The Pennsylvania State University

The Graduate School

Ecosystem Science and Management

LAKE TROUT RESTORATION IN THE GREAT LAKES: FROM HATCHERY TO NATURAL REPRODUCTION

A Thesis in

Wildlife and Fisheries Science

by

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Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Master of Science

May 2015

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ABSTRACT

Lake trout populations in the Great Lakes began to decline in the late 1800's as a result of overexploitation, sea lamprey predation and habitat degradation. This decline continued into the 1950's, when lake trout were thought to be completely extirpated from the Great Lakes, with the exception of Lake Superior and limited areas of Lake Huron. Stocking of lake trout began in the 1960's in an effort to restore naturally reproducing populations, with stocking relying on remnant lake trout populations as source populations. These source populations from the Great Lakes, the Finger Lakes, and from introduced populations in the western United States provided the ability to produce numerous hatchery strains. Historically, multiple lake trout strains were stocked in various locations throughout the Great Lakes with the intent that natural selection would allow for survival of the strain best suited to each specific habitat. However, stocking has not yet achieved intended recovery targets of restoring basin-wide natural reproduction, and so hatchery supplementation continues. In an effort to further lake trout restoration goals throughout the Great Lakes, this study had three components: (1) evaluate the efficacy of hormone manipulation to increase hatchery production of the Klondike strain (2) quantify the current genetic status of hatchery strains in production; (3) identify hatchery strain of origin for lake trout reproducing in the Niagara River, NY, as well as their offspring, and to quantify adult lake trout movement into and out of the Niagara River.

The Klondike strain of lake trout is a humper morphotype native to Lake Superior and the production of broodstock is maintained at Iron River National Fish Hatchery in Iron River, WI. The Klondike strain has very poor eye-up rates compared to other hatchery strains raised in similar conditions (3-33% eye-up rates, compared to >70% of many lean morphotypes). Based on previous studies analyzing potential health and environmental changes possible in a hatchery setting, induction of spawning through the use of gonadotrophin releasing hormone analogues

(GnRHa) was explored as a possible hatchery management tool for increasing eye-up rates. The experimental design consisted of two treatment groups of fish (low dose of 10ug/kg body weight and high dose of 20ug/kg bw) and a control group (saline solution injection). Other lake trout, not used in the experiment, but raised in a similar hatchery setting were used for further comparison (i.e., these "baseline" fish were not handled because they did not receive hormone or saline injections). Both the low and high dose injection of GnRHa resulted in similar increases in eye-up rates compared to control fish (~62%) and baseline non-study fish (>98%). Interestingly, the control injection of saline solution also had higher eye-up rates when compared to the baseline group of fish (84% compared to >98% in treatment groups). Beyond an increase in eye-up rates, fish treated with hormones also had a higher number of viable eggs per fish than baseline fish. While this study had some confounding factors that made results for saline-injected control fish difficult to interpret (i.e., hormone injected and control fish were held in the same holding tanks), hormone injection did improve eye-up rates. This improvement in eye-up rate allows the hatchery to produce a greater number of fry, while still maintaining the same number of broodstock fish. Because the Klondike is the only strain of the humper morphotype currently in the federal hatchery system, increased production could result in reaching targeted stocking rates, and possibly allow for increased stocking or stocking in new locations.

Currently, there are six different hatchery strains of lake trout in production in the federal hatchery system. These strains each represent a sample of six different lake trout populations and are used to assist in the restoration goal of establishing naturally reproducing lake trout populations throughout the Great Lakes. Because these strains are a sub-sample of the source population, genetic diversity can become limited if not properly monitored. This can be detrimental to establishment of or developing populations, if stocked fry are inbred, genetically similar, or somehow have reduced survival due to lack of genetic diversity. To understand the current status of lake trout hatchery strains, fin clips were taken from at least two different year

classes of each strain for genetic analysis (only one year class was available for the Lake Champlain strain; three were available for the Seneca Lake strain). For the Klondike strain, a sample of the source population was available and used for comparison. Using a suite of eleven microsatellite loci, each strain was assessed for genetic diversity. All hatchery strains were found to have similar levels of genetic diversity, even though all are genetically distinct from one another. The Klondike strain, however, showed evidence of loss of genetic diversity, specifically allelic richness, when compared to the wild source population. Continued monitoring of the genetic diversity of the Klondike hatchery strain would be beneficial.

Based on sampling by the U.S. Fish and Wildlife Service and anecdotal evidence from recreational fishermen, a naturally reproducing population of lake trout appeared to be using the lower Niagara River for spawning. To better understand movement of this population and to provide further evidence of natural reproduction, fish were captured in the fall 2010 and 2011 before spawning began and implanted with radio transmitters. Tissue samples were also taken to assess hatchery strain of origin of all adults captured. Sampling for naturally reproduced offspring took place in the fall using egg traps, as well as in the spring using nets. Naturally reproduced offspring were genetically sequenced to determine species before microsatellite markers were used to identify hatchery strain of origin. Identical microsatellite protocols were used on adult samples to determine hatchery strain of origin. The majority (86%) of lake trout sampled during this study, adult and offspring, were assigned to the Seneca Lake strain. While radio transmitters were deployed, the data gathered was insufficient for statistical modeling. Based on the findings from this study, the majority of natural reproduction occurring in the lower Niagara River is by stocked lake trout of the Seneca Lake strain.

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ACKNOWLEDGEMENTS

I would like to thank the Great Lakes Restoration Initiative, the Fish and Wildlife Service and the Pennsylvania Cooperative Fish and Wildlife Research Unit for funding for this project. I thank my advisers, Tyler Wagner and Meredith Bartron for all their assistance, guidance and patience. I thank my committee members, Victoria Braithwaite and C. Paola Ferrari for their support and expertise.

I thank all the members of the Fish and Wildlife Service that supported me during this project. The Lower Great Lakes Fish and Wildlife Conservation Office for their help with field work, specifically: Dimitry Gorski, Todd Duvall, Betsy Trometer, Michelle Casto-Yerty, Robert Haltner and all other office staff and interns that contributed to this project. I would like to thank the entire staff at the Northeast Fishery Center in Lamar, PA, especially: Shannon Julian, Jeff Kalie, Greg Jacobs, and Brian Layton. Lastly, I would like to thank the staff at Iron River National Fish Hatchery, particularly Dale Bast, Carey Edwards, and Shawn Saunders. Without the cooperative effort of these people, this project would not have happened.

I appreciate all the help and support of my lab members- Devin DeMario, Tyrell Deweber, Evan Faulk, Megan Kepler, Lori Smith and Bob Mollenhauer- as well as the love and support of my family and friends.

Chapter 1

Lake Trout (*Salvelinus namaycush*) in the Great Lakes

Lake trout biology- Lake trout (*Salvelinus namaycush*) are a large freshwater char native to northern North America (Healey 1978). Preferred habitats include cold, deepwater lakes; however, they can also be found using shallower waters, including rivers, during the spring and fall. Lake trout can live 20 years or more and are generally reproductively mature between the ages of 5-9 (Zimmerman and Krueger 2009). Size at maturity varies from 330 mm to 600 mm, and like age at maturity, is related to factors such as lake size, climate, and exploitation status of the population (e.g., fishing pressure, proportion of stocked fish contributing to the population, and health of the population; Healey 1978; McDermid et al. 2010a). Current lake trout populations throughout the Great lakes consist of three different morphotypes: the lean, humper, and siscowet, which are defined on the basis of phenotypic and life history characteristics (Krueger and Ihssen 1995). These morphotypes are thought to have evolved from the utilization of different habitats and resources throughout the Great Lakes (Page et al. 2004; Janssen et al. 2007; Zimmerman et al. 2009) and are genetically distinct (Krueger and Ihssen 1995; McDermid et al. 2007; Guinand et al. 2012). Variations between the three include habitat, preferred depth, growth rates, body shape, spawning time and buoyancy (Eschmeyer 1957; Rahrer 1965; Zimmerman et al. 2009; Goetz et al. 2010; McDermid et al. 2010a). Lean lake trout are the most prized commercially (Harvey et al. 2003) and have primarily been the focus of restoration efforts (Page et al. 2004; Janssen et al. 2007).

Lake trout reproduction- Lake trout populations in the Great Lakes do not exhibit clear sexual dimorphism during the spawning season, aggression between males, or nest construction, unlike many other salmonid species, such as salmon, brown trout, or brook trout (Gunn 1995; Esteve et

al. 2007; although see Muir et al. 2012 for some evidence of sexual dimorphism in lake trout). Additionally, there is less information available on lake trout spawning behavior compared to shallower spawning salmonids, particularly about any potential variation between different morphotypes (Muir et al. 2012). As it is currently understood, spawning is influenced by photoperiod, temperature, and strong wind and storm events (Esteve et al. 2007; Muir et al. 2012). There is also evidence to suggest that individual lake trout may not spawn each year (Healey 1978). Lake trout broadcast spawn over clean, cobble substrate with eggs incubating in interstitial spaces, and no parental care is provided (Gunn 1995; Janssen et al. 2007). Morphotypes may be unique in spawning time, location, and behavior, factors that contribute to the maintenance of each variation's unique characteristics (Eschmeyer 1957). Currently, however, observations of lake trout spawning generally come from easily observable habitats and may not represent the full breadth of spawning behaviors or habitats (Muir et al. 2012).

Lake trout decline- In the Laurentian Great Lakes, the lake trout decline began in the mid- to late-1800s. This decline was largely attributed to overexploitation, sea lamprey (*Petromyzon marinus*) predation and habitat degradation (Christie 1973; Cornelius et al. 1995; Eshenroder et al. 1995; Hansen et al. 1995; Holey et al. 1995). These factors contributed to the fishery collapse and lake trout were thought to be extirpated throughout most of the Great Lakes basin, with the exception of Lake Superior and limited areas of Lake Huron (Schneider et al. 1983; Page et al. 2003; Zimmerman and Krueger 2009). Stocking of fish and other restoration efforts were initiated to restore lake trout; however, no increase in native stocks was noted (Coble et al. 1990). Because sea lamprey predation on lake trout was identified as an important source of mortality, the Great Lakes Fishery Commission developed methods and programs to reduce the number of sea lamprey (Schneider et al. 1983; Symula et al. 1990; Page et al. 2004). The chemical treatment of sea lamprey spawning and rearing locations, in particular, was successful in reducing

sea lamprey abundance. This reduction in sea lamprey predation resulted in an increase in lake trout survival in many areas of the Great Lakes (Coble et al. 1990). However, the reduction of sea lamprey alone was not enough to successfully restore lake trout populations, as a suite of other factors (e.g., habitat quality and nonnative species) also limit the ability of lake trout to maintain naturally reproducing populations.

Invasive species- As of 2012, more than 180 nonnative species have established reproducing populations in the Great Lakes and connecting water bodies (Mills 1993; Vander Zanden et al. 2010). The establishment of these species is having an impact on lake trout restoration. Nonnative species that most directly affect lake trout restoration are those species that reduce the quality of spawning habitat (e.g., zebra mussel, *Dreissena polymorpha* and quagga mussel, *D. rostriformis bugensis*), egg survival (e.g., round goby, *Neogobius melanostomus*), and fry survival (e.g., alewife, *Alosa pseudoharengus* and rainbow smelt, *Osmerus mordax*).

The zebra mussel was introduced into the Great Lakes in 1986 in Lake St. Clair through ballast water, although multiple introductions are suspected (Griffith et al. 1991; Bronte et al. 2003). A second species of *Dreissena* mussel was found in 1989 in Lake Erie and identified as the quagga mussel in 1991 (Mills et al. 1993). Currently, both the zebra and quagga mussel can be found throughout all five Great Lakes. Due to their filtering capability, these species have caused fundamental changes in the phytoplankton community, and therefore in the cycling of nutrients and energy throughout the Great Lakes (Nalepa et al. 2010). Dreissenid mussels colonize habitats preferred by lake trout for spawning which has impeded natural reproduction. Mardsen and Chotkowski (2001) observed reduced survival of lake trout eggs spawned over substrate fouled with mussels, both in Lake Michigan and laboratory experiments. Wave action may cause eggs to wash over the sharp edges of zebra mussels shells, causing abrasion or even rupture, and spawning lake trout may actively avoid areas with mussels present (observed in Lake

Michigan; Marsden and Chotkowski 2001). In addition to wild spawned eggs, stocked lake trout yearlings may also be negatively affected by dreissenid mussels. O'Gorman et al. (2000) found shifts in capture depth of age-2 lake trout, thought to be associated with shifting prey depths due to changes in phytoplankton community composition as the result of mussel filtering.

Even in good quality egg habitat, predation by nonnative and native species can reduce the survival of lake trout fry. Round gobies are superior lake trout egg predators when compared to native species, specifically the slimy (*Cottus cognatus*) and mottled (*C. bairdii*) sculpin, and crayfish (*Orconectes propinquus*; Chotkowski and Marsden 2001; Fitzsimons et al. 2006). The round goby was first detected in the St. Clair River in 1990 and speculated to have been introduced through ballast water (Jude et al. 1992). According to a laboratory study done by Fitzsimons et al. (2006) round gobies are able to locate eggs better, consume more, and increase consumption with increasing egg density compared to native lake trout egg predators. Jonas et al. (2005) found predator density in Lake Michigan to be twice that of spawning grounds used by naturally reproducing populations in Lake Huron and Lake Champlain. Since lake trout spawning occurs over winter, predation rates may be reduced due to cold water temperatures; however, the length of lake trout egg incubation suggests that predation is still a significant source of egg loss (Chotkowski and Marsden 1999; Jonas et al. 2005; Fitzsimons et al. 2006).

In addition to poor habitat and direct predation, reduced survival of eggs and fry can be attributed to nonnative prey species of adult lake trout, specifically the alewife and rainbow smelt. Both of these species were introduced to the Great Lakes system through the opening of the Erie Canal (Smith 1970). These species have become prey for lake trout and contain thiaminase, a thiamine destroying enzyme (Honeyfield et al. 2005). The consumption of prey containing thiaminase leads to reduced survival in offspring due to what has been called early life stage mortality syndrome (EMS; Honeyfield et al. 2005; Fitzsimons et al. 2007). Beyond causing early mortality, low thiamine levels may have adverse effects on fry which do survive, including

reduced predator avoidance and decreased ability to capture prey (Perkins and Krueger 1995; Honeyfield et al. 2005).

Lake trout in the hatchery system- Lake trout restoration relies heavily on stocking, where wild fish are brought into the hatchery system and maintained to produce a hatchery stock. Fish remain separated by source population and are referred to as hatchery strains. Historically, many strains were stocked across the Great Lakes with the intent that natural reproduction and natural selection would result in locally adapted lake trout most suited for survival given the habitat conditions (Krueger et al. 1989). With the exception of Lake Superior, which had the largest number of remnant populations, stocking has not yet been as successful in re-establishing natural populations as originally anticipated (Stevens et al. 2012). Currently, six hatchery strains are maintained by the U.S. Fish and Wildlife Service for the purposes of stocking, however this number has fluctuated over time (i.e., new strains are created and old strains are phased out). The currently used strains have been developed from native lake trout populations within the Great Lakes (e.g., the Apostle Island, Huron Parry Sound, and Klondike strains), native populations in the northeastern United States (e.g., the Lake Champlain and Seneca Lake strains), as well as introduced populations in the western United States (e.g., the Jenny-Lewis Lake strain from Wyoming lakes, originally stocked with transplanted lake trout from Lake Michigan; Krueger et al. 1989; Page et al. 2005; Tracy Copeland, USFWS, personal communication). In addition, Canada maintains at least four hatchery strains, developed from native lake trout populations (e.g., the Michicopten Island, Tarantorous, Slate Island, and Dorian strains; Great Lakes Fishery Stocking Database; http://www.glfc.org/fishstocking/).

Each strain has an adult population maintained in a hatchery that is used to produce offspring each year for the purposes of stocking. Stocking usually occurs when fish are fingerlings (e.g., approximately 1 in long) or yearlings (e.g., approximately one year old);

however, stocking of yearlings is most common (Elrod et al. 1993). Before stocking, hatchery fish are given two identifications to distinguish them from naturally reproduced fish: (1) the adipose fin is removed, and (2) a coded wire tag is inserted into the snout (Mardsen et al. 1989; Lantry and Lantry 2008). The coded wire tag is a small piece of metal stamped with an identifying number, which provides information on strain, hatchery, stocking location, and date, that will remain in the fish for the rest of its life (>98% retention rate; Dale Bast, USFWS, personal communication). Coded wire tags can only be extracted by lethal sampling methods, but presence can be detected with a small electronic device.

While stocking is an important component of lake trout restoration (Schneider et al. 1983), the process of maintaining hatchery strains represents concerns with regards to genetic diversity. The Lake Trout Broodstock Management Plan (Holey 1997) seeks to minimize any negative impacts of reproduction in the hatchery system by providing guidelines on the collection, mating, and maintenance of hatchery stocks as well as stocking practices of juveniles. Specifically, random mating of unrelated individuals with a 1:1 ratio is used to prevent any artificial selection for fish better suited to hatchery conditions, as these characteristics may not be advantageous in the wild. Gametes are collected from wild fish to supplement mating of hatchery fish, in an effort to maintain the genetic diversity of the strain. Juvenile fish stocked at various locations represent, as much as possible, the total diversity of the hatchery strain, rather than a limited number of family classes (e.g., randomly sampling from the entire year class, not just a limited number of egg batches; for full review, see Page et al. 2005). Maintaining, as fully as possible, the genetic diversity of remnant populations through hatchery stocks is important based on the recorded physiological and genetic evidence for the existence of discrete lake trout stocks/morphotypes throughout the native lake trout range (Krueger et al. 1989; Page et al. 2004; Page et al. 2005; McDermid et al. 2010b).

Lake trout restoration is an ongoing process. After more than 50 years of stocking, only in Lake Superior are self-sustaining populations found and discontinuation of stocking has been recommended (Stevens et al. 2012). Lake trout are economically important for many areas of the Great Lakes, not as a commercially harvested species, but as a recreational fishery and for local industries that rely on fishing-related tourism (Bronte et al. 2006). Three different aspects relating to lake trout restoration throughout the Great Lakes are examined in this document, including hatchery production of eggs from a humper morphotype, a general understanding of the genetic composition of the current hatchery strains, and evidence of natural reproduction in the Niagara River, NY. Objectives are listed within each chapter as it relates to each specific study.

Chapter 2

The Klondike Strain: Hormone Treatment Beneficial to Eye-Up?

Introduction

Fish reared in captivity often exhibit reproductive dysfunction, resulting in lack of final maturation (Zohar and Mylonas 2001). For females, this usually means no final oocyte maturation, ovulation or spawning; whereas, males may have diminished milt volume and/or quality (Podhorec and Kouril 2009). This dysfunction is generally attributed to a lack of natural spawning cues, thought to be the result of rearing in an artificial environment (Lam 1982; Zohar and Mylonas 2001; Podhorec and Kouril 2009). Reproductive success can be improved with manipulations of the captive environment, such as photoperiod, water temperature, or spawning substrate, in an effort to better mimic natural spawning conditions. However, this is not feasible for some species. For example, simulating migration or reproducing natural spawning conditions (e.g., water depth/pressure) may not be possible, or there may be a lack of knowledge about natural spawning environments (Mylonas et al. 2010). When environmental manipulations are not effective or possible, hormones can be used in an attempt to increase spawning of captive species. Houssay (1931) first demonstrated the success of this technique using an injection of freshly dissected pituitary glands.

When fish reproduce in the wild, external stimuli (spawning cues) begin the cascade of hormone production necessary for reproduction. Spawning cues trigger the hypothalamus of the brain to release gonadotrophin releasing hormone (GnRH), which targets the pituitary gland (Lam 1982; Mylonas et al. 2010). The pituitary gland then releases luteinizing hormone (LH) and follicle stimulating hormone (FSH) to the gonads, triggering the release of steroids to induce final maturation (Lam 1982; Mylonas et al. 2010). In an effort to remedy any disruption in the natural hormone process, hormone treatments in captive broodstocks have targeted different sections of

this process. Initially, pituitary glands from donor fish were ground and injected into non-maturing fish in an effort to increase spawning, a technique called hypophysation (Lam 1982). Unfortunately, this did not supply standard amounts of luteinizing hormone or follicle stimulating hormone (i.e., it was dependent on levels in donor fish), and had the potential for disease transmittance (Lam 1982). Due to high cost and variability of hypophysation, mammalian hormone injections, specifically human chorionic gonadotropin, were used in an attempt to stimulate the gonads (Lam 1982). However, with the injection of mammalian hormones an immune response was likely to develop after multiple treatments, rendering the application ineffective. Most successfully used across a number of different captive species (e.g., salmon, catfish, flounder), has been the application of gonadotrophin releasing hormone (GnRH) and its analogues (GnRHa; Billard et al. 1984; Peter et al. 1988; Harmin and Crim 1992). GnRH is relatively universal in fish species (Fernald and White 1999), which reduces the likelihood of immune response and targets a higher link in the hormone cascade (Lam 1982). By using GnRHa, the fish is stimulated to release its own stores of luteinizing hormone and follicle stimulating hormone to ensure reproduction. The primary limitations of this technique include cost, adequate amounts of LH and FSH in the pituitary for release, and it may require a dopamine antagonist in some species to be effective (Lam 1982; Peter et al. 1988; Mylonas et al. 2010). Gonadotrophin releasing hormone analogues are available both in short-term and long-term release, and are generally effective in small dosages (Lam 1982; Breton et al. 1990; Mylonas et al. 2010).

Hormone manipulation using GnRH has been successfully used to induce final maturation of a number of cultured fish species, including the common carp (*Cyprinus carpio*; Peter et al. 1988), nase (*Chondrostoma masus*; Szabó et al. 2002), Atlantic salmon (*Salmo salar*; Crim et al. 1983) and winter flounder (*Pseudopleuronectes americanus*; Harmin and Crim 1992). This technique has been most commonly used to induce spawning out-of-season (Crim et al.

1983; Harmin and Crim 1992; Dasgupta et al. 2009; Wang et al. 2009) or to synchronize spawning or increase production during the normal spawning season (Szabó et al. 2002; Levavi-Sivan et al. 2004; Heyrati et al. 2007). Dosage of GnRH used for induction of final maturation varies according to study species (1 ug/kg bw in loach, *Paramisgurnus dabryanus*, to 125ug/kg bw in Atlantic salmon; Crim et al. 1983; Peter et al. 1988), and many species required a dopamine antagonist for GnRH application to be effective (silver carp, *Hypophthalmicthys molitrix*, mud carp, *Cirrhinus molitorella*, bream, *Pararamis pekinensis*, rohu, *Labeo rohita*, kutum, *Rutilus frisii kutum*; Peter et al. 1988; Heyrati et al. 2007; Dasgupta et al. 2009). Dopamine antagonists may reduce egg quality in certain species and is not advised (e.g., arctic charr, *Salvelinus alpinus*, rainbow trout *Oncorhynchus mykiss*, brown trout *Salmo trutta*, silver perch *Bidyanus bidyanus*; Billard et al. 1984; Gillet et al. 1996; Levavi-Sivan et al. 2004). However, as Breton et al. (1990) suggest, best treatment for induction of final maturation may be specific to each species and set of rearing conditions.

One such species that may benefit from the use of hormone treatments is the Klondike population of lake trout. Introduced into the U.S. Fish and Wildlife Service hatchery system in 1995, the Klondike strain of lake trout (hereafter referred to as Klondikes) was developed from a naturally reproducing population located off Klondike Reef in northeastern Lake Superior. The captive broodstock is maintained at Iron River National Fish Hatchery, in Iron River, Wisconsin, and is comprised of two lines: A and B, differentiated by specific fin clips and represent different founding populations. The Klondike strain represents a humper morphotype of lake trout, which generally inhabit offshore reefs surrounded by areas of >100m depth and spawn in August-September, in contrast to the more widely stocked lean morphotype of lake trout which generally lives in less than 80m of water and spawns in late October- November (Rahrer 1965). Klondikes have exhibited reproductive dysfunction in the hatchery, with eye-up rates (number of alive eggs/total number of eggs) of 3-33%, compared to lean lake trout eye-up rates of more than 70%

(Edwards 2011). The Klondike strain was developed to help introduce morphotype variation back into the other Great Lakes (based on recommendations by Page et al. 2004), which are historically thought to have many different morphotypes, up to twelve in Lake Huron alone (Eshenroder et al. 1995).

Several studies have been performed by the U.S. Fish and Wildlife Service to test specific hypotheses related to the low eye-up rates of the Klondike broodstock. For example, adult fish were fed a special krill diet to determine if fish were receiving adequate nutrition for reproduction, however eye-up rates did not significantly improve (Edwards 2011). Fish were sent to Sullivan Creek National Fish Hatchery to determine if production would improve in a different hatchery, but no significant changes in eye-up rates occurred (Edwards 2011). Changing spawning media and incubation temperatures also did not result in an increase in eye-up rates, and there did not appear to be any deficiencies in egg health (based on thiamine, fatty acid and lipid level tests; Edwards 2011). Male Klondike milt was used to fertilize lean morphotype eggs from the Apostle Island strain to determine if there were any fertility problems with male Klondikes; no fertility problems were evident (Edwards 2011). Based on the results of these past studies, all health considerations associated with the spawning process appear to be normal, and all feasible changes to environmental (rearing) conditions have been exhausted. Therefore, this study was undertaken to evaluate the effectiveness of GnRHa in inducing final maturation in female fish of the Klondike strain of lake trout at the Iron River National Fish Hatchery. Only female fish were evaluated in this study, as male milt was previously determined to be adequate for fertilization. Improved Klondike eye-up rates would facilitate larger production, allowing for more fingerlings and yearlings of this strain to be stocked throughout the Great Lakes.

Methods

Experimental animals- Adult broodstock of the Klondike strain are maintained at the Iron River National Fish Hatchery, Iron River, WI. A group of 100 female fish of the A line were separated from the breeding stock for use in this study. Because hormone application is most effective when fish are maturing, but not fully mature (Lam 1982), study fish were assessed for ripeness, after being anesthetizing using tricaine methanesulfonate (MS-222). Ripe fish, identified as fish expelling eggs with very little pressure, were not used in this study as they were already mature. Mature fish were placed in a holding tank for regular broodstock spawning. Green (or 'hard') fish, identified as fish not expressing any ovarian fluid, were also not used in this study as they most likely did not have adequate levels of LH/FSH for hormone application to be effective. Green fish were returned to the raceway for re-assessment and potential spawning with the regular broodstock or in subsequent years. Thus, only female fish with a soft belly, without being ripe or green, were used in this study.

Treatment and experimental design- The use of GnRHa (Western Chemical, Ferndale, WA) was carefully documented, per Federal regulations. This hormone may be active in human systems, and proper protection was observed by all individuals assisting with application. The GnRHa powder was diluted in saline solution following manufacturer protocol.

Fish were anesthetized using MS-222, total length (mm) and weight (kg) measurements were taken, and then fish were randomly assigned to one of three treatments groups: low dose, high dose, and control. Dosages were determined based on previous usage of the hormone on lake trout at Pendills Creek National Fish Hatchery in 1999, which, to the best of our knowledge, used a ~10 ug/kg body weight (bw) injection dose. Low dose treated fish received an injection of hormone at a concentration of 10 ug/kg bw. High dose treated fish received an injection of hormone at a concentration of 20 ug/kg bw. Because the hormone was diluted to a uniform

concentration initially, this was double the volume of low dose fish (of equal body weights). Control fish were injected with saline solution; equally split between low dose volume, and high dose (double) volume. The assignment of fish was random; however, treatment application was monitored to ensure each treatment had an equal proportion of all size fish. Fish were injected posterior to the pelvic fins (intraperitoneally) and received a treatment-group specific fin clip for future identification. After injection, all fish (low dose, high dose and saline-injected controls) were transferred to a single raceway for holding until spawning. Reproductive condition was checked on all study fish 5 days after treatment. Fish were anesthetized using MS-222, and light pressure was applied to the abdomen to check for the expression of eggs. Length, weight and fin clip were recorded as fish were assessed. Fish expressing eggs were air or hand-spawned following standard hatchery procedure. Fish not expressing eggs were returned to the raceway for future assessment. Non-ripe fish were checked every seven days until found to be ripe. All fish spawned were re-spawned two and seven days post initial spawning to determine if multiple egg collections could be procured from the fish. The process was discontinued when volume or egg quality was no longer acceptable for spawning and incubation. All fish which did not express eggs were sacrificed in an attempt to determine potential causal factors.

Production of eggs- Egg and milt were combined dry, before water was added to activate sperm. The mixture was manually stirred for 30 seconds and allowed to rest for one minute to ensure fertilization. Eggs were then rinsed with water to remove excess sperm and submerged in an iodine bath to remove bacteria and fungus from the exterior of the eggs. After being disinfected, a subsample of fertilized eggs, separated by female, was moved to incubation trays, which provided continuously flowing water following standard hatchery procedure. Remaining eggs were pooled by treatment type and incubated under the same conditions. Additionally, any eggs collected from re-spawns were also pooled by treatment type and incubated under continuously

flowing water following standard hatchery procedure. This allowed for monitoring of individual eye-up rates of different females in each treatment group, while still taking space restrictions into account.

Eggs were allowed to develop for approximately 40 days before being shocked to sort dead from live eggs. Egg shocking was done as part of standard hatchery procedure, a process which causes dead eggs to turn opaque for easier identification. After shocking, pooled eggs were run through the automated picker to remove dead eggs before being returned to incubation trays. Those eggs which were separated by female were assessed for eye-up rates after shocking by sub-sampling three groups of approximately 100 eggs from each batch during one sampling event. Each subsample was counted by a different person. The numbers of eyed eggs, dead eggs, as well as eggs with limited development (non-viable) were all noted during this time. Dead egg counts and non-viable egg counts were combined into one dead category and compared against eyed alive eggs for calculation of an eye-up rate (alive/total). Pooled eggs were enumerated following the displacement method (Piper 1986). Eye up rates of non-study fish (both A and B line fish) were also monitored by hatchery staff, as per normal procedure, and used as another control group for comparison. These eye-up rates were provided as a mean for all fish spawned, (i.e., only a point estimate for all fish with no associated variance).

Statistical analysis- A generalized linear mixed model was used to assess differences in the probability of eye-up (i.e., eye-up rates) for the three hormone treatment groups of female lake trout (control fish [saline injected], high [20 ug/kg body weight] and low [10 ug/kg body weight] GnRHa dosage groups). The analysis was based on eye-up rates of individual female fish to account for variation in the breeding population. Because three subsamples of eggs were assessed for eye-up success from each female fish, an individual fish random effect was included in the model. The model assumed a binomial error distribution and used a logit-link function.

The response variable was the number of eggs, out of the total number of eggs counted, that successfully eyed-up (i.e., the proportion that eyed-up).

Bayesian estimation, using diffuse priors, was used to estimate all parameters. The software program JAGS (Plummer 2011) was used to simulate random samples from the posterior distributions for the model parameters, which were used for inferences. JAGS posterior summaries were performed using the programming environment R (R Development Core Team 2011). Three parallel chains were run with different initial values in JAGS to generate 150,000 samples from the posterior distributions for each analysis, after discarding the first 10,000 samples. Posterior means are presented along with 95% credible intervals (similar to a frequentist confidence interval) in parentheses.

Results

Spawning- All fish treated with hormones (high or low treatment group) were ready for spawning 5 days post application. Only 39% of control fish were ready to spawn 5 days post application (Table 2-2). Three spawning takes were necessary for all control fish to be spawned, spanning 3 weeks. One control fish never produced eggs, and was dissected to determine possible reasons for lack of maturation, following standard hatchery procedure. No specific reason was evident.

Comparison of eye-up rates- The posterior mean probability of eye-up was 0.53 (0.21, 0.82) for the control group, 0.58 (0.37, 0.78) for the high dosage group, and 0.59 (0.38, 0.78) for the low dosage group (Figure 2-1). If the presence of overlapping 95% credible intervals is used to make inferences about group differences, then no differences in the probability of eye-up were present among treatment groups (Figure 2-1). However, comparing overlapping credible intervals

(similar to a null-hypothesis testing approach for inference) may not always be the most informative approach for making decisions. Therefore, the probability that the high and low treatment groups had an eye-up probability greater than the control group's eye-up probability was also calculated. The probability of the high treatment group having a greater eye-up rate than the control group was 61%, while the probability that the low treatment group had a greater eye-up rate than the control group was 63%. So, while not statistically significant (no associated *P*-value to be compared to the standard $\alpha = 0.05$), the treatment of both the high and low GnRHa application had a positive effect on eye-up probability that may be meaningful to the hatchery management of the Klondike strain at Iron River National Fish Hatchery.

The probability that the three treatment groups had a higher eye-up probability compared to that of non-study fish was also calculated. Two point estimates were available for the non-study fish Klondike strain of lake trout, A and B line, which were 0.36 and 0.35, respectively. For both the high and low treatment groups, there was a greater than 98% probability that they had higher eye-up rates than either the A or B line fish. Interestingly, the control fish in this study also had a much higher eye-up rate compared to the non-study fish, although neither group received hormone treatments and thus would be expected to exhibit similar eye-up rates. In fact, the control group had a higher eye-up probability compared to non-study fish spawned in the same year: 84% higher than the A line and 85% greater than the B line. The high eye-up rates of control fish used in this study, compared to other groups of fish that did not receive any hormone treatment, deserves further attention.

Pooled eggs- Eggs that were pooled for incubation (both initial spawn and re-spawns) from all treatment groups had eye-up values within the 95% credible intervals of the model estimates. Total spawn and re-spawns eggs collected from low dose fish had an eye-up rate of 68.6%, high

dose fish eggs of 67.3%, and control fish eggs of 51.2%. The average number of good eggs per female fish was 4,220 for low dose treated fish, 4,546 for high dose treated fish, and 3,182 for control fish (Table 2-3). This is a 32.6% (for low dose) and 42.9% (for high dose) increase in good eggs per female of the treatment groups compared to controls. All non-study fish from the A line averaged 36.3% eye-up and 1,767 eyed eggs per female (Table 2-3). Increase in number of good eggs per female of non-study A-line fish compared to treatment groups was 138.8% (low dose) and 157.3% (high dose).

Table 2-1: Biological Data for Study Fish. Number of fish per treatment group (n), and mean (+/- standard deviation) length (mm) and weight (kg) of Klondike lake trout used to assess eye-up rates with GnRHa application are listed. Mean length and weight did not differ significantly among treatment groups.

Treatment group	N	Average length	Average weight
Hormone: Low Dose	29	668 ± 9.96	9.43 ± 0.56
Hormone: High Dose	28	684.1 ± 8.38	9.5 ± 0.52
Control: Low Volume	14	671.1 ± 10.27	9.36 ± 0.40
Control: High Volume	14	691.1 ± 12.11	10.74 ± 0.55

Table 2-2: Total Volume of Eggs (mL) Collected from Female Lake Trout. Data are separated by treatment group and spawning take. Number of fish ready to spawn at each spawning take, as well as average volume per fish, are listed by treatment type. All fish treated with GnRHa were ready to spawn during first spawning take (September 11, 2012); for control fish injected with saline, three spawning takes were necessary for all fish to express eggs.

	LOW DOSE			**HIGH DOSE**			**CONTROL**		
	Total volume (mL)	Number mature	Average volume (mL)	Total volume (mL)	Number mature	Average volume (mL)	Total volume (mL)	Number mature	Average volume (mL)
Spawn 9-11-12	15272	29	527	16735	28	598	5840	11	531
2 days post spawn	740			1110			375		
7 days post spawn	215			400			100		
Spawn 9-18-12							7680	15	536
2 days post spawn							620		
7 days post spawn							225		
Spawn 9-11-12							1080	2	540
2 days post spawn							80		
7 days post spawn							5		

Table 2-3: Pooled Egg Eye-Up. Eye up rates for eggs collected from all female lake trout during this study. Eggs were combined by treatment group before calculation of reported summary numbers. Eggs from the second re-spawn for all groups were excluded due to extremely low survival (<1%)

Treatment type		Date	Number spawned	Total eggs	Alive eggs	Eye-up (alive/total)	Alive eggs/female
Low Dose	Spawn	9/11/2012	29	169,131	117,859	69.70%	4,064
	Respawn	9/13/2012		9,160	4,520	49.30%	156
	Total		29	178,291	122,379	68.60%	4,220
High Dose	Spawn	9/11/2012	28	175,977	120,432	68.40%	4,301
	Respawn	9/13/2012		13,074	6,864	52.50%	245
	Total		28	189,051	127,296	67.30%	4,546
Control	Spawn	9/11/2012	11	67,317	34,692	51.50%	3,154
	Respawn	9/13/2012		4,187	1,062	25.50%	97
	Spawn	9/18/2012	15	83,078	40,766	49.10%	2,718
	Respawn	9/20/2012		7,184	1,526	21.20%	102
	Spawn	9/25/2012	2	12,120	10,260	84.60%	5,130
	Respawn	9/27/2012		1,046	798	76.30%	399
	Total		28	173,992	89,104	51.20%	3,182
Non-Study A line	Spawn	10/28/2012	18	93,842	52,192	55.6%	2,900
	Spawn	9/4/2012	37	190,380	54,300	28.5%	1,468
	Spawn	9/11/2012	3	6,951	1,120	16.1%	373
	Spawn	9/18/2012	5	18,554	5,450	29.4%	1,090
	Spawn	9/25/2012	1	1547	0	0%	0
	Total		64	311,274	113,062	36.3	1,767

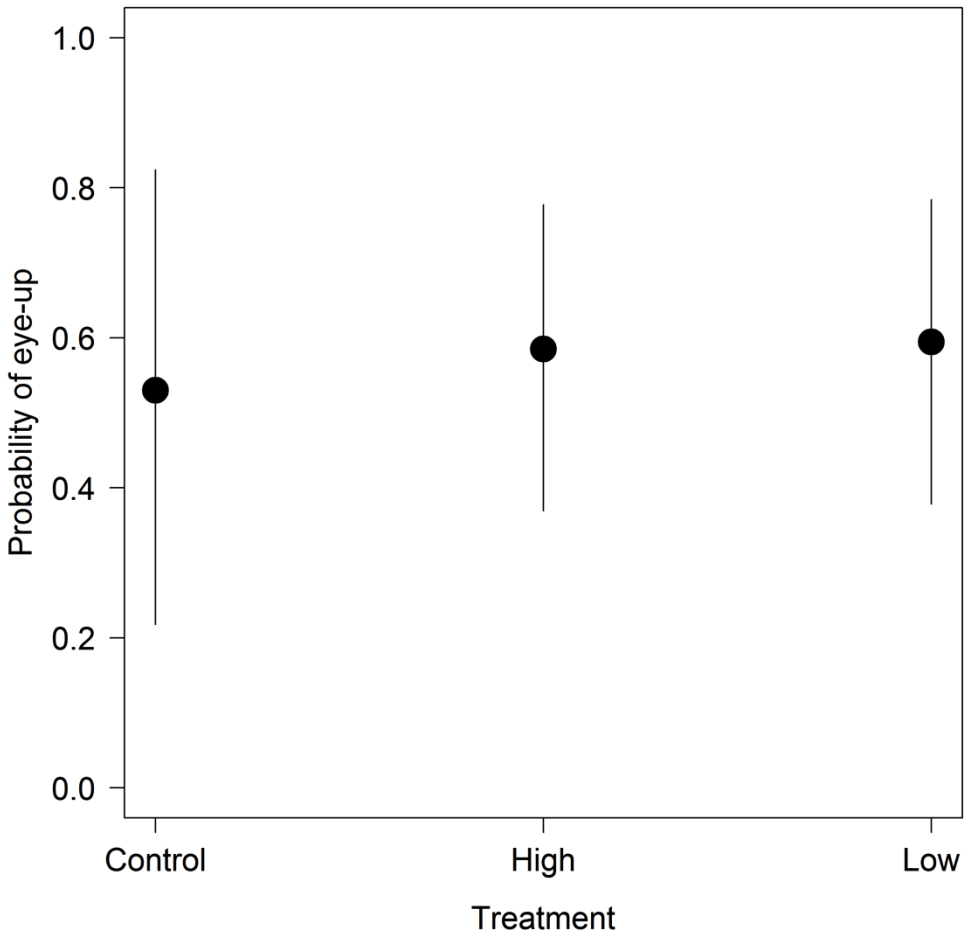

Figure 2-1. Probability of Eye-Up by Treatment. Data are listed by treatment type. Solid circles are posterior means and vertical bars are 95% credible intervals. Control fish received saline solution, high treated fish received an injection of 20 ug/kg body weight (bw) and low treated fish received an injection of 10 ug/kg bw.

Discussion

Acute single-injections of GnRHa were used to both synchronize spawning and increase production of the Klondike strain of lake trout during the spawning season. Using this technique, the Iron River National Fish Hatchery was able to meet production goals for the Klondike strain, and could possibly increase stocking of the strain if the entire broodstock was treated. The low and high dose treatment groups had equally high eye-up rates, and because administering a high dose requires a greater amount of hormone, the use of a low dose treatment would likely provide the greatest benefit when incorporating cost. Alternatively, an intermediate dose may prove useful for application, which could provide the same high eye-up rate, while increasing the number of surviving eggs per female. In both treatment groups, all fish were ready to spawn 5 days post application, which makes spawning more predictable and compact temporally. This was similar to what was found for other salmonids treated with GnRHa. For example, treated female landlocked Atlantic salmon (*Salmo salar*) spawned 7-12 days post application (125 µg pellets; Crim et al. 1983), and rainbow trout in high dose treatment groups (50 ug/kg bw) spawned 5 days post injection (Breton et al. 1990).

Interestingly, control fish eye-up rates were more similar to treated fish than non-study fish. There are two primary potential factors that could explain the difference in eye-up probabilities between the control and non-study eggs: sampling bias or mixing of treatment and control fish allowed for direct or indirect alteration of eye-up. Because control fish did not receive hormones, it would be expected that they would have similar eye-rates to non-study fish. However, all fish used in this study, control or treated, were taken during the peak spawning time and assessed to be partially mature. This may have introduced some sampling bias and altered the eye-up rate, compared to the normal broodstock which may have included a mixture of mature and immature fish. Compounding this issue, treatment fish were unable to be separated from control fish, and instead were held in the same tank for the duration of the experiment.

Because control fish were held in the same tank as hormone-treated fish, the shared environment of the control and treated individuals may have indirectly altered eye-up rates. Potential mechanisms for this may have included hormone-treated fish excreting excess hormones that were absorbed by non-hormone treated fish, or simply having control fish in the presence of mature females (e.g., through behavioral cues) lead to accelerated maturation of the control fish. Further evaluation is needed to determine if presence of treated fish was influencing non-treated fish. Separating treatment groups into separate tanks (or minimally with control fish in the upstream tanks if a flow through system is required) in future studies may help address this issue.

While the eye-up rates of the control fish warrants further investigation, overall eye-up rates in this study were higher compared to non-study fish. Based on the results observed here, GnRHa treatment did not result in a significant increase in eye-up compared to control fish (e.g., probability of higher eye-up in treatment fish compared to control fish was little better than flipping a coin). However, there was an improvement in the number of living eggs produced per fish, >30% compared to controls and more than double compared to non-study fish. This increase in eggs per female will allow hatcheries to produce more eggs while still maintaining the same number of fish.

While hormone treatment may allow for an increase in the number of eggs produced per female, genetic concerns of having an adequate number of breeders should still be taken into account (Page et al. 2005; Jamieson and Allendorf 2012). Increased production from individual fish should not be used as justification for decreasing broodstock numbers. The hatchery system is an artificial environment in which unintended selection might be taking place, and actions should be taken to mitigate these effects. These actions are outlined in the Lake Trout Broodstock Management Plan (Holey 1997), and specifically include actions relating to inbreeding and preserving the genetic diversity of the source population. Additionally, use of hormones to induce spawning may act to artificially alter spawning time, or artificially select for

traits or behaviors that may result in a deviation of traits that were selected for naturally in the wild. Artificial selection may result in decreased fitness in the wild; however, continued decreased productivity leading to a low number of breeders may also have negative genetic impacts as well (Page et al. 2005; Jamieson and Allendorf 2012). Continued monitoring and evaluation of the reproductive output, spawning times, and number of breeders successfully contributing offspring to the next generation is important if the Klondike strain is to be successfully managed for the purposes of stocking throughout the Great Lakes basin.

Chapter 3

The Klondike Strain: A Genetic Comparison

Introduction

Stocking of hatchery fish is commonly employed as a management strategy to supplement declining or extirpated native populations (Nielsen 1993; Page et al. 2003; Araki et al. 2007). While stocking can increase the number of fish in a location, it also presents concerns with regards to the genetic diversity of both the hatchery fish and native fish. These concerns include issues associated with maintaining captive hatchery populations (Holey 1997; Page et al. 2005; McDermind et al. 2010b), considerations of stocking source and stocking location (Halsbisen and Wilson 2009), and interactions between stocked and wild populations (Ryman and Laikre 1981; Fraser 2008; Halbisen and Wilson 2009) including the consequences of introgression (Hindar et al. 1991). Consideration of the potential consequences of hatchery supplementation are particularly important when stocks of hatchery fish have been developed from populations not native to the stocking location; interbreeding between native stocks and hatchery fish generally has negative consequences on performance traits compared to unaffected native populations (Hindar et al. 1991). However, it has also been shown that even in locations following years of stocking, native populations persist and show limited introgressive admixture (Piller et al. 2005; Halbisen and Wilson 2009). If the native population has been extirpated, stocking may be the only viable option to restore a population in a given location.

Maintenance of genetic diversity or avoidance of artificial selection must be a prioritized goal of a hatchery program to avoid negatively affecting the genetic diversity of the captive population (e.g., reduction in effective population size, inbreeding, population bottleneck, etc.; Allendorf and Ryman 1987). In situations where the hatchery program goal is restoration or

recovery, hatchery practices are often closely monitored to limit loss of genetic diversity (Mobrand et al. 2005; Page et al. 2005). The Lake Trout Broodstock Management Plan (Holey et al. 1997) was developed to minimize any negative impacts by providing guidelines on the collection, mating, and maintenance of hatchery stocks as well as stocking practices of juvenile lake trout in the Great Lakes. Random mating of unrelated individuals with a 1:1 ratio is used to prevent any artificial selection for fish better suited to hatchery conditions, as these characteristics may not be advantageous in the wild (Holey et al. 1997). Additionally, gametes are collected from wild fish to periodically supplement the strains in an effort to maintain the genetic diversity within the strain (Holey et al. 1997). Following these management practices should result in hatchery fish that represent, as much as possible, the total diversity of a given strain, rather than a limited number of family classes (see Page et al. 2005). Maintaining the genetic diversity of each strain is particularly important when looking at broader genetic distinctions in the species; specifically, lake trout morphotypes.

Lake trout morphotypes- There are different morphotypes of lake trout, thought to have evolved from the utilization of different habitats and resources within the Great Lakes (Rahrer 1965; Page et al. 2004; Janssen et al. 2007; Zimmerman et al. 2009). Lean, siscowet and humper are the only current morphotypes present in the Great Lakes; however, additional morphotypes may have been present in the past. Lake Huron alone was reported to have had up to twelve morphotypes historically (Page et al. 2003; Page et al. 2004).

From observations of natural populations, the current morphotypes are segregated within each of the Great Lakes based on preferred habitat depth. Lean lake trout typically reside in the shallowest habitats, siscowet reside in the deepest habitats, and the humper morphotype inhabits depths intermediate between the two, utilizing offshore shoals surrounded by deeper waters (Lawrie and Rahrer 1973; Krueger and Ihssen 1995; Guinand et al. 2012). The three morphotypes also differ in physical and biological attributes which correspond to their different

preferred habitats. These attributes include differences in body shape, buoyancy (due to differences in lipid content of the musculature), growth rate and spawning time (Eschmeyer 1957; Rahrer 1965; Zimmerman et al. 2009; Goetz et al. 2010). These differences in habitat utilization may have led to reproductive isolation, as these physical differences were apparent even when fish were raised under identical laboratory conditions (McDermid et al. 2007; Goetz et al. 2010). Additionally, Krueger and Ihssen (1995) confirmed genetic differences between the three forms using chromosomal banding, allozyme data, and mitochondrial DNA analysis. Genetic differences between the morphotypes, along with genetic differences between strains of the same morphotype further support reproductive isolation between morphotypes and geographic source (Page et al 2004). The distinct attributes of these forms, as well as between populations of each form, warrants the close monitoring of these stocks to prevent loss of genetic diversity (Page et al. 2004; Guinand et al. 2012).

The Klondike strain- Introduced into the U.S. Fish and Wildlife Service hatchery system in 1995, the Klondike strain of lake trout was developed from a naturally reproducing humper morphotype population located off Klondike Reef in northeastern Lake Superior. The Klondike strain was developed to introduce morphotype variation back into the Great Lakes (Page et al. 2004). A captive broodstock has been maintained at the Iron River National Fish Hatchery (NFH), in Iron River, Wisconsin. Because of the importance of maintaining morphotype diversity in the Great Lakes, it is essential to understand the genetic relationships between morphotypes. To date, no studies have assessed the genetic relationship of the Klondike strain of lake trout to its source population or to the other hatchery strains currently in production. Therefore, the goals of this study were to (1) determine if the levels of genetic diversity of the hatchery Klondike strain are consistent with wild caught lake trout from Klondike Reef, Lake Superior and (2) compare estimates of genetic diversity of the Klondike strain of lake trout to other strains of lake trout currently stocked in the Great Lakes.

Methods

Genetic data collection- Tissue samples were obtained from the Klondike strain of lake trout maintained at Iron River National Fish Hatchery (NFH). Fin clips were randomly taken from 100 fish of the 2010 year class. Fin clips from the wild source population were collected from Klondike Reef in Lake Superior, WI during wild gamete collection in 2011 by U.S. Fish and Wildlife Service (USFWS) staff. A total of 192 fish were sampled from the wild, for a total of 292 hatchery and wild Klondike samples. All fin clips were individually stored in 95% non-denatured ethanol and sent to the USFWS Northeast Fishery Center Conservation Lab in Lamar, PA.

Other lake trout hatchery strains currently in production in USFWS National Fish Hatcheries were also sampled for genetic comparison. These included five other strains, listed here and followed by source hatchery: the Apostle Island strain (Iron River NFH), the Lewis Lake strain (Saratoga NFH), the Huron Parry Sound strain and Seneca Lake strain (both from Sullivan Creek NFH), and the Lake Champlain strain (Eisenhower NFH). Fifty fin clips were randomly taken from each year class sampled; for most strains two year classes were sampled.

Extraction of DNA from samples was completed using either Purgene (Qiagen Inc., Valencia, CA) or Kingfisher (Thermo Scientific, Waltham, MA) methods, following manufacturer protocol. A random sample of approximately 10% of each batch of extracted samples was analyzes with UV spectrophotometry to assess concentration of DNA. Based on the average of the 10% subsample, all batch samples were diluted to a uniform concentration. Microsatellite loci used for PCR were: Sfo1 (Angers et al. 1995); Ssa85 (O'Reilly et al. 1996); Oneμ10, Oneμ9 (Scribner et al. 1996); Ogo1a (Olsen et al. 1998); SnaMSU01, SnaMSU03, SnaMSU08, SnaMSU10, SnaMSU12, and SnaMSU13 (Rollins et al. 2009). PCR was completed with one primer labeled on the 5' end with a fluorescent dye (FAM, HEX or NED). PCR amplification was performed in a programmable thermocycler (BioRad Laboratories, Inc.,

Hercules, CA) using 10µL reaction volume. The PCR profile for all loci involved a single 2 minute denaturing step at 94ºC, followed by 35 cycles of a 45 second denaturing step at 94ºC, a 45 second annealing step at the primer specific temperature (see Table A-1), and 2 minute extension step at 72ºC. The PCR profile was completed with a 5 minute extension step at 72ºC. PCR products were then visualized on an ABI 3130XL (Life Technologies, Foster City, CA) using internal lane standards, and GeneScan, Genotyper and GeneMapper (Life Technologies, Foster City, CA) software were used to visualize and score the data, however manual verification was performed for accuracy.

Genetic data analyses- Microsatellite DNA variation was evaluated using allele frequency-based methods and individual assignments based on multilocus genotypes. For sample groups obtained from multiple year classes, each year class was evaluated for conformation to Hardy-Weinburg equilibrium using HW-QuickCheck (Kalinowski 2006) and the effective number of breeders was estimated using LDNe (Waples 2006). This was done to avoid combining estimates from multiple year classes for each hatchery strain. GeneClass (ver. 2; Cornuet et al. 1999) was used to assess population classification and FSTAT (ver. 2.9.3; Goudet 1995) was used to calculate F_{ST} values to measure differences in allele frequency between year classes. Determination of the statistical significance of F_{ST} values used Bonferroni corrections for multiple comparisons (Rice 1989). Year classes were combined if F_{ST} values were not significant. Estimates of genetic diversity were calculated using GDA (Lewis and Zaykin 2002) and GenoType Viewer (Kalinowski), including the number of alleles per locus, allelic richness (e.g., number of alleles per locus standardized for sample size), observed and expected heterozygosity, and inbreeding coefficient. Analysis of molecular variance was compared using Genetic Analysis in Excel (ver. 6.5; Peakall and Smouse 2012). A visual structure of population differences was created with the uprooted tree technique, and Cavalli-Sforza and Edwards chords distances (Cavalli-Sforza and Edwards 1967) were calculated using the NEIGHBOR function in PHYLIP (Felsenstein 1993).

The resulting consensus neighbor joining dendrogram was visualized using TreeView (Page 1996).

Results

Year class assessment- Only two sample groups deviated from Hardy-Weinberg equilibrium: the Klondike wild collection and the Klondike hatchery collection ($P<0.001$; Table A-1). Comparisons of differences in allele frequencies did not indicate significant differences between the year classes sampled for each hatchery strain and therefore year classes for each hatchery strain were combined for further analysis (Table A-2).

Klondike hatchery and wild source sample- Basic genetic characteristics were assessed for the Klondike hatchery sample and Klondike wild sample (Table 3-2). Estimates of genetic variation for the Klondike hatchery strain were lower compared to the wild source population, with allelic richness values of 8.59 (hatchery) and 9.38 (wild). Observed and expected heterozygosity and inbreeding coefficient (*f*) were similar for both hatchery and wild samples (Table 3-2). Estimates of the effective population size (N_e) were 88 (95% confidence interval [CI] =59-147) for hatchery fish and 9,685 (95% CI = 975-infinite) for wild fish, although the wild Klondike samples included multiple year classes (Table 3-3).

Among-strain comparisons- Estimates of genetic variation for the Klondike hatchery strain were compared to other lake trout hatchery strains currently in production (Table 3-2). Two year classes were sampled from each hatchery strain, however, only one year class was available for the Lake Champlain strain, and three were sampled from the Seneca Lake strain (Table 3-1). The highest estimates of observed heterozygosity were for the Seneca Lake and Huron Parry Sound strain, while the lowest estimates were for the Lewis Lake and Klondike strains; however, all

estimates were similar (~0.6; Table 3-2). Allelic richness ranged from 7.27 in the Lake Champlain strain to 10.59 in the Apostle Island strain. The largest percentage of molecular variance was found within individuals (87%), rather than among individuals (10%) or among populations (3%). The estimates of pairwise F_{ST} averaged 0.0134 (Table A-2). Estimates of effective number of breeders (N_e) were calculated per year class sampled and ranged from 45 to 280, with varying degrees of estimated uncertainty (i.e., width of confidence intervals varied; Table 3-3). Even within strains, estimates were variable (280 for the 2009 Lewis Lake year class, 51 for the 2010 Lewis Lake year class; Table 3.3). As illustrated by the neighbor-joining tree (Figure 3-1), the genetic affinities among hatchery populations appear to be based on hatchery strain and lake basin of origin. The estimation of genetic characteristics in the hatchery Klondike strain of lake trout was similar to other hatchery maintained strains, and yet sufficient for individual assignment for most strains (see Table 3-4 for percentages of correct assignment).

Table 3-1. Lake Trout Populations Sampled for Genetic Comparison. Hatchery strain, collection location (NFH= National Fish Hatchery), original wild source population location, morphotype, year class sampled, and total number sampled. Fifty fin clips were sampled per year class.

Sample Group	Collection location	Original wild source	Morphotype	Year class sampled	Number sampled
Klondike (wild)	Lake Superior	Lake Superior	Humper	n/a*	192
Klondike (hatchery)	Iron River NFH	Lake Superior	Humper	2010	100
Apostle Island	Iron River NFH	Lake Superior	Lean	2001, 2003	100
Seneca Lake	Sullivan Creek NFH	Seneca Lake, NY	Lean	2002, 2003, 2006	150
Huron Perry Sound	Sullivan Creek NFH	Lake Huron	Lean	2005, 2007	100
Lewis Lake	Saratoga NFH	Lewis Lake, WY	Lean	2009, 2010	100
Lake Champlain	Eisenhower NFH	Lake Champlain, VT	Lean	2011	50

* multiple year classes of the wild source population

Table 3-2: Genetic Characteristics of Populations Sampled. Alleles per locus, expected heterozygosity (H_e), observed heterozygosity (H_o) and inbreeding coefficient (*f*) were all calculated using GDA (Lewis and Zaykin 2001). Allelic richness was calculated using FSTAT (Goudet 1995), and is defined as the number of alleles per locus standardized for sample size.

Sample	Alleles per locus	Allelic richness	H_e	H_o	*F*
Klondike (hatchery)	9.64	8.59	0.61	0.59	0.038
Klondike (wild)	12.28	9.38	0.64	0.61	0.034
Lake Champlain	7.36	7.27	0.62	0.60	0.029
Apostle Island	12.54	10.59	0.61	0.61	0.004
Seneca Lake	9.72	8.07	0.64	0.64	0.003
Huron Parry Sound	9.90	8.83	0.63	0.64	-0.017
Lewis Lake	8.73	8.00	0.61	0.59	0.030

Table 3-3: Effective Population Size. Estimates of the effective number of breeders (N_e) of each hatchery year class and 95% confidence intervals; estimates were based on fin clip genotypes using the program LDNe (Waples 2006).

		95% confidence interval	
Year class	N_e	Lower	Upper
Klondike Hatchery (2010)	88	59	147
Klondike Wild	9685	975	infinite
Lake Champlain (2011)	63	41	114
Apostle Island (2001)	93	68	142
Apostle Island (2003)	219	111	1907
Seneca Lake (2002)	45	33	67
Seneca Lake (2003)	278	118	infinite
Seneca Lake (2006)	211	109	1412
Huron Perry Sound (2005)	193	90	infinite
Huron Perry Sound (2007)	141	78	504
Lewis Lake (2009)	280	121	infinite
Lewis Lake (2010)	51	37	76

Table 3-4: Correct Proportion of Individual Assignment. GeneClass (v.2, Cornuet et al. 1999) was used to assign hatchery samples back to each hatchery strain. Bold numbers indicate proportion of correct assignment. Left column indicates hatchery samples, and top row are the strain they were assigned to.

	Assigned To					
Assigned From	Klondike	Lake Champlain	Apostle Island	Seneca Lake	Huron Parry Sound	Lewis Lake
Klondike	**0.80**	0.01	0.10	0.01	0.01	0.07
Lake Champlain	0.00	**0.62**	0.02	0.28	0.04	0.04
Apostle Island	0.22	0.02	**0.62**	0.01	0.03	0.10
Seneca Lake	0.01	0.14	0.02	**0.79**	0.03	0.01
Huron Parry Sound	0.05	0.02	0.01	0.04	**0.85**	0.03
Lewis Lake	0.05	0.02	0.05	0.02	0.03	**0.83**

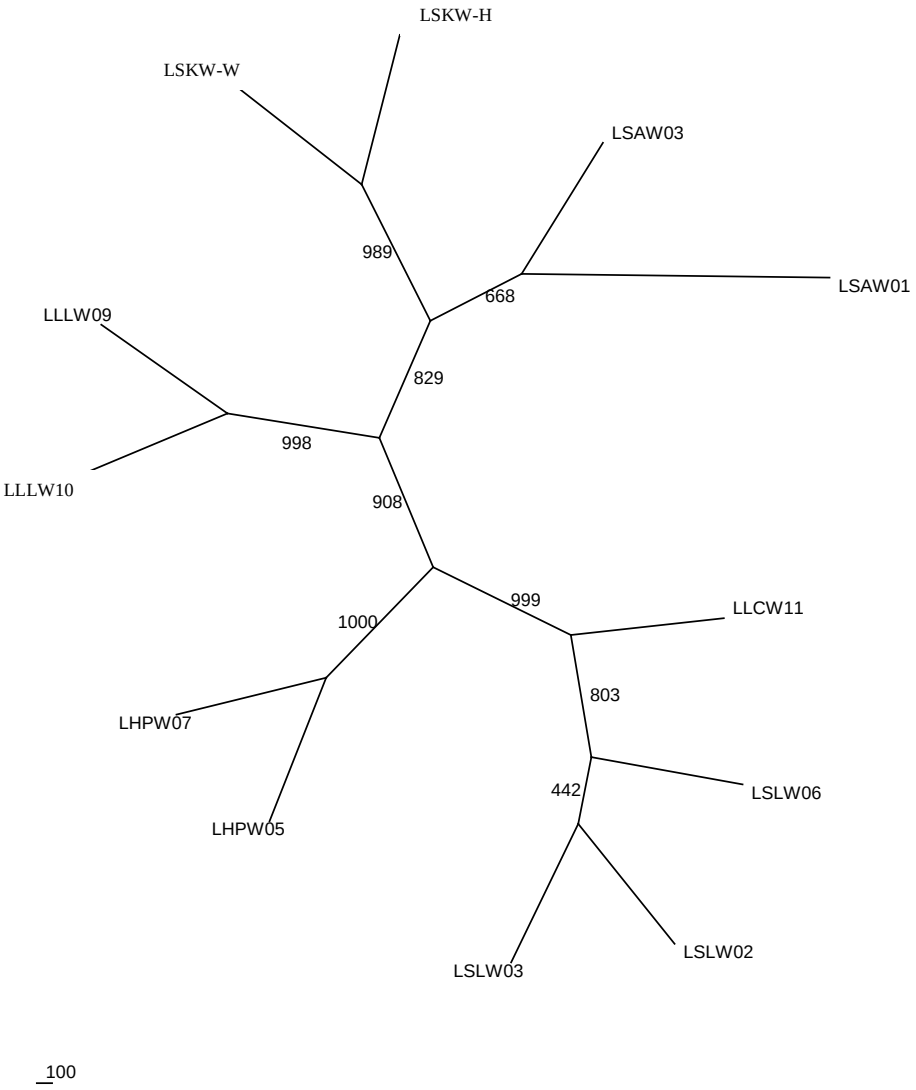

Figure 3-1: Visual Assessment of Populations. Numbers represent bootstrap values over 1,000 replicates that exceed 40%. Abbreviations are four letters for each strain ([LSKW]- Klondike, [LSAW]- Apostle Island, [LLCW]- Lake Champlain, [LSLW]- Seneca Lake, [LHPW]- Huron Parry Sound, [LLLW]- Lewis Lake) followed by year class, or in the case of the Klondike strain, 'W' for wild and 'H' for hatchery population distinction.

Discussion

Estimates of genetic diversity were low across all hatchery strains sampled. In particular, in the Klondike strain of lake trout there appears to be a loss of genetic diversity when compared to the native source population. This is of interest as the Klondike strain is relatively new to the hatchery system (Edwards 2010), and noted as an important strain for the re-introduction of morphotype variability back into the Great Lakes basin (Page et al. 2004). Evaluation of the Klondike strain compared to source population showed a loss of number of alleles per locus, 2-3 alleles per generation currently. This, combined with the normal level of heterozygosity in the sample, may indicate a recent population bottleneck (Petit et al. 1988). Population bottleneck is likely due in part to either: (1) broodstock collection practices, and failing to ensure the sampling of the entire diversity of the population or (2) variable survival of eggs in the hatchery, causing family classes to disproportionately contribute to the next generation. Survival of eggs is of particular note in the Klondike strain, which has been low up until this point (Chapter 2). The effective population size of the Klondike strain is low especially relative to the wild source; however, it is within range of other hatchery stocks, suggesting constraints of hatchery production may be playing a role (Holey 1997; Page et al. 2005). Effective population size was interpreted as effective number of breeders since it was calculated for only one year class for hatchery samples given the spawning protocols for most of the strains, in which a single year class is spawned. This assumption was violated in the Klondike wild sample, as multiple year classes were sampled; therefore, this number may not be an accurate representation to compare directly with the other estimates.

Estimates of genetic diversity were similar for all of the hatchery populations. The low estimates of genetic diversity may be linked to rearing in a hatchery environment, as they were low across all strains. The Apostle Island strain had the highest allelic richness, similar to the findings of Page et al. (2003), whereas the Lake Champlain strain had the lowest. The Lake Champlain strain also fell on the low range of estimates of effective number of breeders. Only

one year class was available from the Lake Champlain strain, which may have contributed to this result, as well as being raised in a different hatchery than all other strains sampled. For all strains with multiple year classes, effective number of breeders was variable, which may be due to a number of factors. Bartley et al. (1992) observed variable estimates of effective population size in different hatchery settings, and hypothesized this was partially based on mating practices. These estimates were most similar to actual number of fish breed when 1:1 ratios were used, as opposed to when fish were allowed to randomly mate or unequal sex ratios were used. For lake trout specifically, introduction of wild gametes during certain years may be affecting estimates of effective population size. The variation in these estimates emphasizes the importance of continuous monitoring of genetic diversity.

While the range of genetic diversity values was similar across strains, each hatchery strain was genetically distinct. Hatchery year classes grouped according to strain, indicating genetic differences between strains, and secondly, according to original wild source population demography. For example, the hatchery strains from Lake Superior (Klondike and Apostle Island strains) were most genetically similar based on F_{ST}, assignment, and CSE distances, demonstrating a shared genetic lineage. The Lake Champlain and Seneca Lake strains are both from outside the Great Lakes region, and are more genetically similar to each other than the strains originating from within the Great Lakes; the Lake Huron sample (Huron Parry Sound) grouped separately from all other. As there was only one humper morphotype sampled, it cannot be evaluated in this study if morphotype variation was an important factor, as was seen in Page et al. (2004).

Preserving and maintaining the genetic diversity of wild populations following local extirpation is difficult and research varies on the suitability and advisement of stocking captive bred individuals for the purposes of restoration (Petit et al. 1988; Araki et al. 2007; Fraser 2008). Currently, hatchery stocking is the predominant tool used to further the restoration goal of establishing naturally reproducing populations of lake trout throughout the Great Lakes

(Markham et al. 2008; Fisheries Technical Committee 2009; Dexter et al. 2011; Lantry and Lantry 2012). Therefore, hatchery rearing and stocking practices will continue, aided by the evaluation of each strain survival and relative contribution to natural reproduction once stocked. However, genetic diversity of each strain and its contribution to maintaining historical diversity is also an important management goal (Holey 1997; Page et al. 2005). The maintenance of the Klondike strain of lake trout is particularly important as this is the only humper morphotype in production. This strain is genetically distinct from other hatchery strains, based on analysis of this study, and needs to be carefully monitored for decreases in genetic diversity. Utilization of the protocols outlined in the Lake Trout Broodstock Management Plan (Holey et al. 1997), including the continued incorporation of eggs from the wild to increase the effective population size and genetic diversity, would be useful in ensuring that restoration management goals are attained.

Chapter 4

Natural Reproduction of Lake Trout in the Niagara River, NY

Introduction

Lake trout populations in the Laurentian Great Lakes began to decline in the late 1800's, which has been attributed to overharvesting, habitat degradation, and sea lamprey (*Petromyzon marinus*) parasitism (Christie 1973; Cornelius et al. 1995; Eshenroder et al. 1995; Hansen et al. 1995; Holey et al. 1995). Eventually lake trout populations were extirpated or greatly reduced, after which stocking was performed in an attempt to re-establish naturally reproducing populations. Specifically in Lake Ontario, stocking was attempted in the 1950's through early 1960's, however was discontinued due to low survival of age-3 and older fish. This low survival was associated with sea lamprey predation and by-catch mortalities (Brenden et al. 2011). It was not until the 1970's that stocking practices were re-established in concordance with the use of lampricide to control sea lamprey populations and the closure of commercial fisheries (Christie 1973; Lantry and Lantry 2008). Stocking targets historically averaged approximately one million annually (1985-1992), yet were reduced due to concerns of predator-prey imbalances (Schnieder et al. 1983; Jones et al. 1993; Brenden et al. 2011). The current annual stocking target for Lake Ontario is 500,000 yearlings (Lantry and Lantry 2011).

To date, natural reproduction by stocked lake trout has not yet led to adequate recruitment in most areas (Reid et al. 2001; Janssen et al. 2006). Recovery of Lake Superior populations has been accelerated compared to other lakes, as fewer populations were extirpated (Swanson and Swedberg 1980; Hansen et al. 1995) and the discontinuation of stocking for all areas of Lake Superior has been recommended (Stevens et al. 2012). In Lake Huron, lake trout restoration has been partially successful. Reid et al. (2001) attributed this success to: (1) controlling sea lamprey abundance, (2) stocking yearling lake trout developed from the remnant

population, (3) creating a refuge from fishing pressures, (4) restricting harvest, and (5) imposing a recreational fisheries size limit. However, in other areas of Lake Huron, such as South Bay, stocking and restoration efforts continue (Morbey et al. 2008). In Lake Michigan, the Mid-Lake Reef Complex (specifically, East and Sheboygan reefs) appear to be supporting naturally reproducing populations of lake trout, albeit at a deeper depth than usually observed (40-60 m as opposed to <30 m; Janssen et al. 2006). In northern Lake Michigan, egg deposition was observed, however at lower rates than in Parry Sound, Lake Huron or Lake Champlain (Jonas et al. 2005). In Lake Erie, recruitment of naturally reproduced lake trout has not yet been observed (Cornelius et al. 1995; Markham et al. 2008). Natural reproduction has been observed in Lake Ontario since 1994, through annual juvenile trawls, with age 1-3 naturally spawned juveniles repeatedly collected near the mouth of the Niagara River (Lantry and Lantry 2012).

In an effort to maintain some of the genetic diversity of different lake trout populations, stocking has utilized many different source populations from across North America (Lantry and Lantry 2008; Page et al. 2004). Collections from these various source populations have been maintained separately to produce hatchery strains. At the present time, six hatchery strains of lake trout are maintained by the United States for restoration purposes. In Lake Ontario, three of those strains have been stocked since 1995: the Seneca Lake, Jenny-Lewis Lake, and Superior strains (Grewe et al. 1993; Lantry and Lantry 2008). Since 2000 however, only the Superior (Traverse Island or Apostle Island strain) and Seneca Lake strain have been stocked into Lake Ontario (Lantry and Lantry 2008).

Because the ultimate goal of the lake trout restoration program is to produce self-sustaining populations, such that stocking is no longer necessary (Lantry and Lantry 2011), understanding hatchery fish reproduction in wild habitats is important. Lake trout biology indicates that fry imprint on their natal reef (Bronte et al. 2002); a behavior which is lost with the stocking of hatchery fish (e.g., no natal reef to imprint on). This may be leading to a shift in spawning sites, from deeper offshore locations to shallower, inshore locations (Gunn 1995;

Marsden and Chotkowski 2001). Managers seek to monitor these shifts in spawning sites to understand changing lake trout behavior, as well as evaluate which hatchery strains are contributing to natural reproduction. This can provide valuable information for informing stocking management practices.

A population of naturally reproducing lake trout appears to be using the lower Niagara River based on: (1) egg collection by the U.S. Fish and Wildlife Service (USFWS) Lower Great Lakes Fish and Wildlife Conservation Office (Trometer 2006), (2) collection of naturally reproduced young in juvenile trawl surveys near the river (Lantry and Lantry 2012) and (3) anecdotal evidence of recreational fishing charter captains catching mature, ripe lake trout. To further evaluate these claims and observations, this study was undertaken to (1) assess the composition of hatchery lake trout strains present in the lower Niagara River, and (2) identify parental strain of origin of naturally reproduced juveniles.

Methods

Study site- This study was conducted on the lower Niagara River, NY (Figure 4-1). The river flows south to north, from Lake Erie into Lake Ontario. Niagara Falls forms a natural barrier, separating the river into an upper and lower section. Sampling took place from the mouth of the river, in Lake Ontario, upstream approximately 17 km, after which point water turbulence from Niagara Falls made conditions unsafe for sampling.

Adult sampling protocols- Adult lake trout were captured in the lower Niagara River during the fall of 2010, 2011, and 2012 (see Appendix B, Table B-1 for sampling date ranges). Sampling effort was not distributed equally or randomly across the study area. Rather, sampling focused on areas where adult lake trout had previously been collected and those areas recommended by local lake trout fishing charter captains. This targeted approach was used due to a limited amount of

sampling time and the large spatial extent of the river. Adult lake trout were caught using hook and line angling. After being caught, all lake trout were held in a live well until processed, no longer than one hour. Immediately before processing, fish were anesthetized using MS-222 and then measured for fork length (mm) and weight (kg). Fish were inspected for the presence/absence of coded wire tags, determined by a hand-held scanner, and whether or not the adipose fin was present in an effort to determine probable hatchery origin. Lake trout were inspected for other fin clips or visible scars, such as lamprey wounds. An approximately 1 cm^2 caudal fin clip was taken and preserved in 95% non-denatured ethanol for genetic analysis.

Collection and culture of eggs- Eggs were collected using egg traps (Trometer 2006) during the fall of 2010 (November 1, 2010 – December 7, 2010) and 2011 (November 2, 2011-December 6, 2011). A total of 11 egg trap lines, with 10 traps per line, were placed in the river. Egg traps were deployed from the mouth of the river to just downstream of the whirlpool (17.5 km upstream) and were concentrated in areas of previously observed spawning activity (Trometer 2006). After deployment, egg traps were checked weekly, weather permitting. During trap deployment, trap location, set time, weather conditions (e.g., clear, overcast, rain, snow, fog) and water depth measurements were recorded. All traps were marked with buoys identifying them as property of the U.S. Fish and Wildlife Lower Great Lakes Fish and Wildlife Conservation Office (USFWS LGLFWCO) in an effort to minimize tampering and to help ensure retrieval if moved from location of deployment. Upon collection of eggs, all dead eggs which were approximately the correct diameter for lake trout (5-6 mm) were immediately preserved in 95% non-denatured ethanol for genetic analysis. All eggs that appeared to be lake trout and still viable were transported on ice in Nalgene (Thermo Fisher Scientific, Inc., Waltham, MA) bottles filled with river water to the USFWS LGLFWCO and raised in a Living Stream System (Frigid Units, Inc., Toledo, OH; Appendix A). Temperature in the Living Stream System was maintained at approximately 8 ºC. The tank was also covered to limit light exposure in an effort to mimic

natural rearing conditions. To keep the collection area and day separate, eggs were placed in egg trays with each batch in individual cups (Appendix B). In 2011, a small sample of hatchery eggs (n=100) were also raised with wild- collected eggs to ensure the Living Stream System was conducive to egg development.

Egg rearing protocols- The Living Stream System tank was disinfected with 35 mL of 5.25% sodium hypochlorite (Clorox) per gallon of water and run for 1 hour before being set up for egg incubation each collection season (fall 2010 and 2011). The system was then drained and rinsed before being refilled. Prior to placing eggs in the Living Stream System, eggs were disinfected by submersion in Argentyne Iodine Disinfectant (Argent Chemical Laboratories, Redmond, WA- 10% polymeric-iodine complex; 90% inert ingredients) for 10 minutes.

Eggs were monitored for development daily during weekdays and when possible on weekends. Non-viable eggs, defined as opaque eggs or when fungal growth became apparent, were immediately removed and preserved in 95% non-denatured ethanol. After hatching, all fry were preserved in 95% non-denatured ethanol. All preserved eggs and fry were transferred to the USFWS Northeast Fishery Center Conservation Genetics Lab, Lamar, Pennsylvania, for genetic analysis. Fry from the hatchery eggs used for testing of the Living Stream System were transferred to another station aquarium for continued rearing for use in outreach programs.

Lake trout offspring sampling- Lake trout eggs and fry were targeted in the spring of 2011 and 2012 using drift nets (Research Nets, Inc., Redmond, WA; 75x50x250cm, 2000 micron mesh with 1000 micron mesh detachable cup) in areas of egg collection, and downstream of suspected spawning locations. In the spring of 2011, drift nets were used from April through May to collect larval fish. Drift nets were attached to an aluminum frame to support open and correct positioning because the variable and high flow conditions of the Niagara River could cause nets to twist and collapse. Nets were set for approximately 24 hours, deployed during the morning,

retrieved the following day, and reset after collecting samples. Location, depth, weather conditions, time, and by catch were recorded for each net set. Any egg or fry collected and identified as possible lake trout were preserved in 95% ethanol for genetic analysis to confirm species and to identify strain.

In the spring of 2012, framed drift nets and ichthyoplankton nets (Aquatic Research Instruments, Hope, ID; 50x200cm, 500 micron mesh) were used to collect juveniles in the Niagara River. Net placement was targeted in areas of successful collection of eggs and fry in 2006, fall 2010, spring 2011 and fall 2011. Sampling began in March (rather than in April, as in 2011) and continued through May, as winter temperatures were higher than average, and no ice cover was observed on the river. Egg and fry preservation followed methods described for 2011.

Genetic species identification- Because lake trout eggs and fry collected from the river had few distinguishable markings, genetic sequencing of the internal transcribed spacer 1 region (ITS-1) was used to determine the species of all egg and fry samples. The ITS-1 region was chosen based on its ability to identify a variety of species based on available sequence data in GenBank (http://www.ncbi.nlm.nih.gov/genbank/). DNA extraction of eggs and fry was completed using Purgene (Qiagen, Inc., Valencia, CA), Kingfisher (Thermo Scientific, Waltham, MA) or Chelex (Sigma-Aldrich, St. Louis, MO) protocols. DNA was amplified using primers flanking the 18S and 5.8S regions of ITS-1, following PCR methods in Pleyte et al. (1992). Products were visualized on a 2% agarose gel to examine banding patterns compared to a control sequence (adult hatchery lake trout). Samples with appropriate banding patterns matching positive controls were then purified using EXO-SAP (Affymetrix Inc., Cleveland, OH). A sequencing reaction was run on EXO-SAP purified PCR products, separately in both the forward and reverse direction, with a BigDye Terminator Kit v3.1 (Invitrogen, Carlsbad, CA). Sequencing reactions were purified using Agencourt CleanSEQ (Beckman Coulter, Inc., Brea, CA), before being visualized on an ABI 3130xl (Life Technologies, Foster City, CA). Sequencher (Gene Codes

Corporation, Ann Arbor, MI) was used to match forward and reverse sequences to obtain a consensus region for each sample. The full sequence was compared to ITS-1 sequences in GenBank using BLASTX (National Center for Biotechnology Information, Bethesda, MD). Top three species matches were recorded, and when in concordance, were identified as the species for that sample.

Analysis of hatchery populations- In order to assign unknown individuals to source population, each hatchery year class was evaluated for conformation to Hardy-Weinburg equilibrium using HW-QuickCheck (Kalinowski 2006) and the effective number of breeders was estimated using LDNe (Waples 2006). FSTAT was used to calculate F_{ST} values between year classes (Goudet 1995). Bonferroni corrections for multiple comparisons were applied to significance values generated for estimates of F_{ST}, (P<0.001; Rice 1989); where appropriate, year classes were combined into hatchery strain populations. GeneClass (ver. 2; Cornuet et al. 1999) was used to determine correct proportion of hatchery assignment. Estimates for genetic diversity for each hatchery strain are available in Chapter 3.

Genetic determination of hatchery strain- All adult fin clips and genetically confirmed lake trout eggs and fry were analyzed to identify hatchery strain of origin. To serve as a baseline for comparison, tissue samples were obtained from hatchery strains of lake trout recently stocked into Lake Ontario that would be the appropriate age to contribute to spawning during the sampling period. Samples were obtained from at least two year classes of hatchery fish per strain (50 fin clips/year class).

All samples were stored in 95% non-denatured ethanol, and processed at the USFWS Northeast Fishery Center Conservation Genetics Lab. DNA extraction was completed using either Purgene (Qiagen Inc., Valencia, CA) or Kingfisher (Thermo Scientific, Waltham, MA)

methods, following manufacturer protocol. A random sample of approximately 10% of the samples from each batch was analyzed using UV spectrophotometry to assess an average concentration of DNA. Using the average concentration, all samples within each batch were diluted with molecular grade water (Sigma-Aldrich Co., St. Louis, MO) to a uniform concentration of ~30mg/uL DNA. Microsatellite loci used for PCR were: Sfo1 (Angers et al. 1995); Ssa85 (O'Reilly et al. 1996); Oneμ10, Oneμ9 (Scribner et al. 1996); Ogo1a (Olsen et al. 1998); SnaMSU01, SnaMSU03, SnaMSU08, SnaMSU10, SnaMSU12, and SnaMSU13 (Rollins et al. 2009). PCR was completed with one primer labeled on the 5' end with a fluorescent dye (FAM, HEX or NED). PCR amplification was performed in a programmable thermocycler (BioRad Laboratories, Inc., Hercules, CA) using 10μL reaction volume. The PCR profile for all loci involved a single 2 min denaturing step at 94ºC, followed by 35 cycles of a 45 second denaturing step at 94ºC, a 45 second annealing step at the primer specific temperature (see Table A-1), and 2 min extension step at 72ºC. The PCR profile was completed with a 5 min extension step at 72ºC. PCR products were visualized using an ABI 3100 or ABI 3130xl (Life Technologies, Foster City, CA) and GeneScan, GenoTyper, and GeneMapper (Life Technologies, Foster City, CA) software were used to visualize and score the data, with manual verification to ensure accuracy.

Samples collected on the river, both adult and confirmed larval lake trout, were assigned to a potential hatchery of origin using GeneClass (ver. 2; Cornuet et al. 1999). Naturally reproduced offspring and adults identified as potentially wild were also assessed for any evidence of hybridization between hatchery strains using NewHybrids (Anderson and Thompson 2002).

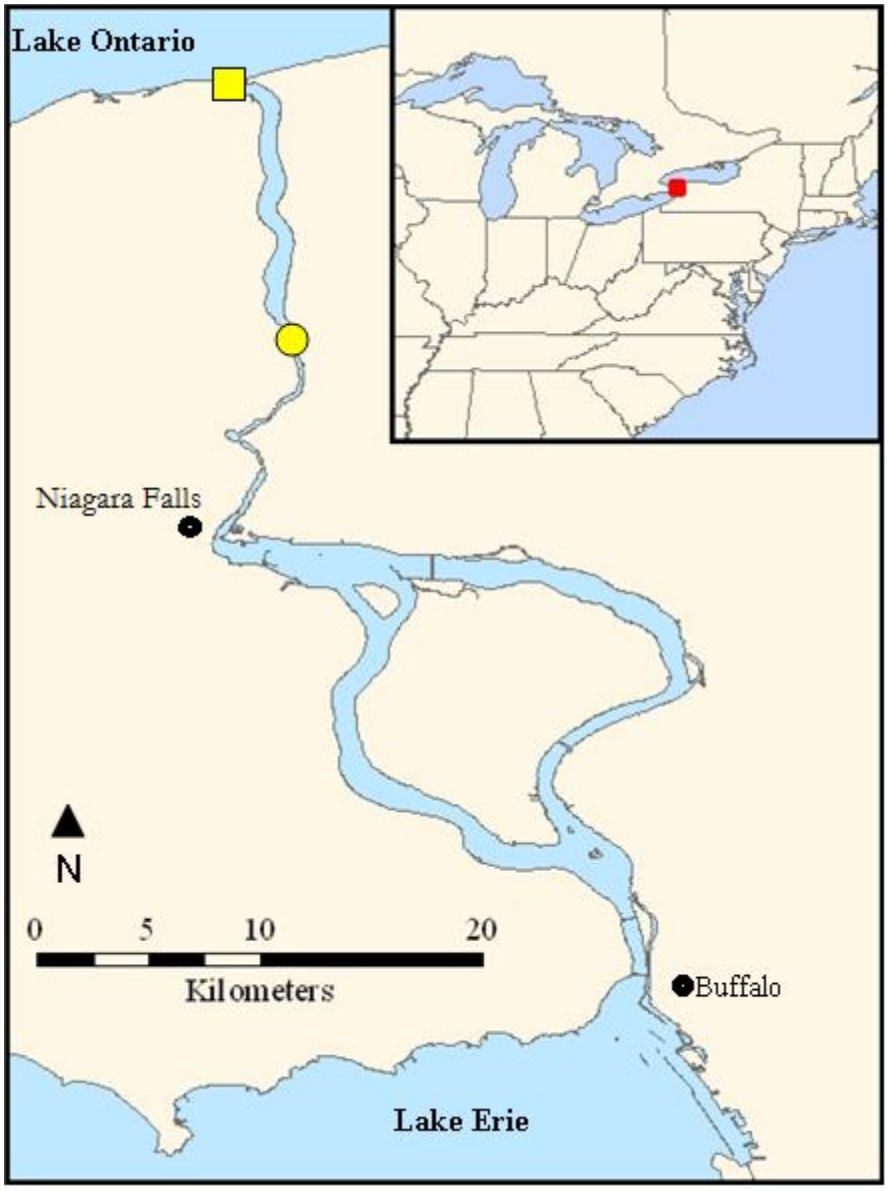

Figure 4-1: Study Location. The Niagara River flows south to north, from Lake Erie into Lake Ontario. All sampling took place on the lower portion of the Niagara River near the confluence with Lake Ontario. Sampling effort was focused on the Niagara Bar at the mouth of the river (yellow square) and upstream near the Lewiston/Art Park area (yellow circle).

Results

Adult collection- A total of 164 adult lake trout were collected during 2010-2012. The majority of the river collections took place in the Art Park/Omega area (Art Park/Omega: 43, other in-river: 7). Total collection per season ranged from 5 lake trout in the spring of 2012 to 70 lake trout in the fall of 2012. Fish ranged in size from 510 mm to 899 mm and 3.2 kg to 14 kg (Table 4-1).

Larval collection- Six eggs were collected in the fall of 2010, four dead and two potentially alive, which were kept for rearing. In spring 2011, 54 eggs and 33 sac fry were collected. Three eggs were collected during the fall of 2011, all of which were potentially alive and kept for rearing. In spring 2012, five eggs and 19 sac fry were collected. See Figures 4-2 and 4-3 for fall and spring collection locations.

Genetic species identification- Of the 120 eggs and sac fry collected, 100 were successfully sequenced. Of those producing adequate length sequences to compare to GenBank data, 76 were confirmed to be lake trout; 43 of these were sac fry, of the 52 total sac fry caught, and 32 were eggs, of the 68 total eggs caught. Sequences which were identified as non-lake trout were primarily fungus/mold (Table 4-2).

Genetic assumption testing- All hatchery populations used for this analysis were found to be adequate for analysis based on ability to successfully genetically differentiate between strains to allow for assignment of unknown origin lake trout to strain of origin (Seneca Lake strain, Apostle Island strain and Lewis Lake strain, Table 4-3 and 4-4; for full results, see Chapter 3).

Hatchery assignment of samples- Hatchery fin clips were provided by USFWS National Fish Hatchery (NFH) personnel for the following strains: Apostle Island strain (Iron River NFH), Seneca Lake strain (Sullivan Creek NFH), and Lewis Lake strain (Saratoga NFH). Of the 164 adult samples of unknown origin, 92% were assigned to the Seneca Lake strain. By collection year, 96.3% of the fall 2010, 91.3% of fall 2011, and 88.6% of fall 2012 were assigned to the Seneca hatchery strain based on microsatellite markers used (Table 4-5). All adults captured in the river in both spring sampling years were of the Seneca Lake strain origin (Table 4-5). The number of fish assigned to the Apostle Island or Lewis Lake strain was small and variable by year. For example, in fall 2010, no fish were assigned to the Apostle Island strain and only one to the Lewis Lake strain. However, in the fall 2011 collection four adults were assigned to the Apostle Island strain and one to the Lewis Lake strain. In fall 2012, two fish were assigned to the Apostle Island strain, and six to the Lewis Lake strain (Table 4-5).

All adult fish sampled that were potentially naturally reproduced (e.g., no coded wire tag and adipose fin present) were assigned to the Seneca Lake strain (n=13; Table 4-5). Additionally, 41 of 43 naturally produced larval samples confirmed to be lake trout through sequencing analysis were assigned to the Seneca Lake strain. Only two fry, one from each spring collection, were assigned to the Lewis Lake strain. No genetic evidence was found of hybridization between strains in any of the naturally reproduced samples (e.g., no unknown origin individuals were assigned as hybrids to either potential parental source; all individuals were classified as one parental strain or another).

Table 4-1. Adult Lake Trout Collected. Total number, mean length, and weight of adult lake trout collected on the Niagara River/Niagara Bar, NY in 2010-2012. Standard deviation is listed in parentheses. Not all fish had fork length or weight measurements available; numbers reflect all available data. Length measurements were based on 72-100% of the data, weight measurements were based on 44-91% of data. Weight measurements were not taken during fall 2012 sampling.

Collection season/year	Adults captured		Fork length (mm)	Weight (kg)
	Niagara River	Niagara Bar		
Fall 2010	22	5	726.6 (76.57)	6.0 (1.65)
Spring 2011	5	11	732.3 (83.30)	4.9 (1.22)
Fall 2011	22	24	703.6 (86.42)	6.7 (2.68)
Spring 2012	5	0	850.5 (36.06)	8.8 (2.47)
Fall 2012	0	70	648.8 (76.93)	N/A

Table 4-2: Non-Lake Trout Sequencing Results. Genus matched to sequencing results from samples collected in the Niagara River (species grouped by genus for summary). Many are forms of fungus or bacteria, which were most likely growing on dead eggs, however two other fish species were identified, which were only able to be classified to genus level.

Genus	Count
Adineta	1
Aphanomyces sp.	5
Paracercomonas	1
Stereum sp.	2
Basidiomycete	3
Spirinchus sp.	4
Saprolegnia sp.	3
Ascomycete sp.	1
Pythiaceae sp.	2
Oncorhynchus	1
Coregonus	1
Total	24

Table 4-3: Correct Hatchery Assignment. The proportion of known hatchery samples assigned to each hatchery population, with bold numbers indicating proportion of correct assignment. Left column indicates hatchery samples and top row are the strain they were assigned to (GeneClass ver. 2; Cornuet et al. 1999)

	Assigned To		
Assigned From	Apostle Island	Seneca Lake	Lewis Lake
Apostle Island	**0.84**	0.04	0.12
Seneca Lake	0.04	**0.95**	0.01
Lewis Lake	0.10	0.02	**0.88**

Table 4-4: F_{ST} Values of Hatchery Populations. To determine if strains were genetically distinct enough to assign unknown individuals to, F_{ST} values were compared. A Bonferroni correction for multiple comparisons was applied (P= 0.0015)

	Seneca Lake Strain	Lewis Lake Strain
Apostle Island strain	0.0167	0.0167
Seneca Lake strain		0.0167

Table 4-5: Adult Niagara River Samples Assignment. Samples are broken down by collection year/season, and potential origin: hatchery or wild. Total numbers of fish collected for each season are listed to the far right; total numbers of fish assigned to each strain are listed along the bottom of the table.

	Seneca Lake strain		Apostle Island strain		Lewis Lake strain		total
Collection season	hatchery origin	potentially wild	hatchery origin	potentially wild	hatchery origin	potentially wild	
Fall 2010	22	4	0	0	1	0	27
Spring 2011	16		0		0		16
Fall 2011	40	1	4	0	1	0	46
Spring 2012	5		0		0		5
Fall 2012	54	8	2	0	6	0	70
Total	137	13	6	0	8	0	164

Table 4-6: Larval Lake Trout Assignments. All eggs and fry confirmed to be lake trout are listed according to hatchery assignment (Seneca Lake, Apostle Island or Lewis Lake strain). Totals for each strain and each collection year are listed at the bottom and far right of the table.

	Seneca Lake strain	Apostle Island	Lewis Lake strain	Total
Spring 2011	24	0	1	25
Spring 2012	17	0	1	18
Total	41	0	2	43

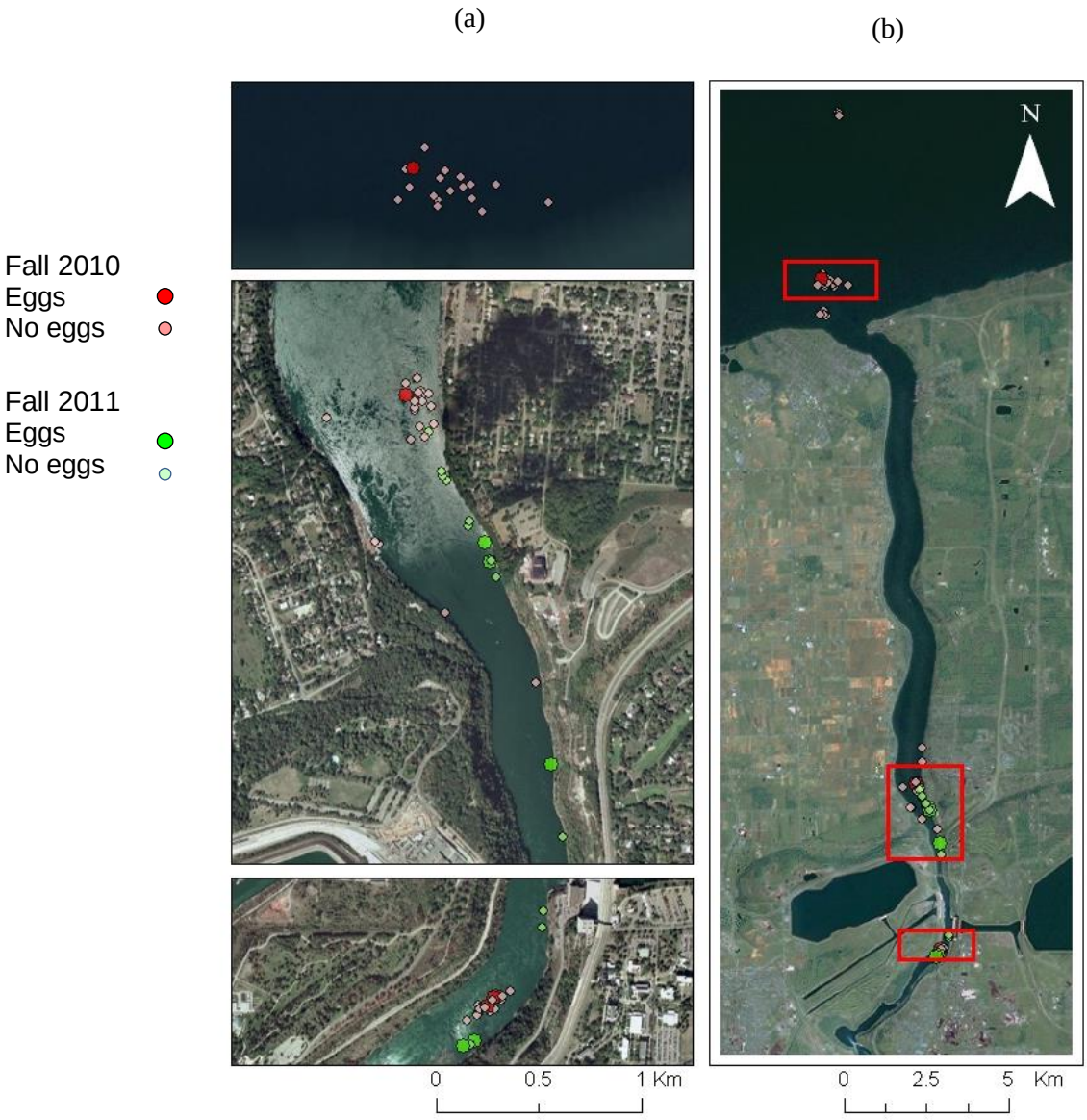

Figure 4-2: Fall Lake Trout Egg Sampling Locations. Successful capture is indicated by darker, larger circles for each year (2010= red, 2011= green). Enlarged views (a) of red boxes highlighted on (b).

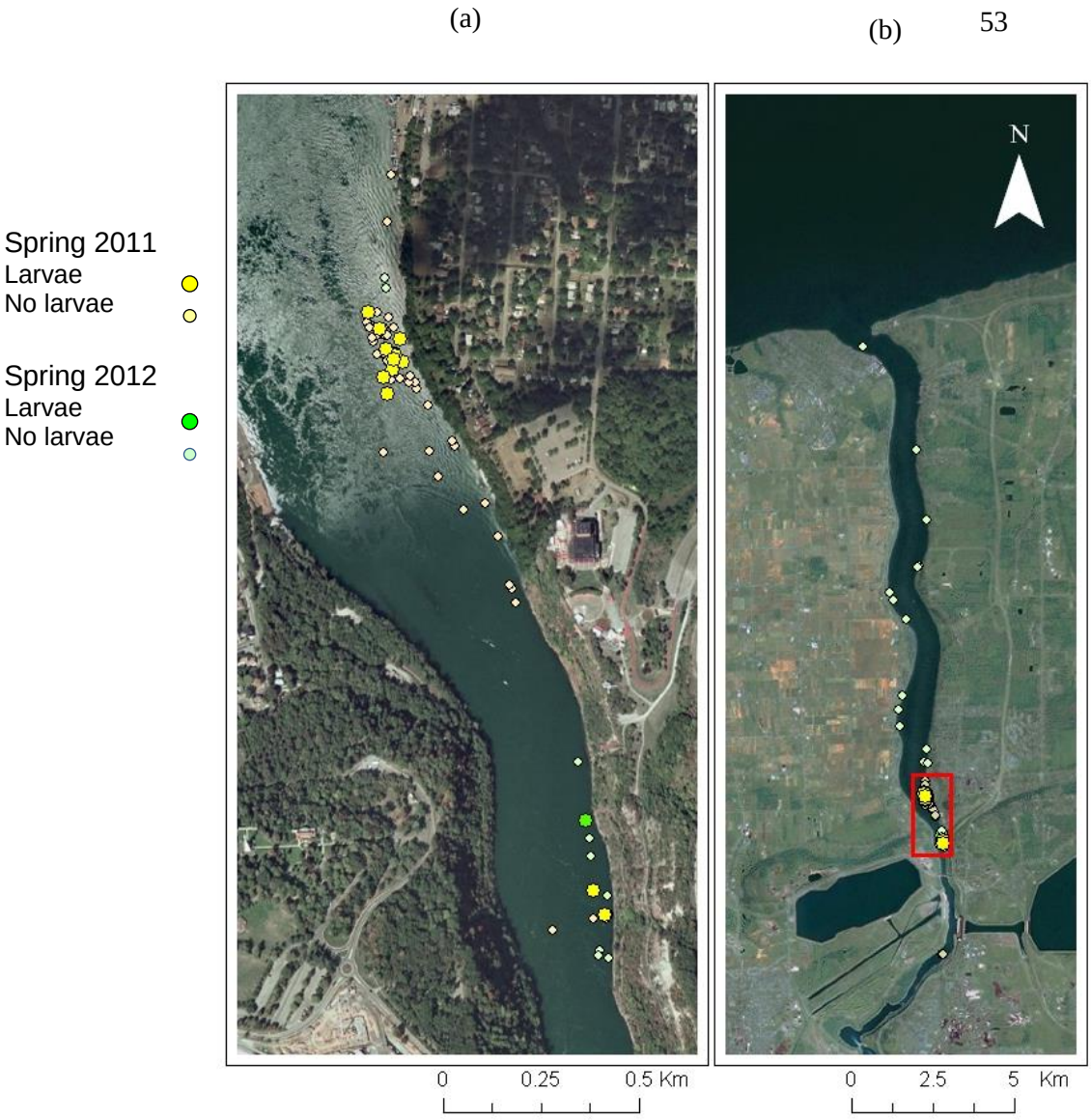

Figure 4-3: Spring Larval Lake Trout Sampling Locations. Successful capture is indicated by larger, darker circles (2011= yellow, 2012= green). Based on previous seasons of sampling, efforts were concentrated from the gorge to Lewiston launch. Enlarged views (a) of red box highlighted on (b).

Discussion

A hatchery stocked population of lake trout is successfully reproducing in the Niagara River, NY. Riverine reproduction has not been reported since 1955 in the Montreal and Dog rivers of Lake Superior (Loftus 1958). In general, reports of natural reproduction by hatchery fish has been limited (Lantry and Lantry 2008; Lantry and Lantry 2011). Interestingly, although Great Lakes origin strains have been stocked into Lake Ontario (Apostle Island strain from Lake Superior, and Lewis Lake strain originally developed from a population in Lake Michigan), it is the non-Great Lakes derived strain that are naturally reproducing. The Seneca Lake strain was developed from a population native to the largest and deepest of the Finger Lakes in New York.

Monitoring of the natural reproduction on the Niagara River was successful in identifying the hatchery strains of adult fish sampled in the river, as well as strain of origin of juveniles naturally produced during the study period. Over 95% of the natural reproduction observed during this study was from the Seneca Lake strain, as seen in previous studies done in Lake Ontario (Marsden et al. 1989; Perkins et al. 1995). Both Mardsen et al (1989) and Perkins et al (1995) used mixed-stock analysis to identify parental stock of naturally reproduced offspring, with Seneca-Seneca reproduction contributing most to samples collected (78% and 73-95%, respectively, for areas closest to the Niagara River). Additionally, Page et al. (2003) found the Seneca Lake strain contributed disproportionately higher to natural reproduction in Lake Michigan than would be expected based on adult population composition. No evidence of hybridization was found in this study, indicating breeding between strains at this location may be minimal. The Seneca Lake strain has made up the largest proportion of lake trout being stocked in Lake Ontario from 2000-2008 (over 50% of target 500,000 yearlings; Lantry and Lantry 2008; Connerton 2009). This, however, has changed based on stock availability (disease concerns relating to the captive Seneca Lake strain limited production). For instance, in 2011, Lake Champlain strain was the only strain stocked by the New York Department of Environmental

Conservation (488,373 yearlings; Connerton 2011). Based on stocking records, it is possible the contribution of the Seneca Lake strain to natural reproduction may be influenced by larger stocking numbers compared to other strains. Additionally, the large contribution of the Seneca Lake strain to natural reproduction in Lake Ontario may be location specific, as Perkins et al. (1995) found Seneca Lake strain reproduction very limited (>10% of total) in two of eight locations sampled. Based on their findings, they recommended the continuation of stocking a variety of strains, which may utilize different spawning habitats than the Seneca Lake strain.

Due to the Marquette strain no longer being in production, and therefore not available for sampling, the Apostle Island strain was treated as a representative sample for Lake Superior lean morphotype stock. In Lake Ontario, these strains were often stocked concordantly, simply with the distinction of "Superior strain" (Lantry and Lantry 2008). The high number of assignments to the Lewis Lake strain may have been a misclassification based on stocking records. Individuals of this strain may still be reproducing, however their numbers would be limited compared to Seneca Lake and Apostle Island strains. The Lewis Lake strain had a relatively lower proportion of correct assignment compared to the Seneca Lake strain (88% and 95%, respectively). The next highest assignment for the Lewis Lake strain was the Apostle Island strain (10%). The Apostle Island strain and Lewis Lake strain are both of Great Lakes origins (Lakes Superior and Michigan), while the Seneca Lake strain is derived from a population of lake trout in the Finger Lakes. Whether the Lewis Lake strain assignments were correct or potential misclassifications of the Apostle Island strain, this study shows the non-Great Lakes origin Seneca Lake strain is contributing most to natural reproduction in the lower Niagara River.

Lake trout were also present in Niagara River in the spring. While lake trout readily move throughout lake-river systems in the western United States (Muhlfeld et al. 2011), there are limited publications available on lake trout river use in the Great Lakes (but see Lake Superior tributaries used for spawning: Loftus 1958; Krueger and Ihssen 1995). It is hypothesized that

lake trout are using the river in spring to feed on mature smelt (Gorman et al. 2000; pers. obs.). Due to the small sample size (n=16 in 2011, n=5 in 2012), few inferences can be made about their behavior without additional information. A diet study of lake trout captured in the river during the spring could lend support to the predation hypothesis. Of the lake trout observed in the spring, all were Seneca Lake strain.

With lake trout stocking not yet producing self-sustaining populations after 40 years of intensive stocking efforts, the monitoring of naturally reproducing stocked populations may be informative to managers. The majority of the fish caught during this study were of hatchery origin; however a few mature, potentially naturally reproduced fish were sampled. It is unknown at this time if pre-extirpation lake trout populations used this location, or mechanisms by which currently lake trout may have begun to utilize this habitat (e.g., hatchery fish followed wild fish to their natal spawning reef; Gunn 1995; Bronte et al. 2002). To assess other locations in the river for lake trout spawning habitat, the current side-scan sonar project being conducted by the USFWS LGLFWCO may be beneficial. Understanding the substrate type of the Art Park/Omega location may aid in identifying other spawning locations. For hatchery managers, this study provides evidence of natural reproduction by the Seneca Lake strain of lake trout, lending support to the importance of the Seneca Lake strain of lake trout in achieving restoration goals.

Chapter 5

Lake Trout Movement in the Niagara River

Introduction

While lake trout generally utilize deep, well oxygenated water within their preferred temperature range (8-12˚C), they will utilize shallower habitats for spawning, and shallower and higher temperature waters for foraging (Morbey et al. 2006). In an attempt to understand these behaviors and preferences, tagging and tracking of lake trout has been done in a number of different North American freshwater systems (Walch and Bergensen 1981; MacLean et al. 1981; Schmalz et al. 2002). Seasonal movement dynamics can be influenced by a variety of factors, for example, summer stratification can limit available habitats (Walch and Bergersen 1981; Blanchfield et al. 2009). Additionally, winter ranges may also be reduced due to light restrictions in foraging habitats resulting from ice and snow cover, which will be dependent on the size of the lake (Blanchfield et al. 2009). While Blanchfield et al. (2009) found significantly larger core use areas for tagged lake trout in the summer rather than winter (Lake Opeongo, Ontario), Schmalz et al. (2002) found no difference in total summer versus winter range in Lake Michigan.

Often, spawning sites are far from foraging locations, and thus fish must travel large distances to reproduce (Walch and Bergersen 1981). Lake trout select spawning habitats with a large wave fetch, and a cobble or rocky substrate, which are most often found nearshore and a few deeper, offshore shoals (Flavelle et al. 2002). In the Great Lakes, there is evidence that lake trout spawning sites are shifting to shallower nearshore habitats, compared to historical deeper offshore locations, associated with changes in behaviors of stocked hatchery fish (Gunn 1995;

Marsden and Chotkowski 2001). Locating and confirming lake trout spawning habitats is important for management for several reasons, including (1) providing evidence of natural reproduction, (2) conserving or restoring spawning habitats, and (3) tracking shifts in lake trout spawning locations. Additionally, understanding lake trout utilization of large river systems is of general interest, as the use of large river habitats by lake trout is not common (see Loftus 1958).

Naturally spawned juvenile lake trout have been repeatedly collected in standard lake trout juvenile index trawls near the mouth of the Niagara River, and represent a high proportion of the naturally reared juvenile lake trout collected during surveying throughout Lake Ontario (Lantry and Lantry 2008). Information on river movements and river habitat use during spawning in the Niagara River can benefit management by improving the understanding of naturally reproducing populations. Therefore, the objective of this study was to quantify movements and river use of adult lake trout during the spawning season in the Niagara River, New York, USA.

Methods

Study site- This study was conducted on the lower Niagara River, NY (Figure 5-1). The river flows south to north, from Lake Erie into Lake Ontario. Niagara Falls forms a natural barrier, separating the river into an upper and lower section. Sampling took place from the mouth of the river, in Lake Ontario, upstream approximately 17 km, after which point water turbulence from Niagara Falls made conditions unsafe.

Tagging of lake trout- Lake trout were sampled using hook and line angling in the Niagara River and Niagara Bar area of Lake Ontario during November 2010 (November 3, 2010- November 9, 2010) and 201l (November 2, 2011- November 19, 2011). The goal each year was to tag 30 lake

trout. To ensure fish were of spawning age, a minimum target length for tagging of 700 mm total length was established (Healey 1978); however, fish expressing ovarian fluid or milt were tagged, even if smaller than the established target.

After fish were caught, they were immediately placed into a freshwater holding tank with recirculating water, before being anaesthetized with MS-222 (fish were not held for > 1 hour). Fork length (mm), total length (mm) and weight (kg) measurements were taken before transmitters (externally mounted radio transmitters [53 mm long, 17mm in diameter, weighing 22 g]; Advanced Telemetry Systems, F2060B) were attached with wire cables through the dorsal musculature below the dorsal fin, and secured with wire clamps. Fin clips were also taken during this time, and information on capture location or any marks (i.e. lamprey wounds/scars) on the fish were recorded. To determine if fish were stocked from a hatchery, the presence of a coded wire tag and adipose fin clip was checked. After tagging, fish were allowed to recover in a freshwater holding tank until they regained equilibrium and were observed actively swimming. Transmitters were checked for proper functioning before fish were released.

Tracking- Lake trout were manually tracked within the Niagara River and Niagara Bar area (using a R2000 Challenger receiver; Advanced Telemetry Systems) twice weekly from boat or shore (weather permitting) by the U.S. Fish and Wildlife Service Lower Great Lakes Fish and Wildlife Conservation Office (USFWS LGLFWCO) staff. Shoreline tracking sites were based on accessibility to the Niagara River (Figure 5-2). Detection of tagged lake trout were recorded as GPS waypoints, either exact location or river transect. In spring of 2011, eight receiver arrays (R4500SD receivers; Advanced Telemetry Systems) were installed along both sides of the river (seven fixed receiver sites on the American side of the river and one site on the Canadian side of the river) in an attempt to continuously monitor fish movement (Figure 5-2). Manual tracking continued approximately twice weekly after the fixed receiver sites were established.

Lake trout detections from the fixed receiver sites were recorded with respect to which section of the river the fish was located using. This approach was used because of the inability to obtain more precise lake trout locations with fixed receivers; i.e., finer-scale assessment of river use was not possible using fixed receiver sites. Accordingly, the river was divided into four sections to assist in describing river-section use by lake trout. River sections included the "bar", which included fish detected just outside of the river and included the shallow shoal known as the Niagara Bar; the "lower river" which was defined as extending from river km (rkm) 0 to 9.55, this section of the river was generally wider than upstream sections; "mid river", defined as extending from rkm 9.55 to rkm 13.8, included the Art Park area and the upper end of this section was defined by the river narrowing and becoming deeper (~50m); and the "upper river" which extended from rkm 13.8 to rkm 17, and was the area of the river above the New York Power Authority (NYPA) discharge. The upper river also differed in hydrology from the lower sections, experiencing large water level fluctuations due to power generation and regulation of water over Niagara Falls.

Transmitter detection- To assess detection ability of fixed receiver stations, a study was performed to determine detection range and depth of fixed receivers. Three sites were tested, Coast Guard, Fox, and Irish (Figure 5-2). At each site, two transects were established along each antenna's azimuth, with three locations per transect (near-shore, mid-channel, and far-shore). A test transmitter was lowered into the water at each location, at 3 m intervals, starting at 1 m depth. Additional depths were taken outside of the 3 m intervals as judged appropriate to determine maximum detection depth. At each depth, the receiver was allowed to cycle through its frequency tables until test transmitter was detected. These detection data were analyzed using logistic regression to quantify tag detection probability, as well as determining site-to-site differences in transmitter detection (Gregory Jacobs, USFWS, personal communication).

Fish movement statistical analysis- Because of the relatively small number of fish tagged and low numbers of fish relocated, the telemetry data were not suitable for making statistical inferences about movement and river use over the duration of the study (i.e., quantitative statistical models were not fitted). Rather, analysis was restricted to generating descriptive statistics in an effort to provide relevant information about general movement patterns and river use observed during the study period.

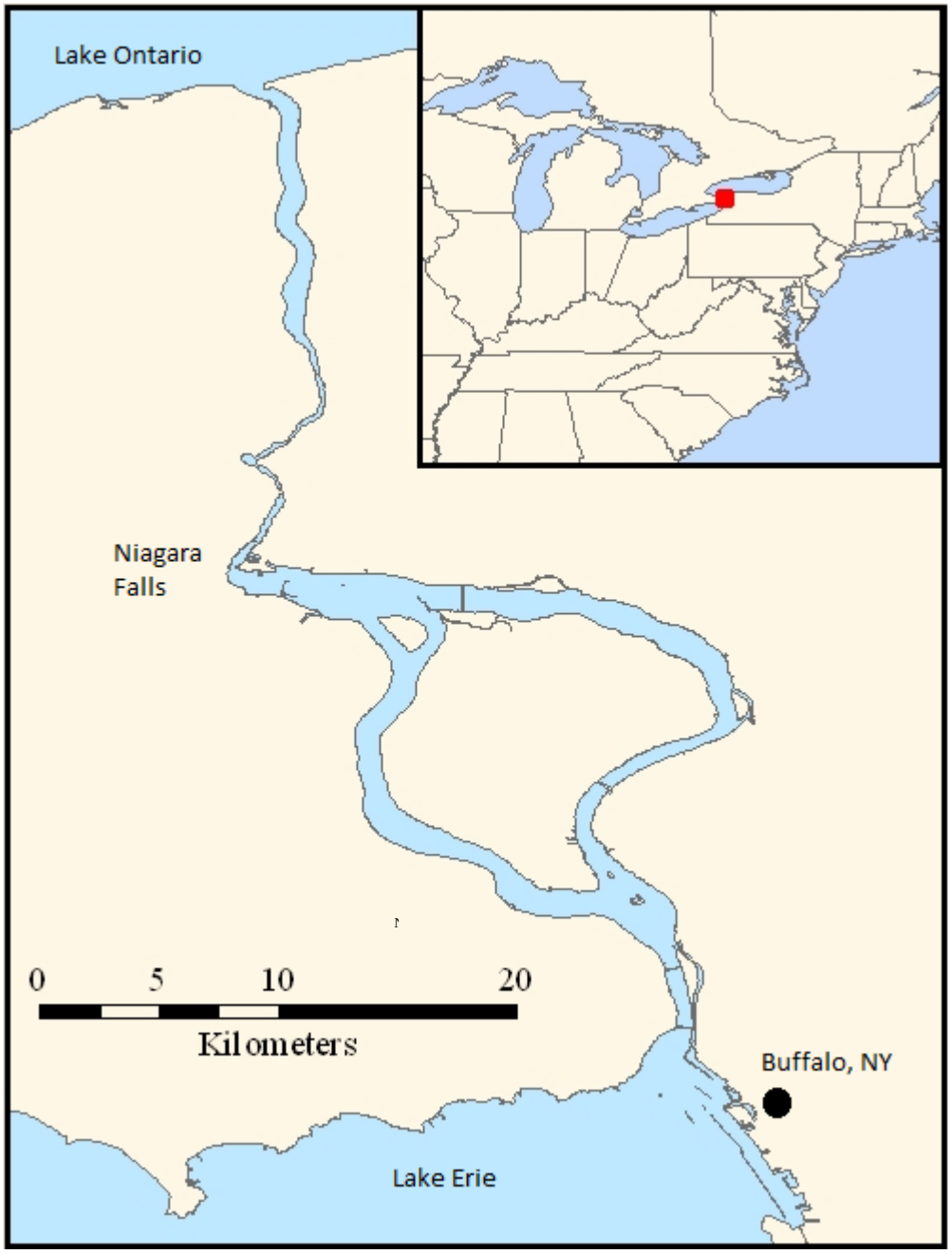

Figure 5-1: Study Area. Sampling took place on the Niagara River, which flows from Lake Erie into Lake Ontario. Niagara Falls naturally divides the river into an upper and lower portion. Sample took place from the mouth of the river in Lake Ontario, upstream approximately 17km.

Figure 5-2: Radio Tracking and Tagging Locations. Yellow circles represent locations of shoreline tracking sites (used during fall 2010, 2011). Green crosses represent locations of fixed telemetry receiver sites (used during fall 2011). Niagara Bar is represented by the black square at the mouth of the river, and Art Park is in the same location as the Omega site; both are sites of targeted lake trout capture. Larger empty circles represent cities along the New York state shoreline

Results

Fish tracking- During the 2010 field season, 27 adult lake trout were captured, 18 of which were radio tagged. Of the tagged fish, two were caught on the Niagara Bar, and 16 were caught in the Art Park/Omega area (Figure 5-2). Neither fish tagged on the Niagara Bar were detected again. Of the 16 fish tagged in the Art Park area, 13 remained within the mid-river area, and three fish were detected in the upper river during the fall 2010 tracking season (November 4, 2010 – December 17, 2010). Average movement per fish was 0.17 rkm/day (SD = 0.32), and movement was approximately equal in the up and down stream directions (51.1% of total movement was upstream; 46.7% downstream). The largest movement observed for a single fish in a day was 3.33 km upstream, from the Art Park area into the upper river section (2010 tracking season; Table 5-1).

Of the 18 fish tagged in 2010, 9 were detected again in 2011. All fish were located in the two lower sections of the river and in the bar area. Detections during 2011 were reduced (14 total) and occurred over a longer time period (May 6, 2011- December 29, 2011). Average movement per day was not calculated for these data because there were too few detections, and interpretation of such a summary statistic may not be representative of typical fish movement during a given time period. Maximum movement for fish tagged in 2010 and located in 2011 was larger compared to fish tracked during fall 2010 (7.93 rkm in one day). In addition, fish movement occurred more often in the downstream direction (79%) than upstream (21%).

During the 2011 field season, 41 fish were captured, 30 of which were radio tagged. Eighteen of the tagged fish were captured on the Niagara Bar and 12 were captured near Art Park. Only 12 fish were detected again, using a combination of manual tracking (November 14, 2011- January 3, 2012) and fixed receiver stations; four of the fish that were re-located were tagged on the Niagara Bar and nine were tagged near Art Park/Omega. Of the fish that were tagged on the Niagara Bar, one fish was detected again on the bar, two fish moved into the lower river, and one

fish was detected in the mid-river section. Of the fish that were tagged in the Art Park area, all were detected in the mid-river area, with the exception of one fish that moved to the bar. Average daily movement was 0.20 rkm (SD = 0.31); however, this is based on manual tracking data only, which only includes three fish. Largest total movement by a fish was 13.1 rkm, over a period of 16 days. No movement information could be gathered from fixed receiver detections, as all fish that were detected on a fixed receiver were only detected at one station.

Transmitter detection- Depth, distance from receiver, and receiver site (Coast Guard/Fox or Irish) were all found to be important in determining the ability to detect radio transmitters with fixed receivers. Overall, detection decreased as distance from receiver increased (Figure 5-3). At the Coast Guard and Fox site, detection probably was 100% at 5 m deep directly in front of the antenna, but dropped to 20% at 5 m, 400 m from the receiver. At the Irish site, detection probability was 80% at 6 m depth when directly in front of the antenna, but detection probability for the same depth dropped to 30% at 600 m from the antenna. These detection probabilities were not sufficient to monitor the deepest available habitat in the river.

Table 5-1: Summary of Manual Tracking. Fish are listed by tagging year and tagging location is noted. Number of detections per fish, average movement per day, largest overall movement and detection date range is listed. All distances are in rkm.

Year	Fish ID	Tagging location	Number of detections	Average movement/day	Largest movement	First detection	Last detection
2010	LTNR-10-001	ART PARK	17	0.04	3.75	11/4/2010	6/21/2011
	LTNR-10-002	ART PARK	14	0.09	0.50	11/8/2010	12/27/2010
	LTNR-10-003	ART PARK	17	0.07	1.29	11/8/2010	12/29/2011
	LTNR-10-004	ART PARK	7	0.64	4.70	11/4/2010	12/27/2011
	LTNR-10-005	ART PARK	9	0.15	14.46	11/8/2010	6/28/2011
	LTNR-10-006	ART PARK	11	0.06	1.20	11/5/2010	6/13/2011
	LTNR-10-008	ART PARK	11	0.14	7.93	11/5/2010	8/4/2011
	LTNR-10-011	ART PARK	7	0.09	13.55	11/5/2010	6/28/2011
	LTNR-10-027	ART PARK	4	0.07	0.36	11/15/2010	11/19/2010
	LTNR-10-014	ART PARK	5	0.12	7.06	11/12/2010	6/28/2011
	LTNR-10-016	ART PARK	7	0.03	0.15	11/17/2010	12/8/2010
	LTNR-10-017	ART PARK	7	0.12	0.51	11/10/2010	11/26/2010
	LTNR-10-020	ART PARK	3	0.45	2.38	11/17/2010	11/19/2010
	LTNR-10-021	ART PARK	1	13.63	13.63	6/24/2011	6/24/2011
2011	LTNR-11B-006	NIAGARA BAR	4	0.01	0.23	11/23/2011	12/29/2011
	LTNR-11B-013	NIAGARA BAR	1	0.42	11.25	11/29/2011	11/29/2011
	LTNR-11B-025	ART PARK	2	0.42	13.12	11/16/2011	12/2/2011

Table 5-2. Summary Table of Fixed Detections. All detections made with fixed receivers for tracking lake trout in the Niagara River. "Fox" is located at 7.52 rkm, and "Omega" is located at 12.12 rkm. Fish ID indicates tagging year (2010= 10; 2011= 11B; LTNR= Lake Trout Niagara River). Original tagging location of the fish, number of detection per fish, detection site using fixed receiver and range of detection dates using fixed receivers are listed.

Fish ID	Tagging location	Number of detections	Detection site	First detection	Last detection
LTNR-10-004	ART PARK	1	FOX	12/27/2011	12/27/2011
LTNR-11B-022	ART PARK	5	OMEGA	11/17/2011	11/21/2011
LTNR-11B-023	ART PARK	7	OMEGA	11/17/2011	11/23/2011
LTNR-11B-033	ART PARK	7	OMEGA	11/16/2011	11/30/2011
LTNR-11B-031	ART PARK	3	OMEGA	11/21/2011	11/24/2011
LTNR-11B-028	ART PARK	12	OMEGA	11/14/2011	11/30/2011
LTNR-11B-003	NIAGARA BAR	1	FOX	1/3/2012	1/3/2012
LTNR-11B-013	NIAGARA BAR	1	FOX	12/27/2011	12/27/2011
LTNR-11B-025	ART PARK	8	OMEGA	11/14/2011	11/26/2011
LTNR-11B-029	ART PARK	10	OMEGA	11/14/2011	11/23/2011
LTNR-11B-042	NIAGARA BAR	5	OMEGA	11/16/2011	11/21/2011
LTNR-11B-024	ART PARK	9	OMEGA	11/14/2011	11/22/2011

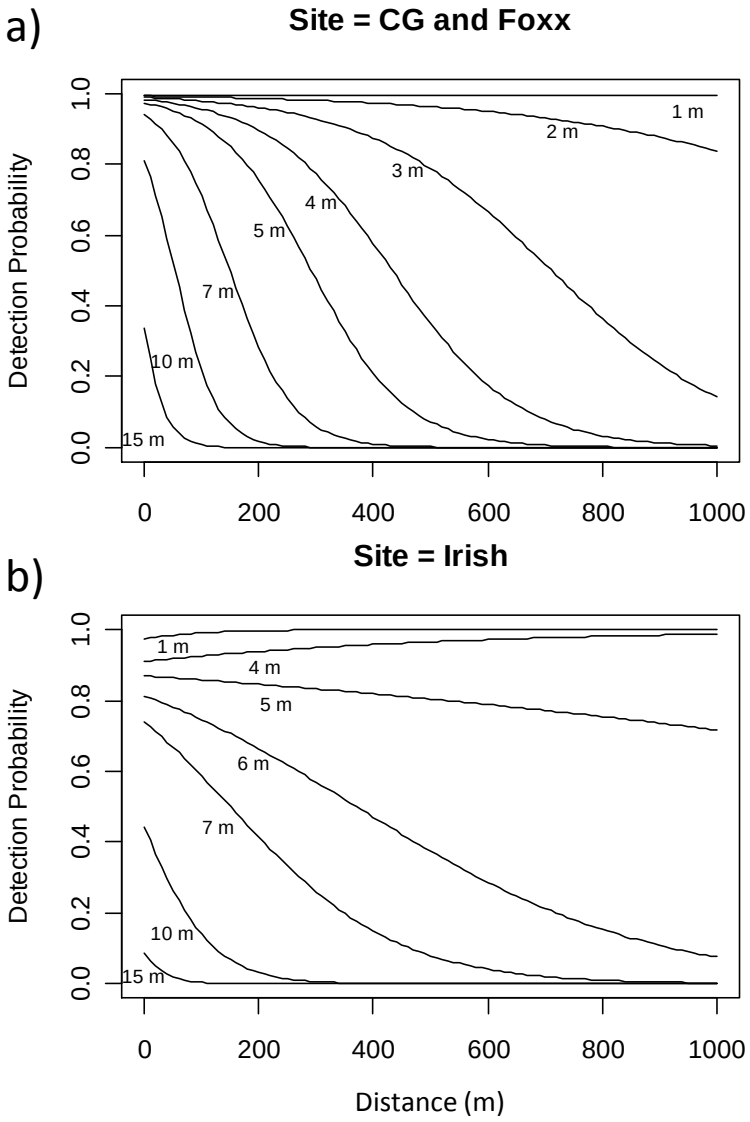

Figure 5-3. Detection Probability. Estimated detection probability contour lines from multiple logistic regression analysis relating detection probability to distance from antenna results at a) Coast Guard (CG) and Mike Fox Property (Fox) sites, and b) the Mary Irish property (Irish) site. Contour lines are for tags set at depths between 1 m and 15 m and plotted against distance from receiver. Maximum river depth at Coast Guard and Fox was between 10-20 m, and 45 m for Irish site.

Discussion

A population of lake trout is using the Niagara River, NY in the fall, presumably for spawning. While spawning was not directly observed, fish captured were sexually mature, based on the expression of eggs and milt. This, combined with the collection of naturally reproduced offspring (Chapter 4), lends support that lake trout utilize the river for spawning purposes. River spawning of lake trout has previously been observed in Lake Superior; however, the population has since been extirpated (Loftus 1958). Many of the relocations of radio-tagged fish during this study were likely during the spawning period, based on time of year, size of fish, and assessment of reproductive maturity (Esteve et al. 2007). The Art Park/Omega area has been suspected to be a lake trout spawning location, based on capture of ripe, mature fish (e.g., by charter captains), and egg collection by Trometer (2006). Lake trout were detected in the Art Park/Omega area for approximately two weeks, which agrees with Schoby et al. (2009) who suggested spawning may take place over a period of 5 to 20 days. After spawning is complete, lake trout may be leaving the river utilizing deeper habitats than this study was able to monitor.

Maximum movement observed in this study was small compared to other lake trout movement studies; however the study area (17 km of river; no tracking in Lake Ontario) was limited. Muhlfeld et al. (2011) recorded a lake trout moving 230 km throughout a river/lake system in Flathead Lake, Montana, during a 136 day tracking period, which ranged from August through December (encompassing the spawning period, as with this study). MacLean et al. (1981) found lake trout movement in Lake Opeongo to be between 7.2-40.8 km during the spawning season. In Lake Michigan, Schmalz et al. (2002) found 90% of lake trout tagged remained within 68.4 km of the tagging location regardless of tagging season, recapture season or population density. Tracking locations would need to be expanded into Lake Ontario for this study to attempt to detect movements of that range.

Limitations- Lake trout in this study were not randomly sampled throughout the river system. For example, fish were tagged in areas suspected of holding lake trout (i.e., the Art Park area and Niagara Bar). Thus, the fish that were tagged may not have been a representative sample, which limits the ability to make broad generalizations about lake trout movement within the Niagara River. In addition, the low detection rates at fixed receivers may have limited this study's ability to assess lake trout movement. The relatively large number detections in Art Park/Omega area may be due to some ability to better detect fish in that location, however this could not be quantified in this study, as detection probability was not assessed in this area. Better detection ability, if present, may be due to the fact that lake trout generally move into shallower waters for spawning. Blanchfield et al. (2005) reported lake trout in 2.0 ± 0.3 m of water during the fall spawning season in the Experimental Lakes Area of northwestern Ontario, Canada. However, the large number of detections in the Art Park/Omega site may also be related to the fact that fish were tagged and released in this area. Currently, no inferences can be made from these data as to when lake trout move into or out of the river, or if there is possibly a resident lake trout population utilizing the river. This is based on the fact that sample sizes are small (only three fish detected with manual tracking in 2011) and their movement may not be representative of the entire population.

Future work- Based on what was learned during this study, several factors should be considered when designing future telemetry studies aiming to quantify lake trout movement and habitat use in the Niagara River. The radio tags and the tracking schedule used were not adequate for quantifying movement or river use. Future studies should take river depth, flows and turbidity into consideration. Transmitters with the capability for being detected in a deep-water environment would be preferred, such as radio tags with underwater antennae or acoustic tags (Flavelle et al. 2002; Morbey et al. 2006; Blanchfield et al. 2009). Lake trout are suspected to

spawn at night (Gunn 1995; Schoby et al. 2009; although see Esteve et al. 2007), so a tracking schedule that includes night tracking could be beneficial, especially if continuous monitoring is not possible through the use of fixed receiver sites. Additionally, in an attempt to understand when lake trout move into the system, lake trout should be tagged earlier in the season (September), although there are associated risks, including: (1) fish tagged this early may be close to the river for other reasons than spawning and may leave the area. Monitoring of some parts of Lake Ontario close to the Niagara River may help clarify this issue, however tag detection related to lake depth must be considered; (2) attempting to target reproductively mature fish may be more difficult if fish are not expressing milt or eggs (sexing fish will be difficult too far in advance of the spawning season), although setting a minimum tagging size may help. While no quantitative model was produced to assess lake trout movement and river use in the Niagara River, there was some support for the Art Park area (rkm 9.5 through 13.8) being important to lake trout using the river. This site is the potential location for future studies further aiming to understand natural reproduction as it relates to stocked hatchery lake trout.

Appendix A

Supplemental Information for Chapter 3

Table A-1. Microsatellites used for population comparison. All microsatellites used in this study are listed below, including locus name, florescence, allele range, primer sequence, running temperature, and developmental source.

Locus name	Allele range	Primer sequence	Temperature (C)	Reference
SnaMSU01	209-321	F-TCACACACCCATTCGTTCAT R-AGCATGGGATAACCACAACC	57	Rollins et al. 2009
SnaMSU03	186-288	F-TGGGCAAATTATTGAAGACAAA R-CAGTATACGTCTCTGCCTGTCTG	57	Rollins et al. 2009
SnaMSU08	117-193	F-AGAGCAGTCGATTGCAGTAGC R-ACTGCCCTCACTGATGGTG	66	Rollins et al. 2009
SnaMSU10	140-256	F-GCACCTCACCACTCACCTTT R-TTATACAGCAGGGCTGAGCA	63	Rollins et al. 2009
SnaMSU12	156-224	F-ATTTTCCACATGCTGCGTCT R-TGAAATAGCTTGGAGCAGTAGC	63	Rollins et al. 2009
SnaMSU13	188-276	F-AGTTTCCAAGGCAGCACTGT R-TGCTACACAGCAAAATGTGTCA	63	Rollins et al. 2009
Oneμ10	170-178	F-ATGGGGAACAGAAGAGGAAT R-CTGTAGGTGTGAAATGTATTTAAA	46	Scribner et al. 1996
Oneμ9	223-231	F-CTCTCTTTGGCTCGGGGAATGTT R-GCATGTTCTGACAGCCTACAGCT	54	Scribner et al. 1996
Ogo1a	142-152	F-GATCTGGGCCTAAGGGAAAC R-ACTAGCGGTTGGAGAACCC	52	Olsen et al. 1998
Sfo1	98-110	F-ACCATAACCCCCCACCAC R-GTCCCTCCGTGGCAGATT	60	Angers et al. 1995
Ssa85	123-137	F-AGGTGGGTCCTCCAAGCTAC R-ACCCGCTCCTCACTTATTC	56	O'Reilly et al. 1996

Table A-2: Test for Hardy-Weinberg Equilibrium. HW QuickCheck (Kalinowski 2006) was used to evaluate deviations from Hardy-Weinberg equilibrium, using a global test and a one-tailed test

	GLOBAL TEST	OBS	EXP	SIGN	PVALUE
Klondike-hatchery	Homozygotes	0	5.2	<	
	Heterozygotes	100	94.8	>	0.001
Klondike-wild	Homozygotes	0	13.5	<	
	Heterozygotes	191	178	>	0.000
Champlain-2011	Homozygotes	0	1.1	<	
	Heterozygotes	45	43.9	>	0.330
Apostle-2001	Homozygotes	0	1.8	<	
	Heterozygotes	49	47.2	>	0.150
Apostle-2003	Homozygotes	0	1.6	<	
	Heterozygotes	50	48.4	>	0.180
Seneca-2002	Homozygotes	0	1.5	<	
	Heterozygotes	50	48.5	>	0.210
Seneca-2003	Homozygotes	0	1.3	<	
	Heterozygotes	50	48.7	>	0.260
Seneca-2006	Homozygotes	0	1.4	<	
	Heterozygotes	49	47.6	>	0.240
Huron-2005	Homozygotes	0	1.6	<	
	Heterozygotes	50	48.4	>	0.170
Huron-2007	Homozygotes	0	1.5	<	
	Heterozygotes	50	48.5	>	0.210
Lewis-2009	Homozygotes	0	1.1	<	
	Heterozygotes	49	47.9	>	0.310
Lewis-2010	Homozygotes	0	2.7	<	
	Heterozygotes	48	45.3	>	0.035

Table A-3: F_{ST}Values for Genetic Comparison of Year Classes. All year classes were assessed to determine if separate hatchery year classes from each strain could be combined into one population for analysis. *P*-values were obtained after 1320 permutations. Adjusted *P*-value for multiple comparisons (using a Bonferroni correction [Rice 1989]) is 0.00041

	Klondike 2011	Champlain 2011	Apostle 2001	Apostle 2003	Seneca 2002	Seneca 2003	Seneca 2006	Huron 2005	Huron 2007	Lewis 2009	Lewis 2010
Klondike 2010	0.00076	0.00076	0.00076	0.00076	0.00076	0.00076	0.00076	0.00076	0.00076	0.00076	0.00076
Klondike 2011		0.00076	0.00076	0.00076	0.00076	0.00076	0.00076	0.00076	0.00076	0.00076	0.00076
Champlain2011			0.00076	0.00076	0.00076	0.00076	0.00076	0.00076	0.00076	0.00076	0.00076
Apostle 2001				0.00076	0.00076	0.00076	0.00076	0.00076	0.00076	0.00076	0.00076
Apostle 2003					0.00076	0.00076	0.00076	0.00076	0.00076	0.00076	0.00076
Seneca 2002						0.00076	0.06667	0.00076	0.00076	0.00076	0.00076
Seneca 2003							0.15076	0.00076	0.00076	0.00076	0.00076
Seneca2006								0.00076	0.00076	0.00076	0.00076
Huron 2005									0.6197	0.00076	0.00076
Huron 2007										0.00076	0.00076
Lewis 2009											0.00076

Appendix B

Supplemental Information for Chapter 4

Table B-1: Adult Lake Trout Sampling Dates. Adult lake trout were targeted on the Niagara River, NY during the field seasons on the dates listed.

	Start sampling	End sampling
Fall 2010	11/3/2010	12/1/2012
Fall 2011	11/2/2011	11/17/2011
Fall 2012	10/24/2012	11/15/2012

Egg tray construction- Egg trays were constructed of 0.5" PVC pipe, 13" long by 12" wide. Metal screen was placed on the bottom, and wrapped up around the sides, being secured by screws and silicone gel. Nine 2" PVC pipes two inches tall were evenly spaced (3X3 grid) on three different constructed trays, to separate eggs from different collection sites. Small holes were drilled in the bottom of each 2" PVC pipe to provide water flow over the eggs, approximately 15 holes per 2" piece of pipe. The 2" PVC pipe 'cups' were secured to the lake trout screen using silicone gel. Small holes were also drilled into the bottom of the egg tray frame, so the structure would sink in the water. Egg trays were held in place by PVC hooks over the edge of the living streams system, keeping the eggs in approximately 1 inch of water. After using this set up for the first season of egg incubation, it was found to be very susceptible to drop in water levels. To prevent any potential desiccation, the structure was modified in 2011. Modification included removing the PVC hooks and moving cups to the other side of the screen; essentially, inverting the structure. This allowed the egg trays to sit on the bottom of the tank,

with small pieces of screen secured by rubber band or ties preventing eggs or hatched fry from moving out of their cups (see Figure B-1). Trays were marked with numerals on one side, and alphabetic characters on the other to prevent from confusing batches of eggs (1, 2, 3 x A, B, C). The trays were distinguished from one other by placement location within the tank, and being labeled 1, 2, and 3.

Figure B-1: Egg Trays in Living Streams. In 2010, egg trays were partially submerged in the tank, as shown above. The cups were not fully submerged to prevent fry from escaping their cup.

This design was susceptible to desiccation, however, so in 2011, egg trays were placed on the bottom of the tank. Eggs and fry were prevented from moving from their cup by screen placed over the opening and secured with rubber bands.

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