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Review

# Larval rearing of mud crab (Scylla): What lies ahead

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#### ABSTRACT

The increasing global demand for mud crabs (genus Scylla) and threats to the wild populations highlight the urgency of fully rearing them in captivity. Despite considerable progress in mud crab production, most crab farms still rely heavily on wild-caught crablets and juveniles while the low and inconsistent success rates of larviculture remain as the main bottleneck impeding the development of mud crab aquaculture. Over the years, numerous studies have been conducted to determine the optimum larval rearing parameters, the ontogenic changes in digestive function and feeding behaviour, and the diets for different larval stages. These data, however, are dispersed and not summarised to inform culture practices. This review provides an update on the current progresses and to pinpoint the gaps in knowledge regarding mud crab larval rearing. We include all four mud crab species under the genus Scylla, i.e. Scylla serrata, Scylla olivacea, Scylla tranquebarica and Scylla paramamosain. Knowledge compiled in this review serves as an important guideline for prospective mud crab larviculture. Future research should gear towards filling in the gaps in our knowledge to advance mud crab larval rearing, thus fully incorporating mud crab into the aquaculture sector.

#### 1. Introduction

Mud crab genus Scylla De Haan, 1833 (Brachyura: Portunidae) is an economically important crustacean species that is widely distributed throughout the Indo-West-Pacific region (Keenan et al. 1998). Its taxonomy has been controversial. Mud crabs were previously recognised as a single species, i.e. Scylla serrata (Forskål, 1775). Estampador (1949) was the first to report three different species (S. serrata, S. oceanica (Herbst, 1796) and S. tranquebarica (Fabricius, 1798)) and one variation (S. serrata var. paramamosain) based on their variation in size, colour and shape. However, due to the unclear differentiation between species and variation, different authors had different taxonomic classifications of the genus Scylla (Serene 1952; Stephenson & Campbell 1959; Perrine 1978; Quinn & Kojis 1987) until Keenan et al. (1998) revised this genus into four distinctive species based on their morphological, morphometric and molecular differences, namely S. serrata, S. tranquebarica, S. olivacea (Herbst, 1796) and S. paramamosain Estampador, 1949. The geographical distribution of S. serrata is the widest, covering the tropics and subtropical coastal area of Indo-West-Pacific region while the other three species are more concentrated around the equator (Keenan et al.

1998; Ikhwanuddin et al. 2011; Alberts-Hubatsch et al. 2016; Fazhan et al. 2017a).

They inhabit intertidal mangrove forests with fluctuating salinity and support the livelihood of local fishery communities (Keenan et al. 1998; Ikhwanuddin et al. 2011; Alberts-Hubatsch et al. 2016). Global capture production of Scylla was above 20,000 t in the last decade (FAO 2018) but the global aquaculture production of mud crabs however, was below 15,000 t since 1980 until 2003, after which it increased exponentially to above 100,000 t with the inclusion of data from China (Fishery Bureau of Ministry of Agriculture of China 2012) and has been increasing steadily ever since (FAO 2018). It is expected that the growth in aquaculture sector will rise due to increasing demand from the global market (Goldburg & Naylor 2005). However, sustainable aquaculture has yet to be achieved for Scylla species since crab farms still rely heavily on wild-caught crabs for seed stock, fattening, and soft-shelled crab production (Ewel 2008; Alberts-Hubatsch et al. 2016; Waiho et al. 2016, 2017; Fazhan et al. 2017b). Large scale aquaculture of mud crabs is currently limited due to very low success rate in the hatchery production of juveniles (Quinitio & Parado-Estepa 2008; Holme et al. 2009a; Waiho et al. 2015). For the past years, attempts for mass rearing

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Crab stage	Description
Zoea (Z) 1	Sessile eyes; abdomen segment = 5; lateral spines present on abdomen segments = 2 & 3 (S. olivacea), 3–5 (S. serrata); ABL = 1.33 mm (S. serrata), 1.12–1.25 mm (S. olivacea) olivacea)
Z2	Stalked eyes; abdomen segment = 5; lateral spines more distinct; ABL = 1.63 mm (S. serrata), 1.47-1.65 mm (S. olivacea)
Z3	Abdomen segment = 6 (plus telson); observable pereopod buds; ABL = 2.09 mm (S. serrata), 1.90–2.17 mm (S. olivacea)
Z4	Elongated lateral spines; distinct pleopod buds at the ventral posterior end on segments = $2-6$ ; ABL = $3.05 \text{ mm}$ (S. serrata), $2.35-2.80 \text{ mm}$ (S. olivacea)
Z5	Pleopod buds increase in size, with setae and are biramous; ABL = 4.05 mm (S. serrata), 3.35-3.80 mm (S. olivacea)
М	First pereopod developed into cheliped; increase in carapace width and size; ABL = 1.79 mm (S. serrata)
CI	Crab-like appearance; carapace margin serrated; ABL = 3.06 mm (S. serrata)

General developmental descriptions and the average body length (ABL) of *Scylla* larvae to crab instar (Ong 1964; Jantrarotai et al. 2006; Lumasag et al. 2007; Yi et al. 2009; Thirunavukkarasu et al. 2014).

Note: Z1 = zoea stage 1; Z2 = zoea stage 2; Z3 = zoea stage 3; Z4 = zoea stage 4; Z5 = zoea stage 5; M = megalopa; CI = crab instar.

of juvenile seeds in the hatchery had been carried out in countries such as Vietnam (Nghia et al. 2007a), Japan (Hamasaki et al. 2011), and the Philippines (Quinitio et al. 1999, 2001; Quinitio & Parado-Estepa 2008).

Crustacean larvae are highly vulnerable to diseases during the initial developmental stages and require specific biotic and abiotic conditions for normal growth and development (Azra & Ikhwanuddin 2015). Commercial hatchery production of crustacean larvae relies on the optimisation of three interrelated factors: rearing conditions, disease and nutrition (Sorgeloos & Léger 1992; Nghia et al. 2007a). The sub-optimal condition of any one of these factors significantly affects the health and growth of larvae, and if left unattended, results in mass mortality of larvae. Works on various aspects of mud crab larviculture and larval rearing technology were reported throughout the years. However, the production of mud crab larvae remained unsatisfactory and most hatcheries encounter inconsistent survival rates of Scylla zoea to crablet stages primarily due to bacterial and fungal infections (Bian & Egusa 1980; Nakamura et al. 1995; Roza & Hatai 1999; Lavilla-Pitogo & de la Peña 2004; Quinitio & Parado-Estepa 2008; Dan & Hamasaki 2015), and molt death syndrome during the transition from zoea 5 (Z5) to megalopa stage (Williams et al. 1999; Hamasaki et al. 2002; Holme et al. 2007; Quinitio et al. 2015; Pates et al. 2017). Another main obstacle in Scylla larviculture is the lack of suitable larvae diets (Quinitio et al. 2001; Baylon et al. 2004; Ruscoe et al. 2004; Holme et al. 2006; Baylon 2009; Holme et al. 2009b; Quinitio et al. 2015). Most hatcheries currently rely heavily on live foods (e.g. Artemia and rotifers) that have inconsistent nutritional values (Tucker 1992; Quinitio et al. 1999, 2001; Quinitio & Parado-Estepa 2008; Baylon 2010) and could also potentially introduce harmful pathogens into the culture (Bentzon-Tilia et al. 2016). This review presents and analyses the current knowledge, research trends and efforts of hatchery practices used in mud crab larviculture, with a general emphasis on the larvae of all Scylla species, if available. The advantages and differences of each aspect were discussed, and recommendations for the optimal hatchery production of mud crab larvae were suggested. The gaps and bottlenecks that need to be overcome in the future were also identified to drive the Scylla larviculture forward.

### 2. Embryonic and larval development

The ten-stage embryonic development of three *Scylla* species, i.e. *S. serrata, S. tranquebarica* and *S. olivacea* has been described in detail by Ates et al. (2012). In general, precleavage and cleavage occur right after egg extrusion, and the embryo enters multicell stage within 1 day. It will then enter the intermediate multicell-gastrula stage where the formation of one or two yolk-free spaces occur. In the following gastrula stage, the yolk-free space increases in size and forms a U-shaped band (germinal disc). Appendage buds are apparent as translucent globular structures in yolk-free space during this stage. A significant decrease in yolk volume (15–20% reduction from 95% in intermediate multicell-gastrula stage) and opacity is expected in the subsequent naupliar stage.

In the eye formation stage, a pair of red eyes as short thin strip can be observed. Then, the embryo undergoes differentiation of the thorax and abdominal regions. Heartbeat is detectable at a rate of < 200 beats per min once it reaches stage 9. The differentiation of the telson at the abdomen and the appearance of chromatophores on the thorax also occur during this stage. During the final prehatch stage, the heartbeat rate is > 200 beats per min and the overall zoeal body are more defined. The ratio of embryo:yolk is about 4:1 of the total egg volume. The colour of the egg mass changes from light yellow to dark orange, then becomes light greyish orange and dark grey before turning to black prior to hatching. With a mean rearing temperature of 28-29 °C, the incubation period of S. serrata (10.3  $\pm$  0.3 days) was significantly longer compared to that of S. tranquebarica (8.7  $\pm$  0.6 days) and S. olivacea (8.6  $\pm$  0.2 days) (Ates et al. 2012). When examined in vitro, the duration of embryonic stages of S. paramamosain increases with the decrease in water temperature, with eggs incubated at 30 °C hatched within 9 days whereas those incubated at 20 °C took almost a month to hatch (Zeng 2007). Similar results were also reported when eggs of S. serrata were maintained in vivo at a range of incubation temperature (20 to 30 °C) (Hamasaki 2003). It is therefore recommended that the incubation temperature of ovigerous females is maintained at 29-30 °C to promote embryonic development and hasten larvae hatching.

The detailed description of larval stages has been reported for the two Scylla species, S. serrata (Ong 1964) and S. olivacea (Jantrarotai et al. 2006). Identification of each larval stage was based on the differences of their appendages, i.e. mandible, maxillule, maxilla including antennule, antenna, maxilliped and telson (Ong 1964; Jantrarotai et al. 2006). In general, a mud crab larva goes through five distinct stages: Zoea 1 (Z1) to Zoea 5 (Z5) (Table 1), each lasts approximately 3-5 days, and megalopa stage which lasts for 7-10 days prior to molting to first crab stage (C1) (Ong 1964) (Table 2). Each zoea stage can be distinguished from each other based on the differences in the number of setation of their maxilla (comparison of S. serrata and S. olivacea larvae in Jantrarotai et al. 2006). Body length during zoeal stage increases by approximately 30% between each molt (Yi et al. 2009). The larval stages of mud crabs are pelagic and dispersive. It was postulated by Webley and Connolly (2007) that they start to return to the nearshore coastal shelf zone once they reach megalopa stage. The pelagic megalopae then metamorphose into benthic crablets and with the assistance of shore currents and changing tides, these crablets return to intertidal zones and grow into adulthood.

In addition to the five zoeal stages, a sixth zoeal stage (Z6) was observed in *S. paramamosain* when feeding conditions are unfavourable, e.g. unsuitable live food (fed solely on rotifers at later larval stages), insufficient feeding and prolonged starvation (Zeng et al. 2004). The main differences between larvae of Z5 and Z6 were the increase in body length and number of setae at all body parts (Zeng et al. 2004). A similar additional zoeal stage was also reported in the larvae of other brachyurans such as the red frog crab *Ranina ranina* (Linnaeus, 1758) (Minagawa & Murano 1993), grapsid crab *Chasmagnathus granulata* Dana, 1851 (Pestana & Ostrensky 1995) and mud crab *Rhithropanopeus* 

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