

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

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

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

TABLE OF CONTENTS

Abstract	VII-2
Table of Contents	VII-3
Lists of Figures and Tables	VII-4
Introduction.....	VII-5
Objectives	VII-10
Methods.....	VII-11
Field collection.....	VII-11
Sample processing	VII-13
Analysis.....	VII-14
Results.....	VII-15
Discussion	VII-27
Acknowledgments.....	VII-33
Literature Cited	VII-34

LIST OF FIGURES AND TABLES

Figure 1 – Map of four sites in the upper Hudson River Estuary	VII-12
Figure 2 – Scatterplot of PCA ordination of environmental variables	VII-16
Figure 3 – Cysts filled with nematode sp. 1.....	VII-20
Figure 4 – Photograph of <i>Creptotrema funduli</i>	VII-20
Figure 5 – Scatterplot of the NMS ordination of parasite abundance at the four sites	VII-23
Figure 6 – Photograph of a gill infection of <i>Myxobolus funduli</i>	VII-25
Table 1 – Environmental parameter data at the four sites	VII-16
Table 2 – Mean abundance with standard error (SE) of parasite species	VII-18
Table 3 – The p-values produced by Mann-Whitney <i>U</i> tests for site pair-wise comparisons.....	VII-21
Table 4 – Parasites present only at one site	VII-21
Table 5 – Sørensen's similarity coefficients (SSC) site comparisons	VII-22
Table 6 – Multi-Response Permutation Procedures (MRPP) <i>p</i> -values for sites' parasite abundance comparisons.....	VII-24
Table 7 – Shannon-Wiener Index (H') for the four sites.....	VII-24
Table 8 – Percent Prevalence of all killifish parasites found.....	VII-26

INTRODUCTION

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

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

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

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

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

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

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

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

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

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

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

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

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

Objectives

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

METHODS

Field collection

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

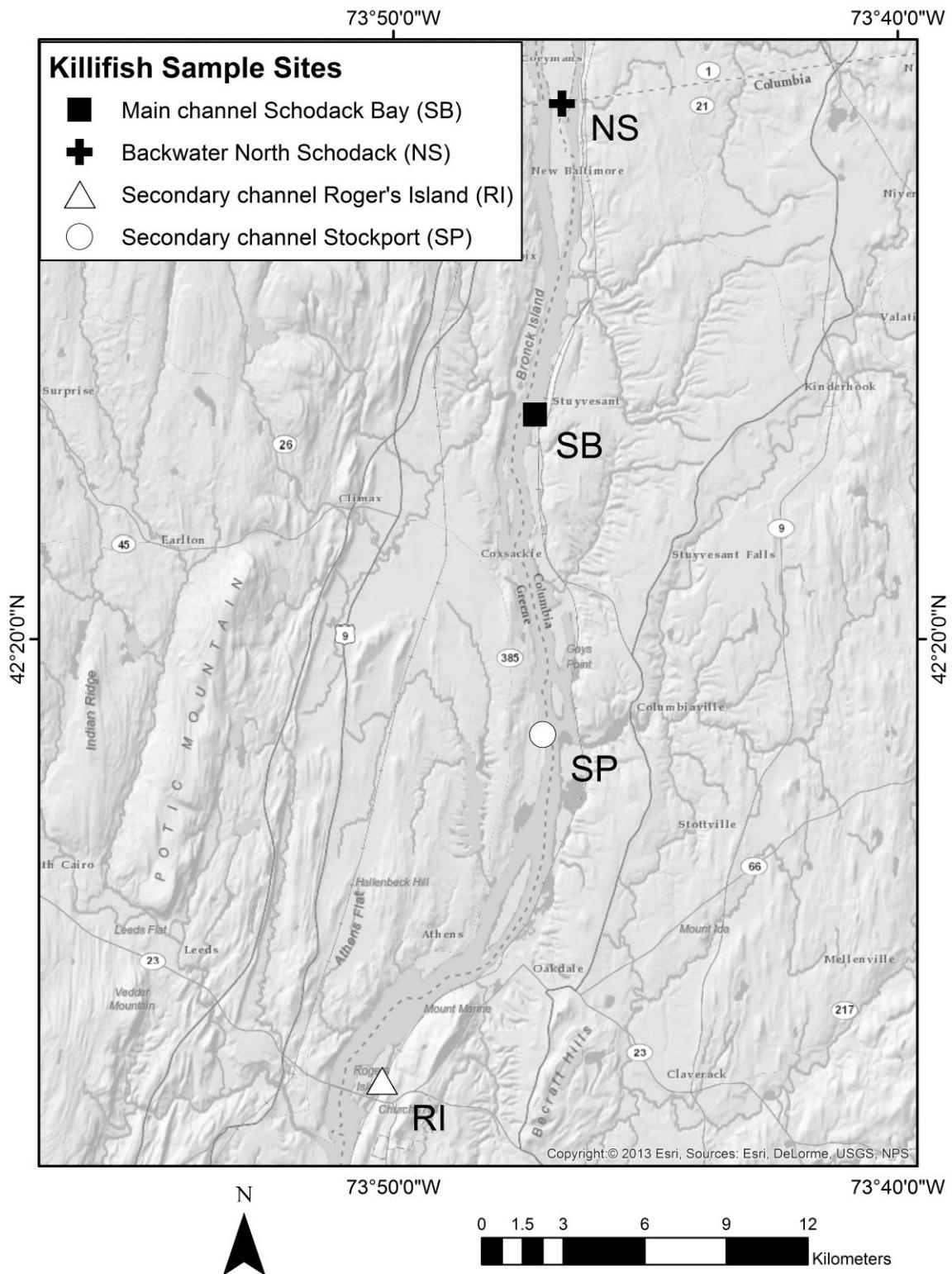


Figure 1. Map of four sites in the upper Hudson River Estuary.

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

Sample processing

Banded killifish were necropsied and the following tissues examined for parasites: the external surface including fins and operculum; esophagus, all gill arches, heart, liver, spleen, kidney, swim bladder, gonads, urinary bladder, intestine, mesenteries, eyes, and gall bladder (when it was intact). Typically, a census count of all individuals of particular species is done, and for most of the digeneans, acanthocephalans, and nematodes this was possible; however, for different parasite groups, it was a matter of necessity to quantify or enumerate them differently. They were preserved using the methods listed by Upton (2005). Nematode sp.1 occurred in cysts of varying sizes that were filled with small nematodes, and usually a multitude of nematodes were present in each cyst (exceeding hundreds inside many cysts). Therefore, the number of cysts full of these nematodes was counted to quantify the abundance of this parasite. Monogeneans found in many of the fish's gills were recorded as present or absent, and were preserved but not counted.

Myxosporea were frozen for preservation for later identification using molecular techniques, recorded as present for presence/absence data, and photographed and measured using Spot102 Basic software (Diagnostic Instruments, Inc. Spot RT Software 4.6 Sterling Heights, Michigan). An exception was with *Myxobolus funduli*, which was

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

Analysis

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

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

RESULTS

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

Table 1. Environmental parameter data at the four sites.

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

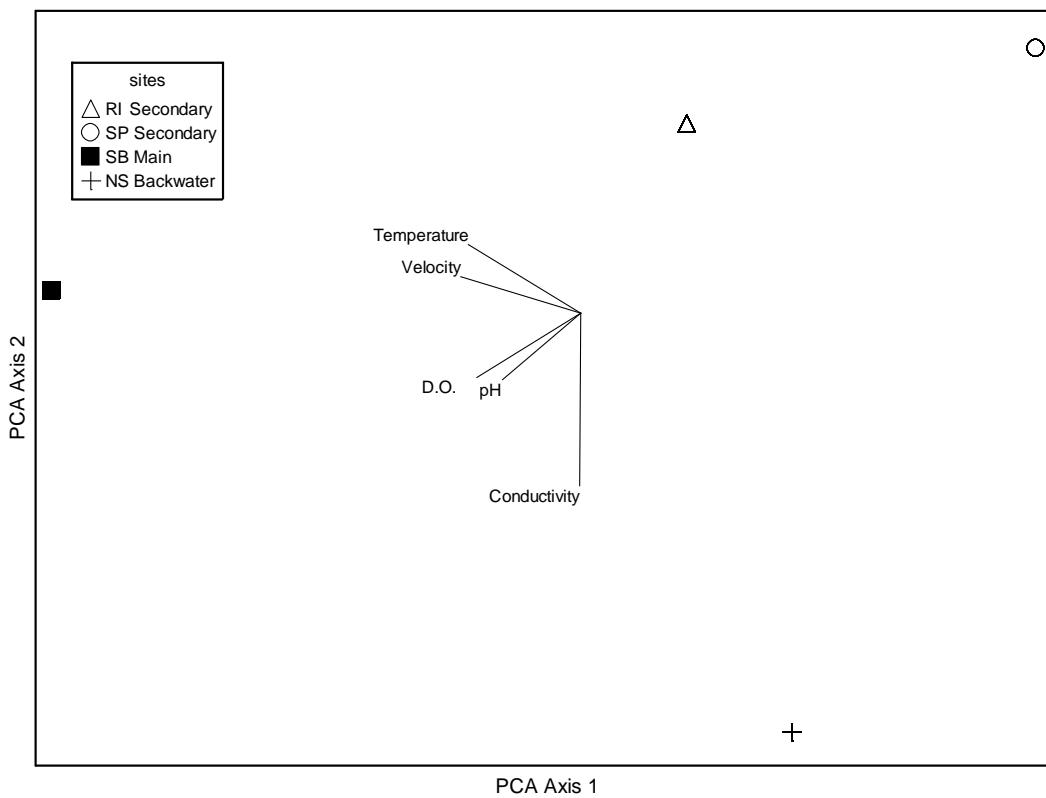


Figure 2. Scatterplot of PCA ordination of environmental variables. Arrangement of four sites—Rogers Island (RI), Stockport (SP), Schodack Bay (SB), and North Schodack (NS)—in two-dimensional space by environmental data variables. Vectors are labeled and illustrated with lines and labeled with the environmental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

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

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

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

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

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

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

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



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

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



Figure 4. Photograph of *Creptotrema funduli*.

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

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

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

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

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

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

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

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

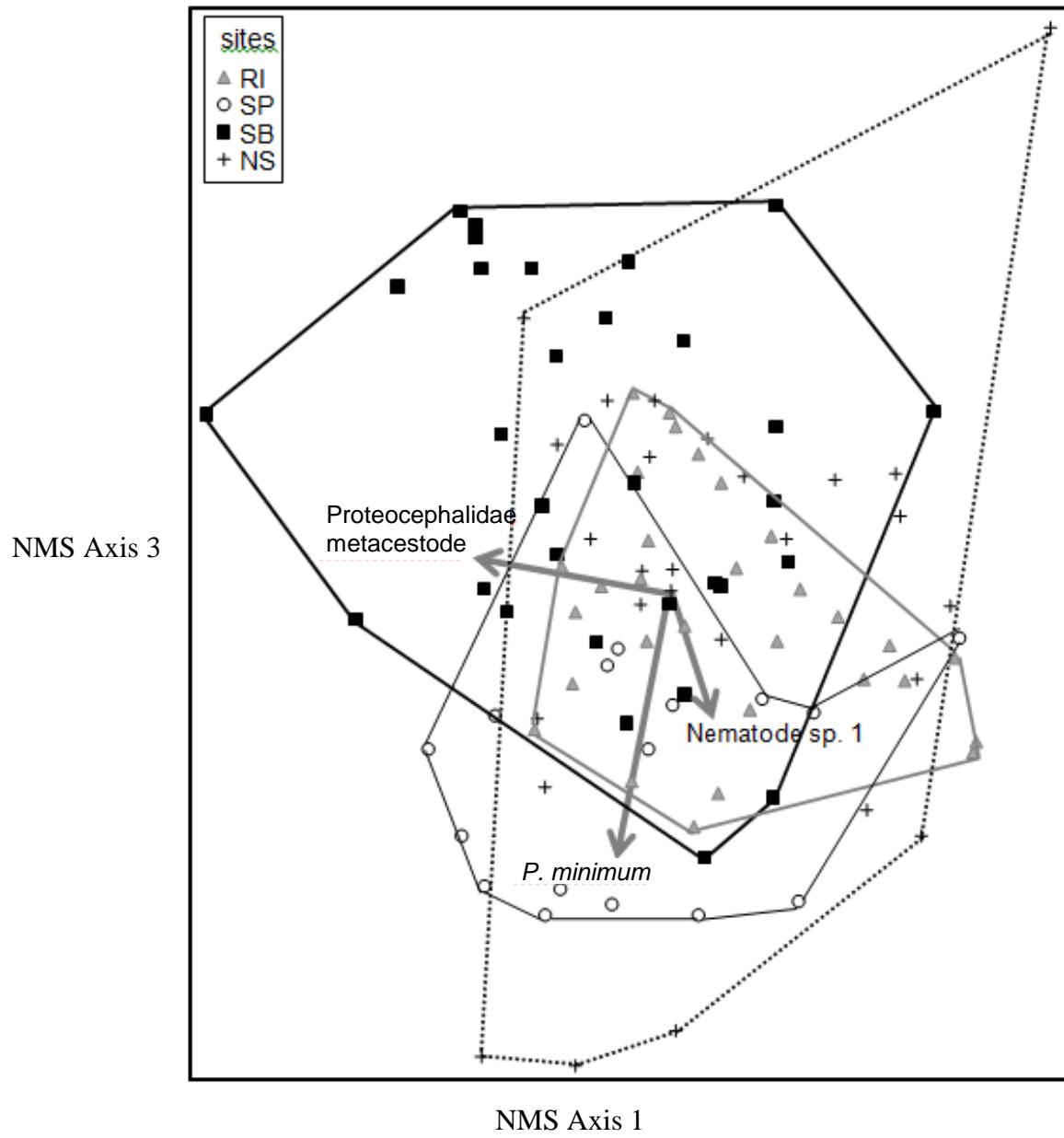


Figure 5. Scatterplot of the NMS ordination of parasite abundance at the four sites. Rogers Island (RI) is pictured as a gray triangle, Stockport (SP) is an open circle, Schodack Bay (SB) is a closed square, and North Schodack is a plus sign. The vectors are labeled with their parasite name and signified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

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

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

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

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

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

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

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

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

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

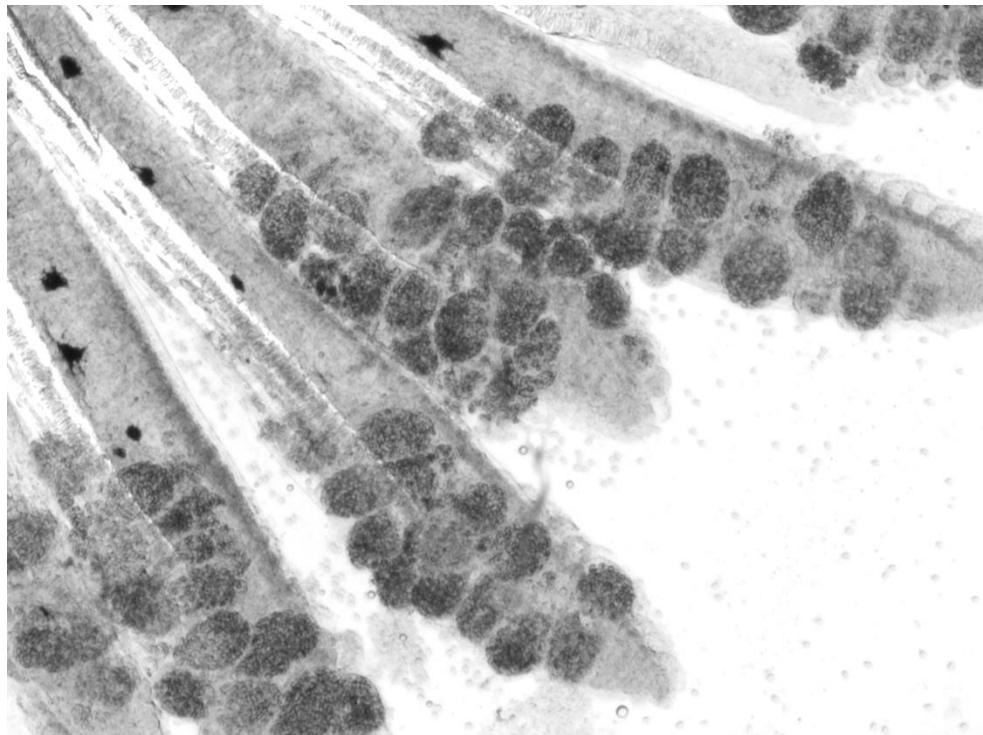


Figure 6. Photograph of a gill infection of *Myxobolus funduli*. The gill filaments are pictured and the darker circular objects are the *Myxobolus funduli* cysts. This was an average, moderate infection for the killifish examined.

Table 8. Percent prevalence of all killifish parasites found.

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

DISCUSSION

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

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

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

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

secondary channel site, had the highest mean abundance of nematode sp. 1 cysts.

Proteocephalidae metacestodes were most abundant at the main channel site (SB).

Because this study examined parasites found within one fish species in one river, it was expected that there would be some similarity between parasite communities; however, the different habitat category types (and to a lesser extent all the different sites) supported distinct parasite communities.

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

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

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

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

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

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

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

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

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

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

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

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

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

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