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RESEARCH ARTICLE

Effect of abrupt salinity change in the survival of Asian green mussel *Perna viridis* (Linnaeus, 1758) spats

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ABSTRACT

Salinity is one of the key environmental factors that affects the growth and survival of marine organisms including mussels. Five different salinity levels (40, 30, 20, 10 and 5 ppt) were used to test the effect of abrupt salinity change in the survival of hatchery-produced spats of Asian green mussel *Perna viridis* (Linnaeus, 1758). Spats were stocked with a density of 30 individuals per 6-L tank. Salinity manipulation was conducted after 2 days from the date of stocking. Based on the results, abrupt change and prolonged exposure to lower salinities particularly of 5 and 10 ppt (up to 5 days) are detrimental to *P. viridis* spats. The critical time for *P. viridis* spats wherein they could seclude themselves from the persistent lower salinities is 28 hours from its exposure. After which, mortality could be high at about 50% and will continue in the succeeding days if low salinity persists. Critically, no single spat can survive until the 4th day of continuous exposure to very low salinity of 5 ppt. Nevertheless, surviving individuals could still recover if salinity will return to optimum levels. Additionally, spats can readily adjust to abrupt change up to 10 ppt from the optimum salinity level as seen in the high survival in 20 and 40 ppt.

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Introduction

Asian green mussel *Perna viridis* (Linnaeus, 1758) is a marine bivalve species belonging to family Mytilidae. The species is widely distributed along the coasts of India in the Indo-Pacific and in Southeast Asia (Rajagopal et al., 2006), and has been introduced in the coastal areas of the western Hemisphere including Venezuela, Trinidad, Jamaica and Florida in the United States (Ingrao et al., 2001). Its habitats range from oceanic to estuarine waters (McFarland et al., 2013), usually at a depth of less than 10 m (Iqbal et al., 2018). Aside from its ecological importance, the species is an important seafood resource and is extensively cultivated for commercial purpose especially in Southeast Asia. This species is now considered a cheap source of protein with high nutritional values and known for its palatable taste (Taib et al., 2016).

Salinity is one of the key environmental factors that affects the growth and survival of marine organisms, particularly in shallower waters where sessile organisms such as mussels occur (Wang et al., 2011; Wu et al., 2018). In the case of *P. viridis,* the optimal salinity ranged from 27 – 35 ppt (Aypa, 1990; Rajagopal et al., 2006). However, in estuarine and coastal systems where these mussels typically occur, salinity fluctuations are considerable. During freshwater influx and heavy rains which decrease the water salinity in these areas, mussel growth and survival are negatively affected (Cheung, 1991; Soon & Ransangan, 2016; Wang et al., 2012). In the Zhoushan islands sea area, mussels often experience short-term salinity and pH fluctuations from 15-35 ppt and 7.3–8.1, respectively, leading to increased mortality of the organisms during wet season (Wu et al., 2018).

In the Philippines, *P. viridis* is widely cultured for commercial purpose (Aypa, 1990). The aquaculture production of green mussel in 2018 reached to 26,302.77 mt valued at 9.8 M USD (PSA, 2019). However, the production of this species in the recent years appeared to be declining (Duncan et al., 2009), and this decline was linked to the decreasing spatfall in the wild (Mero et al., 2019). The supply of seed called spats for the culture of *P. viridis* is dependent from the natural environment in which seed stock is unstable. Sustainable seed supply of cultured mussel species such as *P. viridis* is a crucial component for the success of the shellfish industry. With the aim to augment the seed supply and lessen the reliance of mussel farmers from getting their seeds in the wild, the Philippine government invested for a mussel hatchery in 2014 (Mero et al., 2019).

The natural habitat where *P. viridis* is grown is exposed to constant environmental changes, hence, it is important to know how the hatchery-produced spat may be affected particularly by salinity fluctuations. This study aims to determine the survival of hatchery-produced *P. viridis* spats subjected to abrupt changes in salinity, and determine how long these spats could withstand salinity stress.

Materials and Methods

Experimental Animal and Study site

This study was conducted in September 2019 in the Green Mussel Hatchery and Nursery (GMHN) of the Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo. The *P. viridis* spats used in the experiment were produced in the same hatchery. These spats were one and a half months old with sizes ranging from 5-9 mm shell length (widest part from the posterior to the anterior part of the shell) measured using a plastic Vernier caliper.

Experimental Design

The effect of abrupt salinity change in the survival of *P. viridis* spats was tested using five salinity levels (40, 30, 20, 10 and 5 ppt). Each salinity level served as treatment with three replicates. The 30 ppt treatment being within the optimum salinity range for the species (Aypa, 1990) served as control, so that its salinity was not changed the entire experimental period which lasted for 10 days.

This experiment was conducted in 6-L plastic rectangular tanks. Each tank contained 4 L of water as its final volume having a specific salinity (i.e., 40, 30, 20, 10 and 5 ppt). Nevertheless, all treatments had an initial salinity of 30 ppt. The spats were stocked with a density of 30 individuals per tank. Salinity manipulation (e.g., from 30 ppt to 40 ppt) was conducted after 2 days from the date of stocking.

Following the methods of Ponce-Palafox et al. (1997) and Su et al. (2010), the salinity was increased (for 40 ppt) by progressively adding salt solution using sea salt (bought from the local market) to a separate water stock tank until the desired salinity was obtained, and was left aerated. On the other hand, salinity was decreased (20, 10 and 5 ppt) by adding an aerated tap water to the tanks. The salinities of the stock tanks for seawater as well as the experimental tanks were checked prior to the start of the experiment and in the morning of the succeeding days using a handheld refractometer (ATAGO). The addition of either salt solution or freshwater to the

experimental tanks to abruptly change the salinity was done gradually within 30 minutes (only during the onset of the salinity change). The time frame follows what was recorded by Casila et al. (2017) in Shakuji river estuary, Japan where salinity fluctuated within 30 minutes after freshwater intrusion. Respective salinities (40, 30, 20, 10 and 5 ppt) were maintained for 5 days. On the 6th day, salinities of all treatments except for the control were abruptly returned to 30 ppt and maintained until the end of the experiment to check if the spats could survive or recover after being subjected to abrupt salinity change (lower or higher) and abrupt return to optimal condition. The water stock tanks and the experimental tanks were kept aerated through the centralized aeration system of GMHN until the end of the experiment.

The spats were fed with algae, *Isochrysis galbana* and *Tetraselmis tetrathele* which were given twice daily at 8:00 -9:00 in the morning and 4:00-5:00 in the afternoon. The daily food ration was calculated based on the formula provided by Helm & Bourne (2004) as follow (Eq. 1):

$$V=\frac{S\times 0.4}{W\times C} \quad (1)$$

where V is the volume of algae in liters to be fed, S is the live weight of spat in mg, W is the weight of the required algal cells, C is the algal density (cells per µL), and 0.4 refers to the ration as dry weight of the algae in mg per mg live weight of the spat. The algae were sourced from the Southeast Asian Fisheries Development Center Aquaculture Department and sub-cultured in GMHN following the methods of de la Peña & Franco (2013). With the use of UV filtered seawater, algae were batch cultured in 800 mL glass bottles and to 10 L plastic carboys. All cultures were enriched with Conwy, and were subjected to 24 hours constant illumination with continuous aeration. Water exchange for all experimental tanks was done every after 2 days.

Data Collection and Analysis

Monitoring though direct observation and with the aid of a video recorder was conducted in 1, 4, 6, 8, 12, 24 and 28 hours after salinity had been changed. For the succeeding days, monitoring was conducted once daily (morning), that is before feeding to check for mortalities. Dead individuals if present were removed immediately to prevent degradation of water quality, and were accounted for later analysis. An individual was considered dead if it remained gaping but did not respond within 15 seconds when gently poked by a needle following the method of Segnini de Bravo et al. (1998), Yuan et al. (2016) and Binzer et al. (2018).

Survival rate was calculated by the final number of surviving spats divided by the initial number of spats (stocking density), multiplied by 100 following Vu & Huynh (2020). Gaping percentage was calculated as the number of individuals with valves open divided by the number of surviving individuals during the latest monitoring multiplied by 100. These data are the ones used in the statistical analysis. Gaping as used in this paper referred to the spat which gapes and responds to stimuli particularly to the gentle poke using a needle during a specific monitoring period.

One-way Analysis of Variance (ANOVA) was used to determine significant differences in the survival and gaping among treatments and Tukey's Test for post-hoc. All analyses were done in JASP 0.16.

Results and Discussion

Green mussel spats used in this experiment generally showed a wide range of salinity tolerance similar to other previous reports (Segnini de Bravo et al., 1998; Segnini de Bravo, 2003; Rowchai, 2004; McFarland et al., 2014). The study of McFarland et al. (2014) showed that *P. viridis* were able to tolerate low salinities up to 6 ppt when subjected to gradual salinity change. However, acute salinity changes of 15 ppt or more resulted to low survival. Rowchai (2004) recorded only 26.25% mortality from 15 ppt salinity treatment. Thus, the author suggested to set the lower salinity threshold for *P. viridis* at 15 ppt. Segnini de Bravo et al. (1998) reported a higher salinity tolerance of *P. viridis* under a gradual change of salinity level at 2 ppt every 2 days. The low and high lethal salinities for the species were recorded at 0 and 64 ppt, respectively. The report of Segnini de Bravo (2003) also proven the adaptability of *P. viridis* to salinity fluctuations. The species exhibited physiological compensation to increase (up to 45 ppt) or decrease (up to 15 ppt) in salinity under a change rate of 1 ppt per day.

Survival rate and response in terms of valve opening in different salinities are shown in Figure 1. Generally, survival rate is lower in reduced salinities (i.e., 10 and 5 ppt) and higher in higher salinities (i.e., 40, 30 and 20 ppt). From the time of salinity change, no single individual was observed having its valves open until 24 hours in 5 ppt and 8 hours in 10 ppt. This suggests that the spats from these treatments are negatively affected by such low salinity, prompting them to seclude

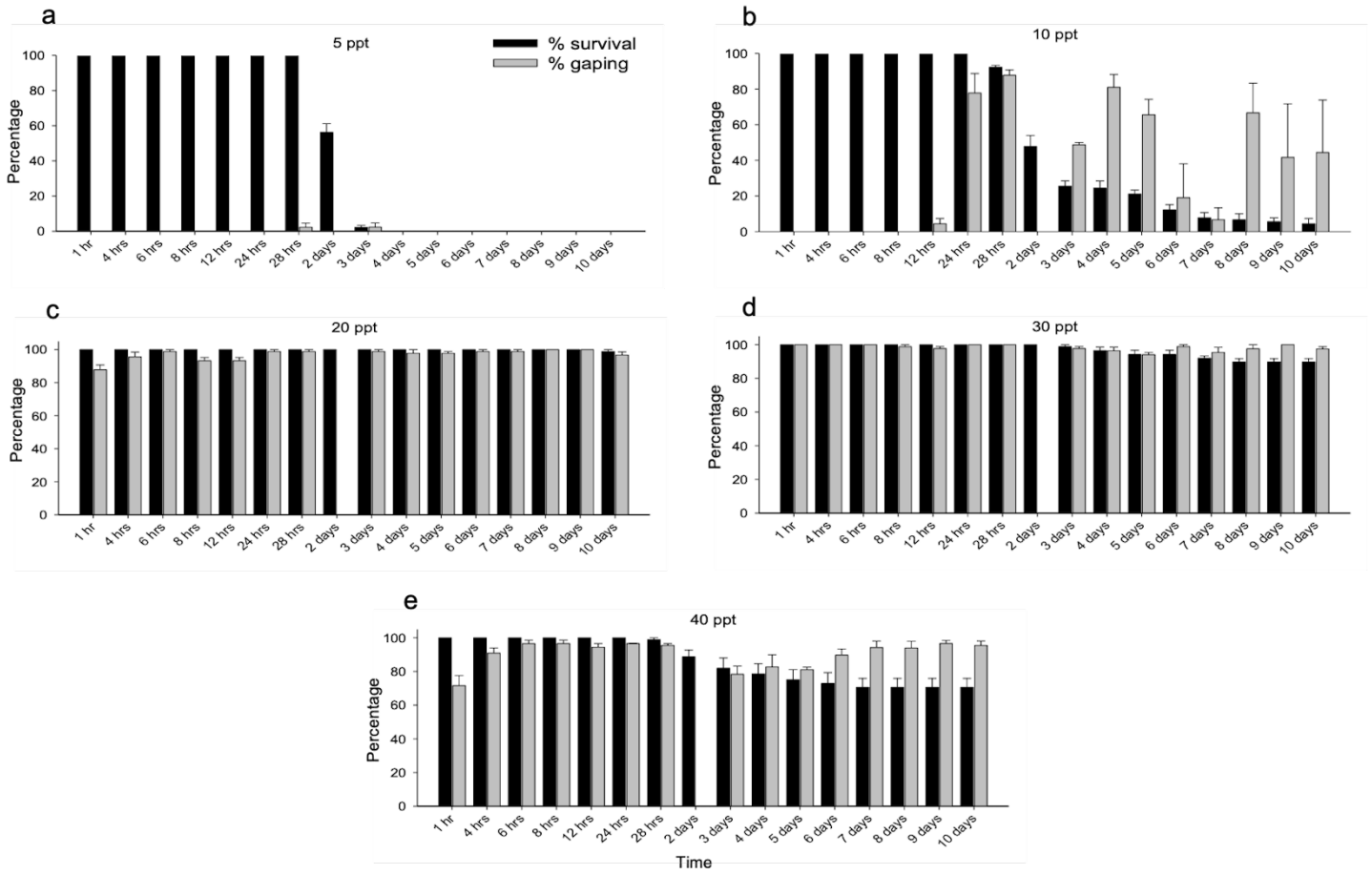

Figure 1. Survival and gaping percentage of *P. viridis* spats (mean±SEM) per salinity treatment. For the first 5 days, salinity was abruptly changed to: a) 5 ppt, b) 10 ppt, c) 20 ppt and e) 40 ppt (30 ppt served as control, thus salinity was not changed). On the 6th day, salinity of all the 4 treatments were abruptly returned to 30 ppt.

themselves by tightly closing their valves. During monitoring on the 28th hour, about 2% of the spats from 5 ppt (Figure 1a) were observed already gaping, and this very low percentage remained until all individuals died on the 4th day. This basically implies, that drastic decrease of salinity into very low level such as 5 ppt would lead to massive mortality of *P. viridis*. This may be true in the wild conditions especially during prolonged heavy rains wherein salinity could drop to certain low level. On the other hand, about 4% were gaping from 10 ppt during the 12th hour monitoring and increased to 77.78% in the 24th hour (Figure 1b). Mortalities started on the 28th hour and continued until only 21.11% remained surviving on the 5th day when salinity remained at 10 ppt. It seems that *P. viridis* could somehow tolerate 10 ppt salinity level but only for a short period of time. Long time exposures could also be detrimental to the organism. It also took about 12 hours adjustment before most could open their valves. When salinity was returned to 30 ppt, mortalities still continued until only 4.44% left surviving on the 10th day. In addition, not a single monitoring time, that spats from 10 ppt were seen 100% gaping. It seems that salinity adjustment ability of *P. viridis* is impaired when it was previously subjected to stressful low salinity such as 10 ppt.

For higher salinity treatments, high percentage of spats remained gaping despite salinity change. In 20 ppt (Figure 1c), 87.78% remained its valves open during abrupt salinity change. Gaping percentage for this treatment remained high varying from 93.33%–100% until the end of the experiment, and despite the salinity was abruptly returned to 30 ppt from 6th–10th day. No mortality occurred in this treatment except for one individual on the 10th day. This shows that 20 ppt is still optimum for the survival of *P. viridis* spats.

For the 30 ppt (Figure 1d), as expected, all spats remained gaping even after water change since the salinity was constant and within the optimum range for *P. viridis*. Though very little percentage (2.22% at most) were observed closed during monitoring periods. On the other hand, neglectable mortalities occurred within the 3rd to 8th day. For 40 ppt (Figure 1e), only 71.61% were observed gaping after salinity was increased. On the 4th hour, gaping percentage increased to 95.55%. High gaping percentage remained high varying from 94.37% to

96.59% until the 28th hour. However, gaping percentage dropped to 80% from the 3rd until 5th day. When the salinity was returned to 30 ppt on the 6th day, gaping increased to 89.67% and remained high. Survival became stable from the 7th to the 10th day, which may imply that the organisms were now adjusted to the present salinity condition.

Effect of Abrupt Salinity Change in the Survival and Valve Opening of P. viridis Spats

In this study, abrupt change of salinities at various levels significantly affected the valve opening and survival of *P. viridis*. On the 1st hour (Figure 2a), gaping percentage was highest in 20 and 30 ppt followed by 40 ppt, whilst individuals from 5 and 10 ppt were not able to open its valves. On the 4th hour (Figure 2b), gaping individuals remained highest in 20 and 30 ppt, while more individuals gaped in 40 ppt making its percentage comparable to 20 ppt, whilst still no individuals gaped in 5 and 10 ppt.

On the 6th, 8th,12th and 24th hour, percentage of gaping individuals did not vary among 20, 30 and 40 ppt, whilst still no gaping individuals were observed in 5 and 10 ppt, except a few from the later on the 12th hour. Similarly, McFarland et al. (2013) observed that *P. viridis* tended to close its valves at lower salinities of 10 and 15 ppt, this in spite of the fact the organisms used in their study were already adults with shell lengths ranging from 90-110 mm.

Stressful environmental condition such as salinities outside the optimum range of bivalves including *P. viridis* would trigger the organism to close its valves to seclude and protect itself from the external environment (McFarland et al., 2013). But this closure as defense mechanism especially when prolonged could lead to serious implications in the organism's being. Valve opening or gaping has a strong influence in many physiological processes in bivalve species (Nicastro et al., 2010). Bivalves have to open its valves for water filtration, feeding and respiration (Ballesta-Artero et al., 2017). Thus, valve closure results to cessation in feeding and gas exchange with the external environment. When the mussel stops feeding, it may use some stored energy which will eventually run out. On the other hand, since the organism can't exchange gas with the external environment, prolonged valve closure would lead to anoxic respiration. These could then lead to fatality of the organism. In the study of Comeau et al. (2018), it showed that raft-cultivated mussel *Mytilus galloprovincialis* gapes most of the time (97.5±1.3%) within the entire 10 days of monitoring on the gaping behavior of the organism. However, the reason of the occasional closures is not clear. It was also noted that valve closures are not synchronized among individuals, similar to *P. viridis* used in this study. Comeau et al. (2018) also noted that valve closure was physiologically regulated rather than environmentally mediated.

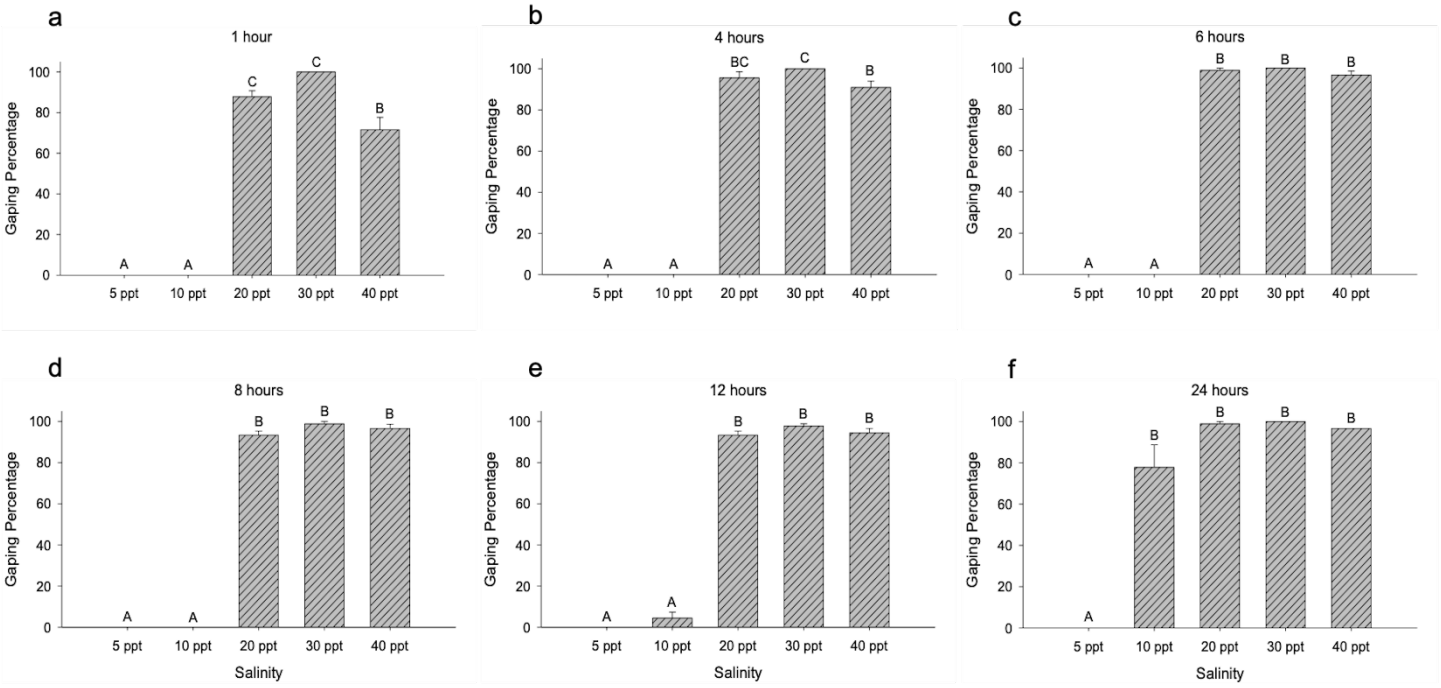

Figure 2. Gaping percentage of *P. viridis* spats (mean±SEM) at different salinity levels after: a) 1 hour, b) 4 hours, c) 6 hours, d) 8 hours, e) 12 hours and f) 24 hours, from the time that salinity was abruptly changed at different salinities, except 30 ppt which served as control. Means of different subscripts indicate a significant difference (p<0.05).

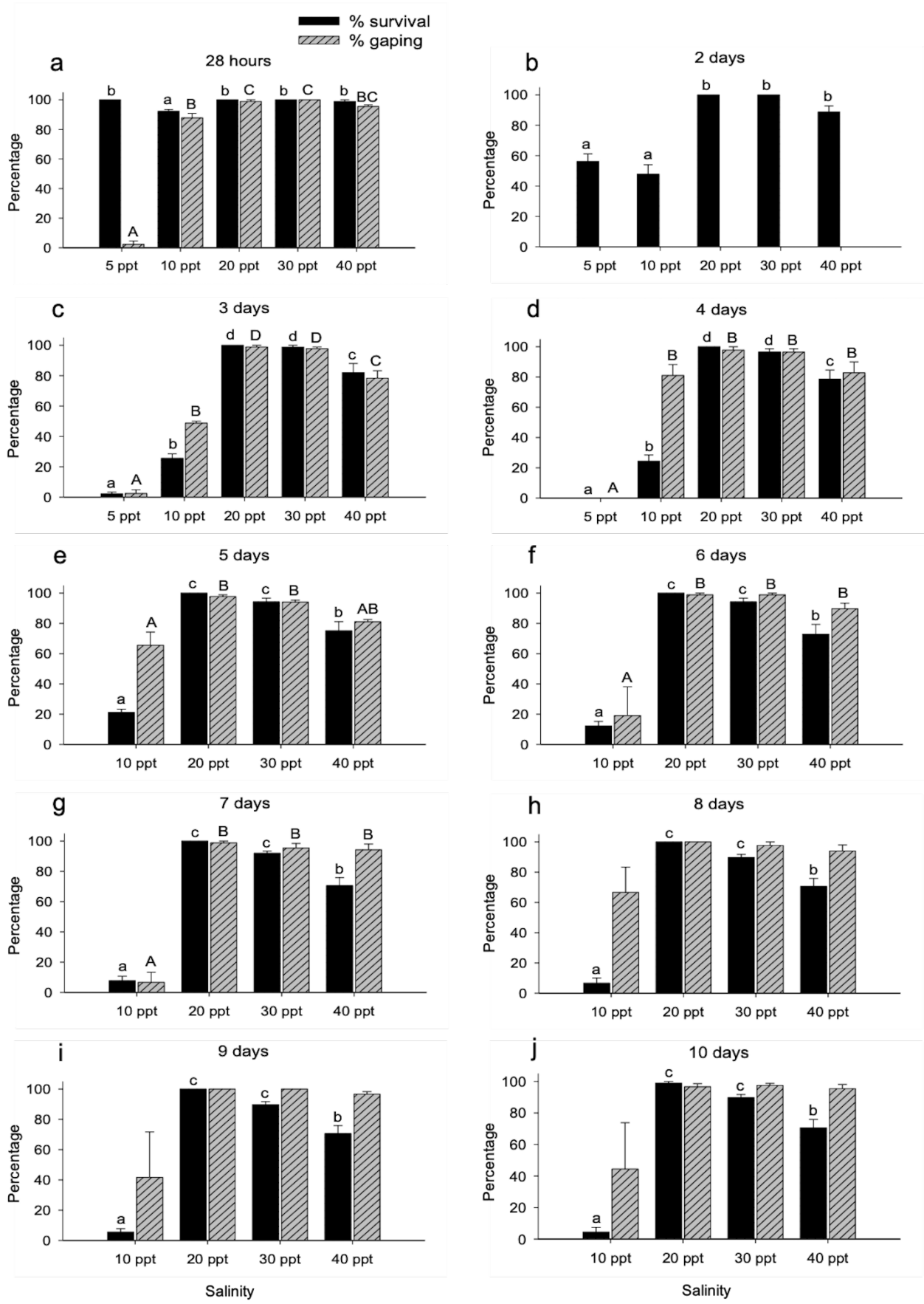

Figure 3. Survival and gaping percentage of *P. viridis* spats (mean±SEM) at different salinity levels after: a) 28 hours, b) 2 days, c) 3 days, d) 4 days, e) 5 days, f) 6 days, g) 7 days, h) 8 days, i) 9 days and j) 10 days. For the first 5 days, salinity was abruptly changed to: 1) 5 ppt, 2) 10 ppt, 3) 20 ppt and 4) 40 ppt (30 ppt served as control, salinity was not changed). On the 6th day, Salinity of the all 4 treatments were abruptly returned to 30 ppt. Means of different subscripts indicate a significant difference (p>0.05).

The valve closure may be induced by a full gut or some likely mechanism. On the other hand, this study noted that a gaping individual once closes its valves re-opens for a split to very few seconds particularly in the higher salinity levels (i.e., 20, 30 and 40 ppt).

Mortalities based on the indicator that valves of an individual are open but unresponsive to stimuli started on the 28th hour (Figure 3a), particularly at 10 ppt. Thus a 100% survival in the first 24 hours was assumed. On the 28th hour, some individuals died in 10 ppt so that its survival rate becomes significantly lower than the rest of the treatments (p<0.01). A significantly lower gaping percentage was also recorded in 10 ppt compared to the 3 higher salinities. On the other hand, a significantly few individuals from 5 ppt were observed gaping during this period. These few individuals may have forced themselves to open their valves as they are already starving or needing for gas exchange, but were not able to withstand such low salinity, thus reclosed and died subsequently. On the 2nd day (Figure 3b), mortalities from 5 and 10 ppt becomes conspicuous at about 50% giving a lower survival rate compared to the 3 higher salinities. From the 3rd until 10th day (Figure 3c- 3j), survival from 20 and 30 ppt remained significantly highest, followed by 40 ppt and 10 ppt. In terms of gaping percentage, this was highest in 20 and 30 ppt followed by 40 ppt, then 10 ppt and 5 ppt. On the 4th day (Figure 3d), gaping percentage in 5 ppt became zero (0) since all individuals were found dead on that day, whilst no significant difference (p>0.05) was found among higher salinities (10, 20, 30 and 40 ppt). The 5 ppt treatment was no longer included in the analysis from the 5th until the 10th day (Figure 3e- 3j) given that there was no more representative sample. These results are similar to the report of McFarland et al. (2014) where mussels that were immediately transferred from 30 ppt to 5 and 10 ppt salinity treatments were all dead within 13 days. Wider salinity difference even within a gradual change scheme also rendered significant mortality up to 100% in 3 ppt within 13 days and 47% mortality in 6 ppt within 28 days. Decreased byssal attachment and increased valve closure where also observed for *P. viridis* at 3 and 6 ppt. McFarland et al. (2013), reported that all individuals in lower salinities (10 and 15 ppt) remained either closed or dead after 120 hours. Rowchai (2004) also observed 100% mortality from 5 and 10 ppt within 72 hours. These mortalities are attributed to the persistent unfavorable water condition such as very low salinity that instigates prolonged valve closure and disables the ability of the organism to remain open long enough for feeding and sufficient gas exchange (McFarland et al., 2013). On the other hand, Segnini de Bravo et al. (1998) recorded a low mortality of *P. viridis* under gradual salinity change at 2 ppt every 2 days. The observed mortalities were only 2% and 4% from 4 ppt and 0 ppt salinity treatments, respectively.

On the 5th day (Figure 3e), gaping percentage was still significantly higher in higher salinities (20 and 30 ppt) except for 40 ppt which did not differ significantly with 10 ppt but comparable with 30 ppt. On the 6th until the 7th day (Figure 3f-3g), when salinities were abruptly returned to 30 ppt, gaping percentage were still significantly higher (p<0.01) among individuals from higher salinity treatments (20, 30 and 40 ppt) than 10 ppt. On the 8th until the 10th day (Figure 3h- 3j), gaping percentages were no longer significantly different among treatments (p>0.05), noting that there were only less than 10% surviving individuals in 10 ppt. Survival and gaping activity generally stabilized in the 7th to the 10th day (Figure 3g- 3j), wherein salinities were already returned to 30 ppt. McFarland et al. (2013) noted that gaping individuals are significantly higher in higher salinities of 25 and 35 ppt. McFarland et al. (2014) observed a high survival of *P. viridis* (more than 85%) despite drastic change provided that the salinity difference is within 10 ppt (i.e., from 30 ppt to 20, 25 and 35 ppt). Given a gradual salinity change, *P. viridis* was more tolerant with 97% survival at salinity treatments of 9 ppt and higher. Comparably, 100% survival at 20 and 25 ppt was obtained in the experiment of Rowchai (2004). In this study, hatchery produced spats can readily adjust to abrupt salinity changes up to 10 ppt.

Conclusion

Abrupt change and prolonged exposure to lower salinities particularly, 5 and 10 ppt are detrimental to *P. viridis* spats. The critical time for *P. viridis* spats wherein they could seclude themselves for the persistent lower salinities is 28 hours from its exposure. After which, mortality could be high at about 50% and will continue in the succeeding days if low salinity persists. On the other hand, if salinity will return to optimum levels, surviving individuals may recover. This study shows that hatchery-produced spat has also a wide range salinity tolerance wherein it may able to survive salinities outside its optimum range as low as 10 ppt and as high as 40 ppt, though a very high mortality may occur in the former. Additionally, hatchery produced spats can readily adjust to abrupt salinity change of up to 10 ppt (e.g., from 30 ppt to 20 ppt and 40 ppt).

The results of this study show the resilience of hatchery produced spats from drastic salinity fluctuations that typically happens in the natural environment. This study also gives idea on the salinity threshold of hatchery-produced spats that will

serve as guide especially in the selection of site where this mussel species is to be transplanted for grow-out.

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Compliance With Ethical Standards

Authors' Contributions

RMPG and LVL conceptualized and designed the experiment. RMPG conducted the experiment, analyzed and interpreted the data, and wrote the draft of the manuscript. LVL reviewed and edited the manuscript. Both authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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