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RESEARCH ARTICLE

Population genetics and conservation implications for the endangered delta smelt in the San Francisco Bay-Delta

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Abstract Over the last two decades, the collapse of the endangered delta smelt (*Hypomesus transpacificus*) in the San Francisco Bay-Delta has resulted in politically charged conservation decisions, including the rationing of valuable Delta water for use in California agriculture and urban centers. A fundamental question remaining in delta smelt conservation is whether current management strategies have been appropriately designed to protect the remaining genetic variation in delta smelt populations, rather than merely mitigating the decline of the species. We used 15 microsatellite markers to characterize genetic variation within and among sampling regions on geographic and temporal scales, to estimate changes in effective population size over time, to determine if a genetic bottleneck exists and to define conservation management units for this species. A genetic bottleneck was detected in each of the four sampling years, and a significant decline in effective population size was observed between sampling years 2003 and 2007. We also detected a weak geographic signal in any given sampling year that was unsupported by temporal consistency of this signal. We assessed two strategies for defining conservation units, and concluded that continuing to manage the species as a single, panmictic population throughout its range is the most feasible management strategy. The results of this study will inform conservation decisions and provide an effective means for genetically monitoring this imperiled species.

Keywords *Hypomesus transpacificus* · Conservation genetics · Effective population size · Bottleneck · Microsatellites · Management units

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Introduction

The number of endangered species of plants and animals continues to rise due to the increasing anthropogenic demand for natural resources (Millennium Ecosystem Assessment 2005). This results in an increasing need to prioritize conservation efforts, as funding and logistical constraints often preclude preservation of a species in its entire range (Faith 1992; Moritz 2002). Conservation prioritization focuses resources on critical populations or habitats to protect existing genetic diversity in order to preserve the ecological and evolutionary processes necessary for species persistence (Crandall et al. 2000; Moritz 2002; Mace and Purvis 2008).

Various methods have been proposed for defining conservation units that are used to prioritize management goals (Mace and Purvis 2008). Traditionally, conservation has been prioritized based on maintaining ecological and evolutionary patterns of diversity (Smith et al. 1993; Myers et al. 2000). More recently, it has been recommended that conservation prioritization should focus on maintaining and restoring evolutionary processes and ecosystem services rather than distinct intraspecific phenotypes (Erwin 1991; Moritz 1995; Rouget et al. 2006). Palsbøll et al. (2007) recommend defining management units based on the amount of genetic divergence at which populations

become demographically independent instead of focusing solely on the rejection of panmixia. Another method involves characterization of ecological and evolutionary patterns of diversity to determine what features need to be conserved in order to maintain evolutionary processes (Moritz 2002). The maintenance of evolutionary processes can be accomplished by maintaining connectedness of populations, ensuring adequate genetic diversity, avoiding inbreeding, and preserving species across a range of native habitats and significant boundary zones (Mace and Purvis 2008).

This study explores the population genetics of the endangered delta smelt (*Hypomesus transpacificus*), an estuarine fish species endemic to the San Francisco Bay-Delta, CA, USA that is at the center of California's water crisis, in order to define conservation management units. Delta smelt are threatened with extinction due to anthropogenic alterations to their ecosystem, including urbanization, non-native species, water diversions, contaminants and the conversion of complex tidal habitats to leveed channels (Nichols et al. 1986; Moyle 2008). Historically, delta smelt were relatively abundant in the Delta, with populations declining dramatically in the 1980s (Newman 2008). They were listed as threatened by both federal and state governments in 1993, and sustained record-low abundance indices prompted their listing as endangered under the California Endangered Species Act in 2010 (USFWS 1993; CDFG 2010b). A major, and very politically contentious, contributor to their decline has been increased water exports from the Delta for urban and agricultural uses (Bennett 2005). Large water pumps at the southern end of the Delta export large volumes of freshwater to supply California's significant agriculture and urban water demands, resulting in altered hydrodynamics of the Delta that degrade delta smelt habitat quality, as well as cause direct mortality of delta smelt through entrainment at the pumps (Bennett 2005).

Because of these extreme anthropogenic alterations to the San Francisco Bay-Delta, the distribution of delta smelt has contracted significantly over the last several decades. Historically, delta smelt were distributed from San Pablo Bay upstream to Sacramento on the Sacramento River and Mossdale on the San Joaquin River, which varied seasonally and with freshwater outflow (Radtke 1966; Moyle et al. 1992; Moyle 2002). Today, large areas of historic delta smelt habitat and designated critical habitat have become unsuitable for some life history stages of the species, even though key environmental characteristics (e.g. temperature, salinity, water depth) of these areas have not changed (CDFG 2003; Miller et al. 2006). Delta smelt disappeared from the southern portion of their historic habitat in the late 1970s, which coincides with substantial increases in the amounts of water exported from the Delta. It is likely that water export operations have a great effect on the distribution, abundance and genetic diversity of delta smelt (Bennett 2005; Simi and Ruhl 2005; Miller et al. 2006).

Conservation managers and scientists are faced with a difficult decision concerning the delta smelt. To protect delta smelt and other fishes, the timing and amount of water exports from the Delta have been altered, which is perceived by some farmers as a threat to their livelihood because of reduced amounts available for irrigation at times (Lund et al. 2010). For this reason, conservation prioritization for this species is essential, as a balance between human needs for water and the needs of this endangered species and its ecosystem must be reached.

In the present study, we analyzed microsatellite data on delta smelt samples collected from their entire remaining geographic range in the San Francisco Bay-Delta over four alternating sampling years to inform conservation management of this species. The goals of this study were to (1) determine the temporal and geographic genetic structure of delta smelt, (2) determine the existence of a genetic bottleneck, (3) estimate the effective population size, (4) define conservation management units, and (5) consider the conservation implications of these findings.

Materials and methods

Population sampling

Delta smelt were collected by the California Department of Fish and Game (CDFG) during the 2003, 2005, 2007 and 2009 Spring Kodiak Trawl Surveys, which were conducted during the delta smelt spawning season from January to May of each year at 39 geographic sampling stations in the San Francisco Bay-Delta (2003: $n = 176$; 2005: $n = 316$; 2007: $n = 336$; 2009: $n = 365$; Fig. 1). We grouped sampling stations into five regions of the Delta by their proximity to one another to facilitate geographic genetic analyses. The southern region of the Delta is not included in our study, as no delta smelt were collected at these sampling stations during these sampling years due to the contraction of their historic range. Abundance indices calculated by CDFG were based on the Fall midwater trawl survey conducted monthly from September through December at 87 sites throughout the Bay-Delta (Stevens and Miller 1983; Sommer et al. 1997; CDFG 2010a). The annual abundance index is the sum of the monthly indices for subareas of the system, and the monthly indices are the average catch per trawl for sites within each subarea multiplied by a volumetric estimate for the subareas, summed across all subareas (Stevens and Miller 1983; Sommer et al. 1997).

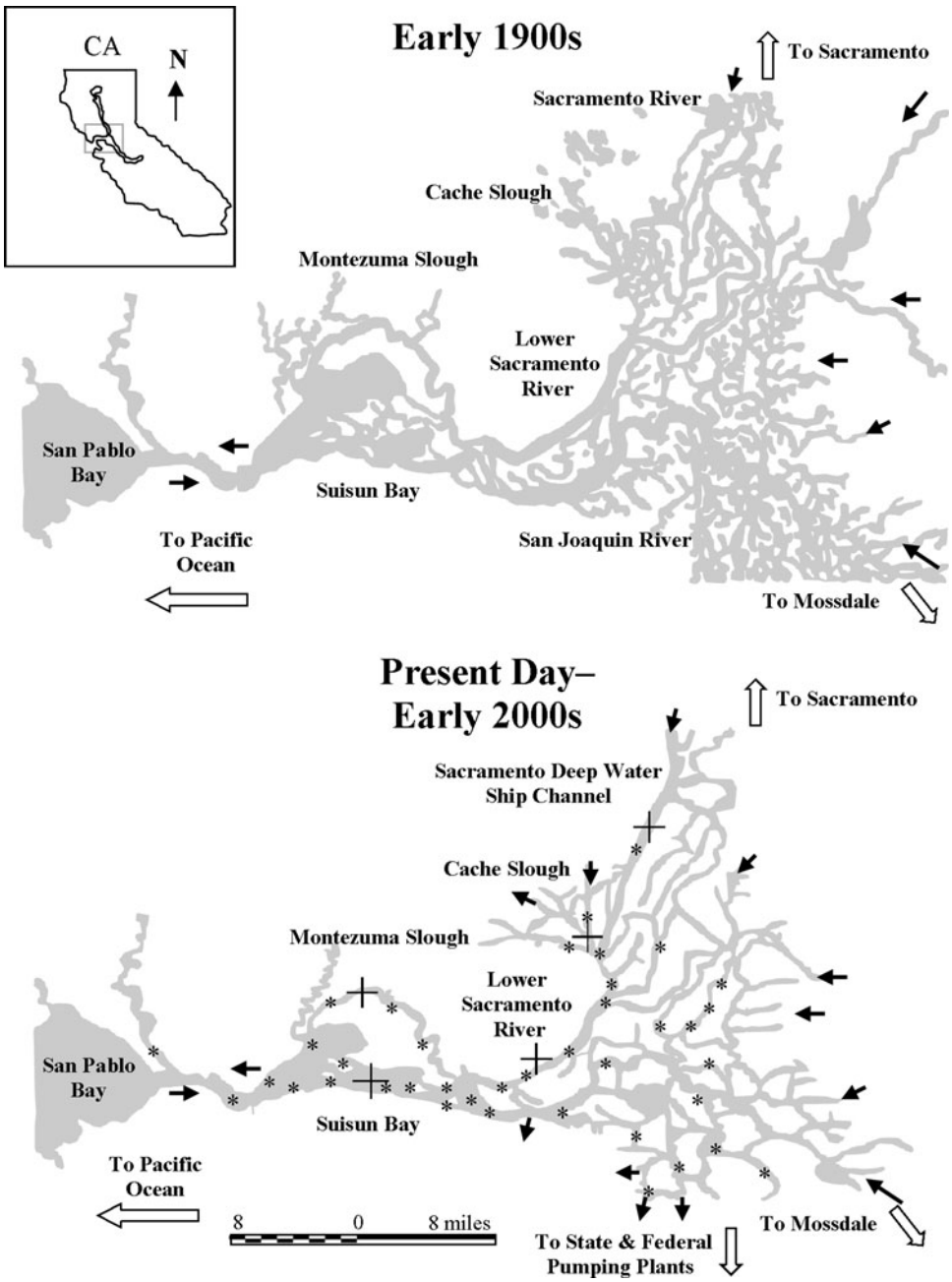

Fig. 1 Map of the San Francisco Bay-Delta, CA. California Department of Fish and Game Spring Kodiak Trawl Survey sampling locations indicated by *, sampling regions indicated by + and hydrodynamic flows indicated by *black arrows*

Microsatellite amplification and genotyping

Fish muscle tissue was sampled from delta smelt heads preserved in 95% EtOH. Genomic DNA was extracted using the DNeasy Tissue Kit (QIAGEN) following the manufacturer's directions, with all samples yielding high molecular weight DNA. We amplified 15 microsatellite loci by polymerase chain reaction (PCR) for all samples using the procedures described in Fisch et al. (2009). We visualized PCR products using an ABI 3730 DNA Analyzer (Applied Biosystems, Inc.) with the LIZ500 internal size standard. Genotyping was performed using ABI's Genemapper™ 4.0 and allele scores were verified manually.

Genetic diversity and differentiation

Genetic diversity was estimated as the number of alleles per locus (A), observed heterozygosity (H_O) and expected heterozygosity (H_E) using CERVUS 3.0 (Kalinowski et al. 2007). Estimations were conducted for all four sampling years independently, sampling regions within years, and sampling regions across years. Allelic richness (A_R) was calculated as a measure of the number of alleles adjusted for sample size using FSTAT 2.9.3 (Goudet 2001) to compare sample sets with different sample sizes. The Wilcoxon signed-rank test was used to determine statistical significance.

The presence of null alleles was determined using MICRO-CHECKER (Van Oosterhout et al. 2004). Exact tests of Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were conducted using GENEPOP 3.4 (Raymond and Rousset 1995) based on the Markov chain method using 1,000 dememorization steps, 100 batches and 1,000 iterations per batch. AMOVA and pairwise comparisons of F_{ST} and R_{ST} between sample sets were calculated and tested for statistical significance with 16,000 permutations in ARLEQUIN 3.1 (Excoffier et al. 2005). The harmonic mean of Jost's D_{EST} across all loci was estimated using the program SMOGD (Jost 2008; Crawford 2010). Significance was determined for F_{ST} and R_{ST} after applying sequential Bonferroni correction (Rice 1989).

We implemented a Bayesian clustering method in STRUCTURE 2.3.3 (Pritchard et al. 2000) to estimate the number of genetic clusters (K) and the proportion of membership of those clusters. Assuming admixture and correlated allele frequencies, we performed 25 independent runs at each K value, assuming $K = 1$ to 10 with 1×10^6 Markov chain Monte Carlo (MCMC) repetitions and a burn-in period of 5×10^5 using no prior information. The steepest increase of the probability of K was measured by plotting the probability of the data [P(D)] and the ad hoc statistic ΔK to determine the most likely value for K (Evanno et al. 2005).

We conducted four sets of analyses in STRUCTURE with the aforementioned parameters. First, we pooled all sample sets over years and sampling sites to determine if temporal and geographic samples were in fact genetically distinct. We pooled samples within each sampling year to assess temporal genetic variation. In addition, we pooled samples across years for each geographic sampling site to assess geographic genetic variation. Finally, we analyzed each year by sampling site to determine within year geographic genetic variability.

Effective population size and bottleneck tests

We used a two-sample method based on temporally separated samples and a one-sample method, based on estimates of linkage disequilibrium, to obtain genetic estimates of the effective population size of the delta smelt population. We used sample sets from every other year, which represents two generations, as delta smelt are an annual fish. Delta smelt are known to live into their second year only in captivity (J. Lindberg, personal communication), so we can assume delta smelt are a strictly annual fish in the wild, allowing this method of N_e estimation to be robust against bias caused by overlapping generations. N_e estimation based on genetic data alone has many limitations and uncertainties, but as we are interested only in relative differences between years and not absolute effective population sizes, these estimation methods are robust to violations of assumptions, as values are compared within the same system (Luikart et al. 2010).

The temporal method (N_e[TM]) operates based on the logic that the difference in gene frequencies between two temporally collected samples from the same population are inversely proportional to the effective population size in the absence of migration and mutation (Waples 1989; Scribner et al. 1997). The linkage disequilibrium method (N_e[LD]) measures the associations between alleles across several loci allowing for the estimation of inbreeding, as a loss of variation is compounded by an increase in linkage disequilibrium, which reduces the frequency of novel gene combinations (Hill 1981; Peel et al. 2004). Both of these methods were implemented in N_eESTIMATOR 1.3 (Peel et al. 2004).

We used an analysis developed by Cornuet and Luikart (1996) to test for recent population bottlenecks in each sampling year and site. This method tests whether there has been a recent reduction in allelic variation in a single population sample based on the loss of rare alleles. We used the program BOTTLENECK 1.2.02 (Piry et al. 1999) to implement this analysis with the following parameters: stepwise mutation model (SMM) and two-phase mutation model (TPM) tested over a range of 0–15% multi-step mutations, as these are the most appropriate for microsatellites (Di Rienzo et al. 1994; Garza and Williamson 2001). We used the Wilcoxon signed-rank test to determine the significance of heterozygosity excess. We calculated combined P-values using Fisher's method and the Z-transform method to test the overall significance of bottlenecks across regions for each mutation model (Whitlock 2005).

We also used the Garza and Williamson (2001) M-ratio test to test for bottlenecks. After a severe bottleneck, M (the ratio of the number of alleles over the range in fragment sizes) is predicted to decline, as the number of alleles should decrease faster than the fragment size range (Garza and Williamson 2001). We used AGARst (Harley 2001) to calculate the mean ratio of the number of alleles to the range in allele size for each locus, M, to detect reductions in population size. We used the program M-CRIT developed by Garza and Williamson (2001) to determine the critical M-ratio below which population declines are inferred using an N_e of 6,000, 10% percent mutations greater than one step and four for the average size of a non one-step mutation. M-ratio values less than M-Crit indicate a recent bottleneck and statistical significance was indicated when $P \leq 0.05$ (Garza and Williamson 2001).

Results

A total of 401 alleles were detected in the 15 microsatellite loci analyzed, which ranged in number of alleles from 6 to 35 alleles per locus. The average expected heterozygosity

for all loci was 0.82. For all years combined, we observed significant departures from HWE for *HtrG107, HtrG118,* and *HtrG126* in 2 of the 5 regions; and for *HtrG114, HtrG115, HtrG119,* and *HtrG129* in 1 of the 5 regions (Table 1). There was not a significant probability of null alleles at any of the loci according to MICRO-CHECKER, as the frequency of null alleles at each locus was less than five percent. GENEPOP indicated no linkage disequilibrium between any of the loci over all sampling regions or within sampling regions. The average allelic richness (A_R) for the four sampling years was 20.8 (SD 0.5), and did not significantly differ between years or regions ($P > 0.05$).

Genetic differentiation

Population divergence, measured as F_{ST}, revealed a weak geographic differentiation signal across sampling years and inconsistent temporal genetic differentiation. Significant levels of differentiation were observed between regions in years 2003 & 2005 and 2005 & 2009 and among regions within years only in 2005 between Suisun Bay and Montezuma Slough ($F_{ST} = 0.007$, $P < 0.001$). After Bonferroni correction, F_{ST} values were statistically significant when comparing geographic samples from Montezuma Slough and Suisun Bay in 2005. Among years, there was not a consistent pattern of significant F_{ST} values between regions after Bonferroni correction (Table 2). AMOVA indicated that the highest variance among samples occurred within individuals, supporting the lack of temporal or spatial population substructure.

A similar genetic differentiation pattern was observed when R_{ST}, a measure of differentiation for microsatellites assuming a stepwise mutation model, was calculated in the program ARLEQUIN (Table 4 in Appendix 1). Significant levels of differentiation were observed between years after Bonferroni correction (2003 & 2007; 2005 & 2007) and between regions in years 2003 & 2005 and 2005 & 2009. After Bonferroni correction, R_{ST} values were statistically significant when comparing geographic samples from Montezuma Slough and Suisun Bay in 2005, and Sacramento Deep Water Ship Channel and the Lower Sacramento River in 2009 (Table 4 in Appendix 1).

Jost's differentiation measure, D_{EST}, a summary statistic based on the effective number of alleles, revealed a similar genetic differentiation pattern in 2005 among regions and similar levels of differentiation observed between all years (Table 4 in Appendix 1, and Table 5 in Appendix 2).

We performed STRUCTURE analyses pooling all regions and years. This analysis revealed that 3 genetic demes were present among the 5 regions sampled over 4 years ($K = 3$; $L(K) = -82{,}000$; $\Delta K = 3.5$). All of the genetic clusters included individuals from all regions and years, indicating lack of consistent geographic or temporal structuring. Given

Table 1 Sample size, allelic richness, levels of heterozygosity and the inbreeding coefficient of all delta smelt sampled in five regions throughout their range in the San Francisco Bay-Delta over four sampling years

	2003						2005						2007						2009					
	N	A_R	H_O	H_E	*HW*	F_{IS}	*N*	A_R	H_O	H_E	*HW*	F_{IS}	*N*	A_R	H_O	H_E	*HW*	F_{IS}	*N*	A_R	H_O	H_E	*HW*	F_{IS}
Region																								
Suisun Bay	11	12	0.83	0.82	0	−0.01	31	12	0.79	0.81	0	0.03	–	–	–	–	–	–	15	12	0.82	0.83	0	0.01
Montezuma Slough	15	12	0.83	0.84	0	0.01	114	13	0.82	0.84	0	0.02	91	13	0.82	0.83	0	0.01	91	13	0.82	0.83	1	0.01
Lower Sacramento River	93	12	0.82	0.82	0	0.01	42	12	0.82	0.82	1	0.01	42	13	0.79	0.83	0	0.04	151	13	0.82	0.83	2	0.01
Cache Slough Complex	57	12	0.81	0.82	1	0.01	87	13	0.79	0.83	0	0.04	60	13	0.83	0.83	0	−0.01	–	–	–	–	–	–
Deep Water Ship Channel	–	–	–	–	–	–	42	13	0.77	0.83	4	0.07	143	13	0.82	0.84	3	0.02	108	12	0.82	0.80	0	0.03
All populations pooled		20	0.82	0.83		0.01		21	0.80	0.83		0.034		21	0.82	0.83		0.015		21	0.81	0.83		0.02
Total	176				1		316				5		336				3		365				3	

N number of individuals, A_R allelic richness[a]; H_O observed heterozygosity, H_E expected heterozygosity, *HW* number of loci with significant Hardy–Weinberg disequilibrium[b], F_{IS} inbreeding coefficient

[a] Allelic richness (A_R) based on a minimum sample size of 156 diploid individuals for the pooled value compared between years and on 20 diploid individuals when compared within and between years for each region

[b] Statistically significant at $P < 0.05$ after Bonferroni correction

Table 2 Pairwise population F_{ST} values for sampled delta smelt in each study region in the San Francisco Bay-Delta collected in four sampling years (lower diagonal) and *P*-values (upper diagonal)

Region & year	2003				2005					2007				2009		
	SB	MS	LS	CS	SB	MS	LS	CS	DW	MS	LS	CS	DW	MS	LS	DW
2003					NS					NS				NS		
SB		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
MS	0.001		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
LS	−0.001	−0.001		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CS	−0.001	−0.002	−0.001		NS	0.017*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
2005	−0.002									NS				NS		
SB	0.002	−0.001	−0.002	0.001		0.001**	NS	NS	NS	NS	NS	NS	NS	NS	0.04*	NS
MS	0.001	0.001	−0.001	0.002	0.007		NS	NS	NS	NS	NS	NS	NS	NS	NS	0.002**
LS	−0.003	−0.006	0.001	−0.001	0.002	−0.001		NS	NS	NS	NS	NS	NS	NS	NS	NS
CS	−0.008	−0.007	−0.007	−0.006	−0.004	−0.007	−0.002		NS	NS	NS	NS	NS	NS	NS	NS
DW	−0.020	−0.005	−0.020	−0.010	−0.008	−0.010	−0.010	−0.004		NS	NS	NS	NS	NS	NS	NS
2007	−0.003				−0.001									NS		
MS	−0.008	−0.009	−0.006	−0.006	−0.001	−0.004	−0.007	−0.001	−0.005		NS	NS	NS	NS	NS	NS
LS	−0.002	0.001	−0.003	−0.002	−0.001	0.001	−0.002	−0.007	−0.010	−0.005		NS	NS	NS	NS	NS
CS	−0.003	−0.003	−0.005	−0.003	−0.001	−0.001	−0.006	−0.002	−0.006	0.001	−0.001		NS	NS	NS	NS
DW	−0.003	−0.004	−0.001	−0.001	−0.001	−0.001	−0.001	0.001	−0.006	−0.001	−0.001	0.001		NS	NS	NS
2009	−0.004				−0.003					−0.006						
MS	−0.020	−0.020	−0.020	−0.020	−0.010	−0.005	−0.020	−0.030	−0.020	−0.030	−0.020	−0.020	−0.020		NS	NS
LS	−0.004	−0.001	−0.001	−0.001	0.002	−0.001	−0.001	−0.009	−0.010	−0.010	−0.005	−0.006	−0.003	−0.010		NS
DW	−0.003	−0.001	−0.002	−0.002	0.001	0.003	−0.001	−0.006	−0.010	−0.006	−0.002	−0.003	−0.001	−0.010	−0.001	

SB Suisun Bay, *MS* Montezuma Slough, *LS* Lower Sacramento, *CS* Cache Slough, *DW* Deep Water Ship Channel, *NS* not significant

Zero values indicate F_{ST} value < 0.001

* Significant ($P < 0.05$) differentiation

** Significant ($P < 0.01$) differentiation after Bonferroni corrections

these results, we performed STRUCTURE analyses for each year independently to determine the existence of independent genetic demes (K) within each year. The analysis revealed that one genetic deme was present in 2003 ($K = 1$; $L(K) = -13{,}100$; $\Delta K = 8$), 3 genetic demes were present in 2005 ($K = 3$; $L(K) = -22{,}750$; $\Delta K = 3.8$), one deme was present in 2007 ($K = 1$; $L(K) = -25{,}900$; $\Delta K = 2.7$), and 5 demes were present in 2009 ($K = 5$; $L(K) = -28{,}000$; $\Delta K = 6.5$). Similar to the results from the STRUCTURE analysis with all years and regions pooled, all of the genetic clusters included individuals from all regions within a year and the majority of individuals were of mixed ancestry, indicating a lack of consistent geographical structuring and high levels of admixture between regions. Geographic differentiation did not coincide with the proportions of demes detected by STRUCTURE.

Effective population size & bottleneck detection

The moments-based temporal method yielded an N_e of 1,430 (95% CI: 970–2328) when all of the samples were pooled over regions and years. The linkage disequilibrium N_e of each year independently was 7,744 (95% CI: 2,736–10,000) in 2003; an N_e of 2,408 (95% CI: 1,821–3,524) in 2005; an N_e of 1,111 (95% CI: 969–1,296) in 2007; and an N_e of 2,435 (95% CI: 1,881–3,428) in 2009 (Fig. 2).

Significant excess heterozygosity, indicating a recent bottleneck, was observed in Suisun Bay and the Lower Sacramento River in 2003 (P-values = 0.03 and 0.04, respectively), in Montezuma Slough and the Deep Water Ship Channel in 2005 (P-values = 0.02 and 0.03, respectively), in the Deep Water Ship Channel in 2007 (P-value = 0.02), and in no regions in 2009 (Table 3). Mean H_{eq}, calculated as the unweighted mean of locus-specific estimates of equilibrium heterozygosity, was 0.82 in 2003, 0.83 in 2005, 0.83 in 2007 and 0.84 in 2009 (Table 3). Using Fisher's method to calculate combined P-values to test the overall significance of bottlenecks across regions, we found significant excess heterozygosity in years 2003, 2005 and 2007 (2003: $P = 0.006$; 2005: $P = 0.001$; 2007: $P = 0.002$; 2009: $P = 0.191$), indicating a bottleneck in these years. Using the Z-transform method, we detected significant excess heterozygosity in all four sampling years, indicating an ongoing population bottleneck (2003: $P = 0.002$; 2005: $P < 0.001$; 2007: $P < 0.001$; 2009: $P = 0.030$) (Table 3). The results remained significant for both mutation models and for all proportions of multi-step mutations.

The critical value for M (M_c) was calculated to be 0.741 using the program M-CRIT. M-ratios averaged across all loci for each year were 0.897, 0.912, 0.844 and 0.876 for 2003, 2005, 2007 and 2009, respectively (Table 3). One locus in

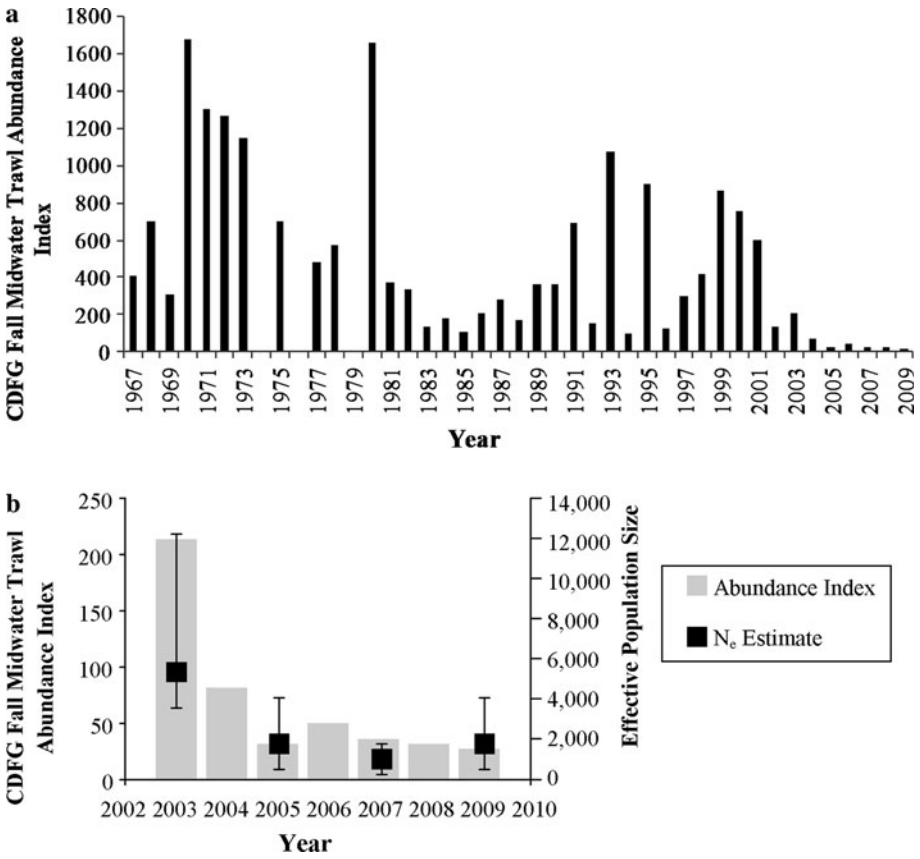

Fig. 2 Delta smelt abundance index and effective population size. **a** California Department of Fish and Game Fall Midwater Trawl Abundance Index from 1967–2009 (CDFG 2010a). **b** Estimates of delta smelt effective population size in years 2003, 2005, 2007 & 2009

Table 3 Results and *P*-values from BOTTLENECK tests implemented in the programs Bottleneck and AGARst for each region and over regions combined within years

Region & year	# Loci[a]	H_{eq}^{b}	*P*-value[c]	Fisher's method[d]	Z-Transform method[e]	M-ratio (variance)[f]
2003		0.82		0.006*	0.002*	0.90 (0.007)
Suisun Bay	13		0.03*			
Lower Sacramento River	11		0.04*			
Cache Slough	11		0.09			
2005		0.83		0.001*	<0.001*	0.91 (0.008)
Suisun Bay	9		0.30			
Montezuma Slough	12		0.02*			
Lower Sacramento River	12		0.10			
Cache Slough	13		0.06			
Deep Water Ship Channel	11		0.03*			
2007		0.83		0.002*	<0.001*	0.84 (0.035)
Montezuma Slough	10		0.30			
Lower Sacramento River	10		0.16			
Cache Slough	10		0.19			
Deep Water Ship Channel	11		0.02*			
2009		0.84		0.191	0.030*	0.88 (0.024)
Montezuma Slough	9		0.35			
Lower Sacramento River	10		0.23			
Deep Water Ship Channel	11		0.16			

* Statistically significant *P*-values at $P < 0.05$

[a] Number of microsatellite loci with with significant excess observed heterozygosity for regions within years

[b] The unweighted mean of locus-specific estimates of equilibrium heterozygosity in each year

[c] *P*-values of bottleneck tests using the Wilcoxon signed-rank test for each region

[d] Combined *P*-values to test the overall significance of bottlenecks across regions using Fisher's method

[e] Combined *P*-values of bottlenecks across regions using the Z-transform method

[f] Garza and Williamson's M-ratio and variance (mean across all loci) calculated in the program AGARst

2003, 2005 & 2009 had an M-ratio less than M_c and three loci in 2007 had M-ratios less than M_c.

Discussion

Genetic differentiation

Our results demonstrate that genetic diversity has been maintained over the four sampling years and between sampling locations within years, as there was no significant difference in allelic richness between years or sampling locations within years and F_{IS} values over all years per collection site and over all samples per collection year are in equilibrium. It is reasonable to see genetic diversity maintained over such a short time period, even in a population that is in the process of a bottleneck, when population abundance has stabilized and the population remains outbred (Fig. 2).

Overall, the genetic data indicate a weak geographic signal among sampling regions, unsupported by temporal consistency in this signal, indicating the existence of a single, panmictic population. In all cases of geographic and temporal genetic differentiation, the F_{ST}, R_{ST} and D_{EST} values were very low (< 0.05 for all pairwise comparisons; Table 2, Table 4 in Appendix 1, and Table 5 in Appendix 2). While some of these values are statistically significant given the large sample sizes, the magnitude of the difference is very small, suggesting a lack of biological relevance.

Levels of genetic differentiation in the population differed between calculations of F_{ST}, R_{ST} and D_{EST} (Table 2, Table 4 in Appendix 1, and Table 5 in Appendix 2). Estimates of F_{ST} revealed less differentiation than did estimates of R_{ST} and D_{EST}, including the lack of significant differentiation between regions in 2009. The genetic differentiation in 2005 between Suisun Bay and Montezuma Slough was statistically significant at $P < 0.001$ after Bonferroni correction using both F_{ST} and R_{ST}, and estimates of D_{EST} for these regions were of higher magnitude than F_{ST} and R_{ST}. We calculated all three estimators to determine if the choice of estimator had an effect on the levels and patterns of population differentiation observed.

Meirmans and Hedrick (2010) advocate using other estimators in addition to F_{ST} when highly variable markers

are used, such as R_{ST} and D_{EST}. R_{ST} is expected to give more accurate estimates of differentiation than F_{ST} if there is memory in the mutation process, although R_{ST} can be less accurate at reflecting population differentiation than F_{ST} due to its high associated variance (Balloux and Lugon-Moulin 2002). According to a meta-analysis of 34 published studies conducted by Heller and Siegismund (2009), D_{EST} appears to be the most appropriate estimator of population differentiation for microsatellite studies and is best suited for describing allelic differentiation among populations, although it is insensitive to population size and may take a long time to reach equilibrium (Meirmans and Hedrick 2010). However, F_{ST} is still useful as a fixation index for measuring levels of inbreeding at different hierarchical levels, and these data are presented here for comparison to other studies (Meirmans and Hedrick 2010).

Bayesian assignment of individuals to genetic demes within years revealed a similar pattern to that of the population differentiation results, as multiple genetic demes were inferred in years 2005 and 2009, but only one deme was inferred in both 2003 and 2007. However, these genetic clusters include individuals from all regions within a year and do not correspond to geographically separated sampling regions within the Delta.

We conclude that the delta smelt population is panmictic, which is expected, as the San Francisco Bay-Delta is a highly connected ecosystem, delta smelt have historically large population sizes and since the majority of delta smelt spawning is thought to occur in the same location (Moyle et al. 1992; Bennett 2005). The ephemeral nature of the population differentiation may be a result of sampling fish during the spawning season, where they are actively migrating from brackish to fresh water to spawn (Moyle et al. 1992). It may also indicate the existence of different migration patterns of subsets within the population, such as resident fish in the Sacramento Deep Water Ship Channel, natal fidelity or high variance in reproductive success. In addition, previously collected samples of wild delta smelt have been analyzed for genetic relatedness, and there is no evidence that random collections of delta smelt include family groups (K. Fisch, unpublished data). This existence of a single, panmictic population is also supported by a previous population genetics study of delta smelt using allozyme markers, although only a few loci were used with a limited geographic sample size (Trenham et al. 1998). However, samples from the San Joaquin River region were not included in either study, as delta smelt were not present at these sampling stations during the survey period due to a reduction in their historic range. The weak geographic differentiation signal may be attributed to anthropogenic homogenization of the species due to this reduced historic range or may be due to sampling artifacts. Future studies examining historic delta smelt samples from the San Joaquin River region are needed to further clarify the historic population structure and patterns of genetic diversity in delta smelt.

Reduced effective population size

The effective population size decreased significantly from 2003 to 2007, indicating a decrease in genetic diversity between these years, even though this pattern was not similarly observed as a decline in allelic richness, as allelic richness may have already been reduced in previous bottlenecks as seen in Fig. 2. The decrease in effective population size is closely linked to the decrease in the abundance index between 2003 and 2007, calculated based on the methods in Stevens and Miller (1983) by the California Department of Fish and Game during the Fall Midwater Trawl (CDFG 2010a) (Fig. 2). In addition, N_e increases slightly from 2007 to 2009, which may indicate a slight increase in genetic diversity between these years. As a population declines, genetic variation is lost, which can be seen as a reduction in the effective population size. The N_e decline detected without a similarly observed decrease in allelic richness may be due to the short sampling period, or as a result of these samples coming from an already declining population with potentially previously reduced allelic richness. Effective population size is an important tool for monitoring genetic variation in threatened populations. Thus, it will be imperative to monitor N_e as an indicator of the success of management strategies for delta smelt (Schwartz et al. 2007; Antao et al. 2010).

Detection of ongoing bottleneck

The presence of a genetic bottleneck was also detected in all sampling years, indicating that the delta smelt population is currently losing genetic diversity as it declines. This signal is expected to persist, as delta smelt currently have sustained low population abundances. This can also be observed as a decrease in census size in the Fall Midwater Trawl abundance index (Fig. 2). The bottleneck signal within sampling regions may inform the pattern of the decline, but may also be due to sampling artifacts. The Cornuet and Luikart method for detecting bottlenecks does not provide an estimate of the timing of the decline (Cornuet and Luikart 1996). However, the genetic signal of the decline, corroborated by the observed census size declines, support the hypothesis that decreases in N_e have likely occurred over the last few decades. This method has been cited as being the most effective at detecting recent changes in N_e (Garza and Williamson 2001; Williamson-Natesan 2005). The M-ratio tests do not indicate a strong bottleneck signal; however, a simulation study comparing the Cornuet and Luikart method with the *M*-ratio test demonstrated that the Cornuet and Luikart method is better at detecting less severe, more recent bottlenecks than the *M*-ratio

test (Williamson-Natesan 2005). Since rare alleles are quickly lost from populations owing to bottleneck events and genetic variation takes longer to change, it is surprising that we detected a decrease in genetic variation over time via effective population size, but not as a decrease in the number of alleles. This may be a result of the existence of previous demographic bottlenecks seen in Fig. 2a.

Statistical tests for bottlenecks assume random mating and no gene flow, and as a result, nonrandom mating or population substructure can produce genealogies that resemble bottlenecks, whereas gene flow may resemble recent expansions (Cornuet and Luikart 1996; Goossens et al. 2006; Busch et al. 2007). The bottlenecks detected for delta smelt may be artifacts of nonrandom mating or gene flow, as there was evidence of statistically significant but low magnitude F_{ST} values between regions. As delta smelt likely comprise a panmictic population, gene flow among sampling regions is high. Gene flow can mimic recent expansion in a population. Since a consistent bottleneck signature was found within regions in spite of the presence of gene flow, we are provided with even stronger evidence for recent reductions in N_e. This may result in the existence of bottlenecks that are more severe than they appear in the analyses (see Funk et al. (2010)). As a result of the observed bottlenecks, delta smelt may become increasingly threatened by reductions in N_e, by experiencing inbreeding depression and the loss of adaptive genetic variation. This may increase the rate of decline through a process known as an extinction vortex (Soule and Mills 1998).

Defining conservation units

These results can be used to define conservation units in two possible prioritization strategies. 1. Managing the species as a single panmictic population throughout its range, or 2. Managing populations in different parts of the Delta as multiple distinct conservation units or preserving only certain subsets of the population due to limited resources. Each prioritization strategy is detailed as follows.

Managing delta smelt as a single panmictic population throughout its range will not alter the conservation management of this species, as this is how it is currently managed (Miller et al. 2006). Resources will continue to be allocated to protect the entire population, and no geographic localities will be favored. This strategy may result in local extinction of some distinct subpopulations, as limited resources or tradeoffs in management decisions may make management of the species throughout the Delta less effective (Taylor et al. 2000). However, if the effective population size is maintained, this strategy will result in the maintenance of the overall genetic diversity, providing the species with the potential to adapt to future environmental challenges.

On the other hand, delta smelt could be managed differently in different parts of the Delta, such as in regions with highly suitable or restored habitat, to prioritize the use of limited resources. By managing delta smelt in different regions independently, local extinction may be avoided, but it may require a significant increase in resources and might be unfeasible (Mace and Purvis 2008). To assess the need for managing the delta smelt population as multiple conservation units, we used the criteria recommended by Palsbøll et al. (2007) that based the delineation of management units on the amount of population differentiation at which populations become demographically independent instead of simply rejecting panmixia. This approach emphasizes the dispersal rate of individuals as the parameter of interest to conservationists instead of the historical amount of gene flow (Palsbøll et al. 2007).

Using these criteria to assess the management of geographically-defined subpopulations of delta smelt, we calculated the amount of genetic divergence among regions as a function of the number of migrants per generation estimated as mN_e, where m is the probability that an individual is a migrant and N_e is the effective population size, assuming selective neutrality and equilibrium conditions (Palsbøll et al. 2007). While this is a simplistic metric based on unrealistic assumptions, this method is used here to simply demonstrate how gene flow and migration rates may be used in conjunction with population differentiation values to inform management decisions. Based on these calculations, using an average N_e of 1,500 over all sampling years and a criterion of at least 10% exchange between sites, regions would be demographically isolated if they exchanged less than $\sim$150 adults (Hastings 1993). This corresponds to an F_{ST} value of 0.016 under a Wright-Fisher island population model. From this, we could conclude that regions constitute separate management units if their genetic divergence exceeds $F_{ST} = 0.016$ (Palsbøll et al. 2007).

None of the statistically significant F_{ST} values between regions or years was greater than 0.016, providing further evidence that this is a panmictic population and should be managed as such. Prioritizing conservation management based on geographic delineations would result in drastic increases in conservation resources required to manage each management unit independently, and if only certain conservation units were protected due to limited resources, the preservation of the species may potentially be jeopardized. In addition, local adaptation can occur even in the face of ongoing gene flow, so it is important to conserve the species throughout its entire range to preserve the evolutionary potential of the species. As a result, we recommend that delta smelt continue to be managed as a single, panmictic population in order to focus efforts on maintaining the effective population size as opposed to maintaining conservation units throughout the Delta.

Preserving the genetic diversity of a single species in an imperiled ecosystem is only one piece of the conservation puzzle. Conservation managers can use this information to develop an ecosystem-wide conservation plan that focuses on mitigating the causes of ecosystem decline in an effort to protect multiple species, by using single species genetic diversity as an attainable goal for their conservation plan. Future conservation plans for delta smelt and the San Francisco Bay-Delta should integrate data on the distribution of species genetic diversity with historical and current ecological data. The survival of this species and the Bay-Delta ecosystem depends upon a balance between water management and anthropogenic water uses that can only be reached through conservation management and habitat remediation.

Conclusions and implications for conservation management

The increasing need for conservation prioritization makes it essential to evaluate strategies for defining management units of endangered species. Many different strategies have been proposed; however, their practical application is often nebulous. The results of this study demonstrate the utility of applying a straightforward strategy for defining conservation units that is based on traditional population genetic methods, but uses more stringent criteria for designating conservation units. The presence of a genetic bottleneck in all sampling years, coupled with a reduction in effective population size over time, highlights the need for careful conservation management and continued genetic monitoring of this imperiled species. Preserving intraspecific genetic diversity is vital to the overall goal of species conservation, as it provides a good indicator of success of protecting the ecological and evolutionary processes necessary for species persistence.

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Appendix 1

See Table 4.

Appendix 2

See Table 5.

Table 4 Pairwise population R_{ST} values for sampled delta smelt in each study region in the San Francisco Bay-Delta collected in four sampling years (lower diagonal) and *P*-values (upper diagonal)

Region & year	2003				2005				2007				2009			
	SB	MS	LS	CS	SB	MS	LS	CS	DW	MS	LS	CS	DW	MS	LS	DW
2003					NS					0.02*				NS		
SB		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
MS	0.002		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
LS	0.002	0.000		NS	NS	0.002**	NS	NS	0.02*	NS	NS	NS	NS	NS	NS	0.04*
CS	0.001	−0.003	−0.002		NS	0.01**	NS	NS	0.04*	NS	NS	NS	NS	NS	0.013*	NS
2005	−0.006									0.01**				NS		
SB	−0.001	−0.001	−0.001	−0.003		0.008**	NS	NS	0.04*	NS	NS	NS	NS	NS	NS	NS
MS	0.001	0.001	0.010	0.010	0.020		NS	NS	0.04*	NS	NS	NS	NS	0.011*	0.001**	0.001**
LS	−0.006	−0.006	0.005	0.000	−0.001	0.006		NS	NS	NS	NS	NS	NS	NS	NS	NS
CS	−0.008	−0.007	−0.006	−0.005	−0.002	0.002	−0.001		0.05*	NS	NS	NS	NS	NS	NS	NS
DW	−0.005	−0.005	0.010	0.010	0.020	0.010	−0.002	0.015		0.001**	NS	0.035*	0.002**	0.016*	NS	0.001**

Table 4 continued

Region & year	2003				2005					2007				2009		
	SB	MS	LS	CS	SB	MS	LS	CS	DW	MS	LS	CS	DW	MS	LS	DW
2007	0.002				0.002									NS		
MS	−0.009	−0.009	−0.007	−0.008	−0.010	0.002	−0.007	0.005	0.030		NS	NS	NS	NS	NS	NS
LS	0.005	0.006	−0.003	−0.001	0.000	0.007	−0.003	−0.002	0.014	−0.006		NS	NS	NS	NS	NS
CS	−0.003	−0.003	−0.001	0.000	−0.004	0.007	−0.001	−0.003	0.010	−0.007	−0.01		NS	NS	NS	NS
DW	−0.005	−0.004	0.000	−0.004	−0.008	0.003	−0.003	0.005	0.020	−0.001	−0.001	−0.003		NS	NS	NS
2009	0.001				−0.002					0.001						
MS	0.000	0.000	−0.008	−0.001	0.001	0.010	0.003	−0.011	0.020	−0.014	−0.002	−0.018	−0.013		NS	0.03*
LS	0.003	0.002	0.009	0.010	0.002	0.020	−0.001	0.002	0.000	−0.002	0.002	−0.004	−0.002	−0.005		0.001**
DW	0.006	0.005	0.010	0.005	0.006	0.010	0.004	−0.003	0.030	−0.013	−0.004	−0.006	−0.001	0.010	0.010	

SB Suisun Bay, *MS* Montezuma Slough, *LS* Lower Sacramento, *CS* Cache Slough, *DW* Deep Water Ship Channel, *NS* not significant

Zero values indicate R_{ST} value <0.001

* Significant ($P < 0.05$) differentiation

** Significant ($P < 0.01$) differentiation after Bonferroni corrections

Table 5 Pairwise population D_{EST} values (Jost 2008) for sampled delta smelt in each study region in the San Francisco Bay-Delta collected in four sampling years (lower diagonal)

Region & year	2003				2005					2007				2009		
	SB	MS	LS	CS	SB	MS	LS	CS	DW	MS	LS	CS	DW	MS	LS	DW
2003					–					–				–		
SB		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
MS	0.001		–	–	–	–	–	–	–	–	–	–	–	–	–	–
LS	0.001	0.000		–	–	–	–	–	–	–	–	–	–	–	–	–
CS	0.000	0.001	0.001		–	–	–	–	–	–	–	–	–	–	–	–
2005	0.0001									–				–		
SB	0.002	0.001	0.001	0.001		–	–	–	–	–	–	–	–	–	–	–
MS	0.000	0.000	0.003	0.001	0.014		–	–	–	–	–	–	–	–	–	–
LS	0.000	0.000	0.002	0.000	0.003	0.002		–	–	–	–	–	–	–	–	–
CS	0.000	0.000	0.000	0.000	0.000	0.001	0.000		–	–	–	–	–	–	–	–
DW	0.000	0.000	0.004	0.000	0.013	0.014	0.007	0.000		–	–	–	–	–	–	–

Table 5 continued

Region & year	2003				2005				2007				2009		
	SB	MS	LS	CS	SB	MS	LS	CS	DW	MS	LS	CS	DW	MS	LS	DW
2007	0.0009				0.001									–		
MS	−0.004	0.000	0.000	0.000	0.000	0.003	0.002	0.000	−0.001		–	–	–	–	–	–
LS	−0.001	0.000	0.000	0.000	0.001	0.001	0.000	−0.002	0.001	−0.001		–	–	–	–	–
CS	0.000	0.000	0.002	0.004	0.003	0.003	0.001	0.000	0.002	0.001	0.001		–	–	–	–
DW	0.000	0.000	0.001	0.002	0.000	0.005	0.003	2.000	0.005	−0.001	0.000	0.001		–	–	–
2009	0.0002				0.001					0.0002						
MS	0.000	0.000	0.000	0.000	0.001	0.006	0.005	0.000	0.000	0.001	0.000	0.000	0.000		–	–
LS	−0.002	0.000	0.004	0.000	0.004	0.002	0.000	0.001	0.005	−0.001	0.000	0.002	0.000	0.000		–
DW	−0.001	0.000	0.004	0.000	0.006	0.007	0.011	0.000	0.003	0.000	0.000	0.001	0.005	0.000	0.000	

SB Suisun Bay, *MS* Montezuma Slough, *LS* Lower Sacramento, *CS* Cache Slough, *DW* Deep Water Ship Channel

Zero values indicate D_{EST} value <0.001

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