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Notes

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

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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 interspecific 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.

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

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(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.

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.

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/μl as described by Ivanova et al. (2007). Reactions were carried out in 25 μl volumes consisting of 5% filter sterilized trehalose, 200 μM of each dNTP, 2.5 mM $MgCl_2$, 5 pmol of each primer, 0.7 unit of ChromaTaq™ DNA polymerase, 1 × 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).