

Embryonic Development, Hatchability and Survival of Early Larval Stage of Mud Spiny Lobster *Panulirus polyphagus* (Herbst, 1793) Under Hatchery Conditions

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ABSTRACT

The embryonic development of mud spiny lobsters *Panulirus polyphagus* was observed, and fecundity, hatching rate and survival rate of early larvae were estimated (n = 18 females). Embryonic development, which occurs on the pleopods of ovigerous lobsters, took 10.5±3.9 days from stage I (bright orange) to stage II-III (orange-dark orange), 7.4±3.4 days from stage III to stage IV-V (orange/brown-dark brown), and then 5.7±0.6 days from dark brown eggs to hatching (24.7±3.8 days from stage I to hatching). Observed water quality parameters included mean salinity 32.1±1.1 ‰, DO 4.0±0.4 mg·L⁻¹, water temperature 26.5±0.3 °C, pH 8.2±0.1, total ammonia 0.14±0.17 mg·N·L⁻¹, nitrite 0.12±0.14 mg·N·L⁻¹ and total alkalinity 144.2±6.8 mg·L⁻¹ as CaCO₃. The mean fecundity (F) was 780,770±340,104 eggs and the mean hatching rate (HR) was 21.9±15.8 %; adult females had a mean carapace length (CL) of 92.3±8.1 mm and body weight (BW) of 665.6±147.1 g. Correlation analysis showed that there were significant, positive correlations between F and CL (r² = 0.57, p<0.01) and between F and BW (r² = 0.69, p<0.01), while there was no correlation between HR and CL or HR and BW. In addition, the survival rates of phyllosomal larvae in starvation and live feed tests were significantly different (p<0.05) throughout the 7-day experiment. No larvae survived in the starvation treatment to day 4, and by day 7, all of the larvae in the rotifer treatment had died, whereas survival for the *Artemia* treatment was 26.6±7.6 % at the end of the trial.

Keywords: Embryonic development, Hatchability, Mud spiny lobster, Survival of larvae

INTRODUCTION

The mud spiny lobster *Panulirus polyphagus* (Herbst, 1793) is an important commercial marine crustacean species. It is distributed throughout the coastal waters of the tropical regions of the eastern Indian Ocean to the Pacific Ocean (Wahyudin *et al.*, 2017; Radhakrishnan *et al.*, 2019). There is high demand for this species for local consumption and export in many countries including Thailand (Bhatiyasevi and Kittiwattanawong, 1994; OAE, 2008; Nitiratsuwan *et al.*, 2017). Unfortunately, due to overexploitation and habitat destruction, the mud spiny lobster is under threat (Radhakrishnan

et al., 2019). To cope with this problem, restocking into existing fisheries and developing the aquaculture of this species are promising solutions. Nevertheless, studies on restocking spiny lobsters remain limited (Phillips and Evans, 1997; Radhakrishnan *et al.*, 2019). Cultivation of the mud spiny lobster is still limited to grow-out activities, especially fattening in floating cages (Charles and Peter, 2003; Vijayakumaran *et al.*, 2009; Solanki *et al.*, 2012), in which the source of seed or juvenile lobsters depends entirely on catches from natural waters (Kittaka, 1994; Jones, 2015). Furthermore, studies on mud spiny lobster production under hatchery conditions are very limited.

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The major bottlenecks in mud spiny lobster hatchery production at a large scale are very low survival rate in the larval stages, difficulty in maintaining individuals from the puerulus stage until they reach the juvenile or marketable size (Kittaka and MacDiarmid, 1994; Williams, 2007; Irvin and Shanks, 2015), and difficulty in obtaining mature broodstock from the wild, particularly males (Ikhwanuddin *et al.*, 2014; Fatihah *et al.*, 2016).

Since commercial hatcheries do not exist for this species, farmers must rely on wild juveniles for grow-out. Currently, the supply of wild juveniles is scanty, and their non-uniform size causes severe cannibalism during culture. Moreover, the harvest of wild juveniles raises concerns about stock depletion. Consequently, the study and utilization of ovigerous female mud spiny lobsters are important first steps for the development of female brooder management and seed production. This should provide better understanding of breeding and larval culture management of this species, both of which are necessary to optimize production. Therefore, the current study focuses on the embryonic development, hatchability and survival of the mud spiny lobster larvae under hatchery conditions. The knowledge gained from the study is useful for production and culture development of this commercially important species.

MATERIALS AND METHODS

Study site and source of mud spiny lobsters

The experiments were conducted at the Klongwan Fisheries Research Station (KFRS), Prachuap Khiri Khan Province, Thailand, during September to December 2020. Ovigerous female mud spiny lobsters (Figure 1) were caught by local fishers using lobster traps in the coastal area of Bang Hin sub-district, Kapoe District, Ranong Province, Thailand (9°35'N, 98°35'E).

Management of ovigerous female mud spiny lobsters for hatching

Mud spiny lobsters were transferred to the hatchery and individually reared in 500-L fiberglass tanks (incubation tank). All lobsters were fed daily at 4:00 p.m. with 4-5 live green mussels (*Perna viridis*) until hatching. During the rearing period, spawning and/or stages of egg development were checked and recorded each morning. When an ovigerous female with a developed stage was identified, the embryonic stage and time interval from the previous stage were recorded. When the egg color changed to dark brown, the female was transferred to a hatching tank (a 500-L fiberglass tank) for spawning.

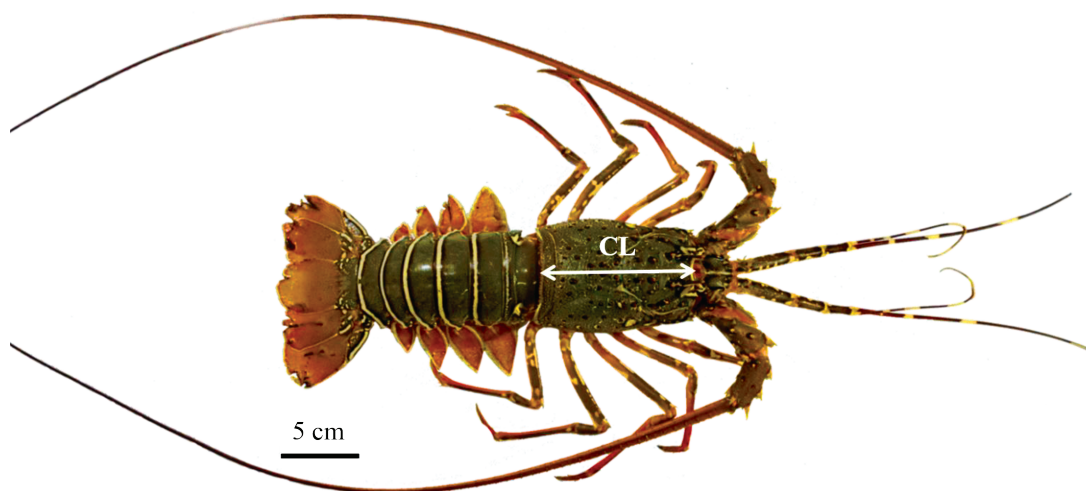


Figure 1. Mud spiny lobster *Panulirus polyphagus* (Herbst, 1793). CL indicates dimension of carapace length.

During the study, approximately one half of the water in the rearing and hatching tanks was exchanged twice a week. To remove uneaten feed and detritus at each water exchange, aeration was temporarily stopped and settled particles were removed from the tank bottom by siphoning. Water quality parameters (salinity, pH, temperature, dissolved oxygen, total ammonia, nitrite and alkalinity) were monitored biweekly. Salinity was determined using a refractometer (Primatech; Jakarta, Indonesia), and the pH of the water was measured using a portable pH meter (pH 11; Cyber Scan; Nijkerk, the Netherlands). Temperature and dissolved oxygen concentration (DO) were measured using an oxygen probe (550A; YSI Inc; Yellow Springs, OH, USA), and the total ammonia, nitrite and alkalinity of the water were determined using the indophenol blue method, the colorimetric method, and the titration method, respectively (APHA *et al.*, 2017).

Data collection

Developmental stages of the eggs, embryos and early larvae of the mud spiny lobsters were investigated under a stereo microscope. Photographs of the embryos and early larvae were taken using a Nikon D7000 DSLR camera with a Nikon DX 18-55 mm lens (modified micro lens). The identification of developmental stages followed Ziegler and Forward (2007).

Each day, a small number of embryos was removed from the surface of the egg mass attached to the ovigerous hairs on the pleopods (located on the abdomen). The cleavage, yolk content, eyespot development and larval formation were recorded according to Ziegler and Forward (2007). The number of days from the appearance of each developmental stage to hatching was also recorded.

The female mud spiny lobsters used in the study were individually measured for carapace length (CL, in cm) using vernier calipers, and weighed to the nearest g. Fecundity was defined as the number of eggs per batch produced by a female and was estimated as follows: the total number of larvae (phyllosoma) produced from a female was estimated by a volumetric method, in which three

100-mL aliquots of water were taken from the hatching tank, the larvae were enumerated, and then totals were averaged across replications. The mean number of larvae per 100 mL was used to calculate the total number of larvae for each female. Fecundity was calculated as the number of newly hatched phyllosomal larvae plus the unhatched eggs. Hatching rate was calculated as percentage of larvae produced to the total egg number.

In addition, the survival rate of the early larval stage was estimated using starvation and live feed tests. Three treatments were assessed: starvation, feeding with rotifers, and feeding with *Artemia* nauplii, with three replicates per treatment. The newly hatched phyllosomal larvae were transferred and reared in a glass aquarium (35×18 cm with 21 cm water depth) at a density of 20 larvae·tank⁻¹ or 4 larvae·L⁻¹ (Sekine *et al.*, 2001). The starvation treatment involved no feeding at all, the rotifer treatment involved feeding with rotifers (*Brachionus* sp.) at a rate of 20-30 mL⁻¹·day⁻¹, and the *Artemia* treatment involved feeding with newly hatched brine shrimp (*Artemia* sp.) at a rate of 5 mL⁻¹·day⁻¹ (Cox and Johnston, 2003). Feeding occurred once a day at 9:00 a.m. The survival rate of phyllosomal larvae was checked and recorded every day until the end of the trial (7 days). Water quality parameters (salinity, pH, DO, temperature, total ammonia, nitrite and alkalinity) were monitored at the start and end of the study period.

Data analysis

The relationships between carapace length or body weight and fecundity or hatching rate were examined using a simple linear correlation analysis and Pearson's correlation coefficient. A probability plot was used to test for normality before performing ANOVA. For the starvation and live feed tests, data on the survival rate of phyllosomal larvae were analyzed using one-way ANOVA, and differences between means were tested using Duncan's multiple range test at the 95 % level of confidence. All analyses were performed using the IBM SPSS Statistics for Windows software (version 21.0; IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

In total, 27 ovigerous mud spiny lobsters were included in this study. Carapace length (CL) ranged from 76-106 mm and body weight (BW) ranged from 372.8-921.4 g. During the study period, 18 (66.7 %) ovigerous females successfully hatched eggs, three (11.1 %) females released eggs that did not hatch, and six (22.2 %) females died. Unfortunately, in most published studies to date, breeding management information for this species is very limited, particularly regarding factors affecting hatching performance and mortality under hatchery conditions. The release of unhatched eggs and mortality of the brooders might have been due to unhealthy condition of the females, perhaps suffering from stress during their transfer from Ranong Province to the KFRS hatchery in Prachuap Khiri Khan (the trip took 5-6 h). Environmental conditions during rearing (water temperature, salinity and feed supply) have been reported to affect brooder quality in other studies on spiny lobsters, such as the longlegged spiny lobster *Panulirus longipes* (Matsuda and Yamakawa, 2000), the Caribbean spiny lobster *P. argus* (Cruz *et al.*, 2001), the pronghorn spiny lobster *P. penicillatus* (Matsuda *et al.*, 2006), and the ornate spiny lobster *P. ornatus* (Smith *et al.*, 2009).

Embryonic development

Egg development of mud spiny lobsters comprised three stages that can be differentiated based on the color of eggs attached to the pleopods: stage I being bright orange, stage II-III being orange to dark orange, and stage IV-V being orange-brown to dark brown (Figure 2 and 3). Table 1 presents the embryonic characteristics and mean length (in days) for stage I to stage V, as observed in this study.

Under hatchery conditions, the eggs of the mud spiny lobster required a long time for hatching. During development, the color of the eggs changed from bright orange to dark brown over 22-29 days (mean 24.7 ± 3.8 days), and then newly hatched larvae (Figure 4a) underwent a pronounced metamorphosis within 1-2 h after hatching (Figure 4b and 4c). Some researchers have described these as naupliosomal and pre-phyllosomal larval stages, respectively (Vijayakumaran *et al.*, 2005; Francis *et al.*, 2014), prior to their development into phyllosomal larvae (Figure 4d). On the other hand, other researchers believe that the early larval development of many spiny lobster species does not include naupliosoma and pre-phyllosomal stages, but only a phyllosomal larval stage after

Table 1. Description and period of embryonic development of mud spiny lobsters *Panulirus polyphagus* reared under hatchery conditions (n = 18).

Stage	Egg color	Description.	Period (days)
I	Bright orange	Cell division not evident; eggs fully filled with yolk or yolk granules throughout.	5-14 (10.5 ± 3.9)
II-III	Orange	Free area of yolk conspicuously larger than in stage I, and yolk occupies about 80 % of embryo.	4-12 (7.4 ± 3.4)
	Dark orange	Yolk occupies about 50–60 % of embryo area and eyespots are present.	
IV-V	Orange-brown	Yolk occupies about 20–30 % of embryo area, outline of embryo visible with embryo appearing whitish and eyespots oval shaped.	5-6 (5.7 ± 0.6)
	Dark brown	Yolk fully depleted, larvae fully formed and hatching imminent.	

hatching (Smith *et al.*, 2009; Ayra and Cruz, 2010); this was claimed to be the case for the mud spiny lobster. However, our results clearly show that the early larvae undergo two metamorphoses before reaching the phyllosomal stage, namely a newly hatched larval (or naupliosomal) stage and a pre-phyllosomal larval stage.

Fecundity and hatching rate

Under hatchery conditions (mean salinity 32.1 ± 1.1 ‰, DO 4.0 ± 0.4 mg·L⁻¹, water temperature 26.5 ± 0.3 °C, pH 8.2 ± 0.1 , total ammonia 0.14 ± 0.17 mg·N·L⁻¹, nitrite 0.12 ± 0.14 mg·N·L⁻¹ and total alkalinity 144.2 ± 6.8 mg·L⁻¹ as CaCO₃), the estimated number of eggs produced by female mud spiny lobsters (n = 18) ranged from 212,676-1,444,185 eggs·batch⁻¹. The mean fecundity (F) was $780,770 \pm 340,104$ eggs for ovigerous females with a mean CL of 92.3 ± 8.1 mm and BW of 665.6 ± 147.1 g. Highest fecundity was recorded for a female mud spiny lobster with CL of 102 mm and BW of 870.2 g, while the female with the lowest fecundity had CL of 83 mm and BW of 513.4 g. In addition, the mean hatching rate (HR) under hatchery conditions was 21.9 ± 15.8 % (range 0.0-47.6 %).

Correlation analysis showed that there was a significant positive correlation between F and CL ($r^2 = 0.57$, $p = 0.00$; Figure 5a), and between F and BW ($r^2 = 0.69$, $p = 0.00$; Figure 5b), while

there was not a significant correlation between HR and CL, or HR and BW.

Egg production was directly related to the growth and body weight of females, with fecundity tending to increase as the size increased, which is in agreement with studies on other species of spiny lobsters (Groeneveld *et al.*, 2005; Linnane *et al.*, 2008; Ayra and Cruz, 2010; Vijayakumaran *et al.*, 2012). However, the hatching rate in the current study (0.0-47.6 %, mean = 21.9 ± 15.8 %) was unrelated to body size. As the mechanisms involved in hatching success in mud spiny lobsters are not known, it is unclear whether the long rearing times in incubation tanks (22-29 days, mean 24.7 ± 3.8 days) observed in this study influenced the hatching rate. Unfortunately, only reports on general hatching management of spiny lobster species have been published, and not data regarding hatching rates (Kittaka and MacDiarmid, 1994; Matsuda and Yamakawa, 2000; Sekine *et al.*, 2001; Johnston *et al.*, 2004; Nelson *et al.*, 2004; Groeneveld *et al.*, 2005; Matsuda *et al.*, 2006; Ziegler and Forward, 2007; Linnane *et al.*, 2008; Vijayakumaran *et al.*, 2012; Francis *et al.*, 2014; Ikhwanuddin *et al.*, 2014; Radhakrishnan *et al.*, 2019). The hatchery management in the current study was similar to the protocol of the aforementioned reports. However, other factors such as water quality, food availability and quality should be further examined, as they may influence hatchability in mud spiny lobsters under hatchery conditions.

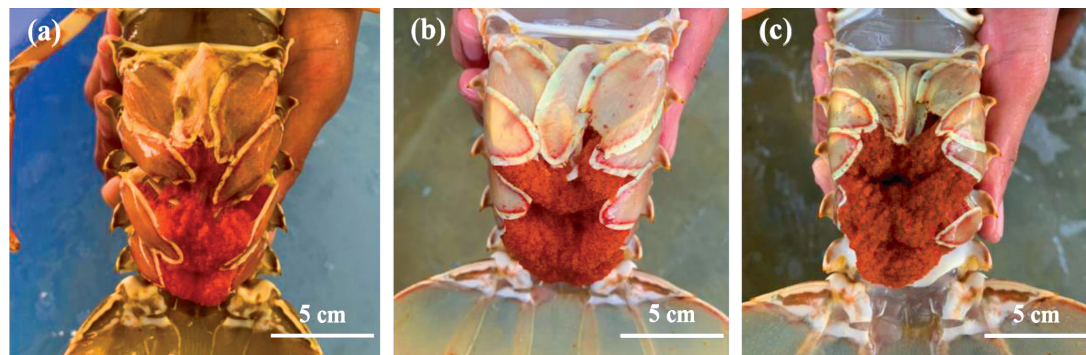


Figure 2. Color characteristics of eggs attached to the pleopods on the abdomen of female mud spiny lobster *Panulirus polyphagus*: (a) bright orange eggs (stage I); (b) orange and dark orange eggs (stage II-III) and (c) orange-brown and dark brown eggs (stage IV-V).

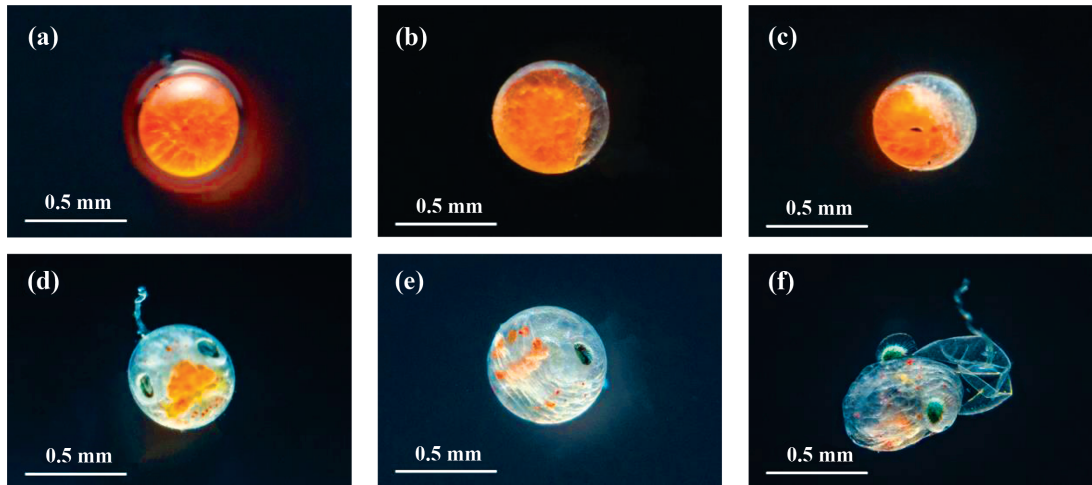


Figure 3. Embryonic development of mud spiny lobster *Panulirus polyphagus*: (a) bright orange egg (stage I); (b) orange egg (stage II); (c) dark orange egg (stage III); (d) orange-brown egg (stage IV); (e) dark brown egg (stage V); and (f) hatching.

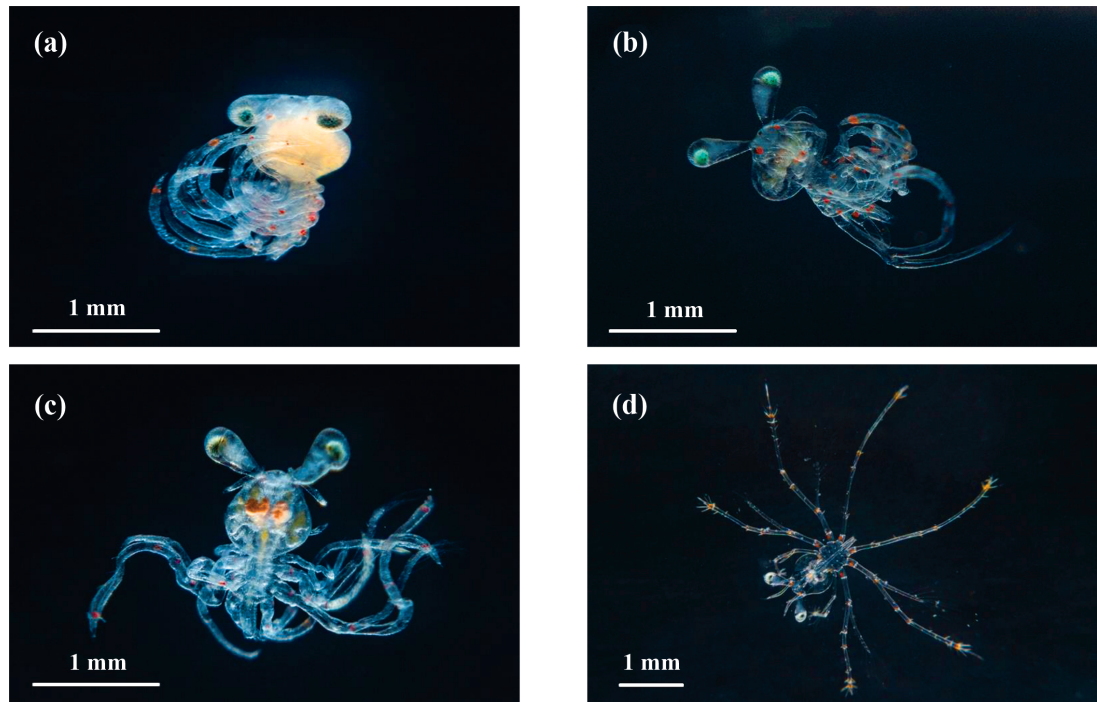


Figure 4. Metamorphosis of mud spiny lobster *Panulirus polyphagus* larvae under hatchery conditions: (a) newly hatched larva (naupliosoma); (b-c) larvae at 1-2 h after hatching (pre-phyllosoma); and (d) larva which has already developed into phyllosoma (primary phyllosomal larva).

Starvation and live feed tests on survival of early larvae

Water conditions (salinity 30-31 ‰, pH 8.32-8.65, DO 3.91-4.70 mg·L⁻¹, water temperature 26.9-27.3 °C, total ammonia 0.00-0.02 mg-N·L⁻¹, nitrite 0.00-0.32 mg-N·L⁻¹ and total alkalinity 134-148 mg·L⁻¹ as CaCO₃) in the glass aquaria were not significantly different among treatments (p<0.05). Mean survival rate of phyllosomal larvae in the starvation treatment (41.6±7.6 %) was

significantly reduced at day 1 as compared to the rotifer- and *Artemia*-fed treatments (86.6±7.6 and 88.3±12.5 %, respectively, p<0.05). The survival of the unfed larvae was lowest throughout the trial and reached zero at day 4. The group fed with *Artemia* had the highest survival rate from day 2-7, but with a declining trend (Figure 6). At the end (7 days) of the experiment, all the phyllosomal larvae in the rotifer treatment had died, while survival in the *Artemia* treatment was 26.6±7.6 %.

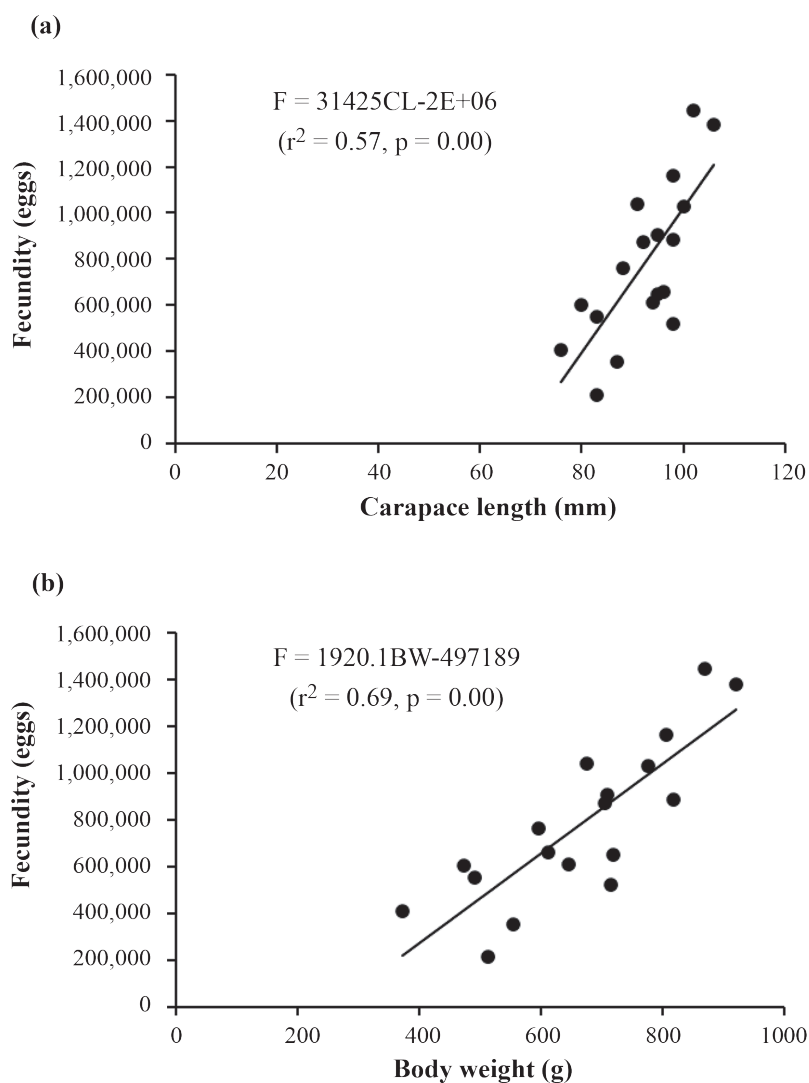


Figure 5. Correlation between (a) carapace length and fecundity and (b) body weight and fecundity of mud spiny lobster *Panulirus polyphagus* under hatchery conditions (n = 18).

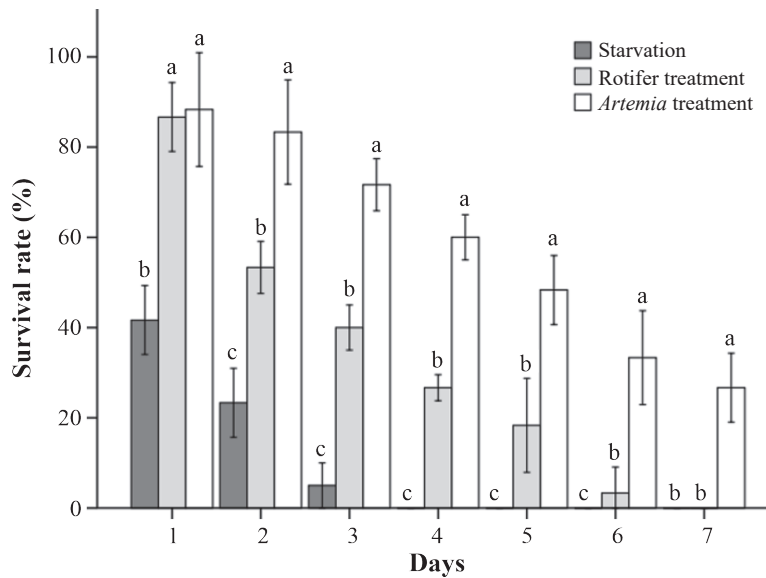


Figure 6. Survival rate of phyllosomal larvae of mud spiny lobster *Panulirus polyphagus* in starvation and live feed tests. Different lower case letters above bars indicate a significant difference between treatments on the same day of observation ($p < 0.05$). Error bars indicate SD.

Normally, many spiny lobster larvae in this period (about 5-7 days after hatching) were primary phyllosomal larvae and then will develop to secondary phyllosomal larvae. The survival of secondary phyllosomal larvae is affected by many factors such as water temperature, salinity and food supply (Smith *et al.*, 2009; Ayra and Cruz, 2010; Francis *et al.*, 2014). The current results show that food availability had a noticeable influence on the survival rates of the mud spiny lobster larvae. Clearly, the phyllosomal larvae should be fed immediately after hatching because the larvae cannot survive starvation. Starvation plays a significant role in determining larval survival in other spiny lobster species, including the Japanese spiny lobster *Panulirus japonicus* (Mikami and Takashima, 1993), the southern rock lobster *Jasus edwardsii* (Johnston *et al.*, 2004) and the tropical spiny lobsters *Panulirus orantus* and *P. homarus* (Smith *et al.*, 2010). The main reasons reported for the mortality of larvae were their lack of nutritional reserves in the eggs from the hatched females, and the lack of access to sufficient food items (Mikami and Takashima, 1993; Johnston *et al.*, 2004; Smith *et al.*, 2010). The resulting nutritional stress decreased or ceased

larval development, with death following (García-Echauri *et al.*, 2019). On the other hand, based on the survival rates of the early larval stage of the mud spiny lobster in the current study, the provision of live feeds allowed greatly improved survival. *Artemia* nauplii was a more appropriate food than rotifers, as the survival rate of larvae fed with *Artemia* nauplii was significantly higher than for those fed with rotifers from day 2 onwards. Thus far, however, attempts to rear mud spiny lobster and other related species from phyllosomal larvae to the puerulus and juvenile stages in mass culture have been largely unsuccessful. Efforts may be hampered by the provision of unsuitable diets during the long phyllosomal larval phase, and currently there is no commercially formulated feed for their rearing (Sekine *et al.*, 2001; Cox and Johnston, 2003; Nelson *et al.*, 2004). The cryptic nature of feeding preferences, complex larval morphology and the length of the larval life cycle are problems affecting their successful rearing under commercial hatchery conditions (Francis *et al.*, 2014; Radhakrishnan *et al.*, 2019). Sekine *et al.* (2001) reported that the duration of the phyllosomal stage was 231-417 days (mean 319.4 days), and

the duration of the phyllosomal stage development in captivity was affected by water temperature and nutritional conditions during the phyllosomal stage (Nelson *et al.*, 2004).

In the current study, the water quality during the hatching and rearing periods were in the following ranges: salinity 30-33 ‰, DO 3.5-4.9 mg·L⁻¹, water temperature 26.0-27.4 °C, pH 8.0-8.6, total ammonia 0.00-0.89 mg·N·L⁻¹, nitrite 0.00-0.66 mg·N·L⁻¹ and total alkalinity 131-155 mg·L⁻¹ as CaCO₃. Ikhwanuddin *et al.* (2014) reported that the optimum water temperature for rearing ovigerous female mud spiny lobsters was 25-30 °C, and that lower temperatures (15-20 °C) could cause mortality and trigger the release of the eggs by the female due to stress. However, optimal levels for the other water parameters have not been clearly defined for successful breeding of this species. In addition, the predominant problems facing mud spiny lobster breeding include low survival rates during the larval stage, the delicate maintenance of numbers in the puerulus and juveniles stages, and the difficulty in obtaining mature broodstock, particularly males (Williams, 2007; Ikhwanuddin *et al.*, 2014; Fatihah *et al.*, 2016). Nonetheless, the production of seed individuals in the hatchery is also important in conservation management strategies that result in the successful release of mud spiny lobster juveniles to the sea. Thus, the development of approaches to improve breeding and larval nursing are interesting and useful topics for future study, and could include research on factors influencing hatchability and improved alternatives to live feed to increase larval survival.

CONCLUSION

The current study indicated that in hatchery conditions, the embryonic development of mud spiny lobsters, *Panulirus polyphagus* took 10.5±3.9 days from bright orange eggs (stage I) to orange-dark orange eggs (stage II-III), and another 7.4±3.4 days to orange/brown-dark brown eggs (stage IV-V). Development from dark brown eggs to hatching required 5.7±0.6 days. Furthermore, the number of eggs increased linearly with increased carapace length and body weight of the female, while the

hatching rate was unrelated to body size. In addition, one of the key factors affecting the early larval survival was food availability. The phyllosomal larvae could not tolerate food starvation, as this caused high mortality at 1 day after hatching and complete mortality within 3-4 days. *Artemia* nauplii could be used for nursing the early larval stage, and showed better success than feeding with rotifers. Our study provides basic information that paves the way for further studies aiming at the development of successful hatchery practices for mud spiny lobster in the future.

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