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Research Article

Effects of Salinity on Fertilization, Hatching, and Larval Performance of Longfin Smelt *Spirinchus thaleichthys*

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Understanding the spawning and rearing habitats of fishes is critical to effective fisheries management and conservation. Longfin smelt *Spirinchus thaleichthys* is an imperiled migratory fish that is believed to spawn and rear in habitats of varying salinities; however, optimal conditions for each stage remain unknown. Here, we examined the effects of variation in salinity on egg fertilization, hatch success, and larval growth and survival. Eggs that were fertilized in freshwater (0.4 ppt) exhibited a significantly higher fertilization rate (81%) than those fertilized in brackish water (62% at 5 ppt), with no detectable effects of fish origin or female size. In contrast to fertilization rates, once the eggs were fertilized, their hatching rates were not affected by the fertilization salinity, incubation salinity, nor their interaction; however, hatching success and larval survival both increased with increasing maternal body mass. Larval growth rate appeared to be independent of salinity and maternal size. Taken together, the results indicate that fertilization is possible at a range of salinities, but optimal at lower salinities for longfin smelt; however, embryos and larvae can perform well across a range of salinities. Furthermore, results indicated that larger mothers produced high-quality offspring, a finding that supports the "bigger is better" paradigm in fisheries science and management. These results likely explain, in part, the spawning and rearing behaviors of wild longfin smelt and suggest that the conservation culture program would likely be optimized by utilizing freshwater fertilization and larger females as broodstock.

1. Introduction

Many fish populations are declining globally due to anthropogenic activities and the intensifying impacts of climate change [1, 2]. An increasing number of species and populations have been listed as threatened, vulnerable, endangered, or extinct, according to local and international biological assessments [3, 4]. One such fish species is longfin smelt *Spirinchus thaleichthys*, which is listed as "threatened" under the California Endangered Species Act in 2009 [5]. Longfin smelt are small anadromous forage fish that were once abundant throughout the San Francisco Estuary (SFE), California

[6, 7]. A rapid decline in this southernmost distinct population [8] has occurred in recent decades, with some studies suggesting that the population may be on the path toward extinction [9]. Therefore, immediate action is needed to prevent further population declines in the wild.

Successful spawning of wild broodstock, hatching of produced eggs, and rearing of larvae are the key features of successful fish conservation hatcheries [10, 11]. Researchers at the Fish Conservation and Culture Laboratory (FCCL) at the University of California, Davis (UCD) and several other departments at UCD have been working for many years to develop suitable captive culture methods for the longfin

TABLE 1: Summary of larvae produced from wild caught longfin smelt *Spirinchus thaleichthys* at the FCCL from 2018 to 2021.

Year	Number of crosses	Fertilization (%)	Hatching (%)	Larval (0–40 dph) survival (%)
2018–2019	45	63.8 ($n = 45$)	94.3 ($n = 15$)	23.9 ($n = 10$)
2019–2020	29	42.8 ($n = 29$)	89.5 ($n = 20$)	68.4 ($n = 16$)
2020–2021	14	37.5 ($n = 14$)	91.7 ($n = 7$)	16.2 ($n = 5$)

Abbreviations: dph, days post-hatch; FCCL, Fish Conservation and Culture Laboratory *Note*. number of samples varied because fertilized eggs were used for other purposes.

smelt [12–15]. However, unpublished data from the FCCL indicate low survival of longfin smelt relative to delta smelt *Hypomesus transpacificus* during the first 40 days post-hatch (dph; Table 1) [16–18]. The spawning of longfin smelt and rearing of their larvae has been challenging, possibly because of their complex migratory behaviors between coastal, estuarine, and freshwater habitats [19, 20].

Fertilization, embryonic development, hatching, and larval growth of fishes are often highly sensitive to salinity. For example, salinity significantly affects sperm performance in capelin *Mallotus villosus* and rainbow smelt *Osmerus mordax* [21]; fertilization rate in Atlantic herring *Clupea harengus* [22] and common carp *Cyprinus carpio* [23]; embryonic development in obscure puffer *Takifugu obscurus* [24] and Siberian sturgeon *Acipenser baerii* [25]; and hatching, larval survival, and deformation rates in common carp and bullseye puffer *Sphoeroides annulatus* [26]. For the endangered delta smelt, incubation salinity significantly affects embryo performance and survival rates [27]. Furthermore, recent research on longfin smelt has shown that their yolk sac larvae are widespread across habitats of varying salinities [12, 20, 28–31] and exhibit the highest growth and survival in brackish water (5 and 10 ppt) relative to freshwater (0.4 ppt), [14]. Since the distribution of larval longfin smelt can range from tidal freshwaters to brackish estuarine habitats [12, 19, 29, 32, 33], a key question has been raised whether egg fertilization, hatching, and larval growth are significantly influenced by salinity gradients throughout the SFE.

To further inform and guide the development of the captive culture and breeding program for this sensitive species, the present study aimed to investigate the effects of salinity during fertilization and incubation and their interaction on fertilization, hatching, and larval performance. The findings of this study are expected to provide knowledge about the key salinity regimes for optimizing longfin smelt spawning and incubation that can be used for the future captive rearing and conservation of this threatened species.

2. Materials and Methods

2.1. Ethics Statement. The study was carried out under the animal ethics approval of UCD Institutional Anima Care and Use Committee (no. 21353).

2.2. Broodstock Collection and Rearing. Mature longfin smelt were collected from the lower SFE, in South San Francisco Bay, by the UCD Otolith Geochemistry and Fish Ecology Laboratory (OGFL) and from Chipps Island in the upper SFE, within the Sacramento-San Joaquin River Delta, by the

US Fish and Wildlife Service (USFWS) between November 29, 2021 and March 18, 2022. A summary of broodstock collection location can be found in Figure 1.

After the fish were transported to the FCCL, they were quarantined in 400-L tanks and received a 3-day prophylactic antibiotic treatment (Pennox 343, Animal Health International, Ceres, CA, USA) in standing water with aeration (20 nephelometric turbidity units, 10 ppt, and 12°C). All ripe females were immediately separated, size measured (fork length in mm and weight in g), and stripped for spawning, if available. The spawned fish were then tagged with FTF-69 Fingerling tags (Floy Tag & Manufacturing Inc., Seattle, WA, USA) and consolidated back with other fish arrived in the same batch for the prophylactic treatment. No feed was provided during the quarantine period. After the treatment, the remaining untagged fish were measured, tagged, and transferred to their assigned broodstock tanks (400-L tanks at 10 ppt and 12°C). All fish were fed five times per day with commercial dry feed (Biovita Starter mash crumble, Bio-Oregon, WA, USA) and newly hatched brine shrimp *Artemia franciscana* (Artemia International, Fairview, TX, USA) and supplemented with adult brine shrimp twice per day to satiation. Tanks were inspected daily and cleaned twice weekly, and any observed mortalities were recorded and removed. Water quality was monitored twice weekly.

2.3. Spawning, Fertilization, and Hatching. A total of 12 crosses were made for this study (Table 2 and Figure 2) from December 02, 2021 to February 18, 2022. During spawning, selected ripe females and males were anaesthetized with 0.1% buffered tricaine methane sulfonate (MS-222, Syndel, ferndale, WA, USA) solution. Fork length, body weight, and tag numbers were recorded. Each female was stripped into an egg bowl, and immediately milt from an expressing male (sometimes more than one male was taken, if the milt quantity was very low) was collected and mixed with the eggs. Six of the 12 crosses were fertilized in filtered source water to the FCCL (0.4 ppt), while the other six crosses were fertilized with 5 ppt saline water prepared using Instant Ocean sea salt (Spectrum Brands Inc., VA, USA) for 5 min before the water was replaced with the filtered source water (0.4 ppt). Temperature was controlled at $12 \pm 1°C$ throughout both incubation periods (first step: 6 days and second step: 10 days; Figure 2). During the first incubation period, eggs were disinfected with Pond Rid-Ich solution (55 mL Pond Rid-Ich diluted with 378 mL water; Kordon LLC, CA, USA) for 1 min daily to alleviate fungal growth [34]. On Day 5, the eggs were mixed with bentonite (Sigma–Aldrich, Saint Louis) to remove the adhesion and coagulation [18]. On Day 6, after the disinfection treatment, live and
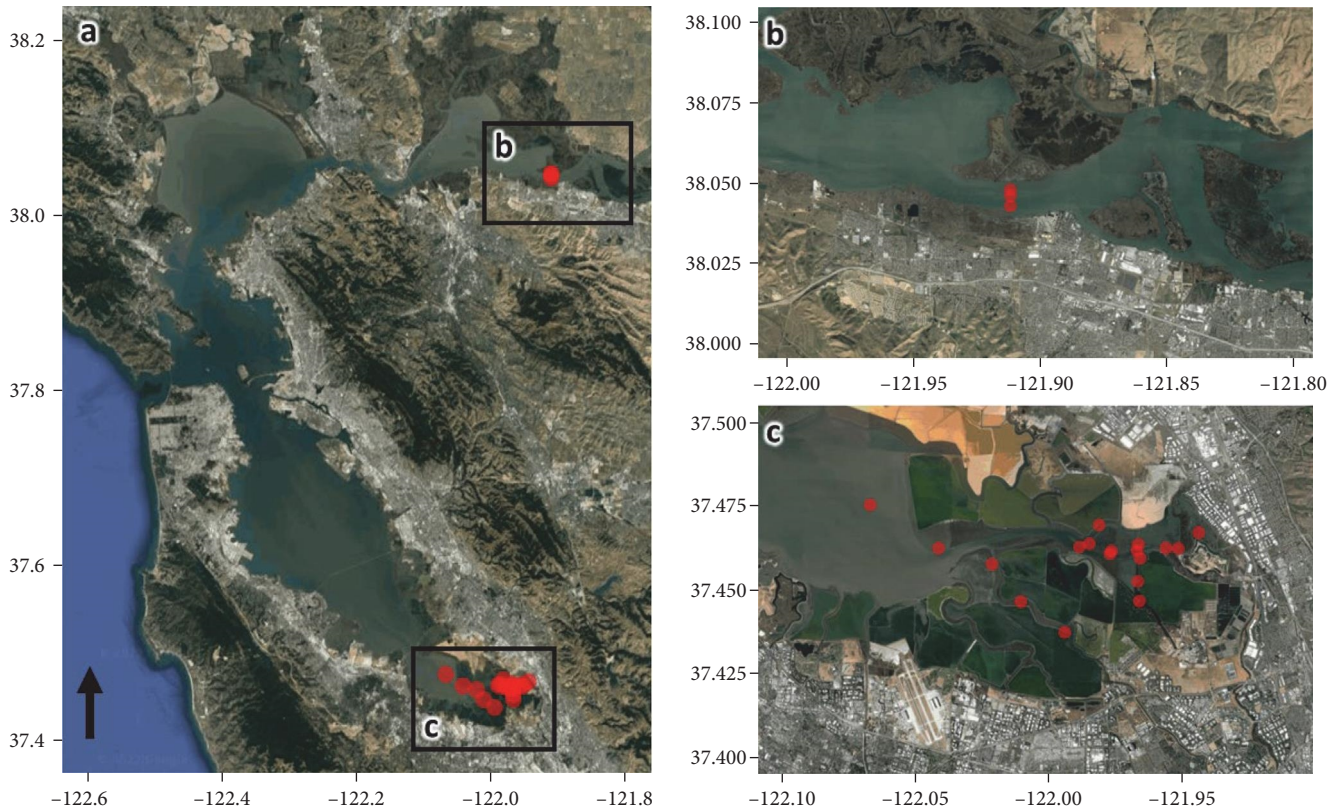
FIGURE 1: (a) San Francisco Estuary, California. The figure shows locations of wild longfin smelt *Spirinchus thaleichthys* broodstock collection, where (b) indicates the Chipps Island and (c) indicates the South Bay. The solid dots represent the location of collection coordinates.

TABLE 2: Numbers, sex, size, and resulting fecundity of wild-caught longfin smelt *Spirinchus thaleichthys* used for spawning.

| Fish source | Collection site | F (n) | M (n) | Fork length (mm) | | Fecundity |
				F	M	
OGFL	South Bay	4	5	104.0 ± 1.5	109.6 ± 2.8	$3{,}661.3 \pm 474.7$
USFWS	Chipps Island	8	12	100.0 ± 1.5	103.4 ± 2.1	$3{,}863.6 \pm 273.9$

Abbreviations: OGFL, UC Davis Otolith Geochemistry and Fish Ecology Laboratory; USFWS, US Fish and Wildlife Service; M, male; F, female. *Note.* Fork length (mm) is presented as mean $\pm$ *SE*.

dead eggs were identified, separated, and number estimated following the protocols for delta smelt developed by the FCCL [35]. The fertilization rate (%) was calculated using the following formula:

$$F(\%) = E_f/E_t \times 100, \tag{1}$$

where F represents the fertilization rate (%), E_f is the number of fertilized eggs, and E_t is the number of total eggs.

The fertilized eggs were equally divided into two groups and transferred to separate column incubators for the second incubation period (10 days) at 0.4 or 5 ppt at $12 \pm 1°C$ until the larvae hatched out (Figure 2). The incubators were inspected daily, and any dead embryos were recorded and discarded.

2.4. Larval Rearing and Trait Assessment. After hatching, the total number of hatchlings was estimated by deducting the dead eggs from the total number of incubated fertilized eggs, and the hatching rate (%) was calculated using the following formula:

$$H(\%) = E_h/E_f \times 100, \tag{2}$$

$$E_h = E_f - E_u, \tag{3}$$

where H represents the hatching rate (%), E_h is the estimated number of hatchlings, and E_u is the sum of dead and unhatched embryos recovered.

Larvae from each incubator were transferred to randomly assigned tanks within four identical recirculating systems and reared at 12°C for 40 days. Each tank held 92 L of 5 ppt water. The range of stocking density was 4–27 larvae per liter (Table 3). During the first 3 days (0–2 dph), larvae were held in the tanks without feeding, and at 3 dph, larvae started to receive their first live prey: rotifers *Brachionus plicatilis*
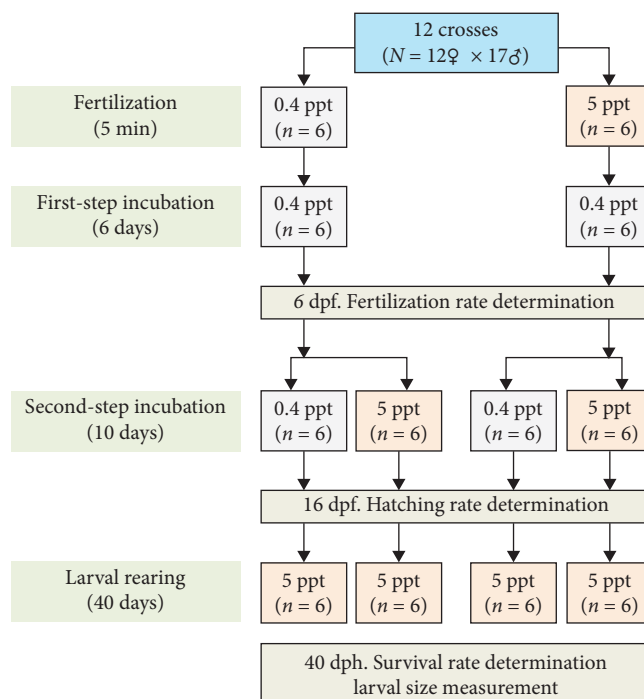
FIGURE 2: Experimental design depicting the fertilization, embryo incubation, hatching, and larval rearing of longfin smelt *Spirinchus thaleichthys* during this study. Here, N = total number of broodstocks used for spawning, n = number of crosses, dpf = days post fertilization, and dph = days post hatching.

TABLE 3: Larval stocking density of longfin smelt *Spirinchus thaleichthys* reared during this study.

Cross	Fish source	Fertilization salinity (ppt)	Incubation salinity (ppt)	Stocking density (larvae/L)
1	USFWS	5	0.4	18
			5	18
2	USFWS	0.4	0.4	16
			5	16
3	USFWS	0.4	0.4	10
			5	10
4	USFWS	5	0.4	12
			5	12
5	USFWS	0.4	0.4	27
			5	27
6	USFWS	5	0.4	10
			5	10
7	USFWS	0.4	0.4	21
			5	21
8	USFWS	0.4	0.4	20
			5	20
9	OGFL	0.4	0.4	22
			5	22
10	OGFL	5	0.4	4
			5	4
11	OGFL	5	0.4	20
			5	20
12	OGFL	5	0.4	17
			5	17

Abbreviations: OGFL, UC Davis Otolith Geochemistry and Fish Ecology Laboratory; USFWS, US Fish and Wildlife Service.

(L-type and size of 210 μm, Reed Mariculture, Pasadena, CA, USA). On the same day, a commercial algae *Nannochloropsis* sp. (Nanno 3,600™ Reed Mariculture Inc., San Jose CA, USA) was added to the systems to increase the turbidity to 10 nephelometric turbidity units. At 10 dph, newly hatched naupalii of *Aretemia* were added to the diet. The co-feeding of rotifers and *Artemia* was continued to 40 dph.

At 40 dph, the total number of larvae was counted, and 20 larvae were haphazardly collected from each tank and euthanized in buffered MS-222 for measurements. Total length (to the nearest 0.1 mm) of each larva was measured using ImageJ v1.53j software (Available online: https://mvnre pository.com/artifact/net.imagej/ij/1.53j). Body weight of each larva was also measured (to the nearest 0.001 g) using an analytical balance (Cole-Parmer, model: TA-224.C, Shanghai, China). The larval survival rate (%) from 0 to 40 dph was calculated using the following formula:

$$S(\%) = 100 \times E_l/E_s, \tag{4}$$

$$E_s = E_h - E_x, \tag{5}$$

where S represents the larval survival rate (%), E_s is the total number of fish that successfully hatched minus those removed for measurements, E_l is the total number of larvae at 40 dph, and E_x is the number of fish sampled for the measurements.

Since the newly hatched larvae were reared at different stocking densities, the density effects on the larval performance were investigated. In addition, since larvae from the two incubation salinities were both reared at 5 ppt, an analysis was also done to explore the effect of salinity shock on the newly hatched larvae when moved from the hatching environment (0.4 and 5 ppt) to the larval rearing environment (5 ppt).

2.5. Statistical Analyses. All analyses were performed using "R" version 4.0.5 [36]. The descriptive statistics (means, maximum, minimum, SEs, etc.) were estimated using the "psych" package [37]. The normality was tested through the Shapiro–Wilk test, while the homogeneity of variance was tested by the Levene's test using the "onewaytests" package [38]. The generalized linear model (GLM) including the "quasi-poisson" family option was used with the "pscl" package [39] for analyzing the effects of fertilization and incubation salinities (fixed factor) on the fertilization, hatching, and larval survival rates as these data did not follow the required assumptions of any parametric model. The "quasi-poisson" option provides flexibility with the required assumptions which considers overdispersion for the dependent variables [40]. In hatching and survival rate models, fertilization salinity, incubation salinity, their interaction, and the source of fish were included as fixed factors, while the size of females and stocking density of larvae (excluded from the hatching rate model) were incorporated as covariates. In the fertilization rate model, only the fertilization salinity and the source of fish were included. The univariate analysis of variance (ANOVA) model using the "car" package [41] was applied to find out any variation in larval size (e.g., total length and weight), where fertilization salinity, incubation salinity,
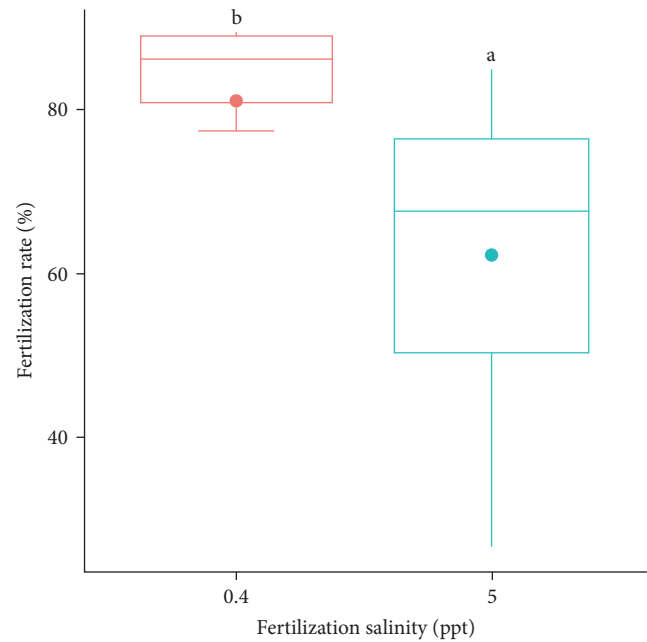

FIGURE 3: Salinity effect on the fertilization rate (%) of longfin smelt *Spirinchus thaleichthys* between 0.4 and 5 ppt treatments. Boxplot shows values of mean (dots), median (horizontal lines), upper and lower quartile, and error bars show maximum and minimum fertilization rates. Letters above each box indicate significant variation between treatments ($p < 0.05$).

fertilization salinity × incubation salinity, and the source of fish were included as fixed factors, and female size and larval stocking density were incorporated as covariates. The linear regression models were run to explore the relationship between female size and spawning performance (i.e., fertilization rate, hatching rate, and larval length and survival rate). To test the significance level and measure the magnitude of effects, the effect size (ES) was estimated [42, 43], where interpretations of ES were based on parameters set by Cohen [42] (i.e., <0.02, 0.02 ≤ to <0.13, 0.13 ≤ to < 0.26 and ≥0.26 for very small, small, medium, and large ESs, respectively, for ANOVA analysis) using the package "effectsize'" [44]. All graphs were prepared using the "ggplot2" package [45].

3. Results

3.1. Fertilization. To evaluate the effect of salinity on fertilization, results of the GLM indicated that fertilization rates at 0.4 ppt (mean ± SE: 80.1% ± 3.6%) were significantly higher than that of 5 ppt (mean ± SE: 62.3% ± 5.8%, $t = 2.18$, $p < 0.05$; Figure 3). Neither broodstock origin ($t = 0.68$, $p = 0.51$) nor female size ($t = 1.38$, $p = 0.18$) appeared to influence fertilization rates. The calculated effect size (ES = 0.28) suggested that there was a 29% difference in relative fertilization rate between 0.4 and 5 ppt treatments due to the fertilization salinity in this study.

3.2. Hatching. Hatching rate was significantly influenced by the female size, with larger females producing offspring with higher hatching rates (77%–96%, $R^2 = 0.57$, $p < 0.001$; Figure 4(a)). Hatching rate did not appear sensitive to fertilization salinity
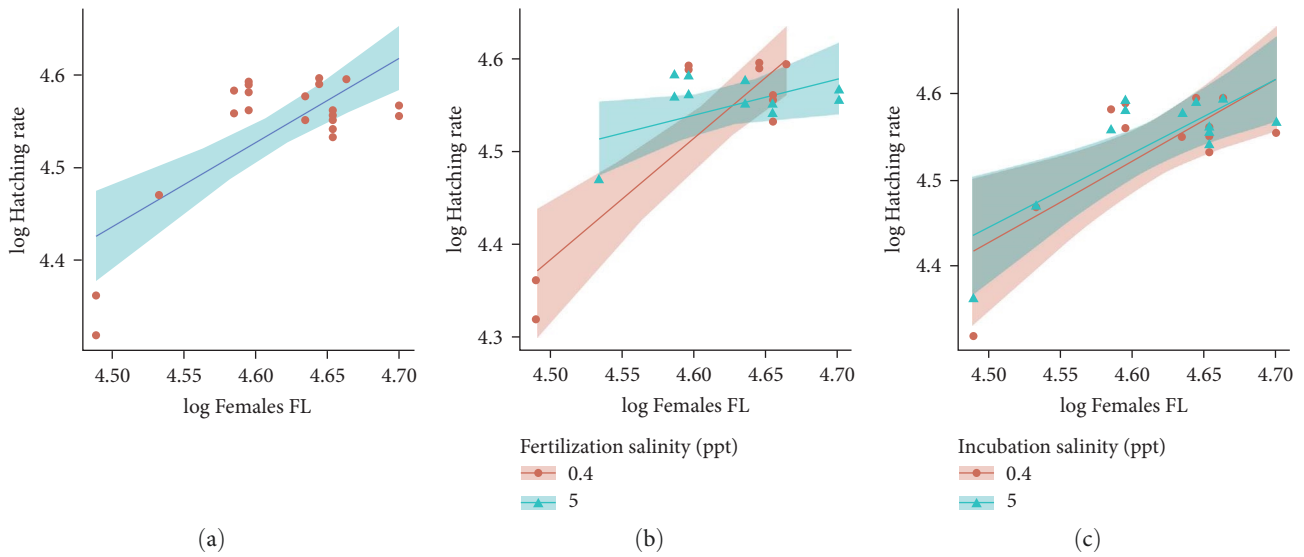
FIGURE 4: The relationship between hatching rate and female size (fork length, FL) of longfin smelt *Spirinchus thaleichthys* in the logarithmic form: (a) The overall relationship between hatching rate and female size; (b) separate relationships based on different fertilization salinities, and (c) separate relationships based on different incubation salinities. Least squares regression line (solid line) and 95% confidence interval (shaded area) are shown for each relationship.
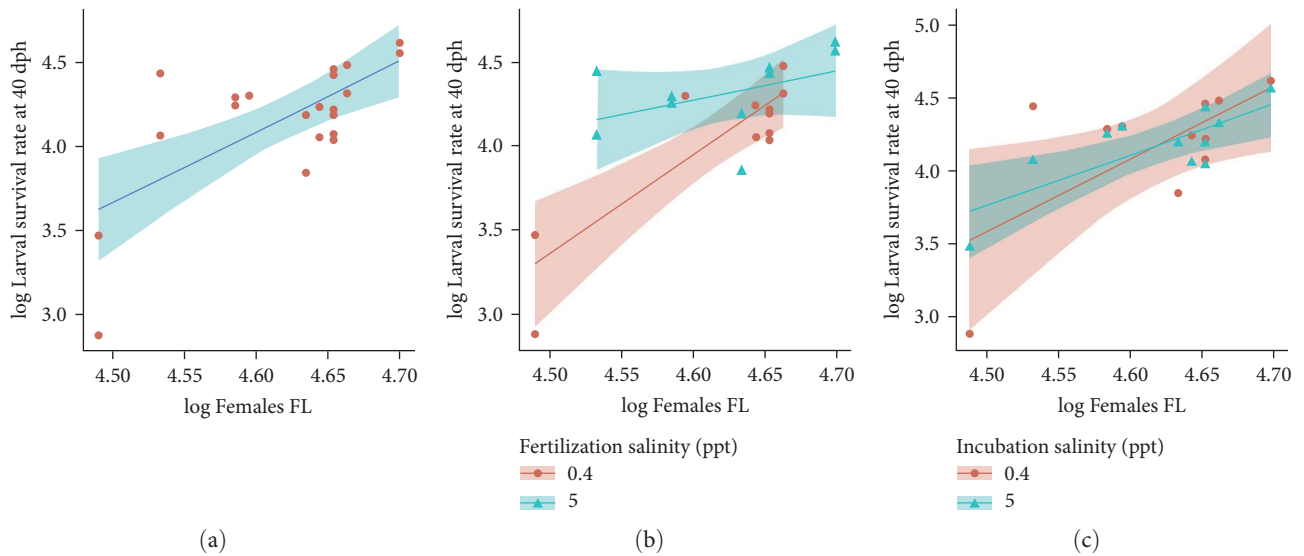

FIGURE 5: The relationship between larval survival from 0 to 40 dph (days post-hatch) and female size (fork length, FL) of longfin smelt *Spirinchus thaleichthys* in the logarithmic form: (a) The overall relationship between larval survival at 40 dph and the size of female broodstock; (b) the separate relationships based on different fertilization salinities, and (c) separate relationships based on different incubation salinities. Least squares regression line (solid line) and 95% confidence interval (shaded area) are shown for each relationship.

($t = 0.19$, $p = 0.85$), incubation salinity ($t = 0.29$, $p = 0.77$), fertilization salinity × incubation salinity ($t = 0.12$, $p = 0.91$), nor the source of fish ($t = 0.34$, $p = 0.74$). The interaction between female size and fertilization salinity indicated that hatching rate increased with female size at lower fertilization salinity ($R^2 = 0.78$, $p < 0.001$), but not at the higher one ($R^2 = 0.32$, $p = 0.06$; Figure 4(b)). In addition, the female size effect on hatching rate was significant and similar at both incubation salinities (0.4 ppt, $R^2 = 0.55$, $p < 0.01$ and 5 ppt, $R^2 = 0.60$, $p < 0.01$; Figure 4(c)).

3.3. Larval Performance. Larval survival from 0 to 40 dph was significantly higher for larvae produced from larger female broodstock ($R^2 = 0.45$, $p < 0.001$; Figure 5(a)). No significant effects of fertilization salinity ($t = 2.1$, $p = 0.053$), incubation salinity ($t = 0.30$, $p = 0.77$), fertilization salinity × incubation salinity ($t = 0.04$, $p = 0.97$), source of fish ($t = 1.7$, $p = 0.11$), and larval stocking density ($t = 0.71$, $p = 0.41$) on larval survival were detected. Fertilization salinity exerted a marginally significant effect on the survival, with embryos fertilized at 5 ppt exhibiting slightly higher larval survival than at 0.4 ppt

TABLE 4: Effects of different salinity, broodstock source, larval stocking density, and female size on total length and body weight of longfin smelt *Spirinchus thaleichthys* larvae reared during this study.

Larval trait	Factors	Sum. Sq.	df	F-value	p-Value
Total length (mm)	Fertilization salinity (ppt)	0.0033	1	0.62	0.45
	Incubation salinity (ppt)	0.0042	1	0.78	0.39
	Fertilization salinity × incubation salinity	0.0009	1	0.17	0.68
	Source of broodstock	0.0100	1	1.8	0.19
	Larval stocking density (ind./L)	0.0088	1	1.62	0.22
	Fork length of female (mm)	0.0079	1	1.45	0.25
Body weight (g)	Fertilization salinity (ppt)	0.000	1	0.02	0.89
	Incubation salinity (ppt)	0.000	1	0.24	0.63
	Fertilization salinity × incubation salinity	0.000	1	0.20	0.66
	Source of broodstock	0.000	1	4.01	0.06
	Larval stocking density (ind./L)	0.000	1	3.59	0.08
	Fork length of female (mm)	0.000	1	4.19	0.06

Abbreviations: Sum. Sq., the sum of squares; df, degrees of freedom; ind., individuals.

($76.3\% \pm 5.3\%$ and $61.3\% \pm 5.6\%$, respectively). Moreover, the calculated effect size (ES = 0.30) suggested that there was a 24% increase in relative survival rate when eggs were fertilized with 5 ppt rather than 0.4 ppt during this study. Larval survival increased with female size at 0.4 ppt fertilization salinity ($R^2 = 0.71$, $p < 0.001$; Figure 5(b)) and at both incubation salinities (0.4 ppt, $R^2 = 0.42$, $p < 0.05$ and 5 ppt, $R^2 = 0.59$, $p < 0.01$; Figure 5(c)). However, no significant relationship between the female size and larval survival was observed at 5 ppt of fertilization salinity ($R^2 = 0.20$, $p = 0.19$; Figure 5(b)). The lack of a difference in survival between fish hatched in 0.4 and 5 ppt (0–40 dph, $t = 0.46$, $p = 0.65$) indicated no detectable effect of "salinity shock" for larvae being transferred from fresh to low-salinity water at 0 dph.

Total length and weight of 40 dph larvae were not significantly affected by any salinity treatments and other associated parameters (Table 4).

4. Discussion

Salinity is one of the most important ecological factors influencing the distribution [46], growth [47], reproduction [48], and larval development [49] of fishes. In particular, salinity can exert strong influence on spawning performance and larval growth [49, 50]. Therefore, by examining responses of the reproductive and early life history of fishes to variation in salinity, we can greatly enhance the effectiveness of conservation culture programs and management practices of wild populations.

Here, we found that longfin smelt can successfully spawn and rear in a range of low salinity (0.4–5 ppt); however, fertilization was highest at lower salinities. Similarly, anadromous pike *Esox lucius* exhibit the best fertilization and embryonic development in freshwater [51], whereas anadromous sea trout *Salmo trutta* [52] and chum salmon *Oncorhynchus keta* [53] also exhibit higher fertilization success at lower salinities (<4 ppt). These studies suggest that lower fertility at higher salinity might be due to intrachorionic osmolality, which requires more energy for eggs to cope with the higher osmotic pressure [54]. The chorion can be

physically altered by salinity that could potentially influence its permeability. Thus, eggs fertilized in saline water could become hardened and may weaken the resistance to internal pressure to prevent successful fertilization [27, 34, 53]. Moreover, evidence showed that high osmotic pressure can inhibit sperm motility [55], which could be another limiting factor for the declined fertilization rate at higher salinity for estuarine species.

Although some studies found significant effects of the fertilization and incubation salinities on the hatching performance in different brackish and marine fish species [52, 56], the present study revealed no variation in hatching of longfin smelt when fertilized eggs were incubated at 0.4 and 5 ppt. Corroborating the findings of this study, several studies also found no fertilization salinity and/or incubation salinity effect on the hatching success in some brackish water species [57, 58]. In addition, high hatching rates were achieved for all the treatments (fertilization salinity: (0.4 ppt: 93.80% and 5 ppt: 94.41%) and incubation salinity: (0.4 ppt: 93.81% and 5 ppt: 94.42%)) in this study. This indicates that the current fertilization and incubation protocols applied at the FCCL are well suited to the needs for culturing of this species in captivity. Furthermore, this suggests that longfin smelt embryos are likely capable of successfully incubating and hatching across a wide range of brackish, low-salinity habitats throughout the SFE, as has been suggested by laboratory [14] and field [20, 31] observations.

The present study revealed a marginally significant effect of fertilization salinity on larval survival from 0 to 40 dph, where the larvae with 5 ppt fertilization salinity had comparatively higher survival rate (76%) than the larvae with 0.4 ppt fertilization salinity (61%). In a recent study, Yanagitsuru et al. [14] demonstrated that the survival rate was highest and notochord length longest in longfin smelt yolk sac larvae in moderately brackish water conditions (i.e., 5–10 ppt), suggesting that these brackish water salinities might be optimal for their larval growth and survival. However, the better fertilization rate of eggs fertilized in freshwater in this study indicates that this might be a key reason that longfin smelt

migrate back to freshwater to spawn. Tana and Tempero [59] used Sr:Ca on the otolith to determine that spawning of common smelt *Retropinna retropinna* occurred in freshwater, while larval growth happened mostly in marine water, and Grimaldo et al. [12] observed high densities of longfin smelt larvae in shallow, low-salinity wetland habitats in the upper estuary. In years with high freshwater outflow, Lewis et al. [20] also observed spawning adults and recruiting larvae of longfin smelt in shallow low-salinity wetland habitats throughout the SFE, including San Pablo Bay and Lower South Bay, CA, United States. Moreover, studies using the otolith strontium isotope for their nursery ground recognition [33] and sampling the physicochemical and biological attributes of SFE [12] suggest that longfin smelt prefer brackish water conditions as their larval nursing grounds. These findings suggest that longfin smelt benefit from spawning in lower salinity habitats and for their larvae to move to moderately brackish water for higher survival and growth.

The size of maternal broodstock is also considered as an important factor for successful fish spawning because it can influence egg quality, fertilization, hatching success, and larval traits. Studies showed that larger and older females can produce high-quality eggs [60], have better fertilization and hatching success [61], and produce good-quality larvae [62]. Evidence suggests that maternal proteins and RNAs can modulate the egg quality [63], while their hormones, such as thyroid and cortisol, can be transferred through the egg yolk to influence larval development [64]. The findings of the present study corroborate these studies and indicate that the hatching rate and larval survival increased with the increase of maternal broodstock size. Similar to the maternal size, studies showed that paternal size also could have significant effects on fish spawning and larval growth [65]. Unfortunately, some males provided tiny amount of milt during this study, and multiple males had to be used to fertilize the eggs for those crosses, which restricted to partition the individual paternal effects on spawning performance and larval development. However, further studies could be done to explore the paternal contribution to these spawning performances in this species.

Lastly, the study found no significant effects of incubation salinity, fertilization salinity × incubation salinity, source of broodstock, stocking density, and salinity shock on any of the measured spawning performances and larval traits. The plausible reasons could be the incubation salinity and fertilization salinity × incubation salinity did not cause any osmotic stress that would lead to the mortality of hatchlings, broodstock collected from two sites might not be genetically different populations, stocking densities in this study might not create any competition for space and food to deter larval growth and survival, and change of rearing water salinity would be tolerable for larval longfin smelt.

5. Conclusion

The population of longfin smelt in the SFE has dramatically declined in recent years, likely due many interacting anthropogenic impacts. The establishment of a conservation culture program is a key component of a multifaceted approach to conserving this imperiled population. Our results highlight that fertilization is highest in freshwater, thus informing optimal culture practices and possibly explaining the anadromous life history of this species. Furthermore, hatching and larval survival were strongly correlated with the female broodstock size, thus highlighting the importance of selecting large females as conservation broodstock. Combined, this work provides new information regarding the responses of longfin smelt reproduction and early life history to variation in salinity, which is key to developing an effective conservation culture program and for guiding the management of the remaining wild population.

Data Availability

Data that support the findings of this study are available from the corresponding author upon reasonable requests.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Md. Moshiur Rahman: Methodology, Investigation, Data curation, Formal analysis, Writing—original draft and editing. Levi S Lewis: Conceptualization, Resources, Writing—review and editing. Nann A. Fangue: Conceptualization, Resources, Writing—review and editing. Richard E Connon: Conceptualization, Resources, Writing—review and editing. Tien-Chieh Hung: Conceptualization, Methodology, Resources, Project supervision and administration, Writing—review and editing.

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