River plume effects on larval yellow perch (Perca flavescens) survival and predation in the western basin of Lake Erie

Lucia Beatriz Carreon Martinez
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RIVER PLUME EFFECTS ON LARVAL YELLOW PERCH
(Perca flavescens) SURVIVAL AND PREDATION IN THE
WESTERN BASIN OF LAKE ERIE.

By
Lucia Beatriz Carreón Martinez

A Dissertation
Submitted to the Faculty of Graduate Studies
through the Great Lakes Institute for Environmental Research
in Partial Fulfillment of the Requirements for
the Degree of Doctor of Philosophy at the
University of Windsor

Windsor, Ontario, Canada

2012

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River Plume Effects on Larval Yellow Perch (*Perca flavescens*) Survival and Predation in the Western Basin of Lake Erie.

by

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6 January 2012
DECLARATION OF CO-AUTHORSHIP / PREVIOUS PUBLICATION

I. Co-Authorship Declaration

I hereby declare that this thesis incorporates the outcome of a joint research undertaken in collaboration with Stuart Ludsin (The Ohio State University) and co-supervisor Timothy Johnson (Ontario Ministry of Natural Resources) under the supervision of Daniel Heath (University of Windsor). In all cases, the key ideas, primary contributions, experimental designs, data analysis and interpretation, were performed by the author, and the contribution of co-authors was primarily in an advisory capacity and through assistance from research collaborators.

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II. Declaration of Previous Publication

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<tr>
<td><strong>Chapter 2</strong></td>
<td>Carreon-Martinez, L. and D. D. Heath &quot;Revolution in food web analysis and trophic ecology: diet analysis by DNA and stable isotope analysis.&quot;</td>
<td>Published in Molecular Ecology</td>
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<td><strong>Chapter 3</strong></td>
<td>Carreon-Martinez, L., T. B. Johnson, S.A. Ludsin and D.D. Heath &quot;Utilization of stomach content DNA to determine diet diversity in piscivorous fishes.&quot;</td>
<td>Published in Journal of Fish Biology</td>
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<td><strong>Chapter 4</strong></td>
<td>Carreon-Martinez, L., T. B. Johnson, S.A. Ludsin and D.D. Heath “Genetic structure and survival of yellow perch (<em>Perca flavescens</em>) in western Lake Erie: the role of river plumes”</td>
<td>Submitted to Canadian Journal of Fisheries and Aquatic Sciences</td>
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ABSTRACT

The western basin of Lake Erie receives input from two main tributaries (Maumee and Detroit River), which differ greatly in their nutrient and sediment loading. The higher turbidity of the Maumee River plume is thought to reduce predation on early-stage juvenile yellow perch (*Perca flavescens*), consequently increasing their survival. For this reason, my overall objective was to evaluate the effect of the Maumee River plume on the overall recruitment of larval yellow perch to the juvenile stage. However, traditional diet analyses are not effective for evaluating larval predation rates. I therefore review genetic and non-genetic diet analysis techniques, and how they have evolved with technological advances, allowing researchers to effectively explore trophic interactions and energy movement in aquatic ecosystems. This provided a framework for my doctoral research for which I used a variety of molecular genetic techniques to estimate survival of larval yellow perch using population genetics and predation rates through stomach content analysis of predator fish.

Using yellow perch microsatellite markers, I measured temporal and spatial genetic structure in larval yellow perch, while Bayesian genotype assignment provided relative larval survival estimates for yellow perch inhabiting the Maumee and Detroit River plumes. Overall, genotype assignment of Age-0 yellow perch establishes that, in the western basin of Lake Erie, larval recruitment to the juvenile stage is significantly higher for fish inhabiting the Maumee River plume relative to those in the Detroit River plume. In addition, I utilized molecular genetic techniques to accurately identify highly digested early-stage juvenile prey to the species level which was not possible with a more traditional approach (visual inspection of gut contents). Specifically, I use polymerase
chain reaction (PCR), cloning and sequencing, to demonstrate the diversity of prey consumed by several freshwater fish species. Finally, using species-specific single nucleotide polymorphism (SNP) assays and microsatellite markers, I quantified predation and cannibalism experienced by larval yellow perch in the Maumee and Detroit river plumes. The combination of markers showed generally higher predation and cannibalism in the less turbid waters of the Detroit River plume, indicative of river-plume effects (possibly mediated through turbidity) reducing larval yellow perch predation mortality. My doctoral research shows the likely mechanism that river plumes in the western basin of Lake Erie contribute to larval yellow perch survival and consequently potential recruitment; however, temporal and spatial variability indicate that other factors also contribute.
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CHAPTER 1

GENERAL INTRODUCTION

The objective of this chapter is to provide a general background in the areas relevant to my research starting from the most general subject to the more specific objectives and goals of my dissertation.

THE GREAT LAKES

Lakes are widely distributed over the planet’s terrestrial surface wherever climatic and geological conditions permit accumulation of standing water. In general, freshwater ecosystems share the same trophic relationships as most other ecosystems. Lakes are best studied as components of their drainage basins because they receive most of their inputs from the terrestrial environment, often delivered by streams and rivers. Hence the physical and biological characteristics of a lake depend on the drainage basin or watershed they are part of (Kalff, 2002).

The Great Lakes basins lie on, or near, the western and southern edges of the Canadian (Precambrian) Shield, a large mass of very old (544 million years) metamorphic and igneous rock. This rock is very resistant to weathering, hence the lakes (or parts of lakes) that lie in the Precambrian area have low concentrations of dissolved solids. On the other hand, lake areas that lie outside the Precambrian area are in basins composed of younger sedimentary rocks that are more easily eroded, and therefore have higher concentration of total dissolved materials (Taub, 1984). Furthermore, the nutrient concentrations and productivity of such lakes are also greatly influenced by the composition of the rock in their drainage basins. For example, Lakes Michigan, Erie,
Ontario and parts of Lake Huron have elevated nutrient concentrations and productivity because their basins are formed with younger sedimentary rocks (Taub, 1984). The Laurentian Great Lakes are the product of complex interactions which make each “Great Lake” unique in its environmental and biological characteristics.

**LAKE ERIE**

Among the five Great Lakes, Lake Erie is the shallowest and most biologically productive. It is divided into three basins which differ in their physical, chemical, and biological properties. Temperature and primary productivity decrease and depth increases, from west to east, the same direction that water flows, therefore the western basin of Lake Erie is the most productive one (Hartman, 1972; Ryan et al., 2003). The northern and southern waters of the western basin are strongly influenced by waters from Lake Huron via the Detroit River and by nutrient-rich waters from the Maumee River, respectively (Charlton 1994). Although Lake Erie receives inputs from many different rivers, the main source of water comes from the upper Great Lakes by way of the Detroit River (contributing ~80% of the total annual water; Bolsenga and Herdendorf 1993). These unique characteristics contribute to make the western basin of Lake Erie a highly productive environment.

Lake nutrient concentration and dynamics affect the composition and structure of the food web, ultimately driving the fish community. Phosphorus, the key nutrient for Lake Erie, varies along a declining gradient from west to east (Ryan et al, 2003), as well as from nearshore to offshore (Bolsenga and Herdendorf 1993). Human impacts have caused extensive changes to the physical and chemical properties of Lake Erie and its tributaries, contributing to the decline in the quality and quantity of fish habitat.
(Trautman 1981; Hartman 1972; Bolsenga and Herdendorf 1993). For this reason, it is important to better understand the interactions between living organisms and their physical environment.

**LARVAL FISH ECOLOGY AND RECRUITMENT**

Fish mortality is highest during the egg and larval early life stages, due to a combination of stochastic events (e.g. storms, flooding, temperature fluctuations, toxicity, etc.) and to biotic factors such as predation, competition and food availability (Houde, 1987), that interact to regulate early life mortality (Houde, 1989; 1991). Starvation and predation on larval fish are considered to be major factors regulating recruitment variation (Hunter 1981, Houde 1987, Leggett and Deblois 1994). Reduced food availability at the onset of larval “first feeding” can cause high mortality due to starvation (i.e. match-mismatch hypothesis; Hjort 1914). Furthermore limited food availability can reduce larval fish growth rate, thus increasing their vulnerability to predation (Shepherd and Cushing, 1980; Miller et al., 1988). Although mortality during the early life stages is clearly critical for understanding fish population dynamics, it is extremely difficult to quantify and is likely to vary enormously from year to year due to the variety of factors that affect it. These complexities lead to very low success in prediction of fish recruitment.

**YELLOW PERCH**

The environmental characteristics of the western basin of Lake Erie, such as depth, temperature and primary productivity, make that water body a very important environment for yellow perch spawning and also as a nursery for larval yellow perch (Goodyear, 1982). Yellow perch spawn in shallow water (depths of 6 m or less) along
Lake Erie shorelines and in tributaries, over sand, mud, and rooted aquatic vegetation (Goodyear, 1982). Eggs are deposited in flat, ribbon-like masses and peak spawning occurs at water temperatures of about 10-12 °C. Egg incubation lasts about 8-14 days, and most hatching occurs in the western basin during early May (Wolfert et al., 1975; Scott and Crossman, 1998).

**Predation on Yellow Perch**

Predator–prey interactions, especially during the early life stages, are very important in shaping the year class of a population. Several studies have reported that yellow perch recruitment success is highly affected by predation (Forney 1971; 1974; Lyons and Magnuson, 1987). Furthermore, low abundance of yellow perch is inversely related to predator abundance for a variety of predator species: alewife *Alosa pseudoharengus* (Brandt et al., 1987; Mason and Brandt, 1996); walleye *Sander vitreus* (Nielsen, 1980; Hartman and Margraf, 1993; Hall and Rudstam, 1999); northern pike *Esox lucius*, largemouth bass *Micropterus salmoides*, smallmouth bass *M. dolomieu* (Liao et al., 2002). In addition, yellow perch adults also have been show to cannibalize yellow perch larvae (Hartman and Margraf, 1993; Truemper and Lauer, 2005).

Often predation and cannibalism are studied by diet analysis using traditional visual methods; however, the identification of larval fish in stomach or gut contents of predatory fish is very challenging and often inaccurate due to their rapid digestibility (Legler et al. 2010), for this reason I used molecular genetic techniques as a tool to identify and quantify larval yellow perch in the stomach contents of predatory fish.
Molecular Genetic Techniques

In the last 30 years, advances in molecular genetic research have provided new tools for use in biology and ecology, providing solutions to previously unanswerable questions. In addition, the field of molecular genetics has evolved tremendously, with state-of-the-art technology that allows the processing of high number of samples in a short period of time at reasonable cost (King et al., 2008; Carreon-Martinez and Heath, 2010). Molecular genetic studies have a tremendous potential in aiding ecological researchers to better understand species ecology and their interactions in the environment. From measurements of genetic diversity and/or genetic structure of a population, to gene expression in differential habitats and ecological forensics, molecular genetic techniques will continue to improve and provide better tools for ecological research (Avise, 1994). In particular, molecular genetic techniques have been used to identify prey to species level in the stomach, gut contents, or feces of a variety of aquatic predators: fish (Rosel and Kocher, 2002; Saitoh et al., 2003), seals (Parsons et al., 2005), penguins (Jarman et al., 2002) giant squid (Deagle et al., 2005), whales (Jarman et al. 2004), among others. Such an approach is particularly valuable in the identification of fish larvae in stomach contents, since traditional visual methods are time consuming and often inaccurate due to the rapid digestion of larval fish in predator stomachs (Brandt et al., 1987; Legler et al., 2010). In chapter 3, I utilized cloning and sequencing for molecular identification of stomach contents, however as I realized the limitations of this technique for the identification of stomach contents, I decided to use an innovative approach (i.e. single nucleotide polymorphisms, SNPs) to identify yellow perch in the stomach contents of conspecifics (chapter 5) and cannibalism (chapter 6).
**River plumes in the western basin of Lake Erie**

River discharge in coastal areas has been directly associated with higher primary production and larval fish abundance (Grimes and Finucane, 1991; Salen-Picard *et al.*, 2002; Morgan, 2005). In freshwater systems, river discharge is also responsible for delivering higher temperatures, nutrients, dissolved organic carbon (DOC), organic and inorganic sediments and phytoplankton (Mortimer, 1987; Moll *et al.*, 1993). Previous research in Lake Erie has demonstrated a strong positive relationship between springtime Maumee River discharge and yellow perch *Perca flavescens* recruitment at age-two in the western basin (Ludsin, unpublished data). However the mechanisms as to how river discharge affects yellow perch population levels (recruitment) are unclear. One possibility is that springtime Maumee River discharge benefits yellow perch recruitment via bottom-up control of food production for pelagic larvae (Grimes and Kingsford, 1991; Hunter and Price, 1992; Chassot *et al.*, 2007) or by reducing predation mortality on larval fish resulting from higher turbidity (top-down; McQueen, *et al.*, 1989). For this reason, the western basin of Lake Erie is an optimal area for studying river plume effects on the population genetic structure and predation effects on yellow perch larvae using sensitive molecular genetics techniques. As part of a larger Great Lakes Fishery Commission project designed to examine river plume effects on yellow perch recruitment in western Lake Erie, my dissertation objectives are to:

i. explore molecular genetic techniques as a possible tool for identifying fish prey items in stomach contents of fish.

ii. quantify genetic population structure in larval yellow perch occupying the Maumee River plume and Detroit River plume habitats;
iii. determine if larval yellow perch occupying the Maumee River plume and Detroit River plume habitats exhibit differential survival to the juvenile stage (Age-0); and

iv. to test whether predation and cannibalism differ between river plumes and if they are a factor contributing to differences in survival between the Maumee and Detroit River plume

Chapter 2 is a review of the evolution and application of molecular techniques used to study trophic interactions in aquatic ecosystems. Chapter 3, I use molecular genetic techniques (cloning and sequencing) to identify fish prey species in the stomach contents of predator fish. In chapter 4, I assess yellow perch larval survival to the juvenile stage (i.e. Age-0) by using population genetics analysis coupled with genetic assignment. Chapter 5 describes the development of a yellow perch specific single nucleotide polymorphism assay (SNP) to compare predation levels between the Maumee and Detroit river plumes. In chapter 6 to better quantify predation experienced by larval yellow perch I analyzed stomach contents of yellow perch to quantify the role of cannibalism in early life mortality using a SNP assay, coupled with genetic microsatellite data to distinguish between predator and prey. In addition I explored the existence of cannibalism in walleye using the same techniques used for yellow perch.

The molecular genetic techniques developed in this work and the quantified levels of predation and cannibalism can contribute in the improvement of fisheries management strategies to better understand and predict the causes of fluctuations in abundance of economically important species such as yellow perch.
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CHAPTER 2

REVOLUTION IN FOOD WEB ANALYSIS AND TROPHIC ECOLOGY:
DIET ANALYSIS BY DNA AND STABLE ISOTOPE ANALYSIS ¹

REVIEW

Characterization of energy flow in ecosystems is one of the primary goals of ecology, and the analysis of trophic interactions and food web dynamics is key to quantifying energy flow. Predator-prey interactions define the majority of trophic interactions and food web dynamics, and visual analysis of stomach, gut or fecal content composition is the technique traditionally used to quantify predator-prey interactions. Unfortunately such techniques may be biased and inaccurate due to variation in digestion rates (Sheppard & Hardwood 2005); however, those limitations can be largely overcome with new technology. In the last 20 years, the use of molecular genetic techniques in ecology has exploded (King 2008). The growing availability of molecular genetic methods and data has fostered the use of PCR-based techniques to accurately distinguish and identify prey items in stomach, gut and fecal samples. In the first issue of Molecular Ecology Resources, Corse et al. (2010) describe and apply a new approach to quantifying predator-prey relationships using an ecosystem-level genetic characterization of available and consumed prey in European freshwater habitats (Fig. 2.1a).

In addition in issue 19 of Molecular Ecology, Hardy et al. (2010) marry the molecular genetic analysis of prey with a stable isotope (SI) analysis of trophic interactions in an Australian reservoir community (Fig. 2.1b). Both papers demonstrate novel and innovative approaches to an old problem – how do we effectively explore food webs and energy movement in ecosystems?

Alternative and imaginative methods for diet analysis have been used since 1946, starting with immunological techniques and continuing with sophisticated DNA-based methods, paralleling technological advances in molecular ecology (Fig. 2.2). Immunological techniques used to identify prey are diverse and include antigen-antibody interactions in solution (e.g. agglutination, precipitation reactions, immunoelectrophoresis) as well as solid-phase techniques (e.g. ELISA, radio-immune assays; Boreham and Ohiagu 1978). Indeed, immunological techniques are still used and are extremely helpful (Fig. 2.2) even though the technique is labor intensive and costly.

Stable isotope (SI) analysis (primarily carbon (reported as $\delta^{13}C$) and nitrogen ($\delta^{15}N$) is another, more widely applied, technique that has been used since the 1970’s to characterize food webs (Deniro and Epstein 1978; 1981). Isotope fractionation events in living organisms most often result in the enrichment of the heavier isotope of nitrogen (increase in $\delta^{15}N$) relative to food items, providing a relative estimate of trophic position. Carbon SI values show less enrichment between diet and organism but often vary between photosynthetic sources (e.g., aquatic phytoplankton vs. terrestrial plants) and habitats (i.e., marine vs. freshwater) and thus can be used to characterize carbon sources of organisms and food webs (see Hardy et al., 2010; Peterson and Fry, 1987). DNA-PCR based techniques have been utilized for the identification of prey items from stomach, gut...
or fecal contents using DNA hybridization (Rosel and Kocher, 2002), cloning and sequencing (Deagle et al., 2005) and presence/absence of diagnostic PCR products on agarose gels (Gorokhova, 2006), among others (see Teletchea, 2009). Molecular genetic techniques used for diet analyses range from straightforward to more complex approaches that utilize cutting edge molecular genetic technology, such as DNA microarrays (Hardy et al., 2010) and high-throughput parallel sequencing (Pegard et al., 2009). However, technically simple and widely accessible approaches such as that developed by Corse et al., (2010) provides a powerful tool for the characterization of complex food webs that can potentially be used by ecologists not familiar with advanced molecular techniques. Furthermore, the approach developed by Corse et al. (2010) is faster and less expensive than more sophisticated molecular genetic techniques, and thus could be used for applied management or conservation purposes. Technological advances are ongoing and new methods may emerge that we have not considered yet, moreover, by combining existing technologies we can overcome limitations inherent in some techniques and gain new and clearer insights into ecological processes.
Figure 2.1. The aquatic habitats used for two studies of diet and trophic interactions that employed molecular genetic and stable isotope analyses. Panel A: Example of Rhone basin habitat (France) where fish diet was determined using PCR to classify prey to a series of ecological clades. Panel B: A weir pool on the lower Murray River (Australia) where food web and prey use was evaluated using a combination of advanced molecular genetic and stable isotope analyses.
Figure 2.2. The number of published studies (per 5- or 10-year interval) using indirect methods for diet determination from gut or fecal content analysis. The solid line shows the number of studies using molecular genetic techniques, while the dashed line shows the studies using immunological techniques.
In Molecular Ecology Resources Corse et al. (2010) describes an innovative technique to analyze trophic interactions in complex food webs via diet analysis. Corse et al. (2010) characterized prey communities by grouping them into ecological clades, where the clades were defined by molecular genetic and habitat similarities. Hence their goal was to characterize diet, and thus trophic interactions, by functional and genetically similar prey groups. They designed 34 sets of 18S ribosomal RNA gene (rDNA) PCR primers that identify all ecological clades in seven different microhabitats in a European river ecosystem. By having a complete database of prey types available in each habitat, they minimized the possibility of underestimating (or missing altogether) diet components not amplified by existing or novel “universal” primers. Furthermore, their work allows analysis of which prey is preferred, that is, predator electivity. Using this approach Corse et al. (2010) successfully show subtly different feeding habits in three closely related species of fish in the same ecosystem.

In a related study in Molecular Ecology, Hardy et al. (2010) combined SI and molecular genetic diet analysis to achieve greater resolution in their food web analysis of a freshwater pool community on the lower Murray River in southern Australia. Their goals were to determine the source of organic matter entering their study ecosystem and whether there was significant seasonal variation in the source of the organic matter. For these ambitious goals, they used two conceptually different approaches; SI analysis and DNA-PCR based diet analysis. SI analysis can detect trophic interactions that are not expected, but often fails to provide specific trophic interactions because isotopic values in potential prey can overlap. Hardy et al. (2010) also used PCR to amplify rDNA subunit
regions and the subsequent sub-cloning and sequencing allowed the authors to identify
diet to available metazoan, fungal, protozoan and plant taxa. The authors also develop a
DNA microarray printed with synthetic rDNA oligonucleotides which is then hybridized
with gut content PCR products to identify prey species. The advantage of a microarray
approach is that it is cost effective and large number of environmental or gut samples can
be screened quickly. Although the technological aspects of Hardy et al.’s (2010) food
web analyses are impressive, of more conceptual interest are their comparisons of the
results from the two approaches. The SI analysis allowed them to identify food web
anomalies that were not evident based on the DNA-PCR approach (i.e., terrestrial carbon
input and seasonal changes driven by the action of methanogens). However, their DNA-
PCR approach provided more specific trophic (predator-prey) interaction information
than would have been possible from SI analysis alone. One of the most important aspects
of this study is the potential for using such a sensitive approach for early detection of
anthropological and natural environmental changes in the ecosystem. Although Hardy et
al. (2010) were able to get a more detailed picture of food web dynamics (transfer of
carbon and energy through the food web) than in Corse et al.’s (2010) study, Hardy et
al.’s (2010) technologically advanced approach is perhaps better suited to experimental
applications to model systems, since it requires substantially greater technical
infrastructure and expertise.

Ongoing change in the environment is inevitable, especially in the face of new
environmental challenges such as climate change or invasive species. It is thus critically
important to have tools to effectively quantify early responses in the community.
Characterizing predator-prey interactions is a very important component of ecosystem-
level studies, particularly because some species will modify their diet in response to environmental change or perturbation. Corse et al. (2010) and Hardy et al. (2010) provide novel and innovative approaches to indirect diet analyses, and both studies highlight the potential for such trophic analyses to detect environmental changes due to anthropological effects. Indirect diet analyses are becoming increasingly common in ecological research (Fig. 2.2) and this reflects the critical need for such information in ecology and conservation. A quantitative understanding of predator-prey dynamics and potential food sources will not only better define trophic interactions and food web structure, it will help us better understand community ecology at a fundamental level.

REFERENCES


CHAPTER 3

UTILIZATION OF STOMACH CONTENT DNA TO DETERMINE DIET DIVERSITY IN PISCIVOROUS FISHES

INTRODUCTION

The direct study of inter-species relationships (e.g. predation and competition) in aquatic ecosystems is logistically difficult, yet accurate characterization of feeding habits can provide useful insight which results in better management of the ecosystem in general. For example, predation during early life stages is reported as a major factor limiting recruitment success for many fish species (Rosel and Kocher, 2002; Saitoh, 2003; Santucci and Wahl, 2003). The study of predation in the early life stages of fish by means of diet analysis is very challenging, mostly because larval and early juvenile prey fish loose all identifiable characteristics within 30-60 min after ingestion (Schooley et al., 2008; Legler et al., 2010). Therefore, traditional visual techniques for diet analysis are often limited in their ability to identify all prey items. Moreover, traditional diet analysis methods often ignore, or proportionally re-allocate (relative to identifiable material), stomach contents which are not recognizable. When digestion is well advanced, traditional approaches cannot identify any prey, potentially omitting important information. In so doing, our understanding of larger-scale processes such as food web interactions and energy flow through an ecosystem can be biased.

Although traditional diet analysis continues to be a useful component in ecological studies due to its low cost and logistical ease (Andraso, 2005; Roseman et al., 2009), in recent years these data have been complemented with other more technologically advanced approaches, including fatty acid analysis, stable isotope analysis and DNA-based diet determination techniques (Schmidt et al., 2009, Corse et al., 2010, Hardy et al., 2010). Fatty acid and stable isotope analyses can provide a broad picture of energy flow through the food web, but fail to give specific information on predator-prey interactions, which often is needed, particularly in more complex ecosystems (Guest et al., 2009; Elsdon, 2010; Hardy et al., 2010). On the other hand, DNA-based techniques have been successfully implemented to identify prey items in stomach, gut or feces samples (reviewed in Symondson, 2002). Moreover, existing molecular databases (e.g. NCBI, BOL) have fostered the implementation of molecular genetic techniques to identify a broad array of possible diet items with often very limited and degraded DNA (Carreon-Martinez and Heath, 2010). One of the advantages of DNA-based techniques for prey identification purposes is that successful amplification can be achieved in samples that usually are not in optimal condition (i.e. feces, gut contents) as it only requires a small amount of tissue for DNA extraction (Teletchea, 2009).

Despite the wide use of molecular techniques to identify stomach contents, there is still limited information regarding the effects of time since ingestion and temperature on the ability of DNA-based assays to identify prey items in vertebrates, particularly in the stomach contents of fish. Saitoh et al. (2003) and Smith et al. (2005) successfully identified prey DNA from unidentifiable stomach contents (mucus and pelleted debris and muscle, respectively) of predatory fish. Rosel and Kocher (2002) detected larval cod
(Gadus morhua L.) DNA in laboratory experiments 12 hours after ingestion by Atlantic mackerel (Scomber scombrus L.). The latter evidence suggests that DNA-based techniques can become the bridge between accurate diet analysis and samples with unidentifiable gut material obtained from long net sets (often 16-24 hours) often used in fisheries science. Undoubtedly, visual diet analysis still has a role in fisheries and fish biology research, but molecular tools increase our ability to identify a more complete determination of diet, and also provide objectivity in the identification of prey items.

The Laurentian Great Lakes represent the largest freshwater ecosystem in the world, and supports a large number of vulnerable and economically important fish species (Fuller et al., 1995). However, the Great Lakes ecosystems have been modified by the introduction and spread of non-native species (Munawar et al., 1999, Steinhart et al., 2004; Roseman et al., 2009; Gozlan et al., 2010; Stokstad, 2010) and climate change (Magnuson et al., 1990; Gozlan et al., 2010). Hence it is imperative to develop rapid and accurate techniques to monitor and characterize species interactions in the Great Lake ecosystems as they change over time. Successful ecosystem management and fisheries forecasting depends on timely and accurate data on species interactions, especially predation and competition.

This paper describes a series of laboratory experiments designed to further explore the value of using molecular genetic techniques (PCR, cloning and sequencing) to identify prey items in stomach contents of predatory fish to the species level, as well as to determine the separate effect of temperature and time post-ingestion on the ability of molecular genetic techniques to accurately identify larval and early juvenile fish DNA in stomach contents of predatory fish. Furthermore, the developed protocol is used to
identify fish and unknown prey material in the stomach contents of four of the primary piscivorous fishes (white bass (*Morone chrysops* (Rafinesque)), white perch (*Morone americana* (Gmelin)), walleye (*Sander vitreus* (Mitchill)) and yellow perch (*Perca flavescens* (Mitchill)) in the Western basin of Lake Erie.

**Materials and Methods**

This section describes two related, but quite distinct protocols. First, we describe laboratory experiments to evaluate how temperature and time separately influence the ability of genetic DNA-based techniques and traditional visual techniques to identify prey items in fish stomach contents, and second the application of the molecular genetic approach to wild-caught Lake Erie predators is described.

*Laboratory experiments.*

*Collection of specimens.*

Three species of adult fish were collected in the Bay of Quinte (Lake Ontario, Ontario, Canada), to use as predators in laboratory studies: bluegill sunfish, *Lepomis macrochirus* Rafinesque, (mean ± S.D. total length (*L*<sub>T</sub>) = 188 ± 13 mm), pumpkinseed sunfish, *Lepomis gibbosus* (L.) (*L*<sub>T</sub> = 170 ± 19 mm) and rockbass, *Ambloplites rupestris* (Rafinesque) (*L*<sub>T</sub> = 197 ± 30 mm). The predators were fasted to clear their digestive tracts prior to experimental trials. Prey consisted of five different species of larval and early juvenile fish: lake whitefish, *Coregonus clupeaformis* (Mitchill) (mean ± S.D. wet mass (*M*<sub>W</sub>) = 0.34 ± 0.28 mg), lake trout, *Salvelinus namaycush* (Walbaum) (*M*<sub>W</sub> = 0.41 ± 0.17 mg), brook trout, *Salvelinus fontinalis* (Mitchill) (*M*<sub>W</sub> = 0.27 ± 0.12 mg), brown trout,
Salmo trutta L. (M_w = 0.32 ± 0.16 mg) and splake, S. namaycush (W.)-X-S. fontinalis (M.) F1 hybrids (M_w = 0.75± 0.28 mg) obtained from White Lake Provincial Fish Culture Station, Sharbot Lake, ON. Prey size differences were significant among species (ANOVA, d.f. = 4, P <0.05). Prey total length (L_T) was estimated from measured wet weight using a regression based on a sub-sample of individuals for each species. Predators were force-fed prey to ensure accurate determination of time post-ingestion. At specified time intervals, predators were euthanized with clove oil and their stomach was dissected and placed in 95% ethanol for further examination.

The laboratory experiments were designed to evaluate the effect of temperature and time on digestion, separately. For temperature trials, L. macrochirus predators were acclimated to three different temperatures (10°C, 20°C and 30°C), fed a single species prey item and allowed four hours to digest (n= 21, 10 and 18, respectively). Our choice of temperatures was based on the expected temperatures (8°C - 20°C) in Lake Erie for early-stage juvenile fish, while newly emerged larvae would experience temperatures near 10°C. To test for the effect of digestion time, sunfish predators (L. macrochirus and L. gibbosus) were also fed a single prey item and held at 20°C for 1, 2, 4, 10, 16 and 24 hours between feeding and euthanasia (n= 5, 15, 10, 13, 5 and 6 respectively). Finally, to test for multiple prey discrimination power, 13 L. macrochirus, 2 L. gibbosus and 12 A. rupestris were fed multiple prey items simultaneously (one individual from two or three different species at a time) and they were given 4.5 hours to digest at 20°C. The identity of prey items in all experiments was not revealed (i.e., blind test) until all stomach content and molecular genetic analyses were complete, to obtain unbiased results.

Laboratory diet analysis
Prior to examining stomach contents, the exterior surface of each stomach was cleared of all possible material that might contaminate the contents (e.g. blood, tissue attached to stomach from predator, etc.). Subsequently, each stomach was opened with clean scissors and tweezers, and the contents flushed into a weighing dish with 95% ethanol. Care was taken to only remove contents by flushing or physical removal with tweezers, to avoid scraping cells from the predator stomach lining. In all cases, all material in the stomach was removed as potential residue of prey fishes.

Visual inspection.

To quantify differences in the state of digestion, stomach contents were visually ranked using a categorical grading system slightly modified from that described in Schooley et al. (2008). The categorical systems is: (5) fully intact (easily recognized as a fish larva/juvenile); (4) partially digested but with identifying characters intact (e.g., lenses still attached to head, melanophores visible, tissues with some identifiable shape or consistency but possibly separated into multiple pieces); (3) extensively digested and exhibiting no intact identifying characters (e.g., lenses detached from head, some sort of matter available but without larval form); (2) some prey material visible but negligible for any physical description or measurable mass; and (1) no material present (fully digested, empty stomach).

The stomach content samples were then further processed for molecular genetic analysis of prey species. When muscle fragments were found, each fragment was patted dry and a small (10-15 mg) tissue sample was transferred to a single well in a 96 well plate for DNA extraction. If there was partially digested matter it was transferred by pipettor to a 0.7 ml tube for subsequent centrifugation (13,000 rpm for 20 minutes) and
DNA extraction. If the stomach was empty, I still thoroughly rinsed the stomach lining with ethanol, and transferred the ethanol to a 1.7 ml tube for centrifugation and DNA extraction.

**Molecular identification of prey items in stomach contents**

**DNA extraction.**

After removal of all the ethanol in the sample, DNA was recovered from stomach contents and/or prey tissue samples following the plate-based extraction method (Elphinstone *et al.*, 2003) and resuspended in 50 uL of Tris–EDTA buffer (10 mM Tris, 1.0 mM EDTA, pH 8.0).

**PCR amplification.**

As the objective of this experiments was to identify only fish prey items, I selected universal CO1 primers that would amplify DNA of all fish species in the Great Lakes; Fish R1 5’ –TAGACT TCTGGGTGGCCAAAGAATCA -3’ and Fish F1 5’ –TCAACCAACCACAAAGACATTG GCAC- 3’ described in Ward *et al.* (2005). The PCR mixture contained 2.5 μL of DNA template, 1×PCR buffer, 1 μl of each primer (0.1 μg μl⁻¹), 1 μl deoxy-nucleoside triphosphate (dNTP) (200 μM) and 0. 5 U Taq polymerase (Sigma-Aldrich; www.sigmaaldrich.com), in a total volume of 25 μl. Positive and negative controls were used each time to test for PCR reaction quality and contamination. The PCR conditions consisted of an initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 10 s, 55°C annealing temperature for 15 s, 72°C for 30 s, with a final step at 72°C for 5 min. PCR products were visualized on 1.8% agarose gels prior to cloning and sequencing to verify amplicon presence and size (655 bp).
Cloning and sequencing.

Approximately 2 µL of PCR product was ligated into a pGEM-T vector (Promega) according to the manufacturer’s instructions, and the ligation was transformed into competent *Escherichia coli* DH5 cells. Eight clones with the correct size insert for sequencing were selected, and DNA was recovered using thermal lysis (Sambrook *et al.*, 1989). This number of clones was chosen after sequencing 30 clones in three different samples and in all cases we found all of the prey species identified in the 30 clone sample (up to five different species) within the first eight clones sequenced. Plasmid DNA and the inserted fragment were amplified using universal M13 primers and the resulting fragment was sequenced using ABI BigDye Terminator version 3.1 on an ABI 3130xl Genetic Analyzer. Sequence data were aligned and manually edited using Mega 4 (Tamura *et al.*, 2007). Three samples with failed PCR amplification (i.e. no visible PCR product in agarose gel) were selected at random and cloned to determine if CO1 amplicons existed at very low concentrations such that they could not be visualized on an agarose gel.

Identification of prey items and sequence similarity analysis.

To classify the prey item sequences to the closest match in the CO1 sequence database (Hubert *et al.*, 2008), all CO1 sequences from prey items were aligned to sequences from the database with Vector NTI 9.0. To determine which species each sequence represented, a similarity analysis between the sequences was utilized, estimated by the Neighbour Joining algorithm (Saitou and Nei, 1987) contained within the program Vector NTI 9.0. Those sequences that had over 95% similarity were considered a
successful match. Successful identification of prey items in laboratory experiments (i.e. time and temperature) using our molecular genetic approach was given a value of 1.0 and failed identifications a value of 0.0 for quantification purposes. In multiple prey species experiments a percentage of prey species successfully identified with the molecular genetic approach from the total prey in each stomach was used.

Statistical analysis.

All data (i.e. predator length, prey size, molecular genetic success and visual ranking data) was explored for normality using a Shapiro-Wilk test in SPSS v11.0. Molecular genetic success and the visual assessment of digestion (ranking) data violated normality assumptions for ANOVA, therefore Kruskal-Wallis non-parametric analyses was used. The effect of predator size (LT) and prey size (MW) on the visual identification of prey items (i.e. rank data) and on the molecular genetic identification success (i.e. binomial data) was tested using non-parametric Spearman rank correlation. Additionally, the effect of predator species was tested using a Kruskal-Wallis non-parametric test when necessary. All the statistical analyses were Bonferroni corrected for multiple simultaneous comparisons.

In the multiple prey species experiments there were no visual ranked data, therefore a Kruskal-Wallis non-parametric test was performed to quantify predator species and predator size effects on the percentage of larvae correctly identified using the molecular genetics protocol.

Field evaluation

Collection of specimens
Piscivorous *M. chrysops, M. americana, S. vitreus*, and *P. flavescens*, were collected for diet analysis in the western basin of Lake Erie. Collections were made with a bottom trawl (7.6-m semi-balloon design, 13-mm stretched-mesh cod-end liner) in the spring of 2006. Trawls were conducted with an average tow time of 18 minutes (range: 5-31 minutes) at a boat speed of about 3-4 knots. Upon retrieval of the trawl, fishes were euthanized with clove oil, stomachs were injected with 100% ethanol to halt digestion, and whole fishes were frozen for future laboratory analysis. In the lab, each fish was thawed, measured (TL nearest 1 mm), and stomachs were removed for diet analysis.

*Diet analysis*

Diet analysis of Lake Erie predators involved separating prey items into major taxonomic groups, under a dissecting microscope (see Legler, 2009). The visual identification of stomach contents entailed all types of prey items, but for the purpose of this study, only samples that contained unidentifiable fish remains or unidentifiable fish remains and other unidentifiable material (e.g. chyme) were used. Prey items and stomach contents were stored in 95% ethanol prior to the molecular genetic analyses. DNA extraction, PCR amplification, cloning, sequencing and sequence similarity analysis were performed in the same manner as explained above.

**RESULTS**

**Laboratory experiments**

Digestion Temperature: The effects of temperature on digestion and the ability to identify stomach contents either by traditional visual analysis and molecular genetic technique were evaluated. The ability to identify remaining prey items 4 h after ingestion
solely by traditional visual analysis was low at 10°C and declined further with increased temperatures (Fig. 3.1). Using the molecular genetic approach, the frequency of failed PCR amplification increased as temperature increased (total n=3, 3 and 11 failed PCRs at 10, 20 and 30°C respectively). The Kruskal-Wallis analysis indicated a significant temperature effect on our ability to identify remaining prey items with traditional visual analysis (Kruskal-Wallis, d.f. = 2, P <0.05). The Spearman rank correlation analysis, after Bonferroni correction, showed no significant effect of prey size or predator size on the visual prey identification at any of the three temperatures (Supplementary Table 3.1). There was a significant effect of temperature on our ability to identify stomach contents using the molecular genetic approach (Kruskal-Wallis, d.f. = 2, P <0.05). On the basis of the Spearman rank correlation analysis, after Bonferroni correction; neither prey size nor predator size had a significant effect on the ability to identify prey using DNA-based techniques at any of the three temperatures (Supplementary Table 3.1). Predator species also had no significant effect on the visual prey identification or on the molecular genetic success based on Kruskal–Wallis analysis. Although a general declining trend with increasing temperature was found in the ability to identify prey remains with both methods, the molecular genetic approach offers better resolution at all of the analysed temperatures, 4 h post-ingestion (Fig 3.1).
Figure 3.1. The effect of environmental temperature on the identification of stomach contents by visual and molecular genetic methods. Left Y axis: Mean rank values of visual prey identification (with standard error) at each temperature treatment. The rank reflects the physical appearance of the prey items after a 4 h digestion time, with 5 being an intact prey and 1 an empty stomach. Right Y axis: The proportion of successful (%) DNA-based prey species identification. Empty stomachs and failed PCRs were scored as unsuccessful molecular prey identification.
Digestion Time: Post-ingestion time had a significant effect on the ability to identify prey in stomach contents using traditional visual analysis (Kruskal-Wallis, d.f.= 5, P <0.05). Additionally, the Spearman rank correlation analysis revealed no significant effect of prey size or predator size on visual prey identification after Bonferroni correction (Supplementary table 3.2). Furthermore, there was no predator species effect on the visual identification of prey items (Kruskal-Wallis analysis; Supplementary material 3.2). In the digestion time experiments, all samples with partially digested remains were identified correctly to species level after one and two hours using the molecular genetics protocol. Treatments of four hours and longer had an increased frequency of failed amplifications as well as a higher incidence of stomachs without any physical remains (Supplementary table 3.2) (Fig. 3.2), hence the decline in our success rate of identifying prey items using the molecular genetic technique. Even though time post-ingestion had a significant effect on our ability to accurately identify prey species using molecular genetic technique, (Kruskal-Wallis, d.f.= 5, P <0.05), the molecular genetic approach provided accurate identification of unidentifiable matter even 16 hours post-ingestion (Fig 3.2). Based on the Spearman rank correlation analysis (after Bonferroni correction) there were no significant effects of prey size or predator size on our molecular genetic prey identification success at any of the sample times. Additionally, no predator species effect was found on the ability to identify prey items using the molecular genetic technique.
Figure 3.2. The effect of digestion time on the identification of stomach contents by visual and molecular genetic methods. Left Y axis: Mean rank values of visual prey identification (with standard error) at each time period. The rank reflects the physical appearance of the prey items, with 5 being an intact prey and 1 an empty stomach. Right Y axis: The proportion of successful (%) DNA-based prey species identification. Empty stomachs and failed PCRs were scored as unsuccessful molecular genetic prey identification.
Multiple Species: To examine species identification resolution in the digestion process, I performed multiple prey species experiments using two or three species of predators. In 18 of 24 trials, the molecular genetic approach correctly identified and discriminated all prey items (Supplementary table 3.3). Predator species or predator size did not affect success in identifying multiple prey species in the digestion experiments, (Kruskal-Wallis, d.f.= 2, P >0.05). Of the 24 trials (predators), 13 were fed three different prey species, and in two cases the molecular genetic technique successfully identified only two of three prey species (in all other cases all prey were correctly identified). The remaining 11 predators were fed two different prey species, and in four samples we identified only one of the two prey species. Typically, the smaller of the prey species were not identified when the analysis failed to identify all prey species present.

The molecular genetic approach did not identify the predator DNA signal in any of the laboratory experiments (n=127 predators). The cloning and sequencing of the subset of “failed PCR’s”, (n=3) yielded short fragments of DNA (approximately 50 bp) whose sequence did not correspond to any CO1 sequence, nor did it BLAST to another gene fragment for any taxonomic group in the NCBI database; therefore, the remaining failed PCR amplification samples were not cloned and sequenced.

Field evaluation results.

The molecular genetic approach improved my ability to identify diet items in piscivorous wild-caught fishes in which original visual inspection failed to identify any identifiable prey remains (i.e. identified as “fish remains” or “other material”). The molecular technique provided much greater taxonomic resolution than the visual analyses of stomach contents. For *S. vitreus, M. chrysops* and *M. americana*, emerald shiners
(Notropis atherinoides Rafinesque) were found to be the dominant food item across individuals (Fig. 3.3). S. vitreus, round goby (Neogobius melanostomus (Pallas)), and M. americana also were found in M. chrysops and S. vitreus stomachs. P. flavescens showed greater diversity in diet, consuming N. melanostomus, trout-perch (Percopsis omyscomaycus (Walbaum)), common carp (Cyprinus carpio L.), and freshwater drum (Aplodinotus grunniens Rafinesque), but no N. atherinoides (Fig. 3.3). Potential cannibalism was identified in all four predator species (Fig. 3.3). Given that predator DNA was not detected in any of the laboratory trials (n = 127), the molecular genetic results probably represent evidence for true cannibalism, although it is possible that at least some of the evidence for cannibalism is due to predator DNA contamination.
Figure 3.3. DNA-based identification of prey items for four species of wild-caught predators taken from western Lake Erie, Canada–U.S.A. (a) *Morone americana* (*n* = 7), (b) *Morone chrysops* (*n* = 20), (c) *Perca flavescens* (*n* = 6) and (d) *Sander vitreus* (*n* = 14), the number of predators with their own species DNA signal in their stomach contents, and thus potential examples of cannibalism. The numbers underneath the identified prey species is the number of fish with that particular prey item in their stomachs. The number of fishes with each prey item adds up to more than the number of predators because some predator fishes had more than one prey item identified.
**DISCUSSION**

This work demonstrates that molecular genetic techniques can identify prey fish species after much longer digestion times than possible with visual methods. The proportion of stomachs with “unknown material” and “unidentified fish” is substantial in most diet studies (30-36%, Legler, 2009; Mullowney, 2001), clearly molecular genetic techniques would have proven useful in identifying prey species in such cases. The diets of wild-caught predators were described with greater taxonomic resolution than possible with the traditional visual methods (e.g. typically “fish” and “chyme”). The enhanced prey species resolution obtained with molecular genetic techniques will prove especially useful in future environmental studies in which accurate and detailed information on predator-prey interactions would be of major value.

Mortality due to predation in larval and early juvenile fishes has been identified as a major contributor to low recruitment success in a variety of fish species (Bailey and Houde, 1989; Hartman and Margraf, 1993; Houde, 1997). However, the detection and identification of small, soft-bodied prey items (such as larval or early-stage juvenile fish) is beyond the ability of traditional visual diet analyses to reliably detect or identify to species (Schooney, 2008; Legler *et al.*, 2010). It is thus common to fail to detect any larval or early-stage juvenile fish remains in stomach content samples (Legler, 2009). While proper characterization of predator diet is important, accurate identification of larval prey (especially larvae of economically important or endangered species) will provide vital information for estimating overall mortality losses and consequently will aid in the management of the species. The laboratory feeding experiments performed here showed that successful identification of small prey items can be achieved even sixteen
hours post-ingestion. Many fisheries programs routinely set gear ‘overnight to one day’ (16-24 hours) which will minimize the likelihood of finding small soft-bodied prey such as larval or early-juvenile fish. Molecular genetic techniques will improve the detection probability for small, young prey fish, as well as add greater taxonomic resolution to the diet analysis.

The success of the molecular genetic stomach content analysis was not affected by prey body size or predator species, and, although the number of failed PCRs did increase with higher temperatures, the molecular genetic approach was less sensitive to the effect of temperature than the visual approach. This represents an important benefit for utilizing molecular genetics for diet analysis in fisheries science. On the contrary, successful identification of larval or small juveniles in stomach contents using traditional visual methods is often limited to a 1-2 hour digestion time (Folkvord, 1993; Schooley et al., 2008; Legler et al., 2010), and the critical digestion time is highly dependant on water temperature (Legler et al., 2010).

The molecular genetic approach clearly shows higher taxonomic resolution in the determination of fish prey items compared to traditional description of stomach contents in the Lake Erie predators. *N. atherinoides* were a common prey in most predatory fish species, which is not surprising given they are an abundant, soft-rayed, and small-sized prey fish in western Lake Erie (Knight and Vondracek, 1993; Ludsin et al., 2001; Tyson and Knight, 2001). Our results, based on the molecular genetic technique, also confirmed the importance of the invasive *N. melanostomus*, and the consumption of less commonly expected food sources such as *A. grunniens*, *P. omyscomaycus*, and *C. carpio* as alternative food sources, particularly for *P. flavescens*. Seasonal variability in the
abundance of prey fish, among other factors, is also a major factor influencing the feeding habits, distribution and ultimately recruitment success of fish species. The Great Lakes are experiencing unprecedented ecological change, and fish diets are responding (Tyson and Knight, 2001; Johnson et al., 2005). Due to the higher sensitivity of molecular genetics analysis, characterization of the diet (and hence diet changes) is attainable using smaller sample sizes strategically collected (e.g. diurnal and seasonal collections) without the need to sacrifice too many fish, since most sampled fish will provide diet data using molecular techniques (i.e., fewer “empty” stomachs). The potential to use gastric lavage instead of lethal sampling was not explored here, but certainly represents a potentially useful approach.

Interestingly, the molecular genetic protocol for diet analysis described in this paper detected predator DNA in the stomach contents of *S. vitreus*, *P. flavescens*, *M. americana* and *M. chrysops* collected in Lake Erie. There are two possible explanations for this observation: 1) contamination from the predator stomach tissue, or 2) cannibalism. Cannibalism has been shown to potentially play an important role in the survival and recruitment success of several species (Persson et al., 2002; Stetter et al., 2007). Although cannibalism has been reported for *P. flavescens* (Thorpe, 1977; Cook et al., 2001; Mullowney, 2001), *S. vitreus* (Chevalier, 1973; Forney 1976; Scott and Crossman, 1998; Mullowney, 2001) and *M. americana* (Scott and Crossman, 1998), the detection of cannibalism in much smaller sample sizes (as is shown here), suggests that cannibalism may be more prevalent in the population than formerly thought. It is unlikely that the detection of the predator DNA in the stomach samples of wild fishes is entirely an artifact due to predator DNA contamination because there was no evidence of predator DNA contamination.
DNA in the 127 fishes analyzed in the laboratory experiments, nor was it detected in all of the wild-caught fish stomachs. However, further investigation of these results with microsatellite markers to test for a genotype match with the predator is underway.

Although the identification of small fish prey is possible using DNA techniques up to 16 hours post-ingestion, the use of shorter net set times is advised to accurately assess predation of rare or early-life stage fish. PCR amplification of smaller DNA size fragments (<600 bp) could improve the success of molecular genetic prey identification past 16-24 hours of digestion, thus allowing longer net sets and providing greater flexibility in the processing of the captured predators. Molecular genetic techniques do not greatly complicate field logistics since stomach contents can be fixed for DNA extraction by the injection of ethanol in the field. Furthermore, the cost of the molecular genetic analysis is not much higher than the labour costs associated with conventional methods of diet analysis. More importantly, this study demonstrates the potential application of molecular genetic techniques for monitoring feeding habits and inter- and intra-species interactions that may drive recruitment success even in large complex ecosystems such as the Laurentian Great Lakes.

REFERENCES


Supplementary Table 3.1

Laboratory experiments to determine the effect of digestion time on our ability to identify prey items using the traditional (visual identification) method vs. the molecular genetic technique of cloning and sequencing. All experiments were conducted at 20°C.

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<th>Prey wet weight (g)</th>
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<th>Mean visual rank ± SD</th>
<th>Molecular success</th>
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BG= bluegill; PKS= pumpkinseed; TL= total length; SD= standard deviation;

Visual rank: rank system of visual identification of stomach contents from 5 (fully intact larvae) to 0 (empty stomach). Molecular success: identification of prey item to species level a value of 1 to a successful identification and value of 0 to a failed identification. NS= no significant effect of predator length or prey size on visual ranking or molecular success and no significant species effect on visual ranking identification or molecular success.
**Supplementary Table 3.2**

Laboratory experiments to determine the effect of temperature on the ability to identify prey items using the traditional (visual identification) method or the molecular genetic technique of PCR cloning and sequencing. All predators had 4 hour digestion time.

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<th>Mean visual rank ± SD&lt;sup&gt;NS&lt;/sup&gt;</th>
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BG= bluegill; PKS= pumpkinseed; TL= total length; SD= standard deviation; Visual rank: rank system of visual identification of stomach contents from 5 (fully intact larvae) to 0 (empty stomach). Molecular success: identification of prey item to species level a value of 1 to a successful identification and value of 0 to a failed identification. NS= no significant effect of predator length or prey size on visual ranking or molecular success, NS= no significant species effect on visual ranking identification or molecular success.
Supplementary Table 3.3

Laboratory experiments with multiple species of prey items fed to a single predator to determine the efficiency of the molecular genetic technique of PCR cloning and sequencing to identify all prey to species level. All predators had 4 hour digestion time.

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<th>Number of species correctly identified$^{NS}$</th>
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<td>RBS 9</td>
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</table>

BG= bluegill; RBS= rock bass; TL= total length; NS= no significant effect of predator length or prey size on visual ranking or molecular success and no significant species effect on visual ranking identification or molecular success.
CHAPTER 4
GENETIC STRUCTURE AND RECRUITMENT OF AGE-0 YELLOW PERCH
(PERCA FLAVESCENS) IN WESTERN LAKE ERIE: THE ROLE OF RIVER
PLUMES

INTRODUCTION

Fish population dynamics are complex processes that respond to a large number of variables, making it difficult to determine what contributes to healthy populations with high recruitment success (Fuiman and Werner, 2002). A better understanding of the interactions between larval rearing habitats and early life survival is critical for effective management and conservation of fish populations, especially those that are commercially exploited. Yellow perch (YP) is an economically and ecologically important species in the Laurentian Great Lakes; however year class strength of YP in the Great Lakes varies considerably from year to year, introducing uncertainty into management decisions (Tyson and Knight, 2001). The western basin of Lake Erie is a particularly important habitat for YP production in the Great Lakes (Goodyear et al., 1982), yet the ecological mechanisms underlying the variation in year class strength are not well understood. One possible mechanism may be the relative difference in nutrient content from two large rivers that discharge into the basin (Maumee and Detroit rivers) creating two distinct water masses (Fuller et al., 1995; Porta et al., 2005). River discharge in marine coastal and freshwater areas has been directly associated with higher nutrient concentrations, increased primary production and increases in larval fish abundance (Mortimer, 1987; Grimes and Finucane, 1991; Salen-Picard et al., 2002). The Maumee River, discharging into the southern part of the western basin of Lake Erie, is characterized by higher
turbidity, productivity, and temperatures compared to the Detroit River which discharges into the northern portion of the basin. It is plausible therefore, that larval fish produced in the Maumee River water mass of the western basin may experience higher age-0 recruitment, and indirectly, this may affect genetic population structure. For this reason, studying the effects of river plumes on larval fish in important freshwater ecosystems, such as YP in Lake Erie, is of great value for fisheries research.

In the last decade, population genetics has become a useful and powerful tool to better understand fine-scale mechanisms in aquatic environments (O’Connell and Wright, 1997; Ward, 2000). Single-species population genetics depend to a great extent on dispersal and gene flow (Slatkin, 1985; 1987). Gene flow could be constrained or enhanced by environmental conditions (e.g., natural barriers, water currents, storms, etc.) (Slatkin, 1987; Parker et al., 2010). Additionally, population genetics can provide indirect estimates of population size, productivity and dispersal, providing data for improved and novel methodologies in fisheries management (Carvalho and Houser, 1994; Begg and Waldman, 1999; Nielsen et al., 2010).

Here I use highly polymorphic microsatellite markers and population genetic analyses to explore the role of river-plume associated turbidity variation on dispersal and survival in early stage YP in the western basin of Lake Erie. The specific objectives of this study were to: 1) test for spatial genetic structure in larval YP, 2) estimate larval recruitment to age-0 in the high turbidity river plume relative to less turbid waters, and 3) test for temporal variation in population genetic structure. My analyses test the hypothesis that larval YP have a recruitment advantage in the high turbidity southern section of the western basin of Lake Erie. Such analyses have important implications for predicting
recruitment success of this economically important species, in addition to improving our understanding of the complex ecology of Lake Erie.

**MATERIALS AND METHODS**

**Study site**

Larval YP were collected weekly at up to 12 sites from late April through mid-June in 2006, 2007 and 2008 within both northern (Detroit River plume) and southern (Maumee River plume) areas of the western basin of Lake Erie (Figure 4.1). Moderate-Resolution Imaging Spectroradiometer (MODIS) 250-m resolution, true colour, near real-time imagery from the Terra and Aqua satellites (http://coastwatch.glerl.noaa.gov/) was used to determine the boundaries of the Maumee and Detroit River plumes based on turbidity, evident in the images.

**Fish collection**

Larval YP were collected using oblique (~1 m from bottom to surface) plankton tows with paired 1 m diameter bongo nets (500 μm mesh) on a weekly basis during late April through mid-June in 2006, 2007 and 2008. Larvae were preserved in 100% ethanol until identification in the laboratory (Auer, 1982). At each collection site, turbidity was estimated using a 5-cm path transmissometer (SeaBird SBE19). Turbidity was used to select sampling sites in the southern and northern areas of the basin, hence providing objective criteria for delineating two distinct larval habitats for the genetic analyses. Additionally, in 2006 I obtained larval YP from Sandusky Bay, OH (Figure 1) collected in the same fashion.

Young-of-the-year (YOY; Age 0+) YP were collected throughout the western basin in late August of each year via bottom trawling (10.7-m headrope; 13-mm cod-end...
liner; 3 km/hr tow-speed). YOY YP were collected from 36, 50 and 48 sites in 2006, 2007 and 2008 respectively, as part of the annual assessment surveys conducted by the Ontario Ministry of Natural Resources and the Ohio Department of Natural Resources (Yellow Perch Task Group 2009). All individuals were humanely euthanized and kept frozen until further laboratory analysis. Juveniles processed for this study in each year were selected from each site in proportion to their catch per unit effort.

**DNA extraction and genotyping**

DNA was recovered from tissue samples following the plate-based extraction method (Elphinstone *et al.*, 2003). Extracted larval DNA samples were re-suspended in 50 µL of Tris–EDTA buffer (10 mM Tris, 1.0 mM EDTA, pH 8.0) while juvenile YP DNA was re-suspended in 100 µL of the same buffer.

Each fish was genotyped at a total of 12 microsatellite loci (Table 1). PCR amplification was performed in 25 µL reactions with the following components: 1.5 µL of template DNA, 2.5 µL 10x PCR buffer, 2.5 µL of MgCl2 (25 mM), 0.3 µL of dNTPs (50 µM of each), 0.2 µL (0.5 µM) of dye labeled primer, 0.2 µL (0.5 µM) of the reverse primer and 0.10 U Taq polymerase (Applied Biosystems, Foster City, CA). PCR conditions were: initial denaturation at 94°C for 2 min, followed by 35 to 40 cycles of denaturing at 94°C for 15 s, annealing for 30s (temperatures locus specific following Li *et al.*, 2007), extension at 72°C for 30 s and a final extension of 72°C for 10 min. Microsatellite allele sizes were determined using a LI-COR 4300 DNA analyzer (Lincoln, NE) and scored using GeneImage IR 4.05 (Scanalytics, Inc., Rockville, MD).

**Categorizing larval fish source**

Larval YP were divided into two spatial/habitat groups following three sequential steps: first, larvae were initially divided based on their geographic (sampling) location
(i.e. northern vs. southern part of the western basin); secondly we used turbidity estimates to select sites belonging to high and low turbidity areas (Figure 1); and third, each group of larval fish was genetically screened for “stray fish” using rank-based self-assignment genotype analysis (Paetku et al., 1995) based on the microsatellite data in GENECLASS 2.0 (Piry et al., 2004). The probability of genotype assignment to either group (high turbidity, south shore Maumee River plume vs. low turbidity, north shore Detroit River plume) was estimated for each larval fish: fish that showed a less than 60% likelihood of self-assignment to its collection group were deemed strays and were eliminated from subsequent analyses. The high turbidity area will be referred to as the Maumee River plume while the area with lower turbidity in the northern section of the basin will be referred to as the Detroit River plume larval group.

Population genetic analysis

Exact tests for Hardy–Weinberg equilibrium (HWE) were performed (20 000 permutations) using Tools for Population Genetic Analysis (TFPGA, v1.3; Raymond and Rousset, 1995; Miller, 1997). Significance values for HWE were Bonferroni corrected for multiple simultaneous comparisons. Genetic differentiation calculations (F$_{ST}$) and pairwise Fisher’s exact tests were conducted to test for differences in allele frequency distributions between larval groups (Detroit vs. Maumee River plumes) within a given year (2006, 2007 or 2008). The same analyses were used to test for temporal variation in allele frequency distributions within larval groups (Detroit or Maumee) among the three consecutive sample years (2006-2008). All genetic differentiation (F$_{ST}$) estimates were calculated in Genepop (version 4.0.7; Rousset, 2008) following Weir and Cockerham (1984). Pairwise Fisher’s exact tests (10 000 dememorizations and 20 000 permutations) were performed using TFPGA. Additionally, for 2006, because we obtained larval YP
(n=35) from just outside of Sandusky Bay, OH (Figure 4.1), we explored genetic differences (as described above) among three larval groups (Detroit, Maumee and Sandusky) in a separate analysis. AMOVA (Analysis of Molecular Variance) was used to partition genetic variance among years, between larval plume groups nested within years, and within larval plume groups using ARLEQUIN 3.0 (Excoffier et al., 2005).

**YOY Genetic assignment**

Larval YP relative survival was estimated by genetic assignment of YOY YP, collected in the western basin of Lake Erie, to their putative larval group (i.e., Maumee or Detroit larval groups), followed by a statistical analysis of relative surviving proportions.

Genetic assignment of YOY was performed using GENECLASS 2.0 (Piry et al., 2004) independently for the three collection years. Our analysis consisted of a two-step procedure (see Beneteau et al., 2009). First, I performed a Bayesian assignment (Rannala and Mountain, 1997) with Monte Carlo re-sampling using Paetkau et al.’s (2004) simulation algorithm (10,000 simulated individuals at an assignment threshold p=0.05). From the Bayesian analysis, I excluded those individuals with probabilities less than 30% of belonging to either one of the larval source populations (Detroit or Maumee River groups). Second, I used the remaining individuals in a rank-based genotype assignment (frequency method, Paetkau et al., 1995). Successful ranked-based assignments were those with probability of 70% or higher of belonging to one group (hence the second group assignment probability would be 30% or lower). Failed assignments (i.e. unknown origin) were those with likelihood between 30% and 70%. In 2006, the YOY were assigned to Maumee, Detroit or Sandusky larval groups using the same two step criteria explained above.
Genetic differentiation ($F_{ST}$) and pairwise Fisher’s exact test for population differentiation were conducted for YOY assigned to Maumee and Detroit River plume groups in Genepop (version 4.0.7; F. Rousset, 2008) and TFPGA (v1.3; Raymond and Rousset, 1995; Miller, 1997) for 2006, 2007 and 2008 independently. Also, I analyzed temporal genetic differentiation within successfully assigned YOY (e.g. Detroit or Maumee River plume groups) by means of genetic differentiation ($F_{ST}$) and pairwise Fisher’s exact test for population differentiation as described above. Hierarchical AMOVA was used to partition genetic variance in successfully assigned YOY among years, between YOY plume groups nested within years and within YOY plume groups, using ARLEQUIN 3.0 (Excoffier et al., 2005).

Additionally, I investigated if YOY successfully assigned to Detroit or Maumee River plume groups were also spatially separated in the western basin of Lake Erie. To achieve this I calculated the mean location of capture (latitude and longitude) for all YOY assigned to Maumee and Detroit River plumes respectively and compared them using a Student’s t-test in SYSTAT v11.0 (Systat Software, Inc).

**Survival assessment**

Weekly average abundance of larvae was calculated for each river plume group (total number of larvae / m$^3$ averaged over all sites sampled in that week). Analysis of variance (ANOVA, SYSTAT v11.0) was used to determine the temporal stability of mean weekly larval abundance within each river plume. The highest weekly larval abundance estimate has been shown to be a good estimator of larval production (Reichert et al., 2010), hence I used the peak values in each group to estimate the YP larval abundance ratio between Detroit and Maumee River plumes. I used the abundance ratio to assess differences in relative survival between Detroit and Maumee larval fish from the larval to
the juvenile stage for each year separately. If the survival of larvae is equal in the Detroit and Maumee River plume larval groups, I would expect the Maumee: Detroit ratio of larval abundance to remain constant as the fish age (i.e. the ratio of assigned YOY from Maumee to Detroit plume areas would be the same as the ratio of larvae sampled in the same water masses). Expected values for the number of YOY fish from each plume in each year were calculated by multiplying the Detroit:Maumee abundance ratio by the total number of YOY successfully assigned for that year. Observed values were the YOY genetic assignment results as explained in the genetic assignment section. We compared expected versus observed estimates using Chi-square ($\chi^2$) tests for each year.

**RESULTS**

The collection sites for larval fish corresponded well with the transmissometry data in discriminating high turbidity (> 6.0 m$^{-1}$) Maumee River waters from the low turbidity (< 6.0 m$^{-1}$) Detroit River sites. Using the 6.0 m$^{-1}$ criterion, only 2 samples from the Maumee River plume area were removed from our initial spatial classification in 2006, and none were removed in 2007 or 2008 (Figure 4.1).

A total of 686 larval fish and 403 YOY were genotyped at 12 microsatellite loci during 2006-08, with five to 23 alleles per locus and observed ($H_O$) and expected heterozygosities ($H_E$) ranging from 0.091 to 0.991 (Table 4.1). Seventeen of 72 tests revealed significant departures from HWE following Bonferroni correction, but none were consistent across larval groups or loci (Table 4.1).

The microsatellite genotype assignment analysis for river plume membership identified a relatively small number of larvae as “strays”: 15% (25 out of 160) and 17% (15 out of 90) from Detroit and Maumee River plume respectively in 2006; 26% (74 out
of 283) and 13% (11 out of 81) respectively in 2007; and 18% (29 out of 153) and 15% (13 out of 86) respectively in 2008. The genetically excluded larval fish were not used in subsequent analyses.

**Population Structure**

Genetic differentiation \( (F_{ST}) \) values between larvae collected in the Detroit versus Maumee River plumes were 0.013 \( (p= 0.0015) \), 0.0068 \( (p= 0.0015) \) and 0.0064 \( (p= 0.003) \) in 2006, 2007 and 2008, respectively (Table 4.2). Genetic differentiation \( (F_{ST}) \) of 2006 larvae collected around Sandusky Bay area were 0.019 \( (p= 0.0003) \) and 0.022 \( (p= 0.0003) \) when compared to 2006 Detroit and Maumee River plume larval groups respectively.
Figure 4.1. On the left, maps of western Lake Erie with larval YP sampling sites during spring 2006 – 2008. On the right, histograms showing the frequency and distribution of turbidity values at each sample site for each year. Turbidity values > 6 indicate high turbidity and differentiate the Maumee River plume (see dark circles on maps and dark bars in histograms) from Detroit River plume waters (see lighter circles on maps and clear bars in histograms). In 2006 eliminated sites (turbidity values <6) are marked with an X; no samples were eliminated in 2007 or 2008. Star symbols represent collection sites around the Sandusky Bay area for larvae obtained in 2006.
Table 4.1. Number of alleles (Na), observed (H₀) and expected (Hₑ) heterozygosity for the twelve microsatellite loci used to genotype larval yellow perch (YP) collected in the western basin of Lake Erie in 2006, 2007 and 2008. Groups are denoted by river plume (Detroit or Maumee) followed by the year of collection.

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Data in bold denotes deviations from HWE (following Bonferroni correction).
Exact tests revealed significant differences in allele frequency distribution between Maumee and Detroit River groups (p < 0.001) in all three years (Table 4.2). Additionally, exact tests revealed significant differences in allele frequency distribution between larval YP collected near Sandusky Bay and both Detroit and Maumee River plume larval groups (p < 0.001) in 2006. There was significant temporal genetic differentiation (FST: 0.006 - 0.01) within each larval fish group among the three sampling years (2006, 2007 and 2008), with Fisher’s exact test for allele frequency distribution being significant (p < 0.001) for all temporal comparisons (Table 4.2).

AMOVA results showed no significant variation among years (2006, 2007, 2008) (df= 2, % variation = 0.43, p = 0.22), while between river plume group variance, nested within years was significant (df= 3, % variation= 0.31, p< 0.001), and within river plume groups explained the majority of the variation and was significant (df= 1366, % variation= 99.26, p< 0.001).

**Genetic Assignment**

The number of YOY that were excluded from both possible source populations based on the Bayesian analysis were 0% (zero out of 119), 8% (13 out of 167) and 25% (30 out of 117) in 2006, 2007 and 2008 respectively (Table 4.3). The remaining YOY were genetically assigned using a rank-based analysis; in 2006, 2007 and 2008 YOY fish successfully assigned to the Detroit River plume group at almost twice the frequency of those assigned to Maumee River plume (Table 4.3). When I included the Sandusky Bay larvae as a possible source population in 2006, we found two YOY that were excluded from all three putative source populations, while eight YOY were assigned to the Sandusky Bay larval group, 24 were assigned to the Maumee River group, 40 were assigned to the Detroit River group; and 45 were failed assignments (unknown origin).
Genetic differentiation ($F_{ST}$) between YOY successfully assigned to Detroit and Maumee River plume groups was 0.015 in 2006, 0.021 in 2007 and 0.057 in 2008 with significant differences in allele frequency distributions (Fisher’s exact test, $p< 0.001$).

Temporal genetic differentiation ($F_{ST}$) between successfully assigned YOY in the Detroit River plume was 0.023 between 2006 and 2007; 0.031 between 2007 and 2008, and 0.057 between 2006 and 2008. Temporal genetic differentiation ($F_{ST}$) in YOY assigned to the Maumee River plume group was 0.016 between 2006 and 2007; 0.067 between 2007 and 2008 and 0.047 between 2006 and 2008. All temporal comparisons for allele frequency distribution differences were significant (Fisher’s exact test, $p< 0.001$).

AMOVA results for YOY successfully assigned to either the Detroit or Maumee River plume showed no significant variation among years (2006, 2007, 2008) ($df= 2$, % variation = -0.35, $p = 0.68$), and significant variation between assigned groups nested within years ($df= 3$, % variation= 1.98, $p< 0.001$), with the majority of the variation being explained by within group effects ($df= 458$, % variation= 98.37, $p< 0.001$).

Based on the Student’s t- test there were no significant differences between mean latitude and mean longitude of collection sites coordinates for YOY assigned to Detroit versus Maumee River plume groups in 2006- 2008, suggesting that there was no spatial bias in either group (i.e. juveniles from both areas were well mixed).

**Survival assessment**

The ratios of peak larval abundance for the Detroit:Maumee River plumes were 73:27 in 2006; 89:11 in 2007, and 70:30 in 2008. No significant difference in larval YP abundance was found between years in the Detroit or Maumee River plumes (ANOVA, $df=2$, $p= 0.281$, $p= 0.641$ for Detroit and Maumee, respectively). Larval abundance ratios were used to calculate the expected ratio of YOY from the Detroit River and Maumee
River larval groups (expected numbers were calculated by multiplying by the total observed YOY that were assigned), while observed values were those obtained using genotype assignment (Table 4.3). Chi-square analysis showed that there were significant differences between the observed and expected values for 2006 and 2007 but not for 2008 (Figure 4.2). Hence, survival of YP was higher in the Maumee River plume than in the Detroit River plume in 2006 and 2007, while we could detect no difference in 2008.
Table 4.2. F_{ST} values between Detroit and Maumee River plume larval groups.

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<th>M06</th>
<th>D07</th>
<th>M07</th>
<th>D08</th>
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<td>0.0165*</td>
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M= sites collected within the Maumee River plume; D= sites collected within the Detroit River plume within the western basin of Lake Erie during three consecutive years. Asterisks denote Fisher’s exact test significance of p< 0.001.

Table 4.3. Genetic assignment results of YOY YP to Detroit and Maumee larval groups collected in the western basin of Lake Erie from 2006-2008. 2006-S: YOY assignment analysis to Detroit, Maumee and Sandusky bay larval groups collected in 2006.

<table>
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<td>2008</td>
<td>-</td>
<td>27</td>
<td>16</td>
<td>44</td>
<td>30</td>
<td>117</td>
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</tbody>
</table>
Figure 4.2. Number of young-of-the-year (YOY) from 2006 through 2008: a) Observed and expected values were calculated based on larval abundance ratio between Detroit and Maumee River plume groups and genetic assignment results; b) residual numbers or observed minus expected. *Significant difference (p < 0.05) between observed and expected values based on Chi square good of fitness analysis. D: Detroit River plume; M: Maumee River plume.
DISCUSSION

I found relatively low, but significant, spatial genetic structure in larval YP in the western basin of Lake Erie between river plume habitats and between river plume habitats and the larval fish collected around the Sandusky Bay area. Within-lake genetic structure has been previously reported for other species in the Great Lakes, although those studies were for adult or young-of-the-year fish (Stepien and Faber, 1998; Miller, 2003; Strange and Stepien, 2007; Sepulveda-Villet et al., 2009). To our knowledge, no previous studies have explored genetic structure of wild larval fish in any of the Great Lakes, although a large body of published literature can be found for marine and estuarine systems (e.g., Lecomte and Dodson, 2004; Christie et al., 2010; Hedgecock, 2010).

Lecomte and Dodson (2004) reported genetic differentiation between two populations of larval rainbow smelt (*Osmerus mordax*) in the St. Lawrence estuary (Canada) and they pointed out the possibility that these sympatric groups are exploiting different environments based on habitat characteristics (e.g. turbidity). Interestingly, the genetic differentiation found in larvae between those two habitats was the same proportion for adult smelt (Lecomte and Dodson, 2004), though they used restriction fragment length polymorphism (RFLP) which may have affected their results; a more sensitive marker, such as microsatellites, might have given a different outcome. Spatial genetic structure has been reported in adult YP in Lake Michigan (Miller, 2003), and Lake Erie (Sepulveda-Villet et al., 2009), as well as for the related Eurasian perch (*Perca fluviatilis*) in Lake Erken in central Sweden (Bodaly et al., 1989; Bergek and Bjoerklund, 2007). Sepulveda-Villet et al. (2009) used mitochondrial DNA (mtDNA) haplotype analysis and found low but significant genetic divergence between adult YP spawning groups within the western basin of Lake Erie (\(\theta_{ST} = 0.12-0.11\)) located around the
Sandusky Bay area and on the northern shore of the western basin. However, due to the nature of the genetic technique utilized by Sepulveda-Villet et al. (2009) it is difficult to directly compare the latter values found in adult YP with the microsatellite genetic data obtained in this study; even so it is important to emphasize the presence of genetic differentiation previously detected in adult fish and now in larval YP.

The genetic structure in larval YP of the western basin of Lake Erie may be attributed to adult YP spawning site fidelity (i.e. homing) (Strange and Stepien, 2007; Parker et al., 2009), likely coupled with the relatively weak swimming capabilities of larval YP (Houde, 1969) that limits active dispersal during the first weeks after hatching. Thus my study may be indirectly describing population genetic structure in the spawning adult YP. The observed year-to-year variation in the genetic structure of larval YP likely reflects variation in environmental conditions of the spawning grounds and rearing sites (Hauser et al., 1998), changes in cryptic barriers such as water currents (Bergek and Olsson, 2009) and differential survival or reproductive success among spawning populations (Sinclair, 1988; Hauser et al., 1998; Shrimpton and Heath, 2003; Bergek and Olsson, 2009). Even though the level of larval genetic structure reported in this study is low, it indicates that genetic structuring can be detected very early in life and that different life stages of fish should be included in genetic studies to better understand the relationship between habitat use and dispersal.

The genotype assignment analyses showed that larval YP from the Maumee River plume experienced significantly higher survival than the Detroit River plume larvae in 2006 and 2007, but not in 2008. The survival advantage for the Maumee River plume larvae may be explained by two different hypotheses: 1) nutrient-rich water from the Maumee River provides a food-rich environment causing a “bottom-up” growth effect on
larval fish indirectly favouring recruitment (Carpenter et al., 1985; McQueen et al., 1989); or 2) high turbidity (i.e. low water clarity) due to suspended sediments and phytoplankton blooms in the Maumee River plume provide protection against visual predators during early life stages (Abrahams and Kattenfeld, 1997; De Robertis et al., 2003). A combination of both hypotheses is also possible.

River discharge into bays, estuaries, and along other coast lines of both marine and freshwater ecosystems typically creates nutrient-rich areas that hold the potential to enhance larval growth and positively influence fish survival and recruitment (Gascon and Legget, 1977; Grimes and Finucane, 1991; Grimes and Kingsford, 1996; Ludsin et al., 2001). Roseman et al. (2005), working in the southern part of western Lake Erie, showed that walleye (Sander vitreus) larvae were found in higher densities in waters with higher zooplankton availability, higher temperatures and lower water clarity. The Maumee River plume is higher in temperature, total phosphorous, and chlorophyll a compared to other areas within the western basin of Lake Erie (Reichert et al., 2010; T. Johengen, University of Michigan, Ann Arbor, unpub. data). Despite these differences, zooplankton biomass and larval YP growth has been shown to not differ between the Maumee and Detroit River plumes in our study years (Reichert et al., 2010, Ludsin S unpub. data). Hence, food availability that enhances larval growth is not likely the dominant factor driving higher survival in the Maumee River plume relative to the Detroit River plume.

Instead, the observed survival advantage to larvae in the Maumee River plume relative to Detroit River plume larvae may be driven by higher predation pressure experienced in the Detroit River plume. Predator abundance in 2006 through 2008 was about 50% higher in Detroit River plume waters (S. Ludsin, unpubl. data). Further, the low turbidity associated with the Detroit River plume in combination with higher larval
abundance in the Detroit River plume makes opportunistic predation more likely, and conversely, lower water clarity and larval abundance (i.e. Maumee River plume) could translate into higher energetic costs in searching for larval fish prey which would reduce opportunistic predation (Abrahams and Kattenfeld, 1997; Utne-Palm, 2002; Pekcan-Hekim and Lappalainen, 2006). In fact, Swenson (1978) reported that high turbidity associated with river discharge in the western arm of Lake Superior favoured lake herring (*Coregonus artedi*) recruitment success by protecting them from lake trout (*Salvelinus namaycush*) that preferred less turbid water. Indeed, higher turbidity levels in the Maumee River plume could be a major factor protecting larval fish from visual predators in the western basin of Lake Erie.

Another possible explanation for the apparently higher larval survival in the Maumee River plume larval fish may be strong currents from the Detroit River (Saylor and Miller, 1987) that could potentially flush large numbers of larvae and/or early juveniles from the Detroit River plume into the central basin of Lake Erie. Such a phenomenon would generate a downward bias in our estimates of relative survival in the Detroit River plume larvae. On the other hand, Maumee River plume larvae would be less subjected to such transport, as currents generated by the Maumee River are much weaker than the Detroit River (Saylor and Miller, 1987; Beletsky *et al.*, 1999). However, the YOY YP that belonged to the Detroit River plume larval group were found to be dispersed randomly throughout the western basin, suggesting that Detroit larval fish were not being systematically displaced towards the central basin.

I do not know the reason why no differences were found in the survival of larvae between the Maumee and Detroit River plumes in 2008. Perhaps other mechanisms besides turbidity and river plume formation were influencing recruitment. Such factors
could include climate variation (Clady, 1976; Collingsworth and Marschall, 2011), water circulation patterns (Beletzky et al., 1999), and changes in habitat quality (Cech et al., 2009) among others (Kane-Sutton, 2010).

On average 17% of the larval fish were excluded from the self-assignment analysis in the Detroit and Maumee River plume larval groups (i.e. strays). These strays were potentially transported by water currents away from their natal site into a new nursery habitat. Even though larval fish remained in the Detroit and Maumee River plumes for several weeks after hatching (Reichert et al., 2010) some larvae (perhaps the weakest swimmers) may have been transported away from their natal areas. Hence it is not surprising to have detected low levels of straying since this study did not collect larval fish from all possible source populations.

Otolith microchemistry is another technique that has been used to assign fish to their larval rearing habitats (Campana, 1999; Campana and Thorrold, 2001; Elsdon et al., 2008). Reichert et al. (2010) identified water-mass specific elemental signatures in the Maumee and Detroit River plume habitats; these elemental signatures were used to assign YOY YP back to their larval rearing area (i.e. Detroit or Maumee River plumes), thus enabling them to estimate recruitment success differences between larvae from Maumee or Detroit River plume waters for 2006 and 2007. Our genetic assignment approach used in this study agreed with the results presented in Reichert et al. (2010) in which the Maumee River plume rearing site had a higher survival in 2006 and 2007 when compared to non-Maumee water mass larval fish. While a quantitative comparison of our findings with Reichert et al.’s (2010) is beyond the scope of this study, both methods appear to be potentially valuable to agencies seeking to discriminate stocks and identify natal origins of recruited individuals.
In conclusion, if food availability and larval growth are similar in the Detroit and Maumee River plumes (Reichert et al., 2010), the higher turbidity in the Maumee River plume is likely contributing to enhanced larval survival through reduced predation pressure. Additionally, the fine-scale genetic structure found in western basin larval and YOY YP suggests that spawning and rearing site quality is playing a role in larval fish survival and hence impacting recruitment of this economically important species. Our results suggest that population genetic structure is present in YP and including early life stages into population genetic studies would provide a better picture of the complex interactions between habitat and YP survival. Nonetheless, the existing contrast in habitat between Detroit and Maumee River plumes is likely contributing to better recruitment and stronger year classes in western basin Lake Erie YP.

REFERENCES


and life history parameters of fishes: hypothesis assumptions limitations and inferences


CHAPTER 5
PREDATION ON LARVAL YELLOW PERCH (*PERCA FLAVESCENS*) IN THE WESTERN BASIN OF LAKE ERIE: THE EFFECT OF THE MAUMEE RIVER PLUME.

INTRODUCTION

River discharge into water basins (*e.g.* estuaries, marine systems or lakes) impacts the water basin with the scale of the impact depending on the river water characteristics such as nutrient content, sediment load, temperature, and discharge rate (Grimes and Finucane, 1991; Harrison *et al.*, 1991; Le Pape, 2003). The mixing areas where the river and the basin waters meet are often clearly distinguishable and are referred to as river plumes. River plumes are recognized as major factors contributing to fish community dynamics, mostly due to the large-scale turbidity and nutrients variation associated with them. Increased turbidity and nutrients, phytoplankton, and zooplankton can seemingly benefit fish via protection from visual predators and increased food availability (Abrahams and Kattenfeld, 1997; Reichert *et al.*, 2010). Early life feeding success and predation are thought to be two of the main factors driving the observed variation in fish recruitment (Cushing, 1975; Bailey and Houde, 1987).

Turbidity is a particularly critical ecological parameter contributing to fish distribution and behaviour (Maes *et al.*, 1998; Trebitz *et al.*, 2007; Chiu and Abrahams, 2010); the importance of turbidity in aquatic ecosystems has been recognized since the late 1930s (Ward, 1938; Doan 1941; Van Oosten, 1945). Previous studies have addressed the possible negative effects that increased turbidity could have on fish (Doan, 1941; Van Oosten, 1945); however turbidity may also have a beneficial effect on fish survival by...
providing protection from visual predators, particularly in the early life stages during which larval fish are highly vulnerable to predation (Bailey and Houde, 1989). Since predation is an important factor for larval survival (Bailey and Houde, 1989; Houde, 1997) turbidity associated with river plumes may have a substantial role in recruitment success through modification of larval predation (DeRobertis et al., 2003; Utne-Palm 2001). Although river plumes and the resulting turbidity variation are expected to be critical for fish recruitment, the actual mechanisms have not been empirically determined.

Predation is generally recognized as a major source of mortality in fish early life stages (Post and Prankevicius, 1987; Anderson, 1988; Govoni, 2005). However, to date, there are no direct analytical methodologies to estimate larval predation. Although visual identification of stomach or gut contents of predator fish could provide such information, it is often inaccurate due to the rapid digestion rate of soft prey tissue such as that found in larval fish (Brandt 1987; Schooley et al., 2008; Legler et al., 2010). For this reason, the application of molecular genetic techniques to study predation on larval fish has been successfully applied in a variety of fish species (Rosel and Kocher, 2002; Saitoh, 2003; Carreon-Martinez et al., 2011). Molecular genetic techniques can be used to identify species-specific DNA sequences, hence facilitating accurate and more reliable predation rate estimates for ecological and trophic studies. Single nucleotide polymorphisms (SNPs) are single base pair DNA sequence differences among species or individuals within a species. The use of SNPs is becoming widespread, primarily because it only requires short DNA fragments (<150 bp) for amplification, which are common in even highly degraded DNA (King et al., 2008; Beja-Pereyra, 2009).

The commercial and recreational fisheries in the Laurentian Great Lakes in general and Lake Erie in particular represent important economic resources (OMNR 2011
The western basin of Lake Erie is a particularly important rearing environment for juvenile yellow perch (*Perca flavescens*), as well as supporting a highly valuable commercial and recreational yellow perch fisheries. The western basin of Lake Erie is the recipient of inflows from two major tributaries, the Maumee and Detroit Rivers (Bedford, 1992; Bolsega and Erdendorf, 1993); these rivers create two areas which differ in turbidity due to the high agricultural nutrient and sediment load in the Maumee River (Bedford, 1992) as opposed to the clearer and less productive waters from the upper Great Lakes arriving via the Detroit River. For this reason the southern part of the western basin, where the Maumee River plume meets Lake Erie, is recognized as having increased turbidity levels compared with the Detroit River plume in the northern part of the western basin. In addition, otolith microchemistry and population genetic analyses (Reichert *et al.*, 2010; Chapter 4) have shown higher mortality in the Detroit River plume and speculated that predation in the Detroit River could be directly influencing survival of Maumee River plume larvae. For this reason, the western basin of Lake Erie is an ideal environment to study the effect of river plume associated turbidity on yellow perch larval survival.

The objective of this project was to study the river plume effect on predation levels of larval yellow perch in the western basin of Lake Erie. My hypothesis is that larval yellow perch will experience higher predation in the Detroit River plume area and less in the Maumee River plume. To address this, predator fish were collected in the spring and summer of 2006, 2007 and 2008 in the Maumee and Detroit river plume areas (i.e. southern and northern part of the western basin of Lake Erie respectively), to determine, via molecular genetic analysis of predator stomach contents, if there was a difference in the larval yellow perch predation rates. In addition, we tested whether the
susceptibility of the early-life yellow perch to predation changed over the sampling period (spring-summer) within years. This study provides the first direct estimate of early-life predation rates for a commercially important species in a large, complex ecosystem.

**Material and Methods**

This section describes two related, but quite distinct protocols. First, we describe the collection of predator fish (walleye, white bass and white perch) in the western basin of Lake Erie, secondly the development and validation of a yellow perch specific single nucleotide polymorphism (SNP) assay, and lastly we describe the application of a SNP-based molecular genetic diet analysis designed to determine the role river plumes/turbidity play in regulating larval yellow perch predation.

**Predator collection**

White bass, white perch and walleye are the most common and abundant predators present in the western basin of Lake Erie (average abundance in collections across all fish species captured: 2006-2008, 15.2%, 37.2% and 10.8% respectively: 63.2% combined), and are hence the most likely to prey on yellow perch larval fish. We thus targeted those species for our molecular genetic diet analysis in the western basin of Lake Erie (Fig 5.1). Collections were made weekly from mid-April through July in 2006 and 2007, and in May in 2008 using a bottom trawl (7.6-m semi-balloon design, 13-mm stretched-mesh cod-end liner) and graded mesh set gillnets (approximately one meter below surface water; random series of consecutive 15m panels of 38, 51, 64, 76, 89 mm stretch monofilament twine). Nets were fished for 1-2 hours once a week from May
through June in 2006 and 2007. In 2008, only bottom trawl sampling was done on May 6, 8, 12, 15, 20 and 23 in the Detroit and Maumee plume areas (Fig. 5.1).

All captured fish were immediately euthanized, stomachs were injected with 100% ethanol to halt digestion, and whole fish were frozen for future analysis. In the laboratory, each fish was thawed, and stomachs were removed for diet analysis.

*Predator DNA extraction and Single Nucleotide Polymorphism assay design and validation*

**DNA extraction**

DNA was recovered from tissue samples from each predator fish (walleye, white bass and white perch) following a plate-based DNA extraction method (Elphinstone *et al.*, 2003), and the resulting DNA was resuspended in 50 uL of Tris–EDTA buffer (10 mM Tris, 1.0 mM EDTA, pH 8.0).

**Yellow perch specific SNP assay design and validation**

Cytochrome oxidase subunit 1 (CO1) mtDNA sequences from every fish species known to be present in Lake Erie (FishBOLD, Hubert *et al.*, 2008) were analyzed to identify a single nucleotide polymorphism that uniquely identified yellow perch from all other species. We identified one nucleotide substitution that was specific for yellow perch (Supplementary material 5.1); that sequence was used to design a *taq-man* (Applied Biosystems) SNP assay (Supplementary material 5.1).

The validation of the SNP assay was divided into three stages; the first step was to determine the range of target DNA concentrations in which the assay amplified consistently. This was achieved by using a wide range of yellow perch DNA
concentrations from 1000, 900, 800, 700, 500, 300 and 100 ng \mu L^{-1}; also 90, 80, 60, 50, 40, 30 and 20 ng \mu L^{-1}, and 18, 15, 10, 8, 5, 3, 1, 0.5 ng \mu L^{-1}. Secondly, to investigate the possibility of cross-amplification with predator DNA, a range of DNA concentrations (20, 18, 15, 10, 8, 5, 3, 1 ng \mu L^{-1}) of each predator (walleye, *Sander vitreus*; white perch, *Morone americana* and white bass, *Morone chrysops*) was used separately as template for the SNP assay. Finally, to test the effect of competing DNA in a single sample, 1.5 \mu L of walleye, white perch and white bass DNA (20 ng \mu L^{-1} each) were each mixed with 1.5 \mu L of low concentration yellow perch DNA (1 ng \mu L^{-1}) and used as template for the SNP assay. We also performed the same analysis, but using a higher concentration of yellow perch DNA (5 ng \mu L^{-1}). Finally, we used a mixture of all three predator DNAs (1.5 \mu L, 20 ng \mu L^{-1} each) without any yellow perch DNA as template for the SNP assay. All of the validation experiments were run in triplicate.

All samples containing yellow perch DNA consistently amplified the target fragment between 18-30 cycles (C_T), and generated a magnitude of the fluorescence signal relative to background (\Delta R_n) of 2.40 (range between 1.8 -3.0 for 0.5 – 20.0 ng \mu L^{-1}), hence the fractional cycle number (C_T) and the \Delta R_n were the threshold parameters used to determine if the sample contained yellow perch DNA. A Student’s t-test was used to test for significant differences in C_T and \Delta R_n for samples containing yellow perch and those with no yellow perch DNA, but containing other species (predator DNA). All statistical analyses were performed using SYSTAT v9.0 (Systat Software, Inc, 2004 v 9.0).
Application of yellow perch SNP assay in wild predators stomach contents

Stomach content sample processing & DNA extraction

Stomach contents were placed in a scincillation bottle with 95% ethanol. Before DNA extraction, stomach contents were sorted to remove potential fish remains from other material (large invertebrates, sediment, plant matter, etc) and well mixed before selecting a random aliquot for DNA extraction. A small subsample of stomach content (0.5-60 mg, wet weight) for each predator fish was aliquoted into a 1.5 mL tube. The tubes were centrifuged at 13,000 rpm for 10 min to precipitate any tissue and DNA in the ethanol. After centrifugation, we removed the supernatant and let the sample air-dry for 12 hours, followed by 10 minutes drying in a Eppendorf Vaccufuge Plus system (www.eppendorf.ca) to ensure all the ethanol was evaporated. After the removal of the ethanol, DNA was extracted from the stomach contents following a plate-based DNA extraction method (Elphinstone et al., 2003), and the resulting DNA was resuspended in 100 uL of Tris–EDTA buffer (10 mM Tris, 1.0 mM EDTA, pH 8.0).

Yellow perch specific SNP assay

Quantitative PCR (qPCR) was done in a 7500 ABI real-time quantitative thermal cycler (www.appliedbiosystems.com). The qPCR reaction (12 µL) consisted of 6 µL of Universal PCR Master Mix (no AmpErase UNG), 0.5 µL of SNP assay (Supplementary material 5.1 for probe concentrations), and 5.5 µL of stomach content DNA. Amplification consisted of one initial cycle at 95° C for 10 minutes, followed by 40 cycles of denaturing at 92° C for 15 seconds and annealing/extension at 60 ° C for 1 minute.
Presence or absence of yellow perch DNA was determined based on the fluorescence ($\Delta R_n$) and $C_T$ values, as determined in the previous section. Stomach samples positive for yellow perch were assigned a value of one and those negative for yellow perch were assigned a value of zero.

**Microsatellite genotyping**

To estimate the number of yellow perch prey items present, stomach content samples that were positive for yellow perch using our SNP assay were genotyped at five microsatellite loci (Y55, Y99, Y96 Y78, Y85; see Li et al., 2007). PCR amplification was performed in 25 µL reactions with the following components: 2.5 µL of template DNA, 2.5 µL 10x PCR buffer, 2.5 µL of MgCl$_2$ (25 mM), 0.6 µL of dNTPs (50 µM of each), 0.4µL (0.5 µM) of dye labeled primer, 0.4 µL (0.5 µM) of the reverse primer and 0.10 U Taq polymerase (Applied Biosystems, Foster City, CA). PCR conditions were: initial denaturation at 94°C for 2 min, followed by 35 to 40 cycles of denaturing at 94°C for 15 s, locus-specific annealing temperature at 60 °C for Y55 and Y78, 55 °C for Y99, Y96, Y85 (Li et al., 2007) for 15 s, extension at 72°C for 30 s and a final extension of 72°C for 10 min. Microsatellite allele sizes were determined using a LI-COR 4300 DNA analyzer (Lincoln, NE) and scored using GeneImage IR 4.05 (Scanalytics, Inc., Rockville, MD).

**Statistical Analyses**

To test if there was a predator species effect on larval yellow perch predation we utilized non-parametric Kruskal-Wallis tests to test for differences among the 3 predator species within each plume for each year separately and comparisons were Bonferroni corrected to account for multiple simultaneous significance tests.
Larval fish are most vulnerable to predation early after hatching (Bailey and Houde, 1989), thus we divided our sampling collection times into “early larvae” (April-May), and “late larvae” for the remaining of the collection times (June-July): this division roughly reflects 0-4 weeks after hatching and 5 - 8 weeks after hatching. We used Kruskal-Wallis non-parametric tests to check for differences in the number of predators positive for yellow perch between collection times (early larvae vs late larvae within plume for a single year in 2006 and 2007, and for differences between plumes (Maumee vs. Detroit) within a collection period for 2006-2008).

To estimate the number of yellow perch prey items in the stomach contents, we used a randomized selection routine that drew genotypes from previously collected Lake Erie larval yellow perch microsatellite genotypes (see Chapter 4) in each of the three sample years. We randomly selected 20 larval genotypes for each microsatellite locus and generated a relationship between the cumulative number of selected genotypes (larval fish) and the total number of alleles observed. This random selection process was replicated 25 times for each locus and for each year and the results were averaged over the 25 replicates (Fig 5.2). The number of prey fish necessary to explain the observed number of alleles in the stomach contents of individual predators was estimated using this relation at each of the five microsatellite loci. The number of prey fish estimated at each locus was averaged across the 5 loci to estimate the total number of yellow perch prey present in the predator stomach contents.

Predation rate (mean number of prey fish divided by total number of predators) was calculated per plume and per collection period for 2006-2008. Parametric Student t-test were performed to check for differences in predation rate between plumes (Maumee vs. Detroit) within year, within a collection period (i.e. early larvae or late larvae) for
2006-2008. We also tested for temporal predation rate differences between plumes by collection period (i.e. early larvae or late larvae) between years. All parametric Student- t test analyses were performed in SYSTAT v9.0.

Figure 5.1. Map showing the predator collection sites in the western basin of Lake Erie. On the left hand, the western basin of Lake Erie with collection sites in the Maumee River plume and Detroit River plume (southern and northern part respectively) are shown. On the right hand, collection site turbidity histograms for the spring-summer of 2006-2008 are shown. Fill pattern denotes US and Canadian territories.
Figure 5.2. Relationship between the cumulative number of selected genotypes (larval yellow perch) and the total number of cumulative alleles observed at five microsatellite loci. This relationship was used to calculate the average number of larval yellow perch present in stomach contents based on the number of alleles present in each sample.
RESULTS

**SNP Assay Validation**

**SNP Yellow perch DNA detection limits**

The lowest DNA concentration at which we consistently detected yellow perch DNA was 0.5 ng µL\(^{-1}\). The highest DNA concentration at which we observed a consistent \(C_T\) and \(\Delta R_n\) value was 20 ng µL\(^{-1}\). The assay performance with yellow perch DNA concentration in the 900 – 1000 ng µL\(^{-1}\) range was similar to the no template control, with no amplification curve detectable. Template DNA concentrations between 0.5 and 20.0 ng µL\(^{-1}\) consistently amplified between 18- 30 \(C_T\) (for 0.5 – 20.0 ng µL\(^{-1}\)) with an average \(\Delta R_n\) of 1.40 (range: 0.9 -1.8 for 0.5 – 20.0 ng µL\(^{-1}\); Fig 5.3). Therefore the most reliable and consistent detection range for yellow perch DNA in stomach samples is between 1.0-20.0 ng µL\(^{-1}\).

Cross-amplification with predator species DNA: To investigate the possibility of cross-amplification with predator DNA we used different concentrations of walleye, white perch and white bass DNA separately at template concentrations ranging from 1.0 - 20.0 ng µL\(^{-1}\), and in all cases the SNP assay showed a \(C_T\) of 35 or higher with a \(\Delta R_n\) signal of < 0.6 indicative of no amplification (Fig 5.3).

The effect of competing DNA mixed in a single sample: The template mixture of yellow perch DNA with DNA from each of the predator species produced a positive amplification signal for yellow perch DNA with \(C_T\) between 20-28 for the lower (1 ng µL\(^{-1}\)) and higher (5 ng µL\(^{-1}\)) yellow perch DNA concentrations; when yellow perch DNA was not present in the mixed template DNA, but predator DNA was, there was no yellow perch DNA signal (\(C_T > 34\) cycles). The template DNA mixtures with yellow perch
DNA yielded $C_T$ and $\Delta R_n$ values that were statistically different than those from the template mixture of predators without yellow perch DNA (Student t-test, $t=14.402$, $df=14$, $p<0.001$ for $C_T$; $t=-11.646$, $df=14$, $p<0.001$ for $\Delta R_n$).

Application of yellow perch SNP assay in wild predators stomach contents

Species effect in predation of larval yellow perch

Kruskal Wallis tests after Bonferroni correction revealed no significant species effect on predation levels in either Detroit or Maumee River plumes for any sample year. We therefore use pooled predator species data for all subsequent analyses.

River plume effect on larval yellow perch predation levels

A total of 790, 751 and 238 predators, were analyzed using the SNP assay in 2006, 2007 and 2008, respectively (Supplementary Table 5.1). Overall, the proportion of predator fish with yellow perch DNA found in their stomach contents in the western basin of Lake Erie was 6.8% (54/790) in 2006 and 5.4% (41/751) in 2007 and 39.9% (95/238) in 2008.

In 2006, we detected yellow perch DNA in 54 predator stomach contents, reflecting 2% (8 out of 363) of the Maumee River plume predators, and 11% (46 out of 427) of the Detroit River plume predators. Kruskal Wallis non-parametric test revealed a significant difference ($X^2= 22.60$, $df=1$, $p<0.001$) in the number of predators consuming yellow perch in the Detroit and Maumee River plumes. In addition, non-parametric Kruskal Wallis statistical analyses also revealed significant differences in predation level between Maumee and Detroit River plumes in both the early larvae period (the 1-4 weeks
after hatching) and the late larvae period in 2006 ($X^2= 21.56$, df=1, $p<0.001$ and $X^2= 4.90$, df=1, $p=0.027$, respectively; Fig 5.4).

In 2007, we detected yellow perch DNA in 41 predator stomach contents; of these, 9 (out of 256; 3.5%) samples were collected in the Maumee River plume while 32 (out of 495; 6.5%) samples were taken in the Detroit River plume. Although the number of yellow perch predators was higher in the Detroit River plume, non-parametric Kruskal-Wallis statistical analysis revealed no significant difference ($X^2= 2.50$, df= 1, $p=0.115$) in the number of predators consuming yellow perch in the Detroit and Maumee River plumes. The effect of river plume on yellow perch predation was statistically significant in the late larvae period (Kruskal-Wallis $X^2= 4.92$, df= 1, $p=0.027$), but not in the early larvae period (Fig 5.4).

In 2008, predators were collected only during the early larvae period (1-4 weeks after hatching), we detected yellow perch DNA in 42 (out of 109; 38%) samples collected in the Maumee River plume while yellow perch DNA was detected in 53 (out of 129; 41%) samples collected in Detroit River plume. Kruskal-Wallis non-parametric analysis revealed no significant difference ($X^2= 0.22$, df= 1, $p=0.637$) in the number of yellow perch predators in the Detroit and Maumee River plumes (Fig 5.4).

Estimation of the number of yellow perch prey items using microsatellite analysis

Using the stomach content DNA from the predators identified as having yellow perch DNA as template for the microsatellite PCR, all five microsatellite loci amplified fragments at expected yellow perch allele sizes based on allele frequency data for western basin Lake Erie larval yellow perch (approximately 150 larval fish per year for 2006, 2007, 2008; see Chapter 4). Fish that did not amplify in all five loci were considered
failed PCR and relatively few of the predators fell into this category (17% (8 out of 46) in 2006, 10% (4 out of 41) in 2007 and 13% (12 out of 93) in 2008). The remaining samples had a wide range of allele size, however only those within the expected size range for yellow perch alleles were considered for prey quantification.

*Predation rate*

Predation rates were calculated by dividing the total number of prey items estimated across all predators by the total number of predators (including those with no yellow perch SNP signal) per plume per collection period for each year; assuming the SNP assay has the same detection window as the molecular genetic technique DNA utilized in Chapter 3 (16 hours), I estimated a daily (24 hour) predation rate (Fig 5.5). In 2006, the predation rate was significantly higher in the Detroit River plume compared to the Maumee River plume in the early larvae period (Student t –test; t= 5.21, df= 285, p < 0.001; Fig. 5.5) and not significantly different in the late larvae period (t= 0.443, df= 494, p= 0.658; Fig 5.5). In 2007, the predation rate was not significantly different between the plumes in the early larvae period (t= -1.195, df= 479, p= 0.233; Fig. 5.5), but was significantly different in the late larvae period (t= 2.20, df=267, p= 0.029; Fig 5.5). Predation rate in 2008 was significantly different between river plumes in the early larvae period (t= 3.30, df=234, p= 0.001; Fig 5.5). Temporal analysis of predation rate for the early larvae and late larvae periods between years was significant for all pairwise analyses (Table 5.1).
Figure 5.3. Results of the yellow perch-specific SNP assay validation experiments. Mean (±SE) ΔRn and CT values for the SNP assays with yellow perch DNA and other species DNA (walleye, white bass and white perch) as template are shown across a wide range of DNA concentrations. Open circles represent ΔRn values for yellow perch and open triangles represent ΔRn values for other predator species (walleye, white perch and white bass). Closed circles represent CT values for yellow perch and closed triangle represent CT values for other predator species (walleye, white perch and white bass). YP stands for = yellow perch DNA; Other stands for walleye, white bass and white perch species.
Figure 5.4. Number of western Lake Erie predators (walleye, white perch and white bass) with and without yellow perch present in their stomach contents identified using the yellow perch-specific SNP assay. *denotes significant differences in number of predators consuming yellow perch between plumes within collection period.
Figure 5.5. Daily predation rate (average number of yellow perch prey per predator per day, including all predators’ walleye, white bass and white perch) by river plume and collection period within year (2006-2008) in the western basin of Lake Erie. Early= predators collected in late April-May; Late= predators collected in June-early July; * denotes significant difference in predation rate between Maumee and Detroit River plumes within collection period per year.
Table 5.1. Mean daily predation rate (± SE) per collection period within plume between years 2006-2008. Early= April-May, Late= June-July. P value denotes significant difference between collection period within plume per year (horizontal; same row within plume). a, b, c= denote significant difference between years within collection period (vertical; within a single column).

<table>
<thead>
<tr>
<th>Year</th>
<th>Detroit Early</th>
<th>Late</th>
<th>P value</th>
<th>Maumee Early</th>
<th>Late</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>2.68±0.48</td>
<td>0.18±0.06</td>
<td>P&lt;0.001</td>
<td>0.08±0.06</td>
<td>0.14±0.06</td>
<td>NS</td>
</tr>
<tr>
<td>2007</td>
<td>0.10±0.04</td>
<td>1.35±0.28</td>
<td>P&lt;0.001</td>
<td>0.21±0.09</td>
<td>0.15±0.09</td>
<td>NS</td>
</tr>
<tr>
<td>2008</td>
<td>3.67±0.48</td>
<td>--</td>
<td>--</td>
<td>1.72±0.30</td>
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</tr>
</tbody>
</table>
DISCUSSION

Using molecular genetic tools, I found that larval yellow perch are being consumed at considerable levels in the western basin of Lake Erie. During our three year observation, predation on yellow perch was present throughout the entire collection season, during both the early larvae collection period (1-4 weeks after hatching) and in the late larvae period (5-8 weeks after hatching). Predation early in the season (i.e. early larvae period) can be attributed to the high abundance of small and vulnerable larval fish (McGurk, 1986; Bailey and Houde, 1989). Brandt et al. (1987) reported peak predation on larval yellow perch in Lake Ontario occurred in the first two weeks after hatching, and that most larvae were completely digested within two hours of ingestion. In Lake Erie, larval yellow perch could hatch as early as the last week of April, continuing through to July, depending on water temperatures (Goodyear et al., 1982). Furthermore, larval aggregates could become targets for ambush predators, since food aggregates require less search and handling time than hunting for single prey fish (MacArthur and Pianka, 1966). In addition, the observed patterns in predation rate between years (higher in 2006 and in 2008 and less pronounced in 2007) coincide with yellow perch Age-0 abundance (YPTG report 2011). Age-0 yellow perch population levels were significantly reduced in 2006 and in 2008 compared to 2007 which was the strongest year class of the three years in the western basin of Lake Erie. Although recruitment levels are often influenced by many biotic and abiotic factors, predation pressure has been known to affect overall recruitment success in many species including yellow perch (Brandt, 1987; Laevastu and Bax, 1991). Therefore it is possible that part of the variation in population abundance observed from 2006-2008 is due to predation during the early life stages of yellow perch. Even though there were no significant differences in the number of predators with yellow perch present
in the stomach contents (frequency within plume and year) between predator species, based on the calculated daily predation rates per species and the abundance of Age-1+ of predators (each species separately) in the western basin of Lake Erie (Supplementary table 5.2; ODNR and OMNR status of fish stock reports 2011 and unpublished results) I estimated that Age 1+ white perch consume on average (2006-2008) 9% of the total larval yellow perch produced in the western basin of Lake Erie in the first 30 days post-hatch. Meanwhile walleye and white bass consumed 0.02 % and 0.35 % of the available larval yellow perch respectively. Indeed high predation rate (particularly for white perch) combined with variation in predator abundance and compositon may be the reason behind the observed year to year variation in yellow perch population levels.

A recent study by Reichert *et al.* (2010) that used otolith microchemistry to estimate larval yellow perch survival to the juvenile stage (Age-0), concluded that larvae in the Maumee River plume survived at a higher rate than those in the Detroit River plume. The authors concluded that the likely mechanism for this differential survival was a likely due to differences in predation and not due to differences in productivity or food supply (Reichert *et al.*, 2010). In addition Carreon-Martinez *et al.* (2011) used population genetics to show that larval yellow perch from the Maumee River plume experienced higher survival, and speculated that turbidity differences between plumes were possibly responsible for the observed differences in survival. Although both studies (Reichert *et al.*, 2010; Carreon-Martinez *et al.*, 2011) speculate that the higher turbidity in the Maumee River plume may be enhancing larval survival, the present study is the first to test that hypothesis. Our results showed that there are lower predation rates in the more turbid waters of the Maumee River plume than in the Detroit River plume. It is likely that the higher turbidity associated with the Maumee River discharge (Reichert *et al.*, 2010;
Grimes and Finucane, 1991) would reduce the likelihood of predators detecting small prey, increase prey avoidance ability, or both (Abraham and Kattenfeld, 1996; Fiksen et al., 2002). Hecht and Vanderlingen (1992) found that the feeding rate of a visual predator (Elops machnata) was significantly reduced in a high turbidity environment. Laboratory experiments have also shown that turbidity increases larval survival by affecting the foraging ability of visual predators (Rowe and Dean, 1998; Ohata et al., 2011).

Another possibility is that lower larval yellow perch abundance combined with lower predator densities in the Maumee River plume (Reichert et al., 2010; Ludsin S. pers com) may reduce the predator-prey encounter rate, leading to the observed lower predation rates. Indeed, predators often avoid turbid water due to reduced foraging success and greater energy costs associated with search effort (Ruiz et al., 1993; Maes et al., 1998). Thus the observed differences in predation rate may reflect predator habitat choice. Nonetheless, turbidity in the western basin of Lake Erie appears to be directly, or indirectly, reducing yellow perch predation rate perhaps by providing protection from visual predators (De Robertis et al., 2003; Pekcan-Hekim and Lappalainen, 2005).

We feel confident that the microsatellite marker-based identification of yellow perch prey is robust because; 1) the PCR amplification yielded non-kin yellow perch allele sizes (Carreon-Martinez et al., 2011), and 2) the microsatellite loci we used do not efficiently amplify other species. Although some yellow perch microsatellite primers have been known to amplify walleye DNA (Cena et al., 2006; LeClerc et al., 2008), the allelic size from the two species are different, and in this case we have only focused on yellow perch allelic size distribution based on our three year population genetics study on larval and juvenile yellow perch fish. It is likely that the yellow perch predation we detected was occurring on larval and early juvenile yellow perch (< 20 mm yellow perch,
June-July; Ludsin unpublished results), and not older fish because we identified DNA from multiple prey items in all stomachs (> 3), and the likelihood of a predator catching three or more juvenile fish (> 50 mm, Age-1) simultaneously is very low. Furthermore our sampling period (hatching to late June/early July) coincides with the time of greatest vulnerability of the young of the year yellow perch (Brandt, 1987; Bailey and Houde, 1989). Finally, it should be noted that the number of yellow perch prey estimated using the microsatellite allele number data is likely underestimated due to the rapid degradation of DNA from small-bodied juvenile fish (Weber and Lundgren, 2009; Carreon-Martinez et al., 2011). Nevertheless, our molecular genetic approach is far more sensitive than traditional visual analysis (Marsh and Langhorst, 1988; Schooley, 2008), given that a visual analysis of larval fish predation was conducted on the fish used for this study, with no larval fish being identified on the stomachs analyzed in this work (Legler, 2009).

In the last decade SNP assays have been used to identify species-specific polymorphisms in a wide variety of applications (Best et al., 2007; Shastry, 2007; Manga and Dvorak, 2010). Although other DNA-based techniques (e.g., species microarrays, next-generation sequencing) are also being used to characterize prey items in the stomach, gut or feces of predators (Bohmann et al., 2011; Deagle et al., 2010), they tend to be expensive and not applicable to large-scale studies at a population level. Even though SNP-based prey identification assays target only one or two prey species at a time, they allow large sample throughput and very high resolution due to the PCR amplification of small DNA fragments (Carreon-Martinez unpublished results). Other molecular genetic approaches to study trophic interactions at an ecosystem level require multiple PCRs, cloning and sequencing, and/or gel electrophoresis (Carreon-Martinez and Heath, 2010; Hardy et al., 2010; Corse et al., 2010) which increase the cost and decreases
the throughput of the analysis relative to a SNP assay. To date, the present study is the first in which a SNP assay has been used to estimate predation rates in a large ecosystem. The potential applications for SNP assays in trophic analyses are numerous; several SNP assays could be developed for various prey species and applied to many predators quickly and at low cost providing reliable and accurate predation data.

In conclusion, this work sheds light onto the mechanisms underlying the effects of turbidity associated with river discharge on predation experienced by larval yellow perch in the western basin of Lake Erie. River plumes can substantially modify the water basin by creating nutrient-rich areas and elevated turbidity that could benefit fish productivity and recruitment. In this case it appears that elevated turbidity, created by the Maumee River plume, provides protection against visual predators for larval fish. Predation levels measured in this work, in combination with other possible factors (i.e. alternative prey availability, predator abundance, etc.), will help explain the observed variation in yellow perch recruitment in the western basin of Lake Erie. The yellow perch-specific SNP assay in combination with yellow perch microsatellite genotyping, were critical in detecting and quantifying yellow perch DNA in predator stomach contents. My work could indeed aid fisheries management to better quantify real predation incidence and to predict recruitment success not only of yellow perch but potentially many other species as well.

REFERENCES


Supplementary material 5.1.

Yellow perch specific single nucleotide polymorphism (SNP) assay primers. SNP assay was designed using CO1 mitochondrial gene sequence. Primer concentrations in parenthesis. VIC and FAM are fluorescent dyes; NFQ: non fluorescent quencher.

YelloPerc1-79YPF CTGGAGCATCTGTTGATTTAACCATT TT (36 µM)
YelloPerc1-79YPR CCCATACGAACAAGGGAGTTTGATA (36 µM)
YelloPerc1-79YPV1 VIC CTCAATTCTAGGAGCTATT (8 µM) NFQ
YelloPerc1-79YPM1 FAM CTCAATTCTAGGTGCTATT (8 µM) NFQ

Sequence- Perca flavescens voucher ROM:ICH:BCF-0214-3 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial. 652 bp

ACCESSION EU524240

1 cctttatcta gtatttgtgt cttgagccgg aatagtgggc actgecctaa gcttgcttat
61 ccgagcagaa ctaagccacgc cgccgctct cctaggagac gaccagatt ataaagttaat
121 tgttacagca catgccttcg taataatttt ctttatagta ataccaatta tgattgcccc
181 cttggaac acgcctagct ccaataattt cttggttct cgttctctgt acatagctt acatagctt acatagctt
241 aaataatatg agcttttggc tcctgcctcc ttctttcctt ctcctcctcct ctcctcctcct
301 agttgaagcc ggagctggta ccggatgaac tgtttatccc cctcttgctg ggaacttagc
361 acatgctgga gcatctgttg atttaaccat tttctctta cacttagccg gggtttccct
421 aattctaggt getattaatt tattaagac atacattaat ataaaaacccc ctcgctctcct
481 ccaaatacac acteccttgt tcgtatgggc tgtattagaat aacggtggct tctctctctt
541 ttcactacct gttcttgccgt cttgtcattc aatgcttctt acatagctt acatagctt acatagctt
601 caatggcctc gatcctgcag gaggccgtgg tccctccttt taccaactt ta
Supplementary Table 5.1

Number of predator stomach contents analyzed with yellow perch specific SNP assay from late April – early July 2006-2008. WA: walleye (*Sander vitreus*); WP: white perch (*Morone americana*); WB: white bass (*Morone chrysops*). Detroit: predators collected in the Detroit River plume; Maumee: predators collected in the Maumee River plume. Note: Variation in sampling weeks among years was based on larval yellow perch abundance in the Detroit and Maumee River plume (Reichert, et al, 2010).

<table>
<thead>
<tr>
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<th>PERIOD</th>
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<td></td>
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<td>WB</td>
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</tr>
<tr>
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<td>4</td>
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<tr>
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<td>82</td>
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<td>21</td>
<td>98</td>
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Supplementary table 5.2

Number of predators (walleye, white bass and white perch) stomach contents analyzed with yellow perch specific SNP assay within plume and year 2006-2008. Daily (24 hrs) predation rate and mean number of yellow perch prey present per species.

<table>
<thead>
<tr>
<th>Year</th>
<th>Plume</th>
<th>Species</th>
<th>Total Number</th>
<th>% with YP</th>
<th>Mean # of YP prey</th>
<th>Daily (24hr) Predation rate ± SE</th>
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<tr>
<td>2006</td>
<td>D</td>
<td>WA</td>
<td>110</td>
<td>7</td>
<td>0.32 ± 0.24</td>
<td>0.48 ± 0.36</td>
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<td>88</td>
<td>15</td>
<td>1.22 ± 0.27</td>
<td>1.83 ± 0.04</td>
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<td></td>
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<td>WP</td>
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<td>0.70 ± 0.17</td>
<td>1.05 ± 0.25</td>
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<td></td>
<td>M</td>
<td>WA</td>
<td>80</td>
<td>5</td>
<td>0.19 ± 0.71</td>
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<td>0.06 ± 0.71</td>
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<td>WP</td>
<td>201</td>
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<td>0.04 ± 0.04</td>
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<td>2007</td>
<td>D</td>
<td>WA</td>
<td>81</td>
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<td>0.16 ± 0.21</td>
<td>0.24 ± 0.31</td>
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<td>0.20 ± 0.14</td>
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<td>WP</td>
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<td>40</td>
<td>2.42 ± 0.36</td>
<td>3.63 ± 0.54</td>
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<td>M</td>
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<td>1.60 ± 0.55</td>
<td>2.19 ± 0.83</td>
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<td>4.50 ± 1.80</td>
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<td>WP</td>
<td>90</td>
<td>35</td>
<td>1.03 ± 0.22</td>
<td>1.55 ± 0.33</td>
</tr>
</tbody>
</table>

D= Detroit River plume; M= Maumee River plume; WA= walleye (*Sander vitreus*); WB= white bass (*Morone chrysops*); WP= white perch (*Morone americana*); SE= standard error.
Supplementary Figure 5.1 Predators (walleye, white bass and white perch) length and number of yellow perch prey detected in stomach contents analyzed using yellow perch specific SNP assay and microsatellite DNA in 2006, 2007 and 2008 respectively.
CHAPTER 6

QUANTIFICATION OF YELLOW PERCH (*PERCA FLAVESCENS*) AND
WALLEYE (*SANDER VITREUS*) CANNIBALISM IN THE WESTERN BASIN OF
LAKE ERIE.

INTRODUCTION

Cannibalism, while not common, is found widely in the animal kingdom, from invertebrates through vertebrates, including fish and mammals (Fox, 1975; Polis, 1981; Smith and Reay, 1991). Although cannibalism occurs broadly, there is no single explanation for the role that cannibalism plays in the diverse species and ecosystems where it is reported. It is generally believed that cannibalism can represent a significant risk to the predator due to ingestion of parasites (Pfenning, 2000). Thus the expectation is that cannibalism should be very rare, and temporally transient. Nonetheless, cannibalism has been shown to persist through time in a wide variety of taxa such as snails, insects, anurans, birds and fish (see review by Fox, 1975 and Smith and Reay, 1991). Previous work suggests that cannibalism confers benefits as well as risks to cannibalistic predators, such as prey with high nutritive content necessary for successful reproduction (Wildy et al., 1998; Meffe and Crump, 1987). Fish and aquatic species tend to exhibit some of the highest incidence of cannibalism, with Fox (1975) reporting that the highest number of cannibalistic species are found in freshwater habitats (53 species listed including invertebrates and vertebrates) while Smith and Reay (1991) reported that cannibalism is found in 9% (36 out of 410) of teleost fish families. Given the scattered occurrence of cannibalism across taxa and ecosystems it most likely results from multiple factors which vary among species and ecosystems; it is therefore important to quantify cannibalism in
aquatic ecosystems, as well as to determine the role it plays in fish population dynamics and ecosystem foodwebs.

Numerous studies in fisheries have demonstrated that fish mortality is highest during the larval period; with early-life predation being one of the most significant contributors to that mortality (Miller, 1988; Houde, 1989; Bailey and Houde, 1989). Curiously, cannibalism is often overlooked as a potentially significant form of early-life mortality, although there is evidence for it in the literature (Chevalier, 1973; Fox, 1975; Smith and Reay, 1991). However, there are only a few studies that specifically quantify rates of larval cannibalism and estimate the potential impact on recruitment. Fluctuations in anchovy (Engraulis capensis) recruitment off the coast of South Africa are likely to be attributable at least in part to cannibalism: Szeinfeld (1991) reported that 6% of the total anchovy egg mortality was the result of anchovy cannibalism. Even a very low rate of cannibalism can cause significant mortality in specific cohorts (Fox, 1975). In a study of adult walleye in Oneida Lake, less than 3% of walleye practicing cannibalism could explain up to 88% of the variation in mortality observed among young fish cohorts (Chevalier 1973, Fox, 1975). Although some studies have shown a strong negative effect of cannibalism on recruitment, little is known of the potential effects of cannibalism on commercially fished species in large freshwater ecosystems such as the Great Lakes. For this reason it is important to characterize the incidence and temporal persistence of cannibalism, particularly for commercially harvested fishes in which population fluctuations represent uncertainty in management efforts.

The western basin Lake Erie is fed by two main river inputs: the Maumee River and the Detroit River. The Maumee River, in contrast to the Detroit River, is characterized as being nutrient-rich (Baker and Richards, 2001; Reichert et al., 2010)
therefore it creates an area of higher turbidity caused by primary production and resuspended sediments. The Maumee River plume has been shown to positively influence larval yellow perch survival (Reichert et al., 2010; Chapter 4) and in Chapter 5, I showed that predation is higher in the Maumee River plume. However, it is not known if cannibalism contributes to larval mortality, nor whether it varies between the two plume environments. Here I quantify walleye and yellow perch cannibalism in the western basin of Lake Erie. Although yellow perch and walleye cannibalism has been reported previously (Fox 1975, Thorpe 1977; Chevalier, 1973), it is not known how big a role it plays in Lake Erie stocks. Traditional diet analysis techniques are not effective in detecting small fish prey due to a rapid digestion rate of soft tissue (Schooley et al., 2008; Legler et al., 2010; Carreon-Martinez et al., 2011), thus I use a combination of molecular genetic techniques to assess walleye and yellow perch cannibalism levels in the early spring and summer of 2007 and 2008. My hypotheses are that cannibalism will be present in yellow perch in the western basin of Lake Erie and that detected cannibalism levels will be higher in the Detroit River plume. My specific objectives were to: 1) develop molecular genetic assays to detect and quantify cannibalism in walleye and yellow perch, 2) test for changes in cannibalism over time and 3) test for plume effects on the rate of cannibalism. The findings of this work will aid in understanding the effect that cannibalism might have on population structure and survival for larval yellow perch and walleye.

**Materials and Methods**

Here I first describe the development and validation of a walleye-specific single nucleotide polymorphism (SNP) assay (the yellow perch specific SNP assay is described
in Chapter 5). Secondly, I describe the detection and quantification of cannibalism in yellow perch and walleye samples taken in two years: over 8 weeks in 2007 and 4 weeks in 2008) using yellow perch and walleye specific SNP-based molecular genetic diet analysis. Finally I quantify cannibalism rates in yellow perch and walleye using microsatellite marker analysis to estimate the number of cannibalized prey items in stomach content samples of both species.

**Walleye SNP assay design and validation:**

Sequences from the Fish Barcode of Life Database (FishBOLD, Cytochrome oxidase subunit 1 (CO1) mtDNA, Hubert et al., 2008) from all fish species known to be present in Lake Erie were screened to identify a single nucleotide polymorphism that uniquely identified walleye and yellow perch from all other fish species. I found one nucleotide substitution that was unique for yellow perch (Chapter 5) and walleye specifically (Supplementary Material 6.1); each single nucleotide polymorphism was used to design a taq-man© (Applied Biosystems) species specific SNP assay (yellow perch: Chapter 5 Supplementary Material 5.1 and walleye: Supplementary Material 6.1).

Yellow perch SNP assay validation was described in Chapter 5. The validation of the walleye SNP assay followed closely that of the yellow perch SNP assay and was divided into three stages; the first step was to determine a range of target DNA concentrations in which the assay provided consistent amplification and fluorescence signal. I used a wide range of walleye DNA concentrations; 0.5, 1, 2, 3, 5, 10, 13, 15, 20, 30, 40, 60, 80, 100, 300, 500, 700, 800 ng µL⁻¹. Secondly to investigate the possibility of cross-amplification with non-walleye DNA, a range of DNA concentrations (0.5, 1, 3, 5, 10, 13, 15, 20 ng µL⁻¹) of other Lake Erie species such as yellow perch, *Perca flavescens*;
white perch, *Morone americana* and white bass, *Morone saxtilus* were used separately as template for the SNP assay. In addition, to test the performance of the walleye-specific SNP assay in the presence of competing DNA in a single reaction, I evaluated the SNP assay with a mixture of DNA composed of 1.5 μL of yellow perch, white perch and white bass DNA (20 ng μL⁻¹ each) without walleye DNA; and with walleye DNA at two different concentrations separately (1.5 μL of 1 ng μL⁻¹ and 5 ng μL⁻¹ walleye DNA). All of the validation experiments were run in triplicate.

All samples containing walleye DNA consistently amplified the target fragment between 18-30 cycles, and generated a mean fluorescence signal of 0.94 (range between 0.44 -1.8 for 0.5 – 20.0 ng μL⁻¹), hence the fractional cycle number (Cₜ) and the Delta Rn (ΔRₚ, magnitude of the fluorescence signal relative to background) were the parameters used as thresholds to determine if the sample contained walleye DNA. A Student’s t-test was used to test for significant differences in Cₜ and ΔRₚ for samples containing walleye and those with no walleye DNA, but containing other species DNA. All statistical analyses were performed using SYSTAT v9.0 (Systat Software, Inc, 2004 v 9.0).

**Yellow perch and walleye cannibalism determination**

Adult yellow perch and walleye were collected in the western basin of Lake Erie and categorized as being from the Maumee and Detroit River plumes (see Chapter 5 for details). Collections were made weekly from late-April through early-June in 2007, and in May in 2008 using a bottom trawl and graded mesh gillnets (approximately one meter below surface water; random series of consecutive 15 m panels of 38, 51, 64, 76, 89 mm stretch monofilament twine). Collections were done for 1-2 hours once a week from May
through June in 2007. Only bottom trawl sampling was done on May 6, 8, 12, 15, 20 and 23 in the Detroit and Maumee plume areas in 2008. Since larval fish are highly vulnerable to predation at the beginning of the larval period (Bailey and Houde, 1989) I divided the collected samples in two collection periods: early larvae (1-5 weeks after hatching) and late larvae (5-8 weeks after hatching) in 2007 or 2008 accordingly. All captured fish were euthanized using clove oil, their stomachs injected with 95% ethanol to halt digestion, and the whole fish were frozen for future analysis. In the laboratory, each fish was thawed, and stomachs were removed for molecular genetic diet analysis. Each processed stomach was emptied into a Petri dish with the aid of tweezers for fragments that needed to be manipulated and by washing the stomach wall clean with 95% ethanol to avoid scraping cells from the stomach lining.

A small subsample of the stomach contents (0.5-60 mg, wet weight) was aliquoted into a 1.5 mL eppendorf tube (as described in Chapter 5) and each tube was centrifuged at 13,000 rpm for 10 min to precipitate any tissue or DNA in the ethanol. After centrifugation, I removed the supernatant and let the sample air-dry for 12 hours, followed by 10 minutes in a dry-vac system to ensure all the ethanol was eliminated. In addition, to differentiate predator DNA from the prey DNA, DNA was extracted from a small section (0.5 x 0.5 cm) of the stomach muscle of all sampled fish. Stomach contents of walleye and yellow perch, separately, were screened for cannibalism using the walleye and yellow perch specific SNP assay respectively.

*DNA extraction & SNP assay:* DNA was recovered from stomach contents and predator stomach tissue samples following a plate-based extraction method (Elphinstone...
et al., 2003) and resuspended in 50 μL of Tris–EDTA buffer (10 mM Tris, 1.0 mM EDTA, pH 8.0).

Quantitative real-time PCR (qRT-PCR) was performed for yellow perch and walleye separately using a 7500 ABI real-time quantitative thermal cycler (www.appliedbiosystems.com). The qRT-PCR reaction (12 μL) consisted of 6 μL of Universal PCR Master Mix (no AmpErase UNG), 0.5 μL of SNP assay probe and primer mix (for primer and probe concentrations see supplementary material 6.1), and 5.5 μL of stomach content DNA. Amplification consisted of a one initial cycle at 95°C for 10 minutes, followed by 40 cycles of denaturing at 92°C for 15 seconds and annealing/extension at 60 °C for 1 minute. Presence or absence of walleye or yellow perch DNA was determined based on the fluorescence value (ΔR_0) and the C_T value as determined in the previous section.

**Microsatellite genotyping and cannibalism rate determination:** To verify the cannibalism in yellow perch and walleye, the stomach content sample DNA was genotyped at three microsatellite loci (Svi5, Svi10, Svi17 from Cena et al. (2006) for walleye; YP78, YP85, YP99 from Li et al. (2007) for yellow perch). PCR amplification was performed in 25 μL reactions, for both predator and stomach content DNA, with the following components: 2.5 μL of template DNA, 2.5 μL 10x PCR buffer, 2.5 μL of MgCl_2 (25 mM), 0.6 μL of dNTPs (50 μM of each), 0.4μL (0.5 μM) of dye labeled primer, 0.4 μL (0.5 μM) of the reverse primer and 0.10 U Taq polymerase (Applied Biosystems, Foster City, CA). PCR conditions were: initial denaturation at 94°C for 2 min, followed by 35 to 40 cycles of denaturing at 94°C for 15 s, locus-specific annealing temperature (according to Li et al., 2007 and Cena et al., 2006 respectively) for 15 s,
extension at 72°C for 30 s and a final extension of 72°C for 10 min. Microsatellite allele sizes were determined using a LI-COR 4300 DNA analyzer (Lincoln, NE) and scored using Genelmage IR 4.05 (Scanalytics, Inc., Rockville, MD).

I compared the stomach content microsatellite genotypes with the predator genotypes at the same microsatellite loci. Alleles present in the stomach content PCRs were compared to the allele sizes generated from the predator DNA, and matching alleles were not included in the estimation of the number of cannibalized prey. The potential number of prey items present in the stomach content was estimated using a relationship between the cumulative number of randomly selected genotypes of 25 individual larval fish and the total number of alleles observed in this sub-sample (as described in Chapter 5). I was therefore able to estimate the number of prey fish likely to explain the number of alleles observed in each stomach content sample for each microsatellite locus. The number of prey fish estimated at each locus was averaged across the estimates at the three loci to estimate the total number of yellow perch prey present in the predator stomach contents. For walleye predator samples we used the same method described above but using the adult walleye allele frequency distribution.

Cannibalism rate was estimated by dividing the average number of larval prey (i.e. yellow perch or walleye separately) present in the stomach contents by the total number of predators collected.

Statistical analysis

I compared the estimated cannibalism rates for the Detroit or Maumee River plumes using the parametric Student t-test statistical analysis, for each species separately. In addition, I tested for possible temporal differences in cannibalism rates between 2007
and 2008, as well between collection periods within year using the same statistical analysis as described above.

**RESULTS**

*Walleye SNP Walleye assay validation*

The lowest sample DNA concentration for which we could consistently detect walleye DNA was 0.5 ng μL⁻¹. The highest sample concentration of DNA in which we observed a consistent ΔRₙ and Cₜ value was 20 ng μL⁻¹. The assay performance with walleye DNA concentration in the 40 – 800 ng μL⁻¹ range was similar to the no template control, with no amplification curve. DNA concentrations between 0.5 and 20.0 ng μL⁻¹ consistently amplified at Cₜ = 18- 30 cycles (for 0.5 – 20.0 ng μL⁻¹) with a mean ΔRₙ = 0.94 (range between 0.5 -1.8 for 0.5 – 20.0 ng μL⁻¹; Figure 6.1). Therefore the most reliable and consistent detection range for walleye DNA in stomach samples is between 1.0-20.0 ng μL⁻¹.

To investigate the possibility of cross-amplification with predator DNA I used different concentrations of yellow perch, white perch and white bass DNA separately at sample concentrations ranging from 1.0-20.0 ng μL⁻¹, and in all cases the SNP assay showed no amplification until around cycle 35, with no residual ΔRₙ signal detected (Figure 6.1). The mixture of walleye DNA with DNA from 3 other predator species produced a positive amplification signal for walleye DNA at cycles between 18-20 at lower 1 ng μL⁻¹ and higher 5 ng μL⁻¹ concentrations. When walleye DNA was not present in the mixture of DNA sample, but other species DNA was, there was no signal positive for walleye DNA (Cₜ > 34 cycles). The DNA mixtures of other predators with walleye DNA yielded Cₜ and ΔRₙ values that were statistically different than those from the
Yellow perch and walleye cannibalism determination

Walleye and yellow perch stomach contents were screened for cannibalism using the respective SNP assay. For walleye 98% of the samples were positive for the presence of walleye DNA (131 out of 133). For yellow perch 93% of the samples were positive for yellow perch DNA presence (173 out of 185). Such high percentages of walleye and yellow perch DNA presence in the stomach contents sample are likely due to predator DNA contamination, therefore I did not use the SNP assay for 2008 samples. From this point on, only microsatellite DNA analysis was used to detect cannibalism and to distinguish between predator and prey DNA signal.

Microsatellite genotyping and cannibalism rate determination

A total of 133 and 55 walleye and 185 and 151 yellow perch stomach content samples for 2007 and 2008 respectively were genotyped at three microsatellite loci. Only stomach content samples that amplified at all three microsatellite loci with at least one allele different than the predator signal were considered positive for cannibalism.

For the walleye stomach content samples taken in 2007 and 2008 there was no indication of cannibalism in any of the samples processed (i.e., the ones that amplified microsatellite alleles matched the predator signal): In 2007 and 2008, 12% (16 out of 133) and 18% (10 out of 55) of the samples amplified all three microsatellites loci respectively; 14% (19 out of 133) and 14% (8 out of 55) amplified two microsatellite loci respectively; and 12% (16 out of 133) and 18 % (10 out of 55) amplified one microsatellite locus.
respectively; 56% (74 out of 133) and 46% (25 out of 55) did not amplify any of the three loci for 2007 and 2008 respectively.

For yellow perch samples, in 2007 cannibalism was detected in 10% (19 out of 185) of the samples; of the remaining samples, 24% (45 out of 185) PCR amplified at only two microsatellite loci, 27% (50 out of 185) amplified just one microsatellite locus, and 39% (71 out of 185) did not amplify any of the microsatellite loci. For the stomach content samples that did PCR amplify one or two loci, only 24% (23 out of 95) had alleles other than the predator alleles present. In 2008, cannibalism was detected in 18% (27 out of 151) of the yellow perch stomach contents; of the remaining samples, 11% (16 out of 151) PCR amplified in two microsatellite loci, 21% (32 out of 151) PCR amplified for just one microsatellite locus, and 50% (76 out of 151) did not amplify any of the microsatellite loci. For the stomach content samples that did amplify for one or two microsatellite loci, 50% (24 out of 48) had more alleles present than those that belong to the predator.

The cannibalism rate was higher for samples collected in the Detroit River plume relative to those from the Maumee River plume in 2007 and 2008. Student t-tests between river plumes within year were not significant in either early larvae or late larvae collection periods in 2007 (Student t-test statistical analysis; t= 1.20, df= 111, p=0.231 and t=0.143, df=70, p=0.887 for early and late larvae periods respectively; Figure 6.2) but was significantly different in 2008 (t= 14.19, df= 1, p < 0.001; Figure 6.2).
Figure 6.1. $C_T$ (panel b) and $\Delta Rn$ (panel a) values for walleye (WA; *Sander vitreus*) SNP assay at different DNA concentrations of walleye and other species DNA (white perch, yellow perch and white bass). In panels a and b: filled circles represent walleye DNA and open circles represent non-walleye species.
Figure 6.2. Cannibalism rate (average number of prey per yellow perch predator per day, including all predators) in the western basin of Lake Erie calculated using microsatellite loci. *: denotes significant differences between plumes within collection period (i.e. early larvae or late larvae) and / or between collection years.
DISCUSSION

Here I describe the development and application of a highly specific and sensitive yellow perch and walleye SNP assay for the detection of cannibalism; however the sensitivity of the assays was such that they ended up detecting the predator DNA signal in almost all stomach contents analyzed. Consequently I do not think that SNP assays are useful tools in distinguishing between predator and prey of the same species. However, I could detect and quantify cannibalism using microsatellite marker amplification in stomach contents; although both the predator and prey DNA PCR amplified, I could distinguish them by allele size. This work is the first to quantitatively estimate cannibalism in yellow perch using molecular genetic methods. Although cannibalism was not detected in walleye, it was relatively common in yellow perch in the western basin of Lake Erie, in particular within the Detroit River plume. Furthermore, many yellow perch stomachs contained more than one yellow perch prey, indicating that the cannibalism was likely not accidental, but rather represents active foraging. However, the estimated cannibalism determined in this work is most likely underestimated due to DNA degradation, as evidenced by the predator samples with only partial PCR amplification of the utilized microsatellite markers. The level of yellow perch cannibalism varied substantially in space (between collection sites) and time (within years and between years), making it difficult to predict or use as a factor in recruitment models. Nonetheless, having identified cannibalism in yellow perch as a potentially significant source of early-life mortality, fisheries management models that include the possibility of cannibalism may be able to better account for larval fish survival and recruitment success.

Cannibalism was detected in yellow perch but not in walleye. The fact that cannibalism was not detected for walleye in our study may be due to a species bias, or it
may be due to the fact that larval walleye were not as abundant as larval yellow perch at our collection sites. The predator sampling targeted areas and times where yellow perch larvae and early juveniles were expected to be abundant. Sampling occurred when the larval yellow perch had left their nursery habitat to start their pelagic stage, making them available to predators (see Chapter 5; Reichert et al., 2010, Legler, 2009). Since walleye larvae hatch earlier and at larger sizes (6–8 mm; Marshal 1977) than yellow perch (5–6 mm; Thorpe 1977), the walleye larvae may have had better predator avoidance behaviour than the larval yellow perch in the same area (Bailey and Houde, 1989). In addition, young walleye might have completed their pelagic phase and moved on to a different habitat (Goodyear, 1982) explaining the absence of young walleye in the stomach contents of walleye predators (i.e. no cannibalism detected). Therefore it is possible I could have detected cannibalism in walleye if more walleye predators were collected at different locations within the western basin of Lake Erie.

The relative high incidence of cannibalism observed in yellow perch may have been due to a combination of factors, such as the abundance of vulnerable victims (i.e. yellow perch prey) and limited alternative food availability. Based on the cannibalism rate calculated in this chapter and the abundance of yellow perch in the western basin of Lake Erie (ODNR and OMNR status of fish stock report 2011) I estimated that, on average (2006-2008), yellow perch is responsible for ~1% of mortality of larval yellow perch in the first 30 days after hatching. Abundance and availability of vulnerable prey is a known factor affecting cannibalism (Fox, 1975; Post and Evans, 1989). Larval and late-larval yellow perch in May through early July are both highly abundant (Reichert et al., 2010) and vulnerable to predation (Baxter, 1986; Bailey and Houde, 1989). In addition, even a small reduction in food availability is enough to increase cannibalistic tendencies
(Fox, 1975; Smith and Reay, 1991). Interestingly, in 2008, when I measured the highest cannibalism rate, the presence of forage fish was well below the long term average (FTG, 2009). In 2007, when cannibalism was not exceedingly high, forage fish abundance was substantially higher (FTG, 2009). Cannibalistic tendencies are exacerbated in the yellow perch congener, the Eurasian perch (*Perca fluviatilis*), in lakes that are nutrient poor (Fox, 1975; Sanderson *et al.* 1999). In addition, I found an increased level of predation (Chapter 5) on young yellow perch in 2008 by other species of predators, suggesting that because food was limited all predators were relying on young yellow perch as prey.

I observed substantial variation in the level of yellow perch cannibalism, with reduced levels after the early larvae collection period in 2007, and with a rise in the levels in the early larvae period in 2008. This temporal change in cannibalism rate could be attributed to the presence of highly abundant and vulnerable larval fish early in the larval period (Fox, 1975; Post and Evans, 1989) as discussed above. On the other hand, the reduction of cannibalism in the late larvae collection period could be due to the migration of late larvae into a benthic habitat (Goodyear *et al*., 1982) or that young yellow perch become better swimmers with improved predator avoidance behaviour as they grow (Blaxter, 1986; Bailey and Houde, 1989). The generally higher cannibalism rate in the Detroit River plume (relative to the Maumee River plume) is likely due to higher water clarity allowing better prey visualization than in the turbid waters of the Maumee River plume (DeRobertis *et al*., 2003; Utne-Palm, 2001). The higher cannibalism rates observed in the Detroit River plume are also in general agreement with the patterns of predation on larval yellow perch larvae discussed in Chapter 5, and predicted indirectly (Reichert *et al*., 2010; Carreon-Martinez *et al.* 2011).
The number of cannibalized individuals in a species' diet is difficult to determine accurately (Fox, 1975; Smith and Reay, 1991); however, I have shown that molecular genetic techniques make it possible to not only identify the presence of specific prey species, but also to estimate the number of prey items consumed by predatory fish. This provided strong evidence suggesting that the high cannibalism levels reported here may explain the high variability observed in yellow perch population levels between 2007 and 2008 since other work has shown that higher cannibalism is associated with reduced recruitment later in life (Chevalier 1973, Fox, 1975, Szeinfield, 1991). Since cannibalism can represent a significant mortality factor, and thus impact yellow perch recruitment success, it should be incorporated into future models of fish population dynamics to better estimate recruitment success. However, this will not be possible until the ecological and trophic factors that drive the high levels of spatial and temporal variation in cannibalism are better characterized and quantified.

REFERENCES


Supplementary Material 6.1

Walleye specific single nucleotide polymorphism (SNP) assay primers. SNP assay was designed using CO1 mitochondrial gene sequence Primer concentrations in parenthesis. VIC and FAM are fluorescent dyes; NFQ: non fluorescent quencher.

Svitr430C_F  TCGACTTAACCATTCTCTCTACATTAGCA (36 μM)
Svitr430C_R  GCGGTATTAATACGGCTCAAAACAA (36 μM)
Svitr430C_V  VIC  ATTAATTGCCTAGAATT (8 μM) NFQ
Svitr430C_M  FAM  ATTAATTGCCCCCTAGAATT (8 μM) NFQ

Sequence Sander vitreus voucher ROM:ICH:BCF-0192-1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial.

ACCESSION  EU524380

1 ccttatcta gtatttgggtg ctttgagcgg gtagtgctct gcctactcat
2 ttctctctct ctctctctct ctctctctct ctctctctct ctctctctct
3 gcctactcat cttgagcgg gcctatgcac atataataat atttctattt
4 atttctattt atataataat atttctattt atataataat atttctattt
5 gcctactcat cttgagcgg gcctatgcac atataataat atttctattt
6 atttctattt atataataat atttctattt atataataat atttctattt
7 gcctactcat cttgagcgg gcctatgcac atataataat atttctattt
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CHAPTER 7
SYNTHESIS AND CONCLUSION

INTRODUCTION

River plume effects on larval survival

Nutrient-rich river runoff into large bodies of water will create river plumes, which are often characterized by increased food availability and higher turbidity associated with increased plankton biomass and suspended sediments. River plumes thus create favourable conditions (i.e. higher food availability) for larval fish and are thought to increase the odds of larval survival (Grimes and Finucane, 1991; Le Pape et al., 2003).

The western basin of Lake Erie is an ideal study site to compare river plume effects on larval fish survival. The Maumee River plume, in contrast with the Detroit River plume, is nutrient-rich and highly turbid due to high primary productivity and suspended sediments (Dolan and McGunagle, 2005; Reichert et al., 2010). A previous study (Reichert et al., 2010) determined that zooplankton availability did not differ between the Maumee and Detroit River plumes. Yet, Reichert et al. (2010) and my Chapter 4 independently showed higher survival of larval yellow perch in the Maumee River plume relative to the Detroit River plume; hence, if food availability is not likely to be creating the difference in survival, the question remains as what is the most likely factor behind the differential survival. Therefore, one of my doctoral objectives was to determine if larval fish were experiencing the same level of predation in the two plumes.
The role of turbidity

Turbidity can potentially affect larval survival by either diminishing or enhancing the contrast between the larval prey and its background (Abrahams and Kattenfeld, 1997; Utne-Palm, 2001), and thus impact the ability of larvae to survive visual predator attacks. Predation is one of the major factors affecting fish survival in early life (Miller et al., 1988; Bailey and Houde, 1989; Leggett and Deblois, 1994). A clear understanding and the quantification of the contribution of predation during early life stages to overall mortality of fish is crucial to better understand fluctuations in population size. Cannibalism (i.e. intraspecific predation) may also play a significant role in early life survival, and it can be an important determinant in overall recruitment success (Chevalier 1973, Fox 1975, Chapter 6).

Yellow perch predation and cannibalism (Chapters 5 and 6) were significantly higher in the less turbid water of the Detroit River plume. These results suggest that turbidity in the Maumee River plume could indeed be protecting larval yellow perch from visual predators. These findings support the importance of the Maumee River discharge associated turbidity in the survival and consequent recruitment of larval yellow perch.

In addition, I found that predation and cannibalism varied within the collection period, both within years, and among years. This temporal variation in predation and cannibalism, apart from the plume effect, will make it difficult for fisheries managers to anticipate year to year variation in predation and cannibalism levels, therefore affecting their ability to predict recruitment success. However, predation and cannibalism, among other factors, could potentially be linked to Age-0 year class abundance. The results of Chapters 4, 5 and 6 showed that the year 2007 was consistently different from 2006 and 2008; it had higher larval yellow perch survival (Chapter 4) and lower predation (Chapter
5) and lower cannibalism (Chapter 6), and this resulted in a stronger year class when compared with 2006 and 2008 abundance numbers (YPTG, 2009). Although predation and cannibalism are not the only factors contributing to strong year classes, careful monitoring of these two factors would aid in the understanding of the underlying patterns behind recruitment variability.

**Innovative techniques for diet analysis**

Successful quantification of predation and cannibalism during the early life stages of larval yellow perch was possible using advanced molecular genetic techniques implemented for diet analysis. There are a variety of molecular genetic techniques that have been used in the field of diet analysis (i.e. cloning and sequencing, primer specific PCR, RFLP, etc; Chapter 2; see reviews by Symondson, 2002; Sheppard and Harwood, 2005; Teletchea, 2009). All of those techniques have their advantages but also their limitations, and often their appropriate utilization depends on the research question and objectives. One of the advantages of the techniques used in Chapter 3 (PCR, cloning & sequencing) was the broad range of prey items that were identified in the stomach contents, but the size of the PCR amplified DNA fragment (~ 600 bp) limited detection due to digestive DNA degradation. Nonetheless, I successfully identified prey to species level even after 16 hrs of digestion time (Chapter 3). On the other hand, the techniques utilized in Chapters 5 and 6 (SNP coupled with microsatellite genotyping) are the first examples of such techniques implemented for diet analysis, and more specifically, to detect cannibalism. The advantage of using SNPs and microsatellite DNA is that both techniques are powerful and accurate in detecting the target DNA, even though the DNA may be highly degraded (because smaller DNA fragments are amplified, ~150- 300 bp). Quantification of prey items in stomach contents has not been explored previous to this
dissertation; Chapters 5 and 6 represent a new and innovative application of microsatellite analysis. However, other molecular genetic techniques such as microarray or parallel sequencing could be evaluated for their potential value for diet analysis in fish.

Ecological and management implications

This dissertation has shown how river discharge associated turbidity could enhance larval survival by mitigating mortality due to predation and cannibalism. Successful management of ecosystems depends on our ability to identify and predict the causes of fluctuations in abundance of economically important species such as yellow perch. Therefore is important to implement ecosystem-based (e.g. multiple trophic levels) fishery management strategies, and to include the interactions of physical and environmental conditions (i.e. climate change, dams, dredging, etc) with biological resources.

CONTRIBUTIONS

In the following text I list my overall contributions to science. First, I present broad and conceptual contributions, and secondly I list more technical and specific contributions of my work.

**Conceptual contributions to science:**

1) I provide tangible results of the mechanism by which river plume associated turbidity is contributing to better survival of larval yellow perch

2) I show the spatial and temporal genetic structure of larval yellow perch in the western basin of Lake Erie.

3) I quantify predation and cannibalism levels affecting larval yellow perch in spring and early summer.
Technical contributions to science

1) Using laboratory experiments, I show how digestion time affects the accuracy of molecular genetic techniques for fish species identification of stomach contents. In addition, I show how higher temperatures does not affect the ability of molecular genetic techniques to identify stomach contents to species level.

2) My research implemented the innovative combination of two molecular genetic techniques (SNP and microsatellite loci) to quantify trophic interactions in complex ecosystems.

Future Directions

My study provided evidence that predation and cannibalism can represent high mortality sources for larval yellow perch. Although my work provided evidence that those two forms of mortality are likely linked to environmental variables such as river-plume effects (e.g., turbidity, food availability), alternative forage fish abundance, relative prey and predator abundance, and other factors (temperatures, river discharge rate, water level, etc), further work on characterizing and quantifying the relationship between environmental factors and variation in predation is needed.

In addition, a real understanding of how predation and cannibalism vary through time (from hatch through Age-1) would help distinguish if mortality is higher during early life stages of larval fish or at the juvenile stage, thus establishing a new paradigm in fisheries science. Once predation and cannibalism in yellow perch early life stages can be quantified and predicted, they should be incorporated into individual fish-based predictive models for fisheries management purposes.

Finally, molecular genetic techniques should be implemented as a tool for monitoring variation in predation and cannibalism under future environmental challenges.
(i.e. global warming, invasive species, anthropogenic effects) that may threaten the stability of not only the Laurentian Great Lakes but other important freshwater ecosystems as well.

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