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Reproduction of females mud crab (*Scylla serrata*) with thyroxine hormone supplementation in traditional ponds from north borneo Indonesia

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Abstract

The purpose of this experiment is to provide information that effects thyroxine hormone development ovary of female mud crab in traditional ponds. A study was conducted in traditional ponds in small islands like Sadau, Tibi, Tias, Mangkudulis and Tarakan islands on the north Kalimantan to evaluate the viability of ecology and biology drive in mature for reproduction females of the mud crab (*Scylla serrata*). The hormone thyroxine supplementation started of mud crabs with vitellogenic stage 2 (maturing). This experiment consists of 3 triplicate treatments and all treatment of supplementation hormone thyroxine are doses; 0ug/BW (control); 0.1ug/BW and 0.2ug/BW. Each treatment was repeated six (6) times. The population structure and maturity stages females of the mud crab (*Scylla serrata*) from traditional ponds are among crustaceans of commercial value. In north Kalimantan, the maturity stages females of the mud crab crabs are landed by artisanal fishermen. Rising population in the coastal areas and high demand for the crabs has led to increased pressure on the crabs. The result of the research is the accumulation of mud crab female mature as 237 with various size; Weight and different levels of gonad maturity. Based on the weight of mature crabs gonad crab caught in ponds can be grouped up; Small size (180-300 g) of about 15% and medium size (301-400 g) of about 62% and large size (401-550g) of about 23%. The stage maturity in traditional ponds of female mud crab have as 45.37% still in the mature ovary stage II and it has as 58.27% in the mature ovary stage III. The supplementation low dose 0.2 µg/BW needed 12 days to mature ovary from vitellogenic stage 2 to vitellogenic stage 3 than the control needed 23 days, while the supplementation dose 0.1 µg/BW needed 13 days. The supplementation thyroxine hormone dose 0.1 µg/BW and 0.2 µg/BW faster to maturity ovary from vitellogenic stage 3 to berried than control. Based on the analysis of variance of the supplementation thyroxine hormone in the female *S. Serrata* indicates that there is a real effect on the acceleration of mature ovarian. The treatments supplementation thyroxine hormone low dose resulted from a significantly ($P<0.05$) time maturity ovarium than control.

Keywords: Maturity stages; mud crab; traditional ponds

Introduction

The mud crab (*Scylla serrata*) is one of the commercially important crabs in Indonesia for local consumption or for the culture which is caught from the mangrove. The mud crab is one of the most valuable crustacean species caught in the traditional pond. The traditional pond has large 5-7 ha area and still have mangrove round pond. The mangrove is the place spawning ground, feeding ground, nursery ground, and place protection. Mud Crab (*Scylla serrata*) is a typical species in the mangrove.

Mature female mud crabs always migrate during spawning and spawning process in nature is dependent on the season (Cynthia *et al.*, 2012). The production and availability of crab larvae is the main problem in the culture of mud crabs. To solve the problem, there is a need to develop a technique to propagate the crab by improving the reproduction of the crab and the survival of the larvae.

The reproduction of female mud crabs is controlled by a variety of hormonal and neuronal factors. These hormones reproduction include the thyroxine hormone such as the gonad stimulating hormone, and the vitellogenin inhibiting hormone which have an agonist-antagonist effect, respectively, on vitellogenesis. The thyroxine hormone in the female mud crab circulation can be transferred to the oocyte, the egg cell, and then into the ovaries (yolksac) before ovulation (Iromo *et al.*, 2014) [5].

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The all of mud crab used in this study are from traditional ponds and yet information about developed stage ovary of female mud crab. The traditional ponds were large than the difficult to control of water quality and environment. In the present study, we investigated effect of thyroxine hormone to development ovary female mud crab in traditional ponds.

2. Materials and Methods

2.1. Animal

The brood stock mud crab (*Scylla serrata*) of various maturation stages were obtained from traditional ponds and mangroves in Tarakan Island, North Borneo, Indonesia. The identification of *Scylla serrata* was conducted according to the description of Kennan *et al.*, (1998). The weights of female mud crabs used ranged from 310 to 400g with the maturity of ovari stage 2.

2.2. Experimental Design

The hormone thyroxine of experiment derived from levothyroxine sodium tablets/Thyrax. Each it contained 100µg of thyroxine. The hormone thyroxine supplementation started of mud crabs with vitellogenic stage 2 (maturing). This experiment consists of 3 triplicate treatments and all treatment of supplementation hormone thyroxine are doses; 0ug/BW(control); 0.1ug/BW and 0.2ug/BW. Each treatment was repeated six (6) times. The supplementation hormone thyroxine with injection is done once the appropriate dose and

carried between the legs of the road and swimming legs used spuit 1.0 ml.

Parameters observed in this study include:

1. Time of ovarian development began from vitellogenic stage 2: Observation of the ovarians maturation of female mud crab was noted after every four days according to John and Sivadas (1978).
2. Time of incubation: Time was the incubation time (days) required for embryo, calculated up to the female when spawning release larvae.
3. Survivalrate of female: Survival rate of female was calculated based on the formula $S=Nt / Nox100\%$ reported by (Effendie, 1997) [2].

2.3. Statistical analysis

Parameters like time of ovarian development, incubation, survival rate of female, were analyzed by the analysis of variance.

3. Results

Females mud crabs (*Scylla serrata*) in this experiment were collected from mangrove on the traditional ponds. Females *Scylla serrata* have been selected and have the maturity level for research. Females mud crabs came from 3 Island locations including are Benion, Tanjung Nyambing and Tibi. Location of the 3 islands located around Tarakan island (Figure 1).

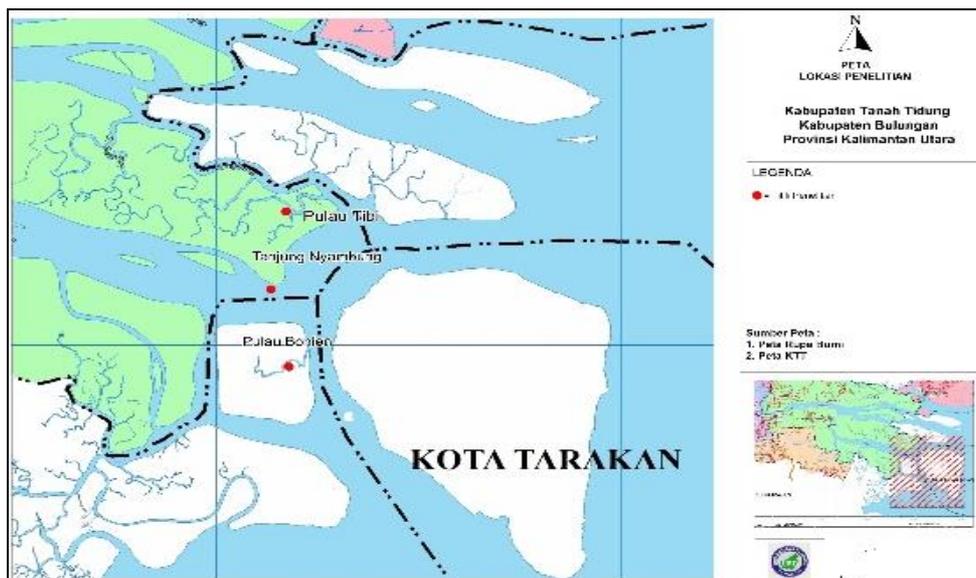


Fig 1: Location tradisional ponds of female mud crab

The results of the three research sites shown that female mud crabs can live and growth in traditional ponds. This mean that the female mud crabs can be tolerance of environmental conditions in the three island areas, or it can be said that

environmental conditions are still within the range of mud crabs. During the research conducted the capture of female mud crabs in traditional ponds (Table 1).

Table 1: Data (mean ± SD) female mud crabs from tradisional ponds

Location	Female mud crab (species)	Weight (g)	Carapace Length (cm)	Stage II (%)	Stage III (%)
A	20	320.7±123.83 ^a	12.1±24.00 ^a	45.5	54.5
B	20	347.2±131.27 ^b	12.9±36.83 ^a	37.4	62.6
C	20	344.1±206.00 ^b	12.4±13.27 ^a	52.3	57.7

Location: A: Benion island; B: Tanjung Nyambing island; C: Tibi island

The mature ovary of female mud crab had various stage and had both a carapace length of 12 cm. It mean that the female mud crab can growth ovaries in the tradisional pond. This is

presumably because the female mud crab can adaption to the environment in tradisional pond and it can develop eggs into embryos and hatch inside there.

3.1. Maturity stages

The maturation stage of the ovary can be grossly observed as it undergoes a series of changes in colour and increases in size during development. The maturity stages of the female crabs examined from three location. The location showed more mature or resting females (Stage II) than the later stages. The percentage of maturing female crabs every location that, A location there are previtellogenic ovary stage II was a 45.5% and stage III was a 54.5 %, B location there stage II are previtellogenic ovary was a 37.4% and stage III there are previtellogenic ovary was a 62.65 % and location C there stage II was a 52.3% and stage III was a 57.7 %.

The developing of previtellogenic ovary (stage II) form as two lines on the upper portion of digestive gland and was easily differentiated from the gland. In the stage II started to form primary oocytes including the presence of vacuolated globules in the cytoplasm and follicle cells on the periphery of

the cytoplasm. The early developing or primary vitellogenesis stage is the initiation of vitellogenesis. The ovary changes to yellow coloration. In the mature or tertiary vitellogenesis stage IV), the ovary enlarged to the maximum and eventually covered the hepatopancreas and the majority of the cardiac stomach.

The results (Table 2) shown ovarium maturation of brood stock *Scylla serrata*, starts from vitellogenec stage 2 to berried. In the treatment supplementation hormone thyroxine dose 0.2 µg/BW was the faster maturity than other. The suplemenation low dose 0.2 µg/BW needed 12 days to mature ovary from vitellogenic stage 2 to vitelogenic stage 3 than the control needed 23 days, while the supplementation dose 0.1 µg/BW needed 13 days. The supplementation thyroxine hormone dose 0.1 µg/BW and 0.2 µg/BW faster to maturity ovary from vitellogenic stage 3 to berried than control.

Table 2: Data (mean ± SD) the ovarium mature of female mud crab

Treatment	Mature of ovarium (%)	Stage II-III (days)	Stage III-Berried (days)	SR (%)
A	55.6 (n=9)	23±13.83 ^a	45±23.00 ^a	66.6 ^a
B	77.8 (n=9)	13±22.00 ^{bc}	30±13.27 ^{bc}	90 ^b
C	77.8 (n=9)	12±14.00 ^c	26±42.31 ^c	90 ^b

Means in the same column with the same superscripts under source of treatments and control are significantly different (P<0.05). Treatment doses; A= 0 µg/BM; B= 0,1 µg/BM; C= 0,2 µg/BM.

Based on the analysis of variance of the supplementation thyroxine hormone in the female *S. Serrata* indicates that there is a real effect in the acceleration of mature ovarian. The treatments supplementation thyroxine hormone low dose resulted a significantly (P<0.05) time maturity ovarium than control. This means that the dose of the supplementation thyroxine hormone can be affect acceleration mature ovarium in the process vitelogenesis.

3.2. Aquatic Parameters

The parameter of water quality measurements during the study are presented in Table 3.

Table 3: Results of measurement of water quality parameters.

Parameters	Rate
Temperature °C	23°C – 26°C
pH	6 – 7
DO	5 – 6,51
Amoniac	0,02 – 1,5
Salinity	20 ppt – 22 ppt

The results of the measurement of water quality in traditional ponds on Tarakan Island show values that are mostly in accordance of survival life and culture of mud crabs. The range of values for incoming traditional pond water quality parameters recorded during the culture experiment (Table 3).

4. Discussion

The traditional ponds have extensive area is very difficult to controlling water quality. However, if condition of sandy clay land and many natural feeds cause be mud crabs can adapt and develop eggs in ponds (Iromo, *et al.* 2010) [4]. The mud crab likes the sandy clay land for grow and if the environmental are not good it's will hide its body into the mud.

According to John and Sivadas (1978), four levels of gonad maturaty were yet mature, premature, mature and salin. Ovarian maturation process (vitelogenesis) on mud crab is the

process absorption vitelogenin by ovary which came from the hepatopancreas. This process is known as vitelogenesis, while the next is the final maturation process in which there is movement to the edge of the egg nucleus, or germinal vesicle break down (GVBD) and ovulation is marked by rupture of the follicle and release of the egg layer into the cavity of the ovaries (Yaron and Sivan, 2006) [9].

Reproduction of male crustaceans is controlled by the endocrine system. The cellular events that occur during the development of the ovary called vitellogenesis process. The female reproduction controlled by a variety of hormonal and neuronal factors (Subramoniam, 2000) [8]. Thyroxine is a hormone to stimulate the growth and development of the gonads. The effect of thyroxine hormone can be absorption vitelogenin by ovary to ovarian maturation. The search for the presence of thyroxine in mud crab initially met with some uncertainty. However, using techniques like radioimmunoassay (RIA) and high performance liquid chromatography (HPLC), were discovered in several organs like hymolimp, ovarium, and heapatopancreas of mud crab. (Iromo *et al.* 2014) [5].

The supplementation of thyroxine hormone illustrated the higher metabolic activity at the maturity level of the female (Iromo, *et al.* 2015) [6]. The supplementation of thyroxine hormone can increase the survival of female maturity than control. That it, thyroxine hormone can improve the function of organs so that the organs will show good performance to survive.

Thyroxine hormone supplementation also affects was the concentration of protein in ovarian maturity level. the hepatopancreas is a storage hormone thyroxine. Hemolimp then transferring it to the ovarian hormones and other targets so that the increased concentration of hepatopancreas ovaries during vitellogenesis. The hormones also play a role to increase the absorption of cholesterol ovaries, fospholipid and protein (Iromo, *et al.* 2014) [5]. During the period of gonad development, protein is needed for growth and developments of the ovaries than to meet the needs of the parent sometimes

have to mobilize fat and protein reserves (Altiner, 2006) ^[1]. The results proved that thyroxine hormone supplementation can be increased the concentrations of the hormone in the hemolymph and absorbed by the ovaries to improve ovarian maturation in female of mud crab.

The results of this study indicate that there is similarity between metabolic patterns with ovarian maturity levels. Increased concentrations of thyroxine hormone in hemolymph crabs are thought to increase metabolism in active cells to synthesize proteins. During the development of gonads, proteins are required for growth and development of ovaries. Thyroxine hormone helps the acceleration of crab metabolism to form the protein needed by the female mud crab for the development of gonad. This is in accordance of Clowes *et al.*, (2003) ^[3] results that the increase of protein and metabolic rate showed effect on reproductive performance.

Conclusion

The supplementation of thyroxine hormone with dose 0.2µg/BW was effective to increase ovarian maturation and survival of female mud crab (*Scylla serrata*) in tradisional pond.

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