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Effect of Water Salinity on Ovarian Maturation Stages and Embryonic Development of Mud Spiny Lobster, *Panulirus polyphagus*

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ABSTRACT

A study was carried out to determine the effect of salinity on ovarian maturation stages (external morphology and histology characteristics of the ovary) and embryonic development (time of embryonic development and size of egg) of mud spiny lobster, *Panulirus polyphagus*. For the ovarian maturation stages, there were three treatments; treatment 1 for 20 ppt (E1T1), treatment 2 for 40 ppt (E1T2) and control treatment for 30 ppt (E1C2) and conducted within 100 days. There was no significant difference ($p>0.05$) between control in 30 ppt at day 1 (E1C1), E1C2, E1T1 and E1T2 noted in the Oocyte Size Index (OSI). Mean oocyte diameter and Gonadosomatic Index (GSI) had significantly differences ($p<0.05$) between E1C1, E1C2, E1T1 and E1T2. For the embryonic development, the berried female was placed in tank with 20 ppt for treatment 1 (E2T1), 30 ppt for control treatment (E2C1) and 40 ppt for treatment 2 (E2T2) and 20-30 eggs were taken for each salinity to observe the embryonic development. Stage 1, 2 and 3 for 20 ppt and stage 1 for 40 ppt cannot be observed the development of embryo. At low salinity of 20 ppt (E2T1), the shortest duration from stage 4 until hatched was about 4 days while at high salinity of 40 ppt (E2T2), the longest duration from stage 4 until hatched was about 8 days. Thus, the low salinity was the shortest time to hatch. The effect on the sizes changes was stronger in the salinities of 30 and 20 ppt than 40 ppt. Therefore, the high salinity was the lowest egg size compared to low and control treatments. In conclusion, the different salinity affected the ovarian maturation stages and embryonic development of *P. polyphagus*.

Key words: Salinity, ovarian maturation stages, embryonic development, mud spiny lobster, *Panulirus polyphagus*

INTRODUCTION

Mud spiny lobster, *Panulirus polyphagus* is widely exported and over-exploited (Phillips *et al.*, 1980). Increasing global demand, a high market value and concern for the sustainability of wild stocks have created significant interest in the development of spiny lobster aquaculture (Radford and Marsden, 2007; Solanki *et al.*, 2012). This high level of exploiting the breeding

population may have an adverse effect on the recruitment of the juveniles, thus affecting the future of entire population. Small or berried females spiny lobster were common in fish markets. This lack of protection of spawners could damage reproductive potential of the lobster population, resulting in recruitment failure and overexploitation and the fact, the average size of the lobster population has been decreasing over the last decade (Chang *et al.*, 2007). In *P. polyphagus* breeding technology, the major problems are low survival rate in the larvae rearing of pueruli stage and hard to maintain from pueruli until juvenile stages (Fatihah *et al.*, 2014a, b; Ikhwanuddin *et al.*, 2014a). In addition, lack of information for the purposes of fisheries management is one of the major constraints in understanding the management and development of mud spiny lobster, *P. polyphagus* (Ikhwanuddin *et al.*, 2014a). No latest information can be shared in terms of physiology, biology, morphology and behavior fisheries of *P. polyphagus* especially in Malaysia based on previous studies (Ikhwanuddin *et al.*, 2014b).

Salinity is one of the most important abiotic factors affecting the growth and survival of aquatic organisms and has complex and wide-ranging biological effects (Sang and Fotedar, 2004), which make it as the most basic parameter of the culture environment for spiny lobster (Phillips *et al.*, 1980). Presently, there is no published information on the salinity tolerance and optimal salinity levels for *P. polyphagus*. Recent reliable data on ovarian specific for maturation are not enough and fragmentary. Recent study on embryonic development was on *P. polyphagus*, where the study was to determine the effect of temperature on ovarian maturation stages and embryonic development (Ikhwanuddin *et al.*, 2014a). The information from this study can help to avoid this species to become endangered species in the future due to over exploitation. The main objectives of the present study were to study the effect of different salinity regimes on ovarian maturation stages (based on the external morphology and histology characteristics of the ovary) and embryonic development (time of embryonic development and size of egg).

MATERIAL AND METHODS

Mud spiny lobster samples: *Panulirus polyphagus* berried females were sampled from Tanjung Sedili Kecil (1°51' N and 104°09' E), Kota Tinggi, Johor, Malaysia. The new hatched females (spent females) were used for the experiment to determine the effect of salinity on ovarian maturation stages. A total of 12 spent females were used with 3 spent females for each treatment and control. Nine berried females were also used for the second experiment to determine the effect of salinity on embryonic development.

Effect of salinity on ovarian maturation stages: Lobsters were individually weighed and measured for Body Weight (BW) and Carapace Length (CL), respectively. Carapace length was measured from rear of eye socket parallel to the centre line of the body shell to the rear of the body shell. Then, lobsters were transferred into 500 L fibre glass tank filled with seawater before being transferred into respective treatment tanks. Upon treatments, three spent female broodstocks were transferred into each treatment tanks. Salinities were adjusted by adding freshwater to produce salinity of 20 ppt for treatment 1 (E1T1) and artificial marine salt was added to produce salinity of 40 ppt for treatment 2 (E1T2). For the control (E1C1), the ambient salinity was used (30 ppt). The experiments were conducted within 100 days and broodstocks were fed twice a day (800 and 1600 h) with chunked squid (*Loligo* spp.) at 5% biomass. Culture tanks were daily siphoned to remove uneaten feed and fecal matters. Water parameters such as dissolved oxygen, salinity and temperature were monitored using YSI 556 multi probe meter. The optimum condition in culturing were pH 6.5-8.5, temperature of 28-32°C, dissolved oxygen at 5-8 mg mL⁻¹ and salinity based on

preferred treatment. After 100 days, broodstocks were dissected to obtain the ovaries which then used to determine the ovary colouration and Gonadosomatic Index (GSI). Gonadosomatic index were determined based on the following equation (Minagawa, 1997):

$$\text{GSI}(\%) = \frac{\text{Ovary weight (g)}}{\text{Body weight (g)}} \times 100$$

After weighing for GSI, ovaries were quickly fixed in Bouin's solution for 24 h. Afterwards, the gonads were dehydrated through an alcohol series. Embedding was aimed to provide an easily handled solid block held in microtome. Cutting was done using rotary microtome for 4-7 μm thickness. Then, floating was done inside the water bath at 38-40°C to stretch the ribbon and placed on top of glass slide after 'fishing'. Glass slide was dried on hot plate at 60°C overnight. Haematoxylin and eosin method were used for staining purpose. Diameter of 100 oocytes were measured from the triplicates of each treatment were measured with image analyzer (Nikon Eclipse 80i). The ovarian maturation stages were also determined through histology characteristics of molecular structure and Oocyte Size Index (OSI). The OSI were also determined based on the equation by Minagawa (1997) as follows:

$$\text{OSI}(\%) = \frac{\text{Oocyte diameter (mm)}}{\text{Carapace length (mm)}} \times 100$$

Effect of salinity on embryonic development: A berried female was also placed for each treatment tank of 20 ppt (E2T1), 30 ppt (E2C1) and 40 ppt (E2T2) with constant temperature (28°C). The berried female lobsters were provided with PVC pipe for shelter. The berried female lobsters were taken to low and high acclimation salinities by gradual change in salinity twice a week by adding either tap water or concentrated seawater as needed. Salinities in the tanks were checked daily using refractometer (MASTER-S28 α). By using dropper, 20-30 eggs were taken from pleopods of lobster in each treatment (20, 30 and 40 ppt) and transferred into the sample bottle to observe for further observation. Sample bottle that contain eggs was fixed with 5% formalin. The developmental stages of the embryos for *P. polyphagus* were determined by Nikon Measuring Microscope MM-800. The diameters of eggs size were measured and the number of days from appearance of each developmental stage was determined. A scheme egg staging embryo development was constructed by observing the complete developmental sequence of the embryos from oviposition to hatching following Tong *et al.* (2000).

Data analysis: Mean oocyte diameter, GSI and OSI were compared using One Way Analysis of Variance (ANOVA). The Independent T-Test was used for further analysis of significant between treatments.

RESULTS

Effect of salinity on ovarian maturation stages: Ovarian maturation stages for different salinity treatments reared within 100 days were classified based on the external morphology and histology characteristics of the ovaries colouration, characteristic of ovary and the oocyte diameter (Table 1, Fig. 1-3). For the external morphology characteristics, the ovary colouration of control in

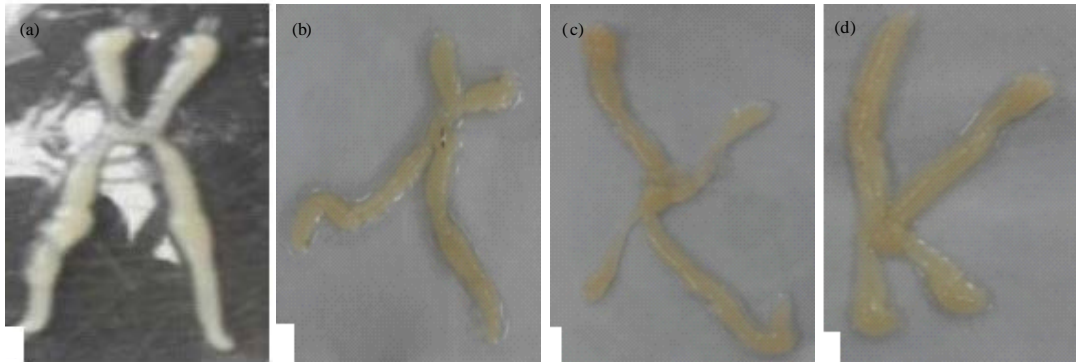


Fig. 1(a-d): External morphology characteristics of ovary colouration of *Panulirus polyphagus* in different water salinity treatments reared within 100 days with all treatments shows the immature ovary stages with transparent and whitish colour, (a) E1C1: Control in 30 ppt at day 1, (b) E1C2: Control in 30 ppt at day 100, (c) E1T1: Treatment 1 in 20 ppt at day 100 and (d) E1T2: Treatment 2 in 40 ppt at day 100

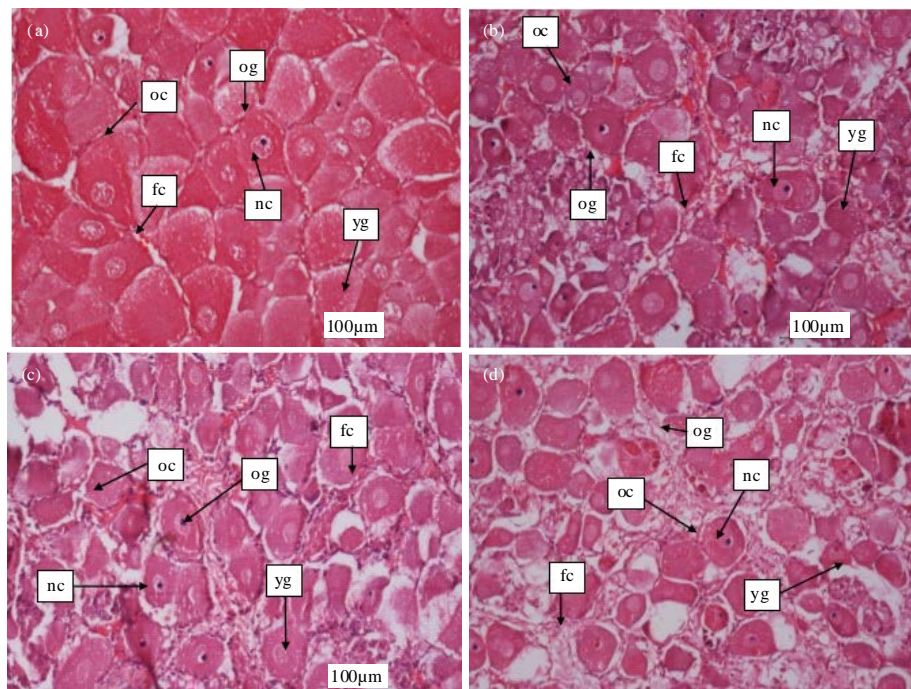


Fig. 2(a-d): Histology characteristics of ovary molecular structure of *Panulirus polyphagus* in different water salinity treatments reared within 100 days, (a) E1C1: Control in 30 ppt at day 1, (b) E1C2: Control in 30 ppt at day 100, (c) E1T1: Treatment 1 in 20 ppt at day 100 and (d) E1T2: Treatment 2 in 40 ppt at day 100, Oc: Section oocyte, nc: Nucleus, og: Ogonia in each lobe, fc: Follicle cell around the oocytes and yg: Yolk globules on large oocytes

30 ppt at day 1 (E1C1), control in 30 ppt at day 100 (E1C2), Treatment 1 in 20 ppt at day 100 (E1T1) and Treatment 2 in 40 ppt at day 100 (E1T2) were transparent and whitish colour.

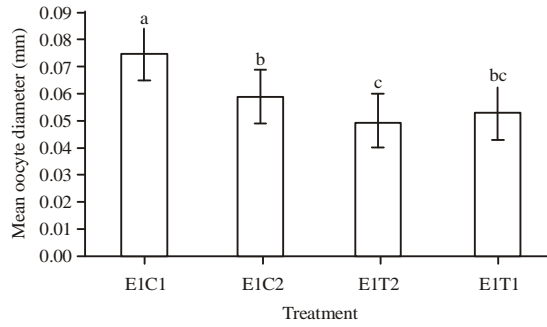


Fig. 3: Mean Oocyte diameter of ovary in different water salinity treatment reared within 100 days of *Panulirus polyphagus*. E1C1: Control in 30 ppt at day 1, E1C2: Control in 30 ppt at day 100, E1T1: Treatment 1 in 20 ppt at day 100 and E1T2: Treatment 2 in 40 ppt at day 100

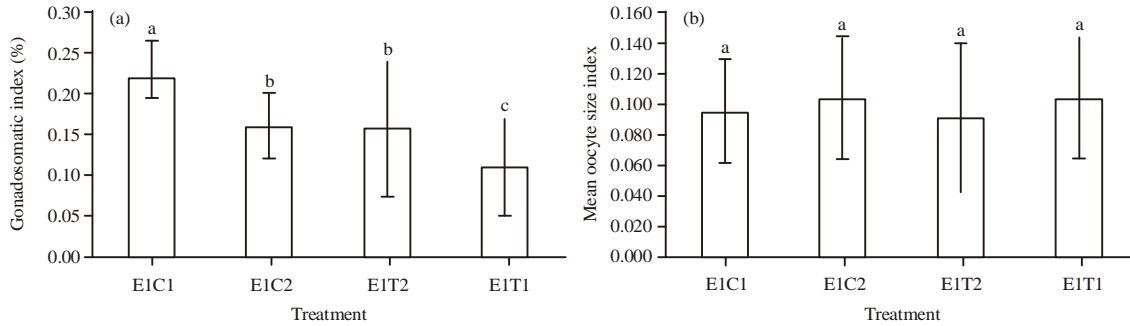


Fig. 4(a-b): Mean (a) Gonadosomatic Index (GSI) and (b) Oocyte Size Index (OSI) of ovary in different water salinity treatment reared within 100 days of *Panulirus polyphagus*. E1C1: Control in 30 ppt at day 1, E1C2: Control in 30 ppt at day 100, E1T1: Treatment 1 in 20 ppt at day 100 and E1T2: Treatment 2 in 40 ppt at day 100

Table 1: External morphology and histology characteristics of ovary of *Panulirus polyphagus* in different salinity treatments

Treatments	Stage	External description	Molecular structure
E1C1	Immature	Ovary thin, flattened, transparent and whitish	Ova transparent with distinct nuclei
E1C2			
E1T1			
E1T2			

E1C1: Control in 30 ppt at day 1, E1C2: Control in 30 ppt at day 100, E1T1: Treatment 1 in 20 ppt at day 100 and E1T2: Treatment 2 in 40 ppt at day 100

Figure 4a-b showed that the highest mean GSI was indicated in E1C1 (0.22±0.04%) at ambient salinity of 30 ppt and the lowest mean GSI was in E1T1 (0.11±0.06%). Meanwhile, mean GSI for E1C2 and E1T2 were 0.16±0.04 and 0.16±0.09%, respectively. The external description of *P. polyphagus* ovaries for E1C1, E1C2, E1T1 and E1T2 were thin, flattened, transparent and whitish colour.

For the histology characteristics, the molecular structure of E1C1, E1C2, E1T1 and E1T2 were ova transparent with distinct nuclei. Based on the Fig. 4b, the highest mean OSI was in E1C2 (0.10±0.04%) and the lowest mean was in E1T2 (0.09±0.05%). Other than that, mean OSI for E1C1

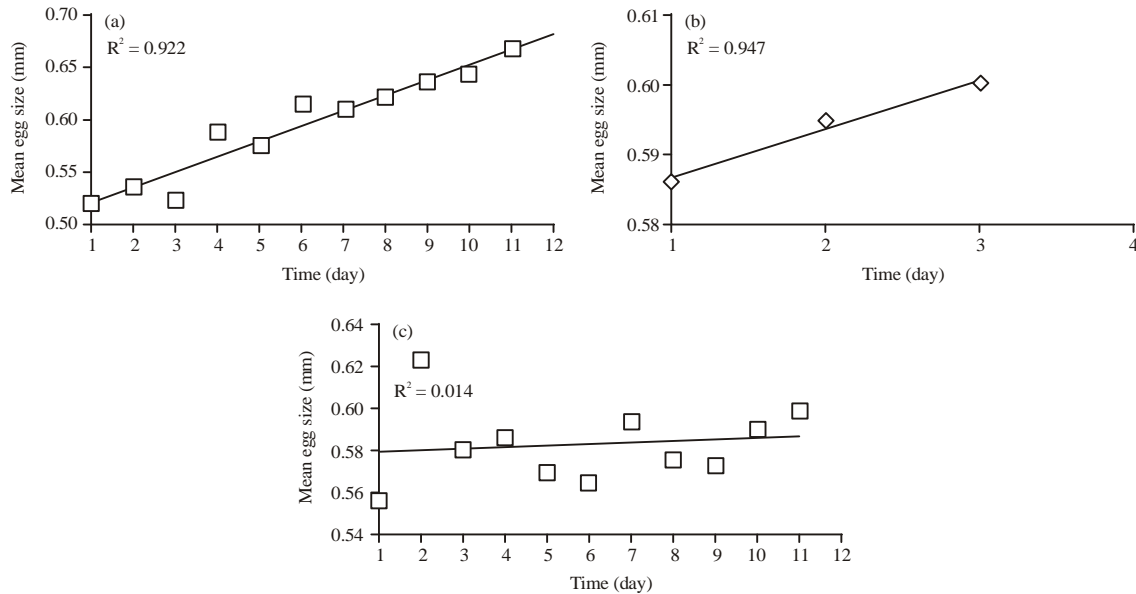


Fig. 5(a-c): Relationship between mean diameter of egg size and number of days taken to hatch of *Panulirus polyphagus* at different salinity, (a) E2C1: Control (30 ppt), (b) E2T1: Low salinity of treatment 1 (20 ppt) and (c) E2T2: High salinity of treatment 2 (40 ppt)

was indicated with value of $0.10 \pm 0.03\%$ and mean OSI for E1T1 was $0.10 \pm 0.04\%$. Based on Fig. 3, the highest mean oocyte diameter was obtained in E1C1 (0.08 ± 0.01 mm) and the lowest was in E1T2 (0.05 ± 0.01 mm). As for E1C2 and E1T1, both showed the mean oocyte diameter of 0.06 ± 0.01 and 0.53 ± 0.10 mm, respectively. There was no significant different ($p > 0.05$) between E1C1, E1C2, E1T1 and E1T2 noted in the OSI. However, mean oocyte diameter and GSI had significant differences ($p < 0.05$) between E1C1, E1C2, E1T1 and E1T2. From the present study, E1C1 recorded the highest mean oocyte diameter, 0.08 ± 0.01 mm with mean CL, 81.79 ± 3.34 mm, mean BW (247.17 ± 46.19 g), GSI ($0.22 \pm 0.04\%$) and OSI ($0.10 \pm 0.03\%$) compared to other salinity treatments.

Effect of salinity on embryonic development

Time of embryonic development: Table 2 showed the times taken to reach the next stage from previous stage until hatching at different salinities during the embryonic development of *P. polyphagus*. All salinities treatments were not in the same stages. For treatment 1 at 20 ppt (E2T1) and treatment 2 at 40 ppt (E2T2), the earlier stage of the embryonic development were not available where E2T2 with 40 ppt has stage 2 till hatching and E2T1 with 20 ppt has stage 4 till hatching. To identify the times taken to reach the next stage from previous stage until hatching at different salinities during the embryonic development of *P. polyphagus*, the comparisons were done only at stage 4 until hatched (Fig. 5). The shortest duration from stage 4 until hatched was at low salinity (20 ppt) of E2T1 with the duration about 96 h (4 days). The time taken for each stages of embryonic development was about 24 h. For the control treatment at 30 ppt (E2C1), the duration from stage 4 until hatched was about 144 h (6 days). Duration of stage 4 and 5 were about 48 h. But, it took 24 h for stage 6 and hatched. The longest duration from stage 4 until hatched was at high salinity (40 ppt) of E2T2 with the duration about 192 h (8 days) as compared the other

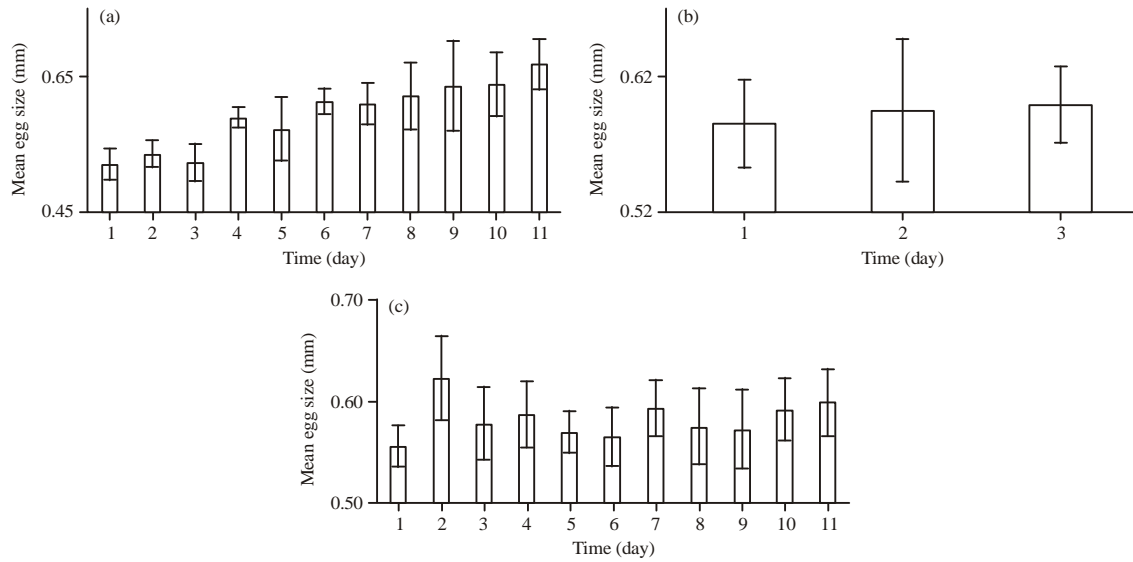


Fig. 6(a-c): Mean diameter of egg size by days till hatching during the embryonic development of *Panulirus polyphagus* at different salinity, (a) E2C1: Control (30 ppt), (b) E2T1: Low salinity of treatment 1 (20 ppt) and (c) E2T2: High salinity of treatment 2 (40 ppt)

Table 2: Times taken to reach the next stage from previous stage until hatching at different salinities during the embryonic development of *Panulirus polyphagus*

Embryonic development stages	Hatch time (h)		
	20 ppt (E2T1)	30 ppt (E2C1)	40 ppt (E2T2)
Stage 1	-	48	-
Stage 2	-	48	24
Stage 3	-	48	72
Stage 4	24	48	72
Stage 5	24	48	48
Stage 6	24	24	48
Hatched	24	24	24
Duration taken from stage 4 until hatching	96	144	192

Dash (-) shows the earlier stage that were not available in embryonic development

salinities treatment (20 and 30 ppt). Duration of stage 4 was about 72 h and duration stage 5 and 6 were taken about 48 h. The embryos took about 24 h to hatch from stage 6 at 40 ppt.

Egg size: Figure 5 showed that the relationship between diameter of egg size and number of days to hatch at different salinity. The value of R^2 for control treatment (30 ppt) shown in Fig. 5 (E2C1) was 0.9226, meaning that there was strong relationship between diameters of eggs size and number of days to hatch. For the low salinity (20 ppt), the value R^2 shown in Fig. 5 (E2T1) was 0.9474, meaning that there was very strong relationship between diameters of eggs size and number of days to hatch. The value of R^2 for high salinity (40 ppt) shown in Fig. 5 (E2T2) was 0.0143, meaning that there was no strong relationship between diameters of egg size and number of day to hatch for *P. polyphagus*. The development of eggs size was not uniformly because at day 2, the egg size was bigger about 0.623 mm compared the other days. Figure 6 showed the relationship between mean diameter of egg size and number of days taken to hatch of *P. polyphagus* at different salinity. Salinities of 30 ppt of control (Fig. 6 (E2C1)) and 20 ppt (Fig. 6 (E2T1)) showed the stronger effect

on the eggs size changes compared to the 40 ppt (Fig. 6 (E2T2)). Besides, to identify the effect of salinities on egg size of *P. polyphagus*, the comparisons were focused only at the late embryos stages from stage 4 until stage 6.

DISCUSSION

Effect of salinity on ovarian maturation stages: Palinurids are mainly restricted to oceanic and near-oceanic water and are generally poikilosmotic over their tolerated salinity range (Phillips *et al.*, 1980) where extreme high and low salinity inhibits the ovarian maturation stages of *P. polyphagus*. Therefore, according to Romano and Zeng (2006), salinity is one of the most important abiotic factors in aquaculture, where some crustaceans' species exhibit some degree of euryhalinity; optimal salinity levels for growth, survival and production efficiency are often species-specific. Salinity is one of a major factor that contributes to the growth rate of crustacean and it has reported to affect the growth of spiny lobster species (Phillips *et al.*, 1980). In present study, *P. polyphagus* was more tolerant to low salinity (20 ppt) as compared to high salinity (40 ppt). Previous study by Kasim (1986) indicates that lobster survive better in lower salinities compared to high salinities. Romano and Zeng (2006) mentioned that salinity range outside 20-35 ppt can significantly reduce survival, growth and development of crustacean. Growth rate of crustacean cultured in higher salinities (40-45 ppt) were significantly less which agreed to present study, where it was clearly shown that the oocyte diameter or ovarian development were less at high salinity water treatment (40 ppt) as compared to other water salinity treatments (20 and 30 ppt). However, other reports have suggested that reduced growth at high salinity conditions may also be attributed to reduce feed assimilation or consumption (Romano and Zeng, 2006).

Crustacean reared at high salinity (45 ppt), were less active upon the introduction of food which may cause reduced growth (Kagwade, 1988b). Previous study by Kasim (1986) mentioned that the rate of oxygen consumption was always lower at optimum salinities and higher at extreme salinities. Ovarian development corresponded well with the changes in GSI and ovigerous percentage (Chang *et al.*, 2007). The colour of ovary changes with ovaries maturation and ovarian development stages (Islam *et al.*, 2010). The cells that constitute the ovaries are three main types: oogonia, oocytes in different stages of development and follicle cells (Santos *et al.*, 2009). The ovarian maturation cycle is differentiated into seven stages; Immature, Early maturing I, Early maturing II, Late maturing, Mature, Semispent and Spent (Kagwade, 1988a). Through microscopic examination, there was only one oogenesis stages out of seven stages were defined in the present study, which was at immature stage. At normal condition, the diameter of the oocyte increased as the ovarian developmental stage progressed (Islam *et al.*, 2010).

According to DeMartini *et al.* (2005), the histological analysis of the developmental stages of oocyte is the most accurate method for determining sexual maturity, because unberried but mature females are indistinguishable from external morphology. Results of histological allowed affirming that the ovaries vary according to gonadal development stage (Santos *et al.*, 2009). Qunitio *et al.* (2007) stated that developing oocytes and the follicle cells or nurse cells are the major cell types found within the ovarian lobes of crustacean, which agreed with the present study of *P. polyphagus*, where the follicle cells are larger and more obvious in immature gonads. The colours of immature ovaries is translucent to yellow and becomes darker yellow to dark orange in mature ovaries due to accumulation of yolk in the oocytes. The yellow colour of the lipoprotein is due to β -carotene, astaxanthin and other unidentified carotenoids (Qunitio *et al.*, 2007). According to Phillips *et al.*

(1980), in immature condition, the ovary is externally white or weakly yellow. Quinitio *et al.* (2007) mentioned that the colour, size and texture of the ovary are closely related to its cellular development. According to Chang *et al.* (2007), the presence of immature and recovery stage ovaries were observed around the year. However in the present study, the lobsters were obtained in October 2012, where in this study the ovarian maturation stage was at the immature stage only.

Effect of salinity on embryonic development: Most palinurids can tolerate wide range of salinities. *Panulirus polyphagus* can endure salinities; 17, 32, 39 and 50 ppt (Kasim, 1986). In the present study, low salinity (20 ppt) was the shortest duration from stage 4 until hatched was about 4 days. It might be the suitable condition for *P. polyphagus* eggs to hatch. For high salinity (40 ppt), duration of developmental stage was longest compared to 30 and 20 ppt. The treatments at 40 and 20 ppt were suitable for marine living organism but might be not suitable for embryos. Duration of embryonic development took 24 h for each stage. In the present study, exhibited the typical low salinity response when exposed for saline solutions lacking chloride but it did not exhibit that response when exposed for solutions that lacked other ions but contained appropriate concentrations of chloride ions. There were no signals of stress that showed by embryos. The result of low salinity on embryos in the present study was almost related to the study by Aiken and Waddy (1986), which explained embryonic development can vary with salinity.

Regulation in dilute media is hyperosmotic in embryos and hyperosmotic conforming in hatchlings and pre-larvae. This change in osmoregulation which accompanies hatch is due to rupture of the relatively impermeable outer egg membrane which may provide some measure of protection for the embryo against low salinity (Charmantier and Aiken, 1987). At 30 and 40 ppt, durations of development from stage 4 until hatched were about 6 and 8 days. Previous study by Sastry and Vargo (1977) noted that lobsters completed development and post-larvae at salinities between 20 and 35 ppt at 15°C and between 15 and 30 ppt at 20°C. The survival was higher at 35 ppt and 15°C and at lower salinities for the higher temperatures (20-30 ppt at 20°C) (Sastry and Vargo, 1977)

Many studies have examined the inter-specific differences in egg size and their impact on larval survival with respect to the evolution of reproductive strategy (Havenhand, 1995) but comparatively few studies have focused on the effect of intra-specific variation in egg size on the viability of crustacean larvae. In the present study, during the final period of embryonic development, egg size increased in low and control treatments, high salinity shows decreased size of eggs, when at stage 5. The increment was generally higher at low salinity. It might be the water salinity treatment at 20 ppt was suitable for the embryonic development and also the egg size. At late embryos stages, the increment of egg size was generally higher at control treatment compared to low and high salinity. High salinity has the lowest egg size compared to low and control treatments. It might be loss of water through the egg membrane.

CONCLUSION

Present study showed that *P. polyphagus* was absolutely adapted to the natural environment compared to extreme high or low salinity on ovarian maturation stages. Within 100 days of treatments, the ovarian maturation was still at immature stage and no significant differences between different salinity treatments. Besides, all berried female *P. polyphagus* had successfully hatched at 40, 30 and 20 ppt. Low salinity treatment is the shortest duration of embryonic development because embryo has lower osmotic pressure and required shorter adaptation time to hatch. The increment of egg size during late embryos stages was generally higher at control treatment (30 ppt).

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REFERENCES

- Aiken, D.E. and S.L. Waddy, 1986. Environmental influence on recruitment of the American lobster *Homarus americanus*: A perspective. *Can. J. Fish. Aquat. Sci.*, 43: 2258-2270.
- Chang, Y.J., C.L. Sun, C. Yong, S.Z. Yeh and W.C. Chiang, 2007. Reproductive biology of the spiny lobster, *Panulirus penicillatus*, in the southeastern coastal waters off Taiwan. *Mar. Biol.*, 151: 553-564.
- Charmantier, G. and D.E. Aiken, 1987. Osmotic regulation in late embryos and prelarvae of the American lobster *Homarus americanus* H. Milne-Edwards, 1837 (Crustacea, Decapoda). *J. Exp. Mar. Biol. Ecol.*, 109: 101-108.
- DeMartini, E.E., M.L. McCracken, R.B. Moffitt and J.A. Wetherall, 2005. Relative pleopod length as an indicator of size at sexual maturity in slipper (*Scyllarides squamosus*) and spiny Hawaiian (*Panulirus marginatus*) lobsters. *Fish. Bull.*, 103: 23-33.
- Fatihah, S.N., J. Safiah and A.B. Abol-Munafi and M. Ikhwanuddin, 2014a. Effect of testosterone undecanoate hormone on sperm and its level in the hemolymph of male Mud Spiny Lobster, *Panulirus polyphagus*. *Pak. J. Biol. Sci.*, 17: 937-941.
- Fatihah, S.N., J. Safiah, A.B. Abol-Munafi and M. Ikhwanuddin, 2014b. Effect of 17 α -hydroxyprogesterone and 17 α -hydroxypregnenolone on sperm quality and sperm quantity in male mud spiny lobster (*Panulirus polyphagus*). *Pak. J. Biol. Sci.*, 17: 1124-1129.
- Havenhand, J.N., 1995. Evolutionary Ecology of Larval Types. In: *Ecology of Marine Invertebrate Larvae*, McEdward, L. (Ed.). CRC Press, Boca Raton, FL., pp: 79-122.
- Ikhwanuddin, M., S.N. Fatihah, A.H. Nurfaseha, M. Fathiah and M. Effendy *et al.*, 2014a. Effect of temperature on ovarian maturation stages and embryonic development of mud spiny lobster, *Panulirus polyphagus*. *Asian J. Cell Biol.*, 9: 1-13.
- Ikhwanuddin, M., S.N. Fatihah, J.R. Nurul, M.Z. Zakaria and A.B. Abol-Munafi, 2014b. Biological features of mud spiny lobster, *Panulirus polyphagus* (Herbst, 1793) from Johor coastal water of Malaysia. *World Applied Sci. J.*, 31: 2079-2086.
- Islam, M.S., K. Kodama and H. Kurokura, 2010. Ovarian development of the mud crab *Scylla paramamosain* in a tropical mangrove swamps, Thailand. *J. Sci. Res.*, 2: 380-389.
- Kagwade, P.V., 1988a. Fecundity in the spiny lobster *Panulirus polyphagus*. *J. Mar. Biol. Assoc. India*, 30: 114-120.
- Kagwade, P.V., 1988b. Reproduction in the spiny lobster *Panulirus polyphagus* (Herbst). *J. Mar. Biol. Assoc. India*, 30: 37-46.
- Kasim, H.M., 1986. Effect of salinity, temperature and oxygen partial pressure on the respiratory metabolism of *Panulirus polyphagus* (Herbst). *Indian. J. Fish.*, 33: 66-75.
- Minagawa, M., 1997. Reproductive cycle and size-dependent spawning of female spiny lobsters (*Panulirus japonicas*) off Oshima Island, Tokyo, Japan. *Mar. Freshwater Res.*, 48: 869-874.
- Phillips, B.F.A., J.S. Cobb and R.W. George, 1980. General Biology. In: *The Biology and Management of Lobsters*, Volume 1: Physiology and Behavior, Cobb, J.S. and B.F. Phillips (Eds.). Academic Press, New York, USA., ISBN-13: 9780121774011, pp: 1-82.

- Quinitio, E.T., J. De Pedro and F.D. Parado-Esteba, 2007. Ovarian maturation stages of the mud crab *Scylla serrata*. *Aquacult. Res.*, 38: 1434-1441.
- Radford, C.A. and I.D. Marsden, 2007. Does morning opposed to night time feeding affect growth in juveniles spiny lobsters, *Jasus edwardsii*. *J. World Aquacult. Soc.*, 36: 480-488.
- Romano, N. and C. Zeng, 2006. The effects of salinity on the survival, growth and haemolymph osmolality of early juvenile blue swimmer crabs, *Portunus pelagicus*. *Aquaculture*, 260: 151-162.
- Sang, H.M. and R. Fotedar, 2004. Growth, survival, haemolymph osmolality and organosomatic indices of the western king prawn (*Penaeus latisulcatus* Kishinouye, 1896) reared at different salinities. *Aquaculture*, 234: 601-614.
- Santos, C.M., G.V. Lima, A.A. Nascimento, A. Sales and L.M.Y. Oshiro, 2009. Histological and histochemical analysis of the gonadal development of males and females of *Armases rubripes* (Rathbun 1897) (Crustacea, Brachyura, Sesarmidae). *Braz. J. Biol.*, 69: 161-169.
- Sastry, A.N. and S.L. Vargo, 1977. Variations in the Physiological Responses of Crustacean Larvae to Temperature. In: *Physiological Responses of Marine Biota to Pollutants*, Vernberg, F.J., A. Calabrese, F.P. Thurberg and W.G. Vernberg (Eds.). Academic Press, New York, USA., pp: 401-422.
- Solanki, Y., K.L. Jetani, S.I. Khan, S.A. Kotiya, N.P. Makawana and A.M. Rather, 2012. Effect of stocking density on growth and survival rate of spiny lobster (*Panulirus polyphagus*) in cage culture system. *Int. J. Aquat. Sci.*, 3: 3-14.
- Tong, L.J., G.A. Moss, T.D. Pickering and M.P. Paewai, 2000. Temperature effects on embryo and early larval development of the spiny lobster *Jasus edwardsii* and description of a method to predict larval hatch times. *Mar. Freshwater Res.*, 51: 243-248.