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RESEARCH

A Qualitative Comparison of Spawning Behavior between Cultured and Wild Delta Smelt (*Hypomesus transpacificus*)

Yi-Jiun Jean Tsai[1], Samantha N. Chase[1], Evan W. Carson[2], Leanna Zweig[2], Tien-Chieh Hung[1]*

ABSTRACT

For many imperiled species, comparisons between wild and cultured populations are invaluable for informing conservation measures, though opportunities to do so may be rare. In this study, we asked whether spawning between and among wild and cultured Delta Smelt varies in terms of behavior or resulting egg fertilization success. We conducted two laboratory experiments in which we allowed wild females to spawn with wild males (wild × wild) and cultured females to spawn with wild males (cultured × wild). Due to small sample sizes, we qualitatively compared our results to published studies of all cultured Delta Smelt (cultured × cultured). Across all three groups, Delta Smelt exhibited spawns that were similar in sequence and manner, varied widely in diel timing, and occurred predominantly between a single female and one or two males. Egg fertilization success was higher in wild × wild trials than in cultured × wild ones, but both fell within the wide range observed among cultured × cultured fish. Thus, spawning was generally similar between cultured and wild Delta Smelt, whether they were in same- or mixed-origin groups. These findings provide rare insight into the spawning behavior of wild Delta Smelt and inform ongoing conservation efforts.

KEY WORDS

spawning, Delta Smelt, behavior, reproduction, cultured, hatchery

INTRODUCTION

Conservation measures designed to mitigate the population decline of imperiled species often rely on a well-researched background of ecology, behavior, and life history (Cooke et al. 2012). For species with prohibitively low population abundance, it can be necessary to rely on a mix of laboratory and field studies of wild and cultured (including captive-, hatchery-, or laboratory-bred; domesticated; farmed; ranched; etc.) populations to obtain such information. In these instances, studies of cultured populations help improve our understanding of species biology and ecology, with the caveat that their behavior,

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morphology, physiology, life history, and genetic characteristics could differ from that of their wild counterparts (e.g., Jonsson 1997; Gross 1998; Price 1999; Weber and Fausch 2003; Huntingford 2004; Jonsson and Jonsson 2006; Champagnon et al. 2012). Behavioral differences between cultured and wild populations are well documented in fish, particularly in terms of anti-predator response, feeding, aggression, reproduction, and migration (Gross 1998; Huntingford 2004; Jonsson and Jonsson 2006; Lorenzen et al. 2012; Marsden et al. 2022), and these differences can translate into fitness-related consequences. For example, in relationship to wild Atlantic Salmon *Salmo salar* males, farmed males are less competitive, have poorer physical condition, and display inappropriate reproductive behavior, resulting in comparatively low breeding and lifetime reproductive success (Fleming et al. 1996, 2000). Thus, while studies of cultured populations can provide much-needed data that would otherwise be difficult to obtain, comparisons between wild and cultured populations are invaluable for informing conservation measures. Although, opportunities for making such comparisons are often rare or impossible for imperiled species.

For Delta Smelt *Hypomesus transpacificus*, a predominantly annual, pelagic fish endemic to the San Francisco Estuary (California, USA), population abundance in the wild has declined significantly (Tempel et al. 2021) due to factors that include habitat alteration, invasive species, and altered hydrology (Moyle et al. 2016). In response, the species is listed as federally threatened (Fed Regist 1993) and state endangered (CNDD 2023). Additionally, a refuge population has been reared in a conservation hatchery since 2008 to help protect the species from extinction (Lindberg et al. 2013). While the cultured population is genetically managed (Fisch et al. 2013; Finger et al. 2018), evidence suggests that it has become adapted to captivity (Finger et al. 2018). Cultured Delta Smelt also differ from wild conspecifics in several ways. For example, cultured females are longer, heavier, and more fecund than wild ones (Ellison et al. 2023). However, the complete extent and nature of these differences has not been fully explored.

Currently, Delta Smelt abundance in the wild is so low that the capture of Delta Smelt during recent monitoring surveys has been rare (Tempel et al. 2021). This has spurred the implementation of several management actions, including the experimental release of ~100,000 cultured Delta Smelt into the wild since December 2021 and a full-scale supplementation effort planned for the near future (USFWS 2019; USFWS et al. 2020; CDFW 2021). As a result of these measures, there is a possibility for cultured and wild (if any remain) Delta Smelt to reproduce together, and for Delta Smelt of cultured ancestry to become the dominant type in nature. Accordingly, understanding if cultured and wild Delta Smelt differ in reproductive behavior and whether they willingly spawn with one another will inform the management of the species during this pivotal point in conservation efforts.

In the wild, most Delta Smelt are thought to be semi-anadromous, moving from low-salinity waters to spawn upstream in fresher waters (Moyle 2002; Bennett 2005; Wang 2007; Merz et al. 2011; Sommer et al. 2011; Bennett and Burau 2015; Hobbs et al. 2019). Though, Delta Smelt life history is complex and may include fresh- and brackish-water resident life-history phenotypes (Hobbs et al. 2019). Spawning occurs during winter–spring (approximately February–June; Bennett 2005) after the first flush of freshwater from seasonal rainstorms (Grimaldo et al. 2009; Sommer et al. 2011). However, it is unknown exactly where or how wild Delta Smelt spawn, as neither eggs nor spawning has been observed in nature (Moyle et al. 1992, 2016; Bennett 2005).

Our understanding of Delta Smelt spawning behavior is derived from presumed similarities to that of other osmerids (e.g., Loosanoff 1938; Hirose and Kawaguchi 1998a, 1998b; Chase 2006; Penttila 2007; Okazaki et al. 2011; Quinn et al. 2012; Peterson et al. 2021), inferences from field studies (Bennett 2005; Merz et al. 2011; Sommer et al. 2011), anecdotal observations (Mager 1996; Lindberg et al. 1997; Moyle 2002; Wang 2007), and more recent laboratory studies (Lindberg et al. 2020; Tsai et al. 2021a, 2021b). In cultured Delta Smelt, spawning is defined by fast, forward-

swimming dashes (known as "bouts" in Tsai et al. 2021a, 2021b) while in direct contact with the substrate and in conjunction with the release of demersal, adhesive eggs (Mager 1996; Moyle 2002; Tsai et al. 2021a). Cultured Delta Smelt exhibit polygynandry (LaCava et al. 2015), with individuals of both sexes engaging in within-clutch serial spawning (Tsai et al. 2021a, 2021b). These fish also exhibit wide variation in the diel timing of, sex and number of participants engaged in, and substrates used for spawning (Tsai et al. 2021b). Briefly, spawning occurs predominantly at night, with diurnal spawns occurring rarely (Tsai et al. 2021b). Most spawns among cultured Delta Smelt occur between one female and one or two males, though they can include up to seven participants (out of five males and five females available, Tsai et al. 2021b). Finally, cultured Delta Smelt can spawn on pebble, sand, and acrylic substrates when not allowed a choice (Tsai et al. 2021b), though it is unclear whether they exhibit substrate preference. Among wild-caught, wild-ancestry Delta Smelt tested in a laboratory environment, females deposit the most eggs on sand and pebble substrates exposed to higher water velocities when provided a choice between varied substrate types (sand, pebble, cobble, artificial and natural Tule *Schoenoplectus acutus*, dead wood, plastic control, and tank floor control) and flow rates (1.4, 8.8, and 15.4 cm s^{-1}; Lindberg et al. 2020). However, no other studies have examined spawning behavior in wild-caught, wild-ancestry Delta Smelt, and it is unknown how their behavior compares to that of their cultured counterpart.

We had the rare opportunity to examine the reproductive behavior of wild-caught, wild-ancestry Delta Smelt. As this study was conducted before the experimental release of cultured fish, we refer to wild-caught, wild-ancestry (F_0 generation) Delta Smelt as "wild" and Delta Smelt of cultured origin and ancestry as "cultured." We asked, *does spawning between and among wild and cultured Delta Smelt vary in terms of behavior or resulting egg fertilization success?* To address this question, we examined spawning behavior in two experiments, in which we allowed wild females to spawn with wild males (wild × wild) and cultured females to spawn with wild males (cultured × wild). We quantified the diel frequency of spawning behaviors, number and sex of participants, and egg fertilization success within each experiment. Though constrained by small sample sizes, we qualitatively compared these results to those of previous studies between all cultured Delta Smelt (cultured × cultured; Tsai et al. 2021b).

METHODS

Experiments were conducted at the University of California–Davis Fish Conservation and Culture Laboratory (FCCL). Facility water used for fish care and experiments was raw surface freshwater pumped from the California Aqueduct near Clifton Court Forebay in Contra Costa County, California, USA that was treated using solids removal and UV disinfection before use (Lindberg et al. 2013). For wild × wild (N = 2 trials) and cultured × wild (N = 3 trials) experiments, mature males and ripe females were allowed to interact freely for 4 days, all behavioral interactions were video-recorded and quantified, and eggs that resulted from spawning were assessed for fertilization success. The protocol for these experiments followed Tsai et al. (2021b) with a few modifications, which we specify below.

Fish

From November 2019 to January 2020, wild, sub-adult Delta Smelt were captured by lampara net in the lower Sacramento River and transported to the FCCL in 80-L carboys. Once at the laboratory, fish were transferred to 1,100-L tanks and maintained at 12 °C and ~0.2 ppt. Fish were quarantined for 3 days after arrival, during which time they were treated once daily with a prophylactic oxytetracycline HCl bath (0.02 g L^{-1} of water at 5 ppt, 4-hr treatment; Pennox 343, Pharmgate Animal Health, Wilmington, North Carolina, USA). After quarantine, fish were tagged with visible alpha-numeric tags and then used as broodstock for the refuge population at the FCCL (Lindberg et al. 2013). Fish were caught before experimental release efforts and were of wild ancestry.

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Wild fish were available for our experiments only after their use as broodstock was completed. Thus, males had been strip-spawned one to three times, and females had been strip-spawned once within the same season before their use here. Males and females were used in these experiments 7 to 91 days and 70 days, respectively, after their last strip spawn as broodstock. One female lost her tag before these trials, so her spawning history was unknown. Males can regenerate milt within 3 days after being strip spawned (unpublished data), so their history as broodstock was not thought to affect milt availability during trials. Few ripe, wild females were available for this study, which limited the number of females that could be used in each trial, limited the number of trials per experiment, and precluded an experiment of wild females with cultured males. Of 20 mature, wild males available for this study, five were used twice: once in a cultured × wild trial and then again in a wild × wild trial 31 to 42 days later. Fish were only used in these experiments if they easily expressed milt or clear, mature eggs during gentle palpation of the abdomen near the vent (Tsai et al. 2021a) immediately before each trial.

For the wild × wild experiment, we observed spawning between two wild females and five wild males from May 19 to 23, 2020 and June 19 to 23, 2020 (N = 2 trials). The upper caudal fins of both sexes were clipped for genetic sampling before this experiment. To visually distinguish between sexes on videos, we also clipped the lower caudal fins of males. Clipping a small portion of both caudal fin lobes did not appear to hinder swimming or spawning behavior (see Appendix A).

For the cultured × wild experiment, we observed spawning between five cultured females and five wild males from May 8 to 12, 2020 and May 19 to 23, 2020 (N = 3 trials). Cultured females were F_{12} generation fish selected from tanks that contained excess production fish from the refuge population (Lindberg et al. 2013). Their domestication indices (a measure of hatchery ancestry, see Finger et al. 2018) were not known, but presumed to be high. Cultured fish were not used as broodstock before testing. The upper caudal fins of wild males were clipped for genetic sampling before this experiment, so we left the caudal fins of cultured females intact to visually distinguish between sexes.

Fish were not fed during the experiment. Fish health was monitored daily. No mortalities resulted from these experiments. All animal care and handling were conducted in accordance with the University of California–Davis Institutional Animal Care and Use Committee.

Experimental Conditions

Fish were observed in a 43.8-cm W × 58.4-cm L × 30.5-cm H (calculated working volume of 78 L) compartment within a 284-L, clear, acrylic, flow-through tank. The compartment was bookended by 0.64-cm plastic mesh. The compartment bottom was a 3 × 4 matrix of black acrylic panels (14.6-cm L × 14.6-cm W × 0.32-cm H, control substrate described in Tsai et al. 2021b), which allowed for eggs that naturally adhered to panels to be incubated after behavioral trials. Flow inlet (40.6-cm-long, 2.5-cm-diameter spray bar) and outlet (3.8-cm-diameter standpipe) were located outside of the compartment and were inaccessible to fish. Two trials were run concurrently within two separate tank systems (Tsai et al. 2021b). All equipment was disinfected with 355 ppt brine solution (FCCL 2020) and then rinsed with freshwater between trials.

Starting approximately 7 days before each trial, freshwater was filtered continuously using 1-µm canister filters to minimize water turbidity. During the experiment, water was set to 15 cm s^{-1} flow velocity (Flo-Mate 2000 portable flow meter, Marsh–McBirney, Inc., Maryland, USA) and maintained at 12.2 ± 0.008 °C. Water quality was monitored according to facility maintenance standards (Tigan et al. 2020).

A video camera (RLC-511, Reolink, Delaware, USA) with infrared capability recorded all behavioral interactions from the side of the tank. We provided visible light on a ~15:9 hr day:night cycle (AquaAir 1200, MicMol, Guangdong, China). Light intensity gradually increased and decreased throughout the day (5:50 to 20:40) to mimic

natural conditions (Tsai et al. 2021b). Infrared light (80 ft IR Illuminator, Tendelux Technology, Guangdong, China) was provided at night to allow for video recording in the dark.

Behavioral Analysis

Videos were observed at up to 2x speed. Every 30 minutes of video footage required approximately 15 to 90 minutes for analysis. Given the intensive time requirement, only the first 24 hours of each trial were analyzed for the cultured × wild experiment. This allowed for direct comparisons of Delta Smelt spawning behavior between this study and Tsai et al. (2021b). Because of the rarity and high conservation value of the wild × wild experiment, we examined the entirety of both trials (96 hours).

For all trials, we recorded the number of spawns and attempts, as defined by Tsai et al. (2021a) and with the minor modifications described in Tsai et al. (2021b; see Appendix A). In brief, spawns were fast, forward-moving, synchronized dashes between multiple fish that included egg release. Egg release could occur at any time during a given spawn and sometimes occurred more than once. Attempts were the same synchronous dashes, but without egg release. During spawns and attempts, fish swam in tandem and in proximity to one another (usually touching and side-by-side or sometimes directly atop one another). A single spawn or attempt could include one or more dashes. The beginning of an interaction was defined as when two or more fish started their first dash. The end of an interaction was defined as when all participants separated (no longer immediately side-by-side or atop one another) or turned away from one another without immediately (≤ 1 sec) engaging in another dash. Individuals could join or leave a spawn or attempt at any time during an interaction. Because of high water clarity and bright lighting, egg release was highly visible and unlikely to be missed. If egg release was ambiguous, interactions were conservatively categorized as attempts. In addition to the number of spawns and attempts, we also recorded the time of day at which the behaviors occurred, as well as the number and sexes of all participants involved.

Egg Fertilization Assessment

After behavioral trials, eggs that adhered to acrylic panels were incubated for an additional 3 days in flow-through egg incubation chambers (Tsai et al. 2021b). These eggs were a subset of the total laid during the trial and were the only eggs used to assess fertilization success. Eggs that were distributed and attached elsewhere could not be safely removed intact for incubation, so were excluded from assessment.

Egg incubation chambers were 22-L plastic bins measuring approximately 48.9-cm L × 28.6-cm W × 19.1-cm H. Water was maintained at a depth of 15.2 cm (20-L working water volume). Inflow (1.3-cm-diameter spray bar) was approximately 5 cm s^{-1}. Outflow (22.5-cm L × 2.5-cm H contracted rectangular weir) was covered in 350-µm mesh that prevented eggs from exiting the chamber. Panels were placed upright into slots in a single row directly in front of inflow. Two egg incubation chambers (one for each of the two trials run concurrently) shared the same water and were held within a 170-L plastic tub (Tsai et al. 2021b). Water was maintained at 12.8 ± 0.03 °C and filtered continuously using 1-µm canister filters.

After incubation, eggs were removed from panels, counted, and assessed for fertilization success following Tsai, Chase, and Hung (2021). To detach eggs, panels were placed into a 0.13% diluted sodium hypochlorite solution, agitated for approximately 3 minutes, and gently brushed with a soft-bristle paintbrush. Eggs were filtered using a 350-µm mesh screen, collected, and rinsed with freshwater. Detached eggs were then assembled into single-layer batches and photographed using a camera (Canon Rebel xTi, Canon USA., Melville, New York) with an 18–55-mm lens. Photographs were then used to quantify and categorize eggs using ImageJ (Schneider et al. 2012) and Fiji (Schindelin et al. 2012). Eggs were categorized as fertilized (and alive) if clear, or dead (including unfertilized eggs) if opaque (Mager 1996; Romney et al. 2019; Tsai, Chase, and Hung 2021).

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Analysis

We examined the diel frequency of spawns and attempts in two ways, depending on whether diurnal spawns were observed. For the wild × wild experiment, both nocturnal and diurnal spawning behavior was observed, so we examined the frequency of spawns and attempts in hourly time-bins. For the cultured × wild experiment, no diurnal spawning was observed, so we used ten 55-minute time-bins that spanned only the duration of night from 20:40 to 05:50 (Tsai et al. 2021b). In one wild × wild trial, one of the two females lost her visible alpha-numeric tag before the experiment. This allowed us to opportunistically examine the number of spawns and attempts for each female.

We also examined the number and sex of participants. Across all trials within each experiment, we created a histogram of spawns and attempts across unique combinations of female and male participants (e.g., one female and one male, one female and two males, two males, etc.). Participant number was calculated as the maximum number of individuals that actively participated in a given spawn or attempt at any time (Tsai et al. 2021b).

To examine egg fertilization success, we calculated the percentage of total eggs fertilized for each trial. We also graphed the total number of eggs counted against the number of eggs fertilized for wild × wild, cultured × wild, and cultured × cultured experiments. Cultured × cultured data (N = 18 trials) were those reported in Tsai et al. (2021b). Cultured fish used in Tsai et al. (2021b) were also F_{12} generation, were not previously used as broodstock, and were derived from multi-family groups with domestication indices that ranged from 6.2 to 9.5. Tsai et al. (2021b) examined the reproductive behavior between cultured males and cultured females on three spawning substrates. No significant differences were found between substrate types in the number of spawns, number of attempts, or proportion of eggs fertilized (Tsai et al. 2021b). We therefore graphed those data across all substrate types.

RESULTS

Wild × Wild Trials

Across both trials, we analyzed 192 hours and 13 minutes of video footage, during which we observed 39 spawns and 121 attempts (see Appendix A, files 1–5). In the first trial, we observed 30 spawns and 64 attempts. Of these, 24 (80.0%) spawns and eight (12.5%) attempts were diurnal, distributed across the first 2 days of the trial (Figure 1). We observed a maximum of seven spawns per hour and 12 attempts per hour. In this trial, females could be individually identified as a result of tag loss in one of the two females. The tagged female participated in 11 spawns and 18 attempts total, with a maximum rate of six spawns per hour and six attempts per hour. The untagged female participated in 19 spawns and five attempts total, with a maximum rate of six spawns per hour and two attempts per hour.

In the second trial, we observed nine spawns and 57 attempts. Of these, one (11.1%) spawn and three (5.3%) attempts were diurnal (Figure 1). We observed a maximum of two spawns per hour and eight attempts per hour.

Across both trials, two to four participants engaged in a given spawn or attempt. Most spawns (29 of 38 spawns in which the number of participants could be identified, 76.3%) and attempts (108 of 121 attempts, 89.3%) consisted of two participants. Spawns were most frequently between one female and one male (29 of 37 spawns in which the sex of all participants could be identified, 78.4%) or one female and two males (7 of 37 spawns, 18.9%, Figure 2A). Attempts were most frequently between two males (82 of 113 attempts in which the sex of all participants could be identified, 72.6%) or one female and one male (19 of 113 attempts, 16.8%). In one instance, a female released eggs without the explicit participation of other fish; other fish were in proximity but did not engage in synchronized dashes alongside the female (see Tsai et al. 2021b).

We found that 1,005 of 1,391 eggs (72.3%) and 827 of 1,021 eggs (81.0%) were fertilized in each trial (Figure 3).

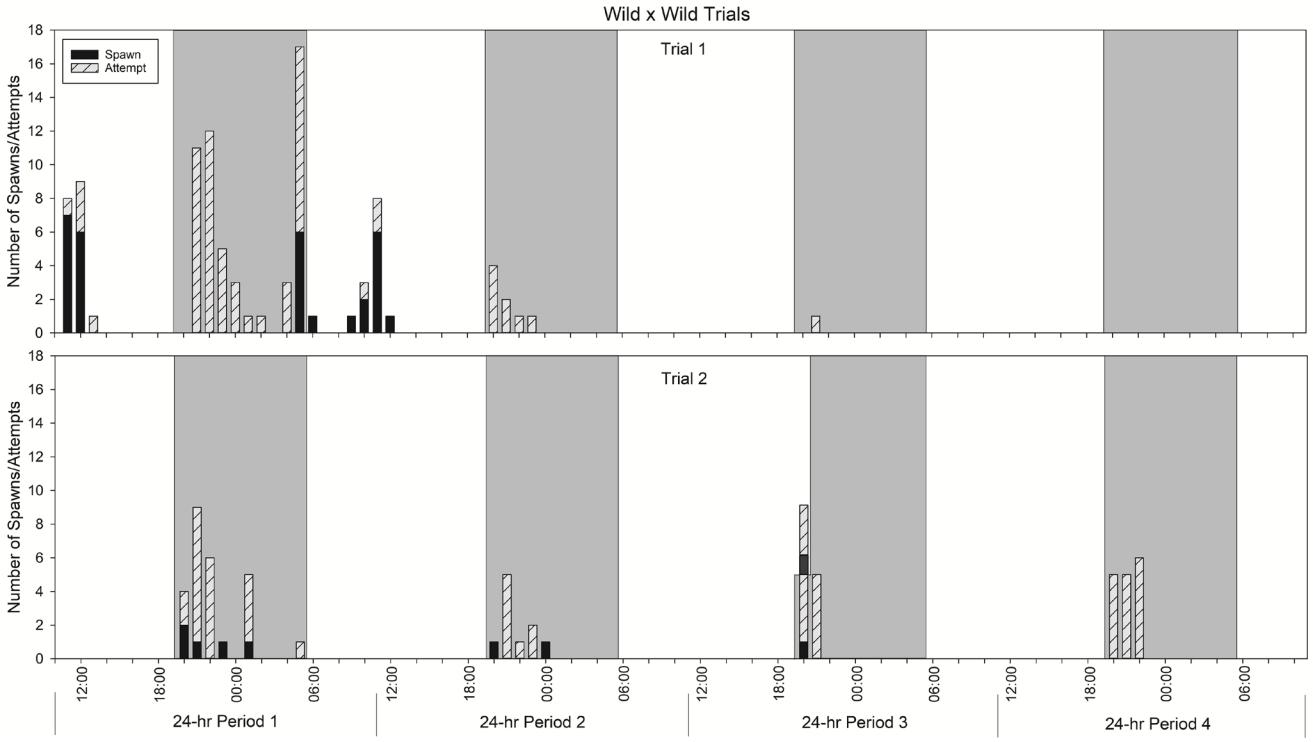

Figure 1 The number of spawns and attempts that occurred per hour in each wild × wild trial (N = 2). The *x*-axis shows the time of day by hour, labeled in 6-hour increments. The *y*-axis is the number of spawns or attempts that occurred. *Black bars* are spawns, and *light grey, hatched bars* are attempts. *Greyed areas* of the graph indicate nighttime when no visible light was available (20:40–5:50). In Trial 2 on Day 3 at 20:00, the greyed area is split to show the number of behaviors that occurred during daylight (before 20:40) and those that occurred at night (after 20:40) within the 20:00 time-bin. Trials started at 11:00.

Cultured × Wild Trials

We analyzed 72 hours and 34 minutes of video footage across all three trials, during which we observed 81 spawns and 54 attempts (see Appendix A, files 6–8). In each trial, we observed nine spawns and 25 attempts, 58 spawns and 21 attempts, and 14 spawns and eight attempts, all of which occurred at night (Figure 4).

Across all three trials, two to six fish engaged in a given spawn or attempt. Most spawns (61 of 81 spawns, 75.3%) and attempts (49 of 54 attempts, 90.7%) consisted of two participants. Spawns were most frequently between one female and one male (61 of 81 spawns, 75.3%) or one female and two males (17 of 81 spawns, 21.0%, Figure 2B). Attempts were most frequently between two males (39 of 54 attempts, 72.2%) or one female and one male (10 of 54 attempts, 18.5%). Females released eggs three times without the explicit participation of other fish.

We found that 55 of 1,952 (2.8%), 1,586 of 3,856 (41.1%), and 1,806 of 4,289 (42.1%) eggs were fertilized in each cultured × wild trial (Figure 3).

DISCUSSION

We had the rare opportunity to examine spawning behavior in wild Delta Smelt. Although our sample size was limited by the low availability of ripe, wild females, these observations allowed us to qualitatively compare the spawning behavior between and among cultured and wild Delta Smelt, drawing from previous studies of all cultured Delta Smelt (Tsai et al. 2021a, 2021b). We found that the spawning behaviors exhibited in wild × wild, cultured × wild, and cultured × cultured trials were similar in the sequence and manner in which they were exhibited (see Appendix A, files 1–8; Tsai et al. 2021b). In all experiments, Delta Smelt demonstrated behaviors that were identifiable

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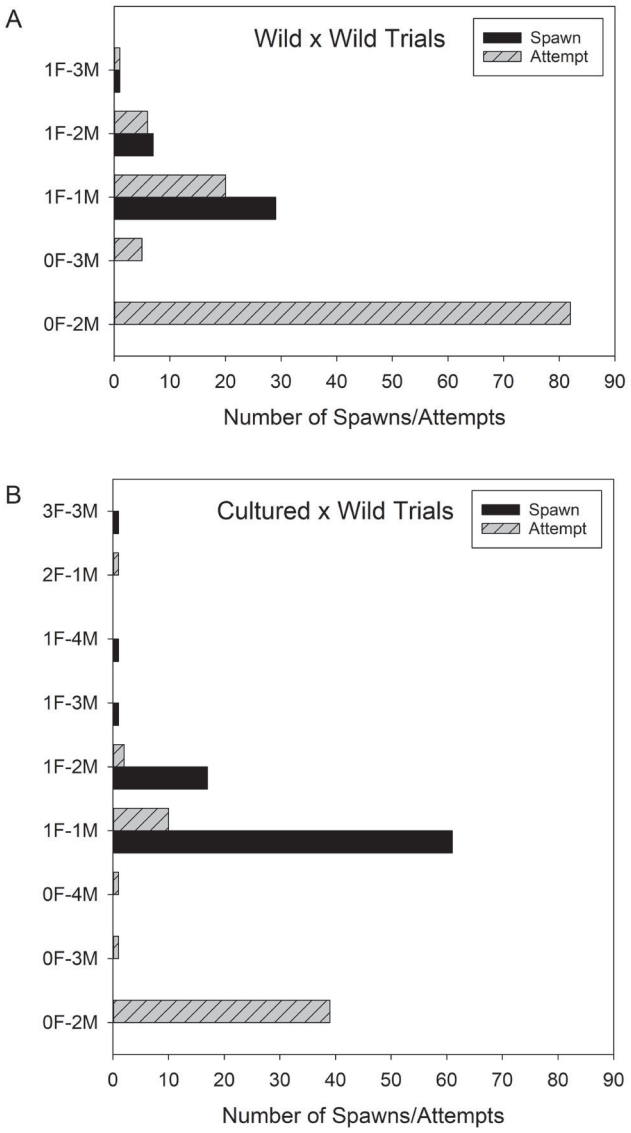

Figure 2 The number and sex of participants that engaged in spawns and attempts across both wild × wild trials (**A**) and all three cultured × wild trials (**B**). The *x*-axis shows the number of spawns or attempts. On the *y*-axis, the number of female participants is followed by F, the number of males is followed by M, and a *dash* separates the sexes. Sex ratios are listed from bottom to top in increasing order of female participants, followed by increasing order of male participants. *Black bars* indicate the number of spawns, and *grey, hatched bars* indicate the number of attempts. Across both wild × wild trials, a total of 37 spawns and 113 attempts occurred in which the number and sex of participants could be identified. Across all cultured × wild trials, 81 spawns and 54 attempts occurred.

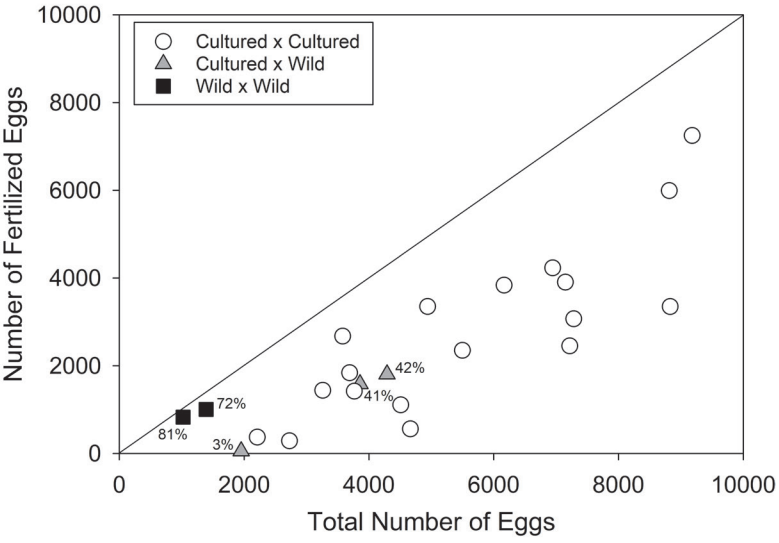

Figure 3 The number of fertilized and total eggs counted in wild × wild (N = 2), cultured × wild (N = 3), and cultured × cultured (N = 18) trials. The *x*-axis is the total number of eggs counted. The *y*-axis is the number of eggs that were fertilized after incubating for 3 days after behavioral trials were completed. *White circles* represent cultured × cultured trials, *grey triangles* represent cultured × wild trials, and *black squares* represent wild × wild trials. The percentage of eggs fertilized is indicated for wild × wild and cultured × wild trials. The *diagonal line* through the graph indicates a fertilization rate of approximately 100%. Data for cultured × cultured trials were derived from Tsai et al. (2021b).

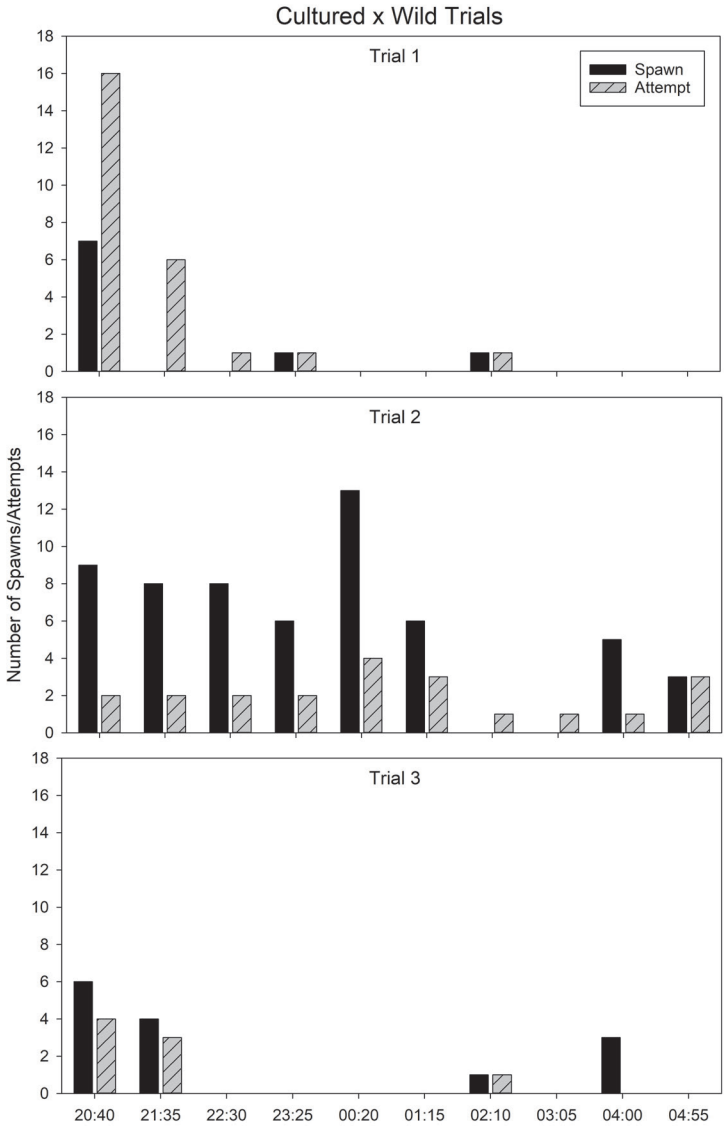

Figure 4 The number of spawns and attempts that occurred per 55-minute time-bin for each cultured × wild trial (N = 3). The *x*-axis is the time of night from 20:40 to 5:50, divided into ten 55-minute time-bins. The *y*-axis is the number of spawns or attempts. *Black bars* indicate spawns, and *grey, hatched bars* indicate attempts. All spawns and attempts occurred at night (no visible light).

as spawns and attempts, as defined in Tsai et al. (2021a). However, we also observed subtle differences in spawning not captured by these behavioral definitions. Spawning often appeared to be less exaggerated (e.g., occurring at slower swim velocities) in wild × wild trials than in cultured × cultured and cultured × wild trials. Though, we cannot rule out the possibility that these perceived differences were the result of the lower female-to-male ratio in wild × wild trials or observation bias, in which we observed comparatively few wild × wild spawns among few individuals. Importantly, unlike in trials of all cultured Delta Smelt, we observed some instances in which wild males appeared to follow wild females by maintaining a close distance behind her and using the same swimming path (see Appendix A, file 9). These "follows" were brief but evident and did not appear to immediately precede or succeed spawning behavior. We cannot yet hypothesize as to the function of this behavior, if any, but suggest that it may occur more frequently among wild Delta Smelt than among cultured Delta Smelt; no such behavior was noted in cultured × cultured trials (N = 17 trials, 170 fish, 409.6 hours of video observed, Tsai et al. 2021b). Further studies utilizing larger compartments could be revealing, because wild Delta Smelt may not be as accustomed to confined spaces as cultured fish and consequently may have truncated or altered such behaviors.

The number and sex of participants engaged in spawns and attempts were similar between all experiments (wild × wild, cultured × wild, and cultured × cultured), in which spawns occurred predominantly between one female and one male, and attempts occurred most frequently between two males (Tsai et al. 2021b). This pattern persisted even when there were fewer available females and a lower female-to-male ratio (2F:5M in wild × wild trials and 5F:5M in cultured × wild and cultured × cultured trials) and is similar to observations reported in other wild osmerids. In Japanese Surf Smelt (*Hypomesus pretiosus japonicus*) of Otsushi Bay, Japan (Hirose and Kawaguchi 1998a)

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and Surf Smelt (*H. pretiosus*) of Puget Sound, USA (Loosanoff 1938), fish collect in mixed-sex aggregations before spawning in smaller groups of one female and a varied number of males. Thus, in Delta Smelt, spawning in small groups of a single female and one or two males may be a relatively consistent participant composition, though studies using more varied sex ratios and group sizes are needed to verify this hypothesis.

Previous studies suggest that the diel timing of spawning activity among cultured × cultured Delta Smelt is highly variable (Tsai et al. 2021b), but predominantly nocturnal (Mager 1996; Lindberg et al. 1997; Moyle 2002; Wang 2007; Tsai et al. 2021a, 2021b), with only 5% of spawns occurring during the day (across 631 spawns in 17 trials, Tsai et al. 2021b). In cultured × wild trials, we observed no diurnal spawning activity, which was consistent with these previous findings. However, for the wild × wild experiment, diel activity differed between trials. Whereas spawning was predominantly (89%) nocturnal in one trial, it was mostly (80%) diurnal in the other. Within the trial with primarily daytime activity, diurnal spawns were distributed across 2 days, suggesting that they occurred naturally and were not induced by the stress of handling or introduction to a new environment. Without a larger sample size, it is difficult to determine whether wild Delta Smelt demonstrate the same patterns in diel spawning activity as cultured Delta Smelt. However, Delta Smelt appear to be capable of exhibiting wide variation in the diel timing of spawning (Tsai et al. 2021b), regardless of their origin or ancestry.

We found that egg fertilization success was higher in wild × wild trials (72.2% to 80.9%) than in cultured × wild ones (2.8% to 42.1%), but both groups overlapped the wide range observed among cultured × cultured fish (10.6% to 79.0%; Tsai et al. 2021b, Figure 3). In contrast, mixed (mean ± SE = 81 ± 3%, range = 10–95%, N = 60) and all-cultured (mean ± SE = 81 ± 2%, range = 0–99%, N = 215) crosses show higher fertilization success than all-wild crosses (mean ± SE = 55 ± 19%, range = 0–80%, N = 4) among Delta Smelt broodstock that are strip-spawned to propagate the refuge population at the FCCL (based on visual estimates of eggs ~3 days post-fertilization in 2017). However, sample sizes for wild × wild crosses were limited in both cases, and fertilization success resulting from spontaneous spawning is not directly comparable to that resulting from artificial propagation (e.g., Di Biase et al. 2016). It is therefore unclear whether fertilization success truly differs between cross types based on these results. Ultimately, the reproductive success of wild and cultured Delta Smelt that spawn in nature may differ from one another due to complex factors, such as:

- **mate choice or preference** (e.g., Atlantic Cod: *Gadus morhua*, Skjæraasen et al. 2010)
- **competitive ability** (e.g., Coho Salmon: *Oncorhynchus kisutch*, Fleming and Gross 1992, 1993; Berejikian et al. 1997; and Atlantic Salmon: Fleming et al. 1996, 2000)
- **offspring survival and fitness** (e.g., Atlantic salmon: McGinnity et al. 2003; Skaala et al. 2012; Wringe et al. 2018; Solberg et al. 2020).

Identifying the role that such factors play in Delta Smelt reproduction would greatly improve estimates of recruitment and thereby increase the efficacy of management strategies (e.g., inform life-cycle models on which such strategies are based), particularly those related to supplementation.

CONCLUSIONS

Our results demonstrate that wild and cultured Delta Smelt exhibit spawning behavior similar to one another and that they willingly and successfully spawn with one another. In light of the recent release and planned supplementation of cultured Delta Smelt into the wild (USFWS 2019; USFWS et al. 2020; CDFW 2021), these findings are useful in predicting and managing the population dynamics of released Delta Smelt. Further studies to better understand the complexities of reproduction in wild Delta Smelt are needed. However, such opportunities are unlikely, considering that the capture of wild

Delta Smelt has been rare in recent years (Tempel et al. 2021), and now that cultured Delta Smelt are being released annually. Thus, our study is likely to remain one of few to describe spawning behavior among wild-origin Delta Smelt of purely wild ancestry (though see Lindberg et al. 2020), and future studies of reproductive behavior in Delta Smelt may necessarily rely on cultured or cultured-ancestry fish.

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