Hatcheries, phenology, and families:
Juvenile steelhead ecology in Forks Creek, Washington

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Table of Contents

List of Tables

Table **Page**

List of Figures

Figure **Page**

Chapter 1: Introgressive tradeoffs in life histories of juvenile naturally-spawned hatchery, wild, and hybrid steelhead (*Oncorhynchus mykiss)*

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Abstract

Propagated organisms may have maladaptive timing for environmental conditions, but an advantage in terms of intraspecific competition. We investigated this tradeoff in a system where hatchery-origin steelhead trout (*Oncorhynchus mykiss*) that have been bred to spawn about four months earlier than wild-origin fish were released into the wild as adults and allowed to breed in common with wild fish. We assigned naturally-spawned offspring from these matings hatchery, wild, or hybrid lineages using a panel of 96 single-nucleotide polymorphisms and then tested for differences in abundance, body size and condition, and geographic distribution. The observed mixture proportions were 0.005 hatchery-linage, 0.313 hybrid, and 0.682 wild-lineage fry. There were significant differences in fork length (hatchery > hybrid > wild), but no difference in body condition. We also modeled emergence dates of the fish we sampled to test for associations between environmental conditions and fish abundance. We estimated that 72.2% of wild-lineage fish emerged after the arrival of temperate spring conditions, compared to 47.3% of hybrid-lineage and 27.0% of hatchery-lineage fish. We hypothesize that the low abundance of pure hatchery-lineage fry may be due to a mismatch between the timing of breeding by the parents and suitable river conditions, resulting in low survival and physical displacement from the system. We draw this inference from the low representation of hatchery-lineage fry despite the preponderance of hatchery-origin parents, and from incubation models indicating that most

hatchery-lineage fish would have incubated and emerged during conditions of high river flows and low temperatures. Thus the advantages in early emergence and large size associated with hatchery origin did not mitigate the disadvantages of breeding early.

Introduction

Timing of breeding affects offspring fitness (Divino and Tonn 2007; Keast and Eadie 1984) and population dynamics (Ludsin and DeVries 1997) in fish. Breeding phenology is thought to be adapted to long-term environmental conditions to prevent a mismatch between offspring development, prey abundance, and suitable abiotic factors (Bailey et al. 2010; Durant et al. 2007). Intraspecific competition may also be affected by the relative timing of emergence (Skoglund et al. 2011). Breeding phenology is thus an important link between adult phenotype and offspring survival, and fish are thought to have evolved spawning dates that optimize competitive and environmental conditions (Murray et al. 1990; Webb and McLay 1996).

The breeding phenology of some cultured populations of anadromous salmonids has been altered through hatchery practices, due in part to the tendency of managers to spawn the first fish that arrive at the facility (Quinn et al. 2002), and in some cases, directed breeding for earlier reproduction (Crawford 1979). Salmonids, like other fishes, face a tradeoff between early and late reproduction. Offspring of early-spawning parents emerge early, which can be maladaptive. One major disadvantage occurs when life history timing is out of phase with suitable river conditions, including river flow during embryo incubation, and food availability for free-living juveniles (Quinn 2005). Scour can result in high rates of mortality for salmon embryos and several authors have speculated that the reproductive timing of wild fish is specifically adapted to avoid such conditions (Lura and Sægrov 1993; Montgomery et al. 1999; Stefanik and Sandheinrich 1999). In addition, emerging early may put individuals at increased risk of predation; Brannas (1995) found that early-emerging Atlantic salmon experienced high mortality from predation by brown trout compared to later-emerging fish.

However, early emergence may also be beneficial. Offspring of early-spawning parents experience a longer growing season, resulting in higher survival through the first year of life (Conover et al. 2003; Divino and Tonn 2007; Keast and Eadie 1984). They may have decreased exposure to gape-limited predators, including cannibalistic conspecifics (Garvey et al. 1998; Huss et al. 2008; Pine et al. 2000), and have larger gapes themselves, providing access to a wider array of prey items (Ludsin and DeVries 1997; Phillips et al. 1995). Combined, these factors may contribute to higher survival for early-emerging offspring.

Emergence timing also affects intraspecific competitive dynamics in salmonids (Tatara and Berejikian 2012). Early emergence offers the benefit of larger size and prior residence in feeding territories, which can contribute to competitive dominance (Einum and Fleming 2000; Einum and Kvingedal 2011; Keeley and McPhail 1998). Larger size at a given date provides an advantage in territorial competition, especially when combined with the advantage of holding the territory when later-spawned fish emerge (Abbott et al. 1985; Rhodes and Quinn 1998). These advantages may be expected to favor early-emerging fry, which would be larger and in possession of feeding territories when later-spawned fry emerge and attempt to compete with them.

Steelhead trout (*Oncorhynchus mykiss*) stocks in Washington State have been intentionally bred to spawn about four months earlier than their wild counterparts (Crawford 1979). This marked phenotypic divergence from wild stocks allows fish to rear in freshwater for only a single year (compared to two or more for wild fish) and, secondarily, was thought to contribute to reproductive isolation between wild- and hatchery-produced individuals. However, when hatchery-lineage fish escape or are released into the wild, their divergent phenology does

not necessarily prevent introgression due to a small overlap in spawning dates (Seamons et al. 2012).

Predicting the ecological and evolutionary effects of the widespread release of propagated fish is important, but far from straightforward. Life history characters, including reproductive timing, are heritable (Carlson and Seamons 2008). Consequently, hybridization between wild and domesticated individuals spawning in sympatry can produce hybrids with traits that are intermediate between those of the parental populations (Seamons et al. 2012). When these fish are released into the wild, natural selection may act on domesticated as well as natural phenotypes, culling individuals with maladaptive traits from the population (Bailey et al. 2010). If hatchery fish have deleterious phenotypes in the wild, intraspecific hybridization could erode local adaptation via outbreeding depression, causing a detrimental shift in breeding phenology (for example, Allendorf et al. 2001).

Here, we investigated tradeoffs experienced by early-spawning hatchery-lineage steelhead, late-spawning wild-lineage steelhead, and hybrids in a natural stream. Using genetic markers, we assigned fry to hatchery, wild, or hybrid lineages. We then tested for differences in abundance, body length and condition, and geographic distribution to assess the relative benefits of emerging early at the risk of being out of phase with environmental conditions, or emerging late, but facing a disadvantage in intraspecific competition. Body condition, a measure of mass to length, can reveal when there are differences in metabolic provisioning between groups of fish that rear in differentially productive habitats (Griffiths et al. 2013). Lastly, we modeled emergence dates of the fish we sampled to look for associations between environmental conditions and fish abundance and body size that might explain differences in the proportions of fry that we observed.

Methods

Background

Forks Creek (near Raymond, WA) is a tributary to the Willapa River on the Washington coast (Figure 1.1). Long-term monitoring was conducted from 1994 – 2011 to study the genetic composition of the steelhead population after commencement of hatchery production (McLean et al. 2004; McLean et al. 2003; Seamons et al. 2012). Historically, the river supported a small population of native winter-run steelhead trout. Hatchery steelhead smolts were introduced from Bogachiel Hatchery on the northern Washington coast in 1994 (Figure 1.1). These fish were of Chambers Creek origin, a domesticated, early-spawning hatchery stock used to enhance recreational fishing opportunities rather than conservation (Crawford 1979; Scott and Gill 2008). The Chambers Creek stock propagated at the Bogachiel Hatchery may have also incorporated some proportion of steelhead native to the Bogachiel River (Mackey et al. 2001). The first hatchery-lineage adult steelhead returned to the Forks Creek Hatchery in the winter of 1995/1996. The majority of these returns were used as broodstock in the hatchery, but surplus individuals were released into Forks Creek, where they were granted access to the spawning grounds used by wild steelhead. Forks Creek, previously naïve to the presence of hatchery steelhead, underwent a major introduction of out-of-basin fish.

Fish counts and radio tracking indicated that the hatchery-origin adults were present in Forks Creek in the winter (mean spawning date: January), about four months earlier that wild fish (Mackey et al. 2001). In the winter of 1995/1996, counts at the weir indicated that 165 hatchery-origin adults and 32 wild-origin adults passed upstream. Hatchery females outnumbered wild females nearly nine-to-one on the spawning grounds (11 hatchery-origin, 90

wild-origin, McLean et al. 2004). Yet even though hatchery females were numerically dominant, they had lower reproductive success, whether measured by number of smolts produced or the number of returning (McLean et al. 2003; McLean et al. 2004). There was a small temporal overlap in spawning date ranges of wild and hatchery fish, and despite differences in median spawning date, a high proportion of hatchery-wild hybrids were observed in both smolts and returning adults in the first cohort of steelhead following the introduction of hatchery fish to the system (Seamons et al. 2012).

Sample collection

Adult steelhead returning to the Forks Creek watershed were collected weekly at a weir downriver from the hatchery during winter and spring of 1995/6 through 1997/8. The origin of these fish (hatchery or wild) was determined based on the absence or presence of an adipose fin, as hatchery fish were marked by removal of the adipose fin prior to their release as juvenile fish. Length, sampling date, and sex were recorded and a fin clip collected for genetic analysis. These adult individuals of known hatchery or wild origin constitute the baseline samples that were used to assign juveniles that were spawned in the wild to hatchery, wild, or hybrid lineages (Table 1.1).

Naturally-spawned/wild-origin steelhead fry, offspring of hatchery- and/or wild-origin adults that returned in the winter of 1995/1996, were sampled. Fry were collected from Forks Creek between July 23 and August 28, 1996 using one-pass electrofishing over 19 km of the stream, including tributaries (Table 1.2). We refer to these naturally-spawned fry as hatchery-lineage, hybrid, or wild-lineage fish depending on their ancestry. A fin clip was collected for genetic analyses and stored at -80º C. Fork length (to the nearest 1 mm) and mass (to the nearest

0.1 g) were recorded. These samples included age-0+ fish, young-of-the-year (YOY), and age-1+ fish (yearlings), distinguished by the bimodal distribution of body lengths with yearlings greater than or equal to 100 mm.

Sampling locations were plotted in ArcGIS 10 (ESRI 2011). We subdivided Forks Creek into stream sections based on hydrology and hatchery practices (Figure 1.1). GPS data recorded in 1996 were only accurate to approximately 60 m, so for the purposes of data presentation, we snapped sample collection locations to the stream using Geospatial Modelling Environment 0.7.2. RC2 (Beyer 2012).

Genotyping and marker selection

Baseline individuals of known origin were initially genotyped at 192 single nucleotide polymorphisms (SNPs). Genomic DNA was extracted from fin clips using a standard protocol for Qiagen DNeasy Blood and Tissue Kits (Qiagen, Valencia, California) with one modification. Small fin clips taken from juvenile steelhead 16 years prior to genotyping yielded low, variable, and in some cases, degraded concentrations of DNA, so we concentrated DNA by ethanol precipitation and also included a preamplification step as described by Smith et al. (2011). All individuals were genotyped using the 5' nuclease assay on a medium-density array as described by Seeb et al. (2009).

From the original panel of 192 SNPs, we selected 93 with the highest F_{ST} for the final genotyping panel (Appendix 1). We confirmed that F_{ST} provided the highest assignment success for our specific populations and marker set over other approaches, specifically BELS 1.0 (Bromaghin 2008) and WHICHLOCI (Banks et al. 2003) based on leave-one-out tests in ONCOR (http://www.montana.edu/kalinowski/Software/ONCOR.htm; Anderson 2008). This is

consistent with the findings of Storer et al. (2012), who evaluated a suite of marker selection approaches and found F_{ST} to be one of the most informative. We identified individuals that had been inadvertently resampled by searching for matching genotypes in CERVUS 3.0 (Kalinowski et al. 2010; Slate et al. 2000). Cutthroat trout (*O. clarkii*) co-occur in Forks Creek and morphologically resemble steelhead fry. The final panel of genetic markers included three diagnostic loci for cutthroat trout described by McGlauflin et al. (2010) so that cutthroat trout could be screened and excluded. The final genotyping panel contained a total of 96 markers. Juvenile steelhead were genotyped as described above at 96 nuclear SNPs and 12% of samples were rerun at all loci to assess data quality. Individuals that failed to genotype at > 6 loci were excluded from further analyses.

Stock identification: individual assignment

Two primary methods are used to identify stock of origin (Manel et al. 2005): individual assignment and mixture proportions. In individual assignment, each fish is assigned back to population of origin, and some individuals may remain unassigned at a given confidence level. To calculate mixture proportions, aggregate proportions of the sample are assigned back to populations of origin. In many cases, mixture proportions are calculated directly (e.g., Anderson et al. 2008), but we followed the methods of Seamons et al. (2012) and calculated true mixture proportions by correcting for known mis-assignment rates from individual assignment.

We first evaluated accuracy and precision of methods to assign individuals to hatchery, hybrid or wild classes using simulated genotypes. We generated 5000 hatchery, wild, and hybrid individuals from our baseline populations in Hybridlab (Nielsen et al. 2006). We then compared assignment success of simulated individuals in STRUCTURE 2.3.3 (Pritchard et al. 2000),

ONCOR (Anderson et al. 2008), GENECLASS2 (Piry et al. 2004), and Discriminant Analysis of Principal Components (DAPC, Jombart et al. 2010) implemented in the ADEGENET 1.3-4 package (Jombart and Ahmed 2011) for R 2.15.1 (R Core Development Team, 2012). DAPC offered the best combination of power and consistency and was used for individual assignment.

First, we performed DAPC on the baseline individuals using population of origin as a prior to establish genetic clusters as recommended by Jombart et al. (2010). Next, simulated genotypes were assigned to classes using the *predict.dapc* function, which assigns new individuals to previously established clusters. Uniform prior probabilities of group membership were applied, and posterior assignment probabilities were used to infer population of origin. This established clusters for pure wild and hatchery lineages. Hybrids were expected to have intermediate assignment probabilities, whereas pure individuals were expected to assign with high probability to either the hatchery or wild lineage. Cutoffs for confident assignment to hatchery, hybrid, and wild groups were determined such that 90% of each simulated group was assigned to the correct category. Mis-assignment rates were determined based on the proportion of each simulated group to hatchery, wild, hybrid, or unknown classes. Finally, wild-origin juvenile fish were assigned to clusters using the *predict.dapc* function and posterior assignment probabilities used to determine hatchery, wild, hybrid, or unknown lineage.

Stock identification: mixture proportions

In addition to assigning individuals to groups, we also calculated the proportion of each group in the juvenile sample. We did so by correcting the observed (raw) proportions from the individual assignment analysis according to the mis-assignment rates of simulated individuals.

We calculated the true mixture proportions using the following equations, as described by Seamons et al. (2012):

$$H_{obs} = (H|M_h) \cdot x + (H|M_w) \cdot y + \left(H\middle|M_{hyb}\right) \cdot z$$

$$W_{obs} = (W|M_h) \cdot x + (W|M_w) \cdot y + \left(W\middle|M_{hyb}\right) \cdot z$$

$$U_{obs} = (U|M_h) \cdot x + (U|M_w) \cdot y + \left(U\middle|M_{hyb}\right) \cdot z$$

In this system of three equations with three unknowns, H_{obs}, W_{obs}, and U_{obs} are observed (raw) hatchery, wild, and unassigned mixture proportions. M_h, M_w, and M_{hyb} are the proportions of simulated genotypes assigned to each group. For example, $H \mid M_h$ is the probability that a pure hatchery genotype is assigned to the hatchery group, whereas $H \mid M_w$ is the probability that a pure wild genotype is mis-assigned to the hatchery group. Lastly, x, y, and z are the unknown variables, i.e. the true proportions of hatchery, wild and hybrid fish, which we solved for using linear algebra in R. Confidence in mixture estimates was evaluated by bootstrapping across individuals; the observed assignments were resampled with replacement and new adjusted mixture proportions calculated. Resampling was repeated 10,000 times. The 95% confidence interval was defined as the 0.025 and 0.975 quantiles on bootstrapped mixture estimates. Mixture proportions were calculated for the total mixture, YOY, and YOY broken down by stream section.

Body length and condition

We tested for differences in fork length and Fulton condition factor (K) between the three different assignment groups. Condition factor is an index of mass to length,

$$K = 10^N \cdot W/L^3$$

where W is mass (g), L is length (mm), and N is 5, a scaling factor to bring value close to one (Cone 1989). For both body length and condition factor, we tested for homogeneity of variance using Bartlett's test and applied a natural log transformation when variances were heteroscedastic. We tested for differences using a one-way ANOVA and, when appropriate, identified differences between groups using a Tukey post-hoc test (Zar 2010).

We also sought to determine whether differences in individual performance or stream productivity during early rearing conditions resulted in differences in metabolic provisioning between hatchery, wild, and hybrid fish. To address this, we evaluated body condition using regression models. Body condition is the relationship between ln(length) and ln(mass), and the preferred method for measuring fish condition according to Cone (1989). Following the methods of Griffiths et al. (2013), we compared four alternative models to determine if there was a difference in the body condition between fish assigned to hatchery, wild, or hybrid groups. For individuals *j* of lineage *i*, the models were 1) common slope and intercept for all individuals, 2) different intercepts according to group and a common slope, 3) common intercept and different slopes for each group, and 4) different slopes and intercepts according to group (Table 1.3). Model selection was performed using Akaike's Information Criterion (AIC, Burnham and Anderson 2002). Support for models 2 – 4 would be interpreted to reflect a difference in body condition between hatchery, wild, and/or hybrid groups.

Emergence model

We hypothesized that the environmental conditions that fish experienced shortly after emerging may explain possible differences in abundance and body condition between hatchery, hybrid, and wild groups. Average stream temperature and flow in Forks Creek from 1995-1998

were reported by Mackey et al. (2001), allowing us to address this question. We obtained the relationship between relative growth rate (percent body weight per day) and temperature for rainbow trout from Bear (2005),

$$r = -0.7691 + 0.4215 \cdot T - 0.0173 \cdot T^2$$

The mass at emergence was not known, but estimated to be 15% less than the lightest fish sampled (0.425 g). We fit this model to our data to account for differences in the age of the fish studied and possible population-specific growth rates. The basic relationship between growth rate and temperature was maintained, but the growth rate optimized to allow the median emergence date for the wild fry, which was the largest sample, to correspond to the median emergence day calculated based on when their parents were in the stream based on redd counts.

$$r = 0.45 + 0.4215 \cdot T - 0.0173 \cdot T^2$$

The resulting model had a maximum growth rate of 3.3% body mass per day, which is appropriate for juvenile steelhead (Wurtsbaugh and Davis 1977b). We calculated the temperature-specific growth rate for each day during the emergence period (March 1 – August 31) and then back-calculated to the emergence date that best explained the observed weight of the fish on the day it was sampled.

This model included the following assumptions: 1) growth rate was independent of the size of the fish, 2) growth rate was independent of lineage, and 3) ration sizes were constant. The effects of temperature on growth rate vary based on ration level, but this parameter is not readily estimated and assumed to be constant (Wurtsbaugh and Davis 1977a; Wurtsbaugh and Davis 1977b). Larger fish grow more slowly than smaller fish at a given ration level, but this effect was not strong on the size range of YOY that we sampled (Wurtsbaugh and Davis 1977a).

Results

Laboratory analysis

We obtained high-quality genotypes for 93 wild and 90 hatchery adult steelhead of known origin and 1022 juveniles of unknown lineage. Two of the juveniles had identical genotypes and both were discarded, yielding a final sample size of 1020 individuals. We observed a genotyping error rate of 0.15% in the 12% of samples that were rerun at all loci. No markers were out of Hardy-Weinberg equilibrium in the baseline populations after Bonferroni correction for multiple comparisons (Appendix 1).

Individual assignment

F_{ST} between hatchery and wild baseline populations was 0.025, providing ample power for differentiating pure wild- from pure hatchery-lineage individuals. F_{ST} between simulated wild and hatchery populations was 0.028. F_{ST} was 0.007 and 0.008 between the simulated hybrids and the simulated pure wild and hatchery populations, respectively. Unsupervised Bayesian clustering algorithms such as STRUCTURE should only be used when siblings are removed from the dataset (Anderson and Dunham 2008; Rodriguez-Ramilo and Wang 2012). We removed full siblings from our dataset, but found that the presence of half siblings confounded the analysis. Half siblings could not be removed because doing so would have dramatically reduced the sample size and because half sibship cannot be used to infer the lineage of other half sibling group members. The pedigree analysis is discussed in detail in Chapter 2. Of the remaining methods (ONCOR, GENECLASS, and DAPC), DAPC offered the greatest power for detecting groups. Consequently, individual assignment was performed using DAPC.

Simulated wild and hatchery genotypes had high posterior probabilities of belonging to their respective populations (Table 1.4). However, simulated hybrids also had high posterior probabilities of belonging to hatchery or wild groups, resembling each of the pure groups in roughly equal proportions (Table 1.4a, Figure 1.2). Cutoffs were determined such that no more than 10% of the 5000 simulated genotypes from each possible lineage were assigned to the incorrect category (Table 1.4b). Individuals with a posterior probability of 0.999996 or greater of belonging to the wild population were identified as wild, posterior probabilities between 0.9585322 - 0.016243 of belonging to the wild population were considered hybrid, and probabilities of belonging to the wild population of less than 0.000006 were considered hatchery-lineage. Yet even with these cutoffs, 31% of simulated individuals assigned as hybrids were of simulated wild or hatchery lineage (Table 1.4b). Applying stricter cutoffs reduced the sample size without improving the mis-assignment rate.

Applying these cutoffs to the juvenile steelhead of unknown lineage, we assigned 602 out of 1020 individuals (59%): 388 wild-lineage, 195 hybrid, and 19 hatchery-lineage fish. The majority of pure hatchery- and hybrid-lineage fish were YOY. However, 14 out of 26 yearlings were identified as hybrids and one appeared to be of pure hatchery lineage.

Mixture proportions

The raw proportions of hatchery, wild, hybrid, and unknown fish in the dataset were 0.019 hatchery-lineage, 0.191 hybrid, 0.380 wild-lineage, and 0.410 unassigned. After correcting for mis-assignment we estimated the true mixture proportions were 0.005 hatchery-lineage, 0.313 hybrid, and 0.682 wild-lineage (Table 1.5). For YOY, these proportions were 0.007 hatchery-lineage, 0.289 hybrid, and 0.704 wild-lineage. Hybrid YOY steelhead were present in

all stream sections (Figure 1.3). The proportion of hybrid fry was highest in Upper Forks Creek, Tributary 1, and Ellis, while the proportion of pure wild fry was higher in Lower and Middle Forks Creek (Figure 1.3).

Body size and condition

Yearlings were identified as longer than 100 mm (mean=121.2, SD=12.9, N=53) and YOY shorter (mean=60.3, SD=11.8, N=967). Fork length and body condition were analyzed for all YOY assigned to a class that had mass and length measurements (N=571). We identified outliers in the relationship between ln(mass) and ln(length) and removed observations that were greater than four standard deviations from the line of best fit (N=4), yielding final sample sizes of 371 wild-lineage, 179 hybrid, 17 hatchery-lineage fish.

There was a significant difference in fork length between wild (mean=55.9 mm, SD=9.91), hybrid (mean=66.3 mm, SD=12.2) and hatchery fish (mean=73.5 mm, SD=12.2; ANOVA, $F_{2,564}$=66.8, p<0.0001, Figure 1.4a). Variances were not homogeneous for raw fork length (Bartlett's K^2=10.4, df=2, p=0.005) so data were natural log transformed to conform to the assumptions of the test (Bartlett's K^2=1.05, df=2, p=0.591). A Tukey test for multiple comparisons indicated hatchery fish were larger than hybrids (p=0.047) and wild fish were smaller than both hatchery and hybrid fish (p<0.0001). Variances were homoscedastic for condition factor (Bartlett's K^2=1.16, df=2, p=0.559) and condition factor did not differ between groups (ANOVA, $F_{2,564}$=0.147, p=0.863, Figure 1.4b).

We evaluated four models (Table 1.3) to determine whether there was difference in the mass-length relationship between hatchery, hybrid, and wild fish. Model selection based on Akaike's Information Criterion indicated that the simple model (Model 1) was the best fit for the

data suggesting no difference in body condition among hatchery, wild, and hybrid groups (Table 1.6, Figure 1.5). We found strongest support for Model 1. The mean standard error was nearly identical for all four models; AIC almost exclusively reflected the penalty for adding additional parameters.

Emergence model

We estimated the emergence date for each YOY fish in the dataset and compared that to the estimated emergence date based on when adults were spawning in the system. The median emergence dates were June 13 for wild fish, May 26 for hybrids, and May 9 for hatchery fish. The date for wild-lineage fish was similar to the June 17 estimate by Mackey et al. (2001) based on when the adults were in the river, as we used this date to fit the model. However, the estimate of April 21 for hatchery-lineage fish was earlier than the estimate based on fry that survived several months after emergence. We noted a marked change in river conditions after May 11, 1996, when flow dropped below 2 m^3/second and temperature rose above 7 ºC. We estimated that 96.0% of wild fish emerged after the change in conditions (Figure 1.6), 80.5% of hybrids had also emerged after May 11, but only 41.2% of pure hatchery fish had emerged after this date. Only 5.88% of the hatchery fish in our sample emerged on or before April 21, their hypothesized median emergence date from Mackey et al. (2001) based on the timing of adult reproduction.

Discussion

Based on mixture proportions, we estimated that only 0.5% of fry in the system were of pure hatchery-lineage, despite the fact that hatchery-origin adults outnumbered wild in the spawning areas (165 hatchery-origin adults, 32 wild-origin adults; Mackey et al. 2001; McLean

et al. 2003). However, the presence of hatchery-origin fish did have a genetic impact on Forks Creek, as hybrids represented 31.3% of the sample, with the remaining 68.2% being of pure wild lineage. We were able to assign pure hatchery-lineage and pure wild-lineage fish to their respective lineages with greater than 90% confidence. However, due to the overlap of genotypes, we were only able to assign hybrid fish with approximately 70% confidence – stricter cutoffs did not reduce the error rate.

Several factors may explain the scarcity of pure hatchery-lineage fry. Hatchery-lineage fish would be older on average than their wild counterparts, and thus would have had more time to experience the cumulate effects of mortality and dispersal. The bulk of mortality for fry occurs soon after emergence; for instance, 65% of Atlantic salmon died in the first 4 months after emergence, and 65% in the first 17 days (Einum and Fleming 2000). Therefore, even if hatchery-lineage, hybrid, and wild-lineage offspring were produced and survived at equal rates per day, we would expect to encounter fewer hatchery and hybrid fish simply because they had more time to die or disperse during the intervening months.

However, given that hatchery-origin adults greatly outnumbered wild fish in the parental generation, it seems unlikely that age-dependent mortality is the only factor that explains their very low abundance in the system. As hypothesized, adverse environmental conditions may have culled the offspring of early-spawning adults, either as embryos or after the young fish emerged from the gravel. Another possibility is that a portion of this cohort was displaced downstream into the Willapa River during high flows and was therefore absent from the system during our sampling. These fish may have returned to natal spawning grounds in Forks Creek as adults. There is some evidence to support the idea that these fish may not have been lost to the system entirely. Seamons et al. (2012) analyzed the mixture proportions for wild-origin smolts and

adults in the same cohort as the fry in this study. Although there were essentially no pure hatchery-lineage smolts in their sample, hatchery-lineage fish comprised 10% of the adults that returned to the system. Perhaps the pure hatchery-lineage smolts, not detected by Seamons et al. (2012), were not actually lost to the population, but by exiting the system early as fry they did not have an opportunity to compete with wild-lineage juveniles.

Hybrid-lineage fry made up about one third of the fish in our sample. Seamons et al. (2012) found 69% hybrids in smolts and, subsequently, 17% as returning adults. As hybrids were likely produced by late spawning hatchery-origin fish mating with early-spawning wild-origin fish, they were probably spawned at an intermediate date. This idea is supported by the finding that hybrids were intermediate in body length, which is a function of age. Based on their abundance as fry and smolts, hybrid fish may have emerged late enough to avoid adverse environmental conditions.

Of 26 yearlings with confident assignment, 14 assigned to the hybrid group, and 1 to the hatchery group. This did not meet with our expectations, as the first wild-spawned fish of hatchery lineage would have emerged in 1996, not 1995. It is possible that some YOY fish were longer than the 100 mm cutoff we determined from the distribution of body lengths and were therefore erroneously identified as yearling fish. Some male hatchery fish may have returned to spawn after only one year at sea or matured with having migrated to sea at all, and in either case could have produced hybrid offspring a year older than most of the fish we sampled. Given the tremendous influence that the Forks Creek hatchery exerted starting in 1994, a combination of the above could explain the observed results.

Geographic distribution

We observed hybrid fry in all stream sections, including in Ellis Creek and Tributary 1, up to 12 km upstream from the hatchery (Figures 1.1, 1.2). Hatchery-origin adults may have ascended the watershed before spawning or their offspring may have dispersed throughout the watershed as fry. Radio-tracking of adults by Mackey et al. (2001) indicated that hatchery-origin fish moved into the upper watershed to spawn, but in smaller proportions than wild-origin fish (7/33 hatchery, compared to 6/10 wild) and stayed approximately 4 km farther downstream than wild-origin fish. Dispersal is also common for juvenile salmonids. Anderson et al. (2013) found that 28% of juvenile coho salmon (*O. kisutch*) were collected outside of their spawning reach, and dispersed up to 6.3 km. Therefore, it is possible that hatchery influence reached the upper watershed when adults were spawning, when hybrid offspring dispersed from downstream spawning reaches, or both.

Hatchery-lineage, hybrid, and wild-lineage fish were not distributed evenly throughout the watershed. We observed the highest proportion of wild-lineage fish lower in the system, where the majority of fish spawn (Mackey et al. 2001). This pattern is the opposite of that observed for the spawning adults. The pattern could reflect age of juveniles – wild-lineage fish were younger and had not had an equal opportunity to disperse or die in the time since they emerged. It is also possible that offspring of hatchery-origin fish that spawned in lower Forks Creek died or moved downstream (i.e., out of the sampling area and into the Willapa River itself) before our sampling was conducted. Yet another explanation is that nests of hatchery females were disturbed by later-spawning wild fish. Redd superimposition is a density dependent factor (Quinn 2005) and could have played a role in survival of hatchery-lineage offspring.

Body length and condition

Fork length was significantly different between all three groups and followed the pattern expected based on the age of the fish: hatchery-lineage fish were the largest, hybrid fish were intermediate, and wild-lineage fish were the smallest. Hatchery and hybrid fish were spawned earlier in the year and so had more time to grow. Yet despite the difference in length, there was no difference in body condition. It is important to note that since our analyses involve individual assignment, up to 30% of hybrids may belong to the wild or hatchery lineage, which may inflate the variance in body condition for putative hybrids. If there was a difference in condition factor, this would be expected to be greatest in the wild-hatchery comparison. The linear models for each of these were virtually identical for all classes, despite difference in sample size and overall body length. In fact, AIC almost exclusively reflected the penalty for adding parameters in the Models 2 – 4, which allowed different slopes and/or intercepts. This further supports the idea that there is no difference in the relationship between mass and length between groups and suggests that the identification error is not obscuring relevant trends.

We conclude that individual variation outweighs effects of lineage and associated differences in early rearing conditions in this system. The difference in survival or residence in the system may be explained by different conditions experienced as embryos and fry, rather than hatchery-lineage induced or seasonal differences in individual performance. Although this finding is observational, it is consistent with that of Tatara and Berejikian (2012), who reviewed the effects of competition between juvenile wild salmonids and released hatchery fish. They found that among the small number of substitutive experiments that had been conducted, there was no difference in competitive abilities between hatchery- and wild-origin fry as measured by growth.

Emergence model

Estimating the date of emergence based on fish mass revealed that most hatchery-lineage fish likely emerged before relatively benign river conditions of late spring (mid-May), with temperatures above 7 ºC and flows below 2 m^3/second. Nearly all wild-lineage fish emerged during higher temperatures and low flows, consistent with the hypothesis that the wild population is adapted to favorable seasonal conditions. This model provides additional insight into why hybrids may have had higher survival than pure hatchery-lineage fish: most emerged after the arrival of temperate spring conditions. The median emergence date we estimated for hatchery-lineage fish, May 9, was later than that calculated by Mackey et al. (2001) based on adult data: April 21. In fact, we estimated that only 6% of hatchery fish we sampled emerged before April 21. These estimates are consistent with the hypothesis that hatchery-origin adults spawning at or before the median spawning date produced virtually no fry that remained in the system through the middle of the summer, and that the hatchery-lineage fry that were present represented those with the latest spawning parents.

We found no evidence to support the hypothesis that early emerging fish have an overall competitive advantage over later-emerging counterparts in this system. This finding differs from that of several other studies. In Atlantic salmon, early emerging offspring had the highest rates of survival and largest final body size, despite experiencing the harshest environmental conditions (Skoglund et al. 2011). Einum and Fleming (2000) also found that selection favored early-emerging fry in Atlantic salmon. Research on coho salmon has shown that prior residents were dominant over intruders of the same size, but that intruders with at least a 6% length advantage were equal competitors (Rhodes and Quinn 1998). Combined, these factors would suggest an

advantage to hatchery or hybrid fry in our system, which were both longer and present in the river before wild fish.

The difference between this study and previous work is likely due to the magnitude of the difference in spawning dates between hatchery and wild steelhead. Wild- and hatchery-origin fish in this system have spawning date distributions that are almost entirely distinct. This is a different case than that of comparing early- and later-emerging fish from the same population. The negative effect of environmental conditions in the early spring may take a strong toll on both survival and displacement of early-spawning fish.

Conclusions

We found that pure hatchery-lineage fry comprised less than 0.5% of the 1020 fish we analyzed, whereas wild- and hybrid-lineage fish were both fairly abundant (68.2% and 31.3%, respectively). We posit that, counter to artificial selection that takes place within many hatcheries for early spawning, natural selection during the first generation of natural spawning may have adversely affected hatchery-origin fish in general. The emergence dates of hatchery-lineage and hybrid fry indicated that conditions favored hatchery-origin adults that spawned later, in many cases those who spawned with wild-origin mates. The difference in the median emergence date for hatchery-lineage fish calculated based on when adults were in the river and the estimated emergence dates of the hatchery-lineage fry we sampled suggests that the original distribution was shifted toward a later date. If this shift reflects survival, and if the adults that return to the system also spawn later, then this may be a case of directional selection against a domesticated trait. According to our best estimate, the emergence dates of the numerous hybrid and wild fish we encountered are coincident with the arrival of benign spring conditions in the system,

suggesting a “match” between ontology and phenology that the pure hatchery-lineage fish did not exhibit.

Tables

Table 1.1. Number and sex of individuals included in baseline hatchery and wild populations. Collection years and totals are shown in bold.

	Hatchery	Wild
1995/6		
Female	24	6
Male	14	16
1996/7		
Female	13	7
Male	24	12
1997/8		
Female	11	32
Male	9	23
Total	**95**	**96**

Table 1.2. Number of young-of-the-year (age-0+, YOY) and yearling (age-1+) steelhead fry collected in each stream section.

Stream Section	YOY	Yearling
Lower Forks	438	7
Middle Forks	78	1
Upper Forks	276	6
Tributary 1	37	7
Ellis Creek	138	32

Table 1.3. Alternative regression models for body condition of young-of-the-year steelhead for individuals, *j*, in assignment classes *i*. Intercept is indicated by β_{0x} and slope is indicated by β_{1x}, where x denotes a unique intercept or slope for each assignment class. Subscripts W, Hyb, and H designate the lineage: wild, hatchery, or hybrid.

Model 1: common intercept and slope
$\ln(mass_{ij}) = \beta_0 + \beta_1 \cdot \ln(length_{ij})$
Model 2: different intercept, common slope
$\ln(mass_{Wj}) = \beta_{01} + \beta_1 \cdot \ln(length_{Wj})$ $\ln(mass_{Hybj}) = \beta_{02} + \beta_1 \cdot \ln(length_{Hybj})$ $\ln(mass_{Hj}) = \beta_{03} + \beta_1 \cdot \ln(length_{Hj})$
Model 3: common slope, different intercept
$\ln(mass_{Wj}) = \beta_0 + \beta_{11} \cdot \ln(length_{Wj})$ $\ln(mass_{Hybj}) = \beta_0 + \beta_{12} \cdot \ln(length_{Hybj})$ $\ln(mass_{Hj}) = \beta_0 + \beta_{13} \cdot \ln(length_{Hj})$
Model 4: different intercept and slope
$\ln(mass_{Wj}) = \beta_{01} + \beta_{11} \cdot \ln(length_{Wj})$ $\ln(mass_{Hybj}) = \beta_{02} + \beta_{12} \cdot \ln(length_{Hybj})$ $\ln(mass_{Hj}) = \beta_{03} + \beta_{13} \cdot \ln(length_{Hj})$

Table 1.4.
a. Proportions of simulated hatchery-lineage (HH), hybrid (WH), and wild-lineage (WW) genotypes assigned to wild (W), hybrid (Hyb), hatchery (H) classes, or unassigned (U).

	W	**Hyb**	**H**	**U**
WW	0.535	0.086	0.000	0.379
WH	0.050	0.384	0.050	0.516
HH	0.000	0.088	0.551	0.361

b. The composition of simulated genotypes (WW, WH, HH) in each of the assignment classes (W, Hyb, H, U).

	W	**Hyb**	**H**	**U**
WW	0.914	0.154	0.001	0.302
WH	0.086	0.688	0.083	0.411
HH	0.000	0.157	0.916	0.288

Table 1.5. True mixture proportions of hatchery-lineage (H), hybrid (Hyb), and wild-lineage (W) steelhead fry with estimated 95% confidence intervals.

	Estimate	2.5% CI	97.5% CI
H	0.005	-0.011	0.023
Hyb	0.313	0.244	0.381
W	0.682	0.620	0.743

Table 1.6. Regression analysis for body condition. Intercept is indicated by β_{0x} and slope is indicated by β_{1x}, where x denotes a unique intercept or slope for each assignment class.

Model	Parameters	MSE	AIC	ΔAIC
1	β_0, β_1	0.009837	-2614.4	0
2	β_{01}, β_{02}, β_{03}; β_1	0.009867	-2608.7	5.704
3	β_0; β_{11}, β_{12}, β_{13}	0.009867	-2608.7	5.722
4	β_{01}, β_{02}, β_{03}; β_{11}, β_{12}, β_{13}	0.009836	-2606.5	7.907

Figures

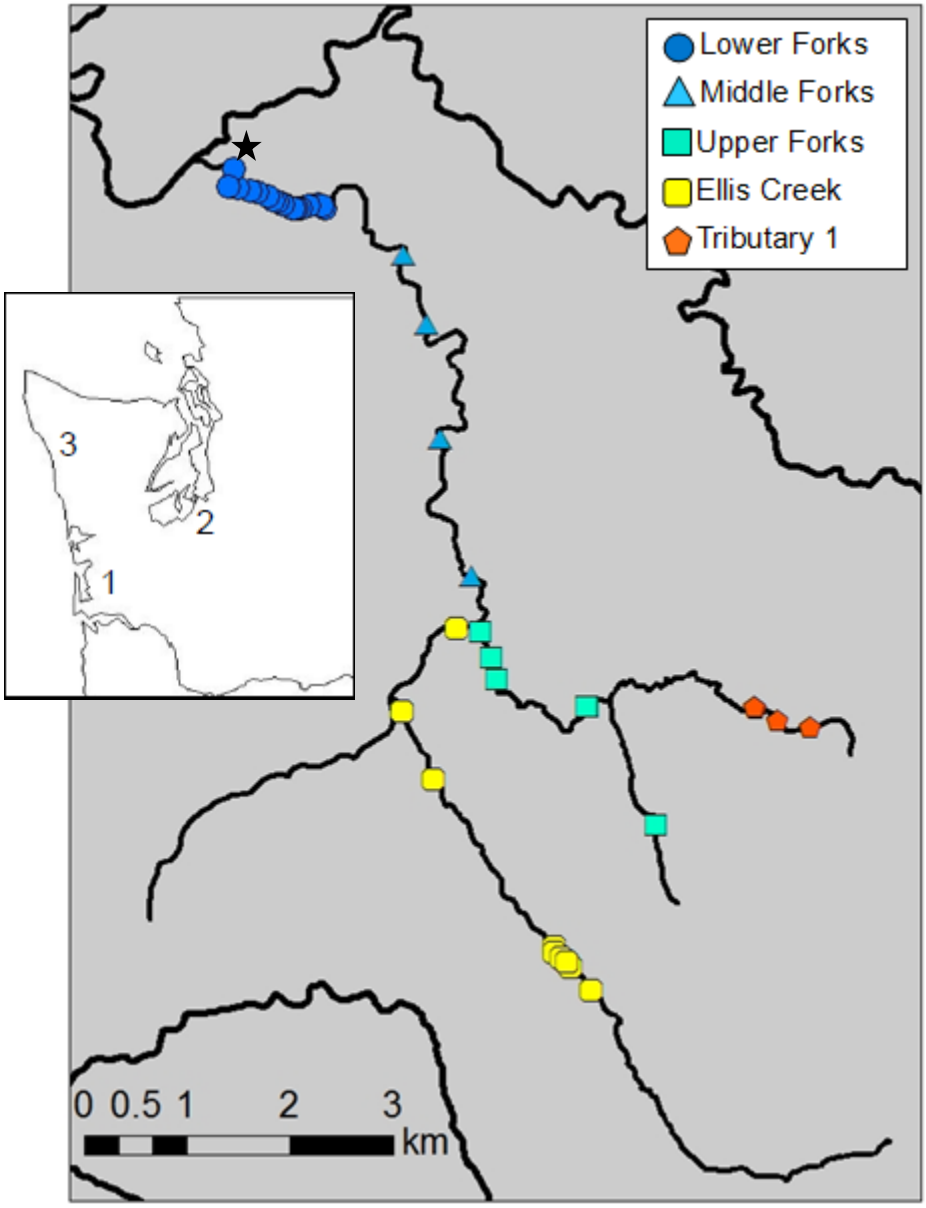

Figure 1.1. Forks Creek Watershed located on the Washington coast. The stream was divided into sections based on hydrology and hatchery activities. The hatchery is marked with an asterisk. The inset of western Washington State shows the location of (1) the Forks Creek Hatchery, (2) the origin of the Chambers Creek hatchery broodstock, and (3) the Bogachiel Hatchery.

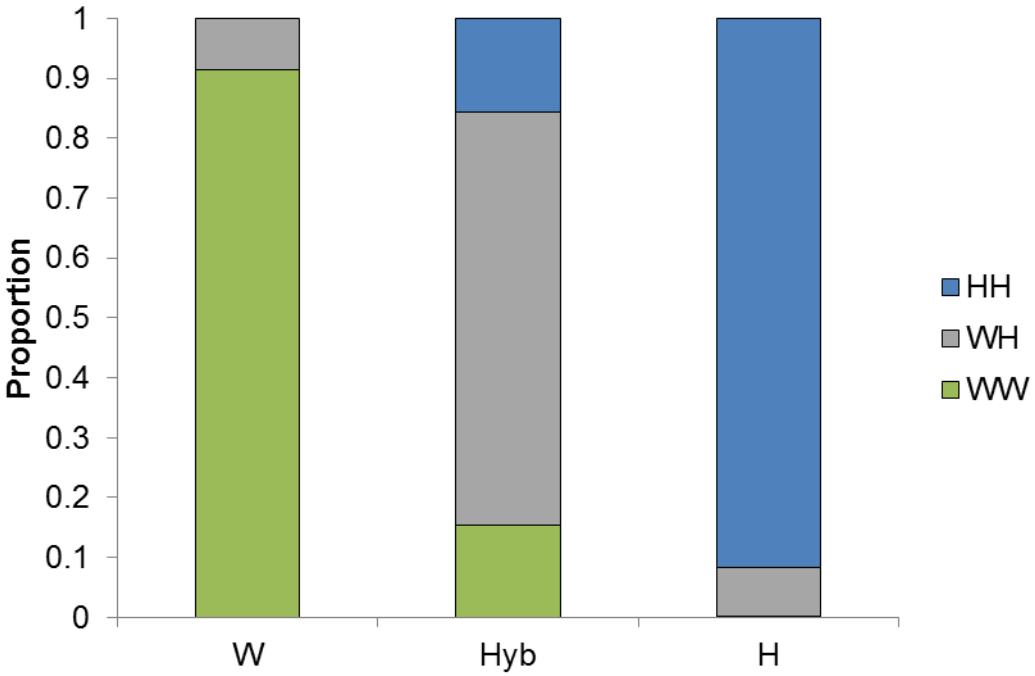

Figure 1.2. Proportions of simulated genotypes of hatchery-lineage (HH), hybrid (WH), and wild-lineage (WW) that assign to each assignment class (W, Hyb, H), excluding unassigned genotypes.

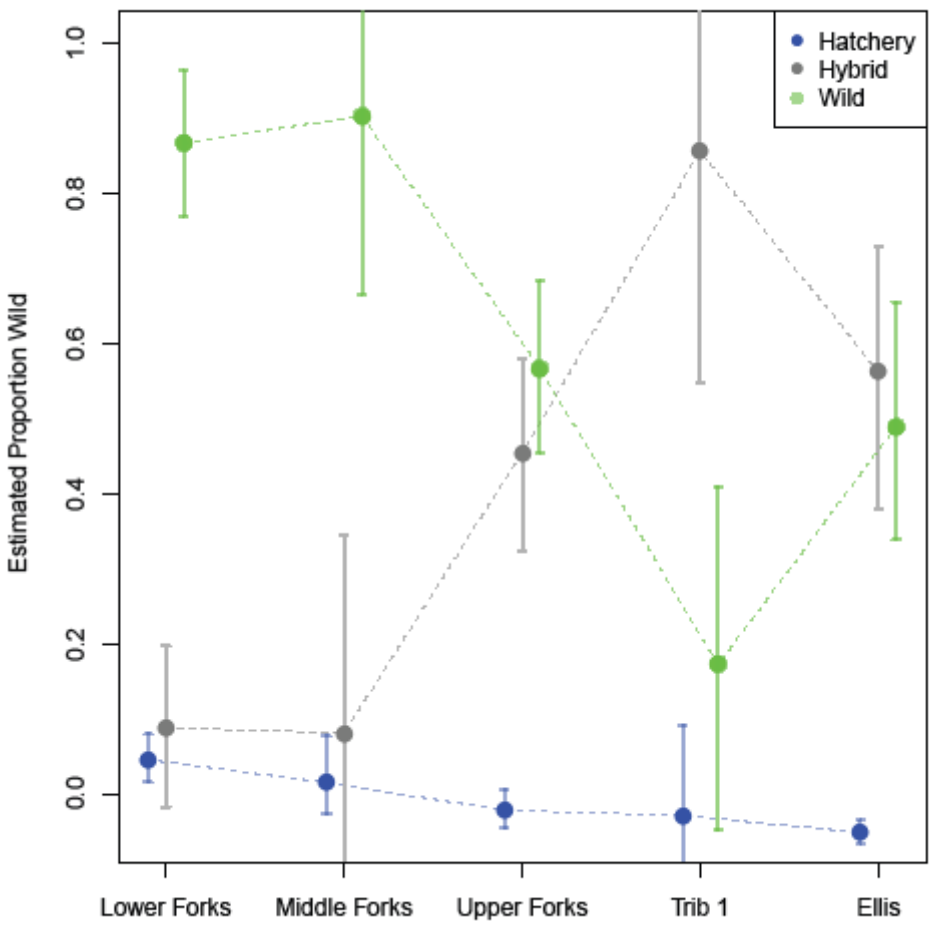

Figure 1.3. Mixture proportions of hatchery-lineage (A, green circles), hybrid (B, gray circles), and wild-lineage (C, blue circles) young-of-the-year steelhead by stream section. Error bars indicate the 95% confidence intervals on each mixture estimate.

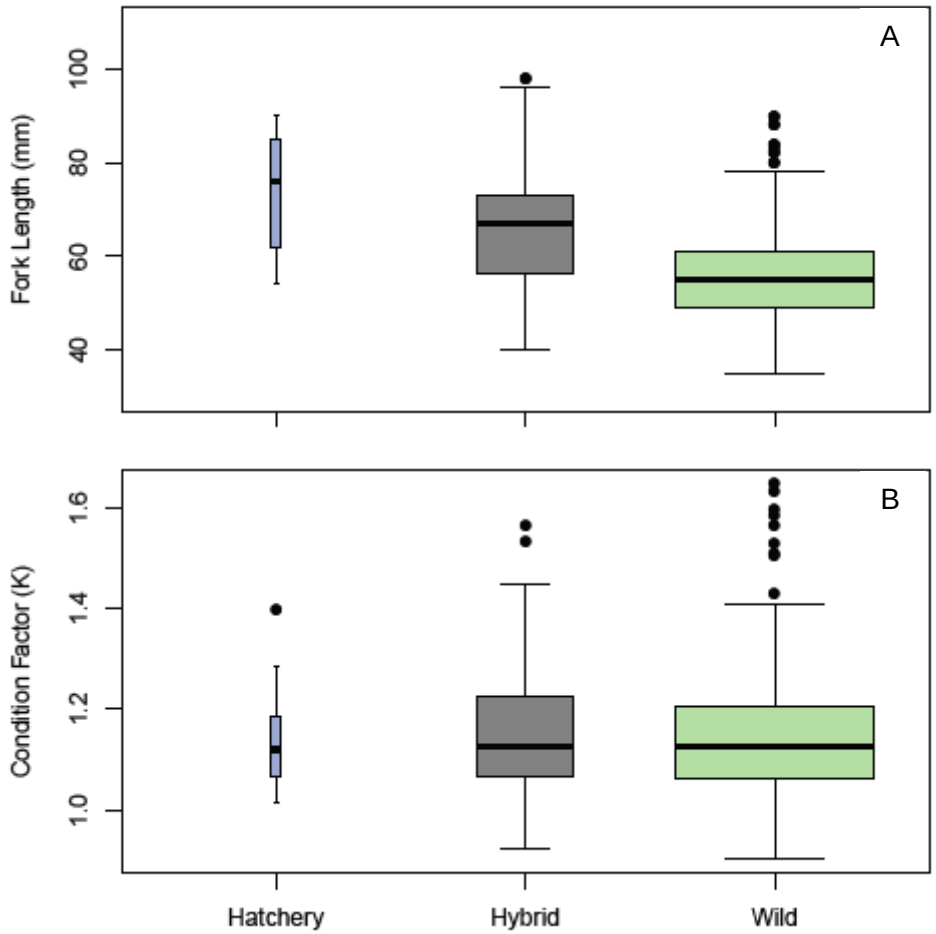

Figure 1.4. Fork length (A) and Fulton's condition factor (B) for hatchery-lineage (N=17), hybrid (N=179), and wild-lineage (N=371) steelhead fry. Box widths are scaled in proportion to sample size.

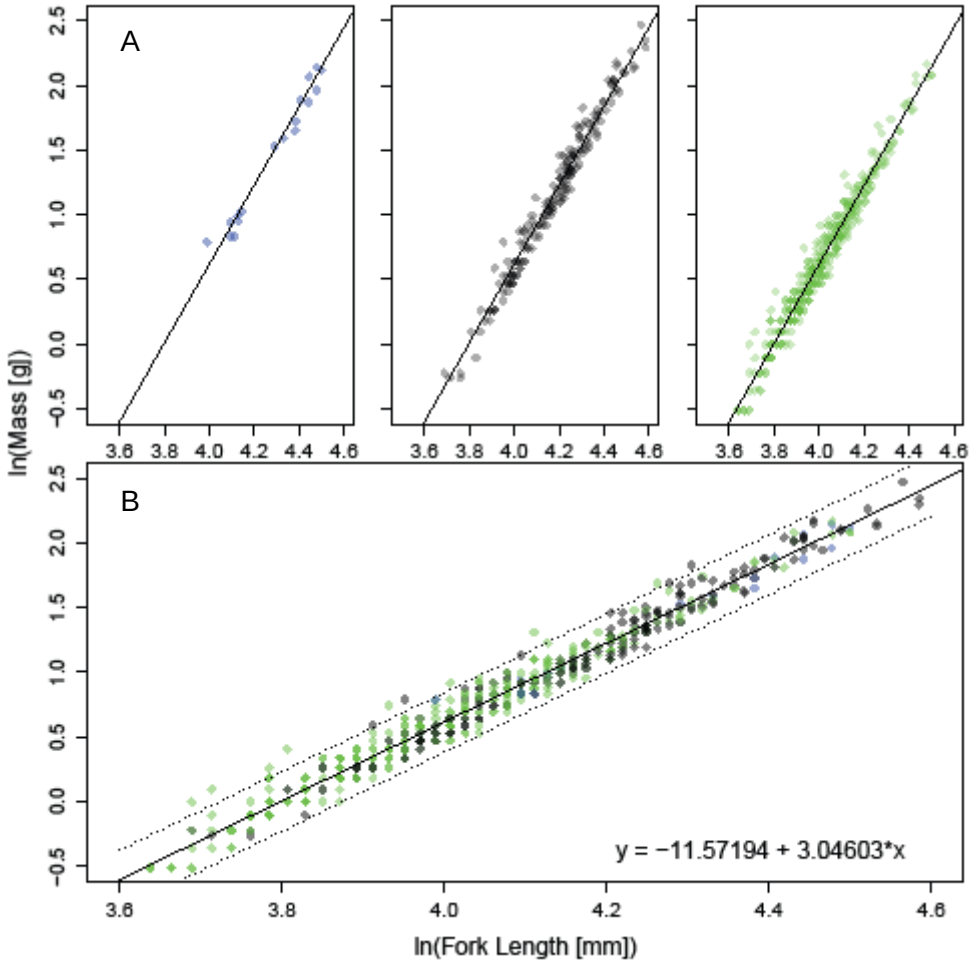

Figure 1.5. Body condition for hatchery-lineage (blue), hybrid (gray), and wild-lineage (green) steelhead. Dots are transparent to accommodate overplotting. Results are shown for each group separately (A) and together (B). Dotted lines indicate the 95% tolerance interval (bound on 95% of the observations).

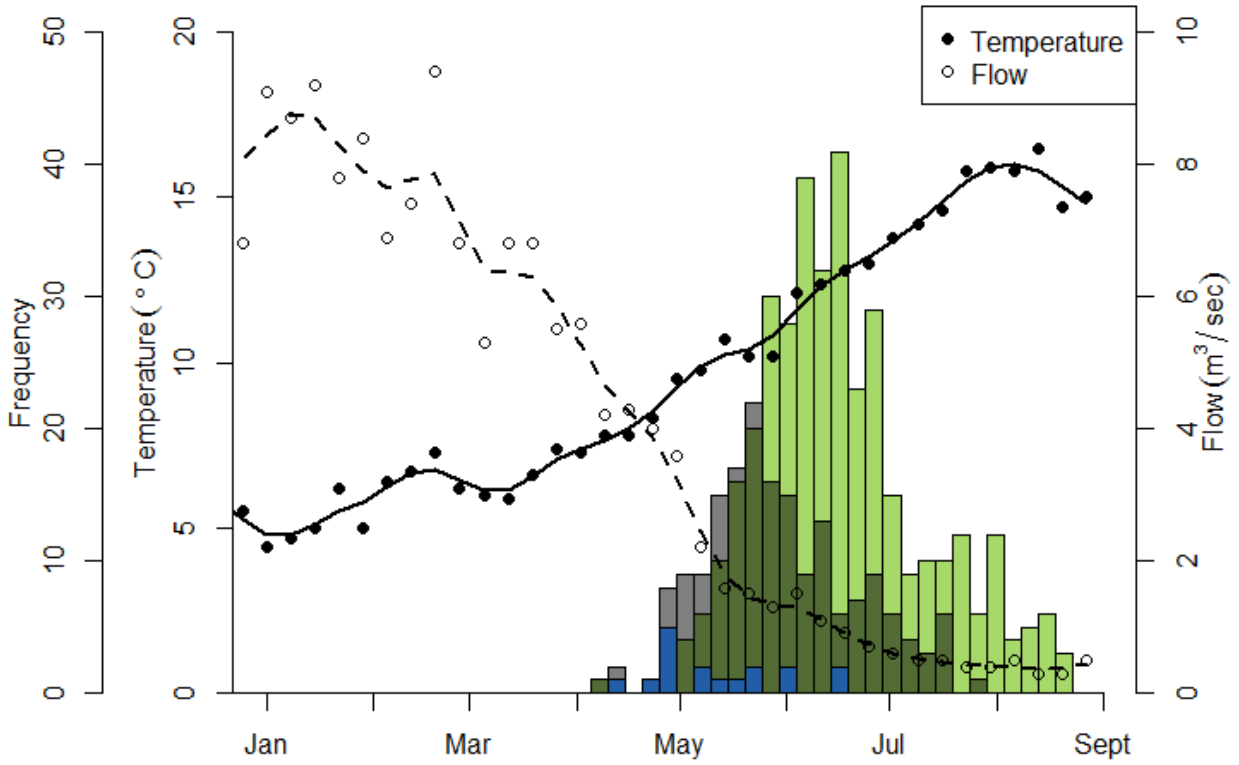

Figure 1.4. Estimated emergence dates for hatchery-lineage (blue), hybrid (gray), and wild-lineage (green) steelhead fry. Bars are transparent to show overlapping distributions. Mean temperature (solid circles, solid line) and stream flow (open circles, dashed line) from 1995-1998 are plotted in their respective units (ºC, m^3/second).

Chapter 2: Relatedness on the landscape: Do juvenile steelhead trout (*Oncorhynchus mykiss*) establish territories near their siblings?

Abstract

Kin selection has been used to explain a wide array of social behaviors across many taxa. Laboratory experiments have indicated that juvenile salmonids not only recognize kin, but also behave less aggressively toward them and tend to congregate with them. However, field studies have been less conclusive regarding whether juvenile salmonids remain in sufficient proximity to interact with siblings in the wild. Here, we analyzed the geographic distribution of juvenile steelhead trout (*Oncorhynchus mykiss*) in a small stream in Washington State to test the null hypothesis that siblings are distributed randomly across the landscape several (1-3) months after they emerged from gravel nests. Age-1+ and age-0+ fish (N=1020) were genotyped at 96 single-nucleotide polymorphisms, and pedigree analysis was performed in COLONY2 to identify full-sibling groups. The median distance between full-siblings was 169 m, compared to 5309 m for the entire sample. Spatial autocorrelation analysis revealed that individuals less than 1 km apart were more genetically similar than expected by chance. The correlation between genetic and geographic distance was highest for pairs of individuals within 0 and 100 m, due in part to the presence of full-siblings at these ranges. Siblings were generally too far apart to interact, but in 18 out of 53 family groups containing 5 or more individuals, the median distance between individuals was 0 m. We conclude that juvenile dispersal is prominent during the first four months after emergence. We suggest that future research focus on characteristics of siblings and environmental conditions associated with sibling groups that remain aggregated versus those that disperse over large or short distances.

Introduction

Since its inception, the theory of kin selection has been used as a framework for understanding social behaviors across many taxa, including mammals (Hurst and Barnard 1992), insects (Queller and Strassmann 1998), amphibians (Pfennig et al. 1999), birds (Krakauer 2005), and fish (Ward and Hart 2003). The theory of kin selection (Smith and Wynne-Edwards 1964) arises from Hamilton's model for the evolution of social behavior (Hamilton 1964), which proposes that an individual can maximize its *inclusive fitness* not only by securing its own survival and reproduction (direct fitness), but also by promoting the survival of related individuals (indirect fitness). Kin selection has been used to explain a wide array of behaviors, notably altruism, but also aggression, cooperation, selfishness, and spite (Griffin and West 2002). However, it can be difficult to establish that kin selection is the mechanism behind an observed behavior and that the behavior plays a meaningful role in the species' ecology. A high degree of relatedness between interacting individuals does not necessarily mean that inclusive fitness and kin selection are the underlying drivers (Griffin and West 2002). Before invoking kin selection as the primary explanation for an observed behavior, there is a need to reconcile theory with empirical data. The capacity to identify kin and the tendency to respond to them in laboratory conditions do not mean that animals in the wild will behave similarly.

For kin selection to operate, individuals must both discriminate kin from unrelated conspecifics and behave differently toward them. Juvenile salmonids of many species maintain territories in rearing streams and can detect conspecifics based on water-born chemosensory cues. Quinn and Busack (1985) established that juvenile coho salmon (*Oncorhynchus kisutch*) could differentiate both familiar and unfamiliar siblings from unrelated individuals and preferred water conditioned by relatives over that of unrelated individuals. Similar abilities were

subsequently demonstrated in other salmonids (Brown and Brown 1996), including Atlantic salmon (*Salmo salar*, Brown et al. 1993), rainbow trout (*O. mykiss*, Brown et al. 1993), brown trout (*S. trutta*, Olsen et al. 1996), and coho salmon (*O. kisutch*, Courtenay et al. 2001).

Laboratory research has provided evidence that juvenile salmonids are less aggressive toward siblings than non-kin conspecifics. In juvenile Atlantic salmon, full siblings shared high-quality feeding territories for twice as long as did pairs of non-kin, and subordinate individuals consumed more food when paired with relatives (Griffiths and Armstrong 2002). Salmon were also greater than 50% more aggressive to unrelated individuals than related individuals (Griffiths and Armstrong 2000). Mortality and competition are high during the juvenile life history stage (Einum and Fleming 2000), so a kin-biased reduction in aggression could shift survival in favor of relatives. These studies suggest that juvenile salmon may behave altruistically toward siblings. However, this work was performed under laboratory conditions; the extent to which kin-biased behavior occurs among salmon and trout in natural streams is not clear.

The first step to determine if kin selection operates for juvenile salmonids in the wild is to answer the question of whether kin are aggregated on the landscape. Full siblings emerge from the same nest (Jonsson and Jonsson 2011; Quinn 2005), so in the absence of dispersal, they would be geographically clustered. Active orientation to relatives could reinforce and enhance kin aggregation over time and operate against the kinds of stochastic processes in streams that tend to disaggregate them. Alternatively, dispersal from natal sites without regard to kin would disrupt the pattern of spatially-aggregated relatives over time as individuals disperse. Lastly, relatives could be farther apart than expected by chance, as predicted by the theory of competition avoidance (Hamilton and May 1977). Competition avoidance may be favored if

individuals compete with neighbors indiscriminately; in this case, inclusive fitness is increased because competitive efforts are not directed at relatives.

Despite experimental evidence demonstrating the capacity for kin recognition and kin-biased behavior in salmonids, field studies have produced mixed results on the subject of kin aggregation. Two families of juvenile coho salmon in an experimental outdoor stream channel distributed themselves such that one family or another numerically dominated the riffle-pool habitats (Quinn et al. 1994). On the other hand, Brodeur et al. (2008) found no association between relatedness and geographic location in wild juvenile Atlantic salmon but acknowledged that average relatedness was low in their sample, which contained only five half-sib families. Migratory brook char (*Salvelinus fontinalis*) displayed kin aggregation beyond the juvenile stages (Fraser et al. 2005), but Atlantic salmon did not (Palm et al. 2008). Carlsson and Carlsson (2002) found that older brown trout were located closer to their relatives while younger trout were farther apart than expected by chance. The authors reasoned that competition for food was fierce in younger fish and may favor competition avoidance; older trout might reduce or cease their food intake before reproduction (Jonsson and Gravem 1985) and compete instead for habitat, perhaps tolerating kin at this stage. In another study, both young-of-the-year and older brown trout were closer together than expected in one stream, but not in another (Carlsson et al. 2004).

Here, we analyzed the distribution of juvenile steelhead trout, the anadromous form of rainbow trout, in a small stream in Washington State to test the null hypothesis that siblings are distributed randomly across the landscape several months after they emerged from the gravel. If relatives were not clustered on a fine geographic scale, then we would conclude that kin interactions do not play a large role in steelhead ecology in this system and life history stage. We

found that the pattern of kin aggregation differed across families and stream sections: some families were tightly aggregated while others had dispersed over several kilometers. We discuss this finding in the context of salmon life history and suggest that, rather than focusing on whether kin aggregation operates uniformly in a population at large, future research should address how genetic and ecological factors influence kin aggregation and dispersal across different families and habitats.

Methods

Study system

Forks Creek (near Raymond, Washington, USA) is a tributary to the Willapa River on the Washington coast (Figure 2.1). The river supports a population of native winter-run (ocean-maturing) steelhead trout. Juvenile hatchery steelhead were introduced to the watershed in 1994 and long-term monitoring was established to determine the impact of hatchery stocking on the wild steelhead. The first hatchery-origin adults returned to the hatchery in the winter of 1995/1996, and some were granted access to spawning areas used by wild fish. For a detailed description of the study system and previous work, see Mackey et al. (2001); McLean et al. (2003); and Seamons et al. (2012).

Sample collection

Adult steelhead returning to the watershed were collected weekly at a weir below the hatchery during winter and spring of 1995/1996. Hatchery or wild origin was determined based on the absence or presence of an adipose fin. Length, sampling date, and sex were recorded, and a fin clip collected for genetic analysis. Adults that had an opportunity to spawn in Forks Creek

in 1995/1996 were considered potential parents of the 1996 cohort used in the pedigree analysis (24 hatchery-origin females, 14 hatchery-origin males; 6 wild-origin females, 16 wild-origin males). Under high flow conditions, an unknown number of adults bypassed the weir (Seamons et al. 2012) so many individuals could not be assigned to known parents.

Wild-origin steelhead fry, offspring of adults that returned in the winter of 1995/1996, were collected from Forks Creek between July 23 and August 28, 1996, using thorough one-pass electrofishing over 19 km of the stream, including tributaries (Figure 2.1). Latitude and longitude were recorded. Locations with unique coordinates were considered distinct sample locations. A fin clip was collected for genetic analyses and stored at -80º C. These samples contained age-0+ and age-1+ fish, distinguished by the bimodal distribution of body lengths, with age-1+ longer than or equal to 100 mm and age-0+ shorter (Table 2.1). The sample contained pure hatchery-lineage individuals (0.5%), pure wild-lineage individuals (68.2%), and hybrids (31.3%). Condition factor (a measure of mass to length) did not differ between hatchery, hybrid, and wild juveniles (Chapter 1), so the origin of the fish was not considered further in our analyses.

Family Groups

We genotyped juvenile steelhead (N=1020, Table 2.1) and their putative parents (N=60) at 96 nuclear SNPs, as described in Chapter 1. Pedigree analysis was performed using the combined full-likelihood pairwise-likelihood combined method implemented in COLONY v2.0.5.0 (Jones and Wang 2010). All juveniles were included as possible offspring. Adults that had an opportunity to spawn in wild were included as potential parents and a genotyping error rate of 0.1% was applied. This was slightly lower than our observed genotyping rate (0.15%); runs were very similar, but the lower genotyping error rate was more conservative, detecting

fewer siblings overall. The final settings in COLONY were a medium run with very high likelihood precision and a marker error rate of 0.15%. Prior probabilities for finding a paternal or maternal parent were 0.2, although preliminary trials indicated that higher and lower values did not affect the number of parents detected. COLONY also provided estimates of the effective population size (N_e) based on sibship assignments (Wang 2009).

Geographic distribution

Collection locations were plotted using ArcGIS 10 (ESRI 2011). We subdivided Forks Creek into stream sections based on hydrology (Figure 2.1). Coordinates were not accurate to a fine spatial scale due to the state of global positioning technology in 1996; many locations appeared on land surrounding Forks Creek. Sampling locations were adjusted by "snapping" the coordinates to nearest location on the stream itself using Geospatial Modelling Environment (Beyer 2012). Pairwise geographic distance between all individuals was calculated in R (R Core Development Team 2012).

A Mantel Test (Mantel 1967) was performed in GENALEX 6.5 (Peakall and Smouse 2012) to test the null hypothesis that no relationship existed between pairwise geographic distance between individuals and relatedness calculated in ML-RELATE (Kalinowski et al. 2006). Relatedness is a measure of similarity so it was converted to a measure of distance by subtracting the value from 1. Relatedness distance was permuted 9,999 times to determine the null distribution of R_{xy}, the Mantel test statistic, and establish a level for significance.

We also performed the spatial autocorrelation test developed by Smouse and Peakall (1999) and refined (Double et al. 2005; Peakall et al. 2003; Smouse et al. 2008) in GENALEX 6.5. We examined the autocorrelation between genetic and geographic distance at different

spatial scales, from 0m to 13km apart. Individuals were randomly shuffled between geographic locations 9,999 times to establish a null distribution on the autocorrelation coefficient (r) under the assumption of no spatial structure. Confidence in r was determined by bootstrapping across individuals within each distance interval 9,999 times. The analysis was performed twice, once on the entire dataset and again with only one representative of each full sibling group to determine the effects of siblings on spatial aggregation.

Results

Family groups

We obtained high-quality genotypes for 22 wild and 38 hatchery adults and 1020 juveniles. Numerous full sibling groups were detected (Figure 2.2). Twelve wild male and two wild female parents were identified in the dataset. A total of 149 juveniles did not have any full siblings. Of 53 age-1+ fish detected, 37 had no siblings. Five groups of two and one group of three siblings were identified in which all were age-1+ fish. In two cases, one age-1+ and one age-0+ individual were identified as full-sibling pairs. One age-1+ individual was identified as part of a group of 19 age-0+ fish.

We were primarily interested in the distribution of individuals with siblings, so we focused our spatial analysis on full-sibling groups that contained five or more individuals, nearly exclusively age-0+ fish. There were 57 large sibling groups, comprising a total of 665 individuals.

Many half-sibling pairs were also detected, indicative of a complex promiscuous mating system (Seamons et al. 2004). Based on simulations involving unrelated individuals, many may have been false positives due to limited marker power. We therefore focused on full-sibling

groups, which were more robust to false positives in detecting siblings. The effective population size assuming random mating was 72 individuals (95% confidence interval = 52 – 98). Assuming non-random mating, the estimate was similar: 68 (95% confidence interval = 45 – 96).

Geographic distribution

Most sampling locations were snapped to the stream within 100 m of their original coordinates (mean = 67.0, sd = 57.9). However, two locations were more than 300 m from the stream; the 24 individuals in these sites were not included in analyses that involved pairwise distance between individuals. Ten additional individuals did not have GPS coordinates, but stream section was recorded. These locations were not included in pairwise distance analyses.

We found evidence for sibling aggregation on the landscape. There were 485605 total pairwise comparisons of geographic distance between individuals (mean = 5112 m, median = 5309 m, Figure 2.3a). Of the 4527 distance comparisons between full sibling pairs that had geographic distances, the median distance between siblings was 169 m and the mean was 316 m (Figure 2.3b). In 28 comparisons, the full-siblings were located >2 km apart (Figure 2.3b). Siblings were generally found within the same stream section (Figure 2.4a). In 18 of out 53 large sibling groups (containing five of more individuals), the median distance between individuals was zero (Figure 2.4b). Four locations contained over 10 individuals from the same sibling groups. However, many groups were dispersed over hundreds of meters (Figure 2.4b). Of the entire data set, including small sibling groups, 72.8% of individuals that had siblings were found in the same location as at least one sibling.

A Mantel test correlating relatedness distance and geographic distance indicated that individuals were not distributed randomly on the landscape ($R_{xy} = 0.159$, $p < 0.001$). The

relationship between geographic distance and relatedness distance was weak ($y = 4.00x10^{-6} + 0.915$, $R^2 = 0.0254$). Spatial autocorrelation analysis indicated that there was a pattern of genetic structure on the landscape, particularly at small spatial scales. Individuals between 0 and 1000 m apart were more genetically similar than expected by chance (Figure 2.5). A similar pattern was evident for the dataset after we removed all but one representative of each full sibling group (N=274); the autocorrelation coefficient was reduced across the intervals from 0 – 50 m, but still higher than the null expectation (Figure 2.5). Starting at intervals of 100m, the pattern of relatedness on the landscape was not strongly affected by the presence of full siblings.

Discussion

Laboratory experiments have demonstrated kin recognition abilities and kin aggregation tendencies in salmonids, but field studies have been less conclusive in demonstrating the level of kin association needed to accrue benefits from kin selection. Here, we analyzed the micro-distribution of 1020 juvenile steelhead to test for kin aggregation on the landscape. We found that related individuals were closer to each other than would have occurred by chance, but we also found evidence for long distance dispersal during juvenile stages (up to 2 km, Figure 2.3). While 72.8% of individuals were collected in the same location as at least one of their siblings, most sibling groups were not tightly aggregated (Figure 2.4b). Most sibling pairs were likely located too far apart for interactions between relatives to play a strong role in their ecology.

Juvenile steelhead trout emerge from gravel nests in the spring and rear for up to several years in freshwater. During this time, they establish dominance hierarchies and defend feeding territories (Fausch 1984). Sampling took place from July 23 – August 29, 1996. Based on the body lengths of the juveniles, the median emergence date for the fish sampled was about June 8

(Chapter 1). Therefore, individuals had emerged between 1 and 3 months prior to sampling. Individuals were collected by electrofishing portions of the stream, up to 50 meters at a time (electrically affecting up to 150 m of the stream). Therefore, collection in the same location does not necessarily mean that fish could interact. However, collection in different locations would suggest that individuals were too far apart for social interactions between them to be likely at the time of capture.

The median distance between siblings was 169 m, which is close to our level of spatial accuracy (approximately 100 m due to inaccurate GPS coordinates measured in 1996). At distances < 100 m, individuals were more genetically similar than expected by chance (Figure 2.5). When sibling groups were removed, individuals 0-50 m apart were still more genetically similar than expected based on a random distribution, but the degree of similarity was lower. We did not remove half sibling pairs from the dataset (doing so would have dramatically reduced the sample size). The presence of half siblings and other relatives such as cousins, second cousins, etc. in the dataset may explain the residual genetic similarity between individuals that were collected close together even after full siblings were excluded.

Most large sibling groups, consisting of almost exclusively age-0+ fish, were located within the same stream section (Figure 2.4a). The distance between siblings differed between stream sections (Figure 2.4b). Lower Forks Creek contained the largest families, with many individuals located within 500 m of one another. In the upper watershed, including Middle and Upper Forks, Tributary 1, and Ellis Creek, there was higher variance, with many individuals in a sib group found in the same location, but a few members up to 2 km apart from the rest of the group.

Distance between individuals in large sibling groups suggests that, while some groups were tightly aggregated up to three months after emergence, the majority dispersed over hundreds of meters. Long distance dispersal is not unusual for juvenile salmonids. Anderson et al. (2013) found that 28% of juvenile coho salmon were collected outside of their spawning reach and dispersed up to 6.3 km (median = 1.5 km). Young Atlantic salmon 2-3 months after emergence also dispersed from high density sites (Einum et al. 2006). Keeley (2001) found that in the absence of emigration, juvenile steelhead experience increased mortality and decreased growth as food competition intensified. Dispersal is therefore an important component of juvenile salmonid ecology, likely helping to reduce density-dependent effects.

The difference in dispersion among sibling groups across stream sections may be due in part to sampling location density (Figure 2.1). Most sampling was conducted in Lower Forks Creek (N=445 fish, Table 2.1) and sampling locations there were more tightly aggregated. Locations in the upper watershed were more dispersed. Samplers were targeting rearing habitat for juvenile steelhead and were limited by access to the stream. We did not find individuals from same sibling group located between 100-200 m apart because sampling locations were farther apart in these areas. Detecting sibling groups was not the design in sampling. Instead, we targeted habitat that was accessible and that was favorable for juvenile steelhead. In the future, we recommend sampling at regular intervals to better characterize spatial distribution.

Our expectation of small sibling groups for older fish was generally upheld. Seven groups of two siblings and one group of three siblings were detected among the yearlings. This is most likely because mortality has decreased the family size and dispersal has made it less likely that related individuals would be sampled together (Keeley 2003). Interestingly, age-0+ fish were more common in the upper watershed (Table 2.1). Older steelhead and other salmonids often

differ in distribution from young-of-the-year fish (Buehrens et al. 2012; McMillan et al. 2013) as the winter period of high flows characteristic of this region is often associated with movement by juveniles (Bustard and Narver 1975; Peterson 1982; Swales et al. 1988).

One age-1+ fish was included in a sibling group of 19 age-0+ fish. True membership in this group is unlikely. Steelhead are iteroparous (capable of spawning in multiple years), but the odds that the same male and female would mate with each other two years in a row are low. It is possible that these individuals are half siblings or unrelated and that the overlap of genotypes resulted in an error in family group composition.

The number and size of family groups we detected were within the range of what is biologically possible for steelhead. Forks Creek has a relatively small population. McLean et al. (2003) reported 11 wild females and 21 wild males present in the river in 1996, although the actual number may have been somewhat higher. Many hatchery females were present as well, although their offspring, both pure hatchery-lineage and hybrid fish, make up only about one third of the dataset. The estimated effective population size determined in COLONY was 72 individuals (95% confidence interval = 52 – 98). We saw considerable variation in sibling group size, with some groups of 30 individuals or more, and many individuals that were not part of large sib groups at all. Research on reproductive success has shown that the majority of adults do not produce any surviving offspring, while a few individuals produce dozens of returning adults (Anderson et al. 2010; Quinn 2005). For this to be possible, some sibling groups would have to be very large during the first and second years of life.

Even in studies that show strong evidence for kin aggregation and discrimination, the effects are not always consistent across families. Quinn and Busack (1985) found that some coho salmon families were more easily detected as unrelated than others, and that a preference for

siblings differed between families. There is also evidence in coho salmon for learned recognition during early juvenile stages, which may help explain why family odors are not always preferred (Courtenay et al. 2001).

Conclusions

We observed that by the end of the summer, one to three months after emergence from nests, juvenile steelhead trout had dispersed substantially from natal sites. The distribution of siblings was non-random, but in the majority of cases, siblings were generally not close enough to interact. The level of dispersal is likely too great for individuals to obtain substantial benefits from kin association of the sort seen in the laboratory. Perhaps future research should focus more on the conditions and characteristics of siblings that do aggregate versus those that disperse over large or short distances. There may be a combination of genetic and ecological factors that influence the decision to stay near natal sites and siblings or to disperse, with potential impacts on survival.

Tables

Table 2.1.Number of age-0+ and age-1+ steelhead collected by stream section.

Stream Section	Age-0+	Age-1+
Lower Forks	438	7
Middle Forks	78	1
Upper Forks	276	6
Tributary 1	37	7
Ellis Creek	138	32

Figures

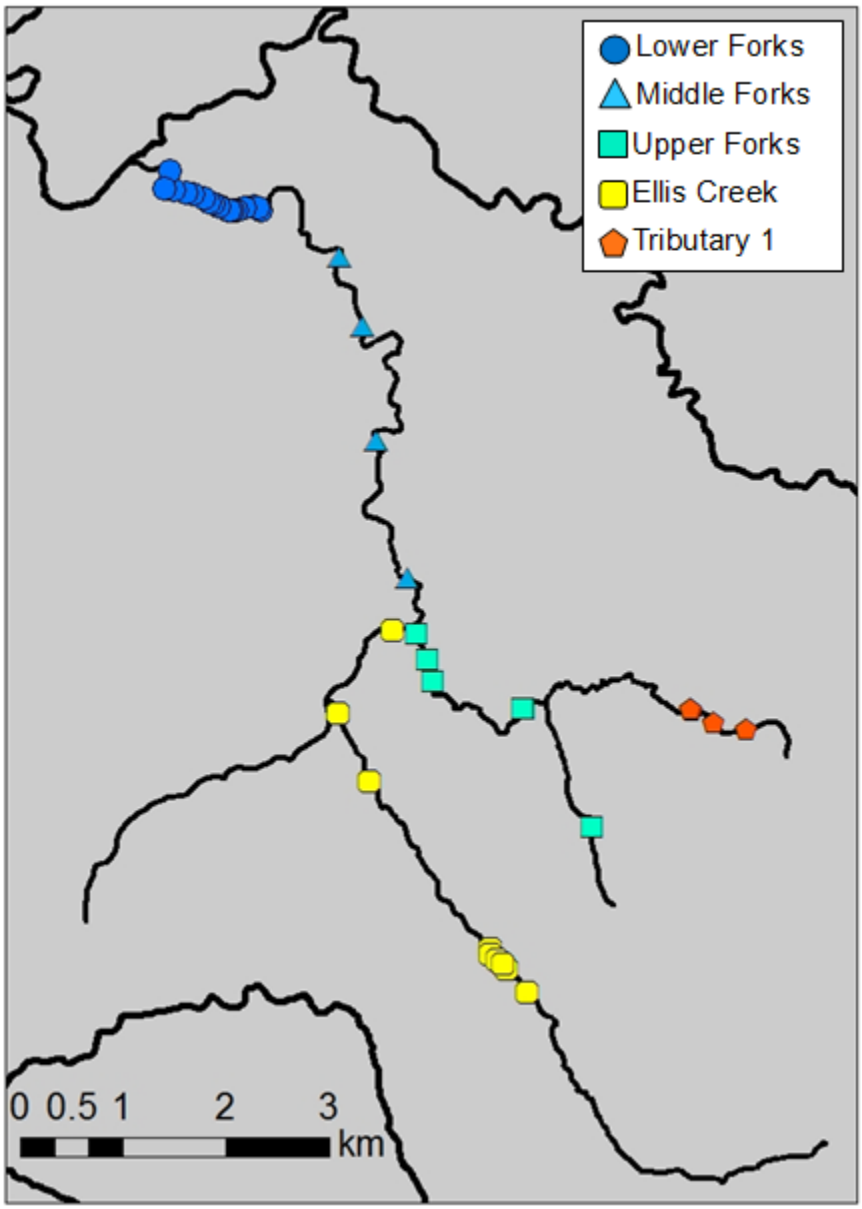

Figure 2.1. Forks Creek Watershed, located on the Washington Coast. The stream was divided into sections based on hydrology and hatchery activities.

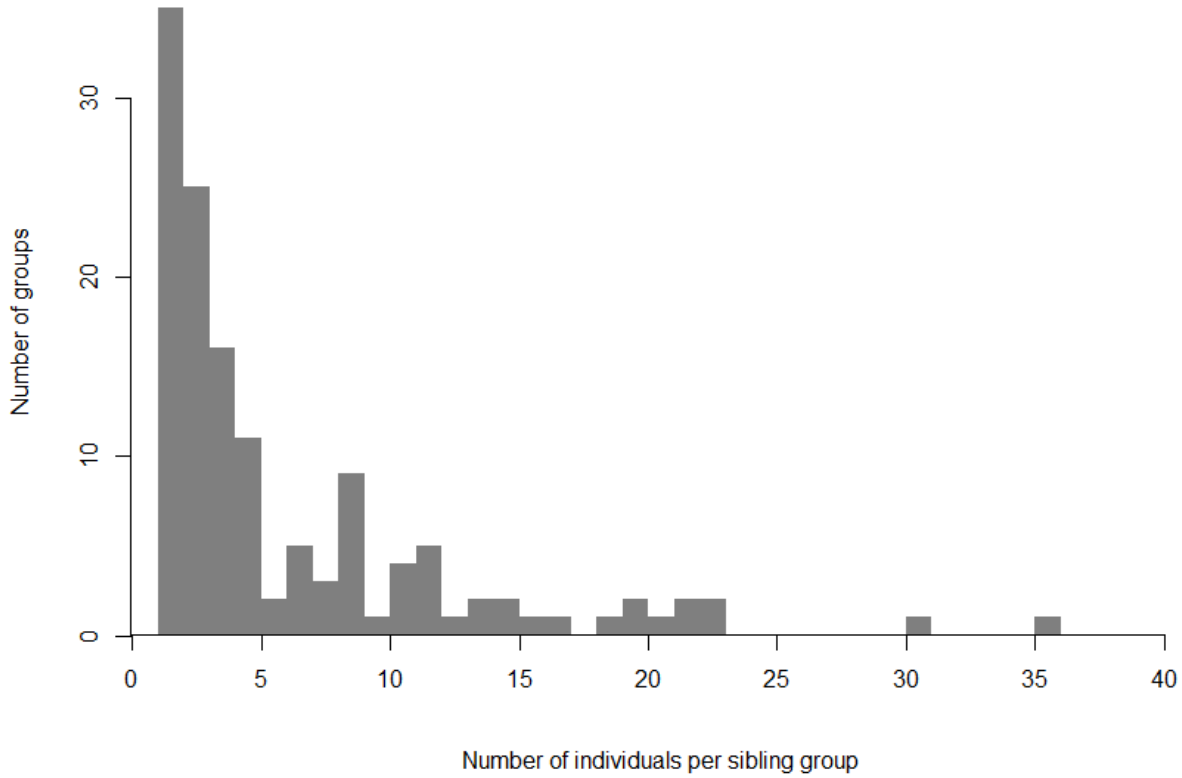

Figure 2.2. Distribution of family group sizes for all individuals (age-0+ and age-1+), excluding 149 individuals with no siblings.

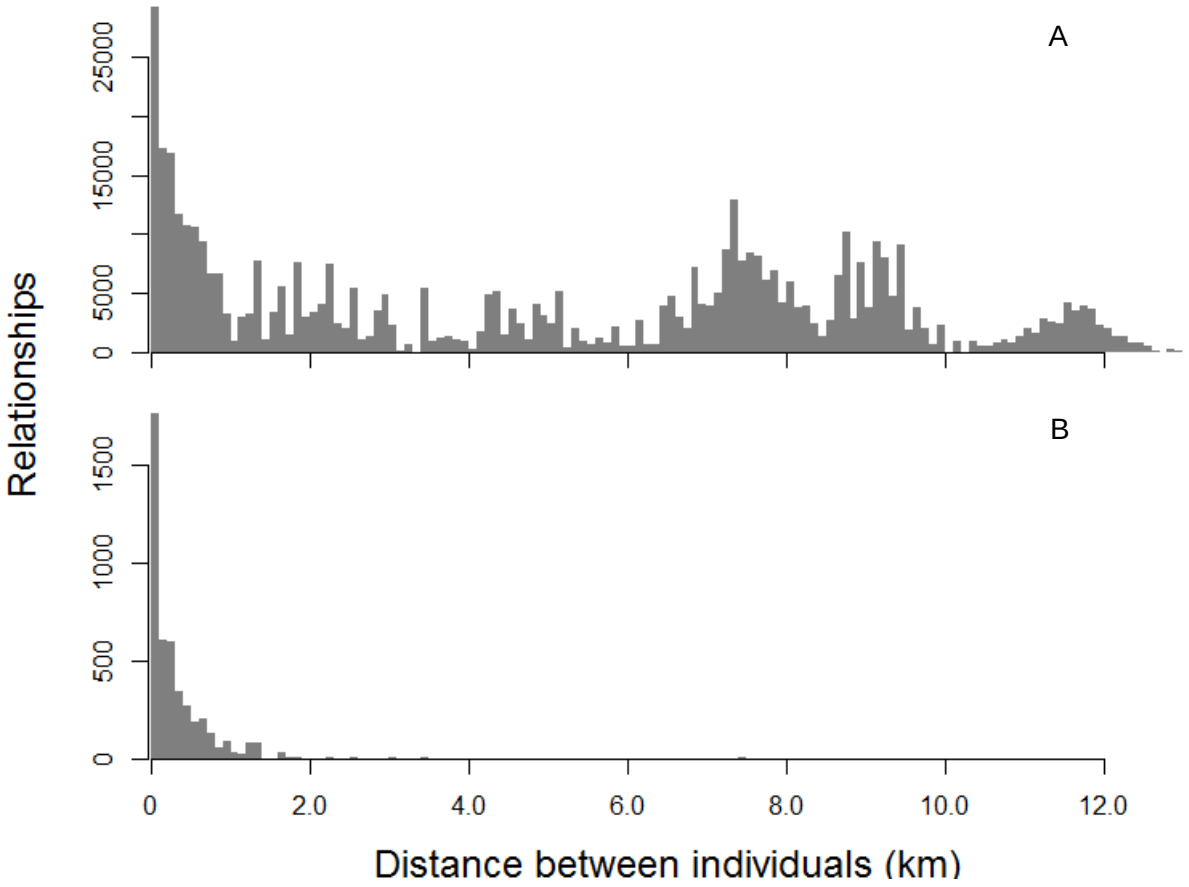

Figure 2.3. Pairwise distance between all individuals (A) and siblings (B).

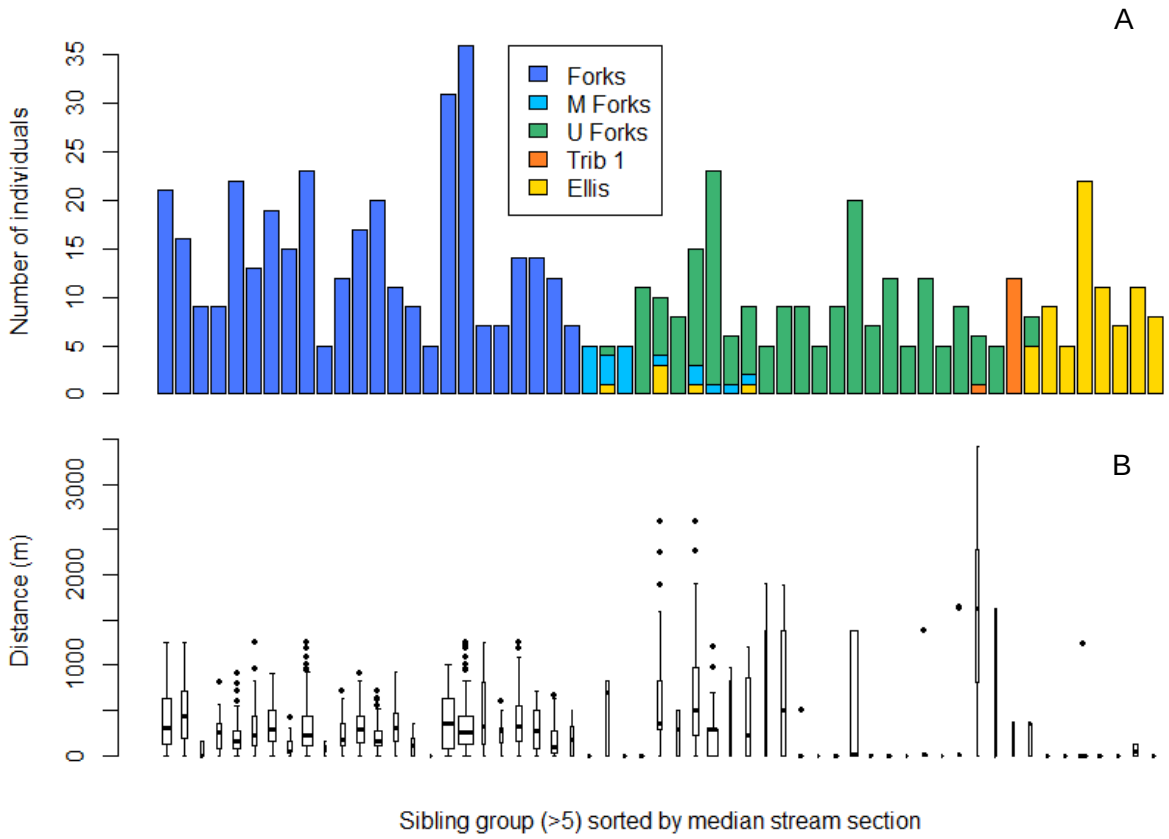

Figure 2.4. a. The number of individuals in large sibling groups, sorted according to median stream section. b. Pairwise geographic distance between all individuals in each sibling group.

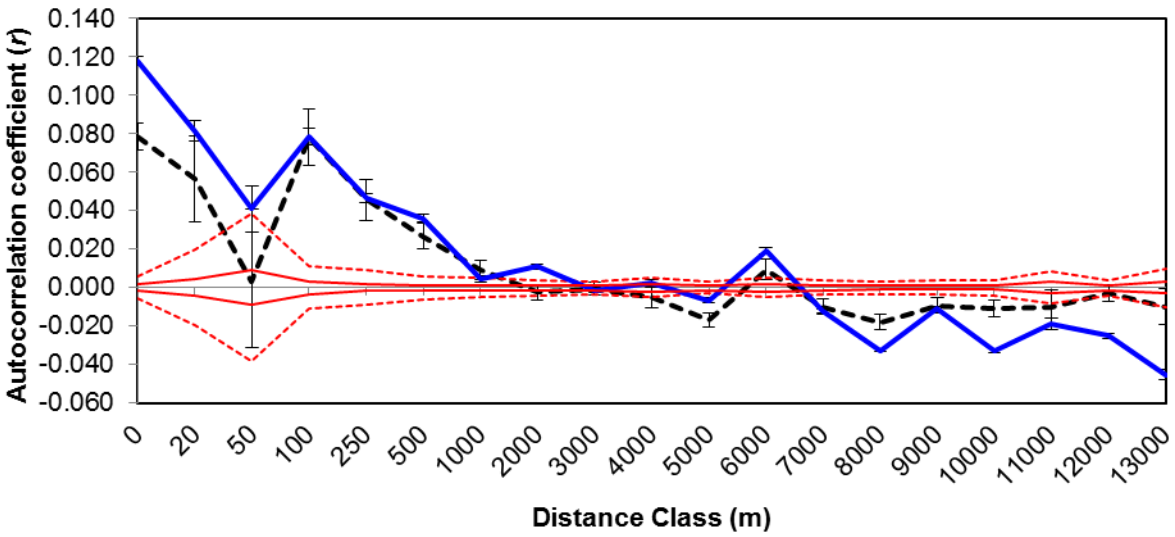

Figure 2.5. Spatial autocorrelation analysis for all individuals (blue solid line) and with full siblings removed (black dashed line). Red lines show the null expectation for all data (solid) and the dataset with no siblings (dashed).

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Appendix 1

Table A1. Locus information and summary statistics. Observed (H_o) and expected (H_e) levels of heterozygosity per locus for known wild- and hatchery-origin individuals, as well as locus-specific F_{ST} values, are shown. The genotyping panel also included three diagnostic alleles for differentiating cutthroat trout (McGlauflin et al. 2010), which are discussed in text.

Marker Name	Reference[1]	F_{ST}	Wild H_o	Wild H_e	Hatchery H_o	Hatchery H_e
OMS00003	a	0.0020	0.500	0.432	0.489	0.460
OMS00006	a	0.0365	0.446	0.474	0.289	0.333
OMS00007	a	0.0006	0.022	0.021	0.033	0.033
OMS00012	a	0.0027	0.011	0.011	0.000	0.000
OMS00014	a	0.0064	0.043	0.043	0.103	0.098
OMS00018	a	0.0180	0.315	0.370	0.211	0.239
OMS00029	a	0.0074	0.054	0.072	0.022	0.022
OMS00040	a	0.0381	0.226	0.233	0.056	0.054
OMS00055	a	0.0120	0.097	0.092	0.022	0.022
OMS00056	a	0.0055	0.312	0.319	0.389	0.386
OMS00057	a	0.0002	0.355	0.481	0.511	0.475
OMS00061	a	0.0001	0.387	0.383	0.375	0.391
OMS00066	a	0.0065	0.194	0.175	0.233	0.255
OMS00067	a	0.0141	0.000	0.000	0.056	0.054
OMS00071	a	0.0112	0.441	0.477	0.404	0.414
OMS00072	a	0.0079	0.538	0.493	0.393	0.457
OMS00074	a	0.0014	0.473	0.497	0.533	0.500
OMS00078	a	0.0103	0.130	0.177	0.267	0.278
OMS00081	a	0.0056	0.000	0.000	0.022	0.022
OMS00087	a	0.0241	0.247	0.217	0.056	0.075
OMS00089	a	0.0001	0.391	0.405	0.416	0.399
OMS00092	a	0.0476	0.280	0.361	0.156	0.143
OMS00105	a	0.0005	0.323	0.383	0.333	0.401
OMS00106	a	0.0005	0.215	0.271	0.289	0.292
OMS00110	a	0.0077	0.011	0.011	0.056	0.054
OMS00116	a	0.0010	0.108	0.121	0.078	0.095
OMS00119	a	0.0057	0.183	0.217	0.244	0.292
OMS00120	a	0.0142	0.355	0.355	0.478	0.448
OMS00121	a	0.0091	0.522	0.500	0.478	0.478
OMS00134	a	0.0023	0.108	0.157	0.122	0.115
OMS00140	a	0.0081	0.032	0.032	0.000	0.000
OMS00153	a	0.0202	0.330	0.304	0.156	0.162
OMS00154	a	0.0000	0.430	0.499	0.500	0.498
OMS00159	a	0.0008	0.022	0.021	0.011	0.011
OMS00164	a	0.0026	0.269	0.248	0.156	0.198

Marker Name	Reference[1]	F_{ST}	Wild H_o	Wild H_e	Hatchery H_o	Hatchery H_e
OMS00175	a	0.0295	0.290	0.344	0.422	0.470
OMS00176	a	0.0021	0.237	0.225	0.256	0.270
OMS00177	a	0.0898	0.264	0.378	0.058	0.078
OMS00180	a	0.0231	0.326	0.340	0.189	0.189
Omy_09AAD-076	b	0.0204	0.151	0.175	0.056	0.054
Omy_1004	c	0.0108	0.462	0.417	0.344	0.478
Omy_101554-306	d	0.0000	0.129	0.121	0.133	0.124
Omy_102420-634	d	0.0180	0.407	0.465	0.352	0.369
Omy_104519-624	d	0.0123	0.376	0.388	0.467	0.464
Omy_105075-162	d	0.0002	0.489	0.444	0.483	0.435
Omy_105401-363	d	0.0054	0.022	0.021	0.000	0.000
Omy_107336-170	d	0.0003	0.065	0.062	0.078	0.075
Omy_107806-34	d	0.0146	0.398	0.496	0.522	0.448
Omy_108007-193	d	0.0289	0.097	0.092	0.256	0.255
Omy_109243-222	d	0.0189	0.489	0.450	0.422	0.499
Omy_110064-419	d	0.0017	0.204	0.200	0.236	0.241
Omy_110078-294	d	0.0244	0.516	0.497	0.460	0.428
Omy_111005-159	d	0.0085	0.366	0.350	0.261	0.260
Omy_111383-51	d	0.0126	0.484	0.441	0.364	0.351
Omy_111666-301	d	0.0009	0.097	0.130	0.111	0.105
Omy_112301-202	d	0.0169	0.419	0.467	0.511	0.500
Omy_112820-82	d	0.0012	0.217	0.243	0.200	0.278
Omy_116733-349	d	0.0006	0.161	0.166	0.156	0.143
Omy_117259-96	d	0.0042	0.355	0.388	0.422	0.437
Omy_117815-81	d	0.0097	0.409	0.412	0.433	0.473
Omy_118205-116	d	0.0071	0.326	0.315	0.244	0.231
Omy_121713-115	d	0.0087	0.366	0.403	0.289	0.320
Omy_127236-583	d	0.0551	0.269	0.292	0.067	0.064
Omy_97077-73	d	0.0157	0.269	0.248	0.356	0.369
Omy_97954-618	d	0.0129	0.228	0.280	0.384	0.386
Omy_arp-630	e	0.0017	0.398	0.448	0.467	0.420
Omy_aspAT-123	e	0.0059	0.097	0.111	0.056	0.054
Omy_cd59-206	f	0.0001	0.484	0.455	0.456	0.448
Omy_cxcr-169	f	0.0027	0.011	0.011	0.000	0.000
Omy_DABc	c	0.0002	0.065	0.062	0.056	0.054
Omy_dacd1-131	g	0.0670	0.312	0.331	0.078	0.075
Omy_g1-103	h	0.0078	0.043	0.042	0.111	0.105
Omy_g12-82	f	0.0202	0.484	0.477	0.523	0.498
Omy_gluR-79	g	0.0336	0.366	0.393	0.556	0.494
Omy_hsc715-80	i	0.0108	0.402	0.443	0.483	0.491
Omy_hsp47-86	i	0.0069	0.387	0.421	0.344	0.352

Marker Name	Reference[1]	F_{ST}	Wild H_o	Wild H_e	Hatchery H_o	Hatchery H_e
Omy_hsp90BA-229	i	0.0249	0.435	0.415	0.300	0.270
Omy_IL17-185	f	0.0214	0.538	0.458	0.289	0.346
Omy_Il-1b-028	b	0.0088	0.538	0.497	0.551	0.494
Omy_IL1b-163	f	0.0017	0.304	0.340	0.416	0.378
Omy_mapK3-103	g	0.0163	0.250	0.266	0.455	0.386
Omy_mcsf-268	f	0.0474	0.391	0.447	0.278	0.255
Omy_metA-161	g	0.0265	0.344	0.499	0.438	0.464
Omy_metB-138	g	0.0084	0.204	0.200	0.122	0.115
Omy_nkef-241	e	0.0356	0.435	0.423	0.533	0.500
Omy_nkef-308	e	0.0160	0.591	0.496	0.478	0.486
Omy_oxct-85	f	0.0063	0.312	0.331	0.333	0.401
Omy_star-206	f	0.0008	0.527	0.461	0.422	0.444
Omy_stat3-273	f	0.0274	0.462	0.408	0.467	0.494
Omy_tgfb-207	f	0.0005	0.452	0.421	0.467	0.437
Omy_u07-79-166	b	0.0045	0.527	0.498	0.427	0.497
Omy_u09-56-073	b	0.0021	0.215	0.271	0.326	0.316
Omy_U11_2a-114	b	0.0623	0.315	0.321	0.078	0.075

[1] a) Sánchez et al. (2009), b) Limborg et al. (2012), c) Hansen et al. (2011), d) Abadía-Cardoso et al. (2011), e) Campbell and Narum (2009), f) Unpublished, J. DeKoning, Washington State University, Pullman, WA, USA, g) Unpublished, N. Campbell, Columbia River Intertribal Fish Commission, Hagerman, ID, USA, h) Stephens et al. (2009), i) Campbell et al. (2009)