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Mini Review
Portunid Crab Breeding and Larval Rearing, Paving the Way for Sustainable Aquaculture

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Abstract
This mini-review focus primarily on the current knowledge and techniques of portunid breeding and larva rearing. Topics include; (i) natural breeding in captivity and artificial breeding for breeding techniques and (ii) environmental conditions, diet requirements and developing factors for larval rearing. Specific knowledge of portunid crab breeding and larval rearing essential for optimizing breeding and culture conditions are shared. On the whole, this work allows for a better understanding of the portunid crab breeding and larval rearing and helps pave the way for future sustainable aquaculture of portunid crabs, among commercially important crustacean family in the world.

Key words: Breeding technology, Crustacean, Domestication, Larval rearing, Seed production

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Crab farming is not a new invention. Wild-caught crabs have been collected and raised in ponds for hundreds of years. However, modern aquaculture of Portunid crabs has only recently taken center-stage as the preferred commercial method. Portunid crabs are one of the main commodities of coastal fisheries as crabbing for portunids has been carried out by fishermen in almost all brachyuran crab-present mangrove regions[1,2]. The knowledge on Portunid crab aquaculture has been acquired by researchers in the Asia-Pacific region including Malaysia allowing for notable rapid progress in culture methods over the past decade. Unrestricted capture, combined with habitat destruction and pollution, inevitably puts much stress on portunid crab populations[3]. Initially, farming of portunid crab (a still common practice in most countries) involves the capture of wild juveniles and growing them out (a process termed as 'fattening') before selling them off[4]. In addition, juveniles are also captured in large volume to induce molting for the production of soft-shelled crab, a delicacy that fetches a much higher price than normal hard-shelled crabs[5]. Furthermore, almost all current hatchery operations obtain wild-berried females for their stocks[6]. This negatively impacts the wild local population and offers no control over the parent's heritage. These factors threaten the sustainability of the portunid crab populations in the wild and eventually lead to population reduction[7]. Thus, the focus has been shifted to the aquaculture production of portunid crab to counter the sustainability issues of their wild populations. The hatchery seed production and nursery culture of portunid crab is still in the experimental stages as high mortality due to cannibalism are often observed when stocking density is increased[8].

Mud crab, genus *Scylla* is the least studied species compared to the other portunid crab species such as *Portunus pelagicus* within the Portunidae family due to its more confined distribution around the Indo-pacific region[9,10]. However, this mud crab species is prevalent and dominant in South East Asia, especially in water bodies around Malaysia[11,12]. Comparing portunids with greater geographical dominancy, not many in-depth studies have been conducted on certain mud crab species, especially *S. olivacea*. The research conducted on mud crab species in Malaysia is still in its early stages, with the most recent research focused on early culture management[13].

However, the lack of knowledge on its broodstock development, breeding techniques and larval rearing can be a hampering factor in understanding an optimum production of portunid crab aquaculture. This review, therefore, was focused primarily on breeding techniques and larva rearing of portunid crab. Numerous inquiries from the general public, researchers and farmers about portunid crab aquaculture over the past couple years has inspired the need for an academic book that describes the knowledge of portunid crab breeding and larval rearing, the foundation for the production of high-quality crablets for sustainable aquaculture production.

BREEDING

Portunid crablet production is primarily dependent on wild-caught animals. Artificial production through *in vitro* fertilization and breeding can be a sustainable source of crablets. Breeding, also known as sexual reproduction that finally produces offspring may include either natural conditions (wild) or artificial conditions (laboratory/captive). Knowledge on breeding especially of portunid crab is important for potential mass culture. Understanding seasonality and breeding conditions are essential for optimizing seed production in captivity and through manipulation could trigger females to reproduce more than once in captivity or breeding season[14]. The term berried female for portunid crab usually refers to females carrying eggs on their pleopods. Artificial breeding especially in portunid crab could enable the production of berried females outside their breeding season. In this chapter, portunid crab breeding aspects of mating, spawning, incubation, hatching, natural breeding in captivity and artificial breeding through *in vitro* fertilization techniques are discussed.

Mating: Male portunid crabs sometimes exhibit dark spots or abrasions on their underside and first walking legs, due to the shell rubbing against the female while they are coupled for the several days preceding the female molt and subsequent mating[12]. These mating scars show that the male has recently mated, thereby providing information on the health of the production facility. The male may mate during its third or fourth intermoult phase after it matures. Females mate once in their lives immediately following the pubertal moult[15].

Spawning: At the time of spawning, the female crab digs a small depression in the sandy bottom with her abdominal flap. The eggs are released into the hole and she proceeds to gather them up with the pleopods. Berried females do not eat while carrying eggs and thus are not caught in traps. Portunid crab eggs are bright orange when first produced but by the time they are ready to hatch they have turned black. The eggs are about 0.3 mm in diameter and a single crab may lay over two million eggs[16]. In spite of this large number of eggs, most larvae and juveniles do not survive to sexual maturity.

Incubation and hatching: Female Portunids carrying an egg cluster are no longer eating and need no feeding. However, even small changes in water temperature of a few degrees can reduce the survival of larvae[17]. Keeping the water temperature in the range of 25 to 32°C seems to be close to optimal for Portunids although even narrower thermoclines may be beneficial. A five, nine or ten-point scale has been developed to monitor embryonic development but the five-point scale seems to be the easiest to use[18]. The importance of preparation of tanks and feed cannot be overemphasized and hatchery personnel must be ready to provide for the larvae right after hatching. They must be trained in recognizing the signs of hatching and the timing from the earlier phases. The condition of the eggs and the developmental stage can be determined by visual inspection of the egg mass. More reliable signs can be observed by removing samples of eggs and examining them using low-power microscopy[19].

Natural breeding in captivity

Mating behaviour in captivity: The entire portunid crab mating process can be divided into four phases, (i) pre-copulation, (ii) molting, (iii) copulation and (iv) post-copulation[12]. The total mating sequence of mud crab in captivity (from pre-copulation to the end of post-copulation) lasts for 82.0±10.8 hrs. With regard to the usual phases of mating, only the length of pre-copulation varies largely among individuals. On account of their importance to future breeding activities of portunid crabs, the effects of sex balance and the number of animals in a certain volume are also discussed in this review.

Inter-species mating: Inter-species mating of portunids was first tested in the genus *Scylla*[20]. It was found that mating among three species of *Scylla*, i.e. *S. olivacea, S. paramamosain* and *S. tranquebarica*, is feasible in captivity and that without the choice of partner, the effect of the different originating population is negligible. Among the three species, *S. olivacea* was the easiest to mate with other species and not much difference was observed in the duration of various mating phases between controls and inter-species trials.

LARVAL REARING

The countries, China, Indonesia and India still focus primarily on wild captures, whereas, the Philippines, Vietnam and Thailand spend most of their efforts on breeding and aquaculture. This has prompted many studies on culturing methods to enhance production through aquaculture. Presently, most of the Portunid culture operations utilize wild-caught larvae that vary in size and age. Historically, the portunid crab culture industry totally relied on wild larva for seed supply. However, recently the industry has begun to depend more on hatcheries, which are more reliable and sustainable. Threats to wild portunid crab populations and growing interest in their use for culture and research have created a need for better methods for rearing and maintaining seed stocks. Various countries have made good progress in seed production of cultured species even though fine-tuning larval and juvenile rearing techniques are an ongoing process with many challenges to develop viable technologies[16]. Improved techniques for larval culture based on careful studies are needed to improve production. Generally, Portunid crabs larvae go through two major larval stages of Zoea and Megalopa before becoming first-day instar crab (C1). In mud crab, there are five zoeal stages that last about 20-30 days before molting into megalopa stage[21]. In the megalopa stage, it usually took six to seven days before the C1 stage was reached. Overall, the mud crab larvae take 21-27 days from zoea to the megalopa to C1 stage.

Water quality parameters: The most significant water quality parameters that influence the growth, survival, physiological processes and physical structure of portunid crab larva are water temperature and salinity. Water temperature is arguably the most important parameter for developing larvae and the best products seem to occur within a narrow thermal differential for portunid larvae[22]. When the water temperature drops below a certain point, larval metabolism slows and growth is retarded[23,24]. Higher water temperatures stimulate metabolism but result in very slow growth and poor survival. The recommended water temperature for *P. pelagicus* larvae is 30°C and thermal fluctuations outside this range will have adverse effects on the development and survival of larvae[16]. Although mud crab zoeae are more tolerance to the fluctuation of temperature, higher survival and growth rate were observed at temperature of 25 to 30°C[25]. The thermosensitivity of larvae is species-specific and more studies are needed on other commercially important crab species to find the best conditions to decrease the cost of temperature control.

The majority of studies on Portunid crab larvae have utilized seawater at a certain salinity or conditions of higher or lower salt content, but salinity can have a range of physiological effects on development. Thus, a general salinity of 28-32 ppt is often observed in most portunid crab hatcheries[26]. Hatcheries need to be concerned about salt effects on larvae if they use seawater outside of the optimal salinity range.

Tank colouration: In addition, the environmental condition of tank coloration was found to affect portunid crab larva, *P. pelagicus*[27]. The studies investigated the effects of various tank colors including black, white, red, orange, yellow, dark grey, blue and brown on Portunid crab, *P. pelagicus* larva. Study by Ikhwanuddin *et al*.[27] described larval tank coloration as having a significant effect on the survival rate and time of molting to reach the megalopa stage. The results showed that white background color gave the poorest results as compared to the other four treatments with no larvae reaching juvenile crab size in white tanks. They also recommended that black background color or dark-grey tanks should be used for larval rearing of *P. pelagicus* to ensure the highest survival, growth and developmental rate. Similarly, in *Scylla* larvae, dark-colored tanks, especially black tanks, resulted in higher survival and growth rate and more synchronized molting[28].

Stocking density: Stocking density is known to affect the general health, growth and survival of portunid larvae. By comparing two different ranges of stocking densities of *P. pelagicus* larvae, (i) low stocking density of 10, 20, 40, 60, 80 and 100 larvae per liter and (ii) higher stocking density of 50, 200, 300 and 400 larvae per liter, Ikhwanuddin *et al*.[29] observed that highest percentage of survival was observed in the stocking density of 20 larvae per liter along with higher developmental rates achieved in low-density trials. A higher optimum stocking density was observed in *Scylla* larvae, i.e. 100-150 Z1/L[26].

Food requirements: The protocol for feeding larva is influenced by both the nutrient reserves in eggs and the food that is captured during culture. Recently various studies have covered different aspects of larval nutrition, highlighting advances in knowledge of different foods and feeding types[30]. Nutrition may be the key to enhancing Portunid larval production. The most common larval feeds were rotifers and brine shrimp[31], while tests of phytoplankton and diatoms did not significantly improve the growth or survival of crab larvae[26]. Additionally, naturally occurring nutrients in earthen ponds or those added such as vitamins and minerals could potentially supplement crab diets. Additional studies will be needed to examine the potential effects of various live foods on growth and development in the early stages of Portunid larvae. Advanced knowledge of larval nutritional requirements will enable higher production and quality of larva and is necessary in order to develop a successful and sustainable crab culture industry[32].

Healthy culture conditions: The ability to produce large numbers of portunid crabs during laboratory or hatchery conditions depends on techniques for reliably rearing healthy larvae. To date, most studies have focused on the use of probiotics to mitigate the early mass mortality of Portunid crab larva[33]. These studies found that the addition of the probiotic, *Lactobacillus plantarum*, altered the nitrogen level and pH of the water and significantly increased the protease and amylase activities in the treated larvae compared to controls. However, raising the amount of added probiotic did not increase the enzymatic activity or survivability of larvae. The addition of calibrated numbers of *L. plantarum* can effectively enhance portunid crabs larval enzymatic activity and survival and improve the quality of water for aquaculture.

CONCLUSION

This review attempts to showcase the current fundamental knowledge on breeding activities and larval rearing of portunid crabs that are paving the way for sustainable aquaculture development in the future. Such knowledge is important for further optimization of culture conditions in most portunid crabs culture in the world[34-40].

SIGNIFICANCE STATEMENT

This study discovers the important aspect for optimization in breeding and larval rearing of portunid cabs that can be beneficial for further sustainable seed production techniques in the future. This study will help the researcher to uncover the critical area of other breeding and larval rearing technologies that many researchers were not able to explore. Thus, a new theory on advanced breeding and larval rearing techniques may be arrived at.

RECOMMENDATION

More advanced studies on the breeding program as well as seed production techniques are required to further enhance the crablet quality of Portunid crabs. This requires further research programs on selective breeding and quality broodstock development to be carried out in collaboration with local industry and international research institutions. Collaborations such as these are promising and expected to contribute greatly to the domestication, farming and larval rearing of portunid crabs, both regionally and locally.

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