



## Notes

## Genetic and phenotypic evidence for splake presence in brook trout and lake trout spawning habitats



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## ABSTRACT

Management of Michigan's fisheries relies extensively on hatchery fish. Hatchery production in Michigan includes splake, a fertile hybrid produced artificially by crossing lake trout (*Salvelinus namaycush*) and brook trout (*Salvelinus fontinalis*) that are stocked in near-shore waters of the Great Lakes. Splake seldom occur naturally because brook trout and lake trout are typically reproductively isolated in space and time. Because some splake are fertile, concerns have been raised over splake stocking based on observations of fish of intermediate phenotype in brook trout and lake trout spawning areas. The overall goal of this project was to determine whether splake are present on brook trout and lake trout spawning habitats. We analyzed 10 microsatellite loci to genotype putative splake collected from two brook trout spawning tributaries to Lake Superior, known hatchery splake, and Michigan Department of Natural Resources (DNR) lake trout and brook trout hatchery strains used in the splake program. Identification of fish of intermediate phenotype as F1 hybrids or either parental species in the two brook trout streams was based on species-specific genotypic differences resolved using Bayesian model-based clustering. Four hybrids were identified among the 15 putative splake from the two brook trout streams. Collections were made at several lake trout spawning reefs to quantify the number and reproductive status of splake, revealing that approximately 56% of captured splake were sexually mature. Results from spawning areas of both parental species confirm that splake were present and may pose a threat to the genetic integrity of spawning populations where they occur.

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## Introduction

Purposeful hybridization of recognized species by management agencies is widely practiced in aquaculture to provide recreational fisheries to targeted stakeholders (Bartley et al., 2000). Hybrids often possess desirable behavioral and phenotypic attributes of both parent species. For example, in Ontario waters of Lake Huron, splake were stocked in an effort to rehabilitate the lake trout fishery that had declined due to sea lamprey (*Petromyzon marinus*) predation (Anderson and Collins, 1995). Managers believed that splake would grow and mature more quickly than lake trout, thereby increasing chances for individuals to reproduce prior to succumbing to sea lamprey (*P. marinus*)

predation. On the other hand, direct hybridization programs are widely believed to be responsible for wide-scale and increasing levels of hybridization in natural habitats (Ryman et al., 1995; Allendorf et al., 2001; Scribner et al., 2001).

Management of Michigan's fisheries relies extensively on hatchery production. The Michigan Department of Natural Resources (DNR) annually stocks approximately 23 million fish from 13 different species and one artificial hybrid into the Great Lakes and inland waterways (<http://www.michigandnr.com/fishstock/>, June 2015). Splake are the hybrid produced by crossing female lake trout (*Salvelinus namaycush*) and male brook trout (*Salvelinus fontinalis*). On average 90,000 hybrid splake are stocked annually (<http://www.michigandnr.com/fishstock/>, June 2015) to provide sport angling opportunities.

Brook trout in Lake Superior and tributaries are the focus of current conservation efforts (Huckins et al., 2008). Specifically, adfluvial or coaster brook trout were once abundant and widespread in Lake Superior but are now found in only a few locations. Fisheries management agencies around Lake Superior are working to restore coaster

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brook trout lake-wide. Lake trout, which have been the focus of extensive rehabilitation efforts for over 60 years, are now nearly recovered (Ebener, 2007). Management agencies have expressed concern that splake stocking in Lake Superior could hamper conservation efforts for brook trout (Newman et al., 2003). Splake are highly competitive and thought to be faster growing and more aggressive than either parental species (Newman et al., 2003).

Pre-zygotic and post-zygotic barriers were believed to be sufficient to prevent splake backcrossing into parental species; however, Buss and Wright (1956) demonstrated that splake are not sterile, and Buss and Wright (1958) demonstrated that a backcross of male splake and female brook trout can produce viable offspring. Brook trout and lake trout do not normally hybridize in the wild because they generally use different habitats for spawning, e.g. Great Lakes reefs for lake trout or streams for brook trout. Lake trout spawn in the Great Lakes during the fall on relatively deep offshore reefs (Goodyear et al., 1982; Gunn, 1995) and brook trout spawn in streams or on shallow coastal reefs. Spawning behaviors of the two parental species also differ. Brook trout typically spawn in streams in October and November in Great Lakes tributaries (Witzel and MacCrimmon, 1983; Huckins et al., 2008). However, brook trout have also been observed spawning on shoal areas of lakes that consist of gravel and smaller rocks at less than 1 m in depth (Fraser, 1985). Lake trout do not construct redds but deposit eggs on reefs with cobble or boulder substrate (Kelso et al., 1995). Splake have been observed spawning in the fall as well, in Great Lakes habitats (Berst et al., 1981) and on redds constructed in gravel habitats in streams (C. Bassett, United States Forest Service (retired), personal communication).

Recently researchers and anglers have noticed putative splake located in brook trout stream spawning grounds as well as on lake trout spawning reefs. If splake are present and attempting to spawn at the same location as brook trout or lake trout, it may be possible for them to backcross. The overall goal of this project was to determine whether splake were present in brook trout and lake trout spawning habitats. If splake were present, the current practice of stocking hybrids may conflict with conservation and management programs for native species.

## Materials and methods

### Sampling in Lake Superior tributaries

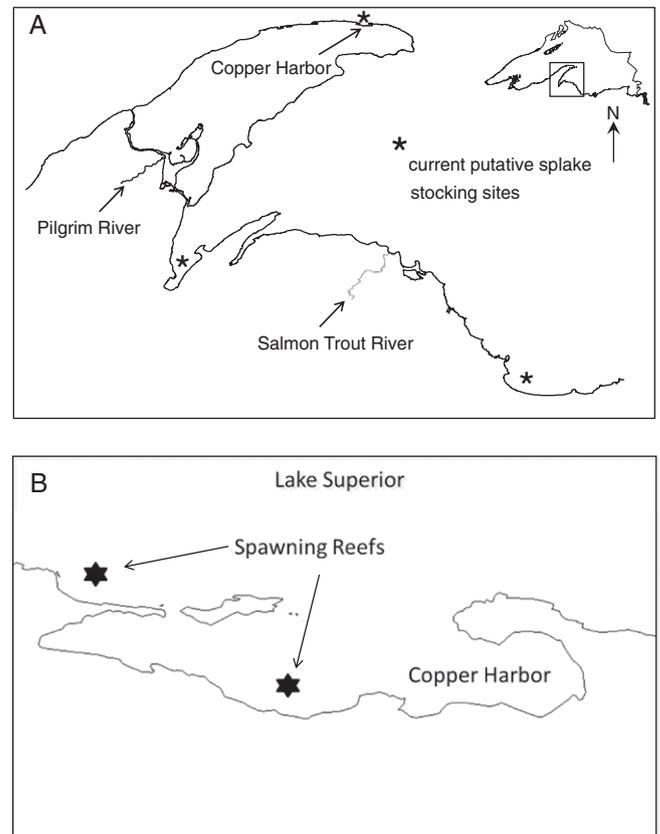
Fifteen fish displaying intermediate phenotypes were caught by anglers in the Pilgrim River and by researchers in the Salmon Trout River, Marquette County, MI. Putative splake and likely resident brook trout from the Salmon Trout River ( $N = 11$ ) and Pilgrim River ( $N = 4$ ) were collected between August and November in 2012 and in 2013 (Fig. 1). Adipose fin tissue samples were dried or stored in ETOH and placed in individual sampling tubes.

### Samples from hatcheries

Samples of known splake, and of individuals from the two parental hatchery strains used in splake production (Assinica brook trout and Lake Superior strain of lake trout) were obtained from the Michigan DNR Marquette State Fish Hatchery.

### Sampling in Lake Superior

The Michigan DNR has been stocking splake annually since 1990 (except 1991) at Copper Harbor. The Great Lakes Indian Fish and Wildlife Commission used gillnets to sample two lake trout spawning reefs (Goodyear et al., 1982) in and near Copper Harbor, MI from 1987 to 2013 (Fig. 1B). Lake trout and splake were identified by external phenotype. Visible phenotypic traits known to be useful for identification of brook trout include reddish spots on dark body, square or weakly forked tail, vermiculations on back and fins with leading white edges. Lake



**Fig. 1.** (A) Locations of Lake Superior tributaries where putative splake were sampled and locations of splake stocking and (B) locations of lake trout spawning reefs where splake were collected.

trout lack reddish spots and vermiculations, and they have a forked tail. Individual fish from Lake Superior and streams with intermediate traits such as dull spots, weak vermiculations, and dusky or no white on fins, coupled with intermediate fork in the tail were labeled as putative splake. Reproductive condition of Lake Superior fish was recorded as immature, mature, ripe, or spent based on the presence or absence of gametes.

### Laboratory genetic analysis

DNA was extracted from fin or scale tissue for both pure and known or suspected hybrid individuals using Qiagen DNeasy kits (Qiagen Inc., Germantown, MD) and the manufacturer's protocols. A NanoDrop 1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE) was used to quantify DNA concentrations for all samples, which were subsequently diluted to a concentration of 20 ng/ $\mu$ l. All fish samples were genotyped at 10 microsatellite loci: SfoD75, SfoC24, SfoC38, and SfoC88 (King et al., 2012), Sfo12 and Sfo18 (Angers et al., 1995), SnaMSU01, SnaMSU10 and SnaMSU11 (Rollins et al., 2009), and Sco19 (Taylor et al., 2001), using PCR conditions reported in the original literature. These microsatellite loci were used because they successfully amplified brook trout, lake trout and splake. PCR products were separated by size on 6% denaturing polyacrylamide gels and visualized using a Hitachi FMBIO-II laser scanner (Hitachi Solutions America, Ltd., San Bruno, CA) or LI-COR 4300 DNA Analyzer (LI-COR Biosciences, Lincoln, NE). Genotypes were scored based on 20 base-pair standards and individual standards of known genotype. All genotypes were scored by two experienced laboratory personnel. Ten percent of all samples were genotyped for all loci and blindly scored to compare to original samples. Genotype error rate was estimated to be <1.0%.

### Statistical analysis

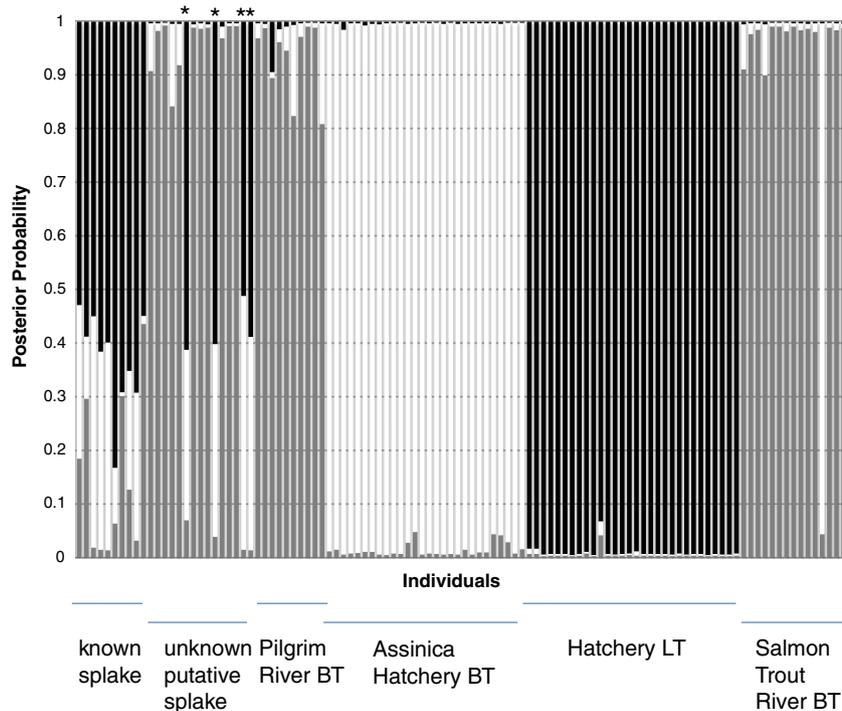
Estimates of allele frequency and measures of genetic diversity (heterozygosity, number of alleles per locus) were obtained using the program FSTAT (version 2.9.3; Goudet, 2001). Analyses were also conducted using the program STRUCTURE (Pritchard et al., 2000) and Bayesian Analysis of Population Structure (BAPS) (Corander et al., 2004) to determine if there was evidence for sub-structuring (representatives from multiple populations or species) in the samples without prior assignment. Ten replicate runs were conducted in STRUCTURE using a 100,000 burn-in and 1,000,000 bootstrap replicates per run to estimate the likelihood of the data under each hypothesized number of genetic clusters ( $K = 1-6$ ). We used the STRUCTURE program to estimate the posterior probability of individuals associated with each genetic cluster and measures of admixture probability. Simulations conducted using the non-spatial genetic mixture analysis of BAPS were used to statistically evaluate whether individuals with intermediate posterior probabilities of group membership had a high likelihood of admixture between members of different clusters, relative to the alternative hypothesis of being a parental genotype. To further define hybrid filial generation if hybrids were detected, we estimated the posterior distribution associated with hypotheses that individuals in our genotyped samples belonged to one of several hybrid categories using a Bayesian model-based clustering approach implemented in the program New Hybrids (method described in Anderson and Thompson, 2002). Genotypic classes evaluated including parental lake trout, parental brook trout, F1, F2, backcross lake trout and backcross brook trout). Analyses were conducted using a 1000 burn-in and 10,000 bootstrap replicates. Uniform priors were assumed for allele frequencies and mixing proportions.

### Results

Brook trout from the two Lake Superior tributaries (Pilgrim and Salmon Trout Rivers) and Michigan DNR hatchery strains of brook trout (Assinica) and lake trout (Lake Superior) were moderately

variable in measures of genetic diversity including expected heterozygosity ( $H_E$  – range 0.585 to 0.777, allelic richness  $A_R$  – range 3.9 to 5.5 alleles per locus; Electronic Supplementary Material (ESM Table S1)). High levels of variation in allele frequency were observed among all wild and hatchery strains of brook trout and lake trout (mean  $F_{st} = 0.274$ ,  $P < 0.001$ ; ESM Table S2). Levels of genetic differentiation among the parental hatchery brook trout strain and brook trout populations from the Pilgrim and Salmon Trout Rivers were likewise high (mean  $F_{st} = 0.117$  and  $0.131$ ,  $P < 0.001$ , respectively). Such high levels of inter-population variance in allele frequency indicated that individual population assignments can be made with high accuracy (Latch et al., 2006). Allele frequencies of known splake from the Michigan DNR hatchery program were intermediate to Assinica brook trout and Lake Superior strain lake trout used in the splake crosses (ESM Table S1).

Analyses using the admixture models in BAPS and STRUCTURE revealed that the number of genetic clusters most consistent with the data was  $K = 3$  (ESM Figure S1). One genetic cluster with a high posterior probability of assignment consisted of stream resident wild brook trout from the Pilgrim and Salmon Trout Rivers (Fig. 2). One cluster was associated with the Assinica Michigan DNR hatchery broodstock of brook trout used in the splake program (mean posterior probability 0.99; Fig. 2), and a third cluster was associated with the Michigan DNR Lake Superior hatchery lake trout strain used in the splake program (mean posterior probability 0.99; Fig. 2). All splake possessed introgressed (lake trout and brook trout) multi-locus genotypes (Fig. 2). Four putative splake possessed multi-locus genotypes indicating possible past introgression between brook trout and lake trout. Program BAPS confirmed that there was statistical support ( $P < 0.05$ ) for these four putative splake being Assinica brook trout–Lake Superior lake trout hybrids (Fig. 2). One of four of these hybrid fish was captured in the Pilgrim River and three of 11 putative splake were captured in the Salmon Trout River. Similarly, the known hatchery splake that served as a validation set of fish were likewise confirmed statistically using program BAPS to be admixed ( $P < 0.05$ ). Additional Bayesian modeling indicated that the highest posterior distribution for each hybrid was associated with the F1 genotype category (0.863,



**Fig. 2.** Posterior probabilities of assignment to each of three inferred genetic clusters for individuals from different wild and hatchery samples. Inferred genetic clusters are hatchery brook trout (white), hatchery lake trout (black), and wild brook trout from Pilgrim and Salmon Trout rivers (gray). Asterisks indicate individuals confirmed to be hybrids. One putative splake examined was from the Pilgrim River and three were from the Salmon Trout River. BT = brook trout, LT = lake trout.

0.809, 0.904, and 0.475 for individuals indicated by asterisks in left to right order; Fig. 2).

The genotype of one individual from the Salmon Trout River had a high posterior probability of assignment as an Assinica brook trout (Fig. 2). Because there is no brook trout stocking in the Salmon Trout River this indicates straying of stocked hatchery brook trout from other locations into the Salmon Trout River. Several brook trout from the Pilgrim River had genotypes indicating possible past introgression of hatchery Assinica brook trout (Fig. 2). However, the estimated posterior distribution associated with brook trout backcross category were all <0.03 for all other resident brook trout from the Pilgrim and Salmon Trout Rivers.

Based on 20 years of GLIFWC sampling data at lake trout spawning reefs near Copper Harbor during the fall lake trout spawning season, 1315 lake trout and 609 splake have been captured (Table 1). During this period, examination of reproductive condition indicated that on average 56% of captured splake were sexually mature and 28% of those were either ripe or had completed spawning. Splake began appearing on the spawning reef outside of Copper Harbor (Fig. 1) in 1992 and in some years splake catch exceeded lake trout catch at the spawning reefs. In addition, the number of splake classified as mature, ripe or spent has also exceeded the number of lake trout in some years (Great Lakes Indian Fish and Wildlife Commission, unpublished data).

## Discussion

The results of genetic analysis confirm that splake are appearing in brook trout spawning streams, which opens the possibility of interbreeding between splake and brook trout. Observational data from lake trout spawning assessments at Copper Harbor indicated that splake and lake trout in spawning condition are also intermixed, which opens the possibility of interbreeding between splake and lake trout. Also, mark and recapture data show that splake tagged at Copper Harbor have been recaptured as far away as Bete Grise Bay, the Montreal River and Black River Harbor (Great Lakes Indian Fish and Wildlife Commission, unpublished data).

**Table 1**

Summary of lake trout and splake captures in assessment fisheries on spawning reefs at Copper Harbor, in Michigan waters of Lake Superior.

Location	Year	Number of fish captured		Number mature	
		Lake trout	Splake	Lake trout	Splake
	1987	139		26	
	1988	110		84	
	1989	69		46	
	1990	81		59	
	1992	84	1	68	0
	1993	5		5	
	1994	57		36	
	1997	83	7	69	5
	1998	80	18	67	4
	1999	48	10	0	0
	2002	67	8	46	5
	2003	76	14	43	10
	2005	118	12	29	5
	2007	27	3	10	1
	2009	37	66	29	53
	2010	22	78	19	55
Copper Harbor—outside	2013	10	1	10	1
	1999	25	6		
	2000	68	10	37	8
	2002	12	0	9	0
	2007	55	152	2	18
	2008	22	19	4	6
	2012	5	56	5	45
Copper Harbor—inside	2013	15	148	13	123

Results from this genetic analysis of putative splake in brook trout tributaries of Lake Superior is counter to *Berst and Spangler (1970)* who concluded that F1 splake stay near the vicinity of stocking sites. The Pilgrim River is approximately 41 km from the nearest splake stocking site and the Salmon Trout River is 48 km from the nearest splake stocking site. The appearance of splake in these areas suggests that stocked splake are dispersing widely from the stocking sites. The appearance of splake in upstream habitat of spawning brook trout streams is particularly troubling for ongoing efforts to conserve and restore populations of coaster brook trout because the Salmon Trout River hosts the last known and verified native adfluvial population of coaster brook trout along the south central shore of Lake Superior. The larger size (*Ayles, 1974*) and potentially higher competitive ability of splake relative to stream brook trout could result in successful backcrossing of splake with brook trout in tributaries where they occur, though we could not document backcrossing in our limited samples.

*Buss and Wright (1958)* demonstrated that a backcross of female brook trout to male splake results in viable progeny. The viability of these eggs is lower than eggs from a pure brook trout cross. From our results, we cannot demonstrate that splake have backcrossed into parental lines. Posterior distributions associated with four identified hybrids (Fig. 2) were highest for the F1 genotype class. We only have definitive evidence that splake are appearing in brook trout spawning areas in the Pilgrim and Salmon Trout rivers, and on lake trout spawning reefs in spawning condition along with lake trout. The genetic analysis, with the number of loci employed, does not conclude that splake are backcrossing into parental lines, but it does confirm that pre-zygotic barriers are not as strong as previously hypothesized when stocked fish are present.

Importantly, not all fish reported to be intermediate phenotype (i.e., putative splake) were genetically confirmed to be splake. Eleven of 15 fish that were suspected to be splake were in fact brook trout (Fig. 2), which raises the question accuracy of public accounts of the magnitude of straying. Direct monitoring efforts by trained agency staff and researchers may be the most appropriate means of quantifying the geographic extent and relative abundance of splake on brook trout and lake trout spawning areas. Further, our results speak to the importance of using genetic data to confirm direct observations.

## Conclusion

The results demonstrate that splake are dispersing widely from Lake Superior stocking locations and are ascending streams that support valuable wild brook trout populations. In addition, splake are spawning on multiple lake trout spawning reefs in Lake Superior, and in some years, splake catch rates equal those for lake trout. Because previous work has shown that splake are capable of backcrossing with brook trout and producing viable offspring, management efforts to release splake into near-shore waters of Lake Superior near native brook trout streams, including the natural population of coaster brook trout should be re-evaluated. Ultimately, the biological and social benefits and costs of any management program must be weighed in order to prescribe future management actions. However, data reported here speak to the need for greater attention to current breeding and stocking practices to ensure that future stocking efforts do not affect the integrity of native brook trout or lake trout stocks or compromise manager's abilities to enforce protective regulations for brook trout.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.jglr.2016.03.006>.

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