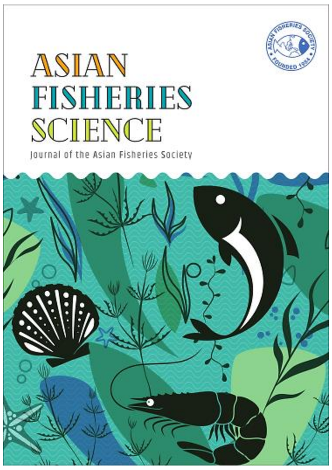

Impact of Short-term Salinity and Turbidity Stress on the Early Developmental Stages of Japanese Flounder, *Paralichthys olivaceus* (Temminck and Schlegel, 1846)

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Abstract

Coastal marine environments are exposed to frequent salinity drop and increase in turbidity due to heavy rainfall. This creates stressful conditions for marine organisms that inhabit the area, especially during their early stages of development. In this study, two experiments were carried out to assess the effects of short-term salinity and turbidity stress on embryonic and early larval stages of Japanese flounder, *Paralichthys olivaceus* (Temminck and Schlegel, 1846). In the first experiment, the embryos of Japanese flounder were exposed to different combinations of salinity (34, 30, 26, 22, 18 and 14 PSU) and turbidity (0, 100, 300, 500, 700 NTU) for a short period of 3 h. Experiment 2 evaluated the salinity tolerance at different developmental stages under the turbidity level of 700 NTU. The results showed that turbidity significantly influenced hatching rate, percentage of abnormality, total length, yolk sac volume, and survival rate, whereas salinity only affected the percentage of abnormality. There was no significant salinity/turbidity interaction of these parameters. Results suggested that embryos were more susceptible to turbidity compared to salinity stress, and embryos and newly hatched larvae stages were more tolerant to environmental stress conditions compared to yolk sac larvae and oil globule larvae stages.

Keywords: heavy rainfall, environmental stress conditions, embryos, newly hatched larvae

Introduction

Coastal marine environments are a major focus of concern regarding the potential impacts of anthropogenic global climate change (Harley et al. 2006). Variations in the environment may affect the physiology and behaviour of marine fish at any life-history stage, and any of these effects may drive population level changes in distribution and abundance (Hollowed et al. 2013; Vaidyanathan 2017). A recent study reported that global fisheries catches of flatfish (Order Pleuronectiformes) have been declining since the 1970s and suggested that climate change may further exacerbate the declining trends and threaten the long-term variability of some flatfish population in the 21st century (Cheung and Oyinlola 2018). According to Rijnsdorp et al. (2009), a prerequisite for population persistence is connectivity among habitats required by successive life stages, allowing the survivors to mature and return to the spawning grounds to reproduce successfully. However, the early life stage of marine fish is severely affected by physical and chemical environment related to climate change (Kim et al. 2013; Sugisaki and Murakami 2018). A recent increasing trend in extremely heavy rainfall due to climate change (Japan Meteorological Agency 2018) is expected to have negative effects on the marine environment. During periods of heavy rainfall, runoffs from rivers and estuaries in coastal waters influence the potential of an increase in turbidity and a decline in salinity (Raper and Braithwaite 2006; Southgate and Lucas 2008; Poloczanska et al. 2009), and these waters are also discharged to offshore areas by tidal currents (Ahn et al. 2005; Clare et al. 2016).

Salinity is an unstable factor in most of the coastal areas during heavy rainfall and may drop from 30 to 15 PSU in short periods (Allen and Pechenik 2010; Morash and Alter 2015). Drop in salinity over a certain period can have particularly strong effects on distribution and survival of fish in such environments (Wang et al. 2015), more specifically because of changes in solute concentration on the efficiency of metabolic processes (Varsamos et al. 2005). In addition, short-term exposure to low salinity may lead to physiologically-stressed situations that have negative effects on organisms (Montory et al. 2014). In white sea herring, *Clupea pallasii marisalbi* Berg, 1923, the developmental rate of embryos and survival of eggs decreased when eggs were exposed to low salinity (Mikhailenko 2000). Moreover, short-term exposure to salinity stress also affected the total length of newly hatched larvae of California grunion, *Leuresthes tenuis* (Ayres, 1860) (Matsumoto and Martin 2008).

Turbidity in water is caused by suspended material such as clay, silts, plankton, particulates organic and inorganic matters, and soluble coloured organic compounds (Boyd and Tucker 1998). Most normal conditions have turbidities less than 50 NTU (Nephelometric Turbidity Units) and rarely exceed 1000 NTU in coastal areas (Boyd 2015). According to Harrod and Theuer (2002), different turbidity levels may lead to a delay in the development and a reduction in the survival of eggs and larvae. For example, short-term exposure to turbidity reduced the percentage of fertilization and larvae hatching of rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792) (Wojtczak et al. 2004). Furthermore, Hasenbein et al. (2013) reported that a combination of salinity and turbidity affected the physiological characteristics in delta smelt fish, *Hypomesus transpacificus* McAllister, 1963. However, there is limited information on how short-term salinity and turbidity stress affect the early developmental stages of Japanese flounder, *Paralichthys olivaceus* (Temminck and Schlegel, 1846) particularly in a framework of environmental change due to heavy rainfall.

Japanese flounder is a common paralichthyid flatfish distributed along the coast of Japan, Korea, and China (Wada et al. 2012; Shigenobu et al. 2013). It is an important commercial species and records an annual catch of approximately 7000 t in Japan (Yamamoto et al. 2004). In natural conditions, Japanese flounder usually inhabits coastal waters at depths of <100 m and spawn pelagic eggs at depths of <50 m (Tomiyama et al. 2008). Although Japanese flounder spawn in offshore banks, eggs and larvae may be passively transported to nearshore nursery grounds by tidal movements, winds, and currents (Bolle et al. 2009; Duffy-Anderson et al. 2015; Gibson et al. 2015). Dou (1995) also reported that the high densities of eggs of Japanese flounder were found in the coastal waters where water salinity and turbidity fluctuated due to heavy rainfall events as mentioned above. In this study, we assessed the effects of salinity and turbidity changes on Japanese flounder during embryonic and early larval stages under laboratory conditions. The results of this study would provide information to better understand the wild population dynamics of this species in the future.

Materials and Methods

Artificially fertilised eggs of Japanese flounder were obtained from a private commercial hatchery in Aichi Prefecture, Japan, of which fertilisation rate and mean egg diameter was 96% and 0.92 ± 0.02 mm (n = 40), respectively. Fertilised eggs were incubated in a 5-L rectangular tank containing seawater of 34 PSU with aeration and temperature of 18.0–19.0 °C. At the gastrula stage, dead and physically damaged embryos were removed using a wide-mouth pipette, and normally developing eggs were used for the experiment. The required salinities were prepared by diluting different proportions of the artificial seawater powder (LIVESea Salt, Delphis Co. Ltd., Japan) with distilled water. To obtain different turbidity levels, kaolin clay ($Al_2Si_2O_5(OH)_4$, approximately 0.4 µm particle size in diameter) was diluted to salinity levels. Chemically inert kaolin clay was used because some suspended particles contain toxic metals, hydrogen sulfide, pathogenic bacteria, and other factors that may have multiple effects on the exposed fish larvae (Isono et al. 1998). In addition, negligible fluctuations of pH at different turbidity levels ranging from 8.00 (0 NTU) to 8.05 (700 NTU) were observed in each salinity treatment. Salinity, turbidity, pH, and dissolved oxygen were measured by a hand refractometer (Iwaki, Japan), HACH 850 (Hach Co. Ltd., USA), Docu-pH meter (Sartorius, Germany) and LUTRON DO-5509 dissolved oxygen meter (Japan), respectively.

Experiment 1: Effects of salinity and turbidity on hatching rate, percentage of abnormality, total length, yolk sac volume, oil globule volume and survival of larvae 6 days post-hatch

Fertilised eggs at the gastrula stage were stocked into six-well plastic microplates at a density of 13 eggs per well. Microplates were disinfected and each well was filled with 5 mL seawater as an original medium (34 PSU - 0 NTU). Embryos were exposed to different combinations of salinity (34, 30, 26, 22, 18, and 14 PSU) and turbidity (0, 100, 300, 500 and 700 NTU) for a period of 3 h. Each of the 30 treatments (6 salinities × 5 turbidities) comprised six replicates. After the 3 h stress period, eggs were transferred to 100-mL glass beakers filled with 50 mL original medium and incubated without aeration under temperature 18 ± 1 °C and photoperiod of 12L:12D light:dark cycle in an electric incubator. Fifty percent of the water in each beaker was exchanged with fresh, original medium daily. The dissolved oxygen and pH of incubated seawater were around 4.7–5.5 mg.L^{-1} and 8.0–8.5, respectively, which were within an acceptable range (Boyd and Tucker 1998).

Hatching rate was calculated as the percentage of stocked embryos that hatched regardless of viability. Twelve hours post-hatch, sixteen larvae from each treatment were randomly collected to measure total length, yolk sac volume and oil globule diameter under a biological stereoscopic microscope with an ocular micrometre at a precision of 10 µm. All larvae were preserved in 4 % formalin solution. Percentage of abnormality in larvae was determined based on the number of normal and abnormal larvae. The larval abnormality was defined as notochord bending, tail deformity, and improper positioning of oil globule. On day 6 post-hatch, the survival rate was calculated. Yolk sac volume (mm^3) and oil globule volume (mm^3) were calculated using the following formula where *L* is the length of the yolk sac; *H* is the height of yolk sac and *D* is the diameter of oil globule

$$\text{Yolk sac volume (mm}^3) = \frac{\pi}{6} \times LH^2$$

$$\text{Oil globule volume (mm}^3) = \frac{\pi}{6} \times D^3$$

Larvae were fed with live rotifers *Brachionus plicatilis* from day 3 post-hatch at a density of 4 rotifers.mL^{-1}. Rotifers were carefully rinsed with seawater at a salinity of 34 PSU to avoid any salinity change.

Experiment 2: Effect of low salinity levels on the survival from embryo to early larval stages under high turbidity level of 700 NTU

Table 1. Developmental stages in Japanese flounder *Paralichthys olivaceus* exposed to low salinity levels and turbidity of 700 NTU.

Stage	Abbre-viation	Period	Description
Embryo	EM	Approximately 26 h after fertilization	Formation of embryo body, appearance of optic vesicles
Newly hatched larva	NL	Day 1 after hatching	Newly hatched larva had a large yolk sac with a little pigmentation, and a single oil globule located in the posterior region of the yolk sac
Yolk sac larva	YL	Day 3 after hatching	The mouth was opened, and eye pigmentation began
Oil globule larva	OL	Day 4 after hatching	Larva with oil globule

Table 1 shows the selected developmental stages (Wang et al. 2015) of Japanese flounder used in this study. Four developmental stages were selected to be exposed to different environmental regimes for a period of 3 h, including embryo (EM), newly hatched larva (NL), yolk sac larva (YL), and oil globule larva (OL). Both embryos (n = 10) and larvae (n = 10) were sampled for the following experimental treatments. Different combinations of salinity (30, 26, 22, 18, 14 PSU) and a turbidity level of 700 NTU were tested. After 3 h exposure, embryos and larvae were transferred to 100-mL glass beakers filled with 50 mL original medium as in Experiment 1. The incubation conditions, food supply, and water exchange were performed according to the same procedure described in Experiment 1. At the end of the experiment, live larvae were counted to calculate the survival rate. The experiment was carried out for 8 days.

Data analysis

All data were analysed using SPSS statistics version 22.0 software. Results were compared using one-way and two-way analysis of variance (ANOVA). Post-hoc Tukey tests were performed to identify which factor levels differed. All analyses were performed at a significant level of $P < 0.05$. Results were expressed as mean ± standard error (SE).

Results

Experiment 1: Effects of salinity and turbidity on hatching rate, percentage of abnormality, total length, yolk sac volume, oil globule volume and survival of larvae 6 days post-hatch

Turbidity had a significant effect on hatching rate ($P < 0.05$), but salinity or the interaction between salinity and turbidity was not observed ($P > 0.05$) (Table 2). At all salinities, the hatching rate of 90% or more were observed for treatments with turbidities of 0 and 100 NTU. At 30 PSU, the rates at 500 NTU and 700 NTU were significantly lower than those at 0 NTU, but there was no significant difference among 0, 100 and 300 NTU (Fig. 1a). As for the percentage of abnormality, a two-way ANOVA analysis demonstrated that there was also no interaction between the two variables ($P > 0.05$), but independently, both salinity and turbidity had an impact on the percentage of abnormality of larvae ($P < 0.05$) (Table 2). At 34 PSU, the percentage of abnormality increased to 3.8, 6.4, 10.3, 12.8, 14.1 % for the turbidity of 0, 100, 300, 500 and 700 NTU, respectively (Fig. 1b).

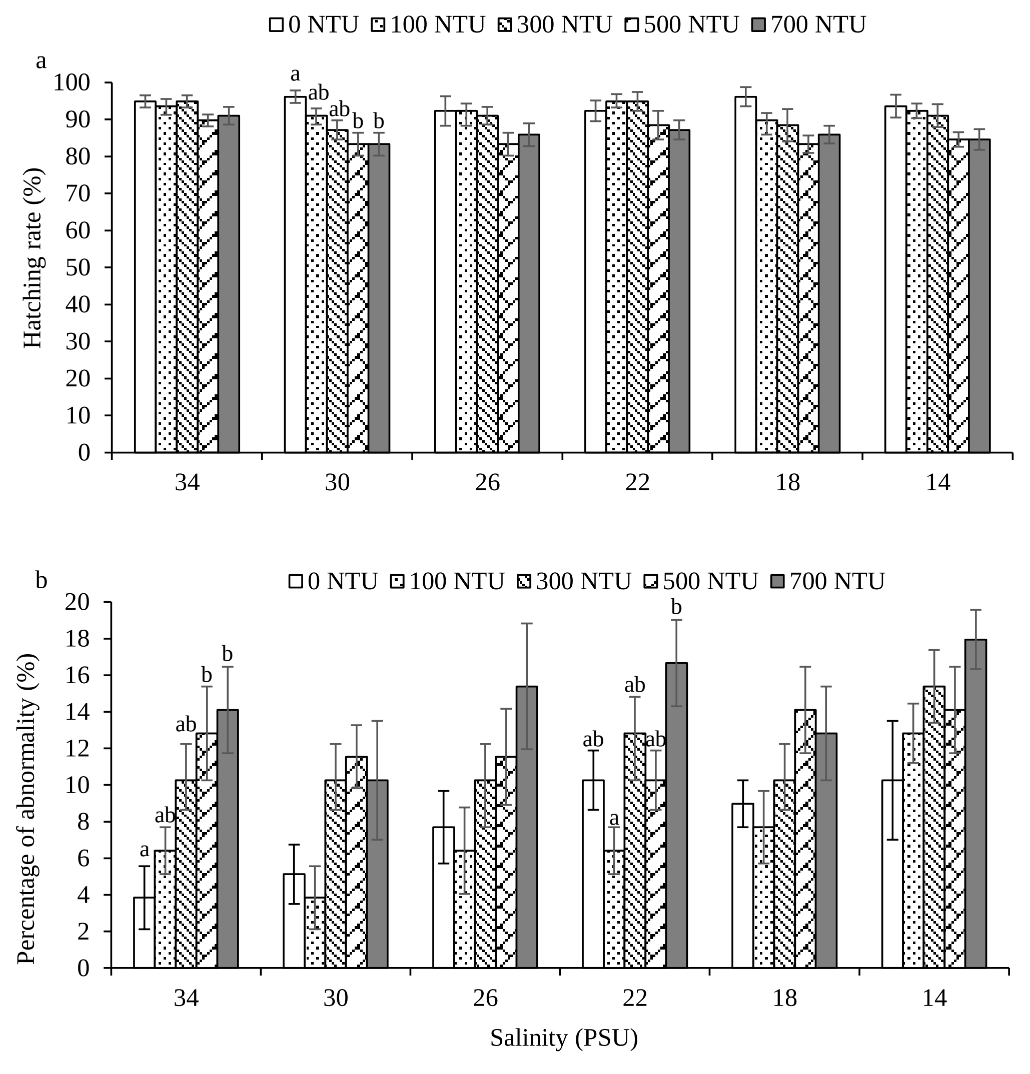

Fig. 1. Hatching rate (a) and percentage of abnormality (b) of the Japanese flounder *Paralichthys olivaceus* obtained by exposing embryos at turbidity levels of 0, 100, 300, 500 and 700 NTU among each salinity level of 34, 30, 26, 22, 18 and 14 PSU for 3 h. Data are shown as mean ± SE. Different letters above the bars indicate significant difference among turbidity levels at each salinity level. Tukey's multiple comparison tests in one-way analysis of variance ($P < 0.05$).

The total length of newly hatched larvae ranged from 2.14 to 2.34 mm in all treatments. Based on the ANOVA results, larval total length was significantly affected by turbidity ($P < 0.05$) (Table 2). At 34 PSU, the sizes of the larvae at 100, 300, 500 and 700 NTU were significantly smaller than those at 0 NTU (Fig. 2A). However, no significant alterations in larval body size were observed in salinity and salinity/turbidity interaction ($P > 0.05$) (Table 2). Similar results appeared for yolk sac volume of newly hatched larvae. There were no significant differences in salinity and interaction between salinity and turbidity ($P > 0.05$), whereas significant effects of turbidity on yolk sac volume were found ($P < 0.05$) (Table 2).

The post-hoc test showed that at 34 PSU, a significant decrease in yolk sac volume occurred in embryos exposed to 300, 500 and 700 NTU compared with those in 0 NTU, whereas there were no differences in yolk sac volume among 100, 300, 500 and 700 NTU or between 0 NTU and 100 NTU (Fig. 2B). Neither salinity and turbidity, nor salinity/turbidity interaction significantly affected the oil globule volume of larvae (Table 2), which recorded a mean value was 0.003 mm^3 at each treatment (data not shown).

With regards to survival rate at the end of the experiment, results showed that there were statistically significant effects of turbidity ($P < 0.05$) on survival of Japanese flounder larvae until day 6 post-hatch, whereas no effects of both salinity and salinity/turbidity interaction were recorded ($P > 0.05$) (Table 2). At 34 PSU, survival rates at 300, 500 and 700 NTU were significantly lower than that at 0 NTU, whereas there was no significant difference among 300, 500 and 700 NTU (Fig. 3).

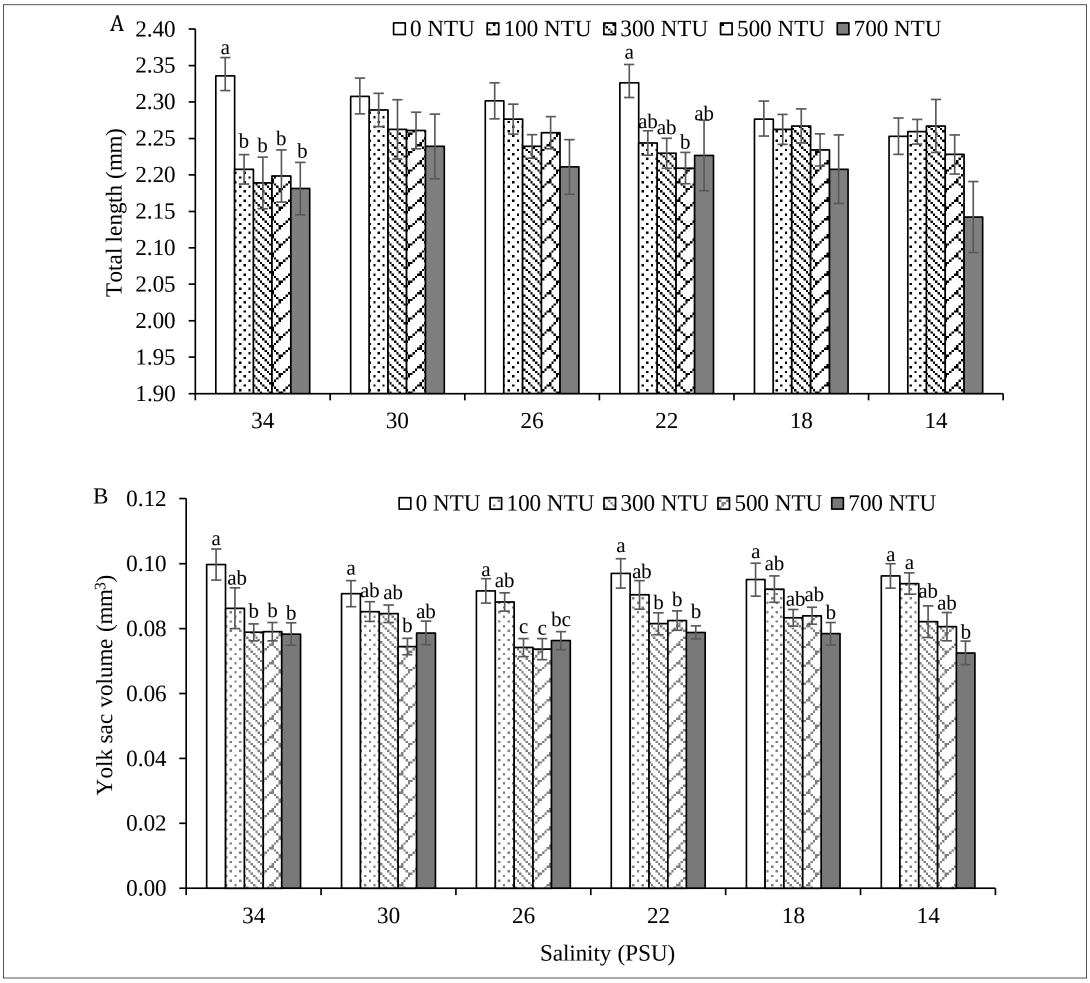

Fig. 2. Total length (A) and yolk sac volume (B) of the Japanese flounder *Paralichthys olivaceus* obtained by exposing embryos at turbidity levels of 0, 100, 300, 500 and 700 NTU among each salinity level of 34, 30, 26, 22, 18 and 14 PSU for 3 h. Data are shown as mean ± SE. Different letters above the bars indicate significant difference among turbidity levels at each salinity level. Tukey's multiple comparison tests in one-way analysis of variance ($P < 0.05$).

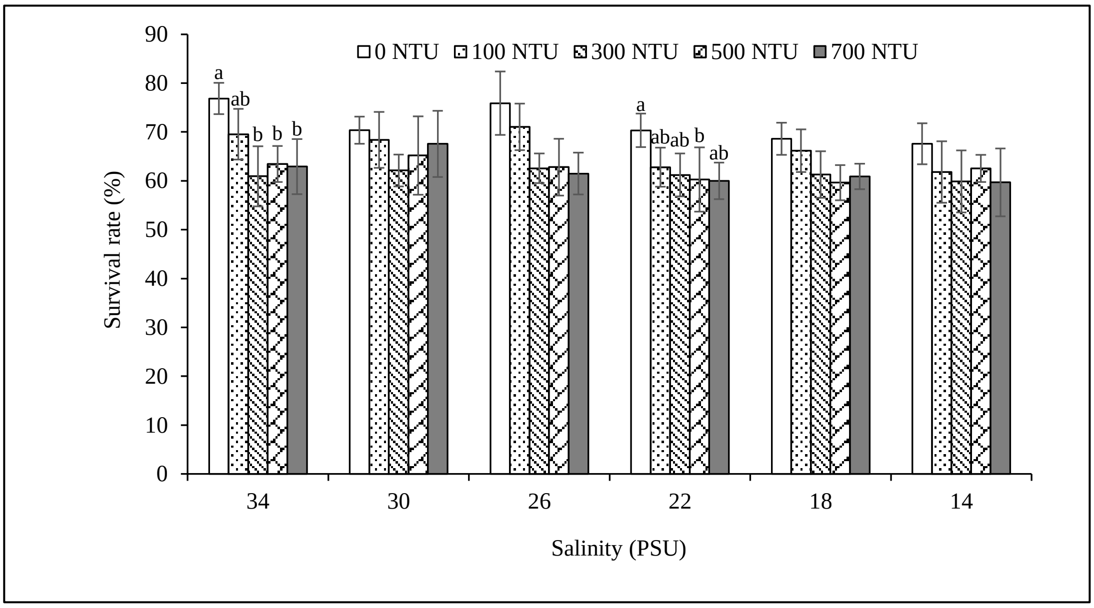

Fig. 3. Survival rate (%) of Japanese flounder *Paralichthys olivaceus* larvae 6 days post-hatch. Data are shown as mean ± SE. Different letters above the bars indicate significant difference among turbidity levels at each salinity level. Tukey's multiple comparison tests in one-way analysis of variance ($P < 0.05$).

Table 2. Summary of two-way ANOVA results on hatching rate, percentage of abnormality, total length, yolk sac volume, oil globule volume and survival rate for Japanese flounder *Paralichthys olivaceus* larvae when embryos were exposed to different salinity (S) and turbidity (T) conditions.

Source	DF	MS	F	P
Hatching rate				
S	5	100.578	2.148	0.063
T	4	528.669	11.291	0.000*
S × T	20	22.65	0.484	0.969
Error	150	48.825		
Total	180			
Corrected total	179			
Percentage of abnormality				
S	5	118.900	4.225	0.001*
T	4	351.724	12.499	0.000*
S × T	20	16.632	0.591	0.914
Error	150	28.140		
Total	180			
Corrected total	179			
Total length				
S	5	0.026	1.836	0.104
T	4	0.128	9.005	0.000*
S × T	20	0.012	0.818	0.692
Error	450	0.014		
Total	480			
Corrected total	479			
Yolk sac volume				
S	5	0.000	1.766	0.119
T	4	0.006	25.844	0.000*
S × T	20	0.000	0.686	0.841
Error	450	0.000		
Total	480			
Corrected total	479			
Oil globule volume				
S	5	5.333E-08	2.182	0.055
T	4	3.215E-08	1.278	0.278
S × T	20	2.000E-08	0.818	0.692
Error	450	2.444E-08		
Total	480			
Corrected total	479			
Survival rate				
S	5	139.603	0.946	0.453
T	4	677.188	4.587	0.002*
S × T	20	28.608	0.194	1.000
Error	150	147.645		
Total	180			
Corrected total	179			

DF = degrees of freedom, MS = mean squares, F = F-ratio, P = probability of significance, * indicates significant difference among treatments ($P < 0.05$).

Experiment 2: Effect of low salinity levels on the survival from embryo to early larval stages under high turbidity level of 700 NTU

Under a single turbidity condition of 700 NTU and 3-h exposure, salinity and developmental stage significantly affected survival rate of larvae ($P < 0.05$), but there was no interaction effect between the two factors ($P > 0.05$) (Table 3). In addition, developmental stage factor had a stronger effect on the mean survival rate of larvae than salinity, as indicated by the mean square value in Table 3. With regards to developmental stage effect, at 18 and 14 PSU, the survival rates were higher and significantly different for larvae at EM and NL stages when compared to those at YL and OL stages. However, there was no significant difference between EM and NL stages. The results also revealed that the lowest survival rate was observed at OL stage in all treatments (Fig. 4).

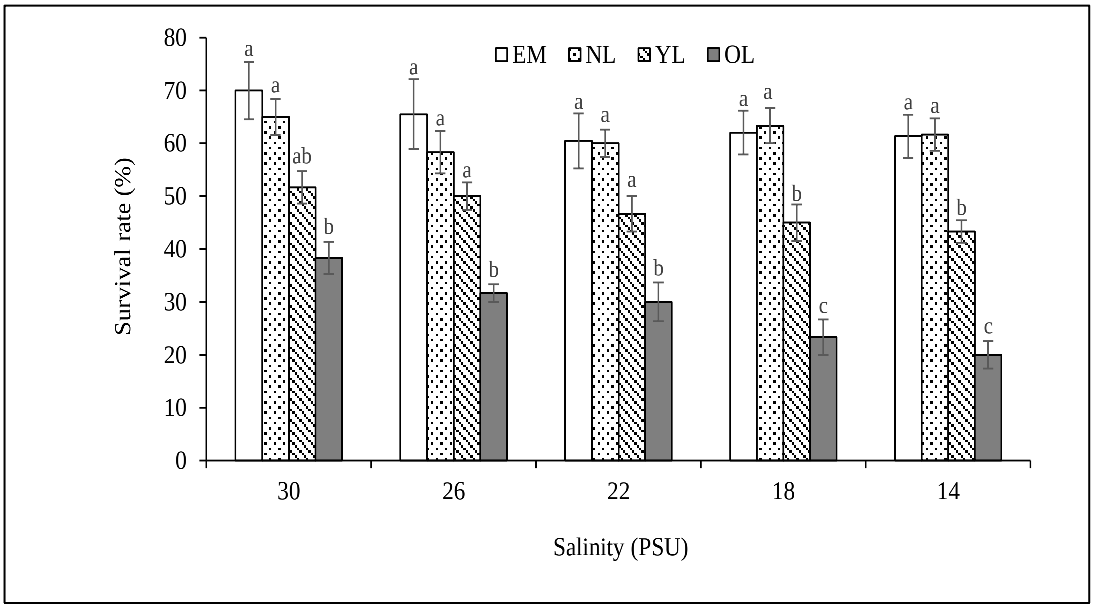

Fig. 4. Survival rate (%) of Japanese flounder *Paralichthys olivaceus* at different early developmental stages exposed to five levels of salinity under high turbidity level of 700 NTU. Data are shown as mean ± SE. EM: embryo; NL: newly hatched larva; YL: yolk sac larva; OL: oil globule larva. Different letters above the bars indicate significant difference among developmental stages at each salinity level. Tukey's multiple comparison tests in one-way analysis of variance ($P < 0.05$).

Table 3. Summary of two-way ANOVA results on the effect of salinity and developmental stage to the survival rate of Japanese flounder *Paralichthys olivaceus* under high turbidity level of 700 NTU. Salinity: 30, 26, 22, 18, 14 PSU; developmental stage: EM: embryo; NL: newly hatched larva; YL: yolk sac larva; OL: oil globule larva.

Source	DF	MS	F	P
Salinity	4	329.030	3.973	0.004*
Stage	3	7906.596	95.464	0.000*
Salinity × Stage	12	62.730	0.757	0.692
Error	100	82.823		
Total	120			
Corrected total	119			

DF = degrees of freedom, MS = mean squares, F = F-ratio, P = probability of significance, * indicates significant difference among treatments ($P < 0.05$).

Discussion

Organisms living in the coastal areas are potentially exposed to short-term fluctuations in environmental salinity and turbidity levels during periods of heavy rain (Poloczanska et al. 2009; Montory et al. 2014). These stressful events may be particularly shocking for animals in the early stages of ontogeny (Montory et al. 2014). The results of this study showed that short-term exposure to low levels of salinity did not affect the hatching rate of Japanese flounder embryos. This finding could explain that the hardened chorion presumably prevents damage to the embryos during short-term exposure to altered salinities (Martin 2015). In addition, Matsumoto and Martin (2008) stated that embryos at later developmental stages might be better able to tolerate exposure to altered salinity by the development of osmoregulatory mechanisms; for instance, the gastrula stage has a higher tolerance to salinity changes than the blastomere stage (Gracia-López et al. 2004). The current experiment using eggs at the gastrula stage showed that they are more tolerant to salinity changes. Conversely, significant effects of turbidity on hatching rate were observed in the present study. The short-term exposure to high levels of turbidity decreased hatching rate of Japanese flounder. Similar results were reported for rainbow trout (Wojtczak et al. 2004) and Pacific herring, *Clupea pallasii* Valenciennes, 1847 (Griffin et al. 2009). During the exposure time, it was clearly observed that the appearance of eggs changed from clear transparent to partially opaque white due to the adhesion of kaolin clay on the surface layer of eggs, which could reduce oxygen intake and cause the mortality of eggs and larvae, like reported previously (Henley et al. 2010; Gray et al. 2012).

Morphological abnormality influences the survival of larvae because of the impairment of swimming and prey catching behaviour (Porter and Bailey 2007; de Soto et al. 2013). In the present study, salinity and turbidity levels significantly affected the percentage of abnormality of newly hatched larvae. Therefore, the majority of the abnormal larvae died gradually after hatching, and only a few larvae survived until the end of the experiment. Under stressful salinity and turbidity conditions, the stored energy is reallocated to cope with stress instead of development (Pérez-Robles et al. 2016) and thereby causing morphological deformities. According to Rodhouse et al. (2014), short-term salinity drops caused by heavy rain resulted in slower metabolic processes and embryos development malformations. Griffin et al. (2009) reported that the increase in larval abnormalities of Pacific herring was observed when embryos were exposed to suspended sediments shortly.

Regarding the size of newly hatched larvae of Japanese flounder, we found no significant difference in the total length when embryos were exposed to short-term salinity stress. However, the total length of newly hatched larvae was inversely correlated to turbidity. A similar result was reported by Griffin et al. (2009) after exposing embryos of the Pacific herring to suspended sediments. Undersized larvae are inefficient predators and more vulnerable to predation due to impair visual and swimming ability (Porter and Bailey 2007). In addition, Bang et al. (2007) also reported that small larvae have fewer yolk reserves to sustain them during the critical transition to exogenous feeding when prey capture behaviour must be learned. Moreover, the high mortality experienced during the larval phase of fishes is widely regarded to be size-dependent (Garrido et al. 2015). Individuals with a larger size at hatching have higher survivorship than those that are undersized at hatching (Vigliola and Meekan 2002).

The oil globule is likely to be the major source of energy sustaining larvae during the difficult transition to exogenous feeding (Berkeley et al. 2004). In the present study, short-term exposure to salinity and turbidity during the embryonic stage had no significant effect on the oil globule volume of newly hatched larvae. Shi et al. (2008) stated that larvae usually depend primarily on yolk sac as their initial energy source, with protein and free amino acids of yolk providing the important sources for enzyme synthesis and metabolism. Thus, the volume of the oil globule remained almost unchanged before the yolk had been fully absorbed (Baras et al. 2018). According to Bloor et al. (2013), the inner yolk sac provides the main energy for growth during the first few days after hatching, and that variations in the size of the inner yolk sac may have an important effect on their capacity for growth and survival during that period. In this study, the effect of high turbidity stress on yolk sac volume of newly hatched larvae were measured and results showed that small yolk sac volumes were found in high turbidity levels, which indicates that larvae can only provide endogenous feed for a short period of time and possibly shorten the leeway in changing from endogenous to exogenous feeding (Ching et al. 2012).

The second experiment pointed out that the effect of salinity differs depending on the early developmental of Japanese flounder under the turbidity of 700 NTU. A higher survival rate of larvae was recorded in EM and NL stages compared to YL and OL stages. As mentioned above, the embryos of fish are protected from the external environment by the chorion, which is an impermeable barrier to the solutes and allows embryos to maintain constant osmoregulatory patterns in different salinity conditions. Nevertheless, after hatching, larvae are exposed to external conditions and depend on their osmoregulatory capacity mechanism for survival (Pérez-Robles et al. 2016). Kucera et al. (2002) revealed that the NL can tolerate a wide range of salinity because of their relatively impermeable skin and the presence of cutaneous chloride cells. Moreover, the pattern of salinity tolerance changes throughout the larval period with the onset of feeding and differentiation of structures important in osmoregulation (Kucera et al. 2002). The present finding is similar to that reported in mangrove red snapper, *Lutjanus argentimaculatus* (Forsskål, 1775) where the NL showed a higher tolerance of abrupt salinity changes than those of older ages (Estudillo et al. 2000). In addition, Estudillo et al. (2000) also indicated that the ability of NL to tolerate a wider range of salinity compared to adults is true to many teleost species. In a review study on the effects of variable salinity on reproduction and early life stages of spotted seatrout, *Cynoscion nebulosus* Cuvier and Valenciennes, 1830, results showed that 1-day post-hatch spotted seatrout larvae could tolerate salinity ranges between 4–40 PSU (Holt and Holt 2002). Moreover, research on tilapia reported that the tolerance of embryos and larvae was influenced by the presence of chloride cell in the yolk sac membrane (Kaneko et al. 2002). Additionally, the density of the chloride cell increased towards hatching and subsequently decreased as the yolk was gradually absorbed, and then increasing again at day 10 post-hatch (Li et al. 1995)..

Conclusion

Japanese flounder eggs are tolerant of short-term exposures to low salinity levels. However, short-term turbidity stress reduced the hatching rate, newly hatched larval size, yolk sac volume and viability of larvae, and could lead to a decline in population in the future because relatively small changes in growth and mortality rates during eggs and larval phases can have a negative impact on the recruitment success of fish population (Rijinsdorp et al. 2009). In addition, Donohue and Molinos (2009) demonstrated that increased turbidity is cited as a putative cause for fish population declines in lakes and reservoirs. Results also indicated that the tolerance of Japanese flounder to environmental stress conditions was dependent on developmental stages, and the most sensitive stage was recorded in OL stage. Therefore, the results of the present study suggest that management of excessive sediment inputs into the marine ecosystem requires more consideration to help conserve the fish population, especially during the spawning season when they are at their most vulnerable stage.

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