

**REPORTS OF THE TIBOR T. POLGAR
FELLOWSHIP PROGRAM, 2014**

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Editors

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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 2014. Major objectives of these studies included: (1) comparison of bud depth and herbivory pressure on the recovery of *Vallisneria americana* in the Hudson River, (2) surveying the current distribution status of goldenclub in the tidal freshwater Hudson River, (3) examination of the benthos to determine the existence of subtidal oyster reefs in the Arthur Kill and western Raritan Bay, (4) evaluation of effects of prey density on foraging efficiency of alderflies and stoneflies, (5) determination of the impacts of PCBs on heart development in Atlantic and shortnose sturgeon larvae, (6) characterization of fish movement into and out of a Hudson River tributary, (7) comparison of feeding interactions between the Oriental weatherfish and native fishes in the Klyne Esopus Kill, and (8) assessment of the effects of dams on densities and sizes of American eels in the Bronx River.

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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 it supports. As such, it presents a major opportunity and challenge to researchers to describe the makeup and workings of a complex and dynamic ecosystem. The Tibor T. Polgar Fellowship Program provides funds for students to study selected aspects of the physical, chemical, biological, and public policy realms of the estuary.

The Polgar Fellowship Program was established in 1985 in memory of Dr. Tibor T. Polgar, former Chairman of the Hudson River Foundation Science Panel. The 2014 program was jointly conducted by the Hudson River Foundation for Science and Environmental Research and the New York State Department of Environmental Conservation and underwritten by the Hudson River Foundation. The fellowship program provides stipends and research funds for research projects within the Hudson drainage basin and is open to graduate and undergraduate students.

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

The work reported in this volume represents the eight research projects conducted by Polgar Fellows during 2014. These studies meet the goals of the Tibor T. Polgar Fellowship Program to generate new information on the nature of the Hudson estuary and to train students in estuarine science.

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FACTORS FOR LOSS AND RESTORATION OF *VALLISNERIA AMERICANA* IN THE HUDSON RIVER - HERBIVORY AND DEPTH IN SEDIMENT

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

Two storm events, Hurricane Irene and Tropical Storm Lee, struck the Hudson River watershed at the end of the 2011 growing season. In 2012, distribution of the most common species of submerged aquatic vegetation (SAV), *Vallisneria americana* (wild celery), in the Hudson River estuary had declined by more than 90 per cent, with no appreciable recovery in 2013 and 2014. Because of SAV's important role in providing habitat for aquatic life and increasing dissolved oxygen, managers and scientists have begun discussing the reasons for the loss, as well as how to assist its recovery through restoration efforts in the estuary.

Two factors, potentially important for the loss, recovery and restoration of *V. americana* in the Hudson River were investigated: sprouting success at varying depths in sediment, and herbivory pressure. Sediment washed into the river by the storm events may have added considerably to the depth at which the overwintering buds of *V. americana* are buried, impeding their ability to sprout. This hypothesis was tested in a greenhouse as well as in the river itself. Sprouting success was found to decrease drastically with depth in the river. This supports the hypothesis that sediment burial was a factor in the 2011-2012 loss of the plant in the river.

Herbivory was hypothesized to be a factor impeding recovery in the river and a potential reason for lack of recovery. Herbivory pressure was tested in the Hudson River using an exclusion cage experiment with controls. No significant difference in growth was found between treatments. There was no evidence to support the idea that herbivory pressure is a significant factor impeding growth of *V. americana* in the river.

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INTRODUCTION

Aquatic life in the Hudson River, like that of many shallow rivers and estuaries, benefits greatly from submerged aquatic vegetation (SAV). The hidden nature and less than spectacular appearance of SAV often makes it underappreciated by the public, but it is in many ways the basis for a productive ecosystem in the river. SAV provides important ecological services to rivers and estuaries (Miller 2013) as well as other aquatic systems, for example, by increasing dissolved oxygen levels in the river, benefiting aquatic life in otherwise low-oxygen areas (Caraco et al. 2006; Findlay et al. 2006). SAV beds also provide habitat (Rozas and Odum 1988) and food (Schmidt and Kiviat 1988; Heck and Thoman 1984) for fish and other aquatic life, with densities of benthic invertebrates in the sediment under SAV beds 3X higher than unvegetated areas in the Hudson River (Strayer and Smith 2001).

This importance to the ecosystem makes the last century's large-scale loss, and sometimes even complete disappearance, of SAV from many aquatic ecosystems (e.g. Orth and Moore 1983) even more disconcerting. This loss is often attributed to agricultural and urban runoff increasing nutrient loading, leading to worsened light conditions, but in other cases attributed to weather events such as hurricanes (e.g. Rybicki et al. 2001; Stankelis et al. 2003) or drought (Harwell and Havens 2003), disease, destruction of habitat, competition with invasive species (Lotze et al. 2006) or by destruction and potential herbivory by aquatic wildlife (Crivelli 1983; Rodríguez et al. 2005). Even when the direct loss of SAV has been attributed to a single disturbance event, the lack of regrowth may still be due to more enduring changes in the ecosystem (Rybicki et al. 2001). The loss of SAV translates into loss of fish and other aquatic life

and a declining health of the system, in turn leading to economic loss for fisheries and tourism (Kahn and Kemp 1985).

Vallisneria americana, an endemic, submerged aquatic species, historically represented the large majority of the submerged aquatic vegetation (SAV) in the lower Hudson River (Findlay et al. 2006), with SAV covering 6% of the total area of the Hudson River below the Troy dam and 18% of shallows (<3 m) in the mid 1990's (Nieder et al. 2004). *V. americana* generally grows in dense beds in the river. It has tape-like leaves that are submerged and rooted but can float on the surface. *V. americana* reproduces sexually as well as asexually, through stolons growing off the main plant and creating new rosettes that later detach, and by producing tubers from which new plants sprout in the next season (Korschgen and Green 1989). The spread and growth of *V. americana* is influenced by light conditions, composition of sediment, flow, competition, climate, salinity, herbivory, human disturbance and storm events (Caraco and Cole 2002; Korschgen and Green 1989; Nieder et al. 2004; Jarvis and Moore 2008; French and Moore 2003; Moore et al. 2010; Rybicki et al. 2001). *V. americana* has been in modest decline from the late 1990s and early 2000s in the Hudson River, losing about a third of its coverage in the river, from approximately 18 km² to 12 km² (Findlay et al. 2014; Nieder et al. 2004; Strayer et al. 2014).

In 2011, the Hudson River experienced two large storm-events: Hurricane Irene (August 21-28), and Tropical Storm Lee (September 1-5). Hurricane Irene produced 30 to 45 cm of rain in some parts of the Hudson River watershed, followed by an additional 8-23 cm of rainfall during Tropical Storm Lee over a 48 hour period (Villani et al. 2012), a massive increase compared to an average monthly precipitation of 10 cm for the months

of August and September (NOAA/NWS 2014). Estimates place the flooding from these events in the 100-500 year recurrence interval (Smith 2012). The precipitation from these events caused a historically high summertime discharge of 8680 m³/s at the mouth of the river (Horowitz et al. 2014). The year after the storm, in 2012, *V. americana* beds had an observed loss of >90% (Strayer et al. 2014). Due to its importance to the Hudson River estuary, the potential assisted restoration of *V. americana* has been discussed by managers and researchers in the years after the loss (Miller 2013; HRNERR 2014).

This study addressed two factors related to the loss and potential restoration of *V. americana* in the Hudson River after storm events in August and September 2011. The first hypothesis tested is that the recovery of this species in the growing seasons after the storm has been hampered by new sediment settling out after the storm and burying the overwintering buds from the earlier season which do not have enough energy stored to reach the surface. The second hypothesis is that regrowth has been hampered by herbivory pressure and destruction by animals on the few remaining *V. americana* beds in the river.

Other potential factors of loss could be light extinction, scouring or a simultaneous event unrelated to the storm such as disease or pollution. Light extinction was considered improbable as the storm event occurred close to the end of the growing season. Scouring would have caused uneven loss depending on flow but the loss in 2012 was consistent through the whole system. No other unrelated disturbance event has been observed or reported.

Lack of Recovery due to Burial by Sediment

The hypothesis of the burial of SAV in the Hudson River is based on the large amount of sediment that was washed into the river during the storm events in 2011. A total of 2.7 million metric tons (MMT) of sediment was washed in from tributaries, especially in the Mohawk Valley, and only 1 MMT were observed passing the monitoring station in Poughkeepsie in the next month (Ralston et al. 2013). The remaining 1.7 MMT would be enough, if spread evenly, to cover the river-bed from the dam at Troy to Poughkeepsie in 3 cm depth of new sediment. The actual amount deposited in SAV beds in 2011 may have been higher as SAV is known to trap sediment as well (Findlay et al. 2006)

For *V. americana*, the most common mode of reproduction is asexual (McFarland and Shafer 2008). When doing so, it relies on energy stored in reproductive structures (tubers) in the sediment to achieve enough stem and leaf growth in early summer in order to reach the portion of the water column with enough light for photosynthesis to take place. If plant structures (tubers or seeds) are buried too deeply, they may lack sufficient energy stores to send up shoots through the sediment. With *V. americana*'s already modest decline in the last decade in the Hudson River, burial by sediment from the double impact of Hurricane Irene and Tropical Storm Lee may have been enough to cover the remaining beds of *V. americana*. Sediment burial as a potential factor of loss has been explored earlier in the Potomac River. There, laboratory experimental results showed a steep drop-off of visible plant growth with tubers of *V. americana* buried beyond 10 cm depth in sandy as well as silty-clay sediment (Rybicki and Carter 1986).

Lack of Recovery due to Herbivory

SAV restoration, including for *V. americana*, has been conducted in multiple rivers in the United States with multi-year projects ongoing by the Virginia Institute of Marine Science (VIMS) in Chesapeake Bay (Kenneth Moore, personal communication, 2014). This work has developed successful methods of transplanting and seeding of plants and puts emphasis on certain factors of success (Moore and Jarvis 2007). One major factor of success has been herbivory protection, with experiments showing no regrowth of new seed beds unless netting is set up to protect the young plants (Moore et al. 2010).

The rate of herbivory on aquatic macrophytes by fish and other aquatic life has been contentious (Lodge 1991) but considering the dramatic difference between exclusion and non-exclusion plots in the Chesapeake Bay (Moore et al. 2010), and the geographic and ecological (including species composition) similarities between the Chesapeake Bay and the Hudson River, herbivory is a valid candidate as a reason for continued lack of recovery in the Hudson River. Blue crab (*Callinectes sapidus*) has been indicated as the possible reason for destruction as the crab clips the plants (Kenneth Moore, personal communication, 2014). Other potential candidates are waterfowl that eat plants or tubers (Sponberg and Lodge 2005; Stafford et al. 2012) and common carp (*Cyprinus carpio*), a well-established non-native species in the Hudson River that has been shown to destroy submerged vegetation in other systems (Crivelli 1983).

METHODS

Study Site

Field experiments on herbivory and depth in sediment were conducted during the growing season of 2014. These experiments were located in the main stem of the Hudson River outside of Tivoli Bays (river mile 98, coordinates $42^{\circ}2'27''N$ $73^{\circ}54'38''W$) near the eastern shore in between Magdalen Island and train tracks, in an area with earlier documented SAV beds according to GIS data based on aerial surveys in 1997, 2002 and 2007 (Cornell IRIS 2011).

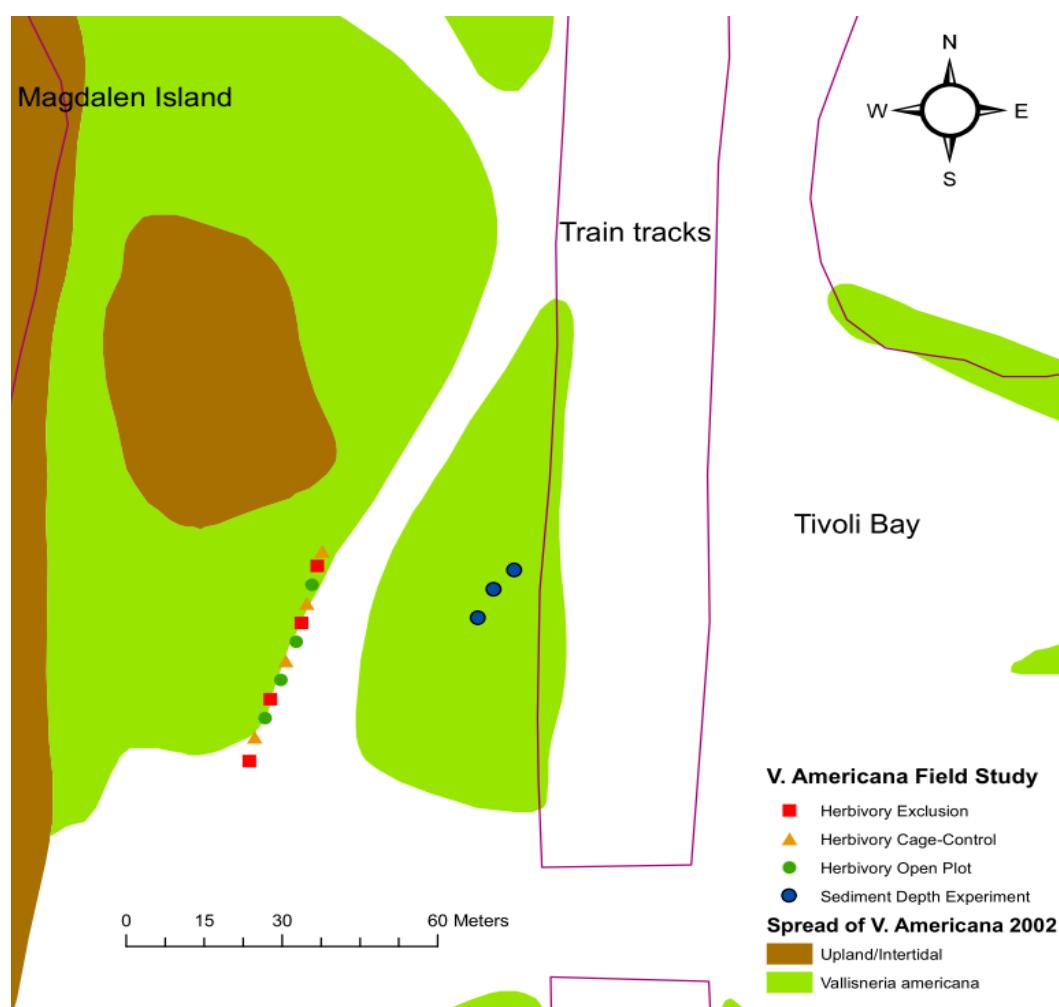


Figure 1: Map of field experiment site

Depth in Sediment - Greenhouse Experiment

Greenhouse experiments were also conducted in 2014. Forty overwintered *V. americana* tubers were purchased from a nursery for this experiment. Tubers were weighed wet and then buried at 5, 10, 20 and 30 cm, with 10 tubers at each depth. Tubers were buried in sediment taken from the lower Hudson River that had been cleaned of any plant material and then compacted by hand to simulate sediment compaction in the river. PVC pipes were used for pots and 2 tubers were put in each pipe providing 10 cm of sediment below the planting depth. Planting was conducted on 5/16/2014. All 20 pipes were marked and placed in a large plastic tub filled with water from the lower Hudson River and kept aerated. Temperature was controlled by opening and closing window sections of the greenhouse. After two months, plants were harvested and sediment was sieved to try to recover unsprouted tubers.

In a separate tub a validity experiment was conducted to test if the nursery tuber sprouting was comparable to those taken directly from the river. Fifteen tubers from a nursery were planted at 2 cm depth and two batches of 15 tubers taken from the river near the experiment site were planted at 2 cm and 5 cm and placed in three pots in a tub. DO and temperature readings were taken for each tub using a dissolved oxygen meter (YSI ProODO Instrument, Xylem Inc.) and iButtons. Both tubs were monitored frequently for sprouting, and date of first sprouting was recorded. Six sediment samples taken from the sediment used were dried and then ashed to determine organic content using the loss-on-ignition method (Schumacher 2002).

Depth in Sediment - Field Experiment

The field experiment was located outside Tivoli Bays in the main stem of the Hudson River. Thirty unsprouted tubers in good condition were excavated from the river near the experiment site, weighed wet and then replanted in individual pots at 2, 5 and 10 cm, with ten tubers at each depth in sediment. Samples were buried in the same sediment used for the greenhouse experiment. Pots were clustered in threes, with one of each depth in each cluster for a total of ten clusters, and each pot was marked by a small flag. Three poles demarcated the area with three clusters each around two of the poles and four clusters around one of the poles. Tubers and pots were buried on 6/16/2014.

Each pot was monitored for sprouting by feel due to low visibility in the river. This monitoring was conducted on 7/8/2014 and 7/21/2014. At the end of the experiment, on 7/31/2014, pots were taken out of the river and sprouted plants were washed, measured for length and the amount of plantlets and tubers or flowers were counted. The plants were then dried and weighed to the nearest 0.01 g. Three sediment samples were taken from the sediment in pots after harvesting, processed and measured to see if the in-river treatment had changed the organic content of the sediment. All data were continuously recorded in Google Docs (Google Inc.) Statistical analyses were performed in Minitab17 (Minitab Inc.). For the sediment burial experiments, binary logistic regression analyses for plants sprouted per treatment were conducted, with depth as a continuous variable and a two-sided confidence interval.

Herbivory Experiment

Twelve plots were chosen and laid out in a North to South pattern (upstream-

downstream) at a depth of between 0.6 and 0.9 m (mean low tide). The plots were square, with each side measuring approximately 1.2 m. Plots were designated either as Open, Exclusion, or Cage-Control. Open plots were marked in each corner with 4 PVC stakes of 1.8 m in length. Exclusion plot cages were made from PVC piping and covered in 2.5 cm metal poultry fencing to a height of 1.2 m with a detachable roof. Each side was constructed with a ‘skirt’ of 30 cm poultry fence lying flat against the ground to prevent potential herbivores from burrowing under the cage (see Moore et al. 2010). Cage-Control plots were set up just as the Exclusion plots but with one side open, without fencing.



Figure 2: Exclusion cage at field site

Plots were maintained, cleaned and repaired once a week. The depth of each cage was measured in the center and adjusted for tide. Light measurements were taken at six plots (two of each type of plot) with HOBO light loggers. Six sediment samples were taken between plots. These were then dried and ashed to determine organic content of the sediment.

Eight peat-pots of sprouted *V. americana*, obtained from a commercial nursery, with two plants in each pot were planted in each plot in a square pattern. Plants had approximately 8 cm of leaf length when planted. Planting and installation of cages were done in summer (6/27/2014) and pots were harvested in three rounds, three pots taken in the first round (7/31/2014), two in the second round (8/28/2014) and the remainder in the third harvest (9/17/2014).

Plants were only harvested if its peat-pot could be identified. Any connected runners outside of peat-pots were harvested as well. After harvest, the length of the three longest leaf blades were measured, plantlets and leaf blades were counted, and plants were divided into aboveground and belowground biomass. Any tubers or flowers were counted. In the third harvest, tubers were weighed wet. Plants were dried for a minimum of 24 hours at 60 C° and dry weight measurements were taken. Measurements of dry weight, blade length, amount of blades and amount of plantlets or tubers produced were compared across harvests to look at the effects of herbivory. For the herbivory experiments a one-way ANOVA test was conducted on each response variable separately for each harvest date with each treatment compared pair-wise through a Tukey-Kramer test if ANOVA showed significant differences. The harvested plants from each pot (two original plants per pot) was the unit of measurement.

RESULTS

Sediment Burial - Greenhouse Experiment

None of the 40 tubers in the greenhouse sprouted during the experiment phase. The sediment was sieved with no tubers recovered. However, sprouting was recorded in the separate validity trial. First sprouting in the validity experiment was recorded on 5/27/2014, 11 days after planting. Mean organic content of the sediment used was 2.94% ($\sigma = 0.24$).

Total number of identifiable separate plants	Total number of rosettes	Total dry weight of sprouted plants	Initial tubers	Depth in sediment (cm)	Source
2	13	2.37	15	2	Nursery
5	10	2.2	15	2	River
1	3	1.41	15	5	River

Table 1. Results of validity trial experiment.

Sediment Burial - Field Experiment

Sprouting in the planted pots was observed in the field (by feel) at the first observation, after 23 days. No further sprouting was found in the next observation or during harvest. All pots were recovered at harvest. The area used for the sediment burial experiment had been recorded as an SAV bed earlier in 2002 and 2007. At the time of planting for the experiment no *V. americana* sprouting was observed, however, by the time of the first observation there was dense *V. americana* growth in the area. Planted tubers could be easily discerned from wild due to the pots.

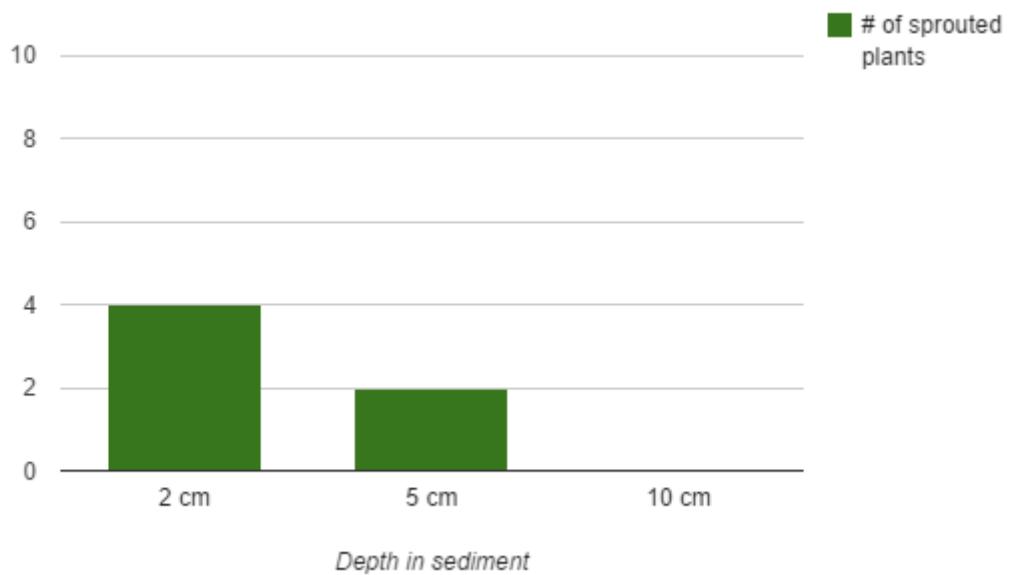


Figure 3. Total number of sprouted tubers in-river

There was a significant difference in sprouting by depth, with 40% of plants at 2 cm sprouted, 20% at 5 cm and none at 10 cm ($p=0.014$). Total and average dry weight of the plants sprouting at 2 cm were higher compared to those at 5 cm (see Figure 5).

Average numbers of blades and rosettes were also higher at 2 cm than 5 cm. Since there was no sprouting at 10 cm no data could be recorded. The mean wet-weight of tubers, measured before planting, of the six successfully sprouted plants was slightly higher (2.5 g) than that of the twenty four non-sprouted plants (2.1 g), but the difference was not significant (two-tailed unpaired t -test p -value = 0.3454).

Average # of rosettes	Average # of blades	Average weight of sprouted plants (g)	Total weight of sprouted plants (g)	# sprouted	# buried tubers	Depth in sediment (cm)
5	20.5	0.59	2.37	4	10	2
3	11	0.31	0.62	2	10	5
-	-	-	-	0	10	10

Table 2. Comparative data on in-river sediment burial experiment

Comparing the wet-weight of the tubers pre-planting to the ones that later sprouted was slightly higher (2.5 g, N = 6) than unsprouted ones (2.1 g, N = 24) but the difference was not significant (two-tailed unpaired *t*-test *p*-value = 0.3454). Pots in the river and in the greenhouse were sieved after the experiment and it was observed that unsprouted tubers did not survive (i.e., no tubers were recovered). It was also observed that most tubers in the river were found at an approximate depth of 0-3 cm. Mean organic content (three samples) of sediment from river pots after harvest was 3.81% ($\sigma = 0.09$), about 1% higher than when buried.

Herbivory Experiment

All cages survived the growing season with only minor repairs needed. Accumulation of floating vegetation, mainly water chestnut (*Trapa natans*), was extensive and required weekly cleaning. Eighty-four plants were harvested out of 96 originally planted; plants were not harvested if the peat-pot could not be found. Five Control plot plants, Four Exclusion plot plants and three Open plot plants were not recovered. Two of the plants, one in an Open plot and one in a Cage-Control plot, were observed to have lost most of their growth, seemingly ripped or pulled but with part of

their peat-pot remaining, these plants were included in analyses. A blue crab claw was found inside one of the Exclusion cages. It was observed that Exclusion and Cage-Control plots seemed to accumulate more sediment on top of the pots than Open plots, although no controlled measurements were taken.

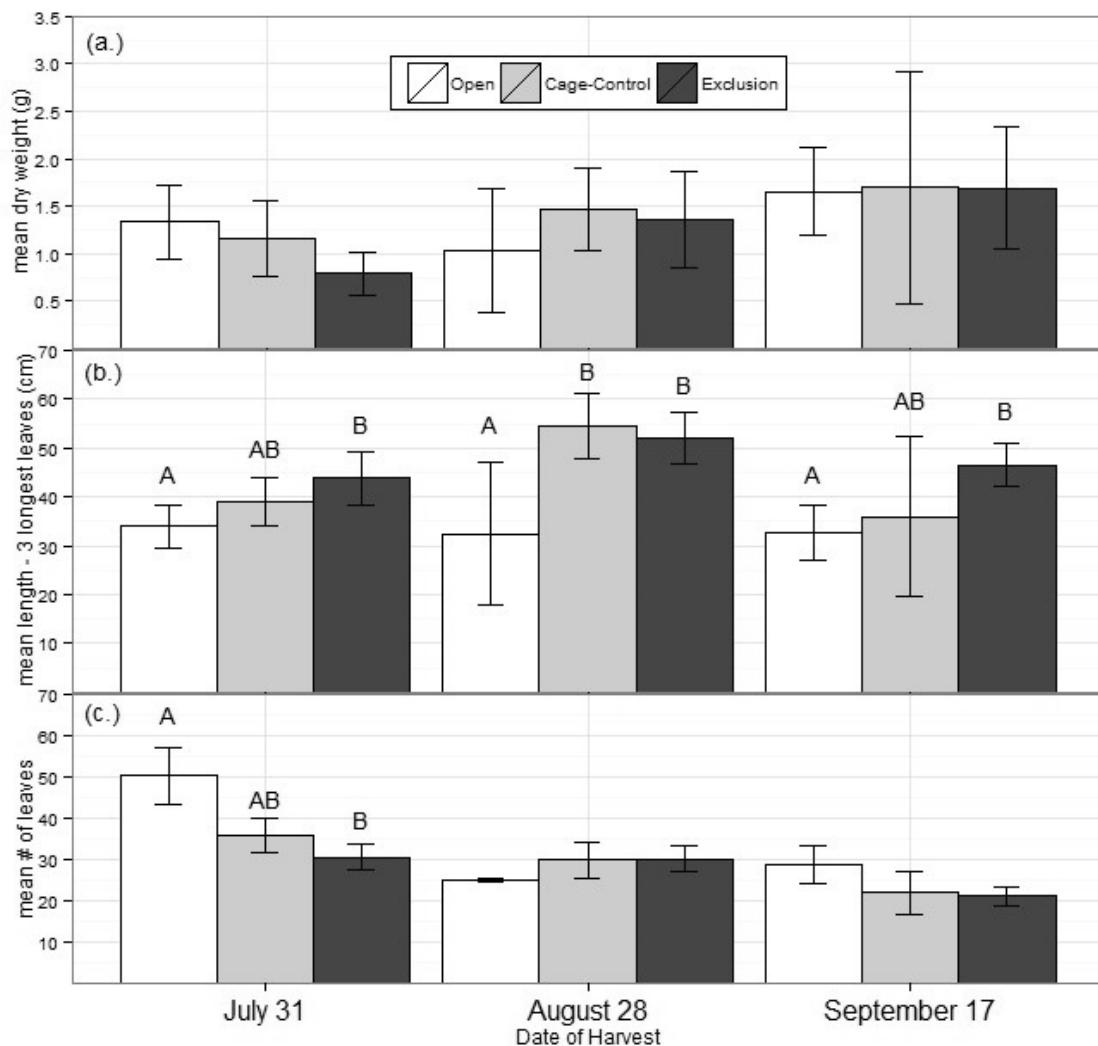


Figure 4. Mean dry weight (a), mean length of three longest leaves (b) and number of leaves per pot (c). Error bars as 95% confidence interval. Differences between treatments at same harvest date as per ANOVA post-hoc Tukey-Kramer method noted above bars if significant (95% C.I.).

The difference between Open plots and Exclusion plots was statistically significant in the first harvest for blade length, number of blades, number of rosettes, but not for dry-weight. There was no statistically significant difference between Exclusion and Control (one side open) indicating a lack of herbivory. Average blade length was the only factor that differed significantly between Open plots and Exclusion plots through all three harvests. Blade length did not differ significantly between Control plots and Exclusion plots.

In the third harvest 20 out of 24 pots had produced tubers, as compared to one pot in the second harvest and none in the first. Neither the weight nor number of tubers differed significantly between the three treatments. Plants were buried in peat-pots but plants sent runners outside the potted area. Mean organic content of sediment between plots was 6.27% ($\sigma=1.03$). Light meter readings were taken over three days but were inconsistent and yielded no dependable results, potentially due to coverage by plants or sediment. There were no correlation between growth and relative north-south position of cages.

Dry weight of plants compared to their N-S plot position

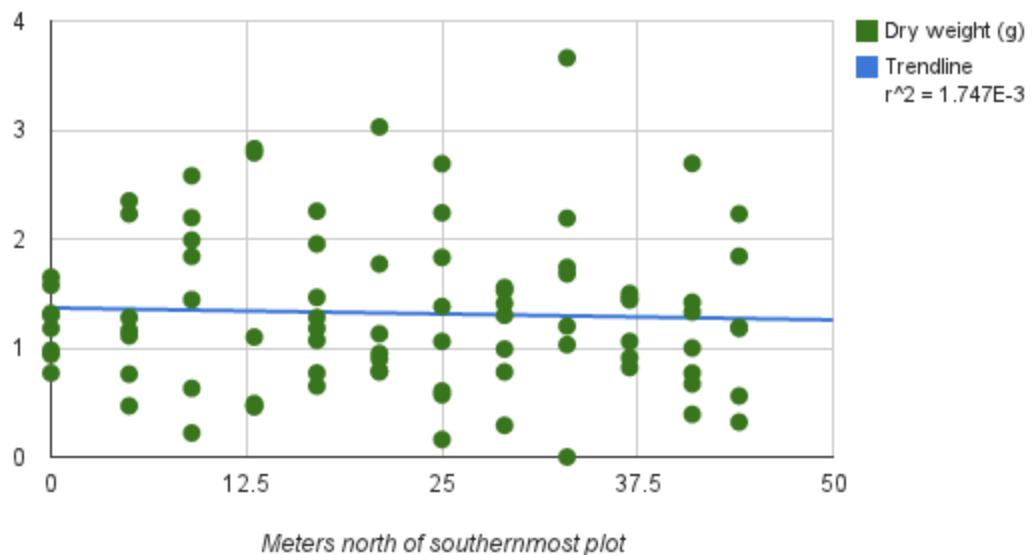


Figure 5. North-south comparison of dry weight of plants.

DISCUSSION

Sediment Burial

The results of the sediment burial experiment lend support to the hypothesis that increased depth in sediment will decrease the likelihood of sprouting for *V. americana* tubers in the Hudson River. Although lack of sprouting in the greenhouse could potentially be attributed to lack of flow or to excessively high temperatures, the tubers in the validity experiment in the same greenhouse room and similar conditions did sprout successfully at 2 cm depth.

The field experiment further showed a clear correlation between depth in sediment and sprouting (see Figure 3) with 40% sprouting at 2 cm, 20% at 5 cm and no sprouting at 10 cm. Following the estimates by Ralston et al. (2013) new sediment in the lower Hudson River may have added 3 cm in depth if spread evenly, and potentially more than that in active SAV beds due to sediment trapping (Findlay et al. 2006). The in-river experiment shows a clear effect of increased depth in sediment on sprouting. If sediment depth of tubers changed from 2 to 5 centimeters, the experiment indicates a halving of successful sprouting, which could be a potentially river-wide effect. During excavation for tubers to be used in the experiments, it was observed that tubers were found mostly in the top 1-2 cm layer of the sediment. Three more centimeters added on to the depth (to 5 cm) may have drastically reduced and potentially slowed sprouting in the season after Irene and in turn affected the available stock to produce tubers for coming seasons; however, this may not solely explain the > 90% loss witnessed in 2012 as compared to 2011.

The depths chosen at the beginning of the experiment were based on a study in

the Potomac River by Rybicki and Carter (1986) where the tubers were planted from 10 to 35 centimeters. These authors found that sprouting declined from about 90% at 10 cm to 0-25% at 25 cm, depending on sediment composition. The difference between Rybicki and Carter's (1986) results and the present experiment could potentially be attributed to the high turbidity of the Hudson River or the more northern latitude, as compared to the Potomac, decreasing light and heat signals for the tubers (Jarvis and Moore 2008; McFarland and Shafer 2008) or a difference in sediment composition. Jarvis and Moore (2008), in a similar experiment in Chesapeake Bay chose 5 depths between 0.2 and 10 centimeters for *V. americana* seeds and found a large difference in oxidation-reduction potential above versus below 2 cm, but they did not find a significant difference in sprouting success.

In the same paper, Jarvis and Moore (2008) reported that higher amounts of organic matter correlates negatively with germination success of *V. americana* seed, and that germination decreased by approximately half between 1.5% and 3% of organic content. Although seed germination and tuber sprouting are different, the high organic content (2.94%) in the sediment used for the greenhouse experiment may have still have affected the tubers. How the sediment burial effect would affect other rivers would depend on the geomorphology of the system and its tributaries; the Hudson River had much of its sediment supplied from easily suspended sediment in the Mohawk River tributary (Ralston et al. 2013), this may not be the case in other systems.

Herbivory

The results from the herbivory experiment in the river provided little to no

evidence that herbivory from larger herbivores was impeding *V. americana* regrowth in the area. This may be due to the Hudson River supporting lower density of herbivores than Chesapeake Bay. For comparison, Chesapeake Bay has a large commercial blue crab fishery (Chesapeake Bay Foundation 2008), as compared to a small (and diminishing) population of the species in the Hudson River (NYSDEC 2015). Low blue crab numbers were reported by local fishermen (personal communication - Stuart Findlay, 2014) and it may be that the drastically lowered stock of SAV in 2012 and 2013 meant that herbivores and animals reliant on SAV have already decreased or changed migration patterns by 2014, as has been shown to happen with both waterfowl (Hansson 2010) and with fish (Winemiller and Jepsen 1998).

The experiment did not test for herbivory from herbivores smaller than 1 inch in body width, such as small fish or invertebrates. There could also potentially be herbivory of the overwintering buds during the fallow season as has been observed by swans and other waterfowl in lakes and rivers in North America (Sponberg and Lodge 2005, Stafford et al. 2012). Plants were observed to have more rosettes in the first harvest (see Figure 6), especially outside the pot. This could be due to quick spread in the beginning of the season and subsequent deterioration of runners from the original rosettes. In the later harvests, more deteriorated and broken connections were observed.

The high organic content in the area around the herbivory experiment would only have a limited effect on the potted plants as most of the underground growth was centered in the peat-pot.

Storm Impact and Restoration

Perhaps one of the more important outcomes of the herbivory experiments is to show that plants from nursery stock can survive and grow in the river with low mortality, rapid spread, and with production of tubers. The apparent lack of a need for herbivory protection may be beneficial as it would decrease costs for any future restoration efforts in the Hudson River.

‘Tuber banks’ have been discussed as being a source from which *V. americana* could recover after a disturbance (Harwell and Havens 2003), but from the results of this experiment, *V. americana* tubers that do not sprout appear to deteriorate and do not create a tuber bank that could last more than one season. This means that the season after the disturbance the potential tuber bank would have to have good enough conditions to sprout and establish a new set of viable tubers. Establishment after a storm event that removes tubers or creates negative conditions lasting more than a season (e.g., increased settled sediment or worsened light conditions) may therefore have to come from sources such as seeds banks, surviving *V. americana* beds in the river and from other nearby populations or via animals, as has been observed in similar species (Harwell and Orth 2002; Rybicki et al. 2001).

From the herbivory experiment it was also observed that almost all tubers were produced at the end of the season between 8/28/2014 (second harvest) and 9/17/2014 (third harvest). This timing corresponds to when the storm would have caused high turbidity in the river (Ralston et al. 2013). This may have led to lower light availability for plants going into the reproductive stage and producing smaller tubers that would have had an even more difficult time sprouting when buried. These “death spirals” with *V.*

americana have been recorded before (Titus and Hoover 1993) as each stage negatively reinforces the next, so that the second year's crop may not have had enough energy reserves to re-establish quickly.

Hurricane season on the Atlantic seaboard coincides with the growing season in the Hudson River (NOAA/NWS 2014) which means most hurricanes affecting the Hudson River would cause both light-extinction and sediment burial issues for SAV. It may be that both effects combined to decrease re-sprouting in the next year, with small tubers, due to low light during the earlier growing season, having to sprout out of deeper sediment, due to sediment burial. The only time this would not have had any effect would be if a storm occurred after or before the growing season but before the river froze over.

Hurricanes can have serious and long-lasting effects on rivers (Strayer et al. 2014) and with climate change predicted to increase the strength of hurricanes and the amount of precipitation over the U.S. Northeast (IPCC 2013), it is important to figure out how hurricanes may affect SAV in the long run. Strayer et al. (2014) proposed a framework for describing disturbances and stresses that lead to long-term change in the river, and a hurricane would represent an abrupt, brief, fast and severe driver. That said, the response may be a long, gradual and slow recovery. Since not much can be done to stop hurricanes, and restoration, as stated before, may be undone easily by the next storm, it is also important to focus on how to make the recovery a little less slow, long and gradual. Rybicki et al. (2001) identified storms as being the reason for loss of SAV in the Potomac, the reason for its slow regrowth was argued to be the low water quality (high nutrient load and high turbidity). Although water quality has improved in the Hudson River in the last few decades (Miller 2013) there is still room for improvement. Lowering

turbidity generally would mean that light extinction and sediment deposition would be less severe during a storm event and recovery could proceed faster due to higher light penetration in successive seasons unless offset by higher growth of phytoplankton. This could create a positive cycle with more SAV, and especially, *V. americana*, in turn also improving water quality (Findlay et al. 2006).

Any future restoration of SAV would have to consider that restoration efforts may be temporary and gains in plant stock from restoration could be wiped out by the next storm. Restoration, even without the need for herbivory protection in the Hudson, may be considered an expensive option if the reality is that the job will be undone in short time. On the other hand, if restoration is not done, multiple storm events returning at close intervals could potentially extirpate the stock of *V. americana*, and SAV in general. If a storm event of the same magnitude as Irene and Lee were to occur in the Hudson Valley in the next few years, the remaining SAV may well be gone. Extirpation of SAV has happened in other systems (Rybicki et al. 2001) and in the Hudson River extirpation of SAV may also affect fish populations and general water quality. Assisted restoration may bring SAV back much more quickly than natural regrowth and limit any effects on important fish species in the river but the cost of restoration should be calculated and weighed against the potential gains both environmentally and economically for the river, its biota and the people that rely on it.

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**THE CONSERVATION STATUS OF GOLDENCLUB
(*ORONTIUM AQUATICUM*) IN THE FRESHWATER TIDAL WETLANDS OF
THE HUDSON RIVER**

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ABSTRACT

The central goal of this study was the conservation assessment of goldenclub (*Orontium aquaticum* L.) in the Hudson River Estuary. It was hypothesized that factors including hydrodynamic stress, sediment disruption, competition from co-occurring species, and increased herbivory had caused a decline in goldenclub abundance and distribution. Survey results were compared to abundance and distribution data gathered between the 1930s and spring 2014. Several historically thriving stands of goldenclub were found to have become diminished or extirpated since the 1970s, while new stands of varying sizes were found to have established in alternate locations in recent years. Although goldenclub abundance seems to be declining in the Hudson River, the discovery of a few large new stands in the spring of 2014 suggests that goldenclub is not in as much danger as was originally expected. Results indicate that exposure to open river, and resulting increased exposure to hydrodynamic stress, may be having a negative impact on goldenclub abundance and stand health, with increased herbivory posing a potential threat to Hudson River goldenclub. These factors should continue to be monitored and taken into account if goldenclub is to be conserved in the Hudson River and potentially used in wetland restoration.

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INTRODUCTION

Goldenclub (*Orontium aquaticum* L.), also known as floating arum and never-wet, is an unusual and beautiful flowering wetland plant belonging to the arum family (Araceae). It is an herbaceous vascular perennial best recognized by its water-repellent, bluish-green, pointed elliptical leaves and thick yellow-tipped flowering stalks, or spadices. Goldenclub grows from a stout and usually deeply sunken rhizome that produces contractile roots used to anchor the plant (Hotta 1971). Examples of healthy goldenclub plants are shown in Figure 1. Goldenclub is the only known living species in its genus, although *Orontium mackii* and *Orontium wolfei* fossils from the Upper Cretaceous and Paleogene of western North America have been identified (Bogner et al. 2007). The closest living relatives to goldenclub are the skunk cabbages *Symplocarpus* and *Lysichiton* (Grayum 1990; Tam et al. 2004). Together, the three genera comprise the subfamily Orntioideae.



Figure 1: Healthy Goldenclub Plants. South Cruger Island, May 27th, 2014.

Unlike other aroids whose inflorescence is composed of a spadix enclosed by a hood-like spathe, the flowerstalks of goldenclub plants lack apparent spathes, and are instead encompassed by small basal sheaths. There is disagreement regarding the nature of these sheaths, having first been classified as spathes due to being the last foliar element before the spadix (Engler 1877), and later as sympodial leaves by Ray (1988), who argued that *Orontium* spadices lack spathes entirely; however, the small sheaths of goldenclub stalks are frequently referred to as spathes in modern literature.

The flowerstalk and rhizome of goldenclub may be eaten if thoroughly cooked or dried (Wilson 1960; Peterson 1978). Nearly all tissues of goldenclub contain minuscule packets of calcium oxalate crystals which, if eaten raw, can result in minute painful lacerations to the mouth or throat, and thus are thought to serve as a defense against herbivores (Wilson 1960).

Upon completion of anthesis, the goldenclub flowerstalk becomes green and grows downward. This allows fruit maturation and abscission to occur near, or in, the water, the spadix disintegrates (Koltz 1992). Germination of the typically green or yellow goldenclub berries may occur on the spadix, although most germinate while floating after detachment from the spadix (Kiviat 1976). Floating is enabled by small air spaces in the pericarp, which becomes waterlogged after approximately one week causing the berry to sink, and after one additional week splits allowing for release of the seed (Ridley 1930; Koltz 1992). A study by Megonigal et al. (2005) found that *Orontium aquaticum* seedlings had higher biomass when exposed to elevated carbon dioxide in both flooded and non-flooded conditions.

Goldenclub is endemic to eastern North America, with its range extending northeast to New York and Massachusetts, south to Florida along the eastern coast, west to Texas, and inland to Tennessee, Kentucky, West Virginia, and Pennsylvania (NatureServe 2013). Due to its aesthetic properties, it is also a desirable species for use in ornamental water gardens. As a result, goldenclub is an introduced species in Sweden, where it was first reported in 1982 and has since become established in a lake in Västergötland (NOBANIS 2009).

Grear (1966) proposed an earlier distribution and possible migration route accounting for goldenclub's present distribution in the eastern United States: as the Araceae is a predominantly tropical family, the prevalence of tropical conditions further northward during the Tertiary may have allowed the range of *Orontium* to extend farther northward than the current boundaries of the tropics. If *Orontium* and related genera migrated from the region of the Arcto-Tertiary flora, then it is possible that they had previously been distributed more widely across North America during the late Cretaceous and early Tertiary, from the eastern coast out to or past Colorado to the West, due to the supportive climate conditions of the era (Grear 1966). Grear further argued that subsequent changes in climate and erosion cycles brought about by the uplift of the old Cretaceous, or Schooley, peneplain during the Miocene, may have led to the spread of *Orontium* to eastern North America as the west became increasingly arid. The recovery location of the *Orontium* fossils described by Bogner et al. (2007), *Orontium mackii* (Maastrichtian, New Mexico) and *Orontium wolfei* (Lower–Middle Eocene, northern Washington and southern British Columbia), as well as the additional Orontioideae fossil described, *Symplocarpus hoffmaniae* (uppermost Maastrichtian of North Dakota and

lowermost Paleocene) is supportive of a previously broader geographic distribution of *Orontium* in North America (Bogner et al. 2007). A Miocene fossilized *Orontium* spadix was additionally discovered in the Rocky Mountain region of Colorado (Cockerell 1926). Because these Orontioideae fossils found by Bogner et al. in Late Cretaceous and Eocene assemblages were thought to have grown under climates ranging from warm subtropical to temperate, authors propose that the relatively cool climatic tolerances of extant Orontioideae evolved no later than the Early Cenozoic (Bogner et al. 2007).

Today, goldenclub occurs on the eastern Coastal Plain of North America and in the Piedmont and Appalachian Highlands (Grear 1966). Goldenclub grows on sandy, muddy, or peaty shores in relatively still and shallow waters (Fernald 1950), and can be found in freshwater tidal wetlands (Tiner 1988), bogs (Radford et al. 1968), marshes (Beal 1977), “sloughs” (Harshberger 1904), rivers (Hellquist and Crow 1982), as well as in streams, ponds, lakes, and swamps (Beal and Thieret 1986), as reviewed by Koltz (1992). Goldenclub is locally abundant in, and arguably characteristic of, freshwater tidal mudflat vegetation in the northern half of its range (Harshberger 1904; Nichols 1920; Muenscher 1937; McVaugh 1958; Kiviat 1976). Goldenclub has affinities for slightly acidic and tanniniferous environments, though has been observed to grow in habitats with pH ranging from slightly below 5 to just above 7 (Beal 1977; Koltz 1992). In New York, goldenclub is reported to grow primarily on freshwater tidal mudflats and in a few New York shrub bogs (NYNHP 2013). It is possible that there are different genotypes present in acidic peatlands and circumneutral fresh-tidal habitats.

Although prevalent in certain locations within the plant’s range in North America, such as the Okefenokee Swamp in Georgia where the plant has been well studied

(Greening and Gerritsen 1987; Patten and Fath 2001), there are many regions lacking information on the distribution and abundance of goldenclub, despite its threatened status in these regions. Goldenclub has reportedly declined in Massachusetts, Connecticut, Pennsylvania, and northern New Jersey (NatureServe 2013) although many other plants of southern affinities are increasing in the northeastern states. Goldenclub has been assigned a global rarity rank of G5, meaning that the species is apparently secure globally, though potentially rare in some parts of its range, particularly at the periphery (NatureServe 2013). In New York and Massachusetts goldenclub is considered a species of conservation concern (NatureServe 2013).

Most New York goldenclub populations are in the freshwater tidal areas of the Hudson River, making the study of goldenclub there critical to conservation at the northern range limits. Although goldenclub was reportedly thriving in the tidal Hudson River in the 1930s (Svenson 1935; Muenscher 1937; McVaugh 1958), it is now considered a threatened species in New York (NatureServe 2013). Since the Hudson River Improvement Fund supported the investigation of goldenclub's status on the Hudson River as a citizen science project, volunteers and workers from Hudsonia Ltd. and the surrounding area have worked towards documenting the abundance and distribution of goldenclub along the Hudson River. Data were collected for many of the remaining goldenclub stands in the Hudson River. Observations from the citizen project suggested that goldenclub abundance along the Hudson River may have declined in stand locations originally reported in the 1930s and 1970s, and that there seems to have been an increase in visible herbivore damage to goldenclub plants at a few of the remaining stands.

The central goal of this study was the conservation assessment of goldenclub in the Hudson River. As a secondary goal, environmental data were collected and analyzed in order to determine which factors may be contributing to the current status of goldenclub in the Hudson River. As it was expected that several factors were involved in determining the current status of goldenclub in the Hudson River, multiple working hypotheses were developed. It was expected that goldenclub had declined on the Hudson River in abundance and distribution since the 1970s with the following factors contributing to the current status of goldenclub: 1) sea level rise, reductions in substrate, and increases in flooding and hydrodynamic energy from storms, wind, waves, and ship wakes inflicting stress on plant populations, 2) competition from other plant species limiting the physical space available to goldenclub, or 3) stress and potentially reduced growth or reproduction resulting from increased herbivory. The null hypothesis was that goldenclub abundance and distribution has remained stable since the 1970s, possibly as a result of the plant having changed locations and remaining abundant overall. Certain rare plant species have been known to disappear from some areas while becoming established in new ones, and it was acknowledged that goldenclub may be growing in regions of the Hudson River that have previously escaped observation, considering the complexity of Hudson River environments.

Goldenclub is a charismatic and unusual aquatic plant with both aesthetic and conservation significance on the Hudson River, although little is known about the ecology of goldenclub there. Conservation research on species at their northern range limits is also crucial to understanding climate change impacts and maintaining unique genetic diversity within species at range margins. Results of this study have implications

in determining the conservation status of this plant in the Hudson River, and in understanding how to best protect remaining goldenclub stands. Many restoration projects are currently being carried out within the range of goldenclub; results obtained through this study may be useful in determining whether goldenclub would be a good candidate for incorporation in wetland management projects. Goldenclub is relatively easy to germinate and grow, at least for the first year or so (Kiviat 1976), and could possibly benefit from such an arrangement if the natural abundance and distribution of goldenclub declines.

METHODS

Remaining gaps in the data collected through Hudsonia detailing the current distribution and abundance of goldenclub on the Hudson River were filled, and distribution and abundance data were compared to historic records. Stations along the river where goldenclub had been described historically were surveyed and surrounding regions were searched for new goldenclub stands. Environmental factors thought to affect goldenclub were also measured and analyzed, although due to the observational nature of this study as well as time and resource limitations, rigorous experimental analyses were not attempted. Through observation in the field as well as through the implementation of camera traps, data were collected on the abundance, distribution, and ecology of goldenclub on the Hudson River.

Historic data were acquired from the New York Natural Heritage Program, literature, and other field biologists and naturalists who visit the Hudson River wetlands. From late April to the beginning of June historically reported goldenclub sites along the

Hudson River were visited, and the current status of the plant relative to the baseline provided by historic data was assessed. Goldenclub flowers in New York from mid-April through the beginning of June (NYNHP 2013), but the best time to observe the plant on the Hudson River is in May, before other growing vegetation impedes detection of goldenclub. Field work was carried out on foot and by kayak. The goldenclub sites surveyed through this study extend north to Schodack Island, and south to the Mudder Kill at Barrytown. Areas along this range were also searched for previously unreported goldenclub stands by surveying environments potentially supporting goldenclub within 100 or more meters from historically reported stands.

Measurements and observations of goldenclub stands include UTM coordinates of stand locations (measured using the Videometer 3.0 application for iPhone 5), counts of individual plants, measurements of stand size and calculation of plant density, identification of major co-occurring species, visual estimates of plant vigor and of leaf tissue removal by herbivores, observations of tracks or other wildlife signs potentially identifying vertebrate herbivores, collection and identification of invertebrate grazers and pollinators, examination of the surroundings for threats such as fill or dumping, fetch measurements (measured using the Google Earth ruler tool), and photographic documentation (Table 1). A “clump” of leaves with or without spadices was considered to be a single plant (genet), as the literature does not mention underground connections among clumps.

Table 1: Variables describing goldenclub stands and number of sites (n) for which these variables were estimated.

Variable Name	Description	n
Abundance, Most Recent Count	Most recent value recorded for the number of individual goldenclub plants within a stand. For most stands, this value was recorded between 2012 and 2014.	33
Abundance 2010s	Ordinal ranking of the number of plants in a stand recorded between 2010 and the present. For ordinal abundance rankings, 0 denotes that no plants were observed at location, 1 denotes very low abundance (between 1 and 4 plants), 2 denotes low abundance (between 4 and 15 plants), 3 denotes moderate abundance (between 16 and 30 plants), 4 denotes high abundance (between 31 and 100 plants), and 5 denotes very high abundance (> 100 plants).	33
Abundance 2000s	Ordinal ranking of the number of plants in a stand recorded between 2000 and 2009.	15
Abundance 1970s	Ordinal ranking of the number of plants in a stand recorded between 1970 and 1979.	14
Abundance 1930s	Ordinal ranking of the number of plants in a stand recorded between 1930 and 1939.	9
Cattail Presence	Categorical variable indicating whether or not cattail was found within three meters of a stand. 1 denotes cattail presence, 0 denotes cattail absence.	27
Distance from Railroad	Distance of stand from nearest railroad (m).	33
Extirpated	Categorical variable indicating whether a once existing stand has since become barren of goldenclub. 1 denotes stand extirpation, 0 denotes a non-extirpated stand.	33
Fetch East	Fetch East of a stand, obtained by measurement of the expanse of open river (in the absence of a dividing land barrier) between the stand and the nearest shore due East of the stand (m).	33
Fetch North	Fetch North of a stand (m).	33
Fetch South	Fetch South of a stand (m).	33
Fetch West	Fetch West of a stand (m).	33
Herbivore Damage Prevalence	Ordinal ranking of the proportion of plants in a stand bearing signs of herbivore damage. 0 denotes an absence of visible herbivore damage, 1 denotes grazing on <10% of plants within a stand, 2 denotes grazing on 10-25% of plants, 3 denotes grazing on 26-50% of plants, 4 denotes grazing on 50-75% of plants, 5 denotes grazing on >75% plants.	18
Herbivore Damage Extent	Ordinal ranking of the average severity of visible damage borne by damaged goldenclub plants at a given stand. 0 denotes an absence of visible herbivore damage; 1 denotes very minimal damage, with damage only visible on one or two leaves, or with stalk tips minimally chewed; 2 denotes that <25% of leaves on damaged plants show some signs of chewing, or that one or two stalks have been bitten off; 3 denotes moderate damage with between ~25-50% of leaves show signs of grazing and/or have at least 50% of flower stalks chewed off, with some stalks remaining; 4 denotes extensive damage, with between ~50-75% of leaves show signs of grazing and/or with most or all of flower stalks chewed off; 5 denotes very extensive grazing, with >75% of leaves showing signs of grazing, with most or all flower stalks missing, some plants chewed to the ground.	18
Maximum Historic Abundance	Ordinal ranking describing the largest abundance value recorded for each stand between the 1930s and 2000s.	33
Mean Fetch	Average of fetch value due North, South, East, and West of a stand (m).	33
Newly Reported	Categorical variable indicating whether or not a stand is considered newly reported (first reported after the year 2000).	33
Plant Vigor	Ordinal ranking of plant size in a stand on a scale of 0-3. 0 denotes an absence of goldenclub; 1 denotes low vigor, with at least 75% of plants being small (approximately 30 cm tall and 45 cm wide or less) with 0-8 stalks; 2 denotes variable plant vigor, with a mix of small (0-9 stalks) and larger plants (10 or more stalks) with neither group exceeding 74% of population; 3 denotes high plant vigor, with at least 75% of plants being large (approximately 31 cm tall and 46 cm wide or greater) with 10 or more stalks.	32
Spatterdock Presence	Categorical variable indicating whether or not spatterdock is found within three meters of a goldenclub stand. 1 denotes spatterdock presence, 0 denotes spatterdock absence.	27
Stand Density	Density of plants within a stand, obtained by dividing the number of plants within a stand by the stand dimensions. For extirpated stands, the stand volume was recorded as zero.	33
Stand Dimensions	Dimensions of a stand measured in meters squared.	19
Sweetflag Presence	Categorical variable indicating whether or not sweetflag is found within three meters of a goldenclub stand. 1 denotes sweetflag presence, 0 denotes sweetflag absence.	27
UTM E	Easting Universal Transverse Mercator (UTM) zone 18T coordinate value for a stand.	33
UTM N	Northing Universal Transverse Mercator (UTM) zone 18T coordinate value for a stand.	33

Images collected from camera traps (COACH, Zoran Corporation) placed at the Cruger Island South stand, set in three springs (2012-2014), were examined in order to determine herbivores potentially responsible for the extensive leaf tissue damage observed on many Hudson River goldenclub plants in recent years. Images from most days between June 19th and approximately July 3rd 2012, between May 1st and June 2nd 2013, and between April 22nd and May 10th 2014 were collected from two cameras placed approximately two meters apart. Although the camera traps captured images during both day and night, nighttime scenes were often not illuminated fully by camera flashes, and thus herbivores grazing at night could not be identified from most photographs, despite movement within the stands having triggered the infrared motion detectors.

Based on observations in the field, data were compiled describing each goldenclub stand (Table 1). Characteristics of declining, stable, or increasing stands and their surroundings were compared to seek clues to causes of decline. Data from thirty-three stands were utilized in this study, representing all confirmed, or previously confirmed, locations of goldenclub stands in the freshwater tidal wetlands of the Hudson River from the 1930s to the present. Stands with only one or two plants recorded recently or historically were excluded from analyses because it is common for plants to establish in a new location but not persist. Stands currently barren of goldenclub plants that contained more than two goldenclub plants historically were included in the analyses. Historic abundance values for many stands are lacking because presence of goldenclub was not documented until more recent years, or documentation of the presence of goldenclub was not available. As a few of these sites were surveyed by other field biologists and naturalists, data from those sites are missing for some of the variables

examined. For extirpated stands, data were not recorded for herbivore damage or stand dimensions. In this study, twenty-five of the thirty-three sites were surveyed in the spring of 2014. The remainder of the stands were most recently surveyed by Erik Kiviat, Hudsonia volunteers, and other field biologists and naturalists between the years 2012 and 2014 with the exception of a few historically reported stands believed to have been extirpated (e.g., Kingston Point) or which had been searched for unsuccessfully in recent years prior to 2012 (Kiviat, unpublished data).

The status of each stand was determined by assigning ordinal abundance rankings to all records of goldenclub abundance in the Hudson River through time, and comparing recent and historic ordinal abundance rankings for each stand. This was done to make some of the historic reports on goldenclub abundance comparable to the recent ones, as authors of historic reports frequently provided subjective descriptions of goldenclub abundance, such as “abundant” or “sparse”, rather than a count. Modern rankings for goldenclub abundance were compared to historic rankings to determine whether a stand had declined, increased, or remained stable in abundance.

Spearman rank correlation analyses were used to compare all non-categorical variables. Mann-Whitney *U*-tests were used to assess binary predictor variables, and logistic regression analyses were used to predict binary dependent variables. For all statistical tests, α was set at 0.1. Statistical and graphical analyses were performed with Statistica version 12 (StatSoft, Tulsa, Oklahoma).

RESULTS

The approximate locations of the 33 goldenclub stands in the freshwater tidal wetlands of the Hudson River are shown in Figure 2. A few of the labels indicate multiple goldenclub stands in close proximity: four distinct stands have been observed in the Rams-Horn Creek region, two in the Saugerties north cove region, two in the Stony Creek region, two in the Tivoli North Bay region, three in the Cruger Island North region, and two in the Mudder Kill region. The Schodack Island east edge goldenclub location marker indicates the location of only a single plant, and thus this stand was excluded from statistical analyses. All other labels indicate individual stands. Goldenclub is currently extirpated from Middle Ground Island, Rogers Island north and west, the Roeliff Jansen Kill mouth, Greene Point, Inbocht Bay, Magdalen Island, Kingston Point, Sleightsburg, and the Storm King cove, as well as one stand in the Stony Creek region, one stand and a small grouping of goldenclub plants in the Cruger Island North region, and one small stand by the mouth of the Mudder Kill. The current status of the Stockport Creek stand is unknown, as explicit information on the stand's location was absent from historic records (Anne Williams, unpublished data 1982). The Stockport Creek stand was excluded from analyses for this reason, and the Stockport Creek label shows an approximated location. Goldenclub may still be present there, or in other unsearched areas of the Hudson River.

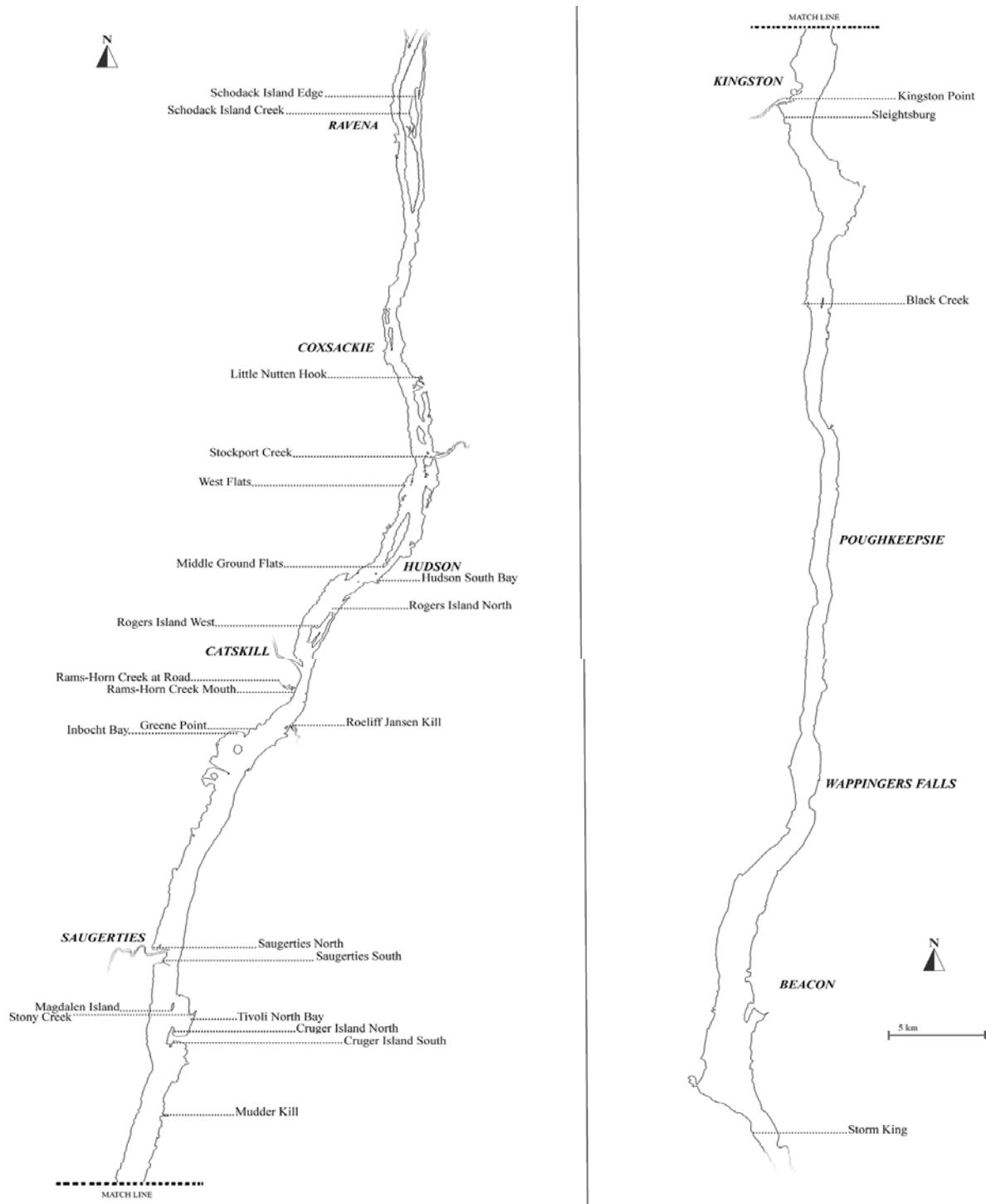


Figure 2: All documented goldenclub locations in the freshwater tidal wetlands of the Hudson River, current and historic.

The current conservation status of documented goldenclub stands on the freshwater tidal wetlands of the Hudson River is described in Table 2. The status (increased, stable, or decreased) was obtained by comparing the earliest available report to the most recent report. For all non-extirpated stands, the most recent report was obtained between 2012 and 2014. Stands marked “New 2014” were first documented (as far as available data indicate) in the spring of 2014 as part of these survey efforts. Because of this, historic abundance could not be compared to the most recent abundance observed for these stands. Check marks in the right-most columns of Table 2 indicate whether a given stand was considered newly reported (first documented between the year 2000 and the present), or found extirpated.

In summary, of the 33 stands examined, 19 stands were found to have decreased goldenclub abundance since the year they were first reported (14 of which are now extirpated), six were found to have increased abundance, five to have stable abundance, and three were first documented in the spring of 2014. Of the 33 stands, 14 were first reported between 2000 and 2014 (Table 5, Appendix for individual stand abundance details over time). Of the 12 stands which were reported to contain goldenclub plants in the 1970s, eight were found to have decreased abundance (four of which are now extirpated), three to have increased abundance, and one to have stable abundance when observed most recently (Table 2, Appendix Table 5). An additional 18 stands were first reported following the 1970s, although it is difficult to determine whether these stands first developed following the 1970s, or if their presence had gone unnoticed or undocumented prior to the 1970s.

Table 2: Status of known goldenclub stands in the freshwater tidal Hudson River.

Site Name	Current Abundance Ranking, Obtained 2012-2014.	Abundance Ranking for First Report	Year of First Report	Status (Since First Report)	Extirpated	Newly Reported (First Observed in 2000 or More Recently)
Schodack Island Creek	4	5	2002	Decreased		✓
Little Nutten Hook	5	2	1937	Increased		
Middle Ground Flats	0	4	1935	Decreased	✓	
West Flats	2	4	1975	Decreased		
Hudson South Bay	4	1	2000s	Increased		
Roger's Island North	0	5	1930s	Decreased	✓	
Roger's Island West	0	5	1930s	Decreased	✓	
Rams-Horn Creek at Road	2	1	2012	Increased		✓
Rams-Horn Mouth Northern Bank	2	2	2012	Stable		✓
Rams-Horn Mouth Southern Bank	4	4	1937	Stable		
Rams-Horn Mouth Rocky Bank	4	4	2012	Stable		
Rams-Horn Mouth New	5	5	2014	New 2014		✓
Roeliff Jansen Mouth	0	1	2001	Decreased	✓	✓
Greene Point	0	5	2000	Decreased	✓	✓
Inbocht Bay	0	4	1937	Decreased	✓	
Saugerties North	2	4	1937	Decreased		
Saugerties North Shore	3	3	2014	New 2014		✓
Saugerties South	5	4	1937	Increased		
Stony Creek East	0	4	1983	Decreased	✓	
Stony Creek West	3	5	1935	Decreased		
Tivoli North Bays Big Bend	5	4	2012	Increased		✓
Tivoli North Bays East Bank	5	5	2014	New 2014		✓
Cruger Island North I	0	5	1956	Decreased	✓	
Cruger Island North II	3	3	2000	Stable		✓
Cruger Island North III	0	2	2012	Decreased	✓	✓
Cruger Island South	5	5	1970s	Stable		
Mudder Kill (Astor Point) Tracks	0	2	2000	Decreased	✓	✓
Mudder Kill (Astor Point)	4	5	1993	Decreased		✓
Kingston Point	0	4	1937	Decreased	✓	
Sleightsburg	0	4	1951	Decreased	✓	
Black Creek	2	1	2000s	Increased		✓
Storm King	0	1	1996	Decreased	✓	
Magdalen Island South End	0	2	1976	Decreased	✓	

In one of the largest goldenclub stands remaining in the Hudson River, near the south end of Cruger Island, camera traps captured images of several animals passing through patches of goldenclub plants, though none was seen eating goldenclub plants. Animals included white-tailed deer (*Odocoileus virginianus*), Canada goose (*Branta canadensis*), beaver (*Castor canadensis*), and muskrat (*Ondatra zibethicus*). Tracks made by white-tailed deer, muskrat, Canada goose, and snapping turtle (*Chelydra serpentina*) were also observed in many surveyed stands that showed herbivore damage. Examples of herbivore damage are depicted in Figure 3. All but one of the existing goldenclub stands surveyed showed signs of herbivore damage to goldenclub leaves and stalks (Appendix Table 6).

Potential pollinating insects were collected from goldenclub flower stalks. These were mostly Diptera (true flies) and included *Sepedon* and *Dictya* sp. (Sciomyzidae), *Coenomyia ferruginea* (Xylomyidae), *Apotropina* sp. (Chloropidae), *Azelia* sp. (Muscidae), and an aphid. The lady beetle *Coleomegilla maculata* (Coleoptera: Coccinellidae) that is both a pollen feeder and predator (Andow and Risch 1985) was also observed.

All variables examined for Hudson River goldenclub stands and their descriptions are listed in Table 1. The results of Spearman rank correlation analyses on all non-categorical variables, excluding cardinal fetches, are in Table 3. Table 4 shows the results of Spearman rank correlation analyses comparing variables thought to be indicative of stand health, such as abundance, stand density, plant vigor, and herbivore damage prevalence and severity with fetch due north, east, south, and west of each stand, and mean fetch, in order to examine possible associations between exposure to hydrodynamic

stress and goldenclub stand health. Figure 4 depicts the relationship between the level of exposure to the open Hudson River a goldenclub stand has to the west and the most recent goldenclub plant count for each stand. The result suggests that stands with a greater abundance of goldenclub tend to have shorter western fetches. All stands with western fetches greater than 73 m, aside from the Little Nutten Hook stand, were found to be extirpated when most recently surveyed (for the majority of stands, this was between 2012 and 2014). Stands containing more than 40 plants, aside from the Little Nutten Hook stand, had minimal or no exposure to the Hudson River immediately to the west. The Little Nutten Hook stand, however, is quite sheltered on three sides.

Results of logistic regression analyses using fetch values as predictors of stand extirpation suggest that mean fetch, western fetch, and northern fetch are positive predictors of goldenclub stand extirpation. Mann-Whitney *U*-test results suggest that spatterdock (*Nuphar advena*) presence is positively associated with herbivore damage prevalence, mean fetch, southern fetch, western fetch, and eastern fetch. Figure 5 depicts the relationship between spatterdock presence and mean fetch for Hudson River goldenclub stands. Sweetflag (*Acorus calamus*) presence was positively associated with mean fetch, northern fetch, southern fetch, eastern fetch, and western fetch. No significant associations were found between cattail (*Typha*) presence and non-categorical variables.



Figure 3: Grazing of goldenclub plants. Tivoli North Bay, Big Bend Stand (Top) May 17th 2014; Cruger Island South, May 27th 2014 (Middle); Saugerties South May 13th 2014 (Bottom Left); Saugerties North Shore (Bottom Right) May 26th 2014.

Table 3: Results of Spearman rank correlation analyses comparing physical characteristics of each goldenclub stand and aspects of the stand environment recorded during the spring 2014 survey, and during historic observations of stands. Each box contains the correlation coefficient (rho) for each comparison. Statistical significance is annotated as follows: $p \leq 0.1 = ^*$, $p \leq 0.05 = **$, $p \leq 0.01 = ***$, $p \leq 0.001 = ****$.

Variable	Abund., Most Recent Count	Stand Dimens.	Stand Density	Plant Vigor	Herbiv. Damage Prevalence	Herbiv. Damage Extent	UTM E	UTM N	Mean Fetch	Distance from Railroad	Max. Historic Abund.	Abund. 2010s	Abund. 2000s	Abund. 1970s	Abund. 1930s	
Abund. Most Recent Count	1.000	**** 0.806	**** 0.819	**** 0.887	0.092	0.063	0.153	0.162	-0.261	0.008	** 0.395	**** 0.992	0.303	0.250	-0.200	
Stand Dimens.	**** 0.806	1.000	* -0.391	0.186	0.043	0.143	0.068	-0.135	0.230	*** -0.626	** 0.530	**** 0.803	0.031	-	-0.775	
Stand Density	**** 0.819	*	1.000	**** 0.851	0.138	-0.228	0.180	0.249	** -0.350	0.254	0.227	**** 0.815	0.258	0.335	0.000	
Plant Vigor	**** 0.887	0.186	**** 0.851	1.000	0.111	-0.102	0.146	0.207	-0.290	0.119	0.229	**** 0.885	0.278	0.266	-0.204	
Herbiv. Damage Prevalence	0.092	0.043	0.138	0.111	1.000	*** 0.659	-0.327	-0.362	0.259	-0.040	-0.190	-0.023	-0.081	0.318	-0.544	
Herbiv. Damage Extent	0.063	0.143	-0.228	-0.102	*** 0.659	1.000	*	** -0.443	-0.511	0.196	-0.163	-0.276	-0.039	0.083	0.281	-0.544
UTM E	0.153	0.068	0.180	0.146	-0.327	*	-0.443	1.000	**** 0.900	0.177	-0.141	0.266	0.158	-0.149	0.145	0.365
UTM N	0.162	-0.135	0.249	0.207	-0.362	** -0.511	**** 0.900	1.000	0.242	0.026	0.195	0.166	-0.166	-	0.183	0.036
Mean Fetch	-0.261	0.230	** -0.350	-0.290	0.259	0.196	0.177	0.242	1.000	-0.069	0.051	-0.285	-0.119	-	0.365	0.115
Distance from Railroad	0.008	*** -0.626	0.254	0.119	-0.040	-0.163	-0.141	0.026	-0.069	1.000	0.125	-0.002	-0.083	-	0.314	-0.456
Max. Historic Abund.	** 0.395	** 0.530	0.227	0.229	-0.190	-0.276	0.266	0.195	0.051	0.125	1.000	** 0.411	0.352	** 0.660	** 0.791	
Abund. 2010s	**** 0.992	**** 0.803	**** 0.815	**** 0.885	-0.023	-0.039	0.158	0.166	-0.285	-0.002	** 0.411	1.000	0.305	0.227	-0.200	
Abund. 2000s	0.303	0.031	0.258	0.278	-0.081	0.083	-0.149	-0.166	-0.119	-0.083	0.352	0.305	1.000	0.625	0.500	
Abund. 1970s	0.250	-0.412	0.335	0.266	0.318	0.281	0.145	-0.036	-0.115	-0.314	** 0.660	0.227	0.625	1.000	0.661	
Abund. 1930s	-0.200	-0.775	0.000	-0.204	-0.544	-0.544	0.365	0.183	0.365	-0.456	** 0.791	-0.200	0.500	0.661	1.000	

Table 4: Correlations between stand health and fetches. Statistical significance is annotated as follows: $p \leq 0.1 = *$, $p \leq 0.05 = **$.

Variable	Mean Fetch	Fetch North	Fetch South	Fetch East	Fetch West
Stand Density	** -0.350	*	*	-0.087	** -0.402
Plant Vigor	-0.290	-0.250	-0.257	*	** -0.363
Most Recent Abundance	-0.261	-0.254	-0.254	0.014	** -0.390
Herbivore Damage Prevalence	0.259	0.145	0.203	** 0.482	-0.003
Herbivore Damage Extent	0.196	** 0.496	0.036	*	-0.276
Abundance 2010s	-0.284	-0.273	-0.264	-0.034	** -0.386
Abundance 2000s	-0.119	-0.378	0.013	-0.139	-0.399
Abundance 1970s	-0.115	0.305	-0.195	-0.099	-0.138
Abundance 1930s	0.365	0.321	0.138	-0.413	*

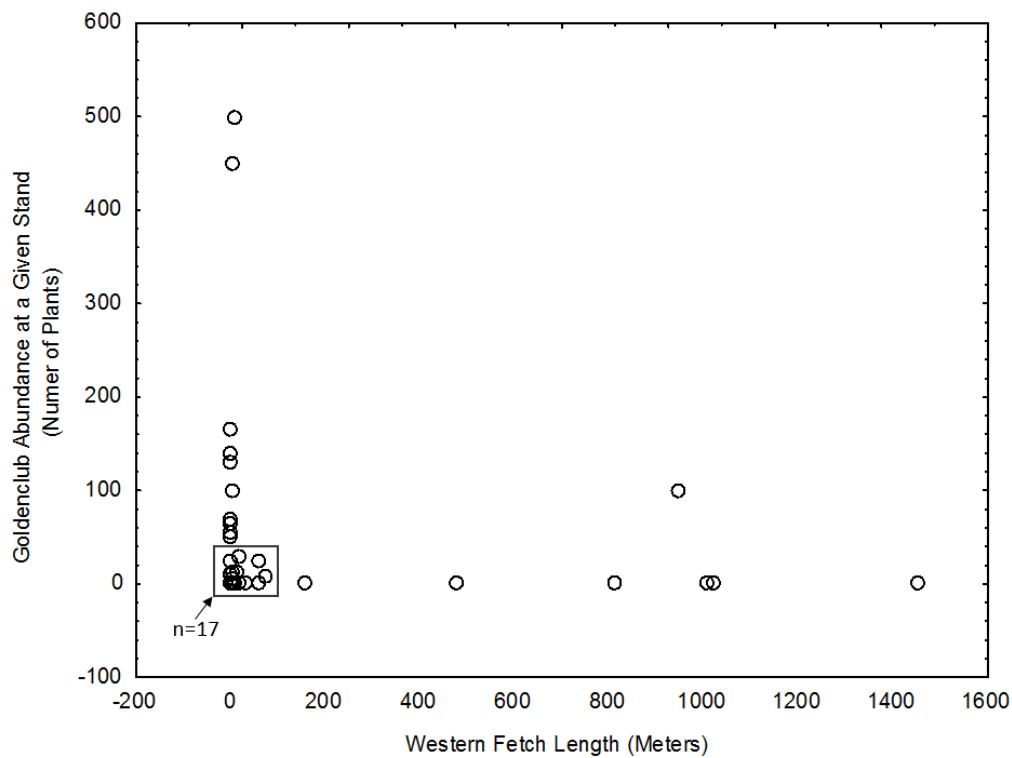


Figure 4: Goldenclub abundance for each stand vs. western fetch.

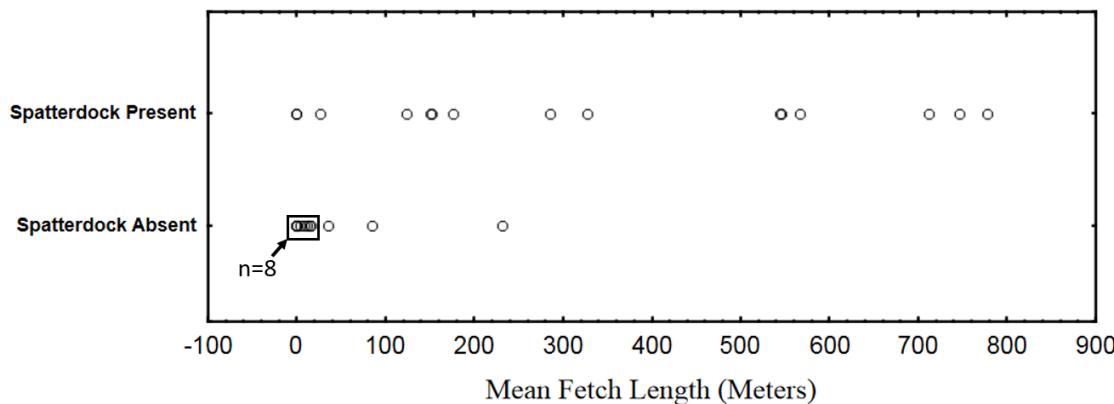


Figure 5: Relationship between spatterdock presence and mean fetch for current and historic goldenclub locations.

DISCUSSION

Observations in 2012-2013 suggested that goldenclub abundance along the Hudson River had declined in stands described in the 1930s and 1970s. For instance, in 1936 Muenscher (1937) estimated the Rogers Island stand to cover approximately 10 ha. In May 1974, however, Kiviat and W. D. Countryman searched the same region to find only two 1 ha stands of goldenclub (Kiviat 1976), and by May 2012 goldenclub had become extirpated from Rogers Island. A population of hundreds of plants at the north end of Cruger Island (Kiviat 1976) was also found to be extirpated when visited by Kiviat in 2012 (Kiviat, unpublished data). Recent photographs of these two locations are in Figure 6. On the other hand, a few goldenclub populations, such as those located at Cruger Island south, Hudson South Bay, Mudder Kill, and Little Nutten Hook appear to be holding their own. Kiviat also observed an increase in visible signs of grazing on goldenclub in the last decade or so, such as at the south end of Cruger Island where leaf damage was observed on 50% of plants in 2012. Through the present study, remaining gaps were filled in the data collected on the abundance and distribution of goldenclub in

the freshwater tidal wetlands of the Hudson River. These data were compared current to historic abundance and distribution records, and a few variables potentially associated with the current status of goldenclub were examined, along with camera trap data revealing possible culprit(s) behind increased herbivore damage.



Figure 6: Examples of locations where goldenclub was previously prevalent, but is now extirpated. Cruger Island North I, Spring 2014 (Left); Rogers Island North, Spring 2014 (Right).

Due to observations prior to the spring of 2014, an overall decline in goldenclub abundance since the 1970s was expected. It was found that, while many stands have become diminished or extirpated, new stands have become established, or were discovered anew, in alternate locations. Results from the spring 2014 survey combined with historic records suggest that more than half of the 16 goldenclub stands first reported in the Hudson River in the 1970s or earlier, some of which were once very large, have declined in abundance or become extirpated. The 10 “new” goldenclub stands reported since 2000 still containing goldenclub (as of 2014 survey efforts) vary in size. Some of these new stands may have existed prior to 2000 and escaped observation. Very few of the stands observed historically have maintained a stable abundance since first reported. Of the 14 total stands first observed in 2000 or more recently, three have increased in

abundance since the year they were first reported, two have remained stable, and six have declined (four of which are now extirpated). Three were first observed in the spring of 2014, and thus an abundance change assessment could not be made. These results suggest that goldenclub may establish at a few favorable locations during one season, and then decline due to changes in environmental conditions or natural aging of the cohort.

Alternatively, there may have been an overall decline in goldenclub following the 1970s, with recent event(s) allowing new stands to become established. Together, results suggest that several of the newly established stands may not be thriving, and thus goldenclub abundance in the Hudson River may still be in danger of decline.

It was hypothesized that sea level rise, erosion, and increases in flooding and hydrodynamic energy from storms, wind, waves, ship wakes, and ice may be inflicting stress on Hudson River goldenclub. Negative correlations between fetch variables and stand density suggest that stands with more exposure to the main river, and thus more exposure to wind, waves, and ship wakes tend to have a lower density of goldenclub plants. Longer western fetch was also found to be negatively correlated with plant vigor, the most recent abundance of goldenclub at a given stand, and ordinal abundance rankings between 2010 and the present, suggesting that stands with longer western fetches currently tend to have fewer and smaller plants. The quantity and strength of these negative correlations for western fetch suggest that hydrodynamic energy from storm winds, which frequently come from the west, may be most negatively impacting goldenclub stands. The lack of significant correlations between abundance rankings from the 2000s and 1970s, and the weak positive significant correlation between western fetch and 1930s abundance rankings, indicate that longer western fetches were historically not

characteristic of lower abundance, or perhaps that 1930s evaluations of stand sized were inconsistent. Severe storms such as Hurricane Sandy (2012) and Hurricane Irene (2011), accompanied by increased frequency and severity of flooding due to more extreme precipitation and sea level rise in the Hudson Valley (NYSDEC 2014), perhaps secondary to global climate change, may have caused greater impacts on stand health in recent years. The presence of fewer or less-severe storms and a decreased risk of flooding may have meant that Hudson River goldenclub was historically not impacted as strongly. That mean fetch, western fetch, and northern fetch were found to be positive predictors of goldenclub stand extirpation in logistic regression analyses further implicates exposure to open river, and resulting hydrodynamic stressors, in the decline of goldenclub stands. In his 1937 survey, Muenscher also commented that the goldenclub was most abundant behind beds of “*Scirpus*,” *Sagittaria*, or other plants where it is protected from the direct wash of the river currents and winds in the Hudson River.

During this survey, goldenclub was observed in the upper, middle, and lower, intertidal zones. Stands in the lower intertidal zone with most immediate exposure to the Hudson River were potentially more susceptible to stress as a result of tidal cycles and waves. With an increase in sea level rise, goldenclub in the lower intertidal zones may be submerged for longer periods of time, and as a result, may be under increased stress from greater respiratory demands. The three major co-occurring plant species that were examined in this survey, spatterdock, sweetflag, and cattail, grow mostly in the lower third, middle third, and upper third of the Hudson River intertidal zone, respectively (Kiviat, unpublished data). That spatterdock presence was found to be positively associated with southern fetch, western fetch, eastern fetch, and mean fetch at current and

historic stand locations could suggest that stands in the lower intertidal zone were impacted most heavily, possibly as a result of downstream effects of storms and sea-level rise. Sweetflag presence was also found to be positively associated with all five fetch variables, although there were no significant associations of cattail presence and other variables. That sweetflag and, most notably, spatterdock presence were found to be positively associated with longer fetches may indicate that stands in the lower and middle intertidal zones are more susceptible to extirpation, or that the relatively robust spatterdock and sweetflag were able to expand in the absence of other species.

Although it was hypothesized that goldenclub in the Hudson River may also be declining due to competition from other plant species, and examined associations between goldenclub stands and three major co-occurring plants (spatterdock, sweetflag, and cattail) no significant relationships were found between any of the co-occurring plants and abundance, extirpation, density, size, or vigor of goldenclub stands. As “presence” of co-occurring species was recorded if a plant was within 3 meters of goldenclub (which could have been at the center or periphery of stands), it is possible that the level of interaction of goldenclub with these other species was limited at some stands, while substantial at others, and thus this data have limited use for determining the impact of competition. Qualitative observations in the field brought to attention a possible biologically significant impact of sweetflag on stand health; the rhizome systems of sweetflag are very dense, and in the stand locations containing both goldenclub and sweetflag, clear borders could be seen between patches of the two species. Expansion of sweetflag in a goldenclub stand may result in a reduction in space for goldenclub. This scenario might be made worse in the case of increased hydrodynamic stress, which could

potentially allow more robust species like sweetflag to persist while goldenclub stand health is compromised.

It was hypothesized that increased herbivory in recent years may also be inflicting stress on goldenclub in the Hudson River. Leaf and flower stalk tissue removal due to herbivores was observed in almost all stands, some of which was extensive. It is possible that more extensive damage may be negatively impacting plants by reducing their ability to photosynthesize and reproduce. Historic photographs and the absence of comments on herbivore damage in historic records indicate less herbivore damage to Hudson River goldenclub in previous decades than was observed in more recent surveys, thus it is suspected that grazing has increased in recent years. Although herbivore damage prevalence and extent were not found to significantly correlate with goldenclub abundance, nor to predict extirpation of goldenclub stands, it is possible that over time extensive herbivore damage will have biologically significant effects on Hudson River goldenclub. Camera traps detected white-tailed deer, beaver, muskrat, and Canada goose in the Cruger Island goldenclub stand. The deer appeared to be foraging; however, it was not possible to distinguish what they were eating. The positive association found between spatterdock presence and herbivore damage prevalence in statistical analyses may suggest that swimming herbivores, such as muskrat and beaver, might be responsible for some of the damage, as goldenclub in the lower intertidal zone would likely be more accessible. As increased eastern fetch was positively correlated with herbivore damage prevalence, and increased eastern and southern fetches were found to be positively correlated with herbivore damage extent, it is possible that increased exposure to the main river, and resulting increased exposure to hydrodynamic stress, may have compromised goldenclub

defense against herbivores. Dr. Timothy Block (Pennsylvania Natural Heritage Program, personal communication 2014) reported seeing both geese and deer grazing on goldenclub, and judged from photographs of Hudson River goldenclub that geese, deer, and possibly muskrat, were responsible for the types of damage observed in recent surveys. The presence of deer, goose, muskrat, and beaver tracks, as well as snapping turtle trails, in many of the stands bearing signs of grazing further implicate these herbivores. Muskrat and deer tracks were the most prevalent in grazed stands among these potential culprits. It is believed that cut spadices within visible teeth marks are the result of muskrat or possibly beaver grazing, while crushed or torn leaves are the marks of deer or goose grazing. The negative correlation between stand dimensions and distance from railroads (i.e. larger stands tend to be closer to railroads) potentially suggests that deer or other herbivores are deterred from grazing in stands near railroads, or that the railroad may serve as a barrier to hydrodynamic energy from the main river for those stands growing on the side of the tracks closest to shore.

Factors other than those described in the hypotheses are likely also affecting the status of goldenclub. Other potential sources of chemical or physical stress include dredging and modifications to flow and sedimentation patterns as a result of residential and industrial development, as several goldenclub stands in the Hudson River are near private residences or are exposed to traffic from recreational boaters. Nutrient loading from inorganic nutrients and treated sewage being emptied into the Hudson River, from other sources of nitrogen and phosphorus such as agricultural runoff, or from atmospheric deposition of nitrogen in conjunction with other factors may also be contributing to the status of goldenclub and other rare plant species in the Hudson River.

The central results of this survey and examination of historic records of goldenclub in the freshwater tidal wetlands of the Hudson River suggest that many stands present in the 1930s and 1970s have declined, while a few new stands seem to have established, with hydrodynamic stress resulting from increased exposure to the main river being one of the most likely causes for abundance decline. That three new, large goldenclub stands were reported in the spring of 2014 suggests that the status of goldenclub in the Hudson River is not in as much danger as was originally expected. The potential effects of herbivory, development, as well as hydrodynamic stress from storms and rising sea levels on Hudson River goldenclub should continue to be monitored and taken into account if goldenclub is to be conserved in the Hudson River and potentially used in wetland restoration.

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APPENDIX

Table 5: Current and historic goldenclub stand locations in the Hudson River and abundance data.

Site Name	GPS(UTM)	Abundance Data
Schodack Island Creek	600481 m E, 4702980 m N	Approximately 70 plants in two groups (this study), 100-150 plants in three groups (NYNHP ca. 2002, pers. comm.)
Little Nutten Hook	600682 m E, 4688975 m N	100+ plants (Stevens ca. 2014, pers. comm.) (Werier ca. 2013, pers. comm.), "large stand" (NYNHP ca. 2013, pers. comm.), "Rare... Newton Hook-Coxsackie" (Muenscher 1937)
West Flats	599756 m E, 4683103 m N	10 plants (this study), "Relative Abundance = Common" (Vance NYSDEC ca. 1970's, pers. comm.).
Middle Ground Flats	598675 m E, 4678795 m N	0 plants (Barbour ca. 2000, pers. comm.), 1 plant (Kiviat ca. 1970s, unpublished data), "Common... south end of tidal mudflat between Hudson City and Athens" (Muenscher 1935)
Hudson South Bay	598384 m E, 4678131 m N	65 plants (this study), "44 small plants" (Kiviat ca. 2012, unpublished data), 4 plants (Barbour ca. 2000, pers. comm.)
Rogers Island North and West	596241 m E, 4676747 m N; 595545 m E, 4675919 m N	0 plants (this study), 0 plants (Kiviat ca. 2012, unpublished data), "acres of patchy distribution" (NYNHP ca. 2001, pers. comm.), "stand of many plants" (Lake ca. 1992), "2 ha" (Kiviat and Countryman ca. 1974, Kiviat 1976), "only a few plants of <i>Orontium</i> " (Countryman ca. 1969, pers. comm.), "no more than 3 ha of goldenclub" (McKeon ca. 1966, pers. comm.), "over 25 acres of tide flats in which goldenclub forms over 90 per cent of rooted vegetation" (Muenscher 1937)
Rams-Horn Creek At Road	593552 m E, 4672601 m N	6 plants (this study), 12 plants (location several meters away where no plants were found in 2014) (Federman ca. 2012, pers. comm.), 3 plants (NYNHP ca. 2000, pers. comm.)
Rams-Horn Mouth I (Northern Creek Bank)	594243 m E, 4672085 m N	12 (this study), 30 (Lukas and Sorensen ca. 2012, pers. comm.), Nonspecific Historic Data
Rams-Horn Mouth II (Southern Creek Bank)	594244 m E, 4672067 m N	100 plants (this study), 150-200 plants (Lukas and Sorensen ca. 2012, pers. comm.), Nonspecific Historic Data
Rams-Horn Mouth III (Rocky Bank)	594244 m E, 4672067 m N	55 plants (this study), 50 plants (Lukas and Sorensen ca. 2012, pers. comm.), Nonspecific Historic Data
Rams-Horn Mouth IV (Southernmost Stand)	594012 m E, 4671596 m N	Approximately 500 plants (this study), Nonspecific Historic Data
Rams-Horn Mouth	No Data	Nonspecific-These records may apply to one or more of the Rams-Horn Mouth stands observed in 2014. See Rams-Horn Mouth I-IV for recent data, "Scattered plants" (Countryman ca. 1971, pers. comm.), 20-30 plants (Goldhammer HRSC, pers. comm.), "Relative Abundance = Occasional" (Vance NYSDEC ca. 1975, pers. comm.). "Frequent" (Muenscher 1937)
Roeliff-Jansen Mouth	594096 m E, 4670339 m N	0 plants (this study), 0 plants (Kiviat and volunteers ca. 2012, unpublished data), 2 plants (NYNHP ca. 2001, pers. comm.)
Greene Point	592395 m E, 4670194 m N	0 plants (this study), 125 plants (NYNHP ca. 2000, pers. comm.)
Inbocht Bay	591530 m E, 4670000 m N	0 plants (this study), "Common" (Muenscher 1937)
Saugerties North	587739 m E, 4658539 m N	8 plants (this study), "Common... fairly abundant, not densely concentrated" (Bard ca. mid-1970s, pers. comm.), 40-50 plants (Smith ca. 1952, pers. comm.), "Common" (Foley and Taber observation 1948, described in 1951 publication), "Common" (Saugerties Marsh Area) (Muenscher 1937)
Saugerties	587919 m E,	30 plants (this study)

North Shore	4658393 m N	
Saugerties South	588251 m E, 4657842 m N	160-170 plants (this study), 50-60 (Hart and Rubbo ca. 2012 pers. comm.), “At least several plants of goldenclub” (Feldman and Dunbar ca. 1961, pers. comm.), “Common” (Saugerties Marsh Area) (Muenscher 1937)
Stony Creek East	589892 m E, 4654976 m N	0 plants (Kiviat ca. 2012, unpublished data), “50-100 plants,” (Kiviat ca. 1983, unpublished data)
Stoney Creek West	589841 m E, 4655033 m N	25 plants (Kiviat ca. 2012, unpublished data), “100+ goldenclub” (Kiviat 1976), “Abundant” (Svenson 1935)
Magdalen Island	588740 m E, 4655210 m N	0 plants (Kiviat ca. 2012, unpublished data), 4 plants (Kiviat ca. 1979, unpublished data), ca 10 plants (Kiviat 1976)
Tivoli North Bay Big Bend	589705 m E, 4654652 m N	130 plants (this study), 50-100 plants (Rogers ca. 2012, pers. comm.), 500+ plants (estimate for entire Tivoli North Bays Marsh System, including Stony Creek and Cruger Island) (NYNHP ca. 2003, pers. comm.)
Tivoli North Bay East Bank of Inlet	589717 m E, 4654598 m N	140 plants (this study)
Cruger Island North I	588859 m E, 4654105 m N	0 plants (this study), 0 plants (Kiviat ca. 2012, unpublished data), 100s of plants (Kiviat ca. 2012, unpublished data), 700-800 plants (Young ca. 1993, pers. comm.), “2000- 2500 plants Cruger Island” (Kiviat ca. 1975, unpublished data, Kiviat 1976), “apparently stable” (Jones ca. 1956-1976, pers. comm.).
Cruger Island North II	588840 m E, 4653997 m N	25 plants (this study), “sparse line of small plants along East side of Island” (Kiviat ca. 2000, unpublished data)
Cruger Island North III	588879 m E, 4653922 m N	0 plants (this study), 6 plants (Kiviat ca. 2012, unpublished data)
Cruger Island South	588808 m E, 4653568 m N	400-500 plants (this study), > 100 plants (Kiviat ca. 2012, unpublished data), “About 500 plants” (Young ca. 1993, pers. comm.), “seems less than in 1970s, but still a lot” (Kiviat ca. 1983, unpublished data), 100s of plants (Kiviat ca. 1970s, unpublished data, Kiviat 1976)
Mudder Kill Tracks	588431 m E, 4649590 m N	0 plants (this study), Goldenclub present at mapped location (NYNHP ca. 2000, pers. comm.)
Mudder Kill	588499 m E, 4649489 m N	70 plants (this study), 100 plants (Varanashi ca. 2013, pers. comm.), “15 to 20 plants in groups of 1 to 3” (Kiviat ca. 2012, unpublished data), 150+ plants (NYNHP ca. 2000, pers. comm.) 105 plants (Young ca. 1993, pers. comm.)
Kingston Point	585578 m E, 4642366 m N	0 plants (Hudsonia volunteers ca. 2012, unpublished data) “None” (Kiviat, Countryman, and Domville ca. 1970s, unpublished data), “none found after quick search” (Domville ca. 1972, pers. comm.), “scattered... quite a bit” (Domville ca. 1964, pers. comm.), “Frequent... Rondout River-Kingston” (Muenscher 1937)
Sleightsburg	585202 m E, 4641315 m N	“None” (Huth ca. 1970s, pers. comm.), one plant (Domville ca. 1964, pers. comm.), “at least several plants” (Feldman ca. 1951, pers. comm.)
Black Creek	586514 m E, 4630779 m N	12 plants (Grant ca. 2014, pers. comm.), 1 plant (Lukas and Sorensen ca. 2012, pers. comm.), 1 plant (Barbour ca. 2000, pers. comm.)
Storm King	584926 m E, 4587137 m N	0 plants (Kiviat ca. 2013, unpublished data), “few plants” (Barbour ca. 1996, pers. comm.)

Table 6: Environmental data collected for each goldenclub stand during recent survey efforts. See Table 2 for variable descriptions. Blank boxes denote an absence of data.

Site Name	Stand Dimensions	Plant Density	Mean Fetch	Fetch N	Fetch S	Fetch E	Fetch W	Dist. from Railroad	Plant Vigor	Herbivore Damage Prevalence	Herbivore Damage Extent	Sweetflag Present	Spatterdock Present	Cattails Present
Schodack Island	195	0.35	0	0	0	0	0	515	2	0	0	0	0	1
Little Nutten Hook	987	0.1	249.75	0	51	0	948	297	2	1	1			
West Flats	8	1.25	0	0	0	0	0	1372	2	2	2	0	1	1
Middle Ground Flats		0	439.5	1112	47	541	58	498	0					
Hudson South Bay	600	0.12	0	0	0	0	0	15	2	2	2	0	0	1
Rogers Island North		0	745.25	1370	200	391	1020	300	0			1	1	1
Rogers Island West		0	567	1160	125	170	813	617	0			0	1	0
Rams-Horn Creek at Road	5.25	1.14	0	0	0	0	0	1368	1	3	2	0	1	1
Rams-Horn Mouth I	110	0.11	151	0	28	576	0	653	2	4	4	0	1	1
Rams-Horn Mouth II	286	0.35	175.75	23	15	665	0	668	1	5	5	0	1	1
Rams-Horn Mouth III	78	0.71	544.25	0	1470	707	0	701	3	3	2	0	1	1
Rams-Horn Mouth IV	350	1.43	546	0	1450	729	5	840	3	4	2	0	1	1
Roeliff-Jansen Mouth		0	712.5	1400	0	0	1450	30	0			0	1	1
Green Point		0	777.5	0	1700	1410	0	1201	0			0	1	0
Inbocht Bay		0	326.75	10	560	260	477	1610	0			1	1	1
Saugerties North	36	0.22	123.25	100	120	200	73	1330	2	3	2	1	1	1
Saugerties North Shore	80	0.38	150	580	0	0	20	1347	3	3	3	0	1	0
Saugerties South	550	0.3	232	82	80	766	0	780	1	2	3	1	0	0
Stony Creek East		0	11.5	20	19	4	3	912	0			0	0	1
Stony Creek West	32	0.78	15.5	0	0	0	62	877	1	2	2	0	0	1
Magdalen Island		0	528.75	0	822	285	1008	216	0					
Tivoli North Bays Big Bend	448	0.29	13.25	23	0	30	0	750	3	3	3	0	0	1
Tivoli North Bays East Bank	264	0.53	2.5	10	0	0	0	765	3	2	2	0	0	1
Cruger Island North I		0	285	1000	100	20	20	50	0			1	1	0
Cruger Island North II	120	0.21	35.75	50	40	53	0	78	2	2	3	0	0	1
Cruger Island North III		0	85	300	0	10	30	31	0			0	0	1
Cruger Island South	750	0.6	26.25	20	20	60	5	70	3	5	3	0	1	0
Mudder Kill Tracks		0	7.5	0	30	0	0	13	0			0	0	0
Mudder Kill	282	0.18	0	0	0	0	0	41	1	4	3	0	0	1
Kingston Point		0	0	0	0	0	0	1680	0					
Sleightsburg		0	231.75	0	754	17	156	1600	0					
Black Creek	200	0.06	17	30	0	25	13	1355						
Storm King		0	85.5	133	159	50	0	47	0					

**SUBTIDAL SURVEY FOR THE PRESENCE OF OYSTER REEFS IN
PORTIONS OF RARITAN BAY, ARTHUR KILL, AND THE HACKENSACK
RIVER OF THE HUDSON RIVER ESTUARY**

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

Eastern oyster (*Crassostrea virginica*) reefs historically occurred throughout the lower Hudson River Estuary (HRE). As a result of anthropogenic pollution and overharvesting during the early 1900s, oyster reefs in the HRE suffered and the population has not yet recovered. Currently, small populations of intertidal oysters have been documented to exist in the HRE. Their presence in the intertidal zone leads to the hypothesis that oysters may also occur in subtidal areas. The goal of this project was to locate subtidal oyster reefs that may exist naturally in the estuary. The study area was focused on the Arthur Kill into western Raritan Bay and northward into the lower Hackensack River. The subtidal survey was conducted by examining the seafloor using a Klein 3900 dual frequency side scan sonar to delineate areas of hard bottom which could be characterized as a subtidal reef or an area that could support an oyster population. Areas of interest were then further investigated using a Video Ray Pro 4 remote operated vehicle to visually determine and characterize the bottom habitat. All data was imported into ArcGIS to visually depict the data. A living subtidal oyster reef was not identified in the study area by means of the underwater methods used; however locations with hard bottom that may support an oyster reef were identified. As part of a shoreline survey of intertidal oysters, single oysters were found on the shores of South Amboy (Raritan Bay), Perth Amboy (Arthur Kill) and Secaucus (Hackensack River). Outside of the study area, a subtidal reef partially exposed during low tide was identified in the East River.

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INTRODUCTION

Eastern oyster (*Crassostrea virginica*) reefs historically occurred throughout the lower Hudson River Estuary (HRE). Historical maps indicate that oyster beds still existed in Jamaica Bay, Raritan Bay, Newark Bay, Upper New York Bay and parts of the Arthur Kill and East River as late as c. 1910 (Metropolitan Sewerage Commission 1912). The New York oyster fishery declined and then ended by the 1930s because of sewage and industrial contamination reducing oyster stocks and rendering the consumption of the remaining oysters detrimental to human health. The current extent of remnant oyster populations in the HRE is poorly documented.

Oysters serve as positive ecosystem engineers and as biological indicators of ecosystem health. Oysters and oyster reefs contribute both biologically and physically to the estuarine ecosystem. Oyster reefs develop as multiple generations settle one upon another and the resulting vertical structure can protect shorelines from wave action. These reefs also provide an important habitat for fish, invertebrates, and macroalgae throughout their distribution (Lenihan and Peterson 1998). These benefits along with their filtering capacity warrant the restoration of oysters to estuaries. In areas like the Chesapeake Bay and Delaware Bay, the maintenance and restoration of oyster reefs is also critical to a commercial fishery. For the HRE, interest in the restoration of oysters is due to their biological importance as filter feeders and for their potential role in protecting shorelines during storm events, as well as their role in maintaining biodiversity within the estuary.

The goal of this study was to locate subtidal oyster reefs that may exist naturally in the estuary. Previous work has identified small populations of living intertidal oysters

in the lower Hackensack River, East River and Hudson River (Medley 2010). Their presence in these intertidal zones suggests that subtidal reefs may exist. It is known that the shell remains of historic oyster reefs still exist buried under sediment. Geophysical mapping conducted on the Hudson River bottom in a five-mile stretch north of the Tappan Zee Bridge imaged oyster beds covering 30% of the river bottom (Carbotte et al. 2004). Live, young oysters were reported to be occasionally recovered during sampling of these fossil oyster beds (Carbotte et al. 2004). More recently, in this same location, an estimated 200,000 oysters were discovered during the analysis of the impact of dredging for the replacement of the Tappan Zee Bridge (Risinit 2013). The Meadowlands Environmental Research Institute also identified five locations in the lower Hackensack River where subtidal oysters were captured during a fish survey (Bragin et al. 2005).

The U.S. Army Corps of Engineers and the Port Authority of NY/NJ has developed the Hudson-Raritan Estuary Comprehensive Restoration Plan (CRP) in conjunction with the New York New Jersey Harbor Estuary Program (USACE et al. 2009). This plan develops guidelines for ecosystem restoration within the estuary, to be utilized by organizations associated with the HRE. One of the eleven Target Ecosystem Characteristics in this plan is to establish oyster reefs. Local organizations such as the Hudson River Foundation and the NY/NJ Baykeeper have undertaken oyster restoration projects to attempt to reestablish this species. Permitting for these restoration projects has been limited by the New York Department of Environmental Conservation and New Jersey Department of Environmental Protection due to health concerns with potential consumption of oysters illegally harvested from restricted waters and the costs of policing

illegal harvesting and monitoring restoration projects. The potential negative impact that the illegal poaching of oysters in contaminated waters could have on the reputation of the otherwise harvestable New Jersey shellfish has led to a ban of oyster gardening and restoration projects in New Jersey waters designated as contaminated (NJDEP 2010). Therefore, reestablishment of the eastern oyster in the HRE may benefit from research focused on the habitat of existing wild oyster populations and the identification of available habitat that can support oyster settlement and future recruitment.

This study examined the benthos of the lower HRE to locate and visualize subtidal oyster populations, and provide insight into restoration efforts of this important species. Side scan sonar was used to survey the study area, as it was the simplest and most efficient method of surveying the study site.

METHODS

Study Area

The subtidal survey area included the entire stretch of the Arthur Kill from Raritan Bay to Newark Bay and the lower Hackensack River. The presence of intertidal oysters suggests that subtidal reefs may also exist in the Hudson River and East River, but the study area was limited due to the restricted time frame and funding of this research project. Dredged channels are present within the interior portions of the study area and were therefore excluded from the analysis.

Intertidal Survey

An intertidal survey of the HRE was conducted to reconfirm the presence of oysters existing in the intertidal zone of previous areas identified in 2006 and 2007 (Medley 2010) and additional intertidal areas surrounding the present subtidal study area. Sampling dates coordinated with spring tides when the lowest water levels would be expected. Exposed shorelines were walked approximately from one hour before low tide until one hour after low tide. In compliance with restrictions listed in collection permits from the New York City Department of Parks and Recreation (NYCDPR), one oyster was collected per every ten oysters found on NYCDPR properties along the East River. For areas on the New Jersey side of the HRE, no such permit restriction existed and all oysters found were collected.

Subtidal Survey

The subtidal side scan sonar survey was conducted over three sampling periods, beginning on 6/4/2014 in the Hackensack River near Laurel Hill County Park, Secaucus, NJ and continuing down the Hackensack River into Newark Bay. The survey continued in Raritan Bay and the lower Arthur Kill on 7/10/2014. On 7/31/14, a final effort was made to further delineate Raritan Bay and return to areas of interest within the Arthur Kill. The Urban Coast Institute of Monmouth University's 27 foot, *R/V Seahawk* was utilized as the research platform for conducting the subtidal survey. Data was collected using a Klein 3900 dual frequency side scan sonar towfish deployed approximately 2 meters below the vessel. A 30m coverage range was set to either side of the vessel, with a depth of 3-30m. Vessel speed and position were monitored using GPS, with a constant

speed of 4-6 knots during sampling to maintain uniformity. Qualitative substrate samples were collected with a petite Ponar grab sampler at areas of interest on hard and soft substrate throughout the study area to confirm bottom type. Sonar records were reviewed using Sonarwiz software, developed by Chesapeake Technology and used to locate areas of interest to further investigate using a remote operated vehicle (ROV) or a Splashcam drop camera. Sonar records were integrated into ArcGIS 10.1 in order to visualize benthic habitat differences.

Bottom types were characterized by the substrate that was found during grab sampling, as well as analyzing sonar and video records. Hard substrate such as rocks and scattered shell was classified as Hard Bottom/Scattered Shell. Soft substrate such as sand or mud was classified as Soft Bottom; these areas would not support a subtidal oyster population. Dredged areas were marked as Channeled, as these areas would be regularly disturbed. Areas that were scanned, but were not of interest for video analysis or grab sampling were designated as Searched/Unclassified.



Figure 1: Intertidal locations where oysters can be found in the Hudson River Estuary.

RESULTS

The intertidal survey re-confirmed the existence of oysters in the previously identified areas of the Hackensack River (HK), Macneil Park (MN), Soundview Park (SV) along the East River, Orchard Beach Lagoon (OB) in western Long Island Sound and the Hudson River at Alpine, NJ (AP) as shown in Figure 1. In addition to these areas, oysters were found in the East River at Castle Hill Park (CH), Bronx, NY, Little Bay Park (LB), Queens, NY, Raritan Bay Riverfront Park in South Amboy (SA) and the Perth Amboy waterfront (PA) in New Jersey (Figure 1). Specifically, four living oysters were found in the Hackensack River location, one at Raritan Bay Riverfront Park and one at the Perth Amboy waterfront. In addition to these living oysters, several recently dead oysters, indicated by both valves remaining attached, were also found at HK, SA and PA. All areas of the East River, with the exception of Little Bay Park, yielded a high abundance of oysters (50+) during each survey event.

Intertidal oysters were found attached to small rocks, other living oysters and bivalves or remaining shells of such. At Soundview Park (SP) and Castle Hill (CH), they were also found attached to tires and other debris. Oysters were collected at NYCDPR properties in the East River by taking one oyster per every ten found. The overall abundance of oysters in these locations was estimated to be, at a minimum, the number of oysters collected multiplied by ten; however, the actual abundance of oysters is likely much greater in these locations since the goal at the time of collection was to collect no more than 50 oysters during each survey event. The CH location particularly contained a visual abundance of oysters that could be seen both exposed in the intertidal zone and submerged in the subtidal zone (Figure 2).



Figure 2: Portions of a subtidal reef exposed at Castle Hill Park, Bronx NY.

Table 1: Intertidal Survey Locations and Number of Oysters collected in the Hudson River Estuary.

Location	Oysters Collected	Date Sampled	Coordinates
Hackensack River, NJ (HK)	4	5/23/2014	40°45'47" N, -74°05'13"W
Raritan Bay Waterfront Park, NJ (SA)	1	5/27/2014	40°28'46" N, -74°16'05"W
Perth Amboy, NJ (PA)	1	6/12/2014	40°30'36" N, -74°15'39"W
Soundview Park, NY (SV)	55	6/16/2014	40°48'35" N, -73°51'46"W
Orchard Beach Lagoon, NY (OB)	65	6/16/2014	40°52'03" N, -73°48'03"W
Alpine, NJ (AP)	21	6/19/2014	40°56'54" N, -73°55'06"W
Macneil Park, NY (MN)	64	7/15/2014	40°47'39" N, -73°51'06"W
Little Bay Park, NY (LB)	32	7/15/2014	40°47'23" N, -73°47'29"W
Castle Hill Park, NY (CH)	91	7/15/2014	40°48'44" N, -73°50'54"W

The subtidal survey did not locate a subtidal oyster reef. Figure 2 shows areas of hard bottom with scattered shell along the western Staten Island shoreline. Video validation in this area depicted scattered oyster shell. Some appeared as entire valves with the potential of being a living oyster, but no oysters were collected by ponar grab sampling. Areas immediately adjacent to SA also consisted of scattered shell, but on a softer bottom. The area immediately adjacent to PA was viewed and showed no evidence of hard substrate or shell.

The subtidal scan in the upper part of the study area did not locate an area of interest to investigate further using video validation (Figure 3). Large rocks could be seen along the eastern Hackensack River shoreline and parts of Newark Bay, but the subtidal substrate in these areas consisted mostly of soft bottom and debris.

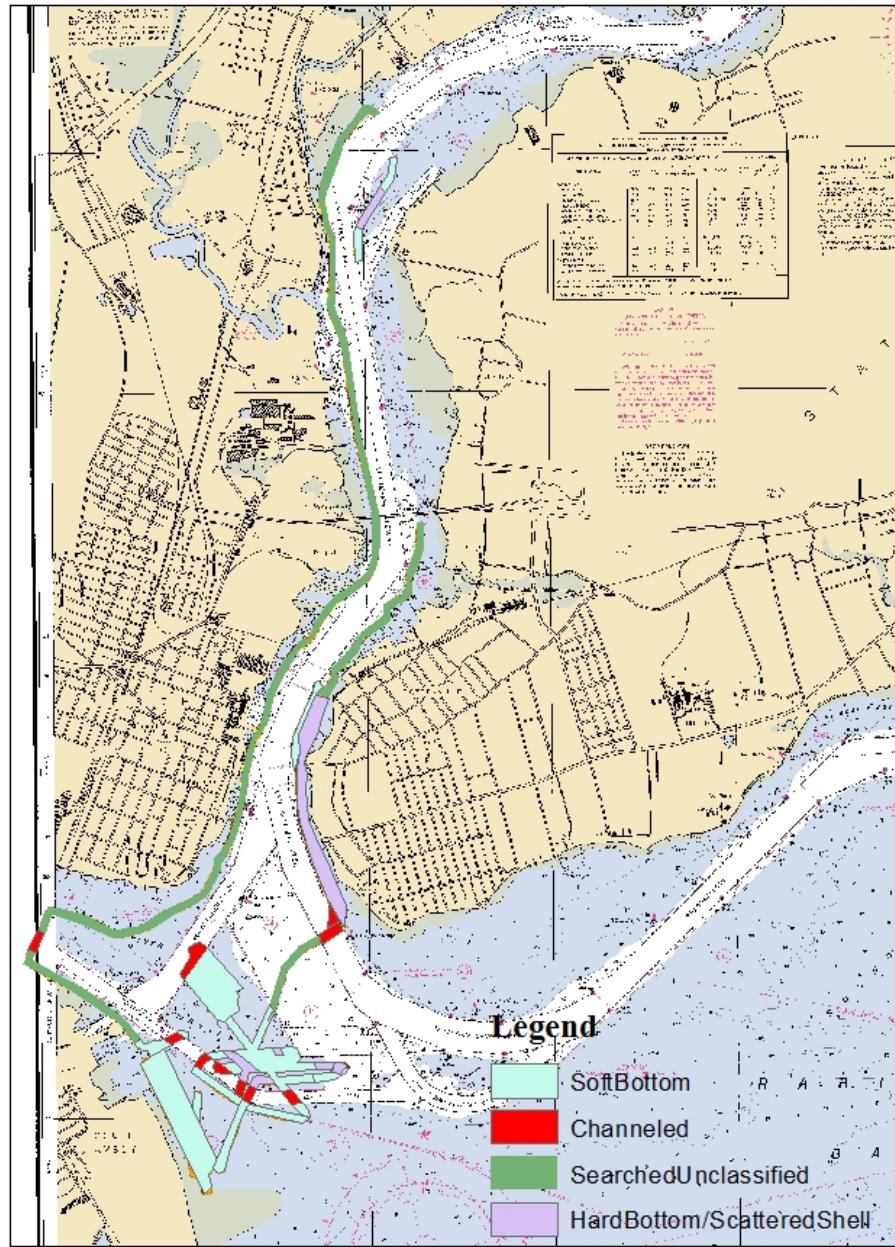


Figure 3: Side scan sonar survey area and delineated bottom types of western Raritan Bay and the Arthur Kill

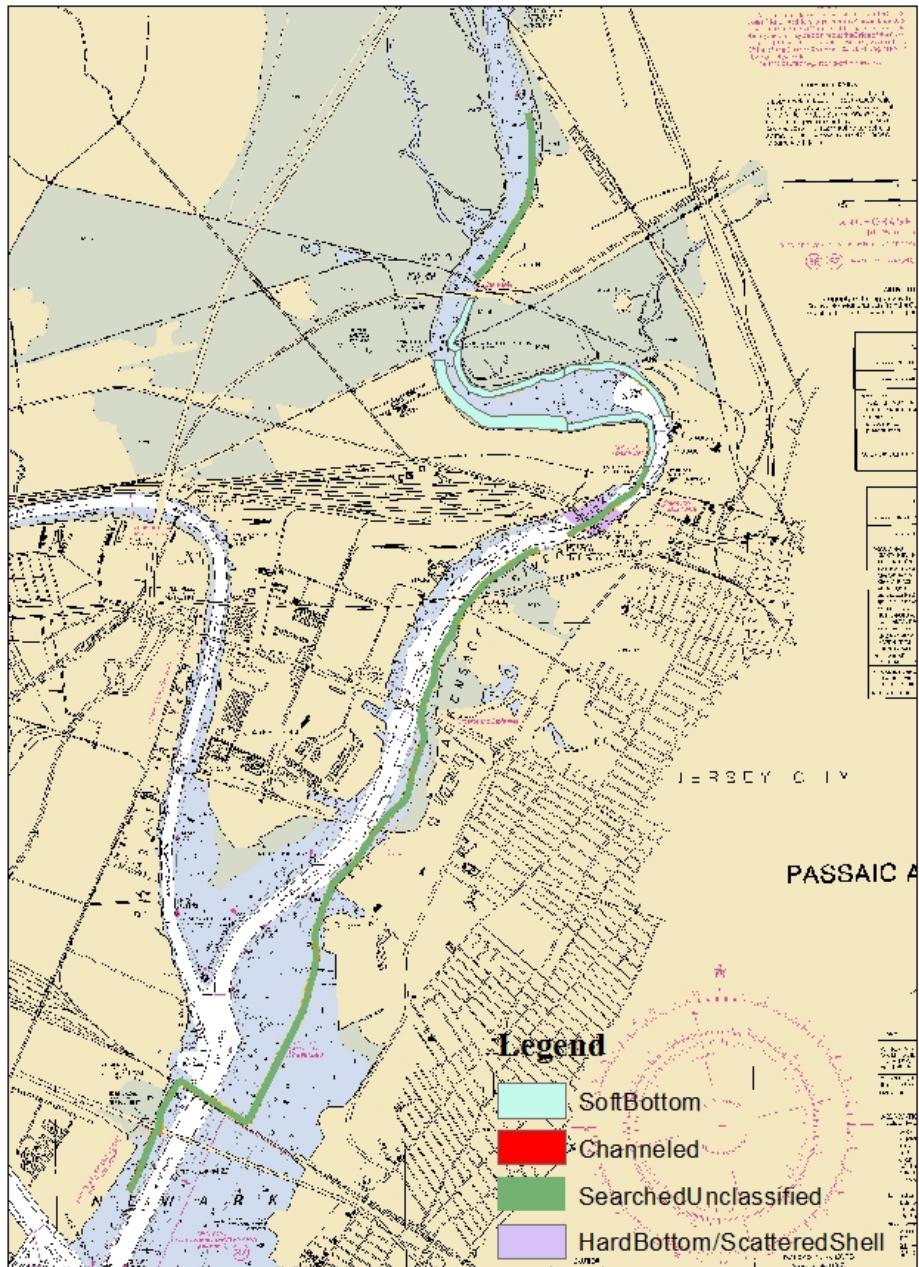


Figure 4: Side scan sonar area and delineated bottom types of the Hackensack River and Newark Bay

Table 2: Subtidal Bottom Type Spatial Data

Bottom Types	Area surveyed (m ²)	% of Survey
Channeled	98866	3.85
HardBottom/ScatteredShell	243928	9.50
Soft Bottom	1036007	40.35
Searched/Unclassified	1189036	46.30

DISCUSSION

The Eastern oyster can be found in both the intertidal and subtidal zones of estuaries along the East Coast of the United States. At the latitude of the HRE, intertidal areas are not ideal for oyster survival due to mortality associated with ice exposure during the winter, so it was expected that the general abundance would be low and individual oysters were more likely to be found instead of intertidal reefs along the shorelines. The identified intertidal locations are geographically separated throughout the estuary which indicates that oyster larvae are present and are distributed to settling sites via tides and currents.

The one living intertidal oyster found in Raritan Bay was large (117.5 mm H x 79.6 mm L) and round in shape which indicates that it grew singly on a hard bottom. The discovery of this oyster, along with another living oyster and several recently dead oysters in the SA and PA locations, suggested that a potential reef could be found in the upper portion of Raritan Bay and the subtidal survey was expanded to include this area. This portion of the HRE is where a mile long oyster bed known as “The Great Beds”

once existed (MacKenzie 1990). However, although scattered oyster shell was identified by ROV, no living subtidal oyster reefs were found.

The subtidal bottom type analysis identified substrate that could support subtidal oyster populations (Figure 2). While the percentage of hard substrate and scattered shell was relatively small, the fact that these areas are present within the HRE is supportive of future oyster restoration or natural recruitment (Table 2). The portion of the sonar record that was unclassified (unverified by ROV), 46% of the entire area surveyed, denotes substrate that would not likely support an oyster population. Large portions of these areas are located near channels that are dredged regularly, disturbing the surrounding bottom habitat. The southeastern side of the Arthur Kill had the most scattered shell and isolated individual oysters found subtidally. This area is located within the pilot location for a proposed “Living Breakwaters” project to be built along the southern shore of Tottenville (Rizzi 2014). The project plans to utilize oysters to construct breakwaters to protect the shores of Staten Island from storm damage.

The waters of the HRE are divided between New York and New Jersey and all oyster restoration projects require approval by the appropriate state regulatory agencies. The “attractive nuisance” that the restoration of oyster reefs will potentially create, requires the State(s) to police the reefs to prevent poaching and public consumption of the oysters (Yozzo et al. 2004); this limits permit approvals. In June 2010, the New Jersey Department of Environmental Protection, under a new administration, banned existing and future research-related gardening of commercial shellfish in waters classified as contaminated. These include portions of Raritan Bay, the Arthur Kill, Hackensack River and Hudson River. The state’s intent is to minimize potential negative impacts to New

Jersey's shellfish industry in non-prohibited waters that could result in public illness due to consumption of shellfish raised in prohibited waters for research or educational projects. The NY/NJ Baykeeper was granted an exception to conduct an oyster restoration project at the Naval Weapons Station Earle in Sandy Hook Bay because that location is continuously supervised by the United States Navy.

With the present restrictions on introduction of live oysters, the future of oyster restoration in the New Jersey waters of the HRE may be limited to encouraging the settlement of existing wild larvae by placing suitable habitat for attachment. The most ideal location for such placement would be in an area where wild oysters are already established. In addition to having adapted to local environmental stresses, the existing populations may have established tolerances to two known protozoan oyster diseases endemic in *C. virginica* throughout much of its range, *Perkinsus marinus*, better known as "Dermo" disease and *Haplosporidium nelsoni*, MSX disease (Coen and Luckenbach 2000). These diseases, along with over-fishing and habitat destruction, are devastating oyster fisheries in the mid-Atlantic region and are hindering efforts in oyster restoration and aquaculture (MacKenzie 1996; Yu and Guo 2006). On a global scale, it was recently estimated that 85% of oyster reef ecosystems have been lost due to disease and overharvesting (Beck et al. 2011) putting additional pressure on preserving wild populations of oysters.

Despite contaminated waters and concerns regarding public consumption by state regulatory agencies, this study confirms that wild oysters occur in the Hudson-Raritan Estuary. The subtidal survey results indicate that there is ample substrate to support oyster restoration and natural recruitment. Although subtidal surveying of the East River

was not feasible as part of this study, the results of the intertidal survey and the relative abundance of live oysters collected strongly suggest that subtidal reefs may be present in this area. The partial subtidal reef exposed at Castle Hill Park, Bronx, NY (CH) during the intertidal survey is evidence that subtidal reefs likely exist but have yet to be identified.

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**PREY DENSITY EFFECTS ON PREDATOR FORAGING:
A COMPARISON OF PREY LOSS AND IMPLICATIONS FOR A NATURAL
INSECT COMMUNITY**

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

Aquatic insect community composition and diversity are strong indicators of stream health; these aspects were examined in the context of disturbance along Wappinger Creek, a lower Hudson River tributary, in order to evaluate changes in community trophic structure. Wappinger Creek was sampled at three locations in late June and early July 2014 to observe diversity changes caused by weather disturbances. Stream health increased with decreasing stream order; the highest stream order site had the lowest diversity. Community diversity and density peaked at the medium stream order site; whereas the headstream site had the most unique insects. Diversity did not generally change between sampling times.

The effects of varying prey density on predator foraging efficiency were studied. The two aquatic insect predator taxa studied were Sialidae (alderflies, Megaloptera) and Perlidae (stoneflies, Plecoptera), chosen for their similar diets, body size and prey recognition methods. Macroinvertebrates for the predator-prey experiments were collected from Wappinger Creek and sorted into containers designated for six treatments with two variable factors: prey density and predator type. While the predators caused prey loss, neither predator's foraging efficiency was significantly affected by changes in prey density. The lack of predator response to prey density ('null numerical response') implies that the predators are limited by intra-predator competition. Predation was not the only factor operating to remove individuals from the experimental communities; intra-guild predation within typically herbivorous larvae may have resulted in density-dependent losses due to excess resource competition within each container. In regard to the Wappinger Creek diversity changes and the reduction of predator abundance, the self-

regulatory actions of low trophic level intra-guild individuals observed in the foraging experiment support the natural community's continued stabilization.

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INTRODUCTION

Lotic ecosystems are predominantly unobserved or overlooked, in part, because their erratic responses to weather and flow across the landscape create variable spatial and temporal heterogeneity across relatively short reaches. Extensive observation, dedication and expense would be necessary to evaluate these systems thoroughly (Barbour et al. 1999; Flotemersch et al. 2006). In contrast, lakes have uniform responses to environmental factors and are evaluated more frequently than streams and rivers. Due to the irregularity of a stream, across its width and along its length, many more samplings of numerous biological, chemical, and physical aspects are needed in an effort to accurately comprehend the aquatic ecosystem, its biodiversity, and ecological niches available (Blocksom and Johnson 2011). Although rivers, such as the Hudson River and other streams in its watershed, are more complicated to assess than lakes, lotic systems fulfill a vital function in the environment that ought not to be overlooked as a result of the challenge that they pose (Limburg et al. 1986).

Biological assessments are utilized by government agencies as well as non-governmental organizations (NGOs) in order to analyze potential sources of pollution and to assess water quality. Based on an aquatic insect's sensitivity to pollution and the community species richness and abundance, the health of the stream ecosystem can be determined through bioassessment. Macroinvertebrates are ideal indicators of stream health due to their strict feeding behaviors, habits and dissolved oxygen requirements which instigate quick responses to environmental changes. Such responses can be utilized to infer a categorical representation of stream health, and the macroinvertebrate community composition can suggest the degree to which the stream is stressed by

pollution (Hilsenhoff 1988; Voshell 2002; Merritt et al. 2008). In order to assist with the correlation between stream health and macroinvertebrates, Hilsenhoff (1987, 1988) classified each genus of common North American freshwater invertebrate species with an empirical value along a scale (0-10) of tolerance. High levels of biodiversity and the presence of especially sensitive families of aquatic insect larvae (Ephemeroptera, Plecoptera, and Trichoptera) are, also, essential factors for stream quality assessments (Hilsenhoff 1987).

In addition to being valuable indicators for bioassessment due to their presence in certain environments, aquatic insects have an intriguing community-level interactions network. Aquatic insects fill crucial ecological niches within the stream ecosystem. For example, most caddisfly larvae (Trichoptera) are filter-feeders or collect fine particulate organic matter from rock surfaces, whereas, dragonfly and damselfly larvae (Odonata) are predaceous (Voshell 2002; Peckarsky et al. 1990). These ecological guilds fill important and distinct roles in the lotic ecosystem. According to the nutrient spiraling concept and river continuum concept, invertebrates are pivotal elements that shred allochthonous coarse particulate organic material (CPOM), which, in increasing stream order, reduces CPOM to fine particulate organic material (FPOM) available for a myriad of invertebrate species, which in turn recycle detritus and decomposing organic matter (Newbold et al. 1982; Vannote et al. 1980). All in all, the aquatic insect niches support a diverse community across long distances and through stream orders.

Many scientists have examined the predator-prey interactions between insectivorous fish and choice prey items (Heck 1981; Ioannou et al. 2009; Baxter et al. 2005; Wesner 2010), but studies of the intra-order aquatic insect predator-prey dynamics

are few. These studies are essential to determine the overall efficiency of predation and effect of predation on the community, specifically whether some prey species are more affected than others, or if the collective prey community is significantly depleted by predators (Benke 1976; Townsend and Hildrew 1978). Peckarsky (1982) stated that the reverse interaction, how prey affects predators, has not been thoroughly examined. A community with high prey density may affect factors such as prey detection, selection of food items, and the success of attacks. The term *confusion effect* is defined as “the reduced rate or success of attacks on prey groups as multiple targets overload the information-processing capacity of predators” (Ioannou et al. 2009). This response has been demonstrated to be dependent on prey density with increasing number of failures as prey density expands and the predator becomes unable to distinguish a vulnerable prey item (Milinski 1977).

In order to test whether the *confusion effect* influences aquatic insect predators; an experiment was designed that compares two common predators’ foraging behavior at different prey densities. The common stonefly (Plecoptera: Perlidae) and alderfly (Megaloptera: Sialidae) larvae have many similarities allowing them to occupy similar niches. Both are known to be voracious generalists and actively hunt their prey. They eat similar prey items, typically small mayfly (Ephemeroptera) and true fly (Chironomidae) larvae (Azam 1969; Peckarsky and Wilcox 1989). These predators choose prey based on an allometric body size relationship. Throughout their development, their prey’s body size directly increases with instar development (Klecka and Boukal 2013). Generalists have strong effects on the community structure and stability of the food webs because they have the ability to switch prey types almost effortlessly depending on the

optimization of their foraging response to varying prey quality and quantity (Murdoch and Oaten 1975).

Community structure is affected by factors such as the environment's productivity and biological interactions. Peckarsky and Dodson (1980) observed the effects of predator-prey interactions on prey recruitment and their aversion to some habitats based on predator and competitor presence; however, they failed to investigate the effects of prey density on predator foraging responses.

Study Objectives

For the purposes of experimentation, there were three major study objectives:

- (1) Assess differences in Wappinger Creek's community structure and species diversity across stream order and time caused by small disturbances,
- (2) Evaluate the prediction that top predators have a major effect in a structurally complex experimental environment; and
- (3) Determine whether aquatic insect predator foraging responses are affected by prey density.

Community structure in the context of stream order was assessed by field sampling. Laboratory experiments were used to understand the role of predator effects as a function of prey density. In the predator-prey density experiment, the preliminary prediction was that neither Sialidae nor Perlidae would be affected by varying prey densities. Considering that both predators respond to hydrodynamic cues, higher prey densities ought not to confuse them, thereby foraging efficiency should remain constant across any prey density. Laboratory tests of these hypotheses were interpreted in order to

predict the persistence of Wappinger Creek's community structure during periods of disturbance.

METHODS

Site descriptions

Wappinger Creek originates from lakes and wetlands in Eastern New York and joins the Hudson River at Wappingers Falls, New York. Three sites along Wappinger Creek (Fig. 1; Table 1) were observed for two consecutive weeks during late June and early July. The sites were accessed through public use lands. The following sites were visited and are listed by increasing stream order: the Town of Stanford Recreation Park in Stanfordville (SRP), Cady Recreation Park in Pleasant Valley (CRP) and Greenvale Park in Poughkeepsie (GVP). The sites displayed physical properties appropriate to increasing stream order (Vannote et al. 1980).

The SRP site is a shallow, calmly moving stream with 90% shade cover; whereas, the GVP site is a meter-deep, slow moving stream with 5% shade cover. In order to perform the predator-prey density experiment, it was necessary to choose a healthy stream that supported a diverse resident community of insect larvae. The SRP and CRP sites can be characterized as fast-flowing lotic-erosional zones containing many riffles and high dissolved oxygen content, and the GVP site is a lotic-erosional zone with low discharge and exhibits the presence of algal mats.

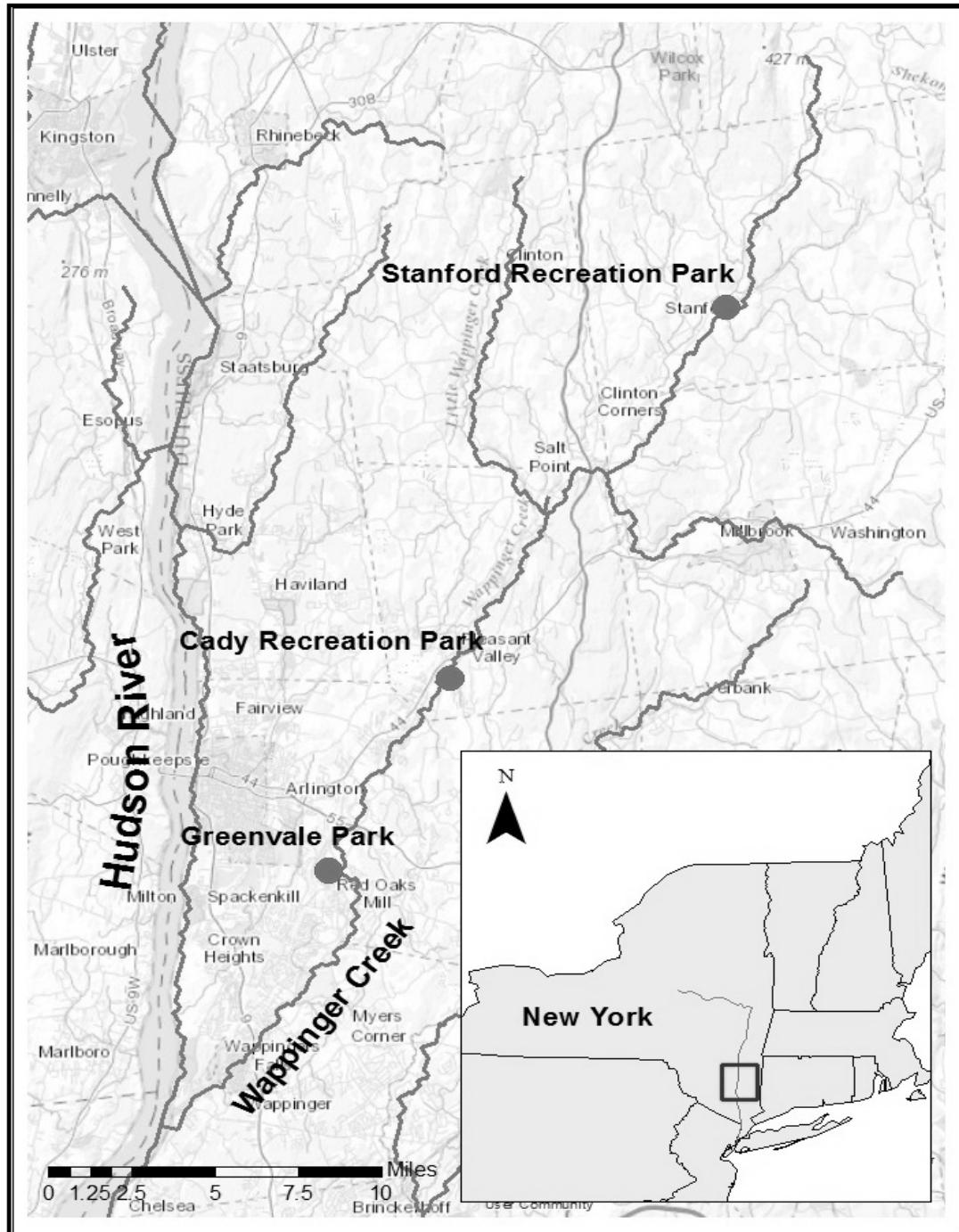


Figure 1: Wappinger Creek site locations for aquatic insect collections. 1A. Stanford Recreation Park at Stanfordville. 1B. Cady Recreation Park at Pleasant Valley. 1C. Glenvale Park at Poughkeepsie. Map courtesy of ESRI ArcMap Online.

Table 1: Wappinger Creek site information including site name, abbreviation, town name, relative stream order and GPS coordinates. Wappinger Creek is located in Dutchess County, New York.

Site Name	Abbr.	Town Name	Relative Stream Order	GPS Coordinates
Stanford Recreational Park	SRP	Stanfordville	Low	41°87'24.40'' N, 73°71'06.67'' W
Cady Recreational Park	CRP	Pleasant Valley	Medium	41°74'20.85'' N, 73°82'87.06'' W
Glenvale Park	GVP	Poughkeepsie	Medium-High	41°66'09.56'' N, 73°87'71.64'' W

Field Sampling

The aquatic insect community was sampled during two consecutive weeks in late June and early July. The macroinvertebrate community was sampled using a dip net with 500 micrometer mesh. Positioning the net facing upstream, approximately one square foot of the stream bottom was agitated by foot in front of the net's mouth, and the contents were placed in a 1-gallon plastic jar filled with 95% ethanol preservation (NYSDEC 2012). This process was repeated three times in order to amass a single kick sample. Three kick samples were collected across a transect line perpendicular to stream flow. The kicks across the transect line were replicated three times for a total of nine composite collection samples per site. All individuals were picked from the samples and identified to family ordination using a dissecting microscope and high intensity illuminator.

Hilsenhoff Biotic Index (HBI), Simpson Diversity Index (SDI), percent Ephemeroptera, Plecoptera and Trichoptera (% EPT), percent individuals based on feeding strategy and species densities were calculated for the composite macroinvertebrate samples at each site.

Prey Density Experiment

Insect samples were collected from the GVP and CRP sites five times during July, August and September 2014. The insects were collected using a dip net and were stored in a 16-quart plastic storage bin. All insects were transported in bins with air pumps and air stones to the laboratory for sorting. Stream water was collected in two liter containers. Four collections were gathered from the CRP site, and two were collected from the GVP site. The predators were collected, separated and kept in solitude within 4 dram snap cap vials until the beginning of the experiment.

In the laboratory, six 4-quart plastic containers were filled with five centimeters of substrate and stream water collected from Wappinger Creek filled to five centimeters above the rock substrata (Fig. 2). The substrate was a mixture of medium-sized cobbles, small pebbles, and sand to increase heterogeneity. A six-outlet commercial air pump was used to aerate the containers; an air stone was placed in the center of each container. Additionally, two layers of mosquito netting covered the openings in order to collect any individuals that emerged as adults.

The containers were



Figure 2: Predator-prey experiment set-up

given labels based on treatment. The treatments were ‘high prey density – Sialidae predator’(HD-S), ‘low prey density – Sialidae predator’(LD- S), ‘high prey density – Perlidae predator’(HD-P), ‘low prey density – Perlidae predator’(LD-P), ‘high prey density – no predator’(HD-N) and ‘low prey density – no predator’(LD-N). Based on insect densities and species composition observed from the field samples, the prey densities and composition for the prey density experiment was determined. The low prey density containers had 20 individuals: 10 Chironomidae, 5 Ephemeroptera and 5 Trichoptera. The high prey density containers had 60 individuals: 10 Chironomidae, 30 Ephemeroptera and 20 Trichoptera. The total prey remaining was counted after a week. The six treatments were performed simultaneously and repeated a total of five times. Average prey consumed by both predators at each density was calculated from the five ‘no predator’ treatment replicates. The ‘no predator’ treatments were used as a control for predation effects.

The insect samples were sorted by hand accordingly. For the final three replicates, the prey body size was divided into size ranges. Large body size individuals were longer than one centimeter, medium body size individuals were between 0.99 centimeters and 0.5 centimeters, and small body size individuals were smaller than 0.49 centimeters. The body length was measured for each predator as well.

At the beginning of the experiment the predators were placed in their designated arenas. The arenas remained undisturbed for seven days. After a week, the water was carefully decanted and replaced with 95% ethanol. The insects were recovered from their containers and those that remained were picked, identified and recorded.

Statistical Analysis

In order to determine the number of individuals lost to predation, the total individuals lost in the five replicates of the HD-N and LD-N treatments were averaged and the standard deviation was calculated. This number was subtracted from the treatments with predators. The additional losses that could not be contributed to the designated predators' activities were observed in all treatments and considered to be lost for other reasons.

Standardized *t*- tests were used to determine the significance of the prey density based on prey consumed for both predators. The null hypothesis was that there was no difference in the proportion of prey consumed at high and low prey density. A one-way Analysis of Variance (ANOVA) determined whether prey consumption was different between the predator treatments. In this case, the null hypothesis was that prey consumption was not significantly different as a function of predator type. Additionally, the relationship between predator body size and prey consumed was graphically represented and fit using linear regression. The relationship between predator body size and proportion of prey lost were also graphed and fit by linear regression.

The changes to the community composition and diversity due to the predator-prey density experiment were quantified by calculating Simpson's Diversity Index ($1 - D$) of the community.

$$D = \Sigma \left(\frac{n}{N} \right)^2$$

The significance of the difference between these diversity values was calculated across the factors, prey density and predator type, using a two-way factorial ANOVA. The proportions of the abundant prey taxa lost (Hydropsychidae, Heptageniidae, Baetidae and

Chironomidae) were calculated, and a two-way factorial ANOVA design was used to calculate the significant differences in proportions between prey density or predator type and the loss of certain taxa. Tukey's HSD *post-hoc* test was performed to identify which taxa were affected more than others.

RESULTS

Wappinger Creek Community

From the samples collected at Glenvale Park (GVP) in Poughkeepsie, Cady Recreational Park (CRP) in Pleasant Valley and Stanford Recreational Park (SRP) in Stanfordville, various descriptive metrics were calculated to describe the aquatic insect community's diversity and indications of stream health during two consecutive weeks in midsummer (Table 2). Notably, due to substantial rainfall between 30 June 2014 and 7 July 2014, the diversity metrics indicated changes. For example, species richness at all sites declined slightly; however, the GVP site's diversity increased from the first observation ($1 - D = 0.5088$) to the second ($1 - D = 0.7922$). The other sites exhibited no change in diversity. The Hilsenhoff Biotic Index remained relatively similar between the observations. Stream health increased with decreasing stream order. Overall, the CRP site had the highest diversity ($1 - D = 0.8254$; SR = 36), as well as the most consistent density (1028.1 - 1338.4 individuals per square meter). The other sites experienced more drastic changes related to the weather conditions. The SRP site's density strongly declined, but the taxa present did not change. In the GVP site, the density increased slightly, yet the species present were radically different. After the rainfall, there were

more individuals present from the pollution-sensitive families Ephemeroptera, Plecoptera and Trichoptera.

Table 2: Descriptive community statistics across the three Wappinger Creek sites. The table includes comparisons of species richness (SR), density (individuals / meter squared), Simpson Diversity Index (1 – D), percent Ephemeroptera, Plecoptera and Trichoptera (% EPT) and Hilsenhoff Biotic Index (HBI).

Site	GVP (Poughkeepsie)		CRP (Pleasant Valley)		SRP (Stanfordville)	
Metric	30 June	7 July	30 June	7 July	30 June	7 July
SR	26	21	36	35	29	26
Density	454.54	602.45	1028.1	1338.4	2153.7	577.2
1 - D	0.5088	0.7922	0.8254	0.7955	0.8234	0.8005
% EPT	17.36	59.95	68.19	45.14	53.96	56.2
HBI	5.62	6.01	5.06	4.79	4.55	4.79

Prey Density Experiment

The estimates for the average prey consumption accounted for a small percentage of the total number of prey lost during the experiment (Table 3; Fig. 3). In the low prey density treatments ($N = 20$), predation accounted for about 55% of total prey loss. Predation contributed to 30% of total prey loss in the high prey density treatments ($N=60$). The residual prey loss was attributed to other community dynamics.

Statistical analysis of the different treatments yielded significant results for certain factors (Table 4). Prey loss did not change across high and low density treatments for either predator ($t_{5.03} = 1.59, P = 0.172$; $t_{4.57} = 1.60, P = 0.178$); however, the differences in prey consumption across Sialidae, Perlidae and absent-predator treatments was significant in high ($F_{2, 12} = 9.93, P = 0.0029$) and low density ($F_{2, 12} = 43.0, P < 0.001$).

Comparisons between Sialidae or Perlidae and the absent-predator treatments were all significant ($P < 0.01$); however, neither Sialidae nor Perlidae had a greater effect on prey loss than the other. These trends applied to both high and low prey density treatments.

Table 3: A comparison of the average predator foraging rate (number of prey consumed per week) and average rate of prey loss for all reasons including predation, intraspecific competition and intra-guild predation during each of six experimental treatments.

Treatment			
Prey Density	Predator	Average Rate of Predator Consumption	Average Rate of Prey Loss
High	Sialidae	7.8 ± 3.7	27.2 ± 3.7
	Perlidae	6.8 ± 3.27	26.2 ± 3.27
	None	-	19.4 ± 1.67
Low	Sialidae	5.0 ± 1.34	8.6 ± 1.34
	Perlidae	4.4 ± 0.71	8.0 ± 0.71
	None	-	3.6 ± 0.55

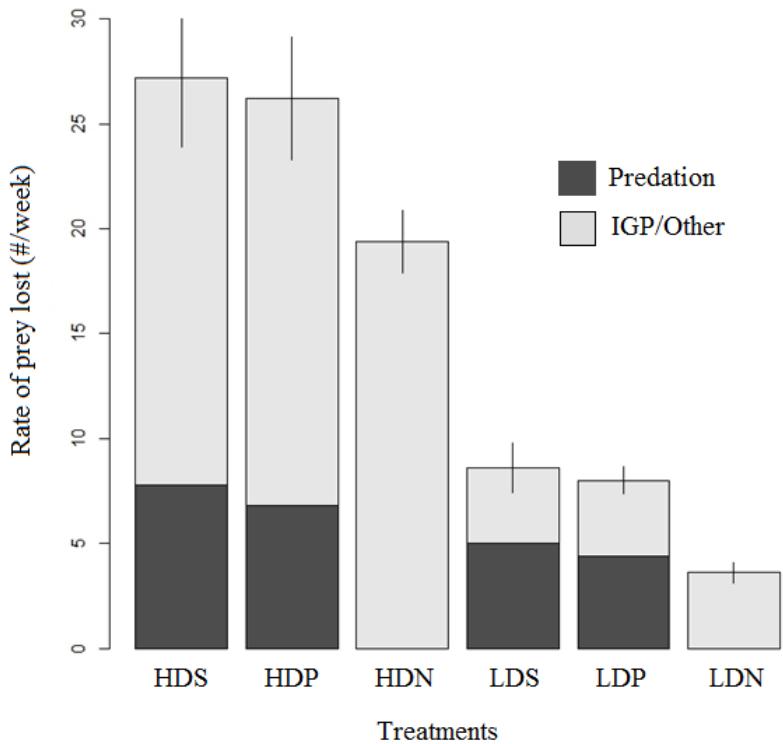


Figure 3: Representation of prey loss rate. The stacked bar plot separates the number of prey lost to predator consumption per week and prey lost to intra-guild predation and for other reasons. The treatments are abbreviated by high (HD) and low (LD) density, and by predator type: Sialidae (S), Perlidae (P) and no predator (N).

The differences between prey loss rates were highly significant between high and low density ($P = 0.001$; Table 4). This alludes to the presence of strong density-dependent losses that are unrelated to the effects of either Sialidae or Perlidae predator additions.

Table 4: Statistical analysis of differences in mean rates of predator foraging A) between high and low density using Standardized t tests, B) among all predator types using One-way ANOVA and C) between two predator types using Standardized t tests. D) Differences in mean rate of prey loss between high and low prey density.

Test	Prey Density	Predator	Statistic	P
A) Differences in predator foraging means for high and low density		Sialidae	$t_{5.03} = 1.59$	0.172
		Perlidae	$t_{4.37} = 1.60$	0.178
B) Differences in predator foraging means among all predator treatments	High		$F_{2,12} = 9.93$	0.0029 **
	Low		$F_{2,12} = 43.0$	<0.001 ***
C) Differences in predator foraging means between predator treatments	High	Sialidae vs. None	$t_{5.57} = 4.29$	0.0061 **
	High	Perlidae vs. None	$t_{5.96} = 4.14$	0.0062 **
	Low	Sialidae vs. None	$t_{7.53} = 7.72$	<0.001 ***
	Low	Perlidae vs. None	$t_{5.3} = 11.0$	<0.001 ***
D Differences in prey loss rates means between high and low density treatments			$t_{18.8} = 3.75$	0.001 ***

Prey Community

The effects on the prey community were calculated through changes to the Simpson Diversity Index (1 – D) after the experiment (Table 5). The Two-way ANOVA yielded no significant change to the prey diversity as a result of prey loss. Neither predator type ($F_{2,26} = 0.98, P = 0.389$) nor prey density ($F_{1,26} = 2.2, P = 0.15$) had significant effect on the species richness or species abundance of the prey community.

In order to determine whether some taxa were negatively affected more than other taxa in the treatments, the proportions of prey consumed for the four most abundant taxa in the prey communities were calculated. The differences in proportions of lost individuals within taxa were evaluated using a Two-way Factorial ANOVA in order to determine whether prey density and predator type had significant effects (Table 5). Prey density ($F_{1,32} = 4.79, P = 0.036$) and predator type ($F_{2,48} = 6.3, P = 0.0037$) had significant results; however, the interaction between prey taxa and prey density was not significant ($F_{3,32} = 0.78, P = 0.512$). The interaction factor between prey taxa and predator type was highly significant, though ($F_{6,48} = 5.25, P < 0.001$). Prey density had a slight effect on the proportions of taxa lost, which relates to the total number of prey lost since more prey were removed in higher density than in lower density (Fig. 3; Fig. 4a). Predator type displayed an effect as a result of the ‘no predator’ treatments being included (Fig. 4b), which further demonstrates that Perlidae and Sialidae contributed to prey loss, but they did not differ in their effect. The prey community experienced heavier losses in the presence of active predators; however, Tukey’s test revealed that Baetidae and Chironomidae experienced higher proportions of loss than Heptageniidae and Hydropsychidae in the presence of both predators, Sialidae and Perlidae (Fig. 5). Baetidae and Chironomidae had higher mean proportions of prey loss than the two other prey families. Baetidae differed most from the other three taxa (all $P < 0.01$).

Table 5: Statistical analysis of prey loss effects on community by assessing A) the difference between the Simpson Diversity Index values ($1 - D$) before and after the prey density experiment. The differences between mean prey loss by taxa in regards to B) prey density and C) predator type as factors were interpreted using a Two-way Factorial ANOVA design. The p values were calculated in R program.

Test	Factor	F	P	
A) Differences among $\Delta(1-D)$ without factor interaction	Predator	0.98	0.389	
	Density	2.2	0.15	
B) Differences in mean loss proportions among prey taxa and density with factor interaction	Prey Taxa	10.16	<0.001	***
	Density	4.79	0.036	*
	Interaction	0.78	0.512	
C) Differences in mean loss proportions among prey taxa and predator type with factor interaction	Prey Taxa	17.27	<0.001	***
	Predator	6.3	0.0037	**
	Interaction	5.25	<0.001	***

The proportion of Chironomidae lost was different from all other taxa, yet only the comparisons of the proportions of Chironomidae lost to proportions of Baetidae and Heptageniidae lost had statistically significant differences. Heptageniidae proportional loss was similar to that of Hydropsychidae ($P = 0.98$). Predator body size relative to the number of prey consumed was graphed in order to determine the differences in prey consumption between treatment replicates (Fig. 6). The number of prey consumed increases with predator body size ($P = 0.053$, $r^2 = 0.326$). More Baetidae and Chironomidae were consumed by larger predators. For example, in an arena with a perlid

predator (body size = 1.15 cm), Baetidae had a 75% loss and Heptageniidae had a 37% loss; however, in a container with a smaller perlid predator (body size = 0.7 cm), Baetidae had only a 53% loss and Heptageniidae had no loss. Predator body size and the proportions of prey consumed from all possible prey had a positive but statistically non-significant relationship ($y = 0.72x + 0.92$; $P = 0.55$, $r^2 = 0.32$; Fig. 7).

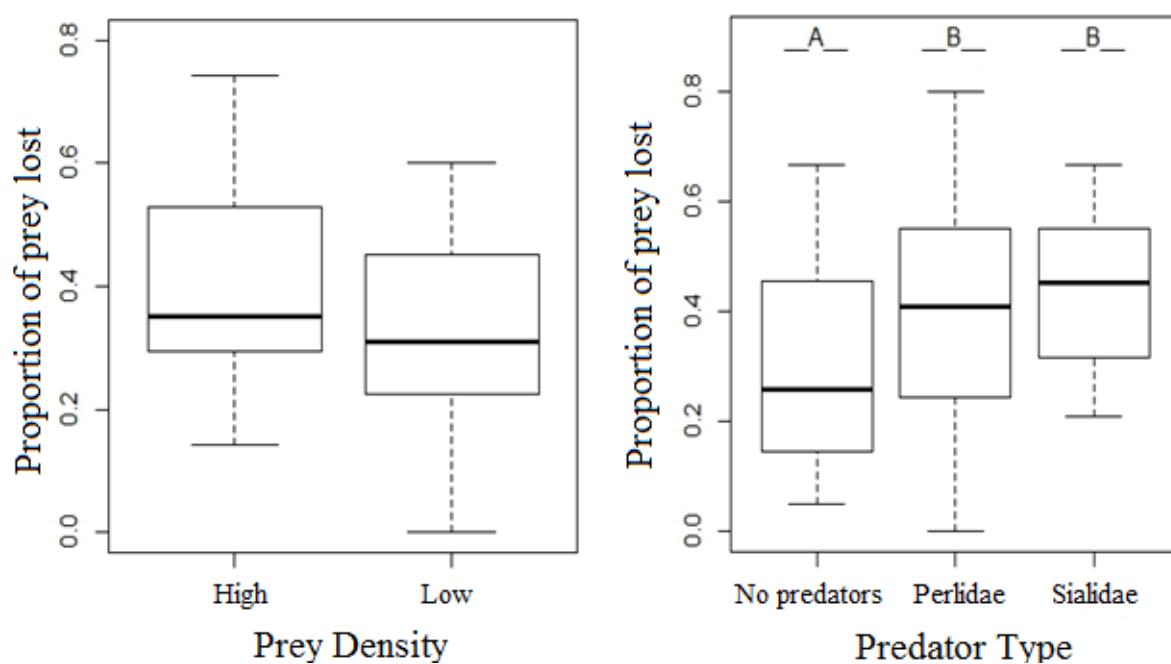


Figure 4: Boxplots showing a) proportions of prey lost in high and low prey density treatments and b) proportions of prey lost in Sialidae, Perlidae and no predator treatments. Significant differences are identified using letters above the boxplots.

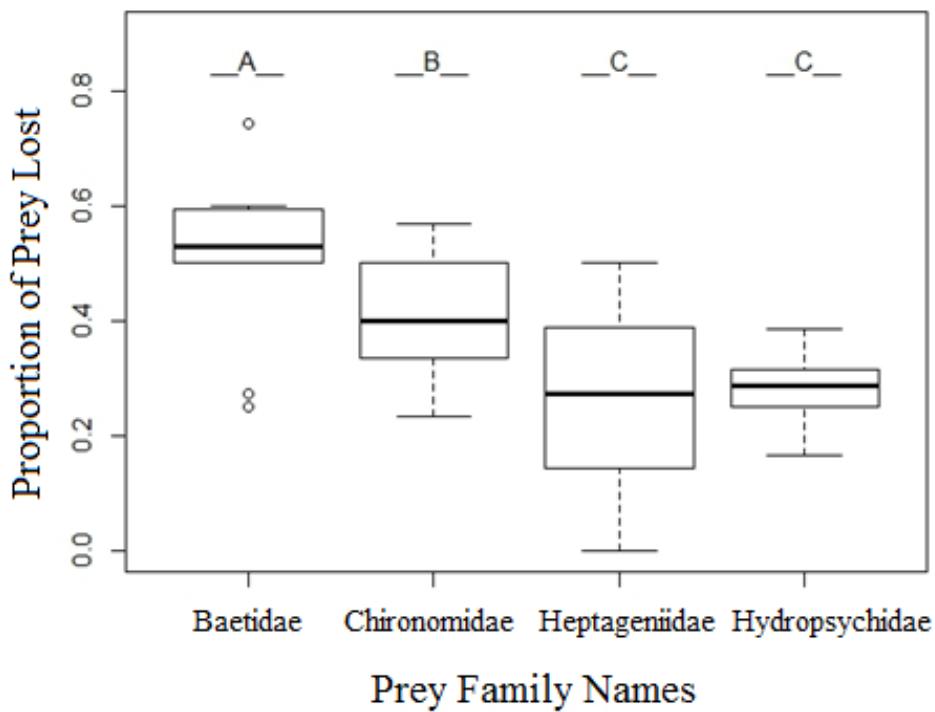


Figure 5: Boxplots showing proportions of prey lost for the prey families Baetidae, Chironomidae, Heptageniidae and Hydropsychidae. Significantly different family means are labeled with letters above each boxplot.

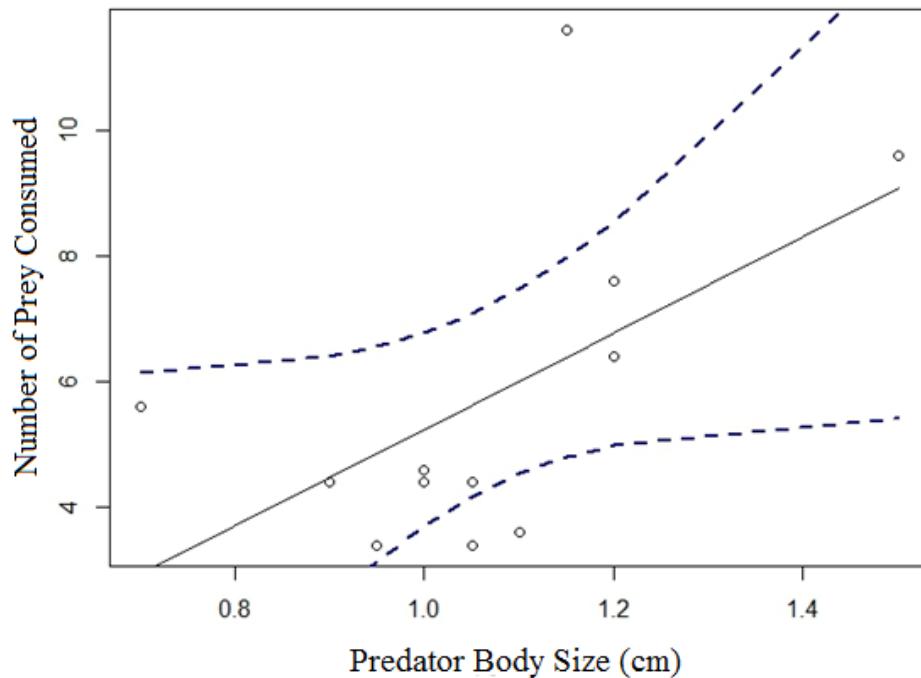


Figure 6: Linear regression representation of predator body size (cm) and number of prey consumed. Linear regression data: $Y = 7.68x - 2.44$, $P = 0.053$, $r^2 = 0.326$.

In a container with a smaller perlid predator (body size = 0.7 cm), Baetidae had only a 53% loss and Heptageniidae had no loss. Predator body size and the proportions of prey consumed from all possible prey had a positive but statistically non-significant relationship ($y = 0.72x + 0.92$; $P = 0.55$, $r^2 = 0.32$; Fig. 7).

Prey body sizes were recorded into three ranges: greater than one centimeter, 0.5 centimeters to 0.99 centimeters and less than 0.5 centimeters. The body sizes of the 55% of the prey individuals were less than 0.5 cm and considered to be ‘small’. Seventy-nine percent of the consumed prey was within the ‘small’ body size range. Of the most abundant prey taxa incorporated in the experiment, both Baetidae and Chironomidae were the smallest taxa and experienced the highest consumption rates (Fig. 5). Prey and predator body size allometry may partially explain the community interactions and losses observed in the predator-prey density treatments.

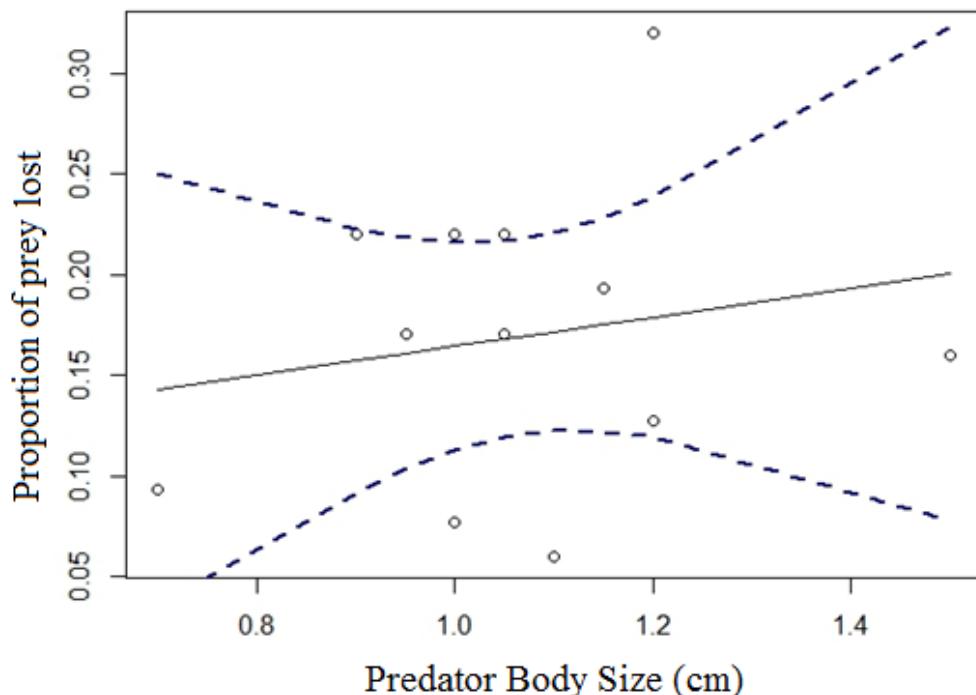


Figure 7: Linear regression representation of predator body size (cm) and proportion of prey community lost. Line of best fit: $Y = 0.92 + 0.072x$, $P = 0.55$, $r^2 = 0.039$.

DISCUSSION

The Wappinger Creek sites resembled the community changes explained by the River Continuum Concept (Vannote et al. 1980) and Nutrient Spiraling Concept (Newbold et al. 1982). These concepts combine insect community's fundamental niches and the cycle of organic nutrients to predict the types of insects and nutrients available throughout all orders of a stream. They predicted that CPOM would be more available in lower orders with higher shade coverage, like the SRP site, and there would be more organisms that can reduce this larger matter into FPOM. Due to the actions of the insects in the lower orders and inflow of allochthonous nutrients, the middle stream orders, like the CRP site, have a highly diverse stream community. Also, a mixture of insects that disassemble large plant material and filterers and collectors of FPOM become prominent features. In the higher orders, like the GVP site, the nutrient load has increased to a point that algal mats ('aufwuchs') cover most of the substrate and little vegetation grows around the stream. At these reaches, the community has reduced diversity becoming composed mainly of filter-feeders. They predict that predator abundance will be congruent throughout the stream orders. The Wappinger Creek sites correspond with these theoretical predictions before the rainfall events between 30 June 2014 and 7 July 2014. The aquatic macroinvertebrate feeding guilds present at the sites changed with increasing stream order based on the availability of coarse and fine particulate organic matter. However, the rainfall caused a disturbance that diminished the diversity of the GVP site in the lower reaches of the creek.

Along with the transformation of the insect functional groups, taxa diversity is maximal at the CRP site where dissolved oxygen and nutrient levels should be the

greatest within the stream. Since taxon diversity changed substantially after the rainfall only in the deep, slow-moving water at the GVP site, this location seemed to benefit from the intermediate natural disturbance. The diversity of the SRP site did not benefit nor suffer from the rainfall, but drastic changes to density were noticed. Considering the number of unique species at this site, frequent disturbances may actively remove them. Percent predators within the community remained constant at all sites, which speaks to the dependence of these communities on their presence acting as a stabilizing factor for community structure across stream order.

In the predator-prey density experiment, all treatments endured losses of prey individuals from the assembled communities. Further losses from the prey community were noticed in all treatments with intentionally-added predators compared to the absent-predator control treatments. This indicates that the predators had a significant, negative effect on the prey community; however, fixed experimental differences in prey density had no effect on either Sialidae or Perlidae consumption rates. According to Peckarsky and Dodson (1980), the lack of predator reaction to shifting prey density is termed a *null numerical response*.

When this response is observed, it is considered that the predator is limited more substantially by factors other than prey availability (Holling 1961; Crawley 1975). Such a factor may be interference competition between conspecific and interspecific predators that occupy the same patch. Since neither Perlidae nor Sialidae encountered other large predators, their foraging response was not reduced between treatments.

Additionally, Sialidae and Perlidae exhibited comparable prey consumption rates. Given their similar body size, voracity and the hydrodynamic sensory cues used to

distinguish their desired prey items, the predators' feeding rates were expected to be comparable. In prey recognition experiments, Peckarsky and Wilcox (1989) determined that *Kogotus modestus* (Plecoptera: Perlodidae) was able to distinguish its preferred prey through mayfly wave patterns made while swimming. This ability allows the predators to identify viable prey items, pursue and attack them after an encounter. The number of prey consumed increased with predator body size, which is supported by Klecka and Boukal's (2013) predator body size allometry results. Furthermore, the proportion of prey lost had no relationship to Sialidae or Perlidae body size. Since the number of prey consumed correlated with predator body size but total prey loss was not, predation most likely is not the main influence on the final proportions of prey loss.

The comparison of predator-inclusion treatments to the absent-predator control treatments showed that large predators only contributed to a partial increase in total prey loss. In other words, factors that were unrelated to predation by introduced Sialidae and Perlidae had either an equal or more substantial influence on determining the prey abundance at the end of the experiment. Since predation by larger predators was not entirely responsible for the disappearance of the larvae, smaller-sized resource competitors may have participated in predator-prey behaviors. This prospective cause of prey loss is called intra-guild predation (IGP). The observation that the predator control treatment-related losses increased from 45% in low density to 70% in high density suggested that the negative interactions between prey led to density-dependent losses.

It has been shown that herbaceous aquatic insects are more likely to colonize available patches with lower competitor densities than those with higher prey densities when predators are present; thereby indicating that their aversion to intra- and

interspecific competition is greater than their response to large-bodied predators (Peckarsky and Dodson 1980). In the predator-prey density experiment, the presence of potential competitors within the same guild had a heavier influence on prey individuals than did the presence of either the Sialidae or Perlidae predators.

The prey taxa that experienced the highest losses were Baetidae and Chironomidae, which belonged to the small prey category (body size < 0.5 cm). This differed from Hydropsychidae and Heptageniidae that, usually, belonged to the medium and large prey categories (body size > 0.5 cm). In addition to the differences in their body size, the prey taxa with greater losses tended to have similar reactions when physically encountered by predators. In streams, Baetidae and Chironomidae (when they are not hidden in self-created organic particle tubes or algal mats) swim in undulating motions that attract undesired predator attention (Peckarsky and Wilcox 1989; Merritt et al. 2008). The other prey taxa do not have this reaction; rather, their habits are to cling to and crawl between rocks in order to escape larger predators. These other prey taxa may not be as efficient competitors as their more active counterparts. Overall, in environments where there are reduced, normal predation pressures on the better competitors, intra-guild predation may have been a way to regulate the frequency of superior prey and influence top-down control to maintain a nutrient-rich ecosystem and reduce the abundance of dominant competitors, which increases aquatic insect species richness and persistence of the community during disturbances.

Holt and Polis's (1997) intra-guild predation module describes the requirements for the coexistence of intra-guild competitors. First, the actions of the intra-guild competitors that predate upon other competitors are inversely correlated with autotrophic

productivity, and second, the intra-guild competitors that are consumed in IGP relationships are superior exploiters of shared resources. Therefore, when productivity is low, better competitors outcompete worse competitors. In order to compensate for the loss of desired resources and to obtain enough energy for their continued survival, the worse competitors tend to become selectively omnivorous and will prey upon the superior competitors. Facultative omnivory, as explained by the IGP module, is a staple in communities with limited resources and numerous competitors of varying exploitative aptitudes (Polis et al. 1989).

In the predator-prey density experiment, small (<0.5 cm) prey were lost with more frequency as a result of regular predation or intra-guild predation. These smaller and faster prey individuals may have been better competitors over resources within the arenas, and were, therefore, consumed by larger, more tentative intra-guild competitors. This would increase the probability of the larger individuals' survival. Due to the lack of allochthonous water inputs such as particulate organic matter during the experiment, resources would have been limited within the treatment containers and would have provoked heightened intra-guild predatory behaviors. Also, the presence of a single Sialidae or Perlidae predator removing dominant competitors may have increased the degree of intra-guild predation, which would act as an additional stabilizing factor within the experimental prey community and allow the survival of some species that are less prone to predation (Gilinsky 1984).

The implications of the predator-prey density experiment can be applied to the communities observed at the Wappinger Creek sites. As noted previously, the large Sialidae and Perlidae predators did not respond to changes in prey density; however, their

non-response implied that their foraging efficiency is probably limited by competition for prey. The Wappinger Creek sites had higher densities of predators (~ 30 individuals/sq. meter) than the density of predators in the experiment. Therefore, an aquatic insect predator's foraging response may change with the interactions with other predators. The experiment supported that a single predator has a slight effect on the prey community; however, the intra-guild predation involving predators of much smaller body size was the dominant cause of prey loss. In a natural community like that of Wappinger Creek, aquatic insect density changed after the rainfall. The GVP and CRP sites increased in density after the rain, and the percent predators decreased; however, the community at the SRP site decreased in density and the percent predators increased. When changes like this occur, predation and IGP are important factors leading towards the restoration of community equilibrium.

Predation acts to organize the community structure and increase diversity. Intra-guild interactions such as competition and predation become important in community organization when obligate predators are few (Hairston et al. 1960; Polis et al. 1989). Severe disturbances like urbanization and pollution instigate trophic cascades throughout the community structure. An upstream shift of slightly poorer water quality, like that of the GVP site, would occur in the lower orders of the stream. Aquatic insect densities and diversity decline, and for some less abundant or vulnerable species, local extirpation is a possibility (Smith and Lamp 2008). Predators, like Perlidae and others that are highly sensitive to pollution, would be removed, and less sensitive predators like Sialidae would replace them. In extreme circumstances the loss of predator diversity will cause a trophic cascade that limits the diversity of the prey community and photosynthetic productivity.

For example, in the GVP site, there was lower predator diversity which corresponded with decreased density and species diversity. In comparison, the CRP site displayed the opposite trends; greater predator richness corresponded with higher site density and diversity. Therefore, predators are crucial to aspects that stabilize the aquatic insect community, especially during disturbances.

Further inquiries about the aquatic insect predators and community structure stem from the predator-prey density experiment. Suggested future investigations include 1) focusing on the actual number and species of prey consumed by the predators through radiometric tagging, 2) testing the foraging responses of other predators found in Wappinger Creek like dragonfly (Odonata: Gomphidae) and dobsonfly (Megaloptera: Corydalidae) larvae, and 3) examining the predator response to increasing predator density in both intraspecific and interspecific competition treatments. Since the prey density did not change the foraging efficiency of the predators, the addition of conspecific individuals and other predator species may affect the focal predator's foraging efficiency. Moreover, further intra-guild predation experiments would provide important information about community structure and stability. If intra-guild predation effects decline in environments with higher proportions of predators to prey individuals, at what predator density or from which combination of predators would this happen?

In order to explore these questions in the future, a similar experimental design to the foraging experiment can be utilized. By setting up multiple treatment arenas simultaneously, the effects to the experimental community can be compared and analyzed under varying predator densities and different organizations of competing predators in the arenas. Accordingly, a series of revised macroinvertebrate foraging experiments can

delve further into understanding the shift from primarily standard predation by obligate predators to intra-guild predation by facultative omnivores in disturbed stream systems.

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**IMPACT OF AQUATIC TOXINS ON HEART DEVELOPMENT IN ATLANTIC
AND SHORTNOSE STURGEON LARVAE**

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

The Hudson River (HR) is teeming with life, and also with toxins. Over the course of 30 years General Electric released approximately 1.3 million pounds of PCB mixtures (Aroclors) into the HR resulting in lasting contamination. While PCBs are chemically quite stable, they are biologically active causing cancer, reproductive, developmental and survival problems. Fish living and breeding in the HR are exposed to these toxins and suffer the consequences. Sturgeon have historically frequented the HR during spawning seasons and with populations starting to increase, more and more sturgeon return to the river each year to lay their eggs in the contaminated sediments. While some information is available on the consequences of toxin exposure to different fish species, little is understood of sturgeon response to PCBs. Proper heart development is essential to survival of many organisms and PCBs are known to impact cardiac development. This study explored the effects of early toxin exposure on sturgeon heart development using molecular tools to visualize the young sturgeon heart. Shortnose and Atlantic sturgeon hearts exposed to PCB 126, TCDD and an Aroclor mixture showed unique cardiac deformities. PCB 126 caused hearts to not loop properly in early heart development, while TCDD and Aroclor treatments resulted in hearts with chambers that did not balloon. In both situations, the fish likely had problems with blood circulation and in most cases, treated fish died soon after hatching. These toxins have a clear impact on development and survival of sturgeon larvae and may hinder the budding recovery of these endangered species.

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INTRODUCTION

Remnant populations of once common native sturgeon species are increasing in the Atlantic Ocean, the result of a fishing moratorium on endangered sturgeon (Bain et al. 2007; Breece et al. 2013). While the majority of the adult life of an individual sturgeon is spent in the ocean feeding, they return to fresh water rivers to reproduce. Both shortnose (*Acipenser brevirostrum*) and Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) spawn in Atlantic coast tributaries, including the Hudson River (HR) in New York, a system characterized by widespread polychlorinated biphenyl (PCB) contamination (Bain et al. 2000; Bopp et al. 1998). While PCBs have limited water solubility, they have accumulated in the river sediment, and cause harm to fish eggs that sink through the water column into the sediment and impact benthic juveniles and adults consuming sediments (Andersson et al. 2001; Bopp et al. 1998; Feng et al. 1998). These eggs undergo early embryogenesis and larval maturation in contaminated riverbeds. Another common aquatic toxin in the HR, 2,3,7,8-tetrachlorodibenzodioxin (TCDD), may also affect developing embryos, juvenile and adult fish living in contaminated waters.

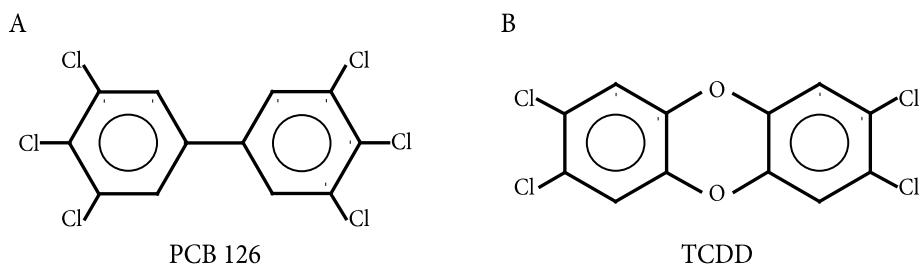


Figure 1: **PCB 126 and TCDD have similar structures**
Both PCBs and TCDD have pairs of carbon rings with attached chlorine atoms. (A) Structure of PCB 126. Other PCB congeners have the same base structure of biphenyl rings, but differ in the number and configuration of chlorine atoms surrounding the rings. (B) Structure of TCDD.

PCBs and TCDD have similar chemical structures and act on the same pathways in cells, causing similar effects in an exposed organism (Figure 1). PCBs and PCB mixtures (produced by Monsanto with the brand name Aroclor) and TCDD accumulate in fatty tissue and are detrimental to survival, development and reproduction of many exposed animals (Daouk et al. 2011; Mayes et al. 1998; Safe 1993; Ulbrich and Stahlmann 2004). These toxins bioaccumulate, resulting in sublethal impacts that may affect the life-span and fecundity of the exposed animal followed by future impacts to their offspring. More directly these toxins often affect development and maturation of specific organs (Antkiewicz et al. 2005; Grimes et al. 2008; Hill et al. 2003; Li et al. 2014; Roy et al. 2011). Toxins and other external disruptions impact the heart, the first functional organ in the developing fish (Hicken et al. 2011; Incardona et al. 2014). Proper development of the heart is essential to continued growth and survival of vertebrates. The importance of heart development to fish survival suggests that studying heart development is important in clarifying long-term consequences of aquatic toxin exposure in fish.

While assessments of water and sediment quality in the HR are common, knowledge of PCB impact on adult fish is limited and to date very little is known about the developmental consequences of toxin exposure on the early life stages of native fish (Bush et al. 1989; Ashley et al. 2003; Monosson et al. 2003; Deshpande et al. 2008). Chambers et al. (2012) established that both shortnose and Atlantic sturgeon treated with PCB 126 and TCDD have reduced survival and growth rate until hatching. This early demise and reduced growth in sturgeon larvae may be due to heart defects as a consequence to toxin exposure. Work in the zebrafish (*Danio rerio*) and medaka

(*Oryzias latipes*) model organisms show persistent cardiac defects as a result of early exposure to TCDD, PCB 126 or Aroclors (Figure 2) (Antkiewicz et al. 2005; Dong et al. 2010; Grimes et al. 2008; Hill et al. 2004; King Heiden et al. 2009; Li et al. 2014; Teraoka et al. 2002). Studies in zebrafish using genetic and molecular tools have established the roles of these and other toxins in causing developmental defects. Unfortunately these tools were often unavailable in native or locally affected fish populations. The genetics of many fish species are evolutionarily conserved, thus there are molecular similarities in proteins expressed in fish hearts. It is now possible to utilize zebrafish protocols on sturgeon larvae to visualize the heart.

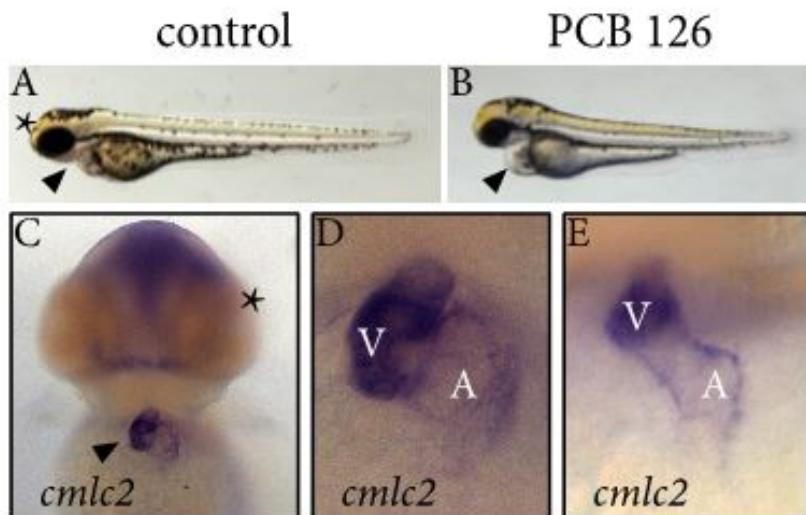


Figure 2: PCB 126 causes heart defects in zebrafish
 (A) Untreated, control fish have normal body development. The fish is oriented laterally, with the heart indicated with an arrowhead and eye indicated with an asterisk for reference. (B) PCB 126 treated fish develop a large pericardial edema, have craniofacial and eye defects and are smaller in body length. (C) Untreated fish in ventral view with marked heart in purple (visualized with heart specific marker, *cmlc2/myl7*, *in situ* hybridization). (D) Magnified untreated fish heart. Control hearts have properly looped and ballooned hearts. (E) The hearts of PCB 126 treated fish do not loop properly, nor do they balloon and thus have reduced blood flow through the heart. A= atrium, V= ventricle, eye marked with asterisk, heart indicated with arrowhead.

The hypothesis tested if shortnose and Atlantic sturgeon treated with PCB 126, TCDD and an Aroclor mixture will develop cardiac defects similar to those seen in zebrafish (Figure 2 & unpublished personal data). To test this hypothesis, shortnose and Atlantic sturgeon embryos were exposed to six concentrations of each toxin, with associated controls for 24 hours at 48 hours post fertilization. To specifically visualize the heart, larvae collected 1 day post hatching (dph) were analyzed using immunohistochemistry (a procedure which marks protein expression). Features of the heart were measured through image analysis to quantify the toxins' effects during cardiac looping (an early necessary stage of heart development). PCB 126 impacts looping of the hearts in sturgeon while TCDD and the Aroclor treatments disrupted chamber ballooning.

METHODS

Lab controlled embryo exposure

Both shortnose and Atlantic sturgeon embryos were acquired from a collaborator, Dr. Chris Chambers (NOAA-NEFSC, J.J. Howard Marine Science Laboratory), who purchased them from hatchery operations on the Saint John River, New Brunswick in late May 2014. Embryos were kept in recirculating 1 part per thousand (ppt) salt water overnight, then sorted into treatment dishes. All toxins were provided by Dr. Issac Wirgin (NYU Langone Medical Center), who also helped to set up treatments. Treatments included various concentrations of PCB 126 (0.1, 1.0, 10, 100, 1000 and 10000 parts per billion (ppb)), an Aroclor blend (0.2, 2.0, 20, 200, 2000 and 20000 ppb), and TCDD (0.0005, 0.005, 0.05, 0.5, 5.0 and 50 ppb) in an acetone carrier. Following 24 hour treatment, embryos were grown in 1 ppt salt water in glass dishes at 15°C. Dead embryos were removed before each of the twice-daily water changes. Hatched larvae were removed twice daily as well, placed in secondary containers and photographed the following day. Photographed larvae were collected and fixed for archiving or for use in immunohistochemical assays.

Immunohistochemistry

Sturgeon larvae are fairly opaque and without additional processing, their hearts are nearly impossible to see post-fixation. Immunohistochemistry protocols allow for visualization of specific proteins expressed in an organism. In fish, the primary antibody MF20 stains the musculature, including the heart (obtained from Developmental Studies Hybridoma Bank). The antibodies, which bind to protein fibers in the musculature of zebrafish, medaka and other fish, is quite robust and also binds to myosin found in

sturgeon hearts (Bader et al. 1982; Guerrero et al. 2004; Grunow et al. 2011). The immunofluorescence protocol was modified from a zebrafish protocol used previously (Singleman and Holtzman 2012). Formalin fixed larvae were bleached in 3% H₂O₂ plus 1% KOH then permeabilised in a proteinase K dilution (10µg/ml). Proteins in larvae were blocked in a 10% sheep serum, 0.2% saponin in 2mg/ml BSA/PBS solution overnight at room temperature. Larvae were then incubated overnight at room temperature in a 1:10 MF20 primary antibody dilution, followed by another overnight wash at room temperature in secondary antibody, AlexaFluor anti-IgG2b-546 (1:500 dilution).

Stained embryos were imaged using a stereoscope microscope (Leica) and camera (Zeiss AxioCamMRc) with its associated programming (Zeiss AxioVision). Photographs were analyzed using ImageJ (NIH) (Figure 3). For each sturgeon heart image the heart was measured for various lengths, perimeter and areas as described in Table 1.

Statistical Analysis

Measurements (see Table 1) taken using ImageJ were analyzed with Principal Component Analysis (PCA) using the program SYSTAT. PCA is an analysis tool that pools the data and determines PC axes that account for the variance of the data. Data is redefined into principal components to explain the variance between samples. To interpret the utility of each PC axis, PC scores for each heart were calculated by multiplying the component loading value for each variable by the original measurement and collecting the sum of all values for each heart individually. These PC scores were used to create graphs summarizing the PC data by dose. Significant variables determined by PCA were plotted against dose using linear regression.

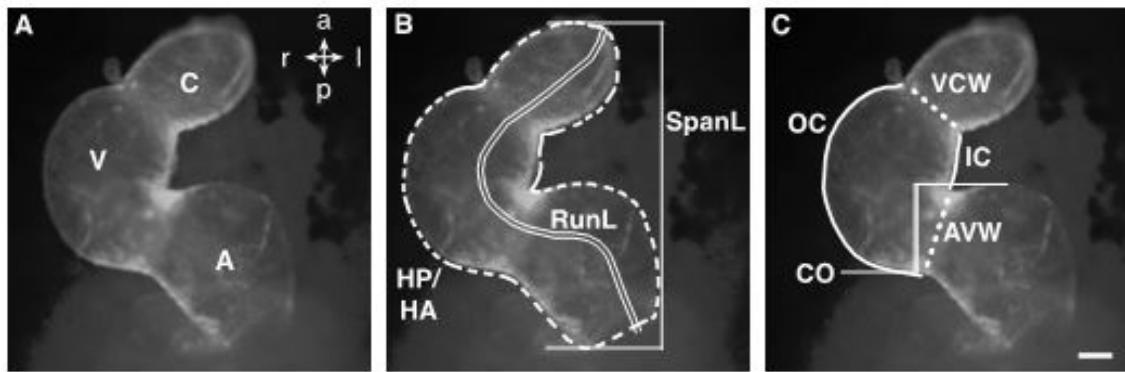


Figure 3: Cardiac Measurements

(A) Atlantic sturgeon heart from a ventral view. C= conus arteriosus, V=ventricle, A= atrium, a= anterior, p=posterior, r= right and l= left. B and C show measurements taken for each measured cardiac variable. (B) Running Length (RunL), Spanning Length (SpanL), Heart Area (HA) and Heart Perimeter (HP) are labeled. (C) Ventricle-Conus constriction Width (VCW), Atrium-Ventricle constriction Width (AVW), Outer Curvature (OC), Inner Curvature (OC), and Chamber Overlap (CO) are labeled. Scale bar = 0.1mm.

Table 1:

Measurement Definitions

Definitions of measurements collected from images of stained Atlantic sturgeon hearts.

Variable	Abbreviation	Definitions
Outer Curvature	OC	Outer curve of ventricle. Length measured from VC constriction to AV constriction.
Inner Curvature	IC	Inner curve of ventricle between conus and atrium. Length measured from VC constriction to AV constriction.
AV Constriction Width	AVW	Measured width of constriction between ventricle and conus. Eventual site of outflow valve.
VC Constriction Width	VCW	Measured width of constriction between atrium and ventricles. Eventual site of atrioventricular valve.
Heart Perimeter	HP	Perimeter of entire heart, including conus, ventricle and atrium.
Heart Area	HA	Area of heart determined by calculation of space within perimeter area.
Chamber Overlap	CO	Overlap of ventricle and atrium measured as distance between apex of ventricle and top of atrium.
Running Length	RunL	Length measured through the heart (path of blood flow) from inflow of atrium to outflow of conus.
Spanning Length	SpanL	Length measured in a straight line from the top of the conus to the lowest edge of the atrium.
Linearity Ratio	Lrat	Linearity ratio calculated by the division of running length by spanning length

RESULTS

Heart Development Stages of Shortnose Sturgeon

While sturgeon have historically been an important commercial fish and are currently under protection to increase populations, little is known about their development. This lack of knowledge reduces the full ability of researchers to study consequences of environmental contaminants on the development of this native fish. Compared to zebrafish, which undergo embryogenesis in about 3 days, shortnose sturgeon have a much longer embryonic development of 12 to 14 days. In zebrafish, first cardiac looping then chamber ballooning are completed at hatching and cardiac rotation occurs shortly following hatching. By 1 day post fertilization (dpf), this heart tube loops, defining the two chambers of the heart: the atrium and ventricle. These chambers balloon as the heart functions, pumping blood through the developing fish. A final rotation of the chambers, placing the ventricle ventral and anterior to the atrium completes early heart development by 5 dpf. While these stages of heart development are well documented in the model organism, little is known about heart development in most sturgeon species, and nothing has been published about shortnose sturgeon. The information available for other fish species, including other sturgeon species suggests that heart development in shortnose sturgeon is similar to development in zebrafish. It was found that at 1 day post hatch (12-14 dpf), the sturgeon heart is looped and in the process of chamber ballooning (Figure 4A). By 2 weeks post hatching (wph) the heart has completed cardiac rotation; later time points, 3 and 6 wph (Figure 4B-D), show the heart still in this configuration; however, the conus arteriosus (muscular outflow tract) has elongated and the ventricle has become more round. Comparisons between zebrafish and sturgeon heart

development shows consistent parallels, supporting the use of zebrafish as a model for sturgeon heart development.

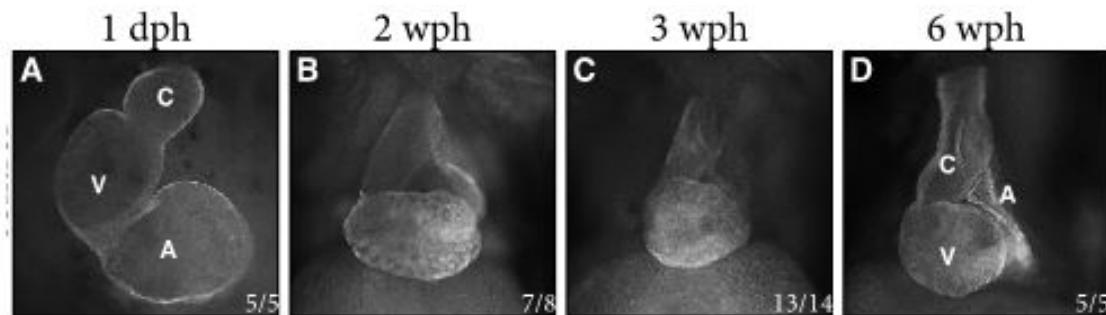


Figure 4: Normal heart development in shortnose sturgeon
MF20 stained sturgeon hearts at (A) 1 day post hatching (dph), (B) 2 weeks post hatching (wph), (C) 3 wph, and (D) 6 wph. A= atrium, V= ventricle, C = conus arteriosus

Toxins Affect Heart Development and Maturation of Shortnose Sturgeon

Shortnose sturgeon and zebrafish have similar heart development stages and likely have similarities in cellular mechanisms during development. Toxins impeded proper heart development in zebrafish during cardiac looping and chamber ballooning (Figure 2) and likely have similar consequences on sturgeon hearts. Sturgeon treated with PCB 126 showed the most severe phenotypes at 1 dph: heart elongation, reduced looping and ballooning, and reduced chamber distinction (Figure 5A). Many hearts are curved slightly, but looping is not completed. Aroclor treated shortnose sturgeon had hearts that were looped by 1dph, but had inconsistent and irregular ballooning of the either atrium or ventricle (Figure 5B; atrium = A, ventricle = V). TCDD treated sturgeon hearts had ballooning defects in fish treated with lower concentrations, while those treated with higher concentrations tended to have hearts with both reduced looping and ballooning (Figure 5C). These phenotypes are similar to those seen in zebrafish (Grimes et al. 2008; personal data unpublished). Samples collected for shortnose sturgeon were too few to run statistical analysis; however, sample number can be increased next season.

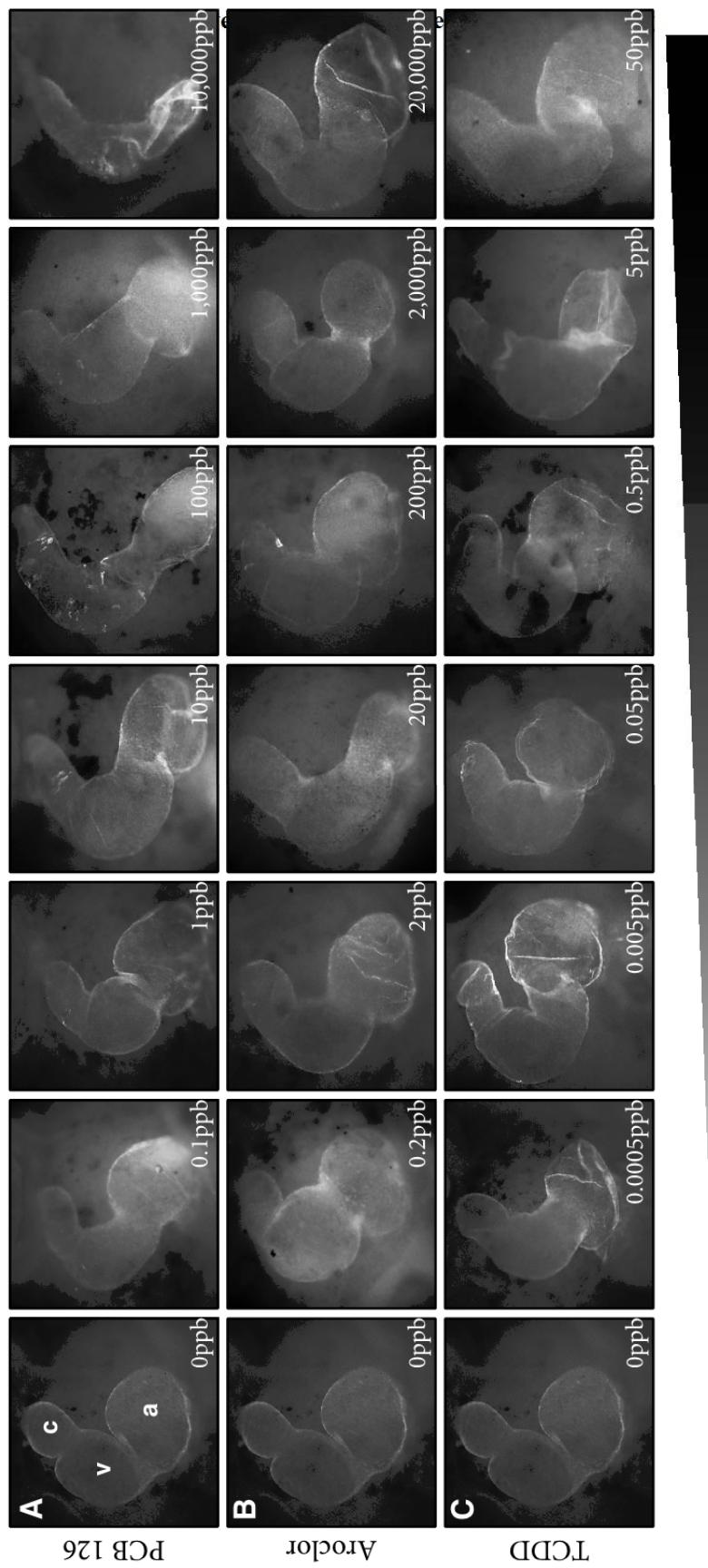


Figure 5: Toxin derived cardiac response of shortnose sturgeon MF20 stained hearts of treated shortnose sturgeon larvae 126 treated fish. (B) Increasing concentration of Aroclor

Survival of shortnose sturgeon larvae was monitored post hatching. Fish with the most severe heart phenotypes, died soon after hatching. Some TCDD and Aroclor treated larvae survived past 3 weeks post hatching (wph). At 2 wph, two groups of TCDD (0.05 and 5 ppb) and two groups of Aroclor (20 and 2000 ppb) treated fish survived. Both groups of TCDD treated fish retained a juvenile chamber orientation (atrium and ventricle side by side) or had a reverse chamber orientation (atrium ventral to ventricle) (Figure 6). Aroclor treated larvae at 2 wph had a variety of phenotypes: normal (ventricle ventral to atrium), juvenile orientation, or reversed orientation, the most common orientation being the reversed orientation. At 3 wph only the Dioxin 0.05 ppb, Aroclor 20 and a small group of Aroclor 2000 ppb remained. Phenotypes of chamber orientation in surviving larvae tended toward a normal configuration (Dioxin 0.05 and Aroclor 2000 ppb); however, few fish also had a reversed orientation (Aroclor 20 ppb).

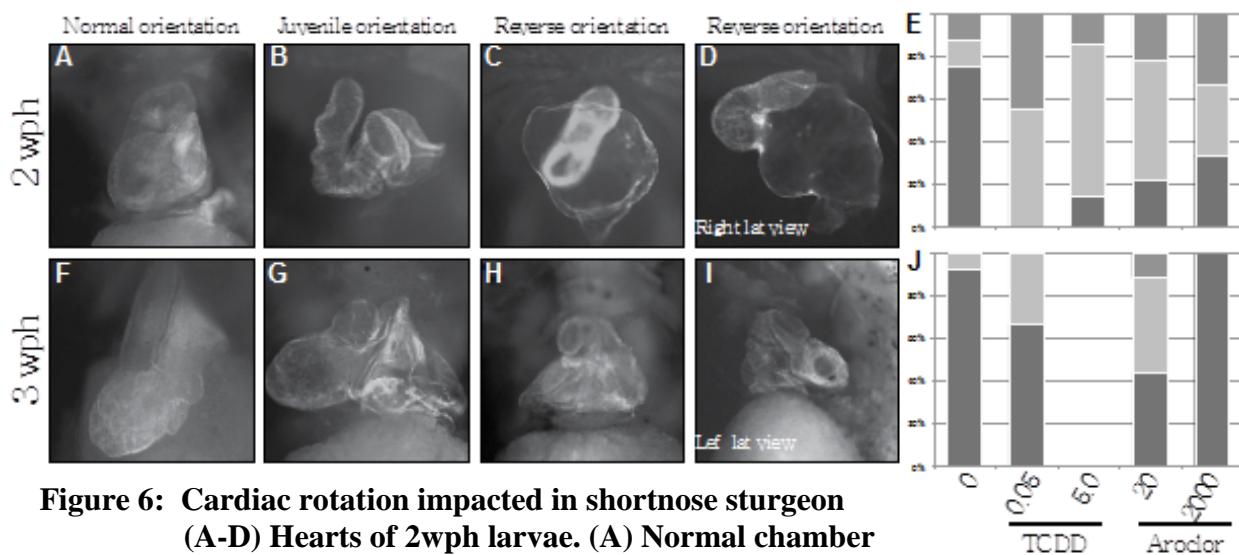


Figure 6: Cardiac rotation impacted in shortnose sturgeon
(A-D) Hearts of 2wph larvae. (A) Normal chamber orientation, ventricle anterior to atrium (B) Juvenile orientation, chambers side by side. (C) Reverse orientation, atrium anterior to ventricle. (D) Right lateral view of (C).
(E) Graph – relative number of differently oriented hearts within 2wph treatments. (F-I) Hearts of 3wph larvae. (F) Normal orientation (G) Juvenile orientation. (H) Reverse orientation. (I) Left lateral view of (H). **(J)** Graph-relative number of differently oriented hearts within 3wph treatments.

Impact of Toxins on Atlantic Sturgeon Heart Development

Atlantic sturgeon spawn in many contaminated waters along the Atlantic coast and have experienced similar population declines as shortnose sturgeon since they live and spawn in similarly contaminated habitats. Further, Atlantic sturgeon showed similar trends in developmental consequences to toxin treatments in comparison to shortnose sturgeon. Each toxin presented different phenotypes in treated fish hearts, likely disrupting cardiac development during different stages of development. PCB 126 treated larvae hearts tend towards linearity with increased treatment concentration. Cardiac looping is disrupted and as a consequence these hearts are also not ballooned properly, and chamber distinctions are difficult to determine (Figure 7A). Both Aroclor and TCDD interfere with chamber ballooning but with different outcomes: Aroclor treated larval hearts tend to have more indistinct chambers and chamber ballooning defects (Figure 7B). Some hearts are more linear than untreated controls, but this phenotype is less pervasive in Aroclor treated fish than in PCB 126 treated fish. TCDD seemed to have the least impact, affecting ballooning of chambers more often than looping or chamber delineation (Figure 7C).

Statistical Analysis of Atlantic Sturgeon Heart Morphology

As a result of the diverse genetic background of wild samples, it is important to account for this variability to clearly identify significant morphological changes caused by toxin treatment. Atlantic sturgeon measurement data were analyzed using Principal Component Analysis (PCA). Each variable impacts each principal component in unique ways (Table 2), which can be visualized in a graph where each variable's contribution for each PC axis is plotted in relation to the other PC axes (Figure 8).

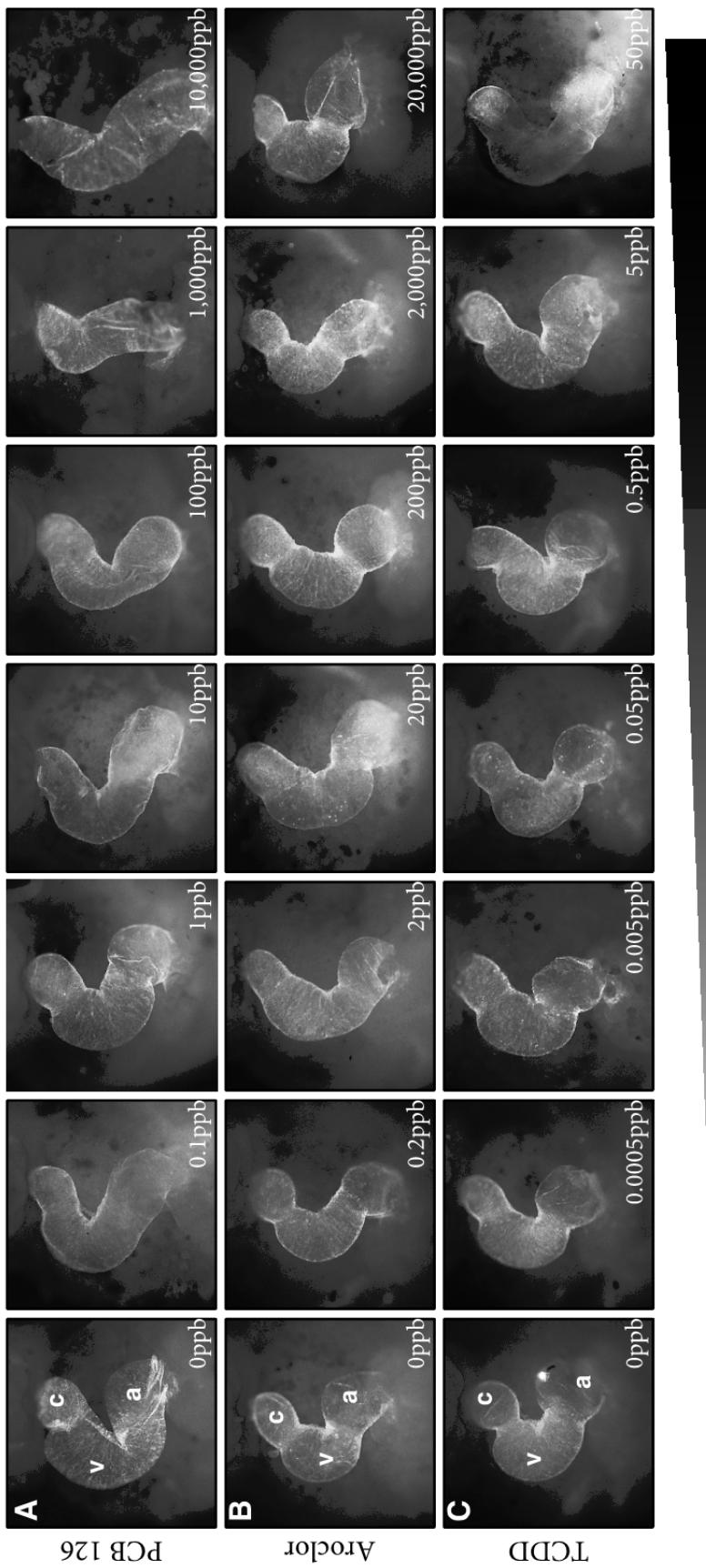


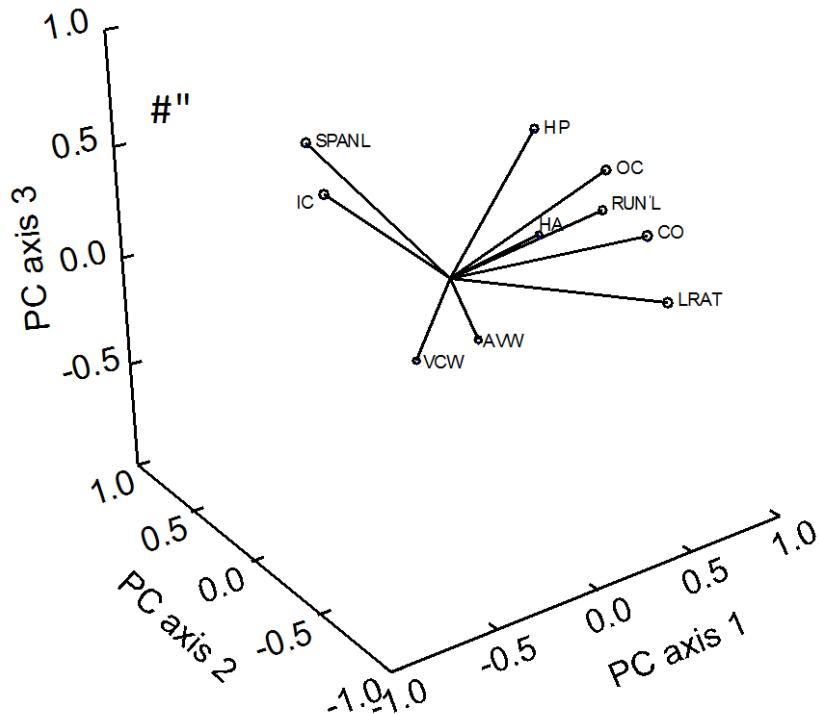
Figure 7: Toxin derived cardiac response of Atlantic sturgeon
MF20 stained hearts of treated Atlantic sturgeon larvae at 1 day post hatch (dph). (A) Increasing concentration of PCB 126 treated fish. (B) Increasing concentration of Aroclor treated fish. (C) Increasing concentration of TCDD treated fish. c= conus arteriosus (conus), v=ventricle, a= atrium

Variable	PC1	PC2	PC3
OC	0.834	0.003	0.300
IC	-0.216	0.623	0.230
AVW	0.447	0.396	-0.550
VCW	0.209	0.551	-0.649
HP	0.836	0.451	-0.142
HA	0.727	0.369	0.412
CO	0.747	-0.373	0.135
RunL	-0.209	0.770	0.422
SpanL	0.830	0.012	0.113
Lrat	0.776	-0.485	-0.138

Table 2: Component Loadings of each morphometric variable Percentage of total variance explained by each principal component is in parenthesis. Component loadings, or weights of each variable's contribution to each principal component is listed, bolded values indicate variables with the most impact.

Figure 8: Variable contribution in relation to PC axes

This graph shows the original variables (see Table 1 for definitions) plotted to the main three principal components.



PCB 126 impacts cardiac development during looping of the heart tube. As the dose increases, 1 dph hearts have a more linear and narrow morphology than untreated hearts (Figure 7A). The pattern of morphological change is reflected in the PCA analysis of data collected for PCB 126 treated fish. Each PC is differentially influenced by each measured variable (Table 2, bolded) and shows trends in morphological changes by dose

(Figure 9). The weight of these variables indicates that PC1, and thus most of the variability, is impacted by how curved the hearts are. Heart curvature decreases by dose ($p=0.09$; Figure 9A). Correspondingly, the hearts become more linear with increasing dose ($p=0.03$; Figure 9B). PC3 is influenced most by the ventricle-conus constriction and atrio-ventricular constriction widths, and the decrease by dose suggests that the hearts become narrower with increasing dose (Figure 9C). This narrowing of the heart is suggestive of the reduced ballooning, in addition to disrupted looping seen in PCB 126 treated hearts at high concentrations (Figure 7A).

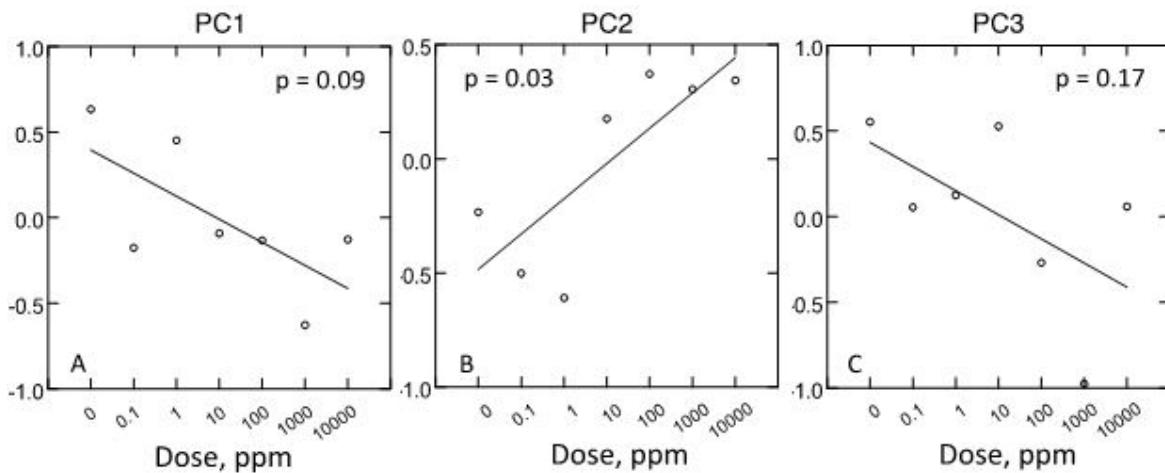


Figure 9: PC scores plotted by dose Independent PC scores were calculated for each heart using the data in Table 1 combined with the raw measurement data. Means were taken by dose for each new variable with graphs produced: (A) PC1, (B) PC2 and (C) PC3.

Of the ten measured variables, three were shown to be significant through regression analysis against dose (inner curvature, $p=0.01$; chamber overlap, $p=0.05$; linearity, $p=0.08$). Inner curvature of the heart expands with increasing dose suggesting that the heart is becoming more linear (Figure 10A). Chamber overlap measures the lateral overlap of ventricle and atrium. Small overlap indicates the heart is more linear

while a large overlap suggests the heart is looped. As the heart loops during normal development, the chambers move from a top-bottom to a side-by-side position, increasing the chamber overlap. The decrease in chamber overlap by dose suggests fish exposed to higher concentrations of PCB 126 have less looped hearts (Figure 10B). Linearity is defined as a ratio of running length to spanning length resulting in a linear heart at a ratio of close to 1 and looped heart ratios significantly greater than 1. The linearity ratio decreases with dose. The control fish have a large linearity ratio indicating a looped heart, while higher concentration of PCB 126 treated fish have linearity ratios closer to 1 indicating a more linear heart (Figure 10C).

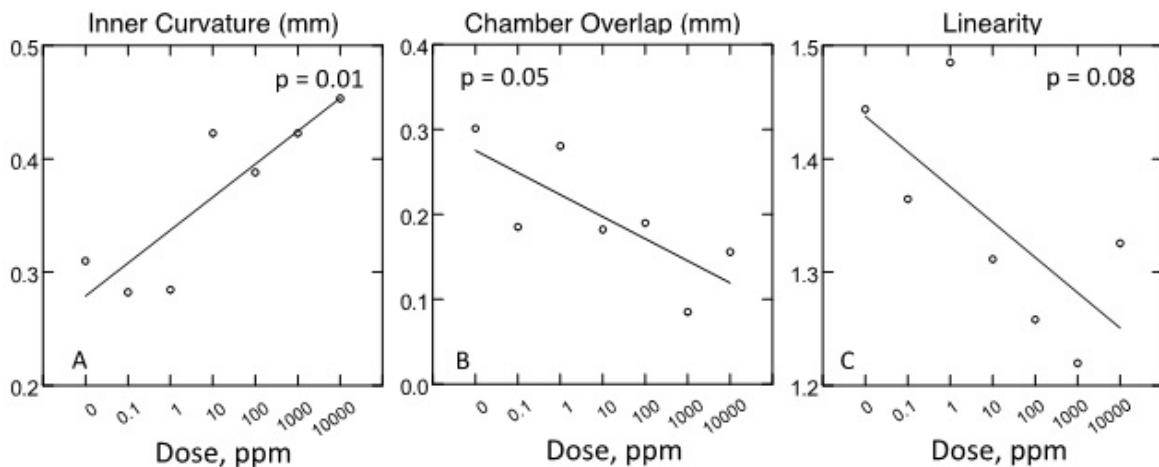


Figure 10: Regression plots of most significant variables by dose. The regression of original variables were plotted by dose. Graphs of three significant variables are shown: (A) Inner Curvature (IC), (B) Chamber Overlap (CO) and (C) Linearity (LRat).

DISCUSSION

As the fish grows, so must the heart to adjust to the increasing blood requirement in the maturing fish. The heart must change shape through cell shape modification, proliferation directed by genetic expression changes, resulting in a heart that is not only looped, but with chambers that are ballooned. These ballooned chambers hold more blood and more efficiently pump the blood through the body. During the three main steps in cardiac maturation (cardiac looping, chamber ballooning and cardiac rotation), valves between the chambers develop and the muscular layer of the heart forms trabeculae, branched structures that increase the force of contraction. Early disruptions in development, including those caused by toxin exposure, inhibit and modify the development of necessary valves and trabeculation, exacerbating heart defects. In both shortnose and Atlantic sturgeon, cardiac looping has taken place and chamber ballooning is underway by hatching in untreated fish. Older shortnose sturgeon larvae undergo cardiac rotation following hatching, similarly to zebrafish (Singleman and Holtzman 2012). Further exploration of processes of heart development in sturgeon may be a valuable course of study for more complete understanding of the similarities in embryonic processes between these important native fish and the convenient laboratory model of zebrafish.

Shortnose sturgeon heart maturation is disrupted by each of the toxins at different stages of development, resulting in heart morphology with different cardiac defects. PCB 126 treated hearts show a clear progression of reduced looping and increased linearity of the heart by dose. The chambers in these hearts do not balloon properly, potentially a consequence of retaining a more juvenile state. This linear

phenotype is also seen in zebrafish embryos treated with PCB 126 at hatching (Figure 2D). Zebrafish embryos with linear hearts have reduced blood flow through the growing fish and die when the blood flow requirements supersede the heart's ability to move blood, typically soon after hatching. Increased mortality in PCB 126 treated sturgeon suggest a similar response to that seen in zebrafish.

While TCDD and Aroclor treated sturgeon showed less consistent phenotypes at 1dph, both exhibited defects in ballooning. While statistical analysis is still underway, the phenotypic data suggests that TCDD and the Aroclor mix have different mechanisms of cardiac disruption as they impact different steps of cardiac development. Most treated fish died within 2 wph, but the heart morphology of those surviving was abnormal. Surviving larvae of TCDD and Aroclor treated larvae suggest that cardiac rotation may also be impacted by treatments. Whether cardiac rotation defects are caused directly by the toxins, or as a secondary consequence to pericardial edema (common in toxin treated fish) is still unknown and warrants further inquiry.

Treatment of Atlantic sturgeon yielded similar trends in 1dph hearts to those seen in shortnose sturgeon. PCB 126 treated fish had the most severe phenotype of linear hearts, and die very soon after hatching. TCDD and Aroclor treated fish exhibited more defects in chamber ballooning than looping.

To properly function, the heart must loop and balloon in the appropriate ways at the correct stages of development. Toxin exposure, especially PCB126, impedes heart maturation, reducing the fitness of the fish and likely hood of survival. PCA analysis showed that that early exposure to PCB 126 results in more linear, misshapen hearts,

indeed higher concentrations exacerbate the linear phenotype. Thus, as the dose increases, hearts are predicted to be more linear (less curved) and thinner (Figure 7).

Previous work using the zebrafish model organism has shown that PCBs and TCDD cause defects in the heart and other developmental processes (Antkiewicz et al. 2005; Grimes et al. 2008; Hill et al. 2003; Li et al. 2014; Roy et al. 2011). The use of zebrafish for many years, coupled with the short life span and high instances of inbreeding have resulted in highly isogenic populations of zebrafish with individuals in a clutch being relatively identical genetically (Nasiadka and Clark 2012). Zebrafish are fed, grown and bred in highly controlled environments making them a useful model. On the other hand, researchers have control over the living and growing environments of wild fish populations. Both shortnose and Atlantic sturgeon used in these experiments come from parents of unknown genetic origin that are likely genetically distinct. The offspring are a heterogenous group genetically and have come from parents whose life history is unknown. These facts may be contributing factors to the variability seen in the heart development of not just treated, but untreated control sturgeon.

Clarification of the effects of aquatic toxins on fish development may be found in a two-fold approach. First, utilizing zebrafish in a controlled laboratory setting will enhance specific response of the developing embryos to toxins, including elucidating the pathways by which these toxins act. Second, enhancing sturgeon experiments by increasing the number of samples and focusing on those treatments with the most severe phenotypes will enable more thorough exploration of the extremes and variety of phenotypes caused by toxin exposure.

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TIDAL COMMUNITY EXCHANGE BETWEEN THE HUDSON RIVER AND A TRIBUTARY

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

Over the course of the summer of 2014, a fyke net was used to sample the fish fauna of the Indian Kill in Staatsburg, New York, during ebb and flood tides. This provided insight into which species were entering and leaving, and when, over the summer in an attempt to characterize their usage of the tributary. A total of 309 individuals representing 17 species were caught. While species richness did not change with tide phase or day/night cycles, a lower Shannon-Weaver diversity index and a less even distribution among the species moving at night suggest that immigrating and emigrating species have a preference for nocturnal movements. More fish left the stream during ebb tide than entered during flood tide, representative of large numbers of a relative few species migrating out of the tributary. Some species previously described as exhibiting potamodromous behaviors, such as the white perch (*Morone americana*) and white sucker (*Catostomus commersonii*) showed similar behaviors in the Indian Kill but require further study to confirm reproduction. In the case of the American eel (*Anguilla rostrata*), movements clustered close to the new moon.

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INTRODUCTION

The health of the environment is dependent on the health of its subsystems.

Though health is a difficult term to define when it comes to a river system, the health of the Hudson River watershed is of utmost importance to the people and animals that live in and around it. There is a popular theory among freshwater ecologists that the health of the system can be determined by the diversity and status of fish fauna (Karr 1981 in North America; Harris and Silveira 2001 in Australia; Pont et. al. 2007 for Europe). For this to be a useful assumption, though, it must first be understood how fish interact with and use the habitats provided by the estuary and its tributaries.

Some species are considered residents in particular habitats, spending significant portions of their adult lives year-round using the resources of a particular area. Other species move daily from habitat to habitat, foraging in one and taking shelter in another (Wooden 1999). Many species only use a given habitat during specific parts of the year: spring spawning runs, for example. Tributaries in particular play a vital role in the life cycle of many fish in the Hudson River. Some anadromous fish, such as alewife, spend a significant portion of their upriver time in tributaries, while the river's only catadromous fish, the American eel, often spends years maturing in the tributaries (Schmidt and Lake 2006). There are many indicators that eels are important players in the ecosystems of the Hudson River. Where they are present, they are often present in high densities, and their tendency towards opportunistic feeding may make them a good candidate for invasive species control (Mount et al. 2011). Unfortunately, though there are areas where their numbers are strong, numbers have decreased substantially along the east coast of North America over the last decades (Velez-Espino and Koops 2010).

The seasonal use of tributaries by fish considered residents in the main river, or potamodromy, is less well understood. While there are two definite examples of species that absolutely rely on seasonal access to tributaries (white suckers and smallmouth bass), there are many species whose presence in tributaries is cyclical but without clear indicators of their use or how critical their time in the tributaries is to their life cycle (Schmidt and Lake 2006).

This study is an attempt at describing general movements of fish into and out of the Indian Kill, a small tributary of the Hudson River, during the summer. The tributary activity of a number of species is well known during the spring, but the post-spawning activity during the summer is less well understood. It is hoped that by observing the summer fish biota further understanding of biota-habitat interactions can be obtained, especially with suspected potamodromous species.

METHODS

The focus of this study was the Indian Kill in Staatsburg, NY, a small stream with sections of rock and mud bottom. The sample site was located at its tidal mouth immediately downstream from the bridge on Thompson Lane in the Mills Norrie State Park, roughly 600 meters upstream from the Hudson River. Samples were taken between June 11th and August 15th, 2014.

The stream was sampled for fish with a fyke net, roughly 12 ft by 12 ft with two trailing zipper bags and a mesh of 0.5 cm, for four-hour intervals between high and low tides, with a temporal buffer of approximately an hour on either side of the tidal extremes. The fyke was anchored with rebar poles pounded into the stream bed, and the

wings of the fyke extended across the entire stream and were anchored on the bottom with rocks. The mouth was open towards the flow of the tides, facing upstream for ebb tide and downstream for flood tide. A durable plastic boat float was placed in the second bag of the net to provide an air pocket to avoid drowning any turtles who wandered into the net.

Over the course of the study period, 32 samples totaling 128 sample hours were carried out. These were evenly distributed between four distinct sample types, 8 samples or 32 hours each of: daytime flood tides, daytime ebb tides, nighttime flood tides, and nighttime ebb tides (See Appendix 1 for a list of sample dates). Sampling was postponed during heavy rain events due to the threat of flooding.

At the end of any particular sample, the bags of the fyke would be accessed through zippers sewn in the top and animals extracted through use of a dip net. If the tide was too high or visibility very poor, the net was removed from its supports and brought on land and the fish would be extracted from there, though this less-preferred method was only rarely used. Fish were placed in a bucket and processed from there. Identification was mainly done on site, though if identity confirmation was needed a picture was taken and provided to a consultant (R.E. Schmidt, pers. comm.). Total length was measured and fish were released afterwards. American eels were anesthetized with clove oil before measurement.

RESULTS

Over the course of two months of sampling, 309 individual fish were caught in the Indian Kill, representing 17 distinct species. Table 1 shows the number caught of each

species and their mean lengths. After the sample periods, it was discovered that there is a private pond upstream from the sample site that may be manually drained periodically into the Indian Kill (R.E. Schmidt pers. comm.). It is unclear how this affected the catch and would be worth further investigation.

It should be noted that the numbers on Table 1 represent fish that were likely moving either upstream or downstream and do not necessarily represent the population that dwell in the Indian Kill. Yellow bullheads were present in the greatest numbers, but were caught as schooling young-of-the-year (YOY) and were largely captured towards the end of the sampling period. Bluegills were present in almost every sample and are therefore ubiquitous.

YOY bluegill, largemouth bass, white sucker, and brown and yellow bullhead were all traveling concurrently with adults during the summer in the Indian Kill. This is reflected in the

Table 1: Total Catch. % Ebb represents the portion of that species caught during an ebb tide.

Common Name (<i>Genus species</i>)	Number	Mean Length/SD (cm)	% Ebb
American Eel (<i>Anguilla rostrata</i>)	21	36.8/17.6	71%
Bluegill (<i>Lepomis macrochirus</i>)	57	11.3/5.2	54%
Brown Bullhead (<i>Ameiurus nebulosus</i>)	17	9.5/5.9	88%
Carp (<i>Cyprinus carpio</i>)	2	71.8/1.1	100%
Fallfish (<i>Semotilus corporalis</i>)	5	20.9/5.2	0%
Golden Shiner (<i>Notemigonus crysoleucas</i>)	6	12.5/6.5	33%
Green Sunfish (<i>Lepomis cyanellus</i>)	10	11.0/1.8	70%
Largemouth Bass (<i>Micropterus salmoides</i>)	9	8.4/12.1	56%
Mummichog (<i>Fundulus heteroclitus</i>)	2	6.7/1.6	50%

Common Name (<i>Genus species</i>)	Number	Mean Length/SD (cm)	% Ebb
Pumpkinseed (<i>Lepomis gibbosus</i>)	11	10.7/2.6	45%
Redbreast Sunfish (<i>Lepomis auritus</i>)	8	16.0/2.5	50%
Smallmouth Bass (<i>Micropterus dolomieu</i>)	1	21.4/NA	0%
Striped Bass (<i>Morone saxatilis</i>)	4	6.6/0.8	75%
Tessellated Darter (<i>Etheostoma olmstedi</i>)	9	6.1/1.0	33%
White Perch (<i>Morone americana</i>)	40	17.5/2.6	37%
White Sucker (<i>Catostomus commersonii</i>)	25	13.5/11.7	88%
Yellow Bullhead (<i>Ameiurus natalis</i>)	82	5.9/5.7	70%

large standard deviations relative to average length present for these species. Striped bass are an anadromous species that typically spawn further downriver in the main body of the Hudson in May and June (Fay et al. 1983), the majority happening between kilometer 54 and 98 (close to Newburgh), though there is evidence of spawning up to Albany (Waldman 2006). They were present in small numbers and exclusively YOY, likely representing dispersal of the new young.

The Shannon-Weaver diversity index was used to compare the diversity of the fish moving through the Indian Kill during different periods because it takes both richness and equitability into account (Peet 1974). The equation for the Shannon-Weaver diversity index is as follows:

$$H' = - \sum_{i=1}^s (p_i \ln p_i)$$

Evenness measures how evenly distributed individuals are throughout the species present. It is calculated by comparing a diversity measurement to a maximum theoretical value. Outputs range from 0 to 1, where 1 is complete evenness and 0 means the population is skewed towards one species. H' and evenness, E_H , were calculated over the entire

summer, and for the total day yield, night yield, ebb tide yield, and flood tide yield independently. These results are shown in Table 2. Evenness is computed as follows:

$$E = \frac{H'}{H'_{\max}}$$

H'_{total} gives an upper limit for the diversity index of any given proportion but gives no information in and of itself – it was used here merely as a way to compare the different subsets. On the whole, the Indian Kill's diversity is distributed relatively evenly ($E_H = 0.88$). Substantially more (171%) individual fish moved at night than during the day. While species richness is comparable between the two, the difference in diversity

Table 2: The Proportion, Richness, Diversity, and Evenness of different sample periods. It should be noted that E_H scales logarithmically, so 0.9 is further from 1.0 than 0.8 from 0.9.

Period	Proportion	Richness	Diversity	Evenness
Total	1	17	$H'_{total} = 2.48$	$E_H = 0.88$
Day	0.269	15	$H'_{day} = 2.21$	$E_H = 0.82$
Night	0.731	15	$H'_{night} = 2.09$	$E_H = 0.77$
Ebb	0.605	15	$H'_{ebb} = 2.17$	$E_H = 0.80$
Flood	0.395	16	$H'_{flood} = 2.26$	$E_H = 0.82$

and evenness mean that a small number of species represent a larger proportion of the night sample. This suggests that transient species are preferentially immigrating and emigrating at night.

Fifty-three percent more fish moved downstream with the ebb tide than moved upstream with the flood tide. This means more individuals were leaving than entering. The lower diversity index and richness of fish in ebb tide samples shows that fewer species were leaving than entering, but at significantly higher numbers; specifically

American eel, brown bullhead, white sucker, and yellow bullhead. This also suggests that transient species were leaving as the summer progressed.

An analysis of weekly catch was done for each species. Since the sampling effort for each week was not consistent (4 hours for one particularly stormy week, up to 20 hours maximum), numbers were normalized by sampling effort (catch per hour of sample time) instead of the raw catch numbers for every week. When analyzed individually bluegill, redbreast, pumpkinseed, and tessellated darter, all of which were named tributary residents by Schmidt and Lake (2006), were consistently caught and should be considered residents of the Indian Kill. Green sunfish fall within this category too, although they were not mentioned in the 2006 paper. Figure 1 shows a comparison of the six non-resident species that demonstrated some periodicity (American eel, largemouth bass, striped bass, white perch, white suckers, and yellow bullheads) with the bluegill, which was chosen to be the representative resident species because of a high population. The remaining species did not have enough data to draw conclusions from.

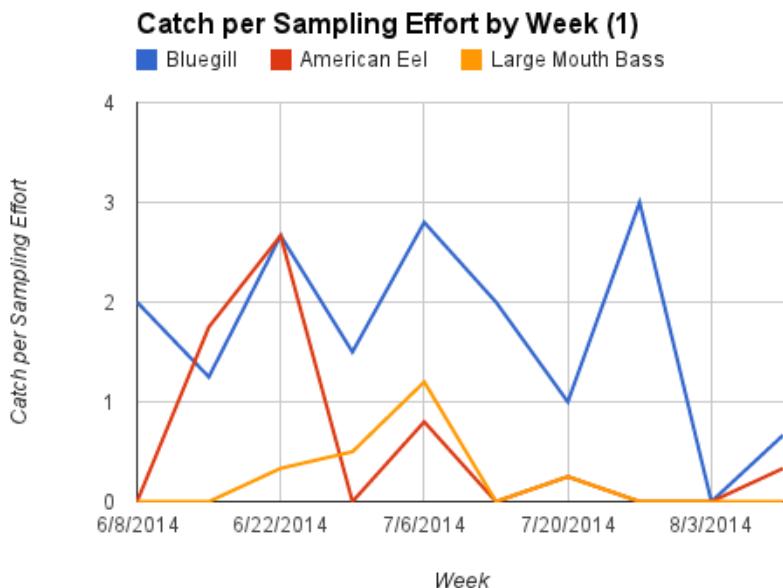


Figure 1A: Catch per Sampling Effort by Week for American Eel and Largemouth Bass. Catch per sampling effort is normalized catch for each four hour sample time per week.

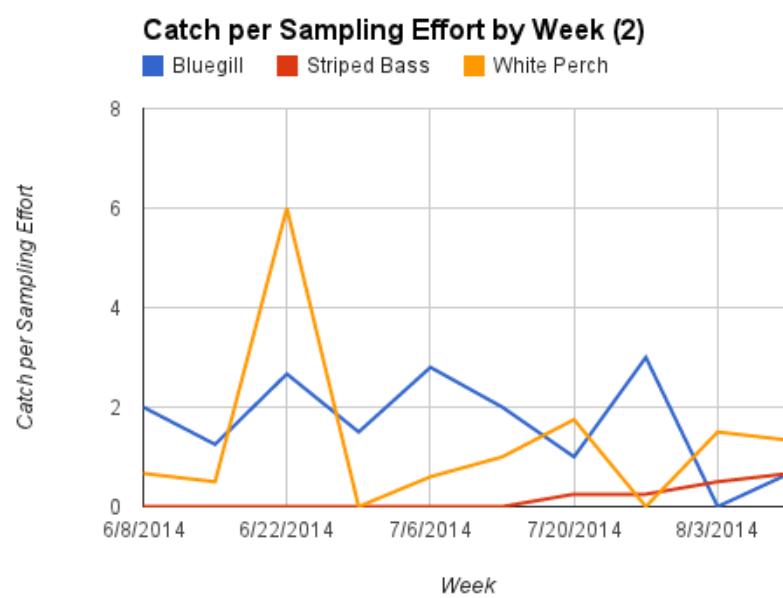


Figure 1B: Catch per Sampling Effort by Week for Striped Bass and White Perch. Catch per sampling effort is normalized catch for each four hour sample time per week.

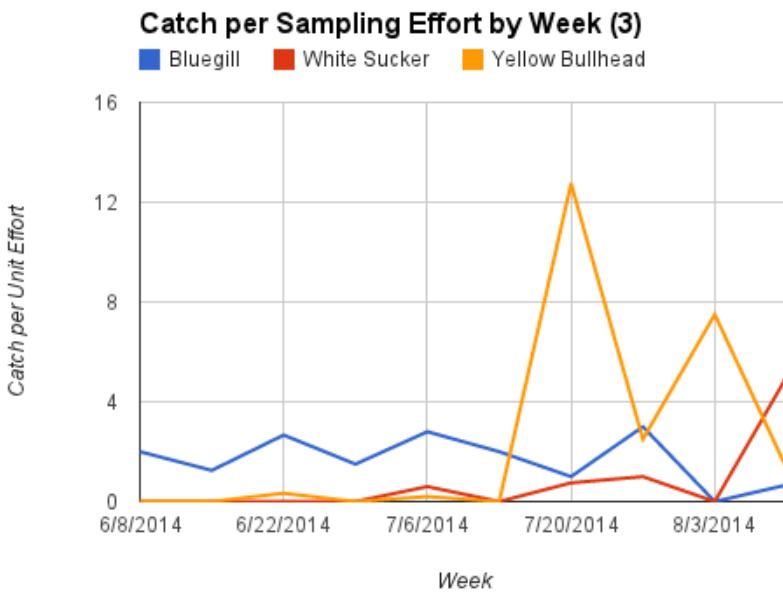


Figure 1C: Catch per Sampling Effort by Week for White Sucker and Yellow Bullhead. Catch per sampling effort is normalized catch for each four hour sample time per week.

Largemouth bass were not caught in large numbers, but catches were clustered around the end of June through July (Figure 1A). One adult was caught and the remainder were obvious YOY. Largemouth bass are clearly reproducing in the Indian Kill but likely not in large numbers. Striped bass, as noted earlier, were only caught as YOY near the end of the summer, likely around the time they were moving north from spawning sites. Yellow bullheads were mostly caught in July and August after a probable spawning period, as most were YOY that were caught as schools, as displayed by the large peaks in Figure 1C.

Schmidt and Lake (2006) outlined species confirmed and suspected of potamodromous behavior. White sucker are listed as completely potamodromous, relying exclusively on tributaries for breeding. Figure 1C shows a dramatic increase in white sucker movement towards the end of the summer in the Indian Kill. The week of August 3rd represents a series of young white suckers leaving the stream, whereas the consistently low numbers caught beforehand were exclusively adults. White perch are listed as partially potamodromous, with the possibility that tributaries provide a significant portion of their breeding habitat. Breeding season is typically in the early spring, though not all tributaries where adults have been present have had observable spawning events (Schmidt and Lake 2006). Figure 1B shows that more white perch were caught towards the beginning of the summer than towards the end, consistent with fish of breeding age leaving after the spring runs. No juveniles were caught, and the methods employed would not collect eggs or larvae, so it is unclear whether the Indian Kill is providing spawning habitat for white perch. Employing plankton nets for the collection of larvae and eggs could represent further research effort.

All American eels captured were sexually immature adult yellow eels that should be considered residents of the Indian Kill (Schmidt and Lake 2006). However, Figure 1A shows a substantial periodicity in their movements inconsistent with their residential nature. There is some correlation between moon phase and eel movements, with eels preferentially moving longer distances on darker nights close to the new moon (Schmidt et al. 2009). Figure 2 shows the catch per sample effort for bluegill (as a control) and American eel related to moon phase over the summer. There is a clear increase in capture of eels per sample effort as the moon phase moves towards the new moon, reinforcing this claim.

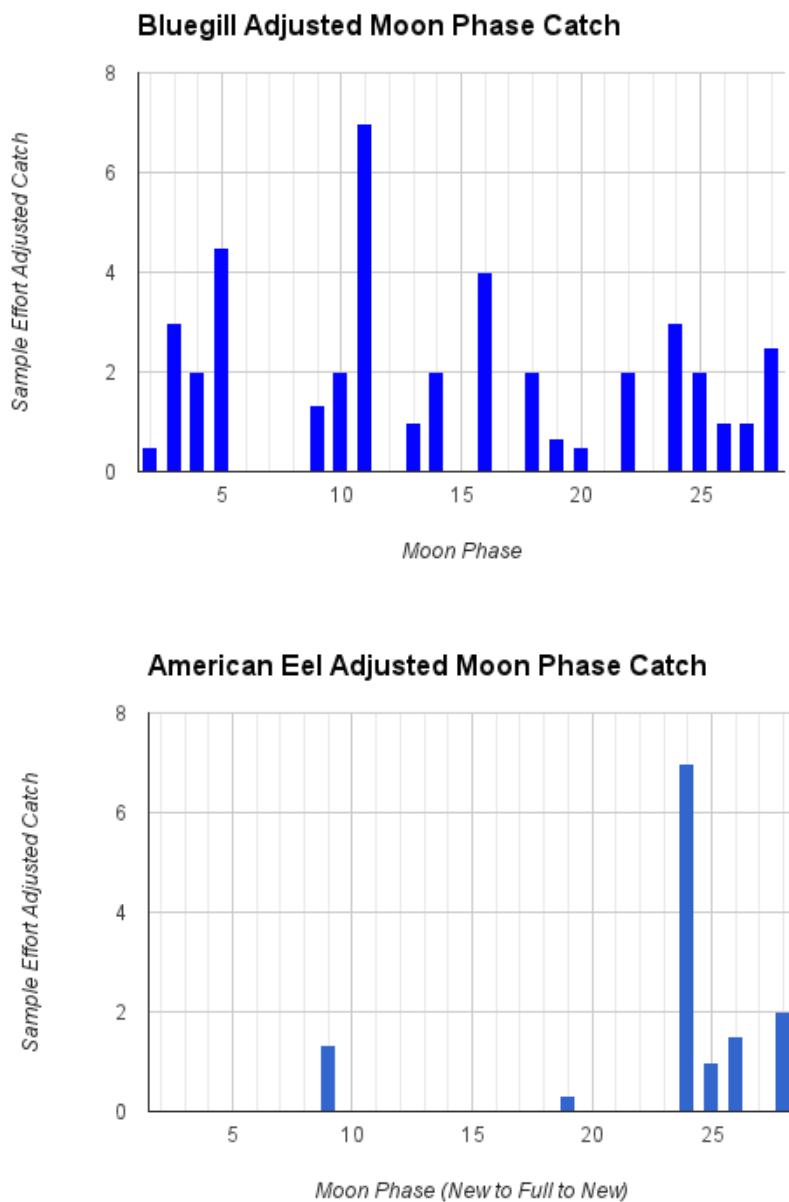


Figure 2: **Adjusted Moon Phase Catch for Bluegill and American Eel.** Moon phase is considered “new” at 0 and 29 and “full” at 15. Bluegill show no particular proclivity for moon phase, but American eel cluster close to the new moon as the nights get darker.

Two species of economic and ecological importance are using the Indian Kill as nurseries in which their young can mature safely away from larger predators: American eel likely year-round, and striped bass at least during the summer. Though it's unclear what the true

Yellow bullhead,
brown bullhead, white
sucker, largemouth bass,
and golden shiners all
showed discrete divisions
in size categories present
(Appendix 2). This likely
represents a division in
usage of the Indian Kill by
different age classes of
these species. This gives
further evidence that these
species are using the
Indian Kill at least as a
refuge for young-of-the-
year, if not spawning.

The data displayed
here shows that the Indian
Kill provides spawning
habitat for many species.

population of American eel is in the Hudson River, it is clear that their numbers have dropped significantly in the past decades (Velez-Espino and Koops 2010). Knowledge of how they use their habitat before a serious population crash occurs is vital.

This study did not seek to compare the Indian Kill and other tributaries of similar size. Further research can use similar methods for diversity assessment to compare other tributaries, though each tributary would need to be sampled in the same year to avoid any year-to-year differences. The diversity index from this year can be compared to other years of the Indian Kill to track how movement changes in future years.

Table 3: Findings by Species

Species	Usage
American eel <i>(Anguilla rostrata)</i>	Large, continuous size range and lack of sexually mature silver eels suggests refuge for maturing eels (Appendix 2). Consistent with catadromous designation. Greater numbers left than entered over the summer (Table 1) with a preference for moving at night close to the new moon (Figure 3).
Bluegill <i>(Lepomis macrochirus)</i>	Broad size range (Appendix 2) and lack of overall movement (Table 1) suggest bluegill are residents, consistent with current thought.
Brown Bullhead <i>(Ameiurus nebulosus)</i>	Age class split between young and adults (Appendix 2) suggest non-residency, consistent with a greater presence during ebb tide samples (Table 1).
Carp <i>(Cyprinus carpio)</i>	Numbers were very low and individuals were large and leaving (Table 1). Unclear how tributary was used, consistent with Possible Insignificant potamodromy designation in Schmidt and Lake (2006).
Fallfish <i>(Semotilus corporalis)</i>	Low numbers, even size distribution. Only caught in flood tides but numbers too low for significant analysis (Table 1).
Golden Shiner <i>(Notemigonus crysoleucas)</i>	Low numbers (Table 1) of unclear activity consistent with observations in Schmidt and Lake (2006). Age classes split between small and large (Appendix 2).

Green Sunfish <i>(Lepomis cyanellus)</i>	Less ubiquitous than bluegill (Table 1) with normal size distribution (Appendix 2). Should be counted as resident, though more were caught during ebb tides.
Largemouth Bass <i>(Micropterus salmoides)</i>	Almost exclusively YOY but for one adult (Appendix 2). Entered and left with equal frequency (Table 1), primary activity earlier in the summer (Figure 2).
Mummichog <i>(Fundulus heteroclitus)</i>	Too few caught to draw conclusions (Table 1). More common in marsh areas than streams.
Pumpkinseed <i>(Lepomis gibbosus)</i>	Stable number of a range of sizes (Table 1, Appendix 2). Entered and left with equivalent frequency, resident - consistent with Schmidt and Lake (2006).
Redbreast Sunfish <i>(Lepomis auritus)</i>	Stable number of a range of sizes (Table 1). Entered and left with equivalent frequency, resident - consistent with Schmidt and Lake (2006).
Smallmouth Bass <i>(Micropterus dolomieu)</i>	One caught, leaving on ebb tide (Table 1). Labelled as fully potamodromous in Schmidt and Lake (2006) though activity cannot be confirmed here.
Striped Bass <i>(Morone saxatilis)</i>	Low numbers of YOY caught (Table 1), clustered at end of summer (Figure 2). Possibly seeking refuge.
Tessellated Darter <i>(Etheostoma olmstedi)</i>	Few caught, of a consistent size (Table 1). Residents as per Schmidt and Lake (2006). Smaller sizes would not be caught by the mesh size used.
White Perch <i>(Morone americana)</i>	Large catch (Table 1) representing one age class (Appendix 2) mostly towards beginning of summer (Figure 2) might represent tail end of breeding run, consistent with partial potamodromous behavior in Schmidt and Lake (2006)
White Sucker <i>(Catostomus commersonii)</i>	Three distinct age classes were caught (Appendix 2), mostly on ebb tide (Table 1), majority towards the end of summer (Figure 2). Dependent on tributaries for spawning, would need larval confirmation.
Yellow Bullhead <i>(Ameiurus natalis)</i>	Highest number caught (Table 1) though concentrated at the end of the summer (Figure 2). Age class split between YOY and breeding adults (Appendix 2). Schooling behavior often caused large single catch.

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I'd like to thank the Hudson River Foundation and the Hudson River National Estuarine Research Reserve for supporting this project. Thank you to Dr. Karin Limburg for her patience and leadership, and to Dr. Robert Schmidt for his advice. And finally thank you to Kathy Schmidt, Riley Sexton, and Annie Frank for helping in the field, and Chris Bowser for lending me his fyke for a summer and helping out in the field.

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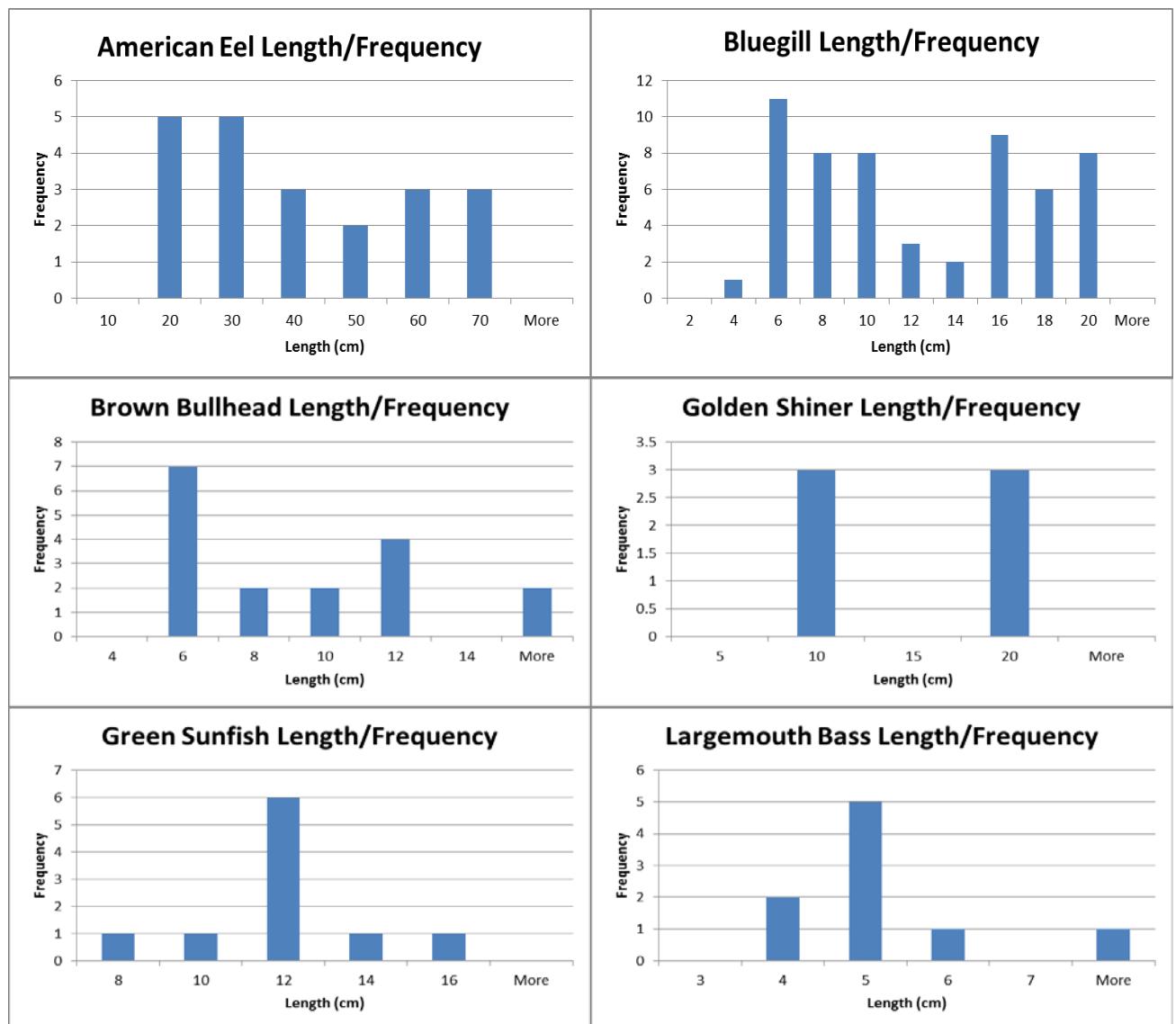
APPENDICES

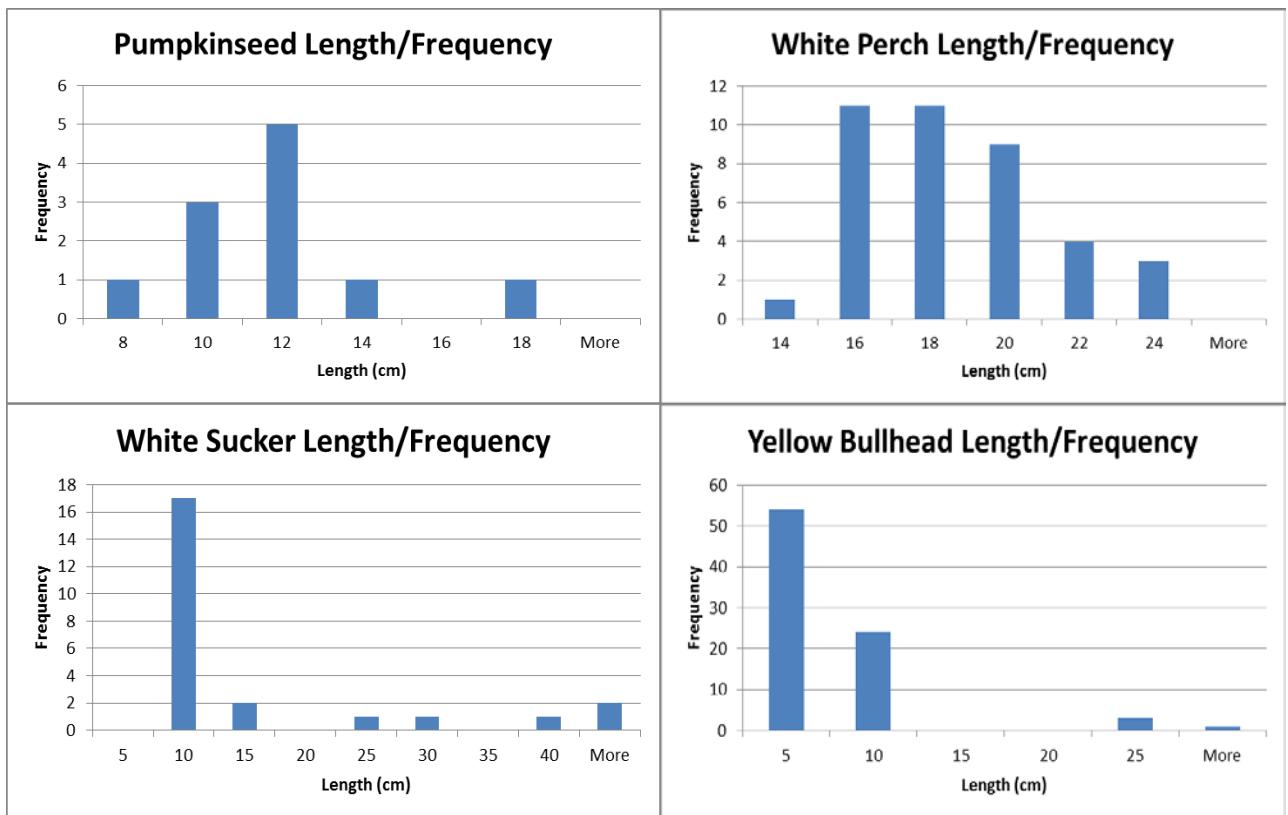
Appendix 1: Sample Dates, Times, and Tidal Stage

Date	Time	Tidal Stage
6/11/14	13:00-17:00	Daytime Ebb
6/12/14	9:00-13:00	Daytime Flood
6/13/14	15:15-19:15	Daytime Ebb
6/17/14	6:15-10:15	Daytime Ebb
6/17/14	12:15-16:15	Daytime Flood
6/19/14	15:00-19:00	Daytime Flood
6/20/14	16:00-20:00	Daytime Flood
6/21/14	22:00-2:00	Nighttime Ebb
6/22/14	18:00-22:00	Nighttime Flood
6/23/14	24:00-4:00	Nighttime Ebb
6/25/14	19:15-23:15	Nighttime Flood
6/29/14	11:15-15:15	Daytime Flood
7/1/14	12:30-16:30	Daytime Flood
7/6/14	21:00-1:00	Nighttime Ebb
7/7/14	17:00-20:45	Daytime Flood
7/8/14	23:00-3:00	Nighttime Ebb
7/9/14	12:00-16:00	Daytime Ebb
7/10/14	19:30-23:00	Nighttime Flood
7/16/14	12:45-16:45	Daytime Flood
7/22/14	18:15-22:15	Nighttime Flood
7/23/14	12:50-16:50	Daytime Ebb
7/23/14	18:50-23:00	Nighttime Flood
7/24/14	20:00-24:00	Nighttime Flood
7/25/14	2:00-6:00	Nighttime Ebb
7/29/14	16:30-20:30	Daytime Ebb
7/30/14	5:00-9:00	Daytime Ebb
8/1/14	13:00-17:00	Daytime Flood
8/1/14	19:00-23:00	Nighttime Ebb
8/5/14	21:00-2:00	Nighttime Ebb

8/5/14	3:30-7:30	Nighttime Flood
8/14/15	19:15-23:15	Nighttime Ebb
8/15/14	0:40-4:40	Nighttime Flood
8/15/14	7:00-11:00	Daytime Ebb

Appendix 2: Length/Frequency Histograms





INTERACTIONS BETWEEN THE ALIEN ORIENTAL WEATHERFISH (*MISGURNUS ANGUILLICAUDATUS*) AND NATIVE FISHES IN THE KLYNE ESOPUS KILL, A HUDSON RIVER TRIBUTARY

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

The alien loach, Oriental weatherfish [*Misgurnus anguillicaudatus* (Cantor)] was sampled from the Klyne Esopus Kill, a small Hudson River tributary south of Kingston, New York. American eel (*Anguilla rostrata*) and Creek chub (*Semotilus atromaculatus*) were the only other common species in the area. Comparisons of food habits and habitat among the three species suggested that Oriental weatherfish have little effect on the native fishes in this stream. Oriental weatherfish preferred silty habitat or areas in rocky streams with a detritus substrate. American eel preferred rocky habitat and were absent from extensive silty areas. Creek chub were found in all habitats but were an order of magnitude less dense than American eel (0.14-0.17 individuals/m² and 1.16 individuals/m², respectively). In rocky habitats, Oriental weatherfish fed exclusively on algae and therefore had little to no overlap with sympatric American eel or Creek chub. In silty habitats, Oriental weatherfish supplemented their algal diet with animal foods, but did not overlap at all with sympatric Creek chubs. Because of habitat and diet segregation among Oriental weatherfish and the two native stream species, there does not appear to be any negative effect of this alien species in this Hudson River tributary. Localities that have abundant silty substrate could, however, support a large population of Oriental weatherfish and thus could have negative effects on native species.

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INTRODUCTION

The Oriental weatherfish (*Misgurnus anguillicaudatus*) is an Asian loach, family Cobitidae, which has been known from North America for about 50 years (Schultz 1960). The first known population in the Northeastern United States was discovered in Connecticut in mid-November, 2014 (Robert Jacobs, personal communication). There has been a proliferation of newly discovered populations of this species in the last two decades, including four in the Hudson River watershed.

Whereas there is general concern about the effects that alien aquatic organisms may have on ecosystems, little information exists on how this species may be altering local systems. Schmidt and Schmidt (2014) provided information on habitat, distribution, and food habits of this species in a Hudson River tributary but they did not address the issue of interactions between Oriental weatherfish and native fishes.

In 2013, R.E. Schmidt and C.H. Bowser discovered a new Hudson River population in the Klyne Esopus Kill, a tiny Hudson River tributary. At that time, Oriental weatherfish were easily collected and subjectively were the most abundant fish in that part of the stream running through a wet meadow. Given the abundance of the weatherfish at this site, and ease of access, a study on the ecology of Oriental weatherfish in this stream seemed warranted. The purposes of this study were to sample all the common fishes in the Klyne Esopus Kill to determine their distribution, habitat, and food habits and compare these data to the Oriental weatherfish. These comparisons may help determine what interactions may be occurring between native fishes and the Oriental weatherfish.

METHODS

Study Area

The Klyne Esopus Kill is a very small Hudson River tributary located in Ulster Park, Ulster County, New York. The study area extended from the tidal mouth in Esopus Meadows upstream about 1.1 km. Sampling was done at four locations (Figure 1). One location was in a wet meadow characterized by relatively low gradient and clay substrate. A silty backwater located on the north edge of the meadow was identified as likely habitat for Oriental weatherfish (Schmidt and Schmidt 2014). The other three sampling sites (Figure 1) were higher gradient rocky stream segments characterized by overhanging forest and gravel and cobble substrate.

Field Methods

Fishes were collected with a backpack electroshocker. Fishes were identified and counted in the field. At two sites, the backwater in the Wet Meadow and Rocky Stream #1, fish population size was estimated by a three-pass depletion technique (Carle and Strub 1978). Block nets were put in place (3/16 inch knotless nylon mesh seines). A team of field workers moved upstream with the shocker and as many fishes as possible were removed and placed in a large container. These fishes were then counted and identified. This process was repeated three times. The length and several widths of the stream were measured with a tape.

All Oriental weatherfish collected were preserved (in 50% isopropanol or 10% formalin) for laboratory analysis. Additionally some specimens of other species were also preserved and returned to the laboratory.

Laboratory Methods

Preserved fishes were dissected to remove the anterior third of the gut. Food items were teased out from the gut and identified as far as practical with a dissecting microscope. Food habits for each species were summarized as the percent of individuals with a particular food out of the total number of individuals with food in the stomach. Since several types of food items could be present in a given stomach, these percentages can sum to more than 100.

RESULTS

Three collections were done in this study. Frequent rains made the visibility in the Klyne Esopus Kill so poor that electroshocker was ineffective. Rocky Stream #1 was sampled on June 16, 2014; the Wet Meadow on June 26; and Rocky Stream #2 on August 12, 2014.

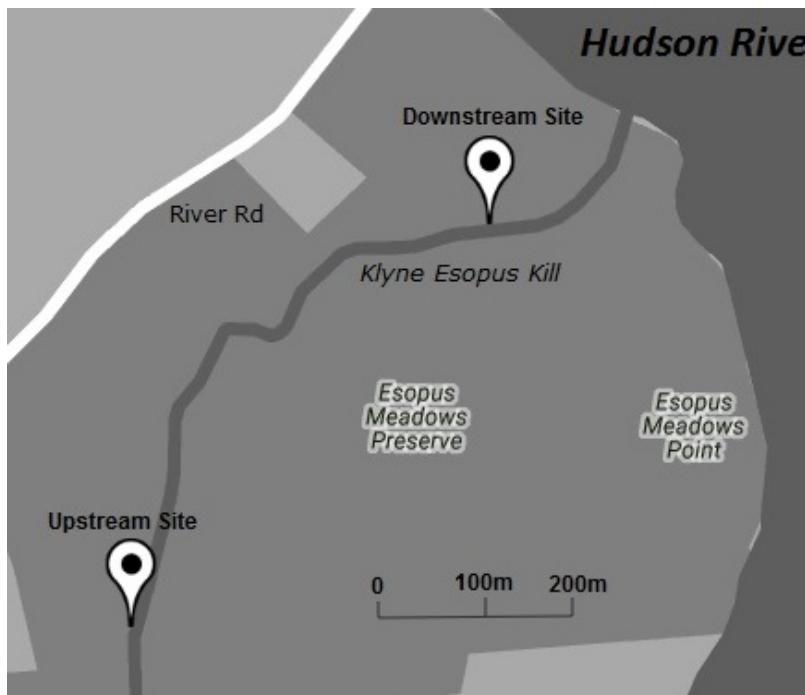


Figure 1: Map of sampling sites on the Klyne Esopus Kill, Ulster Park, NY. The distance between the stream mouth and the most upstream site (Rocky Stream 3) is about 1.1 km.

Habitat

There was relatively clear habitat segregation between American eel and Oriental weatherfish (Table 2). Eels preferred the rocky stream habitat whereas Oriental weatherfish were most abundant in the silty wet meadow location. Even when collected in the rocky stream areas, Oriental weatherfish were found under undercut banks or among tangled branches where dead leaves and silt accumulated. The Rocky Stream #3 sample was not included in this analysis since no Oriental weatherfish were captured in that location. Creek chub are more evenly distributed in the samples, overlapping with both American eels and Oriental weatherfish (Table 2).

Triple pass depletion estimates were done during the Rocky Stream #1 and Wet Meadow samples. The area of the former sample was 43.8 m² and the latter was 147.8 m². Confidence intervals (95%) could only be calculated for the American eel in Rocky Stream #1. For all other observations, the estimated population size equaled the observed number of fish caught (Table 3). American eel had a density one or two orders of magnitude greater than the other species.

Species	RS 1	RS 2	Wet Meadow
American eel	40	6	0
Creek chub	6	4	10
Oriental weatherfish	4	8	10

Table 1: Numbers of individuals collected by electroshocking in the Klyne Esopus Kill, 2014. RS indicates “Rocky Stream”, see Figure 1.

Population	Confidence	Density		
<i>Sample</i>	<i>Species</i>	<i>Estimate</i>	<i>Interval</i>	<i>#/m²</i>
Rocky Stream #1	American eel	51	42-69	1.16
	Creek chub	6		0.14
	Oriental weatherfish	4		0.09
Wet Meadow	Creek chub	25		0.17
	Oriental weatherfish	10		0.07

Table 2: Estimates of population size, 96% confidence interval (for American eel), and density (#/m²) from triple pass depletion sampling in Klyne Esopus Kill, 2014.

Diet

The food habit analysis indicated that there was very little overlap between Oriental weatherfish and American eel in the rocky stream habitat (Table 1). Overlap that was visible was due to plant material (algae) seen in both species. American eel is not known to consume algae (Machut 2006) and the presence of this material in eel guts is probably incidental. Nothing but algae was found in the Oriental weatherfish, which suggests that this material is important in the diet. Schmidt and Schmidt (2014) also reported algae in the Oriental weatherfish diet.

Food	Rocky Habitat			Silty Habitat	
	Ow	Ae	Cc	Ow	Cc
Algae	100	17	0	50	0
Annelids	0	67	0		
Insects	0	33	100	0	100
Beetles				17	0
Salamander				0	17
“Eggs”	0	0	17	17	0

Table 3: Summary of food habit data from fishes occupying two habitats in the Klyne Esopus Kill. Abbreviations are: Ow = Oriental weatherfish, Ae = American eel, and Cc = Creek chub. Numbers are percent of individuals having that item out of all individuals with food in their gut.

In the rocky stream habitat there was overlap between Oriental weatherfish and the native Creek chub in that both consumed insects. However, Creek chub are sight feeders and take insects from the surface or the water column (Hartel et al. 2002) whereas the burrowing habit and presence of substantial barbels indicate a benthic feeding strategy for Oriental weatherfish (Schmidt and Schmidt 2014). In the silty backwater habitat, Oriental weatherfish showed no overlap in food items with Creek chub (and no eel were collected). Again, the predominant food item in Oriental weatherfish was algae. Creek chub is known to be the top predator in small streams and feeds opportunistically on aquatic insects, mollusks (Hartel et al. 2002), or other fishes in the case of large individuals (Etnier and Starnes 1993). Overlap indices were going to

be calculated to compare diets among the three species found in the Klyne Esopus Kill, however, the minimum amount of overlap observed in this study made calculation of overlap indices superfluous.

DISCUSSION

In 2014, Oriental weatherfish was the least common species in the Klyne Esopus Kill (ignoring single individuals of White sucker and Blacknose dace collected). This was unexpected since previous observations (in 2013) indicated a high abundance for this species. Cause(s) of this change are not known.

Oriental weatherfish were found in habitats containing cover and silt. This is similar to the habitats reported by Schmidt and Schmidt (2014). Therefore there was very little overlap with American eel that preferred a rocky stream environment. In fact, American eel density in the Klyne Esopus Kill is close to the highest density reported for this species ($1.2\text{-}1.4 \text{ eels/m}^2$, Schmidt et al. 2006). In either habitat, creek chub was more abundant than Oriental weatherfish (Table 3).

There was almost no overlap between Oriental weatherfish diet and the other two native species. Certainly, Oriental weatherfish are not interacting with American eel in terms of competing for habitat or food items. There is more overlap in habitat and food items between Oriental weatherfish and creek chub, but creek chub outnumber the alien species and feed differently; picking organisms from the water column or substrate surface whereas Oriental weatherfish are mostly feeding beneath the substrate surface.

Despite concerns about the spread of Oriental weatherfish locally and nationally, this species does not seem to be negatively affecting the native fishes in the Klyne Esopus Kill. This

assessment may change under conditions of high population density or in environments where the substrate is mostly silt.

ACKNOWLEDGEMENTS

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THE EFFECTS OF DAMS ON DENSITIES AND SIZES OF AMERICAN EELS IN THE BRONX RIVER

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

Most American eels (*Anguilla rostrata*) move up rivers to mature before migrating to their natal region of the Sargasso Sea to spawn and die. It is well known that dams impede, but don't always prevent the upriver movements of eels. The effects of a series of dams on the densities and size structure of eels in the highly urbanized Bronx River, a tributary to New York Harbor, was examined. Eels were sampled by electrofishing and trapping. It was hypothesized that the sequential impedance of upriver movements would result in fewer and larger eels progressing from the first to the third dam above tidewater. In the first round of electrofishing, the eel population density was found to decrease traveling upriver from 0.212 eels/m² at the 182nd Street Dam, to 0.0765 eels/m² at the Twin Dams, and to 0.040 eels/m² at the Snuff Mill Dam. While eel population density decreased from downstream sites to upstream sites, median eel length increased from 240 mm to 272 mm to 520 mm via electrofishing. With recent declines in American eel populations, the findings of this research may be useful in gaining support for the installation of eel ladders at dams in this watershed to increase the ability of eels to travel to reaches farther upriver.

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INTRODUCTION

American eel (*Anguilla rostrata*) are found in a variety of aquatic habitats along the Atlantic coastline from Brazil to Greenland (U.S. Fish and Wildlife Service 2011). This species also extends inland as far as 1,000 km in systems such as the Mississippi River. Eels occupy a variety of habitats, including streams, rivers, and lakes, as well as oceans, coastal bays, and estuaries. With an elongated form and benthic preferences, eels take shelter in such places as burrows, rock crevices, vegetation and sunken lumber. Eels are mostly nocturnal feeders, and during winter eels burrow in mud and remain inactive (U.S. Fish and Wildlife Service 2011).

The American eel has an unusual and complex life cycle (U.S. Fish and Wildlife Service 2011). As a catadromous species, American eels are born in salt water, migrate to and spend most of their lives in fresh water, and then return to salt water to reproduce. Their eggs are spawned in the Sargasso Sea near the middle of the North Atlantic. Little is known about the conditions in which this takes place, and this process has never been observed. After hatching, the leaf-shaped larvae are passively transported west to the North American coast by the Gulf Stream. Once the larvae have reached the coast, they become glass eels, which possess the typical eel shape and are transparent. Glass eels then start to move up estuaries and begin to behave as a benthic organism. When glass eels become more pigmented, they are termed elvers. Elvers are generally over 100 mm in length, with some pigmentation, and spend most of their time traveling upstream. As eels become larger and fully pigmented they begin to turn yellowish on their bellies; at this stage they are called yellow eels. The majority of growth, as well as sexual differentiation, take place during this stage. As yellow eels mature reproductively, they

become silver eels. This maturation includes a metamorphosis that prepares the silver eel for its migration back to the Sargasso Sea to reproduce (U.S. Fish and Wildlife Service 2011).

Eels that do not migrate far upstream and remain in an estuarine environment complete their life cycle much more quickly. Eels that migrate farther upstream into fresh water tend to live longer and grow larger, increasing their potential reproductive output. An additional benefit of extensive upstream migration is greater dispersal within a watershed, which reduces intraspecific competition. Recent declines in the American eel population have largely been due to commercial fishing, specifically, the harvesting of glass eels and elvers, as well as degradation of habitat and migration corridors (U.S. Fish and Wildlife Service 2011).

The Bronx River is an ideal site for determining the effects of dams on eel populations because it is extensively dammed. Since the Bronx River is a fairly small system with many dams spread over a short distance, it is easy to sample in comparison to a larger system with a greater flow rate. In addition, it was known prior to sampling that each dam site within the Bronx River was passable by eels to some degree, so populations were expected to be found at every sampling site.

The objectives of this study were to estimate eel abundance and population density for each section (separated by the dams), as well as to assess the differences in size structure of the eel populations in each section. Determining the abundance, density, and differences in size structure of this population will help to better understand the overall effects of an array of small dams on inland penetration by American eels. In

addition to helping support the construction of more eel passes, this data could aid in strengthening the conservation status of eels.

It was hypothesized that sections farther upstream would host smaller populations but contain larger eels, while the sections farther downstream would consist of larger populations but contain smaller eels. The rationale behind this hypothesis is that each dam acts as a physical barrier that prevents eels from traveling upstream, so fewer eels will be able to reach each upstream section. Since fewer eels will inhabit the site farther upstream there will be less competition for food, so these eels will be able to thrive. In addition, it can be assumed that the eels found farther upstream are larger because they are older and have spent more time traveling upstream than those eels found downstream.

Sampling Locations



Figure 1: Aerial view of sampling locations (City of New York, 1996)

182nd Street Dam:

METHODS

The 182nd Street Dam is located just outside of the southwest corner of the Bronx Zoo property. This is among the most urban areas that the river runs through, and is also the closest of the sites sampled to the mouth of the Bronx River. There are no dams or obstructions between this sampling site and the mouth of the Bronx River. The stream channel at this site is primarily composed of riffles that are approximately 0.5 m depth as well as low-gradient sections that are approximately 1 m in depth. The banks are fairly littered and there is some heavy wood, as well as garbage debris including things such as rusted bicycles, street signs, and other bulk items in the river.



Figure 2: Ground view of the 182nd Street Dam.

Twin Dams

The Twin Dams site is located on the Bronx Zoo property and consists of two dams that are adjacent to each other, separated by land acting as a natural barrier. Underneath the larger dam is a small area of very shallow water, followed by a deep pool between the two dams, followed by a 30-m section that is narrow and approximately one meter deep.



Figure 3: View of the larger dam at the Twin Dams site

Snuff Mill Dam

The Snuff Mill Dam is located in the New York Botanical Gardens. The river is narrow at this site and flows more rapidly than it does at the other sites. Directly below the dam is a deep plunge pool followed by a large bedrock outcrop, with the depth varying from 0.15 m to 2.0 m. After the section immediately below the dam, there is a 40 m stretch that is approximately 1.0 m in depth, followed by a narrow section of rapidly flowing riffles. Below this section, there is great variation in river characteristics (depth, substrate type, gradient, etc.).



Figure 4: View of the Snuff Mill Dam in the NYBG

Electrofishing

A Halltech battery-powered electrofisher (model HT-2000) set to 150 Volts and 60 Hz was used as the primary sampling gear to collect eels. Block nets of sufficient length were deployed to section off the area of the river being sampled. At each site, an attempt was made to sample the area of the river closest to the dam (downstream of the dam). Block nets could not be placed equidistant from each other at each of the three sites due to differences in the structure of the river, which led to differences in the total area sampled at each site. A team of at least three people netted eels that were stunned by the electrofisher. Two passes were conducted at each site with one person operating the shocker and three or four netters walking in a serpentine pattern. Two samples were taken to allow for the calculation of a population estimate through two-pass depletion.

Trapping

For trapping, approximately 30 baited eel traps were used. All traps were spaced 30 meters apart from each other at each site. At each trap location, depth and substrate type (silt, sand, gravel, pebble, cobble, boulder) were measured. The traps were baited with frozen menhaden (*Brevoortia tyrannus*) once per week and were checked for two consecutive days after being baited. All traps were kept in the same location throughout sampling. Some traps were sunk using rocks, others were also tied to trees.

The initial width of the eel trap entrances was 3.0 cm, which led to the capture of only small eels. Upon dropping the first two traps into the water with bait, very large eels were observed but seemed unable to enter the traps. After the first week of trapping, the trap entrances were widened to 3.8 cm.

Measuring/Tagging

All captured eels were anesthetized with clove oil; eels were placed in a 5-gallon bucket half filled with water with 13 drops of clove oil for approximately five to ten minutes. Eel length was measured; if the eel was over 275 mm, a judgment based on girth was made as to whether the eel could be safely tagged. Eels were tagged with a needle (sanitized with alcohol wipes) and 12 mm passive integrated transponder (PIT) tags. If the eel was captured via electrofishing, it was released in the same section where it was captured. If the eel was captured via trapping, it was released at least two traps away from where it was caught in order to ensure population mixing.

Statistical Analysis

Descriptive statistics were calculated for each site and sampling method used, including minimum eel size, first quartile, median, mean, third quartile, and maximum eel size. In order to compare the size structure of eels captured at different sites, three adjacent box plots were created to illustrate eel size patterns. Differences in size structure were inferred based on observed differences in the sample median.

To estimate population abundance at each site, two electrofishing passes were conducted. By using the number of eels captured from each separate round, an estimate of the eel population and a 95 percent confidence interval were calculated using a two-pass depletion approach (Lockwood et al. 2000).

RESULTS

Trapping took place during the weeks of 7/7, 7/21, 7/28, and 8/18 in 2014. Nine traps were set at the 182nd Street Dam, six traps were set at the Twin Dams (limited access on Zoo property as well as depth of river limited the number of traps that could be placed), and nine traps were set at the Snuff Mill Dam.

Two-pass electrofishing took place two times throughout sampling, once in July and once in August, and the same areas of each site were sectioned off both times. At the 182nd Street Dam the nets blocked an area that measured 640 m², at the Twin Dams the nets blocked off an area that measured 418 m², and at the Snuff Mill Dam the nets blocked off an area that measured 326 m². For both electrofishing events, the 182nd Street Dam site had the most captures and the Snuff Mill dam site had the fewest (Table 1 and Table 4). Capture data from the two pass electrofishing was used to calculate population estimates, of which the 182nd Street population was the largest and the Snuff Mill population was the smallest (Table 2 and Table 5). The population estimates and electrofishing sampling area sizes were used to calculate population densities for each site, with 182nd Street having the largest population density and Snuff Mill having the smallest population density (Table 3 and Table 6).

In addition, the size structure of eels from each of the three sites was compared for each electrofishing event. Few eels were captured at the site the farthest upstream with population estimates of 1 and 13 eels; however, the eels at this site were much larger in size with a median length of 520 mm. Many eels were captured at farthest downstream site with population estimates of 139 and 79 eels; however, the eels at this site were relatively small in size with a median length of 240 mm. Eels captured in the

middle site fell between the other sites with population estimates of 27 and 14 eels and a median length of 272 mm. Data comparing the size structure of eels at each site was used to generate box plots (Figure 5 and Table 7).

First Electrofishing Captures

Site	Captures
182 nd Street Dam	82 individuals
Twin Dams	26 individuals
Snuff Mill Dam	8 individuals

Table 1: Total individuals captured during the first round of two-pass electrofishing.

First Electrofishing Population Estimates

Site	Population Estimate
182 nd Street Dam	139 individuals (95% C.I. [49, 228])
Twin Dams	27 individuals (95% C.I. [24, 30])
Snuff Mill Dam	13 individuals (95% C.I. N/A)

Table 2: Population estimates with confidence intervals calculated from the first round of two-pass electrofishing.

First Electrofishing Population Densities

Site	Population Density
182 nd Street	0.212 eels/m ²
Twin Dams	0.065 eels/m ²
Snuff Mill Dam	0.040 eels/m ²

Table 3: Population densities calculated from first round population estimates and sampling area sizes.

Second Electrofishing Captures

Site	Captures
182 nd Street Dam	51 individuals
Twin Dam	14 individuals
Snuff Mill Dam	1 individuals

Table 4: Total individuals captured during the second round of two-pass electrofishing.

Second Electrofishing Population Estimates

Site	Population Estimate
182 nd Street Dam	79 individuals (95% C.I. [27,130])
Twin Dams	14 individuals (95% C.I. [13,15])
Snuff Mill Dam	1 individual (95% C.I. N/A)

Table 5: Population estimates with confidence intervals calculated from the second round of two-pass electrofishing.

Second Electrofishing Population Densities

Site	Population Density
182 nd Street	0.124 eels/m ²
Twin Dams	0.033 eels/m ²
Snuff Mill Dam	0.003 eels/m ²

Table 6: Population densities calculated from second round population estimates and sampling area sizes.

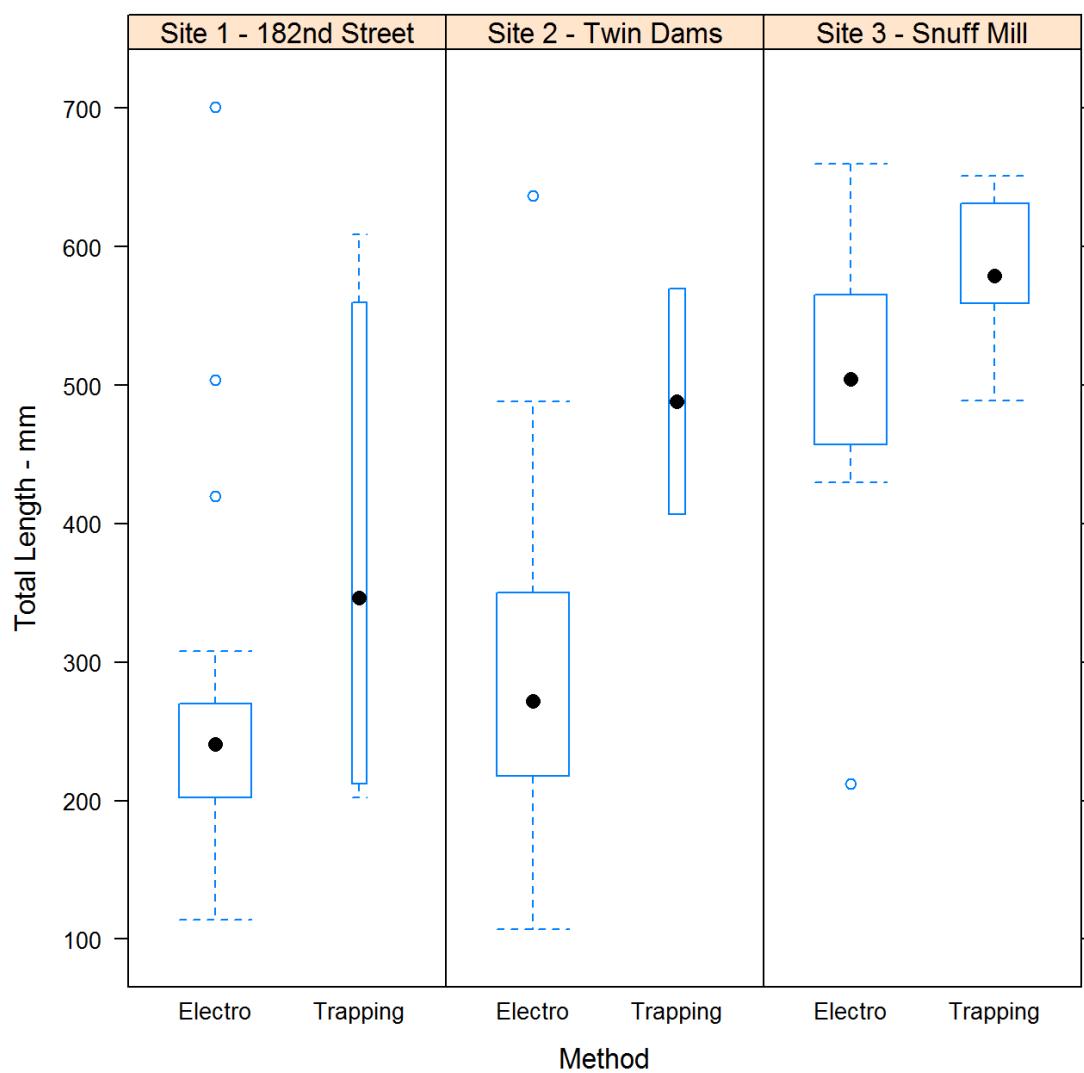


Figure 5: Box plots illustrating total length (mm) of American eel captured by trapping and electroshocking in the Bronx River, NY. Sample sites are ordered left to right from farthest downstream to farthest upstream. The dot represents sample median.

Sampling Type	Location	Min.	Q1	Median	Mean	Q3	Max.
Electrofishing	182nd Street	114	202	240	235.6	270	504
Trapping	182nd Street	202	238.8	346.5	379.3	513.5	609
Electrofishing	Twin Dams	107	220.8	272	292.3	350.2	637
Trapping	Twin Dams	407	447.8	488.5	488.5	529.2	570
Electrofishing	Snuff Mill	212	484	520	514.1	596	701
Trapping	Snuff Mill	489	559	579	585.6	631	651

Table 7: Size data (mm) of eels captured at each site via electrofishing and trapping.

DISCUSSION

As hypothesized, eel densities decreased along the upriver axis, and median eel lengths increased along the upriver axis. Eel populations along the upriver axis decreased from 0.212 to 0.650 to 0.040 eels/m² in the first round of electrofishing, and decreased from 0.124 to 0.033 to 0.003 eels/m² in the second round of electrofishing. While eel population density decreased from downstream sites to upstream sites, median eel length increased from 240 mm to 272 mm to 520 mm via electrofishing. Thus, the hypothesis could not be rejected. This means that with partial barriers in fresh waters, fewer eels are making it to upstream environments that would otherwise be more densely inhabited by this species. The life history of eels drives the species towards more upstream habitats; preventing eels from reaching these upstream habitats could result in reduced reproductive capacity of the species.

Bednarski et al. (2013) studied the effects of the removing two out of four dams on eel abundance in the Mill River system of Taunton, Massachusetts. Their study utilized mark-recapture analysis to calculate an estimate of total population size in preparation for these removals. Within Lake Sabbatia, the headwater impoundment of the

Mill River system, eel population density was estimated between 0.75 and 2.21 eels/hectare compared to averages of approximately 215 eels/hectare at the most upstream site and 1680 eels/hectare at the most downstream site of the Bronx River. Eel population densities estimated at other locations include 182-232 eels/hectare in Fridaycap Creek, Georgia, 232-636 eels/hectare in Lake Champlain, Vermont, 875 eels/hectare in Sippewissett Marsh, Massachusetts, and 1-30 eels/hectare in the Hudson River, New York (Bednarski et al. 2013).

However, when comparing the data from these systems it must be taken into consideration that Lake Sabbatia, the other systems, and the Bronx River all differ in depth, flow rate, substrate composition, and overall structure. In addition, when comparing these population densities it is important to consider that the sampling method used in the Mill River, Sippewissett Marsh, and Fridaycap Creek systems consisted of trapping while the sampling method used to calculate population densities in the Bronx River consisted of electrofishing. Eel population density estimates in the Bronx River appear to be very high compared to other systems.

These high values may be attributed to the fact that sampling took place in areas directly following dams, the areas where eels tend to congregate. Dam removal in the Mill River system took place in 2012 and 2013, with more planned for the future, as well as the insertion of eel passes (Bednarski et al. 2013). With baseline data having been recorded, the effect of dam removal on eel abundance will be studied carefully in years to come. This research will serve a similar purpose, as baseline data for future studies. In a study conducted in headwater streams in Shenandoah National Park, Virginia, American eel demographics were estimated before and after the removal of a large dam located

downstream (Hitt et al. 2012). Following the dam removal in 2004, eel abundances in headwater streams increased significantly. In addition, it was observed that eel abundances increased consistently from 2004 to 2010. Researchers also discovered that the minimum size of eels found in headwater streams had decreased following the dam removal. Significantly more eels measuring less than 300 mm in length were captured in headwater streams following the removal of dams, meaning that dams had previously been hindering smaller eels' ability to travel upstream to headwater streams (Hitt et al. 2012). This study showed that impediment of the river in the form of a dam is the primary factor effecting eel demographics, not predation, river structure, sediment type, or any other environmental factors.

In the Hudson River, researchers studied human impacts on eel populations in tributaries with a primary focus on dams (Machut et al. 2007). It was determined that eel population densities within tributaries are much greater than those found in the main river. Additionally, it was found that eel population densities in areas upstream of barriers were approximately a tenth of those in areas unimpeded by barriers, and the eels upstream of barriers had a smaller mass than those in unimpeded areas. Based on the size structure of eels captured in tributaries, it was hypothesized that tributaries are very important to the growth of immature American eels. Because of the important role American eels play in the balance of the food web and nutrient composition in rivers and tributaries, barrier removal as well as installation of eel ladders could help lessen the negative impacts of human interference in river systems and maintain biodiversity (Machut et al. 2007). Because tributaries tend to serve as “nurseries” for immature eels, and because eels play an important role in balancing food webs and nutrient

compositions, providing eels with passage upstream of dams would likely help in the restoration of the Bronx River to a more natural condition.

With restorations taking place in other systems such as the Mill River and rivers in Shenandoah National Park, it is important to learn more about the success and impacts that these restorations are having on eel populations and the species overall. Protocols for dam removals and methods for constructing eel passes that have proven to be successful in other systems should be used as guidelines for future restorations in systems such as the Bronx River. The resulting eel population data from current restorations can also be used to determine which systems require the most immediate attention, so that the species as a whole can be recovered and its full range can be restored.

In future studies, using smaller tags would allow more eels to be tagged, generating a larger sample size for statistical analyses. In addition, more manpower would allow for more traps to be deployed at additional sites so that more data could be collected. Sampling the full river as opposed to just three small sections would provide a more comprehensive understanding of the eel population in the entire system. For example, while it is unclear whether or not eels tend to congregate in stretches of the river immediately below the dams, or inhabit full stretches between dams in equal densities, the former is more likely based on previous studies of eel populations in Hudson River tributaries (Machut et al. 2007). Electrofishing appears to be a much more efficient way to collect data than trapping because electrofishing takes away much of the chance involved in trapping; however, trapping is very useful in areas where electrofishing is not possible because of a lack of accessibility or water depth. In

addition, trapping is easy to conduct with a minimum of two individuals whereas electrofishing requires a team of at least four.

Although a substantial effect of dams on eel demographics was not found, it was also noted that it is not apparent what the density and size distribution of eels would be in an undammed river of similar size. To better understand the effect of dams on eel population densities and sizes, these data collection methods must be repeated in a system similar to the Bronx River but without any barriers. The comparison of eel demographics in an unimpeded system and eel demographics in a heavily dammed river would reveal the extent at which dams inhibit the upriver movement of eels.

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Pictured (left to right): Julia Les, Anastasia Frank, Alec Schmidt, Erik Bugenhagen, Richard DeMarte, Katherine Guild, and Corinna Singleman. Not present in photo – L. Jonas Hamberg.

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