



Abstract—Heavy rain can decrease salinity and increase turbidity of the water in coastal areas, negatively affecting the development of organisms, particularly during their early life stages. In this study, the effects of salinity and turbidity on embryos of the bamboo sole (*Heteromycteris japonicus*) were evaluated to improve understanding of its tolerance to global climate change. Three experiments were carried out over a 7-d period. In the first experiment, embryos of bamboo sole were exposed for 3 h to 1 of 6 salinity levels (14–34). Low salinity levels (14 and 18) resulted in significantly shorter total lengths of newly hatched larvae in comparison with larval sizes in treatments with higher salinities, but no significant differences were observed in hatching rate and larval survival rate among treatments. In the second experiment, embryos were exposed to turbidities of 0, 100, 300, 500, and 700 nephelometric turbidity units for 3 h. Turbidity significantly decreased hatching rate, survival rate, and total length and increased onset hatching time and percentage of abnormality. In the third experiment, embryos were exposed to different combinations of salinity and turbidity. The interaction effect of salinity and turbidity on total length of newly hatched larvae was significant. These findings indicate that embryo development of bamboo sole was more affected by changes in turbidity than by changes in salinity.

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Effects of salinity and turbidity on development of bamboo sole (*Heteromycteris japonicus*)

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Flatfish species are found globally in marine habitats and are important food fish to humans (Gibson et al., 2015). However, the global catch of flatfish in fisheries has decreased gradually since the 1970s, from about 3 million metric tons (t) to below 2 million t in the 2000s, and is predicted to decline by 20% by the year 2100 (Cheung and Oyinlola, 2018).

Although overfishing has resulted in decreases in flatfish populations, there is another related reason for these reductions: climate change (Cheung and Oyinlola, 2018). In fact, in many studies, fluctuations in fish populations have been found to be closely associated with climate change (Lehodey et al., 2006; Brander, 2010; Holsman et al., 2012; Barange et al., 2014). Baudron et al. (2014) also reported that negative changes in environmental conditions of habitats due to climate change can directly affect fish population dynamics through physiological effects.

One of the effects of climate change in coastal areas is the increased intensity and frequency of heavy rainfall, and those changes in rainfall reduce

salinity and increase turbidity as a result of runoff from the land and rivers (Thrush et al., 2004; Poloczanska et al., 2009). According to Allen and Pechenik (2010), salinity in the upper water column may fluctuate from 30 to 15 in short periods during heavy rainfall events, leading to pelagic fish eggs likely being exposed to low salinity during development. In a recent study, Fukuda et al. (2021) found that, after heavy rains, the salinity level was less than 28 in Hakata Bay, which is located in the city of Fukuoka on the northern end of Kyushu in Japan. In the natural environment, turbidity is usually less than 50 nephelometric turbidity units (NTU) (Boyd, 2015); however, heavy rainfall events cause high runoff to flow more directly into coastal areas, resulting in turbidity values that can be greater than 50 NTU. During heavy rains, the turbidity of outflow can exceed 130 NTU (Zhou et al., 2015) and even reach levels as high as 467 NTU (Zheng et al., 2016), but it rarely exceeds 1000 NTU (Boyd, 2015).

Seawater salinity and turbidity are 2 important ecological variables that

have individual or interactive effects on development, growth, and survival of fish at any life history stage (Bœuf and Payan, 2001; Hasenbein et al., 2013; Phan et al., 2019). In particular, drastic changes in coastal salinity and turbidity, such as those due to extreme weather events, can profoundly affect egg fertilization and incubation, yolk sac absorption, early embryogenesis, and larval survival (Berlinsky et al., 2004; Bilotta and Brazier, 2008). For example, the short-term effect of low salinity (<3) was to lower embryonic survival and egg hatching in a study of Pacific herring (*Clupea pallasii*) (Mikhailenko, 2000). Gray et al. (2012) found that short-term experimental elevation of turbidity had a significant effect on the hatching success rate of embryos of the spotted gar (*Lepisosteus oculatus*), a species that was then considered threatened and is now considered endangered in Canada (COSEWIC, 2015). Phan et al. (2019) reported that increased larval abnormality was recorded in bastard halibut (*Paralichthys olivaceus*) when embryos were exposed to high turbidity (>100 NTU) or low salinity (<30). Although prior to the study we describe herein the short-term effects of salinity and turbidity on the embryonic stage of bamboo sole were unknown, these findings indicate how the bamboo sole (*Heteromycteris japonicus*) might respond to climate change.

The bamboo sole is a small soleid flatfish distributed along southern Japan, in the Yellow Sea, and in the East China Sea (Nakabo, 2002). It is a common species found on sandy seabeds in the Seto Inland Sea of Japan (Yamamoto and Katayama, 2013). Although the bamboo sole is a noncommercial species, it plays an important ecological role in coastal areas. As reported by Jawad (2021), the early life stages of many noncommercial species are essential components of the diets of important commercial species. The spawning season for bamboo sole is from May through June, and their pelagic eggs are often caught in estuaries or bays (Yamamoto and Katayama, 2013), where salinity and turbidity fluctuate because of heavy rainfall events. Therefore, the objective of this study was to evaluate the effects of salinity and turbidity changes on various characteristics of the early stage of bamboo sole. Specifically, we examined the hatching time and hatching rate of embryos, the percentage of abnormality, size, and yolk sac volume of newly hatched larvae, and the survival rate of larvae.

Materials and methods

Collection and preparation of fertilized eggs

Embryos of bamboo sole were obtained in July 2019 from the National Research Institute of Aquaculture, which is part of the Fisheries Research Agency of Japan and located in the Mie Prefecture. The fertilized eggs were collected and transferred to the Shallow Sea Aquaculture laboratory, Mie University, in oxygen-filled plastic bags. Then, eggs were placed in a 5-L glass tank at a salinity of 34 and a temperature of 23°C, with gentle aeration. Only

viable (buoyant) embryos were used for our experiments. The fertilization rate of eggs was 80.2%, and the mean diameter of eggs was 1.02 mm (standard error of the mean [SE] 0.04). For experiments, we created seawater at different levels of salinity by diluting artificial seawater powder (LIVESea Salt¹, Delphis Co. Ltd., Itami City, Japan) with distilled water and checking the salinity with a handheld refractometer (Master-S/Mill Alpha, Atago Co. Ltd, Tokyo, Japan). To achieve the desired turbidity, kaolin clay (with the chemical composition $Al_2Si_2O_5(OH)_4$ and a particle diameter of approximately 0.4 μm) was dissolved in water with a salinity of 34. Turbidity was measured by using a HACH DR/850 portable colorimeter (Hach Co., Loveland, CO).

Experimental design

The experimental conditions were set up to mimic, for a short period, the stressful conditions that organisms typically are exposed to in bays, estuaries, and coastal areas during heavy rains, and then conditions were returned to normal (Phan et al., 2020). Salinity and turbidity are the 2 major variables for water quality in coastal areas of the ocean during and shortly after heavy rainfall events (Phan et al., 2020). In this study, 3 experiments were conducted to examine the following: 1) effect of low salinity levels (6 treatments with salinities of 34 [control], 30, 26, 22, 18, and 14) on embryos of bamboo sole, 2) effect of high turbidity levels (5 treatments with turbidities of 0 [control], 100, 300, 500, and 700 NTU) on embryos of bamboo sole, and 3) combined effect of salinity and turbidity (8 treatments with combinations of salinities of 14 and 18 and turbidities of 100, 300, 500, and 700 NTU) on embryos of bamboo sole.

All experiments were conducted in 6-well plastic microplates, so that each treatment had 6 replicates. When embryos developed to the gastrula stage, 13 normal embryos were removed from the 5-L tank and randomly allocated into each of the 6 wells of a plastic microplate, with each well containing 5 mL of artificial seawater (with a salinity of 34). The embryos were exposed to single and combined salinity and turbidity levels for 3 h. Then embryos were transferred to 100-mL glass beakers with seawater at a temperature within 1°C of the target temperature of 23°C, a salinity of 34, and a turbidity of 0 NTU under a natural photoperiod (embryos were exposed to 12 h of daylight per day) in an electric incubator. Embryos remained in the beakers in the incubator until the end of the experiment. Gradual changes in salinity or in combined conditions of salinity and turbidity were achieved by using an automatic pipette and a water exchange rate of 1 mL until the desired environmental condition was reached. During the incubation period, 50% of the seawater in all treatments was exchanged daily, and dead embryos and larvae were removed by using a wide-mouthed pipette. Embryos

¹ Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

were determined to be dead if morphology had collapsed (embryos appeared white or opaque) or the heartbeat had stopped. Seawater in the beakers had dissolved oxygen of 5.2–5.7 mg/L and pH of 8.0–8.5. Larvae at 3 d post-hatch were fed with live rotifers, specifically with *Brachionus plicatilis* (4 rotifers/mL). All experiments were carried out for a period of 7 d.

Measurements and calculations

Onset hatching time, hatching rate, percentage of abnormality, survival rate, total length, and yolk sac volume of embryos and larvae were recorded. Onset hatching time was determined as the total number of hours from fertilization to the time of hatch. Hatching rate was calculated on the basis of the number of hatching embryos compared to the number of stocked fertilized eggs. The larval survival rate was determined as the total number of live larvae at 5 d post-hatch. The percentage of abnormality at 24 h post-hatch was calculated by using the numbers of normal and abnormal larvae. Individuals were considered abnormal if the notochord was bent or the tail tip was deformed. Only a few abnormal larvae appeared across all treatments in the first experiment; however, all abnormal larvae died by 24 post-hatch. Therefore, the percentage of abnormality was not recorded. Shortly after hatching, 18 larvae from each treatment were randomly collected and preserved in 4% formalin solution, and total length and yolk sac volume of newly hatched larvae were measured under an inverted microscope (CKX53, Olympus Corp., Tokyo, Japan) with an ocular micrometer that had a precision of 10 μm . Yolk sac volume (in cubic millimeters) of newly hatched larvae was calculated by using this formula:

$$\text{Yolk sac volume} = \frac{\pi}{6} \times LH^2,$$

where L = the length of the yolk sac (in millimeters); and H = the height of the yolk sac (in millimeters).

Statistical analysis

The software SPSS Statistics (vers. 22.0, IBM, Armonk, NY) was used for statistical analyses. All data are expressed as means with their SEs. Results were analyzed by using 1-way (first and second experiments) and 2-way (third experiment) analysis of variance (ANOVA). Before analysis, percentages in data were arcsine transformed. Significant treatment effects were detected by using the Tukey's honestly significant difference test, a post hoc multiple-range test. The significance level was 0.05.

Results

Effect of low salinity levels on embryos

In the first experiment, 468 embryos of bamboo sole were exposed to a control level of salinity or to 1 of 5 different salinities (78 individuals for each of 6 treatments). Embryos had great tolerance to a gradual decrease in salinity. In fact, salinity had no effect on onset hatching time (range: 39.3–39.7 h) (1-way ANOVA: $F[5, 30]=46.39, P>0.05$) or on hatching rate (range: 91.0–97.4%) (1-way ANOVA: $F[5, 30]=0.649, P>0.05$). Larval total length was positively related to salinity, ranging from 1.69 mm at a salinity of 34 to 1.61 mm at a salinity of 14. The total lengths of newly hatched larvae in the treatments with salinities of 14 and 18 were significantly smaller than those of larvae in the control treatment with a salinity of 34, but no significant differences in the total lengths of larvae were recorded between the treatments with salinities of 14 and 18 or among treatments with salinities of 22, 26, 30, and 34. Yolk sac volume ranged from 0.100 to 0.110 mm^3 across all treatments, but no significant differences were observed among treatments.

On day 5 after hatching, there were no significant differences in survival rates among all salinity treatments ($F[5, 30]=1.626, P>0.05$) (Table 1).

Table 1

Mean measurements of characteristics of the early stage development of bamboo sole (*Heteromycteris japonicus*) in laboratory experiments conducted at Mie University in Japan in July 2019 to examine the effect of salinity on development. Embryos were exposed to 1 of 6 levels of salinity for 3 h. After treatment, embryos were transferred to a controlled environment and monitored for 7 d. The survival rate of larvae was measured 5 d post-hatch. The different letters after values indicate that the means are significantly different ($P<0.05$) among treatments. Standard errors of the means are provided in parentheses.

Salinity treatment	Onset hatching time (h)	Hatching rate (%)	Survival rate (%)	Total length (mm)	Yolk sac volume (mm^3)
34 (control)	39.3 (0.09) ^a	97.4 (1.6) ^a	80.0 (2.6) ^a	1.69 (0.01) ^a	0.110 (0.004) ^a
30	39.3 (0.07) ^a	93.6 (3.1) ^a	78.3 (4.0) ^a	1.68 (0.01) ^a	0.100 (0.003) ^a
26	39.5 (0.08) ^a	92.3 (4.0) ^a	75.0 (2.2) ^a	1.67 (0.01) ^a	0.101 (0.004) ^a
22	39.5 (0.10) ^a	93.6 (2.4) ^a	71.7 (3.1) ^a	1.67 (0.01) ^a	0.103 (0.004) ^a
18	39.7 (0.18) ^a	91.0 (2.4) ^a	73.3 (3.3) ^a	1.62 (0.01) ^b	0.105 (0.004) ^a
14	39.5 (0.11) ^a	91.0 (3.7) ^a	70.0 (2.6) ^a	1.61 (0.01) ^b	0.103 (0.003) ^a

Effect of high turbidity levels on embryos

In the second experiment, 390 embryos of bamboo sole were exposed to a control level of turbidity or to 1 of 4 different turbidities (78 individuals for each of 5 treatments). Turbidity had a significantly positive effect on the onset hatching time of bamboo sole eggs (range: 39.3–41.8 h) (1-way ANOVA: $F[4, 25]=84.932$, $P<0.05$) (Table 2). The times to onset hatching in turbidities of 300 and 500 NTU were significantly longer than the time in the control treatment with a turbidity of 0 NTU and in the treatment with a turbidity of 100 NTU and were significantly shorter than the time in the treatment with a turbidity of 700 NTU ($P<0.05$). Hatching rate was also affected by turbidity (range: 85.9–96.2%) (1-way ANOVA: $F[4, 25]=4.667$, $P<0.05$). The lowest hatching rate was observed in the treatment with the turbidity of 700 NTU and was significantly lower than the rate recorded in the control treatment and the treatment with a turbidity of 100 NTU. However, no significant differences in hatching rate were found among treatments with the control, 100-, 300-, and 500-NTU turbidities. As for abnormality rates, the results of statistical analysis indicate that there were significant differences between the control treatment and the other treatments (with turbidities of 100, 300, 500, and 700 NTU), but there were no significant differences among those other treatments.

The total length of newly hatched larvae significantly decreased with increasing turbidity levels. The size of larvae in the treatment with a turbidity of 700 NTU was significantly lower than the size of larvae in the other treatments. No significant differences were recorded among the treatments with turbidities of 100, 300, and 500 NTU ($P>0.05$). Yolk sac volume in the treatment with 700-NTU turbidity was significantly smaller than that in the control treatment ($P>0.05$), but significant effects were not found among the control treatment and the treatments with turbidities of 100, 300, 500 NTU. At the end of the experiment, the survival rate of larvae in the treatment with a turbidity

of 700 NTU was significantly different from the rate in the control treatment, but no significant differences were found in the larval survival rate between the treatments with turbidities of 500 and 700 NTU or among the control treatment and the treatments with turbidities of 100, 300, and 500 NTU.

Combined effect of salinity and turbidity on embryos

In the third experiment, 624 embryos of bamboo sole were exposed to 1 of 2 levels of salinity and to 1 of 4 levels of turbidity (78 individuals for each of 8 treatments). Although both salinity and turbidity affect the hatching of embryos of bamboo sole, turbidity had a greater effect on embryo development and larval survival rate than salinity (Tables 3 and 4). The results of 2-way ANOVA analysis indicate that turbidity had an effect on onset hatching time ($F[3, 40]=20.933$, $P<0.05$) but that the effects of salinity ($F[3, 40]=1.458$, $P>0.05$) and of interactions of salinity and turbidity ($F[3, 40]=0.972$, $P>0.05$) were not statistically significant. The same pattern was observed for hatching rates, percentages of abnormality, and survival rates. Neither salinity nor the interaction between salinity and turbidity affected hatching rate (2-way ANOVA: $F[3, 40]=0.047$, $P>0.05$), percentage of abnormality (2-way ANOVA: $F[3, 40]=0.218$, $P>0.05$), or survival rate (2-way ANOVA: $F[3, 40]=0.278$, $P>0.05$) of bamboo sole eggs and larvae. However, turbidity had a significant effect on each of these characteristics (Table 4). A similar pattern was observed for yolk sac volume of newly hatched larvae. Turbidity alone affected volume ($F[3, 136]=2.768$, $P>0.05$), but there were no significant differences in volume among treatments with different salinities ($F[3, 136]=0.062$, $P>0.05$) or among treatments with interactions of different levels of turbidity and salinity ($F[3, 136]=0.049$, $P>0.05$). Total length of newly hatched larvae in all treatments ranged from 1.53 to 1.65 mm. The results of 2-way ANOVA indicate

Table 2

Mean measurements of characteristics of the early stage development of bamboo sole (*Heteromycteris japonicus*) in laboratory experiments conducted at Mie University in Japan in July 2019 to examine the effect of turbidity on development. Embryos were exposed to 1 of 5 levels of turbidity for 3 h. After treatment, embryos were transferred to a controlled environment and monitored for 7 d. The survival rate of larvae was measured 5 d post-hatch. The different letters after values indicate that the means are significantly different ($P<0.05$) among treatments. Standard errors of the means are provided in parentheses. NTU=nephelometric turbidity units.

Turbidity treatment (NTU)	Onset hatching time (h)	Hatching rate (%)	Percentage of abnormality (%)	Survival rate (%)	Total length (mm)	Yolk sac volume (mm ³)
0 (control)	39.3 (0.10) ^a	96.2 (1.7) ^a	2.6 (1.6) ^a	81.7 (1.7) ^a	1.69 (0.01) ^a	0.110 (0.004) ^a
100	39.6 (0.06) ^a	94.9 (1.6) ^a	18.8 (3.2) ^b	75.0 (2.2) ^{ab}	1.64 (0.01) ^b	0.094 (0.004) ^{ab}
300	40.9 (0.07) ^b	91.0 (2.4) ^{ab}	19.9 (2.0) ^b	70.0 (3.7) ^{ab}	1.63 (0.01) ^{bc}	0.090 (0.006) ^{ab}
500	40.8 (0.16) ^b	88.5 (1.7) ^{ab}	24.6 (2.5) ^b	65.0 (3.4) ^{bc}	1.60 (0.01) ^{bc}	0.090 (0.006) ^{ab}
700	41.8 (0.12) ^c	85.9 (2.4) ^b	27.0 (3.3) ^b	56.7 (4.2) ^c	1.59 (0.01) ^c	0.089 (0.004) ^b

Table 3

Mean measurements of characteristics of the early stage development of bamboo sole (*Heteromycteris japonicus*) in laboratory experiments conducted at Mie University in Japan in July 2019 to examine the combined effect of salinity and turbidity on development. Embryos were exposed for 3 h to 1 of 8 treatments that combined 2 levels of salinity and 4 levels of turbidity. After treatment, larvae were transferred to a controlled environment and monitored for 7 d. The survival rate of larvae was measured 5 d post-hatch. Standard errors of the means are provided in parentheses. NTU=nephelometric turbidity units.

Salinity	Turbidity (NTU)	Onset hatching time (h)	Hatching rate (%)	Percentage of abnormality (%)	Survival rate (%)	Total length (mm)	Yolk sac volume (mm ³)
18	100	40.6 (0.06)	91.0 (5.0)	15.4 (2.8)	70.0 (7.7)	1.63 (0.01)	0.098 (0.004)
	300	41.2 (0.12)	82.1 (2.6)	20.5 (3.2)	58.3 (6.0)	1.64 (0.01)	0.095 (0.005)
	500	41.4 (0.07)	80.8 (4.3)	25.6 (3.2)	55.0 (4.3)	1.65 (0.01)	0.089 (0.005)
	700	41.4 (0.15)	79.5 (4.3)	26.9 (4.8)	53.3 (4.9)	1.62 (0.02)	0.089 (0.005)
14	100	40.9 (0.12)	91.0 (3.1)	19.2 (3.3)	71.2 (3.1)	1.62 (0.02)	0.099 (0.003)
	300	41.3 (0.08)	83.3 (3.7)	26.9 (3.3)	53.3 (6.7)	1.61 (0.02)	0.093 (0.004)
	500	41.4 (0.07)	82.1 (4.7)	26.9 (4.3)	46.7 (6.7)	1.54 (0.02)	0.089 (0.003)
	700	41.4 (0.05)	78.2 (3.7)	28.2 (4.3)	48.3 (4.0)	1.53 (0.02)	0.087 (0.004)

significant effects of both salinity ($F[3, 136]=18.653$, $P<0.05$) and turbidity ($F[3, 136]=3.441$, $P<0.05$) on total length, with a significant interaction between these variables ($F[3, 136]=3.156$, $P<0.05$).

Discussion

Shallow coastal areas are some of the most vulnerable systems that will be affected by climate change (Phillipart et al., 2003). Findings from numerous studies indicate that fish in their early life stages were more susceptible than adults to fluctuations in environmental conditions and that they may be particularly sensitive to climate change (Pankhurst and Munday, 2011; Sugisaki and Murakami, 2018). However, the tolerance levels of marine fish in early developmental stages to changes in environmental conditions can vary among fish species (Wang et al., 2002; Shi et al., 2009). Torres et al. (2021) reported that organisms exposed to short-term fluctuations in environmental conditions may experience irreversible damage to body structures and eventually suffer mortality at higher rates if the range of variation is wider than the range of tolerance. In our study, simulating the sudden, short-term changes in salinity that occur after a rainstorm, we found that exposure to low salinity did not affect onset hatching time and hatching rate of eggs and did not affect survival rate of larvae after hatching. These findings are similar to those of our previous study, in which embryos of Japanese seabream (*Pagrus major*) were subjected to stress from low salinity (Phan et al., 2020).

Salinity tolerance of fish embryos has been reported to have improved with advancing development; for example, cleaving embryos were more sensitive to salinity change than blastulae, and blastulae were more sensitive to change in salinity than gastrulae (Farhana et al., 2019). Ord (2019) reported that embryos in the late developmental

stages have more advanced osmoregulatory capacities. In the natural environment, coastal fish species are considered to be euryhaline. Therefore, their embryos can tolerate a wide range of salinities, as has been found for the European seabass (*Dicentrarchus labrax*) (Conides and Glamuzina, 2001), black sea bass (*Centropristis striata*) (Berlinsky et al., 2004), bastard halibut (Phan et al., 2019; Manuel et al., 2021), and Japanese seabream (Phan et al., 2020).

In our laboratory experiments with bamboo sole, short-term exposure to stress from turbidity significantly prolonged onset hatching time and decreased hatching rate of embryos and decreased survival rate of larvae after hatching. In nature, increased turbidity tends to coincide with decreases in dissolved oxygen, which could lead to lack of oxygen during development in individuals of many fish species (Mueller et al., 2017). In addition, turbidity impedes oxygen supply and the normal growth activity of embryos as kaolin clay coats egg membranes (Phan et al., 2020). Del Rio et al. (2019) revealed that oxygen significantly influenced the hatching process in fish embryos, in which low levels of oxygen led to delays in embryonic development and in the time of hatching as well as to a decrease in hatching success rate. Results of our study indicate that the percentage of larval bamboo sole that had abnormalities was higher when embryos were exposed to turbidity levels of 100–700 NTU than when embryos were exposed to no turbidity. The morphological abnormalities most commonly found in our study were notochord bending and tail-tip deformity. An increase in the proportion of larvae that were abnormal because of exposure to high turbidity has also been reported for other fish species, including the Pacific herring (Griffin et al., 2009), bastard halibut (Phan et al., 2019), climbing perch (*Anabas testudineus*) (Nurulnadia et al., 2020), and Japanese seabream (Phan et al., 2020). According to Cahu et al. (2003), such abnormalities are often associated with growth depression and

Table 4

Results from the 2-way analysis of variance in measurements of early stage development of bamboo sole (*Heteromycteris japonicus*) from laboratory experiments conducted at Mie University in Japan in July 2019 to examine the individual and combined effects of salinity (S) and turbidity (T) on development. Embryos were exposed to treatments with different levels of salinity and turbidity for 3 h and then monitored for 7 d. An asterisk (*) indicates that the means are significantly different ($P < 0.05$) among treatments for that factor (or source of variability).

Variability source	df	Mean squares	F	P
Onset hatching time				
S	1	0.083	1.458	0.234
T	3	1.197	20.933	0.000*
S × T	3	0.056	0.972	0.416
Error	40	0.057		
Total	48			
Corrected total	47			
Hatching rate				
S	1	1.233	0.013	0.910
T	3	333.251	3.475	0.025*
S × T	3	4.520	0.047	0.986
Error	40	95.907		
Total	48			
Corrected total	47			
Percentage of abnormality				
S	1	123.274	1.488	0.230
T	3	249.836	3.016	0.041*
S × T	3	18.080	0.218	0.883
Error	40	82.840		
Total	48			
Corrected total	47			
Total length				
S	1	0.082	18.653	0.000*
T	3	0.015	3.441	0.019*
S × T	3	0.014	3.156	0.027*
Error	136	0.004		
Total	144			
Yolk sac volume				
S	1	2.025×10^{-5}	0.062	0.803
T	3	0.001	2.768	0.044*
S × T	3	1.582×10^{-5}	0.049	0.986
Error	136			
Total	144			
Survival rate				
S	1	208.333	1.096	0.301
T	3	1075.000	5.658	0.003*
S × T	3	52.778	0.278	0.841
Error	40	190.000		
Total	48			
Corrected total	47			

high mortality rates of late larvae and early juveniles due to impairments in prey capture and swimming behaviors.

Yolk sac volume plays an important role during the endogenous feeding period that serves as the major source of energy for larvae, providing protein, free amino acids, lipids, and carbohydrates for maintenance, differentiation, growth, and other activities (Subhan et al., 2020). The results of our study indicate a significant downward trend

in yolk sac volume of newly hatched larvae as turbidity increased, but no significant differences in volume were observed among salinity treatments or among treatments with different interactions between salinity and turbidity. Increased turbidity during the early developmental stages of embryos could decrease yolk sac volume, resulting in starvation of larvae before the mouth is fully formed for feeding and a high risk of mortality. Kamler (2008)

suggested that endogenous factors like yolk sac volume are one of the major causes of early mortality in fish. In addition, Subhan et al. (2020) reported that yolk sac volume is a main factor in determining the quality of larvae in the postlarval stage that is critical to adaptation in the natural environment.

Total length of newly hatched larvae is the only characteristic for which individual and combined effects of salinity and turbidity were found. Although stored energy is usually used for growth and organ maturation in normal conditions, under stressful conditions, such energy is reallocated to cope with stress instead of being used for development (Pérez-Robles et al., 2016). In our study, total length of newly hatched larvae decreased when embryos were exposed to combinations of low salinity and high turbidity, indicating that embryos in non-optimal salinities may be unable to maintain their osmoregulatory capacity because of decreasing availability of metabolic energy, thereby decreasing growth in development. Fish larvae with small sizes (<2.57 mm total length) at hatching have a lower chance of survival than larger larvae because they have fewer energy reserves, less feeding ability, and a slower, weaker response to predators (Garrido et al., 2015). Furthermore, larvae that are smaller than other larvae at hatching cannot grow and survive because of a lower metabolic rate and higher cost of development.

Conclusions

Results from analysis of all characteristics of embryos and larvae measured in this study indicate that, although embryos of bamboo sole are quite tolerant and are able to withstand conditions of low salinity, they are sensitive to stress from high turbidity. As the frequency of heavy rainfall events continues to increase, reductions in the hatching rate of embryos and the size and survival rate of larvae of bamboo sole could lead to detrimental effects at the population level.

Resumen

Las lluvias intensas pueden disminuir la salinidad y aumentar la turbidez del agua en las zonas costeras, lo que afecta negativamente al desarrollo de los organismos, especialmente durante sus primeras etapas de vida. En este estudio, se evaluaron los efectos de la salinidad y la turbidez en los embriones del lenguado bambú (*Heteromycteris japonicus*) para comprender mejor su tolerancia al cambio climático global. Se realizaron tres experimentos durante un periodo de 7 d. En el primer experimento, los embriones de lenguado bambú se expusieron durante 3 h a 1 de los 6 niveles de salinidad (14–34). Las longitudes totales de las larvas recién eclosionadas a niveles de salinidad bajos (14 y 18) fueron más pequeñas respecto al tamaño de las larvas en los tratamientos con mayores salinidades, aunque no se observaron diferencias

significativas en las tasas de eclosión y de supervivencia de las larvas entre los tratamientos. En el segundo experimento, los embriones se expusieron a turbideces de 0, 100, 300, 500 y 700 unidades nefelométricas de turbidez durante 3 h. La turbidez disminuyó significativamente la tasa de eclosión, la tasa de supervivencia y la longitud total, y aumentó el tiempo de eclosión y el porcentaje de anomalía. En el tercer experimento, los embriones se expusieron a diferentes combinaciones de salinidad y turbidez. El efecto de interacción de la salinidad y la turbidez sobre la longitud total de las larvas recién eclosionadas fue significativo. Estos resultados indican que el desarrollo embrionario del lenguado bambú se vio más afectado por los cambios de turbidez que por los de salinidad.

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Literature cited

- Allen, J. D., and J. A. Pechenik.
2010. Understanding the effects of low salinity on fertilization success and early development in the sand dollar *Echinarrhynchus parma*. *Biol. Bull.* 218:189–199. [Crossref](#)
- Barange, M., G. Merino, J. L. Blanchard, J. Scholtens, J. Harle, E. H. Allison, J. I. Allen, J. Holt, and S. Jennings.
2014. Impacts of climate change on marine ecosystem production in societies dependent on fisheries. *Nat. Clim. Change* 4:211–216. [Crossref](#)
- Baudron, A. R., C. L. Needle, A. D. Rijnsdorp, and C. T. Marshall.
2014. Warming temperatures and smaller body sizes: synchronous changes in growth of North Sea fishes. *Glob. Change Biol.* 20:1023–1031. [Crossref](#)
- Berlinsky, D. L., J. C. Taylor, R. A. Howell, T. M. Bradley, and T. I. J. Smith.
2004. The effects of temperature and salinity on early life stages of black sea bass *Centropristis striata*. *J. World Aquac. Soc.* 35:335–344. [Crossref](#)
- Bilotta, G. S., and R. E. Brazier.
2008. Understanding the influence of suspended solids on water quality and aquatic biota. *Water Res.* 42:2849–2861. [Crossref](#)
- Bœuf, G., and P. Payan.
2001. How should salinity influence fish growth? *Comp. Biochem. Physiol. C* 130:411–423. [Crossref](#)
- Boyd, C. E.
2015. *Water quality: an introduction*, 2nd ed., 357 p. Springer Int. Publ., Cham, Switzerland.
- Brander, K.
2010. Impacts of climate change on fisheries. *J. Mar. Syst.* 79:389–402. [Crossref](#)
- Cahu, C., J. Z. Infante, and T. Takeuchi.
2003. Nutritional components affecting skeletal development in fish larvae. *Aquaculture* 227:245–258. [Crossref](#)

- Cheung, W. W. L., and M. A. Oyinlola.
2018. Vulnerability of flatfish and their fisheries to climate change. *J. Sea Res.* 140:1–10. [Crossref](#)
- Conides, A. J., and B. Glamuzina.
2001. Study on the effects of rearing density, temperature and salinity on hatching performance of the European sea bass, *Dicentrarchus labrax* (Linnaeus, 1758). *Aquac. Int.* 9:217–224. [Crossref](#)
- COSEWIC (Committee on the Status of Endangered Wildlife in Canada).
2015. COSEWIC assessment and status report on the Spotted Gar *Lepisosteus oculatus* in Canada, 32 p. COSEWIC, Ottawa, Canada. [Available from [website](#).]
- Del Rio, A. M., B. E. Davis, N. A. Fangue, and A. E. Todgham.
2019. Combined effects of warming and hypoxia on early life stage Chinook salmon physiology and development. *Conserv. Physiol.* 7:coy078. [Crossref](#)
- Farhana, T., F. Haque, F. B. Amin, M. M. Zahangir, and M. S. Islam.
2019. Developmental pliability in zebrafish: an experimental enquiry of acute salinity stress on the early life of zebrafish. *Aquac. Rep.* 14:100189. [Crossref](#)
- Fukuda, A., T. Tabata, K. Hiramatsu, and M. Harada.
2021. Analysis of salinity behavior in Hakata Bay after heavy rainfall using a three-dimensional σ -coordinate model. *Jpn. Agric. Res. Q.* 55:137–146. [Crossref](#)
- Garrido, S., R. Ben-Hamadou, A. M. P. Santos, S. Ferreira, M. A. Teodósio, U. Cotano, X. Irigoien, M. A. Peck, E. Saiz, and P. Ré.
2015. Born small, die young: intrinsic, size-selective mortality in marine larval fish. *Sci. Rep.* 5:17065. [Crossref](#)
- Gibson, R. N., R. D. M. Nash, A. J. Geffen, and H. W. van der Veer (eds.).
2015. Flatfishes: biology and exploitation, 2nd ed., 576 p. John Wiley and Sons, West Sussex, UK.
- Gray, S. M., L. J. Chapman, and N. E. Mandrak.
2012. Turbidity reduces hatching success in threatened spotted gar (*Lepisosteus oculatus*). *Environ. Biol. Fishes* 94:689–694. [Crossref](#)
- Griffin, F. J., E. H. Smith, C. A. Vines, and G. N. Cherr.
2009. Impacts of suspended sediments on fertilization, embryonic development, and early larval life stages of the Pacific herring, *Clupea pallasii*. *Biol. Bull.* 216:175–187. [Crossref](#)
- Hasenbein, M., L. M. Komoroske, R. E. Connon, J. Geist, and N. A. Fangue.
2013. Turbidity and salinity affect feeding performance and physiological stress in the endangered delta smelt. *Integr. Comp. Biol.* 53:620–634. [Crossref](#)
- Holsman, K. K., T. Essington, T. J. Miller, M. Koen-Alonso, and W. J. Stockhausen.
2012. Comparative analysis of cod and herring production dynamics across 13 northern hemisphere marine ecosystems. *Mar. Ecol. Prog. Ser.* 459:231–246. [Crossref](#)
- Jawad, L. A.
2021. The importance of non-commercial and small-sized fish species: a proposal for an additional revenue to Iraq. In *Tigris and Euphrates rivers: their environment from headwaters to mouth* (L. A. Jawad, ed.), p. 1611–1623. Springer, Cham, Switzerland.
- Kamler, E.
2008. Resource allocation in yolk-feeding fish. *Rev. Fish Biol. Fish.* 18:143–200. [Crossref](#)
- Lehodey, P., J. Alheit, M. Barange, T. Baumgartner, G. Beaugrand, K. Drinkwater, J.-M. Fronmentin, S. R. Hare, G. Ottersen, R. I. Perry, et al.
2006. Climate variability, fish, and fisheries. *J. Clim.* 19:5009–5030. [Crossref](#)
- Manuel, A. V., T. C. T. Phan, S. Yamamoto, N. Tsutsui, and T. Yoshimatsu.
2021. Effect of simulated sudden thunderstorm flood on the early stage development of Japanese flounder *Paralichthys olivaceus*. *Aquac. Res.* 52:6464–6471. [Crossref](#)
- Mikhailenko, V. G.
2000. Salinity-stress-induced changes in the resistance of embryos of the White Sea herring *Clupea pallasii marisalbi* to freshwater. *Russ. J. Mar. Biol.* 26:370–372. [Crossref](#)
- Mueller, J. S., T. B. Grabowski, S. K. Brewer, and T. A. Worthington.
2017. Effects of temperature, total dissolved solids, and total suspended solids on survival and development rate of larval Arkansas River shiner. *J. Fish Wildl. Manag.* 8:79–88. [Crossref](#)
- Nakabo, T.
2002. Soleidae. In *Fishes of Japan with pictorial keys to the species*, English ed. (T. Nakabo, ed.), p. 1383–1387. Tokai Univ., Tokyo, Japan.
- Nurulnadia, M. Y., A. B. Noradila, and G. Adiana.
2020. Turbidity effects of suspended bauxite in embryonic stage of climbing perch (*Anabas testudineus*) post fertilization. *Egypt. J. Aquat. Res.* 46:181–186. [Crossref](#)
- Ord, J.
2019. Ionic stress prompts premature hatching of zebrafish (*Danio rerio*) embryos. *Fishes* 4:20. [Crossref](#)
- Pankhurst, N. W., and P. L. Munday.
2011. Effects of climate change on fish reproduction and early life history stages. *Mar. Freshw. Res.* 62:1015–1026. [Crossref](#)
- Pérez-Robles, J., F. Diaz, L. Ibarra-Castro, I. Giffard-Mena, A. D. Re, L. E. R. Ibarra, and J. A. I. Soto.
2016. Effects of salinity on osmoregulation during the embryonic development of the bullseye puffer (*Sphoeroides annulatus* Jenyns 1842). *Aquac. Res.* 47:838–846. [Crossref](#)
- Phan, T. C. T., A. V. Manuel, N. Tsutsui, and T. Yoshimatsu.
2019. Impact of short-term salinity and turbidity stress on the early developmental stages of Japanese flounder, *Paralichthys olivaceus* (Temminck and Schlegel, 1846). *Asian Fish. Sci.* 32:93–103. [Crossref](#)
- Phan, T. C. T., A. V. Manuel, N. Tsutsui, and T. Yoshimatsu.
2020. Impacts of short-term salinity and turbidity stress on the embryonic stage of red sea bream *Pagrus major*. *Fish. Sci.* 86:119–125. [Crossref](#)
- Phillippart, C. J. M., H. M. van Aken, J. J. Beukema, O. G. Bos, G. C. Cadée, and R. Dekker.
2003. Climate-related changes in recruitment of the bivalve *Macoma balthica*. *Limnol. Oceanogr.* 48:2171–2185. [Crossref](#)
- Poloczanska, E. S., C. J. Limpus, and G. C. Hays.
2009. Vulnerability of marine turtles to climate change. *Adv. Mar. Biol.* 56:151–211. [Crossref](#)
- Shi, Z.-H., S.-M. Peng, Y.-Q. Yin, H.-Z. Luo, and M.-L. Ni.
2009. Morphological changes of embryo and yolk sac larvae of barred knifejaw (*Oplegnathus fasciatus*) under salinity stress. *Chin. J. Ecol.* 28:471–476.
- Subhan, U., Iskandar, Zahidah, and I. M. Joni.
2020. Efficiency yolk sac utilization of endogenous feeding larvae striped catfish (*Pangasianodon hypophthalmus*) in the environmentally rich fine bubbles (FBs). *AIP Conf. Proc.* 2219:090003. [Crossref](#)
- Sugisaki, H., and K. Murakami.
2018. Impacts of climate change on the marine resources of Japan. In *Climate change impacts on fisheries and aquaculture: a global analysis*, vol. 1 (B. F. Phillips and M. Pérez-Ramírez, eds.), p. 121–128. John Wiley and Sons, Hoboken, NJ.

- Thrush, S. F., J. E. Hewitt, V. J. Cummings, J. I. Ellis, C. Hatton, A. Lohrer, and A. Norkko.
2004. Muddy waters: elevating sediment input to coastal and estuarine habitats. *Front. Ecol. Environ.* 2:299–306. [Crossref](#)
- Torres, G., K. Anger, and L. Giménez.
2021. Effects of short-term and continuous exposure to reduced salinities on the biochemical composition of larval lobster, *Homarus gammarus*. *Zoology* 144:125885. [Crossref](#)
- Wang, H.-S., Q.-S. Fang, and L.-Y. Zheng.
2002. Effects of salinity on hatching rates and survival activity index of the larvae of *Epinephelus akaara*. *J. Fish. China* 26:344–350.
- Yamamoto, M., and S. Katayama.
2013. Age, growth, and spawning period of bamboo sole *Heteromycteris japonica* in the Seto Inland Sea, Japan. *Aquac. Sci.* 61:359–365. [Crossref](#)
- Zheng, L., Z. Qui, Y. Zhou, D. Sun, S. Wang, W. Wu, and W. Perrie.
2016. Comparisons of algorithms to estimate water turbidity in the coastal areas of China. *Int. J. Remote Sens.* 37:6165–6186. [Crossref](#)
- Zhou, Z.-Z., T.-L. Huang, W.-X. Ma, Y. Li, and K. Zeng.
2015. Impacts of water quality variation and rainfall runoff on Jinpen Reservoir, in Northwest China. *Water Sci. Eng.* 8:301–308. [Crossref](#)