Five of the crustaceans found in the pellets were soft-shelled forms of crabs or other crustaceans. The others were crabs very small in size (carapace

width <10 mm) that likely were found as a result of secondary consumption, which has been shown to be a source of error with pellets (Blackwell & Sinclair 1995). Species such as blue crabs, *Callinectes sapidus,* are spiny, but the fact that all large individuals found in the pellets were soft-shelled lends evidence to another factor being responsible for their appearance in the pellets. Although the carapaces of these species were soft and often damaged beyond a point where species recognition was possible, there was always enough material left to fill a pellet, and it is likely that this lends support to the hypothesis that boniness is also an important factor in pellet production.

A further complication is the existence of empty pellets. While a number of pellets had nothing in them which could be used to identify prey species, two apparently intact pellets found were entirely empty, consisting only of the outer gelatinous coating. Their presence indicates that the factors influencing the production of pellets are more complicated than simply the morphology of the prey. It is known that young birds tend not to produce pellets (Derby and Lovvorn 1997), and one possibility is that pellet production is a process mediated in part by a bird's metabolism, hastened by the collection of hard material but occurring even without it.

Diet Analysis

Due to reasons discussed later in this section, an accurate picture of the birds' diet is easiest to obtain through consideration of bolus samples alone. For this reason, the analysis of the birds' general diet will cover the results obtained from boli only.

The diet of the local population, as indicated by the diets observed on the three islands visited, was shown to be broad, with a Shannon-Weaver Index value of 3.03.

This takes into account not only the number of species found but also the proportions of each species within the samples. The number of species and their evenness varied considerably by day, with Swinburne showing more diversity overall. However, the disparity between the number of visits to Swinburne and South Brother may account for the differences in estimates of diversity and species abundance.

Several species stood out as constituting a particularly large proportion of the diet. Black seabass was the most common species, making up 14.4 % of all food items identified. Scup was also frequent, making up 12.9% of boli. Other common species included mummichog, *Fundulus heteroclitus* (11.44%), Atlantic menhaden, *Brevoortia tyrannus* (5.5%), and white perch, *Morone americana* (5.8%) (Table 2). Raw proportions alone though, are not sufficient to understand the importance of different species in the local population's diet. For example, some species such as bay anchovy were present in large numbers (Table 1) but this is mediated by the fact that all the fish were contained in one aggregate bolus. With that taken into consideration, the species' estimated importance in the local diet or even in the diet of the South Brother colony diminishes.

Total species diversity did not change much over time, although the occurrence of particular species did. The main prey species identified, black seabass, scup, menhaden and the two searobins, were each present on four days, each only being absent from the samples collected on one of the two trips to South Brother where those species were not as common. The limited number of trips to South Brother makes accurate evaluation of temporal changes in prey impossible.

The Schoener Index value of 0.337 indicates that the two colonies partly overlap in their diets. Looking at the species compositions for each area gives a more detailed

picture. Immediate differences were observed as to which species made up the largest proportions of food items in each area. In contrast to Swinburne where black seabass and scup were the most common species, on South Brother, white perch (18.9%) and mummichog, *Fundulus heteroclitus* (20.8%) were the most common (Table 1). Swinburne showed more diversity with 32 species compared to 20 species at South Brother. The differences are important, possibly reflecting the relative positions of the colonies, with South Brother being located much deeper into the estuary than Swinburne. This is possibly reflected in the greater proportion of food items found in cormorants at the South Brother location that were derived from regional fresh waters, including the sunfish species bluegill, *Lepomis macrochirus,* and pumpkinseed, *Lepomis gibbosus*. The exact locations of the fresh water foraging areas is unknown but there are numerous city ponds which could be utilized as well as the Hudson River itself.

Although the Schoener Index is scaled to take into account different sample sizes, it does not consider the numbers of specimens within individual samples. This is a possible source of error as seen in the large number of fish with small body sizes found on South Brother. Three species – bay anchovy, mummichog and white perch, were found in high numbers but had small body mass throughout. While the abundance of these species suggest they are important to the colony's diet, their small sizes (largest of these was a mummichog of 13.1 g) likely exaggerate their significance. To compensate for this, the Schoener Index was computed without those species, yielding a value of 0.416, which indicates a slightly higher amount of overlap, though still in the midrange of the index. More samples from South Brother would likely have decreased the biases associated with these species.

Finally, studies conducted in 2006 and 2007 (Grubel and Waldman, unpublished data), indicated that black seabass were a much smaller part of the local diet. Overall, the data from those years show a similar range but different make up of prey species. In those years, cunner was found to be a much more important part of the diet, making up 24% of the boli in 2006 and 17% of boli in 2007, while seabass was much less important, making up 1% and 3% of the boli respectively. A wide range of factors including recruitment, fishing, environmental quality, water temperature, and competition with other species can affect which fish species are to be found in the harbor as well as in what proportions (Waldman 2006). Future research will help us understand the exact of nature different species in the diet over time.

These results were obtained through the use of bolus samples only. This is of major importance when we take into account the results of the first part of the study – the comparison of fish assemblages in pellets with those in boli. Failing to properly consider this bias while analyzing the diet would have caused us to overestimate the importance of spiny species. For example, searobins would have been estimated to make up 17.9% of the diet instead of 10.1%, and black seabass would have been estimated at 22.2% instead of at 14.4%. Conversely, the importance of non-spiny species such as herrings would have been estimated to make up only 6.35% of the diet instead of 8.31%, and oyster toadfish would have been estimated at 2.1% instead of at 3.8%. These differences are large enough to potentially affect the decisions of resource managers and others who rely on such diet studies to evaluate the ecological impacts of cormorants.

CONCLUSIONS

Continued research into the diet of the local Double-crested Cormorant population is important for the proper management of the species in the New York City region. Of particular interest is the extent to which certain fish species are exploited by the cormorants. Neither of the species valued by humans, commercially and recreationally, were common in the samples, indicating that the local cormorant population is not a threat to the local populations of those species.

This research has uncovered a likely cause for the observed differences in both the species representation and the time in which pellets are produced. The differences observed between boli and pellets seem to be upheld by the hypothesis that spininess is an important factor in the formation of pellets. This is seen in the different proportions with which spiny and non-spiny species are found in boli and pellets. Although our results indicate that spininess is an important factor in pellet production, we cannot estimate exactly how important a component it is. Controlled feeding trials would help answer this question. In addition, it is unlikely to be the only morphological factor affecting pellet production and species representation in pellets and to that end, further research into other factors is needed as well.

The results of this study will be useful for those parties with an interest in managing the local marine resources as well as those concerned with managing Double-crested Cormorant populations elsewhere. One of the primary concerns of government agencies is the impact which cormorants have on fish populations and, through that, on the people who depend on those fish for recreation and livelihood. Thus, it is extremely important that the needs of local fishermen and others who rely on the fish must be

considered, however, to proceed in instituting policy without first obtaining the required data will only lead to problems later on.

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