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SHORT COMMUNICATION

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Effect of water salinity on embryonic development of longfin yellowtail *Seriola rivoliana* larvae

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Coastal regions comprise spawning areas for most marine fishes of the world (Sundby & Kristiansen, 2015). Thus, several marine fish species spawn in offshore areas, relying on ocean currents to disperse their progeny. Dispersal mechanisms often bring eggs and larvae into areas of varying salinity and temperature (Hart & Purser, 1995). External environmental conditions could affect early development processes, compromising survival and future growth. In fact, according to Bœuf and Payan (2001) there are very few fish species whose development and growth were not influenced by salinity changes. In addition, salinity affects the buoyancy of eggs and larvae and this can impact the ability of larvae to get to the water surface to inflate their swim bladder (Fielder, Bardsley, Allan, & Pankhurst, 2005). Longfin yellowtail (*Seriola rivoliana*, Valenciennes, 1833) is a carangid species and excellent candidate for aquaculture due to its fast growth, flesh quality and high market value (Kissinger, García-Ortega, & Trushenski, 2016; Mesa-Rodríguez et al., 2018; Quiñones-Arreola et al., 2015; Roo et al., 2014). However, information regarding the effects of specific environmental conditions (e.g. salinity) on the incubation of eggs and yolk-sac larvae of longfin yellowtail is scarce. Therefore, the purpose of this study was to examine the effects of water salinity on the embryonic development, and survival of longfin yellowtail larvae.

Fertilized eggs were obtained from four natural spawnings of two different broodstock groups (BG) held in captivity at National Centre of Aquaculture and Marine Science of ESPOL Polytechnic University 'CENAIM-ESPOL' (Table 1). During the symmetrical cleavage-blastula stage (approximately 6 hr post fertilization, HPF), eggs were transferred directly to incubation tanks containing 50 L of water with different experimental salinities. Before stocking of eggs, the experimental water salinity (15, 20, 25, 30, 35, 40 and 50 g/L) was adjusted by adding either dechlorinated fresh tap water (<1 g/L; tap water was dechlorinated with the use of sodium thiosulfate (15 mg/L) or brine solution (a stock of 80 g/L water was established mixing ambient sea water and brine). The concentration of stock brine solution as well as the experimental salinities was confirmed using a portable refractometer Vital Sine^{TM} SR6 (Pentair Aquatic Eco Systems Inc.). Each experimental salinity was randomly assigned to triplicate tanks. Stocking density was 200 eggs/L. Diameter of whole egg and oil globule was measured with the aid of a conventional MITUTOYO PJ-A3000 profiler (Mitutoyo Corporation Inc.). Ambient seawater temperature ranged between 25.2 and 26.1°C; and pH between 7.97 and 8.31 (pHTestr30, Eutech Instruments^{TM}, Thermo Fisher Scientific®). One airstone was placed in each tank to maintain the dissolved oxygen concentration between 5.78 and 6.90 mg/L, evaluated by an oxygen meter (YSI 550A, YSI Incorporated).

Spawns/S-152 and S-158 were used to explore the salinity tolerance of eggs and 24-hr yolk-sac larvae survival (Trial 1), whilst/S161 and S-8 were used to evaluate salinity effects on embryonic development, buoyancy of eggs, hatching and survival up to 24-hr of yolk-sac larvae (Trial 2). The tests of eggs buoyancy were performed with a batch of eggs placed in a 500-mL cylinder containing evaluated water salinities.

TABLE 1 Description of the four-spontaneous spawning of longfin yellowtail used for the trials

Trial	Spawning (#)	Source of eggs	Date	Fertilization (%)	Eggs diameter (mm)	Oil globule diameter (mm)	Broodstock tank	
							Temperature °C	Salinity g/L
1	S-152	BG1	7-Feb-18	93	1.124 ± 0.023	0.257 ± 0.013	25.4	35
1	S-158	BG1	7-Mar-18	87	1.101 ± 0.033	0.306 ± 0.026	25.6	30
2	S-161	BG1	2-Apr-18	97	1.090 ± 0.017	0.273 ± 0.018	25.1	35
2	S-8	BG3	7-Apr-18	100	1.167 ± 0.034	0.258 ± 0.024	25.1	35

Abbreviations: S, number of spawn; BG, Broodstock groups #1 and #3.

Thereby, water and eggs were mixed by inverting the cylinder and then (after 10 min) the numbers of floating eggs and on the bottom were recorded. Embryonic development was determined at 2 hr intervals, from stocking of eggs to completion of hatching. Changes in eggs over time were observed with a dissecting microscope (OLYMPUS CX31, Olympus America Inc.), and developmental stages were grouped according to Thompson and Riley (1981) methodology cited by Geffen, Fox, and Nash (2006). Newly hatched larvae (notochord length, NL and yolk-sac volume, YSV) were measured according to Bustos and Silva (2011). Dry weight of yolk-sac larvae (DWL) was determined by placing three samples of about 20 larvae on individual trays of aluminium foil. Larvae were rinsed with distilled water and then oven-dried at 65°C for 24 hr. After drying, each sample was weighed on an electronic balance METTLER AE240 (±0.01 mg; Mettler Toledo). Larval survival was determined by counting the remaining larvae after 24 hr post hatching. All data were subjected to Kolmogorov–Smirnov test and Bartlett test to verify the normality and homoscedasticity respectively. A one-way analysis of variance ANOVA (trial 1) and two-way ANOVA (spawn sources and salinity; trial 2) were used to compare the effects of salinity on eggs and yolk-sac larvae. Survival and hatching data (in percentages) were arcsine-transformed prior to analysis. Significant differences were determined with Tukey's test for multiple comparisons at a significance level of 95%. Differences of eggs buoyancy in trial 2 were determined by a Kruskal–Wallis analysis at 95%. The XLSTAT®2016.5 (Addinsoft) software was used.

Longfin eggs developed and hatched at all salinities tested. However, below 30 g/L, hatching success was <8%. In trial 1, the highest hatching rate was registered at 50 g/L being compared only with 35 and 40 g/L ($p < .05$). However, 24-hr survival of yolk-sac larvae was around 40% only at 35 and 40 g/L (Table 2). Similar results (in terms of statistical meaning) were registered in trial 2. Low-salinity water has been used to improve growth rates in many species for mass seed production (Bœuf & Payan, 2001). Blacio, Darquea, and Rodríguez (2003), reported better survival of *S. rivoliana* larvae when cultured at 25 g/L (lower salinity than spawning tank, 35 g/L) from 2 to 30 days post-hatch (DPH). In our study, 100% of mortality of yolk-sac larvae occurred below and beyond salinities of 35 and 40 g/L respectively. Conversely, yolk-sac larvae of *Paralichthys olivaceus* showed better tolerance to lower salinity in comparison with 4- to 14-day-old larvae after hatching (Hiroi, Sakakura, Tagawa, Seikai, & Tanaka, 1997). Similar results were observed between mid-stage larvae versus late-stage larvae of *Epinephelus bruneus* (Inoue, 2016). Faulk and Holt (2006) stated that typically the tolerance of marine fish larvae to changes in salinity is higher for newly hatched larvae compared with first feeding larval stage. Following the onset of exogenous feeding, larval salinity tolerance increases with age as a result of the development of osmoregulation structures. However, the survival results from this study plus previous findings for *S. rivoliana* contradict these statements. Apparently, older *S. rivoliana* larvae (>2 DPH) appear to be more tolerant to brackish water condition of 25 g/L compared with newly hatched larvae. On the other hand, no differences were found according to spawn origin (Table 3). Like other marine fish species, embryonic development of longfin yellowtail follows the usual developmental stages. Time to hatch was not related to salinities, as most hatching occurred between 26 and 28 hr after stocking (Figure 1). In our study, after hatching, salinity was the

TABLE 2 Mean per cent of hatched longfin yellowtail larvae and survival at 24 hr for all experimental salinities

Spawnings	Parameter	Salinity (g/L)						
		15	20	25	30†	35†	40	50
S-152	Hatching rate (%)	1.0 ± 1.7^{b}	–	3.7 ± 5.5^{b}	–	80.7 ± 20.0^{a}	–	100.0 ± 0.0^{a}
	24-hr Survival rate (%)	0.0 ± 0.0	–	0.0 ± 0.0	–	43.5 ± 12.6^{a}	–	6.3 ± 2.6^{b}
S-158	Hatching rate (%)	–	7.4 ± 5.6^{b}	–	43.1 ± 32.5^{b}	–	90.6 ± 4.0^{a}	96.6 ± 5.9^{a}
	24-hr Survival rate (%)	–	0.0 ± 0.0	–	7.0 ± 2.0^{b}	–	49.6 ± 17.7^{a}	4.1 ± 2.5^{b}

Note: Values (mean ± *SD*) in the same row with different superscript letters are significantly different (Tukey's test, $p < .05$).

Abbreviation: S, number of spawn.

†Ambient sea water.

TABLE 3 Effect of water salinity on eggs and 24-hr larvae of longfin yellowtail, *Seriola rivoliana*

	Hatching rate (%)	NL‡ (mm)	YSV‡ (mm^3)	DWL‡ (mg)	24-hr Survival rate‡ (%)
Spawning					
S-161	37.3 ± 23.9	2.631 ± 0.239	0.094 ± 0.012	0.124 ± 0.024[a]	37.4 ± 21.8
S-8	37.7 ± 25.3	2.749 ± 0.185	0.096 ± 0.014	0.084 ± 0.016[b]	28.6 ± 12.0
Salinity (g/L)					
20	2.5 ± 5.0[b]	–	–	–	–
35†	48.0 ± 15.9[a]	2.768 ± 0.204	0.098 ± 0.010	0.109 ± 0.037	31.6 ± 12.8
40	58.4 ± 10.6[a]	2.612 ± 0.182	0.092 ± 0.014	0.098 ± 0.020	34.5 ± 13.5
50	41.2 ± 11.3[a]	–	–	–	–

Note: Values (mean ± *SD*) in the same column (up subdivision line) with different superscript letters are significantly different (Tukey's test, $p < .05$).
Abbreviations: DWL, dry weight of larvae; NL, Notochord length; S, number of spawn; YSV, yolk-sac volume.
†Ambient sea water.
‡Mean values were made with 35 and 45 g/L treatments.

primary factor determining immediate larval survival. Thus, our results suggest that larvae of *S. rivoliana* can tolerate a range of salinities between 35 and 40 g/L, and it is believed that spawning occurs at salinities encountered offshore. Spawning sites of longfin yellowtail are not well documented. However, spawning most likely occur offshore at salinities similar to that of oceanic water (Faulk & Holt, 2006). Generally, eggs and larvae of marine teleost pelagic fishes hatching from eggs spend planktonic life in offshore areas (Hiroi et al., 1997). Limited information is available on salinity tolerances of other *Seriola* fish species during early development. According to Olivieri-Velázquez and Neal (2018), metabolic demand of larvae increases in hyperosmotic environment as they attempt to maintain homeostasis of body fluids and it is necessary to divert more energy into osmoregulation than to growth or development. Mortality of yolk-sac larvae after 24 hr in this study was most likely associated with

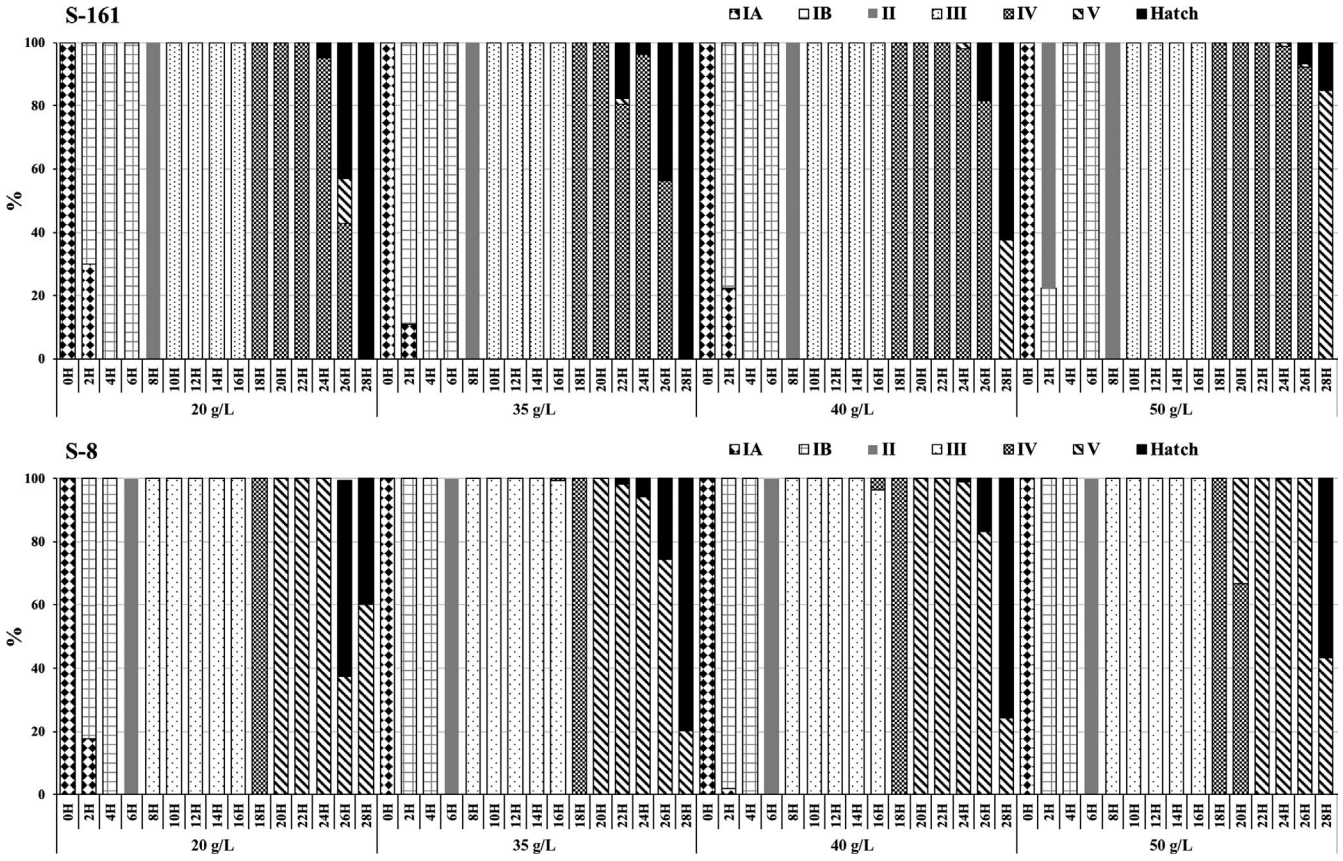

FIGURE 1 Embryonic development of longfin yellowtail at different water salinities. Embryonic stages are based on Geffen et al. (2006)

TABLE 4 Percentage of buoyancy of longfin yellowtail eggs at different water salinities

	Salinity (g/L)			
Buoyancy (%)	20	35†	40	50
S-161	0.8 ± 1.4[a]	75.4 ± 4.1[ab]	81.7 ± 11.2[ab]	86.1 ± 0.5[b]
S-8	16.8 ± 7.6[a]	77.3 ± 29.1[ab]	91.4 ± 7.0[ab]	97.2 ± 2.0[b]

Note: Values (mean ± *SD*) in the same row with different superscript letters are significantly different (Kruskal–Wallis test, $p < .05$).

Abbreviation: S, number of spawn.

†Ambient sea water.

energy cost for osmoregulation. Larvae are relatively undeveloped at hatching and do not possess the osmoregulatory abilities of juvenile fishes such as gills, gut, kidneys and urinary bladder (Faulk & Holt, 2006). In addition, newly hatched larvae in higher salinities had greater survival, but showed also a larger incidence of deformities. Larval deformities of fish cultured in unsuitable salinities have been reported in others studies (Smith, Denson, Heyward, Jenkins, & Carter, 1999).

As expected, eggs and newly hatched larvae of *S. rivoliana* were positively buoyant at salinities above 35 g/L (Table 4). Most of the larvae accumulated and became trapped at the water surface (direct observations).

Our findings provide valuable understanding regarding longfin yellowtail larvae production. However, it is necessary to evaluate the effect of salinity on physiological adaptability, growth and survival of late-stage larvae and juveniles, in order to maximize industrial scale hatchery production of this species.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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