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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 2007. Major objectives of these studies included: (1) determining whether the relationship between nitrite and oxygen under laboratory conditions was also present in the natural environment of the Hudson River estuary, (2) quantifying fecal bacteria in recreational water to determine its origins and suggest potential mitigating policies and practices, (3) studying riparian wetlands to determine impacts on water quality, (4) understanding how the metabolite of phthalate di-esters, phthalate mono-esters, can be used to get a more accurate understanding of phthalate di-ester distribution in the Lower Hudson River Estuary, (5) applying an effect-directed strategy to identify unrecognized toxic chemicals or mixtures of chemicals in upper Hudson River sediments, (6) studying the assimilation of dietary mercury by grass shrimp that were collected along and environmental impact gradient, (7) determining the possible effects of endocrine disrupting chemicals on the terrapins of Jamaica Bay, and (8) using passive and active fish sampling methods to sample fishes in non-vegetated, emergent, and submerged aquatic vegetation sites in New York's Great Swamp.

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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 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 2007 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 2007. These studies meet the goals of the Tibor 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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SIGNIFICANCE OF ESTUARINE HYPOXIA TO ALTERED NUTRIENT CYCLING AND TOXIC NITRITE ACCUMULATION

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

Past laboratory studies have shown that low oxygen conditions disrupt the coupled relationship between ammonia-oxidizing bacteria and nitrite-oxidizing bacteria, leading to nitrite accumulations in low-oxygen aquatic environments. Nitrite is a known fish toxin and is thus of concern in aquatic ecosystems. The goal of this research was to determine whether the relationship between nitrite and oxygen under laboratory conditions was also present in the natural environment of the Hudson River Estuary. Sampling of surface water was carried out across several locations in the brackish southern estuary and the freshwater northern estuary to collect oxygen, nutrient and molecular information during April-September and November, 2007. These data demonstrated no apparent relationship between oxygen and nitrite in the Hudson River Estuary, and nitrite was not found to accumulate in low-oxygen environments as predicted. Both low oxygen conditions and high nitrite concentrations were observed in the estuary, but these conditions did not occur in the same place at the same time. Since nitrite does accumulate in the estuary, chiefly in the vicinity of wastewater treatment plant discharge points, we may conclude that external nutrient inputs, rather than internal nutrient cycling, are a significant source of nitrite in the Hudson River Estuary. These occasional high nitrite concentrations indicate that conditions hazardous to the health of aquatic organisms exist in this estuary. The existence of both low oxygen and high nitrite conditions in the estuary could possibly exert multiple stresses on aquatic organisms, but the synergistic effects of these environmental conditions have not been adequately studied.

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INTRODUCTION

The most significant nutrient inputs into American waterways come from wastewater treatment plant discharges and non-point source land runoff (Yao et al. 2004). These discharges typically contain large amounts of nutrients because they are not treated before release. Despite improvements in water quality and reductions in nutrient discharges into the Hudson River Estuary (HRE) since passage of the Clean Water Act in 1972, anthropogenic nutrient inputs are still a significant problem in the HRE because they lead to localized eutrophication and hypoxia (Steinberg et al. 2004).

Nitrification is the process by which ammonium (NH_4^+) is oxidized into nitrite (NO_2^-) and then into nitrate (NO_3^-) (Ward 2000). These oxidation processes are performed by phylogenetically different groups of nitrifying microbes, or nitrifiers: ammonia-oxidizing bacteria (AOB) oxidize ammonia into nitrite, while nitrite-oxidizing bacteria (NOB) oxidize nitrite into nitrate. Nitrification is important for maintaining water quality in estuarine environments because it links the formation of ammonia from the organic molecules in an environment to denitrification, which releases gaseous nitrogen from a system (Ward 2000). Disruption of any step in the nitrogen cycle may degrade water quality due to a breakdown of nutrient cycling (de Bie et al. 2001).

High amounts of nutrients such as nitrogen can over-enrich aquatic systems and lead to eutrophication (Yao et al. 2004). High rates of microbial activity in eutrophic systems, coupled with the oxygen-demanding decomposition of excess organic matter, depress dissolved oxygen concentrations and often create hypoxic conditions where dissolved oxygen is less than 3 mg/L (Brosnan and O'Shea 1996).

Most aquatic organisms rely on dissolved oxygen (DO) because, like humans, they require oxygen to respire. According to the EPA, 2.3 mg/L or 20% saturation is the minimum DO concentration to which marine organisms can be exposed for more than 24 hours without experiencing increased mortality (Steinberg et al. 2004). The effects of low-oxygen exposure on affected organisms include low growth rates and decreased functioning ability (Brosnan and O'Shea 1996).

The number and diversity of microbial species identified as autotrophic NOB are limited; their study and phylogeny have received much less attention than AOB (Ward 2000). Species composition of NOB assemblages are known to vary along salinity gradients in estuaries such as the Chesapeake Bay (Francis et al. 2003), but have not yet been investigated in the Hudson River Estuary.

The intense stress of hypoxic conditions likely alters NOB nutrient cycling in estuaries. Hypoxia impairs the functioning efficiency of these aerobic microbes, profoundly affecting nutrient cycling and their ability to mediate nutrient inputs.

The decreased functioning ability of NOB under hypoxic conditions allows nitrogenous compounds such as nitrite to build up in aquatic systems. When DO concentrations are high, this buildup does not occur because ammonia and nitrite oxidation rates are highly coupled. Studies carried out in laboratory bioreactors demonstrate the degree to which the functional ability of both AOB and NOB are impaired in hypoxic environments. Growth rates of both AOB and NOB slow as DO decreases, but the growth rate of NOB decreases faster than AOB (Bernet et al. 2001). NOB are thus more sensitive than AOB to low oxygen conditions and the greater sensitivity of NOB to these environmental changes causes the coupling in rates between

ammonia and nitrite oxidation to easily become disturbed. NOB in natural environments are expected to be affected in the same way, but the effects of hypoxia on this microbial group have not been adequately documented.

A significant effect of the decoupling of ammonia and nitrite oxidation under hypoxic conditions in laboratory bioreactors is nitrite accumulation, because the oxidation of nitrite into nitrate could not occur. Under hypoxic conditions nitrite may accumulate to concentrations much higher than natural levels (Bernet et al. 2001).

Elevated nitrite levels are problematic in aquatic habitats, because nitrite-rich waters create environments which are highly toxic to fish and other aquatic organisms, and may even lead to large-scale fish mortality (Alonso and Camargo 2006). Nitrite is hazardous because it prevents oxygen from bonding in the bloodstream. After uptake from water, nitrite enters blood plasma and diffuses into red blood cells. Inside the red blood cells, nitrite oxidizes the iron in hemoglobin to a different oxidation state, forming methemoglobin which lacks the ability to bind oxygen to blood. Nitrite thus decreases the oxygen-carrying capacity of blood, a condition which over time leads to the internal anoxia and eventual death of an affected organism (Kroupova et al. 2005).

Of the multiple physiological effects of nitrite, the most toxic is the creation of methemoglobin. Past studies have demonstrated a relationship between the nitrite concentration of an environment and the amount of methemoglobin produced in an organism's bloodstream. A study on trout determined that as the nitrite concentration of a fish's external environment increases, its blood plasma nitrite concentrations also increase (Bath and Eddy 1980). As plasma nitrite concentrations increase, the percentage of methemoglobin in an organism's bloodstream also increases (Jensen 2003).

Many studies have investigated the effects of elevated nitrite concentrations on specific fish species, including the striped bass (*Morone saxatilis*) and shortnose sturgeon (*Acipenser brevirostrum*), both of which are found in the Hudson River. The largest population of the endangered shortnose sturgeon is in the HRE, which is estimated to contain over 60,000 adult individuals (Bain et al. 2007). The 96-hour LC50 for shortnose sturgeon, which is the concentration at which 50% mortality occurs after 96 hours of exposure, is 10 mg N/L (Philips et al. 2002). The 24-hour LC50 for striped bass, which are the subject of intense ecological study in the HRE, is 50 mg N/L (Russo 2006). In addition, a study of shortnose sturgeon young exposed to nitrite over a 5-day period found that plasma nitrite concentrations were more than 63 times the initial environmental concentration at the end of this period (Russo 2006).

In contrast to toxic nitrite concentrations, low-level nitrite exposure does not necessarily lead to death. However, it may cause other significant physiological effects, most notably the disruption of multiple regulatory functions such as the respiratory, endocrine and excretory processes (Jensen 2003). Clinical observations of fish experiencing stress due to nitrite exposure included erratic swimming, loss of equilibrium, lying on their sides, and reduced growth rates (Russo 2006).

The goal of this research is to assess the functioning ability and diversity of nitrite-oxidizing bacteria along oxygen gradients in the Hudson River Estuary, determine how NOB activity is altered along these oxygen gradients, and measure nitrite concentrations to determine the conditions leading to poor water quality and hazardous environments in the HRE. Whether the same microbial response to hypoxia that is observed in wastewater bioreactors occurs in the HRE will also be assessed.

METHODS

Field Sites

Over the summer and autumn of 2007, sampling occurred at two transects in the Hudson River Estuary. One transect was located in New York Harbor and consisted of the Battery, East River and Newtown Creek (Figure 1). The second transect was centered around Piermont, New York and included the pier in Piermont, NY, the vicinity of the Orangetown, NY wastewater treatment plant outfall and mid-channel under the Tappan Zee Bridge (Figure 2). Sampling was also conducted at a freshwater *Trapa natans* (Eurasian water chestnut) bed near Germantown, NY on September 21, 2007. Data were collected over these two transects once per month during May-September and November, 2007 to create a time series for changes in oxygen conditions, NOB community functioning and nitrite concentrations.

The lower New York harbor is characterized by elevated salinity, high nutrients, high bacterial cell counts and episodic oxygen depletion. An assessment of NOB under these conditions and in these locations is an important step towards understanding how natural microbial systems operate and respond to oxygen-depleted aquatic ecosystems. The relationship between oxygen and nitrite was studied along the New York Harbor transect because of wide ranges in typical DO across its four sites: environments range from a relatively healthy and well-mixed system found at the Battery to a highly variable and poorly mixed system in Newtown Creek. This oxygen gradient was believed to provide ideal conditions to test the hypothesis that nitrite accumulates in low oxygen environments.

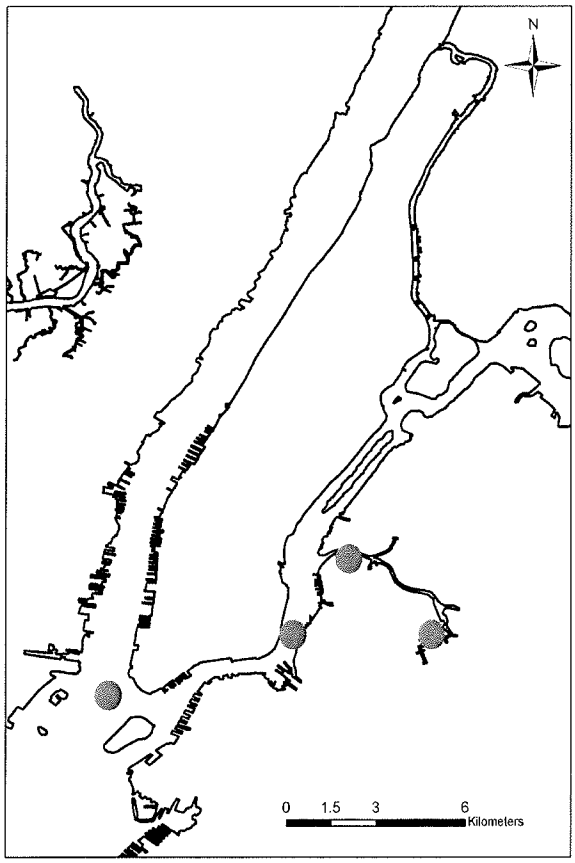

Figure 1: New York Harbor transect. The sites in this transect are (from left): the Battery, East River, Mouth of Newtown Creek, Interior of Newtown Creek.

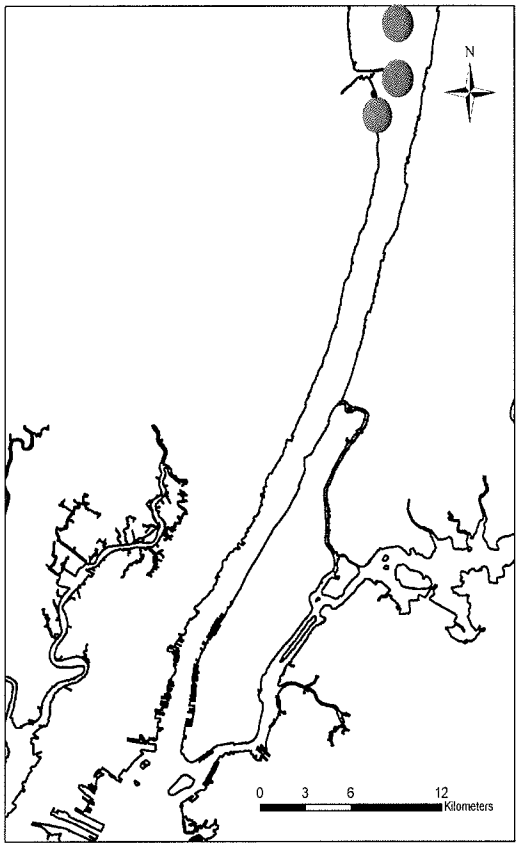

Figure 2: Upriver transect near Piermont, NY. The sites in this transect are (from bottom): Orangetown Wastewater Treatment Plant Outfall, Piermont Pier, Tappan Zee Bridge.

Rain events are associated with alterations in the nutrient inputs and oxygen status in Piermont Marsh. Environments located at HRE margins, such as Piermont Marsh, were investigated to identify whether they served as a point-source of nitrite into the estuary. Based on past studies which observed high nitrite concentrations in this environment in conjunction with rain events during the autumn of 2006, there may be a functional decoupling of ammonia and nitrite oxidation following rain events, leading to nitrite accumulation in the marsh. The time series carried out in the marsh as part of this research sought to determine whether nitrite concentrations observed in Piermont Marsh

during the summer of 2007 were comparable to the elevated concentrations observed during the autumn of 2006.

Further upriver, freshwater margins of the HRE are often dominated by beds of *Trapa*. The water column under this invasive plant experiences reduced light penetration, altered gas exchange and reduced mixing with mid-channel waters due to the dense canopies of floating vegetation growing in thick beds (Caraco and Cole 2002). Hypoxia therefore occurs under beds during low tide. The system is re-oxygenated with the flooding tide by mixing with mid-channel aerobic waters, but when low tide returns the oxygen levels drop once more. Unlike locations in New York Harbor where hypoxia occurs due to anthropogenic nutrient discharges, hypoxia in the *Trapa* beds is caused by an invasive plant. Microbial communities whose respective hypoxic conditions are created by different factors may thus be compared. Nitrite concentrations were predicted to vary with the oxygen status of this variable environment, due to alterations in tidal mixing with mid-channel aerobic waters.

Field Sampling

Water samples were collected across both transects on the Hudson Riverkeeper's boat *R. Ian Fletcher* during May-September and November 2007. Samples were collected using a peristaltic pump connected to a tubing system which drew water up from over the side of the boat, through the pump, through a Hydrolab sensor and back out into the river. Sample bottles were rinsed with water from the station and water was collected for samples using the outflow portion of the tubing system. The Hydrolab sensor collected real-time measurements for oxygen, temperature, salinity, chlorophyll and turbidity for

all locations across the two transects. After collection, samples were immediately transported to the laboratory for storage or analysis.

Nutrient Collection

Water samples for nutrient analysis were collected in a plastic 60 mL syringe. A 0.2 μm Sterivex capsule filter was attached to the end of the syringe, and 50 mL of sample was pushed through the filter into a labeled collection tube. Nutrient samples were filtered prior to storage in order to preserve the actual nutrient concentrations. Removing organic materials and other particulates from water samples prevented microorganisms from continuing to cycle nutrients within the sample bottle and altering the environmental concentrations of all nutrients. Nutrient samples were stored on ice after collection, immediately transferred to a freezer (-20° C) upon return to the laboratory and remained frozen until analysis.

Nitrite Analysis

Standard colorimetric methods were used in the analysis of nitrite from filtered environmental samples. This assay spectrophotometrically determined nitrite concentrations by diazotizing with sulfanilamide and coupling with N-(1-napthyl)-ethylenediamine dihydrochloride (NED) to form an intensely colored pink azo dye. The amount of azo dye formed in a sample is proportional to the initial concentration of nitrite over a range of 0-10 μM, with a precision of about +/- 0.02 μM (Parsons et al. 1984; Grasshoff 1999). After thawing a nutrient sample, 2.5 mL of the sample was measured into a 1 cm cuvette to prepare for spectrophotometric analysis. 500 μL of 60 mM sulfanilamide was added to each sample and allowed to react for approximately 2 min before adding 500 μL of 60 mM NED. This reagent was allowed to react for about 5 min

before the sample was transferred to the spectrophotometer. Samples were read at 543 nm, the wavelength of maximum absorption for nitrogen.

East River Mesocosms

The goal of the mesocosm experiment was to compare patterns of nitrite accumulation under different artificial oxygen treatments in the laboratory, where all biotic and abiotic factors other than oxygen were held constant. Water was collected from the East River in the New York Harbor transect on May 17, 2007. This water was transported back to the laboratory where it was separated into nine 20 L bottles. The nine bottles were further separated into three different oxygen treatments: 10% (low), 50% (medium) and 100% (aerobic) saturation. Oxygen canisters were attached to these bottles to maintain the appropriate saturation. Water samples for nitrite analysis were collected from each of the nine mesocosms throughout the first 50 hours of the experiment.

Piermont Marsh Time Series

Nitrite concentrations in Piermont Marsh were measured over 12 weekdays from July 18, 2007 to August 2, 2007 in relation to precipitation events. Samples were filtered with a 0.2 μm Sterivex capsule filter to remove organic matter and prevent cycling of nutrients by microorganisms. Fifteen mL of each filter-sterilized sample was frozen until analysis, when nitrite concentrations were determined spectrophotometrically.

***Trapa* Bed Tidal Survey**

Nitrite concentrations and dissolved oxygen fluctuations were measured during a survey at the *Trapa* beds on September 21, 2007. Oxygen measurements were collected using the Hydrolab sensor. Beginning in mid-channel, samples were collected in open water and then moved toward the shore. Samples were collected at the outer edge of the

Trapa bed near mid-channel and approximately 20 and 30 m into a *Trapa* and duckweed bed. Samples were also collected farther south in the *Trapa* bed: in the open water near that location, at the edge of the bed, and approximately 30, 200, 600 and 800 m into the thick bed, gradually moving toward land from mid-channel. Samples collected at this location were analyzed for nitrite using spectrophotometric methods.

Molecular Analysis: DNA Extraction

DNA samples were collected across both transects, in addition to the *Trapa* site, to characterize spatial variation in NOB genetic diversity and species composition throughout the HRE. The goal of this diversity survey was to determine the presence of NOB across these locations and to classify the species found at each site. Water samples for DNA characterization of NOB diversity were collected in 4 L bottles rinsed with water from the site before sample collection. Using a peristaltic pump, volumes of approximately 1 L water from a site were run through a 0.2 μm Sterivex capsule filter. It was critical for particulate matter to be collected from these water samples as rapidly as possible to get the most accurate characterization of the microbial community at that site. Once water could not pass through the filter, it was sealed and placed in liquid nitrogen until return to the laboratory, where filters were stored at -40° C until analysis.

DNA was extracted from these samples using the Qiagen DNeasy Tissue Kit. DNA concentrations were determined using the Qubit Quant-iT dsDNA HS (High Specificity) Assay Kit. Due to the restricted nature of AOB phylogeny, it is possible to detect and evaluate NOB community structure based on analysis of their group-specific 16S ribosomal DNA (rDNA) genes. PCR analysis targeted identification of the nitrite oxidoreductase gene, because the presence of this gene indicates the presence of NOB.

Amplification of the 16S gene using universal primers 1522R and 27F indicated that the 16S gene was successfully amplified across several sites.

Molecular Analysis: Genus-Specific Amplification

After amplification of the universal 16S gene using universal primers 1522R and 27F, the genus-specific primers Nbacter 1050R and Nspira 705R were used in nested PCR to determine the presence of the *Nitrobacter* and *Nitrospira* genera in these environments (Freitag et al. 2005).

Molecular Analysis: Sequencing

In addition to amplifying samples using genus-specific primers, samples amplified using only the universal primers 1522R and 27F were sequenced. These amplified products were gel purified using a Qiagen Gel extraction kit. Samples were then cloned using a TOPO TA Cloning Kit, and sequenced by Columbia Genome Center staff using an ABI capillary sequencer. Sequences were generated from DNA extracted from samples collected in April 2007 at the Gowanus Canal, George Washington Bridge and *Trapa* sites in the HRE. These sequences were edited by Sergey Kalachikov and Irina Morozova at the Columbia Genome Center.

Molecular Analysis: Bioinformatics

Based on past studies of the phylogenetic relationships of NOB and sequences (Teske et al. 1994), several AOB and NOB sequences with which to create a database for the HRE were identified. Select sequences representing the diversity of NOB culture collection and dominant marine environmental strains were downloaded from the NCBI GenBank using the FASTA format.

Together with staff from the Columbia Genome Center, these sequences were compared to a database of universal bacterial 16S sequences. This database was created by compiling all of the sequences found at three HRE sites – the Gowanus Canal, George Washington Bridge and *Trapa* – collected in April 2007. Using a modified BLAST search, which aligns environmental sequences with published sequences to determine the percentage of base pairs that are identical across both sequences, environmental HRE sequences were compared to NOB and AOB sequences downloaded from GenBank. The database was queried with a sequence identity cutoff of 90%, so that only environmental sequences whose base pairs were 90% or more identical to database sequences were determined to be the most similar to published sequences. These sequences were then used to construct a distance based, neighbor-joining phylogenetic tree.

RESULTS

Nitrite and Oxygen Dynamics

Contrary to our predictions for both oxygen and the relationship of oxygen and nitrite, only a weak relationship ($p > 0.05$) exists between nitrite and oxygen in the Hudson River Estuary (Figure 3). Throughout the HRE, nitrite concentrations in locations below 50% saturation were much lower than in better-oxygenated environments in the 60-100% saturation range.

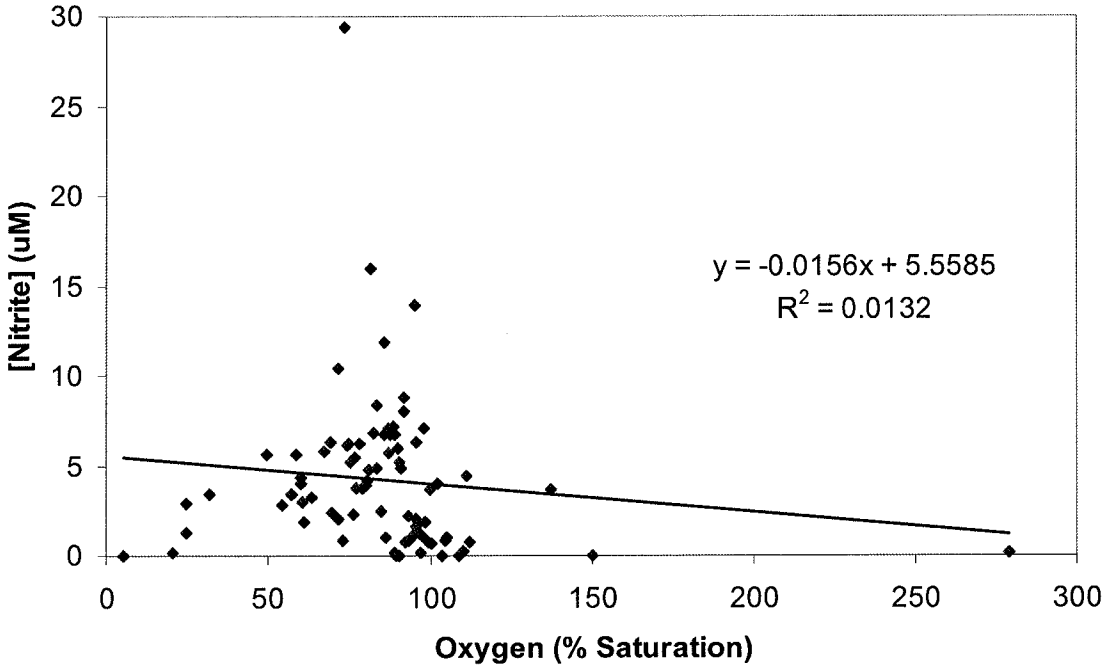

Figure 3: Comparison of dissolved oxygen (% saturation) and nitrite concentration (uM) across 14 locations in the Hudson River Estuary, May-September and November, 2007 (n=81) (p>0.05).

Environmental Transect: New York Harbor

July environmental conditions provided an ideal gradient to test our hypothesis of a relationship between low oxygen and high nitrite conditions: surface DO was 5.3% saturation in the interior of Newtown Creek, compared to about 90% at the mouth of the creek and a well-oxygenated larger channel in the East River. However, there was no increasing trend in nitrite associated with decreasing oxygen (Figure 4). Nitrite was not detected in the most oxygen-depleted environment in the interior of Newtown Creek (Newtown 2): surface oxygen saturation was 5.3%, but nitrite measured there was 0 μM.

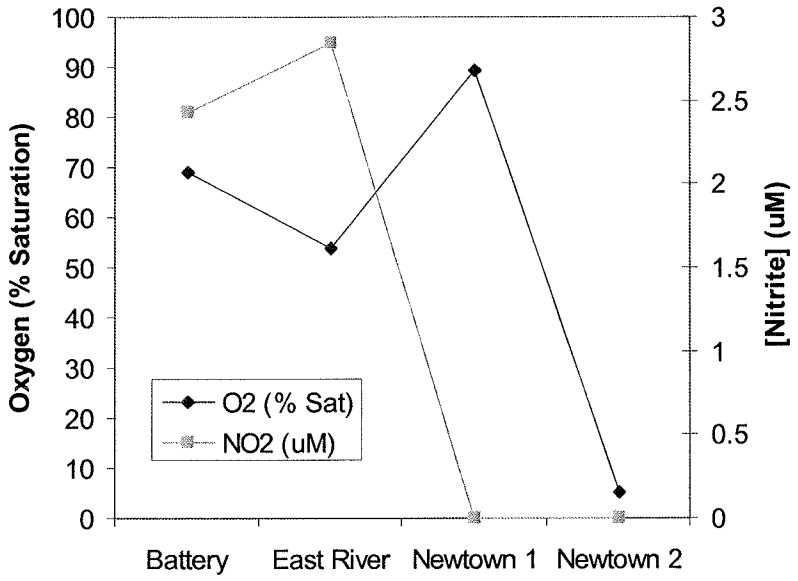

Figure 4: Oxygen and nitrite in the New York Harbor transect observed on July 11, 2007.

Experimental Transect: East River Mesocosms

Despite the different oxygen treatments, nitrite concentrations in the three sets of mesocosms followed essentially the same pattern and the amount of nitrite present was not significantly different across the oxygen treatments (Figure 5).

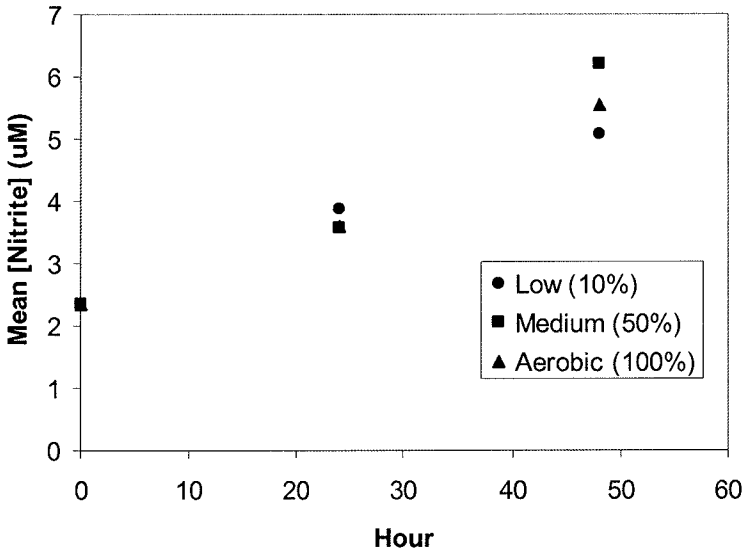

Figure 5: Comparison in nitrite concentrations across three different oxygen treatments.

Environmental Transect: Piermont

Environments with significantly elevated nitrite concentrations were observed in the Hudson River Estuary during the summer of 2007 (Figure 3). Nitrite concentrations analyzed over the Piermont transect displayed a tremendous difference in concentration across a small spatial distance (Figure 6). Concentrations in the vicinity of the Orangetown, NY wastewater treatment plant outfall were consistently greater than concentrations at the Piermont pier, located only a few hundred feet to the north, and under the Tappan Zee Bridge, located at mid-channel further upriver.

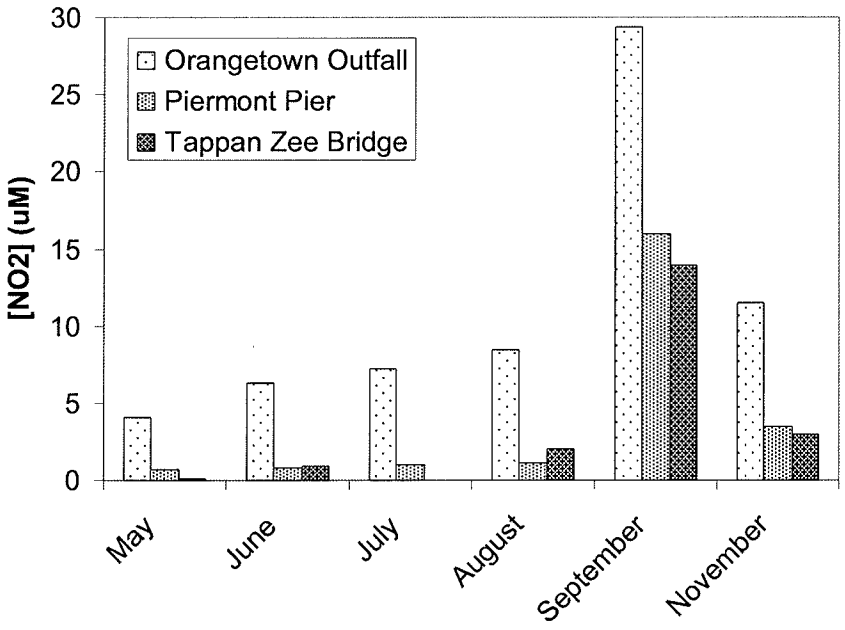

Figure 6: Comparison of environmental nitrite concentrations (in uM) across the Piermont Transect, May-September and November, 2007.

Rain-Associated Nutrient Cycling: Piermont Time Series

Rain events during the 12-day time series were followed by increases in nitrite concentrations in the Piermont Marsh environment (Figure 7). Precipitation information is from Woodcliff Lake, New Jersey, located approximately 16 km from Piermont. The

highest nitrite concentration observed in the summer of 2007 was 1.18 μM on July 21, far below the concentrations of over 40 μM observed during the autumn of 2006 in conjunction with rain events (O'Mullan and Sambrotto, unpublished).

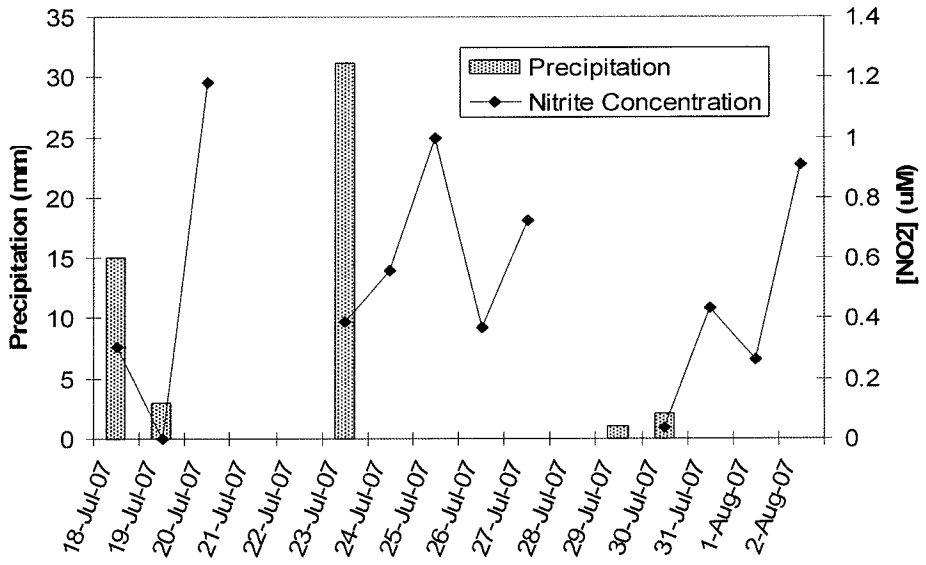

Figure 7: Comparison of precipitation events and nitrite concentrations in Piermont Marsh over a twelve-day sampling period in July-August 2007.

Dissolved Oxygen Fluctuations According to Tidal Cycles: *Trapa* Bed

There appears to be a weak positive relationship between nitrite and oxygen under the *Trapa* beds (Figure 8). Oxygen values were comparable to trends observed in literature where lowest DO occurs under the beds and highest DO is observed in the well-mixed waters free of *Trapa*.

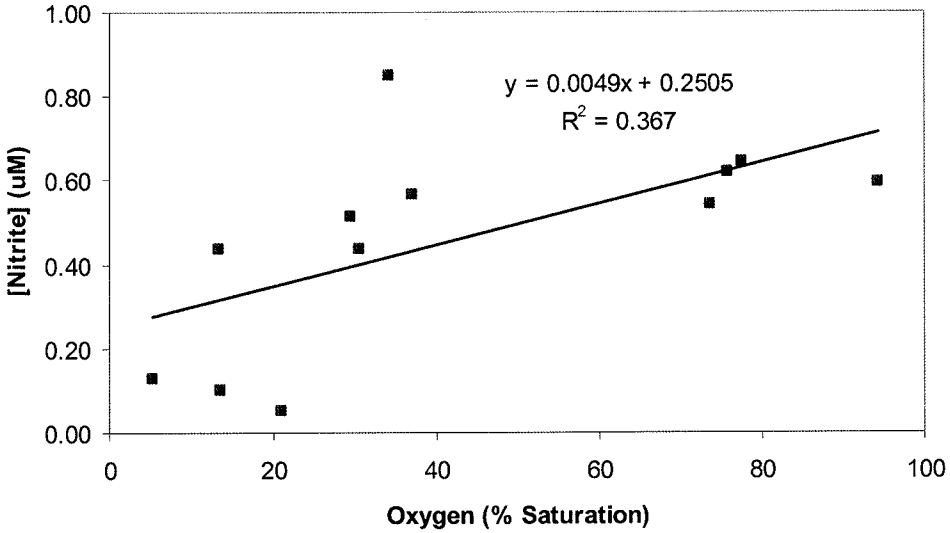

Figure 8: Comparison of dissolved oxygen (% saturation) and nitrite concentrations (µM) in the *Trapa* beds near Germantown, NY based on data collected September 21, 2007 (p=0.05).

Molecular Analysis: NOB Genus-Specific Amplification

We successfully amplified the 16S gene in samples collected in June 2007 from Gowanus Canal, East River, Buttermilk Channel, Newtown Creek and Orangetown Outfall using universal primers 1522R and 27F. Nested PCR using genus-specific primers Nbacter 1050R and Nspira 705R, targeting the genera *Nitrobacter* and *Nitrospira* (Freitag et al. 2005), were used to determine the presence of these two NOB genera across the six sites (Table 1). While the 16S gene was successfully amplified across all sites, as was *Nitrospira*, *Nitrobacter* was only amplified from half of the sites during June.

Site	Universal (1522R)	*Nitrobacter* (Nbacter 1050R)	*Nitrospira* (Nspira 705R)
Newtown Creek 1	+	-	+
Newtown Creek 2	+	+	+
Gowanus Canal	+	+	+
East River	+	-	+
Buttermilk Channel	+	-	+
Orangetown Outfall	+	+	+

Table 1: Results of genera-specific amplifications of 16S rDNA from six HRE sites, based on data collected in June 2007. "+" indicates a successful amplification, while "-" indicates that genus was not found at that location at that time.

Molecular Analysis: Sequencing

Based on environmental samples collected in April 2007, 97 sequences were identified from the George Washington Bridge, 70 sequences were identified from the Gowanus Canal and 94 sequences were identified from the *Trapa* environment.

Molecular Analysis: Bioinformatics

One *Nitrosospira*-like sequence was identified from the Gowanus Canal, and one *Nitrosomonas*-like sequence was identified from the *Trapa* site. *Nitrosospira* and *Nitrosomonas* are 2 genera of AOB. Apart from these AOB-like sequences, which comprise approximately 1% of total sequences in both the Gowanus Canal and *Trapa* environments, no NOB sequences were identified. No AOB-like or NOB-like sequences were found in the George Washington Bridge environment. A phylogenetic tree was created with the two AOB-like sequences, together with database sequences representing the diversity of known AOB and NOB (Figure 9). The two putative AOB sequences share approximately 85-90% sequence identity to known AOB.

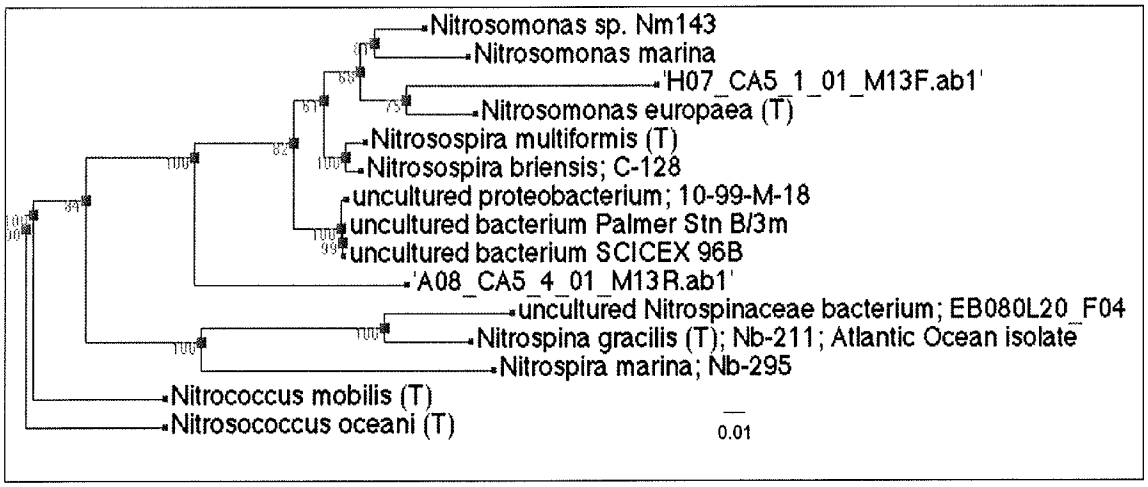

Figure 9: Phylogenetic tree showing the relationships between two sequences of putative ammonia-oxidizing bacteria found in the Hudson River Estuary and database sequences which represent the overall diversity of known ammonia-oxidizing bacteria and nitrite-oxidizing bacteria. The *Nitrosomonas*-like "H07" sequence was found in the *Trapa* environment, while the *Nitrosospira*-like "A08" sequence was found in the Gowanus Canal.

DISCUSSION

Nitrite and Oxygen Dynamics

The focus of this research was to determine the relationship between hypoxic conditions and the buildup of nitrite in the Hudson River Estuary. Based on past studies of wastewater bioreactors, low oxygen levels were predicted to break the coupled relationship between ammonia and nitrite oxidation, leading to significant accumulations of nitrite in hypoxic environments.

However, a comparison of environmental oxygen and nitrite concentrations clearly demonstrated no apparent relationship between oxygen and nitrite in the HRE. These findings are supported by data collected both in the river's brackish regions of the New York Harbor and Piermont transects (Figure 3) and in the freshwater upper regions at the *Trapa* location (Figure 8), in addition to the East River mesocosm experiment (Figure 5). Both the environmental and experimental oxygen gradients demonstrate that different oxygen levels are not linked to different nitrite concentrations, and specifically that low oxygen conditions do not automatically lead to elevated nitrite concentrations in these environments. In addition, based on data from the lower part of the estuary, high nitrite concentrations are found where oxygen is at a relatively high saturation, rather than in hypoxic environments (Figure 3).

The relationship between nitrite and oxygen seems slightly stronger within the freshwater *Trapa* beds than in the brackish portion of the estuary, but the overall relationship in *Trapa* beds between oxygen and nitrite is only weakly positive (Figure 8). Nitrite in the *Trapa* environment did not accumulate to levels as high as observed in the New York Harbor or Piermont transects downriver from the *Trapa* site.

Nitrite did not accumulate in natural environments such as Piermont Marsh in conjunction with precipitation events. These data are highly variable and changes in nitrite concentrations in the marsh were not always associated with precipitation. Due to the fluctuations in this highly dynamic environment it is difficult to quantify the relationship between nitrite and precipitation in Piermont Marsh and to determine whether the predicted decoupling between ammonia and nitrite oxidation occurs there.

The Piermont transect provided invaluable information for locating sources of elevated nitrite concentrations in the HRE. This transect clearly demonstrates that nitrite significantly accumulates in the vicinity of wastewater treatment plant outfalls. We can therefore conclude that wastewater treatment plants are significant sources of nitrite in the HRE. In addition, since none of the environments with elevated nitrite concentrations were hypoxic, we may conclude that internal nutrient processing at these locations is not a significant source of nitrite in the Hudson River.

Therefore, based on analysis of data from the Piermont transect, in addition to other data collected throughout the estuary, it is clear that external inputs, rather than internal microbial nutrient cycling, are a more significant source of elevated nitrite concentrations in the HRE.

Microbial Diversity

Although the NOB genus *Nitrospira* was found to be more widely distributed than the NOB genus *Nitrobacter* across six HRE locations in June 2007 (Table 1), this does not necessarily indicate that *Nitrospira* is more abundant in the estuary. Rather, the composition of AOB and NOB communities varies over time.

Figure 9 demonstrates the phylogenetic relationships between the 2 putative AOB sequences identified in the HRE and our database of known AOB and NOB sequences. These environmental sequences are considered as putative AOB because of their 85-90% sequence identity to known AOB sequences. However, they are also distinct from AOB sequences published in literature. Therefore, these AOB-like sequences could represent 2 new groups of AOB which may be important in estuaries but have not yet been described. This is a significant finding, because it adds to our existing knowledge of AOB phylogeny and suggests that nutrient cycling in the HRE is affected by the activity of microbes which remain to be fully characterized.

Ecosystem Effects of Nitrite and Oxygen

This study demonstrated that both high nitrite and low oxygen environments were observed in the HRE during the summer of 2007 (Figure 3). While the relationship between these variables is still under investigation, it is important to note that both of these potentially hazardous conditions occur in the HRE. Whether or not they exist simultaneously, high nitrite concentrations when coupled with low oxygen conditions in an estuarine ecosystem exert multiple stresses on aquatic life.

Individual environmental conditions observed in the HRE may not create environments toxic enough to cause organism death. However, the combined effects of high nitrite and low oxygen may interact to increase an organism's susceptibility to its environment. Oxygen concentrations affect nitrite toxicity, because nitrite reduces the oxygen-carrying capacity of blood. A reduction in oxygen supply in the external medium therefore exacerbates the oxygen problem within the organism (Kroupova et al. 2005), increasing susceptibility to low oxygen conditions in other parts of the ecosystem.

The implication of multiple stressors which simultaneously exist in spatially different locations is an increased level of fish toxicity. Nitrite does not often accumulate in the HRE to levels which are highly toxic (i.e., the LC50) for most fish species, but many nitrite concentrations observed in 2007 exceeded levels which may exert stress on aquatic organisms. In the case of the shortnose sturgeon and striped bass, the nitrite LC50 values for both species are far above concentrations observed during 2007. However, LC50 values quoted in literature are levels at which 50% of the fish in the study die; fish begin to experience stress in these environments long before they begin to die. Although nitrite does not accumulate in the Hudson up to the LC50 value for either species, it is possible that low-level nitrite exposure over a prolonged period may have lasting effects on organisms which are comparable to LC50-level exposure.

The effects of multiple stressors on aquatic organisms, particularly low oxygen and high nitrite conditions, remain largely unexplored. Studies have shown the effects of one variable or the other on aquatic life, but not the combined effects of both stressors. This lack of knowledge about the interactions (if any) between these different stressors is of concern because it means that the synergistic effects of two potentially hazardous conditions which both exist in the Hudson River remain largely unknown. This work warrants further examination of the multiple stresses exerted by combined high nitrite and low oxygen conditions.

CONCLUSIONS

Two main conclusions may be made based on field and laboratory measurements. The first conclusion, that excessive nitrite accumulation was not observed under low

oxygen conditions, is derived from the results of field sampling in the brackish and freshwater portions of the Hudson River Estuary as well as laboratory experiments. The second conclusion, that inputs to the river rather than internal processing appear to be the major determinant of nitrite concentration, is derived from the results of the Piermont transect where the highest concentrations of nitrite were consistently found in the vicinity of a wastewater treatment plant rather than in an area of low oxygen environment.

These findings strongly indicate that the periodic hypoxia and elevated nitrite concentrations that occur in the Hudson River Estuary create an environment where aquatic organisms are consistently exposed to multiple stresses. Despite the many improvements in water quality and overall ecosystem health that have occurred in recent decades, hazardous conditions still exist in the estuary that should be of concern for the future survival of the many species found in the Hudson.

RECOMMENDATIONS

This investigation primarily focused on oxygen and its influence on nutrient cycling in the HRE, but future investigations should take more variables into consideration in order to accurately reflect the complex and dynamic nature of this ecosystem. In particular, research must investigate the activity of phytoplankton, which produces high quantities of nitrite in light-limited marine environments (Lomas and Lipschultz 2006). Since the turbid HRE environment limits light below the surface, light limitation of phytoplankton may be responsible for nitrite accumulating in the HRE, rather than a breakdown in microbial nitrogen cycling induced by low oxygen conditions. Future work must also refine the preliminary AOB phylogeny presented in this research.

Future work must also further investigate the synergistic effects of the high nitrite and low oxygen conditions which exist in the HRE. Since nitrite is toxic to fish and other aquatic organisms, its sources and sinks must be identified so that we can understand the conditions leading to its accumulation in the environment and thus degradation in water quality. In the past few decades, the Hudson River Estuary has met with great success in improving overall water quality, reducing anthropogenic nutrient loading and rehabilitating natural environments to make them more hospitable for the wide variety of life found in the estuary. In order to maintain these improvements, further investigations must be made to understand the many circumstances that contribute to poor water quality.

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This work would not have been possible without the assistance, patience and guidance of many people. I extend my deepest thanks to the Hudson River Foundation for providing an unparalleled opportunity to conduct this research, as well as to the Center for Environmental Research and Conservation at Columbia University for additional financial support. Many thanks to John Lipscomb, captain of the Hudson Riverkeeper's boat *R. Ian Fletcher*, for providing the opportunity to collect water samples. Thanks also to Sergey Kalachikov and Irina Morozova at the Columbia Genome Center for sequencing environmental samples and producing the phylogenetic tree. Finally, many thanks to my senior thesis academic advisors in the department of Ecology, Evolution, and Environmental Biology at Columbia University and the Environmental Science department of Barnard College.

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MEASUREMENTS OF *ENTEROCOCCI* BACTERIA IN THE HUDSON RIVER: ENVIRONMENTAL HEALTH ISSUES AND POLICY RECOMMENDATIONS FOR COMBINED SEWER OVERFLOW

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

Assessing the water quality of the Hudson River where it is used for recreational purposes is essential to ensure the health and safety of residents. The aim of our project was to quantify fecal bacteria in recreational water, determine its origin(s), and suggest potential mitigating policies and practices. Two general areas of the Hudson River were studied: West 125th Street in Harlem, NY, where the Harlem Piers are currently under construction, and various sites in Piermont, NY, near a highly used recreational pier. Samples were taken from shore throughout the spring and summer of 2007. *Enterococci* bacteria were counted using an EPA-approved membrane filtration method. *Enterococci* counts were found to increase during and after heavy rainfalls. High measurements recorded at sampling sites during dry and wet periods revealed that combined sewer overflows were not solely responsible for failure to meet water quality standards. Additional factors that contributed to the persistence of bacteria in estuarine waters included: salinity, exposure to UV light, grazing communities, sediment attachment, tributary inputs. The complexities of city infrastructure and hydrology make it difficult to detect sources of contamination. We propose that in addition to traditional end-of-pipe solutions, sustainable best management practices and low impact development, such as green roofs and porous pavements, be implemented in areas where stormwater management has been less than effective.

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INTRODUCTION

When heavy rainfalls occur, raw sewage legally flows into the Hudson River. These outflows of sewage are caused by combined sewer overflows (CSOs). Combined sewer systems are designed to collect a combination of building waste and street drainage. Precipitation can create an overwhelming volume of wastewater that often exceeds a treatment plant's capacity for processing. When this occurs, the excess is diverted directly to the river without treatment.

Raw sewage contains various forms of fecal bacteria, including *Enterococci.* These microbes are not dangerous themselves, but are used to indicate the presence of pathogens such as *Salmonella*, *Shigella* and *Escherichia coli (E. coli)*, which can cause gastrointestinal disease and other ailments in humans. Combined sewer overflows expel the sewage during heavy rainfalls, but *Enterococci* can still be detected during dry periods. It is possible that the concentrations remain high due to re-suspension or sedimentary deposition of bacteria that is released in combined sewer overflows.

The development of the Hudson River waterfront, including the study area near West 125th Street, encourages people to use the river recreationally for kayaking, fishing and other activities. This type of recreation, deemed "secondary contact" by the New York State Department of Environmental Conservation (DEC), may involve moderate contact with contaminated water and could cause health problems for those who participate. For example, in 2003 the swimming segment of the New York City Triathlon in the Hudson River was cancelled because of increased health risks caused by heavy rainfalls (Rodriguez 2003). Levels of bacteria which violate federal standards are often found in New York City's waterways because the concentration of bacterial indicator

organisms is not sufficiently monitored and regulated. The ultimate goal set by the Clean Water Act is fishable, swimmable waters. Unfortunately that goal is still far from being achieved in New York City and nearby waters.

Combined Sewers

In New York City, close to 70% of the sewers are combined, meaning that they carry contents from street drainage as well as household and business wastewater (NYCDEP 2005). In the event of a combined sewer overflow stormwater overwhelms treatment plants, or publicly owned treatment works (POTW), causing raw sewage and street drainage to be expelled into one of the city's natural waterways (Figure 1).

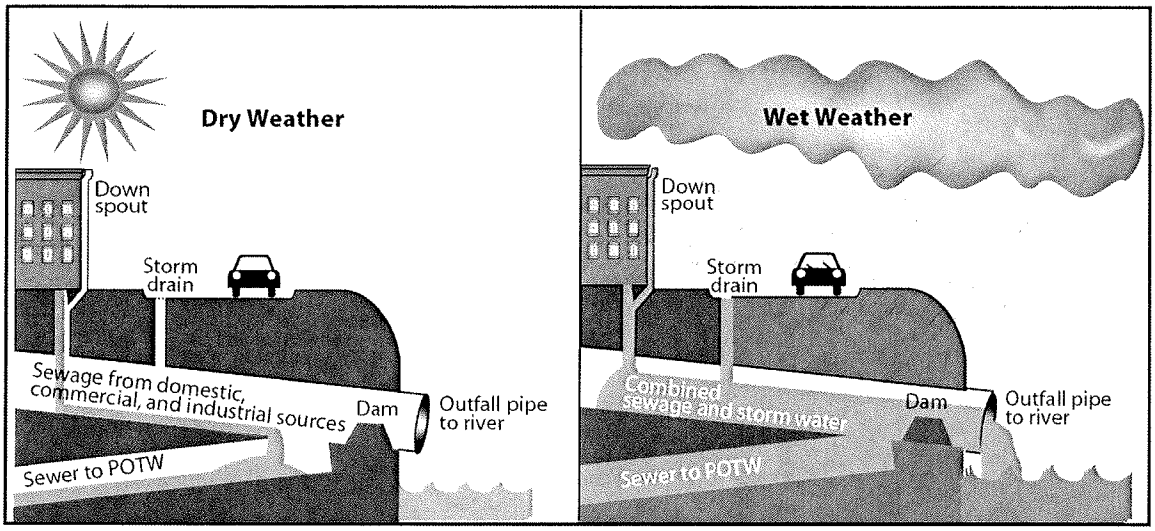

Figure 1. Sanitary sewers are linked to storm sewers in a combined sewer system, which causes overflows to occur in periods of heavy rain (USEPA 2004).

The concept of combined sewers was introduced in 1855 (Tibbets 2005). Prior to that date, all waste was dumped raw and unprocessed into local waterways. Eventually, treatment plants were developed to process the wastewater before releasing it into a nearby stream or river. The combined sewer system allowed the street runoff to be treated along with household wastewater, instead of having a separate pipe for street

runoff that would drain into a natural waterway untreated. However, precipitation can render the combined sewer system ineffective in its channeling of street runoff to treatment plants. As little precipitation as one-twentieth of an inch can cause overflow from combined sewer systems (Plumb 2008). Currently, 40 million people in 32 states live in cities with combined sewers; 772 communities in the United States use combined sewer systems, most of which are in the Northeast and Midwest (Tibbets 2005). The United States Environmental Protection Agency (EPA) estimates that 850 billion gallons of sewage are discharged from CSOs every year nationwide (USEPA 2004).

Enterococci Bacteria and Disease

The presence of raw sewage, as well as the pathogenic organisms it contains, is indicated by various bacteria. *Enterococci* are found in the intestines of humans and other mammals in numbers as high as 108 colony forming units (CFUs) per gram of feces (Tendolkar et al. 2003). Though *Enterococci* are not pathogens, they are strong indicators of pathogens that cause gastrointestinal disease in humans. For this reason *Enterococci* are used as the federal standard for water quality in marine recreational waters such as the Lower Hudson River.

The presence of *Enterococci* bacteria indicates the presence of fecal matter and therefore the presence of pathogens like *E. coli* 0157:H7. *E. coli* 0157:H7 infects the body and causes gastrointestinal ailments such as diarrhea (New York State Department of Health 2006). Contact with the skin or an open wound, or ingestion of contaminated water, can cause various physical ailments from diarrhea to vomiting to blood infections or deep tissue infections in extreme cases. Submersion in water exposes swimmers to toxins, pathogens and irritants that can enter the body through any orifice and may irritate

the skin. Any toxins in the water can be absorbed by the skin through dermal sorption, which can result in the rapid dissemination of toxins throughout the body, causing immune suppression or increased toxicity (Henrickson et al. 2001).

Recreational water illnesses (RWIs) or swimmer illnesses are most dangerous for people with weak immune systems, such as pregnant women, children, and the elderly. This demographic currently accounts for 20-25% of the total U.S. population (Dorfman 2004). Cryptosporidium, or "Crypto", is one type of fecal bacterium which can be life-threatening to individuals with weakened immune systems (CDC 2007). Harmful impacts on human health are not the only potential consequences of fecal contamination in water used for recreational purposes. There are also potential economic costs associated with illnesses caused by polluted water. Sickness can result in loss of time at work plus the cost of medical care. In one study, the economic cost of dealing with gastrointestinal disease in one person was found to be $36.58 per day (Dwight et al. 2005).

Water Quality Standards

In 2004 the EPA altered its federal recreational water quality standards and recommended that states use concentrations of *Enterococci* as a standard in marine and estuarine waters. This change occurred because of epidemiological studies that showed a much higher correlation between swimmer illness and *Enterococci* than between swimmer illness and fecal coliform, the previously recommended standard (Dufour 1984). The presence of excessive amounts of *Enterococci* in recreational waters, as measured by local governments, serves as a parameter for regulating beach closures and issuing public advisories.

The New York City Beach Monitoring and Surveillance Program, run by the Office of Public Health Engineering, routinely checks *Enterococci* levels at New York City swimming beaches (Office of Public Health Engineering 2006). When measurements are found to exceed standards officials issue advisories or close beaches, depending on the level of bacteria found. In the United States from 1988-1994 25% of the 12,000 beach closings were due to microbial contamination (Barton and Fuller 1995). There are no designated public beaches near the New York City study site for this project; the Hudson River at 125th St. is not regularly monitored for bacteria.

The Helicopter Monitoring Report has collected *Enterococci* data in the New York Bight since 1996 (EPA 2005). Samples are collected from 26 sites along the Long Island coast and 44 sites in New Jersey. The New York Bight does not include the area of the Hudson River sampled in this study, but the data from the Helicopter Monitoring Report show very few samples exceeding the EPA maximum of 104 CFUs/100 ml.

Regulations have helped to restore overall water quality in the Hudson River due to the improvement of wastewater treatment processes. The Clean Water Act implemented strict standards for wastewater treatment plants, mandating that plants perform at least secondary treatment. New York City produces about 1.4 billion gallons of wastewater per day (NYCDEP, 2005). All of this wastewater is transported to one of 14 sewage treatment plants throughout New York City. At these plants, the process of treatment begins. The wastewater treatment process currently involves settling (primary treatment), aeration or activated sludge process (secondary treatment), disinfection through chlorination, and the dewatering of sludge. In 1968, treatment plants removed only 65% of all pollutants (NYCDEP, 2005). The current process is designed to remove

90% of pollutants from the wastewater, a mandate of the Clean Water Act of 1972. We will assess the effectiveness of the current process at several recreational sites and determine factors that may contribute to elevated levels of *Enterococci*.

METHODS

Sampling

The majority of water samples were collected from two main study sites in the Hudson River: West 125th Street in Manhattan and the Piermont Pier in Piermont, NY. Values for rainfall were recorded based on proximity of the precipitation data collection stations provided by weather resources to the sampling points. For Harlem, precipitation data from Central Park, NYC was used; for Piermont, precipitation data from Woodcliff Lake, NJ was used.

Harlem

The main area of study is near the construction site for what will soon become the Harlem Piers; the latest projected completion date for the development is spring 2008. The shore is rocky and sloping, and serves as a northern starting point of a biking/walking/running path along the Hudson. A combined sewer discharge point, under the bulkhead where construction is underway, releases raw sewage into the Hudson during heavy rain events.

Data was collected from the Harlem sampling site in June 2007 and August 2007. Samples in August 2007 also have correlating salinity and temperature data, but these data were not collected earlier in the summer due to a lack of proper equipment. Records of daily precipitation were kept for reference in relation to understanding combined sewer overflow, as posted on the Weather Underground website from the location closest to the

sampling site. Multiple sample plates were created from each water sample in order to create duplicate filtrations for statistical significance of the data. In addition to collecting from shore, samples were also obtained from the Riverkeeper research vessel.

Piermont

Samples were collected throughout summer 2007 from three different points off the Piermont Pier and from two locations in a tributary of the Hudson River, the Sparkill Creek (Figure 4). More frequent sampling occurred from the end of pier site and from the Sparkill site below the dam. Temperature and salinity data were also collected at the point of sampling and a few samples also have correlated oxygen levels.

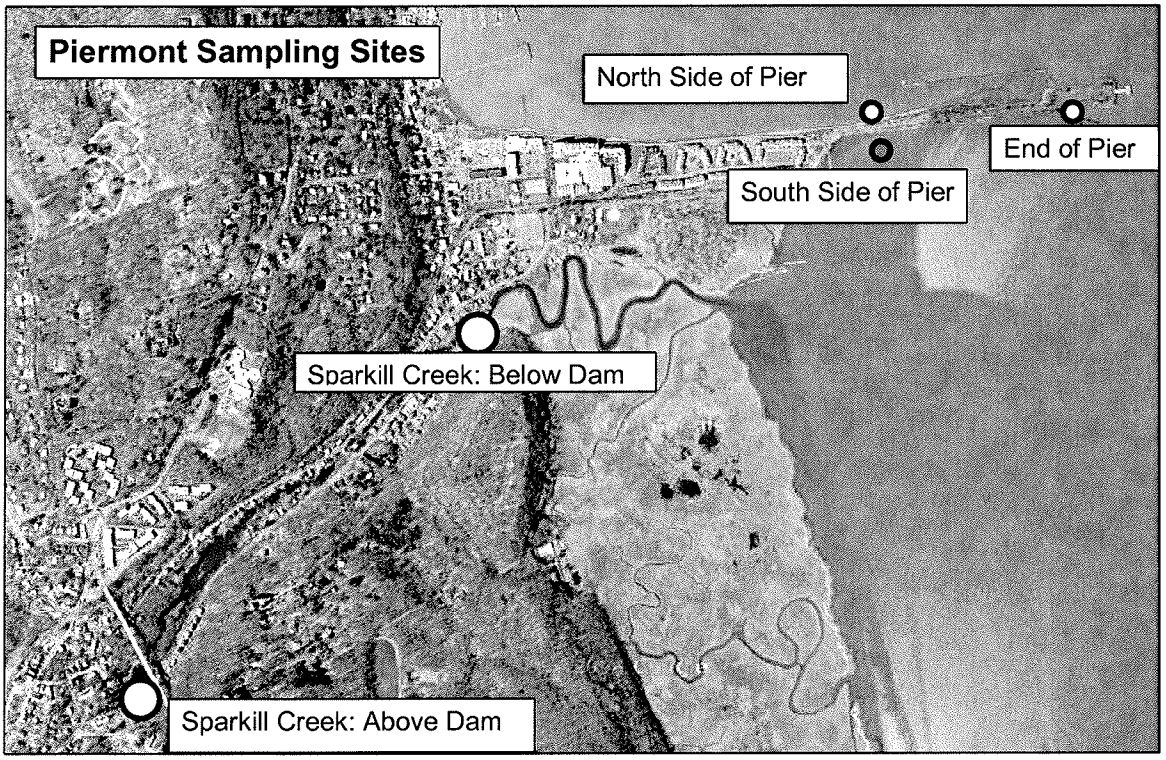

Figure 2. Sites in Piermont, NY that were sampled throughout summer 2007.

Procedure

The procedure for membrane filtration was performed according to EPA Method 1600: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus Indoxyl- D-Glucoside Agar (mEI) (USEPA 2002). The EPA sampling protocol calls for the preparation of agar from a dried medium. As an alternative to this method, pre-made mEI agar plates ordered from a scientific supply outlet were used for samples processed during spring 2007.

Samples were diluted according to the EPA protocol, including a buffer-only dilution to ensure the sterility of the equipment. We deviated slightly from the protocol to account for various levels of bacteria in our water samples. For example, on a heavy rain day when counts were expected to be high, a 100-ml river water sample would be somewhat useless because colonies would be too numerous to count. In this case, a 10 ml or 30 ml sample would be more appropriate and readable.

Sampling from the Riverkeeper research vessel occurred four times between April and July. Samples were taken on East-West transects across the Hudson River near the 125th Street outfall and also at the 79th Street Boat Basin and near 23rd St. on the East River. As with all the samples, records of tidal patterns and rainfall were kept.

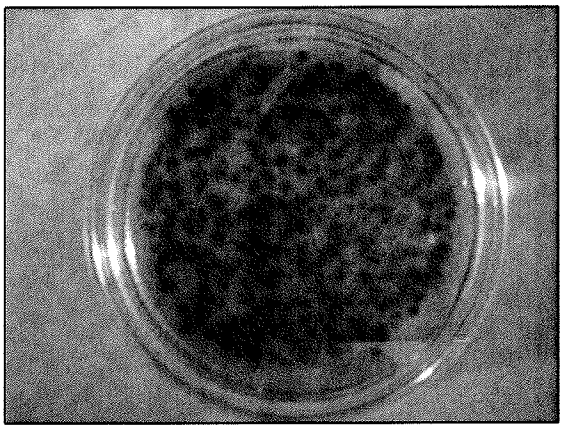

Figure 3. Agar plate after incubation: Blue colonies indicate *Enterococci* bacteria (colony forming units, or CFUs). Above image shows high concentration of bacteria from an undiluted sample of Hudson River water.

After the incubation period, the plates were removed from the incubator and colonies were counted using a dissecting microscope. Colonies which had a blue halo were identified as *Enterococci* colonies (see Figure 3, above). Concentrations were expressed in colony forming units (CFUs) per 100 ml.

RESULTS

125th Street

The values for samples taken at 125th Street (Figure 4) show increased levels of bacteria coinciding with rain events. When more than one inch of rain fell on June 3, 2007, the measured bacteria increased. The high levels were sustained for two days after the rainfall, and bacteria counts returned to below standards on June 6, 2007. The distinct peak in the graph in Figure 4 coincides with the rainfall amount and the bacteria count, which demonstrates the correlation between the two.

Samples were also collected from street drains in order to assess the concentration of these inputs and how they may affect overall concentrations at the sample site. See

Figures 5 and 6 for images of the sites where street drainage was tested and images of the agar plates with bacteria growth from those sites. Concentrations filtered at the lowest dilution for both sites were well above the EPA maximum, greater than 1500 colony forming units per 100 ml.

The East-West transect from the Riverkeeper patrol boat was sampled twice: once in May and once in July. The July transect was performed during a rainfall, and all values were high, whereas the May transect was sampled during dry conditions did not produce any results with high concentrations.

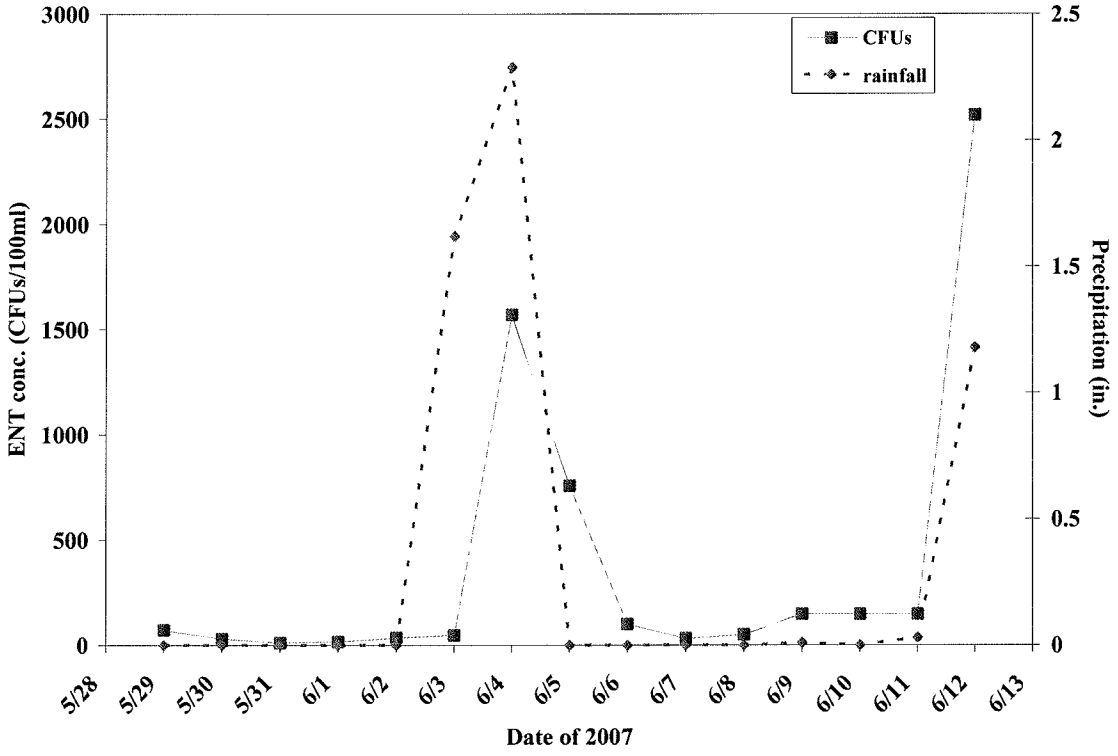

Figure 4. Samples at 125th Street were collected daily between May 29, 2007 and June 12, 2007. Rainfall records indicated above were measured from Central Park monitoring station. Results show significant spikes in *Enterococci* during periods of rain and relatively low concentrations during dry periods.

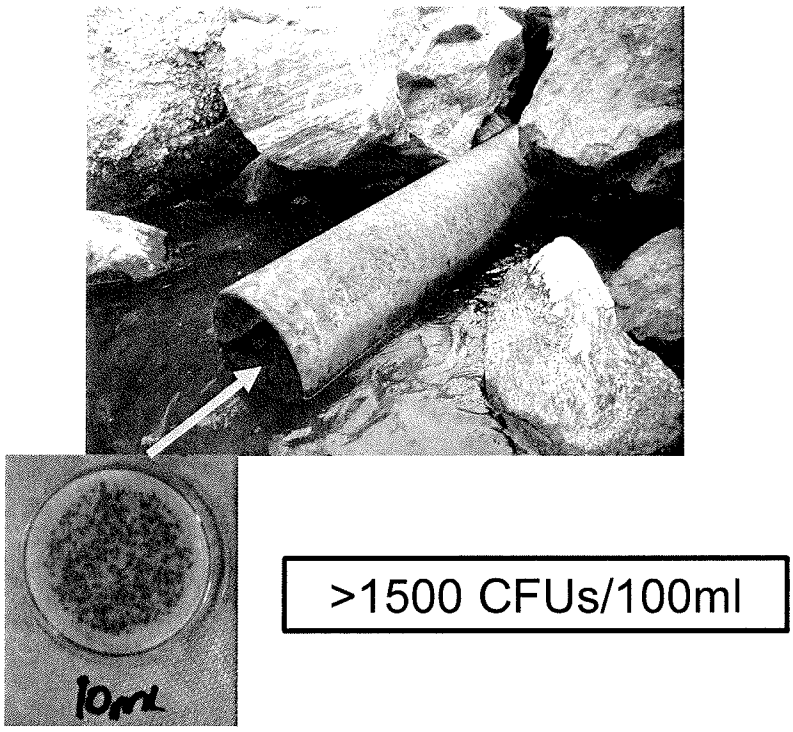

Figure 5. Sample was collected from pipe believed to contain street runoff, emptying directly into Hudson River near sampling site at 125th St. This outflow contained extremely high concentrations of *Enterococci*, exceeding 1500 CFUs/100ml.

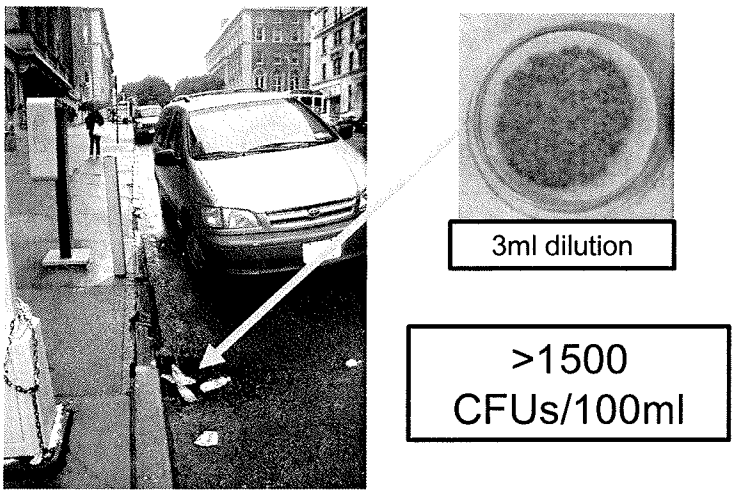

Figure 6. During rainfall, a water sample was collected from a street drain near 116th St. and Broadway which drains to the Hudson River. Concentrations were extremely high, exceeding 1500 CFUs/100ml: Image of filter shows saturation at the lowest dilution.

Piermont Pier

Samples from the Piermont Pier did not correlate directly with rainfall (Figure 7). The highest concentration at the pier, 650 CFUs/100ml, was measured two days after a heavy rain, but bacteria counts for a sample taken 3 days after heavier precipitation resulted in only 200 CFUs/100ml. The day after 0.6 inches of precipitation fell on 7/18/07, no bacteria was detected in the water sample taken on 7/19/07. The day following another major rain event on 7/23/07, bacteria detected was within standards (50 CFUs/100ml). We hypothesized that there would be a direct correlation between rainfall and bacteria measurements, but based on the rejection of this hypothesis, we suggest that factors other than rainfall can also contribute to the fluctuations in *Enterococci* concentrations.

We tested water samples from above and below a dam in the Sparkill Creek, with the hypothesis that a lack of mixing above the dam would contribute to higher bacteria concentrations. We were unable to confirm this hypothesis, but average results did show higher concentrations above the dam (Figure 8).

The Orangetown plant effluent, an input we believed could be detected from the South side of the pier (Figure 9), proved to be highly contaminated. Rainfall did not directly coincide with bacteria measurements, and concentrations in these samples were consistently higher than those measured at 125th Street in Harlem. A paired t-test was performed to test the statistical significance of the difference, and based on the p-value we concluded that the samples taken from the river on the South side of the pier, closer to the plant, were higher than those taken on the North side of the pier (Figure 10).

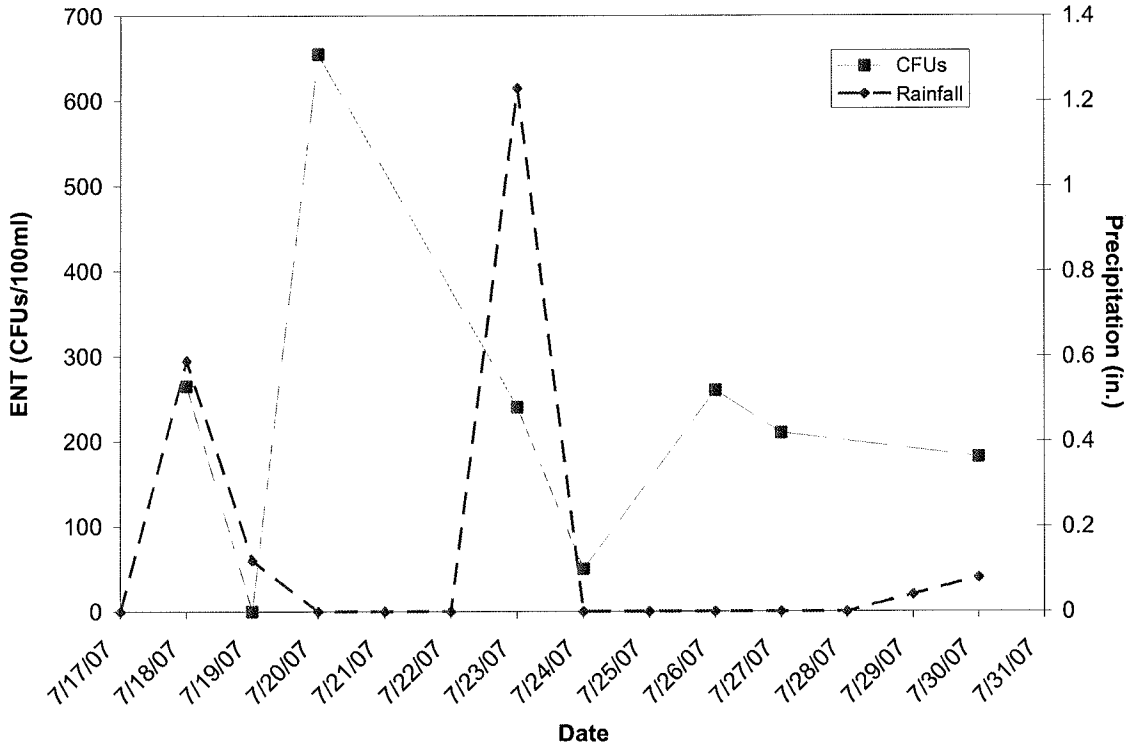

Figure 7. Samples from July 2007 compared with rainfall as measured in Woodcliff Lake, NJ (the closest monitoring station to Piermont, NY).

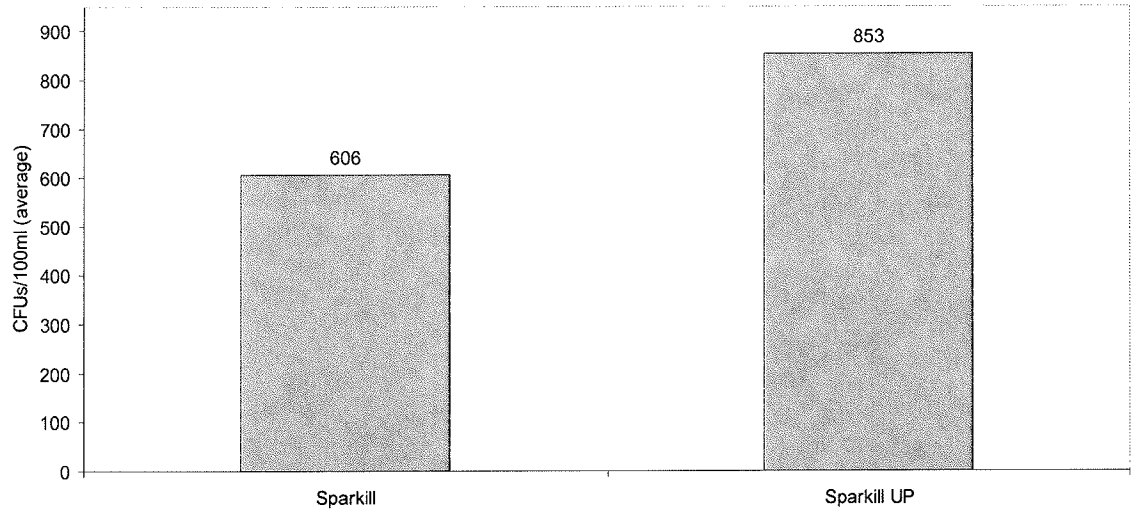

Figure 8. Geometric means from Sparkill Creek below the dam and Sparkill Creek above the dam ("Sparkill UP") from July 2007 show a difference in *Enterococci* concentrations, though not statistically significant (p-value=0.18). Water from above the dam may be susceptible to more inputs via leaky septic tanks.

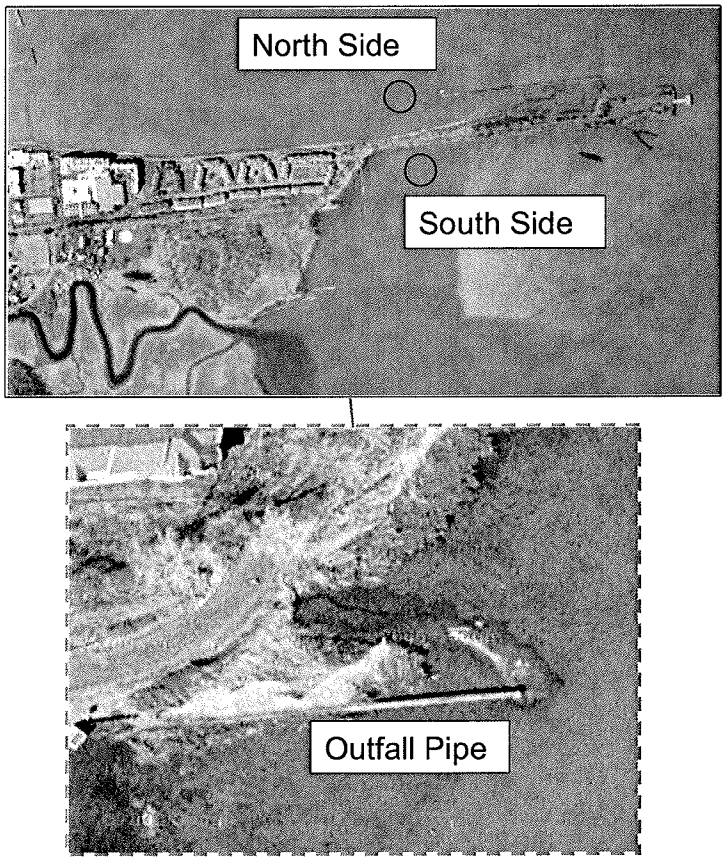

Figure 9. Sampling sites on the North and South side of Piermont pier. Location of outfall pipe on the south side is enlarged.

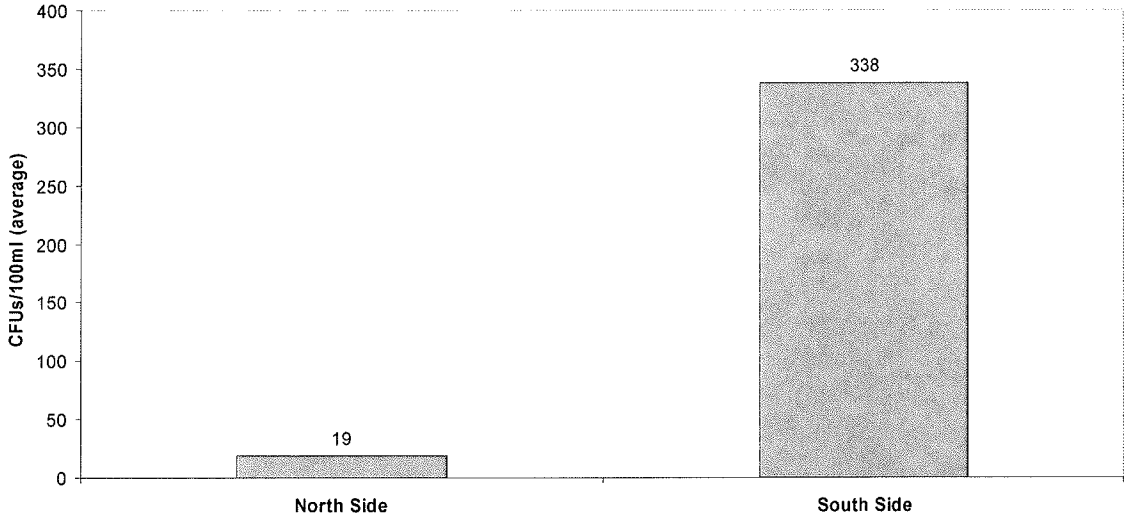

Figure 10. Comparisons for samples collected north of the pier versus south of the pier show a statistically significant difference, p-value = 0.007.

DISCUSSION

125th Street/ Harlem Piers

Heavy rain caused combined sewer overflow that resulted in increased levels of *Enterococci*. The street drain samples indicate that eliminating combined sewers would not effectively solve the problem of contaminants entering the Hudson River. Untreated street drainage also contains fecal contamination and these inputs are designed to flow directly into the river under separate sewer systems.

The development of the Harlem Piers is an exciting and beneficial addition to the neighborhood, an area which lacks any acceptable waterfront access and which consists of a predominantly minority population. However, the water quality of the Hudson River in this location needs to be addressed if the public are going to be encouraged to use and enjoy the river at this waterfront park. In particular, the street drain samples taken from 116th and 125th Streets indicate that eliminating combined sewer would not effectively solve the problem of contaminants entering the Hudson River. Untreated street drainage also contains fecal contamination and these inputs are designed to flow directly into the river under separate sewer systems. In addition, samples from the river transects near 125th Street indicate that rainfall leads to contamination not only on shore, but also in adjacent parts of the Hudson River.

Piermont Samples

The fact that samples collected from Piermont, NY had a much lower concentration of *Enterococci* relative to samples from Harlem and the adjacent part of the Hudson River suggests that upgraded and fully-functioning wastewater treatment plants can be effective the prevention and control of fecal contamination. The fact that *Enterococci* concentrations in the Sparkill Creek were consistently higher above the dam

where the creek flows through residential areas suggests that regulation and monitoring of septic tanks on private properties can also prevent and control fecal contamination.

Additional Sources of Fecal Pollution

In addition to discharges of raw sewage from combined sewer overflows, street drains and improperly sealed septic tanks, there are a number of other sources of fecal bacteria in the Hudson River. Boat basins could contribute to water pollution through illegal dumping of human waste from boats. Bacteria can also remain active in intertidal sediments and be re-suspended in the water column. Sediment polluted with litter, biological waste, and various toxins and chemicals can enter the river during rainstorms. Catch basins designed to capture and store polluted water and sediments are often used but can not capture 100% of these contaminants. The shore of the site near West 125th Street is rocky and has a mild decline towards the river, allowing waste from the path and rocks above to get into the river. Tides and currents can then transport contaminants to other locations.

Previous Studies

Previous studies have found high levels of fecal bacteria in other areas of the Hudson River and New York Bight. These high levels of fecal bacteria are correlated with rain events. The River Project monitored *Enterococci* at Pier 26 in Lower Manhattan and obtained results showing strong correlation between rainfall and high bacteria counts (Rosa 2002). The location sampled by the River Project lacks a number of contaminant sources that are found in our sampling site in Harlem. However, the study did determine that after heavy rainfall, *Enterococci* counts increased to levels exceeding the EPA maximum. Results showed a high positive correlation between

rainfall and *Enterococci* from three sampling points and indicated that raw sewage was being discharged into the sampling area (Rosa 2002). The study also found that when rainfall was below $1/6^{th}$ of an inch, *Enterococci* counts fell to within the EPA standards (Rosa 2002).

A study conducted in the surf zone of Huntington Beach, California found a number of factors that could affect fecal bacteria measurements (Boehm et al. 2002). Though the Hudson River estuary is not a surf zone, it does experience tides, which Boehm et al (2002) found to be highly correlated with bacterial concentration. Not only tides but also time of day that samples were collected was a factor in bacterial concentrations found (2002). Early morning sampling was recommended to obtain the most accurate counts because irradiation can destroy the bacteria. Boehm et al. (2002) also attributed variation in *Enterococci* concentration to seasonal changes, El Niño events, and changes in wastewater treatment and disposal. In addition to seasonal changes and changes in wastewater treatment, the Hudson River has the added complexity of mixed salt and freshwater that could affect *Enterococci* concentrations.

RECOMMENDATIONS

This study provided a small glimpse of the high bacterial contamination near 125th Street, and sampling was not consistent and extensive. In order to make conclusions with confidence, more data should be collected at the same site, with specificity as to time and tidal patterns. In addition data on salinity, exposure to UV light, grazing communities, sediment attachment and tributary inputs should be collected.

Daily sampling is recommended and, when possible, multiple samplings per day at the main study area.

Mitigating the Impacts of Combined Sewer Overflow

The future Harlem Piers are currently under construction. Warning signs should be placed near the waterfront once construction is complete, informing the public of the presence of raw sewage in the area. Kayaking and other water activities should not be encouraged and those who desire to fish from the piers should be sufficiently warned about the raw sewage in which the fish may be swimming while researchers work to find solutions for this problem.

There are two main approaches to solving the problem of combined sewer overflow. One approach is to collect the mixed sewage and storm water runoff in a holding tank and process the excess as soon as the wet weather passes. The DEP and DEC are currently in the process of evaluating plans for every waterbody/watershed in New York City and determining the most cost-effective methods for curbing CSOs.

The alternative approach involves using rainwater as a resource, rather than disposing of it as a waste product. Sustainable alternatives to decrease combined sewer outflow include: greenroofs, green streets, green trees, rain barrels and porous sidewalks. In addition to addressing combined sewer overflow, these methods also serve to counteract the warming effect of urbanization, also known as the urban heat island effect. Streets and pavement tend to absorb sunlight rather than reflect it, causing city temperatures to be an average of 0.5 to 4.0° C warmer than immediately surrounding areas (Alexander and Fairbridge 1999). Policies which fall under the umbrella of the

"alternative" approach may prove to be more sustainable and cost-effective with further research.

Further research should investigate the possible mitigating effects of sustainable practices suggested by Riverkeeper, especially in light of Mayor Bloomberg's "PLANYC". The plan sets a goal to reduce combined sewer outflow by 185 million gallons a day by 2030 (PLANYC, www.nyc.gov/2030). Tentative plans include providing incentives for greenroof installations and new design guidelines for "green" parking lots.

The data presented in this study indicate that the study sites in and near Harlem are in violation of EPA standards for recreational marine water quality. Further action may be taken to contact the EPA or DEP and establish communication regarding these violations. Some possible solutions to decreasing contamination and its effects, given the established sources, include: enforcing proper boat waste disposal, improving the wastewater treatment system and sewer system, employing sustainable initiatives like green roofs, and modifying discharge of street runoff.

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EFFECTIVENESS OF RIPARIAN WETLANDS IN IMPROVING WATER QUALITY IN AN URBAN STREAM

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

Ecological function in urban riparian systems has been relatively little studied and little is known about the potential of these riparian systems to buffer the kinds and levels of contamination associated with urban ecosystems. We studied two riparian wetlands along the Patroon Creek in Albany, New York, a tributary of the Hudson River that is heavily impacted by urban development and carries substantial contamination loads. Our study was designed to assist planning for restoration projects in the watershed. We hypothesized that the water chemistry, bacteria counts, and macroinvertebrate communities would indicate an improvement in water quality as the stream passes through riparian wetlands. We sought to demonstrate trends by using sequential samples. Water samples were collected from above, below and twice within two sections of wetlands (a total of eight sampling sites) from June to September 2007. Multi-plate samplers to collect aquatic invertebrates were placed at the same sites. We analyzed the concentrations of major dissolved ions and bacteria (total coliform, heterotrophic plate count or HPC, and *Escherichia coli*) counts in stream water samples and evaluated the invertebrate communities for richness and diversity. Despite substantial variation between sites, we detected a reduction in ammonia and sulfate after contact with the wetlands. The aquatic macroinvertebrate communities, although quite heterogeneous in composition, showed no spatial trend, indicating that they may be responding to unmeasured factors. Bacteria data provided some evidence of riparian wetland function. An apparent increase in total coliform from upstream to downstream within the wetland sections was accompanied by a drop (n.s.) in *E.coli*. The high level of impervious

surface and the numerous culverts and overland flow from commercial and industrial sites that bypass these buffers may limit function that is observable.

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INTRODUCTION

Only 2% of the river kilometers in the U.S. are free of human impact (Palmer et al. 2007). The extensiveness of the human impact on rivers and streams has led to a deluge of restoration activity, with expenditures estimated at over $1 billion per year (Palmer et al. 2007). Yet a synthesis of river and stream restoration practice found that the ecological effectiveness of restoration projects is inadequately documented and that scientific knowledge is poorly translated into restoration practice (Bernhardt et al. 2007). Our study in the Patroon Creek Watershed, Albany, NY was designed to assist in restoration planning that integrates ecological information and restoration goals at the earliest stages.

Urban stream ecosystems are typically degraded in both ecological function and biodiversity. Riparian buffer creation and maintenance is one of the most widespread activities in stream restoration and the most effective stream restoration programs have focused on improvements to stream banks and floodplains (Roy et al. 2006). The challenge of planning restoration for urban streams is compounded by the dearth of information on riparian buffer function in an urban context (Groffman et al. 2003).

Studies of riparian ecosystem function have concentrated on agricultural and forested watersheds (Groffman et al. 2003) and the impacts of urbanization on stream ecology have received less attention, although this is changing (Paul and Meyer 2001). In rural stream ecosystems it has been shown that riparian zones filter and transform a number of contaminants present in water thus enhancing water quality (Castelle et al. 1994, Clausen et al. 2000, Correll 2000). Riparian areas can serve as buffers by removing soluble nutrients and trapping or releasing suspended sediments (Correll 2000). Restoration projects have improved water quality and habitat in urban streams (for example, Charbonneau and Resh 1992). The lack of information on urban riparian buffers makes it difficult to set realistic ecological goals for restoring urban streams.

Proposals for a multi-use trail and potential restoration activities have been in progress for over a decade in the Patroon Creek watershed. Our objective was two-fold: to increase the understanding of remnant wetland function in an urban stream and to establish ecological information to assist in restoration planning. The focus on function in an urban stream and the connection to restoration planning sets this work apart from

previous published work. Our results should be of interest to restoration planners in other watersheds as well as those studying urban stream ecosystems. We sought to evaluate the ecological function of remnant riparian wetlands along the Patroon Creek in Albany, NY. Evidence of improved water quality downstream of the wetlands would support restoration activities that enhance or expand remnant wetlands along the creek. We hypothesized that water quality would improve as water passes through the remnant wetlands. Specifically, after passing through the remnant wetland zones, measurable changes from upstream to downstream would be found in 1) biotic indicators (indices derived from benthic macroinvertebrate composition), 2) levels of total coliform (TC), *E.coli* and heterotrophic plate count (HPC), and 3) water chemistry (major dissolved ions), dissolved oxygen, and conductivity.

METHODS

Site description

A heavy influence of urbanization is evident in the Patroon Creek, which flows 10 km from its headwaters in the Albany Pine Bush to join the Hudson River through a culvert in Albany's Corning Preserve. For about one-third of its length, the creek drains an unconfined aquifer of fine, deep post-glacial dune sands (Landry and Rosenberg 1976). For much of the remainder, the creek is channelized, deeply incised or otherwise out of contact with its natural floodplain, riparian soils and vegetation. The stream channel was altered by the creation of reservoirs for drinking water in the 19th century and about one-third of the stream was moved and/or buried in culverts to make way for railroad and highway transportation routes in the 19th or 20th centuries.

The watershed today is characterized by urban and suburban land use, with approximately 37% of the surface area occupied by impervious surfaces which are mainly roads, parking lots, and roofs (Audette 2004). Studies in other watersheds have shown alterations in a number of features with increasing impervious surface cover, including stream hydrology, biology, geomorphology (stream channel form) and physical characteristics (water temperature) (Paul and Meyer 2001). Significant sources of contamination in the Patroon Creek watershed include stormwater, leaking sanitation

sewers, and an industrial zone that includes two federal Superfund sites. Earlier research indicates that concentrations of sodium and chloride are high year-round (Erickson 2004), and are higher relative to rural streams (Madden & Robinson 2004). The concentrations of these ions increase from upstream to downstream (Erickson 2004). However, the creek also passes through three nature preserves and a multi-use recreational trail running parallel to the creek has been proposed. In addition to concerns for wildlife and human health within the watershed, the creek pollutes the Hudson River Estuary without abatement.

We have assembled a 4.5 year record of background data on hydrology and water quality in the Patroon Creek watershed. Included are records from a stream gage installed by the US Geological Survey (USGS) in 2002, which makes real-time data available online (http://waterdata.usgs.gov/ny/nwis/current/?type=flow) and is maintained at one of our sampling sites on the creek. The Patroon Creek hydrograph is characterized by an extremely rapid rise in discharge at the onset of a storm event followed by a more gradual decrease (Figure 1). When road de-icing chemicals are applied during storms, there is a significant increase in specific conductance recorded at the gage (Arnason and Robinson 2006). In contrast, during storms when de-icing chemicals are not used (in warmer months) there is a significant decrease in conductance caused by dilution of base flow by precipitation. The inputs of surface runoff are significant flowing into the stream directly and through numerous culverts.

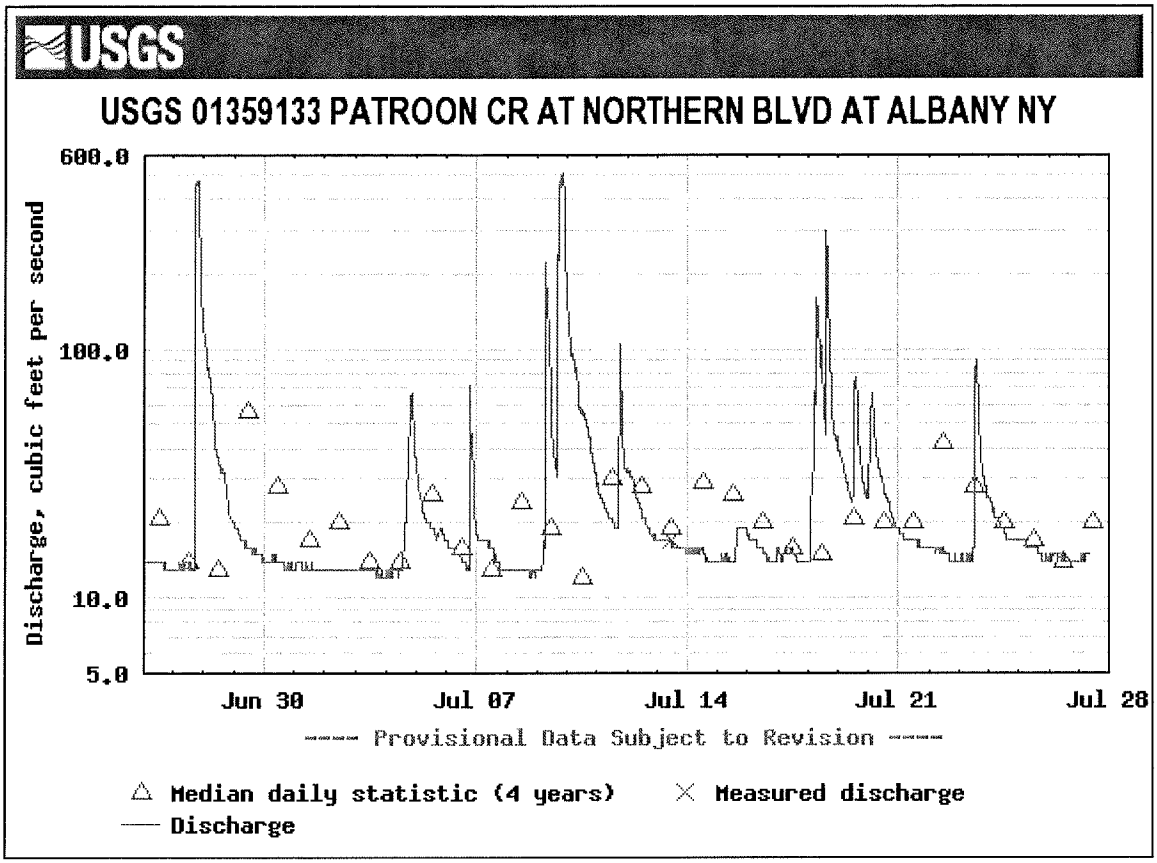

Figure 1. Patroon Creek discharge at the USGS stream gage, June-July 2007. Triangle symbols indicate means for four previous years.

For four years samples have been collected at seven sites along the Patroon Creek for coliform analysis [total coliform (TC), *E. coli*, and heterotrophic plate count (HPC) conducted by the laboratory of the Albany County Department of Health]. Although quite variable in time, there is a general trend toward higher values downstream (Figure 2). Means and maxima appear to be diminishing, but remain quite high (2005 TC mean plate counts = 18,000, maximum = 116,000; 2005 *E. coli* mean plate counts = 2,300, maximum = 2,000). At these levels, the creek may represent a public health hazard for community residents when in contact with the water.

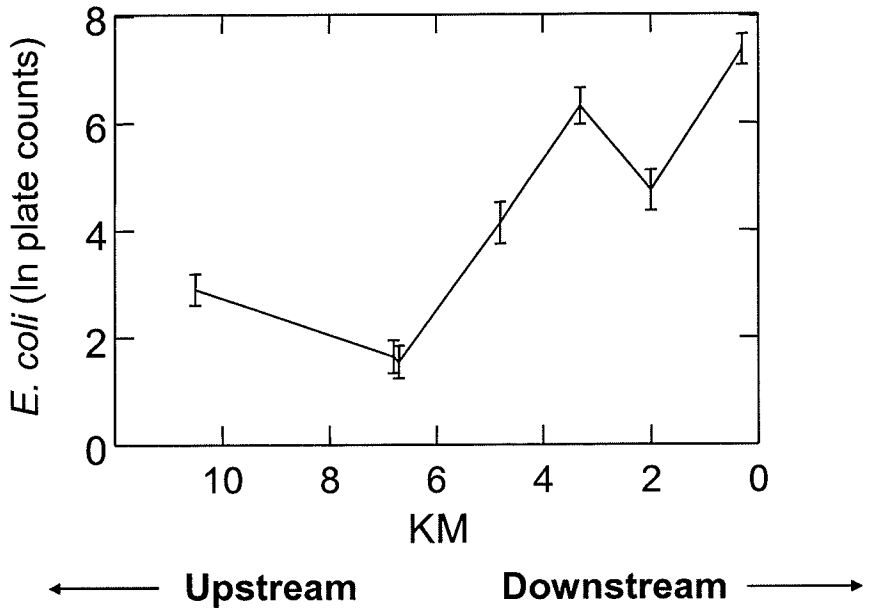

Figure 2. Counts of *E.coli*, means of monthly water samples taken from 2002-07.

The aquatic macrocinvertebrate community is an important indicator of stream water quality. Aquatic macroinvertebrate communities have been studied more than other communities in urban systems (Paul and Meyer 2001). The reduced diversity and abundance seen in urban systems has been correlated with the level of impervious surface and other features of urbanization (Moore and Palmer 2005, studies summarized in Paul and Meyer 2001). Previous biological assessments by Audette (2004) of the Patroon Creek using benthic macroinvertebrates have categorized the water quality as moderately to severely impacted, matching or exceeding those of 1994 New York State Department of Environmental Conservation studies (Bode et al. 2002). However, when comparing sites above and below remnant riparian zones, the indices showed increases in water quality. In addition, when plotted against the amount of upstream impervious surface, the biotic indicators rose indicating substantially "cleaner" water (Audette 2004).

The present study was prompted by previous research (Audette 2004) regarding two remnant wetland areas around Patroon Creek which extend more than 1 km along the stream channel. This earlier work focused on sites above and below the remnant wetland areas. Chemical assessments had shown that mean concentrations of major ions are typical of urban streams, with moderate nutrient levels and high concentrations of sodium

and chloride. All major ions were lower downstream of the two remnant riparian areas. Significant reductions were found in concentrations of nitrate, sodium, calcium and potassium. Controls (samples taken at each end of an underground culvert flow) showed no changes in concentrations (Audette 2004).

Water chemistry and bacteria study

In this study, we sought to repeat previous tests, adding intermediate sampling points within the riparian zones in an attempt to establish rates of change. Sampling was conducted at eight sites. Sites were established above and below the remnant wetland sections plus two sites within each section. Sampling sites U1-U4 are located in section 1, and D1-D4 are in section 2, moving from Upstream to Downstream in the creek. Two of the sampling sites (numbers D1 and D4) were at locations that had been sampled previously (Audette 2004), while the other four were newly selected for this study. A water sample was collected at each site for analysis of major dissolved anions and cations. A separate sample for bacteria analysis was collected at four of the sites, those above and below each remnant wetland zone. The bacteria analysis was conducted for this project by the Albany County Health Department under contract with the City of Albany. Because the number of sampling sites is limited by the sampling agreement between the County and the University we were precluded from sampling bacteria at all eight sites. Dissolved oxygen, temperature, specific conductance and pH were measured in the stream at each sample site when the water samples were collected.

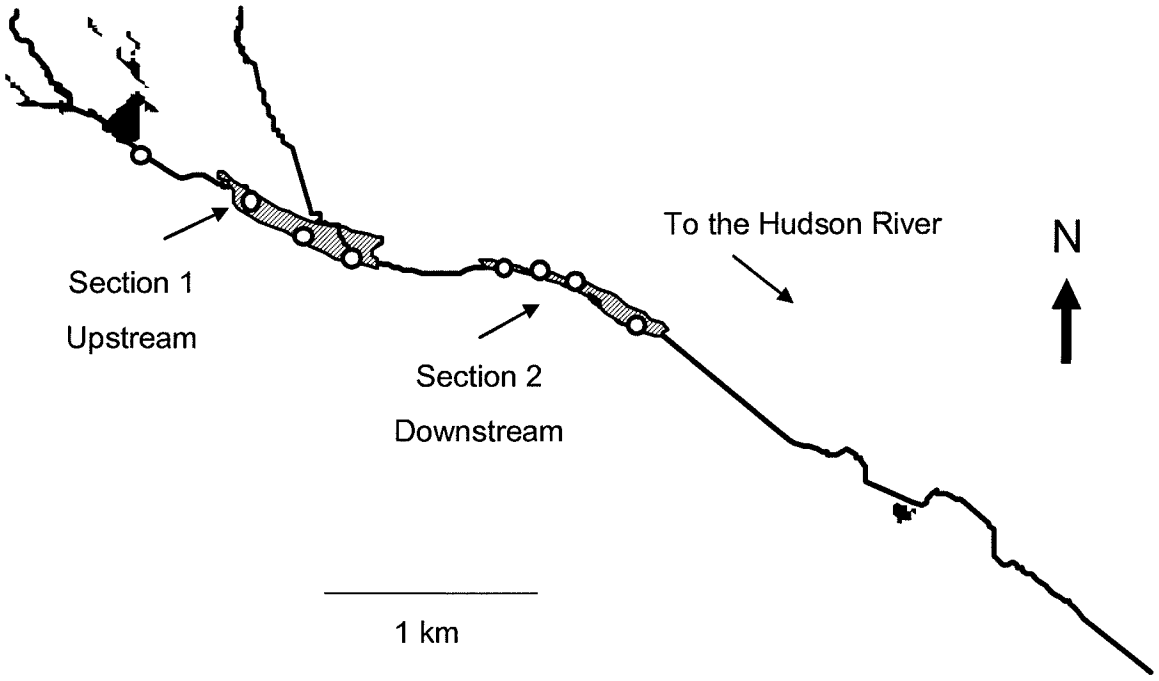

Figure 3. Study sections and eight sampling sites reported in this study along Patroon Creek.

Each site was sampled monthly from May to September 2007 during the second week of the month. Grab samples were taken in sterile Nalgene bottles, stored in a cooler during transportation to the lab, and refrigerated until analyzed. Five milliliters of each sample was filtered and analyzed in an ion chromatograph (Dionex DX-120 and ICS- 90 systems run with Chromeleon™ software) for anion (Cl^-, NO_3^-, Fl^-, and SO_3^-) and cation (Na^+, Ca^{++}, Mg^{++}, and NH_4^+) concentrations.

Quality control for IC data

The level of detection (LOD) and level of quantification (LOQ) were calculated for each ion using the quantities detected in samples of deionized water and known standards (6 cation standard, 5 anion standard, 5 ion standard) that were analyzed along with the water samples. For fluoride, phosphate, and sulfate, values from previous runs with the same machines were used because these ions were either not detected or were seldom detected in the deionized water (DI) in our runs. The LOD and LOQ were calculated using the mean and standard deviations across the six different analysis runs. The water sample

ion data were adjusted using these LOD and LOQ. Other protocols followed US EPA Method 300.1.

Macroinvertebrate analysis

Samples were collected on multi-plates at each of the locations identified above. Multi-plates were used instead of kick sampling due to the nature of the substrate in the creek. The substrate is generally soft, mostly a mix of Pine Bush sand and clay to the west, changing to primarily clay bottom to the east, mixed with organic sediment and other sedimentary materials, natural and anthropogenic. Rocky substrate occurs in some locations, probably of artificial origin except where the creek has deeply incised and lost contact with older floodplains. The plates installed in August and were left in the water for five weeks or more. The last plates to be collected were retrieved eight weeks later (September 22). The samples were removed from the plates and processed following the guidelines in Bode et al. 2002. For each sampling site, half of the sample was analyzed, rather than one-quarter as called for by Bode et al. (2002). This was due to the relatively few organisms observed in the quarter samples at all locations. Because of this modified approach to the analysis, our results should not be used to directly compare the Patroon Creek to data reported for other streams.

For each sampling location, following Bode et al. (2002), the sample with the larger amount of sediment was analyzed, with one exception. The smaller sample for position 1 in the upstream section was analyzed, because the larger sample had to be discarded. The quantity of material analyzed was similar to the amounts for other sampling locations. Position 4 in the downstream section was over-sampled: one-fifth extra material was reviewed, so the data were adjusted for this sample by randomly eliminating one-fifth of the organisms.

For the few organisms that we were unable to identify beyond order, we grouped the organisms as "other" within the order. The "other" categories likely contain organisms from more than one taxon within an order. Rather than distinguish among them, we left them grouped, for example, as "other Ephemeroptera." This conservative approach underestimates the diversity of organisms.

Invertebrates were identified to Family using the pictorial key published by the New York State Department of Environmental Conservation and *A Guide to the Study of Fresh-water Biology* by Needham and Needham (1962) with assistance from staff in the DEC Stream Biomonitoring Unit. Family richness and Ephemeroptera, Plecoptera and Trichoptera (EPT) richness were calculated for each site (Bode et al. 2002). Invertebrate diversity among sample sites was compared using the Jaccard Index of similarity (based on presence-absence of the 14 taxa) and the Curtis-Bray Index (based on both shared composition and abundance) (Magurran 2004). In addition, we calculated site diversity scores using the Simpson Index (Magurran 2004). Matrices of Jaccard and Curtis-Bray similarity were also used to construct cluster diagrams (hierarchical clustering; SYSTAT 11©). The results of cluster analysis using the Curtis-Bray Index were virtually identical to those using the Jaccard Index, thus we report only the Jaccard results.

Statistical analyses

Analysis of variance (ANOVA) was used to test for statistically significant differences between mean values of all water chemistry and coliform measurements for pooled upstream and downstream sites for each of the two zones at α=0.05. For those zones with internal samples, regression was used to test for trends (linear or nonlinear, according to data shape). Because an earlier study (Erickson 2004) indicated that concentrations of chloride and sodium increase in the creek from upstream to downstream, both one-way and two-way tests were used. The two-way analysis was used to test the effect of the upstream versus downstream position of the wetland as well as the effect of site position within a section. The two factors for the two-way analysis were position reported in the tables as POS (within a wetland section, ranging from 1-4) and section reported as SEC (the upstream or downstream section).

RESULTS

Water quality analysis results

Two samples taken during September had nitrate values that were in excess of any previously reported values; these were treated as outliers and they are excluded from analyses in this report. Chloride varied significantly in time and space (Figure 4). Chloride was found to be highly correlated with specific conductance and sodium. The ratios of sodium to chloride and specific conductance to chloride are shown in Figure 5 and indicate that the primary source is sodium chloride (NaCl). The analysis of variance for chloride, by month and by section (section one is the remnant wetland located upstream and section two is further downstream) showed that chloride varied significantly between the two sections and among the months ($p < 0.001$). The dip in the value for July corresponds to high rainfall levels (Figure 1), which appear to contribute substantial variability to much of our data.

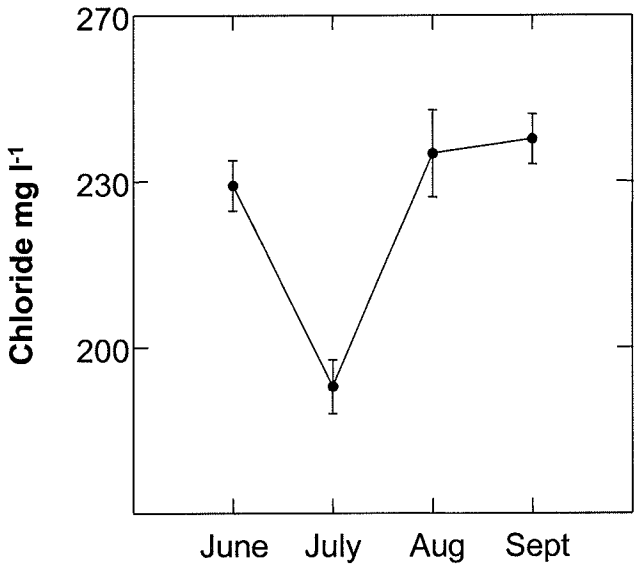

Figure 4. Chloride concentration, averages of eight sample sites, June – September.

Table 1. Two-way ANOVA table comparing chloride ion concentrations in laboratory water samples (Fig. 4). Starred results were significant at $p<0.05$.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	p
POS	482.320	3	160.773	0.145	0.932
SEC	6658.621	1	6658.621	5.992	0.024*
POS*SEC	1552.311	3	517.437	0.466	0.710
Error	21113.084	19	1111.215		

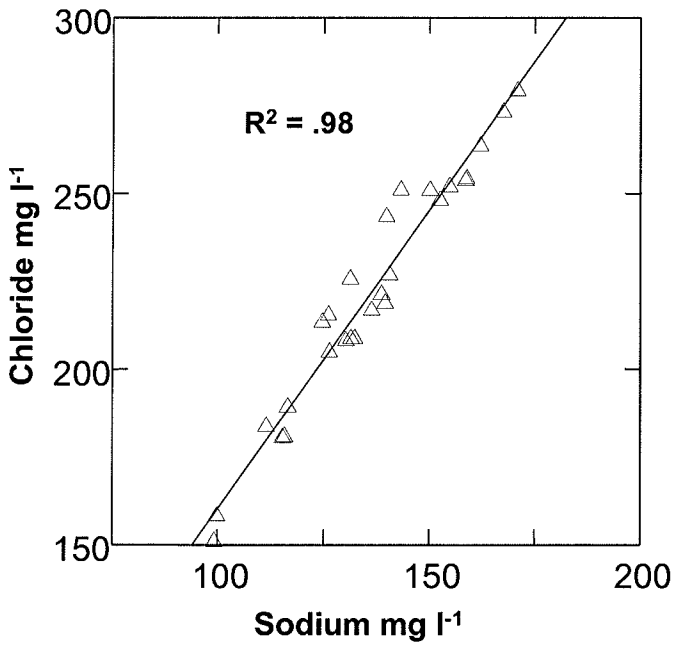

Figure 5. Ratio of concentration of chloride to sodium, averaging the monthly samples taken at the eight sampling sites.

Table 2. Pearson correlation matrix for major ions at all sample locations (N = 18). Starred values indicate significant Bonferroni probabilities ($p < .05$)

	FL	CL	NO3	SO3	NA	NH4	K	MG	CA
Fl	1.000								
Cl	0.327	1.000							
NO_3	0.107	0.222	1.000						
SO_3	0.465	-0.052	0.696*	1.000					
NA	0.277	0.990*	0.274	-0.015	1.000				
NH_4	0.109	-0.497	-0.139	0.238	-0.486	1.000			
K	0.003	0.256	0.454	0.160	0.282	0.245	1.000		
Mg	0.461	0.858*	0.319	0.273	0.865*	-0.238	0.206	1.000	
Ca	0.462	0.876*	0.234	0.204	0.885*	-0.274	0.146	0.939*	1.000

Specific conductance and chloride levels in the creek (averaged for the May to August samples) increase somewhat moving downstream, although the error bars are larger around the higher values (Figure 6).

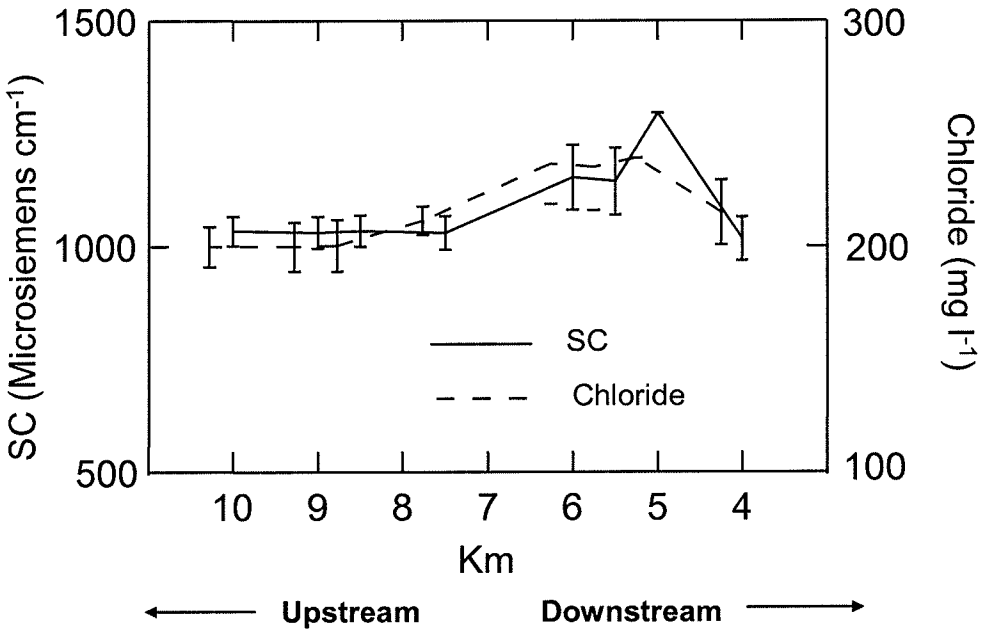

Figure 6. Specific conductance and chloride concentrations in Patroon Creek. Means of monthly samples taken at eight sites from May to August 2007.

Ammonium was not significantly different between zones (Table 3). Although higher mean ammonium values were returned for the most upstream position in the wetlands compared to the sampling point located furthest downstream (Figure 7). Sulfate declined with position and the difference between sections was statistically significant (Table 4).

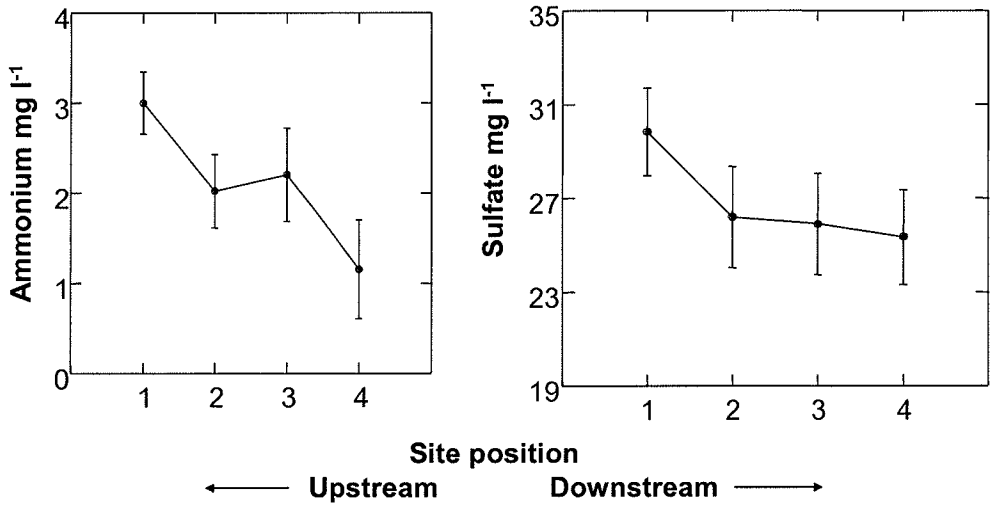

Figure 7. Variations in major ions, ammonium and sulfate, averaged for each wetland position (1-4) across the Upstream and Downstream sections.

Table 3. Two-way ANOVA table comparing ammonium ion concentrations in laboratory water samples (Fig. 7).

Source	Sum-of-Squares	df	Mean-Square	F-ratio	p
POS	7.250	3	2.417	3.012	0.076
SEC	2.411	1	2.411	3.004	0.111
POS*SEC	2.683	3	0.894	1.115	0.385
Error	8.827	11	0.802		

Table 4. Two-way ANOVA table comparing sulfate ion concentrations in laboratory water samples (Fig. 7). Starred results were significant at $p<0.05$.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	p
POS	94.303	3	31.434	1.125	0.364
SEC	188.848	1	188.848	6.756	0.018*
POS*SEC	49.158	3	16.386	0.586	0.631
Error	531.124	19	27.954		

Dissolved oxygen, pooled among the two sections, varied with position, showing a decline and then an increase at the most downstream sampling site (Figure 8). Mean dissolved oxygen was significantly different among positions (Table 5).

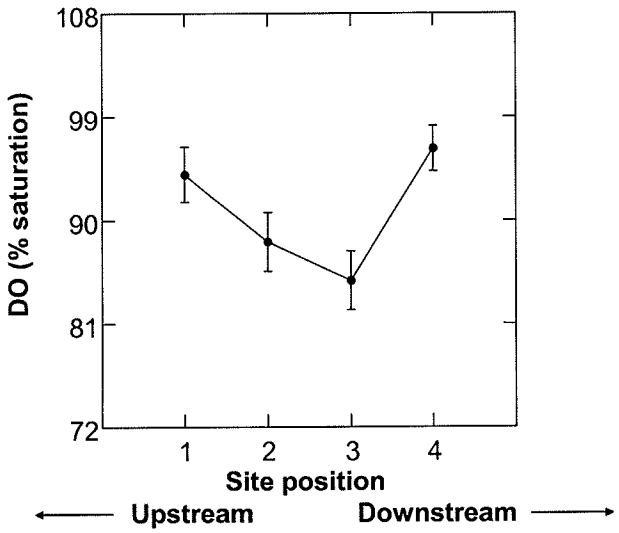

Figure 8. Variation in dissolved oxygen, values averaged for each position (1-4) in each of the two wetland sections pooled.

Table 5. Two-way ANOVA table comparing dissolved oxygen concentrations in field water samples (Fig. 8). Starred results were significant at $p<0.05$.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	p
POS	605.165	3	201.722	5.138	0.008*
SEC	90.484	1	90.484	2.305	0.144
POS*SEC	241.820	3	80.607	2.053	0.137
Error	824.508	21	39.262		

Macroinvertebrate analysis

The total number of organisms found at a sampling site ranged from 23 to 118. The number of families of aquatic macroinvertebrates found at each site varied from 2 to 9. No Plecoptera (stoneflies) were found at any site. Ephemeroptera and Trichoptera were found at six of the eight sites (U1-4, D1, D4). Few species of Ephemeroptera or Trichoptera were found; the highest EPT richness found was 3. A list of taxa is included in Table 5.

Family and EPT richness were greater in the upstream wetland than the downstream wetland. Moving downstream within the two wetland sections showed different patterns (Figures 9, 10). In the upstream section, the family richness was similar through positions 1-3, whereas it dropped slightly in position 4. In the downstream section, family richness dropped considerably moving to positions 2 and 3, and then increased by position 4. EPT richness showed the same pattern, none of these taxa were found in position 2 and 3 of section 2.

Table 6. Taxa of aquatic macroinvertebrates in multiplate samples, Aug-Sept 2007. Taxa marked with an asterisk were also found by Audette (2004).

Class	Order	Family	Genus
Crustacea	Isopoda*		
	Amphipoda*		
Insecta	Ephemeroptera	Baetidae	
		Unidentified	
	Diptera	Simuliidae* (black flies)	
		Chironomidae* (true midges)	
		Empididae (dance flies)	Hemerodromia
	Trichoptera	Hydropsychidae*	
	Coleoptera	Elmidae (riffle beetle)*	
Other Phyla	Gastropoda (snails)*		
	Annelida		
	Nemertea		
	Platyhelminthes		

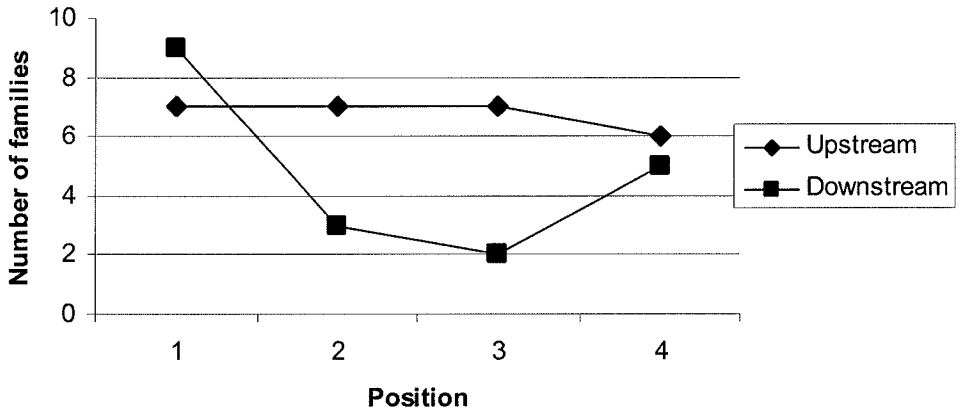

Figure 9: Family richness of aquatic macroinvertebrates for positions in the Upstream and Downstream wetland sections, moving from upstream to downstream (1-4).

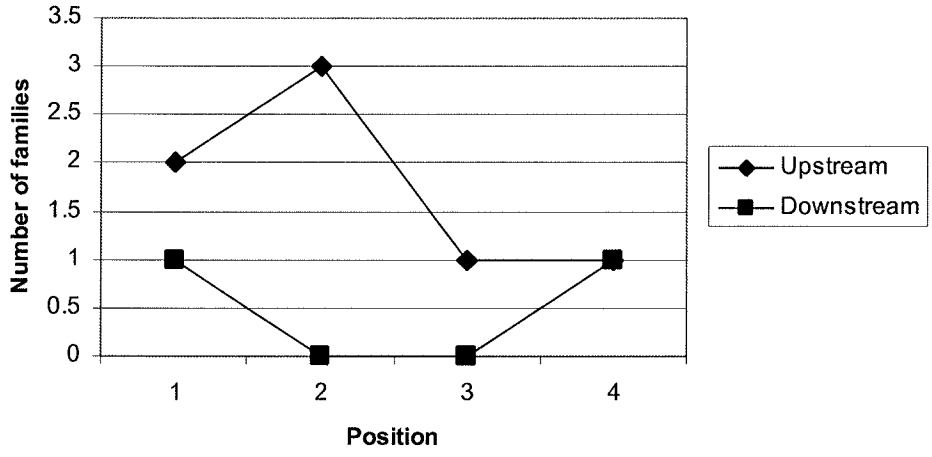

Figure 10: EPT richness for each wetland position in the Upstream and Downstream wetland sections, moving from upstream to downstream (1-4) within the sections.

The Simpson Index shows that the upstream wetland has a higher diversity than most locations within the downstream wetland (Figure 11). The diversity within the downstream section is lower within the wetland than at the entrance or furthest downstream point. Interestingly, the positions with the highest diversity are the most downstream point in the upstream section and the most upstream point in the downstream section.

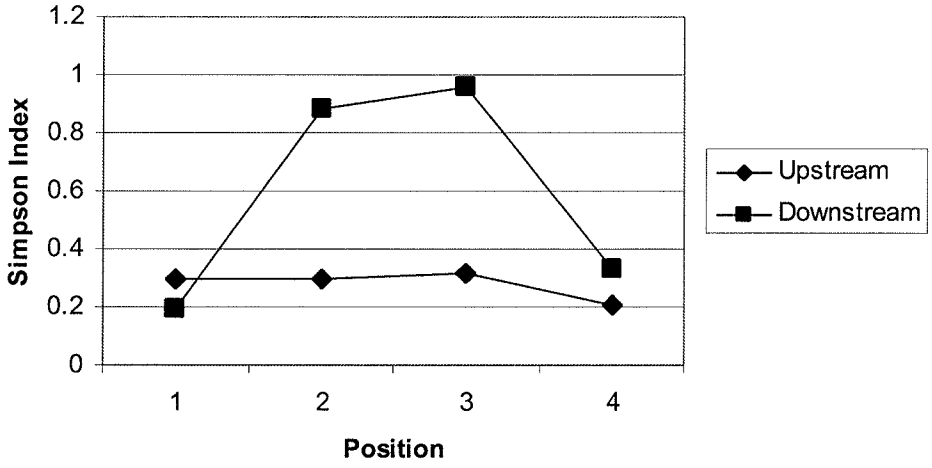

Figure 11: Simpson index calculated for each sampling site (position) in the Upstream and Downstream wetland sections.

The diversity and richness may be underestimated due to the effect of sampling because some of the sampling plates were partially covered with sediment. Clustering based on the Jaccard coefficients showed that the sites are different from each other, with values higher than 0.3 for all pairs (Figure 12). The most similar sites are positions 2 and 3 within the downstream section.

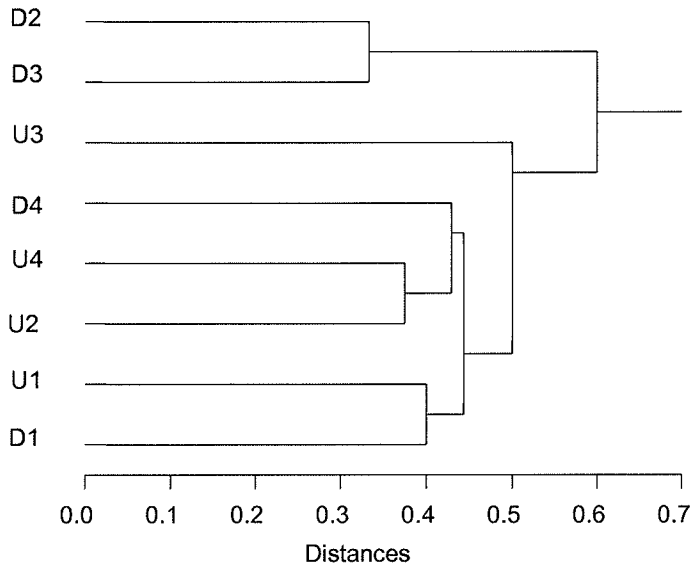

Figure 12. Hierarchical clustering based on a matrix of Jaccard coefficients (presence – absence). Higher values for pairs or clusters indicate greater dissimilarity. U= Upstream section, D= Downstream section, 1-4 indicate the position with the wetland.

Bacteria analysis results

Plate counts for total coliform, *E. coli*, and HPC were returned for positions 1 and 4 within each wetland -- the most upstream and most downstream. Total coliform increased from upstream to downstream, while *E. coli* and HPC decreased (Figure 13). The results for *E.coli* were not significant at α=0.05 ($T = 1.51$, $df = 6$, $p<0.10$).

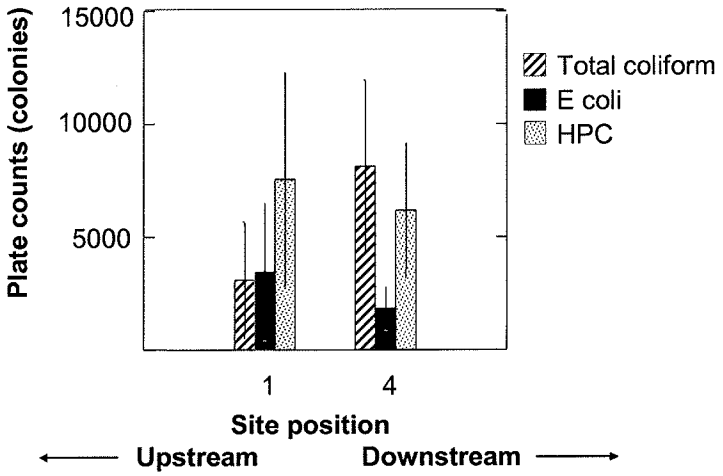

Figure 13. Bacteria plate counts, total coliform, *E. coli*, and HPC, averaged at the two wetland sections. Samples for bacteria analysis were taken only at positions 1 and 4 in both wetlands.

DISCUSSION

We predicted that measurable differences would be found in the biotic indices, bacteria levels, and water chemistry moving from upstream to downstream through the remnant wetlands. The results of the macroinvertebrate study do not show a clear trend, and do not support our hypothesis that water quality improves by passing through remnant wetland areas. The most striking result was that most of our sites had distinct communities. The chemical data do not suggest a cause for these differences and we conclude that the macroinvertebrate communities at these sites are influenced by factors that were not included in the study. Moreover, the benefits of the riparian wetlands to aquatic macroinvertebrates could be insufficient to counteract the influence of the very high level of impervious surface within the watershed. This would explain the absence of a clear trend. One study reviewed by Paul and Meyer (2003) found that in streams with impervious surface cover above 45% the aquatic macroinvertebrate community did not benefit from forest cover in the catchment; all sites showed a loss of biotic integrity. The

Patroon Creek watershed has somewhat less impervious surroundings, but the remnant wetlands have no distinguishable influence on the macroinvertebrate communities present in the creek.

The bacteria and major ion results are the most suggestive of wetland function. The increase in total coliform observed from the upstream to the downstream positions could be due to the bacteria in the soil and sediment in the wetlands and their comparative biotic richness. The decrease in *E. coli* suggests that the increase in bacteria is of types other than those associated with fecal contamination and indicates an improvement in water quality downstream of the wetlands. These results were not significant at the 5% confidence level, however. The bacterial data in this study are lower than means from previous summers for sites that were repeated. Sources of contamination may be fewer than in prior summers or higher stream flow may have had a diluting effect. The progressive change in dissolved oxygen observed in samples from upstream to downstream within the wetlands suggests biological activity within these sections. This would support our hypothesis that the riparian wetlands are buffering water quality.

We hypothesized that the water chemistry would show measurable changes from upstream to downstream within the wetland sections, assuming that the two sections would be similar enough to be pooled. Our results were too variable for chloride and associated ions to allow the planned comparisons of positions. Chloride is not expected to be held in the remnant wetlands, but to pass through these areas. The correlations found here between specific conductance and chloride as well as sodium and chloride, suggest a high variability across sampling sites for sodium and other ions directly associated with chloride. This could be due to the continuous inputs of contaminated stormwater along the creek channel. High chloride levels in warm seasons appear to be a growing phenomenon in areas where road salt is applied in winter (Kelly et al. 2008). The variability precluded establishing rates of change in ion concentrations within the remnant wetlands. Because sulfate concentrations were significantly correlated with the wetland section rather than with position, this correlation supports the evidence that the position within the stream (upstream or downstream) is important to the concentration of ions. Ammonium, the one ion that could be pooled across the two sections, showed a

decrease with position within the wetlands, but this result was not significant at $p<0.10$. Several heavy rainfall events occurred the days that we collected water samples in July (samples taken on July 10 and 13), and this rainfall may be the reason for much of the variability in the ion data. The excessive runoff could have diluted the discharge in the stream. Alternatively, by causing more erosion, the storm events could have put more contaminants into solution.

The results of the dissolved oxygen (DO) sample analysis suggest some ecological function within the wetland sections. The decrease in DO moving from position 1 through position 3 within the wetland buffer areas may be due to biological activity in the wetland soils.

The differences in ion concentration between the two sections could be due to the influence of an increasingly urban landscape moving downstream and the downstream section receiving a greater portion of surface runoff. Also, a tributary that drains a large commercial and industrial zone enters the Patroon Creek between the two wetland areas, and this may also contribute to the differences observed between the two sections. The sections may have different rates of flow that could contribute to differences in the water quality results. A slower flow would allow more opportunity for contact with soil and vegetation and this would affect the transformation of nutrients.

Further research into the soils and riparian vegetation in the remnant wetland areas would provide additional information about the level of current and potential function. The level of the water table and the nature of the soils are important to denitrification and nitrification in wetland soils (Groffman, et al. 2003). The nature of the plant community in the remnant wetlands could influence the level of function in improving water quality (e.g. removal of nitrate; Groffman et al. 2003).

In the Patroon Creek watershed the buffering function of these remnant wetlands is certainly circumvented by entry of runoff from culverts all along its channel directly into the creek. These flows bypass the buffer areas altogether. Numerous culverts enter both sections of wetland, delivering an unknown amount of runoff from Interstate 90 and other surfaces directly to the creek. Restoration efforts would likely reroute these flows into buffer areas. An experimental study in which culverts are re-routed to vegetated buffer areas would yield more useful data for evaluating the effectiveness of buffer

restoration. Our study may have failed to capture the functional capacity of the remnant wetlands because there are too many inputs circumventing the buffers.

Riparian buffers will continue to be important areas of restoration work in streams and rivers. Our findings suggest that in urban streams, restoration planners seeking evidence of ecological function need to look at factors beyond water chemistry and bacteria levels. In streams that receive high proportions of overland flow and discharge from culverts, the position of the wetland buffer within the watershed (upstream versus downstream) will be important to water quality.

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NEW TOOLS FOR ASSESSING THE EXPOSURE OF PHTHALATE ESTERS IN THE LOWER HUDSON ESTUARY

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

Pthahlate di-esters are ubiquitous environmental contaminants used as plasticizers throughout the world. They have recently gained attention for their potential endocrine disrupting as well as carcinogenic effects. Current methods employed to study phthalate di-esters in the environment tend to be flawed due to the abundance of phthalates di-esters in glassware, lab air and solvents. This study is the first in a series to understand how the metabolite of phthalate di-esters, phthalate mono-esters, can be used to get a more accurate understanding of phthalate di-ester distribution in the environment. Samples were analyzed from Jamaica Bay and New York Harbor, New York. Diethylhexyl phthalate was the dominant phthalate di-ester found in sediment with concentrations ranging from 0.115 to 4.914 μg/g sediment, while dibutyl phthalate was the also common with concentrations ranging from 0.033 to 1.560 μg/g sediment. The highest phthalate di-ester concentrations appear to correlate with the presence of combined sewage outflows (CSOs) where untreated sewage combines with storm water under high flow conditions rather than with sewage treatment plant outflows. Due to the predominance of its parent compound in the environment, monoethylhexl phthalate was the only phthalate mono-ester presented in this study. Concentrations in water samples from Jamaica Bay and New York Harbor ranged from 2.91 to 24.02 ng/L. Further studies are needed to fully understand the relationship between phthalate di-esters and phthalate mono-esters in the environment.

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Table 3. MEHP sample concentration .....IV- 16

INTRODUCTION

Dialkylphthalate esters (here referred to as phthalate di-esters; see Figure 1) are ubiquitous and relatively persistent environmental contaminants that are widely produced and used throughout the United States as well as globally (USEPA 1979; Staples et al. 1997b; Blount et al. 2000). They have been primarily incorporated as plasticizers in many products, but have also been utilized as ingredients in other materials such as personal care products (Staples et al. 1997a; Mackintosh et al. 2004). Worldwide, 4.3 million tons of phthalate di-esters are produced each year, making the fate and transport of phthalates a serious concern (Schwarzenbach 2003; Mackintosh et al. 2004). Giam et al (1978) were among the first to recognize phthalates as marine pollutants. Since then, phthalates have received considerable attention, but certainly not nearly the attention they deserve because they have proven to be so difficult to accurately measure without severe and irreproducible blank problems (Blount et al. 2000; Lin et al. 2003; Schwarzenbach 2003; Brownawell 2006). These blank problems exist due to contamination of samples during sample collection or laboratory analysis. Due to the ubiquity of phthalate esters in other laboratory supplies and equipment, background contamination (blank problems) can come from many sources and be irreproducible. This makes it hard to identify the source of phthalates measured.

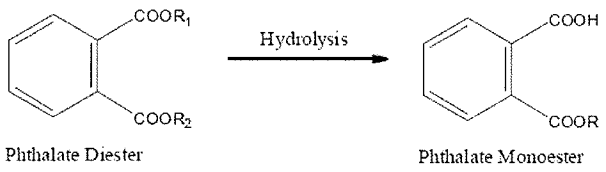

Figure 1. General structure of phthalate di-esters and their mono-ester metabolites (Hauser and Calafat 2005).

Phthalate esters can be rapidly metabolized by organisms and are converted to their monoester forms by microbes in aqueous and sediment exposures (Hashizume et al. 2002; Horn et al. 2004). They are also potentially hydrolyzed abiotically, but only under extreme pH conditions or very long time-scales.

The acute and chronic toxicity of phthalate di-esters to aquatic organisms has been reviewed (Rhodes 1995; Staples et al. 1997a; Call et al. 2001). However, more attention has been recently paid to the potential role of phthalate di-esters as endocrine

disrupting compounds in aquatic wildlife (Shimada and Yamauchi 2004; Patyna et al. 2006) rodents (Silva et al. 2006b) and humans (Colon et al. 2000; Akingbemi et al. 2004; Toda et al. 2004), in addition to their possible carcinogenic properties (David 1999; Silva et al. 2006a). In these studies, a wide variety of mechanisms for potential endocrine disrupting effects has been observed or considered, including thyroid hormone effects, weak estrogen-receptor binding-based estrogenecity, anti-androgenic effects, as well as indirect influences on steroid hormone regulation. For the lower molecular weight phthalates (e.g., di-methyl, di-ethyl, di-propyl), acute and chronic toxicity has been discovered in microorganisms, algae, aquatic invertebrates, and fish (Staples et al. 1997a). Toxicity of the mono-esters has also been shown to increase as the carbon chain length of the alcohol moiety increases (Scholz 2003).

Attracting great interest was the hypothesized connection between phthalate di-esters and hormonal consequences in humans, as seen in a group of girls in Puerto Rico with early onset of puberty (Colon et al. 2000). Many scientists are also concerned about the exposure of infants to phthalates through plastic IV tubing used when they are in the hospital (Calafat et al. 2004). Multi-generational studies with exposures of the most hydrophobic di-isononyl phthalate (DiNP) and di-isodecyl phalate (DiDP) di-esters to the fish model Medaka (Patyna et al. 2006), which examined development, reproductive fitness and endocrine disrupting endpoints showed no or modest effects in food dosed experiments; however, the same scientists have shown elevation of the estrogenic marker vitellogenin in medaka exposed to more soluble dibutyl phthalate (DBP) and diethyl phthalate (DEP), as well as multi-generational effects that resulted in greater sensitivity to phthalate exposure in later generations (Cooper 2006). Due to these potentially harmful effects of phthalate di-esters, it is most important to accurately determine the level of exposure of marine organisms to these compounds in order to conduct meaningful risk assessments that can be related to ecological or human health endpoints.

Phthalates are widely used, volatile, and ubiquitous both in the environment and in analytical systems used to measure them. This makes them difficult to measure and prone to serious contamination issues. Gloves, plastic tubing, solvents, and even the air in the laboratory may contain phthalates that can produce false readings. For this reason, many previous reports of phthalates are most likely inaccurate, and many environmental

chemists have avoided working on the environmental behavior and toxicology of this class of compounds due to these difficulties (Blount et al. 2000; Lin et al. 2003; Schwarzenbach 2003; Brownawell 2006). Often the high level of contamination in blanks creates irreproducible method detection limits that are sometimes far above that which is measured in environmental samples.

Phthalate esters are moderately (DEP) to very (diethylhexyl phthalate (DEHP) and longer chain lengths) hydrophobic and have variable tendencies to bioaccumulate or be removed from surface waters and absorbed onto suspended and superficial bottom sediments (USEPA 1979; Mackintosh et al., 2004). This group of contaminants has already caused problems in sediment in Puget Sound, Washington. Levels of phthalate esters are above those required for sediment quality criteria values, which are in the tens of μg/g (Rude 2006). Concentrations in sediments of one of the common hydrophobic phthalate di-esters DEHP have been measured in many freshwater samples from urbanized waterways or sediments from areas proximate to sewage treatment plants at levels up to 29.7 μg/g near the outflow and 6.5 μg/g 300 m away from the outflow (McDowell and Metcalfe 2001). Taiwanese scientists have even measured levels up to 71.9 mg/kg in local freshwater sediments (Kao et al. 2005). Marine measurements of phthalates have been made in a marine inlet in Vancouver, British Columbia in Canada, with DEHP having the highest measured levels at 1 μg/g in sediment (Lin et al. 2003). The studies conducted by Canadian investigators (Lin et al. 2003; Mackintosh et al. 2004) employed liquid chromatography/ electrospray ionization mass spectrometry to analyze phthalate levels, while attempting to reduce purification steps and handling time to minimize outside phthalate exposure. Still, difficulty in conducting realistic field blanks for sediment samples (e.g., defrosting frozen sediments in lab air) pose problems in requiring the controls to account for contamination issues.

Phthalate di-esters and mono-esters may be introduced to urban harbors and estuarine settings from industrial and municipal wastewater sources. Bruce Brownawell's lab has discovered some of the highest levels of sewage-derived neutral nonylphenol ethoxylate metabolites (nonylphenol or lower ethoxylated metabolites) measured worldwide (many sites in excess of 10 μg/g and several in excess of 40 μg/g in areas of the very highly urbanized New York/New Jersey (Ferguson et al. 2000, 2001a, 2001b,

2001c; Ferguson and Brownawell 2003; Yin 2006)). These metabolites are intermediate in hydrophobicity, and accumulate and persist in some more reducing/suboxic sediment in areas like Jamaica Bay. I hypothesize that the more hydrophobic phthalate esters (e.g., DEHP and DiNP) will exist at similarly high levels in the lower Hudson River and their distributions in sediments can be assessed by innovative means of conducting sediment and water extractions in the field. These techniques can be combined with new analytical approaches that have been applied for measuring phthalate di-ester exposure in humans developed originally by investigators at the Centers for Disease Control and Prevention (CDC) in Atlanta (Blount et al. 2000).

In the past six years, investigators at the CDC have published no less than 36 peer reviewed papers related to both the measurement of phthalate mono-esters and their oxidation products and the monitoring of human or rodent exposure to phthalate di-esters. These papers have already been cited over 650 times. Due in part to much lower volatility and contamination in lab air and lab products, the measurement of phthalate ester metabolites/degradates (mono-esters and mono-ester oxidation products) is much less susceptible to laboratory or field contamination issues and methods have been developed to look at urine, serum, semen, and saliva matrices (Blount et al. 2000; Silva et al. 2005; Kato et al. 2006; Silva et al. 2006a; Silva et al. 2006b). Kato et al. (2005) have worked to correlate the metabolites to their corresponding di-esters in human urine and have determined that the metabolites provide a good approximation of the exposure to the di-esters.

In the following report I have shown our ability to successfully extract and measure phthalate di-esters and mono-esters from sediment and water samples, respectively. Preliminary conclusions have been made as to the rationale behind the distribution of phthalate di-esters and mono-esters within the environment with future research proposed to answer additional questions addressed. These studies will provide the information needed to potentially enable the use of phthalate mono-ester concentrations as an accurate indicator of phthalate di-ester exposure in the environment.

MATERIALS AND METHODS

Materials

A mix of phthalate di-ester standards including bis(2-n-Butoxyethyl) phthalate (BBEP), benzyl butyl phthalate (BBP), dipentyl phthalate (DPP), dibutyl phthalate (DBP), dicyclohexyl phthalate (DCHP), diethyl phthalate (DEP), dihexyl phthalate (DHP), di-isobutyl phthalate (DiBP), dimethyl phthalate (DMP), dinonyl phthalate (DNP), di-n-octyl phthalate (DnOP), Bis(2-ethyoxyethyl) phthalate (BEEP), bis(2-ethylhexyl) phthalate (DEHP), bis (2-methoxyethyl) phthalate (BMEP) and bis(4-methyl-2-pentyl) phthalate (BMPP) were obtained from Crescent Chemical Company (Islandia, NY). Monoethylhexyl phthalate (MEHP) and C13 labeled MEHP were obtained from Cambridge Isotope Laboratories, Inc (Andover, MA).

Preparation

All glassware was rinsed with acetone, hexane, and dichloromethane (DCM) and dried for one minute in a drying oven. Glass vials were rinsed with Teflon lined lids on. Teflon centrifuge tubes were sonicated with acetone and hexane for 20 minutes each. The sonic probe was cleaned with acetone and hexane between each use. Spatulas were solvent rinsed with acetone and hexane and a new spatula was used for each sample. One liter amber bottles were rinsed with methanol and then heated in a muffle furnace for six hours at 450°C. No plastic containers or pipettes were used. All glassware and solvents were covered in aluminum foil when not being used directly.

Sediment Phthalate Di-ester Extraction

Samples from a stored 2003 Regional Environmental Monitoring and Assessment Program (REMAP) sample collection in New York Harbor and Jamaica Bay as shown in Figure 2a were extracted for sediment analysis. Samples were selected based on proximity to sewage treatment plants (STPs) and combined sewage outflows (CSOs). Location labels are abbreviations for location: Upper Harbor (UH), Bowery Bay (BB), Jamaica Bay (JB), Williamsburg Bridge (WB) and Newark Bay (NB). Sediments were thawed with their lids on to minimize contamination from air.

For extraction, wet sediment (0.5 grams) was placed into a 30 mL Teflon centrifuge tube and 7mL of GC^2-MS grade methanol was added to each tube. Samples

were placed in an ice bath and sonicated using a Cole Palmer Ultrasonic Homogenizer (4710 series) for 10 minutes using pulse mode and then centrifuged at 1000 rpm for 8 minutes. Methanol (5 mL) was removed using a solvent rinsed glass syringe and added to a volumetric flask (50 mL) containing 1.5 mL hexane. The flask was filled to 50 mL with solvent (DCM) cleaned water and the hexane removed to be measured for phthalate di-ester concentration using GC-MS. The remaining sediment samples were dried for at least twelve hours and the dry weight was obtained.

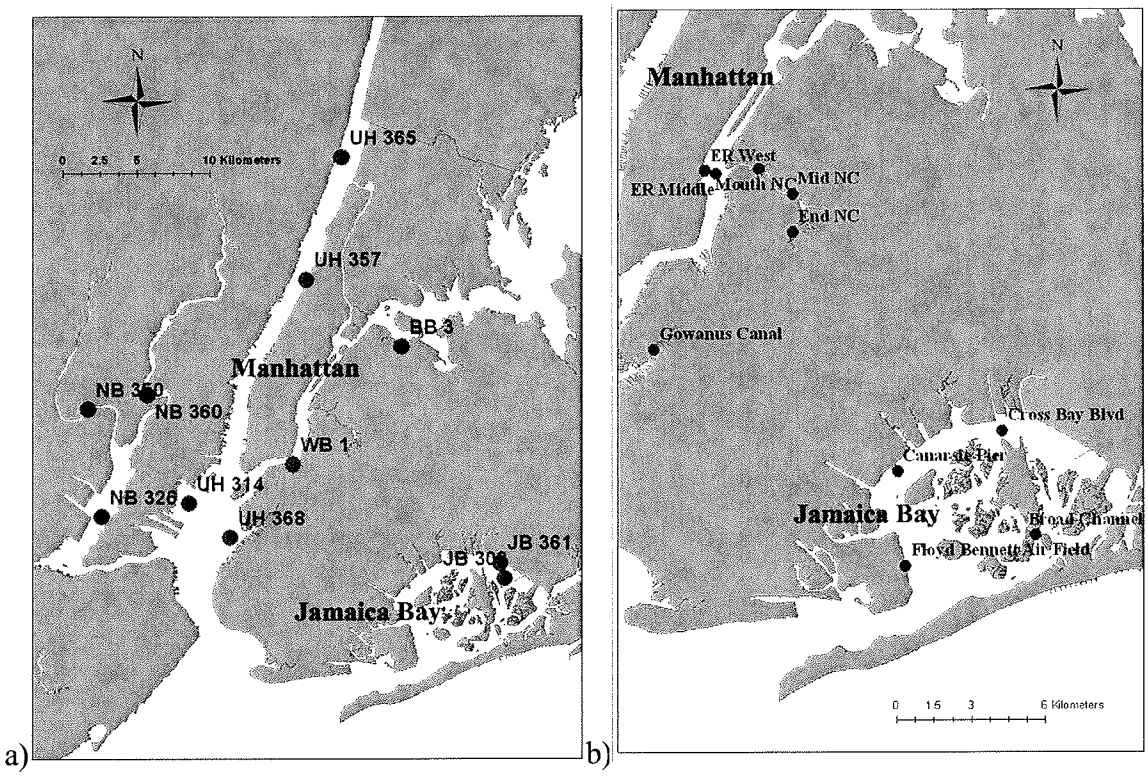

Figure 2. Map of metropolitan New York. a) sediment sample locations; b) water sample locations.

A New Method for Phthalate Mono-ester Extraction

Liquid-liquid extraction of seawater samples rather than solid phase extraction methods were developed to allow for extraction of larger volumes of seawater as well as to minimize co-extractions of interfering compounds. Samples were obtained in locations indicated in Figure 2b in New York Harbor and Jamaica Bay in August and September, 2007. One liter amber bottles were filed with 25 mL of hexane prior to collection. Water samples (about 900 mL) were obtained using a stainless steel Kemmerer sampling bottle (Wildco Instruments) with Teflon end seals. In the lab,

samples were acidified to pH 2.5 – 3.0 using sulfuric acid (5-8 drops), shaken for 30 seconds and then allowed to settle in a refrigerator overnight. Fifteen milliliters of hexane were removed using a glass pipette and measured into a graduated cylinder. The hexane was placed into a cleaned test tube and blown to dryness using nitrogen gas. The sample was re-dissolved in 1 mL of MilliQ water and stored until analysis.

Instrumentation

Phthalate di-esters were measured by gas chromatography-mass spectrometry (GC-MS) with an Agilent 50890 GC and a VG Quattro. The inlet temperature was 300°C and the transfer line was 250°C. Measurements were taken in positive electrospray ionization mode with select ion monitoring (SIM) of masses 104.00, 149.00, 163.00, and 167.00. Initial temperature was 80°C with a one minute hold. Increase to 160°C at 30°C per minute, then to 320°C at 15°C per minute, followed by a 1.50 minute hold.

Phthalate mono-esters were measured with a LC time of flight (TOF) MS, Waters 2695 HPLC and Micromass LCT. Samples were run using negative electrospray with a 25 minute method (Table 1) and a flow rate of 0.2 mL/min. Mobile phases were C18 filtered MilliQ water and acetonitrile (ACN). A Betasil 100 x 2.1mm 3μm phenyl column was used. All samples were run with leucine enkephalin for accurate mass analysis. The initial gradient is shown in Table 1a. After small changes in accurate mass and peak shapes were analyzed, it was hypothesized that nonylphenol poly-1-ethoxy carboxylate (NP1EC) was present and a second gradient (Table 1b) was devised to separate the compounds and samples were re-run using the revised gradient.

a.

Time	% C18 filtered H_20	%ACN
0.00	90	10
4.00	90	10
10.00	5	95
20.00	5	95
21.00	90	10
25.00	90	10

b.

Time	% C18 Filtered H2O	%ACN
0.00	90	10
3.00	90	10
16.00	15	85
18.00	90	10
27.00	90	10

Table 1. HPLC gradient a) before revisions and b) after revisions.

RESULTS

Di-esters

It was determined that using fresh solvents and reducing air exposure were some of the best ways to reduce blanks. To do this, air exposure was limited in extraction procedures as well as by covering all solvents and glassware with aluminum foil. Gloves were determined to not be a source of phthalate contamination to samples. The grade of solvent did not appear to have a significant result on contamination as long as GC grade solvents were used. However, as discussed, more work on sample preparation and identification of sources of blanks is needed.

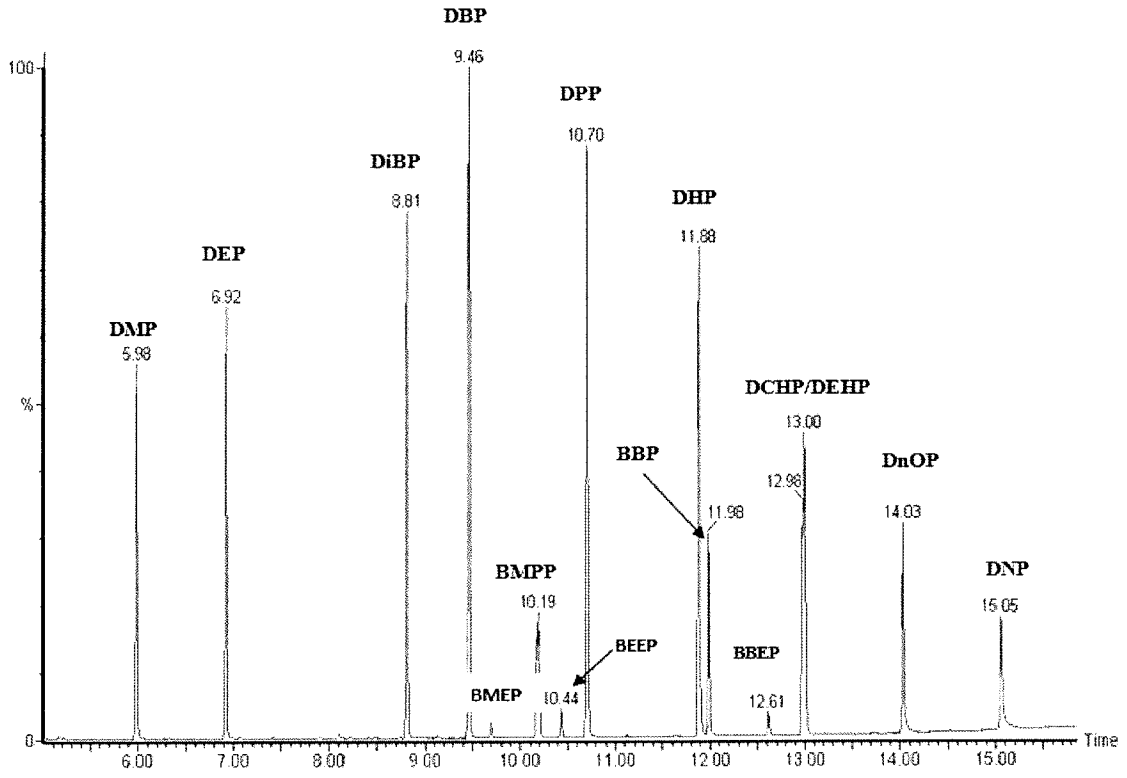

Figure 3. Chromatogram of di-esters analyzed on GC-MS.

A GC-MS method was developed to successfully analyze and separate all of the components of a mixed standard of phthalate di-esters (Figure 3). DCHP and DEHP elute at 12.98 and 13.00 respectively and therefore were treated as one peak with the assumption that DEHP would be the predominant di-ester in environmental samples. Table 2 shows the range of concentrations seen in sediments and blanks of both DBP and DEHP. The blanks are significantly lower than phthalate concentrations for at least four sites including NB 326, NB 350, BB3 and WB1(see Figure 2a). The sites with the highest phthalate di-ester locations coincide with sites near CSOs as opposed to STPs.

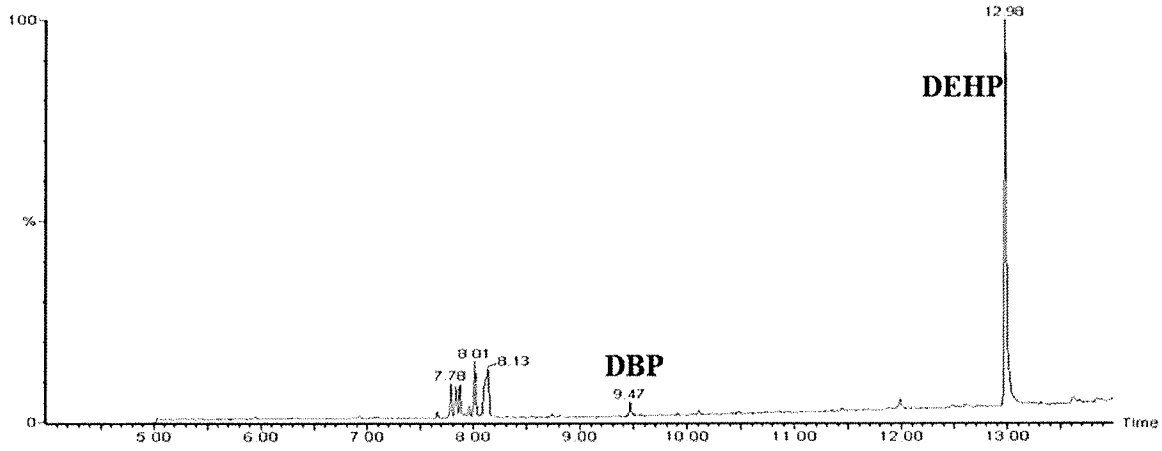

Figure 4. Chromatogram of di-esters in Bowery Bay 3 sample. Peaks between 7.78 and 8.13 minutes derive from contamination during analysis and are not of related to phthalate

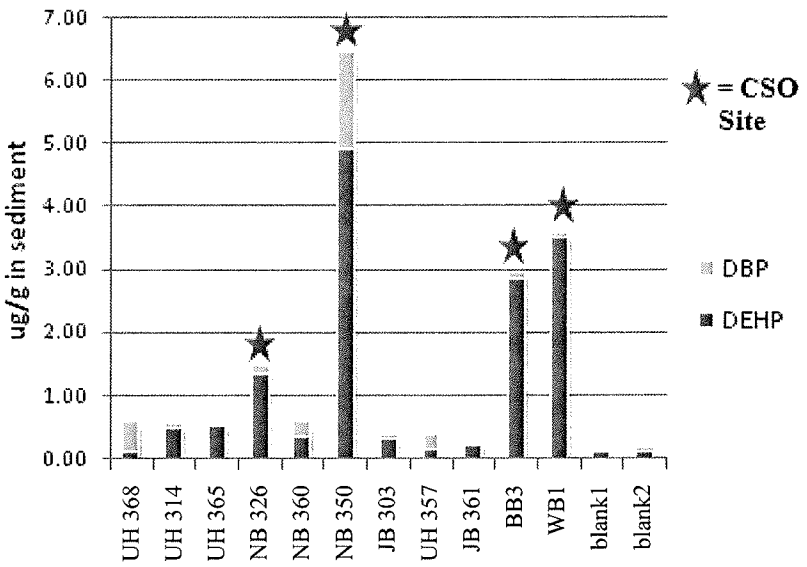

Figure 5. Combined DEHP and DNP concentrations from sediment samples.

Sample	DEHP ug/g	DBP ug/g
UH 368	0.115	0.470
UH 314	0.480	0.068
UH 365	0.517	0.039
NB 326	1.345	0.147
NB 360	0.347	0.234
NB 350	4.914	1.560
JB 303	0.310	0.088
UH 357	0.133	0.252
JB361	0.213	0.033
BB3	2.874	0.084
WB1	3.521	0.077
Blank1	0.099	0.054
Blank2	0.131	0.055

Table 2. DEHP and DNP concentrations in samples.

Mono-esters

For this study we worked only with MEHP due to restrictions with standards and the hypothesis that it would be the most prominent mono-phthalate present in the environment. An initial method was developed for extraction and analysis of MEHP and samples were collected and analyzed. Utilizing an earlier HPLC method we measured

high levels of MEHP, but concentration ratios between sites did not vary significantly and the mass chromatography peak appeared to shoulder. In addition, the accurate mass was heavier than expected. A hypothesis was formulated that a primary hydrophobic and acidic contaminant nonylphenoxyacetic acid (NP1EC) might be interfering with the MEHP signal. A new method was developed (Table 1b) that was able to successfully separate MEHP and NP1EC. By narrowing the mass window to 0.05 amμ and using software correction, the NP1EC interfering peak was able to be successfully separated (Figure 6).

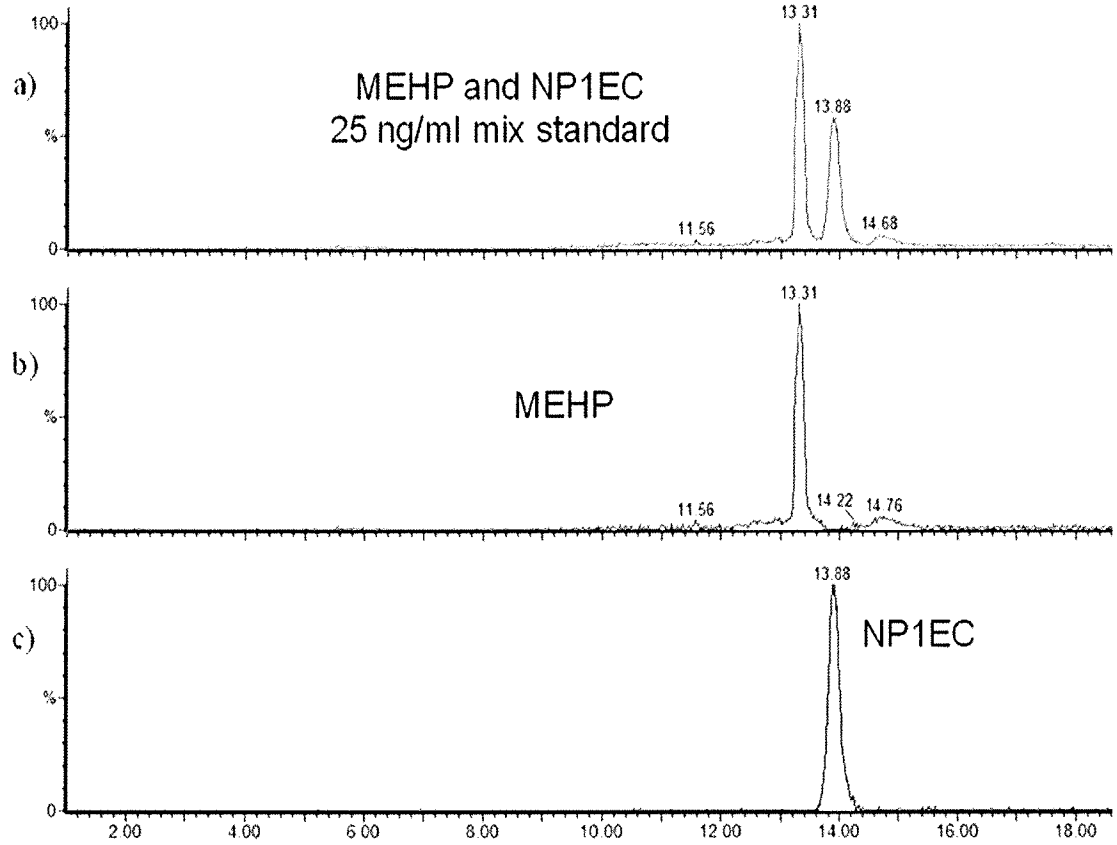

Figure 6. Chromatogram of MEHP and NP1EC mixed standard to illustrate the effect of using the mass accuracy of TOF MS to separate these compounds of similar molecular weight: a) MEHP and NP1EC, 0.20Da window; b) MEHP, 0.05 Da window; c) NP1EC, 0.05 Da window.

A subset of samples was originally analyzed with the first method and all samples were run using the refined method to ensure complete separation from NP1EC. As is evident from Figure 7, a large difference was observed by refining the method to fully separate NP1EC and MEHP. The calculated concentrations using the new method are

presented in Figure 8 and Table 3. The sample taken at Cross Bay Bridge in Jamaica Bay, NY has the highest concentration of 24.02 ng/L. This site is close to a large STP. The lowest concentrations are observed in Newtown Creek near Manhattan, NY. Blank levels were below detection limits.

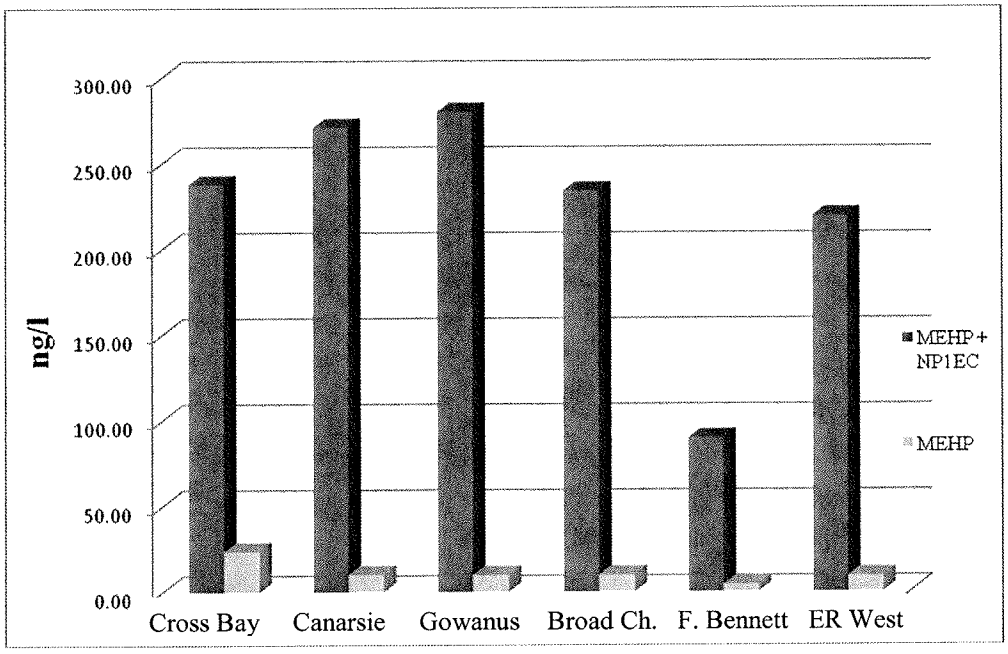

Figure 7. Calculated MEHP concentrations before and after separation from NP1EC.

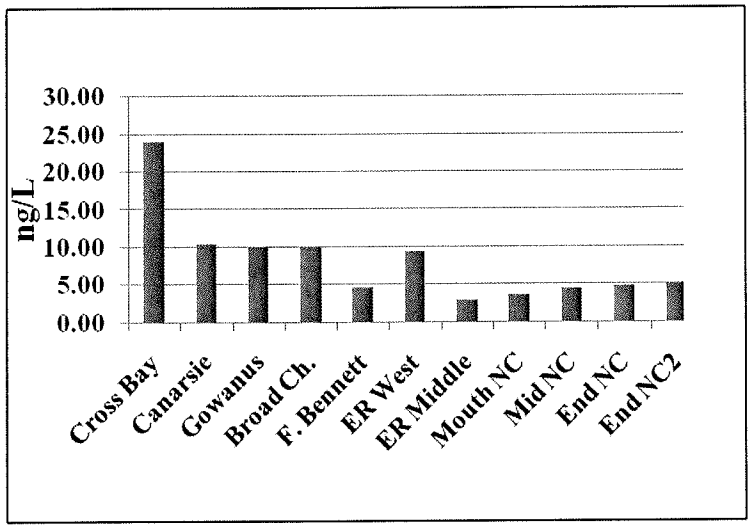

Figure 8. MEHP concentrations in water samples.

Sample Name	ng/L
Cross Bay	24.02
Canarsie	10.41
Gowanus	10.00
Broad Ch.	10.11
F. Bennett	4.56
ER West	9.31
ER Middle	2.91
Mouth NC	3.55
Mid NC	4.44
End NC	4.80
End NC2	5.17

Table 3. MEHP concentrations in water samples.

DISCUSSION

Di-esters

One of the biggest obstacles to overcome when working with phthalate di-ester samples are the blanks that are associated with them. In our work, we found that DBP was one of the most prominent phthalates prone to contamination from outside sources, most likely from lab air. Due to the issues associated with blanks in measuring di-esters, it was important that the extraction procedure developed minimized solvent use and air exposure. While the method currently in use is able to produce results that far exceed blanks, work is still needed to reduce air exposure, particularly during sonication. Additional steps in method development include expanding the method to extract compounds less hydrophobic than DBP.

Sediment extraction results show DEHP/DBP concentrations significantly above blanks in four sites, particularly those closest to CSOs. The current hypothesis for these findings is that transformation of di-esters to mono-esters occurs less often in CSO systems than in STPs. The bacteria in STPs may be a major component in the conversion of di-esters to mono-esters. Therefore, sites where CSOs are the main source of input would theoretically be dominated by phthalate di-esters. This hypothesis needs to be further explored.

As expected from other studies (Hwang et al. 2006; Sha et al. 2007; Teil et al. 2007), DEHP was the most prominent phthalate di-ester found in environmental samples, followed be DBP. The levels of phthalate di-esters found in sediment samples are within the broad range of observed DEHP and DBP values. In the Netherlands, DEHP values were as low as 0.0674 μg/g (Peijnenburg and Struijs 2006). American salt marsh values were 0.28 – 32 μg/g (Hwang et al. 2006). China sediment concentrations as high as 85.16 μg/g were observed for DEHP (Sha et al. 2007). As hypothesized, these levels are also in line with levels of sewage derived nonylphenol ethoxylate metabolites (10μg/g) found in similar environments.

Mono-esters

We have presented the first environmental measurements of MEHP. In the process of method development it was determined that one of the most important steps in

the process is the acidification of the sample to a pH between 2.5 and 3.0. Samples were run before and after acidification and MEHP was only detected after acidification.

Another important step in method development was realizing that NP1EC was interfering with MEHP measurements. The ability to completely separate the two compounds was made possible by the TOF used during analysis. By utilizing accurate mass corrections and a window of 0.05 Daltons, the peaks of the two compounds were able to be completely separated (Figure 6) ensuring measurement of MEHP concentrations only. Further verification of MEHP will be obtained through the use of HPLC-MS-MS. The use of tandem mass analyzers (MS-MS) provides more structural specificity that aids in the confirmation of compound identity.

Levels of phthalate mono-esters observed from Jamaica Bay samples (including Cross Bay Bridge, Canarsie Pier, Broad Channel and Floyd Bennett) were as expected, with Cross Bay Bridge having the highest MEHP levels due its proximity to a large STP. Newtown Creek samples were lower than expected. This is most likely due to the record-low sewage detected in Newtown Creek at that time (O'Mullan 2007), which would reduce the input of phthalate di-esters and mono-esters to the area.

CONCLUSION

This work represents the beginning stage in an effort to understand and accurately quantify the presence of phthalates and their metabolites in the environment. Further research is needed to fully understand the explanations for variance seen in MEHP concentrations and how they correspond to DEHP. Future work is also needed to identify other mono phthalates in the environment. This work will involve refining the current method to allow extraction and detection of less hydrophobic monoesters such as monoethyl phthalate.

ACKNOWLEDGEMENTS

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APPLYING AN EFFECT-DIRECTED STRATEGY TO INDTIFY PREVIOULSY UNRECOGNIZED TOXIC CHEMICALS IN HUDSON RIVER SEDIMENTS

A Final Report of the Tibor T. Polgar Fellowship Program

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Abstract

Hudson River sediments highly contaminated with PCBs, PCDDs, and PCDFs are a concern for the watershed and humans that live in the surrounding area. Potential health risks to humans from these compounds are due to fish consumption and contaminated water. For this reason, recent research has focused on the behavior of mixtures of toxic chemicals rather than individual chemicals to get a better assessment for potential toxicity. Previous studies have shown that the biological responses from fractions with known concentrations of estrogenic compounds or dioxin-like compounds do not correspond. This indicates that there are either unrecognized toxic chemicals or mixtures of chemicals that behave differently from individual chemicals. An effect-directed strategy was developed to distinguish between these two hypotheses. Four sediment samples from the upper Hudson River were analyzed using the effect-directed strategy. We performed a sequential extraction using ASE to initially separate non-polar, semi-polar, and highly polar compounds found in the sediment. All extracts inhibited *Vibrio fischeri* metabolism, indicating the presence of toxic compounds in the extracts. MeOH extracts for River miles 152.7 (36-40 cm) and 188.6 (28-32 cm) exhibited high dioxin or dioxin-like activity and high estrogenicity, respectively, leading to the conclusion there are highly polar compounds present that exhibit toxicity. A di-chlorinated compound and tri-chlorinated compound were found in the MeOH extracts of River mile 152.7 (36-40 cm) and River mile 188.6 (28-32 cm) using liquid chromatography mass spectrometry (LC-MS). Further investigation of these compounds is needed.

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Introduction

Over a span of 30 years, ceasing in the 1970's, the General Electric Company (GE) released as much as 1.3 million pounds of polychlorinated biphenyls (PCBs) into the upper Hudson River from its two capacitor manufacturing plants located in Hudson Falls and Fort Edward, New York. High concentrations of PCBs are still present in the sediments of the 40 miles of the upper Hudson River. In addition to the PCB contamination, the Hudson River receives runoff from both agricultural waste and human sewage sources that contribute a variety of other organic contaminants such as pharmaceuticals, detergent metabolites, fire retardants, and other compounds that may cause endocrine disrupting effects on fish species and other wildlife. High concentrations of these compounds can persist in sediment. In addition to the known compounds above, metabolites and degradation products from weathering, photolysis, and hydrolysis may also contribute to the ecotoxicity of commonly known organic compounds. As a result, there is concern for the effects of fish consumption and contaminated water supplies on human health and for the health of the river and its surrounding watershed (McArdle et al. 2004).

A previous study of Hudson River sediment conducted by Dr. Richard Bopp of Rensselaer Polytechnic Institute (RPI) indicates a range of PCB, PCDD and PCDFs in sediment samples taken from the upper Hudson River at River miles 152.7, 163.6 and 188.6. In fact all sediments had part per million (ppm) levels of PCBs and high part per trillion (ppt) levels of polychlorinated dibenzo dioxins (PCDDs) and polychlorinated dibenzo furans (PCDFs) (Table 1) (Personal correspondence with Dr. Bopp, Bopp et al. 1982, Bopp et al. 1998).

Sediment Sample	Total PCB (ppm)	Total PCDD (ppt)	Total PCDF (ppt)
163.6 (2-4 cm)	7.7	388	1,268
163.6 (36-40 cm)	64	3,600	32,322
152.7 (36-40 cm)	40	33,610	928,863
188.6 (28-32 cm)	460	9,510	74,647

Table 1. Previously collected PCB, PCDD, and PCDF concentrations for all sediment samples.

Considering these previous data collected (Personal correspondence with Dr. Bopp, Bopp et al. 1982, Bopp et al. 1998), the focus in this study was on analyzing the polar extracts from the same sediment samples for degradants and metabolites of PCBs, PCDDs and PCDFs, because these compounds tend to be more polar and more toxic than their precursors. Studies have shown that concentrations of known toxic chemicals in sediment extracts fail to correspond to biological response of fractions from these extracts (Suzuki et al. 2004, Schlenk et al. 2005). This indicates that there are either unrecognized toxic chemicals remaining undetected by current analytical techniques or mixtures of chemicals that behave differently from individual chemicals. An effect-directed strategy was developed to distinguish between these two hypotheses.

Methods

Suzuki et al. (2004) showed non-additive increase in toxic effects from extracts and fractions of compost extracts containing polycyclic aromatic hydrocarbons (PAHs). As a result, conventional methods of determining toxicity of mixtures, such as taking toxic equivalency factors (TEF) of individual compounds to calculate the toxic equivalent

quantity (TEQ) for a mixture, were found to be ineffective for assessing the risk associated with environmental samples (Burgess et al. 1999). Brack et al. (1999), Brack and Schirmer (2003), Suzuki et al. (2004), Sundberg et al. (2005), and Schlenck et al. (2005) determined that different assays measuring different toxic effects need to be utilized to determine the full extent of toxicity within a sample. Based on these studies we developed a strategy incorporating assays to measure dioxin or dioxin-like activity, estrogenicity, and energy metabolism inhibition in environmental samples.

Our effect-directed strategy was selected to facilitate the identification of toxic compounds present in Hudson River sediments. This strategy has several key steps: sequential extraction, biological assay and, finally, separation/mass spectrometric analysis (Figure 1.). A combination of these three analysis techniques not only allows for identification of contaminants but also provides toxicity data on the sediment extracts before and after separation. This strategy, illustrated in the schematic diagram shown in Fig. 1, first starts with three sequential extractions of the sediment sample using solvents with increasing polarities for initial sub-fractionation of contaminants based on their polarity. After concentration and cleanup, extracts are tested for toxicity using several different bioassays. Extracts that indicate any degree of toxicity are analyzed by liquid chromatography ion trap mass spectrometry (LC-ITMS) and gas chromatography mass spectrometry (GC-MS). Fractions are also collected from the LC and subjected to bioassays to determine individual peaks that exhibit toxicity. Fractions that are toxic are analyzed further by LC-ITMS and GC-MS to determine if more fractionation and bioassay analysis is necessary. Once individual components have been isolated, MS^2 data are collected as necessary for structural elucidation and identification.

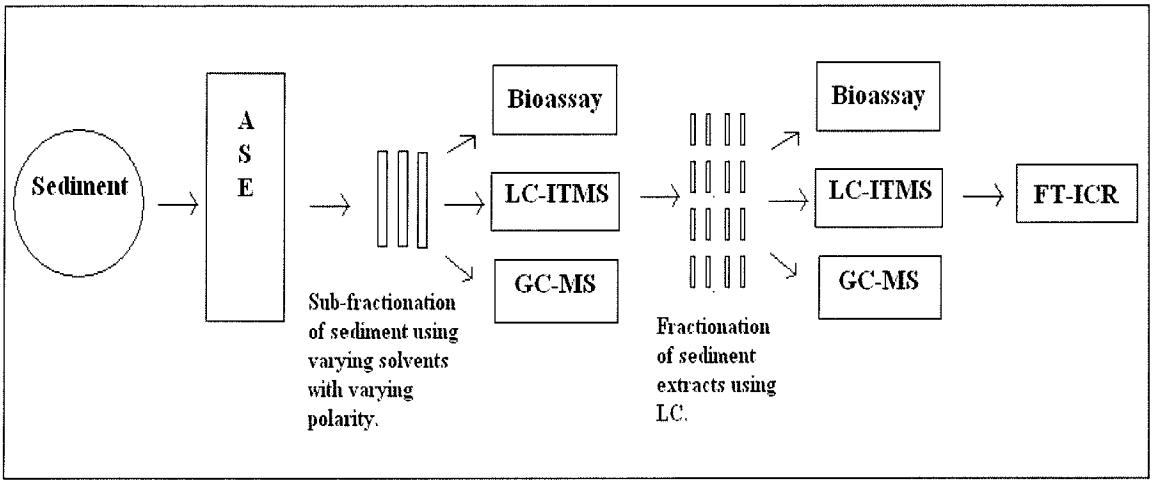

Figure 1. Detailed schematic of effect-directed strategy.

Sediment Extraction & Cleanup

Accelerated Solvent Extraction (ASE) was utilized for sediment extraction (Houtman et al. 2006). A Dionex ASE-200 System was used for extraction. Two grams of sediment and 10 grams of anhydrous sodium sulfate were placed in the extraction chamber. Three extraction solvents were used sequentially. First, hexane extracted nonpolar compounds. Second, a 50:50 mixture of hexane: isopropranol (IPA) extracted semi-polar compounds. Finally, methanol (MeOH) extracted highly polar compounds. The other operating conditions were as follows: pressure of 70 bar, temperature of 100°C and time of 5 minutes. After extraction, extracts were concentrated to ~3mL and passed through an activated alumina column to remove humic acids and other interfering compounds.

Bioassay Analysis

Several bioassays were used to assess the toxicity of sub-fractions from the ASE and fractions collected from the High-Performance Liquid Chromatograph (HPLC): the luciferase reporter gene bioassay, yeast estrogen screen (YES) assay and a rapid toxicity

screening assay. The luciferase reporter gene bioassay is able to detect 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) levels as low as 1 picoMolar (Gasiewicz et al. 1996, Kostyniak et al. 2005). The analysis was performed by Dr. James Olson and Barbara McGarrigle from the Toxicology Department at the State University of New York at Buffalo. A yeast estrogen screen assay (YES) was used to determine estrogenic activity for all of the extracts from River mile 188.6 (28-32 cm) sediment sample. This colorimetric estrogen reporter gene assay uses *Saccharomyces cerevisiae* (yeast) transfected with human estrogen receptor to measure estrogenicity of samples compared to 17β-estradiol (Routledge and Sumpter 1996, Holbrook et al. 2002). Approximately 2mL of sediment extract was evaporated to dryness and shipped to Dr. Katharine Knowlton at Virginia Tech University for analysis.

The Abratox^{TM} assay is a rapid toxicity screening assay. This assay, manufactured by Abraxis Kits LLC, is a luminescence assay which utilizes *Vibrio fischeri* for the detection of toxic compounds in samples (Brack et al. 1999). Toxic compounds inhibit the bacteria from luminescing by interrupting energy metabolism. Dilutions of sediment extracts were made at 2.5% of extract in 1.5mL of nanopure water. For extracts, a 2.5% solution of acetonitrile (ACN) or MeOH in nanopure water was used for the negative control. The positive controls used were a solution of zinc chloride provided in the assay kit and a 400 parts per billion (ppb) solution of triclosan. Triclosan was selected as an additional positive control for its structural similarities to common organic contaminants found in Hudson River sediments.

Liquid Chromatography-Ion Trap Mass Spectrometry (LC-ITMS)

LC-ITMS was performed on a Thermo (San Jose, CA) LCQ Advantage system. Approximately 1-1.5mL of extract was evaporated to dryness and reconstituted into 0.5mL of ACN for LC-ITMS analysis. Separation was performed on a reversed-phase column with an injection volume of 15μL, with a gradient starting at 30:70 CAN: water (with 0.3% formic acid), ramping up to 100% ACN over 15 minutes. Ionization for mass spectral detection was done by electrospray ionization (ESI) in positive and negative mode (Maziarz et al. 2005, Kemmochi et al. 2002). Positive mode had a capillary temperature of 205°C, sheath gas flow of 20, spray voltage of 4.5 kV, and a capillary voltage of 4.00 V. In negative mode capillary temperature, sheath gas flow and spray voltage were the same but the capillary voltage was -10.00 V. A full scan from 100-1000 *m/z* was acquired. All extracts were initially analyzed and fractionated by LC-ITMS, however only certain extracts with peaks of interest were analyzed using MS^2 and MS^3. Fractions were collected at 2 minute intervals from all extracts and these fractions were subjected to the Abratox™ assay.

Gas Chromatography Mass Spectrometry (GC-MS)

GC-MS is a common environmental analytical technique used for determination of organic contaminants in environmental samples, especially those that are relatively nonpolar and difficult to detect by LC-MS (Ryan et al. 2005, Focant et al. 2001). An Agilent (Palo Alto, CA) single quadrupole GC-MS (model 5973) was used for sediment analysis. Approximately 1mL of the extract was evaporated to dryness and reconstituted with 0.3mL hexane for GC/MS analysis. The GC injection port was at 250°C. A Restek (Bellefonte, PA) RTX-5MS column (40.0m x 250μm x 25μm) with stationary phase of

5% diphenyl/95% dimethyl polysiloxane was used for separation. The method used helium as a carrier gas and the oven started at 50°C held for 1 min, then ramped to 250°C at 20°C/min held for 4 min, and finally ramped to 325°C at 10°C/min held for 4 min with a total run time of 26.50 mins.

Results

The Abratox™ assay showed that all extracts were inhibiting the energy metabolism pathways of *Vibrio fischeri* (Fig. 2).

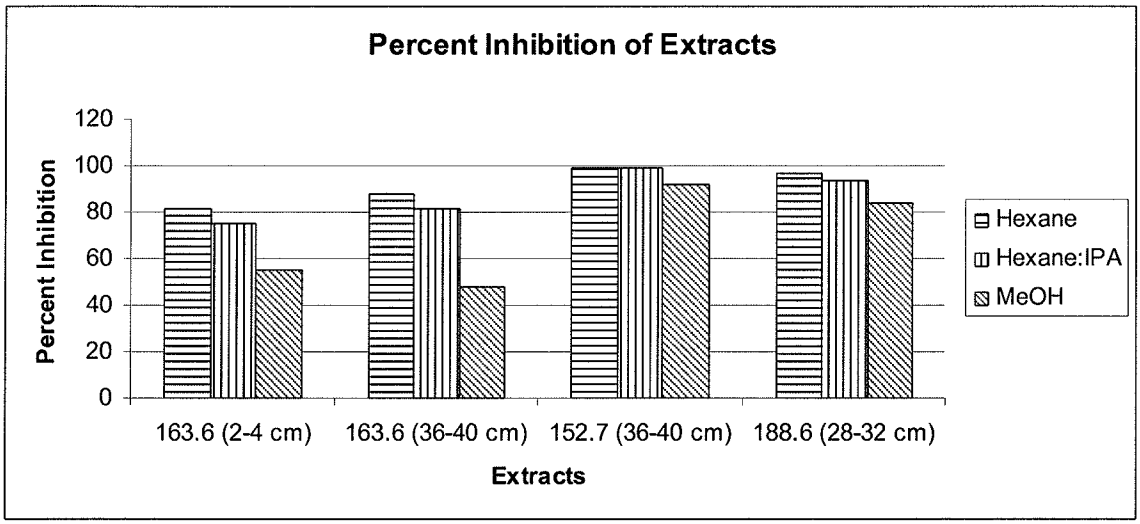

Figure 2. Percent inhibition of luminescence from *Vibrio fischeri* for all sediment extracts.

The MeOH extracts were least toxic of all the extracts within the Abratox™ system, second were the hexane: IPA extracts and, finally, the hexane extracts were most toxic to *Vibrio fischeri*. However, not all extracts showed dioxin or dioxin-like activity in the luciferase reporter gene assay as shown in Fig. 3. Three of the four hexane extracts were below the limit of detection for toxicity; however the hexane extract for River mile 152.7 (36-40 cm) showed dioxin or dioxin-like activity. All hexane: IPA extracts showed dioxin or dioxin-like activity. River mile 152.7 (36-40 cm) showed the highest toxicity,

then River mile 188.6 (28-32 cm) was the next highest, River mile 163.6 (36-40 cm) was third highest, and River mile 163.6 (2-4 cm) was the lowest for hexane: IPA extracts. The MeOH extract for River mile 152.7 (36-40 cm) had the highest toxicity of all extracts. The MeOH extract the River mile 188.6 (28-32 cm) was the second highest toxicity, third was River mile 163.6 (36-40 cm) and, finally, River mile 163.6 (2-4 cm) MeOH extract had the lowest toxicity of the MeOH extracts.

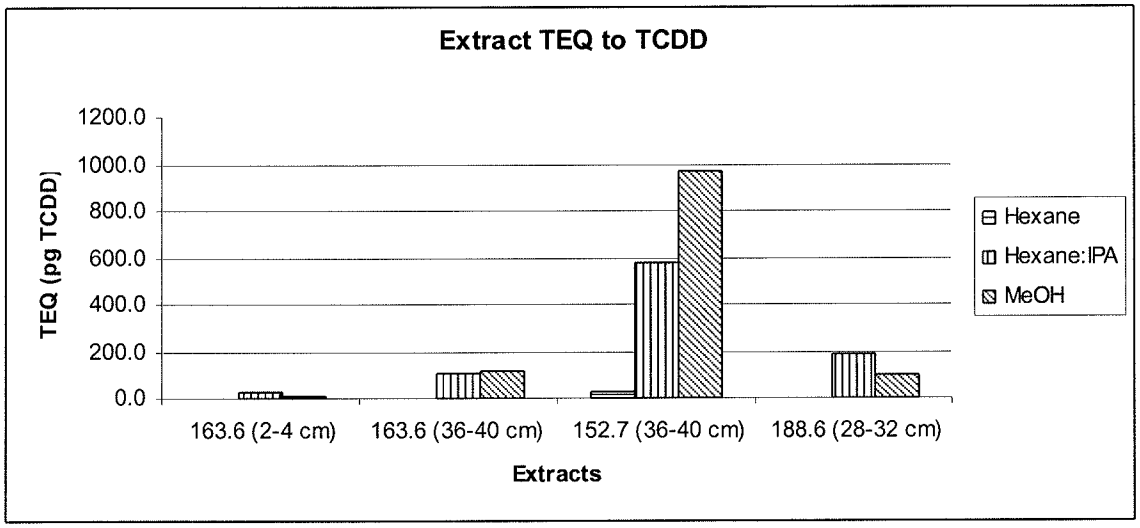

Figure 3. Toxicity equivalents for all extracts compared to pg TCDD.

The hexane, hexane: IPA, and MeOH extracts for River mile 188.6 (28-32 cm) were also run with the YES assay. The MeOH extract had the most estrogenic activity, the hexane extract had the second highest activity, and the hexane: IPA extract had the lowest estrogenic activity seen in Figure 4.

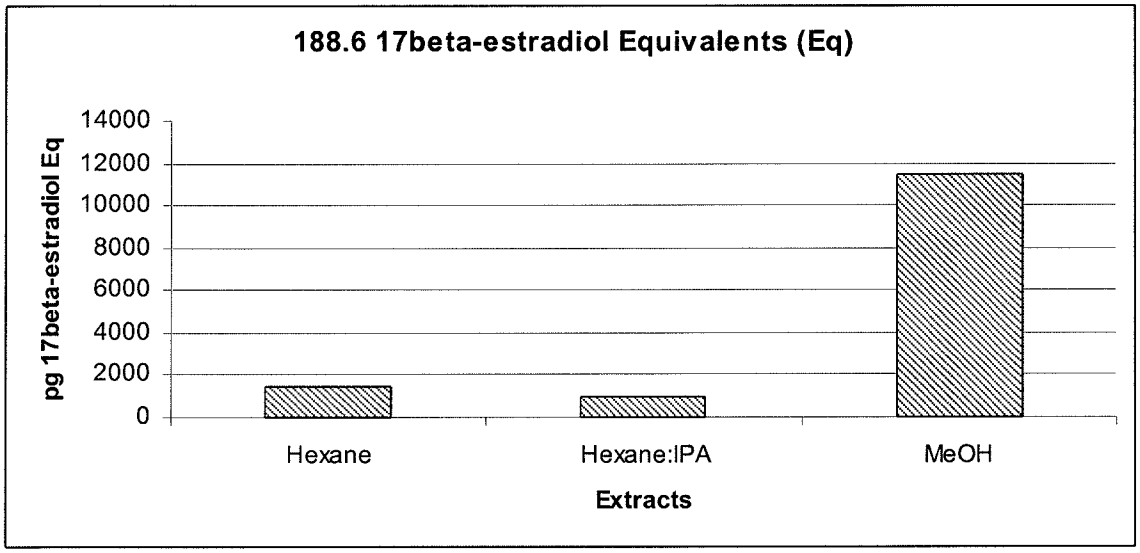

Figure 4. Estrogenicity of River mile 188.6 (28-32 cm) extracts compared to pg of 17β-estradiol.

Once toxicity of extracts was determined they were analyzed by LC-ITMS in positive and negative mode. Fractionation was performed to determine which peaks to focus on. Below in Fig. 5 is an example of a positive mode chromatogram and Fig. 6 is negative mode for the MeOH extract of River mile 188.6 (28-32 cm).

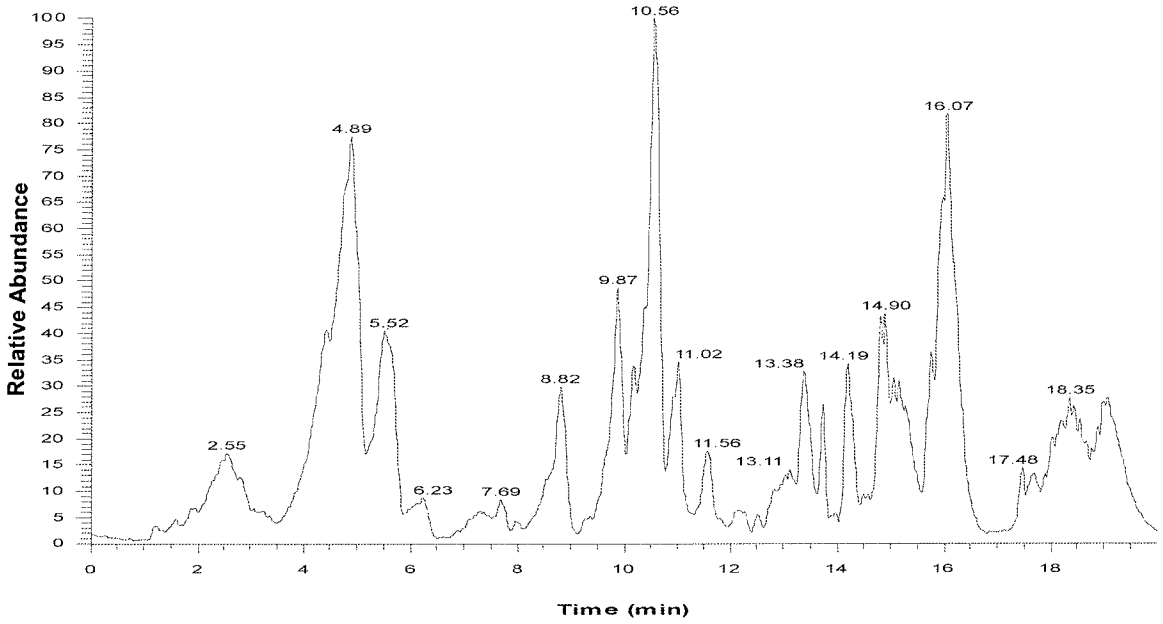

Figure 5. Positive mode LC chromatogram from River mile 188.6 (28-32 cm) MeOH extract.

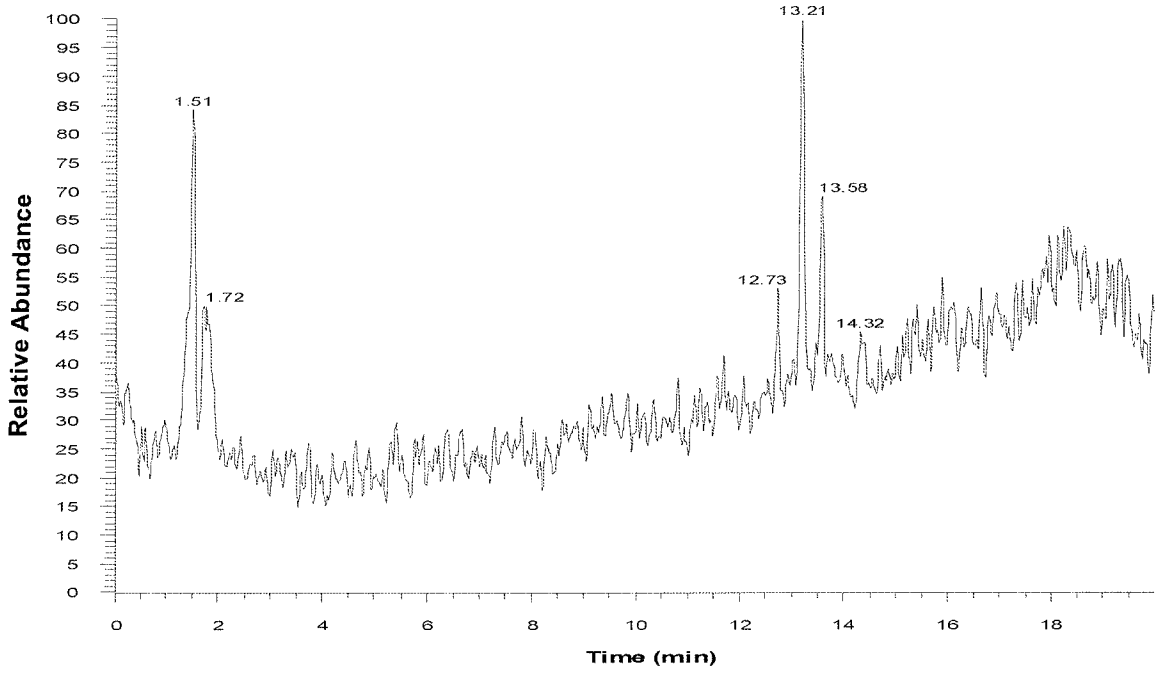

Figure 6. Negative mode LC chromatogram from River mile 188.6 (28-32 cm) MeOH extract.

Although positive mode had more ionizable compounds, negative mode showed two compounds of interest, tri- and di- chlorinated compounds at approximately 13.1 and 13.5 minutes from several of the extracts, specifically the hexane:IPA and MeOH extracts of River miles 152.7 (36-40 cm) and 188.6 (28-32 cm). Shown below in figs. 7, 8, and 9 is a chromatogram and mass spectra of the chlorinated compounds from the MeOH extract of River mile 152.7 (36-40 cm). The two peaks in River mile 152.7 (36-40 cm) MeOH extract correspond to the toxic effect of the fraction from this extract at 12-14 min shown in Fig. 10.

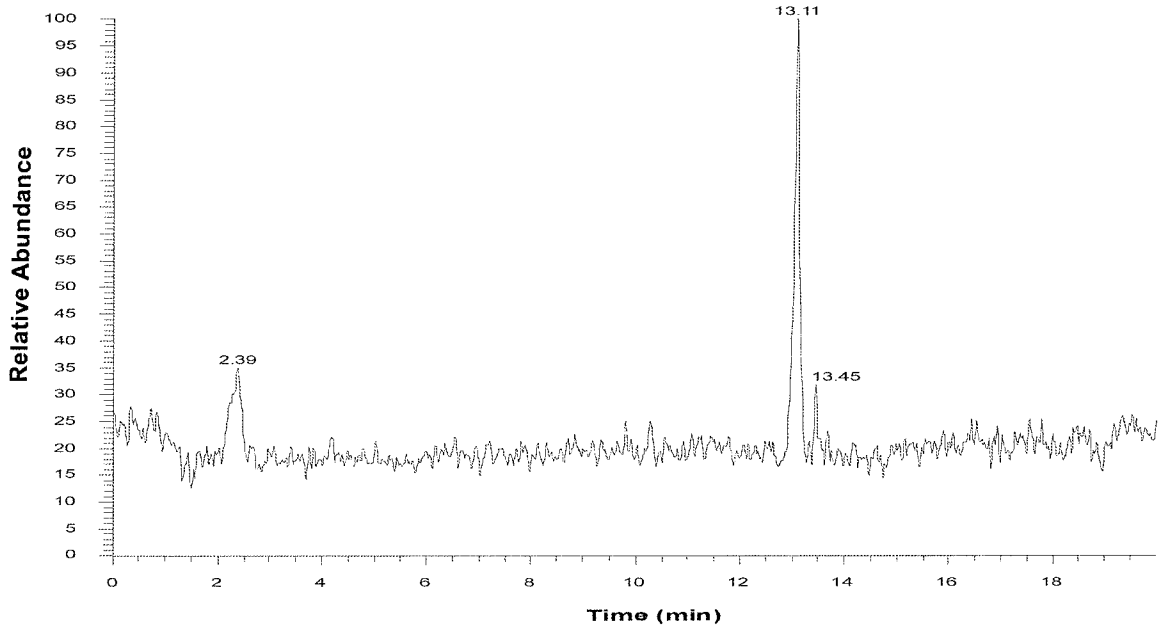

Figure 7. Negative mode LC chromatogram of River mile 152.7 (36-40 cm) MeOH extract.

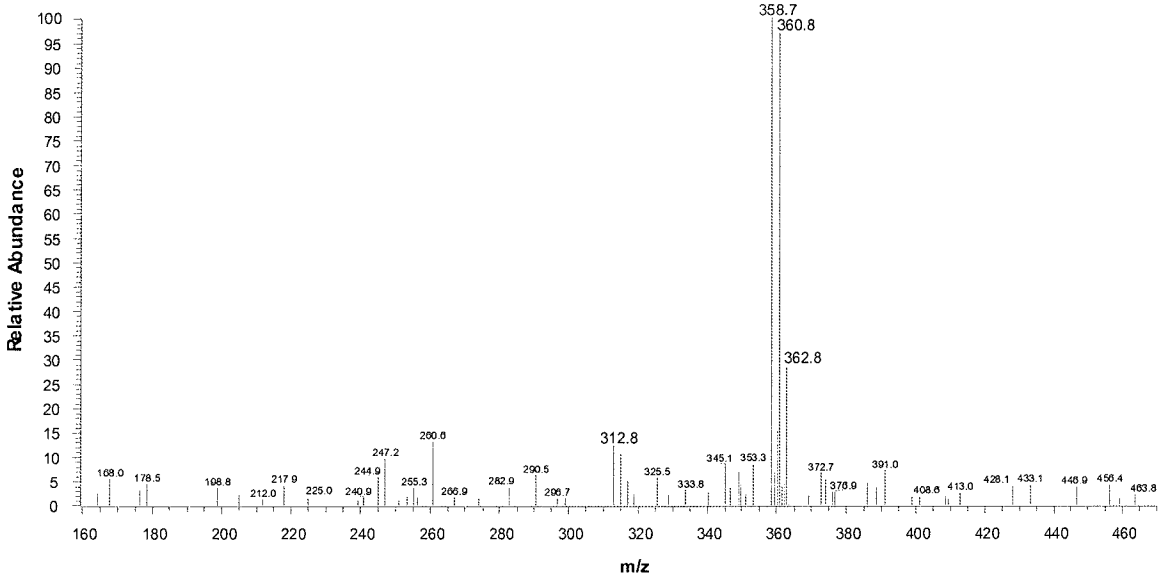

Figure 8. Mass spectra of peak at 13.11 min in Fig. 9 (trichlorinated compound)

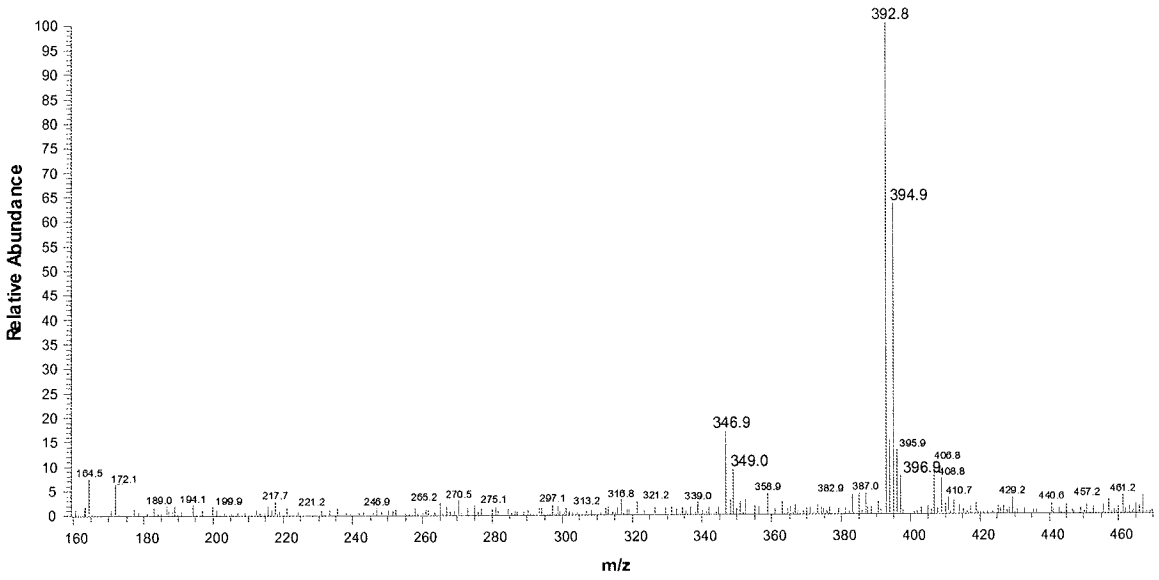

Figure 9. Mass spectra of peak at 13.45 min in Fig. 9 (dichlorinated compound).

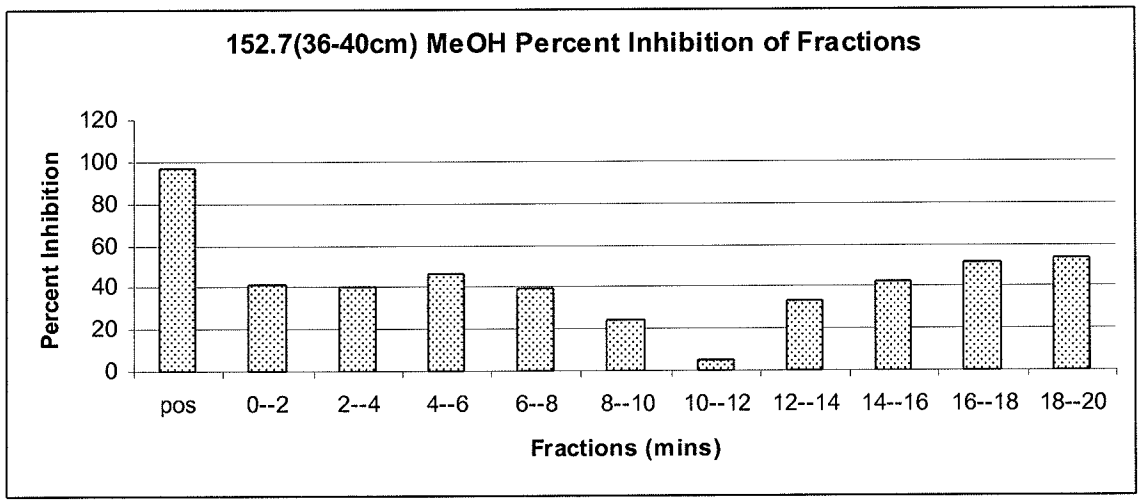

Figure 10. Percent inhibition of *Vibrio fischeri* by fractions from MeOH extract of River mile 152.7 (36-40 cm).

In Fig. 6 the tri-chlorinated compound is also observed at 12.73 minutes, which is confirmed by the mass spectra in Fig. 11.

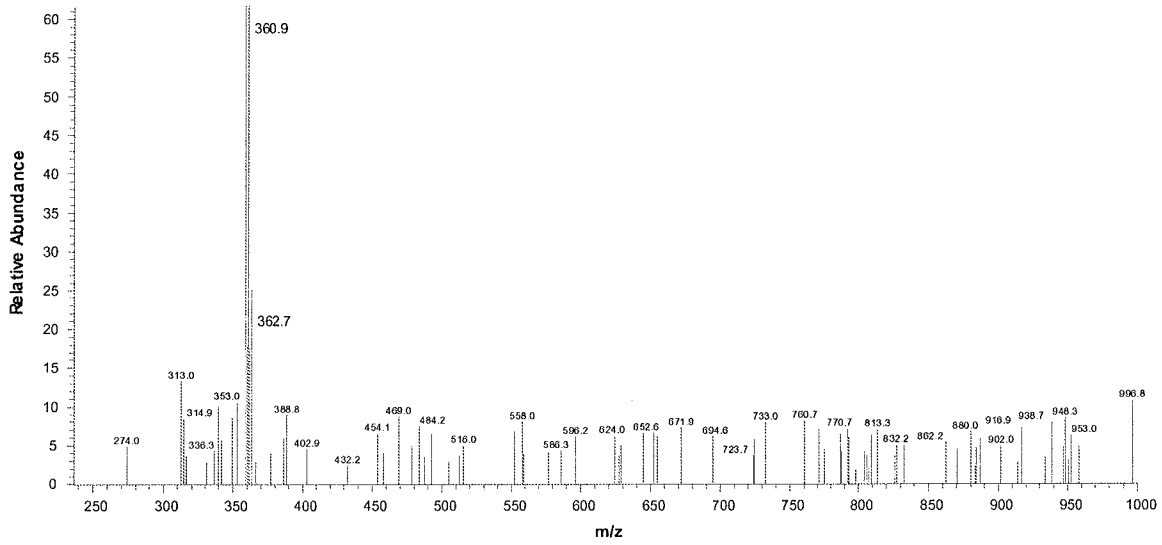

Figure 11. Mass spectra of peak at 12.73 min in the MeOH extract at River mile 188.6 (28-32 cm)

There is also a toxic response seen for the fraction 12-14 min from the MeOH extract of River mile 188.6 (28-32 cm) (Fig. 12).

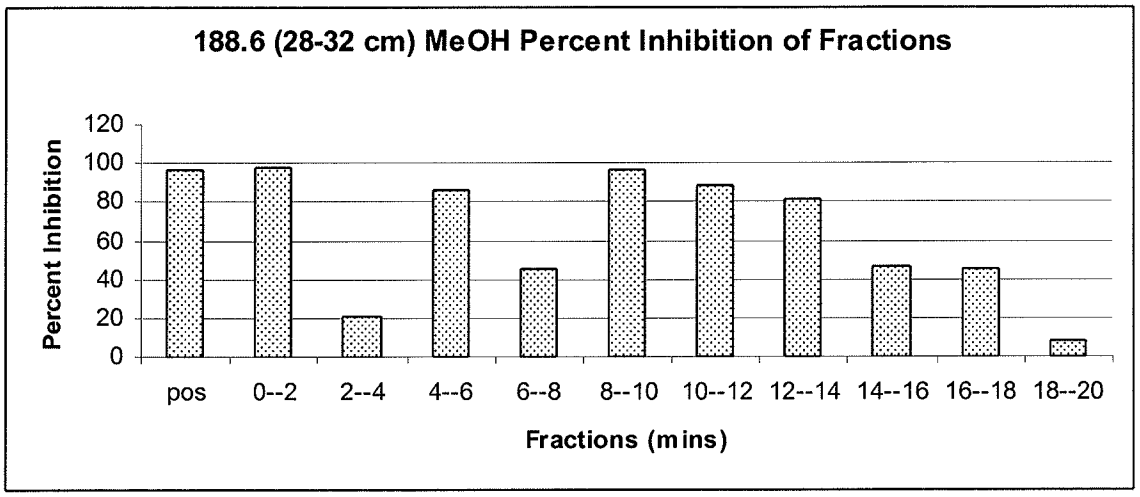

Figure 12. Percent inhibition of *Vibrio fisheri* by fractions from MeOH extract of River mile 188.6 (28-32 cm)

GC-MS analysis was also performed on all sediment extracts. Several peaks of interest were found. A library search provided possible matches for these peaks. Below

is Fig. 13 showing a GC chromatogram from the hexane extract of River mile 163.6 (2-4 cm). One peak of interest at 3.5 min is a 94% library match for 1-chloro-4-(trifluoromethyl)-benzene (Fig. 14).

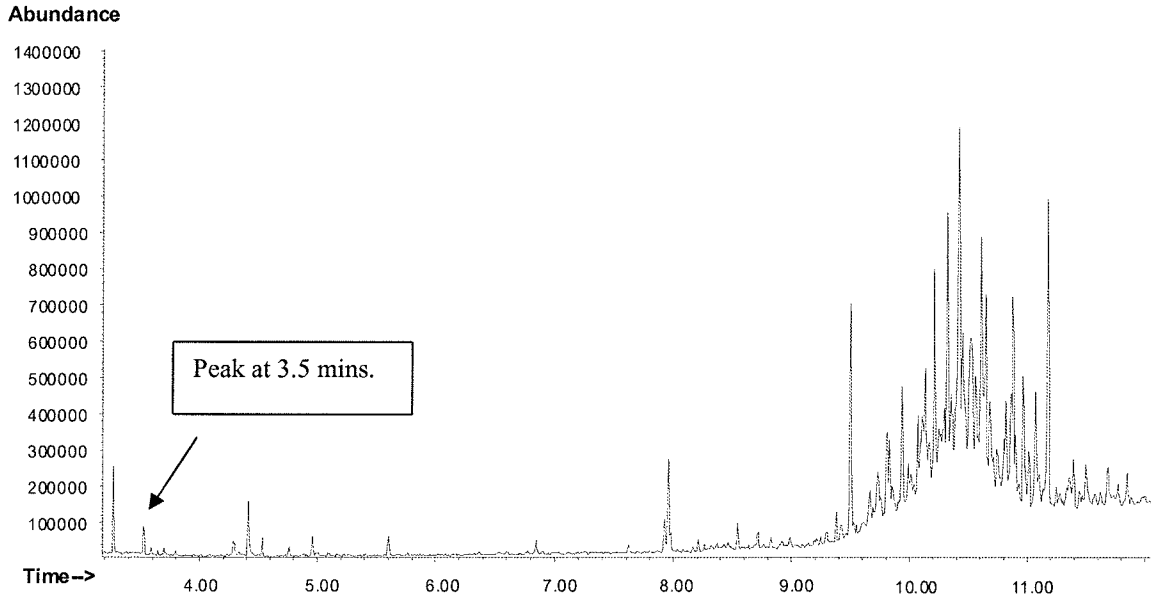

Figure 13. GC chromatogram from hexane extract of River mile 163.6 (2-4 cm).

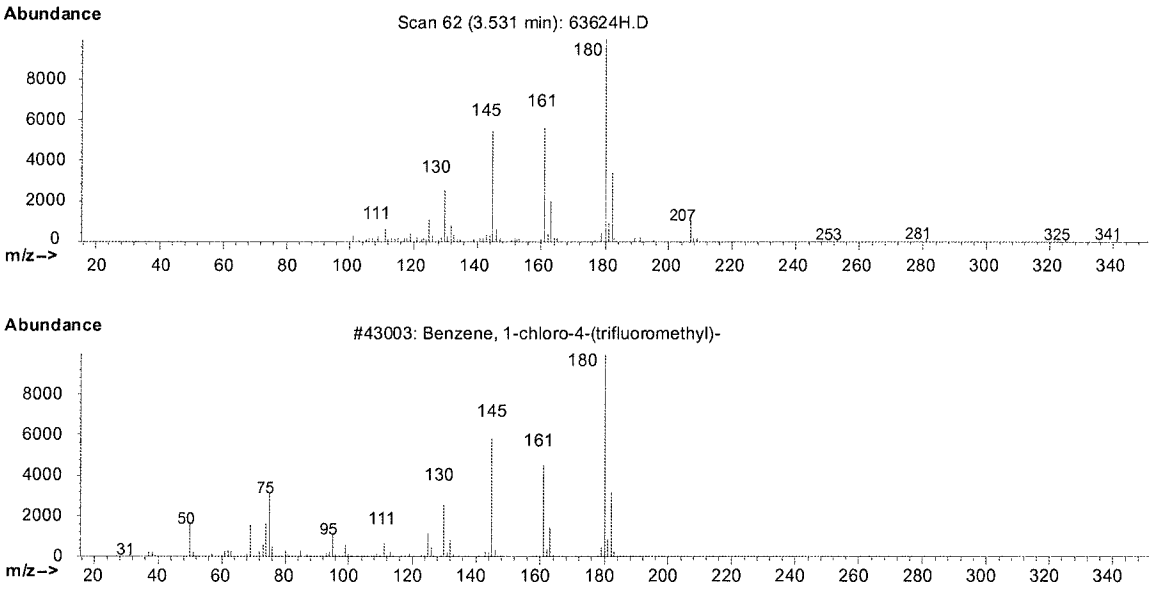

Figure 14. Mass spectra and library match for peak at 3.5 min in Fig. 15 (hexane extract of River mile 163.6 (2-4 cm)).

During GC-MS analysis PCBs were also found in the extracts. Mono- to penta-chloro biphenyls were detected. Below in Fig. 15 is a chromatogram of the hexane: IPA extract from mile 163.6 (36-40 cm). The peak at 8.9 min corresponds to a dichloro biphenyl which is confirmed by the mass spectra in Fig. 16.

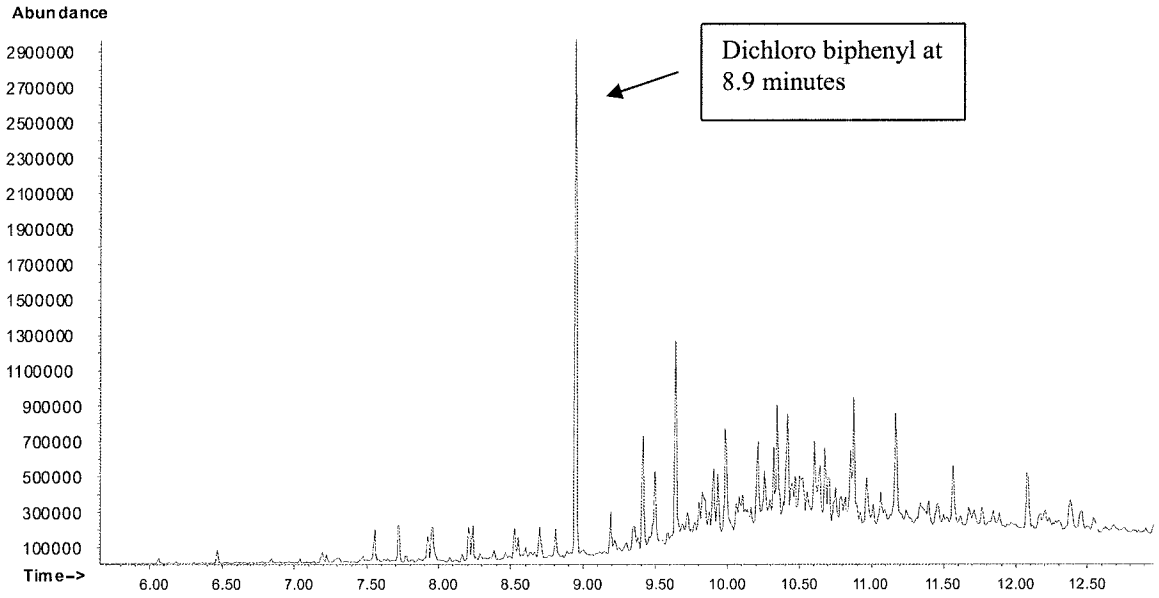

Figure 15. GC chromatogram of the hexane:IPA extract from River mile 163.6 (36-40 cm). Peak at 8.9 min. corresponds to a dichloro biphenyl.

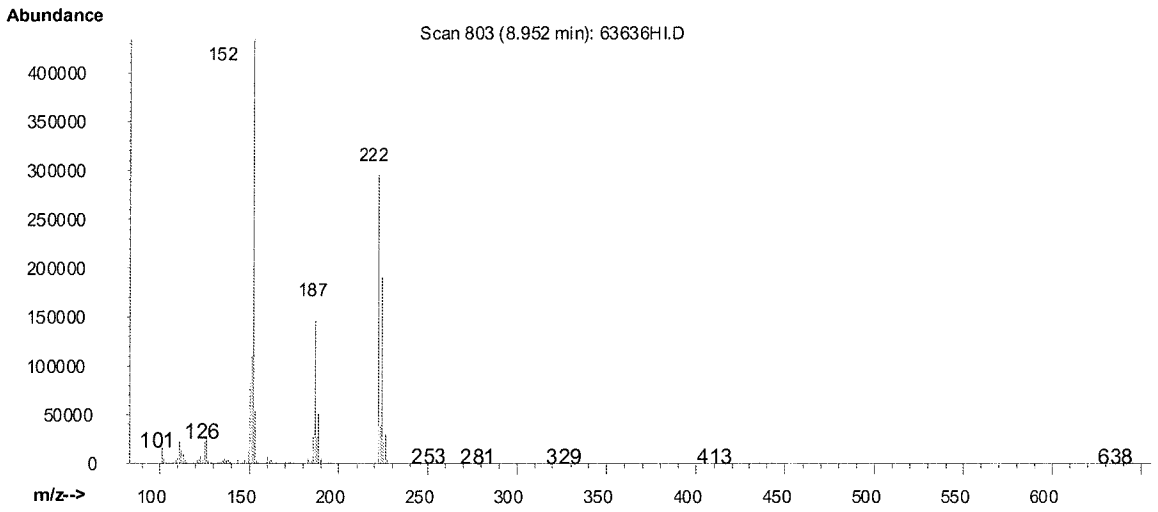

Figure 16. Mass spectra of dichloro biphenyl peak at 8.9 min in Fig. 17 from the hexane: IPA extract at mile 163.6 (36-40 cm).

Discussion

Our results indicate that extracts from Hudson River sediments are toxic in varying ways. Some extracts are highly estrogenic such as the MeOH extract of River mile 188.6 (28-32 cm), while others show dioxin or dioxin-like activity like the MeOH extract of River mile 152.7 (36-40 cm). However, all extracts inhibited the energy metabolism of *Vibrio fischeri* (Fig. 2). The Abratox^{TM} assay is mainly for water soluble compounds and the hexane extracts contain non-polar compounds that are unlikely to be water soluble. Some of the inhibition could be due to the presence of sulfur in the extracts (Brack et al. 1999), particularly in the hexane or hexane: IPA extract. As a result, all hexane and hexane: IPA extracts showed higher inhibition compared to the MeOH extracts due to the possible presence of sulfur. Sulfur can be removed by running extracts through a copper powder column.

The highly estrogenic extract (Fig. 4) from mile River 188.6 (28-32 cm) does not exhibit high dioxin or dioxin-like activity (Fig. 3); however it does exhibit high inhibition

to *Vibrio fischeri*. This indicates that it is likely the MeOH extract for River mile 188.6 (28-32 cm), does not have a large concentration of dioxins or similar compounds but has a high concentration of highly polar, water soluble compounds that are exhibiting high estrogenicity. Further analysis for estrogenicity needs to be conducted on fractions from the MeOH extract to identify the estrogenic compounds.

The fractions from River mile 188.6 (28-32 cm) extract exhibit increased inhibition for *Vibrio fischeri* (Fig. 12) indicating that there is more than one component in the extract that is toxic and that the toxic effects are non-additive, similar to what was seen by Suzuki et al. (2004), Sundberg et al. (2005) and Schlenk et al. (2005). Further analysis of fractions by combining them to determine fraction and mixture toxicity needs to be completed.

Schlenk et al. (2005) found that high concentrations of estrogens did not correspond to biological response. It was thought that there was co-extraction of anti-estrogenic and androgenic compounds. Also, they found that fractions did not yield any identification of sex-steroids as potential toxic agents but the occurrence of polar compounds in extracts did produce a biological response. Taking this into account, it is possible that the toxicity seen in the more polar fractions from the MeOH extract of River mile 188.6 (28-32 cm) could be due to degradation products.

Brack et al. (1999) determined that photodegradation products are possibly more phytotoxic and produce photo-induced ecotoxicity where water depths allow for photolysis of contaminants. This occurrence results in the possibility of these sediment samples having more toxic and polar degradation products due to photodegradation.

However, we found that extracts contained other toxics but we could not identify those compounds.

The tri-chlorinated compound found in the MeOH extract from River mile 188.6 (28-32 cm) at 12.7 minutes (Fig.6 and Fig. 11) corresponds to a toxic effect for fraction 12-14 minutes from the HPLC (Fig. 12). However, further analysis needs to be completed before this compound is identified.

A di-chlorinated compound was found in the extract from River mile 152.7 (36-40 cm) at 13.5 minutes (Fig. 9) which could contribute to the toxicity exhibited in the corresponding fraction from this extract (Fig. 10). More MS^2 and MS^3 data need to be collected for these peaks and also for peaks in positive mode before identification can be made.

GC-MS revealed more possible compounds through library matches. For example, the peak in the GC chromatogram for the hexane extract from River mile 163.6 (2-4 cm) (Fig. 13) at 3.5 minutes provides a library match of 1-chloro-4-(trifluoromethyl) benzene (Fig. 14) at 94%, indicating that some non-polar compounds are not detected by LC-ITMS.

In addition, PCBs were detected in the extracts by GC-MS analysis. Confirmation was seen in the mass spectra for a di-chloro biphenyl from the hexane: IPA extract for mile 163.6 (36-40 cm) at 8.9 minutes in the chromatogram in Fig 15 and Fig. 16. Further investigation needs to be done for the GC-MS data to determine which compounds are present.

Future work for this study involves not only more fractionation of extracts until single components are isolated for identification by Fourier transform Ion Cyclotron mass

spectrometry (FT-ICR) but also determination of toxicity relative to that of the mixture. Also, further inquiry into the role of pH and adsorption, or *in-situ* conditions, in toxicity needs to be conducted. Adsorption and pH could affect bioavailability for aquatic organisms and humans that use the river. Once bioavailability is determined further exposure studies can be conducted to determine the real risk to humans and wildlife from these contaminants.

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ASSIMILATION AND SUBCELLULAR DISTRIBUTION OF DIETARY Hg BY GRASS SHRIMP, *PALAEMONETES PUGIO*, COLLECTED ALONG AN ENVIRONMENTAL IMPACT GRADIENT

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

Chronic exposure to polluted field conditions can impact the bioavailability of metal within prey organisms and the assimilation of dietary metal by predators. The present study investigated the assimilation of dietary Hg by grass shrimp, *Palaemonetes pugio,* collected along an environmental impact gradient within the New York/New Jersey Harbor Estuary. Amphipods, *Gammarus lawrencianus*, were exposed for 2 d to seawater containing the radiotracer ^{203}Hg. A subsample of amphipods was subjected to subcellular fractionation and a compartment containing trophically available metal (TAM) (i.e., metal associated with heat-stable proteins [HSP – e.g., enzymes], heat-denatured proteins [HDP – e.g., metallothionein-like proteins] and organelles [ORG]) was reconstructed to estimate the percentage of ^{203}Hg potentially available to predators. Adult grass shrimp were collected from five Staten Island, New York study sites, fed radiolabeled amphipods and subjected to assimilation efficiency (AE) analysis and subcellular fractionation. TAM-^{203}Hg% in amphipods was ~72.5%. A gradient in AE-^{203}Hg% from ~67% to ~85% was observed, with the highest value for shrimp collected from Travis Avenue, a site located along a tributary of the heavily polluted Arthur Kill. The percentages of assimilated ^{203}Hg associated with the subcellular fractions containing metal-sensitive HDP and ORG as well as cellular debris did not vary for grass shrimp from the study sites. Partitioning of assimilated ^{203}Hg to the biologically-detoxified HSP fraction was not observed in contrast to the partitioning of assimilated ^{109}Cd observed previously in field-collected shrimp. Population differences in the assimilation of metal and metal-specific variability in the partitioning of assimilated metal may have important toxicological implications for impacted grass shrimp populations.

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INTRODUCTION

Organisms within heavily industrialized estuaries such as the New York/New Jersey (NY/NJ) Harbor Estuary are typically exposed to suites of metal, organic and chemical pollutants through multiple pathways (i.e., dissolved, sediment and diet) (Feng et al. 1998, Wang and Fisher 1999a, Steinberg et al. 2004). Concentrations of total and bioavailable metals (e.g., Cd, Cu, Hg, Pb and Zn) in sediments can vary considerably within urban estuaries and may have implications for metal toxicity for resident biota and metal cycling (e.g., trophic transfer) within impacted communities (Berry et al. 1996, Wolfe et al. 1996, Ward 2002, Nichols 2004). For example, population differences in metal accumulation, tissue concentrations of metal-binding metallothionein-like proteins (MTLP), sublethal toxicity (e.g., metabolism, prey capture and predator avoidance) and genetic resistance may be related to high contaminant loads in the field (Kraus and Kraus 1986, Klerks and Levinton 1989, Couillard et al. 1993, Khan and Weis 1993, Paulson et al. 2003, Perez and Wallace 2004, Manyin and Rowe 2006). Chronic exposure to impacted field conditions can also influence the bioavailability of metal within prey organisms and the assimilation of dietary metal by predators (Wallace and Lopez 1996, 1997, Wallace et al. 1998, Rainbow et al. 2004a, Seebaugh and Wallace 2005).

Several recent studies suggest that the transfer of metal along estuarine food chains may be more closely related to the subcellular compartmentalization of metal rather than to whole tissue burdens within prey (Wallace et al. 1998, Wallace and Luoma 2003, Seebaugh and Wallace 2004). For example, a ~1:1 relationship between metals (i.e., Cd and Zn) stored within specific subcellular fractions (i.e., heat-stable proteins [HSP - e.g., MTLP], heat-denatured proteins [HDP - e.g., enzymes] and organelles [ORG]) has been

shown for a variety of soft-bodied estuarine invertebrates (e.g., oligochaetes, bivalves, brine shrimp and amphipods) and metal assimilation by grass shrimp predators (i.e., *Palaemonetes pugio* and *Palaemon macrodatylus*) (Wallace et al. 1998, Wallace and Luoma 2003, Seebaugh and Wallace 2004). Due to this potential bioavailability to predators, metals stored within these subcellular fractions within prey may be considered collectively as a subcellular compartment containing trophically-available metal (TAM) (Wallace and Luoma 2003).

The assimilation of ingested elements (e.g., organic carbon and metals) has been characterized for a wide variety of estuarine invertebrates (e.g., bivalves, polychaetes and crustaceans) as well as fish (Reinfelder and Fisher 1991, 1994a, 1994b, Wang and Fisher 1996, Wang et al. 1996, Baines et al. 2002, Nunez-Nogueira et al. 2006). Assimilation efficiencies (AE) may be influenced by a number of biological and environmental factors including diet composition, the partitioning of elements within food organisms (e.g., metal partitioned as TAM), predator digestive physiology (e.g., gut residence time, gut pH, digestive enzyme activities and absorption by the gut epithelium) and previous exposure to contaminants (Reinfelder and Fisher 1991, Wang and Fisher 1996, Wallace and Lopez 1997, Reinfelder et al. 1998, Wallace et al. 1998, Blackmore and Wang 2002, Chen et al. 2002, Rainbow et al. 2004b). The toxicity of ingested metal may be related to the subcellular partitioning of metal following assimilation (Wallace et al. 2003, Cheung et al. 2006). For example, metal bound to MTLP or sequestered as insoluble metal-rich granules (MRG) is biologically-detoxified and would not be expected to impact cellular functions (Hopkin and Nott 1979, Wallace et al. 2003, Amiard et al. 2006). Conversely, metal associated with metal-sensitive intracellular components (e.g., organelles and

enzymes) may be expected to elicit toxicity at the cellular level (Wallace et al. 2003).

In previous work, Seebaugh and Wallace (2005) observed a gradient in the assimilation of dietary Cd among grass shrimp collected from salt marshes within the NY/NJ Harbor Estuary. Enhanced assimilation of Cd was observed in grass shrimp collected from sites adjacent to a heavily polluted waterway relative to shrimp from a relatively 'clean' site. Grass shrimp were also analyzed for the subcellular partitioning of assimilated Cd (Seebaugh and Wallace unpublished). The percentages of assimilated Cd associated with biologically-detoxified HSP (e.g., MTLP) and metal-sensitive HDP (e.g., enzymes) varied considerably among shrimp from these study sites, which may have important implications for Cd toxicity and detoxification (Wallace et al. 2003). Despite differences in the partitioning of assimilated metal, the percentages of Cd partitioned to the TAM compartment (i.e., the fraction of metal potentially available to predators) in grass shrimp was similar at ~65% to ~73% (Seebaugh and Wallace unpublished).

Study organism

The daggerblade grass shrimp, *Palaemonetes pugio* Holthius 1949 (Decapoda, Caridea), is abundant in estuaries along the eastern coast of North America and can persist under a wide range of ecological conditions (e.g., dissolved oxygen, salinity, temperature and vegetation) (Wood 1967, Nixon and Oviatt 1973, Welsh 1975, Alon and Stancyk 1982, Kneib 1987, Tashiro et al. 1994). This species has an omnivorous diet that can vary according to seasonal abundances of food items and may include diatoms, epiphytic microalgae, plant detritus and an assortment of invertebrates (e.g., copepods, mysids, postlarval crabs, nematodes, oligochaetes and polychaetes) (Welsh 1975, Bell and Coull 1978, Morgan 1980, Chambers 1981, Kneib 1985, Olmi and Lipcius 1991,

Tashiro et al. 1994, Gregg and Fleeger 1998, Quinones-Rivera and Fleeger 2005). Grass shrimp are also important in the diets of several common finfish species, including the mummichog, *Fundulus heteroclitus*, and striped bass, *Morone saxatilis*, and may serve as a vector of metals to higher trophic levels (Nixon and Oviatt 1973, Kneib and Stiven 1982, Posey and Hines 1991, Davis et al. 2003, Carson and Merchant 2005).

Mercury uptake and toxicity in grass shrimp may be influenced by ecological factors, including previous field exposure and parasitism (Kraus and Kraus 1986, Kraus and Weis 1987, Kraus et al. 1988, Bergey et al. 2002). For example, grass shrimp collected from a clean reference site exhibited differences in predator avoidance following exposure to $HgCl_2$ or methylmercury (Kraus and Kraus 1986). Previous exposure to pollutants in the field can also impact the tolerance of larval and adult grass shrimp to subsequent Hg exposure as well as the induction of MTLP (Kraus et al. 1988). Perez and Wallace (2004) observed that grass shrimp, collected along an environmental impact gradient within the NY/NJ Harbor Estuary, exhibited impaired prey capture that may be related to exposure to high levels of sediment-bound contaminants, including metals (e.g., Cd, Cu, Hg, Pb and Zn) and organic pollutants. Grass shrimp exposed to sediment contaminated with metal-rich coal combustion residues accumulated higher whole tissue concentrations of Cd and Se, yet had lower Hg burdens relative to controls (Kuzmick et al. 2007).

The purpose of the present study was to investigate intraspecific variation in the assimilation and subcellular distribution of dietary Hg by grass shrimp collected along an environmental impact gradient within the NY/NJ Harbor Estuary. This work was conducted using radiotracer (^{203}Hg) pulse-chase feeding experiments and subcellular

fractionation techniques. The specific goals of this study were 1) to investigate the influence of field conditions on the assimilation of dietary Hg by grass shrimp, 2) to determine the relationship between Hg assimilation by field-collected grass shrimp and the partitioning of Hg to the TAM compartment within amphipod prey and 3) to examine differences in the subcellular partitioning of assimilated Hg within field-collected shrimp.

MATERIALS AND METHODS

Seawater

Prior to radiolabeling, amphipods were maintained in seawater obtained from the Rutgers University Marine Field Station, located near the mouth of Mullica River in Tuckerton, NJ, USA (TK) (Figure 1). This location has served as a reference site in previous ecotoxicological studies (Weis et al. 2001, Perez and Wallace 2004). Field-collected grass shrimp were maintained and subjected to assimilation efficiency (AE) analysis in site water from their respective collection sites. Site water was diluted with deionized water and filtered as described below.

Field sampling

Adult amphipods, *Gammarus lawrencianus*, (5 to 10 mm in length) were collected by dip net from Great Kills Harbor, Staten Island, NY (GK) and maintained in aerated, filtered TK seawater (1.0 μm filter, 10 ppt, 18-19 °C) (Figure 1). Water filtration was provided by an aquarium filter equipped with a siphon lined with nylon filter material to prevent entrapment of amphipods. Amphipods were fed daily on commercial fish flakes, but were held in a 1-mm screen and allowed to clear their guts for 24 h prior to radiolabeling.

Grass shrimp for this study were collected from five salt marshes surrounding Staten Island, NY, located within the Harbor Core Area of the NY/NJ Harbor Estuary (Figure 1) (Steinberg et al. 2004). Three of these sites are located immediately adjacent to the Arthur Kill (AK), which separates the western shore of Staten Island from New Jersey and connects Newark and Raritan bays. Waters within the AK have long residence times and low rates of flushing and have been subjected to metal, organic pollutant (e.g., fuel oil spills) and hazardous chemical discharges from industrial activities, oil refinery operations and combined sewer overflows over many decades (Olsen et al.1984, Oey et al. 1985, Gillis et al. 1993, Gunster et al. 1993, Crawford et al. 1995, Bergen et al. 2000). GK is located along the southeastern shore of Staten Island. This small harbor is not impacted by industrial activities, but may receive contaminants from runoff, marina operations and a nearby sewage treatment facility. Lemon Creek (LC) is a small tidal creek on the southern shore of Staten Island that empties directly into Raritan Bay and is ~4 km east of the confluence between the AK and the Raritan River. Pollution impacts at LC are low compared to the sites located immediately adjacent to the AK, although sources of contamination may include urban runoff, marina operations and remediation of the former site of dental manufacturing facility adjacent to the creek (Ward 2002). Travis Avenue (TA) (also known as Main Creek) is located along a tributary that extends ~3 km from the AK and may be susceptible to leachate contamination from nearby landfills. Meredith Marsh (MM) lies along Neck Creek, which extends ~0.6 km eastward from the AK. Nassau Creek (NC) (also known as Mill Creek) is a small tidal creek that extends ~0.5 km from the lower portion of the AK and

~1 km from Raritan Bay. NC was subjected to metal discharges from a smelting facility from the mid-1930s until the 1970s (Ward 2002).

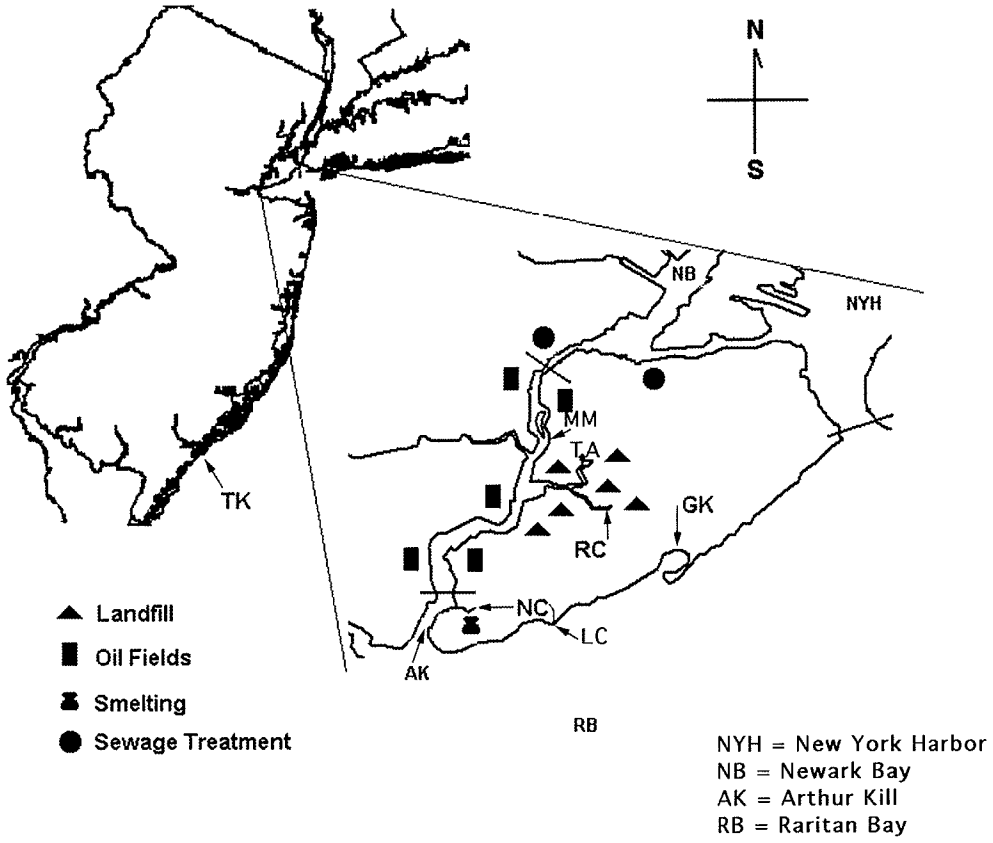

Figure 1. Map indicating the location of collection sites for seawater, amphipods (*Gammarus lawrencianus*) and grass shrimp (*Palaemonetes pugio*) (Great Kills - GK; Lemon Creek – LC; Nassau Creek – NC; Travis Avenue – TA; Meredith Marsh – MM; Richmond Creek – RC; Tuckerton, NJ – TK). Adapted from Ward 2002.

Adult grass shrimp (~3 cm in length) were collected by dip net from each study site 3 d prior to AE analysis, during a 16 d period from mid July to early August 2007 (Figure 1). Gravid females were identified in the field and excluded from analysis (Bauer and Abdalla 2000). Shrimp were maintained in 38 l of filtered, aerated seawater from their respective collection sites (63 μm, 10 ppt, 18-19 °C). When necessary, site water was adjusted to 10 ppt with deionized water. To prevent ingestion of fecal strands, grass

shrimp were housed above a 3-mm mesh partition positioned ~10 cm above the floor of the aquarium. Shrimp were fed once on fish flakes following collection and allowed to clear their guts prior to feeding on amphipods.

Radiolabeling of amphipod prey

Amphipods were exposed for 48 h in 4 l glass bottles containing 3 l of aerated, filtered TK seawater diluted with NANOpure® (Barnstead) deionized water (0.4 μm, 10 ppt, 18-19 °C) and spiked with $^{203}HgCl_2$ in 1N HCl (~0.04 amphipods ml^{-1}) (Isotope Products). The ^{203}Hg activity of the radiolabeling solution was 9.25 kBq l^{-1} (~0.005 μM Hg) and was verified through radioanalysis of 5 ml samples. Acidification of the radiolabeling solution resulting from the addition of radioisotope was offset by the addition of 1N NaOH. Aeration was provided by a 1 ml (35 cm long) glass pipette tip connected to an aquarium air pump via flexible tubing. Following exposure, amphipods were transferred to a 63-μm screen using a peristaltic pump, rinsed three times with clean TK seawater (10 ppt) and stored frozen (-80 °C) in scintillation vials.

AE analysis

Following clearance of gut contents, grass shrimp were transferred to beakers (4 beakers per study site; 4 shrimp per beaker) containing site water (63 μm, 10 ppt, 18-19 °C) and allowed to feed on 5 to 6 ^{203}Hg-labeled amphipods for ~30 min (before the release of ^{203}Hg-labeled fecal strands). Following feeding, shrimp that emitted strong ^{203}Hg signals (i.e., shrimp that consumed sufficient quantities of amphipod tissue) (n = 8 to 11) were rinsed three times with clean site water (10 ppt), transferred to scintillation vials containing site water and analyzed for ^{203}Hg activity (time [t] = 0) (Wallace et al.

1998, Wallace and Luoma 2003). Grass shrimp were maintained in individual mesh-lined chambers within a 38 l aquarium containing filtered site water (63 μm, 10 ppt, 18-19 °C), where they depurated ^{203}Hg for 7 d (Wallace and Luoma 2003, Seebaugh and Wallace 2005). Chambers were suspended above the floor of the aquarium to prevent the consumption of fecal strands (Johannes and Satomi 1966). During depuration, aquarium water was filtered through activated carbon to reduce exposure to dissolved ^{203}Hg from radiolabeled fecal strands. A portion of the water overflow from the aquarium filter was distributed among the individual chambers. ^{203}Hg activities in the aquarium water were monitored through radioanalysis of 5 ml samples and remained at background. Shrimp were fed daily on commercial fish flakes. Individual grass shrimp were removed from the aquarium and analyzed for ^{203}Hg activity at t = 2, 4, 8, 12 and 24 h and approximately every 24 h thereafter for 7 d. A linear regression was fit to the physiological loss component of each retention curve (t > 24 h) (Wang and Fisher 1999b). The *y*-intercept of each regression was used to estimate ^{203}Hg assimilation efficiency (AE-^{203}Hg%) and the corresponding slope was used to estimate the rate of physiological ^{203}Hg loss by grass shrimp from each study site (Wallace et al. 1998, Wang and Fisher 1999b, Seebaugh and Wallace 2004, Seebaugh et al. 2005, 2006).

Subcellular fractionation

Subsamples of radiolabeled amphipods (n = 4; 10 amphipods per replicate) were subjected to a five-part subcellular fractionation procedure to determine the percentages of ^{203}Hg associated with the TAM (HSP, HDP and ORG) and non-TAM (insoluble components [INS – e.g., MRG] and cellular debris [CD – e.g., cell membranes]) fractions (Wallace et al. 1998, Wallace and Luoma 2003). Following AE analysis, grass shrimp (*n*

= 4; 2 to 3 shrimp per replicate) were subjected to this procedure to determine the distributions of assimilated ^{203}Hg among individual fractions (Wallace et al. 1998, Wallace and Luoma 2003). Animals were thawed on ice, wet-weighed and homogenized in 3.3 ml (amphipods) or 5 ml (grass shrimp) of cold Tris buffer (pH 7.6) using a Polytron® (Kinematica) tissue homogenizer. Homogenized tissues were centrifuged at 500 x *g* (15 min at 4 °C). Following removal of the supernatant, the pellet was resuspended in 3.3 ml of Tris buffer and heated at 100 °C for 2 min. An equal volume of 1 N NaOH was added and this suspension was heated at 65 °C for 1 h. The suspension was then centrifuged at 4500 x *g* (15 min at 4 °C) and the supernatant containing CD was removed. The pellet was resuspended in 1 N NaOH and centrifuged a second time (4500 x *g*). Supernatants were combined with dissolved CD collected previously. The pellets containing INS were also recovered (Silverman et al. 1983, Wallace et al. 1998).

The 500 x *g* supernatants were centrifuged at 100,000 x *g* (1 h at 4 °C) to produce a pellet containing ORG. The supernatants were heated at 80 °C for 10 min and cooled on ice for 1 h (Bebianno et al. 1992). The heat-treated cytosol was centrifuged at 38,000 x *g* (30 min at 4 °C) to pellatize HDP, while HSP remained in the supernatant. Isolated subcellular fractions were transferred to scintillation vials and analyzed for ^{203}Hg activity.

Radioanalysis

Radiolabeled samples were analyzed for ^{203}Hg by determining photon emissions (279 keV) using a Wallac Wizard™ 1480 automatic gamma counter equipped with a 7.6 cm NaI crystal. Counting times for live grass shrimp and for subcellular fractions isolated from amphipods and shrimp were adjusted to maintain propagated counting errors of 5% or less (Wallace et al. 1998). ^{203}Hg activities associated with specific

subcellular fractions were used to reconstruct subcellular compartments containing TAM (TAM-^{203}Hg% = HSP-^{203}Hg% + HDP-^{203}Hg% + ORG-^{203}Hg%) in amphipods and grass shrimp and metal-sensitive fractions (MSF-^{203}Hg% = HDP-^{203}Hg% + ORG-^{203}Hg%) in shrimp (Wallace et al. 2003, Wallace and Luoma 2003).

Statistical analysis

The linear regression of the physiological loss component of each ^{203}Hg retention curve was generated using SigmaPlot Version 8.02 (SPSS). The effects of study site on AE-^{203}Hg% values, rates of physiological ^{203}Hg loss and the partitioning of assimilated ^{203}Hg to subcellular fractions (and compartments) were analyzed using one-way ANOVA (fixed effects model) (Sokal and Rohlf 1995, Zar 1999). Percentage fractionation data were arcsine transformed and normality was tested using Shapiro-Wilk's *W* test (Sokal and Rohlf 1995). Homogeneity of variances was tested using Levene's test. Unplanned (i.e., *a posteriori*) comparisons between AE-^{203}Hg% values and rates of ^{203}Hg loss were performed using the Tukey-Kramer multiple comparisons test (Sokal and Rohlf 1995, Zar 1999). AE-^{203}Hg% by grass shrimp and TAM-^{203}Hg% in amphipods were compared using the *t*-test. Statistical analyses were performed using Instat version 3.0a (GraphPad Software) and STATISTICA version 7.1 (StatSoft).

RESULTS

TAM-^{203}Hg% in amphipods

The percentages of ^{203}Hg partitioned to individual subcellular fractions (i.e., HSP, HDP, ORG, INS and CD) within amphipods were determined following homogenization, tissues digestion and differential centrifugation (Figure 2). TAM-^{203}Hg% was ~72.51%.

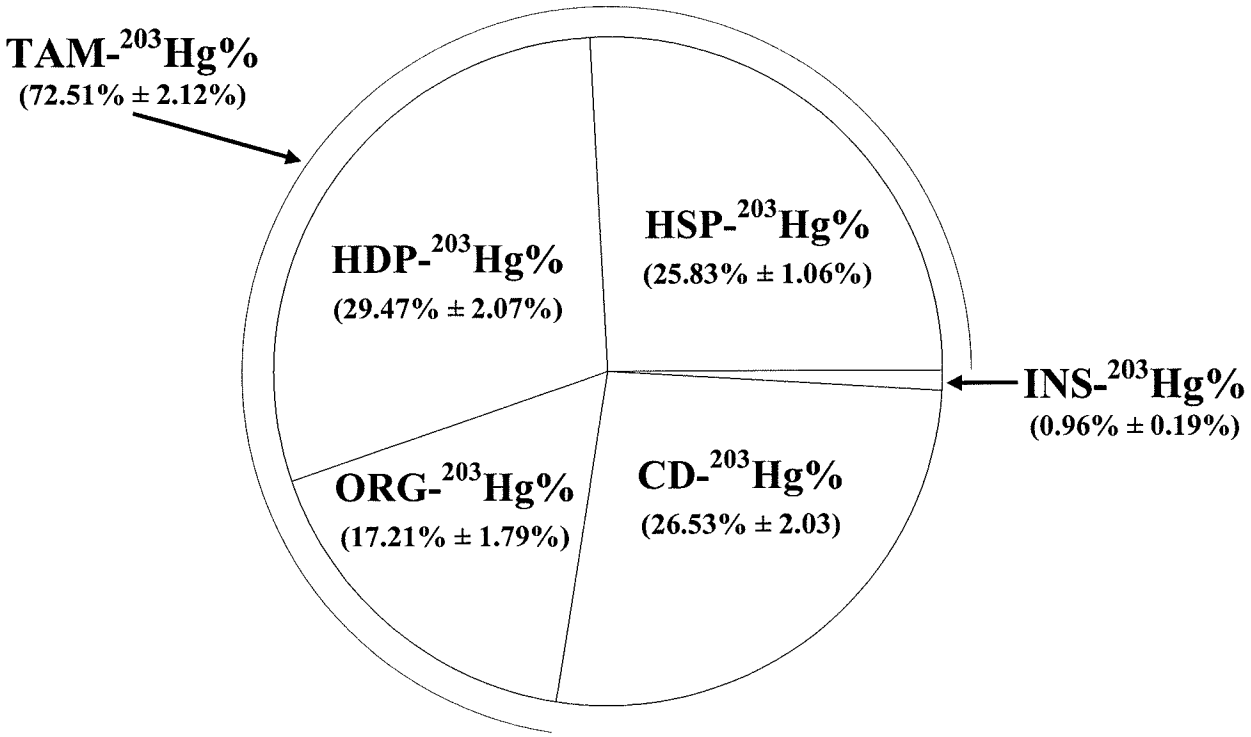

Figure 2. Subcellular distribution of ^{203}Hg in *G. lawrencianus*: heat-denatured proteins (HDP-^{203}Hg%), heat-stable proteins (HSP-^{203}Hg%), organelles (ORG-^{203}Hg%), insoluble components (INS-^{203}Hg%), cellular debris (CD-^{203}Hg%) and trophically-available metal (TAM-^{203}Hg%) (n = 5, 10 amphipods per replicate; mean ± SE).

AE-^{203}Hg% by grass shrimp collected along an environmental impact gradient

AE-^{203}Hg% was determined for grass shrimp collected from each of five Staten Island, NY study sites and pulse-fed ^{203}Hg-labeled amphipods. Depuration of ^{293}Hg by grass shrimp was characterized by a three-compartment loss, with an initial rapid loss of ^{203}Hg resulting from the release of radiolabeled fecal strands from t = 0 to 24 h, followed by a reduced rate of metal loss until ~48 h (Figures 3 – 7). AE-^{203}Hg% by grass shrimp from each site was estimated as the y-intercept of the linear regression of the slowest exchanging compartment of the retention curve representing the physiological loss of assimilated metal (t = 48+ h). A gradient in dietary ^{203}Hg assimilation was observed with

AE-^{203}Hg% values ranging from ~68% and ~67% for grass shrimp from Great Kills Harbor (GK) and Lemon Creek (LC) to ~85% for shrimp from Travis Avenue (TA) (Figures 1, 3 – 8). The assimilation of ^{203}Hg by grass shrimp from Nassau Creek (NC) and Meredith Marsh (MM) was intermediate at ~81% and ~79%, respectively (Figures 5 and 7, 8). AE-^{203}Hg% by grass shrimp from TA exceeded TAM-^{203}Hg% in amphipod prey (Figure 8). Rates of physiological ^{203}Hg loss did not differ among grass shrimp collected from the Staten Island study sites (Figure 9).

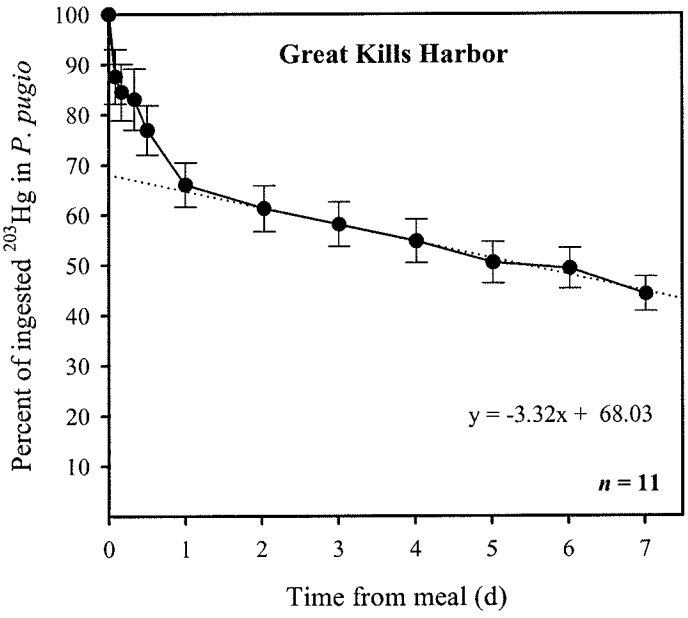

Figure 3. Time course in the retention of ^{203}Hg (n = 11; mean ± SE) by *P. pugio* collected from Great Kills Harbor (GK), Staten Island, New York.

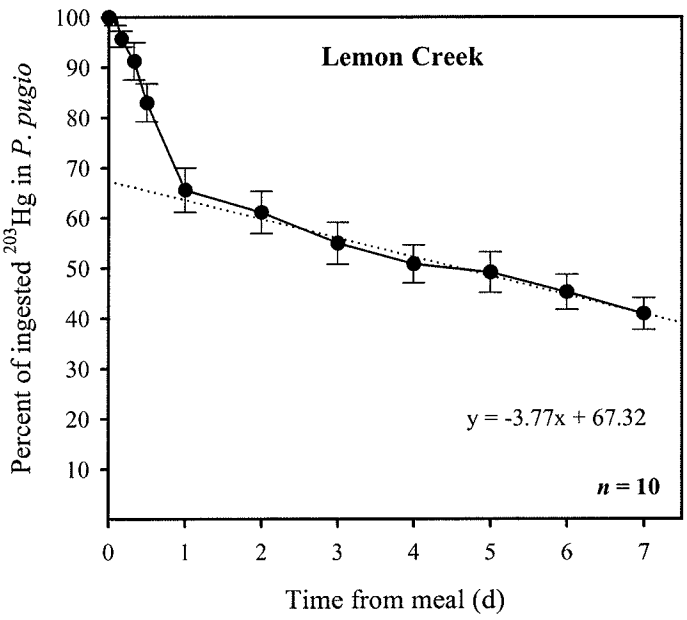

Figure 4. Time course in the retention of ^{203}Hg (n = 10; mean ± SE) by *P. pugio* collected from Lemon Creek (LC), Staten Island, New York.

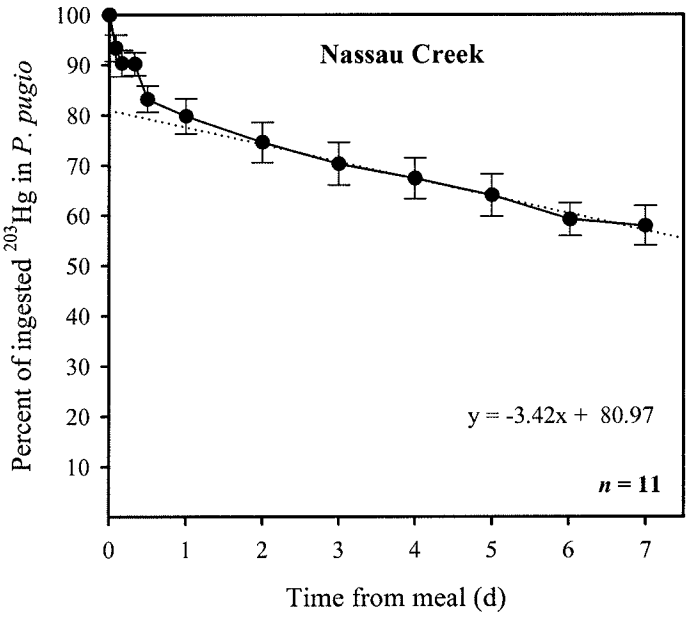

Figure 5. Time course in the retention of ^{203}Hg (n = 11; mean ± SE) by *P. pugio* collected from Nassau Creek (NC), Staten Island, New York.

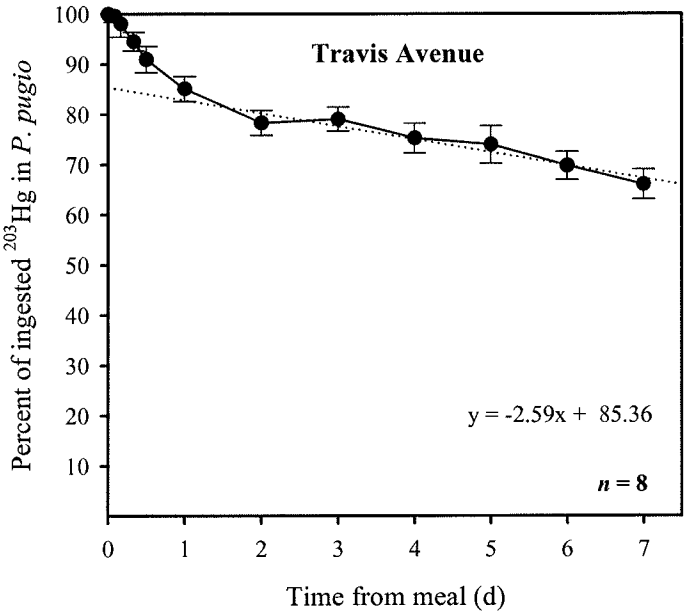

Figure 6. Time course in the retention of ^{203}Hg (n = 8; mean ± SE) by *P. pugio* collected from Travis Avenue (TA), Staten Island, New York.

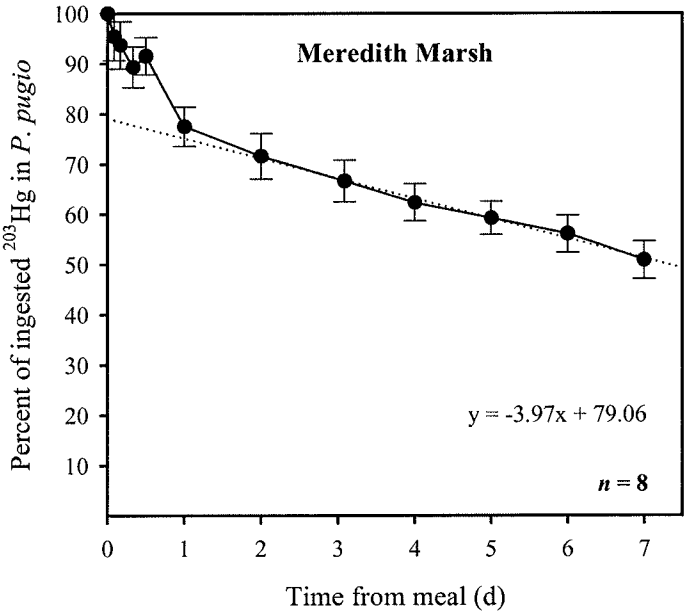

Figure 7. Time course in the retention of ^{203}Hg (n = 8; mean ± SE) by *P. pugio* collected from Meredith Marsh (MM), Staten Island, New York.

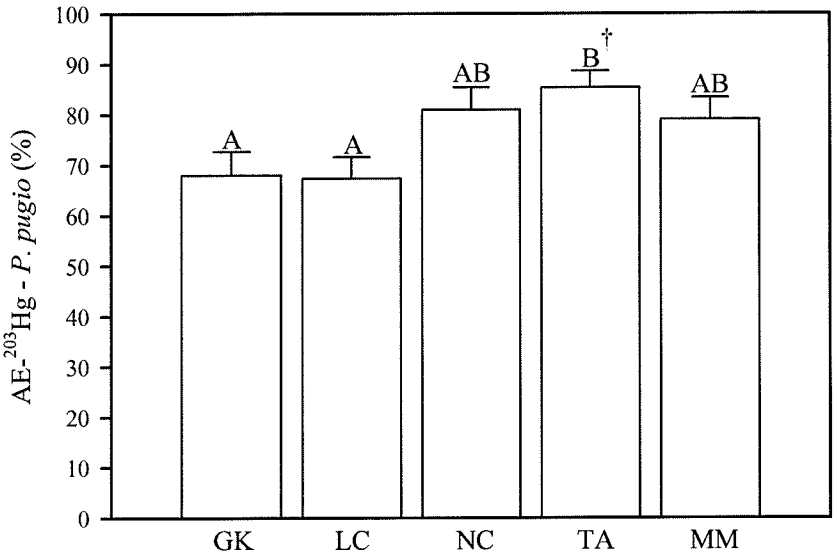

Figure 8. AE-^{203}Hg% by *P. pugio* collected from Staten Island, New York study sites: Great Kills Harbor – GK; Lemon Creek – LC; Nassau Creek – NC; Travis Avenue – TA; Meredith Marsh – MM. AE-^{203}Hg% ANOVA: $p < 0.05$. Significant differences ($p < 0.05$) in AE-^{203}Hg% between sites (Tukey-Kramer multiple comparisons test [*y*-intercepts]) are indicated by different letters. † = AE-^{203}Hg% differs ($p < 0.05$) from TAM-^{203}Hg% in *G. lawrencianus* prey (*t*-test).

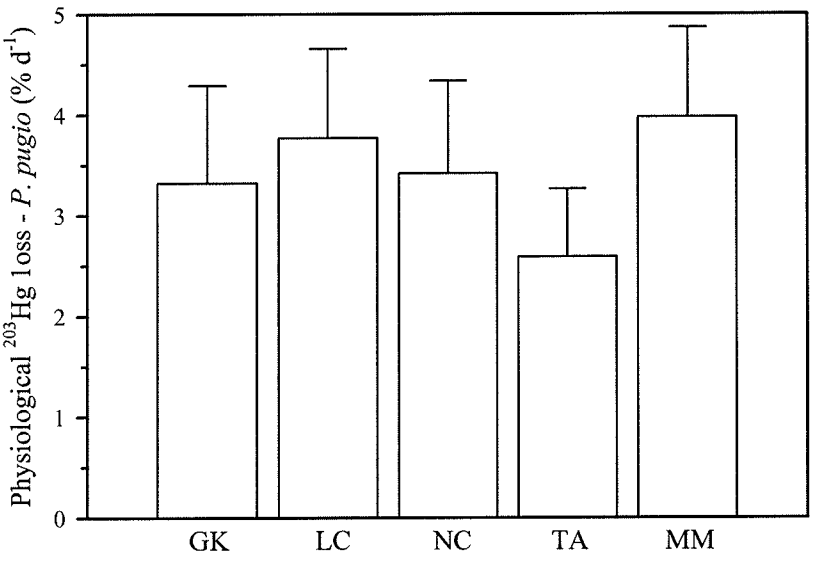

Figure 9. Rates of physiological ^{203}Hg loss by *P. pugio* collected from Staten Island, New York study sites (as per Figure 8). ANOVA: ns

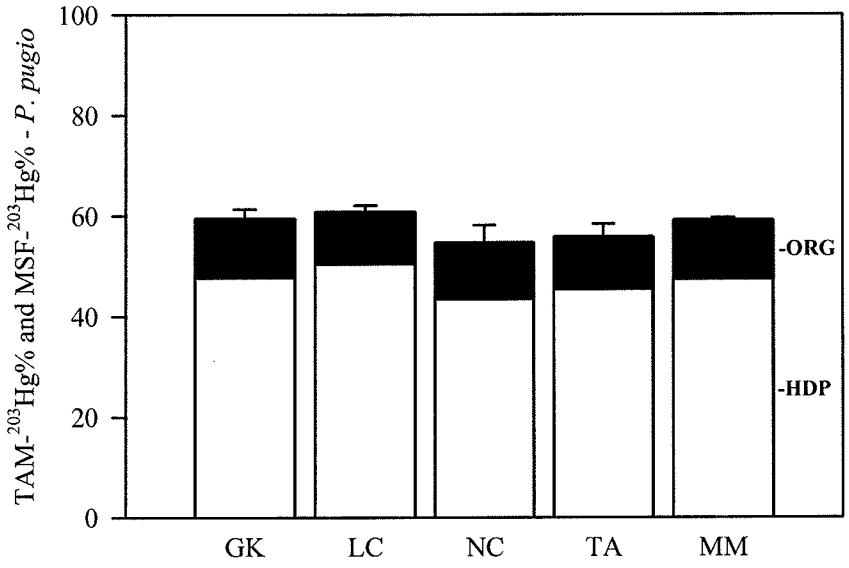

Figure 10. Subcellular distributions of assimilated ^{203}Hg by *P. pugio* collected from Staten Island, New York study sites: Great Kills Harbor – GK; Lemon Creek – LC; Nassau Creek – NC; Travis Avenue – TA; Meredith Marsh – MM. Heat denatured proteins (HDP), organelles (ORG), CD [data not shown], trophically-available metal (TAM-^{203}Hg%) and metal-sensitive fractions (MSF-^{203}Hg%) ANOVA: ns.

Subcellular distribution of assimilated ^{203}Hg in grass shrimp

Following AE analysis, grass shrimp from the Staten Island study sites were subjected to subcellular fractionation to determine the percentages of assimilated ^{203}Hg associated with five operationally-defined subcellular fractions: HSP, HDP, ORG, INS and CD. The percentages of assimilated ^{203}Hg associated with HDP, ORG and CD in shrimp did not vary among the study sites (Figure 10). ^{203}Hg signals for the HSP and INS fractions were below the limits of detection (i.e., could not be resolved from background). Partitioning of assimilated ^{203}Hg to the subcellular compartments containing TAM and MSF was similar (~55% to ~61%) for shrimp from each site.

DISCUSSION

The present study has demonstrated intraspecific variation in the assimilation of dietary Hg by grass shrimp, *Palaemonetes pugio*, collected across an environmental impact gradient within the NY/NJ Harbor Estuary. It must still be determined whether or not adult shrimp populations that inhabit waters within the AK complex and adjacent to Raritan Bay during the summer months represent the same source population. If Staten Island grass shrimp share source populations, variability in the assimilation of dietary Hg may be attributable to differential exposure to pollutant loads in the field (Perez and Wallace 2004, Seebaugh and Wallace 2004). Differences in metal assimilation in impacted populations may also have an underlying genetic component, which may be manifested as intraspecific variation in digestive physiology (e.g., gut residence time, gut pH or digestive enzyme activities]) or internal processing of metal (e.g., detoxification by resorptive [R] cells of the hepatopancreas) following absorption by the gut epithelium (Al-Mohanna and Nott 1987, Klerks and Weis 1987, Lopez 2005, Schlekat et al. 2005).

The pattern of ^{203}Hg depuration by grass shrimp was similar to that observed for ^{109}Cd and ^{65}Zn in previous work and was characterized by a three-component loss, which is consistent with biphasic digestion (Decho and Luoma 1996, Wang and Fisher 1996, Seebaugh and Wallace 2004, 2005). The initial rapid loss compartment (0 to ~24 h) may represent the passage of food particles from the proventriculus, during which some radiotracer is purged before reaching the hepatopancreas due to particle sorting and the transfer of materials to the midgut where it is packaged and expelled in feces (Icely and Nott 1992 and references therein, Wang and Fisher 1999b). The second compartment (~24 to 48 h) may represent the expulsion of residual materials from the hepatopancreas

resulting from intracellular digestion and the loss of blister (B) cells following clearance of the hepatopancreatic tubules in preparation for the next digestive cycle (Al-Mohanna and Nott 1986, Vogt 1993, Wang and Fisher 1999b). The slowest exchanging third compartment (48+ h) reflects the physiological loss of assimilated metal (Penry 1998, Wang and Fisher 1999b). Differences in rates of physiological ^{203}Hg and ^{109}Cd loss by grass shrimp from the Staten Island study sites have not been observed (Seebaugh and Wallace 2005).

Exposure to pollutants through the diet may elicit changes in predator digestive physiology that may be categorized according to whether or not a pollutant has been incorporated into tissues (i.e., assimilated) following absorption by the gut epithelium (Penry 1998, Wallace and Lopez unpublished). Pre-assimilatory toxicity may be induced by ingestion of a pollutant and may include changes in gut retention time, extracellular digestive enzyme activities or absorption of the products of hydrolysis (e.g., from the lumen of the decapod hepatopancreas) during the digestive cycle (De La Ruelle et al. 1992, Icely and Nott 1992, Vogt 1993, Brunet et al. 1994, Chen and Mayer 1998, Chen et al. 2002, Campbell et al. 2005, Wallace and Lopez unpublished). These effects would be related to the solubilization and subsequent availability of the pollutant within the gut fluid and not to pollutant burdens accumulated during previous exposures (Mayer et al. 2001, Campbell et al. 2005, Wallace and Lopez unpublished).

Post-assimilatory toxicity would result from the incorporation of a pollutant into tissues resulting from chronic exposure (e.g., impacted field conditions) (Penry 1998, Wallace and Lopez unpublished). Exposure may result in damage to gut tissues (e.g., the epithelial cells of the hepatopancreas) which may interfere with the synthesis and

discharge of extracellular digestive enzymes or the absorption and assimilation of nutrients (and perhaps pollutants) during subsequent digestive cycles (Icely and Nott 1992, Vogt 1993, Brunet et al. 1994, Nunez-Nogueria 2006, Wallace and Lopez unpublished). Damage to gut tissues may also impact energy reserves (i.e., glycogen and lipid stores within the hepatopancreas) or the transfer of nutrients and pollutants to tissues via the haemolymph (Icely and Nott 1992, Brunet et al. 1994). Assimilated pollutants may also impact mitosis as well as the differentiation of embryonic cells from which enzyme-secreting and absorptive cells of the gut epithelium are derived (Gibson and Barker 1979, Al-Mohanna et al. 1985, Vogt 1993).

Laboratory studies have demonstrated that the assimilation of dietary metal (e.g., Cd) can be impacted by previous or concurrent exposure to dietary metal (Selck et al. 1999, Shi et al. 2003, Rainbow et al. 2004b, Seebaugh et al. 2005, 2006, Seebaugh and Wallace unpublished). The effects of pre- and post-assimilatory toxicity on predator digestive physiology may also be interactive (i.e., additive, synergistic or antagonistic), particularly under conditions where long-term (e.g., field) exposure includes exposure to a pollutant during each digestive cycle (i.e., during feeding) (Selck et al. 1999).

Grass shrimp used in the present study were fed once immediately following collection, acclimated to laboratory conditions and allowed to depurate gut contents for 3 d prior to AE analysis. Since specimens were not fed during the remainder of the acclimation period (i.e., additional nutrients and pollutants were not available for absorption by the gut epithelium), increased assimilation by shrimp from TA may be related to post-assimilatory digestive toxicity resulting from exposure to pollutants (e.g., metals, organic pollutants or leachate contamination from landfills) in the field prior to

collection (Clément et al. 1996, Feng et al. 1998, Ward 2002, Nichols 2004). During acclimation and AE analysis, grass shrimp were maintained in seawater from their respective collection sites and could potentially have been exposed to pollutants through solution. Increased assimilation of dietary Cd and inorganic Hg was observed in the rock oyster, *Saccostrea cucullata*, following pre-exposure to dissolved Cd for 14 d and pulse-fed radiolabeled diatoms (Blackmore and Wang 2004). The assimilation of dietary Cd by the green mussel, *Perna viridis*, pulse-fed radiolabeled diatoms also increased in response to pre-exposure to Cd through solution (Blackmore and Wang 2002). The dissolved Cd concentrations used in these studies were, however, 2 to 3 orders of magnitude higher than dissolved Cd concentrations reported for Lower New York and Raritan Bays and the AK and are not considered to be environmentally realistic (Blackmore and Wang 2002, 2004, Paulson 2005 and references therein).

In previous work, grass shrimp subjected to Cd AE analysis and subcellular fractionation were collected from the Staten Island study sites over a 6 week period and may have included specimens from different clutches, although the extremes in the gradient in Cd assimilation were observed for shrimp from GK (~56%), MM and NC (~78%), which were obtained within a two-week period in mid to late July (Welsh 1975, Alon and Stancyk 1982, Seebaugh and Wallace 2005). In the present study, grass shrimp subjected to Hg AE analysis and fractionation were collected from all sites over a 16 d period from mid July to early August and likely represented the same clutch. It seems, therefore, logical to assume that shrimp were collected during similar development stages and that the observed variability in ^{203}Hg-AE% resulted from differential exposure to environmental pollutants or intraspecific differences in digestion or metal processing.

Grass shrimp collected from sites with high rates of flushing with 'cleaner' waters from Raritan Bay (e.g., GK and LC) may be expected to experience the least amount of impact from contaminants received from runoff, marina operations or nearby impacted waterways (e.g., the AK). Shrimp collected from tributaries within the AK complex (e.g., TA and MM) may be impacted by long-term exposure to contaminants from landfills (e.g., leachate) due to long water residence times and reduced flushing resulting from concurrent tidal surges from Newark and Raritan Bays (Figure 1) (Oey et al. 1985). The influence of this resonance effect on the migration and population dynamics of shrimp within the AK and its tributaries is not clear and must still be investigated (Oey et al. 1985). Shrimp collected from NC may represent transient populations as this creek is located in close proximity to the confluence between the Arthur Kill and Raritan Bay and is subjected to greater flushing than TA and MM, which may also mitigate the potential effects of exposure to high sediment metal (e.g., Cd, Cu and Zn) concentrations on AE-^{203}Hg% (Ward 2002, Nichols 2004).

Perez and Wallace (2004) observed a gradient in prey capture success by grass shrimp collected from GK, NC and a site located within a tributary of the AK, in close proximity to TA (Richmond Creek – RC) (Figure 1). Prey capture rates for NC shrimp were intermediate between GK (highest success) and RC (Perez and Wallace 2004). Interestingly, NC has been subjected to recent (Spring 2007) site remediation efforts which could potentially influence future metal concentrations in sediments and resident biota as well as toxicological impacts (e.g., changes in dietary metal assimilation or prey capture ability) in grass shrimp (Levinton et al. 2003, Perez and Wallace 2004, Seebaugh and Wallace 2005).

Previous studies have suggested that TAM in soft-bodied invertebrate prey may be used to estimate the potential for Cd and Zn transfer to grass shrimp (Wallace et al. 1998, Wallace and Luoma 2003, Seebaugh and Wallace 2004). The direct relationship between AE-^{203}Hg% by shrimp collected from GK, LC, NC and MM and TAM-^{203}Hg% in amphipod prey in the present study provides additional support for the hypothesis that TAM in prey may serve as an estimate of minimum metal bioavailability to grass shrimp. The departure from this relationship between TAM-Hg and AE-Hg% in the case of grass shrimp from TA may be related to digestive physiology or detoxification.

Site-specific differences in the partitioning of assimilated ^{203}Hg to HDP and ORG (and the TAM and MSF compartments) were not observed in grass shrimp in the present study. Additionally, the partitioning of assimilated ^{203}Hg to the biologically-detoxified HSP (e.g., MTLP) and INS (e.g., MRG) fractions was not observed as ^{203}Hg signals associated with these fractions could not be resolved from background. This suggests that tissue concentrations of assimilated ^{203}Hg may not have been sufficient to induce MTLP or to displace metals (e.g., the essential metals Cu and Zn) from the existing pool of MTLP (Amiard et al. 2006). In previous work, the percentage of assimilated ^{109}Cd bound to the subcellular fraction containing HSP (e.g., MTLP) in grass shrimp collected from the Staten Island study sites varied from ~15% to ~46%, with the highest values observed in shrimp collected from LC and NC (Seebaugh and Wallace unpublished). An inverse relationship in the percentage of ^{109}Cd associated with HDP was observed, with the lowest values found for shrimp from LC and NC. Metal-specific differences in the partitioning of assimilated metal may have important toxicological implications for grass shrimp, particularly if a specific metal is not partitioned to the compartment containing

biologically-detoxified metal (i.e., metal associated with HSP and INS) and is available to interact with metal-sensitive intracellular components (e.g., enzymes and organelles) (Wallace et al. 2003).

The potential for metal transfer to predators of grass shrimp (e.g., *Fundulus heteroclitus* and *Morone saxitilis*) may depend upon the extent to which Hg is partitioned to TAM, which can vary according to concentration and duration of dietary exposure (Wallace et al. 2000, Wallace and Luoma 2003, Seebaugh et al. 2005). Although site-specific differences in the percentages of ^{203}Hg partitioned to the TAM compartment within grass shrimp have not been observed, metal assimilation by a predator may also depend upon tissue metal burdens within shrimp as well as predator digestive physiology (e.g., metal solubilization within the gut fluid) (Seebaugh et al. 2005, Goto and Wallace unpublished).

CONCLUSION

Previous studies have investigated interspecific differences in the assimilation of dietary metal among estuarine organisms (e.g., bivalves, copepods and fish) (Chong and Wang 2000, Ni et al. 2000, Xu et al. 2001). In the present study, we have demonstrated site-specific variability in the assimilation of dietary Hg by the grass shrimp collected along an environmental impact gradient within the NY/NJ Harbor Estuary. In addition, Hg assimilation by shrimp from a site located within the heavily polluted AK complex (TA) exceeded Hg partitioned to TAM in amphipod prey. Differences in the assimilation dietary Hg may have toxicological consequences for shrimp from impacted populations (e.g., digestive toxicity, impaired prey capture and predator avoidance) (Kraus and Kraus 1986, Wallace et al. 2000, Perez and Wallace 2004).

Beyond the direct effects of dietary metal on individual organisms, metal exposure at lower trophic levels can impact entire food webs (Croteau et al. 2005). As a dominant epibenthic organism, the grass shrimp plays an important role in nutrient cycling within estuarine communities, is consumed by several ecologically- and recreationally-important species and may influence metal transfer along estuarine food chains (Nixon and Oviatt 1973, Welsh 1975, Kneib and Stiven 1982, Posey and Hines 1991, Davis et al. 2003, Carson and Merchant 2005, Seebaugh et al. 2005). Since population differences in the assimilation and subcellular partitioning of dietary metal by impacted grass shrimp cannot yet be attributed to exposure to specific pollutants in the field, future research efforts will focus on the effects of pre-exposure to specific metals (Cd and Hg) on the assimilation and partitioning of dietary Cd or Hg in this species.

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POSSIBLE EFFECTS OF ENDOCRINE DISRUPTING CHEMICALS ON DIAMONDBACK TERRAPINS (*MALACLEMYS TERRAPIN*) FROM JAMAICA BAY, NY

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

Millions of gallons of toxic chemicals are discarded into America's waterways every day and little is known about their effects on wildlife. Many chemicals are known to disrupt the endocrine systems of some animals, leading to developmental and reproductive disorders. The waters of Jamaica Bay, NY (JB) contain known endocrine disrupting chemicals (EDCs). Jamaica Bay is also home to New York State's largest population of diamondback terrapins (*Malaclemys terrapin*). The purpose of this study was to determine possible effects of EDCs on the terrapins of JB. At least twelve clutches from Jamaica Bay and three comparison sites (Cape May Peninsula, NJ, Peconic Bay, NY, and Barrington River, RI) were collected from naturally laid nests. Eggs were randomly distributed among containers, incubated at a constant temperature, and weighed periodically. Hatchlings were reared and evaluated for 75 days. Body size (mass, carapace and plastron length) was measured routinely for each hatchling. Shell abnormalities were noted for each turtle. Righting response was tested on each turtle at various intervals to determine locomotor performance. Eggs and hatchlings from Jamaica Bay were larger than the other three sites. Cape May Peninsula turtles had the lowest percentage of shell abnormalities. Locomotor performance of turtles from all sites markedly increased with age. Although Jamaica Bay is probably the most heavily polluted site from which turtles were obtained, the results did not indicate that Jamaica Bay turtles were adversely affected. Major effects from EDCs on terrapins may occur over a more extended period of time in nature. Future work to determine other indirect measures of EDCs on the turtles from Jamaica Bay are needed to further understand how EDCs affect diamondback terrapins.

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INTRODUCTION

In 1991 it was proposed that xenobiotic (not usually found in nature) and natural chemicals released into the environment by humans had the potential to affect the endocrine systems of humans and wildlife (Guillette 2006). Numerous studies have shown that endocrine disrupting chemicals (EDCs) are responsible for reproductive disorders, including reduced fertility, reduced hatchability and viability, disrupted hormone secretion and activity, and modified sexual behavior (Guillette et al. 1995). EDCs are a class of pollutants that mimic or block normal hormonal activity in the body. EDCs and their effects have been detected in many species, including humans (Norris 2006). Organisms which have temperature dependent sex determination are particularly susceptible to the adverse affects of EDCs (Willingham 2006).

Life History of Diamondback Terrapins

Diamondback terrapins (*Malaclemys terrapin*) are the only turtles in the world that are strictly endemic to coastal marshes (Ernst and Barbour 1989). They inhabit a thin coastal strip from Cape Cod, MA to Corpus Christi, TX. Diamondback terrapins may be considered keystone species due to their important role as both predator and prey. Their conservation is essential in maintaining the salt marshes where they live and the dunes where they nest. For example, terrapin predation has been shown to dramatically reduce the abundance of periwinkles (*Littorina sp.*) (Levesque and Fauth 1999), which are important predators of salt marsh cordgrass (*Spartina alterniflora*) (Silliman and Bertness 2002). Without terrapins to control populations of periwinkles, mudflats may result. Nesting terrapins also move enormous quantities of nutrients and calories from water to

land. Their eggs feed many predators as well as dune plants, which extract nutrients from terrapin eggs (Feinberg and Burke 2003).

Adult diamondback terrapins exhibit high nest site fidelity, even with suitable habitat nearby, which makes them vulnerable to local population extinctions (Hauswaldt and Glenn 2005). Females are capable of making long excursions to find suitable nesting habitat and tend to return to the same areas year after year to nest (Gibbons et al. 2001; Burke, unpub data). Terrapins are iteroparous and may deposit up to three nests, consisting of 4-18 eggs each, during each nesting season (Palmer and Cordes 1998; Feinberg and Burke 2003). Nesting occurs between April and July and hatchlings typically emerge from mid-August to mid-October (Butler et al. 2004). Diamondback terrapins display temperature-dependent sex determination; warmer nest temperatures result in female hatchlings (Giambanco 2003).

Terrapins have faced population threats since the late 19th century, when they were heavily harvested for soup. During the Great Depression, demand for terrapin meat decreased, allowing populations to rebound in many regions (Hauswaldt and Glenn 2005). Terrapin populations are declining again due to habitat loss and degradation, bycatch, nest predation, renewed consumption and possibly the presence of toxic contaminants. The impact of each factor varies from site to site. For example, where blue crabs (*Callinectes sapidus)* are heavily harvested, many terrapins are killed as bycatch in crab pots (Wood 1997). In heavily urbanized areas, predation by human-subsidized predators (i.e. raccoons) and urban pollutants may be more important (Ner and Burke 2007).

Endocrine Disrupting Contaminants in Jamaica Bay

Jamaica Bay (JB) has been the subject of numerous contaminant studies, including known EDCs (e.g., Tanacredi 1990; Seidemann 1991; Bopp et al. 1993; Bopp et al. 1998; Ferguson et al. 2003; Ferguson and Brownawell 2003; Litten 2003; Reddy and Brownawell 2005). Nevertheless, studies of the biological impacts of Jamaica Bay EDCs are still preliminary. Mena et al. (2005) found that exposure to Jamaica Bay sediments dramatically lowered reproductive rates in the benthic crustacean *Leptocheirus plumulosus*. Zulkosky et al. (2002) found that Jamaica Bay winter flounder (*Pleuronectes americanus*) contained altered levels of sex steroids compared to reference fish and skewed sex ratios overall. It may be important to note that both of these studies focused on short-lived organisms, whereas exposure and accumulation may be even more important to long-lived animals, such as terrapins In fact, recent small-scale studies suggest that, compared to other populations, Jamaica Bay terrapin hatchlings show unusually low levels of sexual dimorphism both internally and externally (Widrig and Madad unpub.), high levels of shell anomalies (Burke unpub. data), and patterns of hatchling mortality at nine months of age similar to those of dioxin-treated snapping turtles (Burke, unpub.; Rowe unpub.). These abnormalities may be due to EDCs, and may have profound effects on the diamondback terrapin population of Jamaica Bay.

Jamaica Bay is known to be highly contaminated with EDCs. I hypothesize that terrapins in JB will significantly differ from those found in reference sites. I predict that JB terrapins will be smaller in size, have more shell abnormalities and will have poor locomotor performance results compared to turtles from control sites.

METHODS

Study Sites

My research focused on Jamaica Bay (40.6°N,73.8°W) where the terrapin nesting population has declined from a high of 2150 nests in 2000 to 1568 in 2006 (Burke unpub.). Jamaica Bay Wildlife Refuge (JBWR) is a 3700ha estuarine refuge located on the southwestern corner of Long Island, New York. JBWR constitutes most of the aquatic portion, the islands and some of the shoreline of Jamaica Bay. JBWR is made up of a main island (Ruler's Bar Hassock) and several isolated islands. JBWR is part of the Gateway National Recreation Area, which is managed by the National Park Service, and terrapins are protected from removal in this area. Jamaica Bay is located at the eastern end of the Hudson-Raritan Estuary.

Cape May Peninsula (39.0°N, 74.9°W) is located on the southern most tip of New Jersey and separates the Delaware Bay from the Atlantic Ocean. Eggs were obtained near the Wetlands Institute, a salt marsh on Cape May Peninsula. Peconic Bay (41.0°N, 72.5°W) is a tidal estuary system located between the North Fork and South Fork of Long Island, NY. Eggs were obtained near an old Suffolk Country Parks housing facility in Flanders, NY. Barrington River, Rhode Island (41.5°, 71.3°W) is a tidal extension of the Runnins River. In RI, eggs were obtained from the Nockum Hill area of Hundred Acre Cove.

Egg Collection

Thirteen to fifteen naturally laid clutches were collected from each site. The eggs were removed from the nest, and three eggs were randomly chosen and frozen for future analysis. Identification numbers were written on the remainder of the eggs with a pencil,

and the eggs were placed into plastic containers containing vermiculite (1 part vermiculite: 2 parts water). Eggs were placed into the incubators at 29°C within three days of being laid. After all eggs were collected, eggs were randomly assorted among containers, shelves, and incubators. Moisture within the vermiculite was monitored weekly and water was added as needed to maintain constant container weight (g).

Egg Weights and Hatching

Eggs were weighed after 3, 5, 10, and 30 days. Moldy eggs were discarded. After 45 days of incubation eggs were put into individual cups with moistened vermiculite and were observed daily until hatching. After the turtle completely exited the shell, the hatch date was recorded and the shell was discarded.

Hatchling Measurements

Hatchlings were rinsed, dried, weighed, measured with digital calipers (carapace and plastron lengths), photographed at 3 days post-hatching, and placed back into individual cups of vermiculite. When the hatchlings were 7 days post-hatching, they were rinsed again, subsequently maintained in individual containers in approximately 2 cm of lukewarm dechlorinated brackish tap water (12-15ppm salt), and exposed to 12 hours of UVA/UVB light each 24 hour period. When hatchlings were 15, 45, and 75 days post-hatching, they were weighed and measured as described above.

Righting Response

When terrapins were 15 days post-hatching, they were towel-dried and placed upside down on a paper towel on a flat surface. A stopwatch was used to measure the time that elapsed when the turtle was placed upside down until the turtle had turned itself right side up. Turtles were allowed a maximum of two minutes to complete each trial.

Three trials were conducted on each turtle with a minimum of 20 second intervals between each trial. The shortest time out of the three trials was used for analysis. Righting tests were always conducted after the turtles received food.

Shell Abnormalities

Shell abnormalities were determined for each turtle by counting the number of scutes on the carapace and denoting any discrepancies from "normal": 1 nuchal scute, 12 left marginal scutes, 12 right marginal scutes, 5 vertebral scutes, 4 left costal scutes, and 4 right costal scutes. Each incident of a missing or additional scute was counted as one abnormality.

Husbandry

After the turtles reached 5 weeks post-hatching, while still in individual containers, they were fed turtle brittle (Nasco) daily to satiation. Two days/week, hatchlings were fed thawed seafood (mysis, silversides, squid, prawn, or krill) supplemented with Reptivite and Herptivite. Turtles were given a minimum of one hour to eat before food was removed and a complete water change was done.

At approximately two months old, turtles were individually tagged for identification. Small shellfish tags were sewn onto the third left marginal scute from the bottom, and secured with monofilament. If the tag was ripped through the scute for any reason, it was attached to the third right marginal scute from the bottom. After turtles were tagged, they remained in individual containers for an additional 24 hours to ensure the tagging caused no ill effects. Turtles were then matched by hatch date and distributed among nine tubs (122cm x 61cm with approximately 1-1.5cm of water). Tubs were equipped with submersible filters, heaters, basking stations, and UVA/UVB lighting.

Turtles were removed from the tubs and placed into the cups daily for feeding. Tubs received 50% water changes and filter cleaning twice a week.

Statistical Analysis

Statistical analyses were performed using SPSS for Windows version 15 and Microsoft Excel. Normality was determined by using One-Sample Kolmogorov-Smirnov tests. Analysis of variance (ANOVA) single factor tests were performed to evaluate the differences in the measurement results (mass, carapace, and plastron growth) among sites. Hatchling and egg size correlations were analyzed using Pearson's Correlation Test. A homogeneity-of-slopes assumption test was used to explore the relationships between egg mass, hatchling mass, and collection site. Chi-square tests were performed to test for differences in hatching success rate, locomotor performance, and shell anomalies among sites. For all tests α=0.05.

RESULTS

Eggs

Fifty-five clutches were obtained from four sites. The average clutch sizes from the sites were not significantly different, although there were more eggs per clutch in northern sites when compared to southern sites (Fig. 1). A total of 567 eggs were collected from all sites combined.

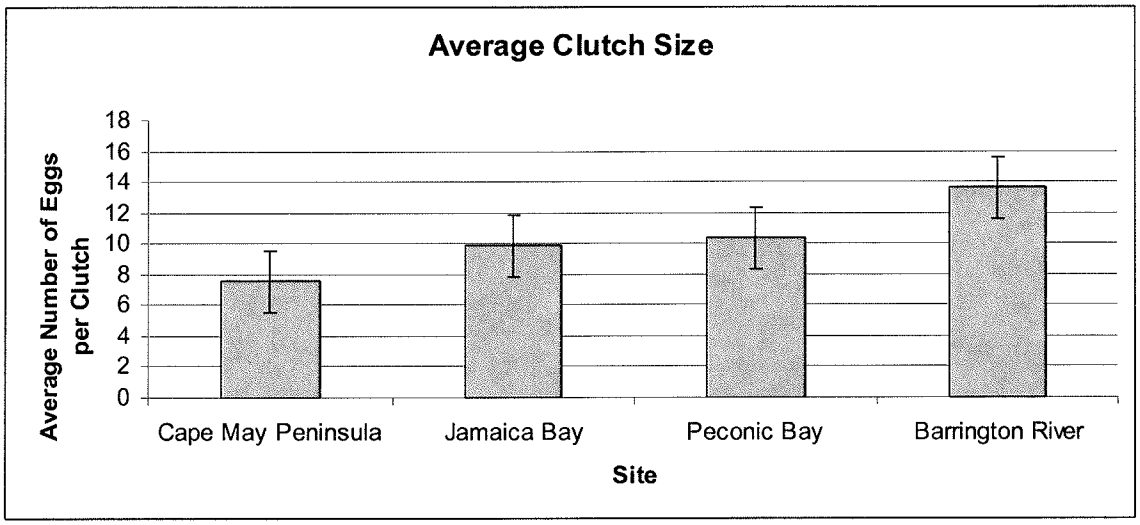

Fig. 1: Average number of eggs per clutch for each site. The three eggs that were frozen from each clutch are not included here.

Eggs from all sites increased in mass over time (Fig. 2). Egg masses differed significantly among the sites at all four measurement times ($p<0.001$) (Table 1), although there was a high amount of variation within each site (Fig 2).

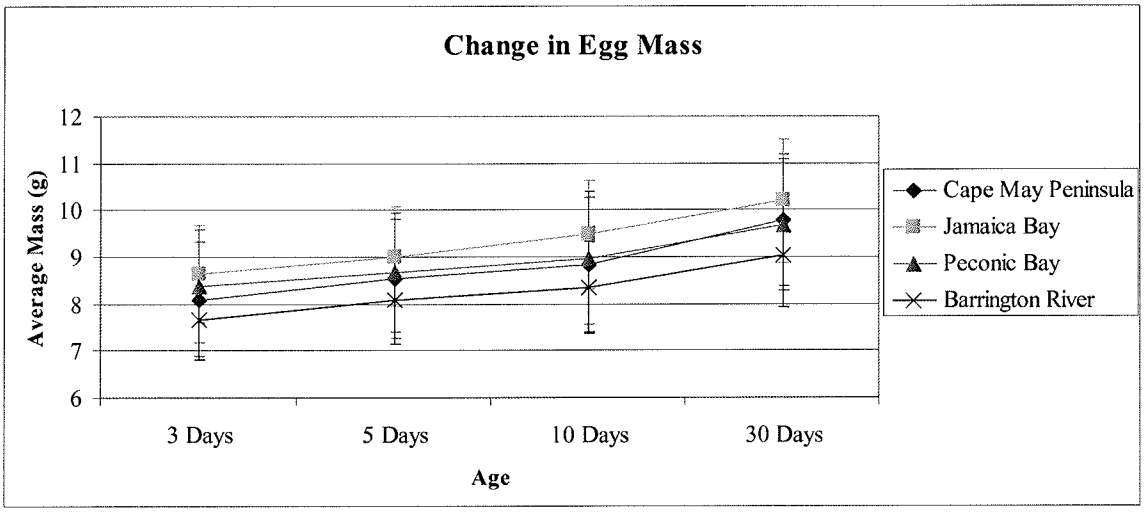

Fig. 2: Change in egg mass from each location. Y-error bars show standard deviation within each site.

Egg Mass	n	Sum of Squares	Df	Mean Square	F	p
3 Days	567	81.772	3	27.257	26.359	5.766E-16
5 Days	567	67.11	3	22.37	19.955	2.6454E-12
10 Days	567	98.96	3	32.987	25.046	3.1894E-15
30 Days	547	106.873	3	35.624	22.377	1.1292E-13

Table 1. Results of ANOVA for egg mass at four different ages across northern and southern sites.

There was an overall hatching success rate of 87.48% (Table 2). The χ^2 test for hatching success rates did not yield significant differences between sites (n= 4; χ^2 =0.98; p =1.0). One entire clutch from Cape May Peninsula failed to hatch, whereas the remainder of all clutches had at least some success. Eggs from Cape May Peninsula had the lowest hatch success rate even when the failed clutch was eliminated from the analysis (83.33%). Eggs from Jamaica Bay had the highest hatching success rate (92.19%).

Site	# of Clutches	# of Eggs	# of Hatchlings	Unhatched Eggs	Hatch Success (%)
Cape May Peninsula	14	106	85	21	80.19
Jamaica Bay	13	128	118	10	92.19
Peconic Bay	15	153	134	19	87.58
Barrington River	13	180	159	21	88.33
Total	55	567	496	71	87.48

Table 2: Egg collection data and hatching success from each site. The number of eggs represented here does not include the three eggs that were frozen from each clutch.

Hatchling Measurements

Because hatchling mass, carapace length and plastron length were correlated with one another, only hatchling mass was analyzed for differences among sites (Table 3).

Hatchlings from Jamaica Bay had larger average body size and mass than hatchlings from other sites at each measurement interval throughout the data collection time (Figs. 3-5), although there were high levels of variation.

Hatchling Mass	n	Sum of Squares	Df	Mean Square	F	p
3 Days	495	45.33103628	3	15.1103	22.2964	1.50E-13
15 Days	491	71.62632619	3	23.8754	33.3217	1.33E-19
45 Days	484	38.21498198	3	12.7383	11.1377	4.41E-07

Table 3: Result of ANOVA testing for differences in hatchling mass among sites.

On average, hatchlings from all sites lost mass between 5 and 15 days old and gained mass between 15 and 45 days of age (Fig. 3). However, between 45 and 75 days of age, hatchlings from Jamaica Bay gained an average of 0.6 g, whereas Peconic Bay gained an average of only 0.1 g, and hatchlings from Barrington River and Cape May Peninsula lost an average of 0.2 g and 0.1 g respectively (Fig. 3). Between 45 and 75 days old, Jamaica Bay hatchlings also had a larger increase of carapace and plastron length compared to the rest of the turtles (Fig. 4, Fig. 5). Turtles from Barrington River generally had a smaller body size and mass compared to other sites, although plastron length slightly was larger than Cape May Peninsula Hatchlings (Fig. 5).

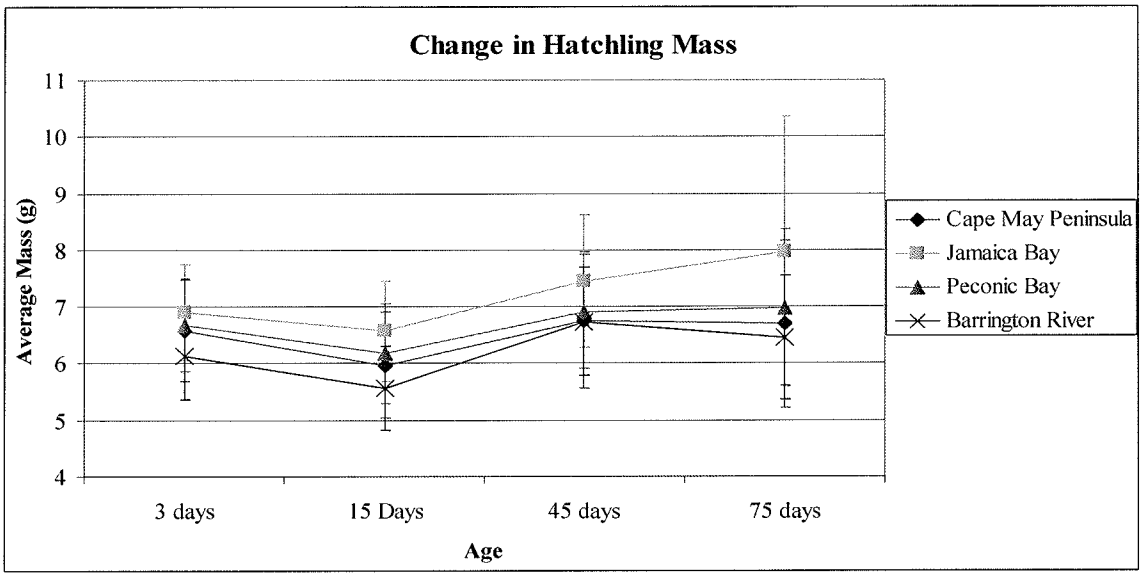

Fig. 3: Change in average mass of hatchlings. Y-error bars indicate high amounts of variation, especially at 75 days of age.

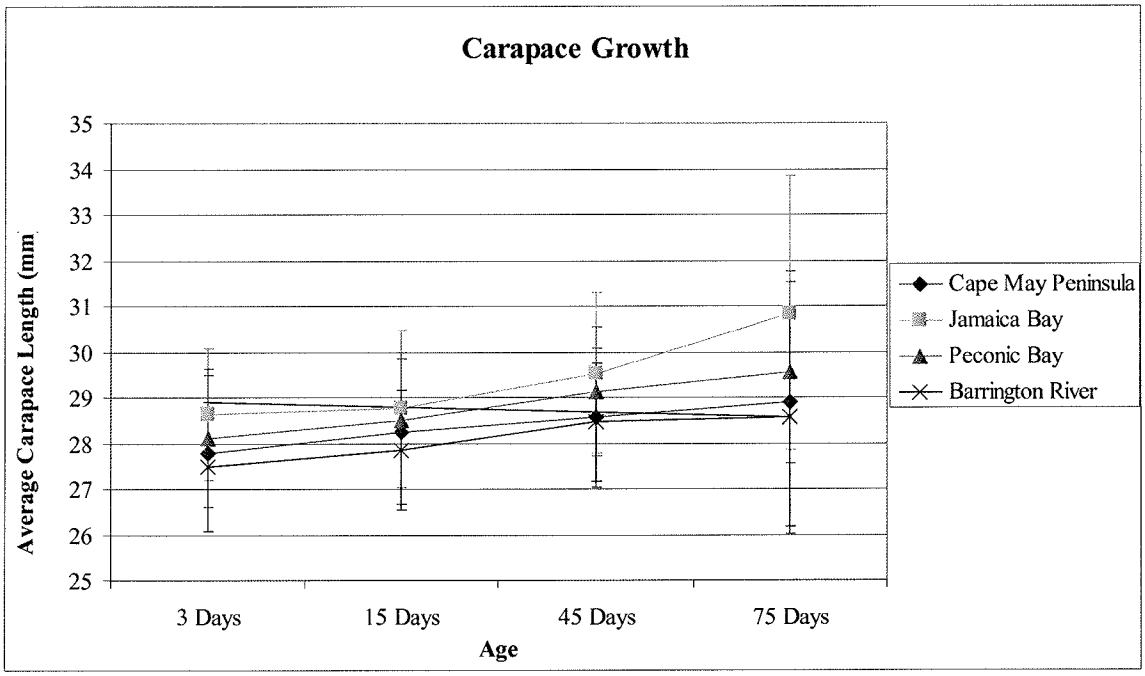

Fig. 4: Average growth of carapace length. Y-error bars indicate a high amount of variation, especially at 75 days of age.

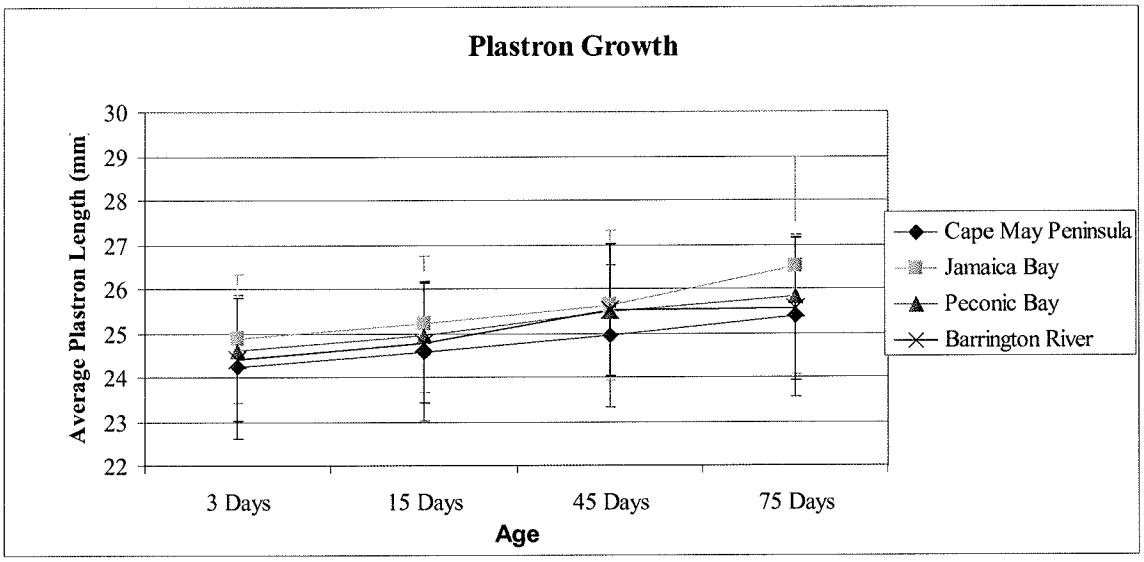

Fig. 5: Average growth of plastron length. Y-error bars indicate a high amount of variation, especially at 75 days of age.

Size Correlations

Hatchling size was significantly correlated with egg mass (carapace length at 15 days (n=488) and egg mass at 3 days (n=567); r =0.757, p<0.001). According to the homogeneity of slopes test the significant correlation between hatchling mass and egg mass varied among collection sites (p=0.001). Carapace length was significantly correlated with hatchling mass (carapace length and mass at 45 days; n=491; df=490; r =0.876; p<0.0001). Carapace and plastron length were significantly correlated (carapace and plastron length at 15 days; n=488; df=487; r=0.828; p<0.0001).

Shell Abnormalities

The majority (57.4%) of all hatchlings had at least one shell abnormality (Fig. 6). Cape May Peninsula hatchlings had the lowest frequency of shell abnormalities (35.8% had abnormalities), followed by Barrington River hatchlings (57.4% had abnormalities). Turtles from New York (both Jamaica Bay and Peconic Bay) had the highest total frequency of shell abnormalities (64.6% and 64.4%). There were significant differences

among sites in the number of shell abnormalities when compared to those with no abnormalities (n=481; df=3; χ^2=11.76; p=0.008).

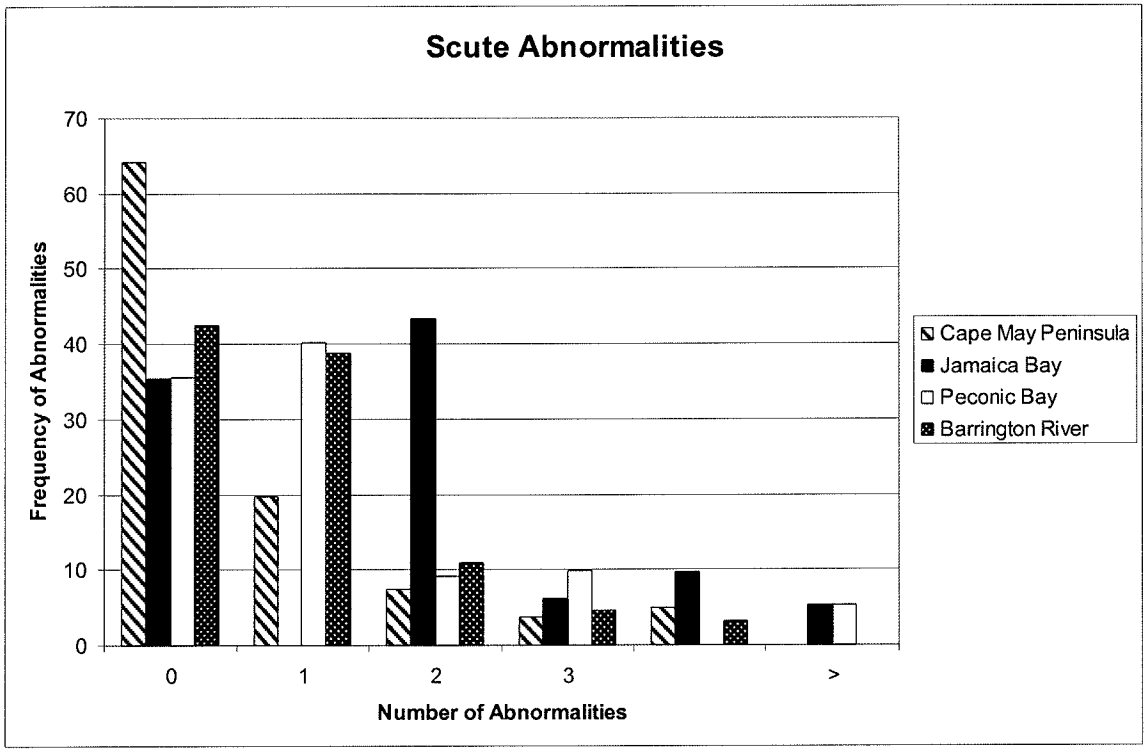

Fig. 6: Percentage of frequency of shell abnormalities for each site.

Locomotor Performance

Righting response performance dramatically improved with age for terrapins from all sites (Figs. 7-9). At 15 days old, over half the terrapins from Barrington River and Peconic Bay did not right themselves within two minutes; Jamaica Bay terrapins had the fastest performance rates (34.8% righted themselves within 30s) (Fig. 7). At 45 days of age, 58.3% of Cape May Peninsula hatchlings righted themselves within 30s, while over half of the turtles from Peconic Bay were still not successful in righting themselves within two minutes (Fig. 8). When the hatchlings reached 75 days old, righting response times were much faster than at younger ages, with Jamaica Bay with the highest

percentage of hatchlings that righted within 30 seconds (Fig. 9). There were some significant differences in righting response times between sites for the fastest and slowest turtles (n=4; df=3; $\chi 2$=8.18; p= 0.04) (Table 4).

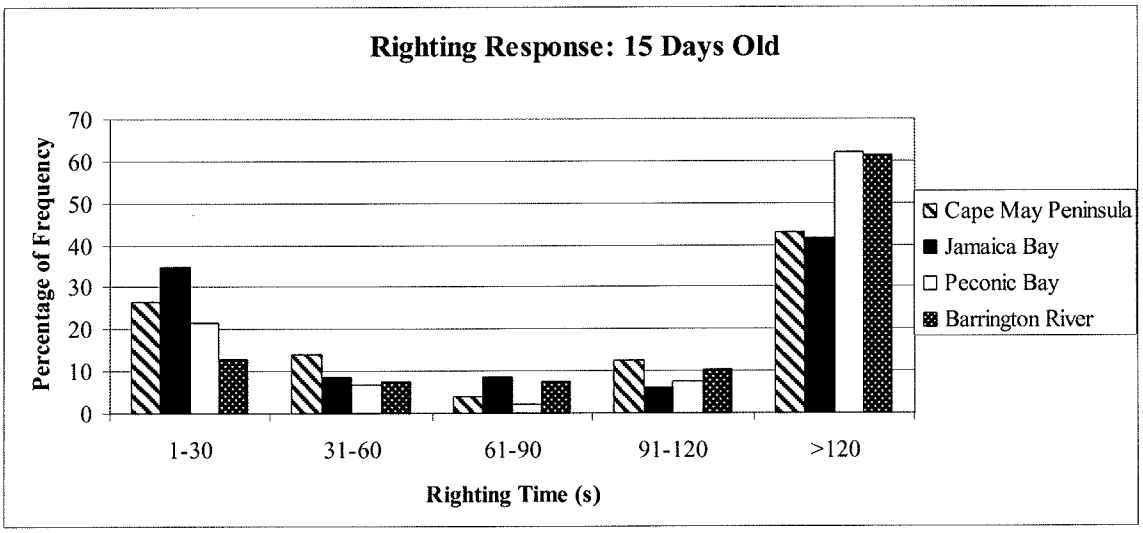

Fig. 7: Righting speed frequencies for each site at 15 days old.

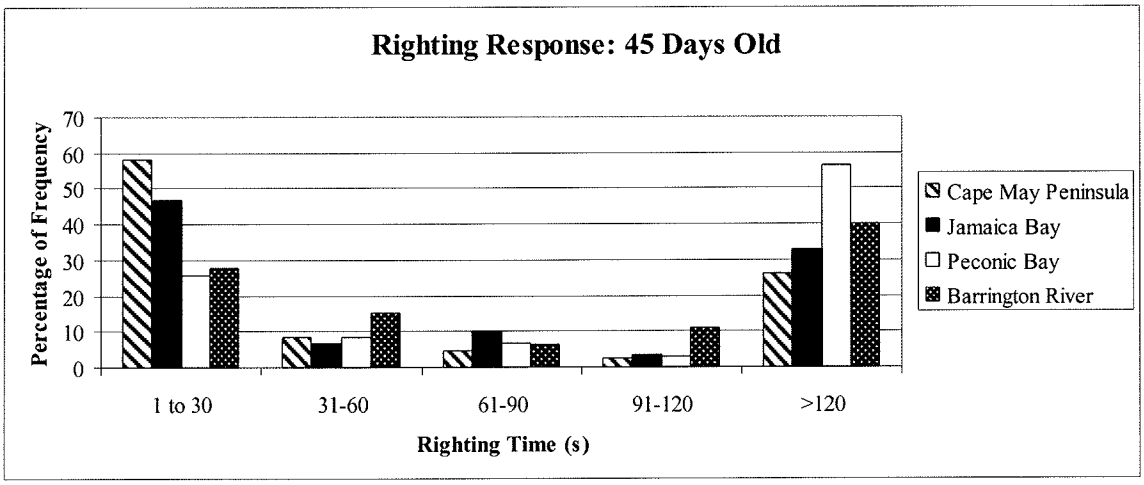

Fig. 8: Righting speed frequencies for each site at 45 days old.

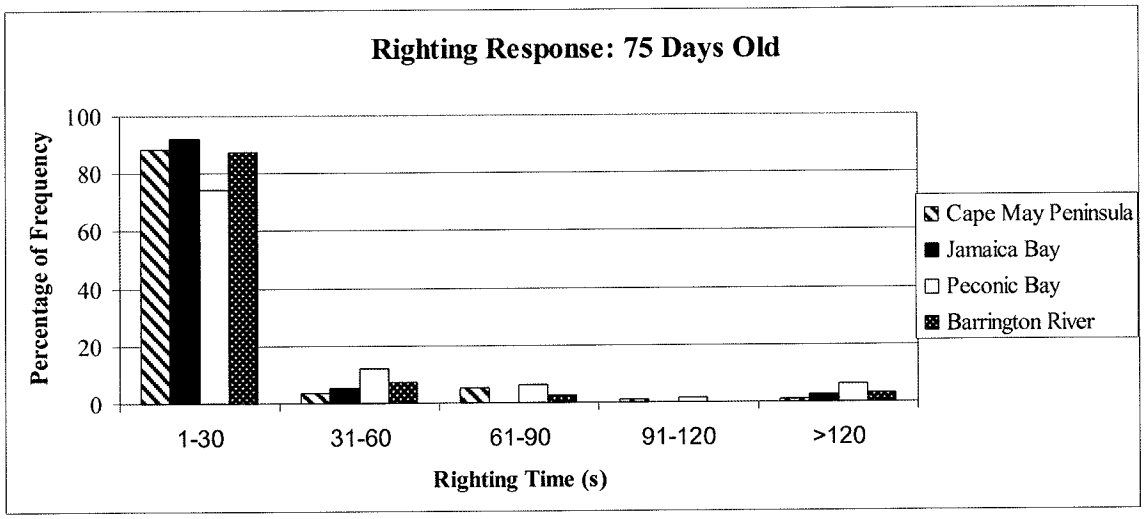

Fig. 9: Righting speed frequencies for each site at 75 days old.

Age	Response Time (sec)	p-Value
15 Days	1-30	0.0142*
45 Days	1-30	0.0003***
75 Days	1-30	0.536
15 Days	>120	0.0655*
45 Days	>120	0.0048**
75 Days	>120	0.2791

Table 4: Significant differences in righting response times between sites.

Age appears to be a factor when determining fitness via righting response. Locomotor performance increased with age at all sites, although turtles from differing sites did not improve at the same rate (Figs. 6-8). Peconic Bay hatchling performance remained poor through 45 days old, and then improved greatly at 75 days old, where as the other groups showed higher levels of improvement at both 45 and 75 days of age.

DISCUSSION

Based on the results, my hypothesis was rejected. Jamaica Bay terrapins are different from turtles at other sites, but not in the predicted manner. Jamaica Bay

terrapins were larger than terrapins from other sites and did not have significantly more shell abnormalities or the poorest locomotor performance scores.

Clutch size increased with higher latitudes, which is consistent with other studies (Allman 2006). Allman (2006) proposed that with decreasing latitude, egg size (and in return hatchlings size) increases. However, in this study, the larger eggs did not come from the southern most population (Cape May Peninsula). The largest eggs and hatchlings came from Jamaica Bay.

There are at least two explanations for eggs and hatchlings from Jamaica Bay being larger than eggs and hatchlings from other sites. The factor causing these unexpected results may be endocrine disrupting chemicals. EDCs have caused antithyroidal consequences in various organisms from exposure to PCBs or nitrates (Guillette 2006), although this has yet to be shown in turtles. Future work should be conducted to determine whether there is a difference in thyroid activity between sites as well as differences in concentrations of EDCs between sites. Current work is being done to determine the differences in aromatase activity (an indirect measure of EDCs) among sites to further investigate the role of EDCs on these terrapins.

Terrapin size (egg mass, hatchling mass, carapace length and plastron length) differed among sites, however the ecological significance of these differences are not quite as apparent. For example, at the age of three days, hatchling carapace length significantly differed among sites, however the average difference between the heaviest (Jamaica Bay) and lightest (Barrington River) turtles was only 0.8g. Another example is plastron length at the age of three days: hatchlings turtles with the largest plastron length (Jamaica Bay) differed from terrapins with the shortest plastrons by only 0.7mm. The

statistical significance was in part due to the large sample sizes that were analyzed. However, as the turtles aged, the differences become more ecologically relevant. For example, when turtles reached 75 days old, hatchlings with the longest carapace length (Jamaica Bay) were 2.2mm longer than the shortest turtles (Barrington River). A difference of 2.2mm in nature certainly could be important when escaping predation. For example, Janzen et al. (2000) found that natural selection favored larger body size; survivorship of smaller hatchlings decreased due to size-dependent predation.

If shell abnormalities are in some way indirectly caused by EDCs, then the data suggest that Peconic Bay and Barrington River are similar to Jamaica Bay. Hatchlings from Cape May Peninsula had substantially less abnormalities when compared to the other sites. It is unclear why hatchlings from Cape May Peninsula had the lowest percentage of abnormalities. Because all eggs were incubated under the same conditions and were randomly distributed, I conclude that shell abnormalities in this case are either caused by EDCs or by some other developmental factor that is not attributed to the incubation environment.

Righting response is significantly affected by some EDCs (Burger et al. 1998). Locomotor performance greatly increased with hatchlings age for all sites. Using righting response as a measure of locomotor performance provided insight into the fitness of the individual. In nature, turtles often find themselves upside down from falling down a slope, for example, and if they are unable to right themselves in a timely manner their chances of predation are greatly increased. Along this line of thought, turtles in this study with the shortest righting response times are considered more fit than those with

longer response times. Therefore, during two of the three periods of locomotor testing, Jamaica Bay terrapins displayed the highest fitness levels.

CONCLUSION

Our hypothesis was not substantiated with the data collected in this study. If Jamaica Bay terrapin hatchlings were affected by higher levels of EDCs than other sites, then the body size, shell abnormalities, and locomotor performance should have reflected that. It is generally accepted that organisms which have been exposed to EDCs are smaller, have higher abnormalities, and have poorer locomotor performance. In this study, turtles from Jamaica Bay had a larger body size, competitive righting response times, and similar occurrences of shell abnormalities to other sites, which are considered "clean" in comparison. It is possible that EDCs may not have an effect on terrapin eggs. EDCs are often lipophilic and bioaccumulate within organisms, especially long-lived organisms, such as terrapins. EDCs may have more pronounced effects on turtles that have inhabited Jamaica Bay for some period of time. Future work to evaluate the effects of EDCs on adult terrapins should be conducted.

The work presented in this study is ongoing. Body size and locomotor performance will be evaluated over one year. Aromatase levels will be evaluated when the hatchlings reach six months of age to determine indirectly if the terrapins are affected by EDCs. It is still expected that Jamaica Bay turtles will have higher levels of aromatase than terrapins from the "cleaner" sites, even though the current body size and performance data may not correlate. The frozen eggs are also going to be analyzed to

determine which contaminants are present within the eggs to provide insight about when and how the effects of EDCs on diamondback terrapins from Jamaica Bay could occur.

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Fish Species-Habitat Associations in New York's Great Swamp

A Final Report of the Tibor T. Polgar Fellowship Program

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Abstract

A comprehensive three-season, multiple gear fish species-habitat association study was conducted in New York's Great Swamp. Sampling sites were chosen based upon their representative characteristics and sampled every third week. Both passive (2' and 3' fyke nets and minnow pots) and active (seine and $1m^2$ throw trap) fish sampling methods were used to sample fishes in non-vegetated, emergent and submerged aquatic vegetation (SAV) sites.

Redfin pickerels (*Esox americanus*) and White river crayfish (*Procambarus acutus*) in the Great Swamp used emergent vegetation as a nursery for young-of-year. Adult redfin pickerels were found in emergent vegetation in spring and autumn, but primarily occupied the non-vegetated open channel of the East Branch Croton River during the summer.

Crayfish species in the Great Swamp appeared to occupy several niches: Ringed crayfish (*Orconectes neglectus*) were dominant in SAV, while White river crayfish were mostly found in emergent vegetation, and Rusty crayfish (*Orconectes rusticus*) were mostly found in non-vegetated areas.

Centrarchids in the Great Swamp were associated with vegetation, while Percids and Golden shiners (*Notemigonus cryoleucas*) were associated with non-vegetated areas. Creek chubsuckers (*Erimyzon oblongus*) were associated with the emergent vegetation/non-vegetated interface. Yellow bullhead catfish (*Ictalurus natalis*) were almost exclusively found in SAV.

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Introduction

The Great Swamp comprises over 6,000 acres of forested and emergent herbaceous wetlands, spanning the towns of Southeast, Patterson, Pawling and Dover, in Putnam and Dutchess Counties, New York. The Swamp drains in both north and south directions, with the division in the village of Pawling, from which the Swamp River flows north to the Housatonic River, and the East Branch Croton River flows south to the Hudson River (Siemann 1999). Historically, prior to man's actions, there was uninterrupted flow from the Swamp to the Hudson River, but this has been curtailed for decades due to the construction of several dams. From 1886 to 1911, there were five dams erected along the East Branch Croton River from the Great Swamp to the Hudson River. Following the flow south from the Great Swamp, there is the Sodom Dam (erected in 1892), the Diverting Reservoir Dam (1911), the Juengst Dam (1886), the Muscoot Dam (1906) and finally the New Croton Reservoir Dam (1906) (United States Army Corps of Engineers 2005).

Removal of small, obsolete or disused dams is gaining popularity as an aquatic habitat restoration measure in many areas (Bednarek 2001), and there is considerable interest among the natural resource management community (including Hudson River National Estuarine Research Reserve) in dam removal along Hudson River tributaries. Baseline information on fish communities upstream of such obstructions represents a valuable information resource for use in the development of future dam removal programs. This study concentrated on the East Branch Croton River, which flows 35 miles from the Great Swamp to the Croton/Hudson River confluence. The study area ranged from where the East Branch Croton River passes under Route 311 in Patterson to approximately 0.5 miles north of the River's Doansburg Rd underpass in the town of Southeast (Figure 1).

The Great Swamp watershed encompasses over 60,000 acres in which over 40,000 people live (Siemann 1999). The land is almost exclusively privately owned, with a few public parks, such as Patterson Environmental Park, that offer access for passive recreation or nature study including kayaking, fishing and bird watching.

There are ongoing efforts to preserve the condition of the Great Swamp, mostly by Non-Governmental Organizations. Groups like Friends of the Great Swamp (also known as FRoGS) and The Nature Conservancy have helped to raise awareness about the Great Swamp's ecology and recreational opportunities.

The Great Swamp provides many ecological services. It is home to many species of fishes, birds, reptiles and mammals, including some that are endangered in New York State such as wild Brook trout (*Salvelinus fontinalis*) and Bog turtles (*Clemmys muhlenbergii*) (Siemann 1999). The Great Swamp stores excess runoff water, and helps purify the water that eventually flows through the Croton Reservoir downstream to the Hudson River.

The Swamp provides several different habitat types for fish including large open channels of the East Branch Croton River, that support submerged aquatic vegetation (SAV). SAV provides nursery habitat for juvenile fish, protects against predation and replenishes water with dissolved oxygen (Mitsch and Gosselink 1993). SAV in the Great Swamp is mostly comprised of Coontail (*Ceratophyllum demersum*), Canadian waterweed (*Elodea canadensis*), Curly pondweed (*Potamogeton crispus*) and Slender pondweed (*Potamogeton pusillus).* The littoral zone is generally made up of steep muddy banks with dense emergent vegetation such as Lizard's tail (*Saururus cernuus*), Arrow arum, (*Peltandra virginica*), and Smartweeds (*Panicum* spp.). The river also has a defined channel, which is non-vegetated in some areas.

While the basic characteristics of the Great Swamp are well known (Siemann 1999) and fish have been sampled in tributaries (van Holt et al. 2006), to date there had not been an extensive survey of the fish communities, nor an examination of fish species-habitat associations in the main stem of the Swamp, the East Branch Croton River.

The goal of the study was to provide information on fish species-habitat associations for use by those who wish to further research the Great Swamp or similar habitats for conservation efforts.

The objectives of the study were: 1) to carry out a comprehensive multi-habitat, multi-season (spring, summer and autumn) sampling program to characterize fish use of vegetated and non-vegetated aquatic habitat 2) to characterize dietary preferences of

common fish species within the Great Swamp and 3) to note the general health of fishes collected.

Materials and Methods

A sampling event was defined as two to four days, consecutive whenever possible, of sampling locations within the Great Swamp every third week, May-September 2007 (Table 1). There were a total of seven sampling events in the study. Sample locations were located consistently through use of a Garmin GPSMap 60CSx unit, and a Geographic Information Systems (GIS) map showing the locations of sample sites were created in ArcMap 9.2 (Figure 1).

Table 1. Sample event dates.

	Sample Event						
	1	2	3	4	5	6	7
Date	May 20-21	June 8-11	June 29-July2	July 19, 21-23	August 8-9, 11-12	August 30-September 2	September 20-23

A variety of gear appropriate to the physical environment (i.e. depth, hydrodynamics, vegetation structure) of the specific sample locations was used to decrease gear selectivity, increase gear effectiveness and ensure accuracy of data (Bagenal 1978).

Non-vegetated sampling locations ranged 2.5' to 3.5' in depth. Emergent vegetation sampling locations ranged 2" to 3' in depth, and consisted mostly of Lizard's tail, Arrow arum, a combination of the two, or Yellow pond lily (*Nuphar luteum*). SAV sampling locations ranged 1.5' to 3.5' in depth, and consisted mostly of Coontail, Canadian waterweed, Curly pondweed and Slender pondweed. Green Filamentous algae (*Cladophora fracta*) was also present at some SAV sites.

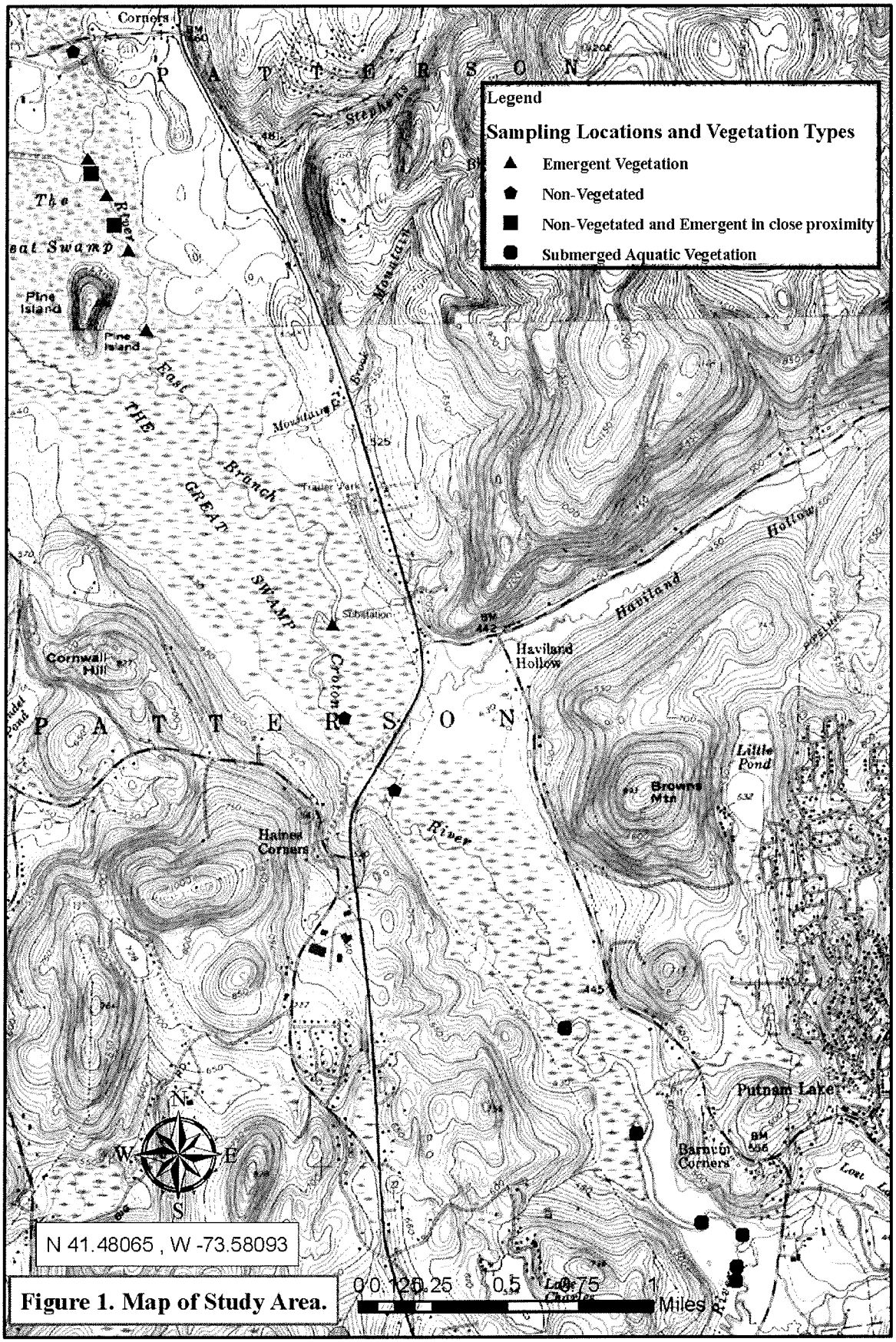

Figure 1. Map of Study Area.

Passive Fish Sampling Methods

2' Fyke Nets

Six locations in shallow water were sampled using 2' fyke nets with ¼" mesh. Due to their physical limitations 2' fyke nets were deemed to be ineffective in the deeper non-vegetated channel and instead were deployed as close as possible to the edge of emergent vegetation. Other sample sites were in SAV beds. Soak times ranged two to nine hours, with a mean of five hours and sixteen minutes.

Data were collected from thirty-seven 2' fyke net deployments at six individual sites. Three sites near emergent vegetation were sampled six times, one site was sampled seven times, for a total of twenty-five deployments. Two sites in SAV were sampled six times, for a total of twelve deployments.

3' Fyke Nets

Six large, open channel areas were sampled using 3' fyke nets, with 1/8" mesh. Both non-vegetated and SAV dominated areas were sampled. Soak times ranged two hours to eight hours and thirty minutes, with a mean of five hours and thirty minutes.

Data were collected from thirty-five 3' fyke net deployments at six individual sites. Two sites in SAV were sampled six times, and a third SAV site was sampled four times for a total of sixteen deployments. Two sites in non-vegetated areas were sampled six times, and a third non-vegetated site was sampled seven times for a total of nineteen deployments.

All 2' and 3' fyke nets were deployed from a canoe and held in place with ten pound mushroom anchors (Murphy and Willis 1996) and rebar hammered into the sediment.

Minnow Pots

Triplicate wire-mesh minnow pot traps (9" diameter x 18" long, ¼" mesh) were deployed at surface, mid-water and bottom depths as near as possible to all fyke net sample locations, hung from snags (Bagenal 1978). Minnow pots had two to nine hour soak times, with a mean of five hours and fifteen minutes, and were baited with small pieces of bread.

Data were collected from seventy-three minnow pot deployments at twelve individual sites. Three sites near emergent vegetation were sampled six times, one site was sampled seven times for a total of twenty-five deployments. Two non-vegetated sites were sampled seven times; one site was sampled six times for a total of twenty deployments. Four sites in SAV were sampled six times; one site was sampled four times for a total of twenty-eight deployments.

The number of individuals, species, total length (TL) (Appendix: Table 3), along with any comments (e.g. external parasites, etc.), were recorded at each sampling location. Fyke net and minnow pot collections were expressed as Catch Per Unit of Effort (CPUE) (number of individuals collected per hour of soak time).

Active Methods

Seine

A bag seine (15' x 4' x 6', 1/8" ace nylon mesh) was used in one shallow, non-vegetated, open area, and two shallow open areas with moderately dense SAV, where there was adequate shore space onto which to pull the seine (Bagenal 1978). The seine was used to actively collect fish within a known area of stream, typically thirty-five yards, by the seine's 15' width. Seining locations were chosen based upon physical parameters (i.e. depth, currents, and substrate) and sampled opportunistically.

Data were collected from a total of thirty-six seine hauls at three individual sites. One non-vegetated site was sampled in triplicate over seven sample events for a total of twenty-one seine hauls. Two moderately dense SAV sites were sampled in triplicate over a combined total of five sample events for a total of fifteen seines. Heavy rainfall caused water heights to rise to levels making seining the SAV locations unsafe during sample events 4 and 5.

Throw Trap

A depth specific, folding aluminum $1m^2$ throw trap was used to sample SAV beds and emergent vegetation communities in eight sample locations, with three replicates per sample location. Throw traps have been shown to be very effective in

sampling densely vegetated aquatic habitats (Freeman et al 1984; Coyne and Trexler 1997). Throw trap sample sites were chosen based upon the presence of emergent or submerged vegetation and three sites were randomly sampled within the specific vegetation community.

Data were collected from 155 deployments at eight individual sites. Four sites in emergent vegetation were sampled in triplicate over seven sampling events for a total of eighty-three deployments (data from one deployment was lost). Four sites in SAV were sampled in triplicate, over six sampling events for a total of seventy-two deployments.

The throw trap was custom designed and fabricated for use in densely vegetated habitats to ensure its durability and accuracy of data. The inside dimension of the throw trap was 1 square meter. It was constructed of 16 gage aluminum sheeting, with piano style hinges in three corners enabling it to be folded flat for transportation to remote sample locations via canoe. The fourth corner was bolted together at the time of deployment.

Fish collected in the throw trap were removed using a custom fabricated bar seine (Rozas and Odum 1987) constructed using a 0.99m wide ¾" aluminum square tube frame, hung with 1/8" Ace nylon mesh, and edged with 1 ½" door sweeps. The door sweeps were hung 0.2" over the bottom edge and both side edges of the snugly fitting bar seine to ensure a tight, yet flexible seal against the throw trap, reducing the likelihood of fish within the trap escaping collection. The throw trap was cleared using the bar seine until three consecutive passes yielded no collection. Fishes and crayfishes collected in the mesh of the bar seine were immediately put into sample containers with fresh water, the containers were labeled and specimens were processed and released as quickly as practical, or kept for further analysis.

The aluminum sides of the throw trap were manufactured to a height of 2' to keep the throw trap's weight below thirty pounds, as it was meant to be portable and thrown with reasonable ease, while maintaining enough weight to sink quickly through dense vegetation. To accommodate water deeper than 2', a deep water extension net was manufactured using a 4' X 13' section of 1/8" ace nylon mesh attached to the top of the throw trap's sides via 2" Velcro strips, allowing the mesh to be removed quickly and easily for shallow and/or emergent use. The deep-water extension net enabled the throw

trap to be utilized in water as deep as 4'. While the dimensions of the throw trap and extension net could reach a depth of approximately 6', the physical limitations of the bar seine and the user became a limiting factor in depths greater than 4'.

The number of individuals, species, total length (TL), along with any comments (e.g. external parasites, etc.), were recorded at each sampling location. Quantitative density estimates were determined from throw trap and seine samples, and expressed as number of individuals/m^2.

Efficiency

Efficiency tests of the throw trap and bar seine were performed in July using typically sized, commonly collected species (Table 2). The throw trap was routinely deployed in a shallow shoreline location with moderately dense SAV, and cleared of all fauna via the method described above. Twenty specimens were released into the cleared throw trap and given one minute to disperse before the bar seine was used to clear the trap via the method described above.

A series of three tests using twenty juvenile Yellow bullhead catfish (*Ictalurus natalis*), ranging in size from 20 to 37 mm, yielded a mean efficiency of 100% in all three tests performed. A test series using a total of twenty White river crayfish (*Procambarus acutus*) and Ringed crayfish (*Orconectes neglectus*), ranging in size from 58 to 91 mm, yielded a mean efficiency of 100% in all three tests performed. A third test series used a total of twenty White river crayfish and Ringed crayfish, ranging in size from 18 to 35 mm, yielded a mean efficiency of 96.5% (Table 2). The high efficiency rates from these tests indicate that the custom fabricated throw trap and bar seine were an extremely reliable sampling method, ensuring accuracy of the density estimates generated from this study.

Table 2. Throw trap efficiency test data.

	Throw 1	Throw 2	Throw 3	Mean Efficiency (%)
Test 1	20/20	20/20	20/20	100.0%
Test 2	20/20	20/20	20/20	100.0%
Test 3	20/20	18/20	18/18	96.6%
			Overall mean efficiency	**98.9%**
Test 1: Total of 20 Yellow bullhead catfish ranging 20-37 mm.				
Test 2: Total of 20 White river crayfish and Ringed crayfish ranging 58-91 mm.				
Test 3: Total of 20 White river crayfish and Ringed crayfish ranging 18-35 mm.				

Dietary Analysis

Fish retained for dietary analyses were kept in a 10% buffered formalin solution until the stomachs were dissected out and contents examined under a binocular dissecting microscope (American Optics 570). Food items were identified to the lowest taxon practical. Food items were grouped by major taxonomic category and counted to determine the percent occurrence and percent composition of total diet for each species. All food items and dissected fishes were preserved in 70% ethanol (Windell 1968).

Preservation

Excluding sub-samples of commonly occurring species retained for dietary analyses, fish were processed in the field and released as soon as possible. In addition, ten representatives of each species were preserved in a 10% buffered formalin solution for taxonomic verification and the preparation of a local voucher collection. The ten representative fish collected from each species were submitted to Dr. Robert E. Schmidt, Associate Curator of the New York State Museum, Fisheries Department, for positive identification and to establish a voucher collection.

Parasitism

Fishes collected were visually inspected for parasites. Representative samples of fishes determined to have parasites were preserved as described above and further analyzed in a laboratory to determine the species of parasite and its affect on its host.

Water Quality

When ever possible, water quality measurements were taken at the time and site of sampling. pH was tested, using an Oakton pH Tester 2. Dissolved oxygen was measured using a YSI 550A dissolved oxygen meter. Temperature and specific conductivity were measured using a YSI 30 multi-parameter meter.

Data Analysis

Differences in species richness and abundance of fishes and crayfishes between vegetation types were tested using a repeated measures analysis of variance (ANOVA) with vegetation type as the between-subjects factor and sampling event as the within-subjects factor. Abundance data were normalized, when necessary, using a log(y+1) transformation (Sokal and Rohlf 1981). Statistical analyses were performed using SuperANOVA software for the Macintosh PC (Gagnon et al. 1989).

Analysis of Variance on 2' fyke net collection data, 3' fyke net collection data and throw trap collection data were conducted for sampling events 2 through 7. No SAV sampling was conducted for these methods during sampling event 1. Analysis of Variance on seine collection data was conducted for sampling events 1 through 3, 6 and 7. No SAV seining was performed for sample events 4 and 5.

Results

Tessellated darter was the most abundant fish collected (N=608; 25.02% of the grand total for the collection) (Table 3). Pumpkinseed and White river crayfish were the second most abundant (N=280; 11.52% of the total). The least abundant fish were *Centrarchid* sp., Pumpkinseed-Redbreast Sunfish hybrid and White sucker (N=1; 0.04% of the total).

Table 3. Rank abundance order and percentage of grand total collection.

Rank	Common Name	Scientific Name	N	% of total	Total Length Range (mm)	Total Length Mean (mm)
1	Tessellated darter	*Etheostoma olmstedi*	608	25.02%	14-66	38.2
T-2	Pumpkinseed	*Lepomis gibbosus*	280	11.52%	27-145	80.2
T-2	White river crayfish	*Procambarus acutus*	280	11.52%	18-110	57.8
4	Yellow perch	*Perca flavescens*	221	9.09%	45-198	77.5
5	Golden shiner	*Notemigonus crysoleucas*	176	7.24%	25-200	60.5
6	Creek chubsucker	*Erimzyon oblongus*	144	5.93%	26-217	56.7
7	Redfin pickerel	*Esox americanus*	138	5.68%	20-280	110
8	Yellow bullhead	*Ictalurus natalis*	112	4.61%	15-245	53.8
9	Bluegill	*Lepomis macrochirus*	103	4.24%	17-148	68.9
10	Rusty Crayfish	*Orconectes rusticus*	92	3.79%	25-110	83
11	Ringed crayfish	*Orconectes neglectus*	87	3.58%	17-102	45.4
12	Largemouth bass	*Micropterus salmoides*	50	2.06%	25-194	58.9
13	Redbreast sunfish	*Lepomis cyanellus*	45	1.85%	21-204	76.6
14	Creek chub	*Semotilus atromaculatus*	44	1.81%	25-122	51.2
15	Crayfish sp.	*Orconectes* sp.	21	0.86%	50-100	83
16	Rock bass	*Ambloplites rupestris*	7	0.29%	40-200	124.5
17	Black crappie	*Pomoxis nigromaculatus*	6	0.25%	31-186	115.5
18	Common shiner	*Notropis cornutus*	5	0.21%	56-130	78.6
19	Brown bullhead	*Ictalurus nebulosus*	4	0.16%	50-235	128.3
20	Fallfish	*Semotilus corporalis*	4	0.16%	164-212	176.3
21	Centrarchid sp.		1	0.04%	12	12
22	Pumpkinseed-Redbreast Sunfish hybrid		1	0.04%	117	117
23	White sucker	*Catostomus commersoni*	1	0.04%	71	71

Passive Fish Sampling

2' Fyke Nets

A grand total of 215 fishes and crayfishes were collected in the thirty-seven deployments, for an overall mean CPUE of 1.068 specimens per hour of soak time. A total of 188 specimens were collected in the twenty-five deployments near emergent vegetation (87% of total 2' fyke net collection), for a mean CPUE of 1.458. A total of twenty-seven specimens were collected in the twelve deployments in SAV (13% of total 2' fyke net collection), for a mean CPUE of 0.339. Repeated measures ANOVA revealed no significant differences in CPUE between vegetation types, or over time (Appendix Table 4).

A total of eight 2' fyke net deployments yielded no collection. Four of these were near emergent vegetation (16% of deployments near emergent vegetation) and four were in SAV (33% of deployments in SAV).

The 215 specimens collected via 2' fyke net were distributed among twelve taxa. Twelve species were collected near emergent vegetation, four in SAV. Near emergent vegetation the number of individual species collected in each 2' fyke net deployment ranged from 0 to 4 with a mean of 1.68. In SAV the number of individual species collected in each deployment ranged from 0 to 3 with a mean of 1.167. Repeated measures ANOVA revealed significant differences in species richness between vegetation types ($p=0.0376$) and time ($p=0.0482$) (Appendix: Table 4).

The most abundant species collected via 2' fyke nets was Pumpkinseed (*Lepomis gibbosus*) (Table 5). A total of 148 were collected (68.8% of total 2' fyke net collection), 134 near emergent vegetation and fourteen in SAV. Repeated measures ANOVA revealed no significant difference in abundance between vegetation types or over time (Appendix: Table 4). A total of seventy-seven Pumpkinseeds were collected near emergent vegetation on a single day in Sampling Event 2. A single net had twenty-seven specimens, one adult and twenty-six juveniles. A second net had twenty-nine adults and zero juveniles. A third net had twenty-one juveniles and zero adults. These collections represent the three highest individual collections of all 2' fyke nets.

Creek chub (*Semotilus atromaculatus*) was the second most abundant species (Table 5), with a total of sixteen collected (7.5% of total 2' fyke net collection). All

sixteen Creek chubs were juveniles collected near emergent vegetation. Creek chubs are known to hatch from nests in gravel (Smith 1985) or sand (Page and Burr 1991). Interestingly, no gravel or sand bottom areas were observed in proximity to sample sites.

The third most abundant species was Yellow perch (*Perca flavescens*) (Table 5), with a total of fifteen collected (7.1% of total 2' fyke net collection). A total of fourteen Yellow perch were collected near emergent vegetation, and one was collected in SAV. A total of thirteen juveniles were collected in a single deployment near Yellow pond lily in sample event 5. This is typical of Yellow perch, which are known to school (Whitworth 1996), especially as juveniles, in clear water near vegetation (Smith 1985).

Table 5. Total 2' fyke net collection.

Species	Sample Event 1		Sample Event 2		Sample Event 3		Sample Event 4		Sample Event 5		Sample Event 6		Sample Event 7	
	EV	SAV	EV	SAV	EV	SAV	EV	SAV	EV	SAV	EV	SAV	EV	SAV
Bluegill		NS											1	
Creek chub		NS							1		15			
Creek chubsucker		NS									1			
Golden shiner		NS			3				1					
Largemouth bass		NS				1			1			2		3
Pumpkinseed		NS	77	2	23	1	9		19	6	6	5		
Pumpkinseed-Redbreast sunfish hybrid		NS	1											
Redbreast sunfish		NS	1		3	1				1		3		1
Redfin pickerel		NS			1				1					
Rusty crayfish		NS	5		3									
White river crayfish		NS			1		1							
Yellow perch		NS							13			1	1	
Totals	**0**	**NS**	**84**	**2**	**34**	**3**	**10**	**0**	**36**	**7**	**22**	**11**	**2**	**4**

NS= Not Sampled

Sample Event 1- A single 2' fyke net was deployed near emergent vegetation and yielded no collection.

Sample Event 4 SAV- No collection. Heavy rains on previous day caused very swift current.

EV= Emergent Vegetation.

NV=Non-Vegetted.

SAV=Submerged Aquatic Vegetation.

3' Fyke Nets

A grand total of 121 fishes and crayfishes were collected in the thirty-five 3' fyke net deployments, for an overall mean CPUE of 0.661. Fifty-nine specimens were collected in the nineteen deployments at non-vegetated sites (49% of total 3' fyke net collection), for a mean CPUE of 0.574. Sixty-two specimens were collected in the sixteen deployments in SAV (51% of total 3' fyke net collection), for a mean CPUE of 0.773. Repeated measures ANOVA on log(y+1) transformed CPUE data revealed no significant differences in abundance between vegetation type or over time (Appendix: Table 4).

The majority of fishes collected in 3' fyke nets, in both non-vegetated sites and SAV were adults. Species varied, with predators and prey alike being collected in both non-vegetated sites and SAV.

A total of eight deployments yielded no collection, six of these were in non-vegetated sites (31.6% of deployments at non-vegetated sites) and two were in SAV (12.5% of deployments in SAV).

The 121 specimens collected via 2' fyke net were distributed among fourteen taxa. A total of thirteen species were collected in non-vegetated areas, six in SAV. In non-vegetated sites the number of individual species collected in each 3' fyke net deployment ranged from 0 to 5 with a mean of 1.4. In SAV the number of individual species collected in each deployment ranged from 0 to 3 with a mean of 1.688. Repeated measures ANOVA revealed no significant differences in species richness between vegetation types or over time (Appendix: Table 4).

The most abundant species collected via 3' fyke nets was Pumpkinseed. A total of fifty-six were collected (46.3% of total 3' fyke net collection), twenty-six at non-vegetated sites and thirty in SAV (Table 6). Repeated measures ANOVA revealed no significant difference in abundance between vegetation types or over time (Appendix: Table 4).

Bluegill (*Lepomis macrochirus*) was the second most abundant species, with twenty-seven collected (22.3% of total 3' fyke net collection). A total of five Bluegills were collected in non-vegetated locations and twenty-two were collected in SAV (Table 6).

The third most abundant species was Rusty crayfish (*Orconectes rusticus*), with nine collected (7.4% of total 3' fyke net collection). All nine Rusty crayfish were collected in non-vegetated locations during sample events 2 and 3 (Table 6).

Table 6. Total 3' fyke net collection.

Species	Sample Event 1		Sample Event 2		Sample Event 3		Sample Event 4		Sample Event 5		Sample Event 6		Sample Event 7	
	NV	SAV	NV	SAV	NV	SAV	NV	SAV	NV	SAV	NV	SAV	NV	SAV
Black Crappie		NS						1		1		1		
Bluegill		NS			4		1	2		2		5		13
Brown bullhead		NS					1							
Creek chub		NS	1											
Creek chubsucker		NS									2	1	2	
Fall fish		NS			1									
Golden shiner		NS		1		1	1	1		1				
Pumpkinseed		NS	1	2	14	4	2	1	1	3	7	12	1	8
Redbreast sunfish		NS							4		2	1		
Redfin pickerel		NS	1		1									
Rock bass		NS			1							1		
Rusty crayfish		NS	6		3									
White river crayfish		NS			1									
Yellow perch		NS					1							
Totals	**0**	**NS**	**9**	**3**	**25**	**5**	**6**	**5**	**5**	**7**	**11**	**21**	**3**	**21**

NS= Not Sampled

Sample Event 5- One net was improperly deployed.

NV=Non-Vegetated.

SAV=Submerged Aquatic Vegetation.

Minnow Pots

A grand total of fourteen fishes and crayfishes were collected in seventy-three total minnow pot deployments (Table 7), for an overall CPUE of 0.037. A total of four specimens were collected near emergent vegetation (29% of total minnow pot collection) for a mean CPUE of 0.031. A total of two specimens were collected at non-vegetated sites (14% of total minnow pot collection) for a mean CPUE of 0.038. A total of eight specimens were collected in SAV (43% of total minnow pot collection) for a mean CPUE of 0.041. Due to low collection rates, statistical analyses for minnow pots were not computed beyond CPUE.

On three occasions the bread bait was completely removed from minnow pots near emergent vegetation, yet there were no specimens in the trap. On one of these occasions there was a school of juvenile fish, small enough to escape the mesh, observed in the immediate vicinity of an empty minnow pot. A sample jar was used to collect one specimen, which was identified as a juvenile Golden shiner (*Notemigonus crysoleucas*) (Schmidt, pers. comm.).

A total of sixty-three deployments yielded no collection. A total of twenty-one were near emergent vegetation (84% of deployments near emergents), eighteen were at non-vegetated sites (90% of deployments at non-vegetated sites), twenty-four were in SAV (85.7% of deployments in SAV).

The fourteen specimens collected in minnow pots were distributed among five taxa. A total of three species were collected near emergent vegetation, one species was collected at a non-vegetated site, and three species were collected in SAV.

Table 7. Total minnow pot collection.

Species	Sample Event 2			Sample Event 3			Sample Event 4			Sample Event 5			Sample Event 6		
	EV	NV	SAV	EV	NV	SAV	EV	NV	SAV	EV	NV	SAV	EV	NV	SAV
Pumpkinseed		1									1	1	1		3
Redfin pickerel	1														
White river crayfish				1						1					
Yellow bullhead									2						
Yellow perch									1			1			
Totals	**1**	**1**	**0**	**1**	**0**	**0**	**0**	**0**	**3**	**1**	**1**	**2**	**1**	**0**	**3**

NS= Not Sampled

There were no minnow pot collections for sample events 1 or 7.

EV= Emergent Vegetation.

NV=Non-Vegetated.

SAV=Submerged Aquatic Vegetation.

Active Fish Sampling

Seine

A grand total of 1363 fishes and crayfishes were collected in the thirty-six seine hauls, for an overall mean density of 0.323 specimens per m^2. A total of 1173 specimens were collected at the non-vegetated site (86% of total seine collection) for a mean density of 0.477 specimens per m^2. A total of 190 specimens were collected in SAV (14% of total seine collection) for a mean density of 0.108 specimens per m^2. Repeated measures ANOVA revealed significant differences in density between vegetation types (p=0.0001) and there was significant interaction of vegetation type and time (.0025). There was no significant difference over time (Appendix: Table 4).

The 1363 specimens collected in seine hauls were distributed among nineteen taxa. A total of seventeen species were collected at the non-vegetated site and a total of fifteen species were collected in SAV. The number of individual species collected in each non-vegetated seine haul ranged from one to ten, with a mean of 5.25. The number of individual species collected in each SAV seine haul ranged from two to ten, with a mean of 4.429. Repeated measures ANOVA revealed no significant differences in density between vegetation types or over time (Appendix: Table 4).

Among the most abundant species collected in the seine were White river crayfish (*Procambarus acutus*), Redfin pickerel (*Esox americanus*) and Pumpkinseed. A total of 109 White river crayfish were collected via seine hauls (8.0% of total seine collection), seventy-seven at the non-vegetated site and thirty-two in SAV (Table 8). White river crayfish were collected in large quantities early in the study, mostly at the non-vegetated seine site (Table 8). Repeated measures ANOVA revealed significant differences in density between vegetation type (p=.0038), time (p=.0016) and a significant vegetation x time interaction (p=.0062) (Appendix: Table 4).

A total of seventy-nine Redfin pickerel were collected via seine hauls (5.8% of total seine collection), fifty-nine at the non-vegetated site and twenty in SAV (Table 8). Repeated measures ANOVA revealed no significant difference in density over time, but significant differences between vegetation type (p=.0099) and a significant vegetation x time interaction (p=.0131) (Appendix: Table 4).

A total of sixty-one Pumpkinseeds were collected via seine hauls (4.5% of total seine collection), thirty at the non-vegetated site and thirty-one in SAV (Table 8). Repeated measures ANOVA revealed a significant difference in density over time (p=.0418), but not between vegetation types (Appendix: Table 4).

A total of 180 Yellow perch were collected via seine hauls. A total of 170 were collected at the non-vegetated site (12.5% of total seine collection), only one of which was an adult. In SAV, three of the ten Yellow perch collected were adults. Yellow perch were collected with increasing abundance as the study progressed (Table 8).

A total of 136 Golden shiners were collected via seine (10% of total seine collection), 108 at the non-vegetated seine site, twenty-eight in SAV (Table 8).

Tessellated darter (*Etheostoma olmstedi*) was the most abundant species collected via seine hauls. A total of 568 were collected (41.7% of total seine collection). A total of 564 were collected in non-vegetated locations, while only four were collected in SAV. Repeated measures ANOVA revealed significant differences between vegetation type (p=.0011), over time (p=.0023) and a vegetation type x time interaction (p=.0047) (Appendix: Table 4). However, these data were determined to be an outlier after graphical analyses and upon research of Tessellated darter nesting habits (Smith 1985) (Lee et al. 1980).

If the outliers of 564 Tessellated darters collected via seine at the non-vegetated site are removed from the data set, the grand total collection via all sampling methods for the study would be 1866 specimens. The grand total for emergent vegetation collection via all sampling methods would be 532 (28.5% of grand total collection). The grand total collection for non-vegetated sites via all sampling methods would be 670 (35.9% of grand total collection). The grand total for SAV via all sampling methods would be 664 (35.5% of grand total collection).

Table 8. Total seine collection.

Species	Sample Event 1		Sample Event 2		Sample Event 3		Sample Event 4		Sample Event 5		Sample Event 6		Sample Event 7	
	NV	SAV	NV	SAV	NV	SAV	NV	SAV	NV	SAV	NV	SAV	NV	SAV
Bluegill	1	10	5	4	13			NS		NS				7
Common shiner			1				2	NS		NS				
Crayfish sp.			19		1			NS		NS				
Creek chub								NS	1	NS	3	1		
Creek chubsucker								NS		NS	2	1	55	
Fallfish						1	1	NS		NS	1			
Golden shiner		3		3			8	NS		NS	36	7	64	15
Largemouth bass								NS	1	NS	1	4	7	10
Pumpkinseed		3	3	6	12	20		NS		NS	5	2	10	
Redbreast sunfish				4				NS		NS	5	1		6
Redfin pickerel	10	8	21		21	7		NS		NS		2	7	3
Ringed crayfish					1			NS		NS	2	3		
Rock bass								NS		NS		2		
Rusty crayfish			23		16		2	NS		NS	1			
Tessellated darter	1		7		179		138	NS	92	NS	131	2	16	2
White river crayfish	14	30	22		37	1	1	NS		NS	2	1	1	
White sucker								NS		NS	1			
Yellow bullhead								NS		NS		6		5
Yellow perch		1	1			1	7	NS	16	NS	54	5	92	3
Totals	**26**	**55**	**102**	**17**	**280**	**30**	**159**	**NS**	**110**	**NS**	**244**	**37**	**252**	**51**

NS= Not Sampled

Sample Events 4 and 5- SAV not sampled due to high water levels.

NV=Non-Vegetated.

SAV=Submerged Aquatic Vegetation.

Throw Trap

A total of 717 fishes and crayfishes were collected in 155 throws, for an overall mean density of 4.63 specimens per m^2. A total of 340 specimens were collected in emergent vegetation (47% of total throw trap collection), for a mean density of 4.08 specimens per m^2. A total of 377 specimens were collected in SAV (53% of total throw trap collection), for a mean density of 5.19 specimens per m^2. Repeated measures ANOVA revealed no significant difference in density between vegetation types, or over time. However, the results do show a significant vegetation type x time interaction ($p=0.0003$) (Appendix: Table 4).

A total of 31 deployments yielded no collection, twenty were in emergent vegetation (24.1% of deployments in emergent vegetation) and eleven of these were in SAV (15.3% of deployments in SAV).

The 717 specimens collected in the throw trap were distributed among twenty taxa. A total of fourteen species were collected in emergent vegetation and a total of eighteen species were collected in SAV. In emergent vegetation the number of individual species collected in each throw ranged from 0 to 4, with a mean of 1.23. In SAV the range was 0 to 6 species, with a mean of 2.04. Repeated measures ANOVA revealed a significant difference in species richness between the vegetation types ($p=0.0055$), but not over time. There was a significant vegetation x time interaction ($p=0.0025$) (Appendix: Table 4).

The four most abundant species collected in the throw trap were White river crayfish, Ringed crayfish (*Orconectes neglectus*), Yellow bullhead catfish and Creek chubsucker (*Erimyzon oblongus*).

A total of 166 White river crayfish were collected (23.1% of total throw trap collection), 145 in emergent vegetation and twenty-one in SAV. White river crayfish dominated throw trap collections early in the study, with 135 collected through the first three sample events. They were rarely present in the last three sample events, with a total of only fourteen collected (Table 9). Repeated measures ANOVA revealed significant differences between vegetation types ($p=.0059$), and over time ($p=.0003$). There was a significant vegetation type x time interaction ($p=.0001$) (Appendix: Table 4).

A total of ninety-nine Yellow bullhead catfish were collected (13.8% of total throw trap collection), one in emergent vegetation and ninety-eight in SAV (Table 9). Repeated measures ANOVA revealed a significant difference between vegetation type (p=.0006), but no significant differences over time (Appendix: Table 4).

A total of eighty-one Ringed crayfish were collected (11.3% of total throw trap collection), four in emergent vegetation and seventy-seven in SAV (Table 9). Repeated measures ANOVA revealed a significant difference between vegetation types (p=.0036), but no significant differences over time (Appendix: Table 4).

A total of eighty Creek chubsuckers were collected (11.1% of total throw trap collection), seventy in emergent vegetation and ten in SAV (Table 9). Repeated measures ANOVA revealed no significant differences between vegetation type (p=.1619), or over time (p=.2657) (Appendix: Table 4).

Table 9. Total throw trap collection.

Species	Sample Event 1		Sample Event 2		Sample Event 3		Sample Event 4		Sample Event 5		Sample Event 6		Sample Event 7	
	EV	SAV	EV	SAV	EV	SAV	EV	SAV	EV	SAV	EV	SAV	EV	SAV
Black Crappie		NS								1		2		
Bluegill		NS	4			1		2		6		3		19
Brown bullhead		NS		1										2
Centrarchid sp.		NS								1				
Common shiner		NS						2						
Crayfish sp.		NS	1											
Creek chub		NS							22					
Creek chubsucker		NS				7	2		22	3	35		11	
Golden shiner		NS				8	3				12	5	1	2
Largemouth bass		NS				11		2	1	3	2			1
Pumpkinseed		NS		1	1	1						3		2
Redbreast sunfish		NS		1				1		3		6		1
Redfin pickerel	11	NS	11	4	6	4	2	4	1			1	8	2
Ringed crayfish		NS		16	2	13	1	36		6		4	1	2
Rock bass		NS								1		2		
Rusty crayfish		NS	27	1	3		1							1
Tessellated darter		NS		1		23		4		4	1	4	1	2
White river crayfish	66	NS	37		28	4	10	7	3	3	1	3		4
Yellow bullhead		NS		2		18		47	1	11		13		7
Yellow perch		NS				2		6	1	1		6		7
Totals	**77**	**NS**	**80**	**27**	**40**	**92**	**19**	**111**	**51**	**43**	**51**	**52**	**22**	**52**

NS= Not Sampled
EV=Emergent Vegetation.
SAV=Submerged Aquatic Vegetation.

Dietary Analysis

A total of 138 Redfin pickerels were collected, ranging in size from 20 mm to 280 mm, with a mean of 110 mm (Table 3). Gut contents analyses were performed on thirty-two specimens. Twenty-two had empty guts. Four contained crayfish; three of these were collected via seine at a non-vegetated site, while the other was collected via seine in SAV. Four contained insects; two of these were collected via throw trap in emergent vegetation, one collected via seine at a non-vegetated site, and one was collected via 3' fyke net at a non-vegetated location. Two contained juvenile fish; both of these were collected via throw trap, one was in emergent vegetation, the other in SAV. This gage is

approximately 0.5 river miles downstream from the most southerly sampling location in this study.

Water Quality

Throughout the study, water temperature ranged from 12.5° C on September 20 to 27.9° C on June 30. Dissolved oxygen ranged from 1.5 mg/l on August 9 to 12.3 mg/l on June 29. Specific conductance ranged from 248 μs on August 12 to 616 μs on May 21. pH ranged from 6.1 on June 9 to 8.3 on July 2. Data from a USGS water height gage in the East Branch Croton River shows the water height on sampling dates ranged 2.4 feet on September 20 to 4.1 feet on August 12, with a mean of 2.9 feet.

Parasitism

Many Redfin pickerel and Golden shiner collected during this study were noted to have Black Spot disease. The black spots on the scales of the infected fishes are metacercaria (encysted stage) of a digenetic trematode. This is a parasite that is thought to not harm the fish, simply using them as an intermediate host (Smith 1985), but effects fish eating birds, such as Kingfishers, Great Blue Herons and Green Herons (Schmidt personal communication), all of which are present in the Great Swamp (Siemann 1999). Smith (1985) states that Creek chubs are particularly prone to Black Spot disease, though none collected in this study were observed to have any.

Discussion

The previous study on the Great Swamp's fish community (van Holt et al. 2006) mainly focused on the Swamp's numerous tributaries and found a greater number of species than my study because it focused on a greater diversity of habitat types and a larger geographic area. This study focused on comparison of vegetated and non-vegetated communities in a small geographic area. The prior study identified twenty-eight fish species, while this study identified eighteen fish species. The only species not identified by the prior study that were collected in this study were Ringed crayfish and a Pumpkinseed-Redbreast sunfish hybrid. The ten species identified by van Holt et al.

(2006) not identified in this study inhabit swift moving streams, characteristic of the Great Swamp's tributaries, but not the East Branch Croton River.

Passive Fish Sampling

2' Fyke Nets

The 2' fyke nets were intended to be used in shallow, non-vegetated areas to compliment the 3' fyke net use. However, the depths for which they are suitable, 15" to 26", were in the littoral zone, either adjacent to or in emergent vegetation instead of intended non-vegetated areas. Because of this, the 2' fyke nets were used near emergent vegetation to compliment emergent throw trap data.

The majority of fishes collected in 2' fyke nets were juveniles, and species that are often preyed upon. Individuals of predatory species [Yellow perch, Redfin pickerel, Largemouth bass (*Micropterus salmoides*)] were observed swimming towards the nets, then changing direction as they neared the wings and/or opening to avoid the net. This indicates that these individual predators were aware of the net. They may have seen the bright white mesh or the tubular shape of the net. Escaping the ¼" mesh would not have been an issue, as very few fish collected throughout the study, by any method, could escape this mesh size. This observation serves to explain why there were very few adult predatory species collected in 2' fyke nets.

The 2' fyke net CPUE was higher near emergent vegetation than the CPUE in SAV due to the high number of Pumpkinseeds collected in sampling event 2, which made up 52% of the total Pumpkinseed collection via 2' fyke net for the entire study. Pumpkinseeds nest near shore, in clear water 6-12" deep. The 2' fyke nets were deployed just outside that range, but certainly within the feeding range of the adult males that would be keeping watch over their nests and the juveniles searching for food (Smith 1985).

3' fyke nets

CPUE was far lower for 3' fyke nets than 2' fyke nets. The 3' fyke nets were deployed in deeper water, some areas non-vegetated. This offers more avoidance

opportunity to fishes, as the channel is wider in these deeper locations, and the nets were have been more visible without the cover of vegetation.

The fact that Bluegills were collected mostly in SAV was expected, as Bluegills are known to prefer SAV habitat (Lee et al, 1980).

Minnow pots

Minnow pots were not a reliable source of data for this study. CPUE was far below that of fyke nets.

Active Fish Sampling

Seine

The large number of Tessellated darter collected was viewed as an outlier. The non-vegetated location that was seined was Patterson Environmental Park in Patterson, NY. A pile of large rocks (1-2' across) was placed along the shore as a way of preventing bank erosion. Most of the rocks are underwater, creating perfect breeding locations for Tessellated darters, which utilize spaces underneath rocks for spawning and nesting (Smith 1985) (Lee et al. 1980). This is the only such location within the study area where there is an abundance of large rocks. The majority of Tessellated darters collected at this site were juveniles, giving more evidence that this is an area of dense Tessellated darter nesting, thus aiding in the determination that these quantities are an outlier.

Yellow perch were collected almost exclusively at the non-vegetated seine site. This is expected, as Yellow perch are known to school in non-vegetated waters, especially juveniles, which dominated collection (Lee et al. 1980) (Page and Burr 1991).

Golden shiners were mostly collected at the non-vegetated seine site. The majority of Golden shiners collected were juveniles, collected in sample events 6 and 7. This is consistent with previous studies, where Golden shiners are reported as late spawners that prefer non-vegetated waters (Smith 1985).

Pumpkinseeds being collected in almost equal numbers at the non-vegetated site and in SAV is to be expected as adult Pumpkinseeds are known to prefer the safety of vegetation, but are also known to guard their nests which require a non-vegetated site (Smith 1985).

Throw Trap

A portion of the Great Swamp's emergent vegetation community is comprised of Smartweed, which has a solid hummock growth pattern not suitable for use of the throw trap. The trap's bottom would not penetrate the lower woody stalks of the Smartweed, and therefore would have large gaps along the bottom allowing fish to escape. In addition, the earthen hummocks rise up causing the bar seine to lose contact with large portions of the sediment, thus allowing fauna within the trap's walls to go uncollected. Visual estimates determined that Smartweed makes up approximately 10% of the Great Swamp's emergent vegetation within the study area.

It was noted early in the study that emergent vegetation, water levels ranging 2" to 24", was used as a nursery for juveniles, particularly Redfin pickerel and White river crayfish. Those were the only two species collected in emergent vegetation in the throw trap during sample event 1. Both species would be in the midst of spring spawning at that time, which takes place in shallow, densely vegetated waters. Redfin pickerels will often spawn again in the fall as water temperatures return to levels similar to those of spring, which explains the presence of adults in such shallow water (Lee et al. 1980). During the warmer summer months Redfin pickerels were rarely collected, but often visually observed in the non-vegetated channel.

Yellow bullhead catfish were found almost exclusively in SAV, which is to be expected. Smith (1985) stated that Yellow bullheads prefer vegetation in clear water, which describes the habitat in which they were collected during this study.

Creek chubsuckers were mainly collected at or near the non-vegetated/emergent interface. This is to be expected as they are known to prefer clear, vegetated water. Smith (1985) states that they have disappeared from much of their former range due to their inability to tolerate silty waters.

Dietary Analysis

The absence of food from the majority of the Redfin pickerels dissected is to be expected from an opportunistic predator. The food types present in these specimens are also typical, as this species is known to prey on crayfish, smaller fish and insects (Smith 1985) (Whitworth 1996). While it was not possible to identify partially digested crayfish

to species, the three Redfin pickerels that consumed crayfish were collected at the non-vegetated seine site where White river crayfish and Rusty crayfish were collected in large quantities. The Redfin pickerel collected via seine in SAV that had consumed a crayfish was collected where Ringed crayfish were collected in large quantities. Two specimens having consumed insects were collected in emergent vegetation, and two were collected in non-vegetated areas. Aquatic insects were observed at all sampling locations: Non-vegetated sites, emergent vegetation, and SAV. Jenkins and Burkhead (1994) stated that invertebrates and crayfish are the dominant food items of the adult Redfin pickerel's diet. The Redfin pickerel containing a juvenile fish that was collected in emergent vegetation was collected at a site where several juvenile Bluegills were collected, but juvenile Redfin pickerels were the dominant species present. The Redfin pickerel containing a juvenile fish that was collected in SAV was collected at a site where the dominant species was juvenile Yellow bullhead catfish. Juvenile Bluegills, Golden shiners, Largemouth bass and Redfin pickerels were also collected at this site.

Conclusions

This study revealed species-habitat associations for certain species within the Great Swamp portion of the East Branch Croton River. Associations are only reported for the eleven species of fishes and crayfishes that individually comprised greater than 3% of the grand total collection.

Crayfishes

Collection numbers for all crayfish species dropped off as the study progressed. River otters (*Lontra canadensis*) and Mink (*Mustela vison*) were observed in the areas where Rusty crayfish had been particularly abundant. Mink were observed preying upon Rusty crayfish on several occasions, and the carcasses of several crayfish species were observed in greater abundance as the study progressed.

A large number of White river crayfish were collected in emergent vegetation (53.2% of total), and in SAV (18.9% of total) for a combined collection total of 72.1% in

vegetation. Statistical analyses revealed a significant difference in favor of non-vegetated water for seine data, and for emergent vegetation for throw trap data.

A large number of Rusty crayfish were collected in non-vegetated areas (55.4% of total), and in emergent vegetation (42.4% of total) for a combined collection total of 97.8%. While the data did not clearly define a direct habitat association for Rusty crayfish, it does strongly suggest that they do not often utilize SAV as habitat. The data suggest that White river crayfish and Rusty crayfish inhabit similar territories. The vast majority of Ringed crayfish (92.0% of total) were collected in SAV. Throw trap collection data provided strong evidence of a Ringed crayfish-SAV association.

Rusty crayfish were observed to dig holes out of the muddy banks for safety when rocks were not present. River otters, Mink and even a Starling (*sturnus vulgaris*) were observed removing Rusty crayfish from these burrows and preying upon them. In addition, the Rusty crayfish's aggressive nature seems to work against it. While other fishes and crayfishes were observed to flee on sight of a human, Rusty crayfish would aggressively approach with their claws displayed. While all three identified crayfish species populations declined as the study progressed, the Rusty crayfish population was most affected.

Centrarchids

Most Bluegills (71.8% of total) were collected in SAV, providing evidence of a Bluegill SAV association. A large number of Pumpkinseeds (48.6% of total) were collected in emergent vegetation, and 30.7% of total were collected in SAV for a combined collection total of 79.3% collected in vegetation. While there were no significant results from statistical tests performed for 2' fyke nets, 3' fyke nets or the seine, the combined vegetation collection percentage provides evidence of a Pumpkinseed vegetation association, except when nesting.

Percids

A large number of Yellow perch (77.4% of total) were collected in non-vegetated areas, providing evidence of a Yellow perch non-vegetated association. Statistical analyses for seine collection data support this association. The vast majority of Tessellated darters (92.8% of total) were collected in non-vegetated areas, providing

strong evidence of a Tessellated darter-non-vegetated association. Repeated measures ANOVA for seine collection data support this association.

Cypriniformes

Though not statistically significant, a large number of Golden shiners (61.9% of total) were collected in non-vegetated areas, providing evidence of a Golden shiner non-vegetated association. A large number of Creek chubsuckers (49.3% of total) were collected in emergent vegetation. However, 42.4% of Creek chubsuckers were collected in non-vegetated areas. There were no statistical analyses run that directly compared collections in emergent vegetation and non-vegetated locations. The similar numbers of Creek chubsuckers collected in emergent vegetation and non-vegetated areas indicate schooling was taking place at the vegetation interface.

Others

As stated above, Redfin pickerels were abundant in emergent vegetation during spring and autumn sampling events, but were abundant in non-vegetated areas during the summer sampling events.

Virtually all Yellow bullheads (99.1% of total) were collected in SAV, providing strong evidence of a Yellow bullhead catfish-SAV species-habitat association. Statistical analyses of throw trap data revealed a significant association.

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APPENDIX

Table 4. Repeated Measures ANOVA comparing fish presence by vegetation over time.
* indicates a significant result.

Source	df	ss	ms	f	p
2' Fyke Nets					
CPUE					
Vegetation Type	1	.300	.300	5.844	.0730
Log(y+1)Time	5	.351	.051	1.875	.1440
Log(y+1) Time X Veg Type	5	.316	.063	1.686	.1841
Species Count					
Vegetation Type	1	2.722	2.722	9.333	.0378*
Time	5	14.278	2.856	2.741	.0482*
Time X Veg Type	5	10.278	2.056	1.973	.1268
Pumpkinseed Collection					
Vegetation Type	1	.766	.766	2.934	.1619
Log(y+1) Time	5	1.639	.328	2.605	.0570
Log(y+1) Time X Veg Type	5	.823	.165	1.309	.3001
3' Fyke Nets					
CPUE					
Vegetation Type	1	.001	.001	.029	.8741
Log(y+1) Time	5	.121	.024	.710	.6233
Log(y+1) Time X Veg Type	5	.212	.042	1.249	.3239
Species Count					
Vegetation Type	1	.028	.028	.025	.8820
Time	5	7.806	1.561	1.033	.4253
Time X Veg Type	5	18.472	3.694	2.445	.0696
Pumpkinseed Collection					
Vegetation Type	1	.061	.061	.558	.4965
Log(y+1) Time	5	.627	.125	1.128	.3778
Log(y+1) Time X Veg Type	5	.352	.070	.633	.6767
Seine					
Density					
Vegetation Type	1	.108	.108	482.53	.0001*
Log(y+1) Time	4	.047	.012	1.714	.1960
Log(y+1) Time X Veg Type	4	.055	.014	1.994	.1440
Species Count					
Vegetation Type	1	8.533	8.533	2.207	.2116
Time	4	51.00	12.750	2.713	.0673
Time X Veg Type	4	39.80	9.950	2.117	.1260
Tessellated Darter Collection					
Vegetation Type	1	4.814	4.814	70.758	.0011*
Time	4	3.051	.763	6.695	.0023*
Time X Veg Type	4	2.605	.651	5.717	.0047*

Source	df	ss	ms	f	p
Yellow Perch Collection					
Vegetation Type	1	.615	.615	9.368	.0376*
Log(y+1) Time	4	2.895	.724	4.845	.0094*
Log(y+1) Time X Veg Type	4	1.291	.323	2.162	.1201
Golden Shiner Collection					
Vegetation Type	1	.045	.045	.207	.6726
Time	4	1.955	.489	2.181	.1176
Time X Veg Type	4	.794	.198	.885	.4948
Pumpkinseed Collection					
Vegetation Type	1	3.131E-4	3.131E-4	.004	.9552
Log(y+1) Time	4	.956	.239	3.188	.0418*
Log(y+1) Time X Veg Type	4	.835	.209	2.784	.0626
White River Crayfish Collection					
Vegetation Type	1	1.197	1.197	36.465	.0038*
Log(y+1) Time	4	2.035	.509	7.220	.0016*
Log(y+1) Time X Veg Type	4	1.512	.378	5.363	.0062*
Redfin Pickerel Collection					
Vegetation Type	1	1.180	1.180	21.301	.0099*
Log(y+1) Time	4	.800	.200	2.790	.0622
Log(y+1) Time X Veg Type	4	1.277	.319	4.453	.0131*
Throw Trap					
Density					
Vegetation Type	1	.576	.576	2.211	.1512
Log(y+1) Time	5	.486	.097	.769	.5743
Log(y+1) Time X Veg Type	5	3.187	.637	5.044	.0003*
Species Count					
Vegetation Type	1	25.840	25.840	9.477	.0055*
Log(y+1) Time	5	5.535	1.107	.852	.5160
Log(y+1) Time X Veg Type	5	25.701	5.140	3.956	.0025*
White River Crayfish Collection					
Vegetation Type	1	.524	.524	9.292	.0059*
Log(y+1) Time	5	1.102	.220	5.173	.0003*
Log(y+1) Time X Veg Type	5	1.230	.246	5.776	.0001*
Yellow Bullhead Catfish Collection					
Vegetation Type	1	1.397	1.397	16.173	.0006*
Log(y+1) Time	5	.432	.086	1.913	.0979
Log(y+1) Time X Veg Type	5	.454	.091	2.009	.0829
Ringed Crayfish Collection					
Vegetation Type	1	.529	.529	10.609	.0036*
Log(y+1) Time	5	.327	.065	1.438	.2165
Log(y+1) Time X Veg Type	5	.214	.043	.940	.4580
Creek Chubsucker Collection					
Vegetation Type	1	.217	.217	6.305	.0199*
Log(y+1) Time	5	.350	.070	1.308	.2657
Log(y+1) Time X Veg Type	5	.378	.076	1.411	.2260