

Salinity Inhabited by Age-0 Splittail (*Pogonichthys macrolepidotus*) as Determined by Direct Field Observation and Retrospective Analyses with Otolith Chemistry

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ABSTRACT

Splittail (*Pogonichthys macrolepidotus*) is a fish species of special concern that is endemic to the San Francisco Estuary. It has been generally accepted that spawning and juvenile rearing occurs during spring in freshwater habitats upstream of the estuary. However, the recent discovery of a genetically distinct population of splittail in the relatively brackish Petaluma and Napa rivers has challenged this assumption. We used a combination of field observations and high resolution sampling of otolith $^{87}\text{Sr}:^{86}\text{Sr}$ ratios to identify the salinity inhabited by young age-0 splittail in the Sacramento, San Joaquin, Napa, and Petaluma rivers. Individual age-0 splittail, two to three months old, were observed in the Napa and Petaluma rivers in salinity as high as 8.5 ppt and 14.1 ppt, respectively, whereas salinity in the San Joaquin and Sacramento rivers was always <1.0 ppt. Otolith $^{87}\text{Sr}:^{86}\text{Sr}$ ratios corresponding to the first month of life suggested that individual splittail in all regions mostly inhabited freshwater, although sev-

eral individuals from the Napa and Petaluma rivers inhabited brackish water up to about 10 ppt. In most instances, there was little intra-individual variability in $^{87}\text{Sr}:^{86}\text{Sr}$ signals, suggesting individuals remained within the natal salinity zone during the first month of life. The exceptions were two fish, one each from the Napa and Petaluma rivers, that appeared to move from freshwater natal to brackish rearing habitats. The apparent ability of age-0 splittail to rear in brackish water almost immediately after being born is one of the fundamental mechanisms supporting splittail production in the Napa and Petaluma rivers.

KEYWORDS

Splittail, *Pogonichthys macrolepidotus*, Cyprinidae, minnow, native fish, San Francisco estuary, Sr isotope ratios, ICP-MS

INTRODUCTION

The question: "How do populations persist in variable environments?" remains a major focus of ecological research. Life-history theory tells us that broad environmental tolerances, behavioral plasticity, or a combination of both enable organisms to cope with various stressors. Information on how early life history traits vary along environmental gradients can

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reveal mechanisms of adaptation for fishes living in dynamic environments such as estuaries and floodplains. To persist, fish species living in such environments must be able to successfully spawn and recruit new individuals to the population in the face of highly variable and uncertain habitat conditions.

The splittail (*Pogonichthys macrolepidotus*) is a native fish species of great concern in the San Francisco Estuary and its watershed (Moyle 2002). Once listed as a threatened species under the Federal Endangered Species Act, it is now considered a “Species of Special Concern” by the U.S. Fish and Wildlife Service, the California Department of Fish and Game, and the CALFED Bay-Delta Program (Sommer and others 2007). Adult splittail generally live in brackish habitats of the upper San Francisco Estuary. During winter and spring flow events the adults migrate to upstream inundated floodplains and river margins for spawning. Larvae and juveniles remain upstream in inundated habitats until they begin to dry and then move downstream to tidal freshwater and brackish portions of the estuary during early summer (Feyrer and others 2005). Peak emigration of age-0 splittail from Yolo Bypass, the primary floodplain of the Sacramento River, varies temporally among years but typically occurs when fish are 30 to 40 mm in length, suggesting an ontogenetic influence on downstream emigration (Feyrer and others 2006). Juveniles will rear in the estuary one to two years, until they become sexually mature, and then initiate their spawning migrations (Daniels and Moyle 1983).

It has recently been discovered that there are two genetically distinct populations of splittail, one represented by fish spawning in the Central Valley and the other by fish spawning in the Napa and Petaluma rivers (Baerwald and others 2007). Whereas the Central Valley population has continuous access to freshwater habitats for spawning and rearing, access for the Petaluma/Napa population may be limited to years when flows are sufficient to freshen the relatively higher salinities of these rivers. This variability in spawning and rearing habitat could potentially affect the productivity and persistence of splittail in the Petaluma and Napa rivers. Understanding how splittail respond to this habitat variability is funda-

mental to understanding the population dynamics of the species.

Both direct observation of fish in the field and retrospective approaches can be used to determine salinity inhabited by individual splittail. One relatively new and rapidly growing tool for retrospective analysis is otolith chemistry (Campana 1999; Campana and Thorrold 2001; Elsdon and others 2008). We previously demonstrated that otolith chemistry effectively discriminates splittail stock structure at a resolution greater than possible by current genetic methods (Feyrer and others 2007a). Here, we build on that work and use the strontium (Sr) isotope ratio technique ($^{87}\text{Sr}:^{86}\text{Sr}$) in otoliths to retrospectively describe salinity inhabited during the first month of life. Otolith strontium isotope chemistry directly reflects ambient water ratios and varies across the freshwater-ocean environment according to conservative isotope mixing properties (e.g., Kennedy 2000, 2002; Faure and Mensing 2005; Hobbs and others 2005; Barnett-Johnson and others 2008). Because strontium isotopes are permanently stored in the otolith and not reworked or re-absorbed, this information can be obtained from fish of any age and used to infer relationships of early life history on recruitment patterns for adult fish.

The goal of our study was to identify the salinity inhabited by age-0 splittail. Our approach was two-fold: (1) direct observation of the salinity inhabited by two to three month-old splittail in the field, and (2) retrospective estimation of salinity inhabited during the first month of life using $^{87}\text{Sr}:^{86}\text{Sr}$ ratios deposited in otoliths. For the first approach, the samples represented the earliest life stage that could be reliably sampled.

METHODS

Age-0 splittail were collected from four primary spawning rivers in the San Francisco Estuary: the Sacramento, San Joaquin, Napa, and Petaluma rivers (Figure 1). Sampling for splittail was conducted May to June with beach seines during a two-year (2002–2003) field study that examined age-0 distribution across the full range of the species (Feyrer and others

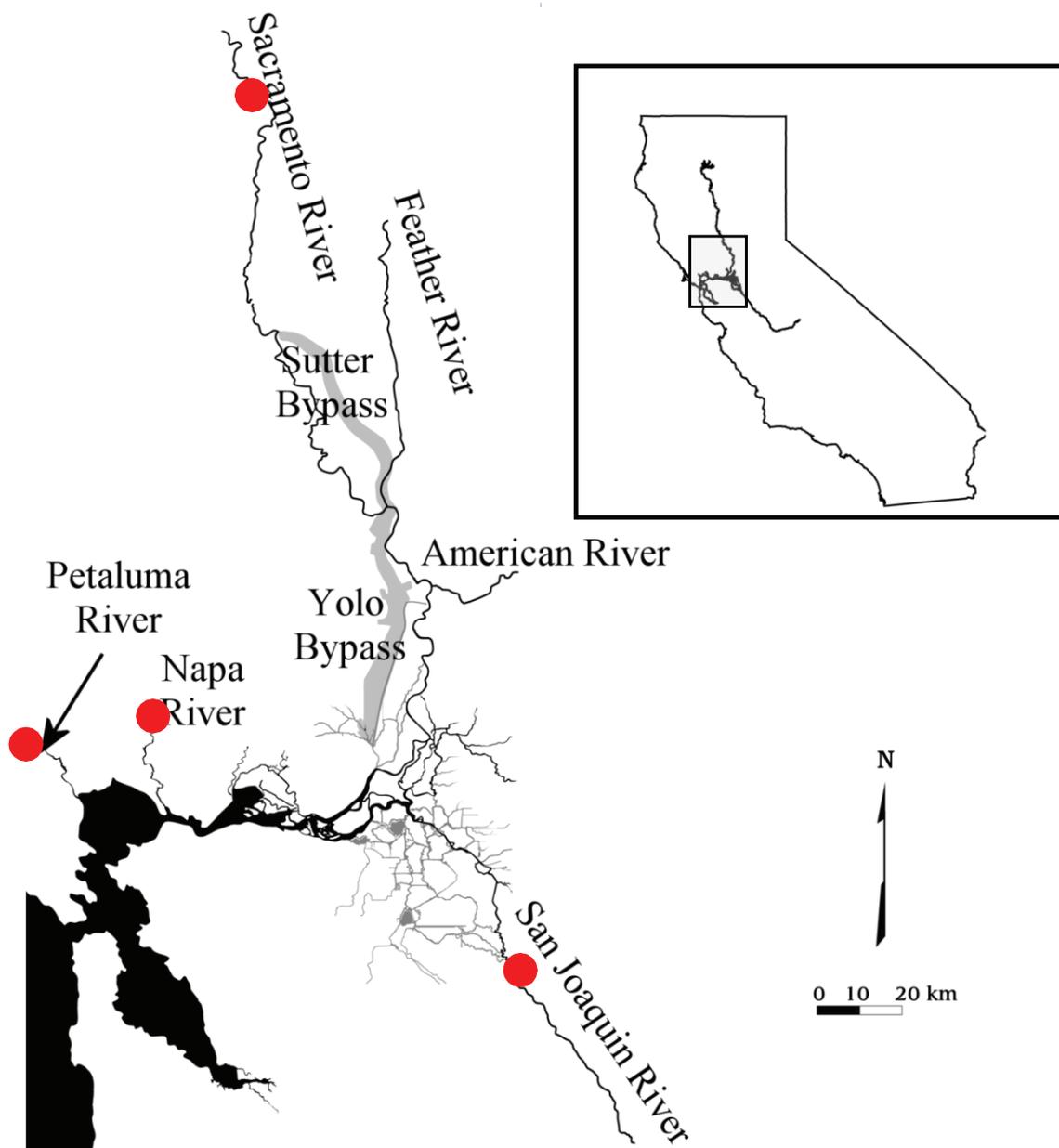


Figure 1 Map of San Francisco Estuary highlighting the approximate locations on the Sacramento, San Joaquin, Napa, and Petaluma rivers where age-0 splittail were collected

2005). Overall, there were over 2,000 individual splittail collected in 181 beach seine hauls taken during daylight hours. Further details on sampling methods, as well as results on spatio-temporal distribution and life history traits, are available in Feyrer and others (2005, 2007b). For our direct field approach, we documented observations of age-0 splittail across the salinity distribution sampled during our two-year

study. The number of samples examined across the rivers was 90, 47, 19, and 25 for the Sacramento, San Joaquin, Napa, and Petaluma rivers, respectively. We used box plots to summarize the range of salinity values observed during field sampling, and for the subset of salinity values when splittail were collected. We used a one-way analysis of variance (ANOVA) with salinity as the response variable and river as the

treatment to test for statistically significant differences. We conducted the ANOVA on all samples, and then a second time for the subset in which splittail were present. Tukey's multiple comparison tests were used to determine differences among locations.

For our second approach, retrospective evaluation of salinities occupied during the first month, we examined the otoliths from a subset of 34 individuals collected during the field study (Table 1). These individuals were collected on the Sacramento River near Butte City, the San Joaquin River near Mossdale Crossing, the Napa River near Kennedy Park (just downstream of the city of Napa), and the Petaluma River just downstream of the city of Petaluma. We deemed these particular individuals suitable for analysis because the sample locations, size, and age of the fish ensured that they were collected from their natal tributaries and did not move among drainages or sampling locations. We generally followed the otolith chemistry methods we used previously for age-0 splittail (Feyrer and others 2007a). Lapilli otoliths were extracted from all fish with acid-washed utensils and rinsed briefly with 10% nitric acid to remove any attached organic tissue. They were then rinsed in deionized water and allowed to air dry. Dry otoliths were individually mounted on glass microscope slides in CrystalBond mounting media, polished to the core with 0.3- μm lapping film, triple rinsed with 5% nitric acid, triple rinsed with milliQ water, and allowed to air dry in a class 100 laminar flow hood.

Strontium isotopic compositions $^{87}\text{Sr}:^{86}\text{Sr}$ were determined with multi-collector LA-ICPMS (Nu Plasma HR

Table 1 Sample size (N) and the mean age (days) and length (mm fork length) of individual splittail used in analysis of $^{87}\text{Sr}:^{86}\text{Sr}$ along otolith transects. Age and length values are estimates that correspond to the endpoint of the otolith chemistry sampling and were generated by the biological intercept method.

River	N	Age	Length
Napa	10	27	25
Petaluma	7	24	24
Sacramento	7	28	26
San Joaquin	10	27	25

from Nu Instrument, Inc.) interfaced with a Nd:YAG 213 nm laser (New Wave Research UP213). Transects across the otolith surface from the core to a distance of 200 μm , corresponding to approximately the first month of life, were ablated using a laser beam size of 60 μm (width), 100% laser power, and 10 Hz repetition rate. Crater depths were approximately 200 to 500 μm (Jones and Chen 2003). Helium was used as a carrier gas in the laser ablation cell to maximize sensitivity and minimize sample deposition at the ablation site. Gas blank and background signals were monitored until ^{84}Kr and ^{86}Kr decayed and stabilized after the sample change (i.e., exposing sample cell to the air) and were measured for 30 seconds. The laser was then turned on, typically for 30 to 60 seconds. Background signals were subtracted from the measured signals automatically. The $^{86}\text{Sr}:^{88}\text{Sr}$ ratio of 0.1194 was used to correct for instrumental fractionation in accordance with exponential law. Peak intensities for ^{88}Sr , ^{87}Sr , ^{86}Sr , ^{85}Rb , and ^{84}Sr were measured simultaneously. Peak ^{85}Rb was monitored to correct for any ^{87}Rb interference on ^{87}Sr , which was negligible. The NIST Sr isotope standard SRM 987 was measured consistently during the course of this study, giving $^{87}\text{Sr}:^{86}\text{Sr} = 0.710253 \pm 9$, compared with the certified value of 0.710258. A laser ablation standard produced $^{87}\text{Sr}:^{86}\text{Sr} = 0.709177 \pm 8$, which is the known seawater value (Brass and Turekian 1974; Faure and Mensing 2005).

The $^{87}\text{Sr}:^{86}\text{Sr}$ data were plotted against distance from the core of the otolith for individual fish for each river and visually examined for any patterns in the variability of $^{87}\text{Sr}:^{86}\text{Sr}$ values. We tested for differences in $^{87}\text{Sr}:^{86}\text{Sr}$ with a repeated measures analysis of variance that had river (*R*) and distance from otolith core (*D*) as fixed factors and individual fish (*F*) as random subjects. We did the analysis in the Minitab software program with the model expression: $F(R) R D R^*D$.

We counted otolith increments, following Feyrer and others (2004), to determine the ages of individual fish and to ensure we characterized only the first month of life with the otolith chemistry. Otoliths were individually mounted on glass microscope slides in thermoplastic resin (CrystalBond, Aremco Products, Ossining, New York). They were polished on one side

with 0.3- μm lapping film to expose a sagittal plane. Digital images of the polished otoliths were taken with a CoolSNAP-Pro cf monochrome camera coupled with a Nikon E400 microscope. The total number of daily growth increments was determined from the digital images with the aid of image analysis software (Image Pro Plus v4.5, Media Cybernetics, Inc.). We used the biological intercept method (Campana 1990) to estimate the age and length of individual fish at the endpoint of $^{87}\text{Sr}:$ ^{86}Sr sampling on otolith transects. Average age estimates at this endpoint ranged across rivers from 24 to 28 days and fork lengths ranged from 24 to 26 mm (Table 1).

To assign salinity values to individuals based on otolith $^{87}\text{Sr}:$ ^{86}Sr we assumed that otolith $^{87}\text{Sr}:$ ^{86}Sr ratios reflect those of the water. Although the application of otolith chemistry as a tool to identify ambient salinity is long standing (Secor and others 1995; Limburg 2001; Lamson and others 2006), the relationship between otolith chemistry and water salinity has not been studied for splittail. Such a relationship has been demonstrated for other species (Kennedy and others 2000, 2002; Barnett-Johnson and others 2008). Further, ambient water was determined to account for 83% of otolith Sr in marine species and 88% for freshwater species (Farrell and Campana 1996; Walther and Thorrold 2006). To this end, we collected water samples in May and June of 2007 to establish a relationship between $^{87}\text{Sr}:$ ^{86}Sr and salinity, and used that relationship to characterize salinity inhabited by splittail based upon the assumption that otolith $^{87}\text{Sr}:$ ^{86}Sr generally reflected that of the water (Kennedy and others 2000). Water samples were collected from four sites on the Sacramento River, two sites on the San Joaquin River, six sites on the Napa River, five sites on the Petaluma River, and at Muir Beach in the Pacific Ocean. The samples were collected in acid-rinsed polypropylene centrifuge tubs, filtered through 45-micron nylon filters, and fixed in 1M nitric acid in the field. Salinity and temperature were measured in the field at the time of water collections with a calibrated handheld YSI 90. Strontium in water samples was purified using a micro-column chromatography technique ("Spec resin," Eichrom Inc.) to remove Rubidium. Purified strontium samples were analyzed in solution phase on the multi-collector ICP-MS for strontium isotopes. Strontium con-

centrations were quantified through liquid aspiration onto the plasma in an Agilent Technologies quadrupole mass spectrometer. We plotted salinity against $^{87}\text{Sr}:$ ^{86}Sr and fit a curve through the data with the assistance of DataFit version 9.0.59 software (Oakdale Engineering, Oakdale, PA, USA) (Figure 2). We allowed the program to select the best fitting model based on minimum residual sum of squares from 298 possible solutions.

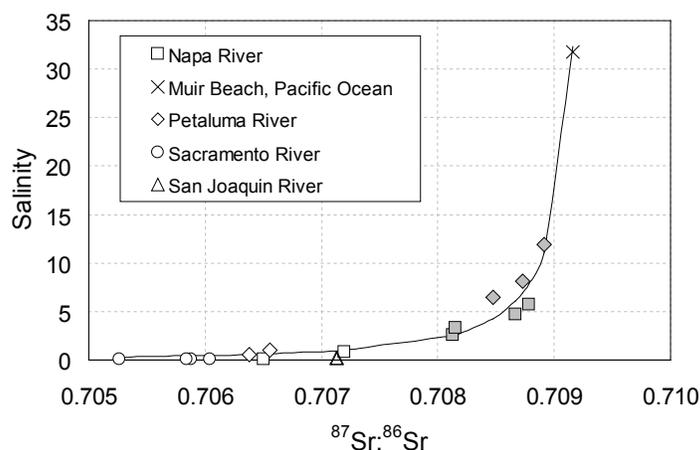


Figure 2 Plot of $^{87}\text{Sr}:$ ^{86}Sr versus salinity for water samples. The curve is defined by $y = 0.000013/(1+2.82*x+1.98*x^2)$ ($r^2 = 0.98$, $P < 0.001$). We characterized the open symbols as a freshwater group and the filled symbols as a brackish group.

RESULTS

Field Observations

Salinity values encountered during the field sampling were significantly different across rivers ($P < 0.001$; $F = 3173$, 177). Salinity in the Napa River was higher than in the Sacramento or San Joaquin rivers, and salinity in the Petaluma River was higher than in the Napa River (Figure 3). While salinity was always below 1.0 ppt in the Sacramento and San Joaquin rivers, it ranged from 0.0 to 8.5 ppt in the Napa River and 5.5 to 14.1 ppt in the Petaluma River. The salinity of the subset of samples in which splittail were observed followed a nearly identical pattern (Figure 3; $P < 0.001$; $F = 2393$, 129).

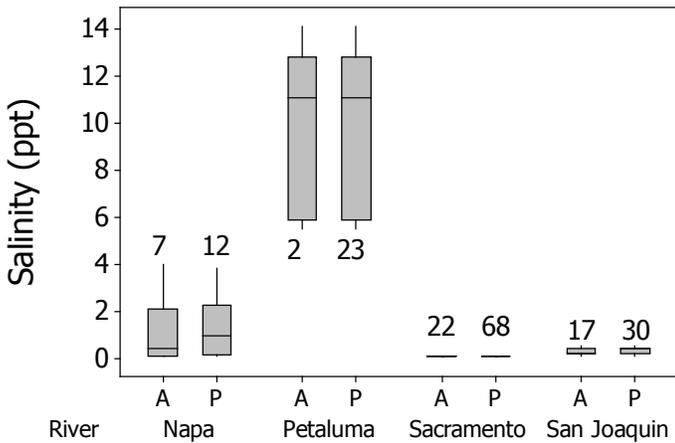


Figure 3 Box plots of salinity (ppt) for all samples (A) and the subset of those in which age-0 splittail were present (P) in four major spawning rivers during May and June 2002 and 2003. Numbers represent sample sizes. The box plots show medians and first and third quartiles. Whiskers show the highest or lowest values in the upper or lower limits, respectively.

Otolith ⁸⁷Sr:⁸⁶Sr

There was a statistically significant difference in ⁸⁷Sr:⁸⁶Sr across individuals and rivers, but not across distance from otolith core, nor the river × distance from otolith core interaction (Table 2). Individuals from the Sacramento River exhibited ⁸⁷Sr:⁸⁶Sr values between 0.7038 and 0.7058, the San Joaquin River between 0.7062 and 0.7078, the Napa River between 0.7035 and 0.7089, and the Petaluma River between 0.7061 and 0.7089 (Figure 4). With the exception of one individual from the Napa River and one from the Petaluma River, there was little intra-individual variation in ⁸⁷Sr:⁸⁶Sr values (Figure 4). The ⁸⁷Sr:⁸⁶Sr

values for the Napa River exception were near 0.708 during the first third of the time series, declined to near 0.704 midway through the time series, and then increased to near 0.706 during the last third of the time series. The ⁸⁷Sr:⁸⁶Sr values for the Petaluma River exception held steady near 0.706 during the first half of the time series and then gradually increased to above 0.708.

Salinity Estimated from ⁸⁷Sr:⁸⁶Sr

There was a statistically significant relationship between salinity and ⁸⁷Sr:⁸⁶Sr in the water samples as described by the equation $y = 0.000013 / (1 + -2.82 * x + 1.98 * x^2)$ ($r^2 = 0.98, P < 0.001$, residual sum of squares = 16.7) (Figure 2). The sample taken from Muir Beach on the Pacific Ocean represented the marine habitat category with a ⁸⁷Sr:⁸⁶Sr value of 0.70916 and salinity of 31.8 ppt. The remaining samples generally appeared to cluster into two primary groups: a freshwater group (<1.0 ppt) with ⁸⁷Sr:⁸⁶Sr values below 0.708 and a brackish group (3.0 to 12.0 ppt) with ⁸⁷Sr:⁸⁶Sr values between 0.708 and 0.709.

Considering both the generally conservative categorization of the water samples (in terms of fresh or brackish), and the more specific method of applying the regression equation relating salinity to ⁸⁷Sr:⁸⁶Sr of the water samples, most individual splittail probably occupied freshwater habitats during the period of time characterized by the ⁸⁷Sr:⁸⁶Sr otolith sampling (Figure 4). However, the data suggested that some individuals from both the Petaluma and Napa rivers probably occupied brackish water habitats, possibly up to about 10 ppt (Figure 4). The data also

Table 2 Results table for the repeated measures analysis of variance in which river and distance from otolith core were fixed factors and individual fish were random subjects

Source	DF	SS	MS	F	P
Individual (river)	41	0.0003	0.000008	69	<0.001
River	3	0.0005	0.0002	22	<0.001
Distance	20	0.000003	0.0000002	1.45	0.09
River × distance	60	0.000005	0.0000001	0.7	0.9
Error	820	0.00009	0.0000001		

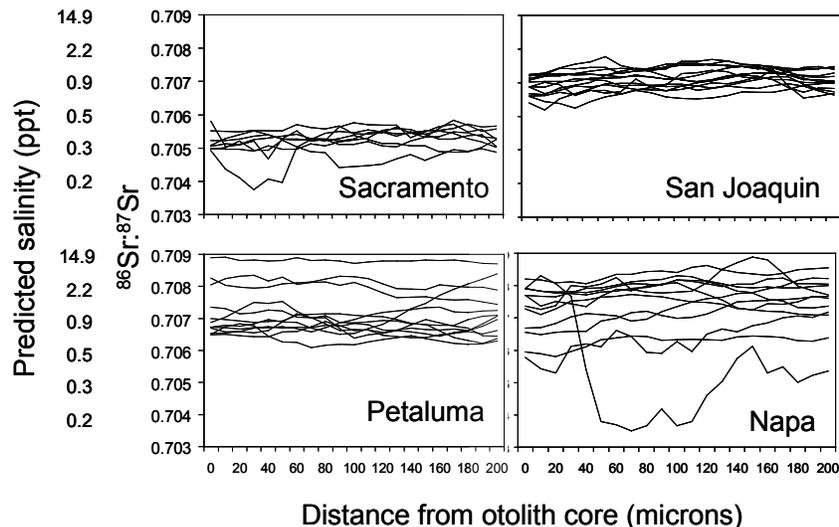


Figure 4 Records of $^{87}\text{Sr}:^{86}\text{Sr}$ in the otoliths of individual age-0 splittail from four major spawning rivers. The salinity scale is provided for reference and was generated from the equation that describes the curve in Figure 2.

suggested that most individuals did not make appreciable movements between freshwater and brackish water habitats. The only exceptions were those noted above in which a Petaluma River individual appeared to have gradually moved to an increasingly higher salinity over a period of days and a Napa River individual may have moved back and forth between freshwater and brackish habitats.

DISCUSSION

Our results demonstrating splittail rearing in habitats of variable salinity provide new insight into the ecology of this special status species. The general conceptual model of splittail early life history (Moyle and others 2004) has been based upon the Central Valley population in which spawning and rearing take place in freshwater habitats upstream of the estuary. However, there is substantially less freshwater habitat available to the Petaluma and Napa river population. Our results suggest that splittail will utilize freshwater habitat in this region if it is available, but that they are also capable of using brackish habitats. Our field observations of age-0 individuals rearing in brackish habitats corroborate laboratory tests that have demonstrated salinity tolerances (loss of equilibrium) up

to 22 ppt (Young and Cech 1996).

The ability of splittail to live in brackish habitats for an extended period of time early in life provides one of the mechanisms supporting the persistence of a genetically distinct population in the Napa and Petaluma rivers. However, Young and Cech (1996) demonstrate that splittail do not tolerate (loss of equilibrium) salinities >22 ppt, which often occur between the outlet of these two tributaries in San Pablo Bay and the upstream population of splittail in Suisun Bay and the delta. Hence, we propose that seawater creates an isolating barrier between the two populations of splittail. This barrier may be “broken” during high flow periods when salinities drop throughout the upper estuary, but the typical geographical separation may also be sufficient to maintain population structuring.

A major assumption of our study is that otolith $^{87}\text{Sr}:^{86}\text{Sr}$ reflects water $^{87}\text{Sr}:^{86}\text{Sr}$. Unlike previous studies utilizing Sr concentration to reflect salinity history, strontium isotope ratios ($^{87}\text{Sr}:^{86}\text{Sr}$) are not physiologically regulated and can therefore provide a precise estimate of salinity exposure (Kennedy and others 2000). Additionally, the relative stability of $^{87}\text{Sr}:^{86}\text{Sr}$ values within individuals, and that indi-

viduals were assayed over the exact same life stage, suggests there were no ontogenetic effects on otolith chemistry (e.g., Toole and others 1993; Fowler and others 1995). The regression equation for the relationship between $^{87}\text{Sr}:$ ^{86}Sr and salinity for the water samples provided us the opportunity to retrospectively assign specific salinity values to individuals under the assumption that otolith $^{87}\text{Sr}:$ ^{86}Sr reflects water $^{87}\text{Sr}:$ ^{86}Sr . However, research on other species suggests that perhaps only relatively broad habitat categories such as freshwater, brackish, or marine can be realistically discriminated (Secor et al 1995; Zimmerman 2005). Indeed, our water samples appeared to cluster into freshwater and brackish groups (Figure 2). We provided a corresponding salinity scale for the otolith $^{87}\text{Sr}:$ ^{86}Sr data in Figure 4, but recognize that broad freshwater or brackish habitat assignments are probably most appropriate given the limitations of the methods. The data support classifying individuals to either freshwater (<1 ppt) or brackish habitats (3 to 12 ppt), which is generally consistent with the direct observations of fish in the field.

Information about variability in habitat use and its effect on individuals can help identify factors affecting population dynamics. Our results have demonstrated that individual splittail born in the Petaluma and Napa rivers must inhabit elevated salinity much sooner in life than those born in the Sacramento and San Joaquin rivers. Likewise, these individuals presumably live in higher salinity habitats most of their lives compared to the Central Valley population. The bioenergetic consequences of this may potentially affect life history traits that can ultimately influence population dynamics. Because $^{87}\text{Sr}:$ ^{86}Sr is permanently recorded in the otolith, the effect of habitat salinity can be examined at any life stage and incorporated into future studies and models. Thus, otolith $^{87}\text{Sr}:$ ^{86}Sr is a promising tool to better understand the effects of habitat variability on splittail population dynamics.

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