CHAPTER THREE: GENETIC INVESTIGATION OF ADFLUVIAL BROOK TROUT (*SALVELINUS FONTINALIS*) IN PICTURED ROCKS NATIONAL LAKESHORE, MICHIGAN, USA.

CHAPTER OVERVIEW

This study focused on two tributaries located in Pictured Rocks National Lakeshore, Mosquito River and Sevenmile Creek. Samples were used from 2011 sampling period (N = 50/site). From 2004 to 2012, streams were sampled once a month and captured brook trout larger than 100mm were implanted with a PIT tag to investigate individual movement patterns. Fifty adfluvial trout were compared to the resident fluvial fish from the open sections of the two tributaries (MOS = 20, SVN = 30). Eight microsatellite loci were used to examine genetic diversity and structure between groups of brook trout. The results showed that adfluvial brook trout were most closely related to the fluvial brook trout from their stream of capture rather than to other designated adfluvial groups, which is consistent with other studies around Lake Superior.

INTRODUCTION

Brook trout (*Salvelinus fontinalis*) are found most often in freshwater streams and small lakes, but anadromous populations are found on the east coast of North America (Behnke 1972; Scott and Crossman 1973), and potadromous brook trout are found in some large lakes such as Lake Superior (Becker 1983; Power 1980). Like many other salmonids, brook trout display differential use of habitat through variation in migratory behavior or morphology associated with different ecological niches (Balon 1980; Power 1980). Features such as body form, feeding morphology, and diet preference vary widely (Power 1980). In the Lake Superior basin, brook trout exhibit a variety of life histories. Fish can spend their entire life within tributary streams (fluvial), spend part of their life within the lake but return to the stream to spawn (adfluvial), or live within the lake and spawn in nearshore areas (lacustrine). Individuals that spend time within Lake Superior (coaster brook trout) may grow larger than stream resident fish and as such are highly sought by anglers (Newman et al. 2003)

Brook trout are culturally important to anglers of all backgrounds and many state, federal and tribal agencies invest significant amounts of funding into brook trout research and management. One of the most unique brook trout forms, the coaster phenotype, is endemic to Lake Superior and is famous for its large size and migratory habits. Until very recently, anglers, agencies, and academics assumed that a coaster brook trout was characterized by large body size and physical presence in Lake Superior for some part of its life. However, Kusnierz et al. (2009) documented brook trout of unremarkable size exhibiting coasting behavior (movement into the Superior basin from a stream) in Pictured Rocks National Lakeshore (PIRO). The adfluvial form of the brook trout is of conservation concern in the Great Lakes region in order to preserve this life history variant.

The adfluvial life history has allowed brook trout to colonize ecosystems in the subarctic regions along the Hudson Bay, temperate areas bordering and east of the Laurentian Great Lakes, and southern coldwater habitats in the Appalachian Mountains of Tennessee and Georgia (Power 1980). In the Lake Superior basin, coaster brook trout were found historically along most of the shoreline (Schreiner 2008; Wilson et al. 2008). The trout provided a high-profile recreational fishery for much of the 19th and early 20th centuries for anglers to catch large numbers of these sizeable brook trout (Newman and Dubois 1997). The ecosystem in the Lake Superior basin has been altered by several anthropogenically induced changes, including overharvesting, stream habitat damage from intense logging, and the introduction of non-native fish. Likely from a combination of these actions, the coaster fishery collapsed and was thought to persist only in isolated remnant populations concentrated near Isle Royale, Nipigon Bay Ontario, and the Salmon Trout River in Michigan (Kelso and Demers 1993, Schreiner et al. 2008).

Historically the adfluvial life history form was found in Pictured Rocks National Lakeshore, but was thought to have disappeared in the later part of the 20th century during the fishery collapse (Kelso and Demers 1993). However, recent studies conducted in PIRO documented individual brook trout moving between watersheds (Kusznierz et al. 2009; Leonard et al. 2013). Currently, a brook trout found within the Lake Superior portion of PIRO is considered to be an adfluvial (migratory) coaster, while those in the streams are presumed to be primarily stream residents. The presence of adfluvial coasters in some streams has also been documented (Kusnierz et al. 2009). In addition, radio tagged brook trout have migrated along the coast of Lake Superior between PIRO streams (Leonard pers. comm.). Brook trout bearing passive integrated transponders (PIT) tags have moved from one stream to another, suggesting that these fish are interacting with brook trout in multiple watersheds, and that gene flow may occur between these groups.

Brook trout found in the Lake Superior basin present significant difficulties for fisheries managers due to the complexity of this species' variability in life history traits that complicate the management of the group (Power 1980, Schreiner et al. 2008). Managers do not ordinarily treat trout from different watersheds as a single stock (population) or a single group of fish that exists separately from other groups of fish of the same species. This default assumption has been the status quo in fisheries management but has failed repeatedly to describe actual circumstances. When the stock of interest is composed of groups of fish displaying different life histories, key management parameters such as suitable habitat availability, stock/population dynamics, effective population size, and harvest pressure assessment are all impacted. Further, there is a need to clarify the delineation of populations (and metapopulations) around Lake Superior using genetic techniques that characterize appropriate management units based upon the amount of genetic divergence between recognized populations. Lake Superior brook trout were managed in the past largely as a single ecotype, and population boundaries were defined by tributary. This resulted in management issues being focused at the watershed level. Estimates of harvest pressure, suitable habitat availability, and other factor affecting the populations did not accurately address the substantial life history variability that exists within brook trout in the region (Newman and DuBois 1997; Newman et al. 2003; Schreiner et al. 2008)

Currently, PIRO brook trout are managed on a stream or watershed basis. State fishing regulations for brook trout vary by stream, and are different from the regulations for the brook trout fishery in Lake Superior. The current regulations do not adequately address the inter-stream movement behaviors that have been documented in PIRO brook trout. Furthermore, variations in daily bag limits and minimum length limits for brook trout in Lake Superior basin are different from stream resident brook trout found in PIRO leading to individual, mobile fish being subjected to multiple regulations. My project addressed the actual population genetic structure found between four tributaries in PIRO. I examined relationships between the Open (downstream) and Restricted (upstream) populations as well as how adfluvial coaster fit into the dynamics of brook trout in the park. These findings can be applied in the discussion about appropriate regulatory strategies for enhancing brook trout success within PIRO to ensure intraspecific biodiversity is retained.

METHODS

STUDY SITE.—Pictured Rocks National Lakeshore was authorized by Congress as America's first national lakeshore by Public Law 89-668 on October 15, 1966, and it was formally established on October 6, 1972. PIRO is situated along the southern shore of Lake Superior in Alger County in Michigan's Upper Peninsula. It extends 62 kilometers (km) between Munising on the west end and Grand Marais on the east end, and is 4.8 km at its widest point. PIRO's boundary extends into Lake Superior out to 0.4 km perpendicular to shore, which protects 2,252 ha of Lake Superior's surface area (Mechenich et al. 2006). PIRO includes 19 named streams and, in general, streams are short and have moderate gradients. Flow discharge generally is highest in the late spring and early summer from a combination of snowmelt and spring rains. PIRO's watersheds and their drainage patterns are determined mostly by the topography of underlying Cambrian rock and surficial Pleistocene and Holocene sediments (Mechenich et al. 2006).

Two Lake Superior tributaries were chosen for this portion of the study, Mosquito River and Sevenmile Creek (Table 3.1; Figure 3.1). These streams were sites of research using passive integrated transponder (PIT) tags, investigating movement patterns of brook trout. There were RFID antennas at these streams that tracked any brook trout immigrating or emigrating from the stream. Mosquito R. and Sevenmile Cr. are both second order streams that flow through a mix of coniferous and deciduous forests. Fish communities in the rivers are dominated by brook trout, daces (*Rhinichthys* spp.), minnows (*Notropis* spp.), suckers (*Castomus* spp.), central mudminnow (*Umbra limi*), and sculpins (*Cottus* spp.) (Boyle et al 1999, Leonard et al. 2013). In addition, a number of exotic aquatic invasive species have been found in PIRO, including steelhead (rainbow) trout (*Oncorhynchus mykiss*), pink salmon (*Oncorhynchus gorbuscha*), coho salmon

(*Oncorhynchus kisutch*), and Chinook salmon (*Oncorhynchus tshawytscha*) (Leonard et al. 2013; Mechenich et al. 2006).

FIELD METHODS.—Brook trout analyzed in this study were sampled using backpack electrofishing during the 2011 field season in the Open (downstream) sections of Mosquito R. (MOS) and Sevenmile Cr. (SVN). Each captured brook trout was measured for total length (TL, mm) and weight (g) (Table 3.2). Caudal fin clips were collected from all captured brook trout for genetic analysis and all fish over 100mm TL were implanted with PIT tags (Cross and Leonard unpublished). PIT tag data was analyzed to determine movement patterns of individual fish (Cross and Leonard unpublished). Fish were designated as adfluvial if they exhibited a last known movement out into Lake Superior. The collection effort in 2011 resulted in a total of 150 brook trout, with 50 classified as adfluvial brook trout. Sevenmile Creek had 50 fluvial and 30 coaster brook trout, whereas Mosquito R. had 50 fluvial and 20 adfluvial. These trout ranged from 102-266 mm TL (mean ± SD: 155.4 ± 30.1 mm) at the time of collection.

Genetic analyses.—Fin tissue samples were taken from the caudal fin and placed in individual sampling tubes containing 95% EtOH. Samples were stored at -20˚C until extractions were performed. DNA was extracted from all samples using QIAGEN DNeasy kits (QIAGEN, Valencia, California, USA). Eight microsatellite markers were employed including Sfo8, Sfo12, Sfo 18 (Angers et al., 1995), C24, D75, C28, C38 (T. King, unpubl. data), and C113, C115 (Sloss et al. 2008) (Table 2.3). Loci were amplified using polymerase chain reaction (PCR) in 10 µl reaction volumes including: 6µl dH20, 1µl PCR buffer (Bullseye), 1µl or 0.6µl 25 mM $MgCl_2$, 0.4µl dNTPs, 0.1µl forward primer with CAG tag, 0.3µl florescent labeled CAG tag (Mullen et al. 2006; Schuelke 2000), 0.4µl reverse primer, 0.2µl of Taq polymerase (Bullseye), and 1µl of DNA. Primers were at a concentration of 10 mM. Loci were amplified on a BioRad

Thermocycler under a variety of primer specific conditions (Table 2.4, Appendix 1). A subsample of the PCR product was visualized on 1% agarose gel to check for quality assurance before pooling 4 microsatellites with different fluorescently labeled CAG tags (PET, VIC, NED, FAM) into 96-well plates. For genotyping, a master mix containing 11.5µl HI-DI formamide and 0.5µl GS600LIZ size standard per well sample was prepared, and 12µl of the master mix was aliquotted to each well on a 96-well plate for fragment analysis, then, 1µl of the pooled PCR product was added to the correct well. Samples were run on an ABI PRISM 3100-Avant Sequencer. Genotypes were scored based on 20 base-pair standard (GS600 LIZ) through the use of GeneMapper Software Program. All genotypes were checked for proper scoring by experienced laboratory personnel.

Statistical Analyses.— The program GeneMapper was used to properly align fragments and score alleles in the eight different loci. Exporting the binned alleles from GeneMapper to Excel, the genetic statistical software program GenAlEx 6.5 (Peakall and Smouse 2006, 2012) was used to calculate frequency-based analyses including *F*-statistics, expected and observed heterozygosity, Hardy-Weinberg Equilibrium (HWE), population assignment and relatedness, as well as distance-based genetic statistical analyses such as Nei's Genetic Identity and Distance analyses (Nei 1972). Adjustment of the HWE P-value utilized sequential Bonferroni corrections for multiple tests as described by Rice (1989). This technique is not as conservative as a normal Bonferroni correction, which can lead to type II errors.

RESULTS

This study resulted in the collection and analysis of 150 brook trout from PIRO. Of these samples, 80 were collected from the Open (downstream) section of Sevenmile Creek and 70 from the Open section of the Mosquito River. After movement analysis, 50 of these Brook trout were designated as coaster or adfluvial fish (Mosquito River = 20; Sevenmile Creek = 30) (Table 3.3).

All eight loci were polymorphic across all populations (Table 3.4) with the total number of alleles ranging from 3 (*Sfo*-C38) to 22 (*Sfo*-8). Significant deviations from Hardy-Weinberg expectations were observed at 16 locus-per-population comparisons. Locus *Sfo*-8 consistently deviated from HW expectations, and even after sequential Bonferroni corrections were performed three locus-per-population comparisons remained significant. I decided to keep *Sfo-8* in order to retain resolution in the study. Deviations from HW expectations can be due to a number of factors including the presence of null alleles, nonrandom sampling, or scoring errors, and it can be difficult to determine the true cause (Castric et al. 2002). Locus *Sfo*-C28 had one comparison remaining significant after corrections. All remaining locus HW deviations were rendered nonsignificant after sequential Bonferroni corrections (Table 3.4).

GENETIC DIVERSITY AND POPULATION DIFFERENTIATION

A consistent level of genetic diversity was observed within and between the sampled populations (Table 3.5). The average observed heterozygosity over all loci was lowest in the samples from Mosquito River adfluvial (0.550) and highest in Mosquito River fluvial (Open) (0.637). The lowest average number of alleles was found in Mosquito River adfluvial (6.25) and the highest in Sevenmile Creek fluvial (Open) (11.125). There was a trend for slightly lower measures of diversity (heterozygosity and mean number of alleles) in the adfluvial designated group than that of the fluvial (Table 3.5). Comparison between the two rivers showed that Sevenmile Cr. had higher observed heterozygosity than that of Mosquito River (Avg. 0.6075, 0.590). The lowest average number of alleles was found in Mosquito River (7.875) and the highest in Sevenmile Creek (9.375).

Fifteen private alleles were observed between the Mosquito River and Sevenmile Creek PIRO brook trout groups, ranging from a low of 3 (MOS adfluvial) to a high of 20 (SVN fluvial) (Table 3.6). There was a trend for lower numbers of private alleles in the adfluvial designated trout than that of the fluvial brook trout (Avg. 5, 14.5). Measures of population subdivision (F_{st} = (H_t - Mean H_e) / H_t) across Mosquito River and Sevenmile Creek groups was 0.046 and between groups 0.027 (fluvial) and 0.015 (adfluvial) (Table 3.8). Significant deviation of F_{st} from zero (indicating genetic subdivision) was observed between all sampled groups ($P<0.001$; 1,000 iterations). Pairwise F_{st} resulted in adfluvial designated trout being most closely related to trout from their stream of capture (i.e. Mosquito adfluvial and Mosquito fluvial) (Table 3.7).

Significant deviations in allele frequency were observed for all pairwise comparisons ($P< 0.01$; 100 iterations). According to Wright (1978) F_{st} values of 0.05 to 0.15 shows moderate differentiation and groups in both sampled populations fell below this range. Inbreeding coefficient (F_{is} = (Mean H_e - Mean H_o) / Mean H_e) across Mosquito River and Sevenmile Creek populations was 0.106 and between groups 0.100 (fluvial) and 0.063 (adfluvial) (Table 3.6). Another frequent way to estimate the genetic relationship between populations is Nei’s Genetic Distance (D) (Nei 1972). Comparing the genetic distance between the four groups (two fluvial, two Adfluvial) found that Mosquito River adfluvial were most closely related to Mosquito River fluvial brook trout (0.133) and Sevenmile River adfluvial were most closely related to Sevenmile fluvial brook trout (0.068) (Table 3.8).

ASSIGNMENT TESTING

Individual assignment tests between the two adfluvial groups showed high accuracy of assignment. For Sevenmile River 97% of adfluvial fish were correctly assigned, while 80% of the Mosquito River adfluvial were correctly assigned (Table 3.9). When the fluvial brook trout from Mosquito River and Sevenmile Creek were compared to the adfluvial group from that river (i.e. MOS fluvial to MOS adfluvial), the assignment accuracy in both adfluvial groups declined (MCST 75%, SCST 90%) (Table 3.10). When all groups were pooled together the assignment test once again declined for the adfluvial groups (MCST 70%, SCST 86%) (Table 3.11). Examining into where the misassigned individuals where being assigned helped to show where possibly shared gene pools and gradients of genetic differentiation were occurring between groups. Within the Mosquito fluvial group, 10/15 were assigned to the Mosquito adfluvial group, 2/15 to Sevenmile fluvial and 3/15 were assigned to Sevenmile adfluvial group (Table 3.12). In the Mosquito adfluvial group, 4/6 of the misassigned were assigned to the Mosquito fluvial group and 2/6 to the Sevenmile fluvial group. Within the Sevenmile fluvial group, 13/21 were assigned to the Sevenmile adfluvial group, 5/21 to Mosquito fluvial and 3/21 were assigned to Mosquito adfluvial group. Looking into the Sevenmile adfluvial group, 3/4 were assigned to the Sevenmile fluvial section and 1/4 to the Mosquito fluvial group.

DISCUSSION

Microsatellite DNA loci were used to evaluate the genetic structure of fluvial and adfluvial brook trout from two major tributaries of Lake Superior located in Pictured Rocks National Lakeshore. Restoring native fisheries has been at the center of research in Lake Superior for many years, starting with the restoration of the lake trout (*Salvelinus namaycush*). Adfluvial brook trout research and restoration has gained more attention since the late 90's and early 2000's. Researching what systems are still producing adfluvial brook trout and how they affect surrounding populations has furthered the understanding of the role adfluvial fish play in metapopulation dynamics.

The results of this study suggest that adfluvial and fluvial brook trout groups are interdependent, with adfluvial brook trout acting as vectors for gene flow among select tributary habitats. This was confirmed in both Mosquito River and Sevenmile Creek by the small genetic distance and the high assignment probabilities of adfluvial groups when compared to the fluvial groups from each tributary (Table 3.8; Table 3.9; Table 3.11). Extensive within-lake movements of potadromous brook trout have been documented in post-glacial lake remnants in Quebec (Fraser and Bernatchez 2005) with individuals being recaptured more than 80km from their spawning sources. D'Amelio and Wilson (2008) suggested that future efforts for conservation should focus on regional populations to determine their spatial extent, productivity, and degree of connectivity with other populations which will help to identify major source sink rivers of metapopulations.

The clear demonstration of shared ancestry between coaster and river-resident brook trout, along with the interdependency of lake and river populations, highlights the need to rehabilitate tributary systems in order to restore adfluvial coaster numbers. Within Mosquito River and Sevenmile Creek, Cross and Leonard (unpublished) found no correlation between condition or total length and the expression of adfluvial movement behavior. Tributaries in Lake Superior vary in the proportion of adfluvial brook trout derived from fluvial populations (D'Amelio et al. 2008). Cross and Leonard (unpublished) also found differences in the number of classified adfluvial fish (MOS n=35 and SVN n=106) which is consistent with the underlying genetic relatedness between Mosquito and Sevenmile brook trout (Table 3.8). If Sevenmile Creek is producing three times as many adfluvial fish than Mosquito River, there would be a higher probability of a brook trout making it into surrounding tributaries (i.e. Hurricane River). This higher presence of adfluvial trout exiting Sevenmile Creek may be why we see the shared gene pool between these two systems.

Varying levels of gene flow among spawning populations in this system further suggests that under normal conditions, brook trout populations in continuous lake-river environments function as a stable metapopulation (Fraser and Bernatchez 2005; D'Amelio and Wilson 2008). Adfluvial brook trout in Lake Superior may link rivers into a network of populations and some rivers produce more coasters, possibly as a consequence of habitat supply or population dynamics (D'Amelio and Wilson 2008). In order to properly manage and conserve native fisheries of brook trout and all life histories in PIRO and around Lake Superior, managers must first understand the underlying dynamics of the populations (i.e. movement and genetics). The results of this study indicate that in order to protect coasters we must protect the fluvial populations from which they derive from and the habitat in those streams.

Table 3.1.—Physical characteristics of two study streams located within PIRO, Michigan.

Stream	**Watershed Area (ha)**	**Length (km)**	**Range discharge (m^3/s)**	**Waterfall distance from mouth (km)**
Mosquito River	3,411	8.5	0.11-1.09	2.36
Sevenmile Creek	2,103	2.5	0.439-0.694	NA

(Handy and Twenter 1985; MIDEQ 1998; Boyle et a. 1999; Mechenich et al. 2006)

Table 3.2.—Brook trout sample size along with range of Total Length (TL), the Mean and the Standard Deviation (STD)

Stream	**BKT N**	**Range TL**	**Mean ± STD**
Mosquito O	50	111 - 231	159.8 ± 27.4
Mosquito CST	20	109 - 209	146.6 ± 28.1
Sevenmile O	50	110 - 266	161.9 ± 27.8
Sevenmile CST	30	102 - 205	153.4 ± 37.2
Total	**150**	**102-266**	**155.4 ± 30.1**

O = Open sections to Lake Superior
CST = “Coaster” or Adfluvial Brook trout

Table 3.3.—Sample distribution of brook trout Collected in PIRO during 2011 season.

Stream/Type	**Open**	**Restricted**	**Total**
Mosquito O	50	NA	50
Sevenmile O	50	NA	50
"Coaster"	50	NA	50
		Total Study	**150**

Open = Access to Lake Superior

Coaster = PIT/RFID data (Cross. Unpublished)

Table 3.4.—Microsatellite loci and descriptions of allelic variation across two populations (four groups) of brook trout from two tributaries located in Pictured Rocks National Lakeshore.

Locus	**Size range (base pairs)**	**Mean number of alleles/ population**	**Ranges of alleles/ population**	**Meets Hardy-Weinberg Expectation?**
Sfo-8	217-294	17.5	13-22	No
Sfo-12	182-306	7.5	4-11	Yes
Sfo-18	161-201	9.25	7-12	Yes
Sfo-C28	177-212	5.5	4-8	Yes*
Sfo-C38	151-164	4.25	3-5	Yes
Sfo-D75	185-245	9.25	6-13	Yes
Sfo-C113	138-172	8.25	6-11	Yes
Sfo – C115	219-275	7.50	5-10	Yes

*Sig different at $P < 0.00625$ for 1 of 4 groups

Table 3.5.—Genetic diversity measures for four sampled groups (two populations) of brook trout from two Lake Superior tributaries located in Pictured Rocks National Lakeshore, Michigan. All populations were genotyped at eight loci; Open = Below Barrier, Coaster = adfluvial brook trout (PIT/RFID data), N = the sample size, and diversity measures for microsatellite DNA loci (observed [H_0] versus expected [H_e] heterozygosity), and A = the mean number of alleles per locus.

Sample	**Code**	**N**	**H_e**	**H_0**	**A**
Mosquito River Fluvial	MOSO	50	0.723	0.637	9.500
Mosquito River Adfluvial	MCST	20	0.656	0.550	6.250
Sevenmile Creek Fluvial	SVNO	50	0.695	0.609	11.125
Sevenmile Creek Adfluvial	SCST	30	0.661	0.606	7.625

Table 3.6.—Private allele list with number found at each loci.

Population	Code	N	Private alleles (number)
Mosquito R. Fluvial	MOSO	50	*Sfo*-8 (1), *Sfo*-12 (3), *Sfo*-18 (2), *Sfo*-C38 (1), *Sfo*-D75 (1), *Sfo*-C115 (1)
Mosquito R. Adfluvial	MCST	20	*Sfo*-8 (2), *Sfo*-C28 (1)
Sevenmile Cr. Fluvial	SVNO	50	*Sfo*-8 (3), *Sfo*-12 (5), *Sfo*-18 (3), *Sfo*-C28 (4), *Sfo*-D75 (3), *Sfo*-C113 (1), *Sfo*-C115 (1)
Sevenmile Cr. Adfluvial	SCST	30	*Sfo*-8 (2), *Sfo*-C28 (2), *Sfo*-C115 (3)

Table 3.7.—F-statistics for sections of tributaries of Lake Superior in PIRO. F_{is} = inbreeding coefficient, F_{it} = Het. of indiv. to population, F_{st} = fixation index

Sample	F_{is}	F_{it}	F_{st}
Total N	0.106	0.145	0.046
Fluvial	0.100	0.124	0.027
Adfluvial	0.063	0.068	0.015

Table 3.8.—Pairwise F_{st} values for fluvial and adfluvial groups.

MCST	SCST	MOSO	SVNO	
	-	-	-	**MCST**
0.058		-	-	**SCST**
0.040	0.041		-	**MOSO**
0.060	**0.015**	0.042		**SVNO**

*all significant at $p < 0.01$ Bold = most closely related

Table 3.9.—Nei's Genetic Distance fluvial and adfluvial groups of four Lake Superior tributaries located in Pictured Rocks National Lakeshore.

MCST	**SCST**	**MOSO**	**SVNO**	
	-	-	-	**MCST**
0.201		-	-	**SCST**
0.133	0.144		-	**MOSO**
0.216	**0.068**	0.151		**SVNO**

Bold = most closely related

Table 3.10.—Accuracy of assignment between adfluvial designated brook trout located in sections of two Lake Superior tributaries in PIRO. N = sample size, A_s = number assigned to self, A_0 = number assigned to other.

Sample	**Code**	**N**	**A_s**	**A_0**	**Accuracy**
Mosquito River	MCST	20	16	4	80%
Sevenmile Creek	SCST	30	29	1	97%

Table 3.11.—Accuracy of assignment between fluvial adfluvial brook trout located in sections of two Lake Superior tributaries in PIRO. N = sample size, A_s = number assigned to self, A_0 = number assigned to other.

Sample	**Code**	**N**	**A_s**	**A_0**	**Accuracy**
Mosquito River	MOSO	50	39	11	78%
Mosquito River	MCST	20	15	5	75%
Sevenmile Creek	SVNO	50	35	15	70%
Sevenmile Creek	SCST	30	27	3	90%

Table 3.12.—Accuracy of assignment between fluvial (Open) and adfluvial designated brook trout located in sections of two Lake Superior tributaries in PIRO. N = sample size, A_s = number assigned to self, A_0 = number assigned to other.

Sample	**Code**	**N**	**A_s**	**A_0**	**Accuracy**
Mosquito River	MOSO	50	35	15	70%
Mosquito River	MCST	20	14	6	70%
Sevenmile Creek	SVNO	50	29	21	58%
Sevenmile Creek	SCST	30	26	4	86%

Table 3.13.—Percentage of adfluvial brook trout assigned between four groups, two in each site of the streams located in Pictured Rocks National Lakeshore.

Site	**Mosquito R. Open**	**Mosquito R. Coasters**	**Sevenmile Cr. Open**	**Sevenmile Cr. Coasters**
MOSO	70	20	10	4
MSCT	20	70	6	-
SVNO	4	10	58	10
SCST	6	-	26	86

Figure 3.1.—Miners River, Mosquito River, Sevenmile Creek, Sullivan’s Creek, Hurricane River and Sable River, Pictured Rocks National Lakeshore, Alger County, Michigan. Inset shows Alger county Michigan. Modified from Leonard et al. (2013)