Should I Stay or Should I Go? The Influence of Genetic Origin on Emigration Behavior and Physiology of Resident and Anadromous Juvenile Oncorhynchus mykiss

Sean A. Hayes [a] , Chad V. Hanson [a b] , Devon E. Pearse [a] , Morgan H. Bond [a c] , John Carlos Garza [a] & R. Bruce MacFarlane [a]

[a] National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southwest Fisheries Science Center, Fisheries Ecology Division, 110 Shaffer Road, Santa Cruz, California, 95060, USA

[b] Department of Forest Science, Oregon State University, 321 Richardson Hall, Corvallis, Oregon, 97331, USA

[c] School of Aquatic and Fishery Sciences, University of Washington, Box 355020, Seattle, Washington, 98195, USA
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Should I Stay or Should I Go? The Influence of Genetic Origin on Emigration Behavior and Physiology of Resident and Anadromous Juvenile *Oncorhynchus mykiss*

Sean A. Hayes,* Chad V. Hanson,[1] Devon E. Pearse, Morgan H. Bond,[2] John Carlos Garza, and R. Bruce MacFarlane

National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southwest Fisheries Science Center, Fisheries Ecology Division, 110 Shaffer Road, Santa Cruz, California 95060, USA

Abstract

We investigated the interaction among genetically identified origin, behavioral tendency to emigrate, and Na^+, K^+-ATPase enzyme activity in recently diverged subpopulations of resident (above-barrier) rainbow trout *Oncorhynchus mykiss* and steelhead (anadromous rainbow trout) in Scott Creek, California. Genetic assignment tests found that the frequency of resident and anadromous origin fish varied by sampling location within the watershed. Individuals immediately below barriers assigned to both above-barrier (37%) and anadromous (63%) subpopulations, and distinct differences in size and age were observed. However, the majority of downstream migration behavior occurred in fish of anadromous ancestry, which represented 97% of the fish sampled as outmigrating smolts. Nonmigratory fish of both life history types and origins typically had low Na^+, K^+-ATPase activity levels throughout most of the year, but significantly elevated levels were observed in individuals from both groups during the spring smolt migration period. Conversely, many fish sampled in the upper watershed with anadromous genotypes were greater than typical smolt size thresholds for this population yet appeared unlikely to migrate based on low Na^+, K^+-ATPase activity. Life history pathways of *O. mykiss* in this population are strongly influenced but not entirely determined by origin in the resident or anadromous subpopulation, and this relationship has implications for recovery of populations impacted by dams, water diversions, and residualized hatchery fish. Further, this work demonstrates the need to consider resident rainbow trout as potentially important resources for recovery of threatened and endangered steelhead populations.

The salmonid *Oncorhynchus mykiss* exhibits tremendous life history variation, ranging from an anadromous form, termed steelhead, to a resident, nonmigratory form, termed rainbow trout (Shapovalov and Taft 1954). In some Pacific salmonids, all individuals in a given region are included in a single management unit referred to as an Evolutionary Significant Unit or a Distinct Population Segment (DPS). Steelhead populations in Washington, California, Idaho and Oregon are divided into 15 DPSs, nine of which are listed under the federal Endangered Species Act as Threatened and two as Endangered.

These listings pertain to "all naturally spawned anadromous *O. mykiss* (steelhead) populations below natural and manmade impassable barriers" (http://www.nwr.noaa.gov/ESA-Salmon-Listings/Salmon-Populations/Steelhead/Index.cfm) (U.S. Office of the Federal Register 2006). However, this delineation is problematic because of the presence of "resident" fish in below-barrier waters of most DPSs (Busby et al. 1996; Zimmerman and Reeves 2002; Pearse et al. 2007; Donohoe et al. 2008) as well as the inability to predict whether a particular juvenile fish will adopt a resident or anadromous life

history strategy. In many cases, it is unclear whether resident and anadromous fish in a stream represent separate populations, evolved through either sympatric or allopatric mechanisms (natural and anthropogenic), or are merely extremes on a continuum of behavioral phenotypes within a single population, with fish of both phenotypes interbreeding and progeny potentially expressing different phenotypes from their parents depending on environmental conditions. As the ratio of the two phenotypes is probably affected by the influence of habitat variation on survival and reproduction (Satterthwaite et al. 2009), this is further complicated by the potential for all of the above to occur and vary among or within DPSs.

Currently, there is limited knowledge on the ability of life history strategies within *O. mykiss* populations to change over ecological time and consequently limited power to predict population responses to actions such as dam removals and water reallocations. This information is critical for recovery planning in habitats where anadromy was lost or inhibited by dam construction and dewatering. In addition, resident life history forms often appear as a "side effect" in artificial propagation of steelhead, where it is referred to as residualization (Willson 1997) and its implications are poorly understood (Kostow 2009). Thus, a better understanding of how ancestry and the environment influence the migratory behavior of *O. mykiss* is necessary for assessment of stock status, conservation, and management.

Additionally, the relationship between resident and anadromous life history forms has intriguing biological implications for understanding influences on evolutionarily stable strategies. As juvenile salmonids mature, their life history pathway may be influenced by genetic and environmental mechanisms (Quinn et al. 2000). When following the anadromous pathway, they undergo the physiological changes associated with the transition from freshwater to saltwater, a process referred to as smoltification. This transformation typically includes changes in body coloration, reduction in body condition factor and, most significantly, changes associated with osmoregulation (Hoar 1976; Hoar 1988). The latter specifically involves a transition in structure of the chloride cells on the external surface of the gill tissue to assist with transitioning from salt retention to salt excretion through the use of Na^+, K^+ pumps (McCormick 1995). The transformation to smolt stage generally culminates in active migration downstream to the ocean. The genetic control over this transformation is complex (McLeese et al. 1994; Thrower et al. 2004b; Nichols et al. 2008; Martínez et al. 2011) and the ability to smolt may remain in resident populations for generations (Thrower et al. 2004a; Thrower et al. 2004b; Thrower and Joyce 2004). This phenomenon requires further investigation to better understand the contribution of resident *O. mykiss* to the downstream-migrating smolts in basins where the two life history forms coexist. Within the Scott Creek basin, a wide range of size-classes (70–250 mm) of juvenile steelhead have been reported to exhibit downstream migration behavior and elevated Na^+, K^+-ATPase levels (for a detailed examina-

tion of juvenile emigration behavior and smolting physiology in southern steelhead, see Hayes et al. 2004; Hayes et al. 2011.)

Here we investigate the genetic origin, physiology, and behavior of resident rainbow trout and anadromous steelhead in Scott Creek, California, where both life history forms of *O. mykiss* are present and share recent common ancestry. Previous work identified genetically distinct resident trout populations above several natural barriers to anadromy in this basin, as well as substantial numbers of resident-origin fish present directly below these barriers (Pearse et al. 2009). In addition, historical records indicate transfer of *O. mykiss* above the barrier waterfalls roughly 100 years ago, where resident trout are observed today (Pearse et al. 2009), providing an experimental analog to populations impacted by dams. We hypothesized that after roughly 30 generations of strong selection against anadromy, resident populations above anadromy barriers would be unlikely to produce smolts, whereas sympatrically breeding populations of both life history types below barriers may show a different trend. To test this, we investigated migration behavior, size at emigration, and Na^+, K^+-ATPase activity levels in fish of resident and anadromous ancestry found above and below anadromy barriers, as well as fish captured in a smolt trap in the lower watershed.

METHODS

Study site.—Scott Creek is a small coastal watershed, approximately 70 km² in area and 100 km south of San Francisco, California (37° 02.5′N). Anadromous fish can access approximately 23 km of stream between the estuary and natural upstream barriers of the main stem and the three main tributaries: Big Creek, Little Creek, and Mill Creek (Figure 1). The main stem barrier on upper Scott Creek is a roughly 10-m drop through carved rock with a single step pool in the lower portion, whereas the Big Creek barrier consists of a single-drop waterfall approximately 35 m high and the Mill Creek barrier is a roughly continuous drop 20-m waterfall. A small estuary is present, which typically becomes a freshwater lagoon during summer and fall, when a sandbar forms at the creek mouth, blocking access to the ocean. Samples were designated as coming from three general areas: (1) above natural anadromy barriers (marked on tributaries in Figure 1), (2) upper shed (below anadromy barriers), and (3) lower watershed, with the separation of the latter two designated by a dotted line on Figure 1. A conservation hatchery has operated in the watershed for several decades, spawning anadromous steelhead and releasing smolt-stage fish each spring. These fish spend very little time in the watershed prior to emigration (Hayes et al. 2004), are all marked by adipose fin clip, and were excluded from this study.

Fish sampling.—Sampling was conducted from March 2002 through June 2008 and involved multiple methods. All fish sampled were grouped into one of three categories: fish above anadromy barriers (AB), upper-watershed fish below anadromy barriers (UW), and smolt trap fish (ST). Fish above anadromy barriers occurred in very low densities and were
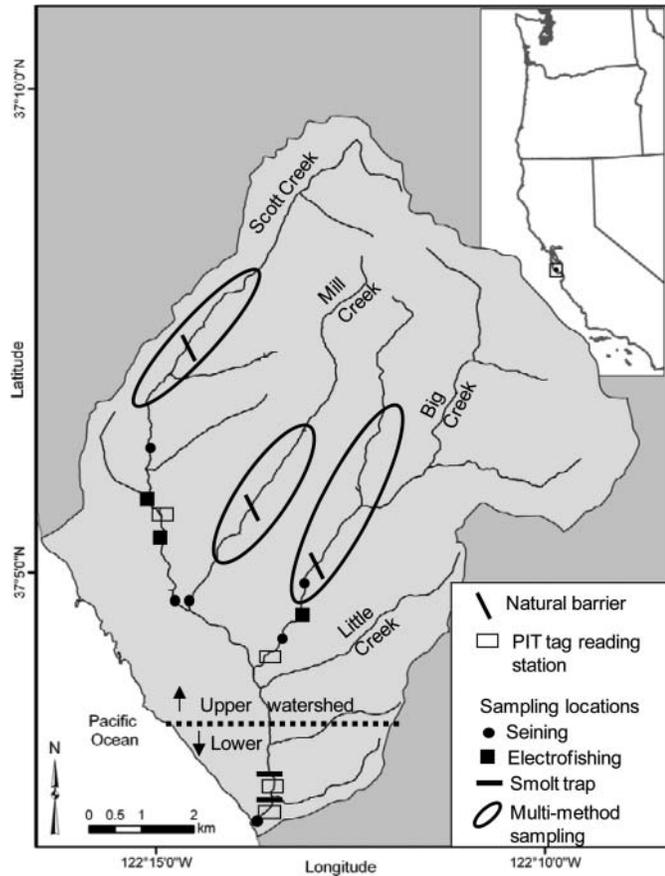
FIGURE 1.   Map of Scott Creek watershed showing locations of sample collection with different sampling methods used. Ovals denote regions opportunistically sampled by multiple methods including seining, electrofishing, and hook and line sampling to acquire additional samples across a larger spatial scale.

opportunistically sampled across several kilometers of stream by multiple methods including hook and line fishing or seine netting (3 m × 1.5 m beach seine, 0.32 cm square mesh) until 2006 and by electrofishing in 2007 (Figure 1). Below-barrier fish in the upper watershed were sampled opportunistically by seine or hook and line, as well as repeated seining at discrete locations from May 2002 through December 2004 (Figure 1). Beginning in 2006, electrofishing surveys were conducted three times per year in four 100-m stream reaches in the upper watershed below the anadromy barriers. From 2002 to 2005, downstream migrating fish were trapped near the head of the estuary by means of a two-chambered square mesh smolt trap with soft mesh or fixed panel wing walls extending to each bank. The trap was operated three days per week throughout the year except during exceptionally high flows associated with winter storms. In 2006, the trap was upgraded to a fixed panel and pipe design with a two-chamber live box and moved approximately 500 m upstream to be collocated with an adult weir trapping station (upper smolt trap sight noted in Figure 1). All mesh spacing was ≤0.635 cm. Effort was made to operate the trap 7 d per week from January through July in 2006, 2007, and 2008.

Fish were handled according to the methods of Hayes et al. (2004). Briefly, all fish were measured for fork length (L_F) and mass. Gill samples (∼5–6 filaments) were collected from approximately 10 fish (>100 mm L_F) from each of the three location categories each month through December 2004 to measure Na^+, K^+-ATPase activity. Small tissue samples from fish ≥55 mm L_F were taken from the caudal fin for genetic analysis. Most fish >65 mm L_F collected after March 2003 received a passive integrated transponder (PIT) tag (11.5 mm FDX-B Glass Transponder; Allflex, Boulder, Colorado) by intraperitoneal injection with a 12 gauge needle. Fish were placed in a recovery container for at least 10 min before release.

Na^+, K^+-ATPase.—Assays that measure activity levels of the enzyme Na^+,K^+-ATPase (hereafter referred to as NKA activity) have become a standard for assessing seawater readiness in salmonid smolts (Björnsson and Bradley 2007). Gill samples were put into individual vials containing SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.3) and placed on dry ice in the field, followed by storage at –80°C until laboratory analysis, which was performed within 3 months of sample collection. Specific NKA activity was expressed as micromoles of ADP per milligram protein per hour (μmol ADP · mg protein^{-1} · h^{-1}) in accordance with McCormick's (1993) nonlethal micro method. Each sample was assayed in triplicate (3 aliquots of the same homogenate) to evaluate within-sample variance. For comparative purposes, samples were combined across years and binned by season.

Fish Movement.—Fish movements were assessed through a combination of instream PIT tag readers (Bond et al. 2007) and opportunistic recaptures. An instream PIT tag reader is located at the head of the Scott Creek estuary and began operation in April 2004. An additional reader was operated intermittently at a downstream location in the estuary during 2005 and 2008. Two additional readers were installed in December 2005, one on Big Creek and one on main stem Scott Creek above the confluence with Mill Creek (Figure 1). Due to variable PIT tag reader detection and trapping efficiencies, as well as periods when readers or traps were inoperable, all reported detection rates represent minimum estimates. For similar reasons, data were pooled across years and binned by month or Julian day unless otherwise specified and interannual variation was not evaluated, due to the confounding effects of inconsistent and changing sampling methods.

Genetic Analysis.—The DNA was extracted from all samples using the DNeasy 96 filter-based nucleic acid extraction system on a BioRobot 3000 (Qiagen, Valencia, California), following the manufacturer's protocols. Extracted DNA was diluted ∼10:1 with dH2O and used for PCR amplification of 18 microsatellite loci previously optimized for use in *O. mykiss* (Aguilar and Garza 2006; Pearse et al. 2007). The PCR products were electrophoresed on ABI 377 automated DNA sequencers and microsatellite genotypes determined using Genotyper 2.1 software (Applied Biosystems, Foster City, California). All genotypes were called independently by two people to ensure

consistent scoring. Discrepancies between the two scores were resolved either by consensus, regenotyping, or by discarding that genotype from the data set.

The computer program GENEPOP (Raymond and Rousset 1995) was used to estimate basic population genetic statistics and test for departures from Hardy–Weinberg and linkage equilibria. Individual assignments were conducted following Pearse et al. (2009) using the allele frequency estimation of Rannala and Mountain (1997) implemented in the program GeneClass2 (Piry et al. 2004). The reference baseline populations were from resident fish above anadromy barriers ($n = 186$) and adult steelhead ($n = 233$) returning from the ocean, with the leave-on-out self-assignment method used for the above-barrier fish in the baseline (Pearse et al. 2009). The reference fish, representing the two life history types in Scott Creek, then established the baseline for assigning the additional juveniles collected for the present study (Pearse et al. 2009). Only individuals assigned to the anadromous or above-barrier resident baseline samples with a probability score of >95% in GeneClass2 were used.

Statistical Analysis.—With the exception of the population genetic analyses described above, the majority of statistical analyses were conducted using SYSTAT 11 for analysis of variance (ANOVA), regression, and nonparametric tests. Analyses which required only *t*-tests were conducted with the data analysis tool pack in Microsoft Excel 2003.

RESULTS

Downstream Migration Trends

Downstream migrant trapping was conducted from January 2003 through June 2008. For the purposes of this study, all fish handled at the downstream migrant trap are referred to as "smolts." Trapping effort and efficiency varied among years due to variable flow conditions, staffing, and equipment resources. Smolt movement peaked in the spring, typically between March and June. Mean L_F for all smolts measured during the course of the study ($n = 3,848$) was 110 mm (SD, 39 mm).

Genetic Influence on Probability of Downstream Migration

Genotype data from a total of 1,675 fish were included in the present study and, of these, 88% were assigned to either the anadromous or resident genetic reference sample with a probability score of at least 95%. Since fish sampled above the anadromy barrier cannot be of anadromous origin, the misassignment of these fish to the anadromous reference sample provides an estimate of the assignment error rate. Of the fish sampled above the anadromy barrier, 92% genetically assigned to the resident reference sample, while 8% assigned to the anadromous population. Conversely, 97% of fish caught in the smolt trap assigned to the anadromous subpopulation, suggesting that nearly all of these fish had an anadromous parental origin. In contrast, the upper watershed samples from below barriers were much more mixed, with 37% of fish

TABLE 1. Population genetic assignment (at 95% confidence) of samples taken from three locations in the Scott Creek watershed.

Location	Resident	Anadromous	Number (%) assigned with 95% confidence
Above barrier	92.13%	7.87%	267 (90%)
Upper watershed	36.82%	63.18%	421 (86%)
Smolt trap	2.62%	97.38%	801 (90%)

assigned to the resident subpopulation and 63% assigned to the anadromous subpopulation (Table 1). Significantly, fish in the upper watershed that assigned to the resident and anadromous subpopulations were of different size, suggesting that most anadromous origin juveniles will migrate by a certain age or size (Hayes et al. 2011), whereas resident genotype fish stay and continue to grow, resulting in their greater representation in the larger size-classes (Figure 2; Kruskal–Wallis test statistic, $n = 421$, $P < 0.0001$ assuming χ^2 distribution with 1 df). The number of fish sampled at the smolt trap that assigned to the resident subpopulation ($N = 21$ or 2.6%) was within the error rate of the assignment test, and too small to determine whether this size difference persists in the migrant fish.

Movement by PIT-tagged (below barrier) fish out of the upper watershed was also directly assessed, as a way of evaluating life history trajectory for individual fish assigned to the two subpopulations with high confidence (Table 2). Specifically, we compared the number of fish redetected migrating through smolt traps and adjacent PIT tag readers in the lower watershed versus fish recaptured in the upper watershed after a full spring "downstream migration season" had occurred. For anadromous fish ($n = 91$), very few individuals (14.3%) were redetected in the upper watershed after a full smolt season had passed, while 85.7% were detected migrating through the lower watershed,
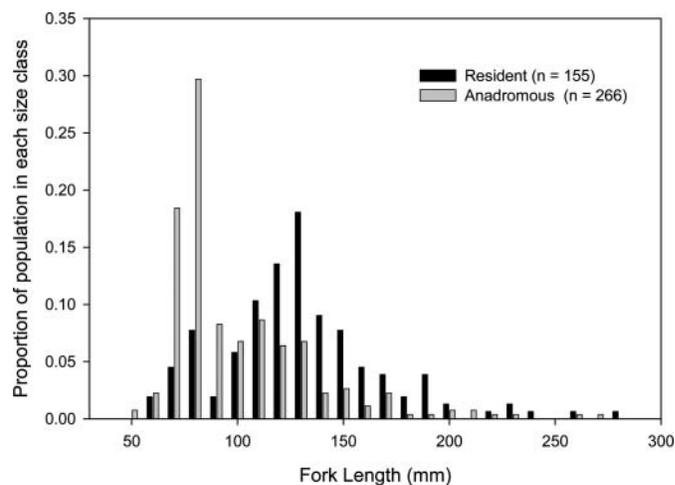

FIGURE 2. Size frequency distributions of fish assigned to the resident and anadromous subpopulation below anadromy barriers in the upper watershed.

TABLE 2. Movement summary of resighted fish tagged in upper watershed and above barriers.

Fish type	Number resighted	Upper watershed n (%)	Lower watershed n (%)
		Location resighted	
Upper watershed fish			
Resident genotype	24	13 (54.2%)	11 (45.8%)
Anadromous genotype	91	13 (14.3%)	78 (85.7%)
Above barrier fish resighted below barrier	9	2	7

TABLE 3. Pairwise Tukey matrix of ANOVA results for comparisons of Na$^+$, K$^+$-ATPase activity by fish type and season with P-values less than 0.3. Winter = January–March, Spring = April–June, Summer = July–September, Fall = October–December, AB = above barrier fish, UW = upper watershed fish, and ST = smolt trap fish. Comparisons for AB by AB and UW, and UW by UW are not shown and had P-values of 0.30 or higher. An asterisk represents the null values of the location and season compared to itself.

Location and season	ST-winter	ST-spring	ST-summer	ST-fall
AB-winter		0.007		
AB-spring	0.002	<0.001	0.173	0.025
AB-summer	<0.001	<0.001	0.126	0.007
AB-fall		0.01		
UW-winter		<0.001		
UW-spring		<0.001		
UW-summer	0.001	<0.001	0.223	0.019
UW-fall	0.001	<0.001	0.238	0.028
ST-winter	*			
ST-spring		*	0.005	0.001
ST-summer			*	
ST-fall				*

presumably on their way out to sea. In contrast, more than half (54.2%) of the resident assigned fish captured in the upper watershed (n = 24) were redetected again in the upper watershed, reflecting their tendency to remain in freshwater. In contrast, 45.8% were detected migrating to the lower watershed.

We placed PIT tags in 398 fish above waterfalls between June 2003 and November 2007 to directly measure the migration rate of known resident fish from above barriers (Table 2). As of October 2008, nine of these tags had been detected in the lower watershed; two fish from above Big Creek Falls were detected by only the upper watershed PIT tag readers, five were detected in the smolt trap, and two more were detected by PIT tag readers downstream of the smolt trap at the entrance to the estuary. This leads to a minimum estimate of the "emigration rate" of fish from above anadromy barriers of 2.7%, with 1.8% exhibiting full downstream migration behavior. It is important to note that the actual rate is likely much greater than this, due to less than perfect detection efficiency by the smolt trap and PIT tag readers, and, more significantly, to the upwards size bias of tagged fish, since many were already mature when tagged and may be less likely to undergo smoltification and migrate than smaller fish.

Na$^+$, K$^+$-ATPase Activity Dynamics

Gill samples (n = 490) were collected between March 2002 and December 2004 and measured for NKA activity (an acceptable measurement required coefficients of variation of <20% between replicate wells of the microplate reader). Pair-wise comparisons by location and season are reported in Table 3. Statistical analysis using ANOVA identified significant differences in NKA activity levels among locations ($F_{2,484}$ = 44.094, P < 0.001) and among seasons ($F_{2,484}$ = 9.820, P < 0.001). Seasonal patterns of NKA activity are shown in Figure 3, with data binned by month for all years. In general, samples collected in the downstream migrant trap showed elevated (typically >2 mmol ADP mg protein^{-1} h^{-1} for this watershed [Hanson 2008]) NKA activity relative to fish in the upper watershed, both above and below anadromy barriers (Figure 3).

Na$^+$,K$^+$-ATPase activity during the smolt out-migration period (February to May) was compared at the three sample

locations and for fish that genetically assigned to the two subpopulations. For two of the locations, AB and ST, genetic assignments were heavily skewed towards either the resident or anadromous subpopulation that was consistent with expectations from observations (i.e., majority resident above barriers). In these two cases, mean NKA activity levels did not differ between the two genetic types (AB, ST; t-test, P > 0.05) and the frequency of assignment to the alternative subpopulation was sufficiently low to potentially be accounted for by assignment test error (Table 1). However,
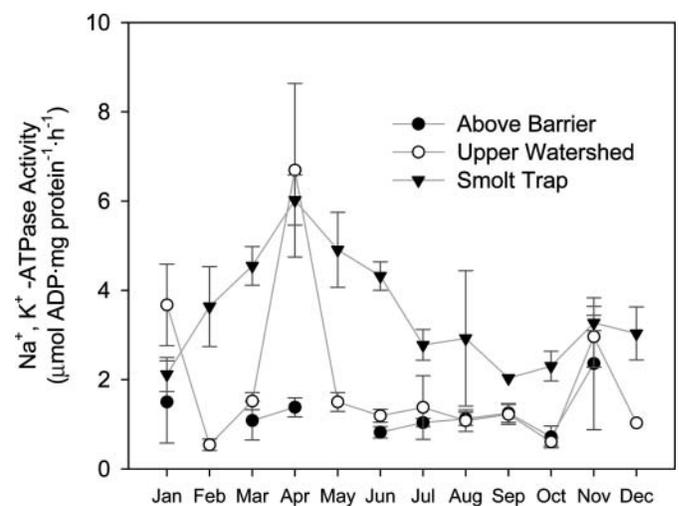

FIGURE 3. Seasonal Na$^+$, K$^+$-ATPase activity levels averaged by month (all years combined, whiskers represent ±1 SE) for fish sampled at the smolt trap and in the upper watershed both above and below anadromy barriers.
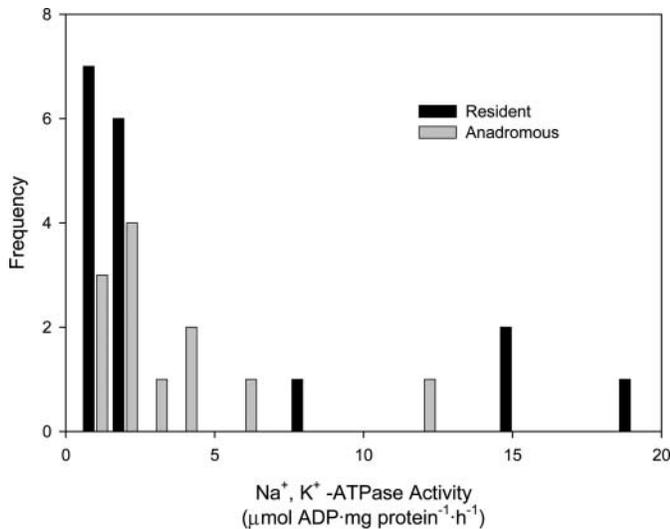
FIGURE 4. Frequency distribution of Na$^+$, K$^+$-ATPase activity levels sampled during the spring smolt season (February to May) for fish of inferred resident and anadromous origin living sympatrically below anadromy barriers in the upper watershed.

in the UW sample, where there were substantial proportions of fish assigned to both subpopulations, anadromous-assigned fish displayed a broad range of NKA levels, suggesting some of these fish were preparing for smolt migration while others were delaying until later in the season or the following year (Figure 4). While most UW fish that assigned to the resident subpopulation showed very low NKA levels, consistent with their resident life history, three of these fish had the highest NKA activity levels observed in the study. In addition, a fish PIT-tagged above Big Creek falls was recaptured at the smolt trap and also had one of the highest NKA levels of the study (22.8 μmol ADP·mg protein^{-1}·h^{-1}). Taken together, these results suggest that resident fish retain the ability to smolt, and rare smolting individuals of resident origins may have a greater magnitude of smolt development relative to below-barrier anadromous fish.

DISCUSSION

Here, we show how residence above barriers to anadromy (large waterfalls) can greatly reduce the tendency to exhibit anadromous behavior. This is presumably due to strong selection against anadromous migration for these fish, which were apparently introduced from the lower watershed during the last century (Pearse et al. 2009; Martínez et al. 2011). However, while most such fish remain in the upper watershed, anadromous behavior, as measured by elevated NKA activity and downstream migration, is still expressed by some fish assigned to the resident subpopulation, albeit at low frequency, and resident behavior is expressed by some anadromous fish as well. In addition, several fish tagged above a 35-m barrier waterfall were found to have migrated downstream, with some detected near the entrance to the ocean. The results of this study help to illuminate the degree of life history plasticity within

a population of *O. mykiss* and provide further evidence that salmonid populations may be able to rapidly shift phenotype ratios in the face of environmental change.

The relative importance of environmental and heritable factors in salmonid life history remains unclear and its resolution is hindered by the challenges of trying to simultaneously integrate disciplines of ecology, physiology, and evolution. There is ample evidence in Atlantic salmon *Salmo salar* that environmentally influenced size thresholds control whether a fish undergoes smoltification in a given year (Thorpe 1977; Thorpe et al. 1982), and the same has been observed for steelhead in Scott Creek, based on field observations and modeling exercises (Satterthwaite et al. 2009). In addition, hatchery-reared offspring of wild steelhead in Scott Creek are larger than wild fish reared under natural conditions and tend to go to sea at younger ages than wild fish (Hayes et al. 2004). Conversely, Thrower et al. (2004b) showed a heritable influence on the proportion of offspring that matured as precocious male parr versus smolted as a function of resident or anadromous parentage.

Our results support the hypothesis that smolt transformation has a strong heritable influence but that the resident and anadromous life history pathways are not fixed phenotypes. The observation of life history variation in Scott Creek, both in the form of anadromous fish residualizing and above-barrier fish migrating downstream, suggests that plasticity can remain within a population for some time. It is unclear at this point whether the low frequency of the alternative life history strategy in the subpopulations dominated by the resident and anadromous pathways is stabilized at a fixed rate, or if it will eventually disappear in the resident subpopulation above the anadromy barrier.

Fish assigned to the anadromous and resident subpopulations are sympatric below anadromy barriers, but it remains unclear to what extent they hybridize. Pearse et al. (2009) reported that hybridization between the two groups occurs at very low frequencies or not at all. However, a greater percentage of fish were of ambiguous genetic origin in the upper watershed area, where the greatest potential for hybridization exists in this system, and these fish reflect ongoing hybridization. Anadromous females likely select males based on size, giving advantage to anadromous males (Schroder 1981; Foote 1989; Berejikian et al. 2000), but the "sneaker" male mating strategy by smaller fish can overcome female mate choice and result in substantial reproductive success by fish that have not been to sea. The "sneaker male" strategy is regularly observed in Scott Creek and other nearby watersheds (Shapovalov and Taft 1954; Hayes et al. 2004) and in many salmonid populations (Seamons et al. 2004).

Prezygotic barriers to fertilization, including differences in the timing of reproduction of resident females and anadromous males (McMillan et al. 2007), may also limit hybridization in some populations. Zimmerman and Reeves (2000) observed a 2-month difference in peak spawner activity (from redd surveys) between anadromous and resident fish in a basin in Oregon, with the latter spawning later. Direct observations of resident fish spawning in Scott Creek have been limited. However, resident

males from Scott Creek were observed expressing milt from early winter to late summer, encompassing the entire anadromous spawning season and several months beyond (S. A. Hayes, unpublished data), suggesting similar delays in resident spawning within this population and a potential barrier to gene flow between resident females and anadromous males. Future work will elucidate patterns of hybridization in this population and its consequences, through a combination of intensive ecological monitoring of life history strategy and advances in genetic techniques, including development of new molecular markers (e.g., Abadía-Cardoso et al. 2011) and parentage studies to assess the pedigree relationships of resident and anadromous fish and their offspring. Breeding and rearing experiments will also help to assess the heritable and environmental components of smoltification and ultimately assess marine survival of fish with different ancestry.

Natural selection may also be acting against hybridization through other mechanisms. Thrower and Joyce (2004) found that *O. mykiss* smolts reared from resident parents (taken from a population above a waterfall ∼25 generations ago) and crosses between resident males and anadromous females, had poor marine survival in comparison to offspring of anadromous fish and anadromous-male-by-resident-female crosses from the same watershed, suggesting selection against both resident fish smolting and hybridization between resident males and anadromous females. Similarly, a pilot study was conducted in Scott Creek with female steelhead crossed with both anadromous and resident males from above barriers and the offspring reared in common garden. Greater marine survival was observed for the offspring of anadromous fathers, although sample sizes were not sufficient to demonstrate a significant difference (S. A. Hayes and D. E. Pearse, unpublished data).

The application of the NKA assay to resident fish above barriers and nonmigratory fish below barriers suggest that resident fish typically show no elevation in NKA enzyme activity during the spring smolt season (Figure 3) and do not show morphological, physiological, or behavioral responses to potential environmental cues, somewhat similar to laboratory results showing reduced seasonal response by steelhead–rainbow trout hybrids in comparison to pure steelhead offspring (McLeese et al. 1994). However, there were several resident assigned fish that had extremely high NKA activity levels (values of 14–22 μmol ADP·mg protein^{-1}·h^{-1}) during the spring migration period, and one was confirmed migrating to the smolt trap from above the anadromy barrier. These results suggest that resident fish retain the ability to smolt but may have an altered NKA activity threshold for smoltification relative to the below-barrier anadromous population. While speculative, there may be some physiological condition where NKA activity reaches very high levels in association with actual life history switching from residency to anadromy, but this will require further work in controlled laboratory conditions. An original goal of this study was to investigate the use of the nonlethal NKA assay for distinguishing resident and anadromous fish.

While the NKA assay appears useful for assessing population trends, it does not provide a reliable "score" at the individual level and is confounded by seasonal fluctuations in NKA levels within *O. mykiss* populations and the high variance of the assay itself.

The results of this study and previous work (Thrower and Joyce 2004) make it clear that both environmental and genetic factors influence the relative ratios of resident and anadromous life history strategies in *O. mykiss* populations. In both studies, there was a strong influence of parental origin on offspring phenotypic expression of life history strategy. It is interesting that both of these populations have been influenced by anthropogenic relocation of anadromous fish above anadromy barriers. In contrast, other populations of *O. mykiss* (where this above-barrier stocking has not been reported) have been observed with both resident and anadromous phenotypes present and no significant genetic divergence between them (McPhee et al. 2007; Heath et al. 2008). McMillan et al. (2007) observed interbreeding between resident males and anadromous females in the Calawah and Sol Duc basins on the Olympic Peninsula during late season spawning when fewer anadromous males were present. In Kamchatka, Russia, interbreeding between fish of both sexes with resident and anadromous phenotype is regularly observed (Kuzishchin et al. 2007). Variation in phenotype ratios across Kamchatka is also observed and has been attributed to habitat within rivers; specifically, resident fish are larger and occur more frequently in more productive rivers (Pavlov et al. 2008). This is likely due to higher fecundity and reproductive success associated with increased growth for resident fish in more productive rivers, whereas marine survival risks are presumed to be constant. Within the Scott Creek watershed, the limited upstream food resources inferred from growth rates (Hayes et al. 2008) and beneficial estuarine life history strategy, which appears to be coupled with the anadromous migratory behavior, result in a similar trend. There is approximately an order-of-magnitude difference in fecundity between anadromous females (50–80 cm length) and resident females (15–25 cm). The resident phenotype in this system may be maintained more through allopatric mechanisms that select for resident behavior above anadromy barriers, with occasional downstream migration over barriers into anadromous waters.

The results of this study and others (McPhee et al. 2007; Heath et al. 2008; Pavlov et al. 2008) suggest that management of *O. mykiss* stocks is more complicated than the current practice of assuming rainbow trout live above barriers and steelhead live below them and the distinction between resident and anadromous stocks remains unclear. It is likely that many steelhead DPSs contain a biological component of resident fish through a combination of phenotypic plasticity and genetic divergence. The effects of artificial propagation on life history switching are also unclear. While residualized hatchery fish have not been observed regularly in this population (Hayes et al. 2004) they are a common management concern in other watersheds (Viola and Schuck 1995; McMichael et al. 1997)

and the observations of anadromous fish residualizing in this watershed (Table 3) emphasize that this life history pathway has the potential to occur and change in frequency.

The above-barrier resident population in Scott Creek is believed to have been derived from anadromous ancestors roughly 30 generations ago (Pearse et al. 2009), and the continued emigration of smolts after this time period has important management implications. While the relatively low rate of smolt production in the above-barrier population may seem trivial, it is likely a significant underestimate of the true emigration rate, and we emphasize its potential importance for management in highly impacted aquatic ecosystems. Notably, the time since divergence of the two subpopulations in this study is similar to that of many *O. mykiss* populations above and below dams, as the resident fish above such dams are typically derived from the steelhead lineage fish trapped there at dam construction (Clemento et al. 2009). Future fish passage and dam removal projects will provide the opportunity for smolting fish from these currently above-barrier populations to complete an anadromous migration and return to their natal stream, as well as for anadromous fish to enter this habitat and potentially interbreed with formerly above-barrier resident fish. Given the uncertainty in the heritable basis of the life history variation in this species and the strong selection against anadromous phenotype experienced by above-barrier populations (Martínez et al. 2011), it is unclear what the outcome of such reintegration might be or how long it would take to achieve a new evolutionarily stable strategy or reexpress anadromy. Nevertheless, the persistence of the ability to smolt and attempt an anadromous migration by fish from a population that has been experiencing strong selection against it for approximately 30 generations indicates that these above-barrier resident populations will be an important resource for recovering threatened and endangered steelhead populations and an appropriate source for reintroduction of steelhead into basins where the anadromous form has been extirpated. From this perspective, it is important to consider all native *O. mykiss* in a basin during conservation planning to ensure the persistence of anadromous steelhead populations within a threatened or endangered DPS.

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