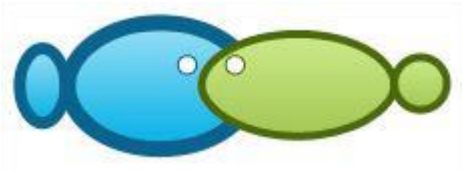

Reproduction of coconut crabs (*Birgus latro*) in Daeo District, Morotai Island, North Maluku, Indonesia

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Abstract. The coconut crab (*Birgus latro*) belongs to a crustacean group that has a very slow growth. This study aimed to reveal the reproduction characteristics of coconut crabs in Daeo, Morotai Island, North Maluku province. The research was conducted from October 2016 to September 2017. Reproduction parameters were observed, including gonad maturity levels (determined by observing gonad developments morphologically), gonadal maturity index, fecundity, egg diameter and size at first maturity. The results showed that the thorax length of females was 28-29.49 mm (stage III), 32.5-33.99 mm (stage IV) at first maturity, while the size of males ranged between 28-29.49 mm. The highest number of mature females (stage III) was found in November, February, May, June, and July. Gonad maturity stage IV in females was found in October, January, and July. The female gonad maturity index ranged from 2.04 to 4.95, while for males it ranged from 0.98 to 1.04. Egg diameter ranged from 0.01 and 0.056 (stage III) and 0.01-0.061 (stage IV). Fecundity ranged from 21463 to 131500 eggs. The first maturity size (Lm) was 48.73 mm for males and 35.33 mm for females.
Key Words: eggs, gonad maturity, size maturity, spawning time.

Introduction. Coconut crabs (*Birgus latro*) are the largest land crustaceans in the world, with a very slow growth rate, needing 3.5 to 5 years to reach gonad maturity and 40 to 60 years to reach their maximum size (Greenaway 2003; Krieger et al 2012; Buden 2012; Drew & Hansson 2014). The growth of coconut crabs is similar to that of other crustaceans, in general. It begins with the process of moulting. All crabs periodically release their exoskeleton, moulting. The moulting process is carried out in a pre-prepared hole that functions as a hiding place from predators (Fletcher et al 1991).

Female crabs with mature gonads will head to the sea to incubate their eggs (Drew et al 2010; Nakasone 2011). During the egg incubation period, the coastal area will be a suitable habitat for females carrying eggs and undergoing vitellogenesis as the seawater has organic and inorganic ions useful for the process (Schiller et al 1991). Egg maturation occurs outside the body and the egg mass attaches to the abdominal part of the female coconut crabs. Environmental conditions, such as drought and contact with freshwater and seawater, are potential threats prior to the release of eggs (Schiller et al 1991; Greenaway 2003). The maturation of the egg requires an average of 4 to 5 weeks before the ovigerous females go to the sea to hatch their eggs and release larvae. After the release of the egg, the coconut crabs migrate back inland in groups. Migration occurs with a periodicity similar to the hatching of eggs, with waves, starting from 4 to 10 days after the release of eggs (Schiller et al 1991).

The reproduction process begins when male coconut crabs transfer sperm packages (spermatophores) to females. The females have no place to accommodate sperm; therefore eggs become fertile soon after copulation occurs. The abdomen at the base of the third periopod opens and fertilization is presumed to occur on the outer surface of the abdomen through which the spermatophore mass passes (Schiller et al

1991). Amesbury (1980) suggested that a sperm mass may be transferred to internal spermatotheca and egg fertilization occurs internally. The female crab will put its eggs in the endopodite and exopodite setae from the third leg pair of pleopods on the left side. The eggs will adhere to give what looks like a string of grapes tightly filling the abdomen. Egg extrusion occurs on the land in cracks or holes near the beach (Sato & Yoseda 2009).

Knowledge of coconut crab reproductive biology is highly necessary in order to comprehend the condition of its stock in nature. Some studies of coconut crab reproduction on Hotama Island have been carried out by Sato et al (2008) on male maturity, sperm count, and size of the relationship of spermatophora to coconut crab; by Sato & Yoseda (2008) on reproductive seasons and the size of maturity of female coconut crabs; by Sato & Yoseda (2009) on the prediction of mating and extrusion time of coconut crab eggs assessed from female pleonal expansion; by Sato et al (2010) on sperm limitation, with possible effects of high selective capture on coconut crab reproduction; by Sato (2011) on variation in sperm stock due to selective capture of coconut crabs; by Sato & Yoseda (2013) on reproductive migration in coconut crabs and by Ohashi et al (2019) on artificial incubation and hatching of embryos of the coconut crab. Several studies on reproduction have also been carried out in Indonesia, including by Refiani & Sulistiono (2009) and Sulistiono et al (2008) in Pasoso Island (Central Sulawesi). Research on the biota in North Maluku has been carried out by Supian et al (2013, 2015) in Uta Island, Widiyanti et al (2015) in Liwo Island, Serosero et al (2016) in Ternate Island and West Halmahera, Serosero et al (2018a) in Daeo, Serosero et al (2018b, 2019) in North Maluku Province, and Abubakar & Ma'sitasari (2019) in West Ternate beach. Research on the reproduction of coconut crabs in North Maluku for an entire year was not carried out, to our knowledge. Therefore, research on coconut crab reproduction is necessary to reveal reproductive information in Daeo, Morotai Island District (North Maluku) and provide information as a basis for coconut crab management, this being the aim of this study.

Material and Method

Sampling location. This research was conducted in a year, starting from October 2016 to September 2017. The research location was in Daeo, South Morotai District of Morotai Island Regency (North Maluku, Indonesia). Observation sites in Daeo area were classified into 3 stations: Station I (Tanjung Tulang), adjacent to residential areas, Station II (Tanjung Soki), a steep area near the beach, and Station III (Mijiu), a shallow and flat area consisting of various vegetation and coconut trees (Figure 1).

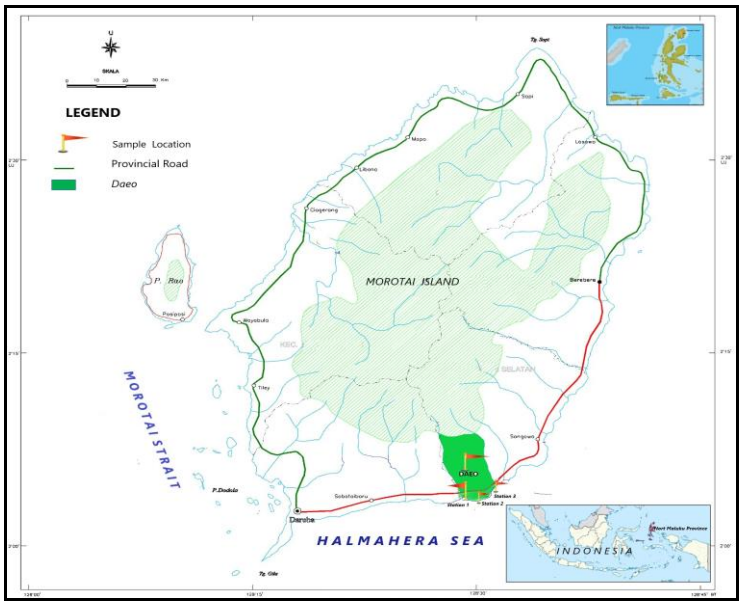

Figure 1. Sampling locations of the coconut crab.

Sample collection. Coconut crabs were obtained through fishing directly, by hand. The bait used was coconut. It was divided into several parts, mashed, and placed in the front of the possible hideout/habitat of the crabs. Arrests were carried out at night. Captured crabs (n=18 individuals per month) were collected, three males and three females (n=6) from each station.

Gonad maturity stage. Coconut crabs were dissected and observed in the laboratory of the Faculty of Fisheries and Marine Sciences, Khairun Ternate University to examine the gonadal development. Determination of male and female gonad maturity stages was carried out by examining the morphological changes of the gonads by referring to Refiani & Sulistiono (2009) (Tables 1 and 2).

Table 1

Morphological characteristics of coconut crab *Birgus latro* male gonads

Maturity condition	*Characteristics*
Immature	A pair of male gonads is found in the abdomen. The gonads are yellowish white, small in size and fill about 5% of the abdominal cavity.
Early mature	Gonad consists of a testicle with ducts of medium size spiral tubes. It is yellowish white and fills about 10% of the abdominal cavity.
Ripe	Gonad consists of a testicle and its ducts of large spiral tubes. It is yellowish white and fills about 30% of the abdominal cavity.
Mature	Gonad consists of a testicle and its ducts of large spiral tubes. It is milky white and fills about 40% of the abdominal cavity.

Table 2

Morphological characteristics of coconut crab *Birgus latro* female gonads

Maturity condition	*Characteristics*
Immature	The surface of the ovary is smooth; the eggs have not formed yet. The ovary has not begun to develop; it is in the form of a pair; its color is light gray. The filling of the ovary in the abdomen is about 25%.
Early mature	The surface of the ovary is soft, egg grains begin to appear, and they are easily crushed if pressed. The size of the ovary increases and expands; the color changes from white to dark gray. The filling of the ovary in the abdomen is about 30%.
Ripe	The surface of the ovary feels rough because the eggs are bigger and more dense; they are not easily crushed if pressed firmly. The ovarian volume is enlarged; it becomes orange. Egg grains are clearly visible, but still coated with oil glands. The filling of the ovary in the abdomen is approximately 60%.
Mature	The surface of the ovary feels rough and dense because the egg grains are bigger and more visible, and they will not be crushed if pressed firmly. Almost all eggs have the same size and round shape. The egg grains are becoming bigger, filling almost the entire abdomen, being clearly visible and dark red in color. They can easily be separated because the oil layers covering them have been reduced. The filling of the ovary in the abdomen is about 80%.

Gonado Somatic Index. Determination of the gonado somatic index was conducted by weighing the gonad and total body net weight of the coconut crab by using a digital hanging scale (accuracy of 0.01 g). The gonad maturity index is calculated by by the following formula (Effendie 1979):

$$GSI=(Wg/W)x100$$

Where: GSI - Gonado Somatic Index (%); Wg - gonad weight (g); W - body weight (g).

Fecundity. The calculated fecundity is the total fecundity of coconut crab gonads reaching the gonad maturity stages III and IV. The gonadal samples observed were preserved with 70% aqueous alcohol solution and analyzed in the laboratory of the

Faculty of Fisheries and Marine Sciences, Khairun University, Ternate. The calculation of egg granules was performed gravimetrically (Effendie 1979).

$F=GX/g$

Where: F - fecundity (grain); G - total gonad weight (g); X - number of eggs from gonad samples; g - weight of the gonad sample (g).

The relationship of fecundity with thorax length was analyzed by simple linear regression (Effendie 1979):

$F=aTL^b$

Where : F - fecundity (grain); TL - thorax length (mm); a and b - constants.

Egg diameter. Observations of egg diameter were carried out using a microscope equipped with an ocular micrometer and an objective micrometer (10X). The eggs measured were from III GMS and IV, with a sample size of 100 individuals (Effendie 1979).

Size of mature gonads. Mature gonad size (Lm) was determined using a frequency distribution of mature gonadal proportions (Nikolsky 1963) and carried out using thorax length data (TL). Thus, the size of mature gonads was determined by using the Sperman-Karber method (Udupa 1986):

$$m=\left[X_k+\left(\frac{X}{2}\right)\right]-(X\sum pi)$$

Where: m - log of the coconut crab thorax length at the first gonad maturity; X_k - log of the class middle value of the last coconut crab thorax length with mature gonad; X - log of the accretion thorax length at the middle value; pi - the proportion of gonad mature coconut crabs in the length class i with the number of crabs at the length interval i; ni - the number of coconut crabs in the length class i; qi=1-pi; M - length of crab first mature gonads as big as antilog m, where anti log m is:

$$\text{anti log } m = m \pm 1{,}96\sqrt{X^2\frac{pi*qi}{ni-1}}$$

Results and Discussion

Gonad maturity stage. The 108 males and 108 females coconut crabs caught were analyzed for reproductive capacity at the 4 stages of gonad maturity (stage I, stage II, stage III, and stage IV). Male coconut crabs were found with varying percentages of gonad maturity. Coconut crabs with mature gonads (stage III) had the highest percentages in all 3 stations: 52.8% (Station I), and 66.7% (Stations II and III) (Figure 2A). Female coconut crabs at the station adjacent to residential areas (Station I) presented a high percentage of gonads in stage I (50%), and no coconut crab was found in stage IV. At Station II, stage I (38.89%) dominated, while Station III was dominated by the coconut crabs in stage II (38.89%). Female coconut crabs with mature gonads (stages III and IV) were dominantly found in Station III, with 33.33% in stage III, and 5.56% in stage IV (Figure 2B).

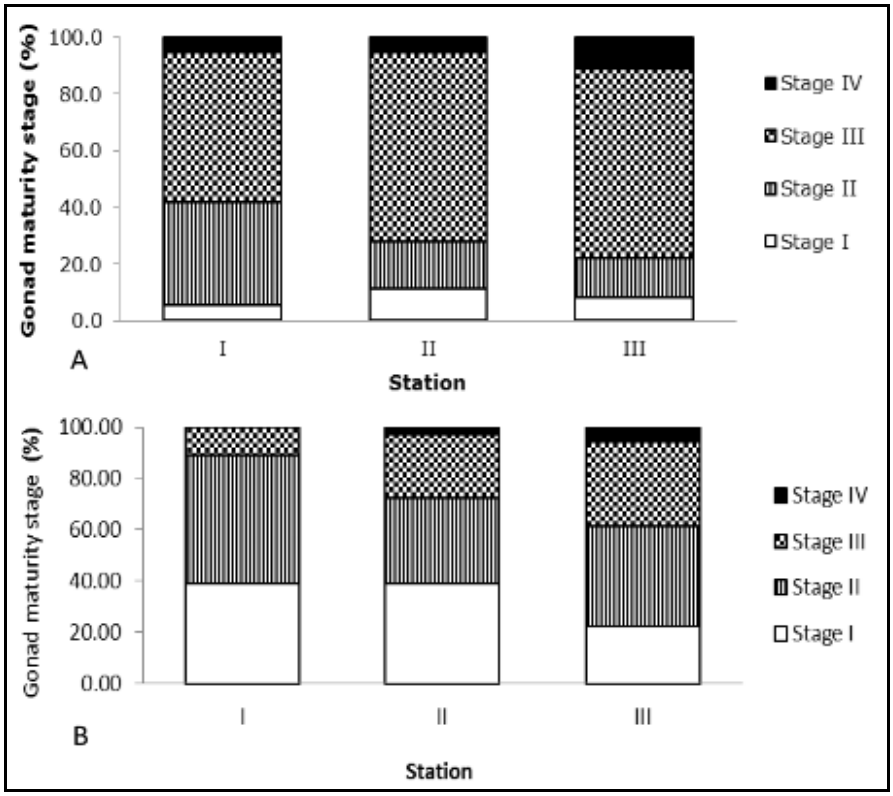

Figure 2. Gonads maturity stage of males (A) and females (B) coconut crabs in each station. Station I (Tanjung Tulang) - adjacent to residential areas; Station II (Tanjung Soki) - a steep area near the beach; Station III (Mijiu) - a shallow and flat area consisting of various vegetation and coconut trees.

Based on time, male coconut crabs (Figure 3A) in stage I were predominantly found in March, July, and August (22.2%), in stage II in September (44.4%), in stage III in June (88.9%), and in stage IV in May (33.3%). The gonad mature coconut crabs (stage III) were also found in a high percentage in October and February (77.8%), November, December, March, April, and July (66.7%). Similarly, gonad mature female coconut crabs (stage III) were found in November, February, May, June, and July (33.3%), and in stage IV in October, January, and July (11.12%). Mature gonad coconut crabs in stage I were dominantly found in April (66.7%) and in stage II in May (66.7%) (Figure 3B).

Gonad maturity is a developmental stage of the coconut crab before and after spawning. The maturity stage is necessary to determine and examine the number of coconut crabs that have mature gonads, the size of the gonads becoming mature, and the spawning pattern in a year. The gonad maturity stage of the male coconut crabs varies between stations during observation. This indicates that the 3 stations are suitable habitats for sustaining coconut crab populations.

Coconut crabs with mature gonads (stage III and IV) were dominantly found at Station III. Female coconut crabs with mature gonads were also dominant at Station III. It is presumed that Station III is a spawning area for coconut crabs, because it is located far from residential areas, and has a variety of vegetation and coconut trees, as well as steep and rocky areas, which are favored by coconut crabs. The low catch of gonad mature coconut crabs at Station I is presumed to be related to the location adjacent to residential areas, being often used as a catching and a degradation area.

The male coconut crabs with gonad maturity were found earlier than females in Daeo. Females with mature gonads (stages III and IV) were found at some observations, indicating that the maturity of coconut crabs did not occur simultaneously, and the spawning process took place continuously. This also shows that the reproductive potential of male and female coconut crabs in the Daeo area is very high and the potential for reproduction exists at any time because of the monthly availability of mature male and

female coconut crabs. Schiller et al (1991) stated that the spawning of coconut crabs in the tropics continues for almost the whole year and can occur several times during long spawning seasons (Refiani 2005; Abubakar 2009).

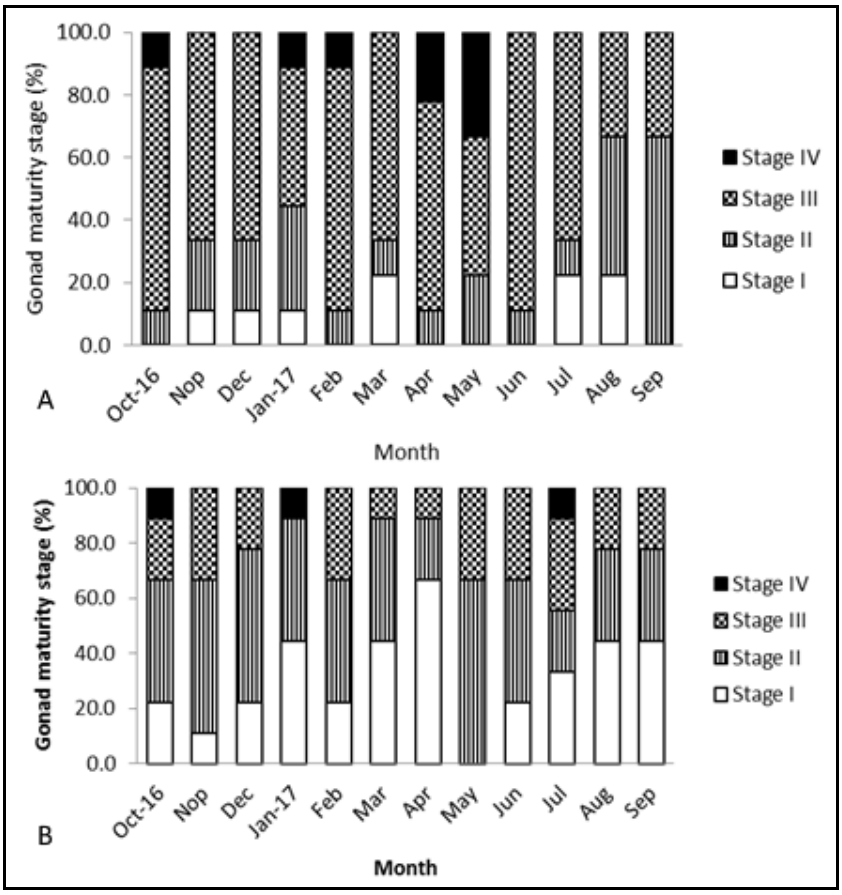

Figure 3. Gonad maturity stage of males (A) and females (B) coconut crabs based on time.

Size at the first mature gonads. Based on the Spearman-Karber method, it was found that the Lm of male coconut crabs was 48.73 mm and first capture size (Lc) was 33.69 mm, while those of the female coconut crabs were 35.33 mm and 25.12 mm, respectively. The Lc value of male and female coconut crabs is smaller than the Lm value. This shows that some of the male and female coconut crabs caught had not reached the size of the first mature gonads.

The ovigerous female coconut crabs in this research were found in September and December at Station III, and in February and November at Station II. The size of the ovigerous coconut crab ranged from 180 to 360 g, with a thorax length ranging from 29.3 mm to 34.16 mm. This value indicates that Station II and III are spawning areas for coconut crabs in Daeo district, Morotai Island. The presence of ovigerous coconut crabs in a population indicates the presence of reproductive activity.

The size at first maturity analysis indicates that male and female coconut crabs had the first captured size (Lc) smaller than the first size of mature gonads (Lm). This shows that the chances of coconut crab reproduction in Daeo are decreased because the crabs are caught before the gonads are mature. Mustac & Sinovcic (2011) state that the Lm values depend on genetic and environmental factors.

The size of the male coconut crabs with mature gonads found in this research was bigger than the value from Niue by 19.7 mm TL (Schiller et al 1991) and in Hotama Island, Japan by approximately 22.2 mm TL (Sato et al 2008). Male coconut crabs with a TL larger than 25 mm are sexually mature individuals (Fletcher 1993; Sato et al 2008). Meanwhile, Sulistiono et al (2009) found that the smallest TL of a mature male coconut

crab is about 30 mm, in Yoi Island, Central Halmahera District. Sato et al (2008) found a relationship between the size and the sperm count produced by male coconut crabs. Male coconut crabs with a larger size have more sperm in their vas deferens and testicles.

The results of this research revealed that 50% of male coconut crabs with mature gonads have a TL of 33.25 mm, and females have 27.75 mm. This value is smaller than that found by Sato & Yoseda (2008) in Hotama Island, who found that all female crabs had a TL bigger than 32.3 mm, out of which 50% had functional maturity, female coconut crabs starting to be functionally mature by 24.5 mm TL. Fletcher (1993) found that female coconut crabs reached their functional maturity at a TL of 25 mm in the South Pacific. Amesbury (1980) found that the average TL of active reproducing female crabs is 42 mm in Mariana Island. Sulistiono et al (2009) found the smallest TL of a female coconut crab with mature gonads, 35 mm. The smallest size of a coconut crab reaching gonad maturation in this research is lower than that found by Sulistiono et al (2009) on Yoi Island, Central Halmahera Regency, which is 35 mm. This shows that the coconut crab population in Daeo, Morotai Island is under more pressure from community activities and habitat degradation than the population in Yoi Island located in areas far away from community activities.

The size of the female coconut crabs at first maturity in this study was smaller than that of the males. This indicates that the utilization level of gonad mature female coconut crabs is higher than that of male crabs. Gonad mature coconut crabs have eggs that taste good, leading them to be popular as food for human consumption (Amesbury 1980; Brown & Fielder 1991; Kessler 2006; Buden 2012).

Helfman (1973) observed ovigerous coconut crabs in May (late spring) in Palau, and Reese (1987) observed ovigerous females in Eniwetak from April (mid spring) to August (late summer). The reproductive season of coconut crabs in the northern hemisphere tropical regions (Solomon Islands) is not seasonal. Sato & Yoseda (2008) estimated the reproductive season of coconut crabs at Hotama Island for three months (starting from June to the end of August). Based on gonad development data on the Daeo coconut crabs, gonad maturity is identified in October-September (except in January) with the discovery of ovigerous coconut crabs in November, December, February, and September. This highlights that the spawning season in Daeo, Morotai Island lasts several months in a year and peaks in February, May, June and July. This highlights that the spawning season in Daeo lasts several months and it is not seasonal.

Gonado Somatic Index. The GSI of female coconut crabs ranged between 2.04 and 4.95, and that of the male coconut crabs ranged from 0.98 to 1.04. GSI values vary based on time (month of observation). The highest male GSI values were found in February, June, July and November, while for female coconut crabs they were found in July, May, June, February and September. The GSI value varies in line with the stage of gonad maturity. Gonads reach maturity for a few months of the year. The GSI value of male and female coconut crabs is relatively the same among the periods of observation, so that the spawning season is not visible. The average female and male GSI values are presented in Figure 4. The highest male GSI value is in February, June, July and November, while the highest female value is in July, May, June, February, and September.

GSI values indicate the stage of gonadal development qualitatively, being an index in line with gonadal development. The female GSI value obtained has a wider range compared with that of male coconut crabs. Female GSI values are generally higher than those of males because female growth is primarily directed to gonadal development. The results of this study found lower GSI values (0.98 to 1.04 for males) and (2.04 to 4.95 for females) than those found by Abubakar (2009) and Refiani (2005). Abubakar (2009) reported that the value of male GSI was from 0.56 to 1.516 and of the female was from 0.375 to 2.949 in Yoi Island. Refiani (2005) found that the highest male GSI value was in November (1.108), and the lowest was in February (0.87) in Pasoso Island. The females reached the highest score in December (6.45), and the lowest in August (0.7). Pillay & Nair (1973) state that the GSI values of female coconut crabs are higher than those of males that only produce sperm. The variation in stages of gonad maturity between

populations is probably caused by several factors, including environment, genetics, body density, and pressure selection from capture or predators.

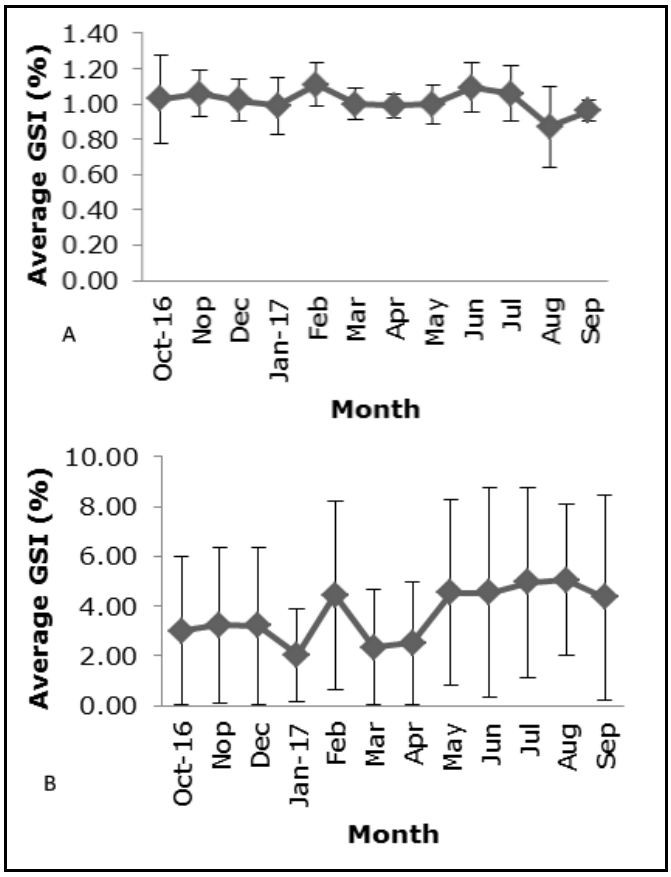

Figure 4. Average Gonado Somatic Index (GSI) of male (A) and female (B) coconut crabs based on month.

Fecundity. Coconut crab fecundity in the Daeo ranges from 21463 to 131500 eggs. Fecundity associated with TL (Figure 5) shows that there was no correlation between TL and coconut crab fecundity with a value of R^2=0.3088 (31%) with the regression equation $F=3.9668TL^{2.6873}$. Referring to this value, the data shows that TL is not the main parameter influencing coconut crab fecundity, as the TL only contributes by less than 35% to fecundity.

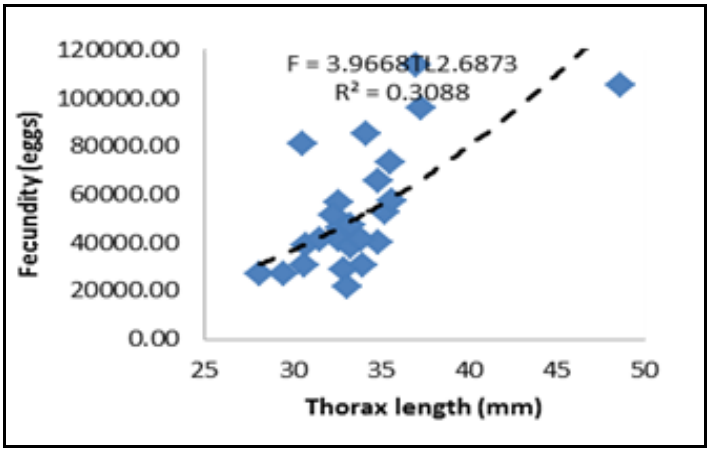

Figure 5. Relationship of thorax length (mm) with fecundity at stage III for coconut crab females.

The fecundity observed in this study is less than the results found by Abubakar (2009) in Yoi Island, Central Halmahera (17698-143210 eggs), and by Refiani (2005) in Pasoso Island, Central Sulawesi (58717-197400 eggs). This research found a correlation between fecundity and body weight. Abubakar (2009) also argued that there is a relationship between body weight and fecundity (R^2=0.694).

Temporally, the number of eggs released in each reproductive season is the same between female coconut crabs with different body sizes, although the number of egg packages issued by females is different in each reproductive season. The number of eggs released varies with body size in some crustaceans, and some larger females can release more eggs (Briones & Lozano 1992). Female crabs with larger sizes will start spawning earlier. Temporal gradients in females with eyed eggs during the reproductive season indicate that female coconut crabs on the island of Hotama only issue one package of eggs in one reproductive season. Schiller et al (1991) also stated that coconut crabs produce only one package of eggs per year in Vanuatu.

Egg diameter. Egg diameter at stage III ranged from 0.01 to 0.056 mm, while at stage IV, it ranged from 0.01 to 0.061 mm (Figure 6). Figure 6 demonstrates that the average frequency of the highest egg diameter is 0.021-0.025 and has one peak. This shows that coconut crabs in the Daeo area spawn completely, without eggs remaining from each spawning.

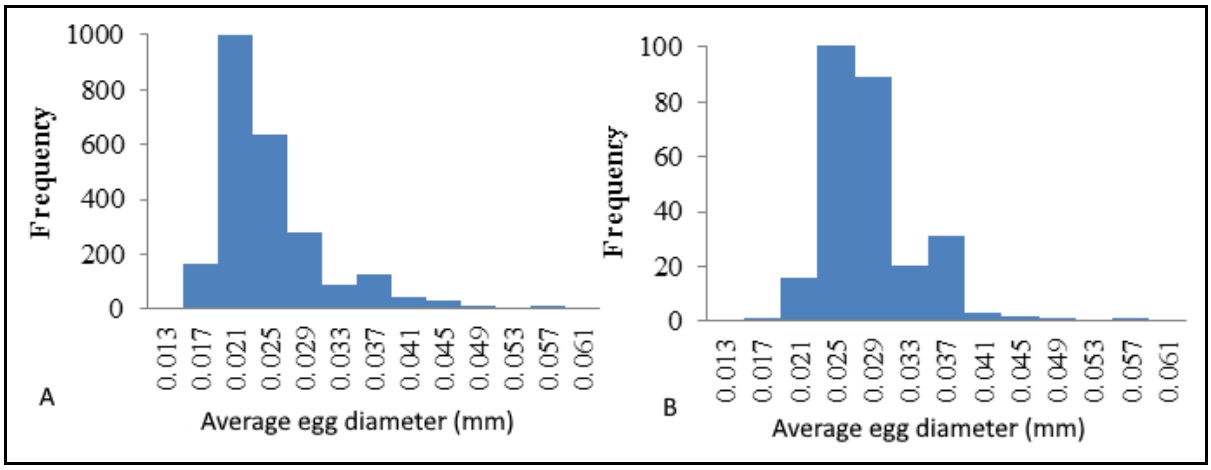

Figure 6. Average diameter of coconut crab eggs in stage III (A) and IV (B).

According to Refiani (2005), the egg diameter of coconut crabs in Pasoso Island, Central Sulawesi ranged from 0.011 to 0.032 mm in stage III, and 0.015-0.035 mm in stage IV. Abubakar (2009) found that the diameter of coconut crab eggs in stage III is between 0.052 and 0.054 mm, and between 0.058 and 0.060 mm in stage IV, in Yoi Island, Central Halmahera.

Conclusions. Male and female coconut crabs at first maturity had an average carapace length of 28.75 mm in stage III, and 33.25 mm in stage IV. However, the frequency of male coconut crabs with an average size between 28.75 mm and 45.25 mm is higher than that of females of the same size. The coconut crabs with mature gonads (stage III and IV) were dominantly observed in 7 months, January, February, May, June, July, October and November, which suggests that coconut crabs spawned over the whole year. Egg diameter in this study ranged from 0.012 to 0.034 mm (stage III) and 0.025-0.048 mm (stage IV), while the fecundity ranged from 21463 to 131500 eggs. The size of coconut crabs with mature gonads studied in Daeo, Morotai Island, tends to be smaller than the size of the coconut crabs captured for food.

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Received: 17 December 2019. Accepted: 01 April 2020. Published online: 02 August 2020.
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How to cite this article:
Serosero R., Sulistiono, Riani E., Butet N. A., 2020 Reproduction of coconut crabs (*Birgus latro*) in Daeo District, Morotai Island, North Maluku, Indonesia. AACL Bioflux 13(4):2002-2013.