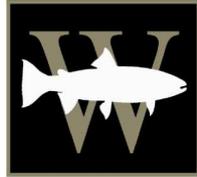


**Characterization of natural coastal cutthroat (*Oncorhynchus clarki*)  
and coastal rainbow (*Oncorhynchus mykiss*) trout hybridization  
across physical habitat gradients in Ellsworth Creek, WA**



**Wild Fish Conservancy**

N O R T H W E S T

S C I E N C E   E D U C A T I O N   A D V O C A C Y

**A report by Wild Fish Conservancy submitted to USFWS**

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## INTRODUCTION

Coastal cutthroat (*Oncorhynchus clarki*) and rainbow (*Oncorhynchus mykiss*) trout have existed in sympatry in Pacific Northwest for millennia, and as a result, natural reproductive-isolating mechanisms such as spatial and temporal segregation of spawning between the species, have evolved to prevent complete genetic mixing of the two species (Campton and Utter 1985; Trotter 1989). Despite these isolating mechanisms, natural hybridization between sympatric coastal cutthroat and rainbow trout populations was first documented more than 20 years ago (Campton and Utter 1985), and has been subsequently observed across the range of sympatry from northern California (Baumsteiger et al. 2005) to southeast Alaska (Williams et al. 2007). Hybridization between the two species was initially of conservation concern because of the harm caused to interior subspecies of cutthroat through introgressive hybridization with introduced rainbow trout (Allendorf and Leary 1988). However, unlike findings of studies completed on interior subspecies, research on coastal cutthroat and rainbow trout has failed to reveal the widespread presence of hybrid swarms, with the only hybrid swarms being documented in isolated resident populations above migration barriers, and in streams where non-native hatchery-origin rainbow trout have been introduced (Johnson et al. 1999). Rather, studies have consistently found a higher frequency of hybridization among younger trout than older trout in several populations, leading to the suggestion that hybrid fitness is reduced in adult lifestages, possibly due to the divergent migratory behaviors of cutthroat (seasonal migrations of modest distance in marine areas) and rainbow trout (full-fledged anadromy and cross-oceanic migrations) in coastal areas (Campton and Utter 1985; Hawkins 1997; Baker et al. 2002; Ostberg et al. 2004). Despite the apparent lack of fitness of hybrids, there is a considerable degree of uncertainty surrounding the behavioral and environmental mechanisms governing hybridization, and under what conditions it might pose a conservation risk to coastal cutthroat or rainbow trout/steelhead populations.

To begin to answer these questions, the relationship between various environmental gradients and the occurrence of hybridization must be established. Ostberg et al. (2004) documented coarse-scale spatial partitioning between coastal cutthroat trout, rainbow trout, and hybrids in two small (500 ha.) coastal streams along the Strait of Juan de Fuca. There, rainbow trout/steelhead were more abundant in the lowest of three study reaches, and least abundant in uppermost reach, with an opposite pattern for cutthroat, and an intermediate pattern for hybrids. Their results suggest a clinal hybrid zone is present, in which coastal cutthroat and rainbow/steelhead maintain separate niches along habitat gradients, with hybridization occurring in transitional areas where niche overlap occurs (Hagen and Taylor 2001). However, Ostberg et al. (2004) did not quantify habitat characteristics, concluding that, “a physical habitat analysis would be the next progression for uncovering the basis for habitat partitioning and the clinal hybrid gradient.” Our study links spatially-explicit pure parental species and hybrid distribution data with quantified habitat data collected throughout the Ellsworth Creek basin in the Willapa Bay area of southwest Washington.

The Ellsworth Creek basin is a 5,000 acre watershed owned and managed in its entirety by The Nature Conservancy (TNC) within the Pacific Northwest Coast Ecoregion in Pacific County, Washington (Figure 1). The watershed, a tributary to the Naselle River at Willapa Bay, ranges from sea level to 1,570 ft. in elevation, includes a 349-acre estuarine emergent marsh at its mouth, and is representative of watersheds in Southwest Washington / Lower Columbia coastal cutthroat trout distinct population segment (DPS). Situated adjacent to the USFWS Willapa

National Wildlife Refuge, the Preserve presents a unique opportunity for conservation and long-term ecological research.

The Nature Conservancy is implementing an ambitious conservation management research strategy for the watershed to study the effects of a range of management and restoration activities that will be monitored over the long-term. As part of the foundation for this research, TNC contracted Wild Fish Conservancy (WFC) in summer 2007 to characterize fish species composition, distribution, and abundance throughout the Ellsworth Preserve. WFC's 2007 documentation of coastal cutthroat trout, rainbow trout, and putative hybrids led to the development of a subsequent study to characterize trout genotype, phenotype, and distribution in 2008.

Although a legacy of anthropogenic impacts remain from past silviculture in the watershed, the preserve contains one of the healthiest fall chum salmon (*Oncorhynchus keta*) populations in Willapa Bay, in addition to coho salmon (*O. kisutch*) and multiple life history forms of coastal cutthroat trout (*O. clarki clarki*), and steelhead/rainbow trout (*O. mykiss*). In addition to characterizing the fish populations, TNC has completed systematic stream habitat, forest structure, hydrological, and road sedimentation surveys, creating a comprehensive baseline against which future watershed changes can be compared. The protected status of the watershed combined with the long-term monitoring component of TNC's monitoring / management plan make the Ellsworth Watershed an ideal location for research and conservation activities.

Here we present the results of genetic analysis of Ellsworth Creek coastal cutthroat trout, rainbow trout/ steelhead, and their hybrids that were collected throughout the watershed in June 2008. The purpose of this study was to 1) characterize the distribution of trout hybridization in the Ellsworth Creek basin and 2) analyze existing stream habitat data to determine which environmental variables correlate with the documented spatial distribution of cutthroat trout and rainbow/steelhead hybridization. The results of this study will improve understanding of the mechanisms governing hybridization between coastal cutthroat and rainbow trout. This information may be considered by resource managers in the development of management and conservation plans. In addition, this information will assist future efforts to accurately identify these species and their hybrids in the field, and can be used to refine species-specific abundance estimates within the Ellsworth Creek Preserve.

## METHODS

### *Fish and Habitat Data Collection*

We used continuous upstream single-pass electrofishing (Smith-Root, Model 12A) to collect coastal cutthroat trout and rainbow trout from June 16-18, 2008. Sampling occurred in discrete 100 meter reaches separated by equal or greater distances of non-sampled channel. We sampled 20 reaches in mainstem Ellsworth Creek from the end of tidal influence upstream for approximately 4.5 kilometers, and 15 reaches in three tributaries from their confluences with the mainstem upstream 0.5-1.5 km. In each reach, we attempted to capture at least two age 0+ trout fry and two older trout. A key assumption of our study was that the distribution of age 0+ trout would closely approximate the distribution of their parental spawning sites because at the time of the study (late June), trout fry would have recently emerged from the interstices and had limited

time and physical capability to move large distances (more than a few hundred meters). Accordingly, sampling in each reach was terminated once the numeric goals were obtained to avoid potential excessive sampling of siblings which would amount to pseudo-replication when relating subsequent genotypes to habitat parameters. In addition, we collected habitat data for each study reach, including latitude and longitude using hand-held Garmin Rhino GPS units, gradient using clinometers, and bank-full channel width (bfcw), which was measured as a perpendicular channel cross section extending between the stream banks from point at which scour stopped and perennial non-aquatic vegetation began.

All trout captured were anesthetized with tricaine methane-sulfonate (MS-222) in order to obtain a caudal fin clip, photographs, and record morphological and meristic data. Fin tissues were dried onto waterproof paper and stored for subsequent DNA extraction. For each individual, we recorded fork-length and five common characteristics of phenotype used to differentiate between juvenile coastal cutthroat trout and rainbow trout: length of maxillary relative to the eye, the number of white dorsal fin interspaces, the number of median dorsal parr marks, the presence and appearance of lower jaw “slashes,” and whether the black pigmentation around the perimeter of the adipose fin was continuous or intermittently interrupted (Appendix 1) (Pollard et al. 1997; Baumsteiger et al. 2005). Each fish was also assigned a species identification “guess” by each of the two experienced field technicians for subsequent comparison with the ‘true’ genetic identity. All fish were released following a brief period of recovery into the habitat units in which they were captured.

#### *DNA Extraction and Analysis*

We completed all genetic lab work at the Molecular Genetics Facility at the University of Washington School of Aquatic and Fishery Sciences. Our molecular analysis generally followed the methodology of Ostberg and Rodriguez (2004) with some modifications. We obtained 2 mm diameter hole-punches of each fin tissue from dried samples and placed them in individual tubes containing T1 lysis buffer and Proteinase K. DNA extractions were performed with a DNeasy Blood and Tissue 96-Well plate kit (Qiagen).

We used single primer PCRs with four primers known to amplify bi-parentally inherited and co-dominant markers and produce polymorphic PCR products that distinguish RBW from CT (Table 1) (Ostberg and Rodriguez 2004). The PCR amplifications used 20- $\mu$ l reaction volumes with 10-50 ng of genomic DNA, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 2.0 mM  $Mg_2Cl_2$ , 0.2% Triton X-100, dNTPs at 200  $\mu$ M each, 1.0 U of Taq Biolase DNA Polymerase (Bioline), and 0.125  $\mu$ M primers. The PCRs were amplified for 35 temperature cycles as follows: initial 94°C dwell for 2 min. (4 min. for OCC 16), followed by 94°C denaturing for 30 sec., followed by the primer-specific annealing temperature for 1.5 min (Table 1), followed by 72°C extension for 1.5 min., followed by 72°C dwell for 3 min. PCR products were visualized on 2% agarose gels stained with ethidium bromide. Band sizes were estimated using a HyperLadderIV 100-bp ladder size standard (Bioline).

#### *Species Assignments*

Species and hybrid assignments followed Ostberg et al. (2004). Individuals displaying homozygous rainbow trout products at all four loci (8 markers), were called rainbow trout, while

individuals displaying homozygous cutthroat trout products for all four loci (8 markers), were called cutthroat trout. Individuals which displayed heterozygous products for all loci (4 cutthroat and 4 rainbow markers), were called F1 hybrids, and all other individuals with mixed marker compositions were called, collectively, Post-F1 hybrids, indicating a mixture of backcross genotypes.

Mitochondrial DNA markers to determine the species of the maternal parent of individuals are currently being processed by Carl Ostberg (USGS).

### *Age Assignments*

Following species assignments, length frequency histograms were constructed to characterize ages of fish from the distribution of lengths for each species (Figure 3). The length frequency distribution including all individuals revealed a trimodal distribution with large (~20 mm) gaps between modes (Figure 3a). We felt the gaps were sufficiently large to allow the assignment of ages to individuals based on their length. Individuals between 27 and 59 mm fork length were designated age 0+, individuals between 82 and 155 mm were designated age 1+ and individuals between 180 and 196 mm were designated age 2+.

### *Statistical Analysis*

In order to relate habitat characteristics to species distribution data, we used a subset of generalized linear models referred to as logit-link GLM, which relates weighted proportions of binary response variables to continuously measured predictor variables with a logit function, similar to a logistic regression.

Because trout can move substantial distances from where they are born (Hoffman and Dunham 2007), analyses were conducted separately for age 0+ trout and older fish. We combined ages 1+ and 2+ because of the small number of age 2 individuals and because there was no reason to separate them since both age classes would have had opportunities to move substantially from where they were born. The proportion of each genotype (rainbow, cutthroat, hybrid) relative to the total number of fish of a given age group collected in a reach was used as the dependent variable in order to accommodate the different sample sizes in each reach. The predictor variables tested included stream gradient; bank-full width (b<sub>fw</sub>) of the channel; and drainage area contributing to each sample reach, which was estimated using ArcInfo 9.3 (ESRI, Inc., 380 New York St., Redlands CA 92373-8100). Separate models were constructed for each species-age class combination (e.g. age 0+ rainbow) because it was expected that spatial distributions between age classes of a species might differ, either due to ontogenetic shifts in habitat preference (Jonsson and Jonsson 1993), or simply due to random dispersal over time. Akaike's Information Criterion for small sample sizes (AIC<sub>c</sub>) was calculated to compare and rank the various models (Burnham and Anderson 2002).

### *Correspondence between Phenotypes and Genotypes*

For each trout sampled, field biologists described each of five phenotypic characteristics in the field: maxillary length, jaw slash intensity, adipose breaks, white dorsal fin ray tips, and median dorsal parr marks. For all age 1+ and older fish, the categorical data collected in the field were

compared with the genotype of each fish sampled. Field staff were unable to consistently collect all the required phenotypic characteristics from age 0+ fish due to the fishes small size; lethal sampling and analysis under a dissecting scope would be required to effectively accomplish this. Additionally, some or all of the traits had not yet developed on most age 0+ fish, and thus analysis of these data would not be informative.

Each team of two biologists made a 'field-call' of each fish being sampled based on all variables available to the biologists; these variables including phenotypic characteristics, location in the watershed, size of the fish, and habitat type where the fish was collected.

## RESULTS

### *Fish Samples and Habitat Characteristics*

We sampled trout and collected habitat data from 20 mainstem and 15 tributary reaches within the Ellsworth Creek basin. Reaches sampled spanned a wide range of habitat conditions, from low gradient unconfined alluvial depositional channels just above the Ellsworth Creek estuary, to high gradient confined hillslope-process dominated transport channels in the headwaters. Basin area above reaches sampled spanned two orders of magnitude (16.67-1660.38 ha), the range of bankfull widths spanned just under one order of magnitude (1.83-13.72 m), and gradients ranged from 1.5 to 7 percent slope (Table 2).

We collected tissue from a total of 130 trout ranging from 27 to 196 mm in fork length, with modes around 40, 120, and 190 mm (Figure 3a). Between 0-5 age 0+ (mean = 1.77), and 0-10 age 1 and 2+ trout (mean = 1.86) were sampled in each reach (Table 2). Trout were field classified as rainbow trout, cutthroat trout, and hybrids.

### *Genetic Results*

Of the 130 samples collected, DNA was successfully extracted from 127 and PCR amplification of all four loci was successful for 120. For the remaining 7 fish, only one PCR failed, so the remaining three successfully amplified markers were used to assign those individuals according to Ostberg et al (2004). Marker frequency distributions were constructed which showed a bimodal distribution of the number of individuals with each number of cutthroat and rainbow markers (Figure 2). The bimodality of the distribution and the presence of individuals with every possible marker count suggested that trout in Ellsworth Creek basin were not part of one hybrid swarm, but rather comprised populations of rainbow and cutthroat trout, between which some hybrids are formed with subsequent later generation hybrids and backcrosses to both parental species. With only four loci, it was not possible to assign hybrid individuals beyond the F1 generation, so they are collectively referred to as "post-F1 hybrids."

The results of the genetic analysis revealed that cutthroat trout, rainbow trout, F1 hybrids and post F1 hybrids were present in the Ellsworth Creek basin. Among 1+ age individuals, all of these genetic groups were present, while among 0+ age individuals, no F1 hybrids were present, and all age 2+ individuals were cutthroat trout (Table 3). The largest proportion of age 0+ fish were rainbow trout (63.1%), while the largest proportion of age 1+ trout were cutthroat trout

(53.4%). Hybrids were a smaller proportion of both age 0+ fish (12.3%) and 1+ age fish (36.2%), with F1 hybrids comprising 7% of the 1+ age trout (Table 3).

#### *Distribution of Rainbow Trout, Cutthroat Trout, and Hybrids*

Separate maps constructed showing the distribution of both age 0+ and age 1 and 2+ cutthroat trout, rainbow trout and hybrids in Ellsworth Creek showed apparent spatial partitioning between the species (Figures 7 and 8). Age 0+ rainbow trout were only found in the mainstem of Ellsworth Creek and in a few cases the lowermost reaches of tributaries, while age 0+ coastal cutthroat were only found in the smaller tributaries and the upper reaches of large tributaries. Few age 0+ trout were identified as hybrids (post-F1 hybrids only, n=8), limiting strength of inferences made about their distribution. All were found in four study reaches; three at the upper limit of rainbow trout distribution and the lower end of cutthroat trout distribution, and one in a tributary within the cutthroat distribution (Figure 7). As expected given the likelihood of their movement over time, spatial partitioning among age 1 and 2+ cutthroat trout, rainbow trout, and hybrids was not as apparent. While the general pattern was similar to that seen in age 0+ trout for rainbow trout, with most being found in the mainstem of Ellsworth Creek, the pattern was markedly different for cutthroat trout, which were found throughout the watershed (Figure 8).

A series of logit-link GLM models were constructed in order to relate the observed spatial distribution patterns to habitat characteristics. There was strong multicollinearity between predictor variables (Table 4), so models were constructed using each variable independently, as well as multiple variable models which included the best variable from the single variable models.

Models developed to correlate the distribution of age 0+ cutthroat trout, rainbow trout and hybrids with habitat characteristics revealed that basin area was an important variable (Table 5). Basin area was the best single variable model for both age 0+ rainbow trout and 0+ cutthroat trout distribution, and was the best overall model when considering all variables for age 0+ cutthroat trout (Table 5). Models including basin area explained between 64.8-82.5% of the variability in age 0+ rainbow trout distribution and 65.7-70.4% of the variability in age 0+ cutthroat distribution (Table 5). For cutthroat trout, bankfull width alone was almost as good a fit as basin area. Although bankfull width and gradient explained a considerable amount of the variability in single variable models, they were highly correlated with basin area and thus added little predictive power to models already containing basin area (Table 5).

Maximum likelihood estimation of model parameter slopes suggested that age 0+ rainbow trout and 0+ cutthroat trout distributions were correlated with habitat variables in opposing directions (Table 6). Age 0+ rainbow trout distribution was positively correlated with basin area, while age 0+ cutthroat distribution was negatively correlated with basin area in all models (Table 6; Figures 4 and 5). Age 0+ rainbow trout distribution was negatively correlated with gradient, while age 0+ cutthroat distribution was positively correlated with gradient in all models (Table 6). Age 0+ cutthroat distribution was consistently negatively correlated with bankfull width, but the relationship between age 0+ rainbow trout distribution and bankfull width was inconsistent (Table 6).

Models developed to correlate the distribution of age 1+ and 2+ trout with habitat characteristics generally revealed basin area to be an important variable, with considerably less support for models not containing basin area (Table 7). The best models for age 1+ and 2+ trout only explained 28.5% and 36.1% of the variability in rainbow trout and cutthroat trout distribution respectively (Table 7), which was about half of the variability explained in age 0+ models. The best fit model for age 1+ and 2+ cutthroat trout also included bankfull width, although this variable in the absence of basin area was not a good predictor of age 1+ cutthroat distribution.

### *Correspondence between Phenotypes and Genotypes*

Age 1+ and older steelhead, cutthroat, and hybrids differed in the non-lethal categorical phenotypic characteristics assessed in the field (Table 8). All steelhead sampled had maxillaries that did not extend beyond the back margin of the eye, while only 87% of the cutthroats' maxillaries extended beyond the eye. The hybrid maxillary metric was intermediate, with 43% of the hybrids' maxillaries extending beyond the eye. Most steelhead had no jaw slashes (85%), while 100% of the cutthroat had dark jaw slashes. Most hybrids had faint (67%) or dark (24%) jaw slashes, only 10% had no slashes. Three-quarters of the steelhead parr had no breaks in the adipose fin margin pigment, while 90% of the cutthroat had one or more breaks. Again, hybrids were intermediate with 48% exhibiting no breaks in the adipose margin pigment. All of the steelhead had 3 or more white pigmented dorsal fin rays, compared to 86% of the hybrids and 55% of the cutthroat. Median dorsal parr marks were present on 85% of the steelhead, 43% of the hybrids, and only 19% of the cutthroat.

Field classification of age 1+ and 2+ trout compared to genetic classification demonstrate that accurate 'field calls' of species were possible for pure cutthroat and rainbow, but more difficult for hybrids (Table 9). Of the 65 individual trout assessed in the field, survey teams of two concurred on the field classification in all but six individual fish (91% agreement); 4 of the 6 disagreements occurred over the field classification of hybrids. When they agreed, field biologists were 100% correct on the field identification of rainbow and cutthroat trout, and 57% correct on the field identification of hybrids (not knowing at the time whether hybrids were present in the watershed). When incorrectly identified in the field, hybrids were most often identified as cutthroat.

## **DISCUSSION**

### *Genetic Results*

Genetic analysis of juvenile trout collected in Ellsworth Creek revealed that pure coastal cutthroat trout, rainbow trout, and hybrids were present, and that hybridization had progressed beyond the first (F1) generation (Figure 2). The presence of multiple age classes for all of these genetic groups with the exception of F1 hybrids (Figure 3), and the presence of post F1 hybrids suggests that hybrid survival and fitness is greater than zero. The lack of age 0+ hybrids in this study and the greater relative abundance of post F1 hybrids among age 1+ fish suggests that initial hybridization events between pure cutthroat and rainbow trout may be infrequent, which is consistent with previous studies (Ostberg et al. 2004; Baumsteiger et al. 2005). Some genetic studies in which hybrids were only found within the 0+ age class have suggested that the first

winter may be a critical period of low survival for hybrids, allowing for seemingly limited geneflow between cutthroat and rainbow trout populations and the maintenance of the two as distinct species (Campton and Utter 1985). The presence of older hybrids in this study and others (Ostberg et al. 2004; Baumsteiger et al. 2005; Kennedy et al. 2009) suggests that other mechanisms may allow for the pure parental species to remain functionally distinct despite the production of hybrid offspring. Such mechanisms might include hypothesized lower survival of hybrids during anadromous migrations (Campton and Utter 1985; Baker 2001); or spatial partitioning leading to reproductive isolation. This is known to occur between other closely related salmonids including bull trout and introduced brook trout (DeHaan et al. 2009), bull trout and dolly varden (Hagen and Taylor 2001), and has been observed between coastal cutthroat trout and rainbow trout (Hartman and Gill 1968; Ostberg et al. 2004). In addition, assortative mating is a likely mechanism, and has been found to be a factor in the partial reproductive isolation of sympatric morphs within a salmonid (Foote and Larkin 1988).

### *Distribution of Cutthroat Trout, Rainbow Trout, and Hybrids*

Fish species can follow a number of distribution patterns within a watershed including continuous longitudinal distributions with abrupt and well-defined limits, continuous longitudinal distributions with more gradual shifts in relative abundance, or even isolated patches associated with specific habitat characteristics. The collection of longitudinally continuous fish distribution, age, and habitat data allows for improved characterization of species distributions and their habitat associations along continuous riverine habitat gradients (Torgersen et al. 2006). Applied to hybridization, such a study design allows for the distinction between clinal and patchy hybrid zones, and for a description of habitat characteristics which may have given rise to, or are associated with, hybridization between two species.

Previous research relying solely on field identification of coastal cutthroat and rainbow trout has shown a general pattern of numerical dominance by rainbow trout in lower reaches of streams and cutthroat trout dominance in upper reaches (Hartman and Gill 1968). However, the movement and dispersal of juvenile trout during their first year of life (Kahler et al. 2001) limits inferences regarding parental spawning distributions, and Hartman and Gill (1968) may have been further hampered by the inadvertent visual misclassification of species that occurs with surprising frequency in studies of coastal cutthroat and rainbow trout (Baumsteiger et al. 2005).

Incorporating genetic analysis, Ostberg et al. (2004) also found a general pattern of numerical dominance by rainbow trout in lower reaches of streams and cutthroat trout dominance in upper reaches, with hybrids more common in the middle reaches. However, spatially discontinuous sampling precluded characterizing the distribution of each species along continuous longitudinal physical habitat gradients, and the timing of juvenile sampling limited their ability to identify species-specific spawning areas due to dispersal from natal spawning sites.

In order to characterize the spatial distribution of coastal cutthroat trout, rainbow trout, and hybrids and determine whether segregation of spawning areas was apparent, we collected trout tissues along longitudinally continuous habitat gradients in mid-June when age 0+ trout were very recently emerged (in fact many still showed signs of the embryonic yolk sac). This ensured that the dispersal of age 0+ juveniles was minimal, likely on the order of 0~100 meters, enabling us to use the distribution of age 0+ juveniles as a proxy for the distribution of adult spawners.

Analyses correlating the distribution of age 0+ trout with physical habitat characteristics revealed spatial segregation between the two species (Figures 4,5,7,8) and inverse relationships between the occurrence of each species and basin area, which was the best predictor of distribution for both species (Figures 6 and 7, Tables 3 and 4). We did not fit statistical models for the distribution of age 0+ hybrids due to a small sample size ( $n = 8$ ), although qualitatively, age 0+ hybrids were found at the upper extent of rainbow trout distribution and the lower end of cutthroat trout distribution. Assuming that the distribution of juvenile age 0+ trout was representative of their parents' spawning location preferences, these results suggest that spatial segregation may play a significant role in limiting gene flow between coastal cutthroat and rainbow trout, and may explain the smaller proportion of hybrids, particularly F1 hybrids, relative to pure cutthroat and rainbow trout.

Although the general patterns of spatial distribution for older trout were similar to those of age 0+ trout, there was more overlap in the distribution of cutthroat trout, rainbow trout and hybrids (Figure 8). The models correlating distribution with habitat characteristics explained approximately half of the variability that the same models did for age 0+ fish (Table 7). Age 1+ and 2+ cutthroat trout and hybrids were widely distributed throughout the basin. Although it is possible that the difference in distribution between the two age classes represents interannual differences in parental spawning distributions, it is more likely the result of downstream movement by older fish, which is common in diadromous and migratory fish species (Jonsson and Jonsson 1993).

#### *Correspondence between Phenotypes and Genotypes*

Like others (Weigel et al. 2002; Kennedy et al, 2009), we found that incorporating phenotypic characteristic and field identification error analyses into data collection and reporting can provide guidance for which traits are more reliable to identify a fish. Knowing which phenotypic traits are most often associated with each species should increase the accuracy of field identification. Non-lethal field identification of 0+ trout was confounded by the small size of the fish. While lethal sampling and phenotypic analysis of 0+ trout may improve field classification, we suggest that consistent and accurate identification of 0+ trout may only be possible by genetic analysis, at least during the early part of the summer before substantial growth has occurred.

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## TABLES

Table 1. Bi-parental PCR primer sets, primer products, and annealing temperatures from Ostberg and Rodriguez (2004).

Bi-parental Primer	Primer Source	Marker Size (bp)	Species	Annealing Temp (°C)
OM-47	(Ostberg and Rodriguez 2004)	275	CT	66
		300	RBW	
OM-55	(Ostberg and Rodriguez 2004)	180	CT	66
		200	RBW	
OCC-36	(Ostberg and Rodriguez 2004)	275-285	RBW	66
		325	CT	
OCC-16	(Ostberg and Rodriguez 2002)	280	RBW	50
		380	CT	

Table 2. Summary statistics for reach habitat characteristics and fish sampling in the Ellsworth Creek basin, WA.

Parameter	Mean	Min.	Max.	St. Dev.
Basin Area (m <sup>2</sup> )	5730113	166647	16603798	4796907
Basin Area (ha)	573.0	16.7	1660.4	479.7
Gradient (% slope)	2.8	1.5	7	1.3
Bankfull Width (m)	7.3	1.8	13.7	2.8
Age 0+ Sampled	1.77	0	5	1.37
Age 1 and 2+ Sampled	1.86	0	10	1.93
Total Fish Sampled	3.63	1	11	2.06

Table 3. Species assignments based on genetic analysis and length frequency histograms for Ellsworth Creek trout by age.

Species	Age 0+	%	Age 1+	%	Age 2+	%	All Ages	%
Cutthroat	13	21.0	27	44.3	4	100.0	44	34.6
Rainbow	41	66.1	13	21.3	0	0.0	54	42.5
F1 hybrid	0	0.0	7	11.5	0	0.0	7	5.5
Post F1 hybrid	8	12.9	14	23.0	0	0.0	22	17.3
All hybrids	8	12.9	21	34.4	0	0.0	29	22.8
Totals	62	100.0	61	100.0	4	100.0	127	100.0

Table 4. Linear correlation matrix for habitat variables used to predict the spatial distribution of rainbow trout, cutthroat trout, and hybrids in Ellsworth Creek.

Variable	BA	CBW	CG
log Basin Area (BA)	1.00	-0.49	-0.65
Channel Bank-full Width (CBW)		1.00	0.83
Channel Gradient (CG)			1.00

Table 5. Model selection results for habitat variables affecting the proportion of age 0+ rainbow trout and cutthroat trout in Ellsworth Creek and tributaries. Models are listed from most plausible ( $\Delta AIC_c=0$ ) to least plausible; k is the number of parameters. The ratio of Akaike weights ( $w_j/w_i$ ) indicates the plausibility of the best fitting model ( $w_j$ ) compared to other models ( $w_i$ ). All single variable and multiple variable models, which include the most important single variable, are shown. Models were not fit for age 0+ hybrids because age 0+ hybrids were collected in only 4 reaches.

Species	Model	Log Likelihood	k	AIC <sub>c</sub>	$\Delta AIC_c$	Relative likelihood	Akaike weight ( $w_i$ )	R <sup>2</sup>	$w_j/w_i$
Rainbow	log basin area, gradient	-9.793	3	26.676	0.000	1.000	0.673	0.806	1.000
	log basin area, gradient, bfw	-9.262	4	28.429	1.753	0.416	0.280	0.825	2.403
	log basin area	-14.399	2	33.320	6.644	0.036	0.024	0.648	27.710
	log basin area, bfw	-13.214	3	33.518	6.842	0.033	0.022	0.677	30.605
	gradient	-20.068	2	44.658	17.982	0.000	0.000	0.454	8031.038
	bfw	-24.526	2	53.574	26.898	0.000	0.000	0.300	693182.797
Cutthroat	log basin area	-11.193	2	26.908	0.000	1.000	0.323	0.657	1.000
	bfw	-11.442	2	27.406	0.499	0.779	0.252	0.647	1.283
	log basin area, gradient	-10.350	3	27.791	0.883	0.643	0.208	0.691	1.555
	log basin area, bfw	-10.700	3	28.490	1.582	0.453	0.147	0.677	2.206
	log basin area, bfw, gradient	-10.030	4	29.965	3.057	0.217	0.070	0.704	4.612
	gradient	-21.723	2	47.969	21.061	0.000	0.000	0.239	37434.574

Table 6. Maximum likelihood estimates of intercepts and slopes for logit-link GLM models correlating age 0+ species distributions with habitat variables in the Ellsworth Creek basin, WA. Models are listed for each species in order of fit starting with the best model.

Species	Model	Intercept	log Basin Area	Gradient	Bank-full Width
Rainbow	log basin area, gradient	32.794	2.567	-1.689	
	log basin area, gradient, bfw	-42.454	3.320	-1.679	-0.223
	log basin area	-39.178	2.618		
	log basin area, bfw	-51.148	3.567		-0.316
	gradient	4.798		-1.420	
	bfw	-3.029			0.513
Cutthroat	log basin area	35.427	-2.461		
	bfw	5.639	-1.095		
	log basin area, gradient	30.798	-2.332	0.795	
	log basin area, bfw	23.077	-1.428		-0.475
	log basin area, bfw, gradient	21.431	-1.524	0.682	-0.347
	gradient	-4.250		0.960	

Table 7. Model selection results for habitat variables affecting the proportion of age 1+ and 2+ rainbow trout and cutthroat trout in Ellsworth Creek and tributaries. Models are listed from most plausible ( $\Delta AIC_c=0$ ) to least plausible; k is the number of parameters. The ratio of Akaike weights ( $w_l/w_i$ ) indicates the plausibility of the best fitting model ( $w_l$ ) compared to other models ( $w_i$ ). All single variable and multiple variable models, which include the most important single variable, are shown. Models were not fit for age 1+ hybrids because no models had reasonably good fit.

Species	Model	Log Likelihood	k	AIC <sub>c</sub>	$\Delta AIC_c$	Relative likelihood	Akaike weight ( $w_i$ )	R <sup>2</sup>	$w_l/w_i$
Rainbow	log basin area	-21.671	2	47.823	0.000	1.000	0.573	0.285	1.000
	log basin area, bfw	-21.540	3	50.081	2.258	0.323	0.185	0.290	3.093
	log basin area, gradient	-21.592	3	50.185	2.362	0.307	0.176	0.288	3.258
	log basin area, bfw, gradient	-21.491	4	52.722	4.899	0.086	0.050	0.291	11.584
	gradient	-25.725	2	55.929	8.107	0.017	0.010	0.134	57.594
	bfw	-26.296	2	57.072	9.249	0.010	0.006	0.098	101.974
Cutthroat	log basin area, bfw	-23.284	3	53.568	0.000	1.000	0.454	0.361	1.000
	log basin area, bfw, gradient	-22.430	4	54.599	1.031	0.597	0.271	0.393	1.674
	log basin area	-25.424	2	55.327	1.760	0.415	0.188	0.280	2.410
	log basin area, gradient	-24.943	3	56.885	3.318	0.190	0.086	0.298	5.253
	bfw	-31.812	2	68.104	14.536	0.001	0.000	0.023	1433.652
	gradient	-32.208	2	68.897	15.329	0.000	0.000	0.023	2131.433

Table 8. Description and composition of categorical phenotypic characteristics for age 1+ and older steelhead, coastal cutthroat trout, and their hybrids in the Ellsworth Creek basin, WA. Phenotypic characteristics were described in the field using non-lethal techniques; species identifications were based on genetic analysis. Values given are percentages.

Phenotype	Description	Steelhead (n=13)	Hybrid (n=21)	Cutthroat (n=31)
Maxillary Extends Beyond Eye	Yes	0.0	42.9	87.1
	No	100.0	57.1	12.9
Jaw Slash Intensity	Absent	84.6	9.5	0.0
	Faint	15.4	66.7	0.0
	Dark	0.0	23.8	100.0
Breaks in Adipose	Absent	75.0	47.6	9.7
	Present	25.0	52.4	90.3
Number of White-Pigmented Dorsal Fin Rays	1 or 2	0.0	14.3	45.2
	3	61.5	28.6	22.6
	4 or 5	38.5	57.1	32.3
Median Dorsal Parr Marks	Absent	15.4	57.1	80.6
	Present	84.6	42.9	19.4

Table 9. Comparison of species identification by field classification versus genetic classification. Field classification was performed by each of two field biologists in each survey team. Values for species identities and error rates are counts and percentages, respectively.

Species	No. fish	Steelhead	Hybrid	Cutthroat	Disagree	Error Rate When Observers Agreed	Error Rate Including Disagreement
Rainbow	13	13	0	0	0	0.0	0.0
Hybrid	21	2	8	7	4	42.9	61.9
Cutthroat	31	0	0	29	2	0.0	6.5

## FIGURE CAPTIONS

Figure 1. Location of the Ellsworth Creek basin in southwest Washington.

Figure 2. Frequency distribution of the number of individuals with each number of cutthroat (a) and rainbow trout (b) markers in Ellsworth Creek.

Figure 3. Length frequency histograms after genotype assignments of all trout sampled (a), rainbow trout (b), cutthroat trout (c), and hybrids (d) from Ellsworth Creek in June 2008.

Figure 4. The proportion of cutthroat trout among age 0+ trout in a reach relative to log basin area, showing the modeled relationship from a single variable logit-link GLM.

Figure 5. The proportion of rainbow trout among age 0+ trout in a reach relative to log basin area, showing the modeled relationship from a single variable logit-link GLM.

Figure 6. The proportion of hybrids among age 0+ trout in a reach relative to log basin area.

Figure 7. Map of Ellsworth Creek, WA and tributaries showing the spatial distribution of rainbow trout (yellow), cutthroat trout (red), and hybrids (brown), as proportions of the total number of age 0+ trout in found in a reach. Empty circles are reaches where no age 0+ fish were captured.

Figure 8. Map of the Ellsworth Creek basin, WA showing the spatial distribution of rainbow trout (yellow), cutthroat trout (red), and hybrids (brown), as proportions of the total number of age 1 and 2+ trout in found in a reach. Empty circles are reaches where no age 1 and 2+ fish were captured.

## FIGURES

Figure 1.

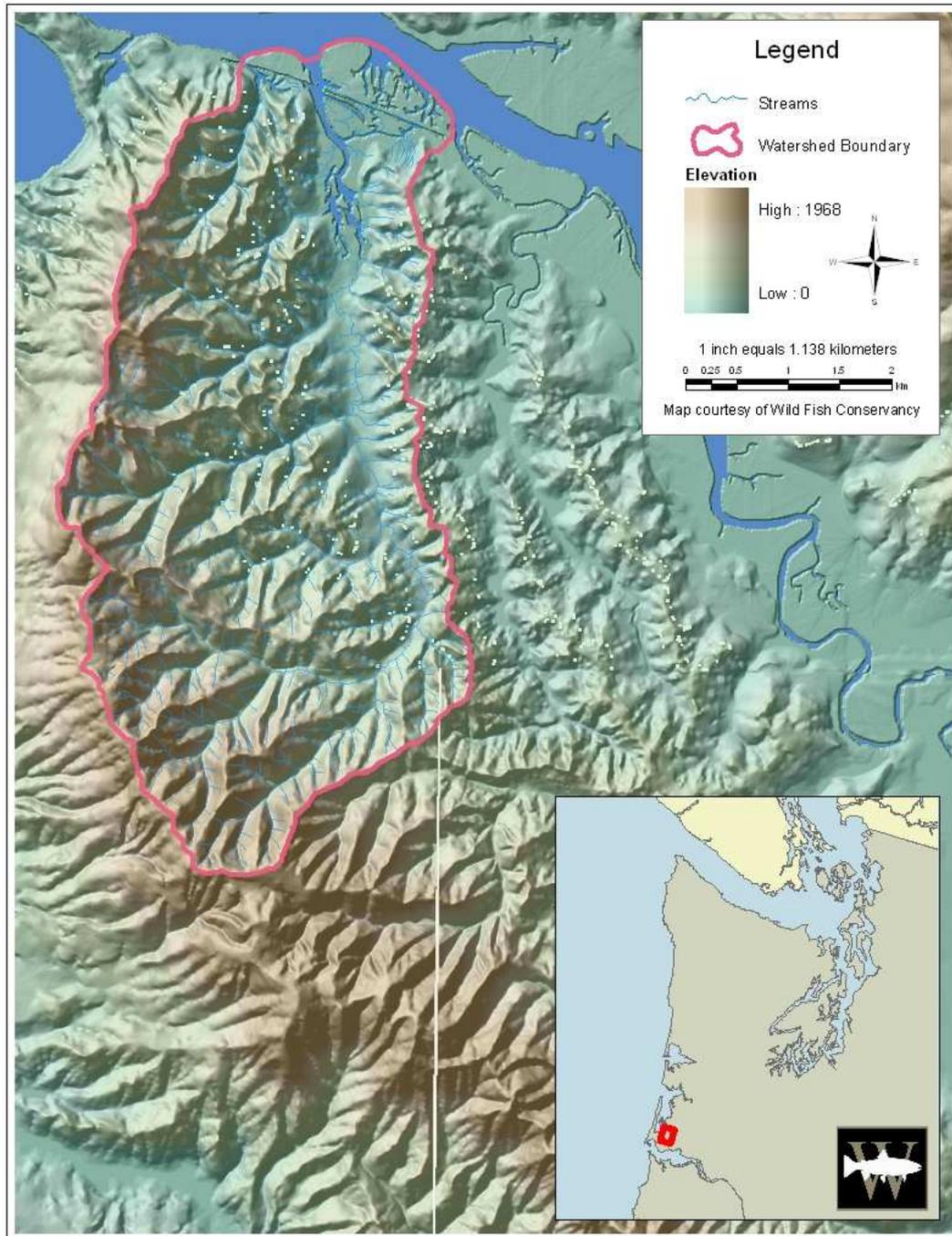


Figure 2.

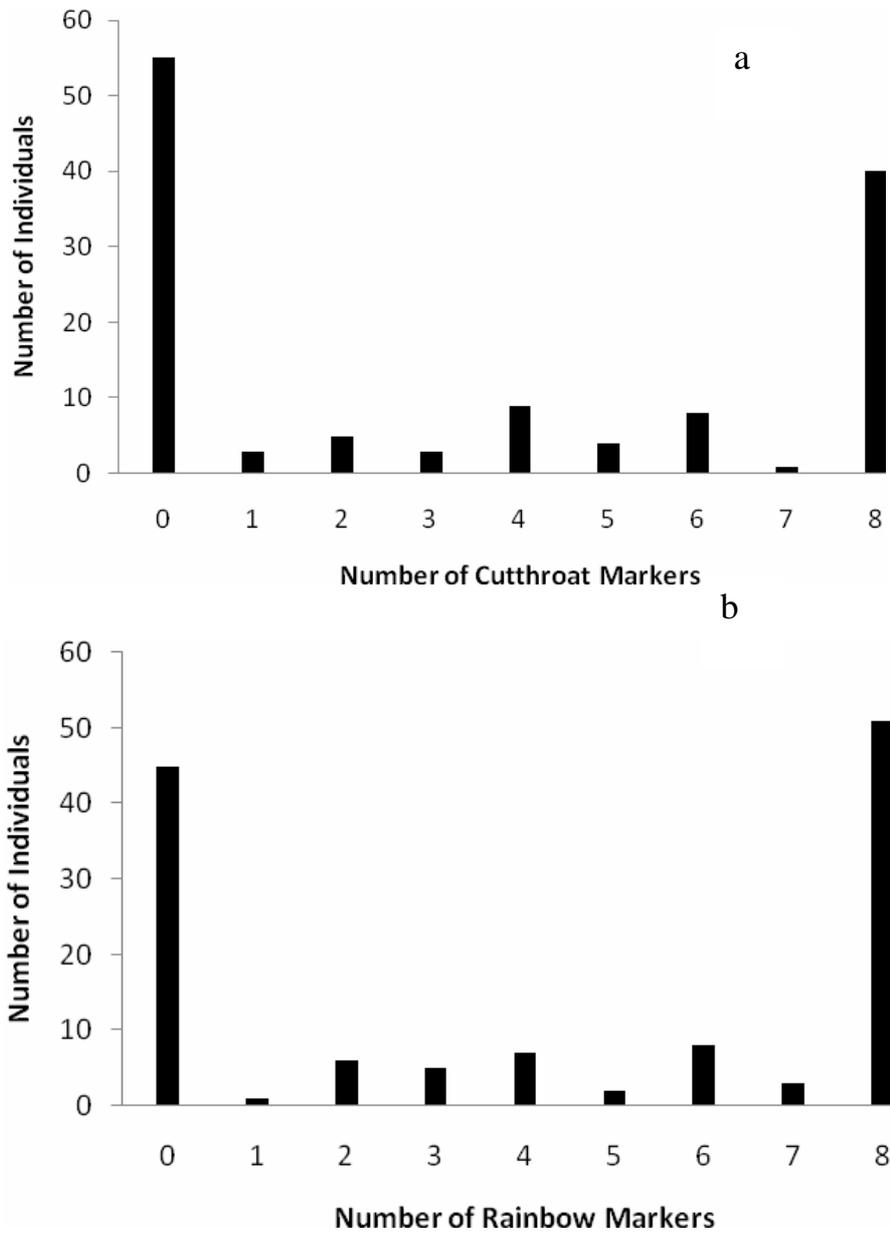


Figure 3.

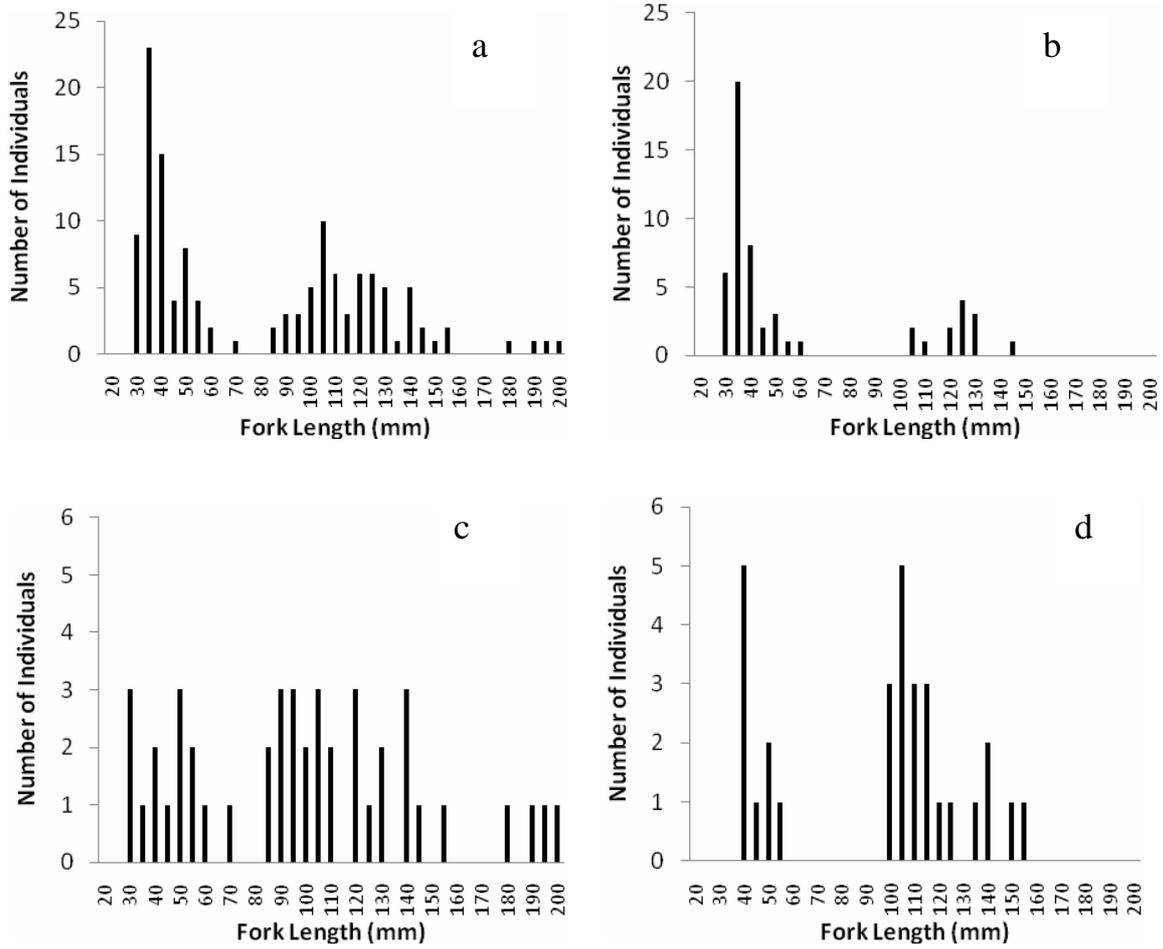


Figure 4.

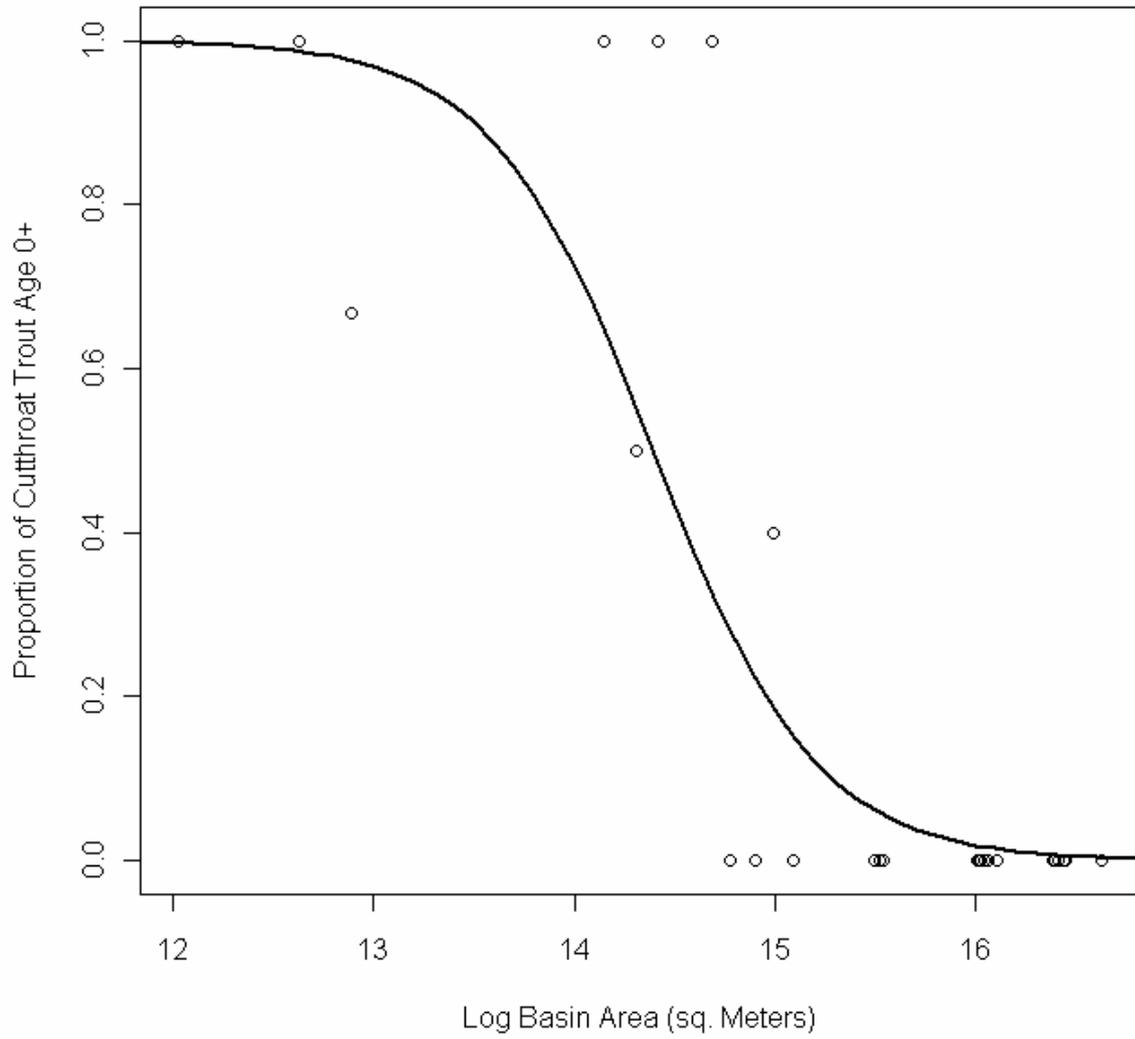


Figure 5.

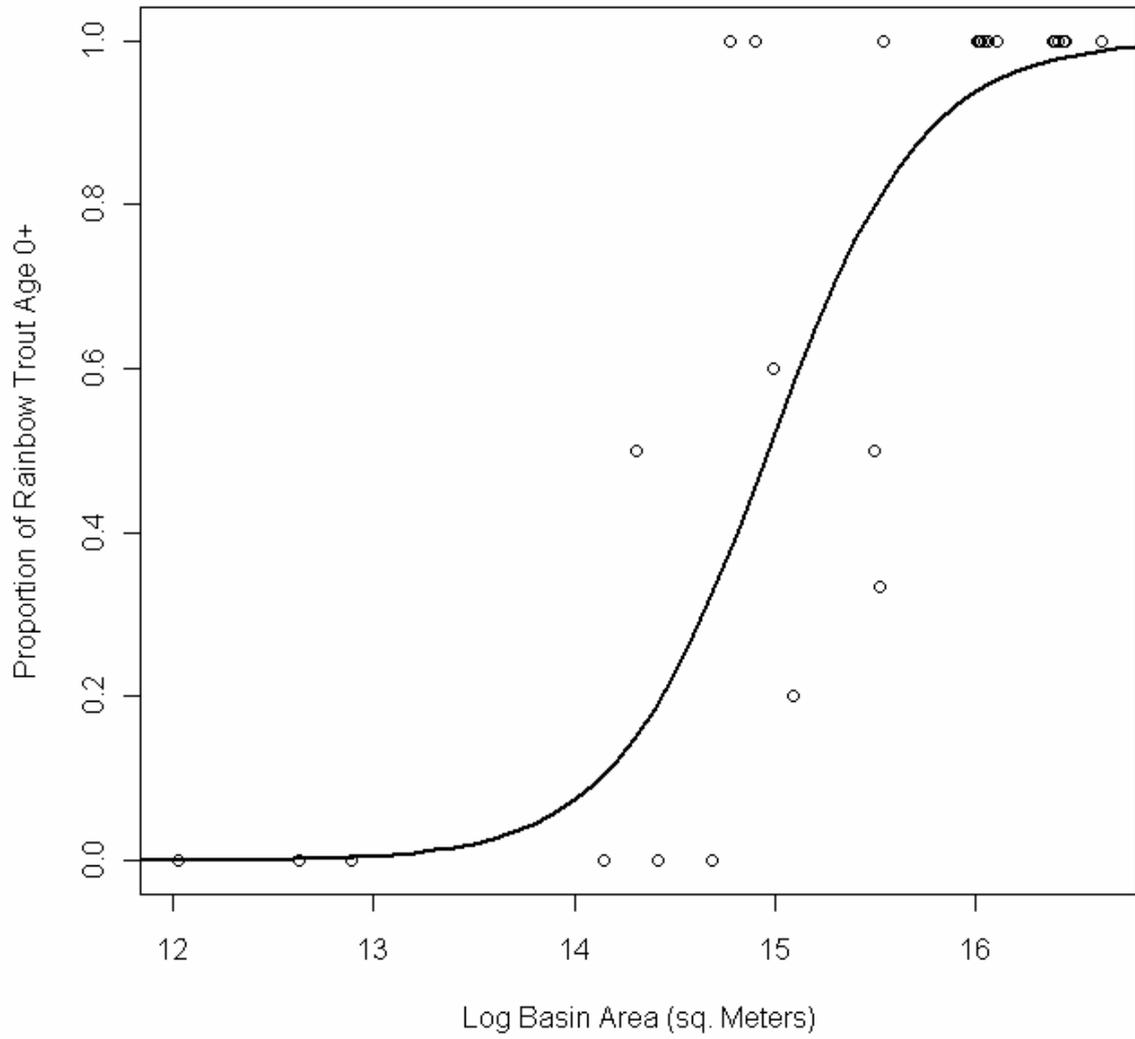


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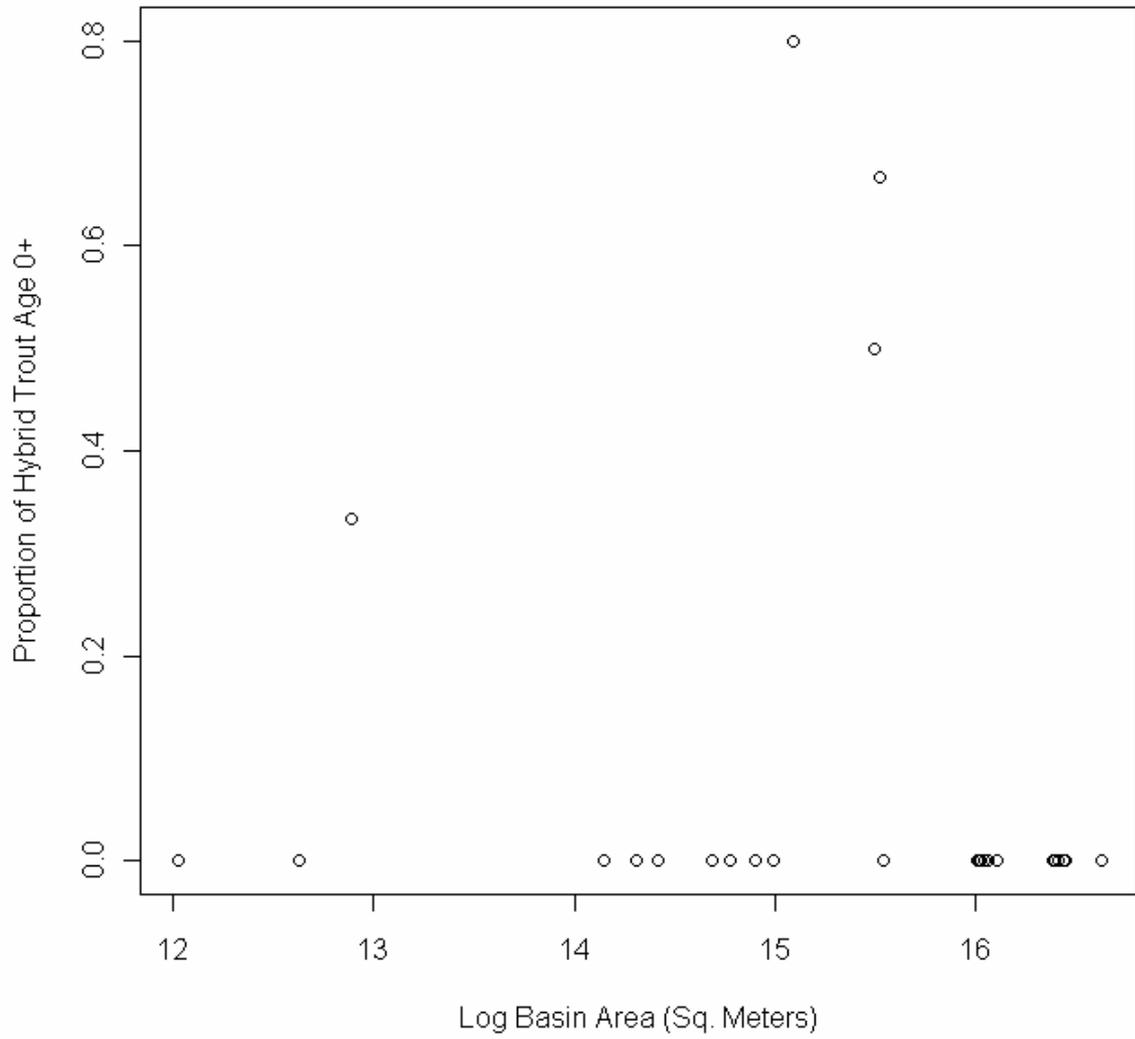


Figure 7.

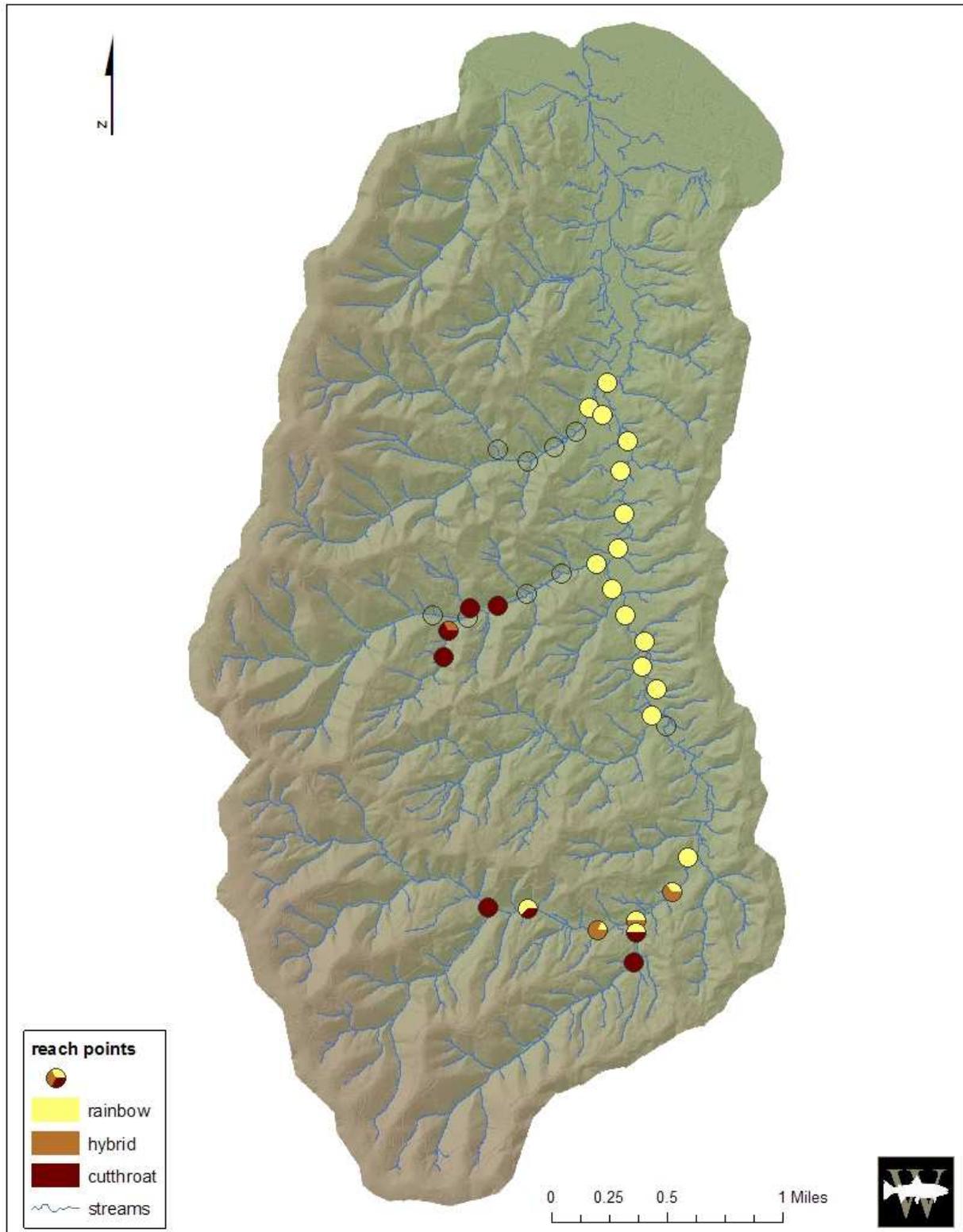
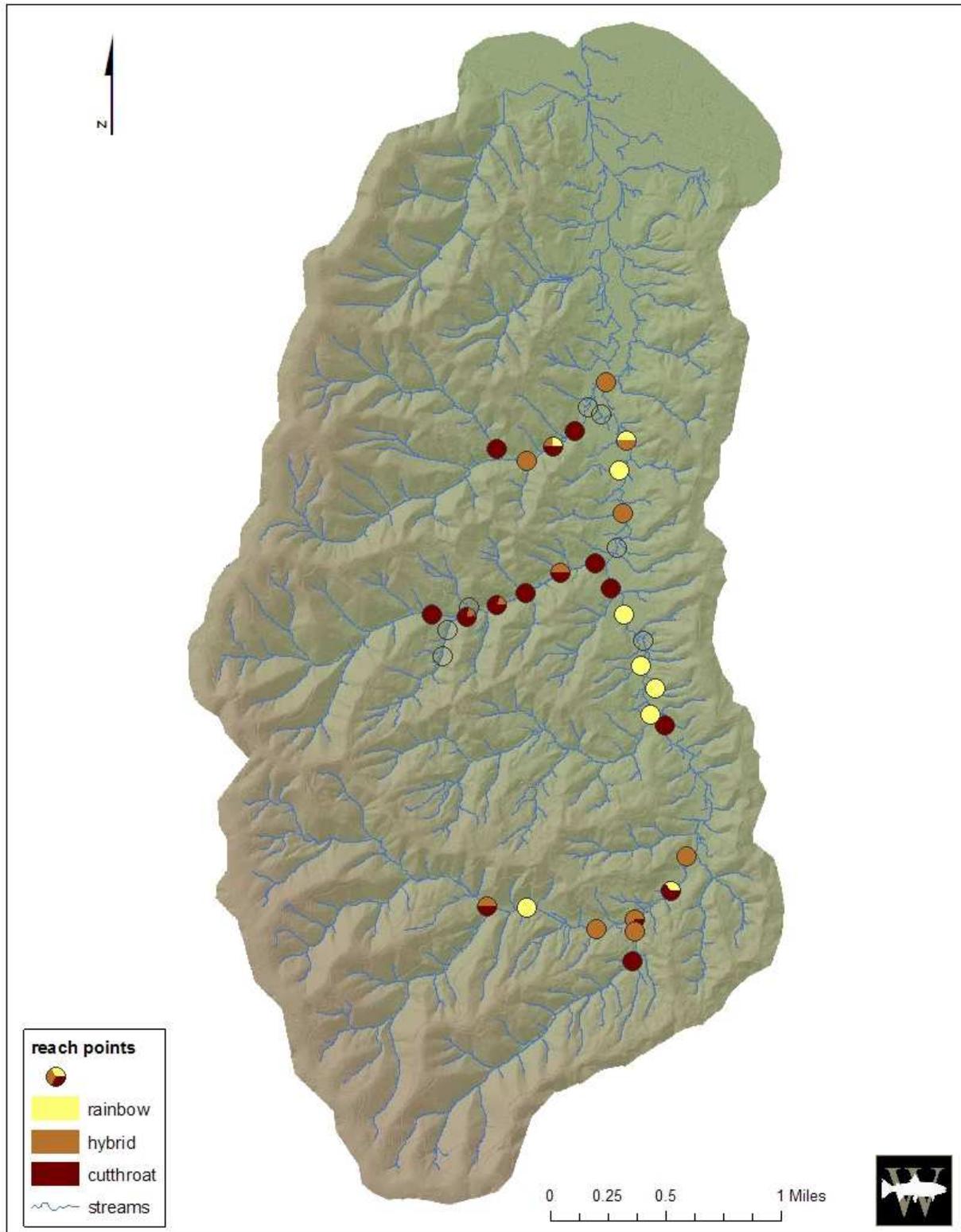


Figure 8.



## Appendix 1

Appendix 1. Phenotype characteristics used for field identification of rainbow, cutthroat, and hybrid trout and for comparisons with genetic results.

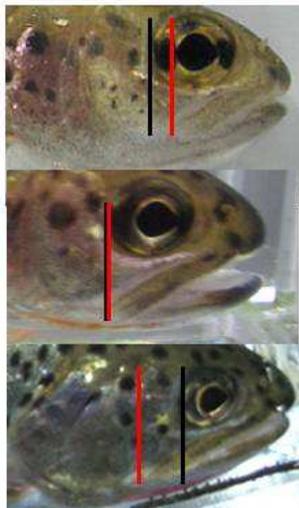
### 1. Jaw Slashes (Dark, Faint, None)



### 2. Adipose fin margin breaks

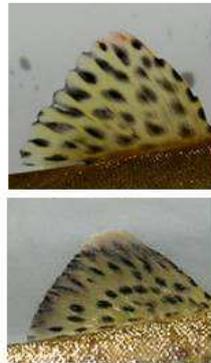


### 3. Maxillary Length



## Visual ID Characteristics

### 4. Number of White Inter-Dorsal Fin Ray Spaces



### 5. Number of Dorsal Parr Marks

