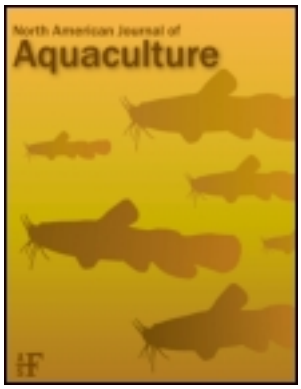

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ARTICLE

Aquaculture Methods for a Genetically Managed Population of Endangered Delta Smelt

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Abstract

In response to Federal listing of the Delta Smelt *Hypomesus transpacificus* as a threatened species in 1993, intensive fish culture techniques were developed to provide a supply of fish for research activities. The Delta Smelt was listed as endangered by the state of California in 2009, and several agencies worked quickly to develop a captive refuge population under genetic management. Captive 2-year-old wild-origin Delta Smelt served as the founding population in 2008. Each year, 250 genetically selected, single pair crosses are made in vitro, and the resultant full-sibling families are combined to rear in multifamily groups. Typically, eight families are reared together from egg to adult stage, with 80% or more of the initial families represented at the adult stage. Multifamily rearing provides an efficient way of achieving a breeding population of 500 in a smaller facility. Juvenile survival increased from 18% in 2009 to 39% in 2010, as facilities and methodologies improved. Growth rate also increased significantly from 2009 to 2010 (from 0.19 to 0.25 mm/d). Subdermal alphanumeric tags identified individuals and allowed spawning of select individuals to preserve genetic diversity in the refuge population. Group marking, by adipose fin clip, provided efficiencies in time and space. Tagging and genetic analyses enabled in vitro spawning of recommended pair crosses each year. At present, we recommend completing the majority of spawning from February to mid-May and continuing to augment the refuge population with wild fish each year. The refuge population provides one type of safeguard against species extinction and provides an example for endangered fish culture.

Managers of species at risk of extinction are often confronted with little time and few options for recovery (Millennium Ecosystem Assessment 2005; Jackson 2008). Although bringing fish into aquaculture settings is not ideal, for many fish species propagation may play an important role in preventing extinction (Nickum et al. 2004). In the current study, the cooperative efforts of several agencies resulted in the development of a safe place in captivity, a refuge for the endangered smelt, and by extension the population is termed a refuge population. Use of the term conservation hatchery does not quite suit the program, as it often implies restocking to the wild. A captive population implies capture of wild animals and rearing them under artificial circumstances, whereas a refuge population includes preservation of the evolutionary potential of a species for many generations – in the captive refuge setting. The refuge population provides one level of protection against species extinction, allowing more time for habitat restoration and improved management. The refuge population of Delta Smelt *Hypomesus transpacificus* was founded in 2007–2008, and fish culture efforts have developed rapidly in conjunction with genetic management (Fisch et al. 2013) of the refuge population.

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Delta Smelt are small, silvery fish endemic to the upper San Francisco Estuary (SF Estuary) in northern California, USA. Predominantly an annual fish, they spend the majority of their life cycle in low saline water of the upper SF Estuary and Suisun Bay (Moyle 2002). They have gained notoriety over the past decade, as their principal habitat is caught in a battle between protecting natural aquatic resources and providing Californians with ample water (Bennett 2005; Sommer et al. 2007; Moyle 2008). Estimates of population abundance declined in 1982 and remained low (Sweetnam 1999; Bennett 2005; Newman 2008). Delta Smelt were federally listed as threatened in 1993 (U.S. Office of the Federal Register 1993) and as endangered under the California Endangered Species Act in 2009 (CFGC 2009). Three additional pelagic fish in the SF Estuary (Striped Bass *Morone saxatilis,* Threadfin Shad *Dorosoma petenense,* and Longfin Smelt *Spirinchus thaleichthys*) also show signs of population decline since 2002, suggesting the SF Estuary problems are widespread (Feyrer et al. 2007; Sommer et al. 2007). Many of the possible causes are anthropogenic in origin (Baxter et al. 2008; Moyle 2008), and returning habitat complexity and the seasonal and interannual variability in salt and freshwater flow conditions to the system may aid native species recovery (Lund et al. 2010; Moyle et al. 2010).

As the risk of extinction for the species increased (Moyle 2002, 2008; Bennett 2005), actions were taken to bring a portion of the wild population into captivity, a refuge, for conservation management of the Delta Smelt. A breeding program was initiated to genetically manage and monitor the refuge population in collaboration with the Genomic Variation Laboratory, University of California–Davis (UC Davis; Fisch et al. 2009b). Fish culture techniques had been previously developed for Delta Smelt, over the past decade, by the Fish Conservation and Culture Laboratory (FCCL), UC Davis, to provide a reliable supply of fish for about 15 research programs annually. The previous culture methods relied on capturing immature wild stock each fall to provide the first filial generation (F_1 generation; all life stages) of fish for research the following year. This method of culture continued until 2007. In 2007, the State of California Department of Fish and Game further restricted collection of wild Delta Smelt due to mounting concern over the low species abundance indices (Sommer et al. 2007). The FCCL proposed to state and federal agencies that the captive population of adult wild fish, spawned in 2007, be reared for another year to serve as the founding population (F_0) of a new refuge population in 2008. With their support, FCCL facilities were expanded and new procedures were developed to establish and maintain a refuge population.

The refuge population was initiated in 2008 with wild-caught 2-year-old Delta Smelt (held in captivity for 1 year). The 328 2-year-old Delta Smelt (birth year 2006, of natural origin) produced 164 full-sibling families to found the refuge population. In each subsequent year, we have selected about 450 1-year-old fish from the refuge population and about 50 wild fish to make a total of 250 single pair crosses (mating of a single male and female). Using the microsatellite genotypes of individuals, a pedigree is reconstructed and pairwise kinship values are calculated (Ballou and Lacy 1995; but see Fisch et al. 2013). Breeding pairs are then selected with the aim of minimizing average coancestry and inbreeding, and to maintain equal representation of founder alleles in the refuge population (Fisch et al. 2013). Delta Smelt are reared well in excess of the annual target population of 500 breeding individuals, starting with over 200,000 eggs, in order to ensure an adequate pool of adult fish (>6,000) from which to select the broodfish for the refuge population and to continue to provide fish for research each year. Using these methods to determine preferred pair crosses, the refuge population has progressed to spawn the F_3 generation in 2011. The captive refuge population could serve as source material to replenish a depleted natural population, if necessary; however, there are no current plans to supplement the wild population with cultured Delta Smelt.

The overall goal of the Delta Smelt refuge population program is to create a captive population that maintains genetic diversity and is representative of the wild population in successive generations. Three primary entities helped initiate the program: (1) the FCCL of UC Davis, with proven delta smelt culture techniques and facilities; (2) the Genomic Variation Lab of UC Davis, which developed the microsatellite markers (Fisch et al. 2009a) and provides the genetic management component of the breeding program annually (Fisch et al. 2009b, 2010, 2012, 2013); and (3) the U.S. Fish and Wildlife Service, which supported the initial genetics work and maintains a smaller population of Delta Smelt at Livingston Stone National Fish Hatchery (LSNFH), Shasta Lake, California, to protect against catastrophic losses at either facility.

There are two main components to developing a successful Delta Smelt refuge population: appropriate fish husbandry techniques to support a genetic breeding program and to rear all life stages of the fish, as discussed in this paper, and the genetic management and monitoring of the population which was described in brief above and in detail elsewhere (Fisch et al. 2013). The fish husbandry techniques are described in three main sections: (1) facility description and rearing, (2) founding population and progeny, F_0–F_1 subadult stage; and (3) new aquaculture techniques in support of the genetically managed population, F_1–F_3. The successful development of a refuge population for Delta Smelt may serve as an example for culture of other endangered fishes.

METHODS

Facilities description and general rearing techniques.—The capacity of the FCCL research facility has increased over the years to about 20,000 adult Delta Smelt. Initially, the refuge population was reared solely in the research facility until the refuge facility became operational. Most of the fish culture techniques developed for Delta Smelt are described in report form

(Baskerville-Bridges et al. 2005); general culture methods are described here, but in less detail.

Research facilities.—The water source for the research facility is raw surface water derived from a man-made reservoir, Clifton Court Forebay, in Contra Costa County, California, and the FCCL is adjacent to the forebay. Water is pumped to three settling tanks (715 × 238 × 75 cm deep; 12,760 L) for removal of larger particles and is then passed through a drum filter (50-μm mesh; PR Aqua Nanaimo, British Columbia; ca. 340 L/min) to remove smaller particles (>50 μm). The water is then treated with ozone (65.1 g/h output unit; Pacific Ozone Technology, Benicia, California) and foam-fractionated before distribution to fish-rearing systems.

Most of the fish-rearing systems are recirculating and biofiltered. Both recirculating and flow-through systems are temperature controlled. Water is circulated by 0.5–1.0-hp pumps through biofilters, UV filters, and a particle filter in recirculating systems. Approximately 10% of system capacity is renewed daily through tank cleaning and water flushing. Water is aerated by central air blowers, or airstones. The research facility has two independent larval-fish rearing systems of 10 tanks (130 L, 68-cm diameter black polyethylene) and a capacity of 1,770 L each. The late-larval stage fish are reared in a recirculating system of 20 tanks (400 L, 68-cm diameter black polyethylene) and a total capacity of 10,460 L. The juvenile-stage through the adult morphology fish are reared in recirculating or flow-through systems with larger tanks (1,100 L; 152-cm diameter; black-interior insulated fiberglass). An indoor recirculating system of 12 tanks (system also includes three larger tanks [1,930 L; 183-cm diameter] used to rear research juveniles, for a total system capacity of 19,570 L) is used to rear the younger juvenile stages (Table 1). Light conditions in the indoor facility where generally late-larval to juvenile fish are reared are approximately 1–2 μmol/m^2/s. The older juvenile to adult stages are reared in an outdoor flow-through system of 13 tanks with temperature control (Table 1), where the ozonated source water circulates between one or two 10-hp heat pumps and a 3,820-L storage tank before passing to the rearing systems. Outdoor tanks have shade-cloth (mesh-fabric) covers.

Refuge facilities.—The new refuge facility was modeled after the FCCL research facility; the main building is 12.2 × 18.3 m. Water (750–950 L/min) from the reservoir (same as for research facility) is sand-filtered (Model SM48-2, 80 PSI max; Everfilt, Mira Loma, California) and is UV treated to supply the egg incubation system, live-prey culture units, and fish-rearing units. For 1 year, 2009, larvae and late larvae were reared in a recirculating system containing both larval and late-larval tanks with the same lighting conditions. However, because the fish appear to have life stage-dependent sensitivities to light, the life stages were separated and light levels were adjusted in 2010 to accommodate the larval and late-larval stages (4–5 and 1–2 μmol/m^2/s, respectively; Table 2). Particle filters (Aquadyne Hartwell, Georgia) were added to each system to remove excess algae and other particulates. The adult rearing systems consist of two recirculating systems of 10 adult tanks per system (as described for the research facility), and these systems are under an awning adjacent to the main building. An effort is made to maintain similar light conditions in the juvenile- and adult-rearing system at both refuge and research facilities. The light conditions in the adult-rearing system are approximately 1–3 μmol/m^2/s. System capacities are similar to those of the research facility, and currently the refuge population is distributed about equally between the two facilities.

Spawning and egg incubation.—Cultured Delta Smelt may spawn from December to August but more typically from January to June in a temperature-controlled environment. The FCCL has opted to manually express gametes and fertilize in vitro rather than allowing mature adults to spawn in their holding tanks. This is performed because the demersal adhesive eggs are difficult to collect from the tanks and to separate from food and feces, and egg release is inhibited in tanks. Manual expression

TABLE 1. Delta Smelt life stages for use in aquaculture rearing and transitions between systems. Categories are general guidelines. Length measurements are TL for larval stage and FL for all other stages. Delta Smelt life stages are defined in more detail in Mager et al. (2004).

Life stage	Days posthatch (dph)	Average length (mm)	Tank volume (L)	Rearing system
Larval	0–40	5–17	130	Recirculating rearing system, black interior tanks
Late larval	41–80	18–23	400	Recirculating rearing system, black interior tanks
Subjuvenile to juvenile	81–199	24–49	1,100	Recirculating or flow-through rearing system, indoor black interior tanks, 80–120 dph; recirculating or flow-through rearing system, outdoor black interior tanks, with tank covers, awning and peripheral shade cloth 121–199 dph
Subadult	200–249	50–54	1,100	Recirculating or flow-through rearing system, outdoor black interior tanks, clear water conditions, shade cloth on tanks; preferably under awning
Adult	>250	>55	1,100	Recirculating or flow-through rearing system, outdoor black interior tanks, or under awning

TABLE 2. Initiation of Delta Smelt refuge population F_0–F_3; description of major changes in fish husbandry over the first 3 years is provided.

		Rearing location of life stage				
Generation	Birth year	Research facility	Refuge facility	Major differences between year-classes	Result	Data
F_0	2006	Wild adults		Wild 2-year-old fish, random mating	High fecundity	Figure 2
F_1	2008	Larvae to subadult	Late subadult and adult	Reared primarily in research facility, cultured 1-year-old, managed mating	Lower fecundity in 1-year-olds	Figure 2
F_2	2009	All life stages	All life stages	Larval and late larval stages reared in one system at refuge with higher light; rotifers grown with *Nannochloropsis* intiated weaning to prepared diet early	Low juvenile survival	Figure 5
F_3	2010	All life stages	All life stages	Larval and late larval separated at refuge, with low light levels for late larval stage; rotifers grown with enriched supplement; weaning to prepared diet delayed	Increased juvenile growth and survival	Figures 5, 6

of eggs results in higher quality and number of eggs, and allows for select pair crosses to be made. A single clutch of eggs (fish can produce several egg clutches per season) is fertilized in a 290–500-mL plastic bowl; larger bowls are used for 2-year-old fish. Water is added to activate sperm, and eggs disperse and adhere to the bottom by means of an adhesive stalk (Mager et al. 2004). Water is replaced, and the bowls of developing embryos are floated in water baths at 16°C for 3 d, which allows staff to monitor and remove dead eggs. After 3 d, eggs are gently freed with fingertips and rinsed with a clay mixture (Bentonite, 16 g/L; Sigma-Aldrich, St. Louis, Missouri) to minimize cohesion. The total number of fertile eggs from each pair cross is volumetrically estimated and recorded along with weights and lengths of parents.

A column-style incubator consists of a vertical clear plastic tube (5 × 42-cm-long Plexiglas) with a 250-μm mesh screen in the bottom to hold a 200-mL mix of coarse (number 7) and fine sand (number 60). At the top of the incubator, a 1.3-cm diameter clear tube extends down to a 9.5- or 19.0-L black bucket with screened standpipe. The incubators receive a recirculating, upwelling supply of filtered water that creates a fluidized sand bed in the columns to keep the eggs moving just above the surface of the sand. At hatch, the larvae swim up, aided by the upwelling water current, and out of the incubator into the bucket.

Rearing, feeding, and fish tank transfers.—From newly hatched larval to adult stage, fish are transferred five or more times between fish-rearing systems to accommodate life stages and breeding program (Table 1). The five development stages (for details see Mager et al. 2004) important to Delta Smelt culture at FCCL are: (1) larval stage (0–40 days posthatch [dph]; 5–17-mm TL; small, transparent, and elongate larvae); (2) late-larval stage (41–80 dph; 18–23-mm FL; elongate form, swim bladder development); (3) juvenile stage (81–200 dph; 25–50-mm FL; metamorphosing into adult fusiform morphology and coloration, and increasing in size); (4) immature stage "subadults" (200–249 dph; 50–54-mm FL; fish have gained weight and are heartier and less sensitive to sunlight); and (5) adults (>250 dph; >55-mm FL; fish begin to develop mature gametes at about 55 mm, and cultured fish can reach 90 mm in the first year). The first three transfers are made water to water to reduce stress.

As Delta Smelt grow, they transition from live prey to a commercial diet. Live prey cultures include brackish water rotifers *Brachionus plicatus* (Reed Mariculture, Campbell, California) and brine shrimp nauplii *Artemia franciscana* (dry cysts available from *Artemia* International, Fairview, Texas). Live prey are fed to fish 6 times/d at a target density of 10 rotifers/mL from 3 to 40 dph. *Artemia* are fed at a target density of 1–3 nauplii/mL from 10 to 120 dph until weaning to a commercial feed. An algal concentrate *Nannochloropsis* (Reed Mariculture) is added to the larval- and late larval-stage rearing systems to increase the turbidity of the water to 9 nephelometric turbidity units (NTU) and promote feeding (Baskerville-Bridges et al. 2004). After fish transition to the outdoor tanks, algae are used for several weeks to help reduce stress by reducing visibility.

To wean fish from live prey, *Artemia* are supplemented with a dry feed mixture at a 2:1 ration of Cyclop-eeze (Argent Laboratories, Redmond, Washington) and Lancy 2/4 (INVE

Aquaculture) 2–4 times/d at 120 dph. After the fish are weaned, they are fed a 2:1 ration of 4/6 NRD (INVE Aquaculture) and 370 Hikari (By-Rite Pet Supply, Hayward, California) 15 times/d at a 1–3% body weight via vibratory feeders. Juvenile fish were transferred to the larger outdoor tanks at about 120 dph and 30–35-mm FL to rear to maturity. To monitor and assess growth, 10 fish per tank in 10–12 tanks were measured biweekly through 140–160 dph. To assess survival, fish were counted when transferred from the "late-larval" tanks at 1,500–3,000 fish per tank and ≥20 mm-FL to the "adult" tanks, and subsequently when stocking density was adjusted. The total population was 48,000–96,000 fish. Analysis of variance was used to compare survival between years, and linear regression analysis was used to compare growth in juvenile fish.

The founding population and progeny, F_0 to F_1 subadult stage.—The founding population of wild Delta Smelt (resident on the FCCL site 1 year) was randomly bred to represent as many wild fish in the new refuge population as possible; 328 fish were mated. The 2-year-old broodfish had an average clutch size of over 4,500 eggs, or three to four times as many eggs as 1-year-old fish, used in subsequent years. Egg contribution was limited and equalized at 1,000 eggs per full-sibling group (FSG); multifamily rearing groups (MFG) were made by combining three to six full-sibling families per MFG in order to rear all families in the limited space available.

To accommodate the 164 families, both the normal larval tanks (130 L) and a system of smaller tanks (70 L, recirculating system) were used; the 70-L tank system was used in this year only. The F_1 generation larval stocking density was adjusted to 3,000 larvae per tank in the 70-L tanks, versus 5,000–6,000 larvae in the larger 130-L tanks (which were used in subsequent years). Following the early larval-rearing phase, the fish were transferred at 40–50 dph to the 400-L tank system, usually with 1,500–3,000 fish per tank, to rear to 80 dph, for the late-larval stage. These MFGs were then transferred into the larger indoor adult tanks. Subadults were combined into 18 of the 1,100-L outdoor adult tanks containing 2–19 full-sibling families per tank in preparation for spawning.

New aquaculture techniques in support of the genetically managed population, F_1–F_3: subadult transfer to off-site location and consolidation using adipose fin clip.—Once Delta Smelt reach the subadult to adult stage (50–60 mm), fish are thinned to 250 fish per each of the 32 MFG in the late fall or winter. A subpopulation of each MFG, 50 fish per multifamily group, are transferred in oxygenated tanks, with 5 g/L of salt, to LSNFH as a safeguard against catastrophic loss. In 2010, and thereafter, two MFGs each with 200 fish were consolidated at the FCCL by use of an adipose fin clip to mark one of the two groups.

Tagging, fin-clipping, spawning, and family recovery assessment.—Broodfish required both an individual identification tag and a fin clip to implement the genetically managed breeding plan. The tagging and fin-clipping process was usually initiated in January or February, prior to the spawning season, starting with 20 fish per MFG. Fish were tagged, anesthetized (100 mg/L tricaine methanesulfonate; Argent Laboratories), allowed to recover, and combined to adult tanks (<400 per tank). Small plastic visible implant alphanumeric tags (Northwest Marine Technologies, Olympia, Washington) were inserted under the skin near the dorsal fin (Figure 1), and two small samples of fin were removed and stored in a 95% solution of ethanol for DNA processing and archiving. Attempts were made to tag an equal number of males and females over the season. As fish mature, males and females are sorted to separate tanks.

Fish from each MFG are also subsampled for weight and length comparisons prior to the spawning season in January and again in April to monitor growth over the spawning season; data are presented for 2010. Wild fish are processed separately during spawning operations.

Managed breeding, family recovery, and wild fish incorporation.—During the spawning season (ca. February through May) the female tank is sorted for ripe females twice a week, tag numbers are recorded and sent to the geneticist, males are recommended (and recovered from the tank housing the tagged males), and spawns are completed within hours. Gender is observed as running milt in males and distended belly and egg development observed at vent in females. In maturing females, eggs can be extracted by mild pressure applied to the abdomen.

Assessment of family recovery of the tagged broodfish pool begins after the initial tagging and fin-clipping operations are complete, but tagging and assessment continue until all spawns have been completed for the season. Recovery is defined as successful parentage assignment of individuals from each full-sibling family in the tagged pool of broodfish. At the end of the spawning season, the number of families recovered in the tagged pool of broodfish are tallied and the percent recovered is calculated based on the number of families initiating each generation. Full-family recovery includes the number of families successfully spawned as a percentage of the initial families for each generation.

In late fall of each year, subadult wild fish (usually greater than 50 mm, some with initial development of gametes) are collected from the lower Sacramento River, where they congregate prior to migrating into fresher waters for the spring spawning season (Moyle 2002). A target population of about 50 wild subadult fish is currently collected (under permit) to become part of the refuge population on an annual basis.

Rearing changes between 2009 and 2010.—Eggs were combined with equal representation of full-sibling families (FSG) by combining 750 eggs from each of eight adult pairs, within 10 d of each other, to make an MFG. Each MFG had 6,000 eggs, and resulting larvae (anticipating 5–10% embryo mortality) are incubated together, as described earlier.

In 2010, several feeding and rearing changes were implemented to help promote growth and survival of the F_3 generation (birth year [BY] 2009) based on observations made in 2009, and to compensate the higher larval stocking density of 6,000 versus 5,000 larvae per tank in previous years (prior to the refuge population; Table 2). Changes include the following:

FIGURE 1. A cultured Delta Smelt with an alphanumeric tag inserted below the dorsal fin. [Figure available in color online.]

(1) rotifers cultured with RotiGrow Plus (omega fatty acid preenriched microalgal blend), considered an enrichment diet for the rotifers and larvae instead of the microalgae *Nannochloropsis*, used prior to 2010 (both from Reed Mariculture), and rotifers fed at higher density in 2010 (17 rotifers/mL/larval tank in 2010 versus 10/mL prior); (2) delayed weaning of fish to a prepared diet mixture (Cyclop-eeze from Argent Laboratories, and EPAC/NRD 4/6 from INVE Aquaculture, 1:2 mixture) from 70 to 120 dph, based on unpublished FCCL data of improved performance; and (3) rearing late-larval fish under low light (1.4 μmol/m^2/s) by separating the larval and late-larval rearing systems, observed to promote better swimming and feeding behaviors from past experience. In addition, light levels of the outdoor adult tanks were reduced at the refuge facility by doubling the shade cloth covers and adding a shade cloth drape to the overhead awning perimeter (1–3 μmol/m^2/s) to help the juvenile fish transition to the brighter outdoor environment.

RESULTS

The Founding Population and Offspring, F_0–F_1 Subadult Stage

Random mating of the 2-year-old wild fish produced 164 F_1 families. Mean fecundity of the F_0 generation was 4,569 eggs per clutch, and mean length of the female was 98.3-mm FL in 2008 (Figure 2).

New Aquaculture Techniques in Support of the Genetically Managed Population, F_1–F_3

Subadult transfer to off-site location.—Fish transfer to LSNFH was successful, with less than 1% mortality.

Adipose fin clip and tagging results.—The new adipose fin clip procedure resulted in labor and space efficiencies. Adipose fins did not regenerate, and the mark was effective in distinguishing between two groups of adults housed together throughout the 5–6-month spawning period. Combining two MFGs in each broodfish tank resulted in significant production efficiencies.

The retention of alpha tags for individual fish identification was generally good (40–100%), but there was a marked effect of fish size on tag retention (Figure 3). All broodfish grew over the spawning season, but fish in the last five MFGs were still significantly shorter in early April than the first five MFGs, averaging 61.9- versus 74.3-mm FL (ANOVA: $P < 0.0002$; Figure 2A). The older and larger F_2 broodfish (hatched earlier in the previous season) exhibited better tag retention, 92% retaining the tag in MFGs 1–20 versus 72% in MFGs 21 and higher (the youngest fish of the season; Figure 3). Wild fish lost 59% of their tags when receiving tags on January 14, 2010; however,

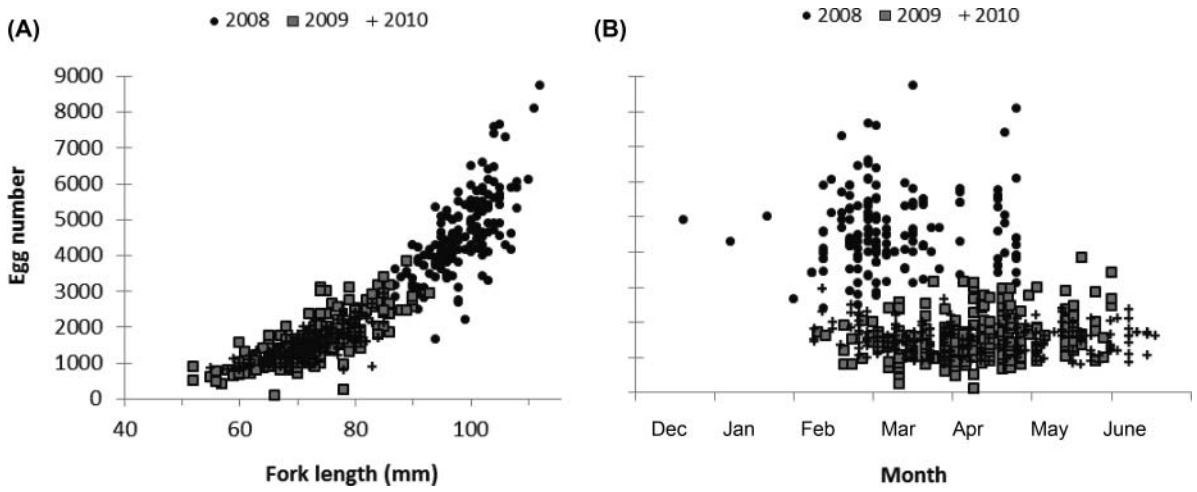

FIGURE 2. Data are illustrated for 2-year-old founding population (2008, black circle) and 1-year-old F_1 and F_2 broodfish in 2009 (gray square) and 2010 (black cross), respectively, in terms of **(A)** fork length and **(B)** date (December through June). Data are for the selected spawns of the tagged broodfish pool in the refuge population and do not capture the full-season reproductive potential of the smelt in terms of timing and frequency of spawns or fecundity of females, as only one egg clutch per female is depicted.

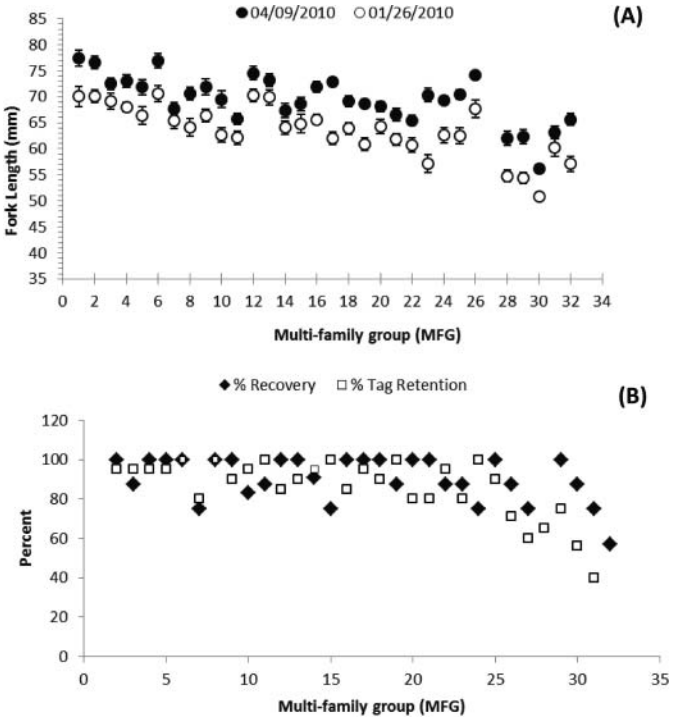

FIGURE 3. Variation in broodfish size, tag retention, and recovery of families within each MFG for F_2 delta smelt in 2010. **(A)** Length of fish (average FL and SE; $n = 20$ fish per MFG) measured on January 26–27, 2010 (open circles) and again on April 7–9, 2010 (filled circles); **(B)** tag retention, represented by number of live fish with tag of total fish tagged (%; $n = 20$ tagged fish per MFG 2 months after tagging [squares]); recovery of full-sibling families from each MFG also shown (% recovered of eight inititial families stocked per tank [diamonds]).

fish survival was high (89.5%), and fish retagged February 11, 2010, were observed to have good tag retention.

Managed breeding, family recovery, and wild fish incorporation.—The F_1 generation (BY2008, spawn 2009) was initiated with 164 families in 2008 (Table 3). At the subadult or adult stage, most fish were combined with two or more multifamilies per tank, resulting in 2–19 full-sibling families per tank in 18 tanks. More than 1,400 broodfish were individually tagged, and parentage was analyzed based on DNA from fin clips. Of the initial 164 full-sibling families, 153 (93%) were recovered in the F_1 adult population in 2009, and 145 of the recovered families (88%) were spawned to create the F_2 generation (Table 3). A total of 508 fish were selected from the tagged F_1 broodfish pool and wild broodfish tanks (53 wild fish contributing) to make 254 pair crosses designed to minimize mean kinship (Fisch et al. 2013; Table 3). The F_1 refuge population was spawned mid-February through May in 2009 (Figure 1). The mean fecundity of the 1-year-old F_1 broodfish of 1,579 eggs per clutch and average female length was 71.9-mm FL (Figure 2).

The F_2 generation (BY2009, spawn 2010) was initiated with 254 families. One tank of larvae, eight families (MFG 27), was lost to a technical problem. The F_2 broodfish were consolidated into 18 adult tanks at the refuge facility. The F_2 generation (BY2009, spawn 2010) broodfish pool consisted of more than 1,800 tagged fish; 219 of the initial 254 full-sibling families were recovered (86%), and 206 of those families (81%) were successfully spawned. From the tagged F_2 broodfish pool, 432 cultured fish and 34 wild broodfish contributed to the refuge population to make 233 total pair crosses (Table 3); average egg clutch size was 1,471, and average female length was 72.0-mm FL. Fish

TABLE 3. Delta Smelt refuge population management at the UC Davis FCCL. Families recovered in broodfish life stage (750 eggs per FSG) after rearing in MFGs (eight FSGs per MFG) are determined through parentage analysis (see Fisch et al. 2012).

Delta Smelt generation	Founder	F_1	F_2	F_3
Birth year (spawn year)	2006 (2008)	2008 (2009)	2009 (2010)	2010 (2011)
Number of tagged broodfish from a population of ca. 6,400		>1,400	>1,800	>1,700
Number of FSGs initiating the generation		164	254	233
Number of FSGs recovered in tagged pool of broodfish (as % of initial FSGs)		153 (93%)	219 (86%)	197 (85%)
Number of FSGs included in successful spawns (as % of initial FSGs)		145 (88%)	206 (81%)	187 (80%)
Number of wild fish supplementing refuge population		53	34	64
Number of select pair crosses made to initiate the next generation	164	254	233	256

spawned early in the previous season (prior to May 4) were larger and spawned earlier—by the end of April (188 of the 250 pair crosses, or 75%); the remaining 25% were smaller, hatched late in the previous season, and required longer to mature and spawn (Figure 4). F_3 progeny of this later-spawning group had lower survival than progeny of early spawners (Figure 4).

The F_3 generation (BY2010, spawn 2011) broodfish pool consisted of more than 1,700 tagged fish; 197 of the initial 233 full-sibling families were recovered (85%), and 187 of those families (80%) were successfully spawned. From the tagged F_3 broodfish pool, 448 cultured fish and 64 wild fish were selected to make 256 pair crosses to produce the F_4 generation.

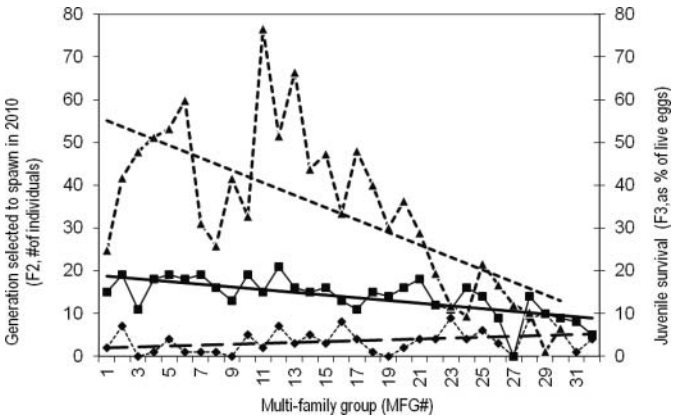

FIGURE 4. Recovery of the F_2 generation and survival of the F_3 generation Delta Smelt juveniles as a function of MFG. Total number of F_2 parents spawned per MFG in 2010 (solid black line) declines with spawn date, which coincides with increasing MFG number as spawning season progresses (from mid-February to through mid-June). The F_2 parents that hatched late in the previous year, 2009 (those with high MFG numbers), tended to spawn late in 2010 season, e.g., not until after May 4 (dashed black line). Survival of F_3 juveniles (at 80 dph; dashed line–triangle marker, secondary *y*-axis) reflects this pattern, as F3 fish hatched later in the spawning season, 2010 (with higher MFG numbers), had poor survival. The low survival of juvenile fish from MFGs 7–10 is attributed to a temporary disease problem in these groups. Lines are included representing the best linear fit of the data.

Rearing changes between 2009 and 2010, comparison of growth and survival of the F_2 and F_3 generations.—Juvenile fish survival improved significantly from the F_2 generation to the F_3 generation, averaging 18% and 36%, respectively (ANOVA: $P < 0.0001$; Figure 5).

Growth rate was also significantly higher in the F_3 generation versus the F_2 generation fish (0.248 versus 0.188 mm/d; 5.2-mm intercept; $P < 0.0001$; Figure 6). Average water temperature varied within 1.3°C across systems (15.4–16.7°C) and between years. Temperature averaged 0.5°C higher for larval and late-larval stages, but 0.8°C lower for juvenile to adult stages in 2010 versus 2009.

In 2009, larval and late-larval stages were reared in one system with the same lighting conditions (4–5 μmol/m^2/s) for both. Under these conditions the larvae were observed to be swimming and feeding actively in the upper water column, whereas individuals in the late-larval stage were lower in the water column and appeared to be more stressed. In 2010 the rearing systems were separated by life stage, reducing incident light levels to 25% of the 2009 levels in the late-larval fish-rearing system, and said fish demonstrated more active and normal behavior at the lower light level (1–2 μmol/m^2/s).

Juvenile fish are also sensitive to light. Reducing the incident light to the outdoor tanks appears to have contributed to decreased juvenile mortality from 970 in 2009 to 219 in 2010 during 3 d of transitioning to outdoor tanks.

DISCUSSION

Successful fish husbandry methods are described that support a genetically managed breeding program for the Delta Smelt refuge population, and no significant loss of genetic diversity has been observed to date (Fisch et al. 2013). Wild fish are

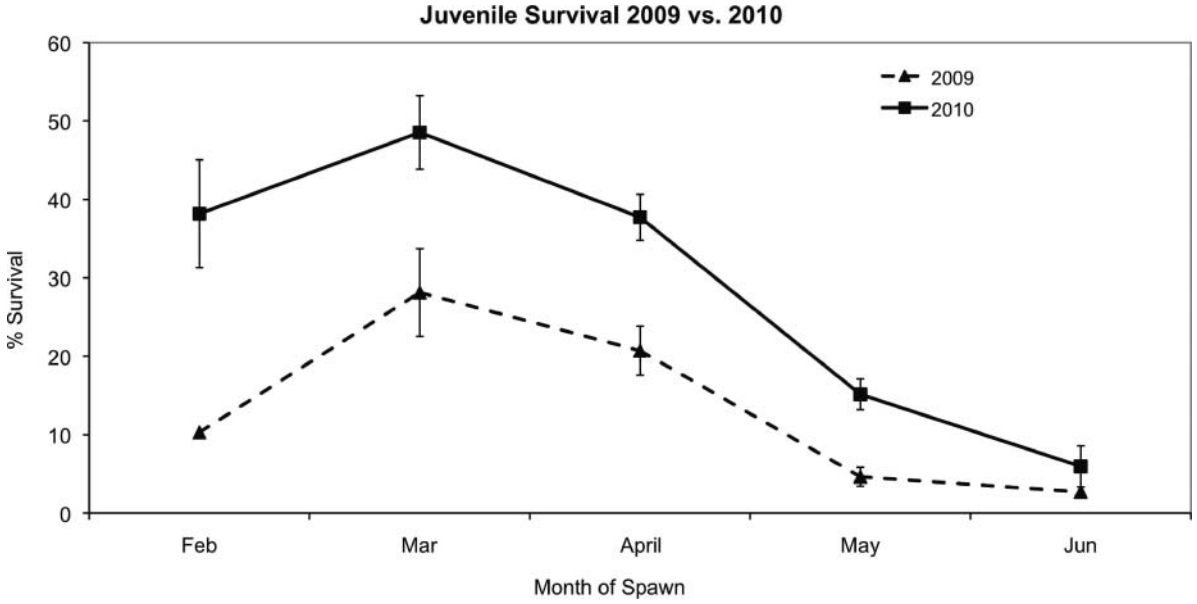

FIGURE 5. Survival of cultured juvenile Delta Smelt spawned in 2009 and 2010. Data represent average survival for all MFGs of juveniles spawned in month indicated at transfer to final adult tanks (ca. 100–120 d posthatch; 2010 data: black line, square marker; 2009 data: dashed line, triangle marker; SE of the average included). Survival was higher in 2010 than 2009 ($P < 0.0001$) and also for the 3 months March–May ($P < 0.009$).

supplemented, as founders, each year to help maintain genetic diversity and minimize genetic drift.

Facility expansion and modifications in rearing techniques contributed to improved fish rearing success over the 3-year period of this study. Improved juvenile survival and growth in the F_3 generation (Figure 6) are thought to be attributable to methodological changes, but several changes were made simultaneously and so weighting importance of each change is not possible. Differential lighting of life stage rearing systems appeared to benefit the late-larval life stage as these fish tended to be more active, feeding, and swimming higher in the water column when the light levels were lowered. An increase in feed quality and quantity, and a delay in weaning to a dry diet may also have contributed to the improved growth and survival observed in 2010; age at weaning may be species specific but is also influenced by diet quality and co-feeding of live and inert diets (Person-Le Ruyet et al. 1993; Chen et al. 2006; Engrola et al. 2009). In addition, Delta Smelt transitioning from the late-larval to the juvenile stage appear to be sensitive to light and crowding, so improved growth and survival may also be due to transitioning the fish into larger tanks earlier and keeping the fish in a darkened indoor environment longer, before moving them to well-shaded outdoor tanks. Slightly warmer temperatures (0.5°C higher mean temperature) for larval to late larval-stage rearing may also have contributed to faster growth and higher survival.

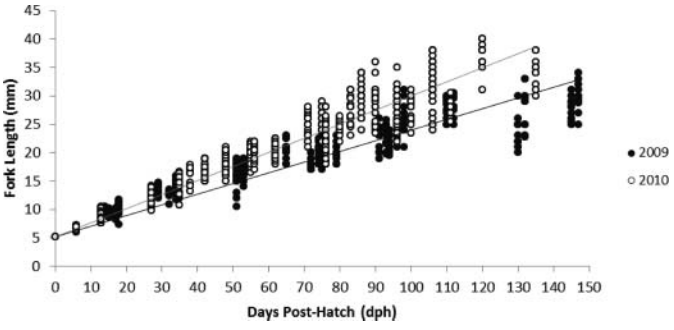

FIGURE 6. Comparison of cultured Delta Smelt growth in length over the first 5 months of life for 2009 and 2010. Daily growth rate is 0.188 and 0.248 mm/d for 2009 and 2010, respectively, and significantly higher in 2010 ($P < 0.0001$); intercept is fixed at 5.20 mm based on average size at hatch (authors' unpublished data).

Adopting the practice of rearing the smelt in multifamily groups (usually eight families per group) has permitted good family retention while reducing labor and facility costs. Loss of families has been less than 20% of the approximate 250 initial families each year. Retention of a high proportion of the families to the adult stage is likely due, at least in part, to equalizing family size at the egg stage (750 eggs per full-sibling group). Equalizing family size has been recommended as a strategy to maximize effective population size and reduce domestication selection for traits more suitable in a nonnatural environment in captive populations (Allendorf 1993; Frankham 2008). However, family representation is worse in those MFGs spawned early or late in the season, as reflected in juvenile survival (Figure 3). Modifying the MFG structure for the larval rearing phase(s) in these early and late MFGs may improve family retention for the most compromised groups. Potential gains may be had by combining half the number of families

(i.e., four versus eight families), increasing representation of each family in the MFG (1,000–1,200 eggs per MFG versus 750 eggs per MFG typically used), and reducing stocking density from 6,000–4,000 larvae per tank. Smaller rearing groups can later be combined to the standard eight families per MFG after the period of high larval mortality has passed.

Removal of adipose fin clip and alphanumeric tagging techniques were both useful in combining families and identifying individuals, contributing to the efficiency and the ability to implement the genetic breeding program. The best fish tagging and fin clip sampling schedule for accomplishing both the fish breeding and the genetic and pedigree analysis may be a mix of tagging 600–700 fish in late January to early February prior to the spawning season, and then supplementing the pool of tagged fish by tagging more fish throughout the season as they become mature. In 2009, fish were tagged and fin-clipped for DNA and parentage analysis throughout the busy spawning season. In 2010 and 2011, a large representative group of fish was tagged and fin-clipped in January. The latter method proved useful in creating a larger pool of fish from which to select breeding pairs, especially early in the season, and also reduced the tagging effort during the busy spawning season. However, the youngest broodfish require more time to reach an adequate size for tagging (Figure 3). Additionally, care should be taken to include in the pool of tagged fish those that mature in mid-to-late season to help minimize imposed seasonal spawning bias.

Spawning.—The wild population of Delta Smelt is thought to spawn primarily from early April to mid-May (Moyle 2002), but the spawning period varies year to year and probably extends from February to June in some years (Wang 1986). Therefore, an effort was made to spawn fish during the full spawning season in the refuge population. However, fish hatched late in the spawning season, from mid-May to mid-June, appear to have limited utility to the refuge population overall. These younger fish are significantly smaller ($P < 0.0002$) and appear to spawn later in the season (F_2 adults, MFG $\geq$ 21; Figure 4), and their progeny do not survive as well as those spawned midseason (F_3 juveniles; Figure 4). Taken together, these factors contribute to a cycle of diminishing family recovery for fish hatched late in the season.

The captive founding population produced 164 families through random gamete fertilization. In the F_1–F_3 generations, close to 250 families were produced each year with 80–88% recovery of one or more individuals from each family (Table 3). Documenting the family recovery is an important component of a genetically managed population, especially over multiple generations, with the intention to monitor and to minimize the potential loss of families (and within-population diversity) in each generation (Williamson 2001). As more fish species become imperiled, developing managed breeding programs will become a more common fish management tool. A well-managed population constitutes a genetic bank and provides one level of security against species extinction.

CONCLUSIONS

The refuge Delta Smelt population has been maintained through the F_3 generation as of 2011. The value of the refuge population lies in the safeguard it provides against extinction and in the additional time it allows for the improved management of its natural habitat.

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