Population Genetics of White Perch (*Morone americana*), an Invasive Species: Using Microsatellite Loci to Identify Source of Invasion in Lake Champlain

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An Honors Thesis by

Jordon Tourville

Advisor:

C. William Kilpatrick

Department of Biology

University of Vermont

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Abstract:

White perch (*Morone americana*) are a common species throughout most of Lake Champlain, however, this species was not known within Lake Champlain prior to 1984. Invasion into Lake Champlain could have been through the Champlain Canal from the Hudson River, via the Richelieu River from the St. Lawrence, or by accidental release from another source. DNA was isolated from tissue samples of white perch collected from the Hudson River, three locations within Lake Champlain, and the St. Lawrence River, and three microsatellite loci were examined. In order for the hypothesis to be examined some genetic differentiation had to exist between the potential source populations of white perch populations. The number of private alleles and significant F_{ST} estimates indicated substantial population differentiation between the two source populations. No private alleles or significant F_{ST} estimates were observed between the Lake Champlain populations and the Hudson River population, whereas five private alleles and significant F_{ST} estimates were observed with the Saint Lawrence River population. Population assignments revealed that most of the Lake Champlain sampled individuals could be confidently assigned to the Hudson River population, however, five individuals could not be assigned to either source population sampled with any degree of confidence. The substantial population differentiation between the St. Lawrence River and Lake Champlain and the absence of differentiation between the Hudson River and Lake Champlain demonstrate that the most likely route of the white perch invasion into Lake Champlain was via the Hudson River through the Champlain canal.

Introduction:

White perch (*Morone americana*) are predominantly an estuarine species; their range extending from coastal rivers in South Carolina to the Miramichi River estuary in New Brunswick, Canada (Scott and Crossman, 1959). They also have the ability to travel great distances each generation and will easily invade freshwater lakes and rivers. White perch may become easily landlocked but survive equally well in isolated freshwater biomes (Carlander, 1997). White perch prefer turbid waters with higher conductivity, and thrive regardless of the presence or absence of competitors that have overlapping habitats (Hawes and Parrish, 2003). Their diet consists of benthic invertebrates, zooplankton assemblages dominated by *Daphnia*, some native and invasive freshwater mussels, and small baitfish such as rainbow smelt (*Osmerus mordax*) (Couture and Watzin, 2008). At certain times of the year however, their diet can consist primarily of fish eggs, mainly of percid fishes such as walleye (*Sander vitreus*) (Schaeffer and Margraf, 1987; Roseman et al., 2006). White perch are prolific reproducers with females spawning 140,000 eggs on average per season (Carlander, 1997). Due to their rapid and almost exponential population growth, predation on native baitfish and eggs, and competition with native species, white perch are classified as an aquatic nuisance species in many states (Kuklinski, 2007).

The first known invasion of white perch outside of their historic range was into Lake Oneida via the Mohawk River and Erie Barge Canal in 1946 (Scott and Crossman, 1959; Scott and Christie, 1963). By 1948, white perch had moved through the Oswego River into Cross Lake and Lake Ontario (Scott and Christie, 1963). From there, white perch had easy access into Lake Erie via the Welland Canal by 1961, and into the remainder of the Great Lakes (Boileau, 1985). Boileau (1985) reports the presence of white perch in the St. Lawrence River system east of

Montreal by 1951, and the USGS collected specimens in the Richelieu River during the 1980's and 1990's, just north of Lake Champlain (USGS, 2012). With known white perch populations known to traverse canals, and with populations located both north and south of Lake Champlain, either access has the potential for migration of this invasive species (Figure 1). Access to Lake Champlain via the Champlain Canal has been possible for white perch since its opening in 1823. The explanation that white perch have potentially only very recently exploited this waterway is thought to be due to the contemporary reduction of high levels of pollution in the canal after the passing of Federal Water Pollution Control Act Amendments of 1972, and adoption of the Clean Water Act in 1977 (Daniels, 2001). Ironically, as a consequence of lowering water pollution in New York, the door may have opened for invasive species to pass into previously unoccupied areas.

Despite its common name, white perch are not actually perch (Family Percidae) but are a temperate bass of the family Moronidae and thus are not closely related to yellow perch (*Perca flavescens)*, a native species of Lake Champlain (Becker, 1983). However, the two fish species do share many of the same resources and habitats. They are both diverse opportunistic feeders that will subsist on minnows, insects, and other invertebrates (Schaeffer and Margraf, 1986). White perch are better competitors due to their average larger body size, and overall more aggressive behavior (Harrell and Webster, 1997). An increase in white perch could lead to the decline of native species including yellow perch and white bass (*Morone chrysops*), because white perch reproduce in an exponential fashion when invading new waterways (Hawes and Parrish, 2003; Kuklinski, 2007). As white perch numbers increase so does the degree of exploitative competition with native species, according to Schaeffer and Margraf (1986).

White perch are known to prey heavily on walleye eggs as a main source of their diet during the spring months and are also strong competitors of yellow perch within Lake Erie (Schaeffer and Margraf, 1987). To date, these trends do not appear to have largely affected the walleye and yellow perch populations within Lake Champlain, but it is hypothesized that white perch in high numbers would have a crippling impact on these species (Hawes and Parrish, 2003; Couture and Watzin, 2008). State fisheries have recorded expenditures on walleye and yellow perch angling by resident and non-resident fishermen; in 1997 for example $6,741,697 and $5,427,056 were expended on these two species respectively (Gilbert, 2000). Additionally, expansion of white perch has led to issues of increased cyanobacteria blooms in some lakes due to intense *Daphnia* grazing by this introduced species (Couture and Watzin, 2008).

In order to address the spread of white perch, it would be helpful to first determine the route of invasion into Lake Champlain. Knowing the source of invasion may allow researchers to understand how the species will spread further into the lake and by what mechanism this might be occurring. Two mechanisms have been proposed to explain how white perch may have spread in Lake Champlain; a northern invasion gradient based on the geographic point of introduction of the species assuming an introduction from the Champlain Canal, or an environmental gradient based on abiotic factors such as water temperature, turbidity, and conductivity within the lake (Hawes and Parish, 2003).

In order to examine how white perch were introduced into Lake Champlain, I have taken a genetic approach utilizing neutral microsatellite markers. White perch have been reported in both the Champlain Canal (Hudson River) and the Richelieu River (St. Lawrence River) (Plosila and Nashett, 1990; USGS, 2009) and both of these are potential sources for the white perch within Lake Champlain. A third potential source of white perch could be from accidental

anthropogenic introduction. Marsden and Hauser (2009) suggested that white perch entered Lake Champlain from the Hudson River through the Champlain Canal, and Hawes and Parrish (2003) indicated that the Champlain Canal was more likely the source of invasion because the first white perch specimens found in Lake Champlain were taken very close to the exit of the canal. If this hypothesis is correct then little genetic differentiation is expected among the Lake Champlain populations and the Champlain Canal and Hudson River populations, and greater differentiation among the Lake Champlain populations and the St. Lawrence and Richelieu River populations would be expected. If an accidental human-mediated introduction occurred, then a reduction in genetic variability corresponding to a small founder event would be expected. The objective of this study is to quantify the degree of genetic differentiation among these white perch populations in order to identify the most likely geographic source of the white perch populations within Lake Champlain.

Methods and Materials:

a. *Sample Collection*

White perch samples were collected from three sites in the Lake Champlain basin, one site in the Hudson River, and two sites in the Richelieu/St. Lawrence River system. Most individuals sampled were reported to be young-of-the-year (aged 0-1) with only a few individuals being over a year old. Geographical coordinates and sampling information for each sample site are given below (Figure 1; Table 1).

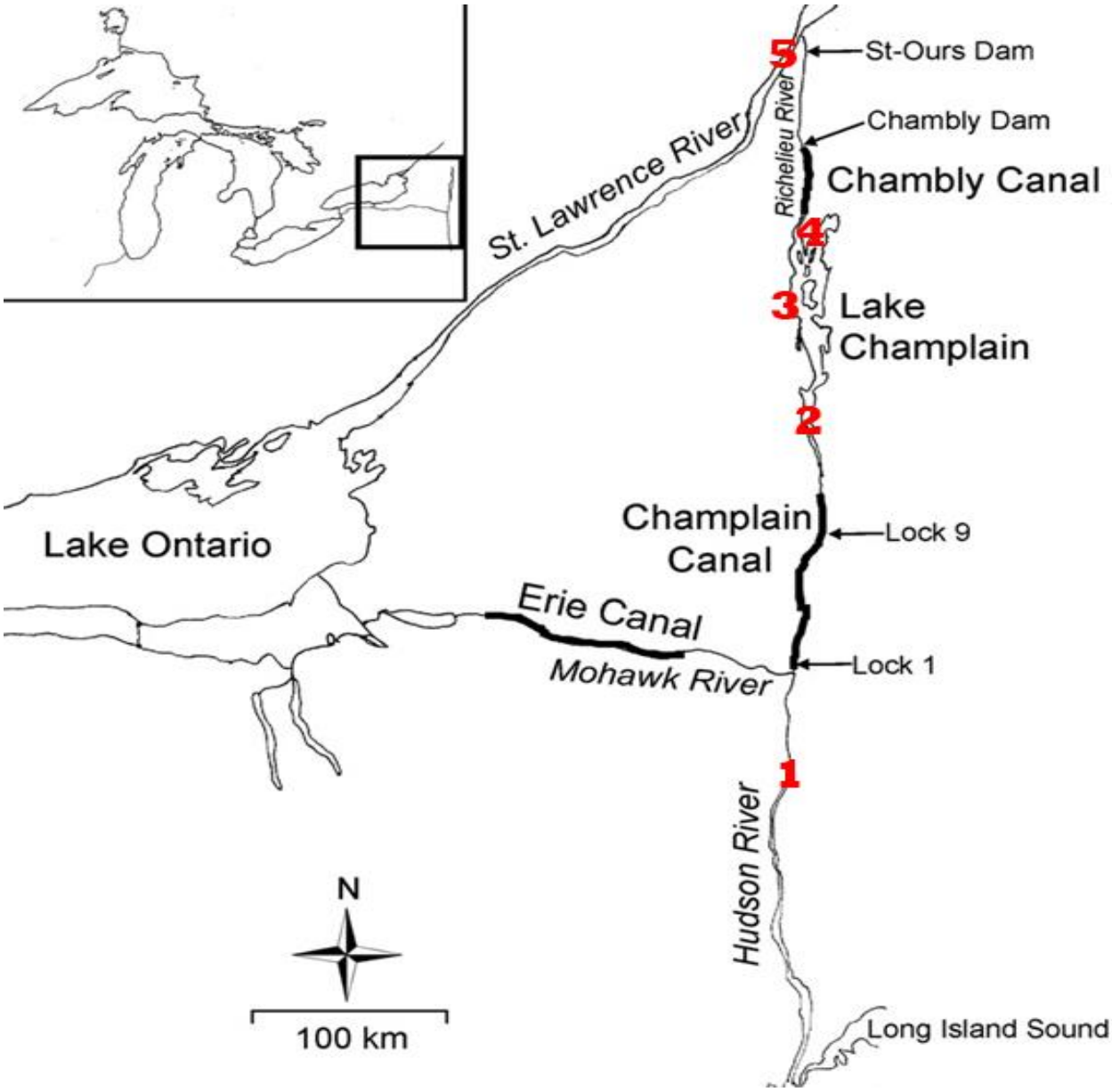

Figure 1: Detailed map indicating the white perch sample locations and relative positions of these sample areas compared to the canal systems entering into Lake Champlain. Note that Lake Champlain is flanked on its northern and southern ends by canals. These highlighted canals represent the two possible pathways white perch could have exploited to gain access into Lake Champlain (Marsden and Hauser, 2009).

Table 1: Information on each sample site including sample coordinates, the number of samples collected, and the method of sample collection.

Site	Physical Location	Latitude	Longitude	Number of Samples (N)	Sampling Method
1 (HR)	Hudson River, Coxsackie, NY	42N 21' 16.257''	73W 47' 42.9072''	36	Seining
2 (BB)	Lake Champlain, Bulwagga Bay	44N 1' 47.913''	73W 26' 22.1418''	21	Angling
3 (MSR)	Lake Champlain, Mouth of the Saranac River	44N 41' 59.195''	73W 26' 47.3238''	20	Seining
4 (MB)	Lake Champlain, Missisquoi Bay	44N 59' 34.6884''	73W 10' 7.5858''	20	Bottom Trawling
5 (SL)	St. Lawrence River, Mouth or Richelieu River	46N 23' 22.4514''	72W 26' 47.7384''	23	Seining

Individuals from site 1 (HR) were obtained by seine-haul fishing from a boat by members of the Hudson River Estuary Program and shipped frozen to the lab. Individuals from site 2 (BB) were obtained using traditional angling methods and transported frozen to the lab. Individuals from site 3 (MSR) were collected using seine-haul fishing by graduate students from SUNY Plattsburg from a boat and sent frozen from SUNY Plattsburg. Individuals collected from site 4 (MB) were caught using bottom trawling methods by boat by biologists from the Vermont Department of Fish and Wildlife. Muscle tissue was extracted and stored in 95% ETOH, and shipped to the lab. Individuals from site 5 (SL) were obtained by 24 seining nets by field research assistants

supported by MRNF (Ministère des Ressources naturelles et de la Faune) based in Quebec. Muscle tissue was extracted and stored in a lysis buffer solution (Qiagen), and shipped to the lab.

Once in the lab, a thin piece of muscle tissue approximately 1cm long was taken from each individual posterior to the dorsal fin on the dorsal and lateral side of the fish. Each sample was placed in a 1.5 mL centrifuge tube filled with 0.3 mL of 95% ethanol for tissue preservation, labeled accordingly, and stored at room temperature.

b. *DNA Isolation and Purification*

Samples were processed 4-6 at a time. Approximately 1/3 of the tissue sample was cut into small fragments, placed in a mortar, and liquid nitrogen was added in order to freeze the muscle tissue before grinding into a fine powder. The sample was placed into a 1.5 mL centrifuge tube and 0.3 mL of a Qiagen cell lysis solution and 1.5 µL of Proteinase K (Qiagen) were added in order to rupture the cells and digest proteins (including DNAses). After incubation in a water bath for 24 h at 55°C, each sample was placed on ice for 1 min and then treated with a Qiagen protein precipitation solution and centrifuged in order to remove the proteins from the sample. The DNA was precipitated by adding the DNA supernatant to 0.3 µL of 100% isopropanol in a new centrifuge tube, centrifuging for 1 min, and then decanting the isopropanol. The DNA was purified by adding 0.3 µL of 70% ethanol to the tube, mixing gently, and centrifuging for 1 min. The DNA was air dried and stored in a refrigerator in 50 µL of sterile distilled water. The quality and quantity of the DNA was examined using agarose gel electrophoresis and via spectrophotometry on a NanoDrop Spectrometer® (NanoDrop).

c. *Microsatellite Amplification and Analysis*

Samples with low quantity DNA under a concentration of 100 ng/µL were excluded from further analysis. Microsatellite loci were amplified using PCR (polymerase chain reaction) on a Perkin Elmer 9600 GeneAmp Thermal Cycler®. Genetic markers were amplified using primers (Table 2) characterized in previous studies of striped bass (Han et al., 2000; Liu and Ely, 2009).

Table 2: Reported characteristics of the 7 microsatellite loci initially tested along with primer information. All SB loci are from Han et al., (2000) and Dla 11 is from Liu and Ely (2009). All loci originate from striped bass (*Morone saxatilis*) but amplify for white perch.

Locus	Primer 1	Primer 2	Annealing Temperature (°C)	Size Range (bp)	Number of Alleles
SB6	ACAGCAAAGATAAACATCTG	TTCATGATGTTTCACCAGG	46	183-247	9
SB8	TGAGGAAGGTTTGAGAGAC	TTCTGCTCCTTAGATGAAC	46	174-218	3
SB11	CACCTCTAATGCTTCCATGC	CGAATGCGCTACAAATCTGC	53	115-195	11
SB13	TGCTGAGCCGGTAATTCAAG	CACACATATGCATGGATGCA	51	129-141	3
SB83	TGGGCCTGATTGGAATCAAAA	GATAGGTTGTATCAATGTTGC	50	163-209	12
SB231	GCAGCTTCATTAAACCAC	ACCTTCACTTATTGGCAG	55	135-147	4
Dla11	CCCAAGCTTGGGCAAGCACACACCT-CTAATGCT	CCGGAATTCCGGCGAATGCGCTACAAATCTGC	53	84-315	4

The presence of the amplified DNA was examined by agarose gel electrophoresis. The loci SB 6, 8, 11, 13, 83, 231 (Han et al., 2000) and Dla11 (Liu and Ely, 2009) were all examined

as potential markers for this study. Each locus was selected for their potential higher level of variation based on the large number of alleles reported in previous studies (Han et al., 2000; Liu and Ely, 2009). All SB loci were simple GT dinculeotide repeats, and locus Dla 11 was more complex. Loci SB 11 and Dla 11 failed to amplify after multiple PCR attempts. The remaining 5 loci successfully amplified white perch DNA. To prepare the DNA for mass screening, labeled primer tests were conducted in order to identify which primers would work together in a multiplex reaction. Fluorescent tags were added to each reverse primer (Table 3). Loci SB 6, 8, and 231 initially worked well together under the same PCR conditions whereas SB 13 also worked well with SB 231, and SB 83 did not work well with any other primer. PCR amplification was performed in 12.5 µL volume reactions containing 6 µL Multiplex PCR Master Mix 2x (Qiagen), 1.25 µL of each forward and reverse primer diluted from a 10 mM stock solution, and 1.0 µL of DNA diluted to 200 ng/µL, and a compensatory amount of sterile water. The reactions were multiplexed in 3 groups: 1) forward and reverse primers of SB 6, 8, and 231; 2) forward and reverse primers of SB 13 and 231 for all samples, and 3) forward and reverse primers of SB83 alone for all samples. All multiplex groups amplified under the same PCR conditions including: initial denture of the DNA at 95°C for 30 sec; 35 cycles of denature at 95°C for 30 sec; annealing at 50°C for 20 sec; extension at 72°C for 15 sec; followed by final extension step at 72°C for 5 min. Nine SL samples were of very low quality and were excluded from the reactions, leaving a total of N= 111. Once completed, 1 µL of a 1:10 or 1:20 dilution of the amplified PCR product was added to an individual well of a 96-well plate with 15.2 µL of a 1:75 mixture of the GeneScan Liz 600 size standard (Invitrogen) and formamide. Amplified DNA underwent automated DNA fragment size determination on an ABI Prism 3130xl Genetic Analyzer® (16 capillary), a fluorescence-based detection system, at the DNA Analysis Facility

at the Vermont Cancer Center. Results of the DNA fragment size determination were viewed using the program GeneMapper v5.1. Allele calls were assigned to each peak for each sample viewed and exported to an ExCEL spreadsheet for further analysis.

Table 3: Observed size range for each locus used in the study along with the fluorescent label associated with each reverse primer.

Locus	Fluorescent Label (Reverse)	Observed Size Range (bp)
SB6	6-FAM (Blue)	185-205
SB8	VIC (Green)	175-187
SB13	VIC (Green)	118
SB83	PET (Red)	159-205
SB231	6-FAM (Blue)	149-183

d. *Data Analysis*

The program GenAlEx6 (Peakall and Smouse, 2006), an ExCEL based genetic software analysis package, was used to calculate observed (Ho) and expected (He) heterozygosities within the populations sampled, as well as the number of observed alleles and allele frequencies by locus and by population. Deviations from HWE were determined using the program GENEPOP v4.2 (Raymond and Rousset, 1995) utilizing the Markov chain method (Guo and Thompson, 1992). The test produced a P-value associated with the probability of Ho being supported and no deviation from HWE occurring within that population. Pairwise F_{ST} values were determined by the program Arlequin v3.11 (Excoffier et al., 2005) and used as an estimator of the mean genetic distance between the source populations and the Lake Champlain populations. The number of migrants per generation between each population was estimated in order to access the level of potential gene flow between populations. Population assignment was performed using the

program GeneClass2 (Piry et al., 2004). The program utilized a Bayesian statistical approach (Rannala and Mountain, 1997) that returned a log likelihood value that each individual was from either the Hudson River or the St. Lawrence River reference populations with a certain level of confidence. Samples MSR 10 and MSR 17 were excluded because the size of the microsatellites were determined with a different size standard that the bulk of the samples.

Results:

The number of observed and effective number of alleles present at locus SB 231 for the HR population with 8 alleles was much larger than the number of alleles found in any Lake Champlain population (Table 4). Additionally, the observed size range of alleles (149-183) for SB 231 was above the size range of 135-147 reported for striped bass (Han et al., 2000). Observed heterozygosities for each locus and over all loci were greater in the MSR, BB, MB, and SL populations than observed for the HR population (Table 4). The allelic distribution was dominated by a few common alleles shared by every population (LC: n=61; HR: n=36; SL: n=14). Comparing the number of private alleles in each of the potential source populations (HR and SL), I found considerable genetic differentiation between the two populations with a total of 13 private alleles, 8 in the Hudson River population and 5 in the Saint Lawrence population (Figure 2).

Table 4: Summary statistics of the alleles identified at each locus. Reported values include the number of observed (Na) and effective (Ne) alleles for each locus at each population, the observed (Ho) versus expected (He) heterozygosities for each locus, the corrected unbiased expected heterozygosity for each locus, and the mean values across loci.

	Locus	Na	Ne	Ho	He	uHe
MSR	SB6	3.000	2.632	0.800	0.620	0.636
	SB8	3.000	1.578	0.150	0.366	0.376
	SB231	5.000	2.432	0.600	0.589	0.604
	Mean	3.667	2.214	0.517	0.525	0.538
BB	SB6	3.000	2.066	0.667	0.516	0.528
	SB8	2.000	1.446	0.286	0.308	0.316
	SB231	4.000	1.492	0.381	0.330	0.338
	Mean	3.000	1.668	0.444	0.385	0.394
HR	SB6	4.000	2.360	0.571	0.576	0.585
	SB8	2.000	1.456	0.222	0.313	0.318
	SB231	8.000	1.385	0.222	0.278	0.282
	Mean	5.000	1.734	0.339	0.389	0.395
MB	SB6	3.000	2.827	0.850	0.646	0.663
	SB8	2.000	1.406	0.050	0.289	0.296
	SB231	4.000	1.606	0.350	0.378	0.387
	Mean	3.000	1.946	0.417	0.438	0.449
SL	SB6	5.000	3.347	0.615	0.701	0.729
	SB8	3.000	2.253	0.462	0.556	0.578
	SB231	3.000	1.815	0.571	0.449	0.466
	Mean	3.667	2.472	0.549	0.569	0.591

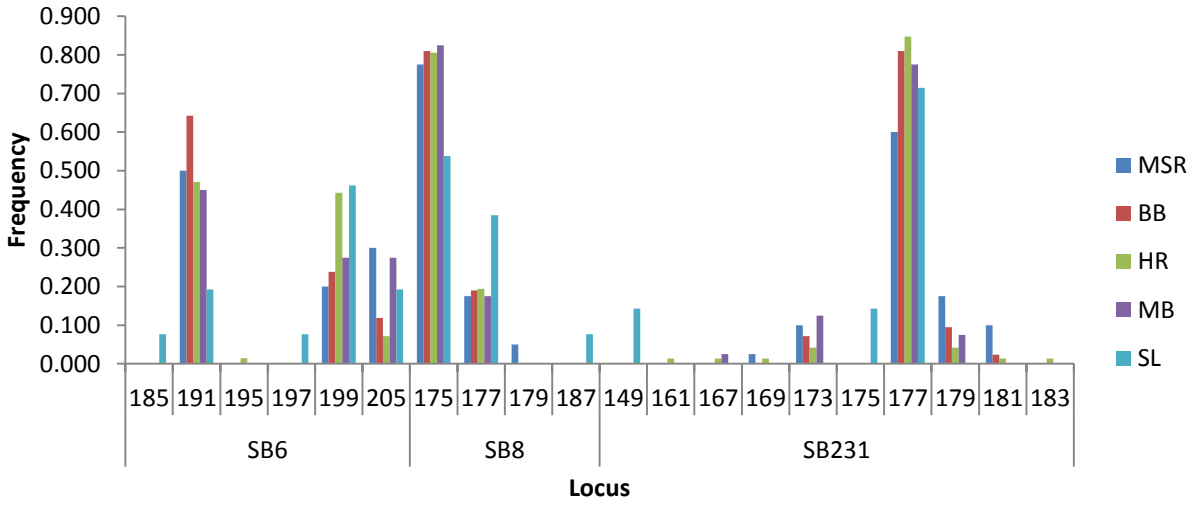

Figure 2: Frequency of alleles at three loci among the five populations sampled.

The MB, HR and SL populations each demonstrate a significant deviation from HWE at a single but different locus (Table 5). Both the BB and MSR populations were in HWE for all loci.

Table 5: P-values associated with a H_o of HWE. **=p<0.01, *=p<0.05.

Locus	MSR	BB	HR	MB	SL
SB6	0.0675	0.5879	0.9661	0.2696	0.0092 **
SB8	0.2709	1.0000	0.0956	0.002 **	0.4707
SB231	0.2246	1.0000	0.0454 *	0.2393	0.7171

The results for pairwise F_{ST} values indicate significant genetic differentiation between the St. Lawrence River population and the Hudson River population (F_{ST}=0.079). There is also differentiation between the St. Lawrence River population and all Lake Champlain populations with a mean F_{ST}=0.0927±0.021345 (Table 6). F_{ST} values between the Hudson River source population and all Lake Champlain populations was below 0.0465 (mean F_{ST}=0.025±0.018134) and all values were not significant, indicating little to no genetic distinction between the Hudson River and the Lake Champlain white perch populations. The number of potential migrants (N_m) per generation between MSR, MB, and BB (Lake Champlain) and the HR source ranged from 7.5 to 19.7 individuals (Table 6). Conversely, the potential N_m per generation between Lake Champlain white perch populations and the SL source population ranged from 3.4 to 5.0 individuals.

Table 6: F_{ST} values are below the diagonal with the significance of the value (designated with an *), and the mean number of migrants per generation (N_m) are above the diagonal.

	MSR	BB	HR	MB	SL
MSR	0	12.971	7.467	22.009	4.172
BB	0.01694	0	19.67	24.158	3.442
HR	0.04650	0.01491	0	18.853	5.026
MB	0.00264	0.00293	0.0149	0	4.632
SL	0.08401*	0.1206*	0.079*	0.07339*	0

The proportion of individuals in each Lake Champlain population assigned to the Hudson River was much greater than the proportion assigned to the SL source population (Figure 4). Out

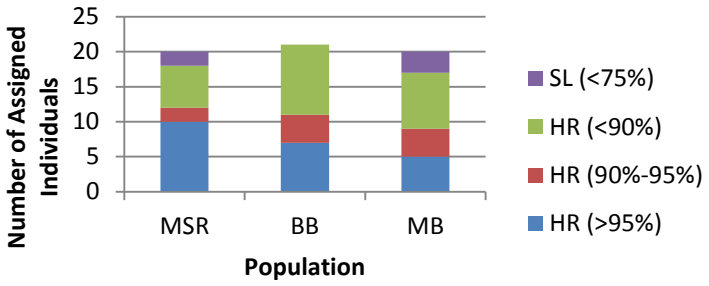

Figure 4: Graph representing the number of individuals in each Lake Champlain population assigned to either the HR or SL reference population and the confidence associated with each assignment.

of 61 individuals sampled from 3 different regions of Lake Champlain, 56 could be assigned to the Hudson River reference population. Approximately 50% (34) of these individuals were assigned to the HR source with a confidence of 90% or greater, and another 22 individuals were assigned with a confidence of greater than 80%. The 5 individuals designated to be more likely

derived from the SL source population were assigned at a confidence below 75% confidence (Table 7).

Table 7: The log likelihood and corresponding confidence level applied to each of the 5 individuals assigned to the SL reference population in the population assignment test.

	SL		HR	
Individual	Log Likelihood	Probability (confidence %)	Log Likelihood	Probability (confidence %)
1 (MSR12)	-2.273	73.57	-2.718	26.43
2 (MSR15)	-2.224	68.04	-2.552	34.96
3 (MB12)	-2.273	73.57	-2.718	26.43
4 (MB17)	-1.091	52.08	-1.937	47.92
5 (MB20)	-2.273	73.57	-2.718	26.43

Discussion:

The exact test for HWE revealed that HR, MB, and SL populations each exhibited at least one locus that was not in HWE whereas all loci were in HWE for the BB and MSR populations. Deviation from HWE indicates a violation of any one of the assumptions upon which this equilibrium is based; small sample size is a likely contributor to this deviation. Guo and Thompson (1992), and Selkoe and Toonen (2006) caution that when sampling populations for genetic study, at least 50 individuals should be used in order to validate any statistical analyses.

The difference in the number of private alleles maintained by the source populations HR and SL indicates substantial differentiation and isolation. The St. Lawrence population displayed 5 private alleles across all loci, contributing 0.08-0.14 proportion of the total allelic frequency. The SL population had fewer private alleles (5) when compared to HR (8), but the SL private alleles were observed at a higher frequency. When compared with the Lake Champlain populations, 5 private alleles were still present in the SL populations but all the private alleles

observed for the HR population were also observed in the LC populations, indicating that those alleles are shared between the Hudson River and Lake Champlain populations, whereas the private alleles in the St. Lawrence population are isolated for the Lake Champlain populations.

In pairwise comparisons of F_{ST} (Table 6), all populations demonstrated significant differentiation from the St. Lawrence population. Conversely, no genetic differentiation was detected among all Lake Champlain populations and the Hudson River population. These findings suggest that there is no population structure or partitioning between white perch from the Hudson River and Lake Champlain. The same cannot be stated for the St. Lawrence population, and there is a clear isolation between the St. Lawrence population and Lake Champlain and Hudson River populations.

Population assignment testing revealed that the vast majority of white perch sampled in Lake Champlain could be confidently assigned to the Hudson River reference population. Two individuals from MSR and 3 from MB were assigned with less than 75% confidence to the St. Lawrence population. While this could indicate a low level of migration from the St. Lawrence River at some time in the past, it could also suggest that a source other than the population currently in the Hudson River may have contributed to the genetic diversity of white perch in Lake Champlain.

In light of the F_{ST} and population assignment results, the dichotomy in the estimated levels of migration (N_m) between the Hudson River population and Lake Champlain could indicate white perch accessibility to the lake from this source. Since population differentiation is present between Lake Champlain and the St. Lawrence River and not between the Hudson River and Lake Champlain given equal geographical distance, a barrier might exist in the St. Lawrence

River, which does not exist in the Hudson River that prevents extensive migration between these systems. The nature of this potential barrier is only speculative, but it could be a physical (i.e. rapids or dams in the Chambly Canal, Quebec), or an environmental (i.e. high levels of pollutants in the Richelieu River) barrier (see Marsden and Hauser, 2009). While the N_m values do not give any useful indication of gene flow by themselves, they do support the findings of the population differentiation and population assignment tests.

The data indicate large genetic overlap between the Hudson River white perch and the Lake Champlain white perch populations while suggesting isolation and genetic distinction between the same Lake Champlain populations and white perch populations found in the St. Lawrence River. Given the results of the population assignment test, introduction of white perch into Lake Champlain most likely occurred from the Hudson River via the Champlain Canal, however, the possibly of an independent introduction cannot be completely ruled out as a source of some white perch.

It is possible a small number of founders of both sexes were introduced to the lake, potentially on multiple occasions either concurrent of after the initial invasion of white perch, from sources other than the two potential source populations examined. Five of the fish sampled from Lake Champlain failed to assign to either of the two potential source populations sampled with a confidence of greater than 75%, suggesting that they were likely derived from some other source. In order to assess this possibility, data from future studies examine more loci in order to make a more confident assignment using population assignment analysis. Additionally, an observation of any private alleles in the Lake Champlain population would signify a small founder event and would point toward an independent introduction. Determining the specific source population may be difficult as population differentiation between many potential source

populations, such as the Hudson River and the Great Lakes, might be very low or non-existent. Data from this study suggests that if an accidental introduction did occur, it may have coincided with, or occurred after white perch invasion from the Hudson River, given the lack of differentiation between the Hudson River population and Lake Champlain.

The ecological and conservation implications of this study are important in a number of respects that deserve discussion and exploration by future studies. The lack of population differentiation indicate that the Hudson River and Lake Champlain white perch populations can be treated as one population limited only to geographic distance easily overcome by their short generation time of about 2 years (Carlander, 1997). Based on this data, it is still not clear if invasion into Lake Champlain in the mid-1980's was an isolated event by a small number of migrants or a continuously repeating process involving large numbers of fish. What prompted white perch to colonize the Champlain Canal and Lake Champlain at that time and not in the prior 150 years the Canal was operational is unknown. Daniels (2001) posits that high levels of pollution prior to the 1980's acted as a barrier to white perch movement into the lake, and that only after the massive environmental movement of the 1970's and the lowering of pollution levels in the New York Canal system did white perch exploit this route. However, this mechanism, and the nature of the barrier to white perch migration in the Richelieu River remains undetermined.

Conclusions:

The findings of this study are unable to refute the hypothesis of Marsden and Hauser (2009), which suggest white perch introduction into Lake Champlain came from the Hudson River. Population differentiation was high between the Hudson River and St. Lawrence source

populations, and the large number of private alleles found in the St. Lawrence population suggests a substantial level of genetic differentiation between the St. Lawrence population and the invasive white perch found in Lake Champlain, while little population differentiation was observed between the Hudson River population and the Lake Champlain populations. Calculated F_{ST} values support this conclusion in that statistically significant F_{ST} values were found in pairwise comparisons between the SL population samples and the Lake Champlain populations, and non-significant values were found between the Hudson River and Lake Champlain populations. Additionally, population assignments placed 56/61 sampled Lake Champlain individuals in the Hudson River reference population supporting the conclusion that invasion of white perch into Lake Champlain was through the Champlain Canal from the Hudson River. The 5 individuals assigned to the St. Lawrence population were assigned with a low level of confidence and could represent individuals derived from a different source. Although the initial invasion may have represented one isolated founder event or a continuous migration, the high number of estimated migrants per generation from the Hudson River, and low level of genetic structuring indicate that white perch populations from the Hudson River and Lake Champlain could be considered a single population.

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