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Genetic identification of two putative world record Michigan salmonids resolves stakeholder and manager questions

Jared J. Homola^{a,*}, Jeannette Kanefsky^{a,1}, Kim T. Scribner^{a,b,2}, Todd G. Kalish^{d,3}, Mark A. Tonello^{c,3}^a Department of Fisheries and Wildlife, Michigan State University, 27 Natural Resources Building, East Lansing, MI 48824, USA^b Department of Zoology, Michigan State University, 13 Natural Resources Building, East Lansing, MI 48824, USA^c Michigan Department of Natural Resources, 8015 Mackinaw Trail, Cadillac, MI 49601, USA^d Michigan Department of Natural Resources, PO Box 30028, Lansing, MI 48909, USA

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ABSTRACT

The ability of fishery managers to quickly and effectively answer stakeholder questions using the best available science is crucial for successful management. The 2009 capture of a potential world record brown trout (*Salmo trutta*) and the 2010 capture of a potential world record land-locked Atlantic salmon (*S. salar*) in Michigan required managers to acquire genetic verification of the species identity. Given the variety of hatchery strains used to maintain Great Lakes fisheries for brown trout and in the absence of physical markings, managers also were interested to determine the strain of origin for information on assessing performance. DNA barcoding techniques using sequences from the mitochondrial (mt) DNA cytochrome c oxidase I (COI) gene and frequency-based analysis of species-specific microsatellite genotypes provided data to establish the species of both fish. The putative brown trout was confirmed to be a new world record specimen. Using individual assignment tests based on maximum likelihood estimators informed by multi-locus microsatellite genotypes, we determined the fish to be from the Seeforellen hatchery strain ($p < 0.01$). Analysis of the COI gene in the putative Atlantic salmon resulted in assignment as a brown trout. The presence of only brown trout alleles at all six microsatellite loci examined revealed that the individual was not an inter-specific hybrid. Given sufficient genetic divergence exists among species, populations, or hatchery strains, the combination of mtDNA barcoding and microsatellite genetic analysis can provide accurate and rapid identification to address stakeholder and management questions.

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Introduction

Fishery managers increasingly are able to use new and innovative techniques to address management questions and to convey scientifically-based information to the public. A manager's ability to convey accurate information to stakeholders in a timely fashion is important to maintain public support for aquatic resource management. When managers can effectively meet the needs of the angling public, angler satisfaction and regulation compliance are likely to increase (Roell and DiStefano, 2010; Spencer, 1993).

Genetic data increasingly are being used to answer questions of interest to fishery managers and stakeholders. Researchers have increased their understanding of fish movements (Burger et al.,

2000), range boundaries (Wenburg and Bentzen, 2001), and effects of anthropogenic activities on aquatic systems (Neraas and Spruell, 2001) using molecular tools and recently developed statistical methods. Tools including microsatellite markers have been used extensively to gain information on broodstock identification (Colbourne et al., 1996), dispersal patterns (Homola et al., 2010), and population dynamics (Cegelski et al., 2006).

Phenotypic plasticity among individuals and life history phases often leads to inaccurate species identification when only morphology is considered (Hebert et al., 2003). Consequently, taxonomic assignments increasingly have become based on DNA sequence analysis (Ward et al., 2009). Over the past decade, DNA barcoding has become advocated as a means of identifying species (Hebert et al., 2003; Hubert et al., 2008; Ward et al., 2005). Barcoding techniques have been shown to separate 93–98% of known fish species, and have been used to characterize over 5000 fish species (Ward et al., 2009), although successful discrimination requires sufficient genetic divergence and lack of introgression (Hubert et al., 2008). The concurrent use of DNA barcoding and multi-locus microsatellite techniques could increase confidence in species assignment, as well as determine population (or hatchery strain) of origin, parentage

* Corresponding author at: Annis Water Resources Institute, Grand Valley State University, 740 West Shoreline Drive, Muskegon, MI 49441, USA.

E-mail addresses: homolaj@mail.gvsu.edu (J.J. Homola), kanefsk1@msu.edu (J. Kanefsky), scribne3@msu.edu (K.T. Scribner), kalisht@michigan.gov (T.G. Kalish), tonellom@michigan.gov (M.A. Tonello).

¹ Tel.: +1 517 432 4935.

² Tel.: +1 517 353 3288.

³ Tel.: +1 517 373 1282.

(Jones and Ardren, 2003), and assess possible hybridization (Vähä and Primmer, 2006).

The accuracy of species assignment especially is crucial in cases involving the identification of potential world record specimens. One such case was the 2009 capture of an 18.8 kg putative brown trout (*Salmo trutta*) in Michigan's Manistee River. News of the catch received international attention (e.g. IGFA, 2010), and required state fisheries biologists to verify species identity. Morphological examination initially was used to identify the fish as a world record brown trout (WRBT) before genetic confirmation could be obtained. Several months later, another potential world record fish was caught in Michigan waters. This putative world record land-locked Atlantic salmon (WRLAS, *S. salar*) was harvested from Torch Lake in Antrim County. Morphological examination using common characteristics of each potential species (Table 1) failed to conclusively identify the species since both Atlantic salmon and brown trout characters were observed and the individual was filleted prior to being examined by biologists. Since traits of both species were present, biologists suspected the individual could have resulted from the hybridization of an Atlantic salmon with a brown trout since both were previously stocked into Torch Lake (MDNR, 2001–2005).

The purpose of this study was to genetically identify the species and strain of two potential world record fish and examine each individual for genetic evidence of inter-specific and inter-strain hybridization. Additionally, we examined the relevance of our findings in contexts relevant to managers and stakeholders. Applications for the simultaneous use of multiple genetic markers also are suggested.

Materials and methods

Study area and sample collection

The putative WRBT was captured in September 2009 on hook and line in the Manistee River, near its confluence with Bear Creek (44°17'38" N, 86°07'12" W) in the northwestern portion of Michigan's Lower Peninsula. The Manistee River is 373 km in length and drains into Manistee Lake before reaching eastern Lake Michigan (Chiotti et al., 2008). The putative WRLAS was captured in March 2010 on hook and line while trolling in Torch Lake (44°57'39" N, 85°18'15" W), a large (7597 ha) and deep (maximum depth 91 m) oligotrophic lake in the Lake Michigan watershed that is located in the northwestern portion of Michigan's Lower Peninsula (Colby and Washburn, 1972). Migration from Lake Michigan into Torch Lake is impeded by a dam in the village of Elk Rapids. Samples were collected from both individuals and stored dry for genetic analysis following phenotypic examination.

Samples used to establish microsatellite genotype baselines for Atlantic salmon were supplied by the Aquatic Research Laboratory at Lake Superior State University. Brown trout broodstock samples for each of the stocked strains (Seeforellen, Gilchrist Creek, and Wild

Rose) were supplied by Michigan Department of Natural Resources (MDNR) hatcheries.

Field analysis

A variety of techniques were used to identify each fish morphologically, including taxonomic keys ("Key to Atlantic Salmon and Brown Trout Identification" [MDNR internal memo, unpublished, N.E. Fogle, 1981, Lansing, MI] and "Key to Salmonidae of Michigan Waters" [MDNR internal memo, unpublished, date and author unknown]), distribution documentation, and historic stocking information. Visually observable morphological characteristics that were used in attempts to determine the species of each individual in the field are detailed in Table 1 (Schrouder, 1975; Scott and Crossman, 1973).

Laboratory analysis

Tissue samples consisting of six scales and an adipose fin sample were provided from the putative WRBT and putative WRLAS specimens, respectively. Adipose fin samples were used to establish brown trout ($n = 294$) and Atlantic salmon ($n = 51$) baselines. All samples were subjected to overnight digestion with proteinase K, followed by a standard phenol/chloroform/isoamyl alcohol DNA purification protocol (Sambrook et al., 1989) for the scale samples or the manufacturer's recommended protocol of the DNeasy Blood and Tissue Kit, (Qiagen, Valencia CA), which utilizes a silica-based spin column method, for the fin sample. The purified DNA was quantified using a Nanodrop ND-1000 Spectrophotometer (Thermo Scientific, Waltham MA) and diluted to a concentration of 20 ng/ul for use in polymerase chain reactions (PCR).

Species assignment analysis was conducted using DNA barcoding, a technique that examines a short region of the mtDNA cytochrome c oxidase I gene (COI). DNA barcoding standardized sequences can assign the species of an individual since genetic variation across species is more pronounced than among individuals within a species (Hebert et al., 2003).

An approximately 700 base pair (bp) region of the COI gene was PCR amplified from genomic DNA isolated from the two specimens using a primer cocktail consisting of four primers, each at a concentration of 5 pmol/ul as described by Ivanova et al. (2007). Reactions were carried out in 25 μ l volumes consisting of 5% filter sterilized trehalose, 200 μ M of each dNTP, 2.5 mM MgCl₂, 5 pmol of each primer, 0.7 unit of ChromaTaq™ DNA polymerase, 1 \times ChromaTaq™ PCR buffer (Denville Scientific, Metuchen, NJ) and 100 ng of template DNA. Reagent concentrations and volumes were slightly modified from those detailed in Ivanova et al. (2007) and thermocycling conditions presented in Ivanova et al. (2007) were used as described. Products were visualized on a 1% agarose gel using ethidium bromide staining.

The PCR products were cleaned with a Qiagen Qiaquick PCR purification kit (Qiagen, Valencia, CA) to remove unincorporated nucleotides and primers. The amplification primers contain 5' M13 sequence tails that allow the products to be sequenced with the corresponding forward and reverse M13 sequencing primers (Messing, 1983). Gene sequences were determined from both strands by automated fluorescent DNA cycle sequencing using the M13 primers and the Big Dye Terminator kit v3.1 (Applied Biosystems, Foster City CA), and visualized on an ABI 3730xl DNA Sequencer (Applied Biosystems, Foster City CA). All sequencing was carried out at the Michigan State University Research Technology Support Facility. Electropherograms were visually inspected for signal strength and sequence quality. Sequences then were aligned by eye and trimmed using the computer program MEGA4 (Tamura et al., 2007). A total of 541 nucleotides of reliable sequence were obtained from each sample and these were queried against the GenBank nucleotide database using the BLAST algorithm (<http://blast.ncbi.nlm.nih.gov/>; Altschul et al., 1990).

Table 1

Differentiated morphological characteristics examined for determination of species between Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) prior to genetic analyses.

Characteristic	Atlantic salmon	Brown trout
Gill rakers on first arch	18–24	14–20
Tongue shape	Fairly pointed	Square
Vomerine teeth ^a	Single row; poorly developed	Two rows; well developed
Maxillary	Usually extends to rear edge of eye or slightly beyond	Usually extends well beyond rear edge of eye
Dorsal fin rays	Usually 11	Usually 9
Caudal peduncle	Narrow and tapered	Thick and stocky
Caudal fin	May be slightly forked	Square and not forked
Adipose fin	May not be spotted	Typically spotted

^a Vomerine teeth characteristics are difficult to ascertain in the field and typically require microscopic examination for definitive results.

To assess potential inter-specific hybridization and assignment provided by DNA barcoding and to determine the strain of the putative WRBT and WRLAS, we performed a second analysis using six variable microsatellite loci. All individuals were genotyped at the following loci: *Ssa197*, *Ssa85* (O'Reilly et al., 1996), *Omy301* (Estoup et al., 1993), *One9* (Scribner et al., 1996), *Sfo1* (Angers et al., 1995), and *Ogo2* (Olsen et al., 1998). PCR was conducted in 25 μ l volumes containing 100 ng of template DNA, 2.5 μ l of LGL buffer (0.1 M Tris-HCl, 15 mM MgCl₂, 0.5 M KCl, 0.1 mg/ml bovine serum albumin, 0.025% Tween-20), 10 pmol fluorescently labeled forward and unlabeled reverse primers, sterile water, 0.5 U Taq polymerase and either 80 μ M (*Ssa197*, *Omy301*, *Ssa85*, *Sfo1*), 120 μ M (*One9*), or 160 μ M (*Ogo2*) of each deoxy-nucleotide-triphosphate (dNTP) was added. PCR was performed using Robocycler 96 thermocyclers (Stratagene, Inc., La Jolla, CA) and the following amplification process: 94 °C for 2 min for 1 cycle, followed by 94 °C for 30 s, locus-specific annealing temperature (56 °C for *Ssa197*, *Omy301*, *One9*, *Sfo1*, and *Ogo2*, and 50 °C for *Ssa85*) for 30 s, and 72 °C for 30 s for 30 cycles, then 72 °C for 5 min for 1 cycle. Amplified PCR products were visualized on 6% denatured polyacrylamide gels using an FMBIO II scanner (Hitachi Software Engineering Co., Ltd., Yokohama, Japan). Allele size was determined by comparison to brown trout samples of known genotype and molecular size standards. Genotype scores were confirmed independently by two laboratory personnel.

Statistical analysis

Allelic frequencies for each species and hatchery strain were estimated individually and further examined using F_{IS} estimates from GENEPOP (v4, Rousset, 2008). Mean allelic richness for each population was estimated using FSTAT (version 2.9.3.1, Goudet, 2001) to compare genetic diversity across sample groups of differing sizes. Genetic diversity was evaluated by estimating observed (H_o) and expected (H_e) heterozygosity within samples, per locus, and averaged over the loci using GENEPOP.

Verification of species assignments from DNA barcoding techniques and hatchery strain assignments was performed using the frequency-based method described in Paetkau et al. (1995) using GENECLASS (v2.0.h, Cornuet et al., 1999). Known brown trout and Atlantic salmon were used as baselines when assigning the putative WRBT and WRLAS to a species. Individuals from each of the three hatchery strains of brown trout (Seeforellen $n = 119$, Gilchrist Creek $n = 60$, and Wild Rose $n = 115$) were used to establish genetic baselines to facilitate strain assignment.

Stocking records provided by the MDNR were used to assess probabilities of each species being captured, as well as each specific strain. We calculated respective probabilities based on the number of individuals stocked from each strain from 2002 to 2009. These dates are consistent with expected age of brown trout in Michigan, as indicated by their age frequency distribution ($n = 1939$, from 1999 to 2009; Tracy Kolb, MDNR, personal communication). For the Manistee River, we included all counties in the Michigan watershed of Lake Michigan and those stocked just into the Manistee River. For the Torch Lake putative WRLAS, we examined stocking records for each strain of brown trout and Atlantic salmon released into the lake itself, or its connecting waters.

Results

Sufficient genetic diversity within and among species and strains required for assignment testing was observed for each of the three brown trout hatchery strains as well as with the Atlantic salmon (Appendix 1). Mean allelic richness ranged from 5.20 (Wild Rose strain brown trout) to 8.01 (Seeforellen strain brown trout), and estimates of expected heterozygosity (H_e) ranged from 0.462 (Atlantic salmon) to 0.753 (Seeforellen strain brown trout). Atlantic salmon

did not amplify at loci *Ogo2* and *Sfo1*, presumably because those loci were developed for other genera (pink salmon, *Oncorhynchus gorbuscha* and brook trout, *Salvelinus fontinalis*, respectively).

The nucleotide sequence obtained from the putative WRBT for use in DNA barcoding was a 100% match (identical) to regions of six existing GenBank sequences (matching 541/541 possible nucleotide sites in the putative WRBT query sequence, with no gaps). Because the six alignments between our query and the top hit sequences were identical, each had identical bit scores (1000) and expect values (0). The bit score is assigned to the alignment of the query sequence and the matching hit sequence; a higher score indicates a better alignment. The expect value (E) indicates the number of matching sequences expected to be observed by chance when searching a sequence database of a specific size, and reflects background noise. The closer the expect value is to zero, the more significant the match. All six perfect matches (GenBank accession numbers FJ907951, EU524356, EU524355, EU524354, AM910409, and EF609450) were identified as COI sequences from *Salmo trutta*, the brown trout. The number of mismatches (37/537) between the top matching brown trout and Atlantic salmon COI sequences from GenBank indicates that sufficient sequence divergence has occurred in the COI genes of these two species to allow unambiguous discrimination. Frequency-based assignment techniques informed by six microsatellite loci also determined the individual to be a brown trout with 100% certainty. Further analysis of microsatellite loci using the program GENECLASS revealed that the WRBT was Seeforellen strain with 99.87% certainty.

The sequence obtained from the putative WRLAS was identical to that of the WRBT, therefore resulting in identical GenBank query results. Microsatellite genotyping of the putative WRLAS revealed species-specific brown trout alleles at all six loci (Appendix 1) and frequency based assignment probabilities also supported the DNA barcoding assignment as a brown trout with 100% confidence. Conclusive determination of strain was unsuccessful, with Seeforellen strain receiving the highest probability (61.1%), followed by Gilchrist Creek (21.8%), and Wild Rose (17.1%).

Strains of brown trout stocked from 2002 to 2009 by the MDNR into the study areas included Seeforellen (2002–2008), Gilchrist Creek (2002–2009) and Wild Rose (2002–2009). From 2002 to 2009, the MDNR released 66,062 (5.18%) Seeforellen strain brown trout, 200,555 (15.72%) Gilchrist Creek strain, and 1,009,403 (79.1%) Wild Rose strain into the Manistee River. When expanding our search to all individuals stocked into Michigan counties in the Lake Michigan watershed, 3,189,786 (33.5%) of Seeforellen strain, 2,562,252 (26.91%) Gilchrist Creek strain, and 3,768,491 (39.59%) Wild Rose strain were stocked from 2002 to 2009. No stocking of brown trout occurred in Torch Lake from 2002 to 2009, however 231,181 Atlantic salmon were released in the lake during that time.

Discussion

Through the use of routine genetic laboratory and analytical techniques, this study was able to promptly answer questions of immediate importance to managers and stakeholders. The detailed information gained through DNA barcoding and microsatellite genetic analysis offered high certainty when traditional morphologically-based taxonomic techniques failed to provide similar levels of confidence. In management scenarios where accurate species identification is essential, especially during instances of international attention or potentially large economic benefit, such as the capture of a world record fish, the methods described here should be employed.

The assignment of the WRBT to the Seeforellen strain was unexpected since relatively few fish from this strain were stocked in the Manistee River and the strain has exhibited reduced survivorship in Michigan waters (Wills, 2006). In the river of capture, Gilchrist

Creek strain brown trout were stocked at a frequency greater than three times that of Seeforellen and Wild Rose at a more than 15 times greater rate than Seeforellen. Wills (2006) found Gilchrist Creek strain brown trout survived at a rate more than 100 times that of Seeforellen strain and more than six times greater than Wild Rose. The putative WRLAS functions as an example of how phenotypic plasticity can potentially misinform taxonomic classification based on traditional morphometric data; however, the individual's strain could not be determined conclusively.

Concurrent use of DNA barcoding and analysis of microsatellite genetic markers is a valuable approach that has many beneficial uses to fishery managers and stakeholders, in addition to the species identification use described earlier. One possible use for these techniques is to investigate the consequences of inter-strain hybridization on the fitness of progeny. Genetic techniques can be used to detect hybridization and distinguish parental strains, clearing the way for more in-depth queries involving the success and fitness of certain crosses. Should the offspring of a specific cross of strains prove to be less fit, managers could choose to avoid stocking the two parental strains in connected waters, consequently maximizing stakeholder benefits from stocking efforts. Another application for the ability to accurately discern the species and strain of wild caught fish using molecular techniques is to quantify the success of stocking programs. This especially is true in situations where hybrid individuals are stocked (e.g., splake (*Salvelinus namaycush* X *Salvelinus fontinalis*)), and morphology is not always reliable for distinguishing species. Another example is if natural reproduction occurs in a stocked species that cannot be clearly distinguish, whereas the stocked individuals may have been tagged (clips, coded wire tags, etc.; Page et al., 2005). The ability to attribute the progeny of stocked strains to their respective lineages could result in long-range assessments of the benefits of stocking various strains.

Supplementary materials related to this article can be found online at doi:10.1016/j.jglr.2011.11.011.

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References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. *J. Mol. Biol.* 215, 403–410.

Angers, B., Bernatchez, L., Angers, A., Desgroseillers, L., 1995. Specific microsatellite loci for brook charr reveal strong population subdivision on a microgeographic scale. *J. Fish Biol.* 47, 177–185.

Burger, C.V., Scribner, K.T., Spearman, W.J., Swanton, C.O., Campton, D.E., 2000. Genetic contribution of three introduced life history forms of sockeye salmon to colonization of Frazer Lake, Alaska. *Can. J. Fish. Aquat. Sci.* 57, 2096–2111.

Cegelski, C.C., Campbell, M.R., Meyer, K.A., Powell, M.S., 2006. Multiscale genetic structure of Yellowstone cutthroat trout in the Upper Snake River basin. *Trans. Am. Fish. Soc.* 135, 711–726.

Chiotti, J.A., Holtgren, J.M., Auer, N.A., Ogren, S.A., 2008. Lake sturgeon spawning habitat in the Big Manistee River, Michigan. *J. Fish. Manage.* 28, 1009–1019.

Colbourne, J.K., Neff, B.D., Wright, J.M., Gross, M.R., 1996. DNA fingerprinting of bluegill sunfish (*Lepomis macrochirus*) using (GT)_n microsatellites and its potential for assessment of mating success. *Can. J. Fish. Aquat. Sci.* 53, 342–349.

Colby, P.J., Washburn, G.N., 1972. Feeding behavior of Lake Whitefish and Lake Herring in Torch Lake, Michigan. *Progr. Fish-Cult.* 34, 151.

Cornuet, J.-M., Piry, S., Luikart, G., Estoup, A., Solignac, M., 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics* 153, 1989–2000.

Estoup, A., Presa, P., Krieg, F., Guyomard, R., 1993. CT_n and (GT)_n microsatellites: a new class of genetic markers for *Salmo trutta* L. (brown trout). *Heredity* 71, 488–496.

Goudet, J., 2001. FSTAT (version 2.9.3): a program to estimate and test gene diversities and fixation indices. (Available from) <http://www.unil.ch/izea/software/fstat.html>.

Hebert, P.D.N., Cywinska, A., Ball, S.L., deWaard, J.R., 2003. Biological identification through DNA barcodes. *Proc. R. Soc. Lond. B Biol. Sci.* 270, 313–321.

Homola, J.J., Scribner, K.T., Baker, E.A., Auer, N.A., 2010. Genetic assessment of straying rates of wild and hatchery reared lake sturgeon (*Acipenser fulvescens*) in Lake Superior tributaries. *J. Great Lakes Res.* 36, 798–802.

Hubert, N., Hanner, R., Holm, E., Mandrak, N.E., Taylor, E., Burridge, M., Watkinson, D., Dumont, P., Curry, A., Bentzen, P., Zhang, J., April, J., Bernatchez, L., 2008. Identifying Canadian freshwater fishes through DNA barcodes. *PLoS One* 3 (6), e2490.

International Game Fish Association (IGFA), 2010. New all-tackle brown trout world record: 41 lb 7 oz (18.8 kg). <http://www.igfa.org/News/post/New-All-Tackle-Brown-Trout-World-Record-41-lb-7-oz-%28188-kg%29.aspx>2010 (Accessed on 24 June 2011).

Ivanova, N.V., Zemlak, T.S., Hanner, R.H., Hebert, P.D.N., 2007. Universal primer cocktails for fish DNA barcoding. *Mol. Ecol. Notes* 5, 544–548.

Jones, A.G., Ardren, W.R., 2003. Methods of parentage analysis in natural populations. *Mol. Ecol.* 12, 2511–2523.

Messing, J., 1983. New M13 vectors for cloning. *Methods Enzymol.* 101, 20–78.

Michigan Department of Natural Resources (MDNR), 2001–2005. Fish stocking database. <http://www.michigandnr.com/fishstock/2001–2005> (Accessed on 24 June 2011).

Neraas, L.P., Spruell, P., 2001. Fragmentation of riverine systems: the genetic effects of dams on bull trout (*Salvelinus confluentus*) in the Clark Fork River system. *Mol. Ecol.* 10, 1153–1164.

O'Reilly, P.T., Hamilton, L.C., McConnell, S.K., Wright, J.M., 1996. Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. *Can. J. Fish. Aquat. Sci.* 53, 2292–2298.

Olsen, J.B., Bentzen, P., Seeb, J.E., 1998. Characterization of seven microsatellite loci derived from pink salmon. *Mol. Ecol.* 7, 1087–1089.

Paetkau, D., Calvert, W., Stirling, I., Strobeck, C., 1995. Microsatellite analysis of population structure in Canadian polar bears. *Mol. Ecol.* 4, 347–354.

Page, K.S., Scribner, K.T., Bast, D., Holey, M., Burnham-Curtis, M., 2005. Influences on the genetic diversity in the lake trout hatchery program used for restoration efforts in the upper Great Lakes. *Trans. Am. Fish. Soc.* 105, 872–891.

Roell, M.J., DiStefano, R.J., 2010. Effects of a conservative rock bass length limit on angler participation, sport fish populations, and crayfish prey in a Missouri Ozark stream. *N. Am. J. Fish. Manage.* 30, 552–564.

Rousset, F., 2008. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Mol. Ecol. Resources* 8, 103–106.

Sambrook, J., Fritsch, E., Maniatis, T., 1989. *Molecular Cloning, a Laboratory Manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Schrouder, J., 1975. *The Atlantic salmon in Michigan*. Michigan Department of Natural Resources, Fisheries Division pamphlet No. 43.

Scott, W.B., Crossman, E.J., 1973. *Freshwater Fishes of Canada*. Fisheries Research Board of Canada, Ottawa, Ontario, Canada.

Scribner, K.T., Gust, J.R., Fields, R.L., 1996. Isolation and characterization of novel salmon microsatellite loci: cross-species amplification and population genetic applications. *Can. J. Fish. Aquat. Sci.* 53, 833–841.

Spencer, P.D., 1993. Factors influencing satisfaction of anglers on Lake Miltona, Minnesota. *N. Am. J. Fish. Manage.* 13, 201–209.

Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* 24, 1596–1599.

Vähä, J.-P., Primmer, C.R., 2006. Efficiency of model-based Bayesian methods for detecting hybrid individuals under different hybridization scenarios and with different numbers of loci. *Mol. Ecol.* 15, 63–72.

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., Hebert, P.D.N., 2005. DNA barcoding Australia's fish species. *Philos. Trans. R. Soc. Lond. Biol. Sci.* 360, 1847–1857.

Ward, R.D., Hanner, R., Hebert, P.D.N., 2009. The campaign to DNA barcode all fishes FISH-BOL. *J. Fish Biol.* 74, 329–356.

Wenburger, J.K., Bentzen, P., 2001. Genetic and behavioral evidence for restricted gene flow among coastal cutthroat trout populations. *Trans. Am. Fish. Soc.* 130, 1049–1069.

Wills, T.C., 2006. Comparative abundance, survival, and growth of one wild and two domestic brown trout strains stocked in Michigan rivers. *N. Am. J. Fish. Manage.* 26, 535–544.