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Evaluating the Performance of Captive Breeding Techniques for Conservation Hatcheries: A Case Study of the Delta Smelt Captive Breeding Program

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Abstract

The delta smelt, an endangered fish species endemic to the San Francisco Bay-Delta, California, United States, was recently brought into captivity for species preservation. This study retrospectively evaluates the implementation of a genetic management plan for the captive delta smelt population. The captive genetic management plan entails tagging fish, molecular data collection, pedigree reconstruction, relatedness estimation, and recommending fish crosses annually in an effort to minimize the average coancestry in the population and limit inbreeding. We employed 12 microsatellite DNA markers to examine temporal genetic diversity in consecutive, discrete generations to determine the effects of intensive genetic management on the population and to quantify the amount of wild genetic diversity present within each captive generation. Wild fish are incorporated into the captive population each generation to minimize genetic drift, and 91% of the original founders are still represented in the F_3 generation. The average mean kinship in the third generation in captivity was 0.0035. There was no evidence of significant genetic divergence of the captive population from the wild population. The results of this study yield management insights into the practical application of genetic management plans for captive populations and conservation hatcheries, in an attempt to preserve the genetic integrity of endangered species.

Key words: *genetic management,* Hypomesus transpacificus, *mean kinship, microsatellites, pedigree reconstruction, relatedness*

The delta smelt, *Hypomesus transpacificus* (Osmeriformes, Osmeridae), is a pelagic planktivorous fish threatened with extinction due to anthropogenic ecosystem alterations (Moyle et al. 1992). The species is endemic to the San Francisco Bay-Delta (California, United States), which has become highly urbanized and affected by the introduction of nonnative species, water diversions, contaminants, and the conversion of complex tidal habitats to leveed channels (Nichols et al. 1986; Moyle et al. 1992). Although relatively abundant prior to 1980, the species subsequently dramatically declined (Newman 2008), due, in part, to increased water exports from the Bay-Delta for urban and agricultural uses and ecosystem alterations caused by both nonnative species and humans (Feyrer et al. 2007; Sommer et al. 2007; Baxter et al. 2008). The species was listed as threatened by both federal and state governments in 1993 and was listed as endangered under the California Endangered Species Act in 2010 (USFWS 1993; CDFG 2010). In response to the species' declining abundance, captive breeding efforts were initiated to establish a captive assurance population of delta smelt at the University of California, Davis Fish Conservation & Culture Laboratory (FCCL) (Lindberg et al. Forthcoming). The goal of the delta smelt captive breeding program is to create a genetically and demographically robust captive population that will act as a genetic bank in the event this species becomes extinct in the wild, as well as potentially serve as a source for supporting wild populations if such a need arises (Fisch et al. 2009a, 2009b, 2010).

Traditionally, the goal of most fish hatcheries has been to boost the wild adult census size by supplementing wild stocks with fish reared for part of their life in captivity (Lichatowich 1999; Hedrick et al. 2000b; Palm et al. 2003; Waples et al. 2007; Naish et al. 2007). More recently, however, hatchery objectives have diversified and now include the

preservation of the genetic integrity of endangered species (Hedrick et al. 2000a, 2000b; Fraser 2008). Because hatchery fish are released by the millions into the wild every year, hatchery practices have the potential to be severely detrimental to wild fish populations if appropriate captive management and reintroduction plans are not implemented (Heard 1995; Augerot and Foley 2005). As a result, many conservation hatcheries aim to preserve populations that are unable to persist in the wild by implementing management practices that attempt to maintain the genetic integrity of the hatchery populations (Utter and Epifanio 2002). The ultimate goal of these conservation hatcheries is similar to zoo-based conservation breeding programs: to maintain genetic variability and fitness within captive populations until these species can be reintroduced to the wild as self-sustaining populations (Utter and Epifanio 2002; Pollard and Flagg 2004; Fraser 2008).

The delta smelt captive breeding program operates under rigorous population management standards similar to successful zoo-based conservation breeding programs. The model adopted by managers of the delta smelt captive breeding program is to control mate selection in an effort to minimize average coancestry in the population, thereby maintaining gene diversity and limiting inbreeding (Ballou and Lacy 1995). Under this model, hatcheries develop genetic management plans for their hatchery and supplementation programs, in an effort to maintain a genetically robust captive population while decreasing the demographic and genetic consequences of supplementing wild populations with hatchery fish (Allendorf and Ryman 1987; Waples and Drake 2004; Araki et al. 2007). The captive assurance population for delta smelt is in its fourth generation as of 2012; our goal for this study was to retrospectively evaluate the founding and subsequent genetic management of the delta smelt captive breeding program. Specific objectives were as follows: 1) evaluate the effectiveness of the past genetic management of a conservation hatchery by calculating founder representations, average mean kinship (*mk*), inbreeding coefficients, effective population size, and the genetic divergence from the wild population; and 2) conduct a molecular analysis to estimate the relatedness of the captive population founders to validate the assumptions of pedigree-based management strategies. The results of this study provide insight into the practical application of genetic management plans for captive populations and conservation hatcheries seeking to preserve genetic integrity of endangered species.

Founding and Historic Management of the Captive Assurance Population

The delta smelt captive breeding program was initiated in 2006 when ~2000 fish were collected from the lower Sacramento River (CA, United States) by the staff at the FCCL for their research propagation efforts. The first captive spawning occurred in 2008 when these fish were 2 years old. A total of 328 of the initially collected wild fish were available for spawning at that time, but only 290 fish successfully produced offspring due to larval mortality in the remaining crosses; these 290 fish represented the F_0 generation. When the F_0 generation was spawned, fish were randomly crossed in single pair crosses (one male and one female) and no individual fish was used twice, resulting in 145 breeding pairs. Fish were spawned at random in this generation because, at the time, these wild-origin fish were assumed to be unrelated and microsatellite markers had not yet been developed to further elucidate relationships.

Following the F_0 generation, the captive assurance population was maintained in discrete generations using 1-year-old fish. Although the F_0 generation was spawned at random, the F_1 through F_3 generations were spawned by methods intended to minimize the average coancestry in the population (Ballou and Lacy 1995; Fernandez and Toro 1999; Sonesson and Meuwissen 2000; Ivy and Lacy 2012). Traditionally, pair selection methods designed to minimize coancestry are based on pedigree calculations of *mk* (Ballou and Lacy 1995), although molecular estimates of relatedness, termed mean relatedness (*mr*), have been proposed as suitable substitutes (Doyle et al. 2001). For the F_1 generation, spawning pairs were based on *mr* calculations; to minimize the average kinship in the next generation, fish with low *mr* values were preferentially selected as breeders. For the F_2 and F_3 generations, spawning pairs were based on *mk* calculations to minimize average kinship in each subsequent generation. A pair selection algorithm designed to identify the group of breeding individuals that would produce the set of offspring with the lowest average kinship was used to preferentially select breeders (Ivy and Lacy 2012); their Ranked MK Selection algorithm). A total of 494 (247 pairs), 466 (233 pairs), and 516 (258 pairs) individuals were spawned from the F_1, F_2, and F_3 generations, respectively. These numbers include additional wild-caught fish captured annually.

Prior to spawning the F_1 through F_3 generations, wild fish were captured from the same location in the lower Sacramento River and incorporated into the captive population (F_1: 54; F_2: 34; F_3: 68). Because wild fish were assumed to be unrelated to the existing captive population, wild fish were preferentially mated with wild fish to create new founding pairs of genetically under-represented individuals. However, as the sex ratio of captured wild fish was unequal, wild fish without a wild mate were paired with a captive fish. Wild fish continue to be incorporated into the population each generation to build an open system that allows gene flow from the wild population into the captive assurance population.

Although delta smelt will spawn naturally in captivity if density is high enough, the staff at the FCCL manually express eggs from a single female and combine them with milt from a single male to create a single pair cross (Lindberg et al. Forthcoming). Approximately, 1000 larvae from each full-sibling family are then combined in tanks (~8 families/tank) due to facility space limitations. Consequently, parentage analyses are needed to reconstruct the pedigree that is then used to calculate *mk* values used for genetic management. Each generation, candidate adult

broodfish are genotyped at 12 microsatellite loci according to Fisch et al. (2009a), and the resulting molecular data are used in conjunction with the software program Cervus (version 3.0; (Kalinowski et al. 2007) to assign parentage to candidate spawners. Briefly, a parent pair (sexes known) parentage analysis is conducted with an empirically estimated genotyping error rate of 0.01, calculated from genotyping replicate samples, and fixed confidence intervals of 95%. Candidate parents are limited to the individuals known to have contributed to the tank of fish that each individual originated from in the previous generation. Across the F_1 through F_3 generations, 98.3% of analyzed fish were conclusively assigned parents from the previous generation to reconstruct the pedigree. Fish that were not assigned parentage had insufficient microsatellite data (i.e., too many missing genotypes) for the analysis and were consequently not used as spawners.

Methods

Sample Collection and DNA Preparation

Tissue samples were collected from the caudal or adipose fin of captive adult fish at the FCCL and preserved in 95% EtOH. Thousands of candidate adult spawners (fish approximately 1 year old) were sampled each generation prior to spawning to facilitate pedigree reconstruction and genetic management (F_1: n = 1400; F_2: n = 1858; F_3: n = 1753). The fish ultimately selected as breeders in each generation were used for our retrospective genetic analyses (F_0: n = 290; F_1: n = 494; F_2: n = 466; F_3: n = 516). Wild samples (SKT2007 sample set) were obtained from muscle tissue of wild adult fish collected by the California Department of Fish and Game during the 2007 Spring Kodiak Trawl Survey and were preserved in 95% EtOH (n = 372; Figure 1). Wild fish were collected during the spawning season from their entire range (Figure 1) and were assumed to be representative of the species in the wild during the time that the captive population was initiated. Genomic DNA was extracted from all samples using the DNeasy Tissue Kit (Qiagen, Valencia, CA, USA) following the manufacturer's directions, with all samples yielding high molecular weight DNA.

Microsatellite Genotyping

A total of 12 microsatellite loci described by Fisch et al. (2009a) were amplified by polymerase chain reaction (PCR) for both captive and wild samples (Table 1). PCR products were visualized using an ABI 3730 DNA Analyzer (Applied Biosystems, Inc., Carlsbad, CA, USA) with the LIZ500 internal size standard. Alleles were scored using ABI's Genemapper™ 4.0 and verified manually (Applied Biosystems, Inc.). To reduce genotyping errors, two control samples with known allele sizes were included in every 96-well PCR plate, allele calls were independently scored by two people, and genotypes with questionable allele calls were re-amplified and scored again.

Founder Relatedness

The performances of different relatedness estimators differ based on number of alleles, allele frequency distributions and numbers of microsatellite loci used in a study, and the composition of relationship categories present in a population (Queller and Goodnight 1989; Ritland 1996; Lynch and Ritland 1999; van de Casteele et al. 2001; Wang 2002; Milligan 2003). To choose the best estimator, sampling variances for three relatedness estimators were calculated for four relationship categories (full siblings, half siblings, parent–offspring, and unrelated) as in Ivy et al. (2009). Relatedness coefficients for 1000 simulated pairs of individuals in each relationship category were calculated using the software program SPAGeDi (Version 1.3a; (Hardy and Vekemans 2002). For each of the four relationship categories, the means and variances of the relatedness coefficients were calculated for each of the following three estimators: r_{xy}QG (Queller and Goodnight 1989), r_{xy}LR (Lynch and Ritland 1999), and r_{xy}-Wang (Wang 2002). Then, the relatedness estimator possessing the smallest variance across relationship categories was used to calculate relatedness among wild-caught fish.

Relatedness coefficients between all pairs of individuals in the F_0 generation (n = 290) were calculated to determine if any close relatives (i.e., full siblings or half siblings) were present among the fish used to initiate the captive population, to validate the assumptions of a pedigree-based management approach. In pedigree-based management, captive population founders are generally assumed to be unrelated and not inbred (Ballou 1983). Pairs of F_0 individuals identified as close relatives were given hypothetical parents in the captive delta smelt pedigree to capture those relationships, rather than assuming those fish to be unrelated. Those hypothetical parents were assumed to be unrelated to all F_0 fish, conceptually making them founders in place of their assigned offspring.

Founder Representation and *mk*

A population founder is an individual that is unrelated to all other living individuals in the population except its own descendants; they are typically the wild-caught individuals used to initiate a captive population. If a wild-caught fish selected for spawning fails to produce offspring, that fish never becomes a founder of the captive population. Furthermore, founders can be lost from a population over time if they have no remaining descendants in a future generation. The software program PM2000 (Pollak et al. 2002) calculates a variety of genetic summary statistics from a population's pedigree. The reconstructed pedigree data for delta smelt were input into PM2000, which we used to calculate the number of founders (i.e., wild-caught individuals) that the fish selected for spawning each generation could be traced back to through the pedigree. PM2000 was also used to calculate the corresponding founder representations, which are the proportion of genes in each generation's cohort of spawners that are derived from each founder. Founder representations are of interest because genetic diversity retention in a captive population is maximized when founder representations

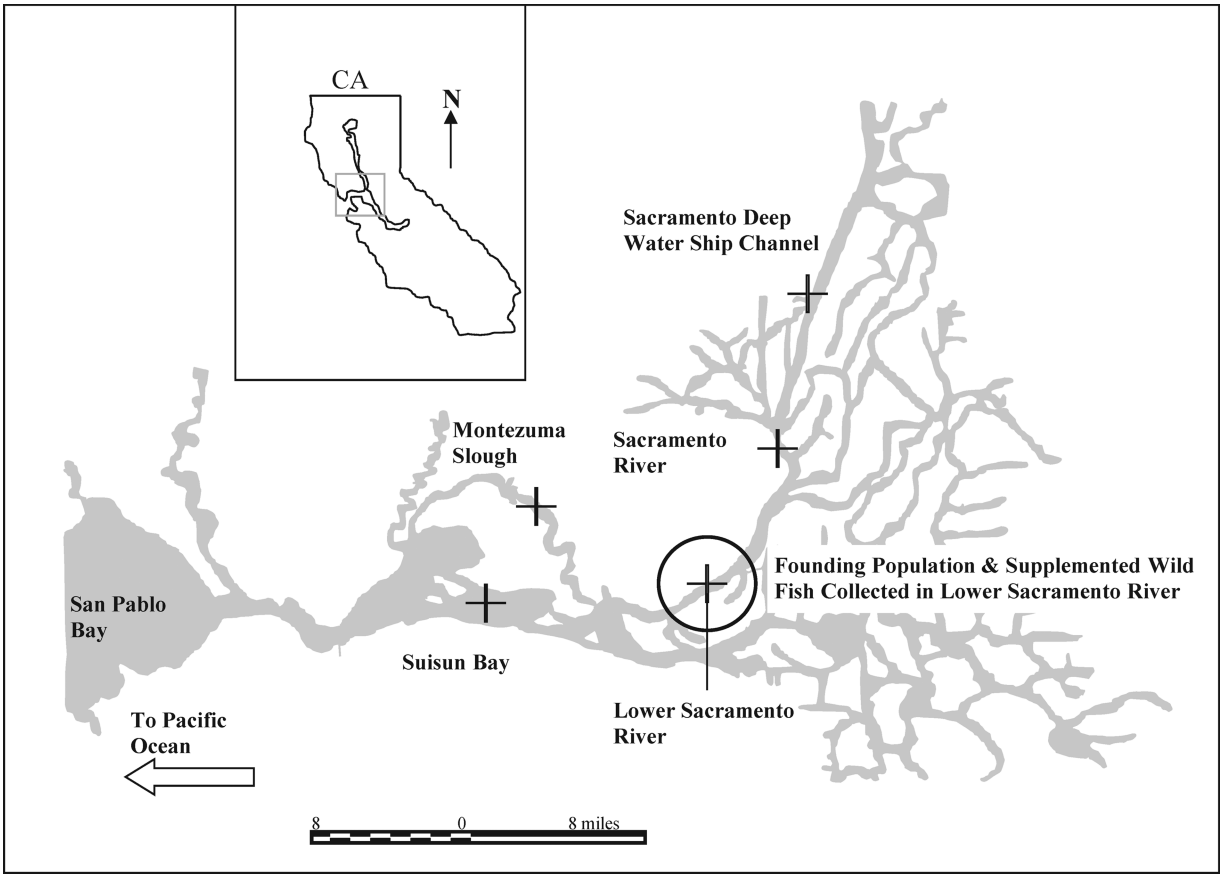

Figure 1. Map of California Department of Fish and Game 2007 Spring Kodiak Trawl Survey sampling locations and collection location of the captive population founders and supplemented wild fish in the San Francisco Bay-Delta, California, United States.

Table 1 Mean relatedness coefficients and variances for three relatedness estimators, based on allele frequencies from the captive delta smelt population

	Relationship Category			
	Unrelated	Half sibling	Full sibling	Parent–offspring
r_{xyQG}	−0.012 (0.010)	0.234 (0.013)	0.487 (0.018)[a]	0.490 (0.004)
r_{xyLR}	−0.011 (0.004)[a]	0.201 (0.014)	0.439 (0.025)	0.445 (0.011)
r_{xyWang}	−0.001 (0.008)	0.243 (0.012)[a]	0.492 (0.019)	0.498 (0.002)[a]

Values were based on 1000 simulated pairs from each of the relationship categories
[a]Smallest variance per relationship category

are equal (Lacy 1989; Lacy 1995). Finally, we used PM2000 (Pollak et al. 2002) to calculate the average *mk* and inbreeding coefficient observed in each captive generation.

Genetic Diversity Statistics

We calculated a variety of molecular and pedigree-based genetic statistics to assess the utility of the historic genetic management of the delta smelt captive breeding program for preserving the genetic integrity of the captive population. Prior to calculating molecular statistics, we used GenePop 3.4 (Raymond and Rousset 1995) to test for Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) using the Markov chain method with 1000 dememorization steps, 100 batches, and 1000 iterations per batch; significance was determined by applying a sequential Bonferroni correction (Rice 1989). We also checked for null alleles with Micro-Checker (van Oosterhout et al. 2004). After these initial analyses were completed, Cervus 3.0 (Kalinowski et al. 2007) was used to estimate genetic diversity for all four captive generations and the wild population (SKT2007 sample set) as the number of alleles per locus (A), observed heterozygosity (H_O), expected heterozygosity (H_E), and polymorphic information content. To compare populations with different sample sizes, allelic richness (A_R) was calculated as a measure of the number of alleles independent of sample size using FSTAT 2.9.3 (Goudet

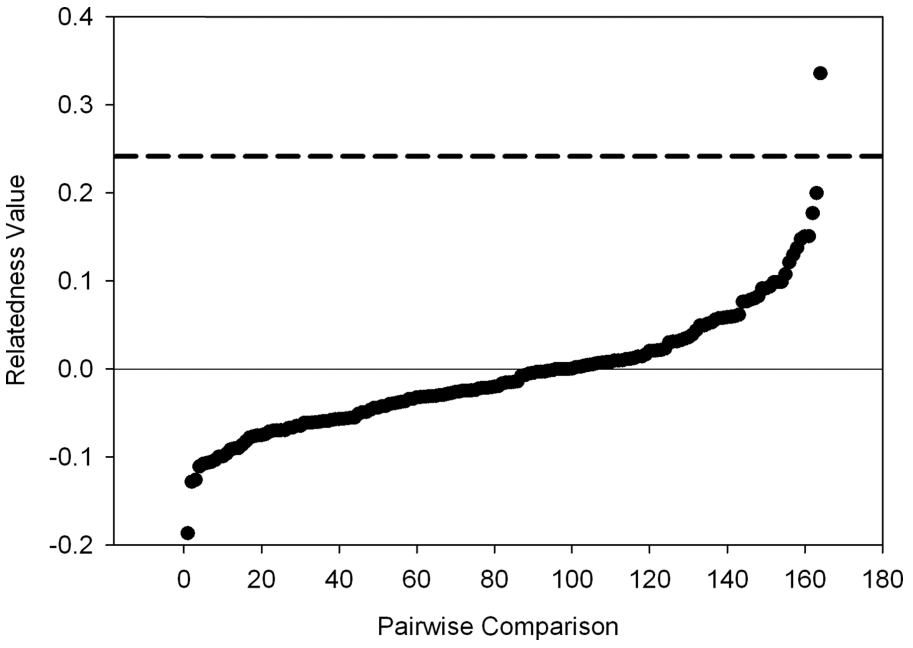

Figure 2. Pairwise relatedness coefficients estimated for the 290 original founders, graphed in order of increasing value. The dashed horizontal line represents the cutoff between related individuals (half siblings) at 0.24 for Wang's r_{xy}. One value was considered to be related based on this cutoff value.

2001); statistical significance was determined using the Wilcoxon matched-pairs signed-rank test. Genetic divergence between the captive and wild delta smelt populations was evaluated by calculating pairwise comparisons of fixation index (F_{ST}) between each captive generation and the wild population and testing for statistical significance with 16,000 permutations in Arlequin 3.1 (Excoffier et al. 2005).

Effective Population Size

We calculated the effective population size of each generation of delta smelt in captivity using both pedigree-based and genetic methods. All of the adult broodfish selected for spawning from each generation were used to calculate the effective population size (N_e) of the genetically managed captive population. Effective population size was also estimated using all sampled adults from each generation to compare with the N_e of the genetically managed captive population.

Pedigree-based Estimates of N_e

The reconstructed delta smelt pedigree was used to calculate the inbreeding effective population size (N_e) using family size as a proxy for lifetime reproductive success. As there are an equal number of female and male parents, N_e was estimated as: $N_e = (Nk - 2)/[k + (V_k/k) - 1]$ with N = total number of parents, k = average family size, and V_k = variance of family size (Crow and Kimura 1970; Herbinger et al. 2006).

Genetic Estimate of N_e

The inbreeding N_e (Ne[LD]) of each parental generation was estimated based on LD among the generation's offspring using the program LDNe (Waples and Do 2008), as it implements the bias correction developed by Waples (2006). This method does not require assumptions about random mating nor information about haplotype frequencies and produces unbiased estimates of N_e over a wide range of samples sizes and true N_e values (Waples 2006). We estimated N_e for each generation in LDNe assuming monogamy (random mating for the wild F_{-1} generation) and excluding rare alleles with frequencies less than 0.02 (*Pcrit* = 0.02).

Results

Founder Relatedness

Using relatedness simulations based on allele frequencies in the F_0 generation, the means and variances of the relatedness coefficients for each of four relationship categories were calculated using three different relatedness estimators (Table 1). Sampling variances ranged from 0.002 to 0.025 across relatedness estimators and from 0.002 to 0.019 for r_{xy}-Wang. The smallest variances were observed for parent–offspring pairs; however, variances were not notably different from one another for the other relationship categories. r_{xy}-Wang possessed the smallest variance across two relationship categories, where the other two estimators had the smallest variance in only one relatedness category each. Although the choice of relatedness estimator likely had little influence on the results, the pairwise relatedness coefficients for the 290 F_0 fish were calculated using Wang's r_{xy}. Given both the distribution of observed relatedness values (Figure 2) and the means and variances observed for the four simulated

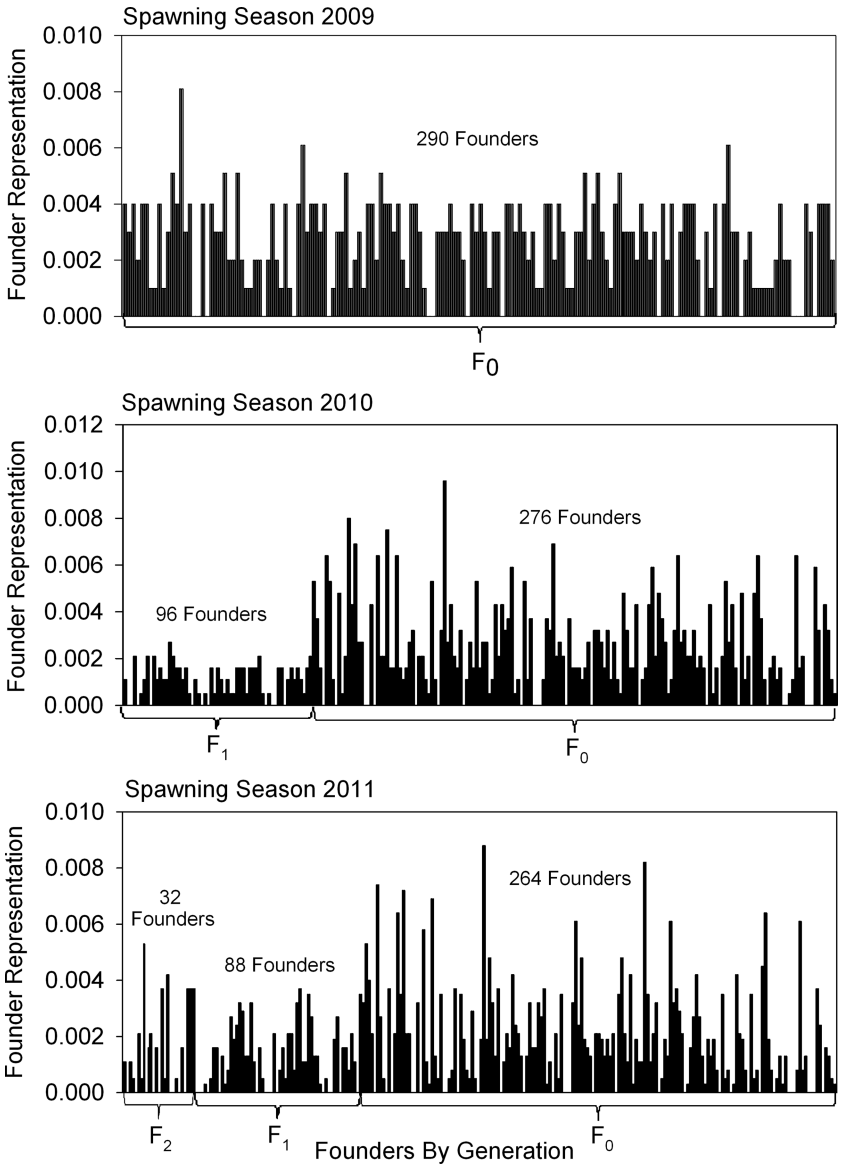

Figure 3. Founder representation and number of founders remaining in each generation of the captive delta smelt population.

relationship categories with this estimator (Table 1), only one pairwise relatedness value indicated what might be a notably close relationship between a pair of founders. One pair of founders with an r_{xy} of 0.34 was assumed to be closely related (Figure 2); although the high variances of the relatedness estimates precluded precise relationship assignment, this pair of founders was assumed to be half siblings to capture a "close relationship" for future pedigree-based management decisions.

Founder Representation and mk

Founder representation varied across generations. Generations F_1, F_2, and F_3 were traced to 408, 442, and 512 founders, respectively. A total of 264 of the original 290 wild-caught F_0 fish (91%) continue to be represented in the F_3 generation. In addition, 248 wild-caught fish incorporated since the F_0 generations are founders of the F_3 generation. Figure 3 demonstrates how founder representations varied both within and across generations over time. The average *mk* for the F_1, F_2, and F_3 generations were 0.0021, 0.0029, and 0.0035, respectively (Figure 4). The inbreeding coefficients for each generation were 0.0000, 0.0000, and 0.0012 for the F_1, F_2, and F_3 generations, respectively.

Genetic Diversity Statistics

A total of 312 alleles were identified for the 12 microsatellite loci in the 2138 samples that were genotyped (Table 2). New alleles (G_{X-X}) were discovered in each captive generation ($G_{0-1} = 10$, $G_{1-2} = 1$; $G_{2-3} = 2$). Eight alleles in the F_1 generation and one allele in the F_2 generation were gained due to the incorporation of wild individuals. Two alleles in the F_1 generation, one allele in the F_2 generation, and two

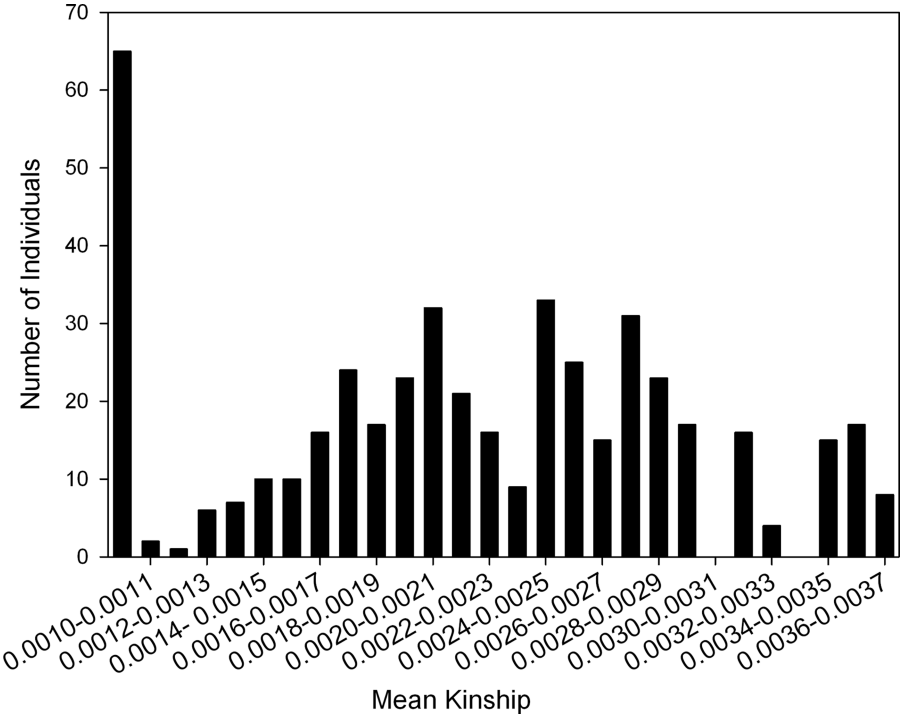

Figure 4. Histogram of *mk* in the F_3 generation of the delta smelt captive population.

alleles in the F_3 generation were possibly gained due to mutation or allelic dropout in the previous generation. Alleles were also lost (L_{X-X}) in each generation ($L_{0-1} = 9$, $L_{1-2} = 15$; $L_{2-3} = 8$). The new alleles gained offset the alleles lost in the F_1 generation, which highlights the importance of bringing wild individuals into captivity to maintain genetic diversity. However, in subsequent generations, the number of new alleles did not offset the number of alleles lost and is likely confounded by the sample size. The number of alleles per locus in the founding population ranged from seven at locus *HtrG104* to a maximum of 33 at locus *HtrG127* (Table 2; Appendix I).

A_R ranged from 7 to 33 alleles at each locus across a combined set of all samples based on a minimum sample size of 156 diploid individuals. When each sample set was analyzed individually, the SKT2007 sample set represented the highest allelic diversity ($A_R = 24.1$), followed by the F_0 generation ($A_R = 22.2$). The allelic diversity of all of the generations in captivity pooled, including the incorporated wild fish, was $A_R = 20.8$, which was not significantly lower than that present in the F_0 generation. When compared across all loci, the difference in A_R between the F_0 generation and wild populations was not significant ($P > 0.05$). However, the F_1, F_2, and F_3 generations had significantly lower A_R than the SKT2007 sample set ($P < 0.02$ for all generations). On average, between the F_0 and F_1 generations, 0.8 alleles per locus were gained and 0.8 alleles per locus were lost. In the F_2 generation, an average of 1.3 alleles per locus were lost from the F_1 generation and 0.1 alleles per locus were gained. Between the F_2 and F_3 generations, 0.2 alleles per locus were lost and 0.7 alleles per locus were gained.

Table 2 Genetic diversity of delta smelt at 12 microsatellite loci, including number of individuals genotyped at each locus (*N*), total number of alleles in each population (A), A_R for each population, H_O, H_E, HWE *P*-values (*P*), and F-statistics within population (F_{IS})

	Captive population				Wild population
	F_0	F_1	F_2	F_3	SKT2007
N	290	494	466	516	372
A	281	282	268	259	289
A_R[a]	22.2	21.4	20.7	20.4	24.1
H_O	0.84	0.84	0.85	0.83	0.84
H_E	0.86	0.86	0.86	0.84	0.86
P	0.29	0.37	0.44	0.51	0.86
F_{IS}	0.01	0.02	0.00	0.01	0.41

[a]A_R based on a minimum sample size of 156 diploid individuals

High levels of average heterozygosity were observed in both the wild and captive populations, although some loci have relatively low heterozygosity. The mean H_E in each of the four generations of the captive population (F_0, F_1, F_2, and F_3) was 0.86 (ranging from 0.53 to 0.96 across loci), 0.86 (ranging from 0.52 to 0.96 across loci), 0.86 (ranging from 0.52 to 0.96 across loci), and 0.84 (ranging from 0.46 to 0.96 across loci), respectively. The mean H_E of the wild sample set SKT2007 was 0.86 (ranging from 0.54 to 0.96 across loci) (Table 2). HWE tests revealed that some of the microsatellite loci deviated from HWE in both the captive and wild populations after sequential Bonferroni correction (Appendix II). The loci deviating from HWE were not consistent over generations, and these departures may be due to the finite size and nonrandom mating in the genetically managed captive

Table 3 Pedigree-based and genetic estimates of N_e in captive delta smelt over three generations for all sampled adults and for only those fish used as broodfish

		All Sampled Adults					Broodfish				
Year	*N*	u_k	V_k	V_k/u_k	N_e†	Genetic estimate (95% CI)	u_k	V_k	V_k/u_k	N_e†	Genetic estimate (95% CI)
F_{-1}	—	—	—	—	—	—	—	—	—	—	−4065 (9757 − infinity)
F_0	290	8.14	63.2	7.76	158	356 (341 − 371)	2.76	2.55	0.92	297	748 (660 − 860)
F_1	453	6.13	40.6	6.63	236	292 (282 − 301)	1.78	1.35	0.76	523	558 (517 − 604)
F_2	439	6.88	36.5	5.31	270	359 (344 − 374)	1.89	3.16	1.67	323	673 (592 − 775)

N, number of broodfish sampled; u_k, average family size; V_k, variance of family size; N_e†, N_e estimate accounting for variance in family size
The LD method was calculated using the software program LDNe 95% confidence intervals (CI) were not calculated for the N_e† method

population. There was no evidence of null alleles in the data set according to Micro-Checker, as the estimated frequency of null alleles at each locus was less than 5%.

F_{ST} values for all captive generations and the wild population indicate little to no differentiation (ranging from −0.035 to −0.001), and the F_{ST} values between generations were also negligible. None of the F_{ST} values were significant ($P > 0.05$).

Effective Population Size

The pedigree-based estimates of effective population size (N_e†) for all of the sampled adults were 158, 236, and 270 for the F_0, F_1, and F_2 generations, respectively (Table 3). The index of variability (V_k/u_k) (Crow and Morton 1955) for all of the sampled adults from the F_0, F_1, and F_2 generations was 7.76, 6.63, and 5.31, respectively (Table 3). The $N_e(LD)$ estimates calculated using LDNe (Waples and Do 2008) for three generations for all of the sampled adults were the following: F_0: N_e = 356 (341 – 371), F_1: N_e = 292 (282 – 301); F_2: N_e = 359 (344 – 374).

The pedigree-based estimates of effective population size (N_e†) for the broodfish of the captive population were 297, 523, and 323 for the F_0, F_1, and F_2 generations, respectively (Table 3). The index of variability for the broodfish from the F_0, F_1, and F_2 generations was 0.92, 0.76, and 1.67, respectively (Table 3). The $N_e(LD)$ estimates calculated using LDNe (Waples and Do 2008) for three generations for the broodfish only were the following: F_{-1}: −4065 (9757 − infinity), F_0: N_e = 748 (660 − 860), F_1: N_e = 558 (517 − 604); F_2: N_e = 673 (592 − 775).

Discussion

Although genetic management guidelines have been established to maintain the genetic health of hatchery populations, their implementation in fish hatcheries is limited. As a result, negative genetic changes in fish hatcheries have been documented, such as high levels of inbreeding, adaptation to captivity, reduced viability and fecundity, and reduced effective population size, all of which may result in decreased fitness of supplemented wild populations (Ryman and Laikre 1991; Araki et al. 2007; Frankham 2008). With intensive genetic management of both supplementation and conservation hatcheries, many of the negative genetic changes to wild fish populations may be mitigated. By implementing a pedigree-based genetic management plan designed to maximize gene diversity and limit inbreeding, the founding gene diversity of the captive population can be preserved, consequently maintaining the effective population size (Lacy 1994; Ballou and Lacy 1995). The winter-run Chinook salmon conservation hatchery provides a good example of a successful genetic management plan. By attempting to equalize founder contributions and ensuring that the hatchery did not produce a large fraction of the next generation, the supplementation of winter-run Chinook salmon into the wild population did not appear to decrease the overall wild effective population size (Hedrick and Hedgecock 1994; Hedrick et al. 2000b). However, conservation hatchery populations have also been shown to accumulate negative genetic changes (Hedrick et al. 2000a, 2000b; Osborne et al. 2006; Fraser 2008). For example, Hedrick et al. (2000a) evaluated the bonytail chub captive broodstock and discovered low genetic diversity due to a small number of founders. In addition, analysis of the Rio Grande silvery minnow propagation program revealed that it maintained allelic diversity but still resulted in higher inbreeding in captive versus wild fish stocks, although a more recent study showed that the program has retained diversity in the captive and wild populations over the past decade (Osborne et al. 2006; Osborne et al. 2012). These results highlight the need for rigorous genetic management of captive populations to preserve their genetic integrity. This evaluation of the genetic management plan of the captive delta smelt population aims to assess its ability to minimize *mk* and minimize genetic divergence from the wild population, in an effort to inform conservation hatchery genetic management plans of other species.

Founder Relatedness

Conservation breeding programs typically assume that wild-caught founders are noninbred and unrelated (Ballou 1983). Research is beginning to emerge that suggests this assumption has little impact on *mk* calculations (Rudnick and Lacy 2008; Ivy et al. 2009), although studies have focused on lower fecundity species and populations with single founding events. However, it is important to evaluate founder relationships and to assess the power of marker-specific molecular-based relatedness estimates, as some conservation hatcheries are beginning to use these relatedness estimators to make

breeding recommendations (Fraser 2008; Kozfkay et al. 2008; Sturm et al. 2009).

Previous research has demonstrated that molecular relatedness estimators are subject to high variance, and as a result, may be limited in their ability to accurately assign individuals to relationship categories (Blouin et al. 1996; Norris et al. 2000; Ritland 2000; Sekino et al. 2004). In this study, relatedness estimates had moderate variances, and when these are converted to 95% confidence intervals, there is large overlap between relationship categories. After calculating the pairwise relatedness values for all F_0 fish, we determined that only one pair was plausibly related at the half-sibling relationship level, with most fish falling somewhere between unrelated and half siblings (Figure 2). The existence of a single, undetected half-sibling pair and other, lesser-related individuals among the 290 fish most likely did not significantly impact genetic management based on results from Ivy et al. (2009) and Rudnick and Lacy (2008). However, as the identity of a half-sibling pair was recognized after molecular analyses, this information was incorporated into subsequent, pedigree-based breeding recommendations. The knowledge that only two out of 290 randomly collected wild delta smelt were found to be closely related suggests that capture techniques and the current size of the wild population support collecting small groups of fish from the wild, as they are likely unrelated.

Genetic Management Maintains Genetic Integrity of Captive Delta Smelt Population

An important goal in captive breeding programs is to maintain the effective population size to minimize genetic drift, which can be accomplished by equalizing founder representations (Allendorf 1993). If each individual contributes exactly the same number of offspring to the next generation, the rate of inbreeding and genetic drift would be approximately half of that produced by random parental contributions in an idealized population, effectively doubling the effective population size (Wright 1938; Wang 1997). It is for this reason that equalizing founder representations may be an important aspect of hatchery programs. In the delta smelt captive breeding program, we observed variable founder representations in each generation (Figure 3). Currently, the variable founder representations result, in part, from an inability to make 100% of recommended breeding pairs. From some families, an insufficient number of offspring are sometimes recovered in the next generation to spawn the optimal number of breeders from those families. In addition, some crosses are not viable (less than 5%), and if the parents are not available for a second spawning, these parents may not be represented in the next generation. Even though equalizing founder representations is beneficial, it does not prevent within-family selection, and important variation in some genes related to performance and survivorship may still be lost due to the lack of mate choice. This may cause existing variation in a captive population to become fixed due to domestication selection and relaxed natural selection in captivity (Bryant and Reed 1999; Rodríguez-Ramilo et al. 2006). In addition, Waples (1999) points out that the expected effect of equalizing family size will only be realized if equalization happens at the time of spawning the offspring. The managers of the delta smelt captive breeding program attempt to equalize family size at the time of spawning by minimizing the variance in number of spawners per family and equalizing the number of offspring cultured from each single pair cross.

The combined results regarding the genetic diversity of the captive delta smelt population indicate that the population was initially founded with and continues to retain high levels of allelic diversity and heterozygosity (Table 2). Neither allelic diversity nor heterozygosity is significantly different between the current captive population (F_3) and the F_0 generation, suggesting the genetic management plan utilized in the captive delta smelt population is effective at maintaining genetic diversity, at least over the short term. The initial founding generation (F_0) was captured from the wild in the fall of 2006 as sub-adults. As delta smelt are an annual fish and live only for 2 years in captivity, the wild adult fish collected by the CDFG in 2007 are part of the same cohort. We compared the 2007 sample set to each generation of the captive population to determine if the captive population had diverged from the wild population. The lack of significant difference in A_R and the high percentage of shared alleles between the F_0 generation and the wild population was not unexpected and indicate that the F_0 fish were genetically representative of the wild population. However, a significant decline in A_R in the later captive generations was observed.

The proportion of the H_E of a wild population that is predicted to be captured by the founders of a captive population is equal to

$$H_f = H_w * \left[1 - \frac{1}{1 - 2N}\right], \quad (1)$$

where H_f and H_w are the mean expected heterozygosities in the N founders and the wild population, respectively, from which the founders were sampled (Crow and Kimura 1970). Thus, the 290 initial founders of the captive delta smelt population were predicted to capture 99.8% of the wild population's heterozygosity. The expected heterozygosities in the SKT2007 and F_0 sample sets were 0.86 and 0.86, respectively, indicating that the initial captive population founders captured comparable H_E to that of the wild population (SKT2007 sample set).

F_{ST} values suggest that the current captive population is an adequate genetic representation of the wild population. Results suggest that there is no evidence of population divergence among the four sample sets, as F_{ST} values were all negative and none were statistically significant. Negative F_{ST} values result from the imprecision of the algorithm used to estimate this value and indicate a value close to zero (Weir and Cockerham 1984).

The predicted loss of gene diversity over time in a randomly mating population arising from the founder effect and subsequent small size effects can be calculated using the equation:

$$H_t / H_0 = \left[1 - \frac{1}{2N_{f0}}\right] * \left[1 - \frac{1}{2N * \left(\frac{N_e}{N}\right)}\right]^{t-1}, \quad (2)$$

where N_{f0} = number of effective founders, t = time in generations, H_0 = founder heterozygosity, N = current captive population size, and H_t = H_E of captive population at time t (Frankham et al. 2002). Given the founding population of 290 individuals with H_0 = 0.86 and a stable population size of 500 individuals, in 100 generations, assuming idealized conditions, this population will retain 90.5% of its initial gene diversity, which meets one of the criteria of a successful captive breeding program (Soulé et al. 1986; Ballou et al. 2006). However, because this projection is based on the assumption of random mating and additional wild-caught fish continue to be incorporated into the captive population, the captive delta smelt population may actually be able to retain higher levels of gene diversity than predicted if careful genetic management continues and genetic diversity in the wild is maintained.

The effective population size of the broodfish from each generation in captivity was relatively consistent with the census size, with the exception of the wild founders (F_{-1}) (Table 3). The founders came from a presumably large wild population, so this result is expected. In addition, the N_e of the F_{-1} generation calculated using LDNe is negative, implying an infinite N_e, as N_e was large enough in the wild founding population that no evidence of LD was detected beyond that which could be explained by sampling error. The N_e/N ratio is relatively high for each generation based on the variance N_e and N_e(LD). From this, we can conclude that N_e is being maintained in the captive population, after the initial bottleneck from bringing the founders into captivity.

By comparing the N_e estimates from the broodfish to those estimated from all sampled adults using both methods, the effect of trying to equalize family size can be quantified. Using all sampled adults, the variance of family size is 5–7 times larger than the average family size and N_e ranges from 158–270. After equalizing family size, variance of family size is smaller than the average family size in 2 out of 3 years, and N_e is approximately twice as large, ranging from 297–523 (Table 3). The index of variability is 5–8 times greater for all sampled adults than for broodfish only. Simply reducing the number of offspring spawned per family will reduce the index of variability; however, the difference in the index of variability observed in this study exceeds that which would come from this effect alone. For example, for the first year of broodstock, if the number of offspring was randomly reduced from u_k = 8.14 to 2.76, V_k would be expected to be reduced to V_k = 9.1 based on the equations in Waples (2002). However, the V_k achieved using genetic management is V_k = 2.6, which is three times lower than the effect of randomly reducing offspring number (Waples 2002).

In addition, the genetic estimates of N_e for all progeny are in better agreement with the estimates of N_e from parentage analysis. This is likely due to the random sample of all progeny meeting the assumptions of the LD method. The broodfish only sample is not a random sample and many closely related individuals have been removed, causing an increase in the genetic estimate of N_e. From these results, we can conclude that equalizing family sizes in captivity will maintain effective population size by decreasing the index of variability. Even when population sizes remain large and the index of variability is minimized, N_e can decline due to unequal sex ratios and nonrandom mating (Ryman et al. 1995; Luikart et al. 2010). Reductions in N_e will then lead to increased genetic drift, rapid loss of genetic diversity, and higher rates of inbreeding, making populations more susceptible to extinction (Saccheri et al. 1998; Waples 2002). The methods employed to manage the delta smelt captive breeding program aim to reduce variance in reproductive success and equalize sex ratios to maximize N_e in order to maximize genetic diversity in the captive population. It is important to monitor N_e in the captive population each generation, as N_e is an important tool for monitoring genetic variation (Schwartz et al. 2007).

Recommendation to Fish Hatcheries to Incorporate Genetic Management Plans

This study demonstrates the utility of using a genetic management plan to maintain a captive fish population. Conservation breeding programs have their origins in zoos, in part, because these institutions manage much smaller populations that are easier to manage than the hundreds to thousands of fish that hatcheries traditionally manage each generation. The implementation of a genetic management plan might be more difficult and cumbersome for fish hatcheries; however, these institutions generally release large numbers of fish back into wild populations and have been documented to negatively impact the genetic diversity and effective population size of wild populations (Ryman and Laikre 1991). Thus, it is important that fish hatcheries implement genetic management plans to prevent detrimental changes to supplemented wild populations.

Because the adoption and implementation of a genetic management plan in a large fish hatchery is costly and labor intensive, genetic management plans can be tailored specifically to each hatchery. Efforts to maintain genetic diversity and prevent genetic response to captive breeding are important to success for both conservation and population supplementation purposes. As a result, it is important for hatchery managers to determine what is cost effective and physically possible to accomplish with a genetic management plan in a hatchery setting. A genetic management plan that includes analyses to attempt to equalize family contributions and allow for breeding schemes that minimize kinship may preserve the genetic integrity of the captive population. This may be accomplished using molecular markers as demonstrated in this study, by keeping family groups in separate tanks or by tagging individuals. Procedures not based on molecular markers may be relatively inexpensive and may still allow for the implementation of a simpler genetic management plan for those hatcheries where using molecular data to reconstruct the pedigree is not feasible. In addition, genetic adaptation in captivity is of particular concern in the delta smelt captive population because the only natural conditions they are exposed to come from the open flow through water system. To reduce genetic adaptation to captivity in other hatcheries, fish may be exposed to simulated natural conditions in tanks or use an open flow through water system that exposes captive fish to conditions in their native habitat. Regardless

of the complexity or limitations of the genetic management plan, the following guidelines are recommended:

1. Pedigree analysis of the captive population should be conducted to allow equalization of family sizes and allow for a breeding scheme that minimizes kinship in the population.
2. Wild individuals should be periodically incorporated into the captive population to allow gene flow between the wild and captive populations in order to minimize genetic drift in the captive population, if possible.
3. Genetic adaptation to captivity should be minimized through exposure to naturalistic conditions.

Conclusion

The results of this study suggest that fish hatcheries utilizing genetic management plans designed to minimize *mk* can preserve genetic diversity in captive populations. Incorporation of wild fish into each generation is also an important component to the success of these programs; however, this is not feasible in highly endangered or extirpated populations, indicating the increased need for careful genetic management. Continued genetic management of the delta smelt captive breeding program may preserve this species should it become extinct in the wild and will serve as a model for fish hatcheries adopting hatchery genetic management plans.

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