

Hybridization between delta smelt and two other species within the family Osmeridae in the San Francisco Bay-Delta

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Abstract Hybridization among closely related species may pose a threat to species persistence, especially between native and introduced species. We analyzed nine microsatellite loci, mitochondrial sequences and 16 species-specific single nucleotide polymorphisms (SNPs) in two native species (delta smelt and longfin smelt) and one introduced species (wakasagi smelt) in the family Osmeridae to describe the extent of hybridization among these species in the San Francisco Bay-Delta, CA, USA. We identified 29 putative hybrids with a microsatellite-based Bayesian assignment method, and we further screened these putative hybrids with the SNP loci and mitochondrial DNA (mtDNA) sequencing. From the Yolo Bypass, 11 % of morphologically ambiguous individuals were F₁ hybrids and 0.1 % of positively identified delta smelt from throughout the San Francisco Bay-Delta were F₁ hybrids according to their SNP genotypes. mtDNA sequencing revealed wakasagi smelt as the maternal parent for all five delta smelt × wakasagi smelt hybrids and longfin smelt as the maternal parent for the single longfin smelt × delta smelt hybrid. Hybridization among these three species appears to occur at relatively low frequencies and may not

be an immediate threat to the persistence of the imperiled native species; however, the presence of hybrid individuals warrants continued monitoring.

Keywords Delta smelt · Longfin smelt · Wakasagi smelt · *Hypomesus* · Microsatellites · Single nucleotide polymorphisms (SNP)

Introduction

Interspecific hybridization with introduced species poses a serious risk to native species; it can result in the loss of genetic integrity, the invasion of nonnative species, and the extinction of indigenous taxa (Miller et al. 1989; Leary et al. 1995; Rhymer and Simberloff 1996; Boyer et al. 2008). The San Francisco Bay-Delta, California, USA (Bay-Delta) is a highly invaded system, with >200 introduced nonnatives (Cohen and Carlton 1998). The co-existence of a rich community of invasive species alongside many threatened species makes hybridization of particular concern in this system, and there have been several known cases of genetic integrity erosion in native Californian species due to introgression (Moyle 2002; Weigel et al. 2003; Cordes et al. 2006).

Two native Osmerid fish species that inhabit this estuarine ecosystem are the endemic delta smelt (*Hypomesus transpacificus*) and the longfin smelt (*Spirinchus thaleichthys*). Although abundant in the past, the delta smelt population has experienced substantial decline in recent years and was classified as a federally threatened species in 1993 and as endangered under the California Endangered Species Act in 2010 (USFWS 1993; Newman 2008; CDFG 2010). The longfin smelt was listed as threatened under the California Endangered Species Act in 2009 (CDFG 2009).

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The decline of these species has been attributed to a number of causes, including entrainment losses to water diversions, reduced water outflow, toxin accumulation and introduction of nonnative species (Moyle 2002).

Though numerous exotic species likely detrimental to delta smelt and longfin smelt populations have been introduced into this system, including predators and competitors, the wakasagi smelt (*Hypomesus nipponensis*) is of particular interest because it is the only nonnative Osmerid in the Bay-Delta. The wakasagi smelt is a Japanese native that was successfully transplanted into reservoirs throughout the Sacramento drainage in 1959 as a forage fish (Moyle 2002). Though the wakasagi smelt is not very abundant in the San Francisco Bay-Delta (Allen et al. 2006), over the past several years, wakasagi smelt and morphologically ambiguous *Hypomesus* specimens have been consistently detected within the Yolo Bypass, a flood bypass for the Sacramento River where all three species' ranges overlap (USGS 2012 and Adib-Samii, personal communication). This mixing zone is worth investigating, as interspecific hybridization has the potential to reduce the genetic integrity of delta smelt.

Limited evidence of hybridization between these two species exists to date. Stanley et al. (1995) concluded that the two species are too genetically distinct for introgression to take place, while Trenham et al. (1998) discovered two F_1 hybrids between delta smelt and wakasagi smelt. May (1996) also identified interspecific hybrids between delta smelt with both wakasagi and longfin smelt by allozyme analysis. Although the extent of the hybridization shown in these studies was minimal, environmental conditions within the estuary have changed over the past decade, making it important to elucidate the current extent of hybridization in the Bay-Delta (May 1996).

We examined hybridization among these three Osmerid species using three genetic methods: (1) We employed a Bayesian assignment method using microsatellite markers to screen a large number of individuals for putative hybrid individuals; (2) We developed 16 single nucleotide polymorphism (SNP) markers to quantify putative hybrid individuals determined by the Bayesian assignment method; and (3) We sequenced mitochondrial DNA (mtDNA) from these putative hybrid individuals to quantify the directionality of hybridization. By elucidating patterns of hybridization among native and introduced Osmerid species, the outcomes of this study will improve species conservation management in the San Francisco Bay-Delta.

Methods

Reference samples were collected for each of the three species by the California Department of Fish and Game

(CDFG; delta smelt: $n = 30$; longfin smelt: $n = 30$; wakasagi smelt: $n = 30$) from throughout the San Francisco Bay-Delta, CA, USA (Table 1; Appendix Fig. 2). Forty-four morphologically ambiguous (intermediate or ambiguous phenotype) Osmerid specimens were opportunistically collected when discovered during normal surveys from the Yolo Bypass ($38^{\circ}21'12.46''N$, $121^{\circ}38'34.71''W$) by the California Department of Water Resources, CDFG, and the United States Army Corp of Engineers in 2009. A reference sample set of 839 delta smelt samples collected by CDFG during the Spring Kodiak Trawls (SKT; conducted throughout the San Francisco Bay-Delta from 2003 to 2007) were also screened for the presence of hybrid individuals.

Whole genomic DNA was extracted from fin clips preserved in 95 % ethanol using the DNeasy Tissue Kit (QIAGEN). We amplified 9 microsatellite loci that co-amplified in all three species by polymerase chain reaction (PCR) for all samples according to the methods described in Fisch et al. (2009). Genetic diversity statistics were calculated with the software program GENETIC DATA ANALYSIS (GDA) version 1.1 (Lewis and Zaykin 2001). Sequential Bonferroni correction was applied to correct statistical significance levels for multiple comparisons (Rice 1989). We calculated allelic richness (A_R) with the software program FSTAT 2.9.3 (Goudet 2001). Pairwise F_{ST} values among species were calculated and tested for significance with 16,000 permutations in ARLEQUIN 3.5 (Excoffier et al. 2005).

We used the assignment method of Pritchard et al. (2000) to detect interspecific hybrid individuals using the program STRUCTURE v2.3.4. We performed a preliminary STRUCTURE analysis of the three groups of reference samples with K values ranging from one to ten, 10 independent iterations, 1×10^6 Markov chain Monte Carlo (MCMC) repetitions, admixture, independent allele frequencies and a burn-in period of 5×10^5 using no prior information. We then performed 10 independent iterations for $K = 3$ to represent the 3 species with the same parameters. We included the reference samples for each species, morphologically ambiguous individuals, and the SKT delta smelt samples. This run of STRUCTURE was used to assign q_i values to all individuals. We ran STRUCTURE a third time to test for recent hybridization according to the procedures in Schwartz and Beheregaray (2008) and Bingham et al. (2012) using the parameters above, with the addition of prior population information.

We simulated 500 individuals of each pure species and hybrid class (F_1 hybrids, F_2 hybrids, and first generation backcrosses (BC_1)) using HYBRIDLAB (Nielsen et al. 2006) to assess the accuracy of parental assignments using STRUCTURE. Individuals were simulated five separate times, and we used STRUCTURE to evaluate each

Table 1 Number of individuals screened, total number and private alleles per species and hybrids detected by sampling year and region for the SNP validation method

Species and year	Training samples			Screened samples														
	Delta Smelt	Wakasagi Smelt	Longfin Smelt	2009	2007	2005						2003						Total
Sampling station	All	All	All	YB ^a	MS	LS	CS	DW	SB	MS	LS	CS	DW	SB	MS	LS	CS	
N	30	30	30	44	91	42	69	143	31	114	42	87	42	11	15	93	59	883
Number of alleles	120	106	75	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Private alleles	72	42	28	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Total hybrids	0	0	0	5 (12)	1 (3)	0	0	0 (6)	0	0 (2)	0	0	0 (6)	0	0	0	0	6 (29)

Hybrids determined by the Bayesian assignment method are displayed in parentheses

SB Suisun Bay, MS Montezuma Slough, LS lower Sacramento, CS Cache Slough, DW deep water ship channel, YB Yolo Bypass

^a All samples collected were from the CDFG SKT with the exception of those sampled at Yolo Bypass, which were collected specifically to identify potential hybrids

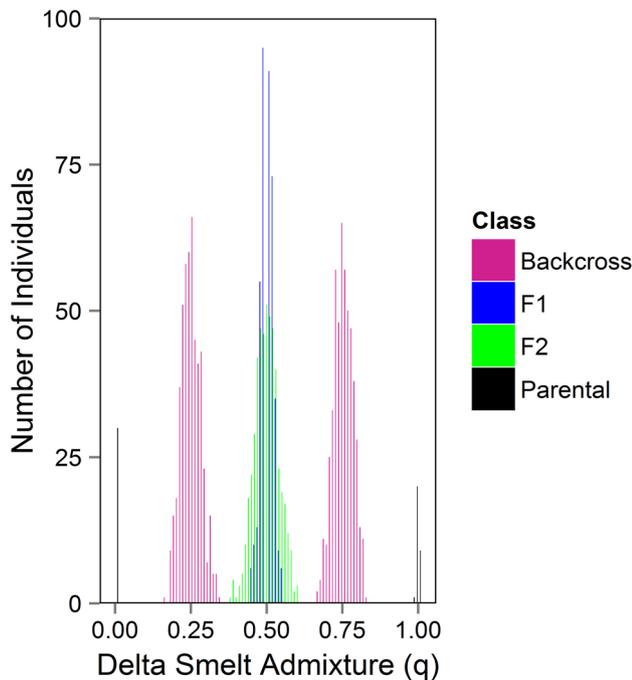


Fig. 1 Distribution of genotypes from the parental and hybrid simulations. The q_i values represent the proportion of genetic contribution from delta smelt (delta smelt = 1.0, other species = 0.0). The graph depicts the range of STRUCTURE-based q_i values of the simulated pure species, F₁, F₂ and BC₁ hybrids generated in HYBRIDLAB. The q_i value thresholds for hybrid detection are 0.001 and 0.998

replicate. We ran STRUCTURE ($K = 3$) with no prior information and the same parameters as above. Based on the q_i values from the STRUCTURE runs and our hybrid class simulation, individuals were classified into hybrid classes as described in Bingham et al. (2012) (Fig. 1).

We developed species-specific SNP assays to complement the microsatellite-based hybrid assignment method (Appendix Supplemental Methods). We screened putative hybrid

individuals identified with the Bayesian assignment method with the SNP panel and sequenced their mtDNA. To distinguish between pure and hybrid individuals among the three species, we identified haplotypes for four nuclear loci (Appendix Table 3). An individual was assigned a haplotype for each nuclear locus when at least two diagnostic SNPs were successfully genotyped (i.e. one SNP with an allele unique to one species and second SNP unique to another species). Individuals with all four haplotypes assigning to the same species were considered pure/non-hybrid, while individuals heterozygous at any locus or possessing conflicting haplotype assignments were classified as hybrids (Appendix Table 4). With four nuclear loci used per individual, the chance of misidentification of any F₁ backcross was <6.25 % (1/16). Samples that did not meet this requirement due to failed amplification were removed from further analysis.

Results and discussion

Delta smelt and longfin smelt, the two native Osmerids in the San Francisco Bay-Delta, have faced population declines over the last few decades, and may be further threatened through interspecific hybridization with the introduced wakasagi smelt (May 1996; CDFG 2009, 2010). Although interspecific hybridization is a natural evolutionary phenomenon, anthropogenic influences may artificially increase rates of hybridization, especially with the introduction of nonnative species (Rhymer and Simberloff 1996; Barton 2001; Seehausen 2004).

Genetic diversity statistics calculated for each species indicated that the allele frequencies of each species are unique, enabling the assignment of individuals to one or more species. Most of these loci contained private alleles for each species, although species differences were largely due to differences in the frequencies of common alleles (Appendix Table 5). The three species were genetically

Table 2 Potential hybrid individual identities based on the Bayesian assignment method and on the SNP-based method

ID	Sample location	Bayesian assignment method					SNP-based method		
		D q_i	W q_i	L q_i	Posterior probability No hybrid ancestry	p	Hybrid Class based on μ sat	Hybrid Class based on SNPs	mtDNA
1	2005 DW	0.977	0.002	0.021	0.273	**	BC ₁ -D/L	DS	DS
2	2005 DW	0.923	0.002	0.075	0.27	**	BC ₁ -D/L	DS	DS
3	2005 DW	0.96	0.026	0.014	0.767		BC _x -D/W	DS	DS
4	2005 DW	0.959	0.018	0.023	0.74		BC _x -D/L	DS	DS
5	2005 DW	0.969	0.002	0.03	0.55		BC _x -D/L	DS	DS
6	2005 DW	0.894	0.102	0.004	0.416	*	F ₂ -D/W	DS	DS
7	2005 MS	0.926	0.002	0.073	0.265	**	BC ₁ -D/L	DS	DS
8	2005 MS	0.964	0.029	0.006	0.474	*	F ₂ -D/W	DS	DS
9	2007 DW	0.946	0.013	0.041	0.844		BC _x -D/L	DS	DS
10	2007 DW	0.804	0.193	0.003	0.752		BC _x -D/W	DS	DS
11	2007 DW	0.702	0.001	0.297	0.023	***	F ₁ -D/L	DS	DS
12	2007 DW	0.443	0.001	0.556	0.437	*	F ₁ -D/L	DS	DS
13	2007 DW	0.974	0.001	0.024	0.214	**	F ₂ -D/L	DS	DS
14	2007 DW	0.901	0.002	0.097	0.3	*	F ₂ -D/L	DS	DS
15	2007 MS	0.89	0.004	0.107	0.609		BC _x -D/L	F ₁ -D/L	LS
16	2007 MS	0.958	0.001	0.041	0.671		BC _x -D/L	DS	DS
17	2007 MS	0.621	0.001	0.378	0.022	***	F ₁ -D/L	DS	DS
18	YB	0.977	0.02	0.003	0.736		BC _x -D/W	–	WS
19	YB	0.959	0.015	0.026	0.811		BC _x -D/W	DS	DS
20	YB	0.885	0.06	0.055	0.642		BC _x -D/L	DS	DS
21	YB	0.662	0.335	0.003	0.622		BC _x -D/W	F ₁ -D/W	WS
22	YB	0.043	0.022	0.935	0.588		BC _x -D/L	LS	LS
23	YB	0.025	0.077	0.898	0.651		BC _x -L/W	LS	LS
24	YB	0.54	0.454	0.006	0.655		BC _x -D/W	F ₁ -D/W	WS
25	YB	0.461	0.535	0.004	0.665		BC _x -D/W	WS	WS
26	YB	0.591	0.404	0.005	0.43	*	F ₁ -D/W	F ₁ -D/W	WS
27	YB	0.642	0.355	0.004	0.129	**	F ₁ -D/W	F ₁ -D/W	WS
28	YB	0.093	0.904	0.003	0.427	*	F ₂ -D/W	F ₁ -D/W	WS
29	YB	0.17	0.232	0.598	0.198	**	F ₂ -D/L	LS	LS

q_i is the mean proportion of admixture within individuals from STRUCTURE. Posterior probability from second run of STRUCTURE for the probability of mixed ancestry

D Delta smelt, W Wakasagi smelt, L Longfin smelt

– Indicates missing data or not enough information

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

distinct, with significant pairwise F_{ST} values after Bonferroni correction ($p < 0.001$; delta smelt and wakasagi smelt: $F_{ST} = 0.13$; delta smelt and longfin smelt: $F_{ST} = 0.16$; longfin smelt and wakasagi smelt: $F_{ST} = 0.21$). The simulation results reveal that the nine microsatellite loci used in this study should allow for the detection of 100 % of all first and second generation hybrids (F₁, F₂, and BC₁) from parental genotypes, as there is no overlap between simulated hybrid and parental species q_i value distributions (Fig. 1).

Levels of hybridization among the three Osmerid species appear to be low based on both the Bayesian and SNP based assignment methods (Tables 1, 2, Appendix Table 4). Our analyses revealed that delta smelt hybridize with both wakasagi smelt and longfin smelt. Five individuals collected in the Yolo Bypass in 2009 were determined to be F₁ hybrids between delta smelt and wakasagi smelt (Table 1). One individual collected in 2007 from Montezuma Slough was determined to be an F₁ hybrid between delta smelt and longfin smelt. Less than 0.1 % of the

individuals from the SKT were classified as F_1 hybrids according to the SNP genotyping method (Table 2, Appendix Table 5). Backcrosses between hybrids and the three species were detected in this study only using the Bayesian assignment method, and may likely be an artefact of using this method with non-diagnostic microsatellite markers, low levels of genotyping error (<5 %, empirically estimated) and/or because of the small number of SNPs screened in this study (Table 2). With SNP validation, no introgression was observed, and only F_1 hybrids were positively identified, suggesting that hybridization is much less prevalent than the Bayesian assignment method would indicate; alternately, the SNP panel may be less sensitive to detecting introgression than the Bayesian assignment method (Appendix Table 5).

These results reveal infrequent hybridization in individuals positively identified as delta smelt by California Department of Fish and Game during the annual SKTs. However, morphologically ambiguous fish are discovered by CDFG, and genetic analysis of a collection of such morphologically ambiguous fish from the Yolo Bypass revealed that a significant proportion of these fish were hybrids (11 %). This is to be expected, as these fish had intermediate morphological characteristics between two species and the Yolo Bypass is a relatively unique area within the Bay-Delta where wakasagi smelt have become established and overlap in range with the other two species (USGS 2012). mtDNA sequencing revealed wakasagi smelt as the maternal parent for all five delta smelt \times wakasagi smelt hybrids and longfin smelt as the maternal parent for the single longfin smelt \times delta smelt hybrid. This result indicates that hybridization is likely unidirectional in this system.

Little is known about the relative fitness of hybrids, making it difficult to predict the evolutionary consequences of hybridization among these three species. An investigation of hybrid fitness is warranted, to assess the reproductive capacity, pathogen resistance, and health of hybrids compared to parental species. Even when introgression is not present, hybridization can contribute to competition with non-hybrids, predation, disease transmission and wasted reproductive effort, which may contribute to altered genetic diversity and reduced viability and productivity of native populations (Laikre et al. 2010). Although hybridization was detected in this study and it does not appear to be an immediate threat to the native Osmerids in the Bay-Delta, it may increase in the future due to declining population abundances, changes in spawn timing due to climate change, and increased invasion of the Bay-Delta by wakasagi smelt (May 1996). Due to these factors, continued genetic monitoring of these native species may be important in the long run to allow managers to implement mitigation strategies if introgression is observed.

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