

SHORT COMMUNICATION

Impact of Short-Term Salinity and Turbidity Changes on Hatching and Survival Rates of Japanese Sea Cucumber, *Apostichopus japonicus* (Selenka, 1867), Eggs

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E-ISSN: 2073-3720

<https://doi.org/10.33997/j.afs.2022.35.1.008>

Abstract

The increased frequency and intensity of extreme rainfall events attributed to global climate change could lead to changes in salinity and turbidity levels in coastal waters which may negatively impact the survival of organisms, particularly during the early developmental stages. In this study, the influences of salinity and turbidity on the early life stage of Japanese sea cucumber, *Apostichopus japonicus* (Selenka, 1867), were studied in a series of small-scale laboratory experiments. During the first half of the experiments, *A. japonicus* fertilised eggs were exposed to various levels of salinity stresses (34: control, 30, 26, 22, 18, 14 PSU) for a single period of 3 h. In the second half of the experiments, following the same duration as the first experiment, fertilised eggs were exposed to different levels of turbidity, 0 (control), 100, 300, 500, and 700 NTU. The results showed that the hatching and survival rates significantly decreased as salinity dropped. As for turbidity, there were significant adverse effects on hatching and survival rates of *A. japonicus*. This study shows that short-term low salinity and high turbidity influenced events could potentially reduce larvae survival of *A. japonicus*. These factors could affect the robustness of future adult populations.

Keywords: heavy rainfall, environmental stresses, coastal areas, early life stage

Introduction

Japanese sea cucumber, *Apostichopus japonicus* (Selenka, 1867), are widely found in the shallow temperate areas of the northwest Pacific, including the Bohai Sea and the Yellow Sea in China, the coast of Russia to the east, and the shores of Japan and Korea (Lu, 2015). In Japan, the spawning season of *A. japonicus* starts from March to May in southern Japan and from May to August in the northern region, when seawater temperature ranges between 18–25 °C (Katow et al., 2015; Lu, 2015). In Asian countries, *A. japonicus* is considered one of the main commercial species and valuable seafood (Lu, 2015). However, wild catches of *A. japonicus* have declined substantially over its distribution area since the mid-2000s (Purcell et al., 2013), with climate change being one of the primary causes. According to Pörtner and Peck (2010), climate change is expected to affect individual organisms, altering species populations at all phases

of their lives. In addition, the early life stages of marine invertebrates are considered the most sensitive when it comes to environmental stresses caused by global climate change, with many species showing low survival rates during development (Peck et al., 2013).

Extreme rainfall events occur more often due to climate change, which may impact coastal marine species during the breeding season (Manuel et al., 2020). Heavy rainfall causes turbidity levels to increase and rapid drops in salinity levels in the intertidal and shallow coastal areas (Raper and Braithwaite, 2006; Poloczanska et al., 2009; Corbari et al., 2016), suggesting that marine invertebrates living in these environments may be exposed to short-term instabilities that could be terminal. A few studies have reported the impact of changes in salinity or turbidity on marine invertebrate species in their early life stages, including the gastropod, *Nassarius reticulatus* (Linnaeus, 1758) (Génio et al., 2008), sand dollar,

Echinarachnius parma (Lamarck, 1816) (Allen and Pechenik, 2010), Manila clam, *Ruditapes philippinarum* (Adams & Reeve, 1850) (Arakawa et al., 2014), gastropod *Crepidatella fecunda* (Gallardo, 1979) (Montory et al., 2014), *Crepidatella peruviana* (Lamarck, 1822) (Montory et al., 2016), the sea urchin, *Heliocidaris crassispina* (Agassiz, 1864) (Mak and Chan, 2018) and disk abalone, *Haliotis discus discus* Reeve, 1846 (Manuel et al., 2020).

However, little is known about salinity and turbidity's influence on the development of *A. japonicus* in its early stages. Therefore, the present study aims to investigate the impact of short-term alterations in salinity or turbidity on *A. japonicus* eggs in terms of hatching success and survival. This study was carried out to best mimic typical conditions in coastal areas during a downpour of rain to which coastal marine species are exposed for a short period before returning to their normal environment.

Materials and Methods

Apostichopus japonicus eggs

Apostichopus japonicus eggs were acquired from the Mie Prefectural Hatchery in Japan. Normal developing eggs about 1 h after fertilisation were collected and transferred to the laboratory at Mie University by car and supplied for the present experiment. The cleavage stage eggs of echinoderm species, including *A. japonicus*, are considered more sensitive to environmental stress than other stages during their embryonic development (Lamare et al., 2014; Qui et al., 2015). Therefore, eggs of *A. japonicus* that developed to the cleavage stage were used in this study. A stereomicroscope (CKX53, Olympus, Japan) was used to observe and determine the developing stage of eggs.

Experiment 1: Impact of low salinity stress on hatching and survival rates of *A. japonicus*

Six levels of salinities (14, 18, 22, 26, 30 and 34 PSU (control)) were tested in six replicates. Seventy-eight eggs (cleavage stage) were stocked in each replicate and were placed into the plastic microplates with six wells containing 5 mL of 34 PSU seawater. Eggs were incubated under a photoperiod of 12 L:12 D (light: dark cycle) and a temperature of 22 ± 1 °C. Commercial sea-salt powder (LIVESea Salt, Delphi, Japan) was dissolved in distilled water to make seawater of various salinities. The short duration of heavy rainfall events that caused low salinity in the coastal areas tends to fluctuate between half an hour and 3 h (Llasat, 2001; Jaroszweski and McNamara, 2014). In a recent study, Marzuki et al. (2022) also reported that the peak time of short-duration rain events is around 3 h. In the present study, exposure of eggs to each salinity level lasted for 3 h, and then kept constant at a salinity of 34 PSU for the remaining time of the

experiment. An automatic pipette was used to achieve a gradual change and recovery of salinity. A stereomicroscope (CKX53, Olympus, Japan) was used to observe and record the hatching and survival rates.

Experiment 2: Impact of turbidity changes on hatching and survival rates of *A. japonicus*

Experiment 2 was also conducted with six replicates to determine the effects of different turbidity levels (0 (control), 100, 300, 500, and 700 NTU (Nephelometric Turbidity Units)) on the hatching and survival rates of *A. japonicus*. Seventy-eight eggs were stocked in each replicate in six-well plastic microplates following the same temperature and photoperiod conditions described in experiment 1. Different levels of turbidity solutions were prepared by dissolving kaolin clay ($\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$, particle with a diameter of 0.4 μm) in seawater (34 PSU). The change in turbidity level and exposure period were conducted the same way as in experiment 1. A shaker (SIA BIOSAN, Latvia) at a speed of 100 rpm was used to maintain the proper suspension of kaolin clay particles in the water column during the exposure time. Hatching and survival rates were also recorded for each of the treatments using the same criteria as in experiment 1.

Results

Experiment 1: Impact of low salinity stress on hatching and survival rates of *A. japonicus*

Hatching rates of the eggs recorded significant differences among treatments exposed to short-term salinity stress. Hatching rates at 30 and 26 PSU were significantly lower than that of the control (34 PSU) and significantly higher than 22 and 18 PSU (Fig. 1A). There were no significant differences between 30 and 26 PSU or 22 and 18 PSU. In the 14 PSU group, only 3.3 % of *A. japonicus* eggs hatched.

Salinity recorded a significant effect on the survival of larvae at the termination of the experiment. Significant differences between the control treatment and most other treatments were obtained. *Apostichopus japonicus* exhibited a 100 % mortality at 14 PSU and only 14 % of the larvae in the 18 PSU treatment survived (Fig. 1B).

Experiment 2: Impact of turbidity changes on hatching and survival rates of *A. japonicus*

The hatching rate of *A. japonicus* was significantly affected by turbidity. Hatching rates were considerably lower in the 100, 300, 500, and 700 NTU treatments than the control treatment (0 NTU) (Fig. 2a). No significant changes in hatching rate were obtained between 300 and 500 NTU treatments. Eggs exposed to a turbidity level of 700 NTU did not hatch.

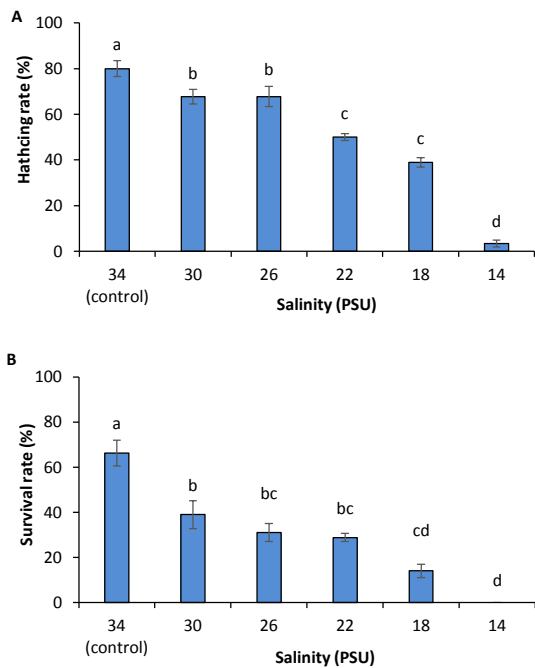


Fig. 1. Hatching rate (A) and survival rate (B) of *Apostichopus japonicus* larvae at 5 days post-hatch. Eggs were exposed to salinity levels indicated for 3 h and then returned to optimum level, control seawater (34 PSU). Values shown are mean \pm SE (n = 6). Different alphabets above the bars indicate significant differences among the groups ($P < 0.05$).

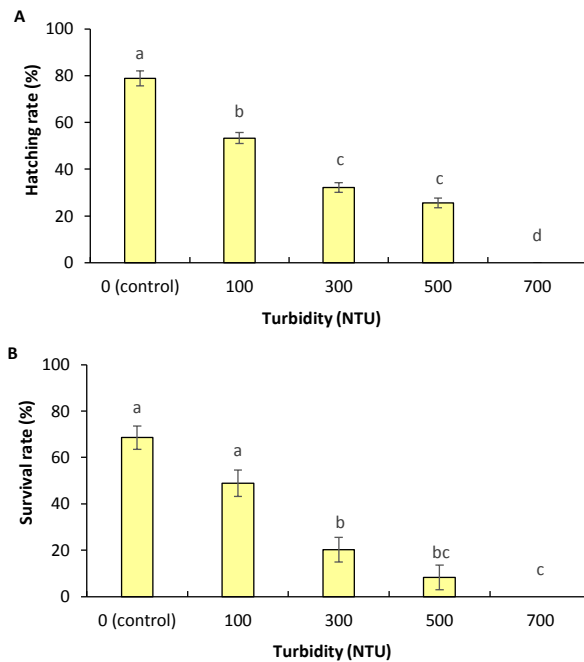


Fig. 2. Hatching rate (a) and survival rate (b) of *Apostichopus japonicus* larvae at 5 days post-hatch. Eggs were exposed to different turbidity levels indicated for 3 h and then returned to optimum turbidity level (0 NTU). Values shown are mean \pm SE (n = 6). Different alphabets above the bars indicate significant differences among the groups ($P < 0.05$).

At the end of the experiment, the survival rate showed a decreasing trend as turbidity increased. The survival rate in the control group was significantly higher than that of all treatments except for the 100 NTU treatment. Furthermore, *A. japonicus* recorded 100 % mortality in the 700 NTU treatment (Fig. 2b).

Discussion

Marine invertebrate embryos and larvae that inhabit coastal areas are affected by salinity drops and turbidity increases during the summer season (Kashenko, 2000; Todd et al., 2015). These factors are especially crucial for the embryonic development of invertebrate species during their spawning season. Chen and Chang (2015) reported that *A. japonicus* is a stenohaline species and decreasing salinity will result in a high mortality rate. In the present study, satisfactory development of *A. japonicus* embryos occurred in conditions close to spawning (temperature of 22 ± 1 °C and salinity of 34 PSU). The results showed that the hatching rate of *A. japonicus* was significantly reduced after short-term exposure to low salinity. Madrones-Ladja (2002) also found that the embryos of the windowpane oyster *Placuna placenta* (Linnaeus, 1758) did not progress through the gastrula stage at 16 PSU. At salinity less than 16 PSU, no development was detected. In addition, normal fertilisation and development of the polychaetes *Alitta virens* (Sars, 1835) (syn. *Nereis virens* Sars, 1835) and *Hydroides elegans* (Haswell, 1883) did not take

place under salinities of 18 and 20 PSU, respectively (Ushakova and Sarantchova, 2004; Pechenik, 2007). Moreover, tolerance to low salinity in *A. japonicus* offspring varied ontogenetically; for example, the blastula stage can survive in a salinity range of between 20.0 and 20.5 PSU (Qui et al., 2015). In the present study, fertilised eggs (cleavage stage) were used at the start of the experiments and almost all the eggs did not hatch at 14 PSU. On day 5 post-hatch, *A. japonicus* survival decreased with decreasing salinity levels.

In the natural environment, increased turbidity is a significant contributor to the decline in the density and biomass of invertebrates (Henley et al., 2000). Abrasion, interference with respiration and ingestion by clogging of filtration mechanisms and preventing the proper egg and larval development are some of the effects of turbidity on invertebrates (Berry et al., 2003). The impacts of increased turbidity are highly species-specific, but invertebrate eggs and larvae are considered the most vulnerable (Todd et al., 2015). The results for *A. japonicus* indicated that turbidity significantly affected hatching success and survival rates in this species, with significantly lower hatching rates under the higher turbidity treatments. In particular, *A. japonicus* eggs exposed to the turbidity of 700 NTU exhibited 100 % mortality. Arakawa et al. (2014) reported that the lower hatching rate of *R. philippinarum* was observed in higher turbidity conditions. Increased turbidity in the water column

resulted in decreased dissolved oxygen levels, which may contribute to hatching failure due to a reduction in oxygen availability for developing embryos, thereby preventing proper embryonic development (Henley et al., 2000).

Conclusion

The present study proves that the embryos of *Apostichopus japonicus* are susceptible to salinity and turbidity changes. Both salinity and turbidity had a detrimental effect on the hatching rate, subsequent embryonic development, and survival of larvae of this species. These impacts resulted in higher mortality in the early developmental stages, resulting in a decline in the population of *A. japonicus* in the natural environment. The Japan Meteorological Agency (2018) predicted that the intensity and frequency of extreme precipitation events have been increasing globally, suggesting that reduced seawater salinity and increased turbidity could become a more common phenomenon. Therefore, future work should seek to identify the potential influence of salinity and turbidity changes on the development, survival, and settlement of larvae of this species.

Acknowledgements

The authors would like to acknowledge the Mie Prefectural Hatchery and its staff for providing fertilised eggs of sea cucumber to carry out this study.

Conflict of interest: The authors declare that they have no conflict of interest.

Author contributions: Phan Thi Cam Tu: Conceptualisation, methodology, investigation, validation, formal analysis, writing - original draft, review and editing. Albert Valdish Manuel: Methodology, investigation, writing - review and editing. Naoaki Tsutsui: Methodology, investigation, validation, resources, formal analysis, writing - review and editing. Truong Giang Huynh: Formal analysis, review. Takao Yoshimatsu: Conceptualisation, methodology, investigation, resources, formal analysis, writing - review and editing, supervision.

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