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Genetic Analysis of the Brown, Brook, and Tiger Trout Populations in the Lake Champlain Basin

Markie Palermo[1,2]*, Sandra Latourelle[3], and Nancy L. Elwess[3]
Student[1]: Essex High School, Essex Junction, Vermont
Intern[2], Mentor[3]: State University of New York at Plattsburgh
*Correspondence: mpalermo@ccsuvt.org

Abstract

The purpose of this pilot project was to assess both the brook trout (Salvelinus fontinalis) and the brown trout (Salmo trutta) populations in the Lake Champlain Basin using six microsatellite DNA loci. Additionally, the DNA was tested to determine whether hybridization between brook trout and brown trout was occurring to produce a proposed tiger trout (Salmo trutta X Salvelinus fontinalis). Moreover, the hypothesis was that the further apart the test sites, the greater the genetic diversity within the trout populations. DNA samples were taken from adipose or caudal fin clippings through live capture and release at various locations in the Lake Champlain Basin. Furthermore, in the field, the rate of the current was determined, GPS longitude and latitude coordinates were found, the different trout species caught were measured in length, and water and air temperature were recorded. Preliminary data suggested that there were genetic differences in the trout populations at the various locations.

Introduction

The Lake Champlain Basin covers 8,234 square miles in Vermont and New York (Figure 1)[1]. The New York State Department of Environmental Conservation (NYDEC) placed genetic monitoring of brook trout, Salvelinus fontinalis as a priority, due to their declining numbers[1]. In addition, brook trout also serve as indicators for the quality of coldwater habitats. Several agencies (NYDEC, U.S. Department of Interior-Fish and Wildlife Division, Lake Champlain Research Institute, and Trout Unlimited) support the genetic monitoring of these fish in order to produce quantitative indicators concerning the health of this ecosystem[1]. Data produced from this pilot project can contribute to planning decisions and protection of habitat that will contribute to a healthy economy and eco-system. At the same time, non-native brown trout, Salmo trutta are being stocked in streams and tributaries of the Lake Champlain Basin. The concern by numerous regional and state agencies is that the native brook trout populations within the Lake Champlain Basin are declining, due to the mating of the female brook trout with the male brown trout (hybridization), thus producing a sterile tiger trout, Salvelinus fontinalis X Salmo trutta[2]. It is known that tiger trout exist in other parts of the country[3] because of this interbreeding, but it is unknown if this hybridization is occurring in the Lake Champlain Basin. This is an important community issue for two reasons: the need to maintain a healthy eco-system and because fishing has a significant economic impact within this geographical region. It was hypothesized that the further apart the test locations, the more genetically diverse the trout populations were. It was also hypothesized that the brook trout and the brown trout populations are interbreeding to produce tiger trout. The purpose of this pilot project was to genetically test the trout from tail clippings, using microsatellite markers to determine if and where this hybridization had occurred. Microsatellite markers are increasingly being used to analyze population genetics[4]. Microsatellites are a group of tandem repeated DNA sequences consisting of di-, tri-, and tetranucleotides. The more tandem repeats within a tested DNA sample, the longer the DNA segment will be. This project also determined genetic diversity using six microsatellite genetic markers for the brown trout and brook trout samples located within the Lake Champlain Basin. The lengths of these segments were measured in terms of base pairs. This genetic survey will serve as a baseline for further genetic monitoring of brook trout, brown trout and possibly tiger trout within the Lake Champlain Basin.

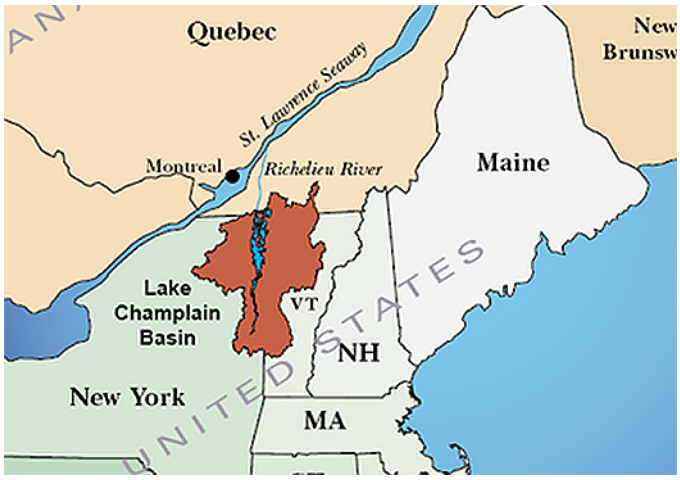

Figure 1. Map of the Lake Champlain Basin found in upstate New York, Vermont, and Southern Quebec. Map retrieved from http://www.lcbp.org/atlas/PDFmaps/nat_region.pdf,edited to focus on the Lake Champlain Basin instead of the entire northeastern United States and southeastern Canada regions.

Materials and Methods

Both brook and brown trout were caught and released in True Brook in Saranac, New York and Great Brook in Plainfield, Vermont by electroshocking the water. The trout (N=26) were scooped up in nets and small tail clippings were taken from the caudal fin or adipose fin and the samples were put in sterile collection tubes. Each fish was measured for length; and weather conditions, GPS satellite positioning and water temperatures were

recorded. Additionally, the collected fish were identified as brown trout, brook trout, and possibly tiger trout (unknowns). Gloves were worn when handling each fish. After collecting the samples at each site, the fish were released before moving on to the next site. DNA isolation using the fin clippings was done following: Animal Tissue Spin Column Protocol from DNeasy® Blood and Tissue protocol from Qiagen. The isolated genomic DNA was then put through polymerase chain reactions (PCR) using PuReTaq Ready-To-Go PCR beads, 200-400 ng of DNA, and 2 µM of forward and reverse primers (Table 1). The thermal cycler was programmed for 35 cycles of 94°C for 30 sec, 56°C for 30 sec, followed by 72°C for 45 sec. The samples were assayed for allelic diversity using six microsatellite markers which were known to be polymorphic; they were Sfo- C129, Sfo-C79, Sfo-C113, Sfo-292, Sfo-262 and MST-85 (Table 1). After PCR, 5 µl of 5x loading dye was mixed with 20µl of each DNA sample for each marker and loaded into a 1.25% agarose gel containing ethidium bromide. Following DNA gel electrophoresis, each gel was visualized and photographed using a gel documentation system (Figures 2-4). Once the DNA was analyzed through DNA gel electrophoresis, further analysis was done using a Agilent 2100 bioanalyzer to determine the exact size of each PCR product (data not shown).

Results

The trout samples examined demonstrated a wide range of values for both the number of alleles and size in base pairs of the PCR products (Table 2). All the microsatellite genetic markers except the MST-85[5] fell within the range of previously published results for these markers[6,7,8,9]. The genetic marker Sfo-292 had the greatest number of alleles for the brown trout (15), brook trout (11) and tiger trout (5), as well as the largest size range in base pairs (197-327 bp) for all samples (Table 2, Figure 2). In contrast, the Sfo-C79 marker had the least number of alleles (2) for each trout tested and also had the smallest size range (100-108 bp) (Table 2, Figure 3). The Sfo-C113 marker exhibited a size range for the tested samples (129-155 bp) with brown trout having three alleles, brook trout six alleles and the unknowns three alleles (Table 2, Figure 4, Figure 5). The Sfo-C129 also had the same allele frequency as the Sfo-C113 marker with a base pair range of 239-259 (Table 2). Finally, the MST-85 marker was not a useful marker due to all the DNA fragments it produced (data not shown).

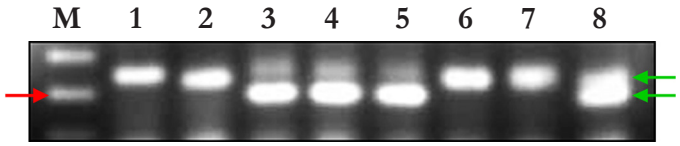

Figure 2. DNA electrophoresis results for Sfo-292 marker. M = 100 bp marker, red arrow =200 bp. Lanes 1-2, 6-7 are brown trout samples; lanes 3-5 are brook trout samples; lane 8 is a suspected tiger trout sample with two bands (green arrows).

Table 1. Genetic Marker Primer Sequences

Marker	Sequences
Sfo-262	Forward 5' CCCATGTCAGTATTGGACTC 3' Reverse 5' CTTCATGGGCAGAATGGAC 3'
Sfo-292	Forward 5' CCTTAGTCCCCTGTGCTTG 3' Reverse 5' CTGAGACCGCACTGGTACAC 3'
Mst-85	Forward 5' GGAAGGAAGGGAGAAAGGT 3' Reverse 5' GGAAAATCAATACTAACAA 3'
Sfo-C 79	Forward 5' CACTGGCCTGGTTTAGTAGG 3' Reverse 5' CTGCTAGCCCCATACATCAC 3'
Sfo-C 113	Forward 5' GGAGCCCAGACTATATTGACG 3' Reverse 5' CCTTGAAGTCTTGCCAGATG 3'
Sfo-C 129	Forward 5' CACGACGTTGTAAAACGACAGTGGGT 3' Reverse 5' AGGTATTCACACCTCAGATTGG 3'

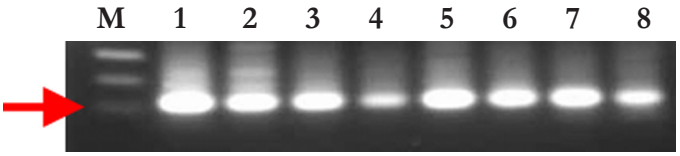

Figure 3. DNA gel electrophoresis results. Sfo- C79 marker using brown trout DNA (lanes 1-3), brook trout DNA (lanes 4-7) and a suspected tiger trout DNA (lane 8). M represents the 100 base pair marker, arrow = 100 bases.

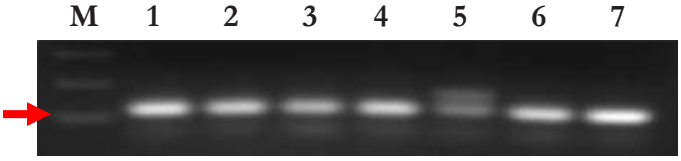

Figure 4. DNA gel electrophoresis results for Sfo-C113 marker using brown trout DNA (lanes 1-4, 6, 7) and a suspected tiger trout DNA (lane 5) with two bands present. M = 100 base pair marker, the arrow is pointing to 100 bases.

Discussion

The improvement of microsatellite genetic markers in recent years has supplied a source of polymorphism needed for genetic identification in fish. The two main purposes of this pilot project was to first, use six microsatellite DNA markers to assess the genetic diversity of brook trout and brown trout populations in the Lake Champlain Basin. Second, these six microsatellites were also used to determine whether hybridization between brook trout and brown trout was occurring and producing a proposed tiger trout.

Initial screening of all DNA samples using each of the six microsatellite genetic markers produced a variety of results, some more easy to interpret than others. The level of polymorphisms produced by the brook and brown trout in this project were comparable to those examined in other studies[4,5,6,8,9]. However, the MST-85 results showed more alleles than those from previous

studies[5] making this particular marker unreliable for this study.

Considering the results from this project, microsatellite markers Sfo-C113, Sfo-262 and Sfo-292 showed promise for identifying genetic diversity as well as identifying tiger trout. The Sfo-C113 marker showed the least number of alleles (Table 2) but did produce two DNA bands for one of the unknown trout samples thought to be a proposed tiger trout (Figure 4, lane 5). The bottom band in lane 5 (Figure 4) for the proposed tiger trout fell within the size range for brown trout, while the top band fell within the range for brook trout (Table 2). On the other hand, the Sfo-292 produced the greatest number of alleles (Table 2) but it also produced two distinct DNA bands for one of the proposed tiger trout samples (Figure 2, lane 8). Here again, one of the two DNA bands (from Figure 2, lane 8) matches both the brook and brown trout DNA bands. Finally, the Sfo-262 genetic marker showed that the brook trout alleles fell within the range of 320-356 bp and 392-412 bp; while the brown trout alleles were measured to be 356-375 bp. The proposed tiger trout had alleles which matched two alleles from brook trout (338 and 350 bp) and one allele from brown trout (375 bp). Further testing on an increase sample size and from more collection sites will need to be done to determine consistent reliability of these markers. As mentioned, the trout were only collected from two sites.

There were several areas of concern with this study. First, it was hoped that more trout samples could be collected, but due to the record snowfall during the winter and record rainfall during the spring, this produced swollen and fast moving streams which had an impact on the number of trout within the stream. Second, most of the brown trout were collected from the Plainfield, Vermont location, while most of the brook trout samples were collected at the True Brook stream near Saranac, NY. This made it difficult to compare any genetic differences between the brook trout between the Plainfield, VT and Saranac, NY locations as well as the brown trout populations.

To expand this project, more trout samples need to be collected and tested to truly evaluate the brook and brown trout populations. Also, more collection sites need to be included from the Lake Champlain Basin. Furthermore, the DNA samples collected for the suspected tiger trout should be sequenced to confirm the findings. These results will be shared with the Lake Champlain Research Institute and the U.S. Department of Fish and Wildlife. It could influence the management and stocking of the streams within the Lake Champlain Basin. Finally, the oldest and most established markers (Sfo8, Sfo12, Sfo18, and Sfo23[4]) should be included, since they have been widely used and have generated familiarity within the 'trout community' to promote consistency.

Table 2. Summary of variations for microsatellite markers

***Sfo-C113* Microsatellite**	**Brown Trout (N=12)**	**Brook Trout (N=11)**	**Unknowns (N=3)**
Allele range in base pairs	*131-137 bp*	*137-155 bp*	*129-149 bp*
Number of alleles	*3*	*6*	*3*
***Sfo-262* Microsatellite**			
Allele range in base pairs	*356-375 bp*	*320-412 bp*	*323-375 bp*
Number of Alleles	*5*	*9*	*4*
***Sfo-292* Microsatellite**			
Allele range in base pairs	*205-327 bp*	*197-292 bp*	*197-293*
Number of Alleles	*15*	*11*	*5*
***Sfo-C79* Microsatellite**			
Allele range in base pairs	*100-104 bp*	*104-108 bp*	*100-108 bp*
Number of Alleles	*2*	*2*	*2*
***Sfo-C129* Microsatellite**			
Allele range in base pairs	*239-247 bp*	*241-256 bp*	*249-259 bp*
Number of Alleles	*3*	*6*	*3*

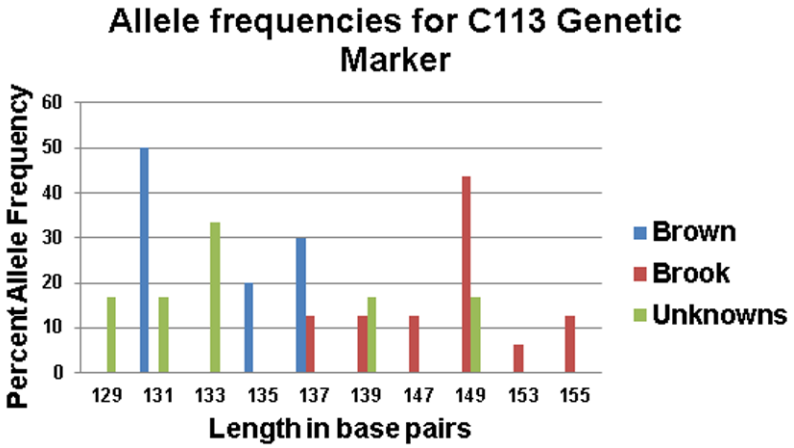

Figure 5. The percent allele frequency for the Sfo-C113 genetic marker is shown for the brook (N=11), brown (N=12), and unknown trout (N=3) samples. The brook trout alleles (red bars) fell within the range of 137-155 bp; the brown trout (blue bars) alleles were measured to be 131, 135, and 137 bp. The unknown trout (green bars) had alleles which matched one allele from brook trout (149 bp) and two alleles from brown trout (131 and 137 bp).

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