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[Article]

Morphological, Physiological, and Genetic Techniques for Improving Field Identification of Steelhead, Coastal Cutthroat Trout, and Hybrid Smolts

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Abstract.—In streams with sympatric populations of steelhead *Oncorhynchus mykiss* and coastal cutthroat trout *O. clarkii clarkii* (hereafter, cutthroat trout), life history descriptions and smolt production estimates may be hampered by misclassification of hybrids as steelhead or cutthroat trout. Additionally, important morphological and physiological differences between hybrid and non-hybird smolts are often unknown. Therefore, we assessed field classification and created classification models to quantify and reduce misclassification rates among migrating steelhead, cutthroat trout, and hybrid smolts. Field misclassifications of smolts with steelhead or cutthroat trout genotypes were low (1% and 2%, respectively). However, field misclassification of fish with hybrid genotypes was high, with 11% of the hybrids being misclassified as steelhead and 42% of the hybrids being misclassified as cutthroat trout. Hybrid smolts were larger, had lower gill Na^+, K^+-ATPase activities, and lower condition factors than steelhead but were similar to cutthroat trout smolts in these same measurements. Additionally, statistical classification analyses using morphological traits including subterminal jaw slash intensity, hyoid teeth presence, maxillary length, breaks of pigment along outer margin of adipose fin, condition factor, and migration date improved classification error rates of hybrids from 53% to 21%. In systems with sympatric populations of steelhead and cutthroat trout, we recommend a thorough evaluation of field-based identification methods with genetic techniques to assess the effectiveness of field-based classification in addition to examining important life history differences among steelhead, cutthroat trout, and their hybrids.

The conservation of native species depends on accurate estimates of population demographics, including population abundance, survival, recruitment, and migration. Often these estimates are based on the identification of individual organisms in the field using one or more phenotypic characters and the assumption of little or no hybridization with other species. In systems where native species are closely related, known to hybridize, or demonstrate phenotypic plasticity, introgression assumptions and field-based identifications may be inaccurate (Baumsteiger et al. 2005). This inaccuracy can lead to improper conclusions on basic life history characters and population demographics, causing poor decisions on proposed endangered species listings, critical habitat designations, and effective conservation plans.

In many Pacific coastal streams, juvenile steelhead *Oncorhynchus mykiss* (anadromous rainbow trout) and coastal cutthroat trout *O. clarkii clarkii* (hereafter referred to as cutthroat trout) are sympatric (Behnke 1972) and phenotypically similar across many traits (Crawford 1925; Hawkins and Quinn 1996). Morphological differences between the two species at juvenile life history stages are limited, and the expressions of phenotypic characters are often variable. For example, the subterminal jaw slash on cutthroat trout is a commonly used character to distinguish between steelhead and cutthroat trout adults (Behnke 1992). However, in some populations, juvenile cutthroat trout develop the jaw slash character at varying points in their juvenile life history (Pollard et al. 1997). This phenotypic variation causes juvenile cutthroat trout to be misidentified as steelhead (Baumsteiger et al. 2005). Additionally, after a thorough literature search, we found no published studies quantifying important morphological and physiological differences between hybrid and nonhybrid fish at the important smolt life history stage. These data are greatly needed as many

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monitoring and research programs rely on accurate identifications at the smolting stage. Furthermore, morphological changes that occur during smolting may influence the ability to use parr characteristics in identification.

In addition to similar morphology, natural hybridization between steelhead and cutthroat trout is present in many systems and can be greater than 85% within a stream, adding to the difficulty of species identification (Campton and Utter 1985; Young et al. 2001; Bettles et al. 2005). The creation of first-generation (F_1) hybrids has led to deeper introgression between these populations in the form of multiple generation (F_{2+}) hybrids and backcrosses (to either parental species; Campton and Utter 1985; Baumsteiger et al. 2005). This introgression is believed to be rooted in postzygotic mating patterns, as little prezygotic differences appear to exist between these species (Campton and Utter 1985; Young et al. 2001). Hypotheses of the postzygotic mating patterns responsible for this natural hybridization include satellite mating, abundance numbers, spawn timing, and overlap in spawning habitat, especially in streams subject to strong anthropogenic effects (Ostberg et al. 2004; Baumsteiger et al. 2005). This introgression further increases the difficulty in correctly classifying individuals to a species.

Despite these difficulties, previous research has shown that field-based phenotypic misclassification of closely related salmonid species at other (nonsmolt) life stages can be quantified with the addition of genotypic identifications and reduced with the creation of classification models (Weigel et al. 2002; Baumsteiger et al. 2005). Field-based phenotypic methods can be used as a primary identification of each species or hybrid. These identifications can then be matched with those made with genotypic approaches to quantify the classification error rates based on a particular species, hybrid, or field biologist. Descriptions of phenotypic characters can then be matched with the genetic identification of individuals through the creation of statistical classification models to examine if the error rate of field identifications can be lowered. Lastly, better classifications can be used to adjust various population assessment parameters to create better conservation plans. Therefore, our objectives were to first identify important morphological, physiological, and migrational differences among migrating steelhead, cutthroat trout, and hybrid smolts that could be used to increase the accuracy of field-based classifications. Secondly, we assessed the field misclassification rate of steelhead, cutthroat trout, and hybrid smolts by using genetic techniques. Lastly, we created classification models to quantify misclassification rates and assess if these rates could be reduced.

Methods

Study site.—Fish were collected from Abernathy Creek, Washington (46°22"N, 123°14"W), a third-order tributary located 87 river kilometers from the mouth of the Columbia River. Abernathy Creek's drainage area is 110 km^2, with a mean annual discharge of 1.94 m^3/s (SE = 0.31) and mean water temperature of 10.1°C (SE = 0.1).

Field measurements.—We collected steelhead, cutthroat trout, and hybrid smolts migrating out of Abernathy Creek in the spring of 2005 (1 April to 30 June) using a rotary screw trap. The screw trap was located 300 m upstream from the mouth of Abernathy Creek and was operated by the Washington Department of Fish and Wildlife. The screw trap was checked each morning (0800 hours), and fish caught in the trap were visually identified by experienced U.S. Fish and Wildlife Service biologists trained to distinguish steelhead and cutthroat trout smolts. Throughout the season, every suspected cutthroat trout, every suspected hybrid, and every fifth suspected steelhead were measured (fork length [FL], mm), weighed (g), gill biopsied, digitally photographed, measured for phenotypic characteristics, and fin clipped for later genetics analysis (total number of fish sampled = 243). Gill biopsies were frozen and taken to the laboratory. Gill Na^+, K^+-ATPase activity levels (μmol ADP$\cdot$mg protein$^{-1}\cdot$h^{-1}) were determined from gill biopsies using the method of McCormick (1993). Digital photographs were imported into the program TpsDig (Rohlf 2001), and 15 landmarks relating to body shape morphology were marked on each fish. Lastly, phenotypic characters that have been used to distinguish steelhead and cutthroat trout at the parr life stage were recorded on each fish (Pollard et al. 1997). These characters included whether the maxillary extended past the back margin of the eye, the presence or absence of hyoid teeth (also known as basibranchial teeth), the presence and color intensity of subterminal jaw slashes (absent, faint, dark), the number of breaks of pigment on the outside edge of the adipose fin (hereafter, breaks in adipose), the number of white pigmented dorsal fin ray interspaces (dorsal pigment), and the presence or absence of median dorsal parr marks (dorsal parr marks). Steelhead parr have a maxillary that does not extend past the back margin of the eye, no hyoid teeth, no jaw slashes, zero or one break in pigment on the outside edge of the adipose fin, 3–5 white pigmented dorsal fin ray interspaces, and dorsal parr marks ($N \sim 5$ marks) present (Pollard et al. 1997). Cutthroat trout parr have a maxillary that extends past the margin of the eye, hyoid teeth, jaw slashes, one or two breaks in pigment on adipose fin, 1–3 white pigmented dorsal fin ray interspaces, and no dorsal parr marks (Pollard et al. 1997). Hybrid parr have a combination of cutthroat trout and steelhead characters (Hawkins 1997).

Genetic identification.—A small section of the pelvic fin (9 mm^2) was clipped and placed in individual vials of 100% solutions of ethanol for genetic analysis. A chelex extraction was used to extract whole genomic DNA from each fish in a 96-well plate format (Miller and Kapuscinski 1996). Less than 1 mm^2 of tissue was placed in 180 μL of a 5% Chelex (Sigma) solution, boiled for 8 min, and vortexed for 30 s to release DNA. Extracted DNA was amplified at eight biparentally inherited, species-specific markers. The first marker (*OCC16*) was developed by Ostberg and Rodriguez (2002), whereas the remaining seven (*OM47*, *OM55*, *OCC34*, *OCC35*, *OCC36*, *OCC37*, and *OCC38*) were developed by Ostberg and Rodriguez (2004). Markers show fixed allelic differences between rainbow trout and cutthroat trout and also have been shown to be inherited codominantly in hybrids. Polymerase chain reaction (PCR) conditions followed those of Ostberg and Rodriguez (2004). Electrophoresis of PCR products was accomplished using an ABI 3100 Genetic Analyzer and resulting electropherograms were analyzed using the program GENESCAN (Applied Biosystems, Inc.). Multilocus genotypes for all fish were determined using the program GENOTYPER (Applied Biosystems). Allowing for occasional missing genotypes, a minimum of five loci was used for each individual. Other studies have shown that two diagnostic loci is sufficient to identify F_{2+} hybrids, while five or six provide sufficient power to be almost exact in hybrid classification (Avise and Van den Avyle 1984; Campton 1990; Verspoor and Hammar 1991; Gregg et al. 1998).

We used the methods of Anderson and Thompson (2002) to assess the power of the species-specific loci to identify the parental species and various classes of hybrids. This method uses Bayesian statistical methods to calculate the probability that an individual fish assigns to each of the six different classes: steelhead, cutthroat trout, F_1 hybrid, second-generation (F_2) hybrid or F_{2+} hybrid, hybrid backcross with steelhead, or hybrid backcross with cutthroat trout. All simulations and power estimates were carried out using the program NEWHYBRIDS (Anderson and Thompson 2002). Final class assignments were based on the category with the highest probability, usually greater than 90%. The use of probabilities allows for a transparent estimation of class in light of possible allelic scoring error and the rare presence of fourth- or fifth-generation hybrids.

Data analyses.—Differences in FL, weight, Fulton's condition factor, gill Na^+, K^+-ATPase activity level, and date of migration past the screw trap among genotyped steelhead, cutthroat trout, and hybrid groups were compared using a multivariate analysis of variance (MANOVA). All variables were log transformed to meet the assumptions of normality and equal variances. If significant, the MANOVA was followed by separate analyses of variance for each variable and comparisons between each group were made with Tukey's multiple comparisons tests. All tests were considered significant at the 0.05 alpha level.

We used canonical variates analysis (CVA) to reduce the dimensionality, aid in the interpretation, and compare the measured characteristics (i.e., maxillary, teeth, jaw slashes, breaks in adipose, dorsal fin pigment, and dorsal parr marks) among the groups (Johnson 1998). Additionally, each phenotypic variable was converted to a percentage and its importance was assessed along with variable importance during statistical classification model building.

Nonbody shape variation in digital photographs relating to fish position, orientation, and fish size was removed with generalized procrustes analysis using the program TpsRelw (Rohlf 2001). Following nonshape variation removal, shape variables (i.e., partial warp scores) were assessed for normality and equality of variances and compared among groups using MANOVA and CVA. Visualizations of shape differences among the groups were created using the program TpsRegr (Rohlf 2001), where deformation grids were created by regressing partial warp scores against the three groups.

Field identification error rates of steelhead, cutthroat trout, and hybrid smolts were determined by comparing the field identifications with genetic-based identifications under the assumption that fish genotypes were created without error. The number of individuals assigned to each group in the field was compared to the number of individuals assigned to each group genotypically using a log-likelihood *G*-test. To determine the error rate for each classification group, conditional classification probabilities were calculated and compared.

One set of statistical classification models was built using phenotypic character data (i.e., maxillary, teeth, jaw slashes, breaks in adipose, dorsal fin pigment, and dorsal parr marks) that also included FL, weight, gill Na^+, K^+-ATPase activity level, condition factor, and date of migration. A different set of models was built for body shape variables (partial warp scores). For all classification models, prior probabilities were set to the proportion of each group found in the sample (determined from molecular markers). This method

TABLE 1.—Probabilities-based multilocus genotypes of individual hybrid smolts from Abernathy Creek, Washington, assigned to each of six different classes: steelhead (STH), coastal cutthroat trout (CCT), first-generation hybrid (F_1), second-generation hybrid (F_2), hybrid backcross with coastal cutthroat trout (BCc), or hybrid backcross with steelhead (BCs). All simulations and probabilities were carried out using the program NEWHYBRIDS (Anderson and Thompson 2002).

Final class designation	Assignment probability class					
	CCT	STH	F_1	F_2	BCc	BCs
F_1	0.00	0.00	0.99	0.00	0.00	0.00
F_1	0.00	0.00	0.99	0.00	0.00	0.00
F_1	0.00	0.00	0.99	0.00	0.00	0.00
F_1	0.00	0.00	0.99	0.00	0.00	0.00
F_1	0.00	0.00	0.99	0.00	0.00	0.00
F_1	0.00	0.00	0.99	0.00	0.00	0.00
F_1	0.00	0.00	0.99	0.00	0.00	0.00
F_1	0.00	0.00	0.99	0.00	0.01	0.00
F_1	0.00	0.00	0.92	0.05	0.01	0.02
F_1	0.00	0.00	0.66	0.24	0.01	0.09
F_2	0.00	0.00	0.00	0.95	0.05	0.00
F_2	0.00	0.00	0.08	0.60	0.00	0.32
F_2	0.02	0.00	0.00	0.58	0.40	0.00
F_2	0.00	0.00	0.27	0.47	0.00	0.26
BCc	0.00	0.00	0.00	0.05	0.94	0.00
BCc	0.00	0.00	0.00	0.05	0.94	0.00
BCc	0.01	0.00	0.00	0.10	0.90	0.00
BCc	0.00	0.00	0.00	0.02	0.87	0.00
BCc	0.11	0.00	0.00	0.16	0.84	0.00

accounted for steelhead and cutthroat trout being found much more often than hybrids (Johnson 1998). Model classification error rates were compared using cross-validation techniques. For classification based on FL, weight, condition factor, date of migration, maxillary, teeth, jaw slashes, breaks in adipose, and dorsal parr marks, discriminant stepwise selection was used to identify important variables for classification. When performing stepwise selection, we used an alpha of 0.15 for entry of a variable into the discriminating set and an alpha level of 0.05 for a variable to remain in the set. Nearest neighbor discriminant analysis ($k = 2$ nearest neighbors) was then used to create the classification model. Often canonical functions classify among groups better than raw variables, so an additional nearest neighbor discriminant analysis ($k = 5$ nearest neighbors) with canonical functions found to be important in the CVA was created. For classification based on body shape variables, linear discriminant analysis was used with all shape variables. Also, linear discriminant analysis with the shape canonical functions found to be important in the CVA was used to create an additional classification model.

Results

Successful genotypes were obtained for 98% (237 of 242) of the samples. The excluded five samples did not meet the minimum five genotyped loci required for analysis in this study. Species class probabilities exceeded 0.70 for each pure class distinction, with 99% of samples having probabilities of 0.95 or greater. Species class probabilities exceeded 0.47 for each hybrid class distinction, with 68% of samples having probabilities of 0.90 or greater (Table 1). The majority of samples gave probabilities consistent with those of pure species, with 92% being pure and 8% being hybrids. Of the 19 hybrids found, 10 (53%) were F_1 hybrids, four (21%) were F_2 hybrids, and five (26%) were backcrossed cutthroat trout hybrids; no back-crossed steelhead hybrids were found.

Fork length, weight, condition, gill Na^+, K^+-ATPase activity, and date of migration were different across at least two of the three groups (Pillai's trace $= 0.61$; df $=$ 10, 404; $P < 0.01$). Cutthroat trout were significantly larger than steelhead ($t = 5.09$; $P < 0.01$), but hybrid fish were not different from either cutthroat trout ($t = 1.37$; $P = 0.36$) or steelhead ($t = 1.44$; $P = 0.32$; Figure 1A). Cutthroat trout also weighed more than steelhead ($t = 3.17$; $P < 0.01$; Figure 1B), whereas hybrid fish did not differ in weight from either cutthroat trout ($t = 1.33$; $P = 0.38$) or steelhead ($t = 0.43$; $P = 0.90$). Steelhead had higher condition factors than both hybrids ($t = 7.86$; $P < 0.01$) and cutthroat trout ($t = 3.98$; $P < 0.001$; Figure 1C), but hybrids and cutthroat trout did not differ ($t = 0.34$; $P = 0.94$). Steelhead had higher gill Na^+, K^+-ATPase activities than both hybrids ($t = 7.36$; $P < 0.01$) and cutthroat trout ($t = 2.95$; $P < 0.01$; Figure 1D), but hybrids and cutthroat trout did not differ ($t = 1.09$; $P = 0.51$). Lastly, average date of migration did not differ between steelhead and hybrids ($t = 0.47$; $P = 0.88$; Figure 2), but cutthroat trout migrated significantly later than the two other groups

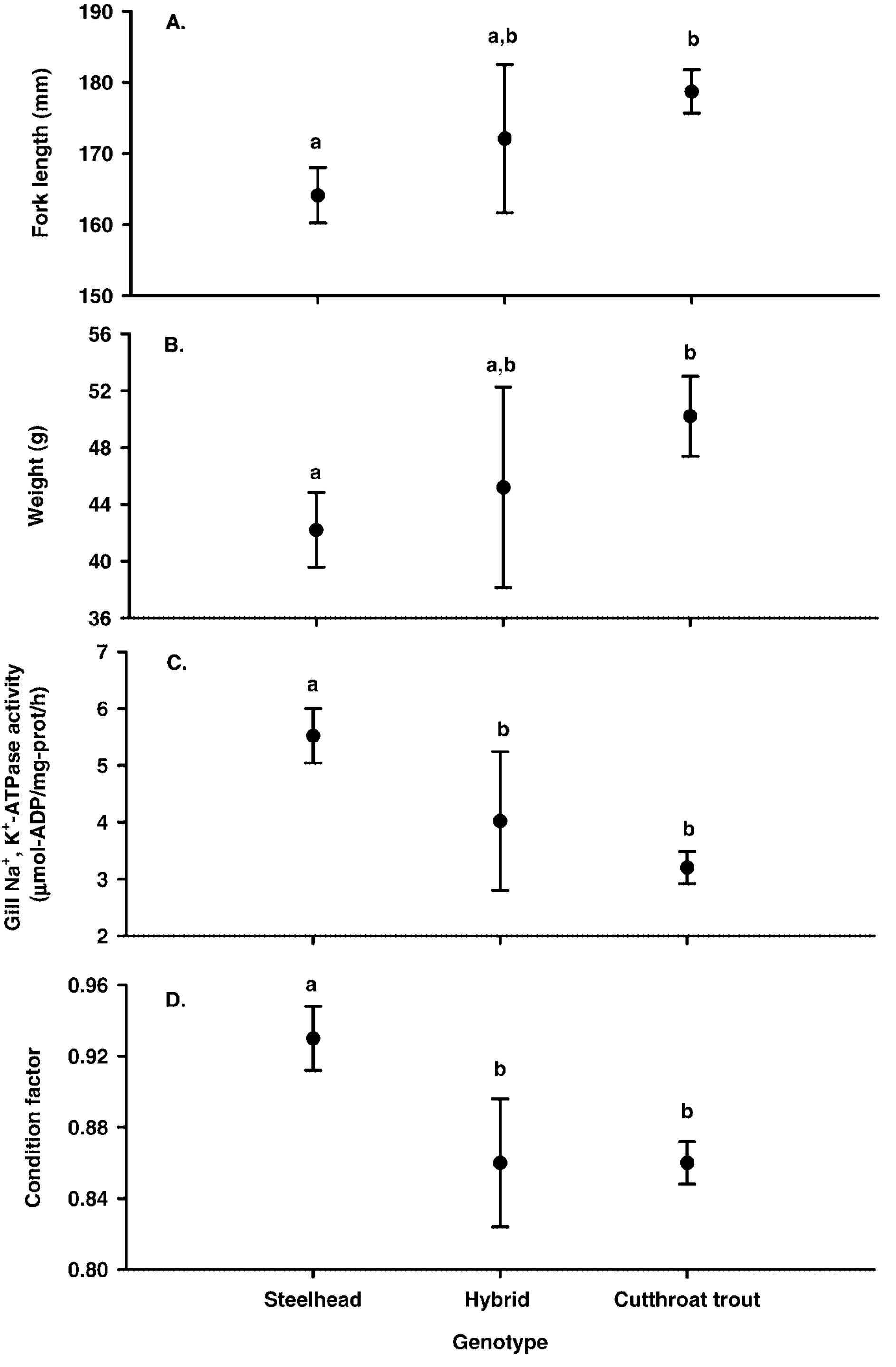

FIGURE 1.—(**A**) Fork length (mm), (**B**) weight (g), (**C**) gill Na^+, K^+-ATPase activity level (µmol ADP• mg protein^{-1}• h^{-1}), and (**D**) Fulton's condition factor of steelhead smolts, steelhead × coastal cutthroat trout hybrid smolts, and coastal cutthroat trout smolts. Different letters represent significance at the 0.05 alpha level.

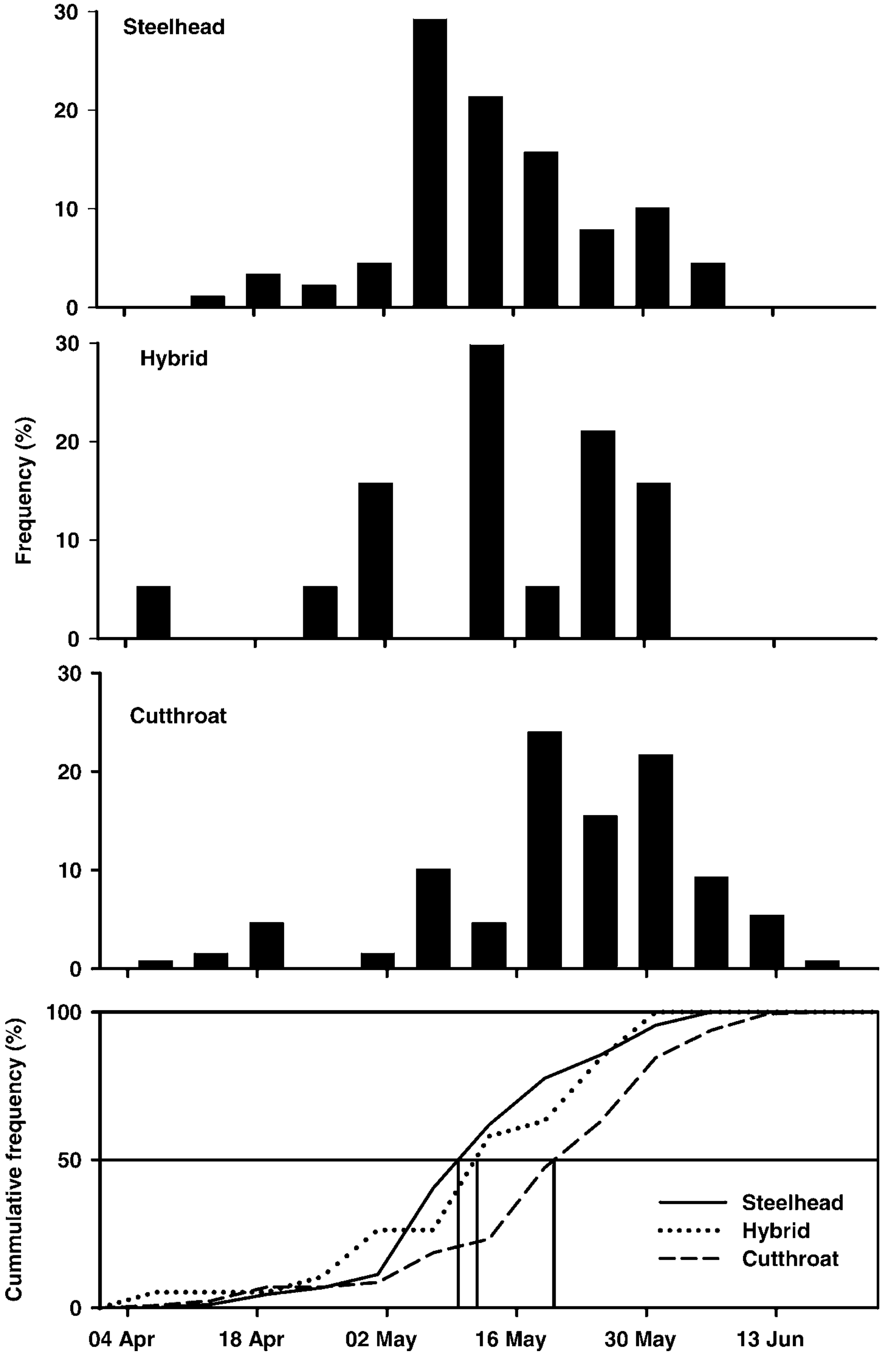

FIGURE 2.—Percent frequency of migrating steelhead smolts, steelhead × coastal cutthroat trout hybrid smolts, and coastal cutthroat trout smolts and cumulative frequency of all smolt groups.

TABLE 2.—Description and composition of categorical phenotypic characteristics for steelhead smolts, coastal cutthroat trout smolts, and steelhead × coastal cutthroat trout hybrid smolts.

		Composition (%) within smolt group		
Phenotype	Description	Steelhead	Hybrid	Cutthroat trout
Maxillary extended past eye	Yes	0	68.4	96.9
	No	100	31.6	3.1
Hyoid teeth present	Yes	1.1	78.9	97.7
	No	98.9	21.1	2.3
Jaw slash intensity	Dark	1.1	26.3	84.5
	Faint	1.2	47.4	14.0
	Absent	97.7	26.3	1.5
Number of breaks in adipose	0	58.6	31.6	9.2
	1	35.6	42.1	26.9
	2	4.6	21.1	53.1
	3	1.2	5.2	10.8
Number of white pigmented dorsal fin rays	0	6.9	10.5	17.0
	1	3.4	10.5	13.2
	2	23.0	36.8	38.0
	3	32.2	31.7	27.9
	4	34.5	10.5	3.9
Median dorsal marks present	Yes	3.5	5.3	0
	No	96.5	94.7	100

(steelhead: $t = 4.05$, $P < 0.01$; hybrids: $t = 2.74$, $P < 0.05$). The day of peak migration occurred on 9 May 2005 for steelhead, 11 May 2005 for hybrids, and 20 May 2005 for cutthroat trout (Figure 2).

Juvenile steelhead, cutthroat trout, and their hybrids differed in many of the measured phenotypic characters (Table 2). All steelhead (100%) sampled had maxillaries that did not extend past the back margin of the eye, while 68% of hybrids and 97% of cutthroat trout did have maxillaries that extended past the eye. Only 1% of steelhead had hyoid teeth, but 79% of hybrids and 98% of cutthroat trout had hyoid teeth. Most steelhead had no jaw slashes (98%), but most hybrids had faint slashes (47%) and most cutthroat trout had dark slashes (85%). Most steelhead had zero or one break in adipose pigment, whereas most hybrids had zero, one, or two breaks and cutthroat trout had one or two breaks in adipose pigment. One-third of steelhead had four white pigmented dorsal fin interspaces, but only 11% of hybrids and only 4% of cutthroat trout had this characteristic. Lastly, median dorsal parr marks were absent on most fish regardless of genotype. Canonical variates analysis indicated that the variation in characters could be reduced to two canonical functions (Can 1 and Can 2). These two canonical variables were significant (Can 1: $F = 65.9$, df = 22, 444, $P < 0.01$; Can 2: $F = 4.82$, df = 10, 223, $P < 0.01$) and accounted for all (100%) of the variability. Plots of mean canonical scores and their 95% confidence interval for each group suggest that steelhead were more distinct from hybrids and cutthroat trout (Figure 3).

Body shape was significantly different among the three groups (Pillai's trace = 0.74; df = 26, 199; $P < 0.01$). Shape deformation grids showed that steelhead had smaller heads and maxillaries (landmarks 1, 2, 4, and 5) than hybrids and cutthroat trout (Figure 4). Steelhead were more robust in overall body depth compared to hybrids and cutthroat trout (Figure 4). Conversely, cutthroat trout had longer maxillaries and were more slender. Hybrids were intermediate in head size and body depth but closer to cutthroat trout than to steelhead. Canonical variates analysis indicated that much of the shape variation could be reduced to one canonical function. This function was significant ($F = 8.87$; df = 52, 396; $P < 0.01$) and accounted for 97% of the total variability. Plots of mean canonical scores and their 95% confidence interval for each group suggest that steelhead were the most distinct, with hybrids and cutthroat trout demonstrating smaller differences (Figure 5).

Overall, field assignments of steelhead, hybrids, and cutthroat trout were significantly different from genotypic assignments ($G^2 = 44.36$; df = 4; $P < 0.05$). Calculated condition classification probabilities between field identification and genotypic identification showed that field misclassifications of steelhead and cutthroat trout were low (1% and 2%), but misclassification of hybrids was high (53%; Table 3). Eighty percent of the misclassified hybrids were identified as cutthroat trout in the field, whereas 20% were misclassified as steelhead. Also, of the five backcrossed cutthroat trout, all five were misclassified as cutthroat trout in the field. Of the 10 F_1 hybrids, four

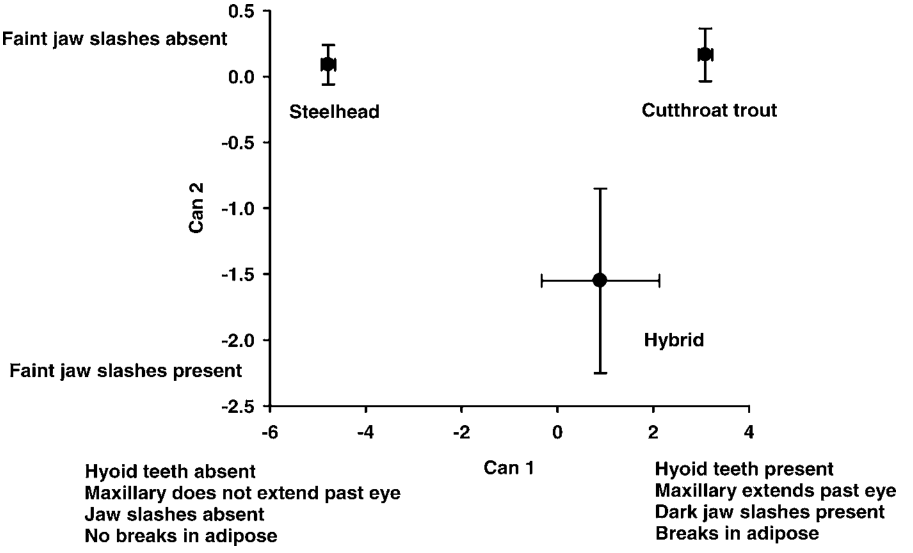

FIGURE 3.—Ordination of mean and 95% confidence intervals of phenotypic characteristic canonical scores along two canonical discriminant functions (Can 1 and Can 2) for steelhead smolts, coastal cutthroat trout smolts, and steelhead × coastal cutthroat trout hybrid smolts.

were misclassified in the field (two as steelhead and two as cutthroat trout). Of the four F_2 hybrids, only one was misclassified as a cutthroat trout.

Classification showed varying results in relation to models with nonbody shape variables versus shape variables. Stepwise selection of phenotypic characters showed maxillary, teeth, jaw slashes, adipose pigment, fish condition, and migration date to be significant predictors of fish classification. Nearest neighbor discriminant analysis of the significant predictors resulted in cross-validated classification error rates (Table 3) that were higher than initial field classifications (Table 4). However, nearest neighbor discriminant analysis with the two canonical functions resulted in error rates that were lower than field identification rates. With this classification model, the error rate of classifying hybrids was lowered from 53% to 21%, while model error rates for steelhead (1%) and cutthroat trout (2%) were similar. Linear discriminant analysis of shape variables resulted in higher error rates than field classifications. Linear discriminant analysis of the shape canonical function was the least effective model, misclassifying every hybrid fish.

Discussion

In our study, the majority of steelhead and cutthroat trout smolts were correctly identified in the field using parr identification characteristics outlined by Pollard et al. (1997). Conversely, when using these same phenotypic characteristics only half of hybrids were correctly identified and those misidentified were mostly misidentified as cutthroat trout. Our results do not concur with previous studies suggesting that cutthroat trout are more readily identified as opposed to steelhead (Hawkins 1997; Baumsteiger et al. 2005). Nevertheless, given that introgression appears common and that hybrids may make up a relatively significant proportion of a population (Campton and Utter 1985; Griswold 1996; Ostberg et al. 2004; Baumsteiger et al. 2005; Williams et al. 2007), misidentification of hybrids may result in skewed demographic statistics and ultimately may negatively affect management decisions. Based upon our results, we encourage fisheries managers to consider identification error rates when determining demographic statistics from which future management decisions are made.

No singular character differentiated steelhead, cutthroat trout, or hybrid trout during field identification. In the field the best characteristics for identifying steelhead smolts from cutthroat trout smolts were maxillary extension past the eye, presence of hyoid teeth, and jaw slashes. Dorsal parr marks were least effective, presumably because salmonids tend to lose this characteristic as they undergo smoltification. Conversely, identification of hybrids was most accurate when a mixture of steelhead and cutthroat trout characteristics were present. For example in our study, hybrids were most easily discerned when they exhibited faint jaw slashes and migrated earlier during the spring. Migration date in particular was important

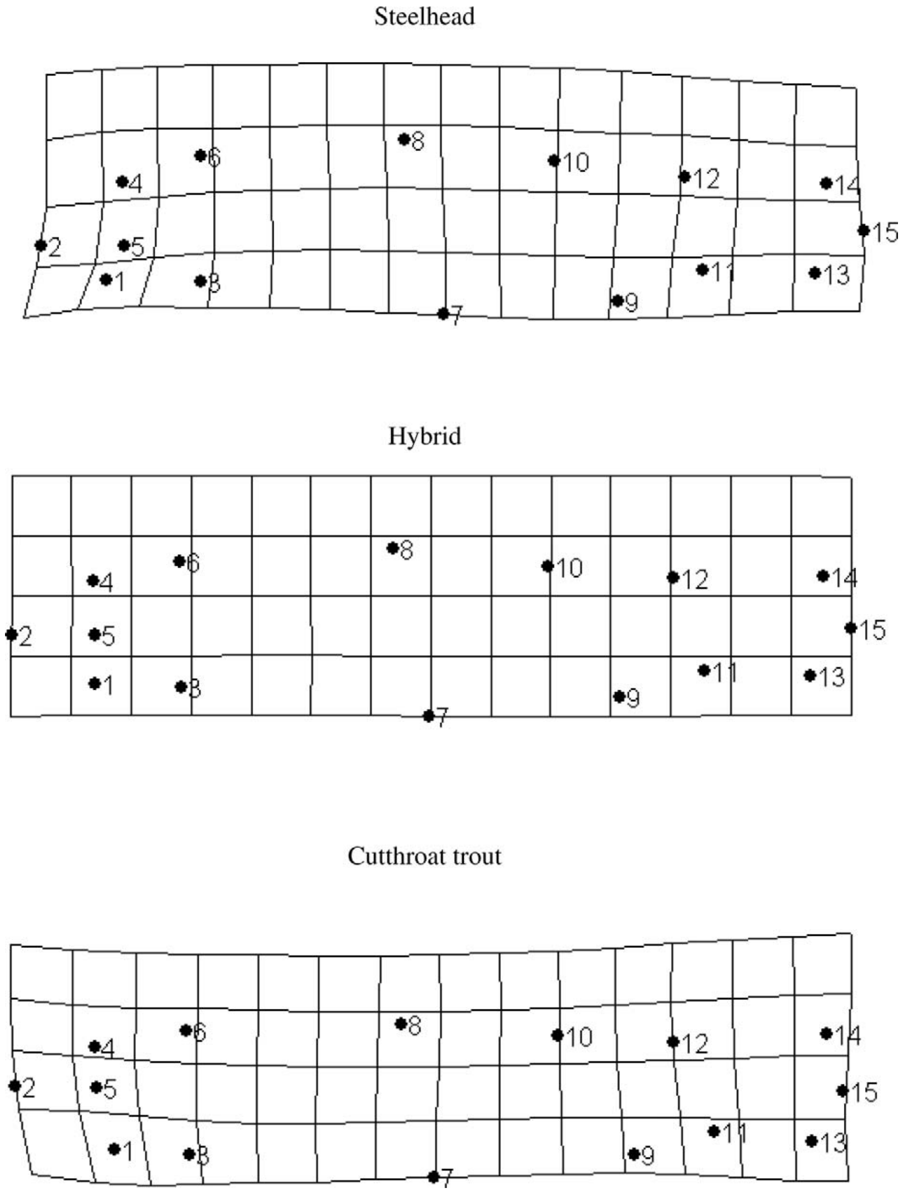

FIGURE 4.—Deformations associated with steelhead smolts, steelhead × coastal cutthroat trout hybrid smolts, and coastal cutthroat trout smolts. The grids were generated with the software TpsRegr (Rohlf 2001) by regressing partial warp scores against dummy variables representing each smolt group.

in differentiating hybrids from cutthroat trout because hybrids generally migrated earlier. Identification of hybrids was least accurate for backcrossed cutthroat trout. These hybrids displayed visual characteristics similar to cutthroat trout and thus were commonly identified as such. Although we did not detect a difference in gill Na^+, K^+-ATPase activity between hybrids and cutthroat trout, it may be used as a means for classification when coupled with migration timing differentiating steelhead from hybrids or cutthroat trout. Lastly, were unable to find any published data comparing gill Na^+, K^+-ATPase activity of steelhead, cutthroat trout, and their hybrids, but our results for Abernathy Creek suggest that hybrids follow a similar smolt physiology trajectory as cutthroat trout.

We found that steelhead, hybrids, and cutthroat trout

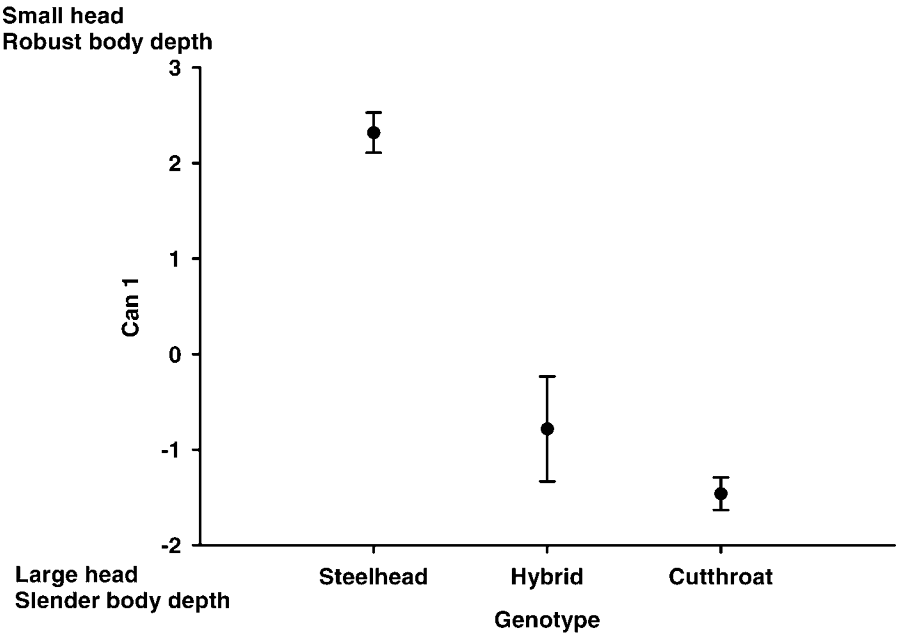

FIGURE 5.—Ordination of mean and 95% confidence intervals of body shape morphology canonical scores along one canonical discriminant function (Can 1) for steelhead smolts, steelhead × coastal cutthroat trout hybrid smolts, and coastal cutthroat trout smolts.

were morphologically distinct at the smolt stage. Morphological differences between steelhead and hybrids were much greater than between cutthroat trout and hybrids. Although few studies comparing steelhead, cutthroat trout, and hybrid morphology exist, previous data suggest juvenile steelhead have a higher critical swimming velocity than juvenile cutthroat trout, with hybrids being intermediate, and these data were correlated with cutthroat trout having narrower caudal peduncles than steelhead and hybrids having intermediate morphologies (Hawkins and Quinn 1996). These results are similar to our body shape results, where we found steelhead to be more robust in overall body shape and to have higher condition factors than cutthroat trout. However, unlike previous studies (Hawkins and Quinn 1996), this study found that hybrids were closer to cutthroat trout in body shape. Differences among studies can arise due to variations in study designs and objectives, like the inclusion or exclusion of F_2 hybrids and backcrossed cutthroat trout versus the use of reciprocal F_1 hybrids created from hatchery parents or the focus on the parr life stage versus the smolt life stage.

The smolting salmonid community in Abernathy Creek was complex, consisting of steelhead; cutthroat trout; possible F_1, F_2, and F_{2+} hybrids; and backcrossed cutthroat trout. This study, in conjunction with other studies, supports the hypothesis that steelhead and cutthroat trout mating selection in Pacific Northwest streams can be unpredictable. Hybrids have been found

TABLE 3.—Cross-classification of field identification of steelhead smolts, coastal cutthroat trout smolts, and steelhead × coastal cutthroat trout hybrid smolts with identification based on genotype.

		Field classification			
Genotypic classification	Number of fish	Steelhead	Hybrid	Cutthroat trout	Field error (%)
Steelhead	89	88	1	0	1.1
Hybrid	19	2	9	8	52.6
Cutthroat trout	129	0	3	126	2.3

TABLE 4.—Classification error rates for steelhead smolts, coastal cutthroat trout smolts, and steelhead × coastal cutthroat trout hybrid smolts.

	Model error rate (%) for smolt group		
Variable type	Steelhead	Hybrid	Cutthroat trout
Phenotypic character variables			
Selected variables	2.3	63.2	2.3
Canonical functions	1.2	21.1	2.3
Shape variables			
All variables	8.3	89.5	6.5
Canonical function	6.0	100.0	2.4

in streams with a high amount of human alteration in California (Baumsteiger et al. 2005), Oregon (Griswold 1996), and Washington (Campton and Utter 1985; Ostberg et al. 2004) as well as in relatively pristine streams in the Copper River basin of Alaska (Williams et al. 2007). Additionally hybridization and introgression have been found in streams exhibiting high gene flow, low gene flow, and directional and reciprocal hybridization (Campton and Utter 1985; Baumsteiger et al. 2005; Bettles et al. 2005). This preponderance of streams with highly variable conditions and levels of hybridization and introgression across a broad geographic range highlights the value of assessing each stream individually, especially where steelhead and cutthroat trout are sympatric and are the focus of research and monitoring studies. Thus, Abernathy Creek appears to be a large source of field identification error, primarily stemming from the introgression between hybrids and parental cutthroat trout.

By incorporating phenotypic and migration variables in statistical classification models, we were able to reduce the field misclassification rate of hybrid smolts from 53% to 21% while keeping the identification error rate of steelhead and cutthroat trout similar to field estimates. A similar study with westslope cutthroat trout *O. clarkii lewisi* and introduced rainbow trout in Idaho was able to use models to reduce field identification error from 39% to less than 14% (Weigel et al. 2002). Although our study involved different subspecies, life history types, and study sites, we both found jaw slash intensity and the presence or absence of hyoid teeth to be useful traits for identification. Conversely, Weigel et al. (2002) found spot shape to be important, while we did not examine this trait; we found maxillary extension past the eye to be useful, while Weigel et al. (2002) did not examine this trait. It is encouraging that some of the traits we examined were also used with success in Idaho. However, we caution that due to the complex and variable phenotypes that steelhead and cutthroat trout exhibit, traits found to be useful in Abernathy Creek may or may not be useful in other areas. We encourage others to repeat our study in other streams throughout the region to determine the robustness of applying the use of phenotypic traits in our study throughout the range of steelhead and cutthroat trout.

Incorporating statistical classification models and field identification error analyses into data collection and reporting can provide quantifiable evidence for which traits should be used to identify a fish. Collecting this type of data can reduce cost and improve the overall products of a given project (see Weigel et al. 2002). For example, classification models can be used to train new biologists, prioritize sample collection efforts, and increase the robustness of population surveys. Ultimately, project objectives dictate the kinds of data validation measures that are necessary for proper inference, and we recommend that assessment of breeding structure and field identification error rate is valuable for a broad array of objectives when working in streams with sympatric populations of steelhead and cutthroat trout.

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