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ARTICLE

Spawning Behavior of Cultured Delta Smelt in a Conservation Hatchery

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Abstract

Understanding reproductive behavior of sensitive species is crucial for their conservation. The Delta Smelt *Hypomesus transpacificus* is a federally threatened, state-endangered fish whose reproductive behavior is poorly understood. We used genetic techniques to investigate the spawning behavior of cultured Delta Smelt in a conservation hatchery. We conducted a natural tank-spawning experiment in a total of four separate tanks during two spawning seasons. Delta Smelt were allowed to spawn in order to investigate spawning patterns using genetic parentage analysis of larvae produced. In total, 2,474 larvae were assigned two parents with >80% likelihood. Of the adults that had larvae assigned to them, males spawned on average 2.8 times and females 1.7 times. The mean number of larvae produced by females was 40.7, while males produced a mean number of 19.2 larvae during a single spawning season. Genetic diversity was reduced from the parent population to the offspring population, as indicated by a small but significant reduction in heterozygosity. Finally, we found no evidence that Delta Smelt preferred to mate with unrelated individuals.

Conservation hatcheries must often balance production considerations with, among other things, domestication selection (e.g., Bryant and Reed 1999; Waples 1999), maximization of overall genetic diversity in the captive population, and minimization of inbreeding (e.g., Ballou 1984; Allendorf and Luikart 2007; Frankham 2008; Fraser 2008). Conservation hatcheries therefore benefit from a full understanding of the biology of the species both in captivity and in the wild. However, it can be difficult to gain knowledge of rare and sensitive species, some of which may have never been observed spawning in the wild. For such species, gaining knowledge of reproductive behavior can inform genetic management both in situ and in a hatchery setting (e.g., Fraser 2008).

One way to indirectly observe reproductive patterns is to perform genetic parentage analysis (e.g., Chakraborty et al. 1988). This requires both parent and offspring genotypes, which are

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then used to assign offspring to parents. Genetic parentage analysis is commonly used to characterize the mating system and reproductive behavior of fish that mass spawn (e.g., White Seabass *Atractoscion nobilis*: Gruenthal and Drawbridge 2012; Atlantic Cod *Gadus morhua*: Bekkevold et al. 2002). In a hatchery setting, genetic parentage analysis has also been used to analyze reproductive success or fitness of hatchery fish (e.g., Baumsteiger et al. 2008; Anderson et al. 2013), assess breeding protocols in captive settings (Rio Grande Silvery Minnow *Hybognathus amarus*: Osborne et al. 2013), and maintain genetic diversity (Delta Smelt *Hypomesus transpacificus*: Fisch et al. 2013). Furthermore, by assigning parents to offspring, researchers can estimate population parameters, such as the likelihood that individuals mate with related individuals, the number of times males and females are likely to spawn in a season, and variance in reproductive success in a population. Genetic parentage analysis is a natural choice for exploring the reproductive behavior of Delta Smelt, which is intensively managed in a conservation hatchery, yet has poorly understood mating patterns.

Delta Smelt, a small fish in the family Osmeridae, is endemic to the upper San Francisco Estuary (SFE), specifically in the Sacramento–San Joaquin Delta and Suisun Bay (McAllister 1963; Wang 1986; Moyle 2002). In 1993, the U.S. Fish and Wildlife Service (USFWS) listed Delta Smelt as threatened under the U.S. Endangered Species Act, and the state of California changed their listing from threatened to endangered in 2010 due to further population declines (USFWS 1993; CDFG 2010). As part of a larger trend, Delta Smelt have been identified as the most serious example of pelagic organism decline (POD) occurring in the SFE, due to the species' rapid decline and small native range (Sommer et al. 2007). The population health of Delta Smelt directly impacts California water politics by affecting the operation of state and federal pumping stations that deliver water to both cities and farms (Bennett 2005 and citations therein). Despite the pivotal role that Delta Smelt play in California water politics, many ecologically and biologically important life history traits, such as reproductive behavior, are poorly understood (e.g., Bennett 2005).

It is commonly thought that Delta Smelt live in the freshwater–saltwater mixing zone in the SFE in turbid water (typically above 12–18 NTU: Feyrer et al. 2007, 2010; Nobriga et al. 2008). Delta Smelt typically live for 1 year and die after spawning, although a small but unknown proportion of 2-year-old fish have been observed (Moyle 2002; Bennett 2005). Spawning behavior in the wild has not been directly observed, but based on capture of the pelagic larvae and adults, it is thought that Delta Smelt spawn in freshwater from February to May (Wang 1986). Exact spawning locations are unknown, as areas where eggs have been deposited have not been found (Bennett 2005). Adult females are believed to spawn at one time or over a short period of time, based on the presence of a large quantity of eggs of nearly identical size and development stage in adult females (Mager et al. 2004). Delta Smelt employ external fertilization, and clutch size ranges from 1,200–2,600 eggs (Moyle et al. 1992). Much of the information about Delta Smelt spawning behavior has come from the University of California (UC) Davis Fish Conservation and Culture Lab (FCCL), where culture techniques for Delta Smelt were developed, and a refuge population provides a genetic bank for conservation purposes (Fisch et al. 2013; Lindberg et al. 2013).

To examine reproductive behaviors of Delta Smelt, we conducted natural spawning experiments at the FCCL where fish were allowed to freely mate in tanks. Herein, natural refers to a situation where gametes are not manually expressed and crossed in vitro (strip-spawned) and adults are not stimulated with hormones. We then used genetic parentage analysis to assign parentage to larvae that were hatched from collected eggs and reared to 7 d. The goals of this study were to answer the following questions: (1) How many individuals within a tank produce viable offspring during the season? (2) Can males and females spawn more than once in a season? (3) What are the relative contributions of adult individuals and different mating pairs to offspring produced during a spawning event? (4) Are Delta Smelt more likely to mate with unrelated individuals? (5) What are some genetic consequences of allowing Delta Smelt to spawn naturally?

METHODS

Hatchery facility and fish rearing.—The FCCL has learned to culture the sensitive Delta Smelt by means of experimentation, observation, and trial and error (see Lindberg et al. 2013 for detailed information on culture techniques). In brief, fish are cultured at the FCCL out of doors in black 1.52-m-diameter (1,100 L) tanks that have bare floors and shade-cloth covers and are supplied by a water flow-through system (8 L/min). For the younger life stages of Delta Smelt, the FCCL "greens" the water to mimic turbidity (see Lindberg et al. 2013). Throughout this experiment, the fish were fed a commercial diet daily at 3% of their body weight, and water quality (checked twice weekly) and temperature (12°C) were kept constant.

The refuge (broodstock) population is genetically managed in collaboration with the Genomic Variation Lab (GVL) at UC Davis. Genetic management is based on maximizing retention of genetic diversity and minimizing kinship in the population (see Fisch et al. 2013 for details on genetic management). Each year, beginning in January and ending in mid-May, adult male and female Delta Smelt are tagged, and fin clips are sent to the GVL for microsatellite genotyping and parentage analysis. Based on parentage analysis, the pedigree is reconstructed and single pair crosses (SPCs) are made to minimize kinship, equalize family contribution, and maintain genetic diversity in the refuge population. Offspring from eight SPCs (16 individual parents) are placed in a tank, and fish in each tank are known collectively as a multifamily group (MFG). Each season, the FCCL houses approximately 30 MFGs (the combined total of offspring from 240 SPCs).

TABLE 1. Spawn date, approximate number of eggs collected, number of offspring genotyped with 80% data, number of offspring assigned to two parents with >80% probability, number of adults assigned parentage (parents), and number of unique adult pairs for Delta Smelt from tank A (300 adults) and tank B (310 adults). Values were combined (rather than summed) in Parents, and Unique Pairs columns, as some parents spawned more than once.

Date	Approximate larvae hatched	Offspring ≥ 80% genotype data	Offspring assigned two parents	Parents	Unique pairs
		Tank A			
Feb 15	480	92	88	33	58
Feb 18	6,000	89	87	36	60
Feb 21	8,800	89	89	34	56
Mar 7	3,200	91	90	35	59
Mar 10	2,000	92	91	40	53
Mar 15	3,280	91	87	33	49
Mar 16	3,200	91	90	31	49
Apr 12	8,000	90	85	39	60
Apr 13	3,800	89	88	36	69
Apr 25	2,500	92	92	44	65
Mean	4,126	90.6	88.7	36	57.8
Combined				184	572
Total	41,260	906	887		
		Tank B			
May 3	560	89	87	30	46
May 10	6,800	84	75	36	51
May 11	1,600	86	81	35	46
May 20	2,400	86	82	31	44
Mean	2,840	86.3	81.25	33	46.8
Combined				98	185
Total	11,360	345	325	132	

Experimental design.—Four individual natural-spawning experiments were performed at the UC Davis FCCL in Byron, California. There were two tanks containing unsexed adults (~9-month-old adults where sex is unknown) during the 2011 season: tank A ($n = 300$ adults) and tank B ($n = 310$ adults) (Table 1). Another two tanks contained sexed ~9-month-old adults during the 2012 season: tank C ($n = 101$ adults) and tank D ($n = 46$ adults) (Table 2). Adults for each tank were collected from two different MFGs from the captive population of Delta Smelt raised at the FCCL (Fisch et al. 2013; Lindberg et al. 2013); adults in tank A were full sibling offspring from two MFGs (32 potential parents), adults in tank B were full sibling offspring from two different MFGs. Tanks C and D were similarly stocked with adult Delta Smelt. In the first year (2011) the experiment was initiated in February, without prior assignment of sex, as fish were sexually immature and sex could not be determined, and ended in April (tank A) or May (tank B). In the second year (2012) we began the experiment in March so that the sexes of all adults were identified. The sex of the fish was determined by applying a small amount of pressure to the abdomen and observing expression of either milt or underdeveloped eggs. Generally, the sex ratio is very close to 1:1 when immature fish are randomly sampled (M. Nagel, unpublished data). Assigning sexes facilitates calculating the effective number of male and female breeders, the number of times a sire or dam can mate, and increases confidence in parentage analysis. All adults were fin clipped for genetic analysis.

Egg collection and incubation.—During the spawning season, FCCL staff checked tanks each morning from Monday to Friday to feel for the presence of eggs by running a hand around the tank walls and floor. When eggs were found, staff wiped the surface of the tanks by hand to loosen the adhesive eggs and then partially drained the tank into a fine mesh net to collect the eggs (T. Stevenson, University of California–Davis, personal communication). In most cases, eggs were only incubated if more than 500 live eggs (less than a full clutch) were estimated. The number of eggs was estimated volumetrically (eggs are approximately 1 mm in diameter, 100 eggs = 1 mL). Eggs were then treated with bentonite clay to make them less adhesive and then incubated in upwelling column incubators (Lindberg et al. 2013) until they hatched, approximately 10 d later. The larvae hatched into buckets, where they were collected, euthanized, and preserved in ethanol for genetic analysis.

DNA extraction and microsatellite analysis.—We analyzed a 1-mm^2 fin clip from each adult, and randomly selected 95 whole

TABLE 2. Spawn date, estimated number of larvae hatched, number of larvae with >80% genotype data, number of larvae assigned sire and dam, number of males assigned as sires (N_s), effective number of sires (N_{es}), number of females assigned as dams (N_d), effective number of dams (N_{ed}), number of unique sire–dam pairs, change in inbreeding (ΔF), demographic N_e, for Delta Smelt from tank C (37 adult females and 64 adult males) and tank D (22 adult females and 24 adult males). Values were combined rather than summed in N_s, N_{es}, N_d, N_{ed}, Unique Pairs, ΔF, and Demographic N_e columns because some adults spawned more than once.

Date	Approximate larvae hatched	Offspring ≥ 80% genotype data	Offspring assigned sire and dam	N_s	N_{es}	N_d	N_{ed}	Unique pairs	ΔF	Demographic N_e
				Tank C						
Mar 15	400	81	79	8	4.47	3	2.27	9	0.08	6.02
Mar 16	400	81	80	12	8.47	4	2.01	18	0.08	6.49
Apr 2	400	86	83	19	10.78	5	4.01	28	0.04	11.69
Apr 3	200	91	90	14	10.74	6	2.73	21	0.06	8.70
Apr 4	200	90	84	14	8.32	5	2.62	25	0.06	7.97
Apr 18	200	87	84	16	13.96	4	2.73	33	0.06	9.14
Apr 20	250	91	90	11	5.51	3	1.09	11	0.14	3.65
Apr 24	300	90	89	9	4.52	2	1.02	9	0.15	3.34
Apr 26	2,000	79	79	12	7.77	3	2.41	16	0.07	7.36
Apr 30	150	94	94	16	6.51	2	1.74	20	0.09	5.50
May 10	200	90	85	11	7.40	2	1.99	20	0.08	6.27
May 14	200	87	84	12	6.04	5	1.46	16	0.11	4.71
Mean	408	87.25	85.08	13	7.87	4	2.17	17.9	0.09	6.74
Combined				51	25.16	23	13.81	194	0.01	36.24
Total	4,900	1,047	1,021					229		
				Tank D						
Mar 26	100	88	88	10	6.46	5	2.42	14	0.07	7.05
Apr 12	150	73	73	7	3.88	2	1.56	10	0.11	4.44
Apr 25	1,500	89	78	11	7.87	3	1.33	15	0.11	4.64
Mean	583	83.33	79.7	10	6.07	3	1.56	13	0.10	5.38
Combined				15	8.19	8	4.99	37	0.04	12.40
Total	1,750	250	239					39		

larvae per spawn date for genetic analysis. Whole genomic DNA was extracted using the DNeasy Tissue Kit protocol (QIAGEN, Valencia, California). Multiplex PCR amplifications were performed for 15 microsatellite loci in five multiplex reactions described in Fisch et al. (2009) (Table 3). The microsatellite markers were designed specifically for Delta Smelt and have been tested for presence of null alleles, linkage disequilibrium, and usefulness for parentage analysis in the broodstock (Fisch et al. 2009). The PCR products were visualized using an ABI Genetic Analyzer 3730xl (Life Technologies, Carlsbad, California) by combining 2 μL of PCR product, 8.8 μL formamide, and 0.2 μL LIZ500 size standard and heating this mixture at 95°C for 3 min. We used Genemapper 4.0 software (Applied Biosystems, Carlsbad, California) to genotype the loci and verified the allele scores manually.

Parentage analysis.—After genotyping, offspring with <80% genotyping data (<12 loci) were discarded, based on a conservative application of the suggestion of Morin et al. (2010). We used the software program MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004) to detect the presence of null alleles and scoring errors in all four tanks. We used COLONY version 2.0 (Jones and Wang 2010) to conduct parentage analysis using a maximum likelihood method. Selected options in COLONY were as follows: polygamous mating system for males and females, medium-length runs, and a probability of 1.0 that the parent was genotyped. For the parents of unknown sex in both tanks from 2011, we included the genotypes of all parents in both the mother and father genotype (even those with <80% genotype data) files to ensure all potential parent crosses could be identified and verified manually to ensure that the same individual adults were never assigned as both sire and dam. For tanks C and D, where sex of the parents was known, we used separate input files for potential mothers and potential fathers.

Parent relatedness.—To estimate relatedness of assigned male–female pairs in tanks C and D, we used COLONY (Jones and Wang 2010). Half-sibling relationships were not possible given the pedigrees of the parent fish, which could only be unrelated, cousins, or full siblings. We calculated the number of

TABLE 3. Fifteen microsatellite loci for Delta Smelt used in this study: number of alleles per locus (N_a), observed heterozygosity (H_o), and expected heterozygosity (H_e) observed in this study when all parent tanks were combined.

Locus	N_a	H_o	H_e
Htr103	16	0.495	0.508
Htr104	7	0.906	0.917
Htr107	22	0.789	0.803
Htr109	14	0.903	0.922
Htr114	23	0.584	0.553
Htr115	26	0.955	0.962
Htr116	7	0.961	0.945
Htr117	24	0.878	0.866
Htr118	4	0.948	0.87
Htr119	47	0.931	0.929
Htr120	14	0.959	0.934
Htr126	23	0.827	0.809
Htr128	36	0.927	0.903
Htr129	5	0.727	0.709
Htr131	25	0.236	0.233
Mean	19.53	0.80	0.79

expected sibling pairs and the number of expected nonsibling pairs of parents based on the calculated genetic relatedness of the parental population. We then calculated the number of observed sibling and nonsibling pairs assigned as parents to the genotyped offspring. We used a chi-square test to test the null hypothesis that sibling crosses are no more likely than nonsibling crosses.

Genetic diversity and effective population size.—Observed and expected heterozygosity values (H_o and H_e, respectively) for adult groups in each tank, analyzed offspring from each spawn date, and total combined analyzed offspring from each tank were calculated using the software program GenAlEx (Peakall and Smouse 2006, 2012). We used a *t*-test assuming unequal sample variances to determine whether changes in observed heterozygosity from parent to offspring tank were statistically significant.

We calculated demographic effective population size (N_e) and the resulting rate of inbreeding for tanks C and D using an equation that takes into account both variation in reproductive success of individual males and females and variation in family size derived from Lacy (1989) following Gruenthal and Drawbridge (2012):

$$N_e = \frac{4N_{ed}N_{es}}{N_{ed} + N_{es}}. \quad (1)$$

In equation (1) the effective number of dams, N_{ed}, and sires, N_{es}, are calculated using the equations (Gold et al. 2008):

$$N_{ed} = \frac{1}{\sum_{k=1}^{n_f} q_k^2} \quad (2)$$

and

$$N_{es} = \frac{1}{\sum_{k=1}^{n_m} q_k^2}. \quad (3)$$

The variables n_f and n_m are the number of females and males, respectively, that contributed to the spawn, and q is the proportion of offspring that individual dams or sires contributed to the spawn. The rate of inbreeding (ΔF) was then calculated using the equation from Falconer (1989):

$$\Delta F = \frac{1}{2(N_e)}.$$

RESULTS

The number of spawning dates analyzed (those where enough eggs were produced to be collected by the FCCL staff) varied from 3 (tank D) to 13 (tank C), and the total spawning period ranged from 17 d (tank B) to 70 d (tank A) (Tables 2, 3). During both spawning years, hatchery personnel incubated and reared from 150 to 8,800 larvae from 29 different spawn dates. From each spawn date, we extracted DNA from 95 offspring, genotyped them using 15 microsatellite loci, and conducted parentage analysis for a total of 2,755 larvae.

We detected no null alleles in our microsatellite markers and conservatively estimated the rate of scoring errors to be ~0.01 per locus. The mean number of alleles per locus ranged from 4 (*Htr118*) to 47 (*Htr119*), with a mean of 19.53 alleles per locus (Table 3). Loci had high heterozygosity (mean $H_e = 0.79$, mean $H_o = 0.80$).

In tanks A and B we estimated that 41,260 and 11,360 larvae, respectively, were raised to 7 d (Table 1), and of those, 906 and 345 larvae were analyzed for parentage with ≥80% genotype data, representing 2.3% and 2.7% of viable larvae, respectively. In tanks C and D, approximately 4,900 and 1,750 larvae hatched, and 1,047 and 250 of those were analyzed for parentage with ≥80% genotype data, representing 21.4% and 14.3% of viable larvae, respectively (Table 2).

In tank A, ≥80% genotype data for all loci were not available for 12 potential parents, and in tank B, ≥80% genotype data were not available for 27 potential parents; however, no parent genotype data were removed from COLONY analysis so that all potential parents could be considered for parentage assignment. For accurate parentage assignments, we discarded offspring with <80% likelihood of being assigned to two parents for tanks A and B (Table 1) and an individual sire or dam for tanks C and D (Table 2). This left 2,474 offspring assigned (with mean likelihood of assignment to an individual parent of 99%), and at least 73 offspring assigned per spawn date (Tables 1, 2).

Number of Spawning Adults

In tank A, 184 out of 300 adults (61.3%) were assigned at least one larvae, and in tank B, 101 out of 310 adults (32.6%)

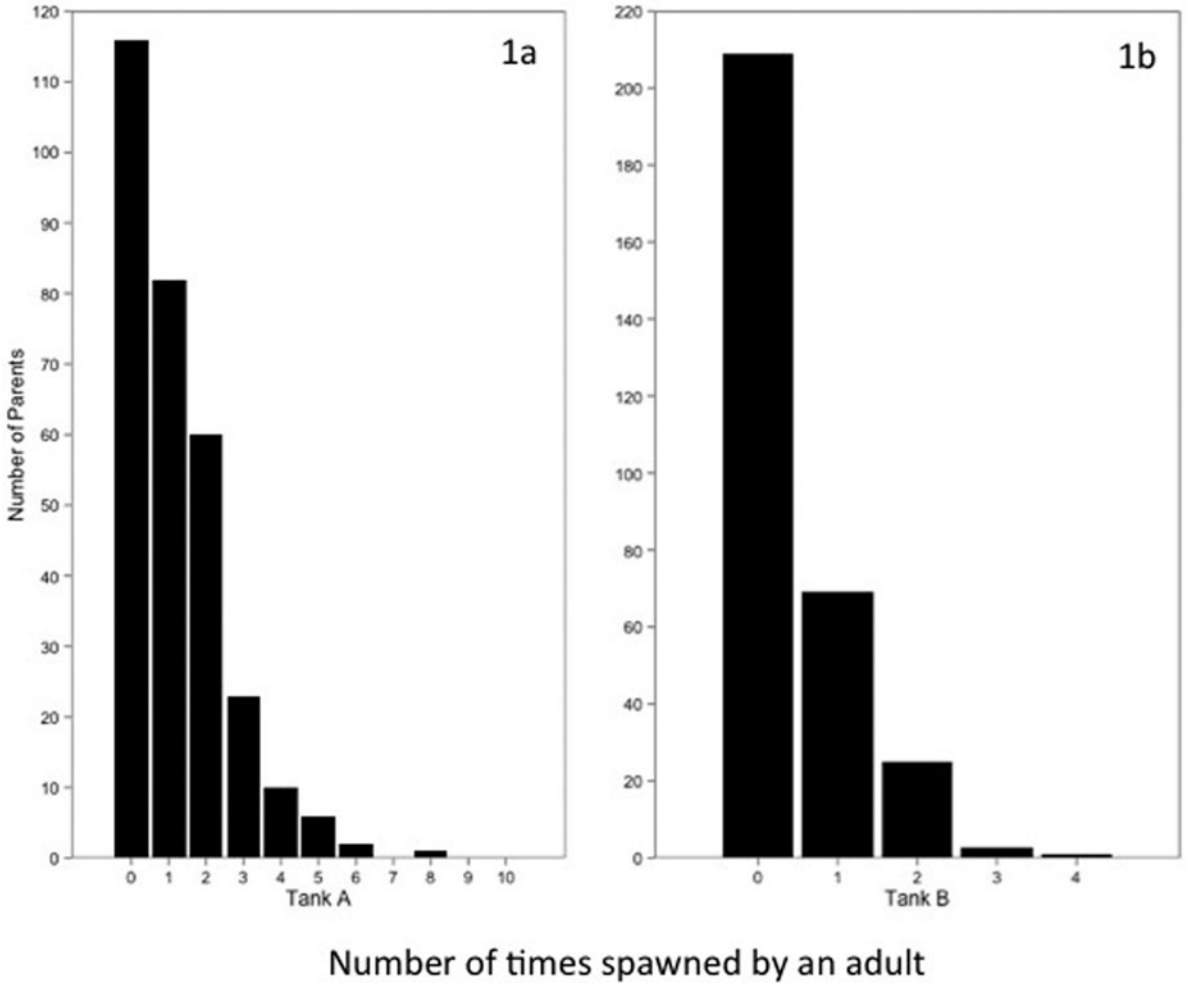

FIGURE 1. (**a**) Number of times (from 0 to 10 individual collection dates) each potential Delta Smelt parent in tank A was assigned a larvae (e.g., 116 adults were assigned larvae on zero times, one adult was assigned larvae on eight times). (**b**) Number of times (from zero to four) each potential parent in tank B was assigned a larvae.

were assigned at least one larvae (Table 1). In tanks C and D, a higher proportion of males were assigned larvae than were females: in tank C, 80.0% of males were sires and 62.1% of females were dams. In tank D, 62.5% of males were sires and 36.4% of females were dams (Table 2).

Spawning Frequency during Season

Individual fish in tank A had at least one larvae assigned to them between one and eight times over 10 analyzed spawn dates, with a mean of 1.96 (Figure 1a). In tank B, adults had at least one larvae assigned to them between one and four times over four analyzed dates, with a mean of 1.35 (Figure 1b). When combined, individual dams in tanks C and D had offspring assigned to them a mean of 1.71 times, while sire had offspring assigned to them a mean of 2.75 times (Figure 2a, b). However, both the number of offspring produced and number of times spawned varied (see Tables S.1 and S.2 in the Supplement available with the online version of this paper for in-depth number of analyzed offspring produced by each parent in tanks C and D on each spawn date).

Relative Contributions of Individuals and Unique Pairs

Of the subsample of larvae analyzed, individual parents in tank A had up to 25 larvae assigned to them when all spawn dates were combined (mean = 9.69) (Figure 3a), and in tank B up to 34 (mean = 6.62) (Figure 3b). In tank C, up to 140 larvae were assigned to a given dam when all dates were combined (mean number of larvae assigned to dam = 44.39) (Figure 4a) and up to 84 larvae to an individual sire (mean number of larvae assigned to sire = 20.14) (Figure 4b). In tank D, dams produced up to 67 analyzed offspring over all spawn dates (mean offspring assigned to dam = 29.86) (Figure 4c), and individual sires produced up to 31 analyzed offspring (mean offspring assigned to sire = 15.93) (Figure 4d). In tanks C and D, we found that the majority of unique male–female combinations contributed ≤5 sampled larvae on a given spawn date. In tank C, 24% of

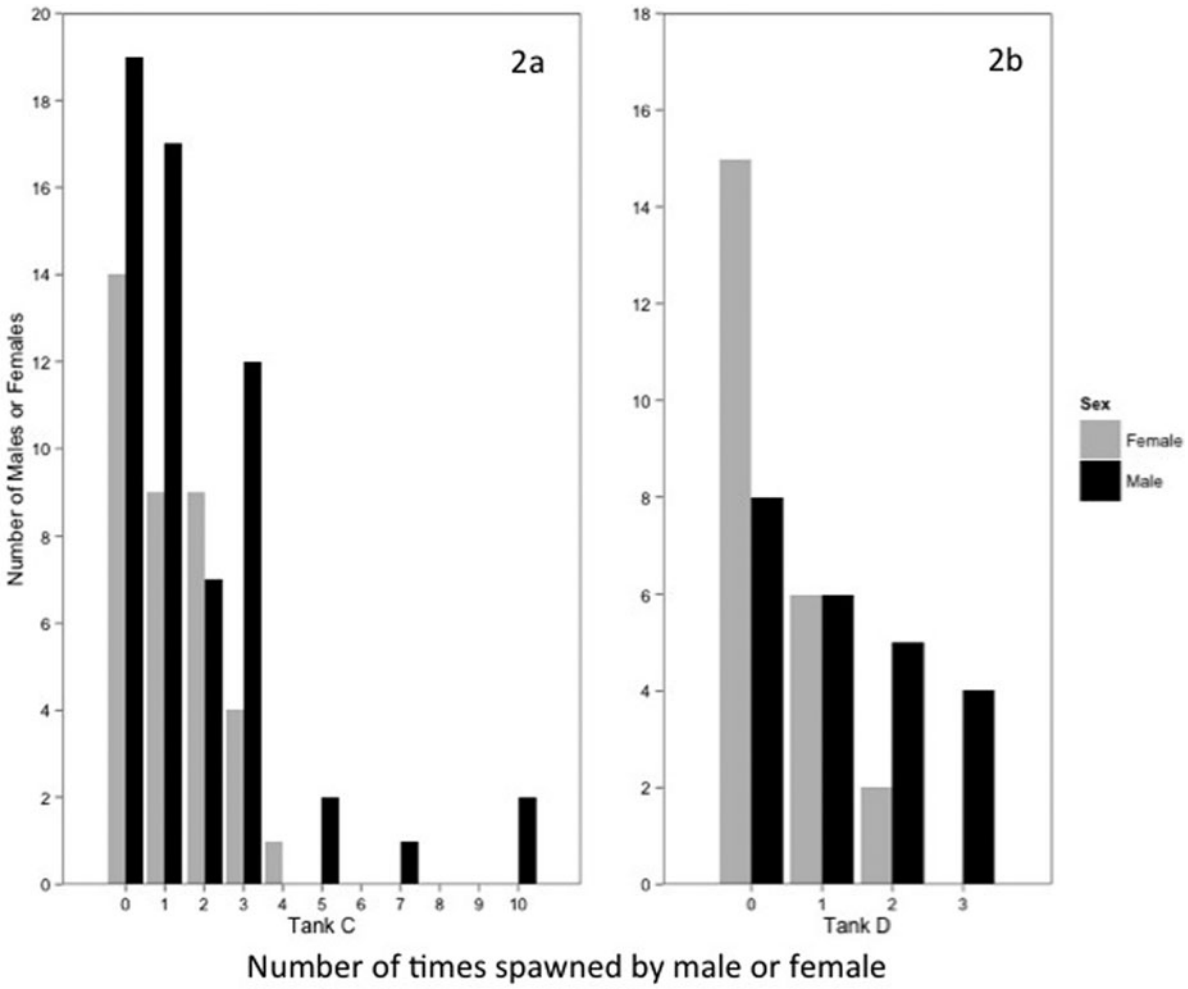

FIGURE 2. (**a**) Number of times (from 0 to 12 individual collection dates) that individual adult male and female Delta Smelt from tank C were assigned larvae (e.g., 19 males and 14 females had larvae assigned to them on zero dates). (**b**) Number of times (out of four total) that individual males and females in tank D were assigned larvae.

unique male–female pairs produced >5 larvae (61.31% of the larvae analyzed). In tank D, 56.4% of unique pairs had ≤5 larvae (20.5% of larvae), while 43.5% of unique pairs had 79.5% of larvae (those that produced >5 larvae).

Preference of Related Mates

Based on relatedness calculated in COLONY, in both tanks C and D nonsibling pairings were no more likely than sibling pairings (see Table 4 for expected and observed number of sibling and nonsibling mating pairs in both tanks). According to chi-square tests for both tanks, the number of sibling–sibling matings did not differ significantly from the number of nonsibling matings ($P > 0.05$) (Table 4).

Genetic Diversity

Observed heterozygosity, H_o, decreased from the mean value in each tank of adults to their mean offspring values (Table 5) and was statistically significant (*t*-test assuming unequal variances: $P < 0.001$ for all four tanks). For example, in tank C, mean H_o of adults was 0.80 and the mean H_o of all offspring analyzed for that tank was 0.74 (Table 5). Mean number of alleles per locus (N_a) decreased from adult tank to analyzed larvae in all four tanks; however, this reduction was only statistically significant in tank D (*t*-test assuming unequal variances: $P > 0.05$ in all tanks except tank D where $P < 0.001$; Table 5).

Demographic N_e

The N_{ed} values in tanks C and D were less than the total number of estimated parents per tank (74 and 23 for tanks C and D, respectively). When all dates were combined, $N_{ed} = 36.24$ in tank C and $N_{ed} = 12.40$ in tank D (Table 5). Mean ΔF per spawn date in tank C was 0.09 (9.0% per generation) and in tank D ΔF was 1.0 (10% per generation). When all dates were combined in each tank, ΔF in tank C was 0.01 (1.0% per generation and in tank D was 0.04 (4.0% per generation).

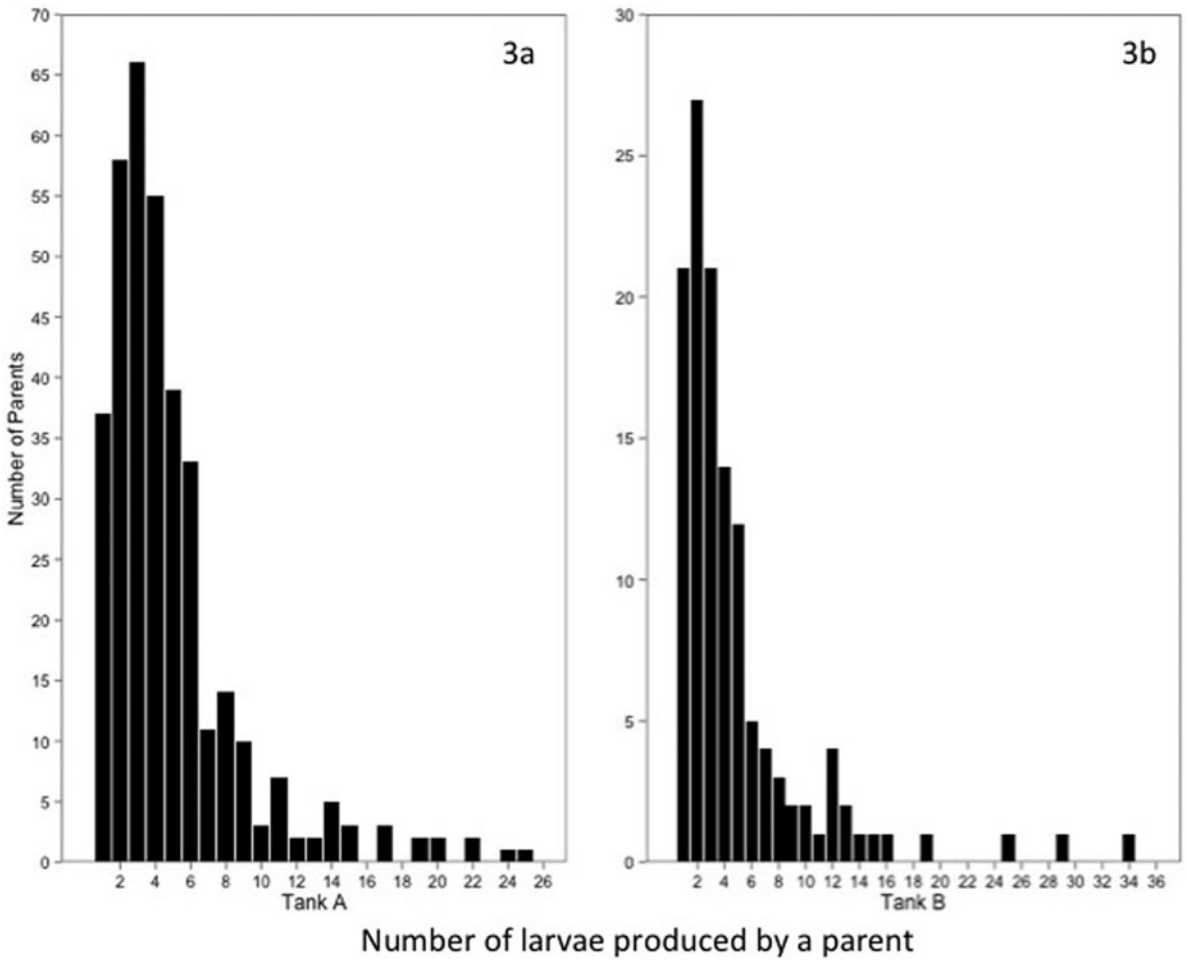

FIGURE 3. (**a**) Number of Delta Smelt larvae assigned to individual parents in tank A (e.g., 37 females only had one larvae assigned to them over the entire season). (**b**) Number of larvae assigned to parents in tank B.

DISCUSSION

This is the first experiment where Delta Smelt spawning behavior, in culture, was indirectly observed using genetic data. Although these experiments occurred in captivity, adult fish were allowed to choose their own mates, i.e., gametes were not expressed manually with in vitro fertilization. Tanks A and B were set up in the first year primarily to determine whether the experimental design was feasible (fish will spawn at that density, eggs can be collected and incubated, and larvae can be assigned parentage with high confidence). In the second year we reduced the number of adults and sexed them to gain more insight. Thus, the discussion is largely focused on tanks C and D.

During both years, we found that a large proportion of adults in tanks A and B had no larvae assigned to them (∼39% and ∼70%, respectively; Table 1). In tanks C and D, where fish were sexed, a higher proportion of females than males did not have larvae assigned to them (Table 2). Several factors may have contributed to these findings. First, some adults will not spawn in a tank environment. Second, the experiments may have ended too early; if the duration of the experiments was extended, later-maturing fish may have spawned. Third, if more spawn dates had been analyzed (including those that produced fewer than 500 eggs) we may have detected additional sires and dams. Fourth, analyzing additional offspring on each spawn date would have improved statistical power, assigning parentage to additional sires and dams that were undetected. Nevertheless, 382 adult Delta Smelt spawned, allowing us to examine several aspects of reproductive behavior.

In the second year, by reducing the number of potential parents, we were able to analyze a greater proportion of the offspring produced (20.83% in tank C, 14.3% in tank D), thereby improving our ability to interpret the data. We found that on each spawn date, individual or small groups of males and females were likely pairing off. In addition, of the offspring analyzed, a few females contributed a disproportionate number of offspring to the next generation. For example, in tank C, female FP60 contributed a total of 140 offspring (13.7% of offspring analyzed), and in tank D, female FP17 produced 57 offspring (19.7% of offspring analyzed).

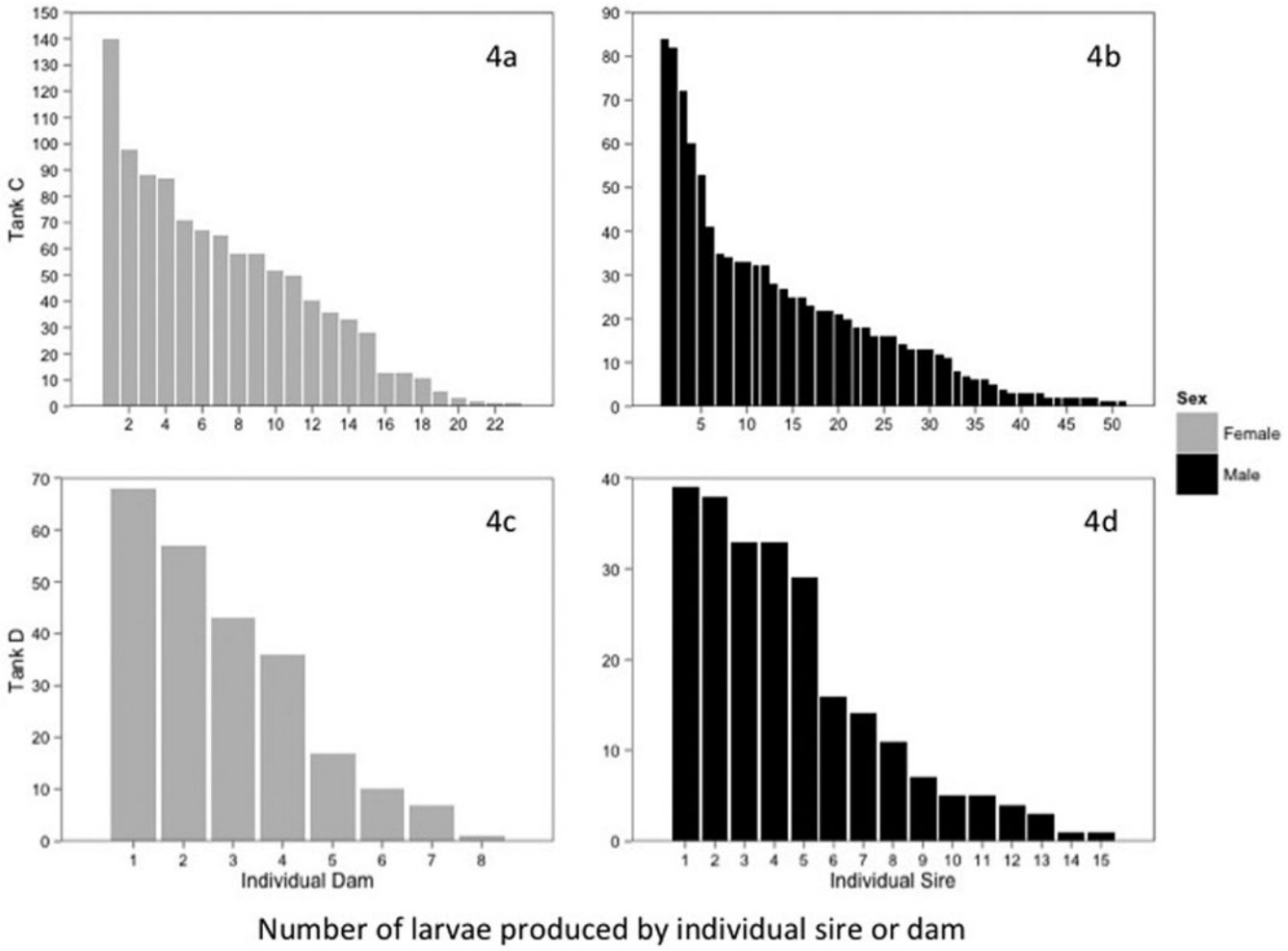

FIGURE 4. (**a**) Number of Delta Smelt larvae assigned to individual females in tank C (e.g., one female had 140 larvae assigned to her, 23 individual females had only one larvae assigned to them). (**b**) Number of larvae spawned by individual males in tank C. (**c**) Number of larvae spawned by individual females in tank D. (**d**) Number of larvae spawned by individual males in Tank D.

Spawning frequency over the course of a season varied between males and females. Males were assigned larvae on up to 10 dates and females on up to four dates. We chose not to group multiple analyzed dates into multiday spawning events because we could not rule out a female or male spawning on each individual analyzed date (and thereby serially spawning), and there was no clear and consistent pattern to follow in order to combine dates (this is apparent in Tables S.1 and S.2). In some cases, sires or dams may not have contributed to a specific analyzed date. In addition, FCCL staff may have missed a small number of eggs on a given date, which were then inadvertently combined with eggs from a later date. For example, in tank C, female FP60, had offspring assigned to her on four different dates: 47 offspring on March 15 and 53 on March 16, which may have comprised a single spawning event. Then FP60 had 39 offspring assigned on May 10, and one on May 14, indicating a second or possibly third spawn. The single egg collected on May 14 may have been left over from May 10, or she may have serially spawned. In future studies, eggs can be examined for developmental stage to determine whether they are from the same or different spawn dates. In addition, future studies could conduct postseason dissections to reveal how many females held their eggs and did not spawn at all (potentially due to the unnatural tank environment).

In addition to the above data for FP60, we found evidence that three more dams (FP65, FP75, and FP54) in tank C may have serially or fractionally spawned over the course of a few days, rather than releasing the whole clutch at once. Female FP65 had 7, 10, and 41 offspring assigned to her on April 2, 3, and 4, respectively. Females FP75 and FP54 also produced large numbers of offspring on April 2 and 3 (23 and 27 offspring, and 24 and 46 offspring, respectively; see Supplement). We did not observe this pattern in tank D as no proximate dates were analyzed.

Effective Population Size and Genetic Diversity

Our findings of reduced heterozygosity and reduced N_{ed} led us to conclude that allowing fish to spawn naturally in the hatchery would increase genetic drift and inbreeding, causing the loss

TABLE 4. Mean number of alleles per locus (N_a) in Delta Smelt. Observed heterozygosity (H_o) and expected heterozygosity (H_e) were calculated using Genalex. Demographic N_e (N_{ed}) was calculated according to equations from Lacy (1989) for tanks where fish were the sex of parents were identified (all parent tanks and tanks C and D). Tank A and tank B parents did not have sex identified, therefore N_{ed} was not calculated for their offspring. Values in bold text indicate offspring value significantly different from parent value (*t*-test assuming unequal variances: $P < 0.001$).

Tank	Mean N_a	H_o	H_e	N_{ed}
Tank A parents	14.13	0.80	0.77	22.12
Tank A offspring	11.81	0.77	**0.76**	
Tank B parents	14.40	0.80	0.78	20.59
Tank B offspring	10.67	0.77	**0.76**	
Tank C parents	14.20	0.80	0.78	16.88
Tank C offspring	8.90	0.74	**0.68**	36.24
Tank D parents	12.20	0.80	0.77	15.89
Tank D offspring	**7.98**	0.73	**0.69**	12.40

of rare alleles and genetic diversity over time (Lacy 1989). It is expected that N_{ed} values for offspring from each tank were lower than the actual number of adults that spawned; not all adults spawned, and a small proportion of individuals and pairs contributed the majority of offspring to the next generation. In the mass-spawning Gilthead Seabream *Sparus aurata*, Brown et al. (2005) found a similar reduced N_e and increased inbreeding due to high variance in family size. Brown et al. (2005) found that lower numbers of males than females spawning also constrained N_e.

The rate of inbreeding in tank D when all spawn dates were combined (4.0% per generation) was higher than the value expected in an ideal population (1.56% per generation: Falconer 1989). Tank C had a lower combined ΔF (1.0%), though mean ΔF per date (9.0%) was still quite high, due to higher variance in family size on each given date. Fessehaye et al. (2006) found a mean ΔF of 3% per generation in Nile Tilapia *Oreochromis niloticus* when allowed to spawn naturally.

Mate Choice

It is unknown whether Delta Smelt select mates in the wild, and if so, whether their selection is based on a morphological or genetic cue that endows offspring with "good genes" (Bateman 1948; Trivers 1972; Brown 1997) that give higher relative fitness to offspring. We did not find evidence that Delta Smelt avoid breeding with siblings during this experiment. However several studies have found that fish species may select mates based on *major histocompatibility complex* (MHC) variation. For example, Landry et al. (2001) found evidence that female Atlantic Salmon *Salmo salar* choose mates with dissimilar MHC genes, but did not practice inbreeding avoidance based on microsatellite data. Forsberg et al. (2007) found that female Brown Trout *S. trutta* were choosing mates with intermediate MHC variation, and Johnson et al. (2010) found that in a wild population of Trinidadian Guppies *Poecilia reticulata*, females both chose and produced more offspring with less-related males. Other studies have found a size-assortative mating system. Bekkevold et al. (2002) found a pattern where certain adults of both sexes have higher reproductive success due to a size-assortative mating system. Further research may reveal whether Delta Smelt are more likely to mate with individuals that have particular morphological, behavioral, or genetic cues.

Management Implications

Delta Smelt are the subject of considerable legal, regulatory, and scientific efforts dedicated to understanding its life history and habitat needs and improving its status in the wild (Bennett 2005). Spawning is considered one of the most critical life periods in Delta Smelt (Moyle 2002; Bennett 2005) and other annual species. If the wild population continues to decline, steps may be taken to introduce captive individuals into the natural habitat, and the genetic health of captive individuals is critical for reintroduction. To date, the FCCL has served its purpose of maintaining a genetically diverse refuge population of Delta Smelt (Fisch et al. 2013). Its continued success is critical for recovery of the Delta Smelt if reintroduction is necessary.

Since its founding, the FCCL has used the conservative minimum kinship method, incorporation of wild fish, and yearly genetic monitoring to maximize genetic diversity and minimize inbreeding over time (Fisch et al. 2013). However, domestication remains a significant concern. In the case of cultured Delta Smelt, gametes are manually expressed from select females and males, in single pair crosses, preventing natural spawning behaviors such as mate choice, spawn timing (for males), or group spawning dynamics. This relaxes natural selection (Bryant and Reed 1999) by allowing less fit individuals to remain in the population, which can undermine the goal of the conservation hatchery to maintain a population Delta Smelt similar to the wild population and fit for reintroduction if the need arises. Anecdotally, we have observed evidence of domestication in Delta

TABLE 5. Comparison of numbers and proportions of expected number of sibling pairs/total possible number of mating pairs and observed siblings pairs/actual number of mated pairs, chi-square value, and *P*-value of chi-square test for tanks C and D.

Tank	Expected sibling pairs/possible mating pairs	Observed sibling pairs/actual mated pairs	Chi-square value	*P*-value
C	141/2,368 (5.95%)	11/194 (5.67%)	0.028	0.870
D	50/528 (9.47%)	4/34 (11.76%)	0.208	0.660

Smelt at the FCCL, based on reduced survival of offspring with wild parents to reproductive age relative to those produced by cultured parents (A. J. Finger, unpublished data). This reduction in survival of offspring from wild parents limits the ability of the hatchery to minimize domestication, which can result in rapid fitness declines when hatchery fish are released into the wild (Araki et al. 2008). Indeed, Christie et al. (2012) found that adaptation to captivity occurred in a single generation in steelhead *Oncorhynchus mykiss*. Further studies are required to explore domestication selection in the refuge population and determine how mate choice and nonrandom breeding may interact with fitness of offspring. Our study provides a foundation for future research into reproductive behavior and information on best practices in the conservation hatchery for Delta Smelt.

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REFERENCES

Allendorf, F. W., and G. Luikart. 2007. Conservation and the genetics of populations. Blackwell Scientific Publications, Oxford, UK.

Anderson, J. H., P. L. Faulds, W. I. Atlas, and T. P. Quinn. 2013. Reproductive success of captively bred and naturally spawned Chinook Salmon colonizing newly accessible habitat. Evolutionary Applications 6:165–179.

Araki, H., B. A. Berejikian, M. J. Ford, and M. S. Blouin. 2008. Fitness of hatchery-reared salmonids in the wild. Evolutionary Applications 1:342–355.

Ballou, J. D. 1984. Strategies for maintaining genetic diversity in captive populations through reproductive technology. Zoo Biology 3:311–323.

Bateman, A. J. 1948. Intrasexual selection in *Drosophila*. Heredity 2:349–368.

Baumsteiger, J., D. M. Hand, D. E. Olson, R. Spateholts, G. FitzGerald, and W. R. Ardren. 2008. Use of parentage analysis to determine reproductive success of hatchery-origin spring Chinook Salmon outplanted into Shitike Creek, Oregon. North American Journal of Fisheries Management 28: 1472–1485.

Bekkevold, D., M. M. Hansen, and V. Loeschcke. 2002. Male reproductive competition in spawning aggregations of cod (*Gadus morhua*, L.). Molecular Ecology 11:91–102.

Bennett, W. A. 2005. Critical assessment of the Delta Smelt population in the San Francisco Estuary, California. San Francisco Estuary and Watershed Science [online serial] 3(2).

Brown, C. R., J. A. Woolliams, and B. J. McAndrew. 2005. Factors influencing effective population size in commercial populations of Gilthead Seabream (*Sparus auratus*). Aquaculture 247:219–225.

Brown, J. L. 1997. A theory of mate choice based on heterozygosity. Behavioral Ecology 8:60–65.

Bryant, E. H., and D. H. Reed. 1999. Fitness decline under relaxed selection in captive populations. Conservation Biology 13:665–669.

CDFG (California Department of Fish and Game). 2010. State and federally listed endangered and threatened animals of California. CDFG, Sacramento.

Chakraborty, R., T. R. Meagher, and P. E. Smouse. 1988. Parentage analysis with genetic markers in natural populations I. The expected proportion of offspring with unambiguous paternity. Genetics 118:527–536.

Christie, M. R., M. L. Marine, R. A. French, and M. S. Blouin. 2012. Genetic adaptation to captivity can occur in a single generation. Proceedings of the National Academy of Sciences of the USA 109:238–242.

Falconer, D. S. 1989. Introduction to quantitative genetics, 3rd edition. Pearson, Essex, UK.

Fessehaye, Y., Z. El-bialy, M. A. Rezk, R. Crooijmans, H. Bovenhuis, and H. Komen. 2006. Mating systems and male reproductive success in Nile Tilapia (*Oreochromis niloticus*) in breeding hapas: a microsatellite analysis. Aquaculture 256:148–158.

Feyrer, F., K. Newman, M. Nobriga, and T. Sommer. 2010. Modeling the effects of future outflow on the abiotic habitat of an imperiled estuarine fish. Estuaries and Coasts 34:120–128.

Feyrer, F., M. Nobriga, and T. Sommer. 2007. Multi-decadal trends for three declining fish species: habitat patterns and mechanisms in the San Francisco Estuary, California, U.S.A. Canadian Journal of Fisheries and Aquatic Sciences 64:723–734.

Fisch, K. M., J. A. Ivy, R. S. Burton, and B. May. 2013. Evaluating the performance of captive breeding techniques for conservation hatcheries: a case study of the Delta Smelt captive breeding program. Heredity 104:92–104.

Fisch, K. M., J. L. Petersen, M. R. Baerwald, J. K. Pedroia, and B. May. 2009. Characterization of 24 microsatellite loci in Delta Smelt, *Hypomesus transpacificus*, and their cross-species amplification in two other smelt species of the Osmeridae family. Molecular Ecology Resources 9:405–408.

Forsberg, L. A., J. Dannewitz, E. Peterson, and M. Grahn. 2007. Influence of dissimilarity in the reproductive success and mate choice of Brown Trout – females fishing for optimal MHC dissimilarity. Journal of Evolutionary Biology 20:1859–1869.

Frankham, R. 2008. Genetic adaptation to captivity in species conservation programs. Molecular Ecology 17:325–333.

Fraser, D. J. 2008. How well can captive breeding programs conserve biodiversity? A review of salmonids. Evolutionary Applications 1:535–586.

Gold, J. R., L. Ma, E. Saillant, P. S. Silva, and R. R. Vega. 2008. Genetic effective size in populations of hatchery-raised Red Drum released for stock enhancement. Transactions of the American Fisheries Society 137: 1327–1334.

Gruenthal, K. M., and M. A. Drawbridge. 2012. Towards responsible stock enhancement: broadcast spawning dynamics and adaptive genetic management in White Seabass aquaculture. Evolutionary Applications 5:405–417.

Johnson, A. M., G. Chappell, A. C. Price, F. H. Rodd, R. Olendorf, and K. A. Hughes. 2010. Inbreeding depression and inbreeding avoidance in a natural population of Guppies (*Poecilia reticulata*). Ethology 116:448–457.

Jones, O. R., and J. Wang. 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources 10:551–555.

Lacy, R. C. 1989. Analysis of founder representation in pedigrees: founder equivalents and founder genome equivalents. Zoo Biology 8:111–123.

Landry, C., D. Garant, P. Duchesne, and L. Bernatchez. 2001. 'Good genes as heterozygosity': the major histocompatibility complex and mate choice in Atlantic Salmon (*Salmo salar*). Proceedings of the Royal Society B 268: 1279–1285.

Lindberg, J. C., G. Tigan, L. Ellison, T. Rettinghouse, M. M. Nagel, and K. M. Fisch. 2013. Aquaculture methods for a genetically managed population of endangered Delta Smelt. North American Journal of Aquaculture 75: 186–196.

Mager, R. C., S. I. Doroshov, J. P. Van Eennaam, and R. L. Brown. 2004. Early life stages of Delta Smelt. Pages 169–180 *in* F. Feyrer, L. R. Brown, R. L. Brown, and J. J. Orsi, editors. Early life history of fishes in the San Francisco Estuary and watershed. American Fisheries Society, Symposium 39, Bethesda, Maryland.

McAllister, D. E. 1963. A revision of the smelt family, Osmeridae. National Museum of Canada Bulletin 191.

Morin, P. A., K. K. Martien, F. I. Archer, F. Cipriano, D. Steel, J. Jackson, and B. L. Taylor. 2010. Applied conservation genetics and the need for quality control and reporting of genetic data used in fisheries and wildlife management. Journal of Heredity 101:1–10.

Moyle, P. B. 2002. Inland fishes of California. University of California Press, Berkeley.

Moyle, P. B., B. Herbold, D. E. Stevens, and L. W. Miller. 1992. Life history and status of Delta Smelt in the Sacramento–San Joaquin estuary, California. Transactions of the American Fisheries Society 121:67–77.

Nobriga, M. L., T. R. Sommer, F. Feyrer, and K. Fleming 2008. Long-term trends in summertime habitat suitability for Delta Smelt, *Hypomesus transpacificus*. San Francisco Estuary and Watershed Science 6:1–13.

Osborne, M. J., T. L. Perez, C. S. Altenbach, and T. F. Turner. 2013. Genetic analysis of captive spawning strategies for the endangered Rio Grande Silvery Minnow. Journal of Heredity 104:437–446.

Peakall, R., and P. E. Smouse. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28:2537–2539.

Peakall, R., and P. E. Smouse. 2006. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288–295.

Sommer, T., C. Armor, R. Baxter, R. Breuer, L. Brown, M. Chotkowski, S. Culberson, F. Feyrer, M. Gingras, B. Herbold, W. Kimmerer, A. Mueller-Solger, M. Nobriga, and K. Souza. 2007. The collapse of pelagic fishes in the upper San Francisco Estuary. Fisheries 32:270–277.

Trivers, R. L. 1972. Parental investment and sexual selection. Pages 136–179 *in* B. Gampbell, editor. Sexual selection and the descent of man 1871–1971. Aldine, Chicago.

USFWS (U.S. Fish and Wildlife Service). 1993. Endangered and threatened wildlife and plants; determination of threatened status for the Delta Smelt. Federal Register 58:42(5 March 1993):12854–12864.

Van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. MICRO-CHECKER: software for identifying and correcting genotype errors in microsatellite data. Molecular Ecology Notes 4:535–538.

Wang, J. C. S. 1986. Fishes of the Sacramento–San Joaquin estuary and adjacent waters, California: a guide to the early life histories. Interagency Ecological Study Program for the Sacramento–San Joaquin Estuary, Technical Report 9, Sacramento, California.

Waples, R. S. 1999. Dispelling some myths about hatcheries. Fisheries 24(2): 2–21.