

## Development of mud crab breeding technology for conservation and communal livelihoods in the Setiu Wetlands, Terengganu, Malaysia

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### Abstract

Setiu Wetlands is located along the Terengganu coastline which comprises various ecosystems providing a suitable natural environment for breeding and grow out of various organisms. The mud crab is considered to be one of the important natural resources and a source of income for the local community in the Setiu Wetlands. The aim of this study is to develop the breeding and larviculture technology of mud crab (*Scylla paramamosian*) for conservation and livelihood activities in Setiu Wetland. The matured broodstock of mud crab was collected from Setiu Wetland and transferred to AKUATROP hatchery in Universiti Malaysia Terengganu for the breeding experiments. After eyestalk ablation, crabs were separately cultured in 50L dark tanks with sandy bottom connected with recirculating systems and fed trash fish and with blood cockle twice a day. After egg spawning, ovigerous crabs were moved to incubated tanks with continuous aeration and not fed until hatching. Strong phototactic larvae were collected and stocked in 500L tanks at 300inds/L of density. The crab larvae were fed *Artemia* from Zoea 1 to Zoea 3, and intergraded artificial feed from Zoea 4. After megalope metamorphosis, larvae were transferred to larger tanks with substrates. The results showed that 80-90% of broodstock spawned eggs after 50 days of maturation culture. The larvae took 23 to 27 days to complete the ontogenetic development with 5 -10% of crablet survival rate. These results could contribute to crab conservation and livelihood activities in Setiu Wetlands.

**Keywords:** Mud crab, Breeding, Larviculture, Conservation, *Scylla paramamosian*

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### Introduction

Mud crab (*Scylla paramamosain*) are widely distributed in Asian countries, especially in mangrove areas of Pacific and Indian ocean regions (Keenan and Davie, 1998). Recently, mud crab aquaculture was expanded due to its nutrient value, good growth

performance and high market demand. Crab farming rapidly increased leading to the overexploitation of wild crablet resources. Several efforts aimed to improve the mud crab hatchery technology for sustainable development of mud crab culture. However, the seed production unmet the demand because of low survival rate and mass mortality



during larval stages (Jithendran et al., 2010, Wu et al., 2016). Previous studies reported that main constraints were nutrition (Holme et al., 2009), diseases (Lavilla-pitogo and Peña, 2004; Jithendran et al., 2010) and water quality (Li et al., 2008; Li et al., 2012) causing a huge economic loss in crab farming and hatchery husbandry as well.

In Malaysia, mud crab breeding and larvae rearing have not been successful which led to low mud crab production (Tan, 1997, Linh et al., 2017). Mud crab have been mainly exploited by local fishermen based on the mangrove forest in estuaries and coastal area (Ikhwanuddin et al., 2011). The mud crab culture in Malaysia started in 1991 and they were cultured in ponds or pen among the mangrove trees (Tan, 1997). Presently, mud crab culture systems commonly practiced are grow out, fattening and production of soft-shell crabs using seed harvested mainly from the wild (Ikhwanuddin et al., 2014). The mud crab production in Malaysia has seen a noticeable decline in the natural habitat from 623 tons in 1995 to 162 tons in 2005 (Shelley, 2008; Linh et al., 2017) due to overexploitation and indiscriminate fishing of juvenile crab by fishermen. However, mud crab seed captured from the wild is not enough to sustain the current status of mud crab production. Therefore, mass seed production technology of mud crab needs to be developed (Holme et al., 2006).

Setiu Wetlands are one of the most important mud crab research sites (Ikhwanuddin et al., 2014), where three dominant mud crab species as *Scylla olivacea*, *Scylla transquebarica*, *S. paramamosain* are present (Zaidi et al., 2011). Zaidi et al. (2011) also demonstrated that the mud crab in Setiu, Malaysia provide good resources for local fishermen. However, artificial breeding and larval rearing are difficult techniques (Keenan, 1999) because, at the hatchery phase, three main issues are encountered: disease outbreak, incomplete rearing techniques, and lack of nutrition requirement (Sorgeloos and Léger, 1992).

However, the research on mud crabs in Malaysia mainly focused on biodiversity, ecology, fisheries and aquaculture (Ikhwanuddin et al., 2011, Ikhwanuddin et al., 2014). Presently, the green mud crab (*S. paramamosain*) is considered a good potential aquaculture species because it can reach the market size (200 to 300g) after 3 months (Christensen et al., 2004). This study aims to improve the technology of mud crab (*S. paramamosain*) breeding and larvae rearing for mud crab conservation and communal livelihoods in the Setiu Wetlands.

## Material and Methods

### Broodstock collection and maturation culture

The matured female crabs with full ovary were selectively harvested from Setiu Wetlands by putting trap with fish baits (Figure 2A). The live specimens were kept in moisture conditioned tanks and transferred to AKUATROP hatchery, Universiti Malaysia Terengganu. To counteract disease infections, crabs were treated with 200ppm of formalin for 15 minutes, then stocked in 200L tanks with sea water (30ppt) and continuous aeration for 3 days to acclimate to the hatchery condition. After acclimation, the artificial breeding was applied by eyestalk ablation, then disinfected with 200 ppm formalin for 15 minutes and transferred to sand bottom tanks separately (Figure 2B). All cultured tanks were kept in dark condition and connected to recirculating system to cycle 300% of water per day. Crabs were fed daily in the morning and evening with marine fish and blood cockle. When the crab laid eggs, berried crabs were sent to incubator tanks with clean water and continuous aeration without food until hatching (Figure 2C and 2D). Every day, water was exchanged 100% to remove excreta and shed out eggs, also avoiding bacterial infection.

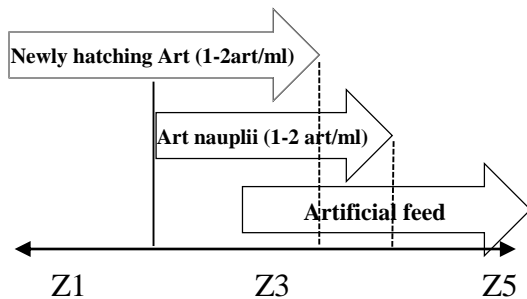
### Larvae collection and rearing

After hatching, strong photopositive larvae were immediately collected and transferred to 100L tanks by mesh net to estimate the number of larvae. The larvae were disinfected again with 200 ppm formalin for 30 seconds. Clean filtered seawater was filled up into 1000L rearing tanks with vigorous aeration (Figure 2E). Larvae were introduced into rearing tanks at 300 inds/L of stocking density. The salinity was maintained at 30ppt, pH 7.5 - 8.5, temperature 28-30 °C, and alkalinity 120 -150 ppm/L. The larvae were fed every 4 hours with umbrella *Artemia* from Zoea 1 (Z1) to Zoea 3, followed by enriched *Artemia* (Selco - INVE, Belgium) and artificial feed from Zoae 4 (Figure 1). When the larvae reach to Megalope stage, the stocking density was reduced to 50 inds/L combined to putting artificial substrates to avoid cannibalism (Figure 2F). From Megalope stage to crablet, crab larvae were mainly fed artificial feeds. Water exchange was conducted every 3 days at 30 % of rearing water. Larvae were daily monitored before feeding. Dead larvae and exuviae were daily siphoned out to prevent contamination. Assessment of each



larval stage was recorded after metamorphosis to determine feeding and survival rates.

Larval stage



**Figure-1: Feeding regime for crab larvae**

Larval Stage Index (LSI) was determined by the formula as:

$$LSI = [(N1 \times n1) + (N2 \times n2) + (Ni \times ni)] / (n1 + n2 + ni)$$

Where: N1, N2, Ni: larval stage

n1, n2, ni: number of larvae

Survival rate of larvae was estimated for each stage by the formula below:

$$\text{Survival rate (\%)} = (\text{number of larvae} / \text{initial stocking number}) \times 100$$

## Results and Discussion

### Spawning and larval development

The female broodstock laid the egg after 30-50 days of maturation culture (Figure 2C) with high spawning percentage (80-90%). Embryo development and hatching time ranged from 10 to 12 days (at 28-30 °C). In which, egg colour gradually changed from yellow, yellow-grey, grey and black colour indicating ready hatching eggs. Different colours of eggs during embryo development were similarly described among *Scylla* crab including *S. serata*, *S. olivacea*, *S. tranquebarica* and *S. paramamosain* whereby from light yellow to dark orange, then becoming light greyish orange and dark grey before turning to black prior to hatching within 8 – 10 days at 30 °C (Hamasaki, 2003; Zeng, 2007; Ates et al. 2012; Waiho et al., 2018). The *S. paramamosain* larvae comprised 5 Zoeal stage (Z1, Z2, Z3, Z4 and Z5), Megalope and crablet and it took from 23 to 27 days to complete the larval development. The zoeal stages are planktonic and photopositive while Megalope and crablet attached on substrates. Cannibalism strongly occurred from Megalope stage. Life cycle and larvae

development of *S. paramamosain* have been reported by several studies (Quinitio, 2011; Linh et al., 2017) and strongly depended on water temperature and salinity (Waiho et al., 2018). However, Zeng et al. (2004) reported that a sixth zoeal stage (Z6) appeared in unfavorable conditions such as unsuitable live food, insufficient feeding and prolonged starvation (Zeng et al. 2004; Waiho et al., 2018). The body length and number of setae at all body parts are the main differences between Z5 and Z6 (Zeng et al., 2004; Waiho et al., 2018). The time to complete its life cycle varies among *Scylla* species: *S. tranquebarica* took 22 days (Thirunavukkarasu & Shanmugam, 2014), 22 to 35 days for *S. serata* and *S. olivacea* (Ong 1964; Jantrarotai et al. 2006). To explain this variation, temperature and salinity were considered important factors affecting larvae growth performance, metamorphosis and survival through influencing the physiological processes of larvae (Hamasaki 2003; Nurdiani and Zeng 2007; Baylon 2010).



**Figure-2: Mud crab larvae rearing in AKUATROP hatchery. A) Broodstock collection in Setiu wetlands; B) Maturation culture systems; C) Berried crab; D) Incubator tank; E) Larval rearing tanks; F) Crablets attaching on the artificial substrates.**



### Larval stage index and survival rate

The survival rate and larval stage index of mud crab larvae are shown in Table 1. The LSI was recorded from 35.2 to 83.2 during larval development. Additionally, the larval survival rate significantly decreased from Z1 to crablet stage with a critical stage between Zoea 5 and megalope metamorphosis. After the complete larval development, the average survival rate was recorded at  $6.3 \pm 3.7$  %. From Megalope stage, the cannibalism was considered one of the factors related to larval survival rate which could daily reduce 10% of larval number (Linh et al., 2017). Previous studies have reported 3 -7 % of survival rate for *S. serata* and 6.9% for *S. tranquebarica* (Thirunavukkarasu & Shanmugam, 2014). Especially, *S. paramamosain* hatcheries in Viet Nam have consistently achieved 10-15% of survival from Z1 to crablet. However,  $6.3 \pm 3.7$  % of survival rate at crablet stage is promising to apply for hatchery husbandry practice in commercial scales. Moreover, crab seed can be directly released to the wild for conservation as crablet adapts well to wild condition.

**Table-1: The larval stage index and survival rate of mud crab larvae during ontogenetic development**

Stage	Larval stage index (LSI)	Survival rate (%)
Zoea 2	$35,2 \pm 2,3$	$92.3 \pm 4.5$
Zoea 3	$72,2 \pm 3,6$	$80.6 \pm 6.2$
Zoea 4	$83,2 \pm 3,3$	$60.8 \pm 5.8$
Zoea 5	$60,7 \pm 3,1$	$52.6 \pm 6.4$
Megalope		$20.6 \pm 9.4$
Crablet		$6.3 \pm 3.7$

### Some issues in mud crab breeding and larvae culture

During the experimental periods, some issues were observed and recorded that strongly affected the broodstock maturation, eggs quality and larval survival rate. Fungus and bacterial infections caused several problems for both broodstock and larvae (Figure 3). Intensive shell erosion and “brown spot” (Figure 3 A and B) were observed due to bacterial infection on broodstock; egg loss and unhatched eggs on berried crab (Figure C and D) (Faizah et al., 2017). Additionally, bacteria, fungus and protozoa (Figure E and F) could lead to mass mortality during larval stages (Lavilla-pitogo and Peña, 2004; Jithendran et al., 2010).



**Figure-3: Some issues in crab breeding and larval culture. A and B) Bacterial infection caused brown spots and shell erosion; C) Egg loss; D) Unhatched eggs; E) Protozoa infection; F) Fungal infection in rearing tank.**

### Conclusion

The primary results of mud crab breeding in Malaysia have been achieved from broodstock maturation culture to larval rearing with 80 – 90 % of spawning and  $6.3 \pm 3.7$  % of crablet survival rate. Improved biosecurity to prevent fungal, bacterial and protozoal infection that caused mortality of broodstock and larvae is required.

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### Contribution of Authors

Khoa TND: Conducted experiments, data analysis and manuscript write up



Faizah SH: Project leader and supervisor, manuscript final reading and approval

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**Conflict of Interest:** None.

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