# Y ORGAN CELLS ACTIVITY BASED ON THE COCENTRATION OF ECDYSTEROID FROM HAEMOLYMPH OF MANGROVE CRAB (*Scylla olivacea* Herbs, 1979)

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## ABSTRACT

*Y organ or ventral gland is an endocrine gland that synthesizes the crab molting hormone (Moulting Stimulating Hormone) or ecdysteroid. Y organ activity produced molting hormone that was associated with molting stages. Molting in the crab consists of four stages, namely intermolting, premolting, ecdysis and postmolting stages. The concentration of circulating ecdysteroid in the haemolymph usually low during intermolting, increased significantly during premolting and decreases dramatically before ecdysis. This study aims to determine the Y organ cell activity based on the concentration of ecdysteroid. Parameters measured were ecdysteroid concentration and histology of Y organ during the five phases of the moon: the dark moon phase, new crescent, the first half, the first convex and a full moon. Analysis of ecdysteroid samples performed using Ultra-Fast Liquid Chromatography (UFLC). The results showed that the pattern of ecdysteroid concentration indicated a low ecdysteroid concentration in the dark phase of the moon, began to rise in the new crescent moon, reaching the peak in the first half, decreased dramatically in the first phases of the moon convex and lower back at the full moon phase. The pattern of ecdysteroid concentration by a reference to the determination of mangrove crab molting stages. Y organs activity synthesize highest ecdysteroid in the first half of the moon phase coincides with the phase premolting middle (Mid premolt), otherwise low ecdysteroid synthesis activity in the dark phase of the moon coincides with intermolting stage and phase of the full moon coincides with postmolting stage.*

**Keywords:** Mangrove Crab, Ecdysteroid, Haemolymph, Molting, Y organ

## INTRODUCTION

Molting in crabs is influenced by internal and external factors (Lockwood, 1967). Internal factors include the ecdysteroid concentration and size of the body that need more space. While external factors are environmental conditions such as temperature and salinity (Brown and Bert, 1993; Chen et al, 1995; Olvera and Peterson, 2004; Samuel and Soundarapandian, 2010), the concentration of oxygen (Gaude and Anderson, 2011; Oesterling, 2012b) , the duration of the light / photoperiod (Aiken et al., 1983), nutrients (Aslamyah and Fujaya, 2010), stressors (Azis, 2008); seasons and cycles of the moon (Quinitio and Lwin, 2009; Fujaya and Nature 2012, Nirmale et al. 2012, Oesterling 2012). External factors will affect the physiological functions of the body through the nervous system and hormonal coordination. The interaction between both internal and external factors will affect the brain and stimulates the organs of Y to produce the molting hormone (Lockwood, 1967: Welsh, 1961).



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Y organ is the source of ecdysteroid molting hormones. This hormone is secreted in the form of ecdyson. In the haemolymph, this hormone is converted into the active hormone, 20-OH-ecdyson by the enzyme 20-hydroxylase, which is contained in the epidermis of organs and other body tissues. Y organ in the crab form a compact mass located on the thorax. Y organ works under the coordination of Molt Inhibiting Hormone (MIH) is generated by the sinus gland that lies in the eye stalk. MIH inhibit the secretion of ecdysteroid by Y organs (Huberman, 2000). Otherwise, an increase in the level or in the haemolymph ecdysteroid concentration will lead to negative feedback that inhibits the release of MIH from the sinus gland. According to Drach (1939), molting cycle in crustaceans including crabs consists of four stages: stage intermolting, premolting, ecdysis and postmolting. Intermolting stage is the longest stage of the molting cycle, which occurs muscle regeneration and accumulation of energy reserves such as glycogen and lipid. The next stage is the preparation premolting to perform ecdysis. At this stage also witnessed the growth of somatic muscles, reabsorption old exoskeleton (cuticle), and the formation of the new exoskeleton (Skinner, 1985). Ecdysis is eksoskleton release through rapid absorption of water from the environment leading to rupture (Skinner, 1985: Chung et al, 1999). While postmolting is water absorption stage to facilitate further expansion of exoskeletons and new exoskeletons hardening process through the mineralized cuticle (Loseke, 2003; Dillamen et al, 2005). Exoskeletons newly formed larger and still pale and soft.

In haemolymph ecdysteroid concentration varies during the molting cycle. In general, low ecdysteroid during intermolting and postmolting. Next on stage premolting, concentration increased and reached a peak shortly before molting (ecdysis) on various species of crustaceans (Soumoff and Skinner, 1983; Skinner, 1985; Chang and O'Connor, 1988; Okumura et al, 2003; Chung, 2010). Along with the increase in ecdysteroid concentration was on stage premolting, Y organ histology showed that secretion is also increased in stages premolting (Gabe, 1956; Uatsumoto, 1962). Y organ inactive and stop producing ecdysteroid the crab *Carcinus maenas* intermolting stage (Nakatsuji and Sonobe, 2004). To reveal changes in mangrove crab Y organ cells based on the concentration of ecdysteroid molting stages and then needs further information. This study aims to determine the Y organ cell activity of mangrove crab (*S. olivecea*) based on the ecdysteroid concentration, month and phases of the molting cycle.

## MATERIAL AND METHODS

Research conducted at the Center for Research and Development of Brackish Water Aquaculture, Faculty of Marine Science and Fisheries, Hasanuddin University (UNHAS) which is located in the village of Bojo, District Mallusetasi, Barru, Province of South Sulawesi.

### Animal Test

Mangrove crab (*Scylla olivacea* Herbst, 1979), about 140 samples width 720-800 mm and weighing 90-100 g, were on intermolting phase. The crabs were put into each box which one crab every subsequent box and then the crabs were reared in ponds. During maintenance, crabs were fed with trash fish with 10% of body weight, given once a day and that on the afternoon before the evening.

### Collection and Extraction of Haemolyph

Collection of haemolymph performed five times during the study: 1) phases of the moon death / dark months, 2) the new moon phase ((New Moon), 3) half moon phase I (first quarter), 4) the convex moon phase I, 5) phase bright moon / full moon (full moon). The number of crabs samples were 3 samples each phase of the moon. Crab haemolymph taken from the base of the



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fifth leg using a 1-mL syringe with a hypodermic needle sized 27. 1 ml haemolymph stored in eppendoft and mixed with anticoagulant at a ratio of 1: 1 [Fujaya & Trijuno, 2007), then the samples stored in the refrigerator (freezer) with -200ºC, and then the sample is ready to be extracted.

**The content of Ecdysteroid titer measurements**

Ecdysteroid content measurement procedure were: 1) residue has dried samples were dissolved with methanol extraction hasl pro UFLC, then put in the autosampler vial UFLC, 2) Samples were analyzed by Ultra Fast Liquid Chromatography (UFLC) Shimadzu LC-20 AD. Ecdysteroid quantification using a standard series of 20-hydroxyecdyson (Sigma). Extraction and measurement hemolimph ecdysteroid content performed in the Laboratory of the Faculty of Pharmacy, Hasanuddin University.

**Collection of Y organ**

Y organ of mangrove crab, a compact mass, totaling pair located on the chest and under the eye stalk or organ X (Figure 1.)

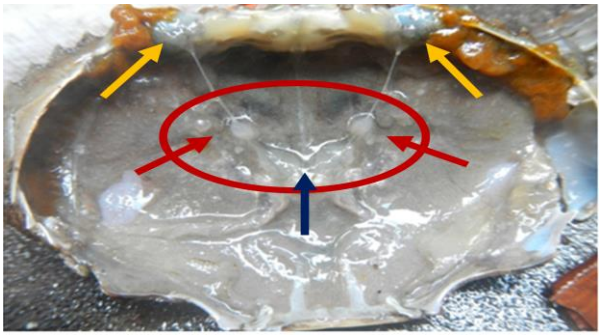

Figure 1. Location of organ Y on mangrove crab (*S. olivacea*), a pair of Y organs (red arrow), a pair of organs X (yellow arrows), hepatopancreas (blue arrows)

Histological observation of Y organs performed in five phases of the moon. Y organ crabs taken by separating the ventral and dorsal carapace. Furthermore, with some caution on the part of the chest cavity organs removed to lift the Organ Y. Te organ then cleaned with normal saline (0.9% NaCl), and stored in microtipe devitson preserved in solution. Y organ samples obtained were processed by routine histological techniques. Samples were cut with a thickness of 4 microns and then stained with hematoxylin eosin staining (HE). Subsequently the samples were observed by using a microscope (Olympus, Japan).

**RESULT AND DISCUSSION**

The pattern of concentration and amount of ecdysteroid molting crab conjunction with moon phases (Figure 2).

Concentration and amount of ecdysteroid molting crabs lower in the dark phase of the moon, began to rise in the new crescent moon phase and reached a maximum concentration in the first half of the moon phases, ecdysteroid concentration decreased sharply in phase I and low convex month on the full moon phase. Ecdysteroid molting occurs when the concentration decreases dramatically triggering ecdysis.



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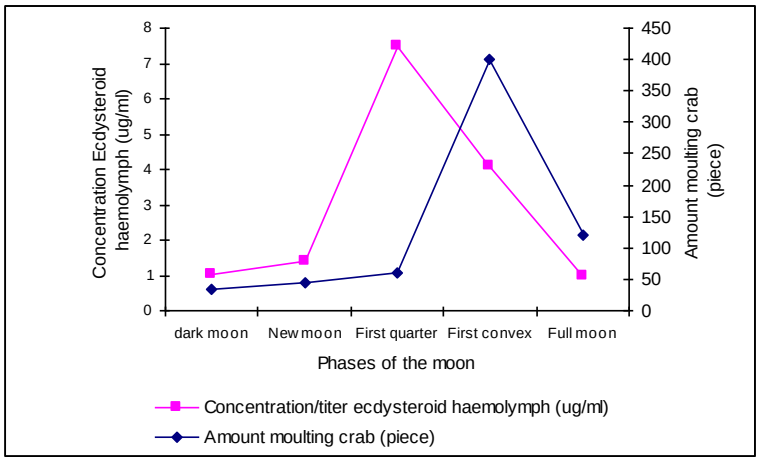


Figure 2. The pattern of concentration and amount of ecdysteroid molting crab, mangrove crab relation with phases of the moon.

Molting in the crab does not coincide with the high concentration of ecdysteroid but occurs when the ecdysteroid concentration decreased dramatically, after reaching a maximum concentration. Therefore the peak of molting occurs on the convex moon phase I. These results are consistent with that proposed by Fujaya & Nature (2012), that the number of crabs are molting in the highest soft shell crab farms in the convex moon phase I. The same is stated by Oesterling (2012), the number of molting crabs has increased a few days before the full moon.

The concentration of circulating ecdysteroid haemolymph typically low during intermolt (stage C1-4), increased during the early premolt (stage D0-1), peaking at mid premolt stage (D1-2), then the concentration decreased sharply at the end of the

premolt stage (late premolt , D3-4), before ecdysis (stage E) and postmolt (stage AB) (Okumura et al., 2003; Chung, 2010). Based on the pattern of ecdysteroid concentration of mangrove crabs and phases of the moon in relation to the molting stage, it is suspected that the dark phase of the moon is intermolting stage, the new crescent moon is an early stage of preparation of molting that early premolt (D0) is characterized by the increase in ecdysteroid concentration. Phase I is the half-moon central or mid premolt stage premolt (D1-2), which reached a maximum concentration of ecdysteroid. Phase I is a convex month stage where there is a drastic decrease of ecdysteroid concentration and is the final stage of preparation of molting is late premolt (D3-4) that trigger ecdysis (E) a discharge of the old cuticle is replaced by a new cuticle. The decline of ecdysteroid concentration occurs in the convex moon phase I, at the same time an increasing number of molting crabs.

Y organ was a glandular organ that synthesize ecdysteroid on the crab. According to Babu (1988), the structure of the Y organs such as the epidermal glands, consisting of a collection of cells that form lobul-lobul. Between lobul with other lobul separated by connective tissue and blood vessels and capillaries. Each lobul covered by loose connective tissue, sometimes hemotosit cells found in the blood vessels and between lobul.



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Histologically, Y organ mangrove crab consists of a collection of cells that form lobul-lobul interconnected and separated by thin connective tissue. In the thin connective tissue found blood vessels and capillaries. Each lobul consists of 10-20 cells, one cell contains a nucleus. Nucleus located acentrik containing 1-2 nucleoli and chromatin contained in the peripheral area. Description organ histology Y mangrove crab is identical to several other types of crustaceans such as Carcinus sp. (Gabe, 1956), Portunus sanguinolentus (Babu, 1988), Varuna litterata (Madhyastha and Rangneker, 1972)

Y organ in the cytology changed every stage of molting. In intermolting stage (Stage C) gland cells appear smaller, homogeneous cytoplasm, blood vessels and capillaries did not appear. The space between lobul-lobul seen clearly. Intermolting stage is the stage where the production is very low ecdysteroid hormones that shrink the gland cells, blood vessels and capillaries as well as hormone transport organs is reduced.

While on stage postmolting (Phase A and B) Y organ, a decline in the volume of the cytoplasm and the emergence of inter-lobular spaces are large. Blood vessels and capillaries are very prominent on the stage premolting disappeared during this stage. Postmolting stage is the stage where the production ekdisteoid cytoplasmic volume decreased so reduced. This phenomenon correlates to the blood vessels and capillaries are narrowed due to hormonal transport is reduced very sharply. According to Babu et. al (1988), P. sanguinolentus crab postmoulting stage characterized by degeneration and a decrease in the volume of the cell cytoplasm. Next comes back interlobular space, blood vessels in organs is reduced due to the transport of hormones to target tissues is very little. Entering premolting early stages (stage D0) many changes. Y organ cytoplasmic volume started to increase and reached a maximum at the middle premolt stage (D1-2), size can be up to two times greater than in the intermolt stage (stage C) and at the stage of final premolt (D3-4). The increase in the volume of cytoplasm in stage premolting also be found in crayfish, Orconectes Limosa (Durand, 1960); Hemigrapus (Matsumoto, 1962); shrimp, Palaemon paucidens, Pandalus kessleri and Procambarus clarkii (Aoto et al., 1974); Portunus sanguinolentus (Babu, 1988). Cytoplasmic volume increased in the early premolt stage and middle premolt Y associated with organ activity in ecdysteroid biosynthesis. According Imayavaramban et al (2007), cholesterol is a major precursor molecule for ecdysteroid biosynthesis in organ Y (steroidogenic cells), cholesterol observed at all stages of molting, but high cholesterol content in the organs of Y at the stage of early premolt (D0), middle premolt (D1 -2) and began to decline in late premolt stage (D3-4). Ecdysteroid biosynthesis is increased in the cytoplasm of glandular cells causes size-lobul lobol enlarged so that the distance between lobul be meeting and huddled together. Dilate blood vessels and capillaries and appears to spread from the interlobular space to the edge of the circle Y. The occurrence of organ enlargement of blood vessels and capillaries is a sign of hormone transport to target tissues is very active on premolting phase. At this stage it was also found that the type of cells containing basophilic cytoplasmic granules are difficult to look at other stages. The characteristics of the Y organ mangrove crab based on the stage of molting can be seen in Table 1.

According Madhyastha and Rangneker (1972), there are two types of cells in organ lobular Y crabs (*Varuna litterata*) which is a type of small epithelial cells was the most numerous and the type of cell contains basophilic cytoplasmic granules but rarely found. Simione and Hoffman (1975) also expressed the same opinion regarding the existence of two types of cells. Types of cells containing basophilic granules were also found in the crab *P. sanguinolentus* (Babu et al., 1988) but not found in the crab *Orconectes Limosa* (Durand, 1960).



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**Table 1. Characteristics of the Y organ cell growth mangrove crab based on the stages of the molting**

| Phases of the moon and Molting Stage | Characteristics |
|---|---|
| Dark phase of the moon<br><br>(Intermolting) | Gland cells, especially the cell cytoplasm decreased in size size is almost homogeneous (red arrows). Cytoplasmic volume decreases due to decreased cell activity. Because the cells shrink so-lobul lobul size also decreases so that the distance between lobul-lobul be tenuous or enlarged (black arrow). Blood vessels and capillaries are not clearly visible but the space between lobul-lobul enlarged so clearly visible (Figure 3a) |
| New crescent phase<br>(Early Premolting) | Gland cells in some lobul began to swell. Cell activity began to increase so that the volume of the cytoplasm begins to increase (red arrows). Distance between lobul-lobul begins to closed each other's Blood vessels and capillaries begin to be seen clearly (Figure 3b) |
| Half-moon phase I<br><br>(Premolting middle / Mid premolt) | Enlarged gland cells, cytoplasmic volume enlarged approximately 2-3 times larger than the cytoplasm intermolting and post molting phase (red arrows). Due to the increased cell activity in the cells lobul-lobul enlarged and the distance between lobul docked (black arrows). Blood vessels and capillaries clearly visible (white arrows). Is cytoplasmic basophilic cell type. (Figure 3c) |
| Convex moon phase I<br><br>(Postmolting) | Gland cells begin to shrink drastically reduced the volume of the cytoplasm (red arrows). Because the activity of cells begins to decrease the size of the cells in lobul-lobul shrink so the distance between lobul-lobul starting dissappear (black arrows). Blood vessels and capillaries begin to shrink (Figure 3d) |



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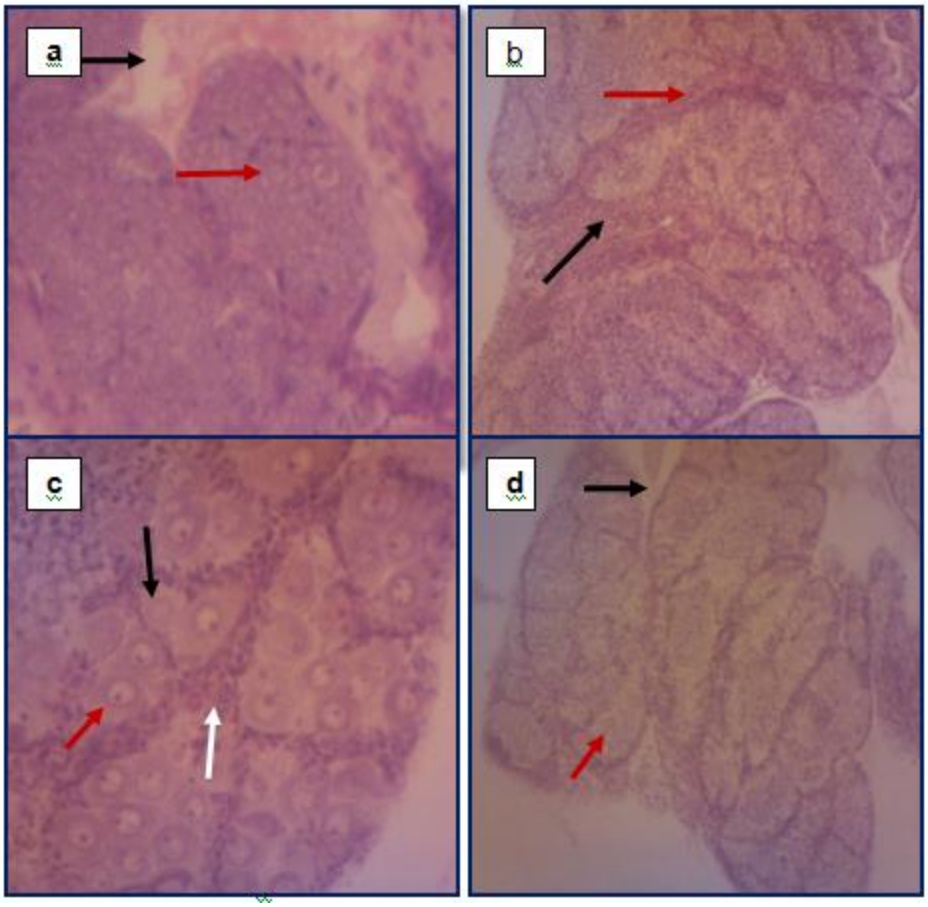


Figure 3. Y organ cell activity of mangrove crab based on the stages of molting. Intermolting stage (a), premolting initial stage (b), premolting middle (c) and phase postmolting (d).

## CONCLUSION

Concentration and amount of ecdysteroid molting crabs lower in the dark phase of the moon, began to rise in the new moon phase and reached a maximum concentration in the first half of the moon phases, ecdysteroid concentration decreased sharply in phase I and low convex month on the full moon phase. Ecdysteroid molting occurs when the concentration decreases dramatically triggering ecdysis.

Y organ cell activity showed differences by molting stages. The highest activity occurs at premolting stage by characterized increased cytoplasmic volume, size-lobul lobul enlarged so that the distance between lobul be meeting and huddled together, dilate blood vessels and capillaries is a sign of hormone transport to target tissues is very active on premolting phase. it was related to the activity of the Y organ ecdysteroid biosynthesis. Postmolting stage is the stage where the production ekdisteoid cytoplasmic volume decreased so reduced. This phenomenon correlates to the blood vessels and capillaries which decreases due to reduced hormone transport is very sharp. The same thing on stage intermolting gland cells appear smaller, homogeneous cytoplasm, blood vessels and capillaries did not appear, the space between lobul-lobul seen



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clearly. Intermolting stage is the stage where the production is very low ecdysteroid hormones that shrink the gland cells, blood vessels and capillaries as hormone transport organs also experienced a reduction

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