PAPER • **OPEN ACCESS**

Concentration of Mud Crab (*Scylla olivacea* Herbst, 1796) Moulting Hormones based on Moon Phase

To cite this article: Hasnidar and A Tamsil 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **253** 012011

Concentration of Mud Crab (*Scylla olivacea* Herbst, 1796) Moulting Hormones based on Moon Phase

Hasnidar and A Tamsil

Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Muslim Indonesia, Makassar 90231, Indonesia

Email: hasnidar.yasin@umi.ac.id

Abstract. Moon phase is one of the external factors that affect the dynamics of crab moulting hormones (ecdysteroids). This study aimed to determine the effect of moon phase on the moulting hormone concentration of the mud crab Scylla olivacea Herbst 1796. Data were collected on the haemolymph ecdysteroid concentration in the eight phases of the moon: new moon, ¼ I, ½ I, ¾ I, full moon, ¾ II, ½ II and ¼ II. Ultra-Fast Liquid Chromatography (UFLC) was applied for haemolymph moulting hormone analysis. The results showed that the pattern of haemolymph ecdysteroid concentration varied based on the phases of the moon. The highest haemolymph ecdysteroid concentration was found in ½ I and ½ II moon phases. During this phase the moon exerts a relatively weak gravitational force. The lowest haemolymph ecdysteroid concentration was found in the new and full moon phases during which the moons gravitational pull is the strongest.

1. Introduction

The mud crab *Scylla* spp. is a crustacean fisheries commodity with promising prospects due to its high economic value [1]. These crabs have a high nutritional value with delicious taste, both of which are factors leading to the high market demand for crabs. Crab products which are widely marketed include fresh (live) crabs, crab eggs, frozen crab meat, and soft-shell crabs.

The length of time required and fluctuations in moulting are problems during crab culture. Due to the high demand, technology is being developed to produce more soft-shell crabs in a short time. Soft-shell crabs are recently-moulted crabs, where the new exoskeleton revealed by the removal of the old carapace is still soft, as it has not yet had time to harden. In addition to making it much easier for consumers to prepare and eat the crab, this commodity allows consumers to consume the whole crab without leaving crab shell waste. However, the success of soft shell crab cultivation has been hampered by the availability of crab seed and feed, the quality of cultivation media, and the induction of moulting.

The availability of soft shell crab has been reported as fluctuating based on the phases of the moon, with an increase in the number of soft shell crabs occurring a few days before and after the full moon (https://www.bluecrab.info/fullmoon.htm). Similar patterns have been reported in the soft shell crab farming industry in several countries, such as in Thailand and Indonesia [2,3]. The number of moulting crabs decreases consistently with every full moon and dark moon phase. When the tide rises, the moulting activity of crabs is affected, however, it increases during a low tide (phase of the moon ½) [3]. Several possible moulting triggers have been studied further; in particular the role of the moon's gravitational force, the intensity of moonlight, and tides caused by the moon phases could affect crab moulting, however the mechanisms are not well known. The cycles of the moon are known to have an influence on hormonal changes in insects and low level vertebrates [4]. The release of neurohormone is triggered by electromagnetic radiation and or the gravitational force of the moon. This study aimed to determine the effect of the moon phase on the concentration of ecdysteroid haemolymph of mud crabs (*Scylla olivacea* Herbst, 1796).

2. Methods

This study was conducted at the Centre for Research and Development of Brackish Water Aquaculture, Faculty of Marine Sciences and Fisheries, University Hasanuddin. This facility is situated in Bojo Village, Mallusetasi District, Barru Regency, South Sulawesi Province, Indonesia.

A total of 140 mud crabs (*Scylla olivacea*, Herbs, 1976) were used in this study. The experimental mud crabs ranged in size from a carapace width of 720mm to 800 mm with a weight range of 90-100 g. All crabs were initially in the inter-moulting phase. Each crab was carefully placed into an individual crab boxes (1 crab per box) for rearing. The crabs were fed with small fish (10% of body weight) once a day in the late afternoon, following [5].

Haemolymph was collected a total of eight times, once during each of the eight following phases of the moon: (i) New moon; (ii) ¼ I; (iii) ½ I /first quarter; (iv) ¾ I; (v) full moon; (vi) ¾ II; (vii) ½ II/last quarter; (viii) ¼ II. At each phase of the moon four crabs from four rearing habitats were selected randomly for measurement. Haemolymph was taken from the base of the fifth leg of the crabs using a 1 mL syringe with a 27 gauge needle. The 1 ml aliquot of haemolymph was stored in a vial tube and mixed with anticoagulant in the ratio of 1:1. The samples were stored in a freezer at -20^{o}C until the sample was ready to be extracted. The haemolymph extraction procedure was as follows: (i) 1mL of haemolymph was added to 3 mL of diethyl ether; (ii) The liquid was then homogenised for 30 seconds using a vortex machine and left (incubated) for 2 minutes; (iii) the top layer was an ether phase containing steroids, and (iv) the remaining residue was re-extracted 3 times before being collected and dried at a temperature of 40^{0}C following [6].

The ecdysteroid measurement procedure was as follows: (i) the dried sample residue was diluted with methanol pro UFLC and placed in a UFLC auto sampler vial; (ii) samples were analysed using a Shimadzu LC-20 AD Ultra-Fast Liquid Chromatography (UFLC) unit with the following parameters/accessories: (a) column: Shim Pack ODS C18 250x4,6 mm; (b) system: reversed phase; (c) mobile phase: methanol-water (80: 20v/v); (d) flow rate: 1 ml/min; (e) column temperature: 40^{0}C; (f) detector: photodiode array (UV) 246 nm; and (g) injection volume: 10 mL. The quantification of ecdysteroid used a 20- hydroxyecdysone (Sigma®) standard series.

3. Results and Discussion

The pattern of ecdysteroid haemolymph concentration of crabs was similar in all replicates. Mud crab ecdysteroid haemolymph concentration showed a marked variation during the 8 moon phases (Fig.1).

The UFLC analysis showed that the low ecdysteroid concentration during the new moon phase started increasing in ¼ I phase, reached its peak in ½ I moon phase, declined gradually on the ¾ I moon phase, before dropping even lower in the full moon phase. The highest concentrations of ecdysteroid haemolymph were recorded during the waxing and waning half-moon phases ½ I and ½ II, while the lowest concentrations were during the new (dark) and the full moon phases.

During the half-moon phases I and II, the positions of the moon, the earth and the sun form an angle of 90^{0}; the combined gravitational force exerted by the moon and sun on the earth is weakest, as they are pulling at right angles to each other, resulting in weaker tidal forces and lower tidal range (neap tides). The results indicate that these conditions triggered the crabs to generate maximum ecdysteroid hormones and induced moulting (ecdysis).

Conversely, when the lowest haemolymph ecdysteroid was recorded, during the new moon and full moon phases, the position of the earth was more or less in line with the moon and the sun. Under these conditions, the moon's gravitational pull is reinforced by that of the sun, exerting a maximum force on the earth, resulting in the highest tidal range (spring tides). The high spring tides have implications including increased water movement and turn-over, so that water quality tends to be better. These environmental factors appear to elicit a response from the crabs, as they started foraging for food after the moulting (ecdysis).

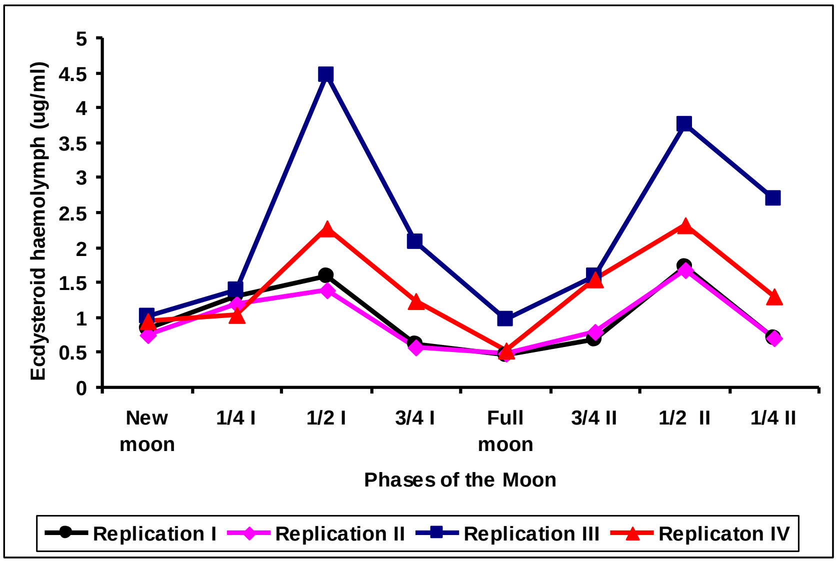

Figure 1. Mud crab (*S. olivacea*) haemolymph ecdysteroid concentration (µg/ml) by moon phase

There is a strong relationship between ecdysteroid hormone concentration and moulting stage. Previous studies have found a low hormone concentration throughout the inter-moult period (C1-4 stage), increased during early pre-moult (stage D0-1), reaching a peak during mid-pre-moult stage (D1-2), then decreasing sharply during the final pre-moult stage (late pre-moult, D3-4), before ecdysis (phase E) and the post-moult stage (phase A-B) [7,8,9]. This study linked this cycle to the phases of the moon. Based on the pattern of haemolymph ecdysteroid concentration dynamics of the mud crabs in relation to the phases of the moon and crab moulting stages, it appeared that the moon phase ¼ I was an early stage of moulting preparation, in which an early pre-moult (D0) was characterized by the increased concentration of ecdysteroid haemolymph. The half-moon phase I (½ I) was a middle pre-moult stage or mid pre-moult (D1-2), where ecdysteroid concentration reached a maximum. The ¾ I moon phase was the stage where there was a drastic reduction in the concentration of haemolymph ecdysteroid and it was also the final stage of moulting preparation called late pre-moult (D3-4) which triggered the ecdysis (E), a discharge of the old exoskeleton cuticles to be replaced by new cuticles. A decline of haemolymph ecdysteroid concentration occurred in moon phase ¾ I, when the crabs appeared to be entering the ecdysis stage.

Moulting (ecdysis phase) of the crabs did not coincide with the high concentration of ecdysteroid; rather, it occurred after the ecdysteroid concentration had dropped dramatically from the maximum concentration. Therefore, moulting can be predicted to occur in moon phase ¾ I. This is consonant with the number of moulting crabs in soft shell crab ponds, which has been reported as reaching the highest level in moon phase ¾ I (before the occurrence of the highest tide), with a rise in the number of moulting crabs a few days before the full moon [3,10].

Haemolymph ecdysteroid concentration was low during the new moon phase. This might be due to the inter-moult phase of mud crabs (*S. olivacea*) at this time. During the new moon phase, the highest and lowest spring tides occurred, leading to the exchange of large volumes of water to provide better water quality. This environmental phenomenon appeared to trigger the crabs to eat more actively, probably to accumulate enough energy in preparation for the moulting process. The inter-moulting stage is a development stage in which the crabs accumulate a certain amount of energy to grow and prepare themselves for the next moulting [8,11]. High protein content and high ash content have been found in crabs harvested during the dark phase of the moon [12]. Furthermore, the majority (70-80%) of mud crabs (*S. serrata*) caught during the new moon have a full fleshy body [13].

The relationship between the concentration of haemolymph ecdysteroid, phases of the moon and the possible moulting stages of mud crabs is illustrated graphically in Figure 2, linking the rise and fall of haemolymph ecdysteroid concentration with moon phase over the first (waxing) half of the lunar cycle, from new to full moon. This diagram indicates that the decrease in ecdysteroid concentration lead to moulting towards the end of this half-cycle, between ¾ I and the full moon.

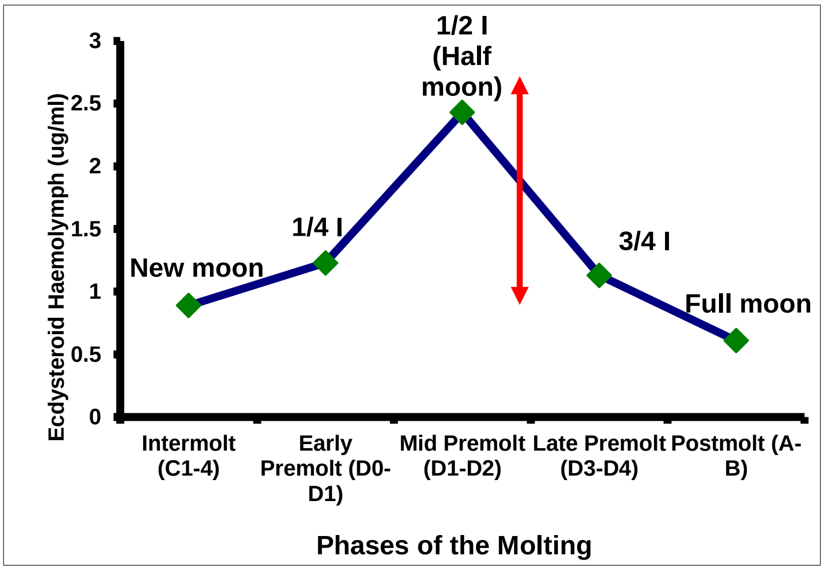

Figure 2**.** Dynamics of mud crab (*S. olivacea*) haemolymph ecdysteroid concentration during the waxing phases of the moon cycle

The ecdysteroid concentration was low during the full moon phase and new moon, at which time the crabs were in post-moult stage. During this stage the exoskeleton or new cuticles harden through cuticle mineralization [14,15]. Cuticle mineralization processes are strongly associated with external conditions especially the calcium content of water. Therefore, this phase requires a good water quality condition. In the post-moult stage, the crabs absorb a large volume of water to facilitate the expansion of the new exoskeleton. As a result, the flesh of post-moult crabs has a high water content. These two moulting stages (expansion and hardening) require appropriate internal and external conditions for the crab to achieve optimal growth and perfect the hardening of the cuticles. Thus, it is widely accepted that availability of food and good environmental quality are crucial factors in the growth of crabs [16]. A similar phenomenon associated with the lunar cycle has been reported in the green crab (*Carcinus maenas*) in the Atlantic Ocean and the Baltic Sea, where moulting occurs more frequently during spring tides than during neap tides; however, in this species, the moulting peak occurs after the new moon or full moon [17,18].

4. Conclusion

The haemolymph ecdysteroid concentration of mud crabs (*S. olivacea*) in our study exhibited dynamics based on phases of the moon, similar to that observed in in the wild, albeit without access to certain possible cues such as moonlight intensity and neap-spring tides. The highest ecdysteroid concentrations were found during the half-moon phases I and II (½ I and ½ II), when the gravitational force of the moon was at its weakest; conversely, the lowest ecdysteroid concentrations were found in the new and full moon phases, when the gravitational force of the moon is at its highest point. The pattern of haemolymph ecdysteroid concentration of during one cycle of the moon appeared to be related to the stages of moulting with inter-moulting stage during new moon phase, early pre-moulting in moon phase ¼ I, mid pre-moulting in half-moon phase I (½ I), final pre-moulting followed by ecdysis in moon phase ¾ I, post-moulting during the full moon, and inter-moulting in the dark moon phase. Further studies are necessary to confirm these results and gain a deeper understanding of the mechanisms influencing and triggering mud crab ecdysis.

References

[1] Millamena O M and Quinitio E T 1999 *Reproductive performance of pond-sourced Scylla serrata fed various broodstock diets* (Mud crab Aquaculture and Biology) Eds C P Keenan and A Blackshaw (Australia: Proceedings of an International Scientific Forum Held in Darwin) **78** 114-118
[2] Quinitio E T and Lewin M M N 2009 *Soft-Shell Mud Crab Farming* (Philippines: SEAFDEC Aquaculture Department Tigbauan, Hoilo)
[3] Fujaya and Alam N 2012 *Effects of water quality, moon cycle, tide on moulting and soft crab aquaculture in commercial ponds* (Paper was presented in the Annual Scientific Meeting of Oceanography Alumni of Indonesia) (http://repository.unhas.ac.id/handle/123456789/2269)
[4] Zimecki M 2006 The lunar cycle: effect on human and animal behavior and physiology *Postepy. Hig. Med. Dosw.* **60** 1-7
[5] Fujaya Y, Aslamyah S, Mallombasang L F and Alam N 2012 Cultivation and Business of Soft Crab (Surabaya : Brillian Internasional) 114 p
[6] Fujaya Y and Trijuno DD 2007 Haemolymph ecdysteroid profile of mud crab during moult and reproductive cycles *Jurnal Torani* **17** 415-421
[7] Skinner D M 1985 *The Biology of Crustacea - Integument, Pigments, and Hormonal Processes* (Moulting and Regeneration Vol 9) ed D E Bliss (New York: Academic Press, Inc)
[8] Okumura T, Kamba M, Sonobe H and Aida K 2003 In vitro secretion of ecdysteroid by Y-organ during moult cycle and evidence for secretion of 3- dehydroecdysone in the giant freshwater prawn, Macrobrachium rosenbergii Crustacea:Decapoda: Caridea) *Invert. Reprod. Dev.* **44** 1–8
[9] Zimecki M 2006 The lunar cycle: effect on human and animal behavior and physiology *Postepy. Hig. Med. Dosw.* **60** 1-7
[10] Fujaya Y, Trijuno D D, Haryati, Hasnidar and Rusdi M 2013 The use of mulberry (*Morus alba*) extract in the mass production of blue swimming crab (*Portunus pelagicus* L.) larvae to overcome mortality due to moulting syndrome *J. Aquat. Sci. Tech.* **1** 1-7
[11] Drach P 1939 Mue et cycle d'intermue chez les crustacés décapodes *Ann. Inst. Océanogr. Monaco* **19** 103–391
[12] Metusalach 2007 Effect of moon phase and body size on rendement, protein content, water and ash content, swimming crab (*Portunus* spp.) *Torani* **17** 223–239
[13] Nirmale V H, Gangan S S, Yadav B M, Durgale P and Shinde K M 2010 Traditional Knowledge on Mud Crab; Ethnoecology of *Scylla serrata* in Ratnagiri coast, Maharashtra *Indian J. Tradit. Know.* **11** 317–322
[14] Loseke L 2003 *All about moulting* (http://www.crabstreetjournal.com/ articles)
[15] Dillaman R, Hequembourg S, Gay M., 2005. Early pattern of calcification in the dorsal carapace of the blue crab, *Callinectes sapidus J. Morph.* **263** 356–374
[16] Passano, L. M. 1960. *Moulting and its control* (The Physiology of Crustacea) ed T H Waterman (New York: Academic Press) **1** 473-536
[17] Naylor E 2001 Marine Animal Behavior in Relation to Lunar Phase *Marine Earth, Moon and Planets* **85-86** 291-302
[18] Wenner L and DeLancey L 2004 *Blue Crabs State of South Carolina's Coastal Resources* (North Carolina: The South Carolina Department of Natural Resources)