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Detecting rainbow trout introgression in Bonneville cutthroat trout of the Bear River basin using field-based phenotypic characteristics

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Abstract.—Cutthroat trout *Oncorhynchus clarkii* × rainbow trout *O. mykiss* hybrids (hereafter hybrids) are difficult to visually distinguish from parental taxa, yet identifying phenotypic traits to separate hybrids from cutthroat trout is needed for conservation and management purposes. We compared phenotypic characteristics against genotype (using 34 species-diagnostic single nucleotide polymorphism loci) for 316 Bonneville cutthroat trout *O. clarkii utah*, rainbow trout and hybrids in Bear River tributaries. Our phenotypic classifications of fish were 91% accurate for Bonneville cutthroat trout but only 68% accurate for rainbow trout and hybrids combined. Classification errors based on phenotype were observed between parental taxa and hybrids but not between cutthroat trout and rainbow trout. The most useful phenotypic traits for distinguishing Bonneville cutthroat trout from hybrids were the absence of a white leading edge on the pelvic fin, the presence of fewer than 7 spots on the top of the head, and the presence of a prominent throat slash. The degree of hybridization in individual hybrids was associated with the number of spots on the top of their head. However, 16% of $>F_1$ hybrids with a higher proportion of cutthroat trout ancestry and 6% of F_1 hybrids exhibited all the phenotypic characteristics of cutthroat trout. The ability to visually detect admixture in hybrids was not related to fish length but was related to admixture level, with logistic regression model results predicting that, for individual hybrids when the proportion of alleles assigned to rainbow trout was >18% (95% CI, 11% to 26%), biologists were more than 50% likely to visually detect *O. mykiss* traits. While we encourage the use of genetic-based assessments for Bonneville cutthroat trout populations when feasible, our results suggest that phenotypic traits can assist in identifying hybridized populations and hybrid individuals, which will benefit the management and conservation of this species. However, our study included Bonneville cutthroat trout from only the Bear River basin, and further work is needed from the southern portion of the subspecies' range to support or refute our findings.

Resumen.—Los híbridos de la trucha degollada *Oncorhynchus clarkii* × y la trucha arco iris *O. mykiss* (de aquí en adelante híbridos) son difíciles de distinguir visualmente de los taxones parentales, pero es necesario identificar los rasgos fenotípicos para separar los híbridos de la trucha degollada con fines de conservación y gestión. Comparamos las características fenotípicas con el genotipo (utilizando 34 loci de polimorfismo de nucleótido único SNPs de diagnóstico de especie) de 316 truchas degolladas de Bonneville *O. clarkii utah*, truchas arco iris e híbridos en los afluentes de Bear River. Nuestra clasificación fenotípica de peces tuvo una precisión del 91% en la trucha degollada de Bonneville, pero únicamente un 68% en la trucha arco iris y los híbridos combinados. Se observaron errores de clasificación basados en el fenotipo entre los taxones parentales y los híbridos, pero no entre la trucha degollada y la trucha arco iris. Los rasgos fenotípicos más útiles para distinguir la trucha degollada de Bonneville de los híbridos fueron la ausencia de un borde delantero blanco en la aleta pélvica, menos de siete puntos en la parte superior de la cabeza y la presencia de un corte prominente en la garganta. El grado de hibridación en híbridos individuales se asoció con el número de manchas en la parte superior de la cabeza. Sin embargo, el 16% de los híbridos $>F_1$ con una mayor proporción de ascendencia de la trucha degollada y el 6% de los híbridos F_1 exhibieron todas las características fenotípicas de la trucha degollada. La capacidad de detectar visualmente la mezcla en híbridos no se relacionó con la longitud del pez, sino con el nivel de mezcla, y los resultados del modelo de regresión logística predicen que, en los híbridos individuales, cuando la proporción de alelos asignados a la trucha arco iris era superior al 18% (95% de intervalo de confianza 11%–26%), los biólogos tenían más del 50% de probabilidad de detectar visualmente los rasgos de *O. mykiss*. Si bien recomendamos el uso de evaluaciones genéticas de las poblaciones de truchas degolladas de Bonneville cuando sea posible, nuestros resultados sugieren que los rasgos fenotípicos pueden ayudar a identificar poblaciones híbridas e individuos híbridos, lo que beneficiará el manejo y la conservación de esta especie. No obstante, nuestro estudio incluyó truchas degolladas de Bonneville sólo de la cuenca de Bear River, y se necesita más investigación en la sección sur del área de distribución para respaldar o refutar nuestros hallazgos.

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The rainbow trout *Oncorhynchus mykiss* is one of the most widely introduced species of fish in the world outside its native range, and the species' ecological plasticity has allowed it to establish self-sustaining populations in lentic and lotic habitats throughout the globe (Crawford and Muir 2008). This plasticity combined with the capacity to hybridize with cutthroat trout *O. clarkii* has arguably made introgression with rainbow trout the biggest threat to most subspecies of cutthroat trout in western North America (Young 1995, Behnke 2002, Budy et al. 2019). Consequently, reducing the extent and severity of hybridization is often a central tenet in management plans developed to conserve cutthroat trout populations (e.g., Coffin and Cowan 1995, Hirsch et al. 2005).

Common goals in cutthroat trout conservation and management plans include identifying pure cutthroat trout populations in order to protect them from future introgression, and reducing introgression in cutthroat trout populations that are already hybridized by manually removing rainbow trout and cutthroat trout × rainbow trout hybrids (hereafter, hybrids). In such removal efforts, assessing the hybrid status of individual fish or populations by using genetic markers is often impractical due to time or cost constraints. For example, in some areas, weirs are operated on spawning tributaries, where cutthroat trout are allowed to pass while nonnative rainbow trout and hybrids are removed (High 2010). In other instances, rainbow trout and hybrids may be gradually removed from streams via repeated electrofishing passes (e.g., Meyer et al. 2017a) or by requiring anglers to harvest any rainbow trout or hybrid that they catch (Heim et al. 2020). In such instances, it is impractical to hold each captured cutthroat trout until genetic analyses can be performed to assess its hybridization status. Likewise, for broadscale status assessments (e.g., Meyer et al. 2006), it is often cost-prohibitive to genetically interrogate cutthroat trout at enough locations to draw firm conclusions about the purity of individual rivers or entire drainages (Della Croce et al. 2016).

Consequently, accurately identifying phenotypic traits associated with introgression would be invaluable for management and conservation of cutthroat trout subspecies. Unfortunately, while differentiating cutthroat trout from rainbow trout parental taxa is relatively straightforward (except for fry; Martinez 1984), distinguishing hybrids from either parental taxa can be challenging, especially for $>F_1$ hybrids (Meyer et al. 2017b), and the difficulty varies greatly among subspecies. For example, phenotypic differentiation of rainbow trout hybrids from coastal cutthroat trout *O. c. clarkii* (Baumsteiger et al. 2005, Kennedy et al. 2009) and westslope cutthroat trout *O. c. lewisi* (Weigel et al. 2002) is particularly difficult, presumably because both of these subspecies evolved in sympatry with rainbow/redband trout and hybridize naturally (e.g., Young et al. 2001, Kozfkay et al. 2007). For other subspecies, such as Yellowstone cutthroat trout, phenotypic differentiation between cutthroat trout and hybrids has been more successful (Seiler et al. 2009, Meyer et al. 2017b, Heim et al. 2020). The latter 2 studies recently identified morphological characteristics that are useful in differentiating Yellowstone cutthroat trout from hybrids. However, the variation observed among cutthroat trout subspecies in coloration and spotting patterns highlights the need for subspecies-specific evaluations to determine whether phenotypic traits can be used to accurately detect introgression in individual fish.

Phenotypic differences between Bonneville cutthroat trout, rainbow trout, and hybrids have not, to our knowledge, been described. Bonneville cutthroat trout from the Bear River basin are closely related to Yellowstone cutthroat trout both genetically (Loxterman and Keeley 2012) and morphologically (Behnke 2002, Seiler and Keeley 2009), presumably because of historical geologic connectivity (Campbell et al. 2011). However, even closely related subspecies of cutthroat trout exhibit morphological differentiation (Seiler and Keeley 2009), further justifying the need for subspecies-specific evaluations. The primary objective of this study was therefore to determine whether diagnostic phenotypic traits used to differentiate Yellowstone cutthroat trout from rainbow trout and hybrids could also be used to reliably differentiate Bear River basin Bonneville cutthroat trout from rainbow trout and hybrids in the field, with an emphasis on characteristics that could be quickly and easily measured. A secondary objective was to evaluate whether the ability of biologists to identify *O. mykiss* traits in an

Table 1. The number of Bonneville cutthroat trout (BCT), rainbow trout (RBT), and cutthroat trout × rainbow trout hybrids captured from several Bear Lake and Bear River tributaries, Idaho, based on genotype. Hybridized fish were categorized as F_1, $>F_1$ with a higher proportion of cutthroat trout ancestry ($>F_1^{BCT}$), and $>F_1$ with a higher proportion of rainbow trout ancestry ($>F_1^{RBT}$).

			Genotype				
Stream	Latitude	Longitude	BCT	$>F_1^{BCT}$	F_1	$>F_1^{RBT}$	RBT
Cottonwood Creek	42.332	−111.773	85	18	1	1	0
Cub River	42.141	−111.686	110	6	2	0	0
Kackley Springs	42.531	−111.795	31	17	13	16	4
St. Charles Creek	42.108	−111.469	5	6	1	0	0

individual hybrid was influenced by fish size and degree of hybridization.

METHODS

Fish Sampling

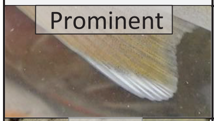
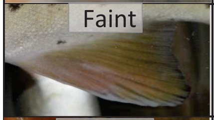
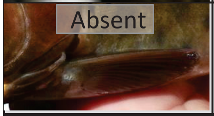
The study was conducted in one tributary to Bear Lake and 3 tributaries to the Bear River (Table 1). The study streams and locations of sampling were chosen because fish populations in these sections of stream were made up mostly of Bonneville cutthroat trout, but they all had an appreciable amount of *O. mykiss* introgression. Fish were collected in July 2020 using backpack electrofishing equipment. Our goal was to collect >300 *Oncorhynchus* for the entire study scattered across all streams to increase the likelihood that the phenotypic traits we identified as most useful for separating taxa would be broadly applicable across the Bear River basin. We did not include age-0 fish in our study for 2 reasons. First, for all 3 taxa (i.e., cutthroat trout, rainbow trout, and hybrids), age-0 fish at the time of sampling were small (i.e., <90 mm TL) and uncommon in our catch. Second, some of the phenotypic traits that distinguish cutthroat trout from rainbow trout are not fully expressed in age-0 fish (Miller 1950, Martinez 1984, Seiler et al. 2009).

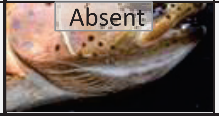
Fish were relaxed in a solution of peppermint oil and water (using a 1:10 stock solution of peppermint oil and ethanol; 0.3–0.5 mL of the solution was used per liter of water) and measured for total length. A suite of phenotypic characteristics were then recorded for each fish (Table 1). The characteristics we included were based largely on previous studies that differentiated Yellowstone cutthroat trout from rainbow trout and hybrids (Meyer et al. 2017b, Heim et al. 2020) because, as mentioned above, Yellowstone cutthroat trout and Bonneville cutthroat trout in the Bear River basin are known to be morphologically similar (Seiler and Keeley 2009). Most of the characteristics are self-explanatory, but head spots require further description. Biologists specifically counted spots directly on the top of the head, above the eyes, and anterior to scale formation (see Table 2). There is often one spot next to each nare on Bonneville cutthroat trout, and, if present, these were not counted. Based on these characteristics, 1 of 2 experienced biologists classified each fish as a Bonneville cutthroat trout, rainbow trout, or hybrid. A small fin clip was collected from each fish for genetic analysis and stored on Whatman sheets.

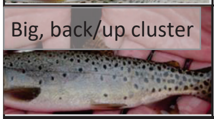
Genetic Analyses

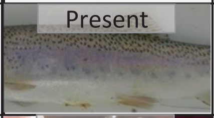
We extracted DNA from all fin clip samples using the Nexttec Genomic DNA Isolation Kit from XpressBio (Thurmont, MD). All samples were genotyped with a panel of 201 single nucleotide polymorphic (SNP) markers (Willis et al. 2020) using the Genotyping-In-Thousands by sequencing (GT-seq) method developed by Campbell et al. (2015). Within this marker panel, 34 SNPs were diagnostic between rainbow trout and Bonneville cutthroat trout from the Bear River (M. Campbell unpublished data), resulting in an approximately 97% probability of detecting rainbow trout introgression of ≥5% at the individual level. Sequencing was performed on an Illumina Nextseq 500 platform. Genotyping was accomplished with scripts modified from Campbell et al. (2015). Primer and SNP information for the SNP marker panel is available on the FishGen database (www.fishgen.net) under the marker set “IDFG CoCut GTseq v3.0 201”. Data summaries and formatting for specific genetic software programs were completed in R version 3.4.1 (R Core Team 2017).

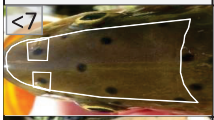
TABLE 2. Description of phenotypic characteristics used to distinguish Bonneville cutthroat trout (BCT) from rainbow trout (RBT) and cutthroat trout × rainbow trout hybrids in several Bear Lake and Bear River tributaries, Idaho, and the frequency of occurrence by genotype. Hybridized fish were categorized as F_1, $>F_1$ with a higher proportion of cutthroat trout ancestry ($>F_1^{BCT}$), and $>F_1$ with a higher proportion of rainbow trout ancestry ($>F_1^{RBT}$). Fins included in (*A*) were dorsal, pelvic, and anal fins. Head spots inside the white boxes in (*E*) were not counted.

(A) White tip on at least one fin	BCT	$>F_1^{BCT}$	F_1	$>F_1^{RBT}$	RBT
	16	17	14	16	4
	72	14	2	1	0
	143	16	1	0	0

(B) Slash	BCT	$>F_1^{BCT}$	F_1	$>F_1^{RBT}$	RBT
	176	23	8	1	0
	55	24	9	14	4
	0	0	0	2	0

(C) Side spots	BCT	$>F_1^{BCT}$	F_1	$>F_1^{RBT}$	RBT
	3	7	10	10	3
	228	40	7	7	1

(D) Rainbow stripe	BCT	$>F_1^{BCT}$	F_1	$>F_1^{RBT}$	RBT
	1	1	2	11	2
	230	46	15	6	2

(E) Head spots	BCT	$>F_1^{BCT}$	F_1	$>F_1^{RBT}$	RBT
	221	36	6	5	0
	10	11	11	12	4

To determine the genetic ancestry of each fish, we analyzed genotype data from the 34 diagnostic SNPs, using the software program *Structure* (Pritchard et al. 2000). For analysis, we used the admixture model with correlated allele frequencies. The Monte Carlo Markov Chain was run for 10,000 steps, following a burn-in period of 10,000 steps. *Structure* was run with K = 2, corresponding to the 2 parental taxa (rainbow trout and cutthroat trout). We classified individuals with 98.5% or greater ancestry to one of the 2 clusters as the parental taxa (cutthroat trout and rainbow trout). We classified individuals with ~50% ancestry of both parental taxa (i.e., 48%–52%) as F_1 hybrids. This was the range of ancestry values observed in known F_1 hybrids sampled from the Idaho Department of Fish and Game's American Falls Fish Hatchery (n = 70). The remaining samples not identified as parental taxa or F_1 hybrids were classified as $>F_1$ with either a higher proportion of cutthroat trout ancestry (hereafter $>F_1^{BCT}$) or a higher proportion of rainbow trout ancestry ($>F_1^{RBT}$). We reported rainbow trout introgression in individual fish as the number of rainbow trout alleles out of the total number of diagnostic alleles examined for that fish.

TABLE 3. The number of Bonneville cutthroat trout (BCT), rainbow trout (RBT), and cutthroat trout × rainbow trout hybrids (HYB) obtained from several Bear Lake and Bear River tributaries, Idaho, categorized by phenotype and genotype. Hybridized fish were categorized as F_1, $>F_1$ with a higher proportion of cutthroat trout ancestry ($>F_1^{BCT}$), and $>F_1$ with a higher proportion of rainbow trout ancestry ($>F_1^{RBT}$).

	Phenotype			
Genotype	BCT	HYB	RBT	TOTAL
BCT	210	21	0	231
$>F_1^{BCT}$	26	21	0	47
F_1 hybrid	1	16	0	17
$>F_1^{RBT}$	0	15	2	17
RBT	0	4	0	4
TOTAL	237	77	2	316

Statistical Analyses

We used logistic regression to determine which phenotypic characteristics best distinguished Bonneville cutthroat trout from hybrids. Differentiating parental taxa from each other was considered immaterial in this study because rainbow trout were never misidentified as cutthroat trout or vice versa. Differentiating between rainbow trout and hybrids was also considered immaterial because detecting either one would confirm the presence of *O. mykiss* alleles in individual fish or any Bonneville cutthroat trout population being evaluated.

We coded our response variable with dummy values of 0 for hybrids and 1 for cutthroat trout. Independent variables were either binary (e.g., spots on side of body were large and clustered dorsally and posteriorly, or not) or ternary (white on leading edge of pelvic fin was prominent, faint, or absent) except for the number of spots on the top of the fish's head, which was a continuous variable. All possible candidate models were tested, and Akaike's information criterion (AIC) was used to identify the best models. The most plausible models were considered to be those for which AIC values were within 2.0 of the best model (Burnham and Anderson 2004). We calculated AIC weights (w_i) to judge the relative plausibility of each of the most plausible models, and the adjusted R^2 for discrete models ($\tilde{R}^2$; Nagelkerke 1991) was used to assess the amount of variation explained by the models. The Hosmer and Lemeshow goodness-of-fit statistic (Hosmer et al. 2013) was used to verify that the most plausible logistic regression models also adequately fit the data.

We used a separate logistic regression to evaluate whether visually detecting *O. mykiss* traits in individual hybrids was related to the length of the fish (i.e., whether smaller hybrids visually exhibited fewer *O. mykiss* traits) and the degree of hybridization in the fish. We used a response variable of 0 for inaccurate and 1 for accurate phenotypic classification of fish genetically confirmed to be hybrids, and the continuous predictor variables were fish length and the proportion of diagnostic alleles identified as *O. mykiss*. For all statistical analyses, SAS statistical software (SAS Institute Inc. 2009) was used, and statistical significance was set at $\alpha = 0.05$.

RESULTS

In July 2020, a total of 316 fish were sampled from the Bear River basin in Idaho for phenotype and genotype comparisons (Table 1). Fish averaged 204 mm in total length and ranged from 92 to 455 mm. Based on genotype, 231 fish were classified as cutthroat trout, 4 were rainbow trout, 17 were F_1 hybrids, 47 were $>F_1^{BCT}$, and 17 were $>F_1^{RBT}$ (Tables 2, 3).

Our phenotypic categorizations of fish were 91% accurate for fish genetically identified as cutthroat trout but only 68% accurate for the combined category of rainbow trout and hybrids (Table 3). Fish misidentified phenotypically as rainbow trout ($n = 2$) were both $>F_1^{RBT}$, with each fish having at least 75% rainbow trout alleles of those that we screened. Fish misidentified phenotypically as hybrids ($n = 25$) were usually Bonneville cutthroat trout, but a few ($n = 4$) were rainbow trout. All but one fish misidentified phenotypically as Bonneville cutthroat trout ($n = 27$) were actually $>F_1^{BCT}$, with an average of only 10% rainbow trout alleles of those that we screened (range 3% to 29%).

Most of the field-based phenotypic characteristics differed between Bonneville cutthroat trout, rainbow trout, and hybrids, but none were completely diagnostic (Table 2). For example, for rainbow trout, F_1 hybrids, and $>F_1^{RBT}$ combined, 89% of fish had prominent white leading edges on both their pelvic and anal fins, with 68% also having a prominent white leading edge on the dorsal

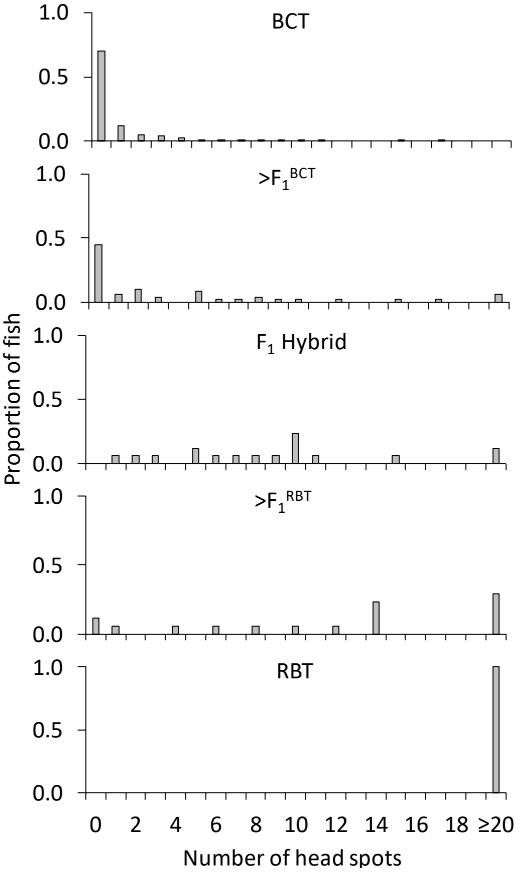


Fig. 1. The proportion of Bonneville cutthroat trout, rainbow trout, and cutthroat trout × rainbow trout hybrids in several Bear Lake and Bear River tributaries, Idaho, with various numbers of spots on the tops of their head. Hybridized fish were categorized as F_1, $>F_1$ with a higher proportion of cutthroat trout ancestry ($>F_1^{BCT}$), and $>F_1$ with a higher proportion of rainbow trout ancestry ($>F_1^{RBT}$).

fin. Unfortunately, white fin tips were also observed on $>F_1^{BCT}$ as well as a surprising number of cutthroat trout. In fact, 34% of $>F_1^{BCT}$ and 7% of cutthroat trout had prominent white leading edges on the pelvic, anal, or dorsal fin, with even higher proportions exhibiting faint white leading edges.

The number of head spots was also a useful characteristic for separating taxa, with 96% of Bonneville cutthroat trout having 6 or fewer spots on the top of their head, while only 29% of rainbow trout, F_1 hybrids, and $>F_1^{RBT}$ had 6 or fewer spots on their head (Table 2, Fig. 1). However, 77% of $>F_1^{BCT}$ also had 6 or fewer head spots. Based on simple linear regression, the degree of hybridization in individual hybrids was associated with the number of spots on the top of their head ($F = 19.9$, $P < 0.001$; Fig. 2), with one additional head spot occurring on average for every 6% increase in rainbow trout admixture.

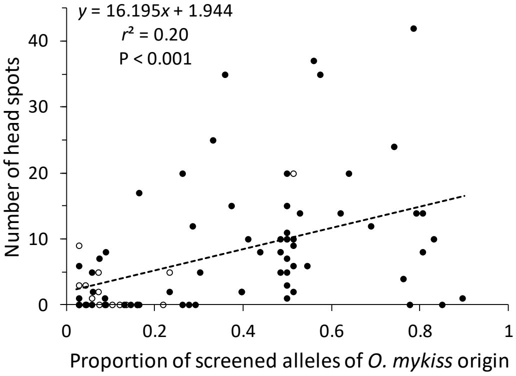


Fig. 2. The relationship between the level of *Oncorhynchus mykiss* introgression in Bonneville cutthroat trout × rainbow trout hybrids and the number of spots on the tops of their head in several Bear Lake and Bear River tributaries, Idaho. The line, equation, and statistics are from fitting a least-squares linear regression to the data. Filled circles represent fish with any white on the leading edges of the pelvic, anal, and dorsal fins, whereas open circles represent fish with no white on any of those fins.

The throat slash, body spotting pattern, and side coloration were also good traits for separation (Table 2). However, when we combined all 7 traits, ten $>F_1^{BCT}$ and one F_1 hybrid exhibited all the phenotypic characteristics of a cutthroat trout, and we phenotypically misidentified all of them.

Phenotypic characteristics included in the most plausible logistic regression model for separating Bonneville cutthroat trout from hybrids were (1) whether the fish had white on the leading tip of the pelvic fin, (2) the number of spots on the top of the head, and (3) the prominence of the throat slash (Table 4). Other models with at least some support (i.e., $w_i \geq 0.01$) also included whether the fish had white on the leading tip of the pelvic fin and the spotting pattern on the side of the body, although these models were much less supported. All the models with $w_i \geq 0.01$ explained more than half of the variation in genotypic differentiation between cutthroat trout and hybrids.

In evaluating whether visually detecting *O. mykiss* traits in individual hybrids was related

TABLE 4. Top candidate logistic regression models that best differentiated Bonneville cutthroat trout from cutthroat trout × rainbow trout hybrids in several Bear Lake and Bear River tributaries, Idaho, based on phenotypic characteristics.

Model	AIC	ΔAIC	w_i	$\tilde{R}^2$
White pelvic fin + headspots + slash strength	226.98	0.00	0.62	0.54
White pelvic fin + headspots + side spotting pattern	229.49	2.51	0.18	0.52
White anal fin + headspots + side spotting pattern	230.25	3.27	0.12	0.52
White anal fin + headspots + slash strength	232.02	5.04	0.05	0.52
White pelvic fin + side spotting pattern + slash strength	236.34	9.36	0.01	0.51

TABLE 5. Logistic regression models evaluating whether visually detecting *Oncorhynchus mykiss* traits in individual hybrids was related to fish length and the degree of hybridization in fish in several Bear Lake and Bear River tributaries, Idaho.

Model	AIC	ΔAIC	w_i	$\tilde{R}^2$
Degree of hybridization	61.03	0	0.55	0.62
Degree of hybridization + fish length	61.46	0.43	0.45	0.60
Fish length	106.75	45.72	0.00	0.01

to fish length (i.e., whether smaller hybrids visually exhibited fewer *O. mykiss* traits) and the degree of hybridization in the fish, the most plausible logistic regression model included only the proportion of rainbow trout alleles observed (Table 5). This model explained 62% of the variation in phenotype accuracy, and predicted that when the proportion of alleles assigned to rainbow trout was >18% in an individual hybrid (95% confidence interval, 11% to 26%), biologists were more than 50% likely to visually detect *O. mykiss* traits (Fig. 3). While the next most supported model did contain fish length, it did not explain additional variation in phenotype accuracy.

DISCUSSION

The results of the present study demonstrate that, in the Bear River basin, simple phenotypic traits such as spotting patterns and body coloration can be used to separate Bonneville cutthroat trout from rainbow trout and hybrids with >90% accuracy, as has also been demonstrated for westslope cutthroat trout (Robinson 2007) and Yellowstone cutthroat trout (Meyer et al. 2017b, Heim et al. 2020). However, while it appears that the same phenotypic traits used to visually identify *O. mykiss* admixture in Yellowstone cutthroat trout can also be used for Bonneville cutthroat trout, the traits appear to be more reliable for the former than the latter. For example, assuming no genotyping errors were made, 58% of the Bonneville cutthroat trout included in the present study had at least one trait considered characteristic of rainbow trout. Most surprising was that 38% of Bonneville cutthroat trout had at least faint white on the leading edge of the pelvic, anal, or dorsal fin, and 7% had at least one fin with a prominent white leading edge. In comparison, <1% of Yellowstone cutthroat trout had any white on their fins (Meyer et al. 2017b, Heim et al. 2020), whereas westslope cutthroat trout appear to lack white fin tips in some populations (Robinson 2007, Al-Chokhachy et al. 2014) but not in others (M. Campbell unpublished data). White fin tips have also been used to distinguish rainbow trout and hybrids from coastal cutthroat trout (Baumsteiger et al. 2005).

Based on both logistic and linear regression model results, the number of spots on the top of the head was one of the best traits for separating Bonneville cutthroat trout from hybrids, as well as for predicting how much admixture existed in any given hybrid. This finding also concurs with recent studies that visually distinguished Yellowstone cutthroat trout from hybrids (Meyer et al. 2017b, Heim et al. 2020). Except for coastal cutthroat trout and westslope cutthroat trout, most subspecies of cutthroat trout have few if any spots on the top of their head, whereas the top of the head on rainbow trout is usually heavily spotted (Behnke 2002); thus, enumerating head spots may help differentiate most subspecies of cutthroat trout from hybrids. However, head spotting characteristics for Bonneville cutthroat trout and $>F_1^{BCT}$ were more variable than was shown for Yellowstone cutthroat

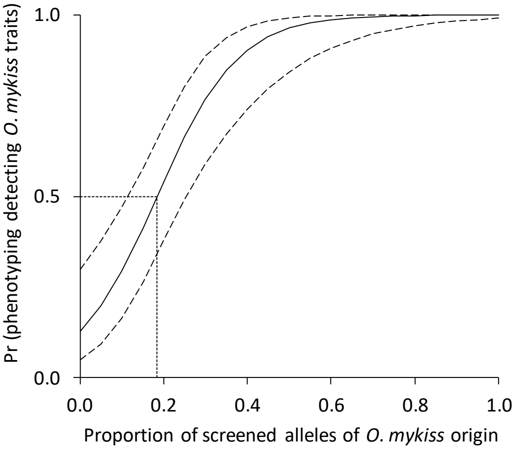


Fig. 3. Logistic regression relationship between the level of *Oncorhynchus mykiss* introgression in Bonneville cutthroat trout × rainbow trout hybrids and the probability of visually detecting *O. mykiss* traits in fish residing in several Bear Lake and Bear River tributaries, Idaho. The wide dotted lines are the 95% prediction limits for the logistic regression relationship. The thin dotted vertical and horizontal lines depict the level of hybridization at which biologists were more than 50% likely to visually detect *O. mykiss* traits in an individual hybrid.

trout, with 10 Bonneville cutthroat trout having at least 7 head spots and almost half of the $>F_1^{BCT}$ having 0 head spots. More evaluations of head spot numbers for other subspecies of cutthroat trout in relation to hybridization levels would help elucidate how diagnostic head spotting patterns are for distinguishing cutthroat trout from hybrids.

Considering all 7 phenotypic traits included in our study, a higher percentage of Bonneville cutthroat trout visually exhibited *O. mykiss* traits than was recently observed for Yellowstone cutthroat trout (Meyer et al. 2017b, Heim et al. 2020). Consequently, we phenotypically misclassified a higher proportion of Bonneville cutthroat trout as hybrids. Such misclassifications may be of concern when conducting selective removals targeting nonnative trout and their hybrids. However, while some accidental removal of Bonneville cutthroat trout is likely unavoidable when using only phenotypic traits, mistaking cutthroat trout for hybrids was far less likely than mistaking hybrids for cutthroat trout, a finding that is consistent with previous studies (e.g., Weigel et al. 2002, Baumsteiger et al. 2005, Meyer et al. 2017b). It could be argued that accidentally culling Bonneville cutthroat trout in a removal project, or accidentally blocking their access to spawning streams, would be better than accidentally releasing or allowing passage to fish with *O. mykiss* alleles, but, unfortunately, our results suggest that the latter type of misclassification was more common. Regardless of the goal of the program, any phenotypically based classification of Bonneville cutthroat trout individuals or populations will result in some level of error, and fisheries managers will have to weigh the risks and benefits of potentially selecting against some natural phenotypic (and potentially genetic) variation while trying to reduce threats from rainbow trout and hybrids. While genotyping errors may also occur, based on Boecklen and Howard (1997) and Rasmussen et al. (2010), detecting low levels of introgression (5%) with a high level of confidence (95%) for individual fish requires the screening of 29 diagnostic markers, which our study exceeded.

Our ability to phenotypically differentiate Bonneville cutthroat trout from rainbow trout and hybrids was not diminished at smaller fish sizes. We questioned whether this was in part due to the exclusion of fry from our study, which were omitted because it is notoriously difficult to distinguish *O. clarkii* from *O. mykiss* at that size (Miller 1950, Martinez 1984, Seiler et al. 2009). However, we did collect a small number of fry, screened them genetically, described their phenotypic traits, and found (1) an equivalent ability to correctly categorize them into taxa; (2) that all the mistakes were incorrect categorization of cutthroat trout as hybrids; and (3) that all the mislabeled cutthroat trout had at least faint white leading edges on their pelvic, anal, and/or dorsal fin. Previous studies have also demonstrated successful phenotypic differentiation of cutthroat trout from hybrids for small fish (e.g., Weigel et al. 2002, Seiler et al. 2009).

The primary limitation of this study was that it only included Bonneville cutthroat trout from the Bear River basin portion of their range; thus, the phenotypic differences we observed between Bonneville cutthroat trout and rainbow trout/hybrids may not be applicable to cutthroat trout from the Bonneville basin. Bonneville cutthroat trout from the Bear River basin have been considered an evolutionarily unique lineage of cutthroat trout for decades (Behnke 1992), and recent genetic analyses suggest they may be more closely related to Yellowstone cutthroat trout than to Bonneville cutthroat trout from the southern portion of their range (Smith et al. 2002, Shiozawa et al. 2018, Campbell et al. 2018). The close phylogenetic connection between Bear River and Snake River cutthroat trout stems from complex drainage reversal patterns among these 2 river basins in recent geologic time (Campbell et al. 2011, Link and Keeley 2018), including as recently as 14,500 years ago when Lake Bonneville breached and flowed north into the Snake River. Until additional investigations are conducted in the Bonneville basin to support or refute our findings, we caution the application of our results to Bonneville cutthroat trout populations in the southern portion of their range without corroborating genetic analyses.

In summary, while our ability to phenotypically separate Bonneville cutthroat trout from hybrids was imperfect, results suggest that certain traits could be used by biologists working in the Bear River basin to quantify the prevalence of *O. mykiss* alleles in a Bonneville cutthroat trout population or to target introgressed fish for removal. Indeed, had we captured all of the study fish at a weir that blocked upstream spawning migration prior to phenotypic interrogation, or with electrofishing equipment during a hybrid removal project, we would have unintentionally released 32% of the fish that we captured that contained some level of *O. mykiss* introgression, but we would have removed 91% of the *O. mykiss* alleles residing in the fish that we captured. Consequently, the use of such phenotypic traits in the field would allow biologists to make instantaneous decisions to remove or release each captured fish rather than individually marking and holding fish for days or weeks until genetic results could be produced. Nevertheless, we caution the use of such phenotypic traits to distinguish Bonneville cutthroat trout from hybrids without concurrent genetic interrogation of at least some fish in the population, or at least some streams in a larger basin being surveyed. And we reiterate our call for future studies in areas of Wyoming and Utah to verify or refute our findings.

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