



Review Article

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A Review of Broodstock Improvement to Brachyuran Crab: Reproductive Performance

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Abstract An improvement in adult brachyuran crabs performance is vital in order to produce better captive broodstock of commercially seed productions. Many factors can control the broodstock performance in brachyuran crabs but of particular importance in aquaculture will be the enhancement of female broodstock that indirectly improve the reproductive performance because of high quality berried females can be used to increase hatchery seed production. This review gives a recent overview on methods to enhance the female's broodstock performance and many of the most important factors affecting female's reproduction, including environmental conditions, nutritional requirements, genetic management, physiological effects and etc. and advise on the future research directions. Eyestalk ablation was the basic procedure to enhance the reproductive performance in most brachyuran crabs. Environmental factors such as temperature, salinity and oxygen affect most brachyuran crab's reproduction such as carapace width, ovarian development and spawning success. For maximal berried female production of brachyuran crabs, a fine balance must be maintained between two distinct nutrient classes (macronutrient and micronutrient) and fresh feed items. The identification of gonadal development is a preliminary attempt to boost the broodstock performance of brachyuran crabs. On the other hand, most chemical used in the acceleration of ovarian maturation are from hormone supplementation or direct injection such as thyroxine hormone, androgenic hormone, steroid hormone and serotonin hormones and was used to exert their effects on target tissue in crabs. **Keywords** Reproduction; Brachyuran crab; Broodstock; Breeding technology; Seed production; Reproductive performance.

1 Background

The true crabs, the Brachyura (Linnaeus 1758) is one of the most diverse animal groups consists of two major group, Podotremata or primitive crabs and Eubrachyura or advanced crabs. They are includes various delicates, commercially and/or important aquaculture crabs such as the blue crab, *Callinectessapidus*, Dungeness crab, Metacarcinus magister, mud crab, Scylla serrata, blueswimmer crab, Portunuspelagicus, Chinese mitten crab, Eriocheirsinensis and fiddler crab, Ucaterpsichores. The recent Fishery and Aquaculture Statistic's report showed that the total marine crab landings increased (FAO, 2014) with development of fishing industry. Thus, to avoid overfishing of commercially brachyuran crabs in the wild, development of crabs' culture in the hatchery should be considered. Producing high quality of female crab broodstock is essential to successful hatchery production. It is also being considered as a first step in hatchery of cultured species. By obtaining broodstock from the wild, it is vulnerable for the improvements of better broodstock into the hatchery. Recent review has pointed to improve reproductive performance and larval quality of captive brachyuran broodstock, as a future alternative for more independent and reliable seed production (Azra and Ikhwanuddin, 2015; Azra and Ikhwanuddin, 2016). However, in the hatchery seed production, the status of the broodstock used and others important criteria such as mating behavior (Waiho et al., 2015; Baiduri et al., 2014; Ikhwanuddin et al., 2014a), size at maturity and fecundity (Ikhwanuddin et al., 2012; Ikhwanuddin et al., 2010), artificial production (Noorbaiduri and Ikhwanuddin, 2015), gonad development (Ikhwanuddin et al., 2014b) as well as natural diet (Ikhwanuddin et al., 2014c) should be firstly known before further culture practices can be taken. Such information on improvement of broodstock performance as well as the vetellogenin production is crucial to produce better seed with a mass production.





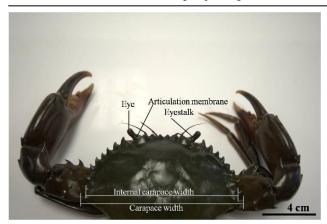


Figure 1 An example of brachyuran crab (genus Scylla) eye, eyestalk, articulation membrane, carapace width and internal carapace width measurement

A suitable method for induction and enhancement of ovarian maturation or reproduction is needed to reduce the wild caught broodstock in the natural habitat.

Several methods or techniques on the induction of brachyuran crab's reproduction were focused on the induction of gonad or hepatopancreas because of this is a crucial organ in the production of embryonic or egg development and it's has proven to be the most important organ synthesizing vetellogenin outside of the ovary (Subramoniam, 2011) under controlled conditions. It is well known that the eyestalk ablation is an easier method to improve and accelerated the reproduction in crustacean as well as brachyuran crabs' species (Khazraeenia and Khazraiinia, 2009). As demonstrated for others crustacean species (reviewed by Green et al., 2014), environmental conditions play a key role in the changes of the brachyuran crab's reproduction and development (Rotllant et al., 2015; Brylawski and Miller, 2006). Studies on food and feeding behavior of brachyuran crabs indicated that nutritional content of food and feeding activities could affecting the reproductive performance of most brachyuran crabs (Sui et al., 2009; Woll and Berge, 2007). It is important to identify the genes that are differently expressed in crab's reproductive performance for better novel methods of reproduction control in selected culture species (Zou et al., 2011). Meanwhile, most recent studies of improvement on brachyuran crab's broodstock were intrigued on their physiological effects of chemicals reaction (Khornchatri et al., 2015; Medesani et al., 2015; Gong et al., 2015) because of their significant effects oncrab's reproduction. On the other hand, few attempts have been made on the culture practices of brachyuran crabs' broodstock as well as by new reproductive strategy (Paterson et al., 2007). In order to improve the efficiency of the crab culture industry as well as other crustacean, appropriate method and techniques on enhancement of the broodstock's performance should be reported in particular for easy application to the culture practices. To our knowledge, the details of this work have not been compiled, organize, and analyzed for brachyuran crab's species.

Thus, this article reviewed recent developments(within 21st century of English full text information) of discussion and/or methods for broodstock improvements at selected species in brachyuran crabs through application and manipulation in environmental conditions, nutritional requirements, genetic selection, physiological effects and others such as culture practices or new reproductive strategy. In addition, the key indicators of reproductive performance will also been discussed in order to give an overview to the future broodstock management. Furthermore, the suitability of such methods was evaluated as tools to improve the female's broodstock for future sustainable of hatchery seed production as well as for mass crab culture.

2 Broodstock Eyestalk Ablation

In the past few years, the broodstock which used either in the research or hatchery culture basically were wild-caught broodstock. Usually, hatchery reared broodstockare sources from the wild is at early ovarian maturation stage or with fully develop ovaries and an improvement in their performance should be a top priority





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Table 1Various effects of eyestalk ablation on brachyuran crabs

Common English name (Species)	Technique(s) and procedure of ESA	Benefit of procedure	Results	Reference
Swimming brachyuran crab (Charybdis lucifera)	 Unilateral Cut the right eyestalk with scissors and wound was cauterized with hot blunt needle Unilateral & bilateral exet the 	Prevent loss of haemolymph and mortality	 Significant effects on biochemical composition except body ash Effectively influence saturated and unsaturated fatty acids Not significantly affected 	Murugesan et al. 2008
Chinese mitten crab (Eriocheirsinensis)	 Unilateral & bilateral – cut the left (unilateral) or both eyestalk (s) and applied burn to the wound 	haemolymph loss and help coagulation	 Not significantly affected digestive enzyme activity of female's hepatopancreas Significantly on serum of lipid 	Wu et al. 2013
Freshwater crab (Potamonpersicum)	• Bilateral – anesthetized (cooling in ice) and excised the eyestalk with scissors and wound was cauterized with hot needle	n/a	 Significantly effects level of glucose and total hemocytes count Accelerated ovarian development and times of molting in females 	Khazraeenia and Khazraiinia, 2009
Freshwater crab (Sartorianaspinigea)	 Unilateral & bilateral – cut the right (unilateral) or both eyestalk (s) with sterile hot scissor and wound was cauterized with hot blunt needle 	Prevent loss of haemolymph and mortality with 2h for next eyestalk	 Mortality occurred after few days of bilateral Significant increase of female gonads by unilateral procedure 	Prasad and Besra, 2012
Freshwater field crab (Oziotelphusasenex senex)	• Unilateral – cut the right eyestalk at its far end and wound was cauterized with hot blunt needle	Prevent loss of haemolymphand mortality	 Affected ovarian growth within 25 days Significantly reduced content of various lipids 	Samyappan et al. 2015
Chinese mitten crab (Eriocheirsinensis)	• Bilateral – clipped the eyestalk using sterile scissors and wound was cauterized	Minimize haemolymph loss and avoid infection	• Strongly induced sex-lethal gene expression in female ovary at day 7 after ESA	Shen et al. 2014
Shore crab (<i>Metopograpsusmes</i> sor)	• Bilateral – excised the base of eyestalk with scissors	n/a	 Effects crab hyperphagia (unsatisfied drive to consume food) at post-ablation Simultaneous enhance the ovarian growth significantly 	Sudha and Anilkumar, 2007
Blue swimmer crab (<i>Portunuspelagicus</i>)	• Unilateral – cut the base of eyestalk (left) at its far end and wound was cauterized with hot blunt needle	Prevent haemolymph loss and mortality	Decrease the percentage of glucose level	Velmurugan et al. 2008

Note: ESA; Eyestalk ablation, n/a; not available

for further used in seed production. The role of eyestalk ablation has been frequently adopted procedure for induced maturation of gonads and spawning of the crabs (Allayie et al., 2011). The eyestalk ablation (ESA) was a removal procedure of the sources of gonad and/or molt inhibiting hormones (GIH and MIH) secreted by the X-organ/ sinus gland complex located in the base of eyestalk or known as a articulation membrane (Figure 1) through bi (both/full) or unilateral (left or right/partial) methods.





Table 2: Examples of calculation formula of some reproductive indicators on selected brachyuran crab species

	Reproductive performance parameter	Calculation formula	Reference
Freshwater crab (Potamonpersicum)	Body mass index	Total weight of crab(including eggs) /Akpaniteaku, 2015carapace width	
	Weight of egg mass	(weight of sample × number of eggs in the ovary) / number of sample	
	Egg mass index (%)	(Weight of egg mass / weight of crab) $\times 100$	
Mud crab (Scylla serrata)	Relative fecundity	Number of eggs extruded / female / batch	Davis et al. 2004
	Total egg mass	Mass of crab at extrusion – mass of crab after hatch	
	Hatching success (%)	(Number of larvae hatched / number of eggs extruded) $\times 100$	
Gazami crab (Portunustrituberculatus)	Berried female successfully hatched (%)	 (Number of females successfully hatched / Wu et al. 2010 total number of surviving berried females) × 100 	
	Female spawned rate (%)	(Number of berried females / total number of surviving females or berried + un-berried females) $\times 100$	
Patagonian stone crab (Platyxanthisdaepatagonicus)	Gonadosomatic indices	Wet weight of the ovary / whole crab wet weight	Dima et al. 2009
	Hepatosomatic indices	Wet weight of the digestive gland / whole crab wet weight	
Chinese mitten crab (Eriocheirsinensis)			Chen et al. 2014
(E. sinensis)	Reproductive effort	(Percentage of egg weight / body weight) \times 100	Wu et al. 2009
Blue swimmer crab Fecundity (Portunuspelagicus)		Total number of the newly hatched larvae (zoea) + total number of un-hatched eggs	Oniam et al. 2012
Blue swimmer crab (<i>P. pelagicus</i>)	Fertilization rate (%)	((Fertilized eggs) / (fertilized eggs + un-fertilized eggs)) ×100	Efrizal et al. 2006

Previous studies showed that this procedure could affects physiological and biochemical metabolism of brachyuran crabs species (Table 1). Multiplication in shellfish is controlled by different neurohormones that are integrated and discharged from the X-organ sinus organ complex situated in the eyestalk of the species (Pervaiz et al., 2011). Meanwhile, the eyestalks of crustacean contain neurosecretory cells that involve in the regulation of molting (Meade and Watts, 2001). In most cases of crustacean especially brachyuran crab, the eyestalk ablation technique showed significant effects on amount of vetellogenin and haemolymphecdysteroids (molting) level (Sudha and Anilkumar, 2007; Tamone et al., 2005). However, this technique is not an appropriate method for enhancing the growth and maturation in certain crustacean's culture due to a failure in recovery of molting and mortality (Venkitraman et al., 2010; Prasad and Besra, 2012). In addition, this technique is a need to induce the gonad development through various methods and techniques for better broodstock production in the future hatcheries seed production. Many factors can control the broodstock performance in brachyuran crabs but of particular importance in aquaculture will be the enhancement of female broodstock that indirectly improve their reproductive performance for further hatchery production.





3 Key Indicator of Reproductive Performance

Based on the previous study, an eyestalk ablation technique were not enough to induce the gonad maturation of brachyuran crab (Kim et al., 2010), thus, an induction of environmental stimuli should be consider for better reproductive output. As described by the study of Wu et al. (2010a), indicators of reproductive performance in brachyuran crab includes of embryonic development (stage), spawning frequency (time), female spawned (percentage), berried female hatched successfully (percentage), carapace width (CW) (crab size), internal carapace width (ICW) (carapace width), wet weights of female without eggs (crab size - body weight), fecundity (total number of eggs per female). In addition, the spawning parameter of brachyuran crabs could also been measured as a time to spawning (days), total eggs mass (g of eggs/female), calculated mass of individual eggs (µg), egg diameter at extrusion (mm), egg diameter prior to hatch (mm), incubation time (hour or days) and percentage of hatching (Davis et al. 2004). Other than studies by Wu et al. (2010) and Davis et al. (2004), the study by Quinitio et al. (2011) also indicated that the reproductive performance of brachyuran crabs can also been indicated asinterval between spawning to hatching (days), number of zoea per crab size (wet weight), number of days from zoea (larval stage) to crab stage and the final survival rate from zoea (larval stage) to crab stage.

Most of the brachyuran have ten embryonic stages which often used which are based on the relative proportion of yolk (Ates et al., 2012; Zeng, 2007). Previous study indicated that crustacean embryos are wrapped in two kinds of layers; thick outer layer and a very thin inner layer, and these structures are distinguishable until hatching occurs (Saigusa et al., 2000). However, the differentiation of embryonic stages was depends on various factors such as environmental conditions or breeding technology of cultured species. Spawning of brachyuran occur between 10-119 days depends on the species and the induction methods used for crabs spawn. The measurement of CW and ICW were shown in Figure. 1. Examples of calculation on key indicator of reproductive performance were shown in Table 2.

4Induction on Reproductive Performance

4.1 Environmental conditions

A significant research effort has been directed towards the identifying of improvement on the reproductive performance for several crustacean species especially crabs. Many studies were conducted to determine the effects of different environmental stimuli on broodstock reproduction of crustacean (reviewed by Green et al., 2014). However, the importance of the environmental effects such as temperature (Brylawski and Miller, 2006; Burmeister and Sainte-Marie, 2010; Fischer and Thatje, 2008; Litulo, 2005; Wu et al., 2010a; Zeng, 2007), salinity (reviewed by Bianchini et al., 2008; Rotllant et al., 2015), oxygen (reviewed by Green et al., 2014), photoperiod (Bembe, 2009), has been only study for some brachyuran crab especially for broodstock which can be use in hatchery culture. The study by Wu et al. (2013), environmental conditions was the main factor affecting the accumulation of energy by hepatopancreas. Thus, the nutrition and energy which stored in hepatopancreas were often consumed soon for gonadal development as well as reproductive performance. In addition, the study by Darnell et al. (2009) also initiated that environmental factors play a major role in determining brachyuran crab size.

4.2 Nutritional Requirements

Nutrient availability is one of the most important factors influencing the broodstock maturation of crabs (Azra and Ikhwanuddin, 2016). Previous review showed that nutrition in aquaculture viewpoint are divided into two distinct classes which are macronutrients, those required in high dietary concentration such as protein and lipids and micronutrients, those required in trace elements (Francis et al., 2014). It is well established that the nutritional content of crustacean crab's broodstock generally reflect the maturation process and the overall viability of seed, through early growth and survival (Oniam et al., 2012). However, the effects of different diets on brachyuran crab reproduction, genus *Scylla* have been done by the previous study (Azra and Ikhwanuddin, 2016); thus, it will be excluded in this present review. Investigations into nutritional classes and ratios have concurrently generated a few of published data corresponding to the nutritional maintenance of broodstock. The nutritional content of diets





showed different effects on reproductive performance of brachyuran crabs such as *P. pelagicus* and *E. sinensis* (Chaiyawat et al., 2008; Sui et al., 2009; Wu et al., 2007).

4.3 Genetic selection

It is important to understand the regulatory mechanism associated with crab reproduction at the molecular and genomic level in more detail. Identifying significant functional genes involved in the gonadal development of crabs indirectly improved the broodstock selection for hatchery used. Genetically induced of oviposition in the brachyuran crabs are not well studied. There are several studies related to the genetic selection such as by Han et al. (2012) and Li et al. (2013) on cyclin B, Ma et al. (2012) on Erk2 and Wang et al. (2012) on vasa gene during ovarian development of brachyuran crabs as our knowledge. These genes usually play different roles in brachyuran ovary such as act as a maturation promotion in the ovarian cycle of brachyuran crabs. More details study should be done on the development of genetic selection especially in the improvement of the brachyuran crab reproductive performance. In additions, the methods of ease-to-obtain of the certain genes were also needed in the future research especially commercial brachyuran crabs culture.

4.4 Physiological Effects (chemical)

To increase viable seed production techniques, better understanding of broodstock selection through chemical effects is considerable new in the future hatchery management. The limited understanding of the physiological effects of several chemical in brachyuran crabs reproduction only allows a much generalized picture of certain broodstock reproduction aspects. Most of the chemicals used in brachyuran crabs cultures areused in the laboratory trials as well as being very expensive. However, several chemicals have been tested as reproduction promotes in aquaculture but they cannot be endorsed for commercial processes due to their residual effects in the body of crabs. The reproductive condition of brachyuran crabs is usually determined by inspection of the gonad. Thus, several methods and techniques are directed to affect the gonad maturation as well as vetellogenin production. Previous studies showed that several chemicals could be used to induce the ovarian maturation and accelerate the reproductive performance of brachyuran crab species such as thyroxine hormone (Iromo et al., 2015), methyl farnesoate (Medesani et al., 2015), steroid hormone, 17-hydroxypregnenolone (Muhd-Farouk et al., 2014) and 17-hydroxyprogesterone (Reddy et al., 2006).

4.5 Others

Research on the culture practices and system is limited and has not been adequately investigated in crab models. In recent years, in-tank and ponds investigation, where the response of broodstock to produced higher female production have been done by the study of Paterson et al. (2007), Oniam et al.(2010) and Wu et al. (2010b). These studies showed that brachyuran crabs reproductive performance can be improved by crab reared in indoor shedding system and in earthen pond culture. Others than that, the improvement in management practices of wild-caught edible crab, *Cancer pagurus* showed acceleration in the spawning of the crab. On the other hand, an interested study by Simeo et al. (2015) indicated that female of spider crab, *Maja brachydactyla* reared without male in the captivity produced four consecutive broods per breeding compared to only three times in the wild. The study by Wu et al. (2010a) showed the different between the first and second female broodstock (same individual with two times spawned) used for hatchery culture. There is no significant different between them, but they found that offspring quality were higher from the first brood compared to the second brood.

5 Future Research Directions and Conclusion

In brachyuran crabs, embryos which develop in broodstock that are carried by the females, they experienced the parental conditions includes environment and nutrition content. The seed produce by the brachyuran crab broodstock usually depend on the broodstock performance. The enhancement of the broodstock is important to the successful of mass rearing of selected crab's species. The development of techniques for the production of improved female's broodstock especially brachyuran crabs could benefit the hatchery seed production. The demand for female's broodstock by crab's hatchery culture, particularly for free-wild caught broodstock, will at some point increasing in the future. Research efforts to enhance the broodstock quality of these delicious





crustaceans in captivity represent the most reasonable way forward in this regard. The present review showed that broodstock reproductive's performance widely differs between crab's species and individually, depending on broodstock spatial and latitudinal aspects, nutritional content and culture system and practices. Generally, external factors such as temperature, salinity, photoperiod and availability of food can determine the length of the reproductive period. The significant induction of ovarian maturation as well as reproductive performance could also been done by the chemicals reaction such as hormone manipulation. It is concluded that the reproductive performance of brachyuran crabs especially female broodstock can be induced by the selected techniques and methods for further used in hatchery seed production.

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