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Ecology of the Young-of-the-Year Emerald Shiner (Notropis Atherinoides) in the Upper Niagara River, New York: Growth, Diversity, and Importance as a Forage Species

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Abstract of Thesis

The emerald shiner (*Notropis atherinoides*) is a relatively understudied Cyprinid that fills a major keystone role in the Niagara River. Little is known about the emerald shiner's early life history, such as the ecology of their larval and juvenile stages, which is the focus of this study. In the upper Niagara River, larvae first recruited into sampling gear in early July at a mean water temperature of 23° C, with larvae appearing into August. Young-of-the-year (YOY) emerald shiners grew an average of 1.5 mm and 31.5 mg a week throughout the growing season with condition peaking during warm water months (August-September). Increased catches of larvae in gyre areas of the river suggest that spawning may occur in these locations with eggs and hatchlings becoming entrapped during early development. However, juvenile stages were most frequently encountered in natural habitats. These natural habitats (marshes, islands, and creekmouths) also displayed a significantly more diverse YOY fish community when compared to developed habitats (i.e. marinas/seawalls) (F $(3,66) = 9.639$, p-value < 0.05). Developed habitats, comprised of little structure and typically lacking vegetation, often times only housed emerald shiners and no other YOY species. Emerald shiners are an essential forage species for native piscivorous fish and birds such as walleye (*Stizostedion vitreus*), steelhead trout (*Oncorhynchus mykiss*), and the New York State threatened common tern (*Sterna hirundo*). Juvenile and adult emerald shiners comprise over half of the diet of walleye and steelhead trout in the river (57% and 59% respectively). Emerald shiners were also the top ranked prey item for walleye (71%) and steelhead (85%) as suggested by the Index of Relative Importance (% IRI). In the Niagara River, the combined effects of YOY fish habitat loss, invasive species, and degraded water quality require that this native forage fish becomes a priority for scientists and fishery managers in order to maintain the ecological integrity of the aquatic food web in the Niagara River.

State University of New York

Buffalo State College

Great Lakes Center

Ecology of the Young-of-the-Year Emerald Shiner (*Notropis atherinoides*) in the Upper Niagara River, New York: Growth, Diversity, and Importance as a Forage Species

A Thesis in Great Lakes Ecosystem Science

By

Jacob Lewis Cochran

Summited in Partial Fulfillment of the Requirements for the Degree of

Master of Arts

August 2017

Approved By:

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Foremost, I must thank my family for their support and inspiration. I fondly remember my grandfather pulling my brother and me out of $2nd$ grade classes under various dismissal "excuses" so we could go fishing with him beneath the railroad trestle. From there, my interest for the aquatic world and the critters that resided within them was peaked. My father fostered these aquatic interests by exposing me to our local waterways, teaching me the basics of aquatic biology and limnology. Fast forward to present day and my mother's passion for fishing is further motivation to continue my crusade to help conserve the fisheries that she and so many others enjoy.

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1. Introduction

1.1 Background

The emerald shiner, *Notropis atherinoides*, is a small planktivorous pelagic fish that is an important forage species in its native habitats. Within Lake Erie, emerald shiners are an important food source for piscivorous fish and birds, such as the New York State threatened common tern (*Sterna hirundo*) (Knight et al. 1984, Bur et al. 1999, Hebert and Morrison 2003). The value of this fish stems beyond its ecological framework, providing an economically important bait fishery (Gordon 1968, Meronek et al. 1997). The Niagara River corridor, which connects lakes Erie and Ontario, has an emerald shiner population that has been poorly studied. Therefore, understanding the ecology of the emerald shiner in this river is crucial to better ensure successful management for this ecologically and economically important species. One of the key factors in determining the population status of this fish in the Niagara River region is to improve our understanding of its early life stages. Aspects of young-of-the-year (YOY) emerald shiner ecology will be the focus of this study, as it has not been documented in the upper Niagara River. This study will examine the first accounts of growth, size-distributions, YOY fish diversity, and the prevalence of YOY emerald shiners as a forage item to walleye (*Stizostedion vitreum*) and rainbow trout (*Oncorhynchus mykiss*) (henceforth referred to as steelhead) within this unique and ecologically important Great Lakes connecting channel.

In teleost fishes, such as the emerald shiner, mortality rates are highest during the early life stages and survival from embryo to adulthood is extremely low (<10% survivorship in some species) (Sogard 1997). Within the larval stage, environmental and ecological challenges naturally lead to high mortality (Dickie et al. 1987). Once emerged from the egg, emerald shiner larvae are highly vulnerable to predation and have around six days to functionally develop and feed before their yolk-sac is reabsorbed (Flittner 1964). Environmental factors such as

temperature can alter the absorption of the yolk-sac and affect survival rates of larval fish (Lasker 1962, Heming and Buddington 1988, Llanos-Rivera and Castro 2006, Wen et al. 2013). Previous literature has documented optimal temperature regimes in both adult and YOY emerald shiners and suggested that the preferred summer water temperature for these YOY's was 22°C-23°C, with temperatures below 15°C considered undesirable for sufficient growth (Reutter and Herdendorf 1974, McCormick and Kleiner 1976). In the upper Niagara River, these temperatures are met and sustained typically from August into September each year (NOAA 2016). Since summer water temperatures in the river are not considered growth-limiting, understanding how YOY emerald shiners grow through time is a main focus of this study since it has not been documented and is important for population recruitment.

In young cyprinid fish, it has been suggested that survival is dependent on growth within the first season (Mills and Mann 1985). Multiple studies have examined YOY emerald shiner size structures and growth in various ecosystems (Flittner 1964, Fuchs 1967, Schaap 1989). At the end of the growing season, YOY emerald shiners in Dauphin Lake (MB, Canada) were 28 mm in 1984 and 21 mm the following year. Schaap (1989) also determined the condition of Dauphin Lake YOY emerald shiners, revealing seasonal and yearly variation, with increasing condition observed during more favorable ecological conditions such as increased water temperatures and zooplankton availability. In Lewis and Clark Lake (SD), larvae were found from late June until early November, with the highest abundance of larvae appearing in early July. In one area of the lake, the average length of YOY emerald shiners at the end of the growing season in October was 52 mm (Fuchs 1967). A study conducted by Flittner (1964) investigated the life history of emerald shiners in Lake Erie (OH). The study found that YOY emerald shiners first appeared in the western basin during August each year of sampling (1958-

1960). YOY Lake Erie emerald shiners accumulated 91% of their growth from August to September, in the first month of life, with little winter growth (Flittner 1964). By the end of the summer, 53 mm was the typical length of what was considered a healthy YOY emerald shiner in Lake Erie. These are some of the few studies that examined YOY emerald shiner population size-structure, condition, and growth in various ecosystems and their findings provide a baseline to compare the growth performance of upper Niagara River YOY emerald shiners.

Other little known aspects of YOY emerald shiner ecology are the type of habitats they use in a river and the various juvenile size ranges that use those habitats. Emerald shiners are generally considered to be a pelagic schooling species having low habitat preference towards vegetation, with hydrology and depth being more influential (Lee et al. 1980, Trautman 1981). However, research is limited on how YOY or adult emerald shiners utilize the upper Niagara River. Emerald shiner eggs are demersal (Crossman and Scott 1979), suggesting that the presence of larvae in an area is most likely the result of spawning near that area unless larvae were carried downstream via water currents. Adult spawning areas are currently unknown and are likely to occur throughout the upper river in open water. However, anecdotal and observational evidence suggests large schools congregate in gyres, such as bulkheads and boat launch sites, in the upper Niagara River. If gyres within the upper Niagara River are used by adults to spawn, larvae could become entrapped in these areas resulting in the delay of larval fish transport to more productive nursery habitats downstream. Markle et al. (2014) found similar larval retention of some catostomids and cyprinids native to Upper Klamath Lake, Oregon, in which strong prevailing winds generated gyres, most notably near shoreline irregularities. In gyre areas in which adjacent current was quick, retention of larval suckers, fathead minnows (*Pimephales promelas*), blue chubs (*Gila coerulea*), and tui chubs (*G. bicolor*) could last from

days to weeks, suggesting hydrology and shoreline topography could act in a way to greatly influence larval fish retention within specific areas of waterways (Markle et al. 2014).

Although larval appearance at various sites is important to determine where spawning areas might occur, the nursery habitats of the river that juvenile emerald shiners and other larval fish select are of high importance for growth and development. Following the absorption of the yolk-sac, larval fish face increased levels of mortality as they switch to exogenous feeding, referred to as the critical period (Hjort 1914, Sifa and Mathias 1987). Therefore, to increase their chances of surviving this critical period, YOY emerald shiners must be physiologically developed enough to feed and also have adequate forage nearby. Siefert (1972) examined the diet of larval emerald shiners from Lake Superior and found that rotifers (*Trichocera spp.)* were the primary food source for hatched fish, and remained important throughout all YOY size classes. As the larvae began to grow, larger zooplankton, such as copepod nauplii, calanoid copepods, and cladocerans were incorporated into their diets. Since zooplankton is an essential food source for YOY emerald shiners, it would be beneficial for these fish to inhabit areas of the river in which these forage items are abundant. Zooplankton diversity and abundance has been linked to increased habitat connectivity, which promotes the undisrupted movement of ecological necessities such as nutrients and conserves habitat complexity (Cloern 2007). This connectivity is lost when artificial shoreline structures replace natural shorelines, such as is the case along the upper Niagara River.

The anthropogenic fingerprint of historic and current development activities can be seen throughout the upper river. The health of the Niagara River has been greatly compromised, resulting in its listing as an Area of Concern in 1987 by the Great Lakes Water Quality Agreement (EPA 2016). Regarding the river's aquatic habitat, 60% of the U.S shoreline is now

hardened (adding seawalls or jetties to a shoreline) with over 30 marinas constructed within the river on the American side alone (Wooster and Mathies 2008). Structures such as marinas, seawalls, bulkheads, and rip-rap have greatly modified many of the littoral habitats within the river. The degradative effects of these habitat alterations stems beyond the emerald shiner, impacting the fish community as a whole. Within a locality, fish species occur together forming assemblages that have complex interactions (Cornell and Lawton 1992). In riverine systems, the biology and habits of many fish species typically result in them remaining in distinct compartments across various habitat types within the system (Rutherford et al. 1987). In large lotic ecosystems such as the Niagara River, adult emerald shiners to utilize pelagic habitats (Trautman 1981, Stewart and Watkinson 2004), yet the literature on YOY emerald shiner habitat is vague.

Littoral habitats have been well documented as nursery areas for YOY fish, and YOY's have frequent fish-fish interactions, such as predation, resource competition, and habitat utilization (Gelwick and Matthews 1990). In YOY fish, it has been suggested that high quality riverine littoral zones with differing habitat types are essential during ontogenetic development, with ecological improvements emerging from the reduction of artificial shorelines in these areas (Schiemer and Spindler 1989). Conversely, information on fish communities and interactions with man-made structures (i.e. marinas, seawalls, etc.) are lacking. It has been shown, however, that some YOY fish species that have a low preference for vegetation are positively attracted to marinas, while species that prefer vegetation are not (Sandström et al. 2005). To better assess the interactions and preferences of YOY emerald shiners in the upper Niagara River, we must know the habitat types they utilize and the species that co-occur with them within those habitats. A study conducted in the upper Niagara River by Kapuscinski and Farrell (2014) examined fish

assemblages as they pertained to muskellunge (*Esox masquinongy*) nursery habitat sites. A canonical correspondence analysis conducted on fish species and habitat variables suggested that emerald shiners, of no documented age class, had strong positive correlations with flow, high macrophyte column density, and coarse substrates within these shallow muskellunge nursery sites. Along with emerald shiners, numerous other species preferred this habitat type, such as spottail shiners (*Notropis hudsonius*), blacknose shiners (*N. heterolepis*), common shiners (*Luxilus cornutus*), and muskellunge (Kapuscinski and Farrell 2014). Unfortunately, species assemblage data is limited for the upper Niagara River and no previous study exclusively examines YOY fish species.

A common component when deciphering the health of a riverine system is to examine its biota. Determining species diversity within an area or habitat is a standard approach when addressing riverine health (Karr 1999). The loss of habitat makes it crucial for restoration efforts to identify fish diversity, an indicator of health, in habitats and areas within the river. This study aims to determine if YOY fish diversity in the upper Niagara River is different among various habitat types categorized as: marshes, creek mouths, islands, and developed sites. The developed sites consist of marinas, seawalls, bulkheads and various other structures that modify the habitat and reduce its connectivity with the rest of the river. Fish diversity can also be affected by the natural changes in the river that occur throughout the summer. Since many fish species in the river typically spawn in early summer (Auer 1982), this influx of YOY fish may boost diversity during the growing season. A similar situation was found in the Indian River (Florida) and its lagoons, where overall fish community diversity was greatest in the summer, attributable to warmer water temperatures and the influx of juvenile fish to the system (Tremain and Adams 1995).

Within Lake Erie, walleye and steelhead have both been documented to predate heavily on emerald shiners (Parsons 1971, Knight et al. 1984, Knight and Vondracek 1993, Clapsadl et al. 2005). These top predators are also some of the most sought-after game fish in the Great Lakes region, contributing to a multimillion dollar recreational fishery in Lake Erie alone (Hushak et al. 1988, Murray and Shields 2004, Melstrom and Lupi 2013). However, little is known about the foraging habits of these two in the upper Niagara River. Although diet studies have been conducted on YOY rock bass (*Ambloplites rupestris*), smallmouth bass (*Micropterus dolomieu*), and muskellunge (*Esox masquinongy*) (George and Hadley 1979, Kapuscinski et al. 2012), no study has yet investigated the diets of walleye and steelhead within this Great Lakes' connecting channel. Therefore, this study aims to determine the diet of these two predators in the upper Niagara River and what role emerald shiners play in it.

Walleye and steelhead both inhabit the upper Niagara River (Yagi and Blott 2012), although research is lacking on how, when, and where they utilize the river or if populations are resident. Observations in the field and anecdotal claims from local fishermen suggest that there is a resident population of walleye in the river while steelhead tend to utilize the river in the fall months to forage before entering surrounding tributaries to spawn. Nonetheless, both predators utilize the river's forage base while they reside in the river. Emerald shiners are one of the most abundant forage fishes inhabiting the upper Niagara River (Yagi and Blott 2012), therefore, it is hypothesized that they will be a main prey item to the diets of these two top predators.

Although it is well documented that emerald shiners are an important forage item for walleye and steelhead in Lake Erie (Parsons 1971, Knight and Vondracek 1993, Clapsadl et al. 2005), most studies group all age-classes of shiners together without identifying whether they are YOY and adult stages. This is problematic because it masks the importance for predators of the

early life-stages of forage fish. This inherently suggests that an abundant and healthy adult forage base is the sole necessity for supporting healthy predator fish populations. This is typically not the case in systems that have abundant and fast growing forage fish such as the emerald shiner. In many reservoirs, YOY gizzard shad (*Dorsoma cepedianum*), another fast growing forage fish, are the most abundant prey item consumed in the fall, supporting various species of piscivorous fish inhabiting these impoundments (Michaletz 1997, Evans et al. 2014). Since emerald shiners are abundant in the upper Niagara River, and walleye and steelhead are frequently documented predating upon them in nearby Lake Erie, my research aims to investigate whether these predators consume YOY emerald shiners in the river.

1.2 Objectives & Hypotheses:

The primary objectives and associated hypotheses for this research are:

Objectives

- Determine the growth, condition, and size distribution of YOY emerald shiners in the upper Niagara River.
- Identify areas in the river used by larval and juvenile emerald shiners as nurseries.
- Investigate the YOY species' assemblage and diversity at various habitat types in the upper river.
- Determine the importance of emerald shiners as a fall forage item for walleye and steelhead.

Hypotheses

- 1. Growth and condition of YOY emerald shiners will be analogous to the nearby Lake Erie population, suggestive of a healthy river population.
- 2. Juvenile emerald shiners will use natural habitat types such as marshes, creek mouths, and island habitats as nursery sites, rather than using human-developed sites with hardened surfaces.
- 3. YOY species diversity will differ between marsh, island, creek mouth, and developed habitat types, and developed sites will have the lowest diversity index.
- 4. Emerald shiners will be an important forage item for walleye and steelhead in the fall.

2. Methods: Young-of-the-year Fishes

2.1 YOY Study Sites

This study was conducted on the upper Niagara River, located between the province of Ontario (Canada) and New York State (US) (Fig. 1). The sites chosen for this study represent the various habitat types found in the river: marsh, island, creek mouth, and developed. The marsh (Burnt Ship Creek, Sandy Creek Marsh), island (Motor Island, Strawberry Island), and creek mouth (Big Six Mile Creek, Spicer Creek) sites are classified as natural sites (Table 1). The fourth habitat type in the study, "developed", is a type of habitat predominantly influenced by artificially man-made structures which are used by YOY fishes and include: Vacant Marina, Ellicott Creek, Isle View Park, Aqua Lane Park, LaSalle Waterfront Park, Big Six Mile Creek Marina, Beaver Island Marina, and Ontario Street (Table 1). This categorization of sampling sites provides a means of comparisons across different general habitat types within the river.

The sampling site at Burnt Ship Creek was located inside a shallow $(< 1 \text{ m})$ embayment surrounded by cattails (*Typha spp*.) with slow average flow velocities (mean \pm SD, 0.13 \pm 0.06 m/s). Aquatic vegetation was generally sparse with *Chara* spp., slender water grass (*Najas gracillima*), nodding water nymph (*Najas flexilis*), and tape grass (*Vallisneria americana*) comprising most species and a substrate consisting of primarily sand. The Sandy Creek Marsh site was located within and along the perimeter of a moderately shallow $(< 1.25 \text{ m})$ slow flowing $(0.17 \pm 0.09 \text{ m/s})$ floating instream marsh comprised primarily of cattails. Sampling occurred within the embayment and also along the outside perimeter of the marsh. Aquatic vegetation was sparse with *Chara spp*., various pondweed species (*Potamogeton spp.*), and tape grass representing the majority of the macrophyte species and a substrate consisting primarily of sand.

 Motor Island, a small (2.5 ha) uninhabited island, is located in the main channel of the river. Sampling at the site occurred around the island's perimeter but was focused in a small

shallow $(< 1 \text{ m})$ nearly stagnant $(0.14 \pm 0.06 \text{ m/s})$ embayment located along the western shore. Aquatic vegetation was moderate, represented predominantly by tape grass and various pondweed species including sago pondweed (*Stuckenia pectinate*) and a substrate consisting of a clay/silt/sand mixture. Strawberry Island is also a relatively small (~10 ha) uninhabited island located in the main channel of the river south of Motor Island (Sweeney 1997). Sampling was conducted within the islands large and shallow $(< 1 \text{ m})$ embayment with minimal flow velocities $(0.25 \pm 0.23 \text{ m/s})$. Aquatic vegetation was also moderate, with *Chara spp.*, various pondweed species, and tape grass comprising most observed species with a substrate composed mainly of clay, gravel, and sand.

 The Big Six Mile Creek sampling site was located at the convergence of Big Six Mile Creek and the upper Niagara River on the west side of Grand Island, NY. Sampling occurred in a shallow area $(< 1 \text{ m})$ upstream of the mouth of the creek and adjacent to a small stone pier. Flow was generally slow $(0.20 \pm 0.12 \text{ m/s})$ at the site, with moderate aquatic vegetation comprised of coontail (*Ceratophyllum demersum*), Eurasian watermilfoil (*Myriophyllum spicatum*), various pondweed species, and tape grass with a substrate consisting of sand and a sand/gravel/cobble mixture. The Spicer Creek sampling site was located downstream of the confluence where Spicer Creek drains into the Niagara River on the eastern edge of Grand Island. Sampling occurred along the nearshore shallow areas $(0.75 m)$ in a section of river with slow flow velocities (0.10 m) \pm 0.04 m/s). Aquatic vegetation was moderate and consisted primarily of coontail and tape grass with a substrate composed of a clay/silt/sand mixture.

The Vacant Marina sampling site was located at an abandoned marina along the east branch of the upper Niagara River. Sampling occurred off concrete docks in deeper water depths (> 1.5 m) with slow water velocities (0.08 \pm 0.01 m/s). Aquatic vegetation within the marina was

minimal with tape grass as the predominant species and a substrate dominated by clay/silt. The Beaver Island Marina sampling site is another marina site which is located on the southern tip of Grand Island. Sampling occurred within and around the operating marina in moderately deeper water (> 1.10 m) with a slow flow (0.16 \pm 0.10 m/s). Aquatic vegetation was moderate with coontail and tape grass being the dominant species and a substrate consisting of clay/silt and sand. The Big Six Mile Creek Marina site is located within Big Six Mile Creek along the western side of Grand Island. Sampling occurred from docks over deeper water $(> 1.8 \text{ m})$ with slow water currents (0.06 ± 0.02 m/s). Aquatic vegetation was extremely sparse within the marina with coontail and tape grass being the only present species observed and a substrate of clay/silt and organic materials.

The Isle View Park sampling site was located along the eastern branch of the upper Niagara River near an active boat launch. Sampling occurred off the docks in deep water $(>1.2$ m) within an area of slow flow (0.16 ± 0.10 m/s). Leafy pondweed (*P. foliosus*), swaying bulrush (*Schoenoplectus subterminalis*), and tape grass represented most of the aquatic vegetation with a substrate primarily comprised of rip-rap and gravel/cobble. The Ontario Street sampling site was also located near a boat ramp but contained a seawall structure. Sampling occurred from the seawall over deep (> 1.8 m) slow flowing water currents (0.15 \pm 0.09 m/s). Aquatic vegetation was sparse with Eurasian watermilfoil and various pondweed spp. representing the majority of the species at the site with a substrate consisting of primarily clay/silt/sand. The Aqua Lane Park sampling site also contained an active boat launch and seawall. Sampling occurred predominantly from the seawall in deeper (> 1.7 m) slow moving water (0.20 \pm 0.07 m/s). The site had moderate aquatic vegetation represented by leafy pondweed, sago pondweed, and tape grass with a bottom substrate consisting of clay/silt and sand/gravel.

The LaSalle Waterfront Park sampling site was located at a public park containing a dock and a concrete overlook structure used for sampling. The site was 1.8 m deep with slow moving water $(0.14 \pm 0.02 \text{ m/s})$ and little to moderate aquatic vegetation. Plant species were predominantly coontail and tape grass with clay/silt substrate. The Ellicott Creek sampling site was located near the confluence of Ellicott Creek and the Niagara River amongst bulkheads and various concrete structures around which sampling occurred. The site was deep ($>$ 3.4 m) with a substantially slow water velocity $(0.08 \pm 0.03 \text{ m/s})$ and little to no aquatic vegetation. Of the sparse vegetation, coontail, Eurasian watermilfoil and tape grass were present with a substrate of clay/silt.

Site habitat descriptions and water velocity data was obtained from the Emerald Shiner Project and collected by Steven Fleck and the Buffalo District United States Army Core of Engineers (USACE). The habitat assessment was done during peak aquatic vegetation in the summer of 2015. Although YOY fish data utilized in this study is from the previous year, observations during the 2014 and 2015 field seasons suggest little, if any, substantial changes in aquatic foliage, substrate composition, depth, and general water velocities at these sites. Due to this timeline discrepancy, this study only utilizes the descriptive data to better represent the sampling sites.

Table 1: YOY fish sampling sites used in this study along with their GPS coordinates and corresponding habitat types.

Figure 1: Map illustrating the location of YOY fish sampling sites used in this study in the upper Niagara River, NY. Natural sites (\triangle) and developed sites (\triangle) are depicted in the legend.

2.2 YOY Fish Collection and Processing

In 2014, larval and juvenile fish were collected weekly from each site from June to the end of August, and then bi-weekly from September to mid-October. Two nets were utilized for YOY seining in the natural sites: a 2.5m x 1m, 500 µm mesh larval seine and a 9m x 1.5m, 3 mm mesh juvenile seine. The larval seine was used in the early season when YOYs were smaller and, as they grew and became more evasive later in the season, the juvenile seine was used more frequently. The sampling of developed sites was conducted by field technicians and collections were made from shoreline areas or anthropogenic structures. Two nets were utilized at developed sites: a 0.5 m diameter 500 µm-mesh long-handled dip net and a 0.5 m diameter, 500 µm-mesh ichthyoplankton tow-net. The sampling effort at both natural and developed sites lasted until ~ 50 individual YOY fish were captured, regardless of species, and represented a random sample of the YOY fish composition. All collected specimens were sacrificed with MS-222 (Western Chemical Inc.) and preserved with 5% sugar-formalin for a few months before being switched to 95% ethanol for long term storage (Buchheister and Wilson 2005). Because preservation usually results in a loss of length and weight in fishes, a pilot study was performed to adjust the length and weight measurements of YOY emerald shiners before analysis (see Appendix A for pilot study).

A total of 6,564 YOY fish specimens were collected during this study and identified to Family level. Of these specimens, 6,228 YOY fish were identifiable to the Genus or Species levels. A total of 1,655 YOY emerald shiners were collected, 77 of which represented the larval stage (\leq 14 mm TL) (Flittner 1964, Auer 1982). YOY emerald shiners were measured in total length (TL) (mm) and weighed (W) (mg). A dissecting scope equipped with a high-resolution Olympus DP21 microscope digital camera (Olympus Corp. Tokyo, Japan) and measuring

software allowed for photos of YOY species to be captured for a reference library and to measure small specimens accurately. YOYs smaller than 20 mm were measured with the camera mounted software, while larger juvenile specimens were measured to the nearest 0.5 mm with a metric ruler due to the limited field of view of the dissecting scope. A top-loading balance was utilized to obtain weights to the nearest milligram of each specimen. Identification keys specific for YOY fishes of the Great Lakes region and other relevant fish keys were used to identify specimens to the lowest possible taxa (Auer 1982, Kay et al. 1994, Werner 2004). If positive species identification was not obtainable, as in a few cases, that specimen was recorded to the lowest taxa but excluded from species-level analyses. The only cases in which this type of higher taxonomic level data were recorded were for YOY redhorse suckers (*Moxostoma spp.*) and common/striped shiners (*Luxilus spp.*), in which species identification during early life stages is extremely difficult due to overlap in distinguishing morphological features (i.e. myomeres/fin ray counts) (Auer 1982, Kay et al. 1994). In circumstances in which samples contained over 50 individual YOYs from a collection event, randomized subsampling with a Folsom plankton splitter (Wildco® Saginaw, Michigan), a precise and widely accepted subsampling method for ichthyoplankton studies, was used to achieve roughly 50 individuals per sample for processing (Van Guelpen et al. 1982).

Water temperatures during the 2014 field season were collected at the natural habitat sites during each seining event. Water temperatures were recorded at the surface with a handheld YSI multiparameter (YSI Inc.). The temperature recordings were typically near shallow shoreline areas, thus potentially resulting in different water temperatures than that of the main channel of the upper Niagara River. These data were used to generate a line graph of the average water temperatures of the sampling sites used in this study throughout the summer of 2014 (Fig. 1).

2.3 YOY Emerald Shiner Size Structure, Growth, and Condition

Growth in fish can be represented in numerous ways, such as length-frequency distributions and average increases in length or weight over time in a given population (Weatherley and Gill 1987, Wetherall et al. 1987). Since YOY emerald shiners have been shown to have distinct cohorts with relatively little age-class overlap (Fuchs 1967, Schaap 1989), length-frequency distributions were used for this study. To further illustrate YOY emerald shiner growth, I calculated growth rates for both length and weight of upper Niagara River YOY emerald shiners following the analysis done in other YOY emerald shiner studies (Flittner 1964, Fuchs 1967, Schaap 1989). Emerald shiners, in general, display isometric growth and juveniles take on the relative body shape of adults early in development (Flittner 1964, Schaap 1989). Therefore, length-weight relationships (Eq. 1) and Fulton's condition factor (Eq. 2) can be appropriately applied to YOY emerald shiners:

(Equation 1) **W = aL^b**

$$
K = 100 \frac{W}{L^3}
$$

Where **W** is whole body wet weight, **L** is total length, **a** and **b** are parameters obtained from the length-weight relationship regression. The factor 100 in the condition factor equation is used to bring K closer to unity (Eq. 2) (Froese 2006).

Various representations of growth, length-weight relationships, and condition factors were used in previous studies that examined YOY emerald shiner populations in Lake Erie, Lewis and Clark Lake, and Dauphin Lake (Flittner 1964, Fuchs 1967, Schaap 1989). I conducted similar analyses so I could compare the YOY emerald shiner population from the upper Niagara River to those in other ecosystems.

2.4 YOY Fish Assemblage and Summer Diversity

To address the hypothesis pertaining to YOY fish species composition and diversity throughout the summer in the upper Niagara River, I examined percent numerical composition overtime and also analyzed diversity values obtained from the Shannon-Wiener diversity index (H'). The Shannon-Wiener diversity index (Eq. 3) is a widely used species diversity index by ecologists (Karydis and Tsirtsis 1996).

$$
\mathbf{H'} = -\sum_{i=1}^{S} \frac{n_i}{n} \times \ln \frac{n_i}{n}
$$

Where n_i is the number of individuals of the *i*th species in the sample, n is the total number of individuals in a sample.

2.5 Data Analysis

Length-frequency distributions were analyzed graphically to depict size structure changes in length (mm, TL) over the summer months (July-October) and to compare each individual sample site in the upper Niagara River cumulatively throughout the summer.

Growth of YOY emerald shiners in the upper Niagara River was determined as changes in length (mm) and weight (mg) throughout the summer. This was accomplished by plotting length or weight (y-axis) *versus* time (number of days, x-axis) from the first larval collection date (day 0). Linear regression analyses were then conducted to determine change in length (mm d^{-1}) and weight per day (mg d^{-1}) via the slope of the fitted regression line for each length or weightat-age (days) model respectively. Seining events conducted at natural sites were pooled by twoweek blocks allowing for comparisons of mean length and mean weight changes over time and length-frequency distributions were generated. These histograms were used to graphically project a growth curve, based on the von Bertalanffy growth equation (Eq. 4), showing the

modeled growth trajectory of YOY emerald shiners throughout the summer using the ELEFAN function in the fisheries program FiSAT II, as described by Pauly and Morgan (1987). Great Lakes specific emerald shiner growth parameters $(L_\infty, K, \text{ and } t_0)$ for the von Bertalanffy growth curve were used as the inputs for the model and were obtained from FishBase.org (Froese and Pauly 2005).

$$
(Equation 4) \qquad \qquad \mathbf{Lt} = L_{\infty} \left(\mathbf{1} \cdot e^{-k(\mathbf{t} - \mathbf{t}o)} \right)
$$

Where **Lt** is length at time, L_{∞} is asymptotic length, *K* is growth rate, and t_0 is the age at which length would theoretically be zero (von Bertalanffy 1938).

The length-weight relationship for YOY emerald shiners collected from natural sites throughout the summer was analyzed by fitting a linear regression after logarithmically transforming the data (base 10). Condition of YOY emerald shiners over the summer was determined by fitting a two-order polynomial trendline to the data.

One-way analysis of variance (ANOVA) tests were used to determine if YOY fish species diversity (H') was different among habitat types throughout the summer and if there was a difference in diversity among summer sampling months (June-October). YOY sampling sites were grouped by their respective habitat type (island, marsh, creek mouth, and developed) to test if diversity differed among these four categories throughout the summer. Also, a one-way ANOVA was conducted pooling all diversity values for each individual YOY sampling site for each month (June-October) to test whether YOY fish diversity differed monthly throughout the summer. For each ANOVA, the Levene's test was used to assess homogeneity of variance, in which none of the models showed significance (p-value > 0.05), indicating homogeneous variances. Tukey's HSD *post-hoc* tests were utilized for each model in which significance was

found. All statistical tests were performed using the statistical program R (R Development Core Team 2016).

3. Methods: Walleye and Steelhead Predation on Emerald Shiners

3.1 Predator Sampling Areas

Predatory fish were collected at Broderick Park, located near the headwaters of the upper Niagara River, NY (Fig. 2), because of its ease of shoreline access for sampling and the typically large concentration of forage fish in this area. The hydrology at this site entraps upstream swimming shiners in an eddy (Allen 2015, Sood 2015). Due to this feature, piscivorous fishes congregate in this area to feed, primarily during low-light conditions (*personal observation*).

A total of eighteen walleye were collected at Broderick Park, where a gyre forms by a recess in the concrete seawall with slower water velocities (Fig. 3) (Allen 2015, Sood 2015). Thirteen steelhead were collected upstream of the Peace Bridge, where water velocities were higher than downstream at Broderick Park (Allen 2015, Sood 2015). Both predator species were collected no further than 100 yards south of the Peace Bridge, and Lake Erie was roughly a half mile upstream from the southern edge of the study area. I observed that steelhead seemed to prefer areas of stronger water currents with large in-river structures, such as boulders, possibly as a means to ambush forage fish attempting to move upstream against the current. Conversely, walleye seemingly preferred to feed in the slower moving areas along the concrete wall and within the Broderick Park gyre in which forage fish congregated in large schools (*personal observation*). Due to these feeding habits and habitat preferences, both predator species were collected within the same study area but not necessarily at the same sites within that area (Fig. 3).

Figure 2: Map illustrating the predator sampling area in the upper Niagara River, NY in relation to New York State. The black box represents the general sampling area and is denoted in the legend

Figure 3: Map illustrating the areas near the inlet of the upper Niagara River in which walleye and steelhead were collected. The solid box indicates the general location in the river in which walleye were obtained while the dashed box represents the general areas steelhead were collected.

3.2 Predatory Fish Collection and Stomach Content Identification

Walleye and steelhead were collected during late summer and fall (August-October) in 2015. Both species were obtained via angling, using artificial baitfish lures during crepuscular feeding activity, as has been documented for walleye sampling (Swenson 1977). Boat based electrofishing during the 2015 field season in various areas of the upper Niagara River resulted in very low catch rates for these two species regardless of time of day, thus the use of angling was adopted for this study. Furthermore, angling has been shown to be a productive method in the Great Lakes region for diet studies of piscivorous fishes (Diana 1990, Clapsadl et al. 2005, Roseman et al. 2014). The predator samples had easily identifiable stomach contents with minimal stomach acid degradation. Sampling events were *ad hoc*, without a particular schedule and depended on weather conditions.

Upon capture, the specimen was immediately sacrificed to prevent regurgitation and labeled. Total length (mm) and weight (g) were recorded in the field using a metric ruler and handheld metric scale. The specimen was then transported to the Great Lakes Center Field Station in the Niagara River (Black Rock Canal) and the predator's stomach was removed, placed in a micromesh bag (Hubco Inc.), and preserved with 70% ethanol for a minimum of one day. Prior to the use of ethanol, some of the first stomachs collected were either analyzed fresh or preserved in 10% formalin; both methods allowed for successful stomach content analysis, but the ethanol was more effective at fixing tissue and less toxic than the formaldehyde. Therefore, 70% ethanol became the standard preservative. No attempt was made to convert preserved weights and lengths to wet weights and lengths of the contents since preservation time, regardless of preservative, was minimal.

Following preservation, stomachs were opened and the contents within the stomach were identified to the lowest taxonomic level (generally species) with a dissecting scope equipped with a high-resolution Olympus DP21 microscope digital camera and fish identification keys (Auer 1982, Werner 2004). In some cases, contents were extremely degraded and identified to group (i.e. shiner group; which represented the genera *Notropis*, *Notemigonus*, and *Luxilus*) or, in extreme cases, unidentifiable. All stomach contents were blotted dry and weighed individually except for prey fragments, which were pooled and weighed collectively. Depending on prey item condition, the specimen was measured in TL or standard length (SL, mm) if the caudal fin rays were absent. Identifiable specimens missing heads or too degraded to measure length reliably were not measured, but were included in species composition counts and also weighed. Recorded lengths were used for determining life stage (YOY or adult) of prey items. Using length-weight data and length-frequency distributions of emerald shiners collected in the upper Niagara River during the 2014 and 2015 field seasons (unpublished data), a 60 mm length was determined to be the maximum length a typical specimen could obtain in its first growing season.

3.3 Data Analysis

Percent numerical composition of prey items in walleye and steelhead stomachs included the life stage, either YOY (≤ 60 mm TL) or adult (> 60 mm TL), of the emerald shiners consumed. A two-tailed independent t-test, which assumed equal variances, was conducted to test whether walleye ($n = 10$) consumed a different size of emerald shiners than steelhead ($n =$ 7). Two dependent t-tests, which assumed unequal variances, were conducted to test whether the predators were of different sizes (length and weight). F-tests were conducted to assess homogeneity of variance for each t-test. All statistical tests were conducted using R statistical software (R Development Core Team 2016)

The index of relative importance (IRI) was used to calculate prey importance in walleye and steelhead diets at Broderick Park over the fall months using stomach contents results. The IRI is a compound index that takes into consideration percent by number (% N), percent by weight (% W), and percent frequency of occurrence (% O) of prey items in a species' diet and allows for a ranking for each prey item (Eq. 5). Since expressing the IRI as a percentage is suggested as a more robust index for greater cross species comparison of prey item importance, % IRI was calculated (Eq. 6), where *n* is the total number of prey item categories within a species' diet (Cortés 1997).

$$
IRIi = (\%Ni + \%Wi) x \%Oi
$$

(Equation 6) $\% \text{IRI}_i = 100 \text{IRI}_i / \sum \text{IRI}_i$ $i=1$

n

To determine if there was a potential life stage preference of emerald shiners in walleye and steelhead diets, YOY and adult emerald shiners had their own category within the IRI. Therefore, emerald shiners represent two distinct prey items within the index for each predator species.

4. Results

4.1 Population Size Structure of YOY Emerald Shiners

Water temperatures at the natural habitat sites in the upper Niagara River during the summer of 2014 were the highest at the beginning of the sampling period on July 1st (23.9 $^{\circ}$ C) and remained relatively constant until September, when the water temperature started to decrease (Fig. 4). Length-frequency distributions of YOY emerald shiners collected in the upper Niagara River during the summer (July-October) illustrate progressive modal (excluding July) growth in length (TL, mm) from larval appearance (July) until the onset of fall (October) (Fig. 5). There is an apparent bimodal distribution observed in the month of July, the first month in which larvae

were captured. The length-frequency distributions of July and August illustrate a drastic growth in length during this time, from an overall mean TL of 17.9 ± 0.26 (SE) in July to a mean TL of 29.6 ± 0.20 in August, an increase of approximately 12 mm. September and October had more similar length distributions with means, respectively, of 37.2 ± 0.30 and 41.2 ± 0.33 (Fig. 5).

YOY emerald shiners were found at each site sampled in this study during the summer except Beaver Island and Ellicott Creek, two "developed" habitat types dominated by man-made marinas. Length-frequency distributions of YOY emerald shiners showed differences among sites (Fig. 6). Developed habitat sites (Fig. 6a) had higher frequencies of larval emerald shiners when compared to sites from the other habitat types. Aqua Lane, however, showed a more even YOY emerald shiner size distribution (larvae and various juvenile sizes) over the summer (Fig. 6a). Few larval emerald shiners were collected from natural habitat types throughout the summer (Fig. 6b-d). Sandy Beach Marsh displayed a predominantly large juvenile emerald shiner distribution (30-40 mm), while Burnt Ship Creek had the fewest individuals collected (Fig. 6b). Motor Island had a relatively even juvenile emerald shiner length distribution structure (20-50 mm) and Strawberry Island had predominantly larger juvenile emerald shiners (35-50 mm) throughout the summer (Fig. 6c). Big Six Mile Creek and Spicer Creek had similar length distributions throughout the summer, with a few post-larvae (15-20 mm) and a broad range of juvenile emerald shiner sizes (20-55 mm) (Fig. 6d). Note the range of sample sizes for each site, which represent weekly (June – August) and bi-weekly (September – October) sampling efforts and are the total number of YOY emerald shiners collected at the site cumulatively over the summer.

Figure 4: Graph depicting the mean water temperatures of the upper Niagara River from natural habitat sites sampled in this study during 2014.

Figure 5: Length-frequency distribution of YOY emerald shiners (n = 1,653) collected in the upper Niagara River from July to October, 2014. Count (y-axis) represents the number of YOY emerald shiners at 1 mm total length intervals (x-axis).

a.) Developed

d.) Creek Mouth

Figure 6: Length-frequency distributions of YOY emerald shiners from each sampling site collected in the upper Niagara River during 2014 (cumulative). Frequency (y-axis) represents the number of YOY emerald shiners in each 2 mm total length interval (x-axis). Histograms are organized by habitat type for visual comparison: (a) developed, (b) marsh, (c) island, and (d) creek-mouth. $n =$ Sample size.

4.2 YOY Emerald Shiner Growth and Condition

YOY emerald shiners collected by seine at natural sites first appeared in the upper Niagara River in early July at a mean total length of 14.83 mm ($SE \pm 0.52$) and grew progressively larger until the last sampling events in mid-October, reaching an average length of 41.22 mm (± 0.33) (Fig. 7). On average, the YOY increased by 5.28 mm (± 0.74) in length every two weeks over the summer, and the observed growth was most rapid during the month of August, accruing an average increase in total length of 7.8 mm during that time. Regression analysis also suggested a significant increase in length over the summer, with YOY emerald shiners increasing an average of 0.22 mm d⁻¹ (y = 0.22x - 9129.3, R² = 0.55, p-value < 0.05, Fig. 8). Growth analysis using a von Bertalanffy growth curve also shows this rapid growth in length during the summer. Growth in length was most rapid from hatch (early July) to late August and then began to slow heading into fall (September-October), with little increase in length projected over the winter months (Fig. 9).

Weight (mg) displayed a similar trend to length, although more variable. The earliest captured YOY emerald shiners weighed an average of 11.9 mg (± 2.44) and reached a mean weight of 427.7 mg (± 11.31) in mid-October (Fig. 10). YOY emeralds shiners increased in weight by an average of 83.16 mg (± 17.82) every two weeks over the summer with weight increase most pronounced during August, the same as length, resulting in an average increase of 132.16 mg during that month. Regression analysis also suggested a significant increase in weight over the summer, with YOY emerald shiners gaining 4.5 mg d⁻¹ (y = 4.5x - 188519, R^2 = 0.46, p $value < 0.05$, Fig. 11).

Initially, YOY emerald shiners grew predominantly in length and began to increase more steadily in weight around 20 mm (TL) (Fig. 12). YOY emerald shiners displayed isometric

growth in body shape ($b = 3.17$, $a = 0.0032$) with length explaining almost all of the variance in weight as determined by a power function curve fitted to the data ($R^2 = 0.98$). A linear regression of the logarithms of weight and length showed that there was a significant positive relationship between the two measurements (F (1, 1,653) = 86,713, $R^2 = 0.98$, p-value < 0.05).

The Fulton condition factor of YOY emerald shiners was lowest post larval emergence (early July), with condition steadily increasing until late August (Fig. 13). Condition peaked in early September, remaining rather constant throughout the month, with a slight decrease in condition observed heading into October. YOY emerald shiner condition became increasingly more variable as the summer progressed with the model displaying a relatively poor fit ($R^2 =$ 0.17, Fig. 13).

Figure 7: Mean length (mm, TL) of YOY emerald shiners (n = 954) collected from seining sites in the upper Niagara River in 2014. Collection events were pooled by two-week sampling blocks throughout the study for temporal comparisons. Error bars, small but present, represent the standard error.

Figure 8: Length-at-age (days) for YOY emerald shiners (n = 954) collected with a larval seine in the upper Niagara River in 2014 (July-October). Day 0 represents the first date in which larval emerald shiners were collected.

Figure 9: Length-Frequency distribution of YOY emerald shiners (n = 954) collected from seining sites in the upper Niagara River bi-weekly throughout 2014 (July-October). Lengths represent 1 mm total length intervals (y-axis). A von Bertalanffy growth curve (*L[∞]* = 105 mm, *K* $= 0.93$ year⁻¹, $t_0 = 0$) was fitted to the histograms illustrating predicted growth in length.

Figure 10: Mean weight (mg) of YOY emerald shiners (n = 954) collected from seining sites in the upper Niagara River in 2014. Collection events were pooled by two-week sampling blocks throughout the study for temporal comparisons. Error bars, small but present, represent the standard error.

Figure 11: Weight-at-age (days) for YOY emerald shiners (n = 954) collected with a larval seine in the upper Niagara River in 2014 (July-October). Day 0 represents the first date in which larval emerald shiners were collected.

Figure 12: Length-weight relationship of YOY emerald shiners (n = 1,654) collected using a combination of gears (seine, plankton net, and dip net) in the upper Niagara River during 2014.

Figure 13: Condition (Fulton's K) of YOY emerald shiners (n = 954) collected using a seine in the upper Niagara River in 2014. A two-order polynomial is fitted to the data. The dotted line highlights the increasing breadth of the variability within the data.

4.3 YOY Fish Assemblage and Summer Diversity

Cyprinids and catostomids represented nearly all of the YOY fish specimens collected at sampling sites in the upper Niagara River during the summer months (Fig. 14), comprising 50% and 46% of all the fish collected respectively. Of the cyprinids, emerald shiners were the most prevalent species captured, accounting for 52% of the fish in the samples (Fig. 15). Bluntnose minnows (*Pimephales notatus*) and spottail shiners (*Notropis hudsonius*) were the second (27%) and third (9%) most numerous cyprinids captured during the study, with various other cyprinid species comprising the small numerical remainder of the family. Cumulatively, 9 families and 29 species were represented in this study, with emerald shiners (27%), redhorse suckers (23%), white suckers (16%), and bluntnose minnows (14%) the most numerous YOY fish species collected at sampling sites in the upper Niagara River (Table 2).

There was a significant difference among habitat types for YOY fish diversity over the course of the summer (one-way ANOVA, $F(3,66) = 9.639$, p-value < 0.05, Fig. 16). However, the only sites that were significantly lower in diversity were the developed habitat sites ($\bar{x} = 0.22$, SE = 0.11). The developed habitat sites had significantly lower diversity than island (\bar{x} = 0.84, $SE = 0.18$), marsh ($\bar{x} = 0.74$, $SE = 0.13$), and creek mouth sites ($\bar{x} = 0.75$, $SE = 0.18$), (*post-hoc* Tukey HSD, p-value < 0.05 , Fig. 16). There was no significant difference found in summer YOY fish diversity among the three categories of natural habitats studied (*post-hoc* Tukey HSD, pvalue > 0.05, Fig. 16). However, the islands had a higher mean diversity in general.

Month (June-October) had a significant influence on YOY fish diversity at the sampling sites within the upper Niagara River (one-way ANOVA, $F(4,65) = 3.648$, p-value < 0.05, Fig. 17). On average, summer YOY fish diversity was highest in July ($\bar{x} = 0.82$, SE = 0.12) and lowest in October ($\bar{x} = 0.18$, SE = 0.09). These mean diversity differences between July and

October were significant (*post-hoc* Tukey HSD, p-value < 0.05, Fig. 17). YOY fish diversity in June ($\bar{x} = 0.33$, SE = 0.07), August ($\bar{x} = 0.5$, SE = 0.18), and September ($\bar{x} = 0.45$, SE = 0.14) did not differ significantly among each other or when compared to July and October (*post-hoc* Tukey HSD, p-value > 0.05 , Fig. 17)

Figure 14: Pie chart representing the percent numerical composition of YOY fish families collected during sampling at the study sites in the upper Niagara River in 2014. Sample size (n) represents the number of specimens collected.

Figure 15: Pie chart representing the percent composition of YOY fish species belonging to the family Cyprinidae collected at the study sites in the upper Niagara River during 2014. Sample size (n) represents the number of specimens collected.

Table 2: YOY fish species collected in 2014 during the study in the upper Niagara River.

Taxonomy, total number collected (n), and percent numerical composition (%) of each species is given. Non-native fish species are denoted (ɬ).

ɬ Species not native to the Lake Erie watershed according to the Great Lakes Aquatic Nonindigenous Species Information System (USGS 2016).

Figure 16: Mean Shannon-Wiener diversity (H') at four different habitat types $(n = 4)$ within the upper Niagara River during 2014. Letters indicate significant differences between habitat types (*post-hoc* Tukey HSD, p-value < 0.05). Sample sizes are shown in the bars and error bars represent the standard error.

Figure 17: Mean Shannon-Wiener diversity (H') by month $(n = 5)$ at YOY fish collection sites in the upper Niagara River during 2014. Letters indicate significant differences between months (*post-hoc* Tukey HSD, p-value < 0.05). Sample sizes ($n = 14$ for each month) are shown in the bars and error bars represent the standard error.

4.4 Importance of YOY Emerald Shiners as Prey

Steelhead that consumed emerald shiners were both longer (mm) and heavier (g) (length: \overline{x} = 623.1, SE = 19.8, weight: \overline{x} = 2,342.3, SE = 148.9) on average than walleye (length: \overline{x} = 552, $SE = 14.6$, weight: $\overline{x} = 1,713.9$, $SE = 165.4$). Over 90% of the walleye's diet at Broderick Park was composed of various species of shiners, with emerald shiners representing 57% of the stomach contents (Fig. 18a). Round goby (*Neogobius melanostomus*), gizzard shad, smallmouth bass (*Micropterus dolomieu*), Johnny darter (*Etheostoma nigrum*), and yellow perch were also present but in low numbers. Of the emerald shiners represented in walleye stomachs, YOY was the predominant life stage consumed numerically at 66%, with adults representing the remainder of emerald shiners present (Fig. 19a). Similar to walleye, emerald shiners were the predominant forage in steelhead stomachs at the site, representing 59% of the numerical composition (Fig. 18b). Unidentifiable shiners and amphipods represented 14% and 11% respectively, with gizzard shad, yellow perch, round goby and dreissenid mussels present but in low numbers. In contrast to walleye, adult emerald shiners were the most consumed life stage in steelhead stomachs at 86% of the diet, with YOYs representing the remainder of emerald shiners present (Fig. 19b). A twosample t-test also suggested that walleye consumed significantly smaller emerald shiners (\bar{x} = 55.45mm, SE = 1.48, YOY size range) than steelhead (\bar{x} = 69.63mm, SE = 1.12, adult size range) at the Broderick Park sampling area (t $(140.43) = -7.62$, p-value < 0.05, Fig. 20).

Collectively, emerald shiners were the most important prey item for walleye during the fall months at Broderick Park (Table 3a). YOY emerald shiners were also the most numerous (40% N) prey item in walleye diets and occurred the most frequently (77% O), although it was second in importance by weight. YOY emerald shiners were the highest ranked (46% IRI) prey item within walleye diets, nearly double the second highest ranked item, adult emerald shiners

(25% IRI). Conversely, adult emerald shiners occurred the most frequently (46% O), represented the largest weight (49% W), and were the most numerous (53% N) prey item within the diets. Adult emerald shiners were the most important (72% IRI) prey item in steelhead stomachs (Table 3b). YOY emerald shiners were present within the stomachs of a third (31% O) of the steelhead sampled, but represented low numerical composition (9% N) and weight (4% W) and were the third highest ranked (6% IRI) prey item in steelhead.

Figure 18: Percent numerical composition of prey items consumed by walleye (a) and steelhead (b) at Broderick Park. N= 18 walleye with 144 individual prey items of various species and $N= 13$ steelhead with 135 prey items consisting of various species. The shiner group category represents prey items that could only be identified to the shiner grouping (see methods).

Figure 19: Percent numerical composition of YOY and adult emerald shiners consumed by walleye (a) and steelhead (b) at Broderick Park. N= 10 walleye which consumed 76 emerald shiners and N= 7 steelhead which consumed 79 emerald shiners.

Figure 20: Mean length (mm) of emerald shiners consumed by walleye $(n = 10)$ and steelhead $(n = 7)$ at Broderick Park. The number of shiners consumed by each species is significantly different and denoted by an asterisk. Error bars represent the 95% confidence intervals. Sample sizes of emerald shiners per each individual predator are contained within the bars and the dashed line represents the juvenile *versus* adult distinction (YOY ≤ 60 mm and adult > 60 mm).

Table 3: Percent number (% N), percent weight (% W), percent frequency of occurrence (% O), and percent Index of Relative Importance (% IRI) ordered by highest corresponding rank of prey items found within (a) walleye ($n = 125$) and (b) steelhead stomachs ($n = 129$).

a.) Walleye $(n = 13)$

b.) Steelhead $(n = 10)$

Prey Item	$\% N$	% W	$%$ O	$%$ IRI	Rank
Adult <i>N. atherinoides</i>	53	49	46	72	
Shiner Group	15		38	13	
YOY N. atherinoides			31	n	ш
P. flavescens		28			IV
D. cepedianum					
Amphipod spp.					
Unidentifiable Fish					VП
N. melanostomus					Ш

5. Discussion

The aim of this research was to obtain a better understanding of the ecology of juvenile emerald shiners in the upper Niagara River and their importance in predator diets. I found that the larvae appear (i.e., are recruited into our sampling gear) in late June when water temperatures are near 23° C, and continue to show up into early August. YOYs appear to be healthy, growing in both weight and length at a rapid rate. Larvae were found inhabiting marinas and back eddies generated by seawalls, often being the only YOY species present in these areas of the river. More natural habitat types within the river seem to be conducive to increased YOY fish diversity from an assemblage perspective. Furthermore, YOY emerald shiners, along with adults, are an important forage item for walleye and steelhead within the river, contributing to over 50% of their diet composition in late summer.

5.1 YOY Emerald Shiner Growth in the Upper Niagara River

Larval emerald shiners in the upper Niagara River recruited into our various sampling gears starting in July and into August (Fig. 5). The first larval emerald shiners detected in this study were collected at an average water temperature of $23.4^{\circ}C$ (Fig. 4). However, these first specimens were late into their post-yolk sac stage. Flittner (1964) determined development from egg to post-yolk sac larvae usually requires less than 7 days, and spawning typically lasts 3-4 weeks. Therefore, the collection of these first specimens which were further along in their larval development, suggests that the first spawning events most likely occurred in late June, with water temperatures closer to the documented spawning temperature of $22^{\circ}C$ (Auer 1982). This observation is similar to what has been reported for Lewis & Clark Lake and Dauphin Lake (Fuchs 1967, Schaap 1989). During July, upper Niagara River YOY emerald shiners reached a mean length of 14.83 mm (Fig. 7), larger than YOY in Dauphin Lake (10 mm, in 1984 & 1985,) but smaller than YOY collected in Lewis & Clark Lake (24 mm) (Fuchs 1967, Schaap 1989). YOY emerald shiners in the upper Niagara River grew progressively bigger in both length and weight throughout their first growing season, displaying average growth rates of 0.22 mm and 4.5 mg per day respectively (Fig. 8 & 11). In mid-September, nearing the end of the growing season, the YOY averaged 37.45 mm in length (Fig. 7). This is larger than Dauphin Lake (1984: 28 mm, 1985: 21 mm), similar to Lake Simcoe (38 mm, 1968), and smaller than what was observed in Lewis & Clark Lake (44 mm, 1965) and Lake Erie (52 mm, 1958) during that same month, suggesting potential early life stage growth differences between these ecosystems most likely induced by climatic and/or environmental characteristics (i.e. temperature, food availability, etc.) (Flittner 1964, Fuchs 1967, Campbell and MacCrimmon 1970, Schaap 1989).

The seasonal growth rate of the upper Niagara River YOY emerald shiners is similar to that in other studies, with August-September being the time of most rapid growth. This is most likely a result of peak water temperatures at that time (Fig. 4), since temperature is an important regulator of growth in emerald shiners, most likely resulting in the differences in size amongst populations from various lakes (McCormick and Kleiner 1976). Interestingly, Flittner (1964) suggested that Lake Erie YOY emerald shiners grew very rapidly and reached an average size of 52 mm by September, 38% larger than the upper Niagara River population during the same month. There are potentially two reasons why the upper Niagara River population does not reflect that of what Flittner (1964) observed for neighboring Lake Erie: 1) Collection methods between the studies were different, therefore influencing different sizes of fish captured in sampling gears and 2) The winter observed in 2014 was colder and more prolonged than normal, thus shortening the growing season and reducing fish growth through later arrival of zooplankton and slowed metabolism. However, Flittner (1964) suggested that his sampling gears may have

resulted in the inclusion of age-1 fish in his YOY analyses, skewing lengths at age (days) towards larger sizes than what most likely represented YOY emerald shiners in Lake Erie. Regardless, upper Niagara River emerald shiners appear to display growth similar to that of other populations, with fast growth during August, a typically productive month for YOY emerald shiners (Fuchs 1967, Schaap 1989). The von Bertalanffy growth model fitted for the YOY emerald shiners in this study suggested that the upper Niagara River population exhibits a similar growth trend as other populations within the region (Fig. 9), with little over-winter growth occurring as was also noted by Schaap (1989) and Flittner (1964).

Length-weight relationship of upper Niagara River YOY emerald shiners suggest that fish grew in length before accruing mass (Fig. 12). This is typical of many fish species and is a result of the necessity of larval fish to develop more complete body features (i.e. mouth, fins, etc.) early in life to sufficiently feed after yolk-sac absorption (Govoni et al. 1986). Condition of the YOY was, therefore, lowest post-hatch, then peaked during August-September and began to decrease slightly into the fall months (Fig. 13). This is a similar trend to what YOY emerald shiners in Dauphin Lake displayed in both 1984 and 1985 (Schaap 1989). The increase in variability in condition towards the end of the growing season suggests that this group of fish included various sizes and shape proportions entering the fall months (Fig. 13).This is possibly the result of a combination of early and late spawning events or the effects of the preservation methods used in this study. The ethanol used to preserve the specimens drastically reduced wet weight (48% decrease in body weight, Fig. A-1) possibly skewing the condition factor of individuals due to this drastic weight loss. However, correction factors were used for both weight and length to bring them back to their average wet weights and lengths, mitigating against the degree of variability the preservative had on the individuals used in this study. Overall, the upper

Niagara River YOY emerald shiners displayed a relatively higher condition than those in Dauphin Lake, most likely a result of higher temperatures and more food availability (Schaap 1989).

Thus, the YOY emerald shiners of the upper Niagara River appear to be healthy and growing well, reaching an average size for over-wintering of roughly 40-50 mm in length prior to the coldest winter months (Fig.9). Research of YOY emerald shiners in other bodies of water suggest that entering the winter months with a length of 38-52 mm is sufficient for high survival rates into the spring (Flittner 1964, Fuchs 1967, Schaap 1989). Since mortality is high during this crucial life stage (Fuchs 1967, Sogard 1997), a healthy YOY population that is not resource limited is suggestive of a productive ecosystem for this species in the upper Niagara River, a nutrient and plankton rich Great Lakes connecting channel (Edwards et al. 1988).

5.2 YOY Emerald Shiner Use of Riverine Habitats

Within the upper Niagara River, YOY emerald shiners utilized various locations comprised of different habitat types (Fig. 6). YOY emerald shiners develop proper swimming appendages rather quickly in early development (Flittner 1964, Auer 1982); therefore, it can be assumed that beyond the larval stage, these fish can choose where to go or stay, rather than happenstance, to maximize feeding opportunities, refuge availability, adequate current velocity, etc. The YOY population size structure at our sampling sites provided insight into the areas in the river in which YOY emerald shiners either: 1) chose to be at a site, 2) chose not to be at a site either entirely or during certain developmental stages, or 3) in the case of larval fish, were transported to a site by water currents, ultimately becoming entrapped (Brown and Armstrong 1985, Lechner et al. 2014).

 The YOY size distributions at specific sites in the river suggest that not all areas of the river are preferred by the same stages of development during the first growing season. The larval stage was predominantly represented at developed sites, such as Aqua Lane and Ontario Street (Fig. 6a). Interestingly, these two sites are in areas of the river in which the strong main channel current meets a seawall, thus generating a gyre. Gyres have been suggested to retain the larvae of marine pelagic species along coastlines, and this hydrologic feature could be the cause of why larval emerald shiners were detected at these sites more frequently than at other areas of the river (Menzies and Kerrigan 1980, Porch 1998). Other developed sites used by larvae were marinas. Within the river, these marinas could potentially function as embayments. Klumb et al. (2003) suggested that in the Great Lakes, embayments are a portion of the body of water separated by the main body of water by a narrow connection, such as an inlet or the entrance of a marina. Thus, these marinas serve as refugia from predators and fast currents and provide increased water temperatures that are beneficial to larval growth (Hall et al. 2003). Since developed sites typically represented gyres or artificial embayments, these two anthropologically-induced hydrologic features may explain why larval emerald shiners were found in these areas more frequently than in other locations in the upper Niagara River. Given the lack of swimming ability of newly hatched fish larvae against currents, it is logical to suppose that spawning may have occurred near these areas or larvae drifted into them resulting in entrapment. This is commonly seen in marine ecosystems where fish eggs and larvae become entrained in large recirculating oceanic eddies and gyres (Porch 1998, Govoni et al. 2013). Although much smaller in size, the upper Niagara River's gyres and eddies may result in larval fish retention.

The larger-size YOY emerald shiners were found mostly at the natural sites (Fig. 6b-c). This observation suggests that once the YOY had reached a sufficient stage of development to be able to swim into faster currents and search for better habitats, they moved to these potentially more suitable areas. Amongst these natural sites, creek-mouth habitats appeared to represent the broadest range of developmental stages throughout the summer (Fig. 6d). This habitat use by YOY emerald shiners has also been observed in the Kanawha River, a tributary of the Ohio River, where they utilized the backwater areas generated by the confluence of creek tributaries as refuge and rearing habitat after the larval stage (Scott 1988). It has also been suggested that tributaries of the upper Niagara River are significantly warmer than that of the main channel (Yagi and Blott 2012). This increase in water temperature, conducive to faster YOY growth (McCormick and Kleiner 1976), may also be a reason why all sizes of YOY appear at these confluence sites of tributaries into the upper Niagara River.

Most sizes of YOY emerald shiners in the post-larval stage were found at the island sites in the river, suggesting that spawning did not likely occur there but the island habitats were preferred by the larger YOY (Fig. 6c). Motor Island and Strawberry Island have shallow bays in which fish collection occurred. These bays potentially represent slack water 'storage' areas in the river in which zooplankton production, such as rotifers and cladocerans (an important food source for YOY emerald shiners) is typically high (Siefert 1972, Baranyi et al. 2002, Thorp and Casper 2003, Pothoven et al. 2009). Food availability in embayments and current refuge from the main channel flow could be why larger YOY emerald shiners used the island sites. Sandy Beach Marsh, a marsh site with slow water currents, could have also served this role, supplying food and providing refuge, as the size-structure of the YOY at the site was similar to that of the islands, primarily representing more developed fish (Fig. 6b). Interestingly, the other marsh site in the study, Burnt Ship Creek, had very low numbers of YOY emerald shiners. This suggested

that the area was not preferred by young emerald shiners, potentially a result of the site's shallow depths and sparse vegetation within our sampling area.

The YOY emerald shiner population size-structure from various sites and habitat types in the upper Niagara River throughout the growing season show: 1) developed sites contained the highest number of fish larvae, 2) marsh and island sites tended to have larger juvenile fish, and 3) creek-mouth confluence sites included almost all developmental stages. Although these results allow for the interpretation of YOY emerald shiner size-structures and their frequencies at these sites, abundance estimations cannot be determined from this data. Larval seine collections, although effective, are notoriously bias in representing population size and also determining accurate measurements of sampling effort. Therefore, information such as YOY emerald shiner abundance at these sites through the season, which was not addressed in this study, would help determine the degree to which these fish may select certain areas and habitats within the river.

5.3 YOY Fish Assemblages and Summer Diversity

From a family perspective, catostomids and cyprinids dominated the upper Niagara River juvenile fish community (Fig. 14). Of the cyprinid species, YOY emerald shiners comprised over half the numerical composition (Fig. 15). This suggests that YOY emerald shiners were potentially the most abundant juvenile. This is supported by a long-term survey of the upper Niagara River fish community undertaken by the Ontario Ministry of Natural Resources, which also found that emerald shiners were one of the most abundant species in the system (Yagi and Blott 2012).

The biodiversity of freshwater ecosystems is in drastic decline and it is urgent to acquire more information on the degree of diversity that still exists and determine the factors that influence diversity declines (Dudgeon et al. 2006). Fish are especially important in this regard,

adding to the resilience of the ecosystem. This is seen through their "portfolio effect", where diversity in species results in increased ecosystem functionality, producing a more stable system in which other fish and animal community members benefit (Tilman et al. 1998, Worm et al. 2006). This study found that YOY fish species diversity was significantly lower at developed sites when compared to more natural sites in the upper Niagara River (Fig. 16). Similarly, Wei et al. (2004) found that within the Great Lakes, fish frequency of occurrence was highest near more natural shorelines, such as marshes, with artificial shorelines representing one of the poorest areas of fish species occurrence. The low diversity at developed sites was most likely induced by the frequent disturbances (i.e. boat traffic and dredging activity) and less preferable habitats (i.e. concrete structures) at these sites, making them less than ideal for many YOY fish species in the river. Furthermore, these developed sites in mygh study had either no YOY fish during a sampling event or only a few species, one of which was typically emerald shiners. Since adult emerald shiners are considered to be attracted to hydrology in the places they prefer (Trautman 1981, Stewart and Watkinson 2004), it is possible that YOY emerald shiners would be one of the few species utilizing these sites on occasion. This finding is important since the synergistic effects of anthropogenic disturbances and climate change are projected to further disorganize fish communities and diversity in rivers in the future (Daufresne and Boët 2007).

Not only is it imperative to determine the habitats within a river in which diversity is poor, it is also important to understand if and for what potential reasons diversity changes throughout the season within the river. In the northern Indian River, Florida, fish diversity has been found to be highest in summer, when water temperatures are warmest, with some of the increase in species richness attributed to the influx of YOY recruits (Tremain and Adams 1995). This is similar to my study in which the majority of the fish species represented in my research

typically spawn in the early portion of the growing season when water temperatures begin to rise, generally during the months of June-August (Auer 1982). My study found that whole river YOY fish species diversity was highest in July (Fig. 17), suggesting that the greatest amount of spawning most likely occurred during this time, and/or the emergence of aquatic vegetation attracted species to these nearshore areas. Since July had significantly greater YOY fish species diversity than June, the new influx of YOY fish species, as a result of YOY emergence during July, could explain the increased diversity within the river during that month. Similarly, the appearance of aquatic vegetation within the upper Niagara River sampling sites during the early growing season most likely influenced further YOY fish migration to these nursery areas during July. This situation has been documented in the Mississippi River (pool 8, Wisconsin), where taxonomic diversity of larval fishes was deemed greatest at sites with emergent vegetation (Dewey and Jennings 1992). Apart from the early portion of the growing season, diversity was significantly lowest in October, the latest sampling month (Fig. 17). This was most likely due to YOY fish species reaching a more physiologically developed stage on average during that time, allowing for easier gear evasion or they joined emerald shiner schools in open water (A. Pérez-Fuentetaja *personal communication*).

 Understanding YOY fish diversity amongst habitats and how this diversity changes throughout the summer in the upper Niagara River is important groundwork for future restoration efforts in the river. Kapuscinski and Farrell (2014) sampled similar sites to those in this study in the upper Niagara River to determine fish assemblages in muskellunge nursery habitats and found that the upper Niagara River fish community was less diverse on average than that of areas sampled in the Buffalo Harbor and the St. Lawrence River. Furthermore, the Niagara River is plagued with both historic and current development, pollution, and

introductions of invasive species, lending to or contributing to the degradation of this ecologically important Great Lakes connecting channel. Therefore, identifying sites in the river in which diversity is lowest could lead to more focused restoration efforts to restore fish assemblages and their habitats.

5.4 YOY Emerald Shiner Importance as a Prey Item for Sportfish

Both walleye and steelhead were collected near the outlet of Lake Erie (head of the Niagara River) within the upper Niagara River during the fall months of 2015. Both species have been documented seasonally foraging in Lake Erie (Parsons 1971, Knight and Vondracek 1993, Clapsadl et al. 2005). However, during fall, when water temperatures are cooler, some individuals appear to venture into the river (*personal observation*) possibly in search of food. The primary forage item of walleye and steelhead collected at the Broderick Park sampling area (Fig. $2 \& 3$) in the upper Niagara River was emerald shiners (Fig. 18). Emerald shiners represented over half of what these predators consumed numerically over the fall, suggesting that emerald shiners were their preferred prey item and/or they were the most abundant prey item the predators encountered at the site during that time. This finding is similar to the diets of these fish reported for Lake Erie (Parsons 1971, Knight and Vondracek 1993, Clapsadl et al. 2005). Interestingly, there was a difference in numerical consumption of emerald shiners by life stage (YOY or adult) between the two predators (Fig. 19). Furthermore, there was a significant difference in the sizes (lengths, mm) of emerald shiners consumed by the predators, with walleye consuming smaller shiners while steelhead consumed larger shiners on average (Fig. 20).

Walleye appeared to predominantly predate on YOY emerald shiners, while steelhead primarily consumed adult emerald shiners (Fig. $19 \& 20$). IRI scores (Table 3) also suggest these life stage preferences between the two predator species. Walleye are suggested to have a narrow

scope for aerobic activity and that may be why they predated primarily on the less developed YOY emerald shiners (Peake et al. 2000, Pratt and Fox 2001). Furthermore, Knight et al. (1984) suggested that in Lake Erie, walleye prefer soft-rayed fishes, such as emerald shiners, and also are size selective eating smaller prey even as adults. In contrast, steelhead appeared to favor adult emerald shiners over YOYs. Results from the IRI analysis suggest that larger, adult emerald shiners were the most important prey item in steelhead diets at this location during fall (Table 2b). The pelagic foraging tendencies and rather efficient swimming capabilities of steelhead may allow them to forage for faster adult emerald shiners (Rowe 1984, Keen and Farrell 1994). However, it is possible that more YOYs were available to walleye than to steelhead due my earlier sampling of walleye (August/September) than of steelhead (September/October). It is also possible that the predators foraged in different habitats (i.e. substrate type, water velocities) that may have influence the size ranges of emerald shiners present in those areas. Regardless, this study provides strong evidence that emerald shiners of various sizes are an important food source for these two sport fish predators in the upper Niagara River.

Uncertainties have been raised about the importance of emerald shiners as a forage item for Great Lakes salmonids (Schaeffer et al. 2008). The results from this study suggest that steelhead, an annually stocked salmonid in Lake Erie, rely on emerald shiners heavily near the inlet of the upper Niagara River, at least in the fall. During sampling an interesting discovery was made, another salmonid species, a male adult coho salmon (*Oncorhynchus kisutch*) was collected. The specimen displayed a kype and was milting, indicative of potential spawning near the study area. Coho salmon were once stocked in Lake Erie by multiple state agencies; however stocking has long ceased, and any reports of coho salmon in Lake Erie are generally considered
strays from the upper Great Lakes (ODNR 2007). The specimen was processed similar to the other predators and inspection of the stomach contents revealed 17 emerald shiners, 13 of which were YOYs. Although Pacific salmon are generally considered not to feed during their spawning runs, the finding of emerald shiners in this salmonid stomach suggests that it is a potential food item for the species during other times of the year. Prior to the elimination of coho salmon stocking in Lake Erie, emerald shiners were considered a staple of their diet (Hartman 1972, Leach and Nepszy 1976). This unexpected finding further illustrates the versatility of emerald shiners in the diet of piscivorous fish species. Therefore, the health and abundance of this native cyprinid is important for piscivorous fishes, especially to recreationally important game fish species in the system.

This study was the first to investigate ecological aspects of the YOY emerald shiner in the upper Niagara River. In doing so, a few novel discoveries were made in this system: 1) YOY emerald shiners appear to be healthy, growing similarly to that of other populations, 2) natural habitat types have a more diverse YOY fish community, with emerald shiners inhabiting all habitat types and, 3) emerald shiners are important forage items for walleye and steelhead during the fall. These major findings not only give a benchmark of the YOY emerald shiner community for future research, but also highlight the importance of the health of the river for this small native cyprinid. Future research and conservation efforts focusing on protecting emerald shiner habitat in the upper Niagara River would benefit nearly every species that resides within this Great Lakes connecting channel. In a time of threatening climate change, habitat loss, introductions of invasive species, and legacy contamination, conservation of keystone species, like the emerald shiner, could help preserve aquatic diversity for future generations.

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7. Appendix A - YOY Emerald Shiner Length and Weight Conversion Factor

7.1 Methods and Results

The effect of the preservation methodology used in this study was assessed to determine changes in YOY emerald shiner length (mm) and weight (mg) over time in order to apply appropriate conversions before analyses. Fifteen YOY emerald shiners were collected, representing both larval and juvenile stages, weighed (nearest 0.0001 g) and measured (nearest mm), fresh.

After fresh measurements were recorded for each specimen, they were transferred to labeled vials containing 5% sugar formalin, similar to what was used in the field to preserve larval samples. Lengths and weights of each specimen were recorded two weeks, one month, two months, and three months after initial placement in the formalin solution. After the third month, specimens were transferred into a 95% ethanol solution for another month, after which final weight and length measurements were recorded, similar to what occurred in this study for the long-term preservation of collected YOY fishes. Lengths were recorded using either a dissecting scope equipped with a high-resolution Olympus DP21 microscope digital camera and measuring software for larval/smaller juvenile specimens, or a metric ruler for larger juveniles due to the small lens of the microscope. All specimens were weighed with the same metric balance.

Schaap (1989) conducted a similar preservation conversion study with adult emerald shiners preserved in 5% formalin and found minute changes in lengths/weights of specimens, in which conversions were deemed unnecessary for the study. This study however, utilized 95% ethanol after formalin fixation, thus prompting the necessity to test the effect of the added preservative to the overall shrinkage of preserved specimens. On average, YOY emerald shiners experienced a 4.59% (SE \pm 0.48) and 48.09% (\pm 7.06) decrease in length and body weight,

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respectively, following the preservation protocol (Fig. A1). Although percent decrease in YOY emerald shiner length was similar to what Schaap (1989) observed and found negligible, the 95% ethanol solution greatly reduced body weight by nearly a two-fold reduction. Due to this finding, the aforementioned mean percent change of both length and weight determined by the preservation conversion study was used to adjust the original lengths/weights of YOY emerald shiners analyzed in this study.

a.) Length (mm)

b.) Weight (mg)

Figure A1: Graph depicting the mean percent change from "wet" conditions of YOY emerald shiner $(n = 15)$ length (a) and weight (b) as induced from the preservation methodology used in this study. Error Bars represent the standard error. Preservatives and the time at which specimens were switched (black bars, x-axis) is denoted.