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Research Article
Effect of Eyestalk Ablation on the Ovarian Maturation Stages of Blue Swimming Crab, *Portunus pelagicus*

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Abstract

Background and Objective: The development of domesticated crab is an important aspect for further sustain ability of seed production and broodstock enhancement. An induction of gonad maturation is important to increase the broodstock used for hatchery seed production. This study was designed to determine the effect of eyestalk ablation on the ovarian maturation stages in *P. pelagicus* in term of the gonadosomatic index (GSI), mean of oocyte diameter and then were compared using one-way analysis of variance. **Materials and Methods:** Three group of treatments were set up included control (without any eyestalk ablation), unilateral and bilateral eyestalk ablation. Crabs were then cultured for 30 days in the culture rearing tank systems immediately after ablation and GSI with mean of oocyte diameter were calculated. **Results:** Mean value of GSI was 2.21, 3.39 and 3.31% in control, unilateral eyestalk ablated and bilateral eyestalk ablated, respectively. In addition, the oocyte development was 175.77, 187.44 and 214.08 μm, respectively. Statistical analysis did not show any difference obtained in GSI of control, unilateral and bilateral ablated ($p>0.05$) but statistically different ($p<0.05$) found in oocyte diameter. **Conclusion:** It was concluded that eyestalk ablation affected the oocyte diameter of ovarian maturation stages of *P. pelagicus*.

Key words: Gonad maturation, crustacean, gonadosomatic index, eyestalk ablation, ovarian maturation, hatchery seed production

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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

Recently, the need to understand and control reproductive process as well as physiological research have been stimulated by the increasing interest in culture of commercially important crustacean such as crab. Blue swimming crab, *Portunus pelagicus* is widely choose as one of the best candidate for aquaculture activity because of their convenient size, easy availability and diversity among the Indo-Pacific region[1-3]. In addition, they are exported to south east Asian countries mostly in frozen and canned forms[4]. However, their hatcheries seed production was fully depend on the wild caught broodstock. Thus, the development of domesticated *P. pelagicus* broodstock should be carried out to prevent the decrease of wild broodstock as well as for sustainability reason.

For developing successful farming techniques for this species in the future, the induction of gonad maturation especially ovarian stages have been a top priority for commercial farmer in many parts of the world[5-9]. Eyestalk ablation is one of the common methods for induce and stimulate the crustacean gonad development[10-12]. These techniques involved the removal of eyestalk with various procedures[13] which causes different effects on species of crustacean. However, there is still no comparative study on the effects of eyes talk ablation on each ovarian maturation stages on commercially important species like *P. pelagicus*. The information of the study could be useful for further improvement in the *P. pelagicus* broodstock culture and management in hatchery seed production.

The main objectives of this study were to determine the effect of eyestalk ablation on the ovarian maturation stage of blue swimming crab, *P. pelagicus* based on external morphological and histological assessments.

MATERIALS AND METHODS

Sample collection, transportation and conditioning: Crab samples of *P. pelagicus* were collected from Tanjung Sedili, Mersing, Johor, Malaysia, in December, 2013. Female crabs were weighed and sacrificed as preliminary study where ovaries were dissected out and weighted to calculate the gonadosomatic index (GSI) and were also used for histological study. In the present study, the spent females of *P. pelagicus* were used as standard in histological study. Carapace width (CW) and body weight (BW) of the crab samples were measured and then were transferred into three 1,000 glass tank filled with sea water.

Water parameters such as dissolved, salinity and temperature were monitored using YSI 556 multi probe meter. The experiments was conducted within 30 days and spent female of *P. pelagicus* fed with cockle twice a day. Culture tanks were daily siphoned to remove uneaten feed and fecal matters. On 30th day, final BW was obtained by weighing the treated and control female crabs. After weighing these crabs (unilateral, bilateral and control group) were dissected and ovaries were taken out and weighed to the nearest milligram to determine the GSI. After taking the ovarian weight, ovary were prepared for histological study.

Eyestalk ablation technique: Three sets of experiments were set up included control (group without any eyestalk ablation), unilateral (experimental group with one eyestalk ablation) and bilateral treatment (experimental group with both eyestalk ablation). The unilateral eyestalk ablation was performed in the experimental female crabs by cutting the right eyestalk at its base with fine and sterile hot scissor and the wound was cauterized immediately with a hot blunt forceps in order to prevent the loss of hemolymph and mortality. For bilateral eyestalk ablation, the second eyestalk was removed after a considerable lapse to time, about 2 min and wound was cauterized immediately with a hot blunt needle in order to prevent the loss of hemolymph and mortality. In case of bilateral eyestalk ablation mortality was observed immediately while performing ablation, so precaution was taken and 2nd eyestalk ablation was done carefully. The crabs were released back to the culture rearing tank systems immediately after ablation.

Histological procedure: Histological analysis basically it consists of a chemical or physical method of killing the tissue and yet retaining characteristic peculiarities of shape and structure. In order to study tissues with a microscope, it must be preserved (fixed) and cut into sections thin enough to be translucent. One way is to make a firm block by freezing fresh or fixed tissue. In study with the light microscope, it is difficult to recognize the various components of cells and tissues without differential staining. All procedure were based on the previous published study by Ghazali *et al.*[14]. The histological analysis of the developmental stages of oocyte is the most accurate method for determining sexual maturity, because un-berried but mature females are indistinguishable from external morphology.

Data collection and analysis: After 30 days, *P. pelagicus* were dissected to obtain the ovaries which then used to

determine the gonad colorations and development stages. The GSI, mean of oocyte diameter and oocyte size index (OSI) were compared using One Way Analysis of Variance (ANOVA). The using ANOVA used for further analysis of significant between treatments. All value was considered significant at level of confidence ($p \leq 0.05$). The gonad somatic index (GSI) was determined based on the Ghazali *et al.*[15].

RESULTS

Effect of eyestalk ablation on ovarian maturation: Mean value of GSI was 2.21±2.31 in control (spent female crab) whereas mean value of GSI was 3.39±1.11 in unilateral eyestalk ablated spent female crab and mean value of GSI was 3.31±1.22 in bilateral eyestalk ablated spent female crab. Statistical analysis did not show any difference obtained in GSI between control, unilateral and bilateral ablated crabs ($p>0.05$). Table 1 showed the BW of the crabs was between 88.37-97.47 g. Their ovary weight ranged between 1.97-3.40 g for all treatment. Although all treatment group was not differ significantly, there are still slightly difference can be observed in these data obtained, slightly difference can be observed in mean GSI as shown in Table 1. In Fig. 1, the results showed that oocyte development was 175.77, 187.44 and 214.08 μm for control, unilateral and bilateral treatment, respectively. There are significant difference on oocyte development as shown in Fig. 1. Oocyte diameter increases in size, small yolk globules start to appear in bigger oocytes, follicle cells around the oocytes (Fig. 2). In Fig. 3, oocytes were small in size and less number of yolk globule were appeared and in Fig. 4, bigger size of oocyte diameter as well as bigger follicle cells around the oocytes. For bilateral treatment, oocyte diameter increases in number and size as shown in Fig. 5. Based on one way ANOVA, there was no significance different noted among control with other treatment but there are still slightly difference can be observed in histological study obtained.

External morphology and histological assessment: For histological ovarian study, it shows growth different ovarian stage for bilateral compared preliminary study during this 30 day period. The Fig. 3 showed the histological assessment of ovarian maturation for stage 2 for control group and Fig. 4 shown ovarian maturation stage for unilateral treatment. Yolk globules occur in the cytoplasm with larger globular inclusions toward periphery, follicle cells hardly recognizable (Fig. 5).

Table 1: Body weight, ovary weight and gonadosomatic index of *P. pelagicus* for control, unilateral and bilateral treatment

	Treatments		
Mean	Control	Unilateral	Bilateral
Body weight of crab (g)	88.37±6.50	97.47±17.41	95.57±12.36
Ovary' weight of crab (g)	1.97±2.04	3.40±1.550	3.27±1.550
Gonadosomatic index (%)	2.21±2.31	3.39±1.110	3.31±1.220

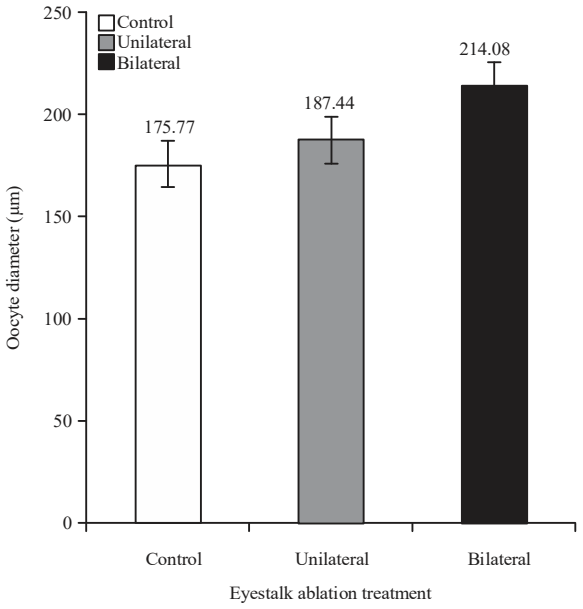

Fig. 1: Mean oocyte diameter (μm) of *Portunus pelagicus* ovarian development for control, unilateral and bilateral treatments

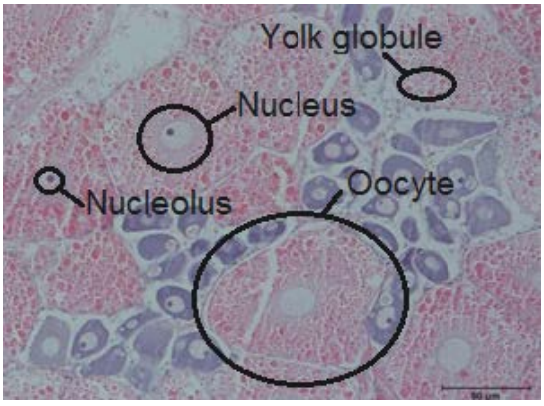

Fig. 2: Stage 2 of early maturing ovaries of *Portunus pelagicus* for initial samples during preliminary study

DISCUSSION

Eyestalk ablation techniques significantly affected the ovarian maturation stages of *P. pelagicus* with bilateral

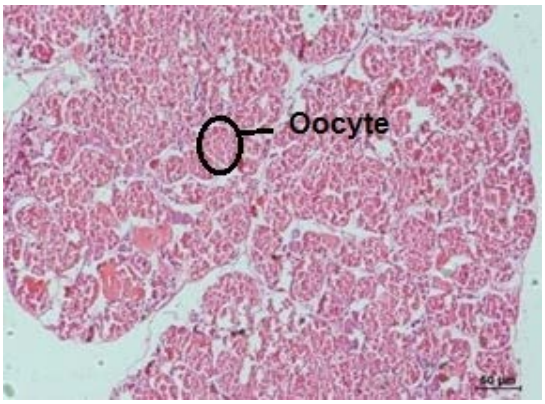

Fig. 3: Stage 2 of early maturing ovaries of *Portunus pelagicus* for control (Oocytes were small in size and less number of yolk globule were appeared)

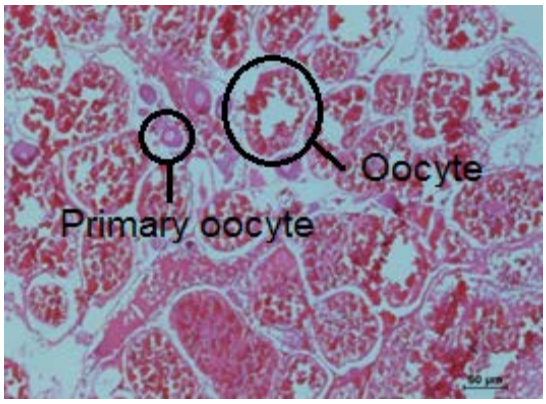

Fig. 4: Stage 2 of early maturing ovaries of *Portunus pelagicus* for unilateral treatment (bigger size of oocyte diameter as well as bigger follicle cells around the oocytes)

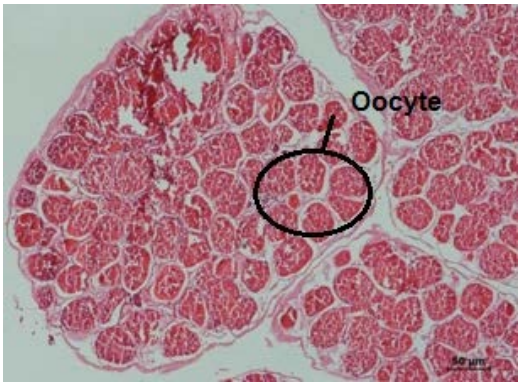

Fig. 5: Stage 3 of late maturing ovaries of *P. pelagicus* for bilateral treatment (oocyte diameter increases in number and size)

treatment resulted in higher size of oocyte development compared to other treatments of eyestalk (i.e., no eyestalk and unilateral eyestalk ablation). As for comparison, studies on eyestalk ablation have been conducted previously by many authors such as Stella *et al.*[16], Ragunatha and Arivazhagan[17] and Wu *et al.*[18]. They found that eyestalk ablation have affected most of the physiological and biochemical compositions and metabolism of brachyuran crabs. It is not unexpected since this technique was significantly contributed in the development and enhancement of crustacean gonad especially crabs[19,20]. Gonad maturation of crustacean is affected by many factors[11,14,15]. In the present study, ovarian maturation of *P. pelagicus* was greatly affected by eyestalk ablation techniques. Eyestalk ablation is usually practiced to induce ovarian maturation in Penaeid shrimp farming as well as in Portunid crab's culture. The effects of eyestalk ablation on gonad development has been reported for a number of species, such as estuarine grapsid crab, *Chasmagnathus granulate*[16], freshwater crab, *Paratelphusa hydrodromous*[17], river crab, *Potamon persicum*[19], Chinese mitten crab, *Eriocheir sinensis*[18] and blood-spotted swimming crab, *Portunus sanguinolentus*[20]. However, the increasing interest of *P. pelagicus* culture, the decreased of *P. pelagicus* broodstock to produce the egg will be a main problem for future aquaculture of this species[2-3,6]. Previous study showed that the eyestalk ablation techniques are successfully induced the ovarian maturation of aquatic animals' especially brachyuran crabs[16-20]. In summary, the results showed that mean value of GSI was higher and oocyte diameter was bigger in size for bilateral eyestalk ablated treatment. This finding would be advantageous to portunid crabs hatchery operators in term of cost production especially by using the spent stage crabs.

CONCLUSION

The results of the present study showed that eyestalk ablation techniques could enhance the development of oocyte especially for *P. pelagicus*. Thus, the eyestalk ablations technique increased the number of broodstock by induce their maturation stage to be used in the *P. pelagicus* hatchery. For suggestion, overall more research needs to be done for baseline study and may be using the same technique but for different crab species. Or use other techniques for this species to enhance the development of ovarian production of female crabs. In addition, may be able to use this technique on male crabs to see the difference stage in gonad development.

SIGNIFICANCE STATEMENT

This study discovered the development of oocyte were bigger through eyestalk ablation techniques that can be beneficial for enhancement of broodstock in the hatchery. This study will help the researchers to uncover the critical areas of domestication that many researchers were not able to explore. Thus a new theory on oocyte development through application of eyestalk ablation may be arrived at.

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