

Down, up, down and “smolting” twice? Seasonal movement patterns by juvenile steelhead (*Oncorhynchus mykiss*) in a coastal watershed with a bar closing estuary

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Abstract: We investigated emigration behavior, habitat use, and Na^+, K^+ -ATPase activity levels of juvenile steelhead trout (sea-run rainbow trout; *Oncorhynchus mykiss*) in Scott Creek, a small coastal watershed with a seasonally closed estuary in central California, USA. Migratory fish moved downstream in the spring with elevated Na^+, K^+ -ATPase activity levels. Larger fish (>150 mm) moved downstream during February and March, leaving the stream, whereas fish moving between April and June were typically smaller and tended to recruit to the estuary. The Na^+, K^+ -ATPase activity levels of estuarine fish varied during the summer as a function of salinity and temperature, but overall levels declined from peak spring values. Many summer recruits were observed retreating upstream into the watershed when estuarine water quality declined in the fall. Rather than entering the ocean when winter storms reconnected the estuary with the ocean in early winter, many migrated downstream several months later during subsequent springs. The largest smolts observed (>190 mm fork length (L_F)) were primarily those that had reared in the estuary the previous summer. Smolts were observed making a single migration from the upper watershed, but they were smaller (~120–190 mm L_F) with potentially reduced marine survival. In summary, we observed fish moving between freshwater and estuarine habitats seasonally and adjusting their osmoregulatory physiology as needed.

Résumé : Nous avons étudié le comportement d'émigration, l'utilisation de l'habitat et les niveaux d'activité de la Na^+, K^+ -ATPase chez de jeunes truites-arc-en-ciel anadromes (*Oncorhynchus mykiss*) dans Scott Creek, un petit bassin versant côtier avec un estuaire qui est fermé une partie de l'année dans le centre de la Californie, É.-U. Les poissons migrateurs se déplacent au printemps avec des niveaux élevés d'activité de la Na^+, K^+ -ATPase. Les poissons plus grands (>150 mm) se déplacent vers l'aval en février et mars et quittent le cours d'eau, alors que les poissons qui se déplacent entre avril et juin sont typiquement plus petits et tendent à se rassembler dans l'estuaire. Les niveaux d'activité de la Na^+, K^+ -ATPase des poissons dans l'estuaire varient au cours de l'été en fonction de la salinité et de la température, mais diminuent en général à partir des valeurs maximales du printemps. On a observé plusieurs des recrues d'été retourner en amont dans le bassin versant lorsque la qualité de l'eau de l'estuaire diminue en automne. Au lieu de pénétrer dans l'océan lorsque les tempêtes hivernales rattachent à nouveau l'estuaire à l'océan au début de l'hiver, de nombreux poissons migrent vers l'aval plusieurs mois plus tard durant les printemps suivants. Les saumoneaux observés les plus gros (>190 mm longueur à la fourche (L_F)) sont en majorité ceux qui se sont développés dans l'estuaire durant l'été précédent. On observe des saumoneaux faire une migration unique à partir du bassin versant supérieur, mais ce sont des poissons plus petits (~120–190 mm L_F) dont la survie en mer est potentiellement réduite. En résumé, nous avons observé des poissons qui se déplacent aux diverses saisons entre les habitats d'eau douce et de l'estuaire et qui ajustent leur physiologie d'osmorégulation selon leurs besoins.

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Introduction

Pacific salmon (*Oncorhynchus* spp.) and steelhead trout (*Oncorhynchus mykiss*, sea-run rainbow trout, hereafter called steelhead) exhibit tremendous life history variation that is typically interpreted as an adaptive response to temporal or spatial variability (Shapovalov and Taft 1954; Hendry and Stearns 2004; Satterthwaite et al. 2009). For example, sockeye salmon (*Oncorhynchus nerka*) exhibit marked polymorphism in their preferred spawning habitat, and this association is thought to exert divergent selective pressures on adult morphology (Blair et al. 1993). Chinook salmon (*Oncorhynchus tshawytscha*), on the other hand, exhibit consistent temporal segregation among spawning runs, with as many as four runs returning to a single river (Fisher 1994). While, Pacific salmon are thought to show comparable levels of variation in earlier portions of their life cycle, until recently logistical challenges have limited the study of this variation.

Of particular interest, but often poorly understood, are the juvenile life history forms, which make use of estuarine habitat (Healey 1982). Estuaries are extremely heterogeneous ecosystems, varying spatially and temporally in their chemistry, physical parameters, and community composition. Thus, myriad trade-offs between opportunities for growth and the risk of predation are all likely to influence the use of this habitat by juvenile salmonids (Wallace and Collins 1997; Roby et al. 2003; Bottom et al. 2005). Because these trade-offs likely underlie adaptive differences within and among populations, they are of particular interest to both biologists and managers.

In coastal California, like many Mediterranean climate regions, streams often have seasonally closing estuaries that form small freshwater lagoons when summer sandbars form at the estuarine mouths (Largier and Taljaard 1991; Schwarz and Orme 2005). The migratory behavior of salmonids in these systems is dictated by the availability of this seasonal habitat. As the marine survival size threshold for steelhead is typically 150 mm (Ward and Slaney 1988; Bond et al. 2008), in California watersheds that lack lagoon habitat, steelhead migrants tend to spend more time rearing upstream and are both older (age 2+) and larger (>140 mm) when they migrate downstream to the ocean (Hanson 2008). In watersheds where lagoons form, juvenile steelhead typically migrate downstream in a broader range of sizes and ages, including many smaller and younger fish (mean fork length (L_F) ~ 100 mm), most of which have elevated Na^+, K^+ -ATPase activity levels indicative of seawater readiness (Hayes et al. 2004, 2008; Hanson 2008). A proportion of these smaller juveniles (~20%) remain in fresh water long enough to be "trapped" by the seasonal closure of the estuary (Bond et al. 2008). These lagoon-reared fish experience very high growth rates for an additional 6 months before winter storms reconnect the stream with the ocean (Smith 1990; Hayes et al. 2008). This additional growth is thought to confer substantially increased survival to adulthood, with estuarine-reared fish producing approximately 60%–90% of the returning anadromous adult steelhead in several California streams (Shapovalov and Taft 1954; Bond et al. 2008; Atkinson 2010). It is commonly assumed that these fish simply enter the ocean once winter storms increase stream flow enough

to break through the sand bar. However, Shapovalov and Taft (1954) observed large numbers of juvenile steelhead migrating upstream during the late fall in Waddell Creek, presumably from the lagoon. The authors suspected that these fish subsequently re-emigrated downstream again, but lacked the equipment to track their fate and ultimately test their suspicion.

Here we sought to develop a more comprehensive understanding of both the physiological and behavioral adaptations that accommodate such a plastic life history in steelhead. Specifically, we investigated the relationships among patterns of movement, habitat quality, and physiological state by combining data from repeated captures, tagging, and a physiological assay for seawater readiness to better understand the seasonal movement into and out of the estuary–lagoon habitat in Scott Creek. This is of particular interest because previous studies suggest that emigrants have decoupled the migratory behaviors and physiological changes associated with smoltification from actual ocean entry (Hayes et al. 2004; Hanson 2008). This is quite unusual for salmonids (Clarke and Hirano 1995; Quinn et al. 2000) and therefore of particular interest. In addition, the timing of when these lagoon-reared fish actually enter the ocean needs clarification, as the potential for a reverse upstream migration may demand connectivity between upper watersheds and estuaries during the driest time of year. Therefore, a better understanding of the physiological ecology of estuarine-reared fish may be critical to management and conservation for these imperiled steelhead populations at the southern end of their geographic range and has implications for undocumented life history strategies in northern stocks. This is especially true given that estuaries and other coastal zones are often heavily impacted by anthropogenic stressors such as development and pollution, and connectivity with upper watersheds is often lost because of dewatering practices.

Materials and methods

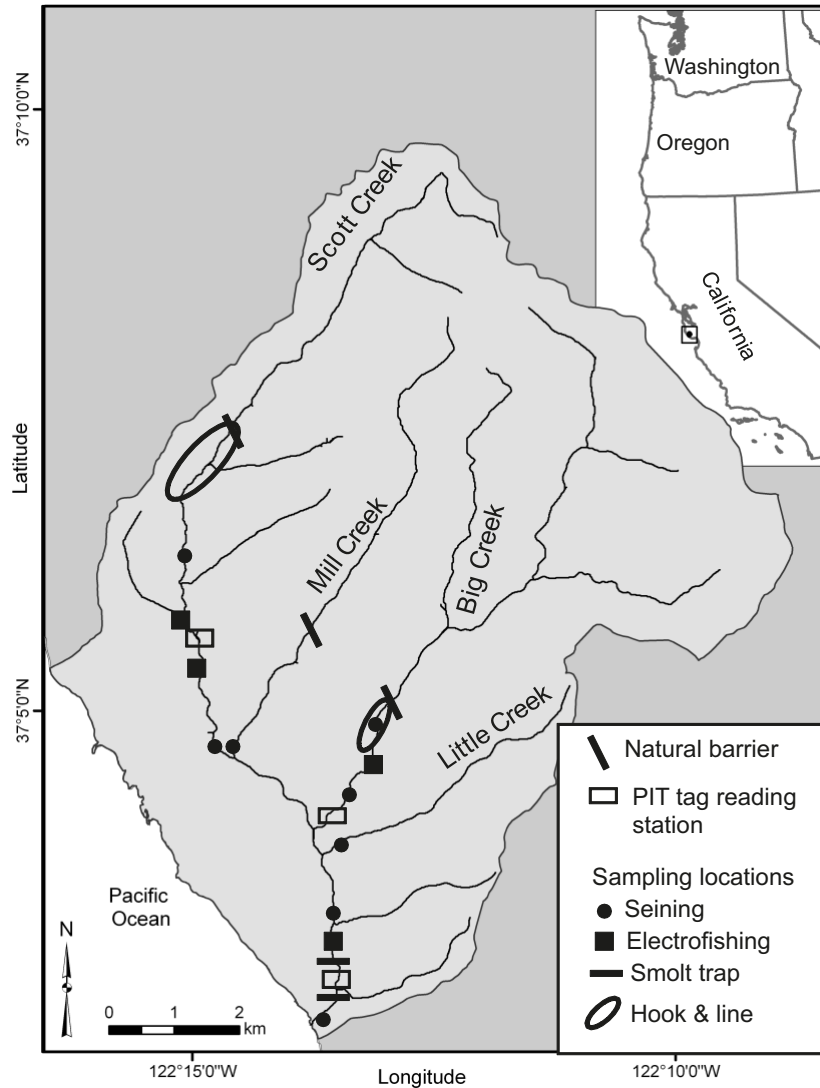
Study site

Scott Creek is a small coastal watershed, approximately 70 km² in area and 100 km south of San Francisco, California, USA (37°02'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 (Fig. 1). 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. A small conservation hatchery operates in the watershed and releases smolt-stage steelhead each spring. These fish are all marked by adipose fin clip and were excluded from this study.

Environmental measurements

Water temperature in the estuary was measured hourly using IB-Cod temperature loggers (Alpha Mach, Mont St-Hilaire, Quebec) from May 2002 to June 2003. These were replaced by two YSI 600 XLM data loggers (YSI Inc., Yellow Springs, Ohio), which recorded salinity, dissolved oxygen, and pH, from July 2003 to the present. To capture the maximum variation in parameters, one recorder was positioned on the bottom and the other floated just below the surface.

Fig. 1. Map of Scott Creek watershed (California, USA) showing locations where different sampling methods were used.



Fish sampling

Throughout the study fish were collected from three sites: in the upper watershed, at smolt traps on the main stem of Scott Creek, and in the estuary. These sites were sampled consistently between March 2002 and June 2008 (Fig. 1). Because of the physical differences of the sites and the availability of sampling equipment, multiple sampling methods were used. Fish in the upper watershed were sampled by seine or hook and line from May 2002 through December 2008. Beginning in 2006, electrofishing surveys were conducted three times per year in four 100 m stream reaches in the upper watershed. Smolt trapping was conducted from 2002 to 2005 by means of a two-chambered square mesh smolt trap with soft mesh wing walls extending to each bank. The trap was operated 3 days per week throughout the year except during exceptionally high flows associated with winter storms. From 2006 to 2008, the trap design was upgraded to a fixed-panel-and-pipe trap with a two-chamber live box and fished 7 days per week from January through July, flow permitting. All mesh spacing was ≤ 0.635 cm. Fish in the estuary (downstream of the smolt trap) were cap-

tured using a 30 m \times 2 m beach seine with 0.95 cm square mesh wings and a 0.635 cm square mesh bag.

Fish were handled according to the methods of Hayes et al. (2004). Briefly, all fish were measured for L_F (mm) and mass (g). Gill samples (about five to six filaments) were collected from approximately 10 fish > 100 mm L_F from each of the three locations each month through December 2004 to measure Na^+, K^+ -ATPase activity. 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 then placed in a recovery container for at least 10 min before release. As described in Hayes et al. (2008), growth rates were calculated for all recaptured PIT-tagged fish based on the change in measured fork length between capture events.

Na^+, K^+ -ATPase

We used McCormick's (1993) nonlethal method of sampling to minimize mortality of these fish, which are listed in the Endangered Species Act, to measure activity levels of the

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enzyme Na⁺,K⁺-ATPase (hereafter referred to as ATPase activity) as a proxy for assessing seawater readiness (Björnsson and Bradley 2007). Gill samples were put into individual vials containing SEI buffer (150 mmol sucrose·L⁻¹, 10 mmol EDTA·L⁻¹, 50 mmol imidazole·L⁻¹, pH 7.3) and placed on dry ice in the field, followed by storage at -80 °C until laboratory analysis within 3 months of sample collection. Specific ATPase activity was expressed as micromoles of ADP per milligram protein per hour ($\mu\text{mol ADP}\cdot\text{mg protein}^{-1}\cdot\text{h}^{-1}$) in accordance with McCormick's (1993) nonlethal micro method. The homogenate of each sample was assayed in triplicate to evaluate within-sample variance.

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 was located in the lower portion of the watershed at the head of the estuary and began operation in April 2004. Two additional readers were installed in December 2005: one on Big Creek and one on the Scott Creek main stem above the confluence with Mill Creek (Fig. 1).

Data analysis

Trapping effort and efficiency varied among years because of variable weather and flow conditions, staffing, and equipment resources and performance. As a result, downstream migrant capture rates were pooled across years and binned by month, with the total number of fish caught per month divided by the number of trapping days that occurred in that month across the course of the study to provide an estimate of the number of downstream migrants captured per daily trapping effort in a given month.

Owing to variable PIT tag reader detection efficiencies, as well as periods when readers and (or) traps were inoperable, all reported detection rates represent minimum estimates. For similar reasons, data were pooled across years and binned by month or day of year unless otherwise specified, and interannual variation was not evaluated because of the confounding effects of inconsistent and improving sampling methods.

We investigated the influence of size and timing of migration through the smolt trap on lagoon recruitment. This was done by assessing the number of fish in a given year that were PIT-tagged when captured at the trap and subsequently detected in the basin after lagoon closure, either by a PIT tag reader or by recapture in the estuary. Fish that were not resighted were assumed to have entered the ocean or died, although it is likely that some fish recruited but were not resighted. Fish that were tagged after lagoon closure in a given year were excluded from analysis.

Results

Downstream migration trends

Fish movement peaked in the spring, typically between the months of March and June. There was a general trend of larger fish being caught between December and March (with a dip in January) and smaller smolts captured during the rest of the year (Supplemental Fig. S1¹). Mean L_F for all down-

stream migrants measured during the course of the study was 110 mm (standard deviation (SD) \pm 39 mm, $n = 3848$). Overall there is a long tail in the length frequency of juvenile steelhead downstream migrants in this system and a wide range of size classes (Fig. 2a).

ATPase activity dynamics

A total of 695 gill samples were collected between March 2002 and December 2004 and successfully measured in the laboratory for ATPase activity, with coefficients of variation (CV) of <20% between replicate wells. ATPase activity levels were log-transformed to achieve normality and analyzed with a linear mixed model that included year as a random variable. ATPase activity levels differed significantly between locations ($F_{[2,681]} = 22.19$, $p < 0.001$) and at the seasonal time scale ($F_{[3,681]} = 21.095$, $p < 0.001$). No interaction was observed between season and location ($F_{[6,681]} = 0.50$, $p = 0.806$). Pairwise comparisons by location and season that proved significant are reported in Supplemental Table S1¹. Seasonal patterns of ATPase activity at the smolt trap and in the estuary are shown (Fig. 2b), with data binned by month regardless of year collected.

Fish sampled in the estuary during spring typically had elevated ATPase activity (Fig. 2b; Supplemental Table S1¹). ATPase activity was typically lower during the period of estuary closure (summer-fall), but it varied among years. As environmental variables were likely to influence this, multiple regression was used to compare ATPase activity of fish on the day they were sampled in the estuary with the 2-week running average of salinity and temperature leading up to that date. A significant effect was observed ($F_{[2,77]} = 41.121$, $p < 0.001$, adjusted multiple $R^2 = 0.516$), with salinity (standard coefficient (SC) = 0.672, $p < 0.001$) appearing to have a greater effect than temperature (SC = -0.279, $p = 0.001$) for the range of environmental conditions observed. Specifically, higher ATPase levels were associated with higher salinities, while lower ATPase levels were associated with higher temperatures.

Estuary recruitment trends

Timing of migration strongly influenced the probability of recruitment to the estuary (Fig. 2c), with later-migrating fish having a higher probability of recruiting to the lagoon ($t_{[345]} = 16.8$, $p < 0.0001$). A comparison of the size frequency distributions for initial tagging at the smolt trap of resighted fish with that of fish that were not resighted revealed a lack of larger fish recruiting to the estuary (Fig. 2a, Kolmogorov-Smirnov two-sample $p = 0.032$, $n = 2296$).

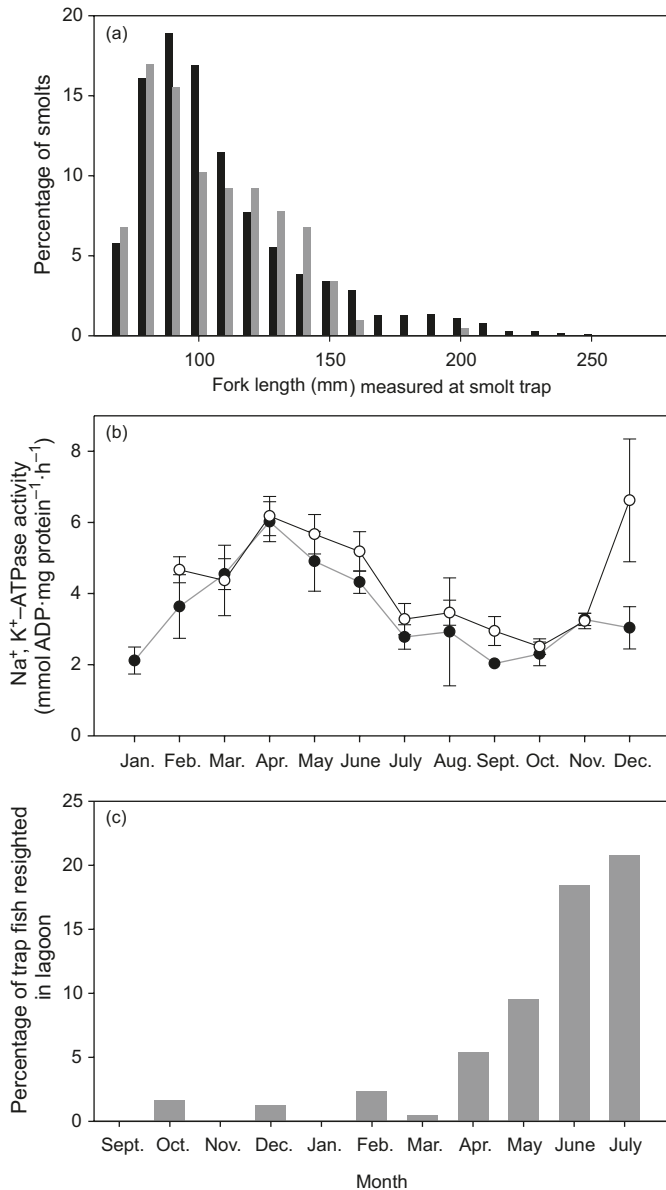
ATPase activity levels were measured for 107 fish that were PIT-tagged prior to estuary closure in 2003 and 2004. A comparison of fish that subsequently recruited to the estuary ($n = 15$) with fish that were not resighted ($n = 92$) found that fish that were confirmed recruiting to the estuary tended to have lower ATPase activity, were significantly shorter in L_F , and migrated later in the year (Table 1).

Post-tagging movement in freshwater (Do some fish smolt twice?)

Many estuary fish moved upstream from the lagoon in fall,

¹Supplementary data are available with the article through the journal Web site (<http://www.nrcresearchpress.com/cjfas>).

Fig. 2. Seasonal dynamics of early and late downstream migrants in a comparison of (a) size distributions of fish tagged at the smolt trap that either were (gray bars) or were not (black bars) resighted in the estuary after sandbar formation between 2003 and 2008; (b) Na⁺,K⁺-ATPase activity levels averaged by month (all years combined, ±1 standard error, SE) for fish sampled at the smolt trap (solid circles) and the estuary (open circles) in the lower watershed; and (c) the percentage of fish tagged at the smolt trap on a monthly basis that were later resighted in the estuary–lagoon after sandbar formation.



typically before winter storms increased flow sufficiently to breach the sandbar. During the course of this study (2002–2008), the mean bar breach date was 7 December, with a range of 14 November to 20 December. Of the fish tagged or resighted in the lagoon after bar closure each year ($n = 1236$), 45% were detected upstream at some point and 28% were detected upstream after bar breach. Upstream PIT tag reader detections of estuary fish peaked in November for the reader a short way above the estuary, often several weeks be-

fore sandbar breach. Detections peaked for the two readers in the upper watershed in January (Fig. 3a).

This upstream movement coincided with deteriorating water quality conditions in the estuary as fall progressed. To reduce noise in temperature and salinity measurements, the rolling 2-week average of each day from July 2003 to December 2008 was calculated and then averaged again by day of year across years. Lagoon salinity increased during fall, as salt water was pushed over the sandbar by large swells from storms in the North Pacific moving down the coast. Dissolved oxygen subsequently dropped, potentially caused by die-off of freshwater algae in response to increasing salinity. Note that while the moving average for dissolved oxygen of the lower sonde hovers around 5 mg·L⁻¹, there are many daily averages during mid-October through November between 0 and 1 mg·L⁻¹, before continuing to decline in dissolved oxygen and increase in salinity during December (Figs. 3b and 3c).

Many of the tagged fish detected migrating upstream spent several additional months in the upper watershed and were captured a second time at the smolt trap during the following spring. A comparison of recaptures at the smolt trap between fish initially tagged in the upper watershed versus the estuary during the previous season shows that downstream movement of estuary fish through the smolt trap peaked in March, while upper watershed fish movement peaked in April ($t_{146} = 4.8$, $p < 0.0001$, Fig. 4a) and that the estuary fish were significantly larger ($t_{147} = 12.7$, $p < 0.0001$, Fig. 4b). However, winter growth rates in the upper watershed for the two groups were not significantly different, with estuary fish having a slightly higher absolute growth rate of 0.22 ± 0.11 mm·day⁻¹ (mean ± SD, $n = 69$) than upstream fish (0.19 ± 0.11 mm·day⁻¹, $n = 94$). In addition, as estuary fish were typically longer to begin with, their specific growth rates (percent change in L_F ·day⁻¹) were lower (0.13 ± 0.08) than fish reared solely upstream (0.16 ± 0.19).

Estuary-reared fish captured at the smolt trap each spring are probably the source of almost all smolts greater than 190 mm in Scott Creek (Fig. 4b). The relative contribution of tagged fish detected at the smolt trap from the estuary versus those on their first migration from the upper watershed is difficult to determine because of varying trap and PIT tag reader detection efficiencies. However, roughly 4% of all upstream-tagged fish were recaptured at the smolt trap. In comparison, 6% of all estuary-tagged fish from the previous year were recaptured at the trap or detected by PIT tag readers the following year, suggesting the estuary is making a major contribution to the subsequent spring’s smolt run each year, particularly in the larger size classes (especially if one adjusts for annual trap efficiencies, which ranged from ~10% to 50%; S.A. Hayes, unpublished data).

ATPase activity appears to differ between fish on the “first” and “second” migration. Unfortunately, the ATPase activity data were collected prior (2002–2004) to acquisition of PIT-tag-based movement data, so the exact life history pathway of larger ATPase-sampled fish moving through the smolt trap is not documented for that time. However, when comparing ATPase activity of fish above and below the 150 mm L_F marine survival size threshold (Bond et al. 2008), it appears that larger fish, which are more likely to enter the ocean after leaving the smolt trap, have higher ATPase

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Table 1. Mean values \pm SD comparing several variables between downstream migrants sampled for Na^+, K^+ -ATPase activity ($\mu\text{mol ADP}\cdot\text{mg protein}^{-1}\cdot\text{h}^{-1}$) that recruited to the estuary for the summer versus fish that were not resighted.

	Estuary recruit	Not resighted	<i>t</i> score	<i>p</i> (two-tail)
Na^+, K^+ -ATPase activity	3.07 ± 2.09	4.09 ± 2.25	2.09	0.097
Fork length (mm)	130.20 ± 38.08	153.47 ± 41.63	2.09	0.037
Timing of migration (day of year)	149.87 ± 39.26	103.52 ± 51.89	2.07	<0.001

Fig. 3. Evidence that steelhead that recruit to the lagoon during summer months migrate back upstream in fall based on (a) monthly detections of estuary fish by instream PIT tag readers located upstream of the estuary in the upper (black bars) and lower watershed (gray bars — detections are limited to one detection per individual fish per day). Further evidence includes declining estuary water quality conditions for two sondes, one floating at the surface (gray line) and one mounted on the bottom (black line), based upon 2-week rolling averages of (b) daily salinity and (c) dissolved oxygen for the period of July 2003 through December 2008. Data are average by day of year across years to display general trends.

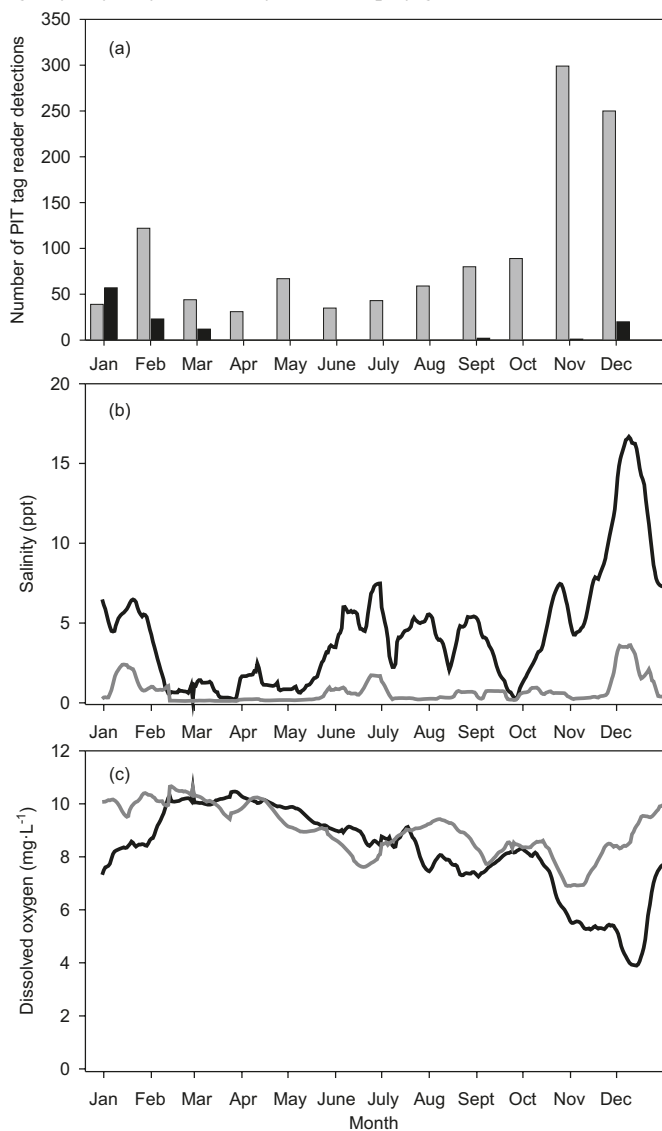
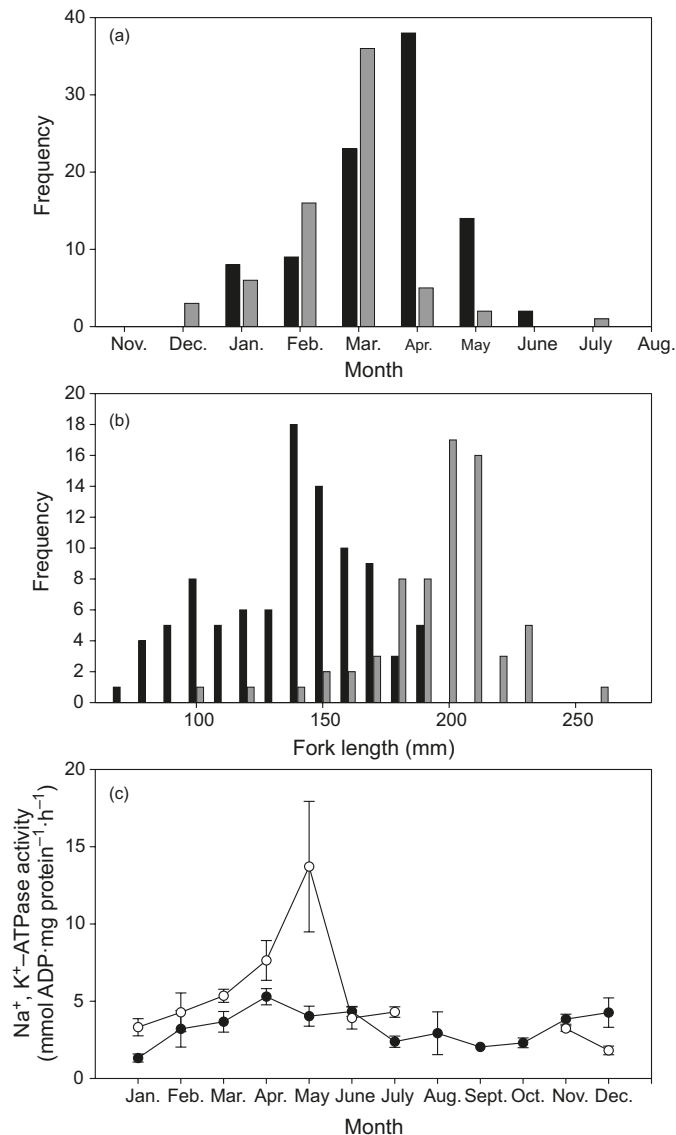


Fig. 4. (a) Fish that migrate upstream from the estuary in fall make a second downstream migration during the spring smolt outmigration period (gray bars), earlier than fish on their first migration (black bars) based on a comparison of the timing of downstream migration through the smolt trap or passive integrated transponder (PIT) tag reader by fish last seen or tagged in either upstream or estuarine habitat (during previous year). (b) Fish on their second migration are larger based on a comparison of the size frequency distribution of fish recaptured at the smolt trap that were last seen or tagged in either upstream (black bars) or estuarine habitat (gray bars). (c) Associated with that are the Na^+, K^+ -ATPase activity levels at the smolt trap for fish <150 mm (solid circles) or ≥ 150 mm (open circles) marine survival size threshold, indicating larger fish are more prepared for seawater entry.



levels (Fig. 4c; $t_{[120]} = 1.98$, $p = 0.001$) during the months of January through May. As 11 of the 37 fish in the larger size class were >190 mm L_F , it is likely that many of these fish were on their second migration and about to enter the ocean.

Discussion

The results of this study help to illustrate the extent of life history plasticity steelhead are capable of exhibiting to make use of available habitat. Downstream-migrating fish in Scott Creek demonstrate a wide range of size classes and degree of smoltification as indicated by Na^+, K^+ -ATPase activity levels. Larger fish (>150 mm) moved downstream during February and March and exited the stream, whereas fish moving during April through June were smaller and tended to recruit to the estuary. Many summer estuary recruits were observed retreating upstream into the watershed when estuarine water quality declined each fall and remained there until performing a second downstream migration several months later during subsequent spring, representing the largest smolts in the system. The observation of regular upstream migration by juvenile steelhead after entering the estuary but prior to ocean entry is rather unusual, with important ecological and evolutionary implications and important consequences for management.

Initial downstream migration and estuary habitat use

Larger fish migrated downstream earlier, typically in February and March, whereas smaller fish were observed migrating in April through June (Supplemental Fig. S1¹). These data are concordant with historical records from the neighboring watershed, Waddell Creek (Shapovalov and Taft 1954). It is now apparent that the larger fish are likely heading directly to sea, whereas the smaller fish are recruiting to the estuary. Corresponding differences in ATPase levels were observed in the present study, with the larger, earlier-moving fish having more elevated ATPase activity. A concurrent laboratory experiment conducted with fish from Scott Creek observed a positive relationship between length and probability of surviving a seawater challenge, indicating that larger fish from this stock have greater osmoregulatory capacity (Beakes et al. 2010).

Water temperatures at the smolt trap are typically between 7 and 13 °C during February and March, with daily highs reaching 17 °C by June. Warm temperatures have been shown to inhibit ATPase expression (Adams et al. 1975), with steelhead typically requiring temperatures < 13 °C to smolt (McCullough 1999; Myrick and Cech 2004). It is likely that smaller size and warmer temperatures contributed to the lower ATPase values observed in the later-migrating fish. However, the ATPase values of these later migrants were still elevated compared with upper watershed nonmigratory fish and suggest an increased level of seawater readiness. In a comparative study of coastal watersheds in central California with and without estuary–lagoon habitat, Hanson (2008) observed a lack of elevated ATPase activity in steelhead juveniles < 150 mm L_F from watersheds without estuaries, demonstrating a population level plasticity in the smoltification process that adjusts to individual watershed habitats.

Elevated ATPase levels observed in the late-migrating

smaller fish suggests a bet-hedging strategy for two reasons. The first is that the estuary–lagoon habitat is not always available, with lagoon formation delayed until late August or September during some years, leaving the fish with a stretch of shallow riffle habitat and limited cover. Few fish are observed recruiting to the estuary in late summer. While their fate is uncertain, it is possible one reason for elevated ATPase levels is they may go to sea when suitable estuarine habitat is not present. Alternatively, they may be consumed by predators before ocean entry (Hayes et al. 2008, 2011). The second reason for potential bet-hedging is occasional spring elevated salinity levels in the estuarine habitat when recruitment is occurring, creating a potential need for enhanced osmoregulatory ability. ATPase activity of fish in the estuary varied in response to environmental conditions, with a substantial drop in activity levels during summers with low salinity and warm temperatures. This is not surprising and has been observed in Atlantic salmon (*Salmo salar*) as well (McCormick et al. 1999).

In Atlantic salmon, the smolt life history pathway is size-related, with a threshold length required to begin the physiological transformation (McCormick et al. 1998). Steelhead in some regions probably have a similar size-based smolting threshold. However, the results of this study and that of Hanson (2008), who observed elevated ATPase levels in migrating fish as small as 70 mm L_F , indicate that steelhead can adapt to local habitat conditions and, under certain circumstances, decouple the minimum size threshold from elevated ATPase levels and migration behavior. Some readers may argue that these smaller fish were not true smolts. It is likely they were not, as the vast majority of downstream migrants in this study exhibited parr coloration and only began to silver after recruiting to the estuary (S.A. Hayes, personal observation), when ATPase levels are decreasing and growth is increasing (Hayes et al. 2008). It was not until these fish retreated upstream and made their second downstream migration that they showed additional signs of smoltification, including complete silverying, reduced condition factor, darkened fin tips, and the highest levels of ATPase activity. At the same time, some of the larger fish coming out of the upper watershed on their initial migration also demonstrated smolt characteristics, indicating that multiple life history pathways are present in this system. We conclude that steelhead have the plasticity to adjust behavioral and physiological processes typically associated with smoltification and spread these processes out in stages to take advantage of variable habitat availability and suitability. The decoupling of the smoltification process from actual ocean entry serves to further complicate the concept of a “smolt”, which was recently described as “a physiological state which continues to defy definition” (Björnsson and Bradley 2007).

Upstream migration and “smolting” twice

Historical studies from the Scott and Waddell creek watersheds during the 1930s and 1940s report almost identical size frequency distributions of downstream migrants as found in the present study, but concluded this was primarily the result of upstream rearing (Shapovalov and Taft 1954). The authors reported upstream movement of juvenile fish in Waddell Creek and suggested that there were probably growth advantages in the estuarine habitat, speculating on the processes

described in our study. Shapovalov and Taft (1954) also lament their inability to track the movements of individual fish through the steps of initial downstream migration, estuarine recruitment, fall upstream migration, and subsequent spring downstream migration. Upstream movement by juvenile salmonids in the parr stage is not uncommon, having been reported in both Atlantic salmon and coho salmon (*Oncorhynchus kisutch*) (Tschaplinski and Hartman 1983; Hutchings 1986; Koski 2009). What is unusual about the data reported here is that this upstream migration takes place after the parr–smolt transformation has begun. Upstream movement may simply be necessary to avoid poor habitat conditions in lagoons. While there are occasional saltwater intrusions during summer, there are typically low salinity levels and extensive freshwater algal production. Storm surges deposit seawater over the sandbar each fall, and the lagoon habitat generally becomes anoxic when freshwater algae dies and reduces oxygen levels in the estuary as it biodegrades. More frequent saltwater intrusions in fall and early winter may make the estuary unsuited to the physiological state of juveniles.

The life history strategy and physiology of “smolting twice” does not appear to have been previously documented in a wild population of salmonids. Atlantic salmon held under laboratory conditions have demonstrated the ability to regress when prevented from migrating and then go through the smoltification process a second time 1 year later (Shrimpton et al. 2000). From a physiological perspective, it is not surprising for an iteroparous anadromous salmonid to undergo this process more than one time. The phenomenon may increase in frequency as one moves south through the steelhead range, where some stocks in southern California may not have access to the ocean for 1 or more years because of dry conditions. In a simple sense, these steelhead have adapted to take advantage of optimal rearing habitat, somewhat like sockeye salmon or Atlantic salmon using lakes for rearing habitat (Hutchings 1986; Quinn 2005). Because of limited watershed size and location of this rearing habitat, they have developed a “circular” migration pattern, moving down and up in the watershed to make maximal use of available habitat. In a recent review, Koski (2009) identifies that coho salmon “nomads”, a life history type previously thought of as displaced fry and parr that do not survive, are actually exhibiting similar movement behaviors in many populations across their range.

What remains a mystery is why estuary fish remain in the upper watershed for so long after upstream migration. Most fish are well over the 150 mm marine survival threshold, and the longest distance they could travel is only about 10 km in this watershed, which they could traverse in a night or two. Yet many delay their actual ocean entry for 2–3 months after the onset of winter storms and the reconnection of the stream with the ocean. Growth was moderate during this time, and the added size may outweigh mortality risks during those months. In addition, with the primary cue for smoltification being photoperiod (Björnsson and Bradley 2007), it may simply be a mechanistic timing issue for these fish to complete the “resmolting” process. Alternatively, this may be indicative of some critical time window for seawater entry to begin their ocean migration. Little is known about steelhead ocean migratory behavior, other than that

they have the largest smolt size requirements of any Pacific salmonid (Ward et al. 1989; Groot and Margolis 1991; Bond et al. 2008), are rarely observed in the California Current ecosystem, and are typically found only in a band along the 50°N parallel (Welch et al. 1998). All of this suggests a major ocean migration that may be coupled in a critical way to synchronizing movements with optimal current patterns. Recent work on the Scott Creek population using archival tags identified a very narrow ocean thermal habitat window and suggests that central California steelhead have the same migratory destination in the North Pacific as more northern populations and are likely to feed in the California Current while transiting north, timing their outmigration from central California to narrow temperature windows between February and April (Hayes et al. 2011).

Our results emphasize the importance of protecting estuarine habitat for steelhead rearing in coastal watersheds. The largest smolts migrating downstream each spring in Scott Creek had previously reared in the estuary and were not products of upstream habitats alone. Despite the intact and arguably high quality of stream habitat within Scott Creek, most of its steelhead production comes from juveniles using the estuary for considerable growth, highlighting the fact that the rearing capacity of these coastal watersheds is limited even under the best of circumstances. Finally, this study highlights the critical need to provide adequate flow in what is a fairly dry Mediterranean climate to maintain connectivity between lagoon and upstream habitats. It is likely that even the most naturally functioning coastal lagoons have seasons when the lagoon habitat becomes degraded. In such cases it is necessary to maintain an exit corridor for steelhead to retreat upstream, away from deteriorating water quality.

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