

## Genetic analysis reveals two distinct Sacramento splittail (*Pogonichthys macrolepidotus*) populations

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**Abstract** The Sacramento splittail is an endemic cyprinid fish of the San Francisco estuary and its tributaries, which is a highly manipulated, constantly changing ecosystem. Splittail is the only extant member of its genus and is listed as a federal and California Species of Special Concern due to uncertainties regarding long-term abundance trends. Determining population structure for splittail is important because unique populations may contain different adaptive genetic variation, which can allow one population to persist through future environmental or demographic stochasticity while others become extirpated. To assess splittail population structure, 13 microsatellite markers were used to genotype 489 young-of-year splittail from five major rivers draining into the estuary: Cosumnes, Napa, Petaluma, Sacramento, and San Joaquin Rivers. Two genetically distinct populations were found to exist within our study region; one largely comprised of splittail collected from the Petaluma and Napa Rivers and the second comprised of splittail collected from tributaries in California's Central Valley: Cosumnes, Sacramento, and San Joaquin Rivers. These results were replicated in two consecutive years with both distance and model-based algorithms. The genetic distinction between these two populations appears

correlated with salinity differences between migratory regions and spawning grounds. Splittail from the Petaluma River exhibited a significantly higher degree of differentiation from the Central Valley population than did Napa River splittail. Our results suggest ongoing monitoring programs are probably highly biased towards sampling splittail from the Central Valley population. Understanding population dynamics of splittail could be improved if monitoring programs were expanded to include all splittail populations.

**Keywords** Cyprinidae · Microsatellites · Population genetics · Sacramento splittail · San Francisco estuary

### Introduction

The San Francisco Estuary is the largest estuary on the western coast of the United States and serves as vital habitat for at least 750 plant and animal species. This estuary provides drinking water for two-thirds of California and supports California's \$28 billion agriculture industry with irrigation water. The ever-increasing demand for water by California's rapidly expanding population has caused the overall health of the aquatic ecosystem to decline. Alterations to the local environment include dams, water diversions, nonnative species introductions, and contaminants. Many of the region's native flora and fauna have experienced dramatic decreases in population abundance and are at risk for extinction.

The freshwater fishes of the family Cyprinidae are highly diverse, with more than 250 species found in North America (Moyle 2002). A large number of genetic studies have been conducted on wild cyprinid species throughout the world using a variety of

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molecular markers. Recent studies have identified population structure (e.g., Mesquita et al. 2001; Tibbets et al. 2001; Mock and Miller 2005), evolutionarily significant units (ESUs) and management units (MUs) (e.g., Hedrick et al. 2001; Salgueiro et al. 2003), new species (e.g., Tang et al. 2004), and phylogenetic relationships (e.g., Ketmaier et al. 2004; Perdices et al. 2003; Stefani et al. 2004).

The San Francisco Estuary and associated tributaries support eight native cyprinid species, including the endemic Sacramento splittail (*Pogonichthys macrolepidotus*), commonly referred to as splittail. Splittail are large-sized cyprinids (>400 mm standard length) that live 5–8 years (Moyle 2002). They are easily recognized from other cyprinids by the enlarged dorsal lobe of their caudal fin, which appears to be split (hence the common name splittail) and are the only extant member of their genus in the world.

Over the last two decades, splittail have exhibited dramatic fluctuations in abundance corresponding to variable flows entering the estuary, which influence the availability of suitable spawning and nursery habitat (Kimmerer 2002; Moyle et al. 2004). Other stressors on the species include non-native fish introductions, altered food webs, dams, water diversions, contaminants, and other human activities (Moyle 2002). The relative abundance and distribution of splittail have been monitored for approximately three decades by several sampling programs, most of which do not specifically target splittail. From 1980 to 1992, splittail abundance reportedly declined by 62% (Meng and Moyle 1995) and in 1999 splittail were federally listed as threatened under the US Endangered Species Act of 1973 (US Fish and Wildlife Service 1999). Splittail were de-listed in 2003 in part because restoration efforts were believed to be addressing the threats to the continued persistence of the species (US Fish and Wildlife Service 2003). However, splittail are still a priority for conservation management because of uncertainty regarding their long-term persistence, ongoing scientific studies using splittail as a reference species for estuarine conditions and processes (such as contaminant exposure) and the hope that by conserving splittail and their habitat, other native fishes will be conserved as well. Splittail are currently a Species of Special Concern for the US Fish and Wildlife Service, California Department of Fish and Game, and a key at-risk species for the CALFED Bay-Delta Program (a collaborative state and federal restoration program). Obtaining a comprehensive understanding of splittail population dynamics will be highly valuable for the development of effective management and conservation strategies to protect the species.

Splittail live in the slightly brackish and freshwater portions of the upper San Francisco Estuary and western Sacramento–San Joaquin Delta (Moyle et al. 2004). During winter, they migrate upstream to freshwater rivers and floodplains for spawning and then return to the estuary and delta in the spring and summer (Daniels and Moyle 1983; Sommer et al. 1997; Moyle et al. 2004). A key uncertainty regarding splittail life history is if they are opportunistic spawners, entering any river system with appropriate flow rates, or if they return to their natal rivers to spawn. Spawning-site fidelity could cause populations to diverge genetically and follow separate evolutionary trajectories. The objective of our study was to determine if splittail spawning in different tributary rivers comprised genetically distinct populations or if the entire group represented a single panmictic population. Given the special status of the species, this information will be critical in determining restoration and conservation objectives.

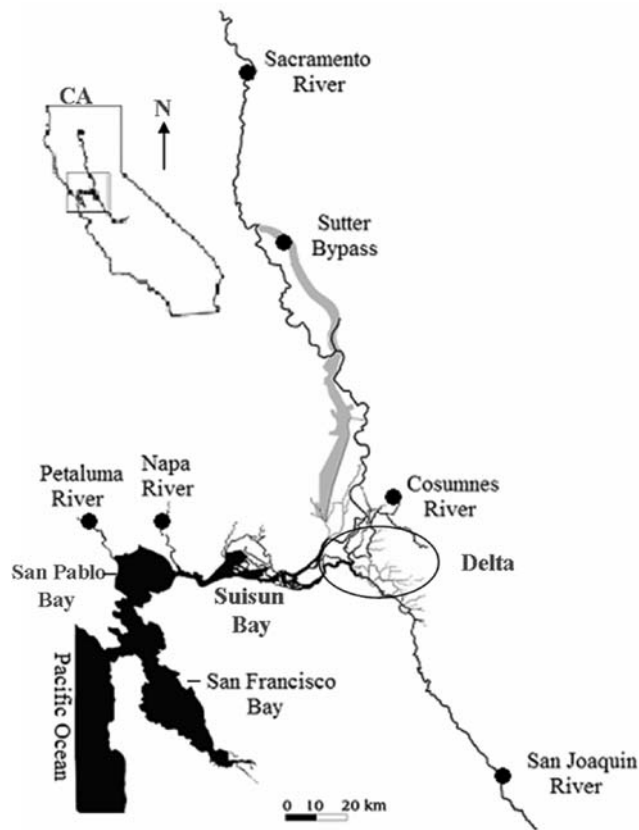
## Materials and methods

### Sample collections

Young-of-the-year splittail (age-0) were collected May–June from several upstream locations in each of the five main tributaries of the estuary: Cosumnes River, Napa River, Petaluma River, Sacramento River (and associated Sutter Bypass), and San Joaquin River (Fig. 1; details of collection methods provided in manuscript by Feyrer et al. 2005). These tributaries were selected to encompass the entire distributional spawning range of splittail. Fish collected from sampling sites located furthest upstream, representing the extreme ends of splittail distribution, were specifically chosen to increase the probability that age-0 splittail collected in each tributary were actually spawned in that particular tributary. Each tributary was sampled for 2 years to test the reproducibility of any observed population structure. All tributaries except the Cosumnes River were sampled during 2002 and 2003. The Cosumnes River was sampled during 2002 and 2004. The sample size for each tributary ranged from 73 to 125 individuals, with a total sample size of 489 individuals.

### Microsatellite genotyping

Genomic DNA was extracted from 5 to 10 mg of caudal fin tissue using either Promega's Wizard® SV 96 Genomic DNA Purification System (cat. no. A2371) or Qiagen's DNeasy Tissue Kit (cat. no. 69506). A suite of thirteen tetranucleotide-repeat microsatellite markers



**Fig. 1** Splittail sampling locations

(CypG3, CypG4, CypG23, CypG25, CypG28, CypG35, CypG39, CypG40, CypG43, CypG45, CypG48, CypG52, CypG53), described in Baerwald and May (2004), were amplified for all samples. Multiplex Polymerase Chain Reaction (PCR) was performed with 20 ng of template genomic DNA, 50 mM Tris-HCl, 10 mM KCl, 5 mM  $(\text{NH}_4)_2\text{SO}_4$ , 2 mM  $\text{MgCl}_2$ , 0.2 mM of each dNTP, 0.3–0.6  $\mu\text{M}$  of each primer (concentration dependent on relative fluorescent intensity of each locus compared to others in multiplex reaction; forward primers were fluorescently end-labeled with 6-FAM, NED, or VIC), and 0.75 units FastStart Taq polymerase (Roche). PCR reactions were performed with Bio-Rad's DNA Engine Dyad thermal cycler under the following conditions: initial denaturation of 4 min at 95°C, 26 cycles of 30 s denaturation at 95°C, 30 s annealing at 58°C, 45 s extension at 72°C followed by a final extension at 60°C for 45 min to produce PCR products with an adenine (+A) addition. PCR products were electrophoresed on 5.5% Long Ranger® (Cambrex) polyacrylamide denaturing gel using Bio-Rad's BaseStation™ in genotyper mode. Alleles were automatically scored for size using Bio-Rad's Cartographer™ software with all automated allele calls verified

manually for accuracy. The following measures were employed to reduce genotyping errors: a Genescan® Rox 400 size ladder (Applied Biosystems) was included in every lane; two control samples with known allele sizes were included in every 96-well PCR plate and gel; allele calls were independently scored by two people; loci with unreliable amplification were removed from study (these loci are not listed above); and any genotypes with questionable allele calls were re-amplified and scored again.

#### Data analyses

Observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosities (unbiased estimates, Nei 1978) and tests for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were performed with the software program Genetic Data Analysis (GDA, Lewis and Zaykin 2001). HWE and LD exact tests (Guo and Thompson 1992) were conducted with 10,000 permutations. Pairwise values of  $\theta$  (an estimator of  $F_{ST}$  that corrects for small sample size; Weir and Cockerham 1984), allelic richness, and average  $F_{IS}$  values were obtained with the software program FSTAT (Goudet 1995, 2001). Pairwise values of  $R_{ST}$  (Slatkin 1995), which assumes a stepwise mutation model, were obtained by using the software package ARLEQUIN (Schneider et al. 2000). Statistical significance for all analyses was determined after a sequential Bonferroni correction for multiple comparisons, using a  $P < 0.05$  cut-off value (Rice 1989).

The software program STRUCTURE (Pritchard et al. 2000) was used to identify the number of genetic clusters ( $K$ ) and assign each individual into the appropriate cluster. This program uses a Bayesian model-based clustering algorithm to group individuals into populations based on allele frequency patterns. Five independent runs of  $K=1-10$  were performed with 100,000 burn-in period and 100,000 Markov chain Monte Carlo (MCMC) repetitions using no prior information and assuming admixture and correlated allele frequencies. A dendrogram depicting the relationships of the sampled groups was created with a neighbor-joining tree, based on Cavalli-Sforza and Edwards chord distances (1967), using the software program PHYLIP (Felsenstein 1993). This tree was visualized using the software TREEVIEW (Page 1996). A Factorial Correspondence Analysis (FCA) was performed using the software program GENETIX (Belkhir et al. 2002) to graphically represent the distribution of genetic variability between splittail from each sampling location in multidimensional space.

Once populations were determined, ability to assign individuals back to their populations was tested in

GENECLASS2 (Piry et al. 2004) using Bayesian methodology (Rannala and Mountain 1997) and Monte Carlo resampling (Paetkau et al. 2004) with 10,000 simulated individuals and a threshold of 0.01. Therefore, if the probability of an individual belonging to any of the sampled populations was  $<0.01$ , the individual was classified as unassigned.

To test for temporal variation, we analyzed pairwise  $F_{ST}$  comparisons, neighbor-joining trees, and STRUCTURE results for all year classes independently.

## Results

A total of 173 alleles were detected among the 13 microsatellite loci. All loci were polymorphic and had normal allelic distributions when the number of alleles per locus was  $\geq 5$ . The number of alleles per locus ranged from two (CypG45) to 26 (CypG40), with an average of 8.3 alleles per locus. Significant genetic differences were not detected between the temporal replicates for each sampling location in pairwise  $F_{ST}$  comparisons, neighbor-joining trees, and STRUCTURE analysis. Therefore, these replicates were combined in all results other than the genetic diversity measurements shown in Table 1 in order to increase sample sizes and the power of the analyses. Splittail groups from all sampling locations and year classes did not appear to be inbred (allelic richness = 6.2–7.8,  $H_E = 0.64$ –0.68,  $F_{IS} = -0.033$ –0.036), with none of the  $F_{IS}$  values found to be statistically significant.

### Hardy–Weinberg equilibrium and linkage disequilibrium

The exact tests of HWE showed that one locus (CypG3) deviated from HWE expectations in three sampling locations (Cosumnes, Sacramento, and San Joaquin). Each of these deviations was due to a deficit

in observed heterozygosity, potentially due to the presence of a null allele at this locus. Three loci deviated from HWE in just one sampling location (CypG53, Cosumnes; CypG43, Sutter Bypass; CypG48, San Joaquin). Among the 78 locus pairs, one pair exhibited genotypic LD and this occurred in only one of the sampling locations (CypG43/CypG40, Sutter Bypass).

### Population differentiation

Pairwise comparisons of  $F_{ST}$  and  $R_{ST}$  both revealed that splittail collected in the Petaluma and Napa Rivers are genetically differentiated from all sampled Central Valley locations (Table 2). Both comparisons also show that the Cosumnes, Sacramento, Sutter Bypass, and San Joaquin sampling locations are not significantly differentiated from each other.

STRUCTURE indicated that the existence of two splittail populations had the highest probability (i.e., is closest to zero with little variance between repetitions) when comparing the likelihood of potential population clusters ranging from 1 to 10 (Fig. 2A). In general, these two populations are comprised of individuals from: (1) Petaluma and Napa rivers and (2) Cosumnes, Sacramento, Sutter Bypass, and San Joaquin rivers (hereafter collectively referred to as the Central Valley population) (Fig. 2B). A few stray individuals found in the Central Valley tributaries genetically assign to the Petaluma/Napa cluster; none of the Petaluma individuals assign to the Central Valley cluster; and several Napa individuals, particularly a group collected on the same day and in the same location, assign to the Central Valley cluster. The neighbor-joining tree (Fig. 3) further corroborates a major bifurcation between the Petaluma/Napa and Central Valley populations, with 100% bootstrap support. The FCA plot (Fig. 4) demonstrates the clear differentiation between splittail collected from the Petaluma River and the

**Table 1** Yearly splittail sample sizes and genetic characteristics

Year	Location	Sample size	Allelic richness	$H_O$	$H_E$	$F_{IS}$
2002	Cosumnes river	75	7.3	0.63	0.64	0.012
	Napa river	32	6.2	0.68	0.66	-0.002
	Petaluma river	36	6.2	0.70	0.68	-0.033
	Sacramento river	59	7.4	0.63	0.66	-0.002
	San Joaquin river	44	7.7	0.64	0.66	-0.028
2003	Napa river	41	6.7	0.67	0.66	-0.010
	Petaluma river	41	6.2	0.68	0.68	0.036
	Sacramento river	21	6.5	0.65	0.64	-0.007
	San Joaquin river	32	7.5	0.65	0.65	0.014
	Sutter bypass	58	7.5	0.64	0.65	0.034
2004	Cosumnes river	50	7.8	0.65	0.65	-0.001

Allelic richness is corrected for sample sizes, observed heterozygosity ( $H_O$ ), expected heterozygosity ( $H_E$ ), inbreeding coefficient ( $F_{IS}$ )

**Table 2** Matrix of pairwise comparisons of  $F_{ST}$  (above diagonal) and  $R_{ST}$  (below diagonal) for all sampled splittail groups

	C	N	P	S	SB	SJ
C	–	<b>0.029</b>	<b>0.050</b>	–0.001	0.002	0.002
N	<b>0.014</b>	–	0.008	<b>0.023</b>	<b>0.031</b>	<b>0.027</b>
P	<b>0.041</b>	<b>0.011</b>	–	<b>0.042</b>	<b>0.050</b>	<b>0.043</b>
S	–0.001	<b>0.012</b>	<b>0.048</b>	–	0.001	0.000
SB	0.001	<b>0.009</b>	<b>0.024</b>	0.006	–	–0.002
SJ	–0.004	<b>0.019</b>	<b>0.045</b>	0.000	0.002	–

C = Cosumnes River; N = Napa River; P = Petaluma River; S = Sacramento River; SB = Sutter Bypass; SJ = San Joaquin River. Values offset in bold indicate statistically significant ( $P < 0.05$ ) differentiation after sequential Bonferroni correction

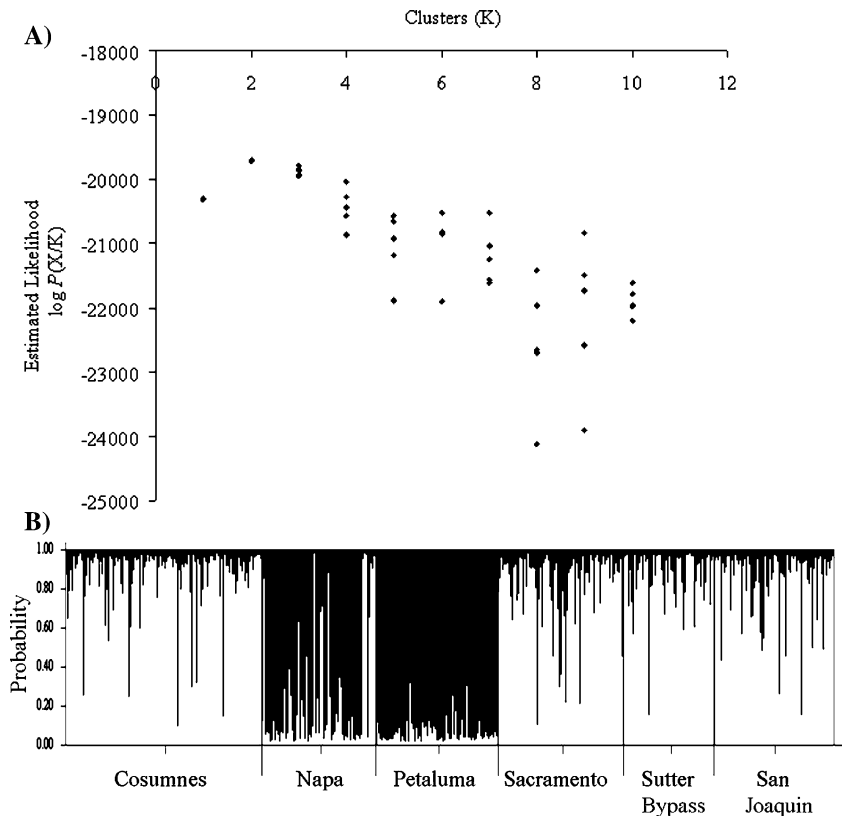
Central Valley population. Individuals collected from the Napa River span across the plot, clustering with both the Petaluma River and the Central Valley population. It is important to note that the majority of Napa River individuals do appear to cluster closer to splittail collected from the Petaluma River. The Napa River individuals clustering with the Central Valley population were, for the most part, the individuals collected on the same day and in the same location that assigned to the Central Valley population in the STRUCTURE analysis. It also appears that while Napa and Petaluma individuals are genetically similar to each other, with a fair degree of intermingling in the FCA plot, there is a trend of genetic distinction between splittail collected

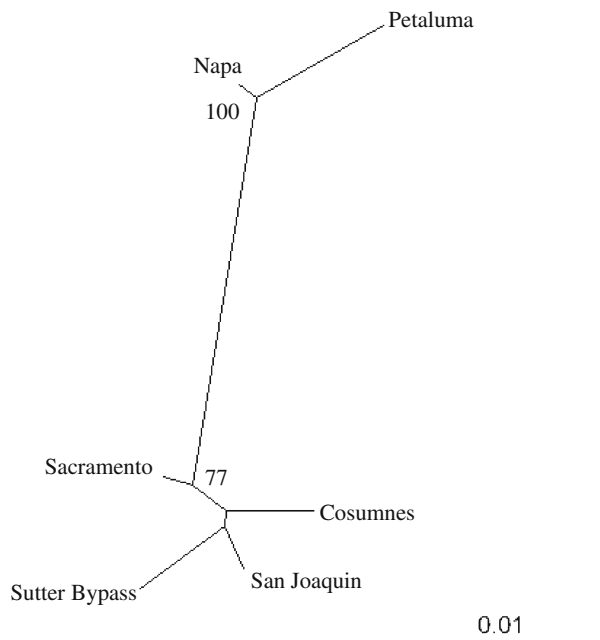
from the two rivers. The majority of Napa individuals cluster above the Petaluma individuals in the plot, suggesting a small degree of differentiation that was temporally replicated during both splittail sampling collection years.

Assignment accuracy

Assignment accuracy is high for the two populations identified in our study when assuming that all splittail populations were sampled. Individuals collected from the Central Valley tributaries assign to the Central Valley population with a 97% accuracy rate and individuals collected from the Petaluma and Napa Rivers

**Fig. 2** STRUCTURE analysis of splittail sampling locations. (A) Optimal number of genetic clusters,  $K = 2$ , was identified by highest estimated likelihood (closest to zero) and lowest amount of variance for five independent runs. (B) Representative display of assignment probability for each individual to the two genetic clusters. Each individual is represented by a thin vertical line

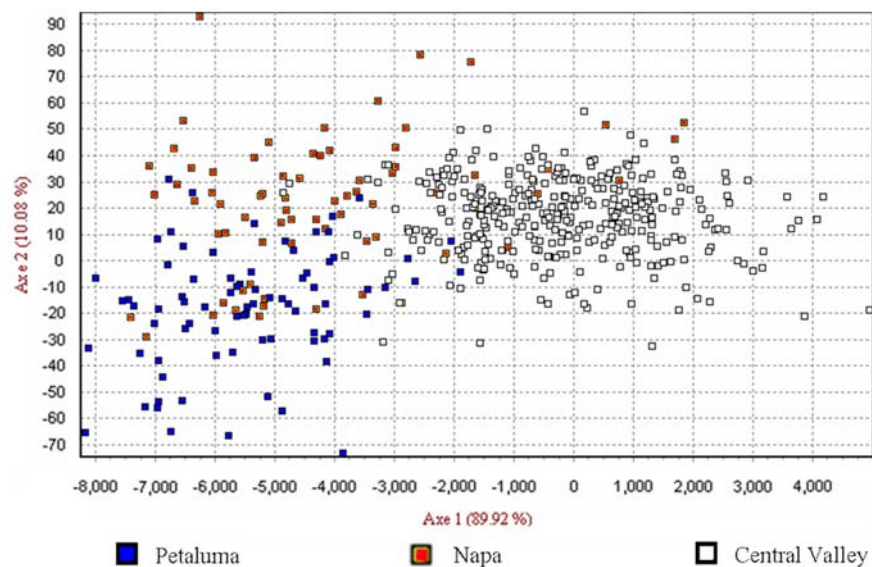




**Fig. 3** An unrooted neighbor-joining tree, based on Cavalli-Sforza and Edwards chord distance measures, displaying the relative relationships between splittail sampled from tributaries of the San Francisco Estuary. Bootstrap support was generated from 10,000 replicates and shown for nodes with support greater than 50%

assign to the Petaluma/Napa population with an 85% accuracy rate. Most individuals collected in the Petaluma or Napa Rivers that do not assign back to the Petaluma/Napa population are from the Napa River and are identified in STRUCTURE as belonging to the Central Valley population.

**Fig. 4** Factorial Correspondence Analysis (FCA) plot showing the genetic relationships between splittail individuals collected from the Petaluma, Napa, and Central Valley Rivers. The majority of Napa individuals clustering with the Central Valley population were caught together on the same date in 2003. A total 100% of the total genetic variation is explained by the first two axes



## Discussion

Maintenance of genetic diversity has become a major component of many species conservation programs, as loss of genetic diversity is one indicator of a reduction in evolutionary potential. In this study, splittail were found to have a high degree of genetic diversity, based on average number of alleles per locus ( $A=8.3$ ) and expected heterozygosity ( $H_E=0.66$ ). These values are comparable to those found in other cyprinid species that have experienced a reduction in historic population size but still retain high genetic diversity, such as the Cape Fear shiner ( $A=8.2$ ,  $H_E=0.70$ ) reported by Saillant et al. (2004) and *Anaocypris hispanica* ( $A=10.3$ ,  $H_E=0.68$ ) reported by Salgueiro et al. (2003). These cyprinid examples are similar to, and even slightly greater than, the average genetic diversity of 78 freshwater fish species ( $A=7.5$ ,  $H_E=0.46$ ) reported by DeWoody and Avise (2000) and are in contrast to the critically endangered cyprinid, *Squalius aradensis*, for which some populations contain relatively low levels of genetic diversity ( $A=4.9$ ,  $H_E=0.45$ ) reported by Mesquita et al. (2005). The observed phenomenon (i.e., high genetic diversity after a reduction in population size) can occur when a species has recently decreased in population size but this reduction has been modest and has not yet been sustained over many generations. Continual reductions of even modest proportions, however, can lead to serious declines in genetic diversity (Frankham et al. 2002) so continued monitoring is recommended. It is important to note, however, that the reported levels of genetic diversity are derived from microsatellite markers, which are presumed to be neutral, and therefore may not always correlate with

the quantitative genetic variation that impacts fitness and determines evolutionary potential (Amos and Balmford 2001). Additionally, the historic levels of splittail genetic diversity have not been determined so it is not possible to know if there has been a significant change in diversity since the population declined.

#### Degree of population differentiation

Results from the  $F_{ST}$  and  $R_{ST}$  pairwise measurements, model-based STRUCTURE analysis, distance-based neighbor-joining tree, and Factorial Correspondence analysis provide a high degree of concordance for the existence of two genetically distinct splittail populations.

These populations, referred to as Petaluma/Napa and Central Valley, have a low to moderate degree of interpopulation differentiation, with values ranging from 0.023 to 0.050. While this differentiation may not be large, it is consistent over consecutive years. Additionally, there appears to be a smaller, but temporally consistent, degree of genetic differentiation occurring between splittail collected from the Petaluma and Napa Rivers. The degree of differentiation between these rivers, however, was not large enough to define them as separate populations.

STRUCTURE results suggest a considerable amount of migration between the populations at all sampling locations, with the exception of the Petaluma River, indicating current gene flow occurring between the populations. All analyses demonstrated that splittail collected in the Napa River were not as differentiated from the Central Valley population as those collected from the Petaluma River. The Napa River may serve as a spawning ground for both of the populations, but it is unclear whether these populations are interbreeding. A Wahlund effect could be occurring in the Napa River but if so, the differentiation between the two populations was not enough to cause Hardy–Weinberg or linkage disequilibrium in either of the two sampled years. The average standard length of all Napa young-of-year splittail captured in 2003 was 34 mm. In contrast, the subset of splittail collected in the Napa River during 2003, which STRUCTURE analysis identified as having a high probability of belonging to the Central Valley population, had an average standard length of 59 mm. While this difference may be coincidental, it is also possible that these individuals are sufficiently large enough to have migrated from another tributary. Alternatively, both populations may utilize the Napa River as a spawning ground but typically do so at distinct times or localities.

#### Potential causes of population differentiation

It is commonly believed that the majority of adult splittail annually return to Suisun Bay, Suisun Marsh, and the western Delta when not spawning since these areas are considered to be splittail's primary rearing and foraging habitat. If splittail from both populations are returning to this central rearing and foraging area, splittail spawning in the Napa and Petaluma Rivers are consistently migrating in the opposite direction (west) versus splittail spawning in Central Valley tributaries, which migrate east. It is not currently known if all splittail migrate back to Suisun Bay and the western Delta after spawning or if the populations intermix in foraging and rearing habitat.

Based on current knowledge of the species and its ecosystem, other potential explanations for the observed population structure include: separate foraging and rearing habitats, differences in salinity tolerance, and high salinity typically creating a barrier to migration. Petaluma and Napa-Sonoma marshes could serve as suitable foraging and rearing habitat at the mouths of the Petaluma and Napa rivers, respectively. These marshes are not consistently part of any splittail sampling programs so splittail rearing in this region may be undetected.

During our splittail collections, we observed significant differences in salinity concentrations between the two populations. Petaluma River had the highest average salinity values with 13 ppt (parts per thousand) for 2002 and 6 ppt for 2003. Napa River had salinity values of 5 ppt for 2002 and 0 ppt for 2003. All other rivers had 0 ppt salinity for both years. For fish that migrate between brackish and fresh water, such as splittail, the physiological demands of tolerating elevated salinity for extended periods of time may limit the amount of energy allocated to growth and reproduction because of increased osmoregulatory demands (Moyle and Cech 2004). Therefore, this potentially strong selective pressure could have caused a divergence between splittail populations, dividing those with a higher salinity tolerance from those that are more susceptible to salinity effects. The majority of individuals collected in the Napa that assign to the Central Valley population were collected in 2003, when the salinity was 0 ppt. It is possible that the Petaluma/Napa population is better able to tolerate increased salinity levels during spawning while the Central Valley population preferentially spawns in freshwater. Alternatively, splittail may migrate through the San Pablo Bay (which is typically extremely brackish) only during years when high precipitation levels lead to a significant reduction in San Pablo Bay's salinity concentration. During typical

years, when the salinity of San Pablo Bay is quite high, splittail spawning in the Petaluma River may be effectively isolated from other splittail due to lack of migration across this bay. While this observed correlation between population structure and salinity concentration is useful for guiding further scientific studies, it is important to not draw conclusions about adaptive genetic variation or adaptive genetic potential from our data. Population genetic studies using neutral molecular markers, such as this study, often do not correlate with the additive genetic variance of a trait, which is necessary for evaluating adaptive genetic potential (Reed and Frankham 2001; Morgan et al. 2001). Therefore, future studies that measure the adaptive genetic variation (e.g., test of salinity tolerance) and adaptive genetic potential (e.g., heritability of salinity tolerance) should be performed within each splittail population to determine if the observed correlation between population structure and salinity differences is scientifically significant.

#### Conclusions and management implications

This study demonstrates the existence of at least two splittail populations, one comprised of individuals found in the Petaluma and, for the most part, Napa Rivers and the other comprised of individuals found in the sampled Central Valley Rivers. The Petaluma/Napa population is likely much smaller than the Central Valley population due to considerable differences in geographic size and suitable spawning habitat but the Petaluma and Napa Rivers are not consistently sampled in current fish monitoring programs. The San Francisco Estuary and its tributaries are part of a highly manipulated ecosystem where the threat of rapid environmental change is constant. It is ideal to preserve as much genetic diversity as possible for a species, both within and among populations, because this diversity acts as an insurance policy against a changing environment. Now that splittail population structure has been determined, these populations should be independently monitored to detect any future substantial reduction in abundance. Management action could then be taken to reduce further population abundance declines, if feasible, so that as much genetic diversity as possible will continue to be preserved. Continued monitoring of population abundance trends would be especially useful for the Petaluma/Napa population, given its smaller size and unique genetic diversity. The population structure revealed in this study should be considered for future management decisions concerning the conservation of the splittail.

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