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MANAGEMENT BRIEF

Validation of Morphological Characteristics Used for Field Identification of Bull Trout × Brook Trout Hybrids

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Abstract

Bull trout *Salvelinus confluentus* are of conservation concern throughout much of their range, in part because of displacement by and hybridization with nonnative brook trout *S. fontinalis*. Hybridization between these species can complicate efforts to control brook trout because hybrids are often difficult to distinguish from parental species. Here, we assess the reliability of five morphological characteristics that were used to identify bull trout × brook trout hybrids among 106 fish collected from Quirk Creek, Alberta. Genetic analysis of up to three microsatellite loci suggested that hybrids were correctly identified 95% of the time and that no bull trout were mistaken for hybrids. Visual inspection of photographs of hybrids suggested that dorsal fin markings were the most reliable morphological characteristic for identifying hybrids. Based on these results, we recommend that field identification of bull trout × brook trout hybrids be based solely on dorsal fin markings.

Bull trout *Salvelinus confluentus* are native to coastal and interior rivers in western Canada and the northwestern United States (Cavender 1978; Haas and McPhail 1991; Reist et al. 2002). Bull trout have been extirpated from numerous subbasins within their range and have declined substantially in many others (Rieman et al. 1997; Post and Johnston 2002). As a result of these declines, the bull trout is a threatened species under the U.S. Endangered Species Act (USFWS 1998, 1999) and is a species of special concern in both Alberta and British Columbia (ASRD and ACA 2009; British Columbia Conservation Data Centre 2011).

Bull trout declines are attributed to anthropogenic stressors, such as habitat degradation (Rieman et al. 1997), fragmentation (Rieman et al. 1997; Neraas and Spruell 2001), exploitation, and displacement by nonnative brook trout *S. fontinalis* (Rieman et al. 2006). The brook trout is a popular game fish that was successfully introduced throughout the bull trout's native range from the late 1800s (MacCrimmon and Campbell 1969) to the early 1990s. Displacement of bull trout by brook trout is relatively common (Buktenica 1997; Shepard et al. 2002; Stelfox et al. 2004) and is thought to result from differences in life history, environmental tolerance, competitive ability, and hybridization (see reviews by Rieman et al. 2006 and DeHaan et al. 2010).

Hybridization between these species is a practical concern because it can complicate efforts to distinguish pure bull trout from bull trout × brook trout hybrids (henceforth, "hybrids"; Buktenica 1997; Kanda 1998). In the 1970s, brook trout began spreading into Quirk Creek, a 12.7-km tributary of the Elbow River in southeastern Alberta (Figure 1; Tripp et al. 1979).

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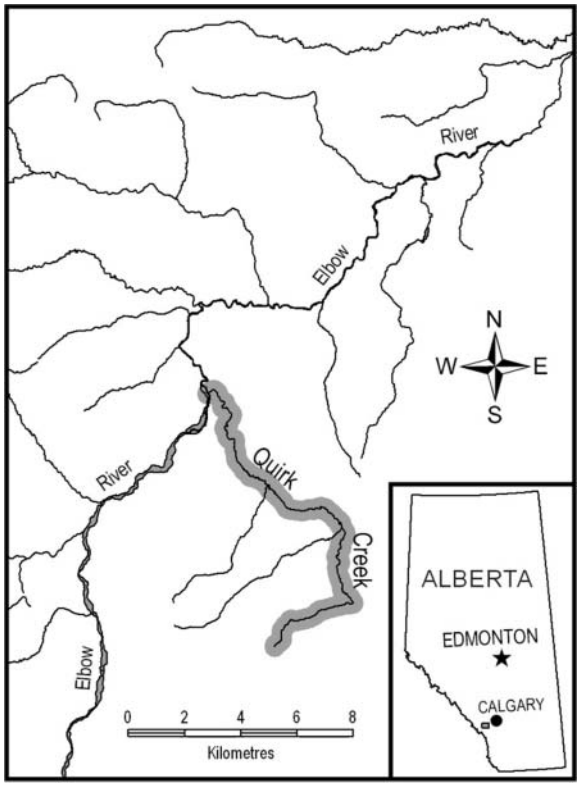

FIGURE 1. Quirk Creek (50°50′19″N, 114°46′55″W), an Elbow River (Bow River subbasin) tributary located southwest of Calgary, Alberta (inset).

By 1995, brook trout had almost completely displaced native bull trout and westslope cutthroat trout *Oncorhynchus clarkii lewisi* in Quirk Creek (Stelfox et al. 2004). In 1998, Alberta Fish and Wildlife and Trout Unlimited Canada initiated an experimental brook trout suppression project on Quirk Creek (Paul et al. 2003). Success of this project hinged on the ability of biologists and volunteer anglers to remove brook trout and their hybrids in sufficient numbers to facilitate the recovery of native bull trout and westslope cutthroat trout. Hybrids were identified by using five external morphological characteristics that were intermediate between those of the parental species. This paper addresses concerns that some of these presumed hybrids were bull trout. Specifically, we used (1) microsatellite DNA to assess whether bull trout were mistakenly identified as hybrids and consequently removed and (2) color photographs to determine which morphological characteristics were most likely to result in correct identification.

METHODS

Morphological characteristics.—After visually inspecting numerous Quirk Creek fish that appeared to be hybrids, Alberta Fish and Wildlife biologists identified five externally visible morphological characteristics that were used to identify hybrids in the field:

1. Dorsal fin markings: brook trout have distinct black markings on the dorsal fin, whereas bull trout do not. Presumed hybrids had pale spots on most of the dorsal fin (Figure 2). Pale spots on the dorsal fin have been used by others (Markle 1992; Buktenica 1997; Rieman et al. 2006) to identify hybrids. These pale spots are not to be confused with the single row of pale spots observed at the base of the dorsal fin in some populations of bull trout (e.g., North Saskatchewan River, Alberta).
2. Dorsal vermiculations: brook trout have distinct, wormlike markings on the dorsal surface of the body. These vermiculations are observed occasionally on bull trout, but when present they are much less extensive. Presumed hybrids exhibited vermiculations to a degree that was intermediate between that of bull trout and brook trout.
3. Dorsal fin shape: the shape of the dorsal fin of presumed hybrids was intermediate between the triangular dorsal fin of bull trout and the trapezoidal dorsal fin of brook trout.
4. Fin stripes: brook trout exhibit a distinct black stripe that is located immediately posterior to the white leading edges of the pectoral, pelvic, and anal fins. Bull trout have no black stripe behind the white leading edges of these fins. Fin stripes on presumed hybrids were intermediate to those observed in parental species; the stripes tended to be thin and gray rather than black.

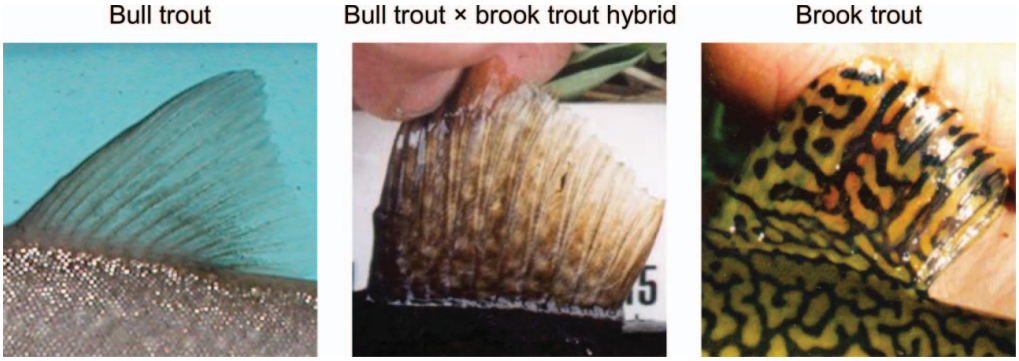

FIGURE 2. Photographs of the dorsal fins of a bull trout, bull trout × brook trout hybrid, and brook trout from Quirk Creek, Alberta, illustrating the differences in fin markings (see morphological characteristic 1 in Methods). [Figure is available in color online.]

5. Lateral spot color: the sides of brook trout exhibit red, orange, or pink spots surrounded by distinct pale-blue halos. On bull trout, these spots range in color from cream to orange, but halos are absent. Presumed hybrids tended to exhibit large orange spots within faint halos.

Sample collection.—From June to October in 1998–2003, biologists from Alberta Fish and Wildlife and Trout Unlimited Canada, and trained volunteers used angling and electrofishing to identify and selectively remove almost 8,000 presumed brook trout and hybrids from Quirk Creek (elevation = 1,530 m; Figure 1; Stelfox et al. 2004). Fin clips were collected from almost all presumed hybrids and from random samples of presumed brook trout and presumed bull trout (20–30 fish/parental species). All fin clips were dried at room temperature for at least 48 h in standard scale envelopes and were then frozen (–20°C). To document the morphological characteristics that were used in field identification, one of us (J.D.S.) photographed all of the presumed hybrids that were captured while he was on-site, and he then inspected these photographs visually to assess qualitatively both the intermediacy and reliability of each of the five morphological characteristics.

Genetics.—We used microsatellite DNA to determine the genetic identity of individuals that were assessed in the field as being bull trout, brook trout, or hybrids. Reference DNA for bull trout was obtained from a DNA library containing data collected throughout North America (E.B.T., unpublished data). Reference DNA for brook trout was extracted from specimens obtained from the Sam Livingston Fish Hatchery in Calgary, Alberta. The original broodstock from the hatchery population can be traced back to the Beity line that originated from the eastern USA during the late 1800s (J. Underwood, Alberta Sustainable Resource Development, personal communication). Genomic DNA was extracted by using Qiagen DNeasy DNA tissue extraction kits. The DNA was extracted primarily from adipose fin tissue; however, if the adipose fin sample was too small to provide the minimum required mass, the anal or caudal fin was used instead. Extracted DNA was stored at –20°C in Qiagen buffer until genetic analyses commenced.

We assayed individuals at up to three microsatellite loci: two were isolated from bull trout (*Sco110* and *Sco216*; DeHaan and Ardren 2005; S. Young, Washington Department of Fish and Wildlife, unpublished data) and one was isolated from brook trout (*Sfo18*; Angers et al. 1995). The three loci show diagnostic size frequency distributions that have been verified in over 1,000 bull trout samples and in a minimum of 50 brook trout. The diagnostic size ranges were (1) *Sfo18*: 148–160 base pairs (bp) for bull trout and 172–192 bp for brook trout; (2) *Sco110*: 191–271 bp for bull trout and 149–173 bp for brook trout; and (3) *Sco216*: 196–294 bp for bull trout and 145–165 bp for brook trout. Loci were amplified by use of polymerase chain reaction and fluorescently labeled primers (for conditions, see DeHaan and Ardren 2005) and were subsequently assayed on a Beckman-Coulter CEQ 8000 automated genotyper.

We defined an individual as belonging to a parental species if it was homozygous for one species' alleles at all three loci. An individual was considered to be a hybrid if at least one allele across the six possible alleles (2 alleles/locus; 3 loci/individual) was heterospecific. If a hybrid was heterozygous for both species' alleles at all three loci, it was classified as an F_1 hybrid. If a fish was heterozygous at some loci but homozygous for one species' alleles at one or more loci, it was identified as a post-F_1 hybrid or backcross. However, because only three loci were used, there was considerable uncertainty with regard to the discrimination between post-F_1 hybrids and parental species and between F_1 and post-F_1 hybrids. For instance, with three diagnostic markers in a system where (1) backcrossing is unidirectional, (2) no $F_1 \times$ backcross matings occur, and (3) all genotypes are equally fecund, 12.5% of all first-generation backcrosses and 66.7% of all third-generation backcrosses will have no heterozygous markers and hence will be misclassified as belonging to a parental species (Boecklen and Howard 1997). Consequently, our genetic analysis was conservative because some fish that were classified as bull trout or brook trout may in fact have been advanced-generation hybrids. Raw allele frequency data are available from E.B.T.

RESULTS

From 1998 to 2003, tissue samples were collected from 104 fish at Quirk Creek. Two presumed hybrids were also collected from the Elbow River, approximately 6 km downstream from Quirk Creek (Figure 1). Of the 106 fish sampled, 24 were identified in the field (i.e., presumptive identification) as bull trout, 23 were identified as brook trout, and 59 were identified as hybrids (note that because sampling was targeted, these data do not reflect the relative proportions of hybrids or parental species in Quirk Creek). According to the genetic analysis, none of the presumed hybrids were bull trout (Table 1). Of the 59 presumed hybrids, six did not possess any of the bull trout microsatellite alleles that were assayed (Table 1); it is likely that these individuals were pure brook trout. Similarly, 3 of the 23 presumed brook trout were genetically identified as hybrids. Of the 56 genetically identified hybrids in this study, 17 (30%) appeared to be post-F_1. Overall, our results suggest that the rates of successful identification based on the use of external morphology were 100% (24 of 24) for bull trout, 77% (20 of 26) for brook trout, and 95% (53 of 56) for hybrids (Table 1). Given that none of the nine misidentifications involved bull trout, external morphology appeared to be a reliable means for distinguishing bull trout from hybrids.

Visual inspection of color photographs of confirmed hybrids suggested that dorsal fin markings (Figure 2) were the most reliable morphological characteristic for identifying hybrids. Pale spots, which were morphologically intermediate with respect to parental species characteristics, were present on the dorsal fins of all confirmed hybrids that were photographed. The remaining characteristics (vermiculations, dorsal fin shape, fin stripes, and

TABLE 1. Identities of 106 fish (bull trout, brook trout, or bull trout × brook trout hybrids) sampled from Quirk Creek and the Elbow River, Alberta, during 1998–2003. Identification (ID) was made from microsatellite DNA (genetic ID; up to 3 loci/individual, 2 alleles/locus) and external morphology (field ID; five characteristics, described in Methods); ID agreement refers to correspondence between the two methods.

Number of fish	Bull trout alleles	Brook trout alleles	Total alleles	Genetic ID	Field ID	ID agreement
4	1	1	2	Hybrid	Hybrid	Yes
1	0	4	4	Brook trout	Hybrid	No
1	1	3	4	Hybrid	Hybrid	Yes
2	2	2	4	Hybrid	Hybrid	Yes
5	0	6	6	Brook trout	Hybrid	No
6	1	5	6	Hybrid	Hybrid	Yes
13	2	4	6	Hybrid	Hybrid	Yes
26	3	3	6	Hybrid	Hybrid	Yes
1	4	2	6	Hybrid	Hybrid	Yes
20	0	6	6	Brook trout	Brook trout	Yes
3	1	5	6	Hybrid	Brook trout	No
24	6	0	6	Bull trout	Bull trout	Yes

lateral spot color) were less reliable in that they varied in the extent to which they were intermediate between bull trout and brook trout.

DISCUSSION

Our results demonstrate a high degree of success in the use of external morphological characteristics to identify hybrids in the field; according to genetic analysis, 53 (95%) of the 56 hybrids in this study were correctly identified, and none of the presumed hybrids was a bull trout. Of the five morphological characteristics that were examined, dorsal fin markings appeared to be the most reliable character for use in identifying hybrids sampled from Quirk Creek. Based on these results, we recommend that field identification of hybrids be based solely on this characteristic.

Although our results suggest that dorsal fin markings were reliable, especially for distinguishing bull trout from hybrids, we note several caveats that should be considered when using this morphological characteristic in the field or when planning and directing future research. First, because dorsal fin markings are not as apparent early in life, this characteristic may be unreliable for young-of-the-year fish. Evidence indicates that a number of young-of-the-year hybrids (<70 mm fork length) that were captured in Quirk Creek during 2005 were misidentified as bull trout (Earle et al. 2010), and the Idaho Department of Fish and Game recommends that dorsal fin markings should only be used to differentiate brook trout, bull trout, and hybrids that are 125 mm or larger (B. L. Gamett, U.S. Forest Service, Salmon, Idaho, personal communication). Second, because our genetic analysis was based on three loci, it is possible that some of the individuals that were genetically identified as belonging to a given parental species were actually post-F_1 hybrids or backcrosses. The presence of post-F_1 hybrids certainly suggests that some hybrids in Quirk Creek were reproductively viable and capable of crossing, backcrossing, or both. We emphasize, however, that the primary purpose of our evaluation was to determine whether bull trout were being misidentified as hybrids and subsequently removed from Quirk Creek. In this context, the field misidentification of some post-F_1 hybrids as bull trout or brook trout is less consequential than the misidentification of bull trout as hybrids. Third, our rankings of the reliability of morphological characters were subjective because they were based on qualitative observations. Objective ranking requires a measure of correspondence between the character of interest and the genetic identity of each fish. We could not measure correspondence in this study because not all of the presumed bull trout, brook trout, and hybrids were photographed. Fourth, external morphology is likely to be less reliable for hybrids that are both genetically and morphologically similar to one of the parental species (e.g., as a result of multiple backcrossings; Allendorf and Leary 1988; Young et al. 2001). For example, introgression might have contributed to the misidentification of three of the hybrids in this study. Finally, the reliability of the five morphological characteristics outside of the Elbow River watershed is unknown. Populations of bull trout may differ in terms of genetics (Taylor et al. 2001; Spruell et al. 2003), morphology (Homel et al. 2008), life history (Brenkman and Corbett 2005), or extent of introgression (Rieman et al. 2006; DeHaan et al. 2010). However, the successful use of dorsal fin markings to identify hybrids in both Idaho (B. L. Gamett, personal communication) and Montana (W. Fredenberg, U.S. Fish and Wildlife Service, personal communication) suggests that this morphological characteristic is reliable for field identification in other systems.

Bull trout in Quirk Creek have co-occurred with brook trout and hybrids for approximately three decades (Stelfox et al. 2004). Although such coexistence has been observed in a number of other systems (DeHaan et al. 2010), it is usually accompanied by a considerable decline in bull trout abundance.

Hybridization contributes to this decline through genetic introgression and a loss of bull trout reproductive effort. Hybrids also complicate bull trout management and conservation efforts that involve the control of brook trout. Using genetic analysis and color photographs, we have shown that dorsal fin markings provide an efficient, simple, and reliable means for researchers, managers, and conservation biologists to identify, quantify, and selectively remove most hybrids.

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