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A pilot study of the performance of captive-reared delta smelt *Hypomesus transpacificus* in a semi-natural environment

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Abstract

A captive breeding programme was developed in 2008 for delta smelt *Hypomesus transpacificus* in reaction to dramatic population decline over several decades. We took 526 sub-adult captive-reared delta smelt and cultured them for 200 days without providing artificial food or water quality management to assess their performance once released in the wild. The results indicated captive-reared sub-adult delta smelt could survive in a semi-natural environment with uncontrolled water quality and naturally produced wild prey through spawning and into their post spawning phase.

KEYWORDS
captive, delta smelt, refuge population, reintroduction, semi-natural environment

Multiple factors have affected aquatic organisms in the San Francisco Estuary since the first European settlement, including habitat alteration, water diversions, flow regime alterations and introduction of exotic species (Bennett & Moyle, 1996; Brown & Moyle, 2005; Moyle *et al*., 2010; Moyle, Katz *et al*., 2011; Sommer *et al*., 2007). Several species native to the Estuary are now listed as threatened or endangered under state or federal status, including delta smelt *Hypomesus transpacificus*, which is federally listed as threatened (US Fish and Wildlife Service, 1993), endangered under the California Endangered Species Act (CDFW, 2017) and Critically Endangered on the IUCN Red List (NatureServe, 2014). There is growing concern delta smelt will face extinction in the next 2–10 years (Moyle *et al*., 2018).

Delta smelt are an annual osmerid fish endemic to the San Francisco Estuary (Moyle, 2002) with silver-like colour and an adult fork length (L_F) of 65–90 mm (Moyle *et al*., 2018). They are a life-long zooplanktivore, consuming larger prey as they grow (Feyrer *et al*., 2003; Lott, 1998; Mager *et al*., 2003; Moyle *et al*., 1992; Nobriga, 2002). Delta smelt have gained attention over the past decade, as their principal habitat has been caught in a battle between protecting natural aquatic resources and providing Californians with ample water (Bennett, 2005; Moyle *et al*., 2018; Sommer *et al*., 2007). In an effort to begin curtailing their potential extinction, a captive refuge population of delta smelt was established at the Fish Conservation and Culture Laboratory (FCCL), University of California, Davis, in 2008 (Israel *et al*., 2011; Lindberg *et al*., 2013). The primary goals of the FCCL are to maintain a refuge population of delta smelt onsite and provide all life stages of delta smelt to academic institutions and governmental agencies for research purposes.

It is challenging to maintain a genetically sound population in captivity with the intention of releasing them into the wild (Crawford & Muir, 2008). Conservation hatcheries strive to slow inevitable evolutionary processes associated with captivity such as genetic or phenotypic changes while capturing, housing and breeding of a species (Finger *et al*., 2017). At the FCCL, culture methods for delta smelt are continuously being improved through research and experimentation. Adults are genetically tested to create a pedigree to determine crossings of least related individuals and to keep equal representation among families with wild-caught delta smelt added into the breeding pool annually (Finger *et al.*, 2018; Lindberg *et al.*, 2013). However, all of these efforts to minimise kinship and keep the genetics of the cultured population as close as possible to the wild do not guarantee success as a recent study found the relative reproductive success of pair crosses with at least one wild delta smelt parent was lower than the ones with two cultured parents (Finger *et al.*, 2017).

Other problems may arise when trying to raise fish in a controlled system for future release into the wild. Fish reared and released for the purpose of rehabilitating populations have been housed in predator-free conventional hatchery environments with plenty of food, allowing better survival through the earliest life stages than nature does. However, these early rearing stages experiences do little to prepare the fish for life outside captivity (Brown & Day, 2002; Salvanes & Braithwaite, 2006). In addition, the ability of captive delta smelt to survive outside captivity is still unknown and warrants investigation (Hobbs *et al.*, 2017; Israel *et al.*, 2011). Consequently, the main goals of this study were to see if captive-reared delta smelt can: (a) learn to find natural prey on their own; (b) forage on natural prey effectively enough to maintain a similar body condition to fish in captivity; (c) have similar survival foraging on natural prey as the control fish reared in the captive environment at the FCCL.

We used 526 sub-adult (297 days post hatch; dph) delta smelt that were the ninth generation (F_9) removed from the wild at the FCCL. All fish were tagged 7 days prior to the study with visible implant alphanumeric (*VIA*) tags (Sandford, Castillo, & Hung, 2019; Northwest Marine Technologies; www.nmt.us). On December 5, 2016, fish were placed into a large trough (length = 7.11 m, width = 2.29 m, depth = 0.81 m) located in the FCCL yard. The fish holding area of the trough was fitted with a bird net (mesh size = 2 cm) to prevent predation by birds. The trough ran flow-through raw water from the California aqueduct, and a stainless steel screen, with openings of 13 cm long and 0.3 cm wide, was placed at the inlet to screen out predators. The incoming water and water in the fish holding area were tested twice per week for pH (PHC201, Hach; www.hach.com), salinity (YSI85, YSI; www.ysi.com), turbidity (MicroTPW, HF Scientific; www.watts.com), dissolved oxygen (LDO101, Hach), total ammonia nitrogen, nitrite–nitrogen and nitrate-nitrogen (DR3900, Hach). Temperature was continuously monitored and logged once per hour (HOBO Water Temp Pro v2, Onset; www.onsetcomp.com).

The trough was siphoned daily and the screens checked for any pinned mortalities. Once a week, 15 arbitrary fish were sampled and anesthetised using 0.1% MS-222 before wet mass (M_T, g) and L_F (cm) were measured and sexual maturity determined. A set of 20 fish cultured under laboratory culture conditions (Lindberg *et al.*, 2013) were marked and sampled repeatedly during the trial as the control group. The control group were maintained indoors and fed a commercial diet (BioVita CRUM #1, Bio-Oregon; www.bio-oregon.com). Fulton's condition factor (*K*) was calculated using the equation: $K = 100 M_T L_T^{-3}$ (Froese, 2006; Fulton, 1904). After the study terminated on day 199, the trough was drained and all remaining fish in the holding area were collected and identified.

Gut contents of dead fish were collected and pooled monthly, preserved with ethanol (200 proof, Koptec, King of Prussia, PA) and used for identification of prey items by shotgun metagenomic sequencing analysis (Kurobe *et al.*, 2018; Jovel *et al.*, 2016). After quality trimming and concatenating pair-end sequences, we obtained a total of 15 million DNA sequences with an average length of 398 bp for the six libraries (Supporting Information Table S1). Relative abundances for five categories of organisms (see Table 1 for each classification) were then estimated by counting the number of DNA sequences at the phylum or class level. Detailed taxonomic information was retrieved using a package taxize in R (Chamberlain & Szöcs, 2013; www.r-project.org). DNA sequences from bacteria were not included for estimating their relative abundance since fish also have gastrointestinal microflora (Wang *et al.*, 2018). In addition, delta smelt are zooplanktivores and bacteria are not their primary diets (Moyle *et al.*, 1992). Similarly, DNA sequences which show similarity to fishes were not included for the analysis since DNA from hosts were expected in the gut contents (Vestheim & Jarman, 2008). Rare taxa, defined as a percentage of DNA sequences less than 0.5% of the total, were not included in the table to conserve space.

The survival of delta smelt was high (81.2%) throughout the first 160 days and was similar to the control group (Figure 1a). Initial

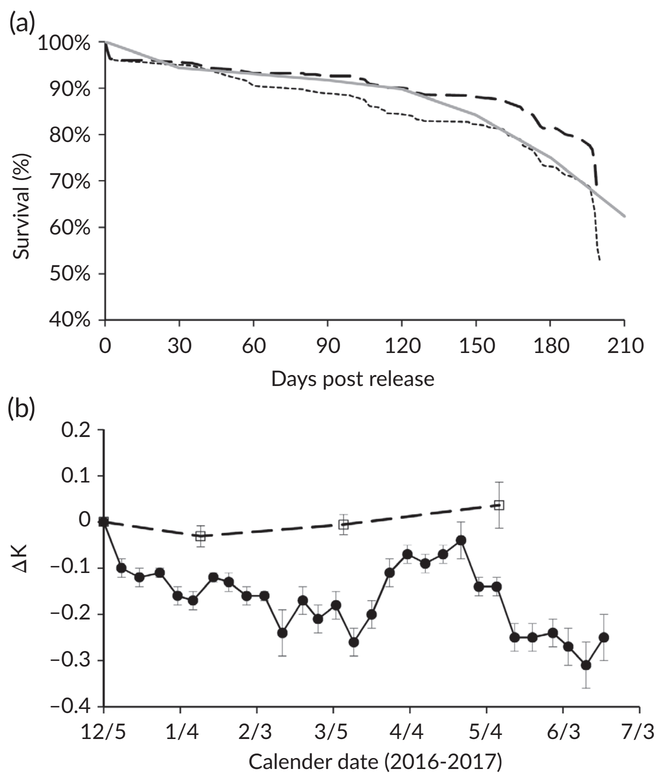

FIGURE 1 (a) Survival of cultured delta smelt *Hypomesus transpacificus* in semi-natural environment and fully controlled environment (------) Study fish (all), (–––) Study fish (handled within 1 week excluded), and (——) Control fish). (b) Mean (± SD) condition Fulton factor changes (Δ*K*) of sampled fish compared with the same fish at the beginning of the study (–●–) Study fish and (–□–) Control fish

mortalities (3.6%) that occurred within 72 h after transportation were considered to be caused by handling stress. The survival trend for cultured fish in a semi-natural environment was similar to the survival of the control group, but a large die-off occurred in the trough between days 197 and 200, which led to the termination of the study. The recovery of delta smelt in this study was 47.1%. Changes in *K* of all fish are shown in Figure 1b. Compared with the control fish, the *K* of the test fish decreased dramatically during the first three samplings (21 days post release) and continued to decline until day 98. The condition factors bounced back to only 0.04 less than the level at day 0 before declining again from day 140. In addition, on day 161, 11 of the 15 fish sampled showed signs of parasite infestation by white-spot disease *Ichthyophthirius multifiliis*.

The turbidity experienced by delta smelt in the trough was much higher (up to 80 NTU) than the turbidity they had experienced previously, showing that captive reared delta smelt can survive in turbidity higher than they experienced in captivity. Various types of organisms were detected in the gut of delta smelt, and their relative abundances changed over time (Table 1). Zooplankton was the major taxon (73.4%) in January, which included copepods, daphnia, amphipods and other taxa. The relative abundance of zooplankton decreased in later months and was replaced with worms (*e.g.*, Nematoda and Platyhelminthes) and insects (*e.g.*, mosquitoes, midges, and fries). Higher percentages of cyanobacteria and phytoplankton were observed in June compared with earlier months. These findings show delta smelt were able to find and consume natural prey in the trough, answering our first question of the study.

Even though samples of the water were not tested to see what organisms were present for delta smelt to choose from, Nobriga (2002) stated larval delta smelt feeding success was related directly to prey abundance. We therefore assumed the highest numbers of prey ingested are what was present in the highest numbers in the trough. Nobriga (2002) also stated that delta smelt mouth width increased continuously throughout the larval period, allowing for ingestion of larger prey. This is reflected in the sub-adult smelt as well as they moved on from purely zooplankton to organisms that are larger, like insects.

The condition factor of delta smelt fluctuated throughout the duration of the study, possibly due to being moved into a semi-natural environment instead of being in a stable laboratory environment. Olla *et al*. (1998) points out that a hatchery provides a plentiful supply of highly nutritious pellets, so there is little need for the cultured fish to actively search for food. Once released, the fish must learn to capture live prey, a task that many fish fail to master (Brown & Day, 2002; Ellis *et al*., 2005; Ersbak & Haase, 1983). The initial decline in fish *K* could be caused by starvation due to the termination of artificial feeding. Their fat storage from the dry feed could have served as an energy source as they physiologically adjusted to new prey items throughout the course of the study. The presence of predators in the trough could also have led to the decline. Hatchery-reared fish generally fail to avoid predators and consequently, suffer higher mortality rates (Brown & Day, 2002; Weber & Fausch, 2001). We tried to minimise the effects of predators but a red swamp crayfish *Procambarus clarkia* (carapace length of 6 cm), > 50 prickly sculpin *Cottus asper* (standard lengths up to 8 cm) and several shimofuri goby *Tridentiger bifasciatus* (standard lengths up to 7 cm) were found in the trough at the end of the study. We assume they entered the trough as larvae through the screen openings and contributed to some of the mortality of delta smelt, but we did not verify this by dissection any of the predators to look for remains of delta smelt.

Changes in the environment (Hammock *et al*., 2017) due to temperature or turbidity could also be causes of the decline in body condition. Hasenbein *et al*. (2013) found that turbidity significantly affected

TABLE 1 Composition (%) of *Hypomesus transpacificus* gut contents by phylum, class and aggregate totals, as derived from shotgun metagenomic sequencing analysis

Classification	Phylum	Class	January	March	April	May	June
	Cyanobacteria	n/a	4.9	4.6	0.4	1.9	23.2
	Arthropoda	Insecta	11.5	43.6	40.5	4.9	25.5
	Bacillariophyta	Mediophyceae	2.0	4.1	0.1	0.0	26.5
	Chlorophyta	Chlorophyceae	1.8	0.5	1.0	0.6	0.1
	Euglenida	n/a	0.1	0.0	7.2	0.0	0.0
	Total phytoplankton		**3.9**	**4.6**	**8.3**	**0.6**	**26.6**
	Annelida	Clitellata	2.1	0.0	0.1	0.0	0.1
	Nematoda	Chromadorea	2.8	25.2	4.8	58.6	0.9
	Platyhelminthes	Cestoda	0.8	2.3	0.8	14.8	0.3
	Platyhelminthes	Trematoda	0.6	6.0	1.0	10.5	0.2
	Total worms		**6.3**	**33.5**	**6.7**	**83.9**	**1.5**
	Arthropoda	Branchiopoda	45.8	4.1	39.6	2.5	7.0
	Arthropoda	Hexanauplia	0.8	8.3	4.1	4.3	0.6
	Arthropoda	Malacostraca	18.0	1.4	0.3	1.2	15.7
	Arthropoda	Ostracoda	8.8	0.0	0.0	0.6	0.0
	Total zooplankton		**73.4**	**13.8**	**44**	**8.6**	**23.3**

Note: n/a: Not available.

juvenile delta smelt's feeding performance, finding the highest feeding rates occurred at low turbidity (<12 NTU). Condition factors could also be influenced by suitable water temperature (Brown *et al*., 2013). Temperature steadily increased from December to July, ranging from 7.7 to 27.8°C. The temperature reached 27°C at the end of the study, which is the thermal maximum for adult delta smelt (Komoroske *et al*., 2014; Swanson *et al*., 2000) and could have caused a quick die-off of the fish in the trough. In addition, flow rate has been shown to have an effect on the condition factor of other species of fish including white perch *Morone Americana* (Gmelin 1789), yellow perch *Perca flavescens* (Mitchill 1814) and channel catfish *Ictalurus punctatus* (Rafinesque 1818) by providing sufficient prey items for fish to consume (Weisberg & Burton, 1993). In this study, the flow velocity in the trough was much slower than the flow in a round tank (control), but a water turnover rate of every 8.2 min was achieved.

Previous studies have shown that cultured fish have a low return rate, meaning that they do not survive as well as their wild counterparts (Jonsson & Jonsson, 2014; Kallio-Nyberg *et al*., 2011; Miller *et al*., 2014). There are various factors that can contribute to their survivability after release including predation (Jonsson & Jonsson, 2006), domestication (Huntingford, 2004), interspecific competition (Houde *et al*., 2017; Miyasaka *et al*., 2003; Mookerji *et al*., 2004), food availability (Kallio-Nyberg *et al*., 2011), health and composition of the environment (Crawford & Muir, 2008; Serrano *et al*., 2009), bodily makeup of the fish (Serrano *et al*., 2009), their athleticism (Zhang *et al*., 2016), size and age (Irvine *et al*., 2013).

The survival rate started to decline after 160 days, possibly due to the fish sexually maturing and spawning. Based on the author's experience and previous studies, the females were usually weak and easily susceptible to disease after a spawning event (Bennett, 2005). Ripe eggs in the collected fish corpses were observed as early as 105 days post release (20 March). The temperature in March was still low (<15°C), which helped the fish to stabilise and recover. Female delta smelt take about 40 to 50 days to produce another clutch of eggs (unpubl. data), but this time, an increase in temperature (*c*. 20°C) and multiple spawning events resulted in higher mortality. This increase in mortality was expected and the trend was similar to the control fish. Other causes of mortality, like the temperature spike at the end of the study and the presence of predators in the trough, combined with the natural mortality delta smelt experience after spawning are all possible factors that contributed to the massive die off that ended our study.

Since hatchery and wild fish grow up in very different environments, differential experience is likely to generate behavioural differences (Brown & Day, 2002). Behaviours learnt early in life are likely to influence behaviour at later stages. Hence, deficiencies generated in early life are likely to affect later success (Salvanes & Braithwaite, 2006). Salvanes and Braithwaite (2005) showed juvenile Atlantic cod *Gadus morhua* L. 1758 benefit from experiencing a spatial landscape during the hatchery-rearing phase, in that they develop flexible behaviour and are capable of enhanced social interactions. This idea would lend itself in the next set of experiments towards releasing delta smelt. If we could increase the complexity of the captive environment for delta smelt, then they might have greater survival if released. There are studies illustrating how simple enrichment of the rearing environment affects learning ability, foraging skills, social behaviour, predator avoidance, aggression and reproductive success (Berejikian *et al*., 2000; Salvanes & Braithwaite, 2005; Brown *et al*., 2003; Fleming *et al*., 1997).

Araki *et al*. (2009) found subsequent generations of founding fish raised in captivity continue to be of detrimental influence on their fitness. Nevertheless, captive breeding programmes have improved over time, employing the most practical methods that take into consideration the importance of maintaining genetic variation within the population, management of epizootic outbreaks across the hatchery, suitable rearing practices for all life stages and reducing effects of domestication (Lorenzen *et al*., 2012; Rowland, 2013). Releasing captive-reared delta smelt along with regulations that protect the fish species in the wild could help mitigate population decline in nature (Jutila *et al*., 2003) and this study demonstrated if delta smelt were raised in captivity to near-adulthood and then either fully liberated or released into field enclosures, the fish would learn to feed themselves and potentially survive in the wild.

Developing policies that allow for *in situ* experiments using cultured delta smelt appears to be a precursor for advancing policies that might allow supplementation actions. Lessard *et al*. (2018) highlight the importance of moving forward to develop a viable and testable supplementation programme for delta smelt conservation. This study is one step in that direction providing information needed to proceed with studies and hopefully 1 day the release of cultured delta smelt into the wild and keep this species from extinction.

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SUPPORTING INFORMATION

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

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