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THE DEVELOPMENT OF LOBSTER PUERULUS (*Panulirus orantus* AND *Panulirus homarus*) IN CAPTIVITY ENVIRONMENT

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ABSTRACT

The present study was conducted to investigate the development of *Panulirus ornatus* and *P. homarus* puerulus larvae into juveniles in a captive environment. Pueruli were collected from the local fishermen and transported by air to the research facilities. Puerulus of each species were stocked into 3 floating plastic compartments (5 cm × 12 cm × 17 cm) with sufficient holes, aeration, and a filtration system until reached their first moulting. Results indicated that both puerulus species were completely moulted after 6 days of stocking with the survival rate of 100% and 93.3%. Moulting began on Day 5 for *P. ornatus* and Day 4 for *P. homarus* pueruli. The weight of *P. ornatus* increased significantly after the metamorphosis ($P<0.05$). The initial and final weights of *P. ornatus* were significantly greater than *P. homarus* ($P<0.05$). In addition, the total and carapace lengths of both species were significantly increased ($P<0.05$). Morphological observations indicated that there was a significant distinguishing feature such as the antenae form and body pattern colour of both species. In conclusion, proper handling of the pueruli according to each species is required to preserve the quality in order to achieve greater success in metamorphosis into juveniles.

KEYWORDS: Puerulus; early juvenile; lobster; *P. ornatus*; *P. homarus*; metamorphosis

INTRODUCTION

To date, the supplies of global fishery products can be classified into capture fisheries and aquaculture. While capture fisheries yields in the last few decades have seen a declining trend, the production of aquaculture activities has remarkably increased. Aquaculture production is around 90 million tons in the last decades and has an increase pattern (FAO, 2022). Of the various types of species caught, approximately 6 million tonnes were crustaceans, including spiny lobster. Currently, the majority of the spiny lobster production is obtained from wild fishing activities with a total production of 74,700 tonnes in 2020. However, the development of lobster cultivation has grown rapidly led by Vietnam and Indonesia (Petersen *et al*., 2013; Anh & Jones, 2015b; Huong *et al*., 2015; FAO 2022) and India (Saleela *et al*., 2017) and still produce only small portions of the global lobster production. With the increasing development of lobster aquaculture, the demand for lobster seed is increasing as well. Lobster seeds for aquaculture are supplied from wild-caught pueruli (Jones *et al*., 2019). It has been reported that even though lobster hatchery technology has been developed in Australia and can produce complete lobster seeds from hatcheries (Smith *et al*., 2009), this technology has not yet been adopted by Indonesian lobster farmers.

The ornate spiny lobster or pearl lobster (*Panulirus ornatus*) and scalloped spiny lobster or sand lobster (*Panulirus homarus*) are the most exploited in Indonesia (Priyambodo *et al*., 2015). Both lobster species

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are abundant in certain Indonesian waters (Chan, 2019; Setyanto *et al*., 2019; Priyambodo *et al*., 2020). Lobster farmers have been cultivating them for decades in small quantities using traditional methods by feeding them trash fish, but research on the development of prepared diets continues to progress (Kurnia *et al*., 2017; Marchese *et al*., 2018; Ridwanudin *et al*., 2018; Amali & Desi, 2020; Arumugama *et al*., 2020; Prayitno *et al*., 2020; Nankervis & Jones, 2022). It has been reported that lobster juveniles can achieve market size after nine months of post metamorphic culture. The development of spiny lobster cultivation technology has also progressed in Vietnam and the Philippines (Anh & Jones, 2015b; Ratunil, 2017), and has begun in Indonesia in recent years (Bahrawi *et al*., 2015). In the natural environment, the life cycle of a spiny lobster requires a long time and complex development, starting from the deep sea, where the adult mate and produces eggs (Radhakrishnan *et al*., 2020). The larvae then develop through several larval instars in a pelagic naupliosoma phase, then migrate towards the shallow water until the completion of several instars within the phyllosoma stage, after which they metamorphose into puerulus larvae or pueruli. The completion of phyllosoma metamorphosis into pueruli requires months (Kittaka & Ikegami, 1988; Kittaka *et al*., 1997; Kittaka *et al*., 2005) or even a year for both *P. ornatus*, and *P. homarus*, making hatchery procedures difficult with inconsistent mortality rates (Kittaka & Kimura, 1989). After an extended developmental period, the pueruli will moult several times and metamorphose into benthic lobster juveniles. Lobster juveniles will continue to grow, involving several instars, before they return to deeper water as mature adult lobsters. Many fishers take advantage of the lobster lifecycle where they catch the pueruli at peak season which falls at the end of the wet season in many Indonesian locations (Bahrawi *et al*., 2015). Lobster pueruli are abundant at that time and can be easily collected by using a special fishing trap (Priyambodo *et al*., 2015).

Fishermen catch puerulus stage larvae to be used as seeds for cultivation, when morphologically they are still clear and unpigmented. At this stage, the pueruli do not show any feeding behaviour (Kittaka & Kimura, 1989), although there are changes in their nutritional composition in comparison to phyllosoma lobster (Kittaka *et al*., 2005; Conlan *et al*., 2014). This phyllosoma to puerulus transition period lasts several days, depending on the species, and is known as a critical phase for successful lobster metamorphosis into juveniles. The pueruli are very vulnerable to predators, and failed metamorphosis during the moulting process into the juvenile stage can result in a high mortality rate. It has been reported that the survival rate from eggs to pueruli of lobster were approximately 6.7% and 13.3% for *J. edwardsii* (Kittaka *et al*., 2005) and 9.6% and 12.6% for *J. verreauxi* (Kittaka *et al*., 1997). Even so, this stage is very ideal in terms of transportation from the field to hatcheries (Shanks *et al*., 2015). In general, lobster farmers keep the puerulus obtained from collectors in floating nets in the ocean until the lobsters grow to market-size harvests. Incorrect handling of the puerulus at this stage has a significant impact on subsequent steps of lobster culture. This is because the puerulus will moult into a juvenile for the first time in a captive environment. Proper handling during this period is required and should be rigorously monitored by farmers who know the process of puerulus metamorphosis into juvenile lobsters.

Although the metamorphosis of several spiny lobster phyllosoma was well documented in some species (Kittaka, 1988; Kittaka & Ikegami, 1988; Kittaka & Kimura, 1989; Abrunhosa & Kittaka, 1997; Kittaka *et al*., 1997; Matsuda & Yamakawa, 2000; Kittaka *et al*., 2005), there is a little known about the development of puerulus into pigmented juvenile of *P. ornatus* and *P. homarus*. The result of the present study may be valuable for fishers, collectors, and lobster farmers in handling lobster puerulus so they can maintain lobster larvae in optimum condition, so that ultimately, lobster cultivation will produce maximum yield. In this study, observations will be made on the development and metamorphosis of the puerulus of two common Indonesian lobster species *P. ornatus* and *P. homarus* into juveniles.

MATERIALS AND METHODS

Animals and experimental system

At the beginning of the experiment, a container box containing 100 L of filtered seawater was prepared (salinity 30 ppt, temperature 30 °C, pH 8.2). A total number of 36 heads of spiny lobster puerulus (*P. ornatus* and *P. homarus*) were purchased from a local collector and land was transported to the experiment facility. Upon arrival, the pack of puerulus were floated in the box for acclimation. The puerulus of each species were then distributed after roughly 30 minutes of adaptation to three compartments. The compartments were made of plastic material (5 cm × 12 cm × 17 cm) with sufficient holes on their sides. Two pipe cuts with diameters 0.5 inches and 4 cm long were provided to each of the compartments. Two continuous air supply and filtration systems were provided to maintain the dissolved oxygen level. The observation duration is from puerulus period until the completion of the first moult or at the juvenile stage. During this observation, puerulus are not fed

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with any diet as suggested by Abrunhosa and Kittaka (1997).

Data collection

After the completion of moulting, the survival, length increment, wet weight gain and moulting rate data were calculated using the formula:

$$\text{Survival rate} = \frac{N_i}{N_0} \times 100\%$$

$$\text{Length increment} = \frac{TL_i - TL_0}{TL_0} \times 100\%$$

$$\text{Weight gain,} = W_i - W_0$$

$$\text{Moult rate} = \frac{M_i}{M_0} \times 100\%$$

Where = Ni = the total number of lobsters that survived, N_0 = the total number of initial pueruli. TL_i = total length of animals at the end of observation, TL_0 = total length of the juvenile at the beginning of the observation, W_i = final wet weight of lobster, W_0 = initial wet weight of lobster, M_i = the number of moulted lobsters, M_0 = the total number of lobsters.

The visual observations during the metamorphosis of the puerulus were conducted and documented using a digital photograph (Nikon Coolpix S2800, Indonesia) with an appropriate scale. The total and carapace length of the lobster were measured using Adobe Photoshop® 21.2.0, by comparing a standard measuring tool with the number of pixels. The weight of the lobster was measured with a digital scale (CX128 TL-series, China). The water quality parameters were collected using a digital water quality tester (Horiba U5000, Japan).

Statistical analysis

All data were analysed using IBM SPSS Statistic for Windows Version 25.0 (IBM, New York, USA). The weight gain, total length increment and carapace length increment of the juvenile lobster were examined using independent t-test analysis at significant level of $P < 0.05$.

RESULTS AND DISCUSSION

The initial and final weights of *P. ornatus* pueruli were significantly higher than the puerulus of *P. homarus*. The biomass weight gain of *P. ornatus* juveniles increased significantly ($P < 0.05$) but not in *P. homarus*. The mean of total and carapace length increment in both species were significantly different after metamorphosis ($P < 0.05$). The survival rate of puerulus lobsters was not significantly different at the end of the development period ($P > 0.05$). The moulting rate of *P. ornatus* puerulus started on the 5th of the days after stocking, while the *P. homarus* puerulus started moulting on day 4 (Table 1). The results demonstrate the development of *P. ornatus* and *P. homarus* pueruli until their first moult into the juvenile stage and show that the *P. ornatus* pueruli are significantly larger than *P. homarus* pueruli, which affects the initial size of the early juveniles of each species. Both species complete their total moulting

Table 1. Morphological measurements of puerulus lobster during the first moult period. Data are expressed as mean ± SE

Parameters	*P. ornatus* (n=21)	*P. homarus* (n=15)
Initial weight (g)	$0.26 \pm 0.04^{a,1}$	0.23 ± 0.03^{2}
Final weight (g)	$0.31 \pm 0.05^{b,1}$	0.24 ± 0.04^{2}
Weight gain (g)	0.05	0.01
Initial total length (mm)	18.33 ± 0.35^{w}	18.84 ± 0.70^{w}
Final total length (mm)	$23.84 \pm 0.48^{x,1}$	$22.00 \pm 0.74^{x,2}$
Total length increment (%)	30.06	16.77
Initial Carapace length (mm)	6.81 ± 0.20^{y}	7.12 ± 0.10^{y}
Final Carapace length (mm)	9.21 ± 0.37^{z}	9.04 ± 0.18^{z}
Carapace length increment (%)	35.24	26.96
Survival (%)	100 ± 0.0	93.3 ± 2.30
Moulting rate D-1 (%)	0	0
Moulting rate D-2 (%)	0	0
Moulting rate D-3 (%)	0	0
Moulting rate D-4 (%)	0	26.7
Moulting rate D-5 (%)	57.1	60
Moulting rate D-6 (%)	100	100

Data in the same column with different superscripts ($^{a,\,b;\,w,\,x;\,y,\,z}$) and data in the same row with different superscripts (^{1,2}) represent significant differences at $P < 0.05$.

rate on the sixth day after the stocking. The difference in size between the pueruli is strongly influenced by several factors, including species and age. Puerulus in the wild may also be collected from different lobster broodstock and have different ages which affect the size and degree of moulting.

Based on the results, the mean sizes of *P. ornatus* pueruli were greater than the *P. homarus* pueruli both at the beginning of the experiment, or after the first moulting into the juvenile and this corresponds to the size of pueruli size collected from the wild in the other studies. Results from the several research indicated that the size of the puerulus ranged between 2.0 and 2.5 cm, but there was not any further explanation about the size and weight of each species (Kittaka & Kimura, 1989; Abrunhosa & Kittaka, 1997; Priyambodo *et al*., 2015; Fadjar *et al*., 2022). The *P. homarus* was reported to have the carapace length ranged between 7-8 mm with weight from 0.25 g to 0.35 g (Anh & Jones, 2015a). Specifically, Fadjar *et al*. (2022) reported that the average size and weight of wild-caught *P. homarus* pueruli for field experiments were 21.6 mm and 0.16 g, respectively. The similar length of *P. ornatus* pueruli was also reported (Ratunil, 2017). The very first report about the size of spiny lobster pueruli was described by Kittaka and Kimura (1989), where the total length of 12 days-old pueruli of *Panulirus japonicus* is 2.3 cm. The similar proximal size of *Panulirus cygnus* pueruli was reported by Abrunhosa & Kittaka (1997). The survival rate of the spiny lobster phyllosoma at the metamorphosis event into the juvenile stage was reported to be very low (Kittaka *et al*., 1997; Matsuda & Yamakawa, 2000; Kittaka *et al*., 2005).

The details complete development of puerulus lobsters are presented in Figure 1-6. There was a distinctive development in the body colouration of the puerulus during the period. Both puerulus experienced noticeable physical development and transformation. Both juvenile lobsters have a clear body part except the eyes. Antennule ends are dark and flattened for the *P. ornatus* puerulus (1C). The end of the antenna of *P. homarus* is sharp, without any distinctive form or colour (1D).

Juvenile lobster exhibit coloration of their carapace edges, pereiopods, upper abdomen, and the base of their antennules. The body becomes whitish to yellow, and pigments started to appear in the whole body (Figure 2). On the third day after stocking, the

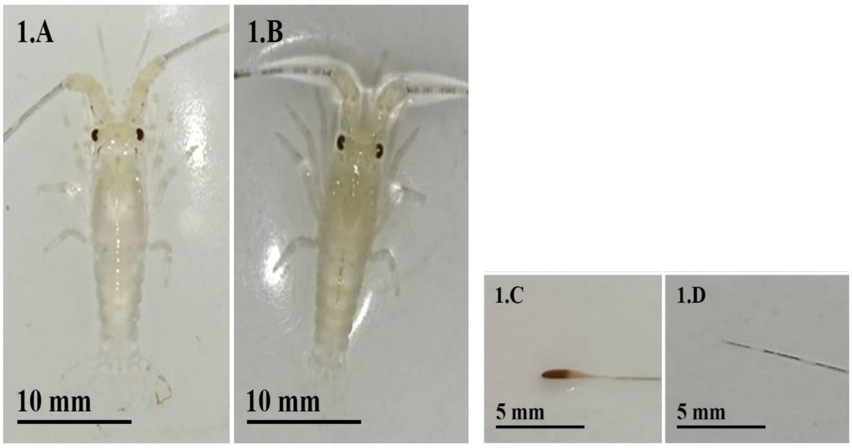

Figure 1. The morphology of puerulus on the first day after collection. (1A) *P. ornatus* & (1B) *P. homarus.*

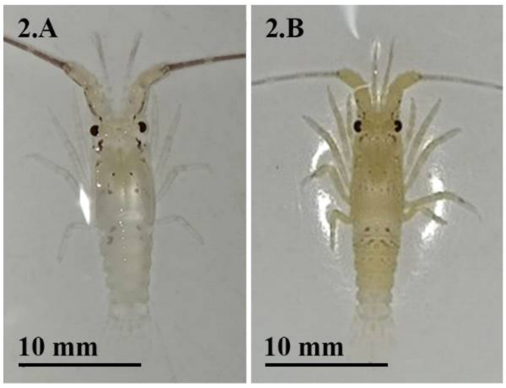

Figure 2. Puerulus *P. ornatus* (2A) and *P. homarus* (2B) after 2 days of stocking.

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coloration extended through all the body parts including the uropod and upper abdomen in puerulus *P. ornatus* (3A) and *P. homarus* (3B). The pigment also spread into the carapace and abdominal segments. The body becomes whitish to yellow (Figure 3).

The *Panulirus longipes* phyllosoma that metamorphosed into the puerulus stage experienced high mortality during observations by Matsuda & Yamakawa (2000). The phyllosoma was dead one by one throughout the culture and cannot achieve the puerulus stage. The mortality rate of the development of phyllosoma into pueruli can be divided into several stages and was found to have a similar pattern across lobster species. High mortality occurred in the first 60 days of the culture of *Jasus verreauxi,* and the rate decreased steadily over the period (Kittaka *et al*., 1997). Under a laboratory condition, the puerulus of *Jasus lalandi* could only survive for 31 days after its metamorphosis and could not achieve the juvenile stage (Kittaka, 1988). The low survival of the puerulus during metamorphosis to the juvenile stage was subject to the overall water quality, food availability and levels of disease infection. However, even at the optimum water quality, the survival rate was also reported to be low (Matsuda & Yamakawa, 2000; Kittaka *et al*., 2005). In *J. verreauxi*, the survival rate of metamorphosed pueruli into juveniles was only 17.2% and the mortalities occurred before, during or the day after metamorphosis (Kittaka *et al*., 1997). Kittaka *et al*. (1997) reported that certain single-celled algae may affect the success rate of *Jasus edwardsii* pueruli metamorphoses into juveniles. With the density of 4,000 to 5,000 cells/mL of *Tetraselmis tetrahele*, the survival rate of pueruli was 13.3% and rate is higher than the survival rate of pueruli reared with *Nanocloropsis oculata* (6.7%). The success or failure of the pueruli transformation into juvenile is highly uncertain. The mortality rate of early juvenile of *J. lalandi* and *Jasus* hybrid were reported to be very high, even adequate food was provided (Kittaka & Kimura, 1989). More research into the nutritional effects on metamorphosis success is needed.

Figure 3. Puerulus *P. ornatus* (3A) and *P. homarus* (3B) after 3 days of stocking, pigmentation started.

In crustaceans, the growth was strongly associated with the moulting or ecdysis event, where the old cuticle or exoskeleton is changed with the new one. The moult of spiny lobster phyllosoma varies according to the species. In general, the time of the intermoult period of phyllosoma instars are extended in the later instars as they progress toward the metamorphosis into pueruli (Kittaka & Ikegami, 1988; Kittaka & Kimura, 1989; Kittaka *et al*., 1997; Matsuda & Yamakawa, 2000; Kittaka *et al*., 2005). In the present study, the intermoult period of puerulus was shorter than any of the previous reports. The most likely reason for the faster intermolt period in our observations than the existing literature is that the puerulus collected from fishers were at the end of the puerulus period. An additional hypothesis for the shorter intermoult period in *P. ornatus* and *P. homarus* may be that other species bay be adapted to cooler waters of subtropical or temperate waters and have inherently slower development (Hoegh-Guldberg & Pearse, 1995). Therefore, they did not require a prolonged period to metamorphose into the next stage. That presumption is based on the morphology character such as a band-pigmentation on the antennule. The features of this stage were also observed in 12 days old of *P. japonicus* puerulus reared under laboratory conditions, where a band of pigmentation was visible on the antennules (Kittaka & Kimura, 1989). In addition, it has been reported that the pueruli of *P. japonicus* moulted into the juvenile stage after approximately 12-15 days (Kittaka & Kimura, 1989). Meanwhile, longer periods of intermolt occurred in *J. edwardsii* and *J. verreauxi* with an intermoult period of 19 days and 25.5 days, respectively (Kittaka *et al*., 1997; Kittaka *et al*., 2005). However, it is difficult to estimate the precise metamorphosis time for pueruli into juveniles. For instance, the pueruli of *J. lalandi* reared under laboratory conditions were unsuccessfully transformed into the juvenile stage even after 31 days of intermolt from the pueruli stage (Kittaka, 1988).

The larval development of several spiny lobster species is complex, involving several metamorphoses and it time-consuming in the hatchery (Kittaka, 1988; Kittaka & Ikegami, 1988; Kittaka & Kimura, 1989; Abrunhosa & Kittaka, 1997; Kittaka *et al*., 1997; Matsuda & Yamakawa, 2000; Kittaka *et al*., 2005). There are so many factors affecting the complete transformation of puerulus (Phillips & McWilliam, 2009). The completion time for metamorphosis from the eggs until the puerulus stage of *J. edwardsii, J. lalandi, J. verreauxi,* and *P. longipes* was approximately 10 months (Kittaka & Ikegami, 1988; Kittaka *et al*., 1997; Matsuda & Yamakawa, 2000; Kittaka *et al*., 2005) and longer time is required for *P. japonicus* (Kittaka & Kimura, 1989). In the present study, the metamorphosis period of puerulus into juvenile is short, only five days and visible features were compared. The instars of several spiny lobsters were well documented, presenting the details of the development of body parts at each instar stage. A subsequent 17 instars were reported to occur in *J. edwardsii, J. verreauxi*, and *P. longipes* (Kittaka *et al*., 1997; Matsuda & Yamakawa, 2000; Kittaka *et al*., 2005) and 15 instars of metamorphosis were recorded in *J. lalandi* (Kittaka, 1988). Under the laboratory condition, the duration of *P. longipes* development from the egg to puerulus was reported to be 281 days when fed with *Artemia* and 294 days when fed with the gonad of mussel *M. galloprovinciallis* (Matsuda & Yamakawa, 2000).

The body colour got darker in several areas and dominated the body form on Day 4 after stocking (Figure 4). In *P. ornatus* juvenile (4A) the dark brown to reddish coloration occurs on the sides and the middle of the carapace. The coloration also occurs on the sides of each segment of the abdomen but in the middle, and the uropod. In *P. homarus* juvenile (4B) the pattern of colour is spreading at each segment with dark brown colour. On the Day 5 of stocking, the colour of the juvenile's body became deeper than before and noticeable formation on the edge of carapace area appeared (Figure 5).

On the Day 6 of stocking, both puerulus completely transformed into a pigmented juvenile lob-

Figure 4. Puerulus *P. ornatus* (4A) and *P. homarus* (4B) after Day 4 of stocking.

Figure 5. Puerulus *P. ornatus* (5A) and *P. homarus* (5B) after Day 5 of stocking, pigmentation started.

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ster. The body colour became darker. The carapace end became rough and showed strong characters (Figure 6).

Differentiating spiny lobster species at the stage of phyllosoma was reported to be difficult (Matsuda & Yamakawa, 2000), while the distinctive features can be seen at the puerulus stage. Even at the puerulus stage, the body has no colour except the eye (Kittaka, 1988; Kittaka & Kimura, 1989; Abrunhosa & Kittaka, 1997), the puerulus of *P. ornatus* and *P. homarus* can be distinguished easily by the shape of their antennae ends. In *P. ornatus*, the shape of the antenna tips is flat with a brown-red colour, whereas in *P. homarus* it does not have this shape and is consistent with a previous report by Priyambodo *et al*. (2015). Those morphological features of puerulus are important, particularly for the fishers and the puerulus collectors in defining the value of puerulus (Hilyana *et al*., 2021). Changes in the shape of some parts of the body and the colour of the pueruli of that two species are visible over time. In *P. ornatus*, a significant development was seen after the third day of stocking, as seen from the colour patterns, especially on the abdomen, while for *P. homarus*, the abdominal pattern is irregular, covering the entire abdomen spaces with brown-colour spots (Priyambodo *et al*., 2015). A post-puerulus of *P. japonicus* was also reported to have a pale brown colour (Kittaka & Kimura, 1989). Abrunhosa & Kittaka (1997) reported the complete transformation of *P. cygnus* from pueruli to juvenile stage. There are ten instars involved in the metamorphosis of the juvenile and distinguished by the development of some body parts. According to that report, the juvenile lobster in the present study is categorized as Instar 1, where the first moult occurred. At this period, both *P. ornatus* and *P. homarus* juvenile show characteristic features described such as alternating band colour in antennule and pereiopods and the strong colouration of the carapace and abdomen.

The quality of water during the observation period is presented in Table 2. All water parameters are within the optimum conditions for spiny lobster aquaculture. The water quality parameter is an important factor in the lobster aquaculture both in sea cage (Junaidi & Hamzah, 2014) or in a recirculation system (Prama & Kurniaji, 2022).

In the present study, all water quality parameters were at optimum condition for spiny lobster culture and a high survival rate was achieved during the metamorphosis from pueruli into the juvenile stage in both species. Since this experiment did not provide any food, optimal water quality was the key factor in providing a good environment for lobster puerulus to survive. Ross & Behringer (2019) reported that the change in the water quality in the environment influenced the protective response of lobster, *P. argus*.

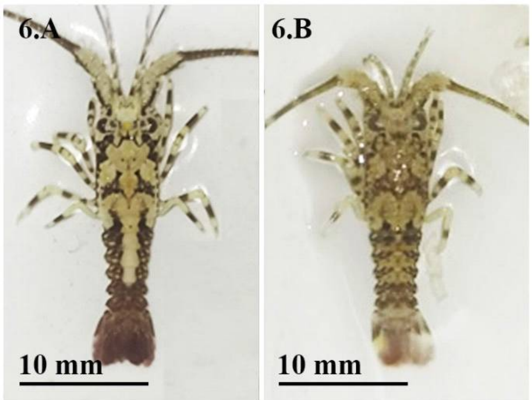

Figure 6. The juvenile *P. ornatus* (6.A) and *P. homarus* (6.B) at 6th days after stocking.

CONCLUSION

In conclusion, the wild-caught *P. ornatus* and *P. homarus* puerulus achieved complete metamorphosis into the juvenile stage under a captive environment with an optimum survival rate. The size of *P. ornatus* was larger than *P. homarus* at the puerulus and juvenile stages. Correct handling method is a critical steps in order to achive a high quality pueruli. As the current study results are preliminary for the species, a further and more comprehensive study is required, particularly on their behaviour.

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