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Original Article

A Conservation Hatchery Population of Delta Smelt Shows Evidence of Genetic Adaptation to Captivity After 9 Generations

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Abstract

Genetic adaptation to captivity is a concern for threatened and endangered species held in conservation hatcheries. Here, we present evidence of genetic adaptation to captivity in a conservation hatchery for the endangered delta smelt (Fish Conservation and Culture Laboratory, University of California Davis; FCCL). The FCCL population is genetically managed with parentage analysis and the addition of wild fish each year. Molecular monitoring indicates little loss of genetic variation and low differentiation between the wild and conservation populations. Yet, we found an increase in offspring survival to reproductive maturity during the subsequent spawning season (recovery rate) in crosses that included one or both cultured parents. Crosses with higher levels of hatchery ancestry tend to produce a greater number of offspring that are recovered the following year. The recovery rate of a cross decreases when offspring are raised in a tank with fish of high levels of hatchery ancestry. We suggest changes in fish rearing practices at the FCCL to reduce genetic adaptation to captivity, as delta smelt numbers in the wild continue to decline and the use of FCCL fish for reintroduction becomes more likely.

Subject areas: Conservation genetics and biodiversity, Reproductive strategies and kinship analysis
Keywords: conservation hatchery, delta smelt, genetic adaptation to captivity

Captive breeding programs may be used as a last resort to prevent extinction when a species is critically endangered. The creation of a captive population has the advantages of preventing the total extinction of a species and providing a source of individuals for reintroduction or supplementation. Captive populations are frequently used for reintroductions of mammals, birds, reptiles, and amphibians with varying success (Frankham et al. 2002). Endangered species of fish are often propagated in a conservation hatchery. Conservation hatcheries can have a variety of different goals, including regular supplementation from the hatchery to the wild population, or maintenance of a completely closed population in captivity (Naish et al. 2007).

Conservation hatcheries, like zoos, strive to maintain a refuge population that can be introduced to the wild for conservation purposes and have beneficial impact on the wild population (Flagg and

1

Nash 1999; Utter and Epifanio 2002; Pollard and Flagg 2004; Fisch et al. 2015). Despite the widespread use of various captive breeding programs for conservation, it is acknowledged that even under the best circumstances these programs can pose risks to the wild populations through genetic or phenotypic changes upon reintroduction to the wild (e.g., Ford 2002; Frankham 2008; Fraser 2008; Laikre et al. 2010). One major concern is that captive populations become genetically adapted to captivity, a phenomenon extensively documented in zoos and conservation hatcheries (Naish et al. 2007; Frankham 2008), including those designed for supplementation of wild populations (Christie et al. 2012). Released individuals may have poor fitness (Christie et al. 2014) and may reduce the overall fitness of a stressed wild population if they mate with individuals in the wild population (Ford 2002; Waples and Drake 2004).

Conservation hatcheries have been widely used since the late 1990s, particularly for salmonids (Naish et al. 2007) despite mixed evidence on their effectiveness. The few nonsalmonid fishes held in dedicated conservation hatcheries vary considerably in species' life history, threats, and captive breeding protocols (e.g., white sturgeon, *Acipenser transmontanus*, Ireland et al. 2002; Rio Grande silvery minnow, *Hybognathus amarus*, Osborne et al. 2012; bonytail chub, *Gila elegans*, Hedrick et al. 2000; delta smelt, *Hypomesus transpacificus*, Lindberg et al. 2013). The delta smelt refuge population at the Fish Conservation and Culture Lab at UC Davis (FCCL) is well suited to examine genetic adaptation to captivity. The FCCL is primarily devoted to maintaining a delta smelt refuge population, which has been intensively genetically managed since 2008 (Fisch et al. 2013; Lindberg et al. 2013).

The delta smelt is a small (5–7 cm) Osmerid endemic to the Sacramento-San Joaquin Delta (Delta). delta smelt generally have an annual life cycle, although they can live for 2–3 years in culture. The species was historically common (Moyle 2002) but has declined precipitously since 1980s (Bennett 2005; Newman 2008; Hobbs et al. 2017) due to a variety of stressors, including habitat alteration, altered river flows due to water exports to cities and agricultural lands, interactions with non-native species, and food web alterations (Nichols et al. 1986; Interagency Ecological Program Management, Analysis, and Synthesis Team (IEP MAST) 2015). The species is now listed by the State of California as endangered and the US Fish and Wildlife Service as threatened (USFWS 1993; California Department of Fish and Wildlife 2017). The critically endangered delta smelt is the focus of intensive conservation efforts (Hobbs et al. 2017), including the founding of the FCCL. The refuge population at the FCCL was developed with 3 main goals: 1) prevent the extinction of the species, 2) provide a source population for the possible release of cultured fish into the wild, and 3) propagate fish for research (Lindberg et al. 2013).

Fish spawned and reared at the FCCL have not yet been released into the Delta for supplementation, in part due to concern that captive delta smelt may pose a genetic threat to the dwindling wild population. Additionally, the Sacramento-San Joaquin Delta is presently generally considered poor habitat for delta smelt (IEP MAST 2015), although large-scale habitat restoration projects are underway (California Natural Resources Agency 2016). The historic drought from 2012 to 2016 in California further exacerbated the critical status of delta smelt (Hobbs et al. 2017). Managers are now evaluating the implications of releasing captive fish from the FCCL into the wild for supplementation. Therefore, it is timely and prudent to review evidence of domestication selection in the conservation hatchery.

The objective of this study is to use FCCL pedigree records and genetic monitoring data to examine evidence of genetic adaptation

to captivity. We calculated the domestication index (DI; level of hatchery ancestry) for each culture-born parent and compared offspring survival to maturity in the spawning season (recovery rate) in various crosses, W (wild) × C (captive), C×W, C×C, and W×W, for several generations. We hypothesized that 1) there are differences in recovery rates of offspring for each cross type in a spawning season, 2) these rates change over time, depending on the number of generations in captivity, and 3) larger DI values correlate with higher recovery rates. We also discuss how patterns in pedigrees and neutral genetic variation inform reintroductions and FCCL protocols.

Methods

FCCL Protocol

Upon initiating the genetically managed delta smelt population, the Genomic Variation Lab (UC Davis; GVL) worked with the FCCL to devise best rearing and mating methods, given spatial and time constraints of both programs. Detailed FCCL protocols and genetic management appear in Lindberg et al. (2013) and Fisch et al. (2013), respectively. The FCCL was designed as a conservation hatchery with substantial resources devoted to rearing and maintaining a refuge population and is operated to replicate the wild population as closely as possible (Lindberg et al. 2013). This is achieved in part through the crossing of wild-caught with cultured fish, minimizing relatedness between cultured parents, and equalizing family sizes at the egg stage (Fisch et al. 2013).

All crosses in this study were made annually at the FCCL from 2008 to 2015. Families were composed of offspring from a single pair cross (SPCs, 1 male × 1 female). SPCs and their genotypes were recorded for subsequent pedigree reconstruction. The refuge population was founded in December 2006 with 2418 wild-caught subadult fish of which 2300 of survived after 72 h (Rettinghouse 2007). In March 2007, 1589 fish had survived (Rettinghouse 2008a), but infrastructure was not in place to make crosses. In 2008, 533 fish survived as 2-year-olds, and 328 (164 females and 164 males) were crossed. The F_1 generation of captive fish born in 2008 was mated in 2009. The F_1 generation is also referred to as birth year (BY) 2008. In 2016, the FCCL created the F_9 generation (BY2016) by crossing wild and cultured fish from BY2015 (Table 1). Reproductive successes for BY2016 crosses were measured via numbers of offspring tagged upon maturation after 1 year in 2017.

Annual Spawning of Refuge Population

Annual spawning of the FCCL refuge population began in late January/early February and ended mid-May/early June. Each week, hatchery personnel sorted through tanks of adult fish from the oldest to the youngest to identify sexually mature individuals (Figure 1; Lindberg et al. 2013). Each tank consisted of 1 multi-family group (MFG) with maturing offspring, and each MFG usually consisted of 8 SPCs from 16 parents mated the previous year. A single MFG is raised until just before the spawning season when 2 were combined. Fin-clip tissue samples were taken from sexually mature individuals for genetic analysis and simultaneously implanted subdermally with an elastomer tag with a unique alpha-numeric ID (tag ID). Tagged fish were then housed in a separate tank for later sorting. Each week, fish were tagged and sorted until about 10 mature males, and 10 mature females were available, depending on the number of mature fish and the timing of the spawning season. Fish were processed in batches from each of the MFGs until 192 fin clips were collected. Throughout a spawning season, an upper limit of 90 fish was tagged

Table 1. RRS and recovery rates (proportion of each cross type that had nonzero recovery) of parents from the F_0 to F_8 generations (parents crossed from 2008 to 2016). For example, RRS for the F_1 generation refers to the reproductive success of parents born in 2008 and crossed in 2009, as measured by their offspring that survived to become mature and be tagged and analyzed for parentage during 2010 spawning season

Gen	Birth year	Year crossed	Year RRS calculated	N tagged	Cross type	N crosses	Mean offspring recovered per cross	Recovery rate	Variance in recovery	RRS
F_0	2006	2008	2009	911	W×W	164	5.28	0.933	63.01	—
F_1	2008	2009	2010	1858	C×C	189	4.06	0.884		1
					(C×W/W×C)	57	3.36	0.877		0.83
					W×W	1	—	0		—
F_1 all									40.46	
F_2	2009	2010	2011	1754	C×C	207	5.17	0.884		1
					(C×W/W×C)	21	2.41	0.571		0.47
					W×W	7	1.64	0.429		0.32
F_2 all									36.37	
F_3	2010	2011	2012	2283	C×C	215	5.12	0.856		1
					(C×W/W×C)	18	2.63	0.667		0.51
					W×W	25	1.73	0.44		0.33
F_3 all									56.69	
F_4	2011	2012	2013	2217	C×C	253	4.24	0.941		1
					(C×W/W×C)	8	1.62	0.625		0.38
					W×W	20	1.13	0.25		0.27
F_4 all									53.86	
F_5	2012	2013	2014	2412	C×C	182	7.13	0.962		1
					(C×W/W×C)	75	3.19	0.68		0.45
					W×W	4	1.19	0.50		0.17
F_5 all									61.40	
F_6	2013	2014	2015	1996	C×C	160	6.12	0.944		1
					(C×W/W×C)	65	3.38	0.692		0.55
					W×W	0	—	—		—
F_6 all									48.33	
F_7	2014	2015	2016	2698	C×C	197	5.81	0.929		1
					(C×W/W×C)	44	2.39	0.795		0.41
					W×W	2	1.73	0.50		0.30
F_7 all									141.12	
F_8	2015	2016	2017	3037	C×C	182	8.18	0.967		1
					(C×W/W×C)	50	4.04	0.86		0.49
					W×W	2	2	0.50		0.24
F_8 all									156.15	
All	—	—		19166	C×C	3164	5.49			1
					(C×W/W×C)	672	3.12			0.57
					W×W[a]	124	1.46			0.27

[a] F_0 removed.

from each MFG. Fin clips were sent to the GVL for genotyping, parentage analysis, and pedigree reconstruction (Figure 1).

Genotyping, Parentage Analysis, and Pedigree Reconstruction

Each year, genetic data were collected and scored at 12 microsatellite markers following Fisch et al. (2009). To reconstruct the pedigree, we assigned tagged individuals to their parents using Cervus (Kalinowski 2007). If only a single parent was identified, no parents were identified, or parents did not match known crosses from FCCL records so that parentage was not determined with high confidence (100%), we used Colony (Jones and Wang 2010) with a reduced subset of possible parents including only the 16 nonsibling parents that contributed to each MFG/tank. Individuals not assigned to both parents according to FCCL pedigree records were discarded. Exclusions were <5% of all individuals and were generally due to poor genotype quality.

Creation of SPCs

Tagging, sorting, genotyping, parentage analysis, and the creation of SPCs occurred concurrently during each spawning season. On Tuesdays and Fridays, FCCL personnel sorted through tanks of tagged individuals and recorded the tag IDs of sexually mature females (Figure 1). The pedigree reconstruction of tagged fish was used by the GVL to select tagged males to mate with specific females to balance a number of factors, including prioritizing crossing of wild fish, minimizing mean kinship (MK) and inbreeding coefficient (*F*) calculated with PMx (Ballou et al. 2011), avoiding overrepresentation of specific families from the previous year's crosses, and allocating remaining tank capacity in the hatchery. A list of recommended crosses was sent to the FCCL, and crosses were made by strip spawning eggs and sperm. Equal numbers of eggs from 8 SPCs (~700 eggs/SPC) were combined into an individual tank, creating an MFG of fish that were reared together (Figure 2). The process continued until about 264 SPCs were created (roughly 32 MFGs, Figure 2). When a
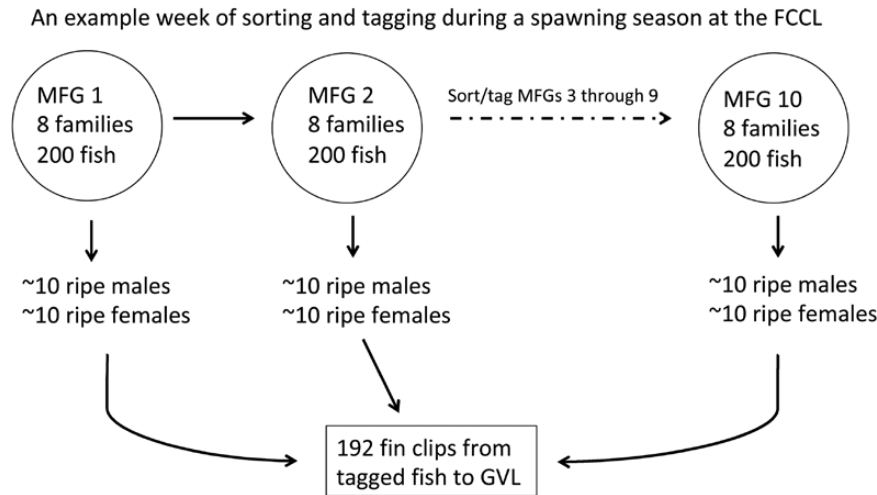
An example week of sorting and tagging during a spawning season at the FCCL

Figure 1. Depiction of how the FCCL personnel sorts through tanks with an MFG in an average week during the spawning season (late January–May 15). Personnel sort through MFGs sequentially, searching for mature individuals. Roughly 10 mature females and 10 mature males are then tagged and fin-clipped until 192 clips are collected. Roughly 10 MFGs will be sorted to reach 192 clips. Fin clips are then sent to the GVL for parentage analysis and pedigree reconstruction.
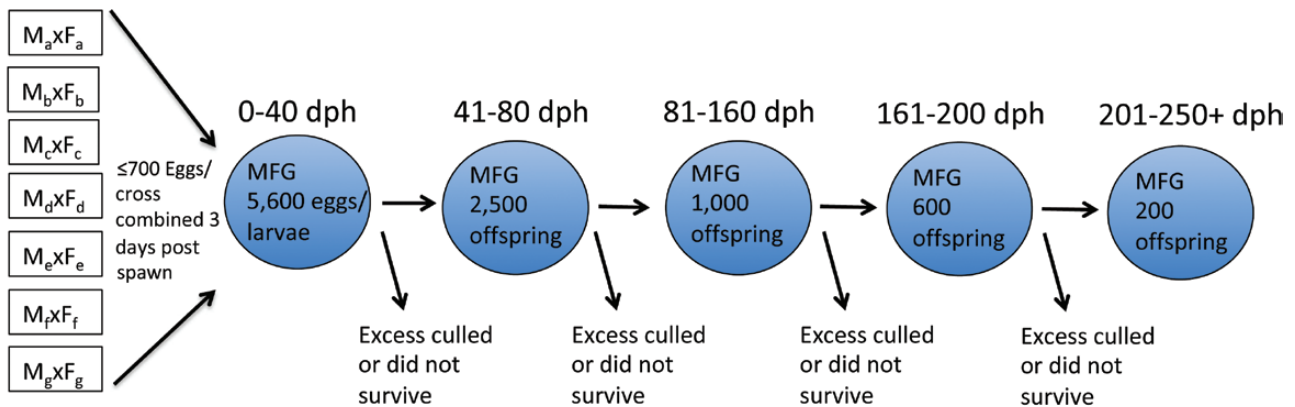
Figure 2. Depiction of how SPCs are combined into MFGs at the FCCL, and thinned over time. For each MFG, 8 single pair crosses are made with a total of 16 parents, each of which could be wild-born or culture-born. These 8 crosses are combined at the egg state, with 700 eggs per cross. MFGs are thinned to 2500 offspring 41 dph, 1000 offspring at 81 dph, 600 offspring at 161 dph, and finally 200 offspring, where they will be kept until and throughout spawning season.

cross was made with a wild parent that was considered successful but had fewer than 700 eggs, those eggs were added to the MFG. There were 4 possible types of SPCs: 1) wild female × wild male (W×W), 2) wild female × cultured male (W×C), 3) cultured female × wild male (C×W), and 4) cultured female × cultured male (C×C). The relative frequency of these crosses depended on the availability of cultured and wild fish and their sexual maturity so that the number of the 4 cross types varied from tank to tank. Growing larvae were thinned to optimal culture density of 200 fish per MFG at 201–250 days post hatch (dph) at 3 life stages when survival was high (Figure 1). Fish were thinned by draining tanks to 6 inches to concentrate the fish, and then haphazardly selecting fish with a net.

Annual Genetic Monitoring

After each spawning season, we monitored cumulative genetic variation and differentiation between generations of parents that were spawned. We estimated overall levels of genetic variability with mean number of alleles (N_A) and observed (H_O) and expected (H_E) heterozygosity with GenAlEx 6.5 (Peakall and Smouse 2012) and allelic richness (A_R) with HP Rare (Kalinowski 2004, 2005).

We calculated pairwise F_{ST} values with the program FSTAT 1.2 (Goudet 1995) to measure genetic differentiation between each generation of parents. We calculated significances using 1000 bootstrap repetitions with a sequential Bonferroni correction (Rice 1989). We also estimated pairwise F_{ST} values between cultured and wild fish separately, within and between years.

We estimated contemporary effective population sizes of the wild and cultured parents used in crosses each generation to monitor levels of genetic variation using NeEstimator 2 (Do et al. 2014). We used the linkage disequilibrium method with the bias-correction, N_{ELD} (Waples 2006; Waples and Do 2010) and removed alleles with frequencies <0.05. Monogamy was selected for analysis of cultured

fish, and random mating was selected for wild fish to reflect the various mating schemes of the cultured and wild populations. Ninety-five percent confidence intervals were calculated by parametric bootstrapping.

We used PMx 1.0 (Ballou et al. 2011) and pedigree records to estimate MK between parents and mean inbreeding (*F*). Results were compiled for each generation of parents.

Recovery Rates

When at least 1 offspring from an SPC survived to adulthood and was tagged, genotyped, and assigned to parents, the cross from which it originated was considered to be "recovered" in the following spawning season. We calculated the recovery rate of each cross type as the percentage of crosses ([W×C/C×W], W×W, and C×C) that had at least 1 recovered offspring each year during the spawning season. Some fish were immature and untagged after the spawning season. These fish may have been from SPCs with no recovered offspring and were considered "unrecovered." We also calculated the variance in the number of recovered offspring for crosses made within years.

Relative Reproductive Success

We calculated relative reproductive success (RRS) of each cross type each year to examine how successful crosses with 1 or 2 wild parents were relative to crosses with 2 cultured parents. RRS is a common method for examining fitness effects of hatcheries (e.g., Araki et al. 2008). We calculated RRS of the W×C, C×W, and W×W crosses compared with C×C crosses each year using the equations $S_{W \times C}/S_{C \times C}$, $S_{C \times W}/S_{C \times C}$, and $S_{W \times W}/S_{C \times C}$, where S is the geometric mean number of offspring surviving to adulthood from W×C, C×W, W×W, and C×C crosses, respectively. The RRS of C×C crosses is always 1 by definition.

Difference in Recovery Success by Cross Type

We used a negative binomial generalized linear mixed model of the RRS of parents from the F_0 to F_8 generations to examine differences in the number of recovered offspring among the 4 cross types (C×C, W×C, C×W, and W×W). This model was constructed using lme4 in R (Bates et al. 2015; R Core Team 2016). Cross type was incorporated as fixed effect and year was included as a random effect. Pairwise comparisons of cross types were made using Tukey's pairwise comparison test with the R package multcomp (Hothorn et al. 2008).

Next, we fitted negative binomial generalized linear models with both cross types and generation (year) as fixed effects using the MASS R package (Venables and Ripley 2002) to examine whether the number of recovered offspring from each cross type changed over time. The global model of cross type, generation, and their interaction term was fitted along with the 4 remaining nested models. Models were ranked using Akaike's Information Criterion corrected for small sample size (AICc). Cross types, W×C and C×W were combined into a single category for this analysis due to a lack of difference in the number of recovered offspring between these cross types. Though fish were not forced to spawn before they were sexually mature, we also fitted these temporal change models excluding individuals born in May or June to determine whether crosses created later, and therefore producing younger offspring the following season, had lower recovery rates.

Effect of DI

The DI is an additive metric that measures the number of generations an individual's genome has spent in captivity. The DI of each individual delta smelt family's offspring was calculated using the

pedigree records with PMx (Ballou et al. 2011). Wild founders or wild annual supplements have a DI of 0, and offspring from 2 founders have a DI of 1, etc. When parents had different DIs, the DI of the cross was the average of their DIs, and the DI of their offspring was the average of both parents plus 1.

We explored the possibility that increasing levels of hatchery ancestry increased recovery rates and reproductive success of individual crosses each season, as well as within each tank. To do this, we fitted a set of negative binomial generalized linear models with the MASS in R (Venables and Ripley 2002) using DI metrics as predictor variables. Predictor variables used for this set of models included the average DI for the parents of each cross, the sum of average DI of parents for all other crosses in the tank, excluding DI from the individual cross analyzed, and the interaction term between the 2. Collinearity between average parental DI and sum of tank DI was relatively high with a Pearson correlation coefficient of 0.82 due to overall increased hatchery ancestry over time in the FCCL population; however, the variance inflation factor for the 2 covariates was within acceptable range at 3.11 (Hair et al. 1995). We then fitted the global model containing all three aforementioned covariates and the four remaining nested models, using recovery number as the response variable. All 5 models were ranked by AICc. We also repeated the analysis using just individuals born before May.

Results

Genetic Monitoring

From 2008 to 2017, 19 166 delta smelt were tagged and genotyped, with 4750 crossed for incorporation into the broodstock at the FCCL (Table 1). Between generations F_1 and F_8, 446 wild fish were crossed into the refuge population.

Over the 9 years of the program, annual values of H_O ranged from 0.83 to 0.85, and H_E ranged from 0.84 to 0.86 (Table 2). A_R dropped slightly over this period, from 23.10 to 20.63 (Table 2), and N_A dropped slightly from 23.58 to 22.17. Pairwise F_{ST} indicated low levels of differentiation (<0.01) between BYs of wild and cultured parents combined (Supplementary Table 1), as well as between wild collections and cultured collections within and among years. However, some values were significant (corrected *P* value after Bonferroni correction = 0.0011; Supplementary Table 2).

The N_{ELD} for wild collections varied considerably and most had upper CIs of infinity (Table 3), signaling that the true N_{ELD} values

Table 2. Genetic monitoring results for each generation of parents at the FCCL

Generation	N	N_A	H_O	H_E	A_R
F_0	328	23.58	0.84	0.86	23.1
F_1	494	24.17	0.84	0.86	23.1
F_2	468	22.42	0.85	0.85	21.1
F_3	512	22.17	0.83	0.84	21.0
F_4	562	22.75	0.84	0.85	21.0
F_5	522	23.00	0.83	0.85	21.2
F_6	448	22.83	0.84	0.85	21.4
F_7	486	22.75	0.84	0.86	21.4
F_8	468	22.42	0.83	0.85	20.8

N is the number of genotyped wild and cultured fish used as parents each year. N_A is number of alleles, H_O and H_E are observed and expected heterozygosity, and A_R is allelic richness. Allelic richness (A_R) was calculated with 404 gene copies, the minimum number of the combined groups.

Table 3. Estimates of contemporary effective population size (N_{ELD}) as calculated with NeEstimator

Generation	N_{WILD}	N_{ELD} wild	N_{CULT}	N_{ELD} cultivated
F_0	328	1542 (7535–52 351)	—	—
F_1	55	503 (115–∞)	439	485 (409–584)
F_2	34	∞ (234–∞)	434	550 (479–638)
F_3	68	1092 (172–∞)	444	425 (366–499)
F_4	45	3520 (123–∞)	514	394 (350–447)
F_5	83	4297 (415–∞)	439	513 (444–599)
F_6	63	∞ (366–∞)	385	843 (683–1076)
F_7	48	∞ (543–∞)	438	1036 (825–1356)
F_8	50	∞ (1093–∞)	416	691 (577–845)

For estimates of N_{ELD} in wild populations, we used the random mating option. For estimates of N_{ELD} of the cultured parents each generation, we used the monogamy mating option.

Table 4. Comparisons of numbers of parents (*N*), MK, average DI, and mean inbreeding (*F*) across generations of parents at the FCCL

Generation	*N*	MK	Avg DI	*F*
F_0	328	0	1	0
F_1	494	0.002	1.88	0
F_2	468	0.003	2.73	0
F_3	512	0.003	3.29	0.001
F_4	562	0.004	4.12	0.002
F_5	522	0.004	4.70	<0.001
F_6	448	0.004	5.18	0.001
F_7	486	0.004	5.73	0.001
F_8	468	0.004	6.01	0.001

All values are pedigree-based, rather than genetic-based, and calculated using the software program PmX version 1.0 (Ballou et al. 2011).

are too large to precisely estimate using this method (Waples and Do 2010). These values were similar to those estimated in Finger et al. (2017) using additional wild samples. N_{ELD} estimates for only cultured parents that were analyzed ranged from 394 to 1036 and were generally larger than the total number of actual spawning parents.

MK among the wild founders in the F_0 generation was assumed to be 0 among. MK from pedigree data increased from 0.002 in the F_1 generation to 0.003 in F_2–F_3 generations and remained constant at 0.004 (Table 4). *F* was consistently lower than 0.002 (Table 4).

Recovery Rates and RRS

In the F_1 generation, in which culture-born fish were first available to mate, the RRSs of crosses with one wild parent (0.83) were already less than C×C crosses (Table 1). In the F_2 generation, RRS of W×C and C×W crosses declined further (0.47). In the F_1 generation, no W×W crosses were made, but from F_2 to F_4, RRSs of W×W crosses declined from 0.32 to 0.27, after which few W×W crosses were made due to low recovery rates. After W×W crosses were discontinued in 2013, the RRSs of W×C and C×W improved moderately reaching 0.49 in 2016 but were never equal to those of the C×C crosses (Table 1). The recovery rates of C×C crosses increased from 0.88 in the F_1 generation to 0.97 in the F_8 generation. Variance in recovery increased dramatically, as some crosses had many recovered offspring and high RRS in later generations, whereas others had a recovery rate of 0 (Table 1). Recovery statistics are summarized in Figure 3.

Difference in Recovery Success by Cross Type

Pairwise comparisons of the recovery of the 4 cross types, C×C, C×W, W×C, and W×W, were significantly different at α = 0.05, with the exception of the comparison between W×C and C×W crosses (Supplementary Tables 4 and 5). A greater number of offspring were recovered from C×C crosses (predicted value of 10.1 recovered offspring) compared with either W×C and C×W crosses or to W×W crosses, with W×W crosses having the smallest number of recovered offspring of all cross types (predicted value of 2.2 recovered offspring; Supplementary Table 4).

The negative binomial model of cross types C×C, W×C and C×W, W×W, generation number, and the interaction effect had the most support based on the smallest AICc value and large Akaike weight (Supplementary Table 6). The model well approximated the actual distribution of reproductive success of C×C delta smelt in the FCCL

(Figure 4). This model indicates a change over time in the RRS of the crosses that varied by cross type. C×C, W×C, and C×W crosses tended to show an increase in the number of recovered offspring over time (Supplementary Figure 1), whereas W×W crosses had lower recovery rates (Supplementary Table 7). All models were refitted with a smaller dataset that excluded fish spawned in the months of May and June. Although parameter estimates for the refitted models differ slightly, the trends remained the same.

Effect of DI

Of the models used to evaluate the effects of DI, the global model containing average parental DI, sum of tank DI, and their interaction term had the smallest AICc value (Supplementary Table 8). Parameters from the best model indicated that crosses with large average parental DIs typically had more offspring mature during the spawning season to be tagged (Supplementary Table 9). The best model also predicted that the presence of other crosses with large average parental DI within a tank reduced the number of recovered offspring in the following year (Figure 5). Overall DI increased over time at the FCCL, indicating that the increase in recovery rate for C×C crosses and the apparent decline of the recovery rates for W×W crosses may be due to the combination of overall increase in DI for C×C crosses and the accumulation of fish with high DI at the FCCL (Figure 6). Refitting the models after removing data from May and June produced the same positive coefficient for average parental DI and negative coefficient for sum of DI in a tank. However, the model without interaction effect had the smallest AICc for models fitted without May and June data (Supplementary Tables 10 and 11). This mismatch and the relatively small ΔAIC_c between the top 2 models on Supplementary Table 8 indicates that the effects between the 2 covariates, average parental DI and sum of tank DI, were more additive than multiplicative.

Discussion

We analyzed 9 years of genetic monitoring and offspring recovery data in the cultured population of the endangered delta smelt at the FCCL conservation hatchery. Annual genetic monitoring using presumably neutral microsatellite markers indicated a minimal loss of genetic diversity and only small amounts of differentiation between the wild and refuge populations. This supports the success of the current genetic management plan for minimizing inbreeding, MK, and differentiation from the wild population. However, we found differences in RRSs and recovery rates of crosses with wild or captive-born
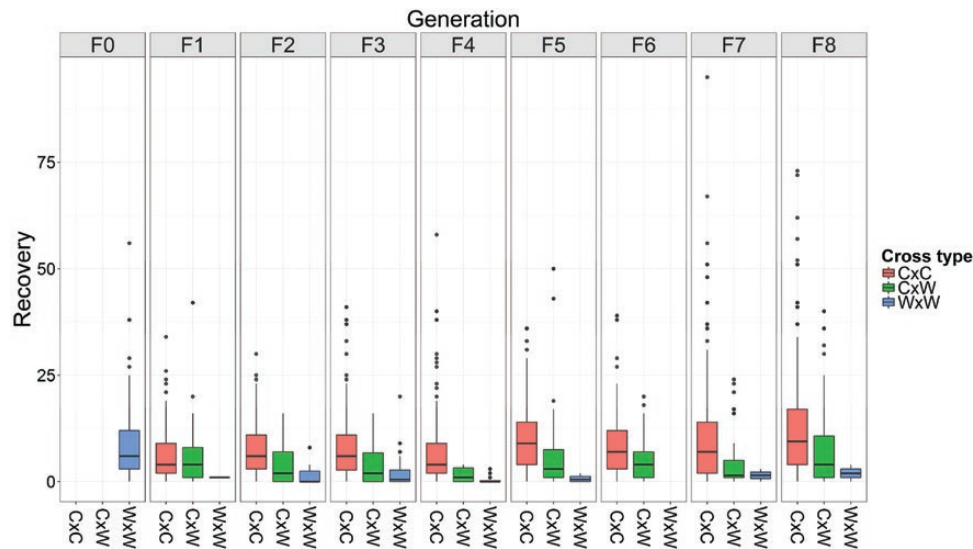
Figure 3. Box plot depicting recovery statistics for each generation (F_0–F_8) by cross type. The F_0 generation was born in 2006, crossed in 2008, and recovery of their offspring was measured in 2009. The most recent generation was the F_8 generation, which was born in 2015, crossed in 2016, and recovery of their offspring was recorded in 2017. See online version for full colors.

parents, and this effect increases with increasing levels of hatchery ancestry of parents. Captive-born delta smelt were increasingly likely to mature during the spawning season and to have more recovered offspring as parents. These results were linked to an increase in DI. The production of mature fish provided an ample number of fish that could be crossed in the hatchery. Our results strongly indicate genetic adaptation to captivity, although other factors may be involved and further research is needed. Here, we explore various mechanisms for our observations and make preliminary recommendations to modify FCCL protocols.

Evidence of Genetic Adaptation to Captivity

Although the majority of wild delta smelt live only 1 year, the founding population survived a year in captivity, so that 2-year-old fish produced the first generation at the FCCL. Older 2-year-old parents may have led to the observed larger recovery rates of the F_0 wild crosses, as larger, older fish are often more fecund (Bennett 2005; Rettinghouse 2008; Baskerville-Bridges and Lindberg 2008). Nevertheless, evidence of genetic adaptation to captivity began immediately after the first culture-born fish were crossed. Offspring of C×C crosses in the early F_1 and F_2 generations had better recovery rates compared with offspring from wild parents, and this trend continued every year. In addition to C×C crosses having better recovery rates than W×W crosses, the recovery rates of C×C crosses increased over time (Table 1). These findings warrant changes in hatchery protocols to reduce genetic adaptation to captivity.

Several lines of evidence point to adaptation to captivity as a primary mechanism for increased RRS and recovery rates of crosses with cultured parents and conversely to lower RRS and recovery rates of 1 and 2 wild-parent crosses. First, the FCCL strives to minimize the genetic effects of small population size by minimizing MK, by equalizing family size as much as possible at egg stage and during the spawning season, and by facilitating gene flow from the wild population. Indeed, genetic monitoring with 12 microsatellites indicates little loss of genetic diversity or an increase in inbreeding. Second, the equalization of family size and the shared environment within tanks isolate levels of hatchery ancestry as a variable that

statistically explains differences in recovery rates. Third, delta smelt born at the FCCL spend their entire life cycle in captivity. Selection by differential mortality occurs at any life stage. Fourth, we show that crosses with higher average parental DI have higher recovery rates, even when one parent is wild. Fifth, we found that the increases in recovery rate of offspring over time for W×C, C×W, and C×C crosses are correlated with an increase in the DI of the cultured parent(s) over time. Finally, we can rule out maternal effects, because we did not find statistically significant differences between recovery rates of W×C and C×W crosses in any year. Culture practices may also play a role in higher recovery rates of offspring from cultured parents. However, culture techniques were consistent among years and would not be expected to change directionally or be associated with DI levels. Parents were crossed in the same way on a given day and in a given season. Taken together, this suggests that recovery rates and RRS are heritable and are subject to directional selection at the FCCL. The trends we observed will likely continue, including an increase in the number of high DI fish.

Even though our results strongly support genetic adaptation to captivity, we cannot rule out other mechanisms, including environmental effects from differences experienced by wild and cultured fish before they are strip spawned. The quality of wild fish may be less than that of culture-born fish, but data on how environmental variables affect spawning success are lacking. We assume that neither the wild nor the FCCL environments changed. We also did not find statistically significant differences between the recovery rates of W×C and C×W crosses. Further research into environmental effects on wild delta smelt success in the hatchery, such as evaluating the quality of wild fish at capture and at spawning, is needed.

Potential Mechanisms for Genetic Adaptation to Captivity

We did not attempt to find genetic or epigenetic changes between culture-born and wild-born fish. Yet, a growing body of research has shown shifts in genetic, epigenetic, behavioral, or fitness patterns in fish exposed in captivity for even a portion of their lives (Reisenbichler and McIntyre 1977; Berejikian et al. 2000; Araki
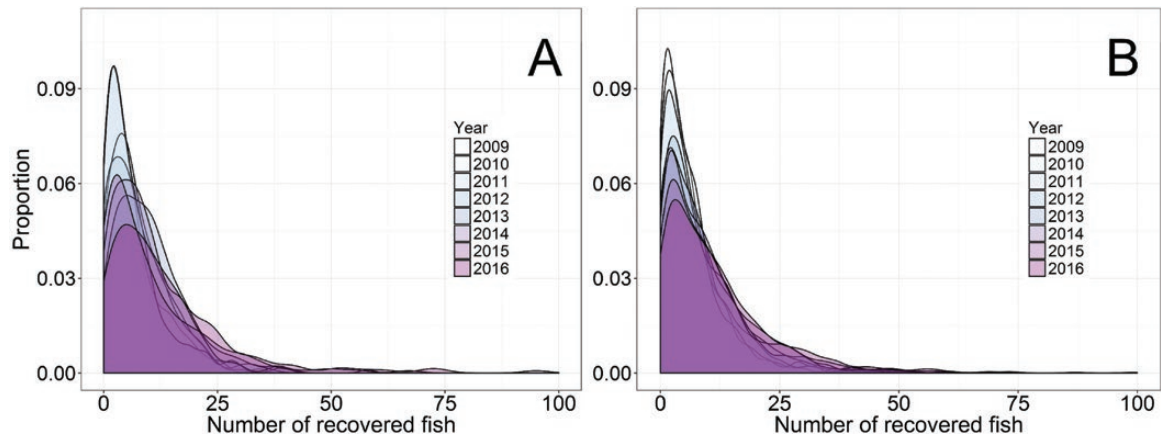
Figure 4. Actual (**A**) versus predicted (**B**) distribution for fish recovery numbers from C×C crosses, demonstrating that our model with linear change in fish recovery number over time fit data well. Predicted distribution on the right is based on the negative binomial model shown in Supplementary Table 7. See online version for full colors.

et al. 2007a, 2007b; Allendorf et al. 2013; Christie et al. 2016; Le Luyer et al. 2017). Selective pressures at the FCCL and in the wild differ considerably: the FCCL is a tightly controlled, predator-free environment with *ad libidum* food availability, whereas the Delta is an estuary with tidal changes in turbidity and temperature, and with larger seasonal and annual changes in temperature and salinity. Adaptation to captivity could cause rapid phenotypic and genetic divergence between wild and hatchery stocks (Einum and Fleming 1997; Lynch and O'Hely 2001; Frankham 2008; Fraser 2008; Christie et al. 2012).

Researchers have recently explored additional mechanisms producing adaptation to captivity, including changes in gene expression or epigenetics in a hatchery setting. For example, Christie et al. (2016) found evidence that hatchery culture produced large-scale changes in patterns of gene expression related to wound healing, immunity, and metabolism in a single generation. Similar heritable changes in gene expression may be associated with increasing levels of DI levels cultured delta smelt. Le Luyer et al. (2017) found parallel changes in DNA methylation in hatchery-reared Coho salmon reared in 2 geographically distant rivers. Similar to wild and FCCL cultured populations, they found no genome-wide genetic differentiation between hatchery- and wild-origin fish from the same river. They concluded that hatcheries might induce epigenetic reprogramming, which may lower the fitness of hatchery-origin fish in the wild. It is possible epigenetic changes lead to variable gene expression in delta smelt at the FCCL, conferring a fitness advantage to offspring of culture-born parents.

Many studies have demonstrated behavioral differences in salmonids raised in different environments (e.g., wild vs. hatchery; Araki et al. 2008; Blanchet et al. 2008; Naish et al. 2007), but few studies have examined interactions between offspring of wild and cultured fish in a hatchery setting where competition for food and territories is ostensibly reduced. Behavioral changes associated with hatchery culture may lead to greater recovery rates and RRSs of culture-born fish. The fish in our study presumably experienced the same rearing environment, as family sizes were roughly equalized at the spawning and egg stages and culture tanks were standardized. Altered behavioral interactions between fish in captivity may have led to differences in family maturation rates within tanks. Wild parents were spawned throughout the season to avoid spawn-timing selection, but SPCs were spawned over a short period and were combined in tanks

sequentially, so that any combination of the 4 cross types could be reared in the same tank. Wild fish crosses were allocated to several tanks, rather than reared separately, to prevent the loss of multiple wild crosses if a tank failed. Our model results on tank effect indicate that the presence of high DI fish in a tank may contribute to the lack of W×W cross after the first generation and is supported by the generally lower recovery and RRS of among offspring of crosses with only a single wild parent. However, there is a paucity of data due to low sample sizes to draw a strong conclusion about the general decline of W×W cross success in the F_0 generation and subsequently. Perhaps a constant environment and similar densities favor C×C offspring behaviorally. Currently, the FCCL is evaluating the effect that tank size and fish density, which may alter behavior, has on recovery rates.

Early Maturity of Cultured Cross Offspring

Research on salmonid hatcheries has shown that the hatchery environment can select for early maturity (Ford et al. 2012), especially when hatchery personnel unintentionally select for early maturing fish. At the FCCL, only fish that have matured can be tagged, and only fish that have been tagged can be spawned. Early in the season, fewer fish are available to tag, meaning that only early maturing fish are crossed. The following year, offspring from these crosses are older and therefore more likely to reach maturity and be tagged. Tagging, sorting, and parentage assessment are labor and resource intensive. The FCCL has tried various protocols to aid in the recovery of offspring from crosses, such as tagging randomly in each MFG without considering sexual maturity or tagging more than 90 fish per MFG. In the former case, many tagged fish never matured during the spawning season and therefore were not incorporated into the refuge population. Indeed, unpublished parentage data from 2016 on untagged fish alive after the spawning season cutoff date demonstrated that the offspring from unrecovered crosses had survived, but had not matured and thus were not recovered.

Consequences of Captive Selection and Recommendations

We offer several recommendations to modify FCCL protocols and to pursue further research in light of our results. First, managers should minimize the time fish spend in captivity (Lorenzen et al.
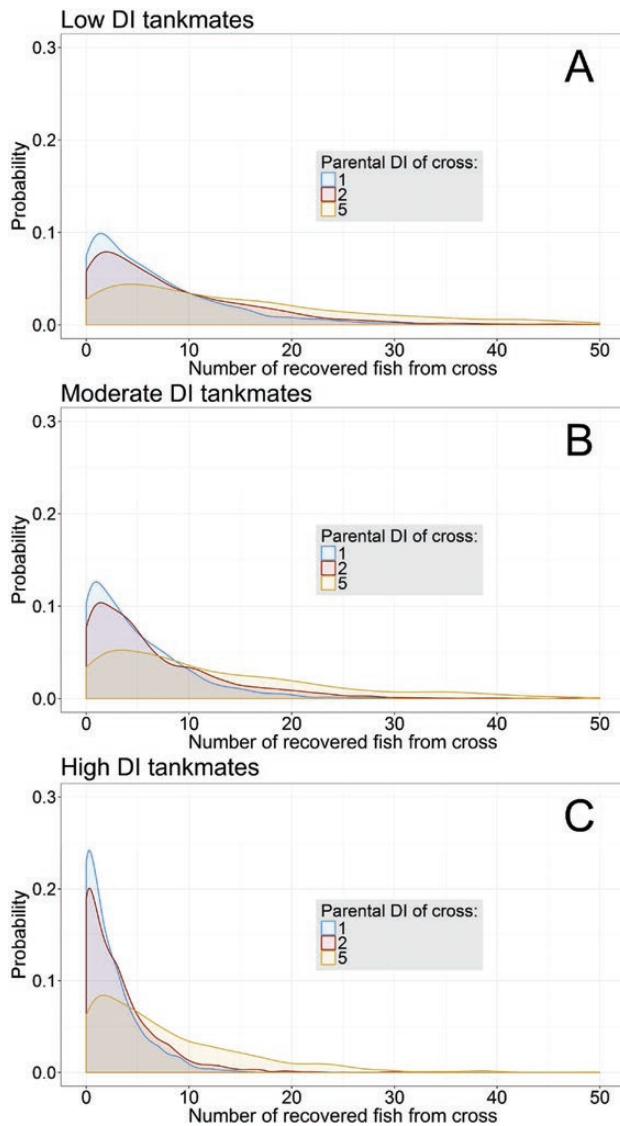
Figure 5. Distribution of number of recovered fish from a cross was found to be a function of the average DI of parents and tank composition based on the negative binomial model in Supplementary Table 9. (**A**) Low DI tankmates = 7 other crosses in tank each with parental DI of 1. (**B**) medium DI tankmates = 7 other crosses in tank each with parental DI of 2. (**C**) high DI tankmates = 7 other crosses in tank each with parental DI of 5. See online version for full colors.

2012) to maintain genetic diversity while preventing domestication selection in the refuge population. However, the FCCL faces significant operational constraints in maintaining a refuge population that minimizes time in captivity, especially the hatchery's small size, the inability to release fish, and the increasing difficulty of capturing wild fish. Estimates of genetic effective population size with large confidence intervals indicate that the broodstock collections (<100 fish per generation) may be only a fraction of total wild population size (Waples and Do 2009). These results are similar to estimates of N_{ELD} in Finger et al. (2017), which used larger sample sizes of wild fish. Therefore, there is no evidence that broodstock collection poses an immediate risk to the wild population. The FCCL should continue to maximize the number of wild fish used to augment the refuge population.
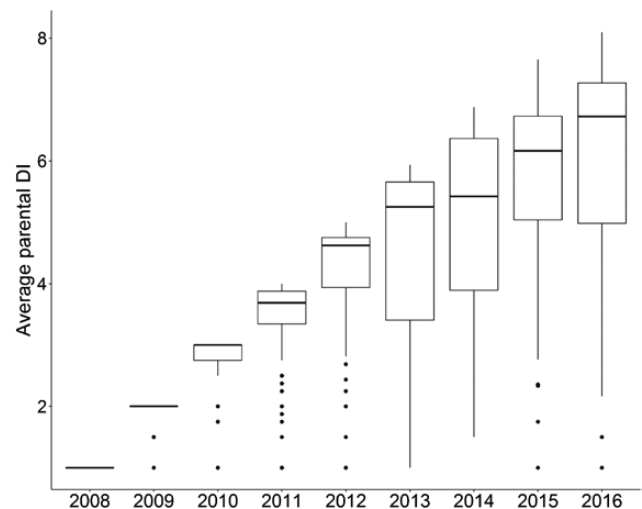

Figure 6. Box and whisker plot displaying average DI between parents for all crosses across years.

A new facility is planned to ameliorate operational constraints through an expansion of the facility, additional personnel, and more science infrastructure, but these changes are several years away. In the meantime, specific recommendations for the current operation include the use of C×W/W×C crosses rather than W×W crosses. The use of C×W/W×C crosses demonstrably improves the chances of incorporating wild fish genes into the refuge population. Another consideration, given that delta smelt fecundity averages 1200–2600 eggs per clutch (Moyle et al. 1992), is to place more or perhaps all eggs from C×W and W×C crosses into an MFG rather than only 700, while still capping C×C cross at 700 eggs. This would likely improve recovery chances for these crosses. We also suggest mating wild fish more than once so that if one cross fails additional matings may be successful. Research has shown that reducing the number of generations in captivity is beneficial for minimizing genetic adaptation to captivity (Frankham 2008). Hence, it may be preferable to accept a small increase in overall *F* and MK in the refuge population by mating wild fish multiple times rather than have lower *F* and more SPCs with high levels of hatchery ancestry. We recommend continuing to retain and mate 2- and 3-year-old wild fish as practiced at the FCCL for the past few years subsequently to this study. We also suggest retaining fish with wild parents that did not mature in later MFGs, as we have found that if the FCCL holds these later MFGs, some of these fish survive and mature in their second year. This would increase recovery statistics for crosses with wild parents.

Future Directions

Our model results indicate that the recovery of offspring from C×C crosses will increase substantially in the near future, as will the recovery of offspring from W×C and C×W crosses to a lesser extent, assuming no extensive protocol changes to the FCCL. If this pattern continues, and fewer wild fish are captured, the FCCL would be able to provide a large number of culture-born fish for reintroduction or supplementation in the future. However, it is questionable whether the release of these fish would result in an overall benefit to the wild delta smelt population given that selection pressures between the field and hatchery differ substantially.

To date, there is no research on survival of FCCL-produced delta smelt in the wild because no fish have been released, as the release

of FCCL delta smelt is not yet permitted. Wild population indices of abundance are so low that we strongly recommend experimental releases to help managers prepare for reintroductions or supplementations (California Natural Resources Agency 2016). These limited releases could be used to estimate the survival of FCCL fish at multiple life stages at several locations in the Delta and to develop field-rearing techniques, including the use of hatching frames to rear FCCL-produced fish in the Delta in a more natural setting. In addition, we also recommend research on phenotypic, genetic, and epigenetic differences between wild and hatchery fish of various ancestries. This may include thermal tolerance, growth and maturation rates, handling stress, predator response, feeding, swimming, and spawning behavior. There has been research on physiology (Jeffries et al. 2016; Komoroske et al. 2016) of cultured delta smelt, but the pedigree and DI values were not considered, and these could affect their performance.

Conclusion

In addition to establishing the FCCL refuge population, managers have responded to the collapse of delta smelt in several ways, including increased surveying, restoration projects, and alteration of water deliveries (Sommer et al. 2007; California Natural Resources Agency 2016; Hobbs et al. 2017). The continued operation of the FCCL ensures that total extinction is prevented if delta smelt disappears in the wild, but the availability of wild delta smelt is a critical component. This is underscored by our finding that RRSs of cultured parents are increasing at the FCCL, indicating adaptation of delta smelt to captivity. These findings fall in line with the majority of conservation hatchery results, suggesting further research, and raising questions about how and when to use FCCL fish for supplementation.

Conservation hatcheries and similar captive breeding programs strive to slow inevitable evolutionary processes associated with captivity while capturing, housing, and breeding a species that may be critically near extinction. In the best-case scenario, refuge populations within conservation hatcheries will be a temporary solution while threats to the wild population are addressed, given the mounting evidence that hatchery-reared fish show less fitness in the wild.

Supplementary Material

Supplementary data are available at *Journal of Heredity* online.

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Data Availability

The primary data underlying these analyses is deposited in Dryad (doi: 10.5061/dryad.6r0p6s2). Primary data includes the full pedigree file with recovery numbers and the wild or cultured status of each cross.

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