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ARTICLE

Assessing captive spawning strategies for supplementation production of Delta Smelt

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Abstract

Objective: To support the declining wild population of Delta Smelt, a conservation hatchery has expanded its mission from maintaining a backup population as insurance against extinction to also producing fish for release into the wild. The substantially higher production demands require a balance between producing large numbers of fish while adhering to conservation genetic principles that maximize retention of effective population size (N_e) and thus overall diversity.

Methods: We performed spawning experiments at the hatchery to evaluate the genetic consequences of two spawning strategies: (1) a pooled strategy where we fertilized premixed eggs from three dams with premixed milt from three sires and (2) a partial-factorial strategy where eggs from three dams were mixed and then apportioned among three containers, each container then receiving milt from one sire. We used genetic parentage analysis of larval offspring to determine the reproductive success of spawners in 10 replicate crosses of each strategy.

Result: The contributions of parents to offspring were more even in partial-factorial crosses and consequently resulted in higher N_e (average $N_e = 5.50 \pm 0.38$; expected $N_e = 6.0$), suggesting its potential for maintaining genetic diversity over time. In contrast, our pooled spawning experiment produced lower and more variable N_e values (average $N_e = 3.86 \pm 1.30$), demonstrating that this more efficient method of production entails high costs in terms of long-term genetic management. Treating our experiments as hypothetical pools of fish for release, we combined the N_e values for pooled or partial-factorial crosses to calculate the effective size of a release population (N_{eR}). Unequal family sizes reduced N_{eR} for our pooled experiment to half of the expected value, whereas the partial-factorial experiment N_{eR} was 88% of the expected value.

Conclusion: We discuss the benefits and risks of each method and how these can be considered when designing a spawning strategy for Delta Smelt supplementation.

KEYWORDS

conservation hatchery, effective population size, endangered species, *Osmeridae*, sperm competition

INTRODUCTION

Genetic management has been an essential component of the captive spawning program for endangered Delta Smelt *Hypomesus transpacificus* since its founding in 2008. Delta Smelt are small, osmerid fish that are endemic to the San Francisco Estuary in California, USA, and have experienced population declines since at least the 1980s (Moyle et al. 2016), leading to listings as threatened under the Endangered Species Act (Endangered and Threatened Wildlife and Plants 1994) and endangered under the California Endangered Species Act (California Department of Fish and Wildlife 2009). In 2008, the University of California, Davis Fish Conservation and Culture Laboratory (FCCL) established a captive population of Delta Smelt to insure against extinction of the species (Lindberg et al. 2013). The FCCL maintains the captive population of this (typically) annual species by strip spawning (i.e., manually expressing gametes and combining them in a bowl for fertilization) single dam–sire pairs. The spawners are selected according to a genetic management plan that emphasizes minimizing the relatedness of spawners, equalizing family sizes, and incorporating wild fish into each generation (Fisch et al. 2013). The FCCL spawns 295 ± 49 single-pair crosses each year (2018–2022) to maintain a captive population of approximately 5400–7600 adult fish. Genetic management has thus far maintained similarity to and retained the genetic diversity of the wild population (Fisch et al. 2013; Finger et al. 2018); however, the wild population of Delta Smelt has declined to near or below levels of detection with current survey gear (Peterson and Barajas 2018). Consequently, the incorporation of wild broodstock has lapsed in recent years.

The decline of Delta Smelt to near extinction in the wild prompted a management decision to implement experimental release (hereafter, "supplementation") of captive-born fish from the FCCL into the wild as the first step toward a potential supplementation program (U.S. Fish and Wildlife Service 2019, 2020, 2021). This management action requires a massive increase in the production of Delta Smelt, with a long-term goal of producing >100,000 fish annually for supplementation (U.S. Fish and Wildlife Service 2019, 2020). A challenge for scaling the production of Delta Smelt for supplementation is to identify one or more spawning strategies that are logistically efficient while maintaining genetic diversity and effective population size (N_e). Although the single-pair crossing strategy that is currently implemented in the hatchery is effective in maintaining a target N_e due to the level of control over the selection of fish to spawn and the ability to equalize family sizes (Fisch et al. 2013), this approach is resource intensive (e.g., personnel, time, and space) and could be impractical for the scale of production that is required for supplementation. In addition, single-pair crosses can result in the complete failure of some spawners for many reasons, including mate incompatibility or poor gamete quality (Busack and Knudsen 2007; Fisch et al. 2015). Thus, reliance on this strategy could, for example, risk the loss of important genetic diversity, particularly given that wild fish remain too limited in abundance to reliably contribute to broodstock. Therefore, evaluations of alternative spawning methods are needed to identify strategies that are consistent with implementation of a conservation-oriented integrated hatchery management plan for Delta Smelt supplementation (U.S. Fish and Wildlife Service 2020).

Impact statement

Delta Smelt are nearly extinct in the wild, so managers are experimentally releasing captive-born fish to supplement the declining population. We experimentally evaluated the genetic consequences of two captive spawning methods to inform production of fish for supplementation.

In this study, we explored two alternatives to the currently implemented single-pair-cross spawning strategy, a pooled strategy and a partial-factorial strategy, both of which employ a multifamily crossing method. Multifamily crosses combine eggs from multiple dams and/or milt from multiple sires, potentially producing fish more efficiently than single-pair crosses and mitigating the risk of poor compatibility between some dam–sire pairs (Fisch et al. 2015). A pooled spawning strategy combines gametes from multiple dams and sires prior to the fertilization process. This method would be more efficient for scaling up production levels but poses a higher risk of reducing N_e, such as when sperm competition results in large variation in family size (LaCava et al. 2015; Beirão et al. 2019). A factorial or partial-factorial spawning strategy involves pairwise crosses between all possible parents or pooling eggs from multiple dams and then applying milt from a single sire (Campton 2004; Fisch et al. 2015). These methods can be resource intensive, but they can reduce the large variation in family sizes that may arise from a fully pooled method, potentially leading to higher N_e values.

We conducted spawning experiments at the FCCL to evaluate the application of these two multifamily crossing strategies to captive Delta Smelt. We tested a pooled spawning method where we admixed milt from three sires and applied it to admixed eggs from three dams (Figure 1A). We also tested a partial-factorial spawning method where eggs from each dam were apportioned among three containers and admixed with eggs from two other dams prior to the fertilization step; each container

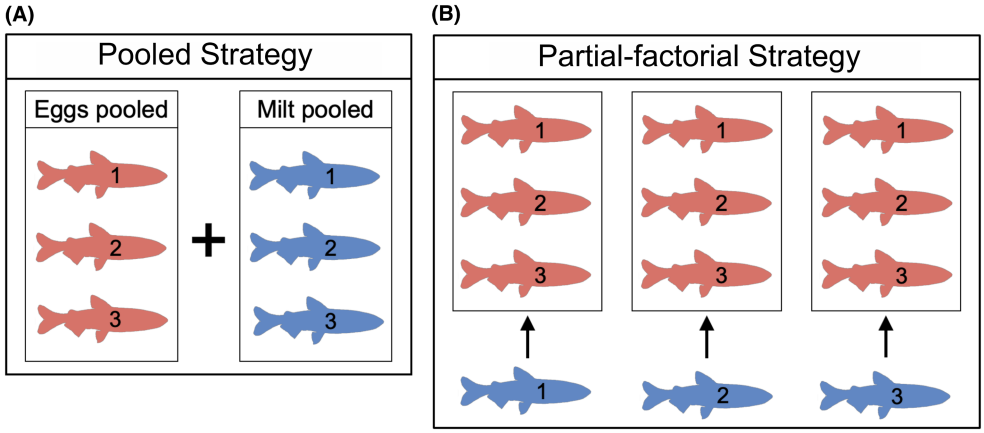

FIGURE 1 A depiction of our experimental multifamily crosses, using (A) a pooled spawning strategy or (B) a partial-factorial spawning strategy, each incorporating eggs from three females (in red) and milt from three males (in blue).

then received milt from a single sire (Figure 1B). The experiments included 10 replicates per spawning method, for a total of 20 multifamily crosses. We used genetic parentage assignment of larval offspring that were sampled at 7–10 days posthatch to determine the relative contribution of each dam, sire, and dam–sire pair to the offspring that were produced in each multifamily cross. Following the framework of Gold et al. (2008) and equations adapted from Lacy (1989), we calculated effective population sizes (N_e) based on the effective number of dams (N_{ed}) and sires (N_{es}) that contributed to offspring in each multifamily cross. Treating our experimental crosses as a hypothetical pool of Delta Smelt for supplementation, we combined the N_e values for pooled or partial-factorial crosses to calculate the effective size of the release population (N_{eR}). We finish by discussing the benefits and risks of each method and the potential application to Delta Smelt supplementation production.

METHODS

Experimental crosses

We performed spawning experiments between April 2021 and June 2022 (Table S1 available in the Supplemental Materials in the online version of this article), using cultured Delta Smelt that are maintained at the FCCL. Detailed hatchery management practices can be found in Lindberg et al. (2013). We selected three ripe females (i.e., sexually mature and expressing mature eggs) and three sexually mature males to spawn in each of our 20 experimental crosses (10 pooled and 10 partial factorial). Sexually mature fish were identified by gently squeezing the abdomen to check for the expression of gametes (Lindberg et al. 2013), and the fish were assigned to crosses according to the mate-selection criteria that are implemented at the FCCL, such as minimizing relatedness (Lacy et al. 2012; Fisch et al. 2013). We spawned a total of 119 unique parents. No parents were spawned in more than one replicate for the same spawning method. However, one female (ID: YT04) was used in a partial-factorial cross and a pooled cross due to the limited availability of ripe females (Table S2). The experimental crosses were made by manually expressing and combining gametes from the fish that were selected for each cross (Ellison et al. 2023). For the pooled crosses, milt from three males was admixed in an extender vial (Rahman et al. 2023) and then added into a container with premixed eggs from three females (Figure 1A). For the partial-factorial crosses, eggs from each of three females were apportioned among three containers and admixed with eggs from two other dams prior to the fertilization step; each container then received milt from a single male (Figure 1B). After the eggs were incubated for 3 days, the three containers for each partial-factorial cross were consolidated. The total number of viable embryos for each multifamily cross was estimated volumetrically (Baskerville-Bridges et al. 2005). The embryos were incubated until they hatched; the larvae were sacrificed at 7–10 days posthatch (at a size large enough for genetic analysis), preserved in ethanol, and sent to the University of California, Davis Genomic Variation Laboratory for processing.

Single nucleotide polymorphism genotyping and genetic parentage assignment

In the Genomic Variation Laboratory, we generated single nucleotide polymorphism (SNP) genotypes for a subsample of 91 ethanol-preserved larvae from each experimental cross. We extracted DNA from whole larvae, using the Qiagen DNeasy Blood and Tissue Kit (Qiagen,

Inc., Valencia, California), following the manufacturer's instructions. We used a Fluidigm SNP Type Assay (Fluidigm Corporation, South San Francisco, California) to genotype 75 biallelic SNP loci that were previously identified by Lew et al. (2015). We used the Fluidigm EP1 system to run 96.96 Dynamic Array Integrated Fluidic Circuits, following the manufacturer's instructions. Each circuit contained 91 samples, three positive control samples (i.e., previously genotyped hatchery Delta Smelt), and two negative controls (i.e., no DNA added to wells). Using the Fluidigm Genotyping Analysis Software, we verified the genotypes by using the positive controls to confirm the accuracy of the genotype groups for each locus. To assign parents to each larva, we used a likelihood-based analysis, with 10,000 simulated offspring and a mistyping rate of 0.001, implemented in CERVUS 3.0 (Kalinowski et al. 2007).

Evenness of parent contributions to offspring

We calculated the total number of larval offspring that was genetically assigned to each parent and to each possible dam–sire pair cross. To evaluate the relative evenness of parent contributions to offspring in each multifamily cross, we performed a Pearson's Chi-square test (Pearson 1900), implemented in R 4.1.0 (R Core Team 2022). For each experiment, we tested for deviations from the null expectation of equal contributions of each parent or pair cross to the total number of offspring. We used a two-sided Wilcoxon rank-sum test (Wilcoxon 1945) to test for a significant difference between the two spawning methods in the proportion of offspring contributed by pair crosses or parents, implemented in R 4.1.0 (R Core Team 2022). Because the pooled experiment was performed over two consecutive years to obtain the full 10 replicate crosses, we performed a Student's *t*-test (Student 1908), implemented in R 4.1.0 (R Core Team 2022), to test for a year effect on the relative contributions of pair crosses and parents to the total number of offspring that was produced in each multifamily cross.

Effective population size

Genetic effective population size (N_e) is determined by the number of parents and the variation in family size among parents that contribute to the offspring pool (Crow and Kimura 1970; Lacy 1989). According to Crow and Kimura (1970), when considering only the number of dams and sires that contribute to the offspring pool, N_e can be calculated as follows (Gold et al. 2008, Equation 1):

$$N_e = \frac{4N_dN_s}{N_d + N_s},$$

where N_d and N_s represent the number of dams and sires, respectively, that produced offspring. When additionally considering variation in family size (Lacy 1989), N_e can be calculated as follows (Gold et al. 2008, Equation 2);

$$N_e = \frac{4N_{\mathrm{ed}}N_{\mathrm{es}}}{N_{\mathrm{ed}} + N_{\mathrm{es}}},$$

where N_{ed} and N_{es} represent the effective number of dams and sires, respectively. We estimated N_{ed} and N_{es} as follows (Gold et al. 2008, Equation 3a and 3b):

$$N_{\mathrm{ed}} = \frac{1}{\sum_{k=1}^{n_f} q_k^2}, \text{and}$$

$$N_{\mathrm{es}} = \frac{1}{\sum_{k=1}^{n_m} q_k^2},$$

where n_f and n_m are the number of dams and sires, respectively, that produced offspring and q represents the proportion of offspring contributed by each dam or sire. We used these equations to calculate N_e, N_{ed}, and N_{es} for each multifamily cross and summarized the results in relation to spawning methods. We then determined the relative influence of failed spawners (i.e., parents that failed to produce any offspring) and variation in family size on the average N_e for each spawning experiment (Gold et al. 2008). We used a Student's *t*-test (Student 1908), implemented in R 4.1.0 (R Core Team 2022), to determine whether the two spawning methods produced significantly different mean N_e, N_{ed}, and N_{es} values.

In addition, we calculated the effective size of two hypothetical release populations (N_{eR}) by combining N_e values across multifamily crosses for each spawning strategy that we tested, using Equation 4 of Gold et al. 2008 (see also Ryman and Laikre 1991):

$$N_{\mathrm{eR}} = \frac{1}{\sum_{k=1}^{n_f} \frac{x_i^2}{N_{\mathrm{ei}}}},$$

where x_{i} is the proportion of offspring contributed by the *i*th multifamily cross to the release population and N_{ei} is the effective population size of the *i*th cross. We additionally calculated N_{eR} considering equalization of offspring from each cross to the release population.

RESULTS

SNP genotyping and genetic parentage assignment

We genotyped a total of 1786 larval offspring, with $n = 881$ and $n = 905$ from the pooled and partial-factorial experiments, respectively (Table S1). The number of genotyped larvae per multifamily cross ranged from 63 to 91 (pooled) and 88 to 91 (factorial). Fewer than the full complement ($n = 91$) of genotyped larvae per cross was obtained for one of the pooled crosses because only 63 viable larvae were produced; we genotyped all the available offspring (Table S1). A subset of 16 larvae assigned to parents from a cross other than the one they were sampled from. Each of the mismatched larvae assigned to two parents from the same incorrect cross, indicating that contamination among crosses occurred after fertilization, likely when the fertilized eggs or larvae were transferred among containers before being preserved in ethanol. The 16 mismatched samples were excluded from further analysis. All the downstream analyses were based on the remaining total of 1770 samples that assigned to two parents from the correct crosses (Tables S1–S3).

Evenness of parent contributions to offspring

The pooled experiments that we performed in 2021 and 2022 were not significantly different in the proportion of offspring that assigned to pair crosses or to parents (Student's t-test, $p = 1.00$); therefore, we present the results from the 2 years combined. We observed both pooled and partial-factorial crosses that deviated from the null expectation of equal contribution of dams, sires, or pair crosses to offspring; however, the deviations were more numerous and larger in the pooled experiment (Table 1; Table S4). Pair crosses and parents in the pooled experiment contributed more variable proportions of offspring, whereas the partial-factorial experiment had a tighter range of contributions that were closer to expected values (Figure 2; Figures S1–S4 available in the Supplemental Materials in the online version of this article). Considering only the percentage of failures within each experiment (i.e., no offspring produced), the pooled experiment had more failed pair crosses (28%), failed dams (13%), and failed sires (7%) than did the partial-factorial experiment (2, 0, and 0%, respectively; Table 2). The difference between the two spawning methods was significant when comparing the rank sums for the proportion of offspring contributed by pair crosses (Wilcoxon rank-sum test, $p = 0.0082$) but

TABLE 1 Percentage of multifamily crosses (of 10 tested in each experiment) with statistically even contributions of pair crosses (PCs), dams, or sires to offspring produced, based on Chi-square tests (full results in Table S4).

Spawning experiment	Even PC contributions (%)	Even dam contributions (%)	Even sire contributions (%)
Pooled	0	10	20
Partial-factorial	30	40	80

TABLE 2 For each spawning experiment, the percentage of pair crosses (PCs; of 90 possible), dams (of 30 spawned), and sires (of 30 spawned) that failed to produce any offspring (full details in Tables S2 and S3).

Spawning experiment	Failed PCs (%)	Failed dams (%)	Failed sires (%)
Pooled	28	13	7
Partial-factorial	2	0	0

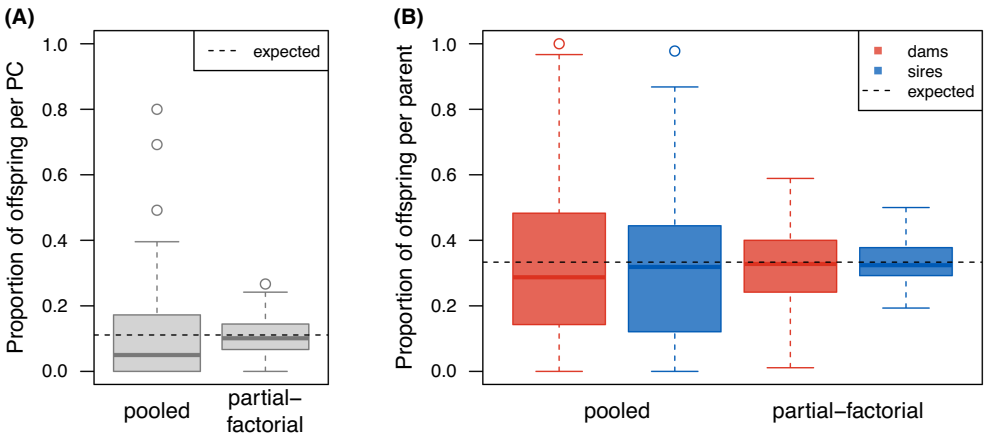

FIGURE 2 The proportion of offspring assigned to (A) each possible pair cross (PC) and (B) each parent for 10 multifamily crosses in our pooled and partial-factorial experiments. The expected proportion for each PC or parent is indicated with a dashed black line.

was nonsignificant when comparing the rank sums for the proportion of offspring contributed by dams ($p = 0.50$) or by sires ($p = 0.77$).

Effective population size

For each multifamily cross, which contained three dams and three sires, the expected N_e value (i.e., assuming all parents contributed to offspring and assuming equal family sizes; Crow and Kimura 1970; Gold et al. 2008, Equation 1) was six and the expected N_{ed} and N_{es} values were each three. The partial-factorial experiment consistently produced higher N_e, N_{ed}, and N_{es} values (Figure 3), with a mean $N_e = 5.51 \pm 0.38$ versus a mean $N_e = 3.90 \pm 1.00$ for the pooled experiment (Table 3; Table S5). The difference in means between the two spawning methods was significant when comparing N_e (Student's t-test, $p = 0.00053$), N_{ed} ($p = 0.026$), and N_{es} ($p = 0.0033$). Although some pooled crosses resulted in N_{ed} or N_{es} values that were close to expected values, the range of values for pooled crosses was much larger than the range of values for partial-factorial crosses (Figure 3B; Table S5). Failed spawners reduced the average N_e for pooled crosses by 13%, whereas no spawners failed in any partial-factorial cross (Table 3). Variation in family size had a larger influence on N_e values than failed spawners in both experiments, causing a 22% reduction in average N_e for pooled crosses and an 8% reduction in average N_e for partial-factorial crosses (Table 3).

When we combined the N_e values from crosses to calculate N_{eR} for two hypothetical release populations (one pooled and one partial-factorial), we found that the partial-factorial $N_{eR} = 53$ (88% of the expected value of 60) was much higher than the pooled $N_{eR} = 29$ (49% of the expected value; Table 4). When we equalized the number of larvae contributed by each multifamily cross to the release population, we observed a higher $N_{eR} = 55$ (91%) for the partial-factorial experiment and a higher $N_{eR} = 36$ (60%) for the pooled experiment (Table 4).

DISCUSSION

Scaling up the production of Delta Smelt under a conservation-oriented integrated hatchery model for

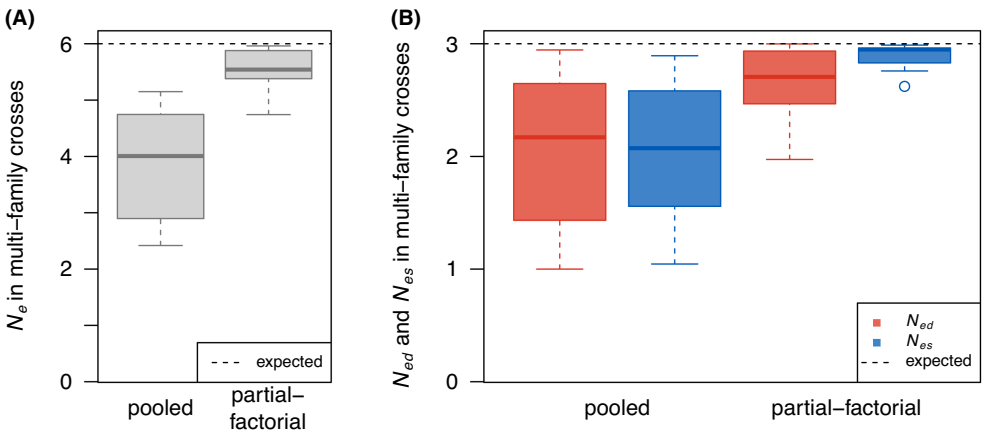

FIGURE 3 For each spawning experiment, the (A) effective population size (N_e) and (B) effective number of dams (N_{ed}) and sires (N_{es}) among 10 multifamily crosses. The expected values are indicated with a dashed black line.

TABLE 3 For each spawning experiment, the expected N_e, N_{ed}, N_{es} in each multifamily cross, the mean observed values (±SD), and the percentage of the reduction from expected to observed values that can be attributed to failed spawners or variation in family size.

Spawning experiment	Metric	Expected value	Mean observed value (±SD)	Effect of failed spawners (%)	Effect of variation in family size (%)
Pooled	N_e	6	3.9 (±1.0)	−13	−22
	N_{ed}	3	2.0 (±0.7)	−13	−19
	N_{es}	3	2.1 (±0.6)	−7	−23
Partial factorial	N_e	6	5.5 (±0.4)	0	−8
	N_{ed}	3	2.7 (±0.3)	0	−12
	N_{es}	3	2.9 (±0.1)	0	−4

TABLE 4 For each spawning experiment, the effective size of the theoretical release population (N_{eR}) expected value, observed value when accounting for variation in larval counts in each multifamily cross, and observed value after equalizing the number of larvae from each cross.

Spawning experiment	Expected N_{eR}	Observed N_{eR} (with larval counts)	Observed N_{eR} (equalized larvae)
Pooled	60	29 (49% of target)	36 (61% of target)
Partial factorial	60	53 (88% of target)	55 (92% of target)

supplementation requires the development of a genetically managed spawning strategy. Here, we experimentally evaluated two multifamily-cross spawning methods to aid in the development of such a strategy. We found that partial-factorial spawning consistently produced high N_e values, suggesting its potential for maintaining genetic diversity and reducing inbreeding in cultured and supplemented populations over time. In contrast, our pooled spawning experiment produced lower and more variable N_e values, demonstrating that this more-efficient method of production entails high cost in terms of the long-term genetic management of cultured and supplemented stocks. The difference in the performance of each spawning method compounded when we combined crosses to form a hypothetical release population.

Combining the N_e values among crosses and accounting for variation in the estimated number of larvae that was produced in each cross revealed that the pooled experiment produced an N_{eR} value that was approximately half of the expected value, whereas the partial-factorial experiment maintained an N_{eR} value that was close to the expected value (Table 4). This suggests that pooled spawning amplifies risk of losing genetic variation as multiple crosses are combined into a release population, whereas partial-factorial spawning buffers against such added loss. Because we sampled larvae soon after they hatched, we captured the reduction in N_e largely due to factors in the fertilization stage. As fish continue to age, competition and other sources of mortality could continue to reduce N_e, though some evidence suggests that the greatest decline in N_e occurs at the fertilization stage, mostly due to failed spawns (O'Leary et al. 2022). Understanding the response in N_e at the subadult to adult life stage, the age at which Delta Smelt are released to the wild (U.S. Fish and Wildlife Service 2020, 2021), would further inform the genetic consequences of these two spawning strategies.

Comparing the N_{ed} and N_{es} values between the two spawning methods that we tested provides insight into the sex-specific mechanisms driving the performance of each method. Variation in sire family sizes caused a significantly larger reduction in the average N_{es} for the pooled experiment (−23%) than for the partial-factorial experiment (−4%; Table 3). A difference of this order of magnitude likely results from the opportunity for sperm competition in the pooled crosses: sperm from multiple males were added to eggs at the same time, likely resulting in competition to fertilize a limited supply of eggs (Rahman et al. 2022), whereas in partial-factorial crosses, sperm from each male was added to eggs in separate bowls. Several studies in hatcheries for other species have similarly found that sperm competition increases variation in the reproductive success of sires (Withler 1988; Cameron Brown et al. 2005; Bartron et al. 2018), demonstrating that sperm competition poses a significant risk of reducing genetic variation in hatchery programs (Beirão et al. 2019).

In contrast to N_{es}, variation in the family sizes of dams caused more similar reductions in N_{ed} for each experiment (−19% for pooled and −12% for partial-factorial; Table 3). This suggests that dam-associated traits such as egg quality affected the relative contributions of dams regardless of the spawning method that was used. For example, only two single-pair crosses with the partial-factorial strategy failed to produce any offspring and they both included the same dam (fish ID: YK70), who produced only one offspring in her third pair cross (Tables S2 and S3). Each of the males she was paired with produced offspring with other females, suggesting that a trait that was specific to this female (e.g., egg quality) could explain the unsuccessful fertilization of the dam's (YK70) eggs. In our experiments, we equalized eggs within females (i.e., for partial-factorial crosses, each female's eggs were split equally into three containers), but we did not equalize eggs among females in the same cross. So it is possible, for example, that one female may have outperformed another simply by contributing more eggs to the cross. Similarly for males, milt was not volumetrically or otherwise equalized among males in the same multifamily cross. Equalizing the gametes that are contributed by each parent to multifamily crosses could reduce some of the variation that we observed in family sizes, which would likely increase N_e values and decrease variability of N_e values.

In populations with high variation in reproductive success, a multifamily-cross spawning strategy can lead to higher N_e values than a single-pair-cross strategy because each parent has multiple opportunities to produce offspring, even if an individual pair cross fails (Fiumera et al. 2004; Busack and Knudsen 2007), as occurred in the case of dam YK70 and her three sires (see above). In contrast, a single-pair-cross strategy links the representation of each parent in a pair cross to its mate, leading to the loss of both parents even if only one is compromised (Fisch et al. 2015). For example, if we spawned three dams and three sires in single-pair crosses, it would

result in three total pair crosses; if one pair cross fails, we lose one-third of the crosses and one-third of the parents. Instead, if we spawned three dams and three sires in a multifamily cross, it would result in nine possible pair crosses; if one pair cross fails, only one-ninth of the crosses would be lost and each of the parents in the failed pair cross would have the opportunity to produce offspring with two other mates, lowering the likelihood of losing the parents completely. For both multifamily-cross methods that we tested, we observed a lower percentage of failed parents than failed pair crosses (Table 2), demonstrating the value of multiple potential mates for each spawner.

As a post hoc comparison with our experimental results (Table 2), data from the regular production season at the FCCL in 2021 show that 9% of the pair crosses (29 of 326) failed at the fertilization stage (T.-C. Hung, unpublished data). Because the hatchery employs a single-pair-cross strategy, that means that 9% of sires and 9% of dams failed to contribute their genetic material to the hatchery population regardless of the reproductive potential of each parent. The FCCL does, however, attempt to cross more than one fish from each family to minimize the loss of genetic diversity due to failed crosses. In a case such as with Delta Smelt, where wild fish are not currently available to contribute to the conservation hatchery, all or most remaining genetic diversity for the species resides within the captive population. This increases the importance of representing as much of that diversity in the captive population and released fish as possible, including by considering a potentially resource-intensive spawning strategy like the partial-factorial strategy that we tested here.

Although we have demonstrated the reduction in genetic diversity that arises from pooled spawning, this strategy requires less time and effort than partial-factorial spawning. Hatchery managers may therefore benefit from a combination of spawning methods to best address the challenge of producing large numbers of genetically managed fish for supplementation. For example, if managers want to employ some pooled crosses, extra pooled crosses would be required to achieve the same target N_{eR} value as is achievable with fewer but more labor-intensive partial-factorial crosses. Similarly, a combination of single-pair crosses and multifamily crosses could be employed to balance production needs. Equalizing gametes at the fertilization step for multifamily crosses and/or equalizing the contributions of different crosses to the release population would also buffer against strategy-specific risk and improve genetic diversity outcomes. Additional experiments testing other spawning methods could also help evaluate the genetic consequences and logistical feasibility of different production options. Identifying a spawning strategy that meets the mandate of balancing production efficiency with conserving genetic diversity requires managers to consider many complex and changing factors, and experiments like this one provide important information to help make these multifaceted management decisions.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data and scripts are available at https://github.com/meflacava/DSAltSpawn.

ETHICS STATEMENT

All the animal handling protocols and rearing operations were reviewed and approved by the Institutional Animal Care and Use Committee (#18081 and #19747) at the University of California, Davis.

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REFERENCES

Bartron, M. L., Sard, N. M., & Scribner, K. T. (2018). Evaluation of effective number of breeders and coancestry among progeny produced using common hatchery mating strategies. *Transactions of the American Fisheries Society*, *147*(1), 185–194. https://doi.org/10.1002/tafs.10013

Baskerville-Bridges, B., Lindberg, J. C., & Doroshov, S. I. (2005). *Manual for the intensive culture of Delta Smelt* (Hypomesus transpacificus). University of California, Davis.

Beirão, J., Egeland, T. B., Purchase, C. F., & Nordeide, J. T. (2019). Fish sperm competition in hatcheries and between wild and hatchery origin fish in nature. *Theriogenology*, *133*, 201–209. https://doi.org/10.1016/j.theriogenology.2019.03.034

Busack, C., & Knudsen, C. M. (2007). Using factorial mating designs to increase the effective number of breeders in fish hatcheries. *Aquaculture*, *273*(1), 24–32. https://doi.org/10.1016/j.aquaculture.2007.09.010

California Department of Fish and Wildlife. (2009). *State and federally listed endangered and threatened animals of California*. California Department of Fish and Wildlife.

Cameron Brown, R., Woolliams, J. A., & McAndrew, B. J. (2005). Factors influencing effective population size in commercial populations of Gilthead Seabream, *Sparus aurata*. *Aquaculture*, *247*(1–4), 219–225. https://doi.org/10.1016/j.aquaculture.2005.02.002

Campton, D. E. (2004). Sperm competition in salmon hatcheries: The need to institutionalize genetically benign spawning protocols. *Transactions of the American Fisheries Society*, *133*(5), 1277–1289. https://doi.org/10.1577/T03-200.1

Crow, J. F., & Kimura, M. (1970). *An introduction to population genetics theory*. Harper and Row.

Ellison, L., Rahman, M. M., Finger, A. J., Sandford, M., Hsueh, C., Schultz, A. A., & Hung, T. (2023). Size, fecundity and condition factor changes in endangered Delta Smelt *Hypomesus transpacificus* over 10 generations in captivity. *Aquaculture, Fish and Fisheries*, *3*(4), 353–365. https://doi.org/10.1002/aff2.116

Endangered and threatened wildlife and plants: Determination of threatened status for the Delta Smelt, 50 C.F.R. § 17. (1994). https://www.govinfo.gov/content/pkg/FR-1994-12-19/html/94-31063.htm

Finger, A. J., Mahardja, B., Fisch, K. M., Benjamin, A., Lindberg, J., Ellison, L., Ghebremariam, T., Hung, T.-C., & May, B. (2018). A conservation hatchery population of Delta Smelt shows evidence of genetic adaptation to captivity after 9 generations. *The Journal of Heredity*, *109*(6), 689–699. https://doi.org/10.1093/jhered/esy035

Fisch, K. M., Ivy, J. A., Burton, R. S., & May, B. (2013). Evaluating the performance of captive breeding techniques for conservation hatcheries: A case study of the Delta Smelt captive breeding program. *The Journal of Heredity*, *104*(1), 92–104. https://doi.org/10.1093/jhered/ess084

Fisch, K. M., Kozfkay, C. C., Ivy, J. A., Ryder, O. A., & Waples, R. S. (2015). Fish hatchery genetic management techniques: Integrating theory with implementation. *North American Journal of Aquaculture*, *77*(3), 343–357. https://doi.org/10.1080/15222055.2014.999846

Fiumera, A. C., Porter, B. A., Looney, G., Asmussen, M. A., & Avise, J. C. (2004). Maximizing offspring production while maintaining genetic diversity in supplemental breeding programs of highly fecund managed species. *Conservation Biology*, *18*(1), 94–101. https://doi.org/10.1111/j.1523-1739.2004.00521.x

Gold, J. R., Ma, L., Saillant, E., Silva, P. S., & Vega, R. R. (2008). Genetic effective size in populations of hatchery-raised Red Drum released for stock enhancement. *Transactions of the American Fisheries Society*, *137*(5), 1327–1334. https://doi.org/10.1577/T07-192.1

Kalinowski, S. T., Taper, M. L., & Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, *16*(5), 1099–1106. https://doi.org/10.1111/j.1365-294X.2007.03089.x

LaCava, M., Fisch, K., Nagel, M., Lindberg, J. C., May, B., & Finger, A. J. (2015). Spawning behavior of cultured Delta Smelt in a conservation hatchery. *North American Journal of Aquaculture*, *77*(3), 255–266. https://doi.org/10.1080/15222055.2015.1007192

Lacy, R. C. (1989). Analysis of founder representation in pedigrees: Founder equivalents and founder genome equivalents. *Zoo Biology*, *8*(2), 111–123. https://doi.org/10.1002/zoo.1430080203

Lacy, R. C., Ballou, J. D., & Pollak, J. P. (2012). PMx: Software package for demographic and genetic analysis and management of pedigreed populations. *Methods in Ecology and Evolution*, *3*(2), 433–437. https://doi.org/10.1111/j.2041-210X.2011.00148.x

Lew, R. M., Finger, A. J., Baerwald, M. R., Goodbla, A., May, B., & Meek, M. H. (2015). Using next-generation sequencing to assist a conservation hatchery: A single-nucleotide polymorphism panel for the genetic management of endangered Delta Smelt. *Transactions of the American Fisheries Society*, *144*(4), 767–779. https://doi.org/10.1080/00028487.2015.1037016

Lindberg, J. C., Tigan, G., Ellison, L., Rettinghouse, T., Nagel, M. M., & Fisch, K. M. (2013). Aquaculture methods for a genetically managed population of endangered Delta Smelt. *North American Journal of Aquaculture*, *75*(2), 186–196. https://doi.org/10.1080/15222055.2012.751942

Moyle, P. B., Brown, L. R., Durand, J. R., & Hobbs, J. A. (2016). Delta Smelt: Life history and decline of a once-abundant species in the San Francisco estuary. *San Francisco Estuary and Watershed Science*, *14*(2), Article 6. https://doi.org/10.15447/sfews.2016v14iss2art6

O'Leary, S. J., Hollenbeck, C. M., Vega, R. R., Fincannon, A. N., & Portnoy, D. S. (2022). Effects of spawning success and rearing-environment on genome-wide variation of Red Drum in a large stock-enhancement program. *Aquaculture*, *560*, Article 738539. https://doi.org/10.1016/j.aquaculture.2022.738539

Pearson, K. (1900). X. On the criterion that a given system of deviations from the probable in the case of a correlated system of variables is such that it can be reasonably supposed to have arisen from random sampling. *The London Edinburgh and Dublin Philosophical Magazine and Journal of Science*, *50*(302), 157–175. https://doi.org/10.1080/14786440009463897

Peterson, J. T., & Barajas, M. F. (2018). An evaluation of three fish surveys in the San Francisco Estuary, California, 1995-2015. *San Francisco Estuary and Watershed Science*, *16*(4), Article 2. https://doi.org/10.15447/sfews.2018v16iss4art2

R Core Team. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Rahman, M. M., Asadi Aghbolaghi, M., & Hung, T.-C. (2023). Evaluate effects of the dilution medium and holding time on various motility parameters of Delta Smelt semen. *Theriogenology*, *197*, 301–309. https://doi.org/10.1016/j.theriogenology.2022.11.029

Rahman, M. M., Hung, T.-C., Badger, M. E., Finger, A. J., & Carson, E. W. (2022, March 22–24). *Assessing sperm quality and competition for paternity success in Delta Smelt* (Hypomesus transpacificus) [Poster presentation]. Interagency Ecological Program Annual Workshop https://tinyurl.com/dsspermcompetition

Ryman, N., & Laikre, L. (1991). Effects of supportive breeding on the genetically effective population size. *Conservation Biology: The*

Journal of the Society for Conservation Biology, *5*(3), 325–329. https://doi.org/10.1111/j.1523-1739.1991.tb00144.x

Student. (1908). The probable error of a mean. *Biometrika*, *6*(1), 1–25. https://doi.org/10.2307/2331554

U.S. Fish and Wildlife Service. (2019). *Biological opinion for the reinitiation of consultation on the coordinated operations of the Central Valley Project and State Water Project* (Service file no. 08FBTD00-2019-F-0164). U.S. Fish and Wildlife Service.

U.S. Fish and Wildlife Service. (2020). *Delta Smelt supplementation strategy* (Interagency agreement no. R20PG00046). U.S. Fish and Wildlife Service, San Francisco Bay-Delta Fish and Wildlife Office.

U.S. Fish and Wildlife Service. (2021). *Delta Smelt experimental release* (Study plan, 7 December 2021).

Wilcoxon, F. (1945). Individual comparisons by ranking methods. *Biometrics Bulletin*, *1*, 80–83. https://doi.org/10.2307/3001968

Withler, R. E. (1988). Genetic consequences of fertilizing Chinook Salmon (*Oncorhynchus tshawytscha*) eggs with pooled milt. *Aquaculture*, *68*(1), 15–25. https://doi.org/10.1016/0044-8486(88)90287-6

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.