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TECHNICAL NOTE

An environmental DNA marker for detecting nonnative brown trout (*Salmo trutta*)

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Abstract Brown trout (*Salmo trutta*) are widely introduced in western North America where their presence has led to declines of several native species. To assist conservation efforts aimed at early detection and eradication of this species, we developed a quantitative PCR marker to detect the presence of brown trout DNA in environmental samples. The marker strongly amplified brown trout eDNA, and produced no amplification of eDNA from 17 other species commonly found in western North America. We field tested this marker and demonstrated positive detections in field samples where brown trout presence was known.

Keywords Salmonids eDNA · Invasive species · qPCR

Brown trout (*Salmo trutta*), salmonid fish native to Europe, northern Africa, and northern and central Asia, have been widely introduced across the globe, including North America. In the western U.S., their presence is associated with the decline of native fish species, including Bonneville cutthroat trout (*Oncorhynchus clarkii utah*) and Apache trout (*O. gilae apache*) (McHugh and Budy 2005; U.S. Fish and Wildlife Service 2009). Determining the presence and distribution of nonnative brown trout is critical for recovery efforts aimed at protecting native fish species or for evaluating the efficacy of chemical and mechanical removal of brown trout before reintroduction of native fish populations. Environmental DNA (eDNA) sampling is gaining popularity as a sensitive method for detection of rare or invading aquatic species (Biggs et al. 2015; Dejean et al., 2012; Siggsgaard et al. 2015; Rees et al. 2014; Wilcox et al. 2016) and for delimiting species distributions (McKelvey et al. 2016). Here, we describe a quantitative PCR (qPCR) marker for eDNA-based detection of brown trout.

First, we compiled DNA sequences published on GenBank of the cytochrome b (*cytb*) region of the mitochondrial genome for brown trout and 13 other salmonids commonly found in the western U.S. (Table S1). We used these sequences and the *DECIPHER* package (Wright et al. 2013) in *R* v. 3.0.1 (R Core Team 2014) to develop a forward and reverse primer set specific to brown trout: forward primer 5′-CGCCCGAGGACTCTACTATGGT-3′; reverse primer 5′-GGAAGAACGTAGCCCACGAA-3′.

We designed a hydrolysis probe (5′-FAM-CGGAGTCG TACTGCTAC-MGBNFQ-3′) by aligning the sequences in MEGA 6.0 (Tamura et al. 2011) and identifying a region specific to brown trout. The probe identically matched seven of the eight brown trout reference sequences, and had a single base-pair mismatch with a one individual from the Adriatic lineage obtained in Turkey (GenBank accession JX960835). The probe has two base-pair mismatches with published sequences of the most-closely related species, Atlantic salmon (*Salmo salar*; GenBank accessions BT044011, FJ435618- FJ435620, and JX960833-

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JX960834.) We assessed the melting temperatures of the primers (forward: 60.4 °C; reverse: 58.8 °C) and probe (70.0 °C) in Primer Express 3.0.1 (Life Technologies). We screened the primers and probe for secondary structures using IDT OligoAnalyzer (https://www.idtdna.com/calc/analyzer).

The marker was tested against DNA extracted from brown trout tissues from 16 different locations and 17 non-target species (Table 1) in 15-µl reactions containing 7.5 µl Environmental Master Mix 2.0 (Life Technologies), 900 nM forward primer, 900 nM reverse primer, 250 nM probe, 4 µl DNA template (diluted 1:100 from extract to roughly ~ 0.5 ng/µl), and 2.75 µl deionized water. We used cycling conditions of 95 °C/10 min [95 °C/15 s, 60 °C/60 s] × 45 cycles on a StepOne Plus Real-time PCR Instrument (Life Technologies). DNA from all brown trout tissues successfully amplified, and there was no measureable fluorescence at 45 cycles for any non-target species.

We optimized primer concentrations following methods outlined in Wilcox et al. (2015) for final concentrations of 600 and 300 nM for the forward and reverse primer, respectively. We tested marker sensitivity by creating a five-level standard curve dilution series (6 250, 1 250, 250, 50, and 10 copies per 4 µl). This standard curve was created by quantifying PCR product (brown trout tissue amplified with the marker) from the above analysis on a Qubit Fluorometer (Thermo Fisher Scientific) and diluting the DNA in TE to the desired concentrations. We ran six replicates of each dilution using optimized primer concentrations to determine the standard curve slope with a resulting amplification efficiency of 99.1 % and $R^2 = 0.99$. On the same PCR plate, we observed 100 % detection across six replicates with 2 copies per 4 µl.

Finally, the marker was screened using eDNA samples collected from four western U.S. sites for which the salmonid community assemblage was known from previous electrofishing surveys (Table 2). Five-liter samples were collected following methods described in Carim et al. (2015), and extracted following a protocol modified from Goldberg et al. (2011). These samples were tested using the same PCR conditions described above, and included an internal positive control to screen for PCR inhibition. The marker performed as expected, with amplification in both samples collected where brown trout were present and in neither of the samples where brown trout were known to be absent.

A brown trout eDNA marker has already been developed for use in parts of Ireland (Gustavson et al. 2015), but was not screened against salmonids common in North America including Atlantic salmon (Tables 1 and S1). To develop the marker we described here, we screened published DNA sequences of brown trout from multiple continents and tested it against tissue and environmental samples from multiple locations throughout the western

Table 1 Species used for in vitro testing of the primers and probe

Common name	Species name	Probe testing sample size	Origin
Brown trout	*Salmo trutta*	16	AZ, MT, NM, WY
Atlantic salmon	*Salmo salar*	1	F
Lake whitefish	*Coregonis clupeiformis*	2	MT
Northern pike	*Esox lucius*	3	MT
Yellowstone cutthroat trout	*Oncorhynchus clarkii bouvieri*	2	ID, WY
Westslope cutthroat trout	*Oncorhynchus clarkii lewisi*	1	MT
Bonneville cutthroat trout	*Oncorhynchus clarkii utah*	1	H, ID
Gila trout	*Oncorhynchus gilae gilae*	4	H
Coho salmon	*Oncorhynchus kisutch*	2	AK, H
Rainbow trout	*Oncorhynchus mykiss*	3	H, ID
Sockeye salmon	*Oncorhynchus nerka*	1	ID
Chinook salmon	*Oncorhynchus tshawytscha*	2	H, WA
Mountain whitefish	*Prosopium williamsoni*	1	MT
Bull trout	*Salvelinus confluentus*	3	MT
Brook trout	*Salvelinus fontinalis*	3	ID, MT, Quebec
Dolly Varden	*Salvelinus malma*	1	AK
Lake trout	*Salvelinus namaycush*	3	MT
Arctic grayling	*Thymallus arcticus*	2	MT

Source refers to the location of wild fish (U.S. state or Canadian province) of fish of hatchery (H) or farmed (F) origin

Table 2 Collection and species assemblage information for eDNA samples used to test the brown trout marker

Waterbody (State)	Latitude	Longitude	Collection date	Salmonids present	Brown trout detected?
Rattlesnake Creek (MT)	46.94572	−113.94522	4/7/2015	BNT, BRK, BUT, RBT, WCT	Y
Copper Creek (WY)	44.71916	−107.45786	7/24/2015	BNT, BRK	Y
West Fork Lolo Creek (MT)	46.66710	−114.57758	7/23/2015	BUT, WCT	N
Black Canyon (NM)	33.17285	−107.97704	9/23/2015	GLT	N

BNT brown trout, *BRK* brook trout, *BUT* bull trout, *GLT* Gila trout, *LKT* lake trout, *LWF* lake whitefish, *MWF* mountain whitefish, *PWF* pygmy whitefish (*Prosopium coulterii*), *RBT* rainbow trout, *WCT* westslope cutthroat trout

United States. Therefore, we expect the eDNA marker described here to work for detection of introduced brown trout in much of North America, and portions of its native range elsewhere, as brown trout present in North America largely originated from Germany and Scotland (MacCrimmon and Marshall 1968).

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