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Distribution of Genetically Differentiated Splittail Populations during the Nonspawning Season

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Abstract.—Genetic assignment of individuals to their population of origin has many management applications, such as forensic identification of protected species, estimation of migration rates, mixed-stock analysis, and assessment of hybridization. In this study, microsatellite markers were used to obtain an overview of population structure and foraging distribution patterns for a migratory cyprinid, the splittail *Pogonichthys macrolepidotus*. We (Baerwald et al. 2007) recently discovered that splittail inhabiting the San Francisco Estuary form two genetically distinct populations, the Petaluma–Napa and Central Valley populations, and individuals reassigned back to their respective populations with high accuracy (98%). In the present study, we genotyped 242 age-0 splittail from previously unexamined locations in Suisun Marsh and the Sacramento–San Joaquin Delta to determine whether they belonged to one of the two established populations or to a yet-unknown population; we also assigned foraging adults to their population of origin to determine whether they exhibited overlapping or segregated distribution patterns during the nonspawning season. We determined that these fish were members of the two established populations. Both populations foraged in Suisun Bay during the nonspawning season, whereas Suisun Marsh was almost exclusively used by the Central Valley population. The Petaluma–Napa population was considerably more abundant in the western portion of Suisun Bay and appeared to preferentially forage closer to its spawning grounds. The results indicate that the distributions of the two splittail populations do not entirely overlap during spawning and foraging; this finding has important implications for the conservation and management of the species.

A vital precursor of effective conservation management is the identification of all essential habitats used by a species during each stage of its life cycle. For instance, protecting spawning habitat for a species will be of limited use if its foraging habitat or migration corridors are being destroyed. Additionally, it is highly valuable from a conservation management perspective to determine whether populations exhibit overlapping or segregated distribution patterns in spawning and foraging grounds. Elucidation of movement patterns and habitat utilization for different populations enables more-effective monitoring, which is extremely important for smaller populations of conservation concern. It is now possible to identify the source population from which an individual probably originated; this is facilitated by using highly polymorphic molecular markers, such as microsatellites (Davis et al. 1999; Hansen et al. 2001) and single nucleotide polymorphisms (Morin et al. 2004; Seddon et al. 2005). These genetic markers do not rely on tagging individuals and are capable of discriminating individuals from separate populations, even when they exhibit spatial overlap. Genetic assignment of individuals is applicable to a variety of fish conservation objectives, such as forensic identification of protected species or populations (Withler et al. 2004; Schwenke et al. 2006), estimation of current migration rates and dispersal (Paetkau et al. 2004; Castric and Bernatchez 2004), estimation of stock composition (Beacham et al. 2005), hybridization (Tranah et al. 2004), and detection of hatchery fish in wild populations (Hauser et al. 2006).

In California, the San Francisco Estuary watershed provides vital foraging and spawning habitat for the splittail *Pogonichthys macrolepidotus* (also known as Sacramento splittail). The splittail has been the only extant member of its genus throughout the world since the extinction of the Clear Lake splittail *P. ciscoides* in

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the early 1970s (Moyle 2002; Moyle et al. 2004). The splittail is endemic to the San Francisco Estuary watershed (Moyle 2002; Moyle et al. 2004), a highly manipulated, constantly changing ecosystem. The upper tidal freshwater portion of this estuary, the Sacramento–San Joaquin Delta (hereafter, the Delta), provides water for approximately 25×10^6 people and over 1×10^6 ha of farmland in California (Mitchell 1996). Approximately 24% of the annual freshwater inflow is pumped out of the Delta primarily by two large diversions, the State Water Project and the Central Valley Project, and is transported to central and southern regions of California (Mitchell 1996; Schaffter and Kohlhorst 1997).

Over the last two decades, splittail have exhibited fluctuations in abundance corresponding to variable flows entering the estuary, which influence the availability of suitable spawning and nursery habitat (Moyle et al. 2004; Feyrer et al. 2006). Other stressors on the species include nonnative fish introductions, altered food webs, dams, water diversions, contaminants, and other human activities (Moyle 2002). The relative abundance of splittail has been monitored for over 30 years by several sampling programs, none of which specifically targeted splittail. From 1980 to 1992, splittail abundance reportedly declined by 62% (Meng and Moyle 1995), prompting the U.S. Fish and Wildlife Service (USFWS) to list the splittail as threatened under the U.S. Endangered Species Act in 1999 (USFWS 1999). Almost immediately, two lawsuits forced the USFWS to reevaluate the listing. After an extended review and public comment period, splittail were delisted in 2003 in part because restoration efforts were believed to be addressing the threats to the continued persistence of the species (USFWS 2003). However, the splittail is still a priority for conservation management because of uncertainty regarding the species' long-term persistence; limited access to spawning grounds during low flow years and a potentially fluctuating population size make the splittail vulnerable to stochastic events in the highly manipulated, highly invaded San Francisco Estuary and Delta. Ongoing scientific studies are using the splittail as a reference species for estuarine conditions and processes, such as contaminant exposure (Stewart et al. 2004; Teh et al. 2004, 2005; Brady et al. 2006). Conservationists hope that by conserving splittail and their habitat, other native fishes will be conserved as well. The splittail is currently listed as a species of special concern for the USFWS and California Department of Fish and Game and is considered to be a key at-risk species for the California–Federal (CALFED) Bay-Delta Program (a collaborative state and federal restoration program).

The majority of adult splittail are believed to undertake an annual spawning migration from brackish estuarine foraging habitat to their preferred spawning habitat in freshwater tributaries and floodplains containing submerged vegetation (Daniels and Moyle 1983; Sommer et al. 1997; Moyle et al. 2004). The presumed central foraging habitat includes Suisun Bay, Suisun Marsh, and the Delta (Moyle et al. 2004). The spawning habitat is considerably broader in geographic range and includes many rivers in California's Central Valley, such as the Sacramento and San Joaquin rivers and two rivers draining into San Pablo Bay, the Napa and Petaluma rivers (Feyrer et al. 2005). In a recent study, we (Baerwald et al. 2007) determined the existence of two genetically distinct splittail populations. One population spawns primarily in the Napa and Petaluma rivers (hereafter, Petaluma–Napa population). The other population primarily uses suitable spawning sites in eastern rivers, such as the Sacramento, San Joaquin, and Cosumnes rivers (hereafter, Central Valley population).

The main objective of the present study was to determine whether the populations exhibited overlapping or segregated distribution patterns in the presumed central foraging grounds during the nonspawning season. To ensure complete conservation management of a species, all critical habitat required by the species throughout its life cycle must be monitored and protected from future degradation. Habitat conservation is a primary component of maintaining genetic diversity so that a species can respond to future environmental change. Therefore, it is important to identify the specific foraging grounds for both populations, particularly the Petaluma–Napa population, which is probably less abundant than the Central Valley population because of its considerably smaller geographic spawning area. We hypothesized that the two populations would exhibit partially segregated distributions dependent upon the proximity of suitable foraging habitat to spawning sites. Our hypothesis was partly based on the premise that increased metabolic costs associated with longer migrations (for review, see Dingle [1996]) might make it energetically unfavorable for splittail to migrate long distances to forage if suitable foraging areas are located closer to their spawning sites. Additionally, we speculated that the populations may have distinct foraging habitat preferences related to environmental factors. Previously, we observed salinity differences between the spawning sites of the Petaluma–Napa and Central Valley populations. The Petaluma–Napa population typically inhabited low-salinity environments during spawning, whereas the Central Valley population spawned primarily in freshwater (Baerwald et al. 2007). We

hypothesized that to decrease energetic costs and possibly remain in a low-salinity environment, the Petaluma–Napa population would preferentially forage near the Petaluma River, Napa River, or western Suisun Bay. In contrast, we hypothesized that the Central Valley population would forage in the Delta and eastern Suisun Bay to decrease energetic costs and remain in a more freshwater environment. Many environmental differences (e.g., temperature, depth, etc.) other than salinity differences between Suisun Bay and Suisun Marsh could also affect the foraging patterns of these unique populations as a possible consequence of adaptive evolution.

The sampling sites originally chosen to identify splittail population structure (Baerwald et al. 2007) are believed to encompass the outer regions of the core distributional spawning range for the species. There is, however, a considerable amount of suitable spawning habitat closer to the presumed central foraging habitat, particularly during years with increased and prolonged flooding. These regions, such as Suisun Marsh and the Delta (Figure 1), were not explored in the initial population structure study. Therefore, to obtain a more-complete picture of the overall structure for the species, this more centralized spawning area was sampled and age-0 fish were assessed for population structure prior to genetic assignment of foraging adults to spawning populations.

Methods

Sampling of age-0 splittail.—Age-0 splittail were sampled using beach seines at several suitable spawning locations in Suisun Marsh and the Delta during the spring spawning season (April–June). Of the 242 age-0 splittail sampled, 240 were captured in 2003 and 2 were captured near Sherman Island in 2002. Analysis of these individuals and the 489 age-0 splittail collected from the outer distributional spawning range (Petaluma, Napa, Cosumnes, Sacramento, and San Joaquin rivers and Sutter Bypass) during the 2002–2004 spawning seasons provided a more comprehensive picture of overall population structure. All collected age-0 splittail were quite small (<50 mm fork length) and were assumed to have been spawned recently (age < 3 months posthatch); thus, we assumed that the fish had not migrated far from the sites where they were spawned. For additional details regarding age-0 splittail sampling and distribution patterns, see Feyrer et al. (2005).

Sampling of foraging adult splittail.—To determine distribution patterns during the nonspawning season, 137 adult splittail (>150 mm standard length) were collected from the Napa River, Suisun Bay, and Delta (Table 1; Figure 1) using gill nets. Adults were collected in August–November 2004, well after the spring spawning period. Splittail at some locations, such as Pacheco Creek and Ryer Island, were collected only one time (October); other locations, such as Chipps Island and the sloughs of Suisun Marsh, were sampled repeatedly. Specifically, Suisun Marsh sampling occurred on a monthly basis in tandem with the University of California–Davis (UC–Davis) Suisun Marsh Fisheries monitoring program. Several attempts to collect adult splittail from various sites in the Petaluma River and San Pablo Bay were unsuccessful, possibly because of a paucity of splittail in these locations during the nonspawning season or because the sampling sites were not located in suitable foraging habitat.

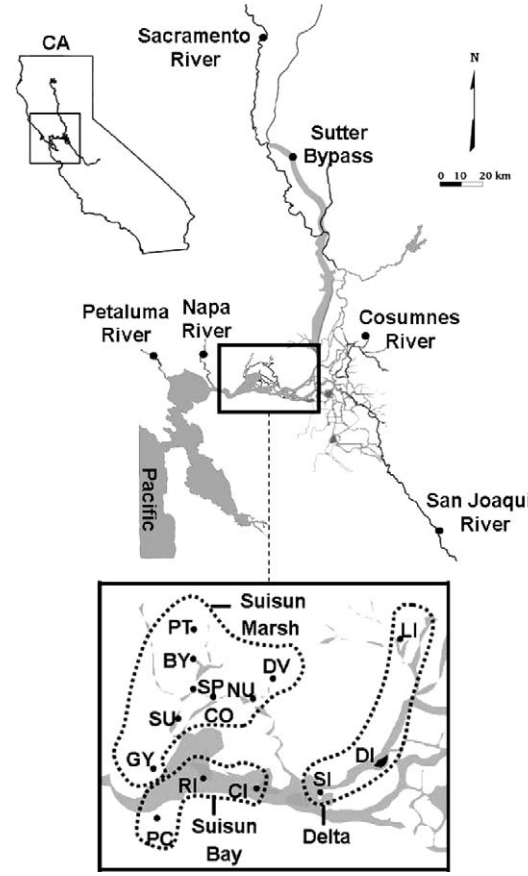

FIGURE 1.—Map of sampling sites in California, where splittail were collected for an analysis of population structure and foraging distribution (see Table 1 for explanation of site codes). Age-0 fish were collected from the Petaluma River, Napa River, Sacramento River, Sutter Bypass, Cosumnes River, San Joaquin River, Suisun Marsh, and Sacramento–San Joaquin Delta; adults were obtained from Suisun Marsh, Suisun Bay, and the Napa River.

TABLE 1.—Sample sizes and expected heterozygosity (H_E), observed heterozygosity (H_O), and inbreeding coefficient (F_{IS}) values from analysis of 13 microsatellite loci in age-0 splittail collected from Central Valley rivers, Suisun Marsh, and the Sacramento–San Joaquin Delta (2002–2004) and adult splittail collected from Suisun Marsh, Suisun Bay, and the Napa River, California (2004).

Region	Specific sample site	Sample code	Sample size	H_E	H_O	F_{IS}
		Age-0 fish				
Outer distribution	Petaluma River	P	77	0.67	0.68	−0.018
	Napa River	N	73	0.65	0.66	−0.014
	Cosumnes River	C	125	0.63	0.62	0.008
	Sacramento River	S	80	0.63	0.62	0.025
	Sutter Bypass	SB	58	0.63	0.62	0.014
	San Joaquin River	SJ	76	0.64	0.62	0.020
Delta	Decker Island	DI	47	0.63	0.62	0.028
	Liberty Island	LI	47	0.64	0.58	0.095
	Sherman Island	SI	94	0.66	0.64	0.018
Suisun Marsh	Several sloughs	SM	54	0.63	0.62	0.020
		Adults				
Suisun Marsh	Boynton Slough	BY	3	0.68	0.65	0.056
	Cutoff Slough	CO	16	0.66	0.63	0.047
	Denverton Slough	DV	17	0.63	0.62	0.016
	Goodyear Slough	GY	8	0.69	0.72	−0.043
	Nurse Slough	NU	9	0.69	0.67	0.032
	Peytonia Slough	PT	17	0.68	0.69	−0.016
	Spring Branch Slough	SP	40	0.65	0.65	0.004
	Suisun Slough	SU	35	0.67	0.66	0.012
Suisun Bay	Chipps Island	CI	23	0.67	0.65	0.021
	Pacheco Creek	PC	22	0.69	0.64	0.076
	Ryer Island	RI	21	0.67	0.63	0.068
Napa River	Coon Island		1			

Data collection.—Genomic DNA was extracted from 10 mg of caudal fin tissue following protocols provided in the kit manuals: the Wizard SV 96 Genomic DNA Purification System (Promega) for age-0 splittail and the Puregene DNA Purification Kit (Qiagen) for adults. A suite of 13 microsatellite markers used to identify splittail population structure in samples from eastern (Central Valley) versus western (Petaluma and Napa rivers) spawning tributaries was evaluated for this study: *CypG3*, *GypG4*, *CypG23*, *CypG25*, *CypG28*, *CypG35*, *CypG39*, *CypG40*, *CypG43*, *CypG45*, *CypG48*, *CypG52*, and *CypG53* (described by Baerwald and May [2004]). Template genomic DNA (20 ng) was amplified by polymerase chain reaction (PCR) using 0.7 units of FastStart *Taq* polymerase (enzyme number 2.7.7.7; IUBMB 1992) and associated 10X buffer (Roche) along with 0.2 mM of each deoxynucleotide triphosphate, and 0.3–0.6 μM of each primer (forward primers fluorescently tagged with 6-carboxyfluorescein, NED, or VIC). The PCR cycling conditions were as follows: initial denaturation of 5 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, final 45 min extension at 60°C. The PCR products were separated by size with 5.5% polyacrylamide denaturing gels using the BioRad BaseStation in genotyper mode. BioRad Cartographer software was used to score allele sizes. To reduce genotyping errors all allele calls were independently scored by two people, all gels contained two control samples with known allele sizes, every lane contained a GeneScan Rox 400-size ladder (Applied Biosystems), and any genotypes with questionable allele calls were reamplified and rescored.

Statistical analyses to assess population structure.—For age-0 splittail, Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) exact tests (Guo and Thompson 1992) were performed with Genetic Data Analysis software (Lewis and Zaykin 2001) using 10,000 permutations to assess significance. Pairwise values of θ (a population differentiation index, F_{ST}, estimator that corrects for small sample size; Weir and Cockerham 1984), and average inbreeding coefficient (F_{IS}) values were calculated using the program FSTAT (Goudet 1995, 2001). Statistical significance ($\alpha = 0.05$) was determined by using a permutation procedure (Goudet et al. 1996) followed by a sequential Bonferroni correction (Holm 1979; Rice 1989). An analysis of molecular variance (AMOVA) was conducted in Arlequin version 3.01 (Excoffier et al. 2005) to partition the molecular variance into within-population and among-population components using 10,000 permutations. The program Structure version 2.0 (Pritchard et al. 2000) was used to genetically cluster individuals without using a priori groupings based on collection sites. The admixture

model with correlated allele frequencies is considered the best model for detecting subtle population structure (Falush et al. 2003) and was the chosen configuration. The optimal number of genetic clusters (k) was determined by selecting the k with the highest probability and the lowest variance across three separate runs. Each run was performed with a 100,000-repetition burn-in period and 100,000 Markov-chain Monte Carlo repetitions. The program CONVERT (Glaubitz 2004) was used to reformat genotypic data into input formats that were suitable for both Genetic Data Analysis and Structure. Microsoft Excel Microsatellite Toolkit software (Park 2001) was used to create input files for FSTAT that could also be used for GeneClass2 (Piry et al. 2004).

Genetic assignment of foraging adults.—The two reference populations consisted of pooled age-0 splittail from the included sampling locations (Petaluma and Napa rivers for the Petaluma–Napa reference population; Central Valley rivers, Suisun Marsh, and the Delta for the Central Valley reference population). To assess assignment accuracy for the reference populations, the likelihood of each age-0 individual assigning back to the reference population from which it originated was calculated using a leave-one-out procedure (Efron 1983), whereby an individual is excluded from its reference population during its assignment to avoid any bias due to small sample sizes.

Genetic assignment of individuals to a population was conducted using GeneClass2. Specifically, a Bayesian algorithm (Rannala and Mountain 1997) was combined with Monte Carlo resampling (Paetkau et al. 2004) to simulate 10,000 individuals. Individuals were classified as unassigned and were excluded from both populations if their multilocus genotypes did not fall within the 95% distribution of simulated genotypes for either reference population. Each individual that was not classified as unassigned was assigned to the reference population in which it had the highest probability of assignment, even if it could not be entirely excluded from the other population. After it was confirmed that each reference population had a high (94%) self-assignment success, the reference populations (age-0 splittail) were used to genetically assign the foraging adult splittail collected in Suisun Bay, Suisun Marsh, and the Napa River to natal populations.

Results

Descriptive Statistics for Age-0 Splittail

Among all genotyped age-0 splittail, 180 alleles were detected from the 13 microsatellite loci (2–25 alleles/locus). Expected heterozygosity (H_E), observed heterozygosity (H_O), and F_{IS} values for age-0 and adult splittail are shown in Table 1. The average H_E across loci was 0.66. The F_{IS} ranged from −0.043 to 0.095 and was not significant for any of the sampling locations after sequential Bonferroni correction, although the P-value for the Liberty Island sample (0.0004) was just above the corrected significance level. Deviations from HWE were tested for the 13 microsatellite markers in age-0 fish from all 10 sampling locations, and significance was determined after sequential Bonferroni correction. One locus significantly deviated from HWE in fish from four sampling locations (*CypG3* in Cosumnes, Sacramento, and San Joaquin rivers and Liberty Island). Three loci significantly deviated from HWE in fish from two sampling locations per locus (*CypG4* in Sherman Island and Suisun Marsh, *CypG43* in the San Joaquin River and Liberty Island, and *CypG53* in the Cosumnes River and Liberty Island). Three loci significantly deviated from HWE in fish from one sampling location per locus (*CypG23* in Sherman Island, *CypG28* in Decker Island, and *CypG48* in the San Joaquin River). Given that *CypG3* did not conform to HWE expectations in fish at 40% of the sampling locations, it was not included in subsequent analyses. The remaining HWE deviations appeared to be biological (specific to sample sites) instead of technical (locus specific), and all other loci were used in subsequent age-0 splittail population structure analyses.

Among 66 pairwise comparisons of loci, 5 displayed genotypic LD after Bonferroni correction (each of the five comparisons exhibited LD at only one sampling location). All observed cases of LD appeared to be biological instead of technical in origin and were specific to Liberty and Sherman islands, except for one locus pair that was specific to Sutter Bypass. Linkage disequilibrium was observed for three locus pairs from Liberty Island fish (*CypG23* and *CypG40*, *CypG48* and *CypG40*, *CypG53* and *CypG40*), two locus pairs from Sherman Island fish (*CypG53* and *CypG43*, *CypG53* and *CypG23*), and one locus pair from Sutter Bypass fish (*CypG43* and *CypG40*).

Population Structure of Age-0 Splittail

The F_{ST} pairwise comparisons (Table 2) showed a clear genetic distinction between splittail sampled from the Petaluma and Napa rivers and those collected from Central Valley rivers (Cosumnes, Sacramento and associated Sutter Bypass and San Joaquin), the Delta, and Suisun Marsh. The Delta and Suisun Marsh splittail were generally not significantly different from splittail sampled in Central Valley rivers. Temporal replication of sampling sites across years showed consistent results for F_{ST} comparisons, and the AMOVA showed that only 0.11% ($P < 0.001$) of the

TABLE 2.—Matrix of pairwise genetic differentiation index (F_{ST}) values representing spatial and temporal comparisons of age-0 splittail collected from Central Valley rivers, Suisun Marsh, and the Sacramento–San Joaquin Delta (Delta) during 2002–2004 (see Table 1 for explanation of sites, represented by letters in each code; numbers in each code refer to sampling year). Values enclosed by triangles represent generally cohesive regions of population structure; values in bold were significant ($P < 0.05$) after Bonferroni correction. Splittail from the Delta and Suisun Marsh were genetically similar to the Central Valley population and genetically distinct from the Petaluma–Napa population (two genetically distinct populations identified by Baerwald et al. [2007]).

	P02	P03	N02	N03	DI03	LI03	SI03	SM03	C02	C04	S02	S03	SB03	SJ02	SJ03
P02	-	–0.003	0.006	**0.013**	**0.046**	**0.046**	**0.041**	**0.047**	**0.052**	**0.055**	**0.041**	**0.056**	**0.055**	**0.047**	**0.051**
P03		-	0.004	**0.009**	**0.040**	**0.044**	**0.035**	**0.041**	**0.044**	**0.045**	**0.032**	**0.046**	**0.048**	**0.038**	**0.043**
N02			-	0.004	**0.034**	**0.039**	**0.027**	**0.035**	**0.040**	**0.041**	**0.031**	**0.047**	**0.045**	**0.038**	**0.038**
N03				-	**0.020**	**0.020**	**0.015**	**0.019**	**0.020**	**0.020**	**0.011**	**0.019**	**0.024**	**0.023**	**0.018**
DI03					-	**0.006**	0.002	–0.001	0.008	0.010	**0.009**	0.009	**0.011**	0.007	0.014
LI03						-	0.005	0.004	0.007	**0.013**	**0.011**	0.005	0.004	0.005	0.010
SI03							-	0.002	0.006	0.007	**0.006**	0.004	0.007	0.003	0.005
SM03								-	0.002	0.004	0.003	0.003	0.003	–0.002	0.006
C02									-	0.004	0.003	0.000	0.003	0.002	0.006
C04										-	–0.002	–0.001	0.002	0.002	0.001
S02											-	0.001	0.003	0.001	0.002
S03												-	–0.004	–0.002	–0.006
SB03													-	0.000	-0.003
SJ02														-	0.000
SJ03															-

observed genetic variation could be attributed to temporal differences, whereas 1.52% ($P = 0.001$) could be attributed to particular sampling sites. When sampling sites were grouped into the two populations (Petaluma–Napa and Central Valley), 3.13% ($P = 0.001$) of the genetic variation was attributable to populations and only 0.44% ($P < 0.001$) was attributable to sampling sites within populations.

Analysis with Structure corroborated the existence of two distinct populations, Petaluma–Napa and Central Valley (i.e., optimal $k = 2$). A considerable minority of age-0 splittail collected in the Napa River had a high probability of belonging to the Central Valley population (21 of 73; 29%). The splittail collected in the Delta and Suisun Marsh genetically clustered with the Central Valley population.

Genetic Assignment of Adults

The HWE and LD tests of the Petaluma–Napa (pooled Petaluma and Napa River samples) and Central Valley (pooled Cosumnes River, Sacramento River, Sutter Bypass, San Joaquin River, Delta, Suisun Marsh samples) reference populations were performed before adult assignment back to these pooled populations. Two loci, *CypG4* and *CypG43*, deviated from HWE expectations in the Central Valley reference population and were removed from the data set before adult assignment. Among the 45 locus pairs (not including *CypG3*, *CypG4*, or *CypG43*), 3 pairs exhibited genotypic LD, each in only one reference population (*CypG25* and *CypG48* in Petaluma–Napa; *CypG25* and *CypG39*, *CypG39* and *CypG48* in Central Valley).

A high percentage (94%) of age-0 individuals assigned back to their reference population in the GeneClass2 self-assignment simulation. A large majority (88%) of adult splittail sampled in Suisun Marsh were assigned to the Central Valley population (Table 3). Only 5% of splittail sampled in Suisun Marsh were

TABLE 3.—Proportion of foraging adult splittail collected in 2004 from Suisun Marsh, Suisun Bay, and the Napa River, California, that were assigned to the Central Valley (CV) or Petaluma–Napa (PN) population (genetically distinct populations identified by Baerwald et al. [2007]) or that were unassigned (UN). Each fish was assigned to the population in which it had the highest assignment probability; UN fish were those with genotypes that deviated from the 95% distributions of simulated genotypes for CV and PN reference populations. See Table 1 for explanation of site codes.

	Assignment		
Location	CV	PN	UN
	Suisun Marsh		
BY	0.67	-	0.33
CO	0.94	0.06	-
DV	1.00	-	-
GY	0.50	0.125	0.375
NU	0.89	-	0.11
PT	0.83	0.06	0.11
SP	0.90	0.075	0.025
SU	0.88	0.06	0.06
	Suisun Bay		
CI	0.74	0.22	0.04
PC	0.45	0.36	0.18
RI	0.57	0.19	0.24
	Napa River		
	-	1.00	-

assigned to the Petaluma–Napa population; the remaining 7% were unassigned.

The one adult captured near Napa River's Coon Island during the nonspawning season was assigned to the Petaluma–Napa population. For collections made outside of the Napa River, the highest percentage of splittail assigned to the Petaluma–Napa population was found in Pacheco Creek (36% Petaluma–Napa assignment). There was a high amount of foraging overlap between the Petaluma–Napa and Central Valley populations; 45% of the fish sampled in Pacheco Creek were assigned to the Central Valley population. To a lesser degree, these two populations also overlapped in foraging distribution for the remaining two sampled Suisun Bay island areas (Ryer and Chipps islands). Although the majority of splittail in these locations assigned to the Central Valley population (57.5–74%), a substantial minority (19–22%) assigned to the Petaluma–Napa population. Therefore, both populations appeared to use Suisun Bay for foraging habitat, whereas use of Suisun Marsh as a foraging area was primarily exhibited by the Central Valley population.

Discussion

Determining the levels of interconnectivity between populations of migratory fish during all stages of the life cycle is essential for monitoring and assessing the impacts of conservation management actions (Metcalfe 2006). Several other California native cyprinids share a similar migration pattern with splittail, migrating from brackish (e.g., Sacramento pikeminnow *Ptychocheilus grandis*; Moyle 2002) or downstream foraging habitats (e.g., hitch *Lavinia exilicauda*; Murphy 1948) to spawn in upstream tributaries or floodplains. With the exception of taxonomic classification (Avise and Ayala 1976; Simons and Mayden 1999), genetic studies have not been performed on any other cyprinid species native to the San Francisco Estuary watershed; thus, the distribution patterns of other native cyprinid populations cannot be compared with those of splittail. Our study showed that the two splittail populations exhibited overlapping distributions in Suisun Bay during the nonspawning season. In contrast, Suisun Marsh appeared to serve as foraging grounds for the Central Valley population almost exclusively.

Hardy–Weinberg Equilibrium and Linkage Disequilibrium Analyses

Age-0 splittail collected near Liberty Island deviated from HWE for three loci and exhibited LD for three locus pairs. Deviations were probably not due to small sample size (47 individuals were collected), a Wahlund effect (not observed during Structure analysis), or null alleles (pattern not found in any other sampling locations). The most likely explanation for the observed deviations is that the age-0 splittail collected near Liberty Island were related to each other. This possibility is corroborated by an almost statistically significant F_{IS} value for splittail collected near Liberty Island ($F_{IS} = 0.095$ averaged across all loci). It is worth noting that the inclusion of a group of related individuals in a reference population has the potential to bias subsequent assignment results. However, we believe that any bias is negligible in the present study because these related individuals constituted a small minority (47 of 581; 8%) of the Central Valley reference population. Additionally, the Rannala and Mountain (1997) algorithm for adult assignment uses allele frequency distributions to calculate genotype probabilities and is less sensitive to bias from related individuals than a method that uses genotype frequencies for assignment.

Population Structure

Results from pairwise F_{ST} comparisons and Structure analysis showed that splittail that were spawned in the Delta and Suisun Marsh were genetically similar to the Central Valley population and distinct from the Petaluma–Napa population. When examining the F_{ST} results, there does appear to be a small degree of genetic distinction between splittail collected in the Sacramento River during 2002 and splittail collected in the Delta (Decker, Liberty, and Sherman islands). Temporal variance is probably responsible for this observation, because the Delta splittail were collected in 2003 and were not genetically distinct from splittail collected in the Sacramento River during 2003. Additionally, Liberty Island splittail had a low level of genetic distinction from Decker Island and Cosumnes River splittail. The speculated relatedness of Liberty Island splittail may have created bias in the data set (as discussed above), and future studies should target representative sampling around Liberty Island to determine whether this result was due to sampling error.

When the significance level was lowered from 0.05 to 0.01, there was a small degree of genetic differentiation between splittail collected in the Delta and Suisun Marsh and those collected in Central Valley rivers. Splittail that are spawned in Suisun Marsh and the Delta may constitute a subtly different group from the Central Valley population, such that a considerable degree of recent or current gene flow exists between the two groups. Currently, there is not sufficient evidence to support the existence of a third genetically distinct splittail population, because the results have not been temporally replicated to determine consisten-

cy and are only considered significant with increased statistical stringency.

Both the Petaluma–Napa and Central Valley populations may use the Napa River for spawning, because a considerable minority of age-0 splittail collected in the Napa River had a high probability of belonging to the Central Valley population (21 of 73 fish; 29%). It is also possible that individuals assigned to the Central Valley population migrated into the Napa River and were not actually spawned there. If migration did occur, then the geographic origin of these fish is probably close to the Napa River (e.g., Suisun Marsh), because the very young age of the fish would preclude substantially long migrations. Regardless of the true origin of these fish, the sampling of both Petaluma–Napa and Central Valley individuals in the Napa River only marginally (1%) decreased the overall success of reassignment to reference populations. Therefore, the inclusion of all Napa River age-0 splittail in the Petaluma–Napa reference population probably did not overly influence the adult assignment results. Future studies should investigate whether the observed intermixing of splittail populations in the Napa River results from spawning by both populations or from migration by the Central Valley population.

Assignment of Adults

When comparing Petaluma–Napa and Central Valley reference populations, overall individual reassignment success was quite high (94%), so it appears that the 10 microsatellite markers used in this study have the ability to distinguish individuals spawned from the Petaluma–Napa and Central Valley populations. It is important to note that age-0 reference splittail were used to assign adult splittail to reference populations. Given that age-0 and adult splittail are temporally separated by at least one generation (2–3 years/generation), slight allelic frequency shifts could affect assignment success, although such an effect would probably be negligible.

We could not reject our hypothesis of preferential foraging closer to spawning sites for the Petaluma–Napa population. Only one adult splittail was captured in the Napa River, and this fish was assigned to the Petaluma–Napa population; it is not known whether both populations use the Napa River for spawning and foraging or whether the river is only inhabited by the Petaluma–Napa population during the nonspawning season. Of all sampling locations (excluding the Napa River), Pacheco Creek had the greatest geographic proximity to Petaluma–Napa spawning sites and contained the greatest number of splittail assigned to the Petaluma–Napa population. Petaluma–Napa adults also were collected while foraging in other Suisun Bay locations (Ryer and Chipps islands) but at frequencies much lower than that in Pacheco Creek; Petaluma–Napa adults were only a small minority of the individuals collected in Suisun Marsh. In contrast, the Central Valley population was found ubiquitously throughout Suisun Bay and Suisun Marsh. The spawning grounds for this population are considerably broader in range than those of the Petaluma–Napa population (Feyrer et al. 2005) and probably support a much greater population. Perhaps the sheer number of Central Valley individuals conceals this population's foraging preferences. The Central Valley population may prefer some foraging sites to others, but its much higher overall abundance may have led to the higher percentage of Central Valley fish than Petaluma–Napa fish among foraging adults in Pacheco Creek. Among age-0 splittail collected in the Napa River, 29% were assigned to the Central Valley population; therefore, it is also possible that the Central Valley adults foraging near the Petaluma and Napa rivers were spawned in the Napa River (or a nearby location) and actually displayed a preference for foraging near natal areas. Alternatively, Central Valley individuals may not have specific foraging site preferences and may simply be opportunistic.

Previous individual-based assignment analysis (e.g., Hendry et al. 2002; Fraser and Bernatchez 2005; Dupont et al. 2007) and mixed-stock analysis (e.g., Ruzzante et al. 2006) of migratory fishes have found distinct populations associated with differences in migratory patterns. These studies have shed light on the complex interplay between environmental factors and life history characteristics that shape genetic diversity. There are many physical differences between the collection sites of Suisun Bay and Suisun Marsh that could prohibit the Petaluma–Napa population from utilizing Suisun Marsh as foraging habitat. For example, during our adult splittail sampling, the sloughs of Suisun Marsh typically had lower salinity (3–7‰) than Suisun Bay (6–13‰). In our previous study (Baerwald et al. 2007), we noted differences in salinity between spawning sites for splittail assigned to the Petaluma–Napa population (average = 0–13‰) and those for fish assigned to the Central Valley population (0‰ at all sites). We speculate that the potentially strong selective pressure of increased salinity could be a factor in the observed population structure. It is possible that any tolerance that the Petaluma–Napa population has for higher salinity levels during spawning continue when this population migrates to foraging sites. Of course, our presumably neutral genetic markers do not detect adaptive genetic differences, so any observed correlation between population structure and salinity should be tested by

comparing physiological salinity tolerance levels or genes influencing salinity tolerance (e.g., Na^+,K^+-ATPase [3.6.3.9] α and β subunits, *Hsc70*) between the two populations. Other studies have found supporting evidence for adaptive population divergence correlated with salinity and thermal shifts between the North and Baltic seas for fish species such as the Atlantic herring *Clupea harengus* (Bekkevold et al. 2005) and European flounder *Platichthys flesus* (Hemmer-Hansen et al. 2007). Other habitat differences between Suisun Bay and Suisun Marsh could also influence foraging distribution (e.g., water flow, temperature, prey type and abundance, vegetation, and sedimentation) for each population. Future studies examining physiological tolerances and preferences for a range of environmental factors could shed light on factors influencing spawning and foraging distributions of the two splittail populations.

Recommendations for Splittail Conservation

Based on sampling collections and total available spawning area (Feyrer et al. 2005), we believe that the total Petaluma–Napa population is considerably smaller than the Central Valley population and is potentially more vulnerable to future stochastic events. Splittail in the primary spawning and foraging grounds of the Petaluma–Napa population should be monitored to ensure future survival of this genetically distinct splittail population. Specifically, we recommend active splittail monitoring of the Petaluma and Napa rivers during the spawning season. During the nonspawning season, splittail in the Napa River, Pacheco Creek, and Suisun Bay should be monitored, and genetic assignment tests should be conducted to ensure that some of the foraging splittail are from the Petaluma–Napa population.

Given that our study examined adult distributions during a single nonspawning season and that some sites were sampled only once, we believe future genetic work should be conducted to examine the temporal consistency (within and between years) in the observed patterns of spatial segregation. Currently, the proportion of adults remaining as residents in the spawning rivers during the nonspawning season is unknown, and future studies to investigate this would be useful for splittail management. Additionally, a recent study validated the use of otolith microchemistry to track splittail migratory patterns (Feyrer et al. 2007). Otolith microchemistry and genetic markers provide complementary information because of they examine migration on different time scales (an individual lifetime versus multigenerational gene flow, respectively). It would be highly informative to determine whether a single individual returns to the same foraging location throughout its lifetime. If this is the case, then foraging by the Central Valley population is probably not simply opportunistic; thus, undetermined environmental cues may be critical to determining foraging site preference. Obtaining a better understanding of the environmental factors affecting site preferences will allow more effective management of the foraging ecosystems. Finally, we believe that accurate abundance estimates for each population are imperative to successfully monitor and protect splittail in the highly manipulated and constantly changing ecosystem of the San Francisco Estuary watershed.

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