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ORIGINAL RESEARCH

Influence of water management, photoperiod and aeration on growth, survival, and early spat settlement of the hatchery-reared green mussel, *Perna viridis*

Fedelia Flor C. Mero · Fiona. L. Pedroso · Mary Jane S. Apines-Amar · Josel F. Cadangin · Donna C. Rendaje · Christine S. Verde · Jean Rose H. Maquirang · Lily Anne G. Piñosa

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Abstract In an attempt to induce early spat settlement and improve mussel seed production, this study aims to determine the influence of water management, photoperiod, and aeration, on the growth, survival and settlement of green mussel (*Perna viridis*). Water in the pediveliger rearing tanks was changed every day, every 3 days and every 5 days for the water-management experiment. Pediveligers were exposed in 24L:0D h (light: dark), 12L:12D h and 0L:24D h conditions for the photoperiod experiment. Three aeration intensities were also tested—mild (10 L h^{-1}), moderate (20 L h^{-1}), and strong (30 L h^{-1}). This study demonstrated that changing water every 3 days was effective in maintaining the rearing water quality and improving the growth and survival of *P. viridis* larvae. Highest growth and survival rates were observed in *P. viridis* spats grown in 0L:24D h photoperiod. There was no significant difference in the settlement rate of larvae exposed to different photoperiods. Mild aeration has shown to improve the growth of *P. viridis* larvae, but higher survival and settlement rates were attained in the strongly-aerated conditions. Therefore, when the larvae start to settle, it is recommended to expose them to darkness, change the water every 3 days and provide a strong aeration to be able to attain high survival and settlement rates, and bigger spats.

Keywords Ammonia · Bivalve · Ingestion rate · Light · Pediveliger · Settlement · Water exchange

Introduction

Asian green mussel *Perna viridis*, or simply green mussel is a large mytilid bivalve present along Southeast Asian region encompassing countries like Thailand, Indonesia, Philippines, and in the Indian peninsula (Sallih 2005; Vakily 1989; Hickman 1992). Its recent global aquaculture production data reaches to 146,815 mt (FAO 2019). In the Philippines, mussels are considered economically important bivalve species with more than 50 years of grow-out aquaculture history (Duncan et al. 2009). According to the latest report of the Philippine Statistics Authority (2018), green mussels contribute 19,208.62 mt of the total aquaculture production of the country valued at 6.93 M USD. However, in recent years, production trend seemed to be declining (Duncan et al. 2009) and some authors (Alfaro et al. 2010; Laxmilatha et al. 2011) associated this decline to the

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F. F. C. Mero · Fiona. L. Pedroso (✉) · M.
J. S. Apines-Amar · J. F. Cadangin · D. C. Rendaje · C. S. Verde · J. R. H. Maquirang · L. A. G. Piñosa
Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, 5023 Iloilo, Philippines
e-mail: fionap_06@yahoo.com.ph

diminishing spatfall in the natural environment. Factors affecting this trend were hypothesized as a result of global warming, pollution, and other anthropogenic activities (Cebu and Orale 2018; Wang et al. 2011; Vijayavel 2010).

In 2014, the Philippine government invested to put the first mussel hatchery in the country to produce seeds for local mussel growers to augment reliance in the wild. With that, there is now a focus to yield hatchery-reared spats. Basic mussel hatchery operation includes spawning, larval rearing including transition stage, and nursery rearing. Mussels are broadcast spawners and gametes are left to fertilize in the water until eggs develop into larvae (Walter 1982). Larval development further continues up to 15–20 days—a critical stage in the mussel life history wherein high mortality occurs in the transition from being planktonic larvae to a sessile young spat which has a characteristic like that of an adult mussel (Power et al. 2004). Larvae ready to settle are called pediveligers (Laxmilatha 2013). Pediveligers are footed larvae that develop at day 12–15 (Manoj and Appukuttan 2003). Pediveligers move by crawling with their foot or drifting through the water using their own mucus as parachutes (Soon and Ransangan 2014).

A plethora of available information from published works suggests factors affecting larval settlement and metamorphosis on mussel and other bivalves (Rittschof et al. 2009; Soon and Ransangan 2014; Alfaro 2005; Alfaro et al. 2006; Rajagopal et al. 1998); however, no known answer was given to solve their complex settlement behavior. In the current paper, three possible factors were investigated to check their effects on growth, survival and settlement rates of green mussel namely, water exchange, photoperiod, and aeration. Water-management strategies during the pediveliger to spat stage have not yet been established. During a water change, incoming water might result in changes in pH, dissolved oxygen and temperature levels which may induce stress to the organisms (Davidson et al. 2009). Frequent water exchange lessens water-quality deterioration brought about the accumulation of uneaten feeds and feces (Kinne 1976); however, it may induce mechanical disturbance to the larvae (Florida Clam Industry 2015). Water-quality parameters outside of the normal range may result in the poor performance of the larvae such as poor shell formation and poor growth (Florida Clam Industry 2015). Meanwhile, photoperiod refers to the time that an organism is exposed to light in a 24-h period and its effect has been tested in some other bivalves (Hastings 2001; Fabioux et al. 2005; Domínguez et al. 2010; Pilate et al. 2014). Moreover, it has been known that photoperiod has shown to regulate physiological changes in invertebrates (Calow 1981). Photoperiod may be particularly useful in hatchery settings as it can be easily controlled and it has a significant impact on the vulnerable early life stages such as larvae and spat. Lastly, aeration is also an important parameter that should be considered although its relationship to larval settlement of *Perna viridis* is not yet clearly established (Quayle and Newkirk 1989). In preliminary trials, aeration lines or airstones were observed to have a large number of attached spats than in any other portions of the rearing tanks. This leads to the hypothesis that air bubbles causing water circulation affect larval settlement and survival.

To date and to the knowledge of the authors, no specific work defines the effects of environmental factors—water exchange, photoperiod, and aeration—on growth, settlement and survival of hatchery-reared green mussel, hence this study.

Materials and methods

Experimental protocol

The study was conducted at the Mussel Hatchery, Institute of Aquaculture Multi-species Hatchery, College of Fisheries and Ocean Sciences, University of the Philippines Visayas (UPV), Miagao, Iloilo. Mussel spawners with shell length (SL) of > 60 mm were harvested in raft cultures of Brgy. Baybay and Brgy. Culajao, Roxas City Capiz and transported to the hatchery.

Upon arrival, mussels were cleaned to remove bio fouling organisms and other debris, kept undisturbed overnight in the conditioning tanks with water temperature of 22–24 °C. Spawning procedures were based and slightly modified from the work of Anil et al. (2017). Spawning was induced through thermal stimulation. Briefly, mussels were desiccated or exposed to air for 60 min and then influx of slightly elevated water temperature at 28–30 °C was used. Gametes released observed to be within 1 h after exposure to the water. Released eggs were incubated in 1-ton capacity fiberglass tanks at a density of 20-30 eggs per ml. Twenty-four

to Thirty six hours post-fertilization, D-shaped larvae were harvested and reared continuously for another 13 ± 2 days in the same tank at a density of 10 larvae ml^{-1} until larvae reached the pediveliger stage. Pediveligers with well-developed foot, swimming and crawling alternately with > 250 µm shell length were used in the experiments.

During the course of all the experiments, pediveliger larvae were stocked at 2 larvae ml^{-1} provided with black nylon nets as substrates. Growth was evaluated by measuring the final shell length and height of the mussel spats having 25 samples per treatment viewed under a compound microscope (4 × magnification). Images were taken using Moticam® Digital 10MP microscope camera and measured (in µm) using Motic® Images Plus 2.0 Software. Shell length was measured along the antero-posterior margin of the shell while shell height was measured along the dorso-ventral axis (Aarab et al. 2013). For all experiments, growth and survival were assessed after 15 days rearing period. Growth, percentage survival, and settlement were noted and recorded as mean ± SEM. The experimental treatments were done in triplicates and followed a Complete Randomized Design (CRD).

Water quality parameters

Some water parameters such as dissolved oxygen, temperature, pH and salinity were measured and recorded daily using YSI multi-parameter instrument. For determination of ammonia level and bacterial count in the water-exchange experiment, water samples were sampled daily before a water change to determine the total ammonia nitrogen (TAN) using the methods described by Fortes (1994) and bacterial total plate count (TPC).

Feeding protocol

For all the experiments, pediveligers were fed with microalgae *Isochrysis galbana* at an initial density of 12,000 cells $larvae^{-1}$ day^{-1}. The quantity of algae to be fed was increased gradually until reaching 100,000 cells $larvae^{-1}$ day^{-1} in day 15 (Wang et al. 2018). The cell concentration of the microalgae was determined by counting a subsample using a haemacytometer (Neubauer Improved Brightline, Marienfield, Germany). The required quantity of feed was taken from the freshly harvested cultures from the algal culture room, acclimatized to the ambient water temperature conditions, passed through a 23 µm sieve and poured uniformly into the rearing tank.

Water management experiment

Experiment on the effect of water management was conducted in 100-l capacity conical fiberglass tanks with each treatment replicated thrice with moderate aeration. UV-filtered seawater was used. To compare growth and survival of pediveligers to spat, 70% of water was changed every day (Sahavacharin et al. 1988), every 3 days (Helm and Millican 1977) and every 5 days (Florida Clam Industry 2015). A 120-µm sieve was used as a filter to siphon water and excess feeds, leaving the larvae inside the tank.

Photoperiod experiment

The effect of photoperiod was conducted in 30 L capacity plastic tanks containing 20 L of UV-filtered seawater. Each treatment was replicated thrice. Moderate aeration was provided in these experimental set-ups and water change was done every 3 days. To compare the settlement, survival, and growth of settling *P. viridis* larvae, photoperiod in light: dark (LD) cycles of 0:24 h, 12:12 h (natural lighting/control) and 24:0 h were evaluated. In the treatment receiving 0 light, the setup was covered with black canvas to prevent the entry of light. On the other hand, 24:0 LD treatment was supplied with 300-lux (Toledo et al. 2002) LED bulb lighting for 12 h. The desired light intensity for this treatment was measured using the probe of a light meter (LI192SA Underwater Quantum Sensor; LI-COR Inc., Lincoln, NE, USA) positioned at the middle layer of the rearing water. Settlement in light: dark (LD) cycles of 24:0 h, 12:12 h and 0:24 h was evaluated in 6 days using petri dishes filled with 20 ml seawater stocked with of 2 larvae ml^{-1}. The settlement was assessed by counting the larvae that have attached to the petri dish by means of secretion of their byssal threads. The samples were viewed under the Motic® dissecting microscope.

At the same time, using indirect method, filtration and ingestion rates of green mussel pediveligers were compared under light and dark conditions for 24 h using *Isochrysis galbana* as feed. Treatments under the light condition were provided with artificial light while those under the dark condition were completely covered with aluminum foil to prevent light penetration. The experiments were conducted in a temperature-constant room and replicated three times.

Briefly, mussel pediveliger larvae, unfed for 24 h were stocked at 2 larvae ml^{-1} in 500 ml plastic beaker filled with UV-filtered seawater. Algal cell concentration was determined at 100,000 cells ml^{-1}. Another set of containers that received algal concentration without larvae were put to correct changes in cell concentration caused by algal sedimentation. In estimation of filtration and ingestion rates, a 10 mL subsample from the culture water was sampled and algal cell count was counted in a haemacytometer (Neubauer Improved Brightline, Marienfield, Germany) three times.

Data were evaluated by a regression line described by Sprung (1984) using the formula:

$$F = \frac{v}{n}\left(\frac{\ln C_0 - \ln C_t}{t} - A\right)$$

$$A = \frac{\ln C_0 - \ln C_t}{t}$$

$$I = FC$$

where F is the filtration rate in ml h^{-1}; C_0 and C_t are the initial and final cell concentration in cells ml^{-1}; t is the duration of the experiment (h); n is the number of larvae in a given volume v; A is the correction for any changes in the control group; I is the ingestion rate; and C is the mean cell concentration.

Aeration experiment

To compare the survival and growth of pediveligers to spat, three aeration intensities were tested: mild (10 L h^{-1}), moderate (20 L h^{-1}), and strong (30 L h^{-1}), each treatment replicated thrice. Aeration study was conducted in 1 L capacity plastic beakers stocked with 1000 larvae per container, replicated three times. The aeration level was adjusted using a flow meter (DFG-4T6T Darhor Flow Meter, Hangzhou Darhor Technology Co., Limited). A single airstone connected to the main blower line using a rubber tube and plastic regulator was positioned at the bottom center of each tank.

Statistical analyses

Statistical analyses of the actual growth rate data, square root transformed percentage survival data, and square root transformed percentage settlement data were analyzed through one-way Analysis of Variance (ANOVA) with SPSS Version 16 software. In cases where the F value of the treatments was significantly different ($P < 0.05$), Duncan's Multiple Range Test (DMRT) was used to determine specific differences between pairs of means.

Results

Water management

Attached spats from pediveliger stage in tanks with every 3 days water exchange exhibited a significantly high growth regarding shell length (1579.8 ± 28.91 μm) and shell height (1142.6 ± 36.51 μm) compared to other treatments (Fig. 1). In contrast, settled spats in daily water change exhibited the lowest growth but with the highest survival rate of 4.69 ± 0.72%. However, no significant difference was observed in mussels subjected to daily and every 3 days water exchange regarding their survival (Fig. 2). Measured total ammonia nitrogen gradually increased towards the end of the culture period especially in the tanks with 3 and 5 days water-

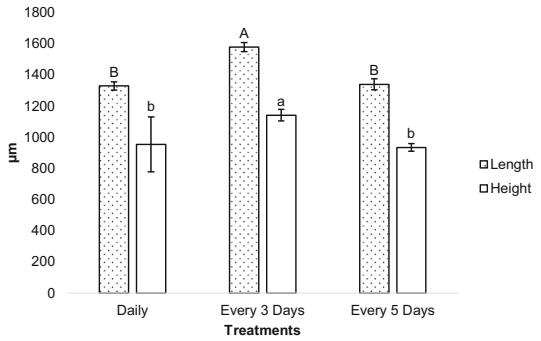

Fig. 1 Final length and height of *P. viridis* at different water-exchange frequencies after 15 days. Mean values ± SEM in each treatment not sharing the same superscript were significantly different ($P < 0.05$)

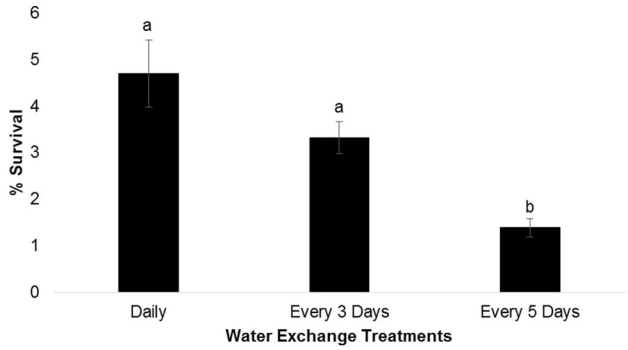

Fig. 2 Survival rate of green mussel *P. viridis* at different water-exchange frequencies. Mean values ± SEM in each treatment not sharing the same superscript were significantly different ($P < 0.05$)

exchange interval (Fig. 3). Total bacterial count increased for the first 4 days of culture but remained constant until Day 15 (Fig. 4).

Photoperiod

Continuous darkness had a significant effect on growth of mussel spats (Fig. 5). Spats from the dark condition treatment (0L:24D h) exhibited a significantly highest mean final shell length and height ($P < 0.05$) of 2190.0 ± 55.24 and 1581.5 ± 42.85 µm, respectively, compared to spats reared in 24L:0D h (1511.7 ± 38.07 and 1133.3 ± 22.9 µm) and 12L:12D h (1492.2 ± 31.47 and 1040.7 ± 33.19 µm) photoperiod regimes. Figure 6 summarizes the survival of mussel larvae to spat after 15 days of culture. The 0L:24D h photoperiod treatment has a twofolds higher survival rate (6.34 ± 0.56%) compared to the

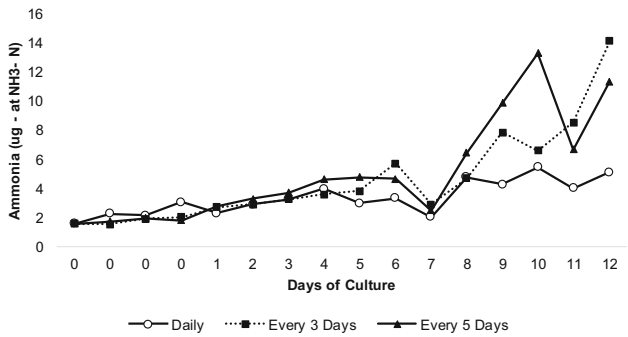

Fig. 3 Total ammonia nitrogen of rearing tanks of *P. viridis* cultured in 15 days at different water-exchange frequencies

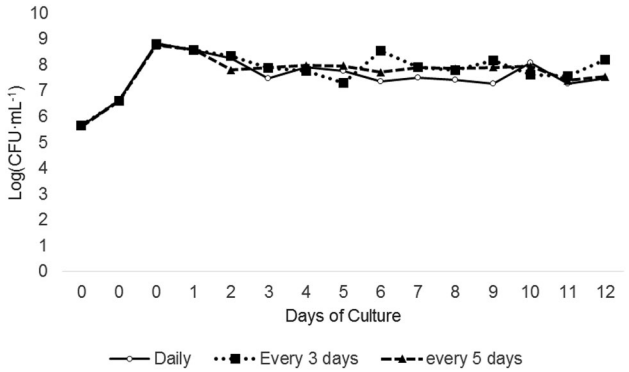

Fig. 4 Total bacterial count of rearing water of *P. viridis* cultured in 15 days at different water-exchange frequencies

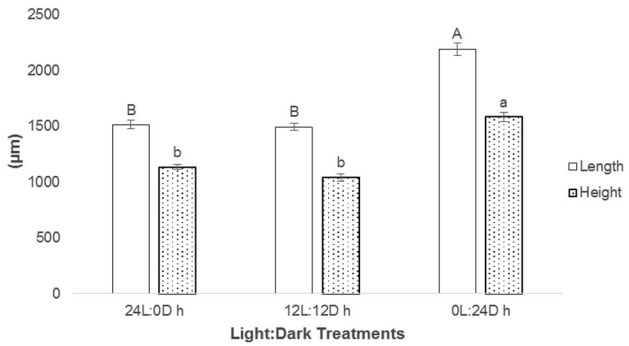

Fig. 5 *Perna viridis* growth (μm) after 15 days at different photoperiod regimes. Mean values ± SEM in each treatment not sharing the same superscript were significantly different ($P < 0.05$)

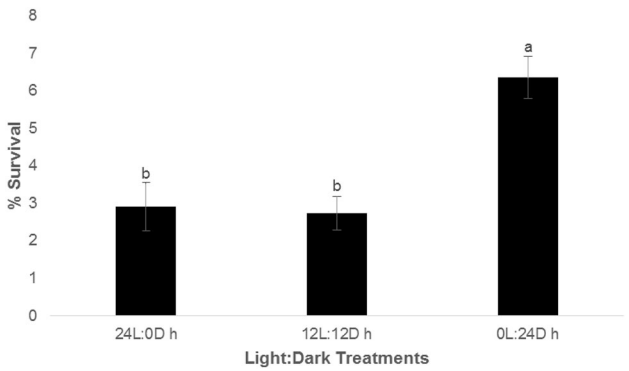

Fig. 6 Percentage surival of hatchery-reared spats after 15 days. Mean values ± SEM in each treatment not sharing the same superscript are significantly different ($P < 0.05$)

treatments receiving light (2.91 ± 0.64% and 2.74 ± 0.44% for 24L:0D and 12L:12D h, respectively). After 48 h after stocking, numbers of settled individuals in petri dishes were counted. It was found out that no significant difference on the settlement rate of the larvae between the treatments exposed to continuous light (24L:0D h) and continuous darkness (0L:24D h) was observed as shown in Fig. 7. Significantly lower settlement rate, however, of 64.90 ± 0.90% was observed in 12L:12D h condition after 48 h but no evident significant difference in the settlement rate in all the treatments was found.

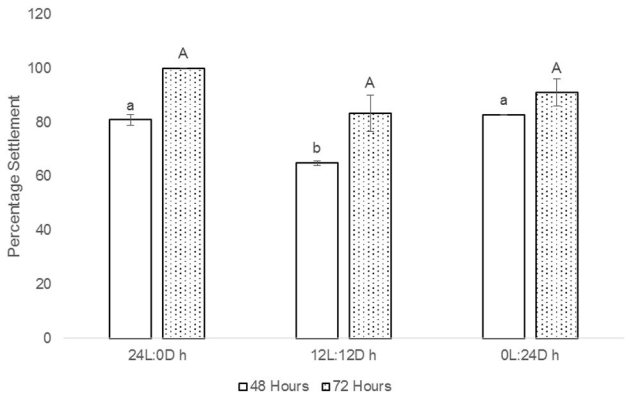

Fig. 7 *P. viridis* settlement rate at different photoperiod regimes after 48 and 72 h. Mean values ± SEM in each treatment not sharing the same superscript are significantly different ($P < 0.05$)

Furthermore, to support growth-related data, larval ingestion and filtration rate experiments were conducted under light and dark conditions. Significantly higher ingestion rate of 2885.20 ± 74.53 cells larvae^{-1} h^{-1} was observed in the dark condition compared to the lighted condition with an ingestion rate of 716.14 ± 84.72 cells larvae^{-1} h^{-1} (Fig. 8). Similarly, the filtration rate in the dark condition (0.103 ± 0.003 mL h^{-1}) was also significantly higher compared to the light condition (0.026 ± 0.003 mL h^{-1}) as shown in Fig. 9.

Aeration

Hatchery-reared larvae from Culajao breeders were used for the aeration experiment. Significantly higher growth in terms of mean shell length was observed in treatments with mild (10 L h^{-1}) and moderate (20 L h^{-1}) aeration compared to strong aeration (30 L h^{-1}). However, there was no significant difference in terms of their shell height (Fig. 10). In terms of survival, heavily aerated tanks produced the highest (0.2046 ± 0.03%) followed by mildly (0.044 ± 0.002%) and moderately (0.0438 ± 0.002%) aerated tanks (Fig. 11). Strongly-aerated conditions were significantly different from other tested aeration levels. Highest settlement rate after 72 h of stocking was observed in moderately-aerated condition (86.13 ± 4.35%) but not statistically significant from that of strongly-aerated (77.1 ± 3.99%) condition (Fig. 12). Non-aerated and mild-aerated conditions had significantly lower settlement rates.

Other measured water parameters in the rearing systems in the three experiments were within the optimum range for rearing the green mussel culture and is given in Table 1.

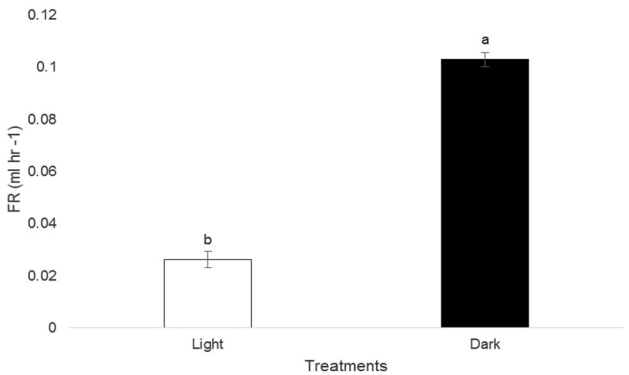

Fig. 8 *P. viridis* filtration rate (ml h^{-1}) exposed in light and dark conditions. Mean values ± SEM in each treatment not sharing the same superscript are significantly different ($P < 0.05$)

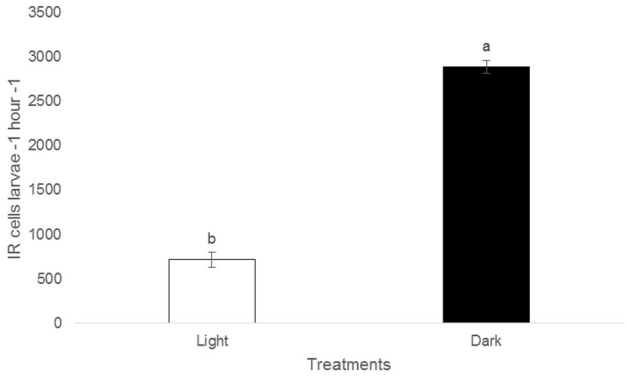

Fig. 9 *P. viridis* ingestion rate (cells larvae^{-1} h^{-1}) exposed in light and dark conditions. Mean values ± SEM in each treatment not sharing the same superscript are significantly different ($P < 0.05$)

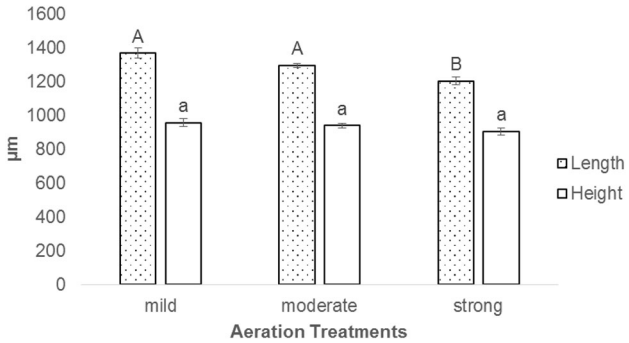

Fig. 10 Final shell length and height of green mussel *P. viridis* at different aeration intensities for 15 days. Mean values ± SEM in each treatment not sharing the same superscript were significantly different ($P < 0.05$)

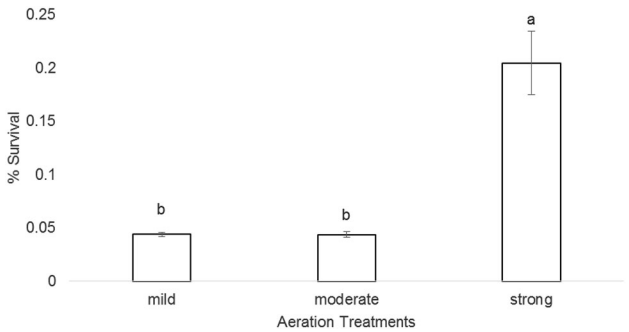

Fig. 11 Survival of *Perna viridis* larvae to different aerated conditions for 15 days. Mean values ± SEM in each treatment not sharing the same superscript were significantly different ($P < 0.05$)

Discussion

With the current efforts to produce hatchery-reared seeds of green mussel in the Philippines, the present study focused on various environmental factors that affect the crucial stage of larval setttlement and metamorphosis, growth and survival.

Little information is available on the effect of water management on bivalve larval settlement, growth and survival from pediveliger to spat. According to Utting (1987), to hold pediveligers during their settlement stage, an airlift downwelling recirculating system is needed. While this kind of system is not yet tried in the hatchery, the current practice of water exchange involving replacement of rearing water is still being practiced. In this method, larvae are drained from the culture tank into fine mesh screen and returned after the tank

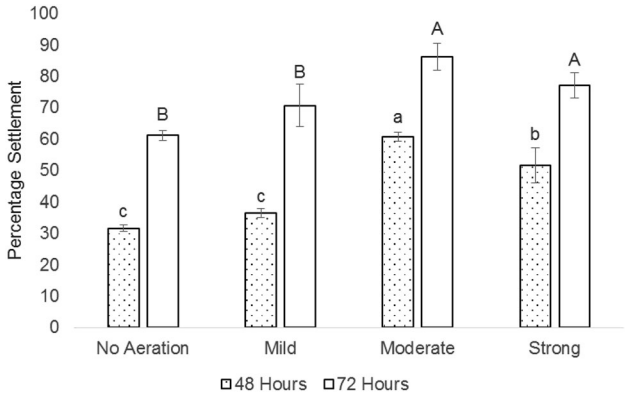

Fig. 12 Percentage settlement of *Perna viridis* spat at different aeration conditions after 48 and 72 h. Mean values ± SEM in each treatment not sharing the same superscript were significantly different ($P < 0.05$)

Table 1 Water-quality parameters for the water management, photoperiod, and aeration experiments

Water parameters	Water exchange	Photoperiod	Aeration
Dissolved oxygen (mg/L)	4.915–6.740	4.65–6.90	3.81–6.05
Temperature (°C)	24.9–27.2	26.2–28.1	26.6–28.7
pH	7.96–8.15	7.96–8.15	7.63–7.90
Salinity (ppt)	29.18–30.83	29.18–30.83	30.17–30.62

is cleaned and refilled (Southgate and Ito 1998). The earlier work of Helm and Millican (1977) on oyster *Crassostrea gigas* showed that slow growth was attained when water exchange was observed daily than water changed at 48 and 72-h interval. Since then, water change every 48 h has been routinely used in commercial hatcheries (Ibarra et al. 1997; Ponis et al. 2003; Labarta et al. 1999; Doroudi and Southgate 2000).

In conformity with the present results, the work of Yan et al. (2006), showed that growth was enhanced in *Ruditapes philippinarum* after changing water every 72 h. In accordance, Oliveira (1998) also found the highest survival of *C. gigas* larvae by changing water every 48 and 72 h. Furthermore, the results of the present study are similar to the work of Antonio et al. (2009) in which highest larval growth and survival performance of mangrove oyster, *Crassostrea rhizophorae* were observed when water was exchanged at either 48 or 72 h.

In this study, it is possible that the mechanical disturbance of handling the larvae more frequently especially when sieving and washing was responsible for the reduction in growth observed in the treatment with a daily water change (Antonio et al. 2009).

Survival was lowest in tanks that received water change every 5 days. From the daily-monitored ammonia levels, an increase in total ammonia nitrogen in tanks with 3 and 5 days water exchange was found. Increased ammonia level can be attributed to the accumulation of larval or spat wastes and or uneaten feeds causing water-quality deterioration. High concentrations (13.0 mg 1^{-1}) of ammonia is toxic and could be tolerated by *P. viridis* only up to 48 h (Reddy and Menon 1979). The biological consequences of ammonia accumulation include secretion of considerable quantities of mucus and pseudofaeces showing gelatinous consistency (Reddy and Menon 1979).

In contrast to increasing ammonia levels when water was changed less frequently, bacterial population in all treatments exhibited similar growth patterns and thus, no significant relationship was established whether bacterial flora affected larval settlement or growth or survival. However, the result of Ganesan et al. (2010) confirmed that bacteria flora composed of *Macrococcus* sp., *Bacillus* sp. and *Pseudoalteromonas* sp. improved settlement rates of *P. canaliculus* by providing a sticky matrix containing cells and exudates for larval attachment and nutrition. Further studies to identify and characterize the bacteria present during the rearing period may explain the behavior of the mussel larvae during settlement. Studies to confirm whether or not these bacteria are harmful or beneficial to the larvae have to be elucidated.

The influence of the three light regimes—light: dark (LD) cycles of 24:0 h (continuous light), 12:12 h (natural) and 0:24 h (continuous darkness) on the survival, growth, and settlement of the hatchery-reared green mussel *P. viridis* were compared in this study. The results of the current study similarly correlate from the work of Brito-Manzano and Aranda (2013) which revealed that continuous darkness and feeding favored growth of fighting conch, *Strombus pugilis* Linné 1758, while continuous light and feeding had a negative effect on growth and survival. The present findings also agreed with previous works in other bivalve species, *M. edulis* (Stromgren 1976a, b) and *M. modiolus* (Stromgren 1976b), which reported that exposure to continuous darkness resulted in higher growth regarding length of *M. edulis* and increased the growth rate of *M. modiolus* significantly. The higher growth of *M. edulis* when exposed to continuous dark conditions was attributed to higher feed intake and higher defecation rate, signifying a heightened feeding activity whereas reduced feeding was observed when organism is exposed to continuous light (Nielsen and Stromgren 1985).

In the present study, similar observations were also noted. High filtration and ingestion rates signifying high feed intake were recorded in larvae exposed to dark conditions (0.103 ± 0.003 mL h^{-1} and 2885.20 ± 74.53 cells·larvae^{-1} h^{-1}) than those exposed to lighted conditions (0.026 ± 0.003 mL h^{-1} and 716.14 ± 84.72 cells larvae^{-1} h^{-1}). This, in turn resulted in high or low growth rates of the organisms.

Although earlier reports and current result of this study suggest that higher growth rates of bivalves were observed when exposed to darkness, the physiological mechanism involved in the perception of photoperiod remains unclear. The fact that reproduction of bivalves is influenced by photoperiod suggests that bivalves are capable of perceiving photoperiod at some point of their development (Utting 1987; Couturier and Aiken 1989; Paulet and Boucher 1991; Numata and Udaka 2010).

The basis for photoperiod perception includes the need for a photoreceptor, transmission of external signals to the internal organs of the organism, and a mechanism for time measurement (Sumpter 1990). In mammals and fish, photoperiod is perceived through the retina and pineal organ while in crustaceans, a pineal analogue was suggested (Withyachumnarnkul et al. 1990; Withyachumnarnkul 1992). Retinal perception in pectinids was possible through eyes located in their mantle. Non-eyed bivalves perceive light through siphon retraction (Hecht 1919, 1920; Kennedy 1960), which is initiated through the photosensitive nerve elements in the pallial nerve (Kennedy, 1960), and influenced by light on cilia activity via photosensitive pigments (Paparo 1986). With that said, presence or absence of light can be a physical cue for settlement of pediveliger larvae. In the present study, settlement is evident after 48 h but not after 72 h. Since bivalve larvae have tiny brains and poor memories, it is suggested that environmental cues rather than larval choice determine where the larvae would settle (Rittschof et al. 2009).

The survival of the pediveliger in this aeration experiment contradicted the results obtained by Loosanoff and Davis (1963) and Bayne (1965) who reported that moderately-aerated and non-aerated treatments gave better survival rates in *Crassostrea virginica* and *Mytilus edulis*, respectively. Moderate aeration had been routinely used in mussel larviculture of *P. viridis*, *P. indica*, *M. edulis*, *M. californianus,* and *M. gallo-provincialis* but its effects were not mentioned (Tan 1975; Sidall 1979, 1980, 1982; AQUACOP 1979, 1983; Appukuttan et al. 1984, 1988; Taylor and Beattie 1985; Eyster and Pechenik 1987; Sreenivasan et al. 1988). However, the results of this experiment were similar to those of Dharmaraj and Shanmugasundam (1999) that states that the necessity of agitating the rearing medium was based on the larvae's ecological adaptations. As the mussels inhabit shallow waters, the larvae are able to adjust to the rough sea conditions (Dharmaraj and Shanmugasundam 1999).

In this study, the highest growth was observed in mildly aerated conditions. The results of this study contradict with Dharmaraj and Shanmugasundam (1999) who reported that growth rate of *Pinctada fucata* larvae is better in non-aerated conditions when compared to moderately and strongly-aerated conditions. Since the pearl oyster larvae thrive in the deeper waters on seabed, the disturbance in seawater is minimal, hence there is greater feeding activity in non-aerated conditions (Dharmaraj and Shanmugasundam 1999). In the present study, mildly aerated conditions may have promoted growth as there is minimal water flow enough to distribute the food in the culture tank (Sanchez-Lazo and Martinez-Pita 2012). Meanwhile, heavily aerated conditions in this study may have inhibited the proper feeding mechanism as water flow caused by air pressure disperses food rapidly and larvae cannot filter in maximal capacity.

In a laboratory experiment, it was found that *Ostrea edulis* larvae tend to spend a proportion of their time close to or at the bottom of glass beakers and suggested that during this time, the larvae may not be feeding or that they may be feeding at a reduced rate (Walne 1966). It was further suggested that the metabolism of the

larvae may be insufficiently rapid to permit continuous swimming and that they may in the natural habitat rely in part on turbulence and the depth of the water column to keep them afloat.

Furthermore, settlement rates were also recorded as a result of various aeration levels tested. Results showed that pediveligers prefer moderately and strongly-aerated than mildly and non-aerated conditions. These results were similar to the findings of Sidall (1982) where enhanced settlement was recorded in strongly-aerated conditions. The present finding also conforms to the results of Alfaro (2005) where settlement of *P. canaliculus* was increased with increasing water flow and oxygen concentrations. However, Trevelyan and Chang (1983) found that increased aeration had a negative effect on *M. californianus* settlement. It is believed that higher settlement rate in strongly-aerated conditions was not due to the increased oxygen concentration in the rearing water but to the increased frequency of contact between the larvae and the substratum (Trevelyan and Chang 1983). Lower water flows enable larvae to explore their surroundings, detaching and re-attaching several times while high water flows prompt the larvae to settle firmly during their first encounter with a substratum (Alfaro 2005; Alfaro and Jeffs, 2002; Eyster and Pechenik 1987).

Loosanoff and Davis (1963) made reference that fertilized eggs and larvae of bivalves can withstand vigorous mechanical disturbance without apparent ill effect and cited an example from the field where the high-intensity settlement of *Crassostrea virginica* larvae was observed to occur immediately following a hurricane.

Under laboratory settings, settlement was enhanced in *Mytilus* larvae with increased water flow, but this effect was related to increased flux of the larvae to surfaces when exposed to strong turbulence (Eyster and Pechenik 1987; Eckman and Duggins 1998), and the effect of turbulence on larval behavior is still unclear. In the natural environment setting, there was heavy settlement of *P. indica* larvae on granite stones constantly lashed by waves (Appukuttan et al. 1984).

Mussels preferred a mildly aerated condition for better growth, but in terms of survival and settlement, strong aeration is necessary. A static water condition may lead to accumulation of toxic wastes which would have been oxidized if heavy aeration is provided (Walne 1966; Helm and Spencer 1972).

Conclusion

This study demonstrated that changing water every 3 days was effective in maintaining the rearing water quality and improving the growth and survival of *P. viridis* larvae. Highest growth and survival rates were observed in *P. viridis* spats grown in 0L:24D h photoperiod. There was no significant difference in the settlement rate of larvae exposed to different photoperiods. Mild aeration has shown to improve the growth of *P. viridis* larvae, but higher survival and settlement rates were attained in the strongly-aerated conditions. Further studies that include regular sampling of survival and growth may be conducted to determine in what particular stage within the 15-day culture period has the highest mortality rate. For water management, studies to identify and characterize the bacteria present during the rearing period may be conducted to explain the behavior of the mussel larvae during settlement. Studies to confirm whether or not these bacteria are harmful or beneficial to the larvae have to be elucidated. Therefore, when the larvae start to settle, it is recommended to expose them to darkness, change the water every 3 days and provide strong aeration to be able to attain high survival and settlement rates, and bigger spats.

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Compliance with ethical standards

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

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