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Note

Observations on the effect of natural diets on ovarian rematuration in blue swimming crab *Portunus pelagicus* (Linnaeus, 1758)

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ABSTRACT

This study was carried out to evaluate the effect of natural diets on ovarian maturation in the blue swimming crab *Portunus pelagicus*. A total of 25 female crabs in the spent condition were collected from Johor, Peninsular Malaysia. Five animals were used for histological examination of the ovary and the rest twenty were used for the feeding experiment. Four dietary groups each comprising 5 crabs, were fed with four different diets *viz*., *Loligo* sp., blood cockle, *Anadara* sp., fish, *Decapterus* sp. and polychaete, *Neries* sp., @10% of body weight daily. Throughout the experimental period, the number of days taken by the females to remature and become ovigerous again as well as the number of larvae produced in each treatment was recorded. The results showed that crabs fed with polychaetes rematured and became oveigerous faster (14.4±7.7 days), followed by crabs fed with blood cockle (14.8± 1.12 days), fish (21.2±11.2 days) and squid (26±12.2 days). However in contrast, squid was found to be the best diet in terms of producing more number of larvae (329240±193390 larvae), compared to crabs fed with scad fish (323500±118096 larvae), followed by cockle (263444±153,021 larvae) and polychaete (256520±160677 larvae).

Keywords: Blue Swimming crab, Natural diets, Ovarian maturation, *Portunus pelagicus*

The blue swimming crab *Portunus pelagicus* (Linnaeus, 1758) (Family: Portunidae) is one of the most commercially important species of crabs in the Indo-Pacific region and the global annual production of the species is 20 t (FAO, 2013). It holds promise as a possible alternative cultivable species to shrimps (Ikhwanuddin *et al*., 2012a). Availability of *P. pelagicus* in the markets is largely based on wild caught crabs, and the sharp increase in the landings of the crab over the past decade suggests that wild stocks of this species are likely to be unsustainable (FAO, 2010). The wild resources are under heavy exploitation with modern gear such as set (gill) and drop nets replacing the conventional crab pots (Kangas, 2000).

To improve the aquaculture of *P. pelagicus*, there is need to optimise reliable production of crab seed and research needs to be oriented towards understanding the reproductive physiology, spawning behaviour and nutrient requirements of the broodstock in captivity (Ikhwanuddin *et al.*, 2014). There are limited studies available on feeding protocols of *P. pelagicus* broodstock (Djunaidah *et al.*, 2003) and so far there has been no study on the effect of different natural diets on ovarian maturation, especially on rematuration of broodstock. Female *P. pelagicus* are capable of multiple spawning from a single insemination or mating (Kangas, 2000). It is important to assess the effect of broodstock maturation diets to increase the production of quality larvae from berried females in the hatchery (Azra and Ikhwanuddin, 2015). The present study was conducted to observe the effects of different natural diets on ovarian rematuration of *P. pelagicus*.

Twenty five female *P. pelagicus* in spent condition were sampled from Gelang Patah, Johor, Peninsular Malaysia. The average carapace width (CW) measurements of the crabs was12.52 ± 1.23 cm and the average body weight (BW) was 145.34 ± 45.80 g. The rearing experiments were conducted at the Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, Terengganu, Malaysia.

Gonads of five female crabs were fixed in Bouin's fixative for histological investigations to confirm that the female crabs used for the study were in spent condition. Remaining 20 crabs were stocked in the experimental tanks. Four treatment tanks (1 t capacity HDPE tanks) prepared for static culture with aeration were provided with PVC pipes as shelters and sand as substrate to aid in spawning. When spawning occurs in *P. pelagicus*, the female settles into sand with her abdomen extended outwards and the eggs are extruded which attach to hairs on the abdomen (Kangas, 2000). Four different natural diets were used in this study *viz*., squid, *Loligo* sp., blood cockle, *Anadara* sp., fish, *Decapterus* sp. and polychaete worm, *Nerries* sp. Each rearing tank was stocked with five female crabs and the tanks were designated as treatment A, B, C and D for crabs that were fed with squid, blood cockle, fish and polychaete respectively The natural diets were minimally processed so that only flesh was used for squid, fish and polychaete worm, while shell of the blood cockles were removed. Crabs were fed twice daily at 08.00 and 20.00 hrs, @ 10% of the total BW (wet weight). The condition of the crabs was observed and recorded daily during each feeding time. To maintain the water quality, 100% of the rearing water was exchanged manually every two days with pristine seawater. Any leftover feed and faecal matter was siphoned out before each feeding time. Salinity of the rearing water was maintained at 29-31‰ and temperature between 25-28°C. The feeding experiment was conducted for a period of 60 days.

For histological studies, the gonads fixed in Bouin's fixative were dehydrated in ascending grades of ethanol, cleared in xylene and infiltrated in paraffin wax. The processed tissues were then embedded in wax and sectioned at 5µm thickness. Sections were mounted on glass slides, stained using hematoxylin and eosin (Santos *et al*., 2009), examined under light microscope and photomicrographs were taken.

All four natural diets were analysed for proximate composition. Lipid level of natural diets was determined using Soxhlet extraction method with petroleum ether as solvent (Zaher *et al*., 2009). For protein analysis Kjeldahl method was followed (AOAC, 2002). Protein level was calculated using 6.25 as conversion factor. Moisture content of the samples was determined by drying the sample in an oven at 105°C for 12 h, until it reached constant weight. Number of days the crabs took to remature and to become ovigerous and the number of larvae hatched were recorded in each treatment, The data were analysed using one-way analysis of variance (ANOVA) to determine significant difference if any between treatments ($p<0.05$). Significance between treatment means were identified using Tukey's *post-hoc* test.

Spent stage (stage 5) ovary of female crabs had oocytes of irregular shape, having appearance of being reabsorbed, along with oocytes in different developmental stages with recognisable unexpelled yolk cells, indicating continuous spawning cycles (Fig. 1). The stage 5 ovary of the spent females observed in the present study were almost similar to stage 2 or smaller than stage 3 of *P. pelagicus* ovarian maturation stages as described by Ikhwanuddin *et al*. (2012b). The ovary appears yellow to light orange, occasionally with dark orange colour in some parts.

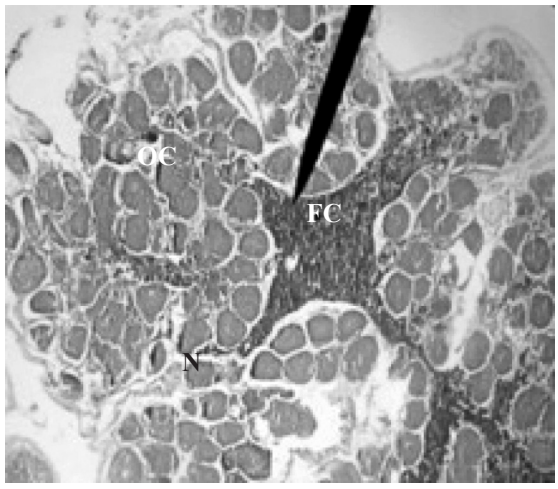

Fig. 1. Histological section of spent stage (stage 5) ovary of *P. pelagicus*. FC : Follicle cell, N : Nucleus, OC : Ocyte cell

Polychaete worm diet induced faster rematuration of spent females (within 14.4±7.7 days), followed by blood cockle (14.8±11.12 days) (Fig. 2.). Crabs fed with fish rematured within 21.2±11.2 days and squid fed crabs rematured in a 26±12.2 days (Fig. 2.). The results showed that there was a significant difference between the treatments in the number of days taken by female crabs to remature ($p<0.05$), with the exception of female crabs fed with blood cockles and polychaetes.

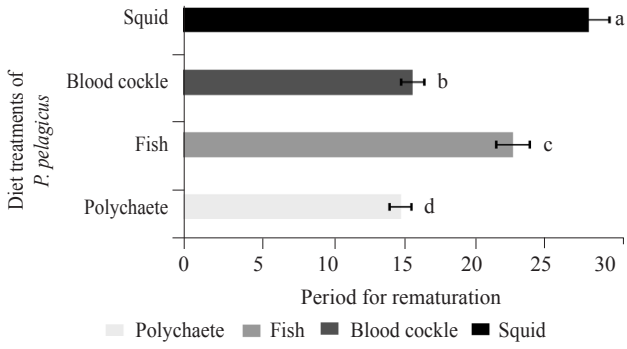

Fig. 2. Number of days taken by females of *P. pelagicus* to remature and become ovigerous in different diet treatments

The number of larvae produced ranged from about 200000 to 500000 individuals in every 100 l. The maximum number of larvae was produced by females fed with squid (329240±193390 larvae), followed by those fed with fish (323500±118096 larvae), cockle (263444 ± 153021 larvae) and polychaetes (256520 ± 160677 larvae) (Fig. 3). Mean larval count was significantly different ($p<0.05$) between treatments except between blood cockles and polychaetes.

Analysis of proximate composition of the diets showed that there was no significant difference ($p>0.05$) in moisture, protein and lipid levels of the diets, except for polychaetes. Polychaetes had the highest moisture content (80.77%) followed by cockle (77.40%), squid (71.58%) and fish (68.63%). Protein content (dry basis) was found to be highest in fish (74.60%), followed by squid (73.15%), cockle (67.86%) and polychaete (67.88%). Lipid content (dry basis) was highest in polychaete (17.75%), followed by fish (7.84%), squid (4.83%) and cockle (4.63%) (Fig. 4).

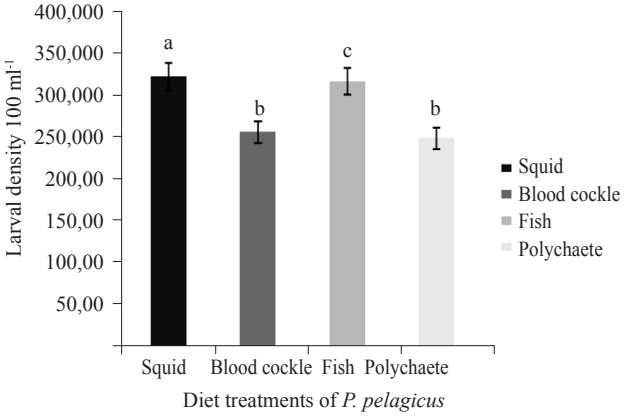

Fig. 3. Mean larval density (per 100 ml) of *P. pelagicus* in different diet treatments

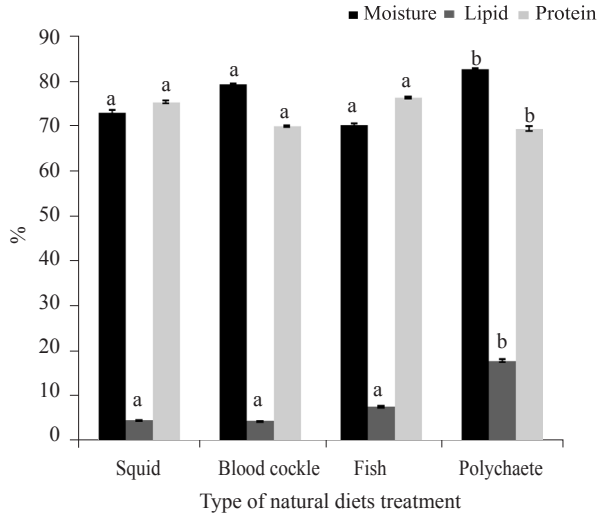

Fig. 4. Proximate composition of different natural diets used for rearing spent female *P. pelagicus*

Juvenile and adult *P. pelagicus* are opportunistic scavengers, cannibals and bottom feeding carnivores (Kangas, 2000). They are also nocturnal species that actively forage and feed after sunset (Grove -Jones, 1987). Natural diets used for the present study *i.e.*, squid, cockle, fish and polychaetes were selected based on the above information and the crabs were fed at 10% of body weight. The daily ration was split into two and fed twice daily in the morning and at night to avoid any compounding effects and to maintain water quality.

Rahaman (1966) stated that *P. pelagicus* is a continuous breeder with three peaks of maximal gonadal indices in November, January and June. However, the reproductive cycle of this species is not concurrent and they have extended breeding season (Krishna and Balakrishnan, 1971). The reproductive activity of this species is believed to be influenced by favourable salinity and availability of planktonic food for the larvae (Krishna and Balakrishnan, 1971). In the present study, histological investigations were carried out to confirm that the female crabs used were in spent condition, charaterised by irregular shaped oocytes. All the spent females stocked in the four experimental tanks rematured and became ovigerous again within the 60 days study period. The number of days taken by the crabs to become ovigerous differed between diet treatments. Polychaetes were found to be comparatively better diet since the time taken for the females to remature and become ovigerous was relatively lesser. The higher concentration of lipid content in polychaete probably helped to enhance the ovarian maturation cycle and lipid metabolism in *P. pelagicus*. The information generated from this study could prove useful in developing feeding protocols for *P. pelagicus* and in enhancing reproductive performance of *P. pelagicus* in captivity through diet manipulation.

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References

AOAC, 2002. *Official methods of analysis,* 17th edn. Association of Official Analytical Chemists, Washington DC, USA.

Azra, M. N. and Ikhwanuddin, M. 2015. A review of maturation diets for mudcrab genus *Scylla* broodstock: present research, problems and future perspective. *Saudi. J. Biol. Sci*., DOI: 10.1016/j.sjbs.2015.03.011

Djunaidah, S., Wille, M., Kontara, E. K., and Sorgeloos, P. 2003. Reproductive performance and offspring quality in mud

crab *(Scylla paramamosain*) broodstock fed different diets. *Aquacult. Int.,* 11: 3-15. DOI: 10.1023/A:1024188507215.

FAO 2010. *Fishstat Plus: Universal software for fishery statistical time series. Portunus pelagicus 1950-2009*. FAO Fisheries Department, Fisheries Information, Data and Statistic Unit http://www.fao.org/figis/servlet/t?ds=Capture&k1=SPECIES&k1v=1&k1s=2629&outtype=ht (Accessed 21 April 2011.)

FAO 2013. *Fishstat Plus: Universal software for fishery statistical time series. Portunus pelagicus 1950-2007*. FAO Fisheries Department, Fisheries Information, Data and Statistic Unit. http://www.fao.org/fi/stat/summary/b-1.pdf. (Accessed 20th December 2014).

Grove-Jones, R. 1987. Catch and effort in the South Australian blue crab (*Portunus pelagicus*) fishery. *South Australian Department of Fisheries, Discussion paper,* September 1987, 45 pp.

Ikhwanuddin, M., Azra, M. N., Sung, Y.Y., Abol-Munafi, A. B. and Shabdin, M. L. 2012a. Live foods for juvenile production of blue swimming crab, *Portunus pelagicus* (Linnaeus, 1766). *J. Fish. Aquat. Sci.*, 7(4): 266-278. DOI: 10.3923/jfas.2012.266.278s.

Ikhwanuddin, M., Azra, M. N., Siti-Aimuni, H. and Abol-Munafi, A. B. 2012b. Fecundity, embryonic and ovarian development of blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758) in coastal water of Johor, Malaysia. *Pak. J. Biol. Sci.,* 15(15): 720-728. DOI: 10.3923/pjbs.2012.720.728.

Ikhwanuddin, M., Liyana, A. N., Azra, M. N., Bachok, Z. and Abol-Munafi, A. B. 2014. Natural diet of blue swimming crab, *Portunus pelagicus* at Straits of Tebrau, Johor, Malaysia. *Sains Malays,* 43(1): 37-44. URL: http://fst.ukm.my/jsm/pdf_files/SM-PDF-43-1-2014/05%20M.%20Ikhwanuddin.pdf.

Kangas, M. I. 2000. Synopsis of the biology and exploitation of the blue swimmer crab, *Portunus pelagicus* (Linnaeus), in Western Australia. *Fish. Res.,* 121: 1-22. URL: http://www.fish.wa.gov.au/Documents/research_reports/frr121.pdf.

Krishna, K. P. and Balakrishnan, N. N. 1971. The annual reproductive cycles of *Uca annulipes, Portunus pelagicus* and *Metapenaeus affinis* (Decapoda: Crustacea) from the south-west coast of India. *Mar. Biol.*, 11(2): 152-166. DOI: 10.1007/BF00348765.

Rahaman, A. A. 1966. Annual changes in the gonad and hepatic indices of the starfish, *Oreaster hedemanni* of the Madras coast. *Bull. Dept. Mar. Biol. Oceanogr. Univ. Kerala.* 2: 1-4.

Santos, C. M., Nascimento A. A. A., Peracchi, A. L., Mikalauskas, J. S., Rocha, P. A. and Sales, A. 2009. Distribution of the endocrine cells in the gastrointestinal tract of nectarivorous and sanguivorous bats: A comparative immunocytochemical study. *Tissue Cell,* 41(3): 222-229.

Zaher, U., Buffiere, P., Steyer, J. P., and Chen, S. 2009. A procedure to estimate proximate analysis of mixed organic wastes. *Water Environ. Res.,* 81(4): 407-415.

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