



Review Article

Larval culture and rearing techniques of commercially important crab, *Portunus pelagicus* (Linnaeus, 1758): Present status and future prospects

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Abstract

For consistent seed production, better understanding of larviculture and rearing techniques is crucial to maximize production of high quality and healthy larvae of cultured species. There are many different larval rearing methods in the world due in part to the geography, climatic patterns, species culture, and feeding regimes. This review provides available information on the present status of hatchery techniques in aspect of larval production, identifies husbandry techniques, recognized the main bottlenecks of current hatchery operations and identify likely future technique for consistent production of blue swimming crab, *Portunus pelagicus*. It is important to simplify larval rearing methods to develop easy-to-use and efficient systems for the mass rearing of healthy crabs. The information on this review will be useful as a guideline to culture others Portunid crab as well as a reference to the academicians, aqua-culturists, and the industry that indirectly support the sustainable aquaculture development for *P. pelagicus* crab.

Keywords: crab, crustacean, larviculture, *Portunus pelagicus*, rearing techniques

1. Introduction

Blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758) is widespread across the Indo-Pacific, including Southeast Asia, and it is one of the more valuable commodities across many countries. It is relatively expensive in comparisons to other sea fishes consumed locally. Exploitation of *Portunus* sp. has rapidly spread to selected countries such as Indonesia, Thailand, Malaysia, and most recently India. Therefore, aquaculture is a potential solution for increasing crab's seed on the natural stock. Most captured and cultured Portunid crabs are of relatively high commercial value such as *Portunus* sp. (Wu *et al.*, 2010), *Charybdis* sp. (Baylon and Suzuki, 2007) and *Scylla* sp. (Quinitio *et al.*, 2011).

Presently the *P. pelagicus* culture operations have to depend solely on seed collected from the wild, which will vary in size, age and with season. Steady development in the past had totally relied on wild *P. pelagicus* juvenile for seed supply, but it is becoming more and more dependent on hatcheries, which is a more reliable source for the future. Hatchery culture techniques are of primary significance for developing a comprehensive technology for sustaining crab production. Threats to wild *P. pelagicus* population and a growing interest in their use for cultural and research have prompted demand for improved techniques to rear and maintain the seeds. Improvements in seed production of *P. pelagicus* technology were made by various authors (Soundarapandian *et al.*, 2007; Ikhwanuddin *et al.*, 2013; Ravi and Manisseri, 2013), and fine tuning larvae and juvenile husbandry technique is an ongoing process with uncertainty over viable technology. As *P. pelagicus* hatchery development of a small-commercial scale has only occurred in a few

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countries, crab farming in most countries depends on wild caught stocks.

For further aquaculture industry development, a better understanding of larval culture techniques is necessary to optimize its production. However, details of this work have not been compiled, organized, and analyzed. The aim of this review is to determine the relative success of a variety of published techniques and broadly share this information with the community including researchers, managers, and educators. We surveyed a comprehensive literature of all rearing attempts for this species to date, including a likely way forward for pilot scale and hence commercial restocking operations.

2. Broodstock Management and Hatching

Usually broodstock were collected from the wild and held in the laboratory for further experiment because of the hatching success was high in wild collected berried females when compared to the laboratory produced berried females (Anand and Soundarapandian, 2011). In addition, the majority of studies have worked with larvae released naturally by captive broodstock (Redzuari *et al.*, 2012; Talpur *et al.*, 2012; Ikhwanuddin *et al.*, 2013). Since the species is not particularly robust, it seems sensible to collect, transport, and maintain captive broodstock with great care to avoid mortality and excessive loss of eggs during incubation. Once in captivity, the berried females usually fed with natural fed such as prawn, mussel, or squid (Andrés *et al.*, 2010) and provided with sand substrate and mild aeration (Talpur and Ikhwanuddin, 2012).

To eliminate the microbial infection, different levels of chemicals such as potassium permanganate (Talpur *et al.*, 2011) or formalin (Soundarapandian *et al.*, 2007) were used to the berried females. The broodstock used for study usually have size at maturity between 10.4 cm to 16.2 cm carapace width (CW) (Maheswarudu *et al.*, 2008; Oniam *et al.*, 2012). The broodstock usually caught using trawl net operation, gill nets or from local fisherman (Trisak *et al.*, 2009; Nitirat-suwan *et al.*, 2010). The fecundity of the *P. pelagicus* broodstock usually is between 400,000 eggs and more than 1,500,000 eggs depend on the feeding and the crab size (Oniam *et al.*, 2012; Maheswarudu *et al.*, 2008). Usually, the broodstock was eyestalk ablated in order to increase their spawning time and development of their gonads (Bhat *et al.*, 2011) and stocked at one crab/tank (Castine *et al.*, 2008; Ikhwanuddin *et al.*, 2013; Ravi and Manisseri, 2013). After hatching, the larvae will be determined according to the study by Josileen and Memon (2004) or Arshad *et al.* (2006). Table 1 showed the summary of broodstock management and hatching at different countries and authors.

Table 1. Summary of broodstock management and hatching of *Portunus pelagicus* at different countries and authors.

Country	Sources of broodstock	Crab size	Treatment for broodstock	Feeding	Water quality for broodstock	Water quality during hatching	Water exchange (%)	Others	References
India	Wild caught – trawler	n/a	n/a	Fresh clam meat	Temperature at 28±2°C, salinity at 35±1 ppt and 8.2±0.1 for pH	Temperature at 28±2°C, salinity at 35±1 ppt and 8.2±0.1 for pH	50	n/a	Josileen and Menon, 2004
India	Wild caught – n/a	n/a	200 ppm	Oyster meat formalin	Temperature at 34±1°C, salinity at 29.5±1.5 ppt, 7.725±2.5 for pH and 5.5±0.5 mg/L for DO	Temperature at 29.5±1.5°C, salinity at 34±1 ppt and 5.5±0.5 mg/L for DO	50	Photoperiod – 12hL:12hD	Soundara-pandian <i>et al.</i> , 2007
Australia	Wild caught – baited pots	n/a	100 µL/L formalin	n/a	Temperature at 27.5±1.5°C and salinity at 34±1 ppt	Temperature at 28±1°C and salinity at 22ppt	n/a	1 µm filtered and UV treated seawater	Castine <i>et al.</i> , 2008
Australia	Wild caught – baited traps	n/a	50 µL/L formalin	Prawns, mussels and squid	Temperature at 28±2°C and salinity at 32±2ppt	Temperature at 26±1°C and salinity at 34±1.5ppt	10	n/a	Andres <i>et al.</i> , 2010

Table 1. Continued

Country	Sources of broodstock	Crab size	Treatment for broodstock	Feeding	Water quality for broodstock	Water quality during hatching	Water exchange (%)	Others	References
Malaysia	Wild caught – local fisherman	n/a	n/a	n/a	Temperature at 27.5±0.5°C, salinity at 30ppt, 7.45±0.45 for pH and 5ppm for DO	Temperature at 29±1°C and salinity at 29±1 ppt	50	Sand substrate – 3cm	Ikhwanuddin <i>et al.</i> , 2011
Malaysia	Wild caught – gill net	n/a	n/a	Chopped fish	Temperature at 29.5±0.5ppt, 8.35±3.5 for pH and 6 mg/L for DO	Temperature at 29±1°C, salinity at 29±1 ppt	100	n/a	Ikhwanuddin <i>et al.</i> , 2012b
Malaysia	Wild caught – local fisherman	124–138mm	120 µl/L formalin and 2ppm KMNO ₄	Not fed until hatching	Salinity at 31±2 ppt, 7.7±0.3 for pH and 7.34±0.55 for DO	n/a	50	Sand substrate	Talpur and Ikhwanuddin, 2012
Malaysia	Wild caught – local fisherman	n/a	120 µl/L formalin and 2ppm KMNO ₄	Not fed until hatching	Temperature at 30°C, salinity at 32.5±2.5ppt, 7.75±2.5 for pH and 5mg/L for DO	Temperature at 30°C, salinity at 30ppt, 8.35±3.5 for pH and 6mg/L for DO	100	Sand substrate – 3cm	Ikhwanuddin <i>et al.</i> , 2012c
Malaysia	Wild caught – gill net	n/a	n/a	Chopped fish meat	Temperature at 30°C, salinity at 30ppt, 8.35±3.5 for pH and 6mg/L for DO	Temperature at 30°C, salinity at 30ppt, 8.35±3.5 for pH and 6mg/L for DO	100	Moderate aeration	Ikhwanuddin <i>et al.</i> , 2012d
Thailand	Domesticated broodstock – pond reared	10.76±0.97cm	n/a	Minced trash fish	Temperature at 32.15±2.15°C, salinity at 33±2ppt, 8.735±0.555 for pH and 4.93±2.465 mg/L for DO	n/a	20-30	n/a	Oniam <i>et al.</i> , 2012
Malaysia	Wild caught – n/a	n/a	n/a	Fresh squid	Temperature at 27.5±0.5°C and salinity at 30ppt, 7.45±4.5 for pH and 5ppm for DO	Temperature at 29±1°C and salinity at 29±1 ppt	50	Sand substrate – 3cm	Ikhwanuddin <i>et al.</i> , 2012e
India	Wild caught – n/a	140–160mm	200 ppm formalin	Raw clam and cuttlefish meat	Temperature at 28±0.1°C, salinity at 35ppt and 8.1±0.1 for pH	n/a	70	Photoperiod – 12hL:12hD	Ravi and Manisseri, 2012
India	Wild caught – n/a	140–160mm	200 ppm formalin	Raw clam and cuttlefish meat	Temperature at 28±1°C, salinity at 35ppt, 8.1±0.1 for pH and 5mg/L for DO	n/a	10	Sand substrate – 10 cm and photoperiod – 12hL:12hD	Ravi and Manisseri, 2013

*n/a, not available; DO, Dissolved oxygen; KMNO₄, potassium permanganate; hL, hour light; hD, hour dark

3. Larval and Juvenile Production

3.1 Feeding requirements, culture system and turbulence

Nutrition can be the dominant factor influencing the larval production in term of increasing the growth and survival of *P. pelagicus*. Recently, because of its importance in production of *P. pelagicus*, dietary aspects have been studied by various authors such as Soundarapandian *et al.* (2007), Castine *et al.* (2008), Ikhwanuddin *et al.* (2011), Ikhwanuddin *et al.* (2012a), Ikhwanuddin *et al.* (2012b), and Redzuari *et al.* (2012). The most commonly offered feed among the culture studies reported was rotifers, *Brachionus* sp. and brine shrimp, *Artemia* sp., which are abundant and commercially available. In one case, supplementing the micro-bound diet with four different dietary protein sources (fish meal, squid meal, krill meal and soybean meal) increased the growth of *P. pelagicus* (Castine *et al.*, 2008). In other studies, the use of phytoplankton or mixed diatom species may not optimize larval growth and survival of *P. pelagicus* (Ikhwanuddin *et al.*, 2012a; Ikhwanuddin *et al.*, 2013). Thus, it showed that more studies are needed to analyze the various type of feed (phytoplankton or zooplankton) that suitable for better survival in *P. pelagicus* production. The summary on the different feeding requirement of *P. pelagicus* larvae to crab stages is shown in Table 2.

The systems used for cultured *P. pelagicus* could enhance better water quality that indirectly improves the quality of seed production. There are several of cultured systems used in *P. pelagicus* study such as in captivity (Josileen and Memon, 2005) and in earthen ponds (Oniam *et al.*, 2010). The hatchery produced of *P. pelagicus* in captivity showed that the crab has 16 stages of moulted shells with the mean growth increment in CW increased steadily from the juvenile phase (Josileen and Memon, 2005). Their results also showed that the crabs mean weight gain was 0.006 to 210 g BW within 275 days. On the other hands, crabs cultured at earthen ponds gains more weight compared in captivity with weight gains range from is 0.09 to 105 g BW within 180 days (Oniam *et al.*, 2010). Other nutrient content in the earthen pond could be an additional food for the crab. Low survival rates in *P. pelagicus* larval stages are mostly due to phototaxis behavior, thus they are trapped at the water surface. Management of water flow rate on the rearing tank may be able to reduce their mortality. There is only one published study that has used water flow on the rearing tanks of *P. pelagicus* larvae (Rejeki, 2007). He mentioned that the water flow rate in the holding tanks could stabilize water temperature, dissolved oxygen (DO), and could keep the zoea in the suspension position. Apparently, much more research is required to examine the potential effects of flow rate on larval growth and development in the early larval stages of *P. pelagicus*.

3.2 Water quality

Temperature is considered to be one of the most important factors effecting growth and survival, and changes in temperature can influence both physiological processes and the physical structure of larval invertebrates. It is well established that temperature has potential influences on larval development and that optimal performances is obtained within a narrow range of temperature for *P. pelagicus* (Bryars and Havenhand, 2006; Ikhwanuddin *et al.*, 2012c; Ravi and Manisseri, 2012; Talpur and Ikhwanuddin, 2012). Below the optimal temperature range, metabolic activity decreases as well as growth and survival. Above temperature range, larvae have higher metabolic rates, resulting in slower growth and lower survival rate. Bryars and Havenhand (2006) observed that temperature had an important influence on survivorship in *P. pelagicus* larvae. The result showed that the percentage of survival was greatest at 25°C at both constant and varying temperature. At constant temperatures of 22.5 and 25°C larval survival was greater than at lowest temperatures as low as 17°C, and the developmental period of the larval period was inversely related to (constant) temperature. Ikhwanuddin *et al.* (2012c) investigated the effects of temperature on larval of *P. pelagicus* reared at two temperatures (30°C and ambient between 24-28°C). They found that larvae reached megalopa stages at 30°C in day 13-14, but all larvae dead in day 6-7 day at ambient temperature between 24-28°C. They also recommended that the optimal water temperature of the larvae rearing of *P. pelagicus* is 30°C. Talpur and Ikhwanuddin (2012) tested four different temperatures (30, 35, 40 and 45°C) on larval survival rates of *P. pelagicus*. Larvae were reared for 12 hr-time period against control with ambient temperature 28°C. Talpur and Ikhwanuddin (2012) showed that a temperature of 30°C produced highest survival and elevated temperature stress adversely affected larvae and no survival was achieved at temperature 40°C and 45°C in early larval stages (Zoea 1 and 2 stages). Any intervention causing adverse alterations to the larval environment such as temperature will badly affect the larval development and consequently the overall survival of *P. pelagicus*. More detail on the study of various temperatures was shown in Table 3. Temperature effects will be species and origin dependent and further experiments will need to be conducted on others commercially important Portunid's crabs to optimize larval growth or survivorship and to minimize the cost of cooling and heating seawater.

Most scientific research on the growth, survival and development of larval and juvenile of *P. pelagicus* has been done with filtered seawater at ambient, tested or extreme salinity. The developmental patterns of *P. pelagicus* are influenced by variations in salinity (Ikhwanuddin *et al.*, 2012c; Ravi and Manisseri, 2012; Talpur and Ikhwanuddin, 2012). Ikhwanuddin *et al.* (2012c) examined the combined

Table 2. Summary on feeding requirements of *Portunus pelagicus* larvae to juvenile stages.

Country	Experimental diets	Crab stages	Summary	Conclusion	Reference
India	Rotifers, <i>Brachionus plicatilis</i> , <i>Artemia</i> nauplii & bivalve meat	Larvae to megalopa	<ul style="list-style-type: none"> • Low survival were observed from megalopa to 1st crab instar • Fast growth at earlier zoea stages compare to late zoea stages 	Lower survival from last zoea to megalopa	Soundarapandian <i>et al.</i> , 2007
Australia	Micro-bound diet (protein based diet: fish meal, squid meal, krill meal and soybean meal), live <i>Artemia</i> nauplii & unfed treatment	Megalopa to crab stage	<ul style="list-style-type: none"> • Higher survival of crab fed with fish meal micro-bound diet compared to live <i>Artemia</i> • <i>Artemia</i> resulted shorter larval development & greater body weight and carapace length 	Soybean meal potentially provide dietary amino acids & replace live food	Castine <i>et al.</i> , 2008
Malaysia	Mixed diatom, <i>Artemia</i> nauplii & rotifer	Larvae to 1 st day juvenile crab	Better survival & development when crab fed with combination diet of rotifers and <i>Artemia</i> compared to addition of mixed diatom	Food type influence crab growth & survival	Ikhwanuddin <i>et al.</i> , 2012d
Malaysia	Individual ingestion rates of crab for <i>Artemia</i> sp. Nauplii & rotifers, <i>Brachionus</i> sp.	Early larval stages	<ul style="list-style-type: none"> • Early zoea stages crab fed more rotifers, <i>Brachionus</i> sp. than <i>Artemia</i> sp. nauplii • Late zoea stages crab fed more <i>Artemia</i> sp. nauplii than <i>Brachionus</i> sp. 	Presence of <i>Brachionus</i> sp did not influence the consumption of <i>Artemia</i> sp. nauplii	Ikhwanuddin <i>et al.</i> , 2012e
Malaysia	Instant frozen, encapsulated & artificial encapsulated feed	Larvae to 1 st day juvenile crab	Best survival, rapid development & highest number of juvenile crab when fed with combination diet of frozen food, rotifer & <i>Artemia</i> nauplii compared to the additional artificial diet	Food type influence crab growth & survival	Ikhwanuddin <i>et al.</i> , 2013

Table 3. Summary of various water quality parameters tested for *Portunuspelagicus* studies.

Country	Crab stages	Water quality parameters and optimum value	Summary	Conclusion	Reference
Australia	Larval stages	Temperature (25°C)	Higher number of larvae reaching each stage from hatching & low stage of development period at higher temperature	Maximum hatching at lower temperature & better survival at higher temperature	Bryars and Havenhand, 2006
China	Larval stage (Zoea 1-4)	Ammonia-N (<16.86 mg/l) Nitrate (<53.34 mg/l) Temperature (30°C)	Increased ammonia-N concentration - decrease larval vigour Increased nitrate concentration - decrease larval survival & molting Higher water temperature - better mean survival & juvenile production compared to the ambient conditions Higher salinity - better growth & survival	Over 16.86 mg/l caused significant decreased of survival & molting rate Over 53.34 mg/l caused significant decreased of larval vigour Temperature affected survival & molting of larvae	Liao <i>et al.</i> , 2011
Malaysia	Larval stage to 1 st day juvenile crab	Salinity (30ppt) Temperature (30°C)	Higher salinity - better final survival but decrease the stage-wise development Higher salinity - better final survival rate & lower salinity - lower stage development	Lower salinity is highly sensitive to the larval rearing Significant results on survival & development at higher or lower temperature Lowest salinity affected both molting and survival	Ikhwanuddin <i>et al.</i> , 2012c
India	Larval stages (Z1-M)	Temperature (30°C) Salinity (35ppt)	No survival at highest temperature (up to 40°C) & lowest survival at ambient temperature Zero survival at the 0 salinity & the highest salinity up to 60ppt No survival at lowest oxygen as low as <0.5 mg/L & better survival at >6 mg/L No survival at pH 4, 6, and 10 & pH 8.2 produced higher survival.	Temperature directly influence larval rearing with higher temperature caused detrimental larval survival Salinity cause primary stress to the crab early larval stages DO not only for respiration, but also for maintain required chemical & hygienic Acidic pH and higher alkaline pH have adverse effect on mortality at early stage Better survival when crab reared pH at 8.0 lowest pH (5.0) is harmful for larvae	Ravi and Manisseri, 2012
Malaysia	Larval stage (Zoea 1-2)	Temperature (30°C) Salinity (<40ppt) DO (>6 mg/L) pH (8.2)	No survival at highest temperature (up to 40°C) & lowest survival at ambient temperature Zero survival at the 0 salinity & the highest salinity up to 60ppt No survival at lowest oxygen as low as <0.5 mg/L & better survival at >6 mg/L No survival at pH 4, 6, and 10 & pH 8.2 produced higher survival.	Temperature directly influence larval rearing with higher temperature caused detrimental larval survival Salinity cause primary stress to the crab early larval stages DO not only for respiration, but also for maintain required chemical & hygienic Acidic pH and higher alkaline pH have adverse effect on mortality at early stage Better survival when crab reared pH at 8.0 lowest pH (5.0) is harmful for larvae	Taipur and Ikhwanuddin, 2012
India	Larval stages (Z1-M)	pH (8.0)	No survival at highest temperature (up to 40°C) & lowest survival at ambient temperature Zero survival at the 0 salinity & the highest salinity up to 60ppt No survival at lowest oxygen as low as <0.5 mg/L & better survival at >6 mg/L No survival at pH 4, 6, and 10 & pH 8.2 produced higher survival.	Temperature directly influence larval rearing with higher temperature caused detrimental larval survival Salinity cause primary stress to the crab early larval stages DO not only for respiration, but also for maintain required chemical & hygienic Acidic pH and higher alkaline pH have adverse effect on mortality at early stage Better survival when crab reared pH at 8.0 lowest pH (5.0) is harmful for larvae	Ravi and Manisseri, 2013

*Z1, Zoea 1; Z2, Zoea 2; Z4, Zoea 4; M, Megalopa; DO, Dissolved oxygen

effects of salinity and temperature on larval of *P. pelagicus*. Trials were carried out at high and low water salinity 30ppt and 20ppt. They reported that salinity significantly affected survival of the crab larvae. Similar observations were made by Ravi and Manisseri (2012) for larvae *P. pelagicus* when tested at various salinities (25, 30, and 35ppt). Ravi and Manisseri (2012) reported that among the salinity tested, the highest mean survival rate and the lowest mean development period were obtained at 35ppt. Talpur and Ikhwanuddin (2012) examined the effects of four level of salinity (0, 40, 60, 80ppt) on the survival of *P. pelagicus* larvae. The result showed that no survival of larvae was observed in challenge groups treated at salinity 0, 60, and 80ppt except for salinity 40ppt where a low survival rate has been observed. From a commercial-hatchery perspective, the effect of salinity on larval survivorship would only be of concern if the facility was using full-strength or low-strength seawater.

The environmental conditions including the physico-chemical characteristics of the larval rearing medium are of extreme significance since these makes up the environment of the larvae. Talpur and Ikhwanuddin (2012) carried out two trials to assess the potential influence of two common physico-chemical parameters (pH and dissolved oxygen) on the early stages (Z1 and Z2) larval survival of *P. pelagicus*. Both conducted the test for 0-4 hrs and controls contained larvae with aerated sterilized seawater. The results showed that no survival was achieved in treated groups. Oxygen in treated tanks was $<0.5 \text{ mg L}^{-1}$ and in control it was $>6 \text{ mg L}^{-1}$. They also tested four different pH ranges (4, 6, 8, and 10) against the control (natural pH) and only pH 8 produced highest survival of Z1 and lowest in Z2, which were statistically significant ($p < 0.05$). Therefore, the physico-chemical parameters such as pH and dissolved oxygen have been discovered to affect the larval survival, growth, development or molting rate of *P. pelagicus* larvae (Talpur and Ikhwanuddin, 2012). Meanwhile, Ravi and Manisseri (2013) showed that the mean overall survival rates and developmental period among different pH treatments were not significant. The study also showed the significant variance when compared with the larval survival rates at the control pH 8.0, the survival rates at other pH values such as 7.5 and 8.5. Table 3 shows the summary of various water quality parameters tested for *P. pelagicus* studies.

3.3 Feeding environment

Talpur and Ikhwanuddin (2012) tested the starvation experiment of *P. pelagicus* larvae for Z1 and Z2. During an 48 hr-starvation test, the result showed that there were no Z1 survived in treated group and Z2 survived in challenge group was not statistically significant. The results showed that the larvae of *P. pelagicus* were not resistant to starvation because of their less nutritional reserves until the Z2.

Stocking density is a key factor in larviculture; without an optimal stocking density, overall survival can be affected. The optimum stocking density during larval rearing is crucial

because overcrowding can affect access to food resources (reducing both larval growth and survival rates) and the quality of the rearing water and other environmental factors. Studies in *P. pelagicus* larval development usually maintain the larval rearing density in the range of 10-400 larvae L^{-1} (Soundarapandian *et al.*, 2007; Maheswarudu *et al.*, 2008; Ikhwanuddin *et al.*, 2012d; Ikhwanuddin *et al.*, 2012e). Soundarapandian *et al.* (2007) concluded that medium-density (50 larvae L^{-1}) culture has apparent advantages and would decrease the overall cost of seed production. Similar observations were made by Maheswarudu *et al.* (2008) when tested at two different densities (50 and 100 larvae L^{-1}) in a 1,000L tank. They reported that the highest survival and differences in statistical significant was achieved when larvae were stocked at density 50 larvae L^{-1} than 100 larvae L^{-1} . Ikhwanuddin *et al.* (2012d) examined the effects of six different stocking densities (10, 20, 40, 60, 80, and 100 larvae L^{-1}) on larval survival and molting period of *P. pelagicus* larvae. At the end of the experiment, they concluded that the highest percentage survival was observed in dark grey tanks where the stocking density of larvae was 20 larvae L^{-1} . In one case, the rearing concentration has been substantially higher, in which a density of 50-400 larvae L^{-1} has been used (Ikhwanuddin *et al.*, 2012e). They tested four different stocking densities (50, 200, 300, and 400 larvae L^{-1}). There were no significant differences among the three stocking densities in terms of survival except for treatment 200 larvae L^{-1} . The lowest survival, highest larval mean BW and Specific Growth Rate (SGR) were achieved in the lowest treatment (50 larvae L^{-1}). They concluded that the high stocking density affected the survival rate, growth and development of *P. pelagicus* larvae. Obviously, the effects of stocking density are important in the larval rearing of *P. pelagicus*. The lowest stocking density showed very fast growth, survival and development rate, which was caused by more space and enough food, compared to the highest stocking.

Photoperiod and tank colorations were among the techniques practiced for the larval rearing of *P. pelagicus*. Both were considered as an abiotic factors in terms of light and utilizing a light that can substantially affect the larval performance of crabs, including swimming, feeding behavior and growth (Rabbani and Zeng, 2005; Andrés *et al.*, 2010). Only one published study has examined the potential effects of photoperiod on larval *P. pelagicus* growth, survival and development: Andrés *et al.* (2010) developed a method for the intensive hatchery culture using static seawater, with 600mL glass beakers filled with UV-filtered seawater, for the culture of larvae *P. pelagicus*. They setup five different photoperiod conditions, 0L: 24D, 6L: 18D, 12L: 12D, 18L: 6D, and 24L: 0D (L= hours of light and D = hours of darkness), which were created by fluorescent light tubes and connected timers. They concluded that photoperiod significantly affected the survival, development, and growth of *P. pelagicus* zoeal larvae. Andrés *et al.* (2010) recommended that the constant darkness led to the lowest larval survival and developmental rate, while a photoperiod regime of 18L:6D appeared to be

the most suitable condition for the rearing of *P. pelagicus* larvae. However, the study by Ravi and Manisseri, (2013) indicated that 12hL:12hD is a better photoperiod ratio for rearing the earlier larval stage of *P. pelagicus*.

Azra *et al.* (2012) compared the effects of five different tank colorations (black, white, red, orange, and yellow) on the survival, growth and development of *P. pelagicus* larvae. They reported that there was statistically significant difference in the survival of *P. pelagicus* larvae reared in black background color tank. They concluded that black background color is favorable for *P. pelagicus* larvae rearing to ensure the highest survival, growth and development rate. Ikhwanuddin *et al.* (2012d) also reported on the effects of tank colorations on larval development and molting time and tested the development and survival of *P. pelagicus* using four different color treatments which are white, dark grey, blue, and brown. The results showed that none of the replicate tanks with a white background color larvae reached the juvenile crab (C1) stage; zoea stage larvae only survived until day 4. They concluded that the best survival was observed in dark-grey-colored tanks for larval rearing of *P. pelagicus*. It shows that the darker background color tank were the best color for the rearing of *P. pelagicus* larvae. Thus, photoperiod and tank colorations affected larval rearing of *P. pelagicus* compared to control group without photoperiod and tank colorations.

The efficient and reliable rearing of healthy larvae and metamorphose is important in the production of large number of *P. pelagicus* during laboratory or hatchery conditions. Most culture and research examining larval rearing of *P. pelagicus* has made use of water treatment and water exchanges, however there were two studies regarding with water treatment and water exchanges (Soundarapandian *et al.*, 2007; Ikhwanuddin *et al.*, 2012d). Soundarapandian *et al.* (2007) examined the effect of water treatment on larval rearing of *P. pelagicus*. The result showed that higher survival was achieved in Z1 and lowest in Z4 when larvae were treated with Calcium hypochlorite and sodium thiosulphate. Ikhwanuddin *et al.* (2012d) tested four different water exchanges (0%, 25%, 50%, and 100%) on larval survival and development of *P. pelagicus*. The results showed that none of the larvae from replicate tanks with 0% water exchange did reach the C1 stage; zoea stage larvae only survived until day 4. They recommended that at least 50% daily water exchange can be performed for any larvae-rearing works. Table 4 shows the different of feeding environment on *P. pelagicus* culture with the summary of culture practices includes starvation test, tank coloration, stocking density, photoperiod, water treatments and water exchanges.

4. Main Obstacle in Hatchery Mass Production

4.1 Available of berried broodstock for larviculture in hatchery

A literature review shows that a number of studies

have been conducted, in providing a good understanding of the larval and juvenile culturing of *P. pelagicus* which includes feeding requirements and environment, culture systems and turbulence and water quality requirements. However, the difficulties in obtaining berried broodstock from hatchery has been one of the factors that promoted researches for developing a methodology for hatchery mass production of seed because of most berried females were caught from the wild (Josileen and Menon, 2004; Soundarapandian *et al.*, 2007; Castine *et al.*, 2008; Andres *et al.*, 2010; Ikhwanuddin *et al.*, 2011; Ravi and Manisseri, 2012; Ravi and Manisseri, 2013). The problem is *P. pelagicus* broodstock are commonly sourced from buying station or directly from the collectors (Ikhwanuddin *et al.*, 2012a;b;c;d;e). Thus, there is a need to maintain the berried broodstock in the hatchery for easy larviculture of *P. pelagicus*.

4.2 Mass mortality at early and late larva stages

A major bottleneck to the development of commercially *P. pelagicus* aquaculture is a lack of understanding of the mass mortality during larval stages of the larvae (Talpur *et al.*, 2011; Talpur and Ikhwanuddin, 2012; Talpur *et al.*, 2012). Laboratory and hatchery cultures of *P. pelagicus* larvae often suffer severe mortality from disease, cannibalism, bacteria, fungi, molting syndrome, and various unknown causes (Hamassaki *et al.*, 2011). Obviously, much more research is required to test other potential techniques of larval rearing on growth, survival and development of *P. pelagicus* (Ikhwanuddin *et al.*, 2013). Alternative techniques were truly needed to increase larval survival, growth and development and indirectly able to diversify the culture techniques of *P. pelagicus*.

5. Future Perspectives

Since the natural resources of *P. pelagicus* are decreasing (FAO, 2010), there is a genuine demand for cultured *P. pelagicus*. There is no doubt that hatchery production is the best model for seed supply in many countries where people realize that natural resources cannot be relied upon forever. However, such work will be particularly crucial for the development of commercial-scale hatcheries for *P. pelagicus*. In the present decades, most literature focused more on development of technologies in larval rearing techniques of *P. pelagicus*. The developments in culture of *P. pelagicus* are more focused in increasing the larval survival and growth. Findings such as optimal rearing temperature, appropriate flow and water management, suitable feeding regimes are basic for future research (Table 2, 3, and 4). Other than manipulation of environmental conditions and diet requirement, other techniques such as using probiotic (Wu *et al.*, 2014) and manipulation in indoor and outdoor system (Cheng *et al.*, 2008) can be model techniques for rearing of *P. pelagicus* larvae. Genetic selection for improved growth (He *et al.*, 2014), the use of molt inhibiting hormone

Table 4. Various types of feeding environment on *Portunuspelagicus* culture.

Country	Crab stage	Feeding environment	Experimental design	Summary	Reference
India	Larvae	Stocking density	50 & 100 larvae/l	Survival highest at lowest stocking density at each zoea stage (Z1-Z4) and vice versa	Maheswarudu <i>et al.</i> , 2008
Australia	Larvae	Photoperiod	0L:24D, 6L:18D, 12L:12D, 18L:6D & 24L:0D	Photoperiod at 18L:6D is most suitable & significantly affected growth & development	Andres <i>et al.</i> , 2010
Malaysia	Larvae	Live prey ingestion	<i>Artemia</i> only, rotifer only & <i>Artemia</i> + rotifer with 30, 60 & 30+60 individual/tubes	Larvae ingested more <i>Artemia</i> after 24 hrs at late zoeal stage as compared to the initial zoeal stage	Ikhwanuddin <i>et al.</i> , 2011
Malaysia	Larvae	Feeding regimes	Rotifer only-Z1 to M, <i>Artemia</i> only-Z1 to M, rotifer-Z1 with <i>Artemia</i> -Z2 to M, rotifer-Z1 to M with <i>Artemia</i> -Z3 to M & rotifer-Z1 to M with <i>Artemia</i> -Z4 to M	Rotifer-Z1 with <i>Artemia</i> -Z2 to M was the suitable feeding regimes with effected survival & development	Redzuari <i>et al.</i> , 2012
Malaysia	Larvae	Tank coloration	White, orange, yellow, red & black	Black color tank - better survival & red colour tank revealed better development	Azra <i>et al.</i> , 2012
Malaysia	Larvae	Tank coloration	White, dark grey, blue & brown	White color tank - worst result and dark grey resulted better growth & survival	Ikhwanuddin <i>et al.</i> , 2012d
Malaysia	Larvae	Stocking density	10, 20, 40, 60, 80 & 100 larvae/l	Highest survival at stocking at 20 larvae/l	
Malaysia	Larvae	Water exchange	0, 25, 50 & 100%	50% water exchange - better results	
Malaysia	Larvae	Antibiotic administrative	Treated & non-treated oxytetracycline	No significant in growth & survival with addition of antibiotic	
Malaysia	Larvae	Stocking density	50, 200, 300 & 400 larvae/l	Mass mortality at highest density & lowest density resulted better survival	Ikhwanuddin <i>et al.</i> , 2012e
Malaysia	Larvae	Starvation test	Feed (rotifer & microalgae) & un-feed	No survival at unfed larvae	Talpur and Ikhwanuddin, 2012
India	Larvae	Photoperiod	6L:18D, 12L:12D & 18L:6D	Photoperiod at 12L:12D - highest survival but low development	Ravi and Manisseri, 2013

*Z1, Zoea 1; Z2, Zoea 2; Z3, Zoea 3; Z4, Zoea 4; M, Megalopa; L:D, light:dark

(Shrivastava and Princy, 2013), various lipid level and feed utilization (Zhao *et al.*, 2015) can be an alternative option for an improvement of the survival rate and increase of the crab production for commercial re-stocking operations. Improved efficiency and effectiveness of captive rearing will support sustainability and stock enhancement efforts in Asia, but also throughout world, where crabs stocks have been critically depleted.

6. Conclusions

In conclusion, the appropriate larval culture and rearing techniques for the optimal growth, survival and development were stocking density between 20 to 50 larvae/l, salinity at 30-35 ppt, temperature between 25-30°C, pH at 8.0, dissolved oxygen is more than 6mg/l, feed and feeding with rotifer at early larval stages and *Artemia* at late larval stages with darker tank coloration can provide better hatchery seed production (Table 2, 3, and 4).

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