A study on the reproduction aspects of windowpane oyster (*Placuna placenta*) within the Kulisusu Bay, Indonesia

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Abstract. Indonesia has enormous coastal and ocean resource potential, including the Kulisusu Bay, located in North Buton Regency, Southeast Sulawesi, which is a habitat of *Placuna placenta* oyster. This species is commonly consumed as food, but excessive consumption of mature oysters tends to influence the wild population's structure and regeneration. Therefore, this study aims to determine the reproductive aspects of *P. placenta*, including the sex ratio, gonad maturity level, gonadosomatic index (GSI), fecundity, and size at first sexual maturity. The study was conducted from October 2017 to October 2018 in the waters of Kulisusu Bay. Sampling was performed using the quadratic transect method, and 40 oysters were collected per location. Subsequently, the sex ratios, gonad maturity level, GSI, and fecundity were analyzed using non-parametric tools including the Chi-square test, quantitative descriptive statistics, and the Mann-Whitney U test. Meanwhile, fecundity and size at first sexual maturity were examined linearly and non-linearly. The results showed that the sex ratio between the males and females was not significantly different at 1.01:0.99. *P. placenta* experienced peak maturity in April, while spawning occurred between late May and July. In addition, the GSI values ranged from 1.57 to 15.7 and 8.55 to 24.44 in males and females, while the fecundity ranged from 126,973 to 1,621,217, and the sizes at first sexual maturity were 6.7 cm and 7.2 cm respectively.

Key Words: fecundity, gonad maturity, sexual maturity, sex ratio.

Introduction. Indonesia is an archipelago consisting of 17,508 islands with a coastline of 81,000 km and a sea area of about 3.1 million km^2 namely 0.3 million km^2 of territorial and 2.8 million km^2 archipelagic waters. Therefore, there is enormous potential for coastal and ocean resources (Dahuri 2001). One of the coastal areas is Kulisusu Bay, located in North Buton Regency, Southeast Sulawesi Province. This bay has become potential for economically critical natural resources, one of which is windowpane oyster (*Placuna placenta*). It is also called kalapenda by the people of North Buton or kapis (Philippines) and methyl (India) (Campbell 2006). Additionally, this oyster can be found in Bintulu, Malaysia (Hamli et al 2015). The people of Southeast Sulawesi are familiar with several names, for example, Kerang simping (North Buton) and kalampese (Tolaki).

The people consume *P. placenta* as food, and it is a commodity with important economic value, for example, the Kulisusu people sell it for IDR 7,500 per kilogram. Although the consumption is still limited, its shell has been used as decoration in several places (Agustini et al 2011).

Pressure on *P. placenta* resources indirectly influences its reproduction biology aspects (Yonvitner 2011). This condition is feared to cause a wild population decline, both in quantity and quality (Yonvitner 2011). Meanwhile, complete scientific information to support management measures is not yet available. A study conducted by Yonvitner (2011) in the waters of Kronjo revealed that the decrease in the potential of *P. placenta* was reflected in the reduced size and production. Certain supporting information is needed in the process of making management policies, one of which is the aspects of reproduction. Knowledge of population dynamics is related to biological processes, especially reproductive systems (Caddy 1989; Knights 2012; Costa et al 2013; Maunder & Deriso 2013; Ayache et al 2016; Delgado et al 2016; Dublinowska et al 2016). Several studies have been conducted on the life-cycle reproduction of bivalves since the 1930s specifically on the reproductive cycles and the size at first sexual maturity in *Amusium japonicum* (Son & Chung 2009), gametogenesis histology and gamete development cycles in *Anodonta gabillotia pseudodopsis* (Sereflisan et al 2009), gonadal development (Son & Chung 2009), as well as gonad and reproductive cycle of *Barnea davidi* (Jeon et al 2012).

Meanwhile, investigations on *P. placenta* include studies on its distribution (Anti et al 2014), utilization (Yonvitner 2011), and ecobiology (Madrones-Ladja 2002). The reproductive aspects have not been studied. Adequate information is quite essential to support the management of resources in the future. Therefore, this study aims to determine the reproductive aspects of *P. placenta*, including sex ratio, gonad maturity level, gonadosomatic index (GSI), fecundity, and size at first sexual maturity.

Material and Method

Research methods. This study was conducted in the waters of Kulisusu Bay (Figure 1) from October 2017 to October 2018. *P. placenta* samples were collected at low tide from all the areas where these oysters were found. More specifically, the samples were collected in two stations by hand up to 40 individuals.

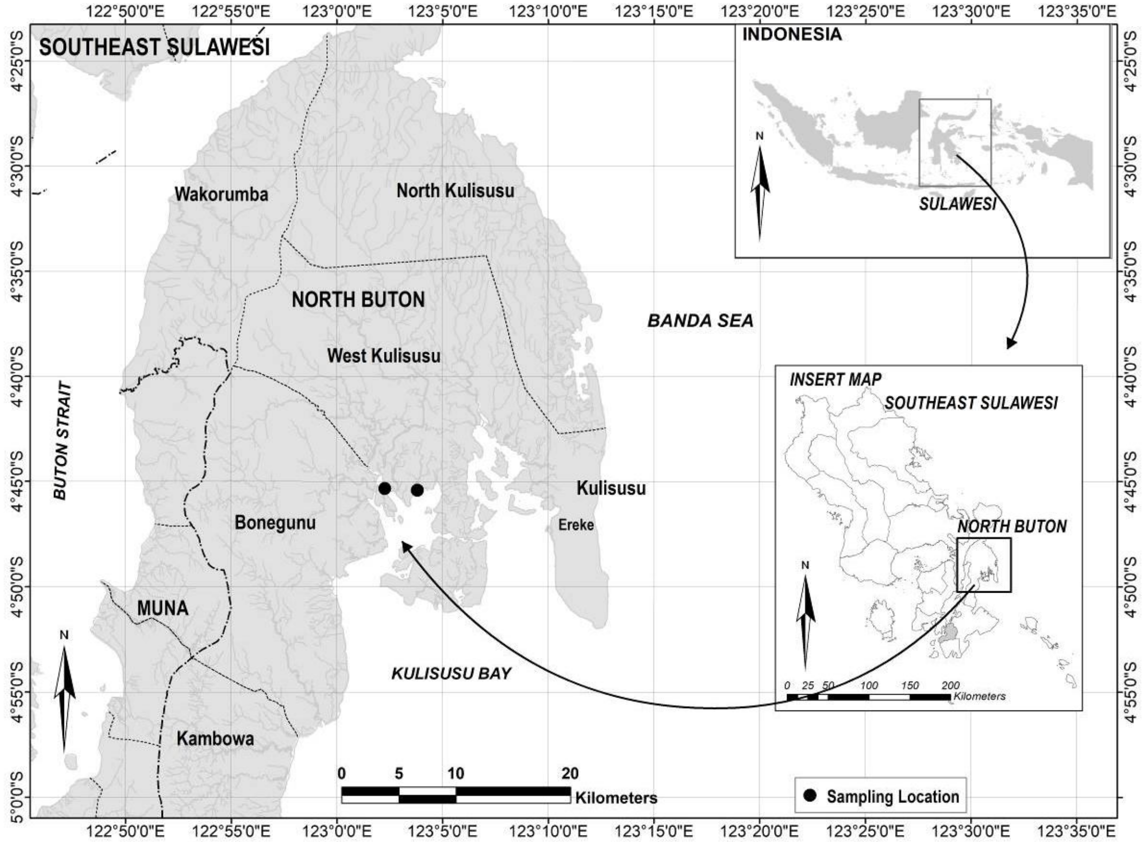

Figure 1. Sampling location of windowpane oyster (*P. placenta*) in Kulisusu Bay.

Analysis of reproduction aspects of sampled *P. placenta* was conducted in the laboratory of the Faculty of Fisheries and Marine Sciences, UHO. After dissection, it was observed that the color of the male gonads was generally white, while the female gonads were orange. GSI observations referred to Sastry (1979), while body and egg weight were measured using a digital scale with an accuracy of 0.0005 g. The shell width was measured by using a caliper with an accuracy of 0.1 mm (El-Sabbagh et al 2016).

Sex ratio. Sex ratios were calculated by using the following equation:

$$X = \frac{F}{M}$$

where: X = sex ratio; M = number of male; F = number of female.

To test whether the number of males and females was balanced, an evaluation was carried out by using the Chi-square (X2) test as follows (Mzighani 2005):

$$X2 = \sum_{i=1,2,3}^{s} \frac{(f1 - f2)^2}{F}$$

where: X2 = value of sex distribution; fi = value of observation to I; F = value of expectation to i; i is 1, 2, 3; S = number of observations.

Gonadosomatic index (GSI). GSI was measured after determining the level of gonad maturity. The data obtained were then analyzed using calculations by Sastry (1979):

$$GSI = \frac{Bg}{Bt} \times 100$$

where: GSI = gonadosomatic index; Bg = gonad weight (g); Bt = body weight including gonad (g).

The GSI value of *P. placenta* between observations was analyzed by a non-parametric Mann-Whitney U Test using Minitab software (Steel & Torrie 1989).

Fecundity. The number of eggs was calculated based on the equation proposed by Effendi (1976):

$$F = n \times \frac{G}{g}$$

where: F = fecundity; n = number of eggs from part of the gonad (g); G = total gonad weight; g = individual gonad weight.

The correlation between fecundity with the width and weight was analyzed by using regression analysis (Steel & Torrie 1989).

The size of P. placenta at first gonad maturity. The 50% chance of gonad being mature was determined by using a non-linear regression function on logistic curves (Arocha & Barios 2009) with the help of SigmaPlot 6.0. The function is as follows:

$$Y = \frac{a}{1 + e^{\frac{-x + xo}{b}}}$$

where: Y = probability of gonad maturation (%); e = natural exponential; a = intersection of line (intercept); b = slope; x, xo = measure of width i (cm).

Statistical analysis. To determine the relationship between the number of male and female individuals, a Chi-square test was performed. To determine whether there was a significant difference in the average GSI value between one month and another, a statistical test, the Mann-Whitney Test, was carried out.

Results

Sex ratio. Male and female *P. placenta* can be distinguished by the color of the gonads, the male gonads are milky white, while those of the females are orange. The samples observed were 1,284, comprising 639 males and 645 females (Table 1).

Table 1

Sex ratio of windowpane oyster (*P. placenta*) in the waters of Kulisusu Bay

Number of shell	*Number of individuals*		*Sex ratio*	
	Female	*Male*	*Female*	*Male*
1,284	645	639	1.01	0.99

Based on the Chi-square test at a level of confidence of 0.05 db (n = 0.05), the number of males and females was proportional in each month, ranging from 53 to 71%, except during April-July 2018 (Figure 2). The analysis results showed that the number of males was not significantly different from females

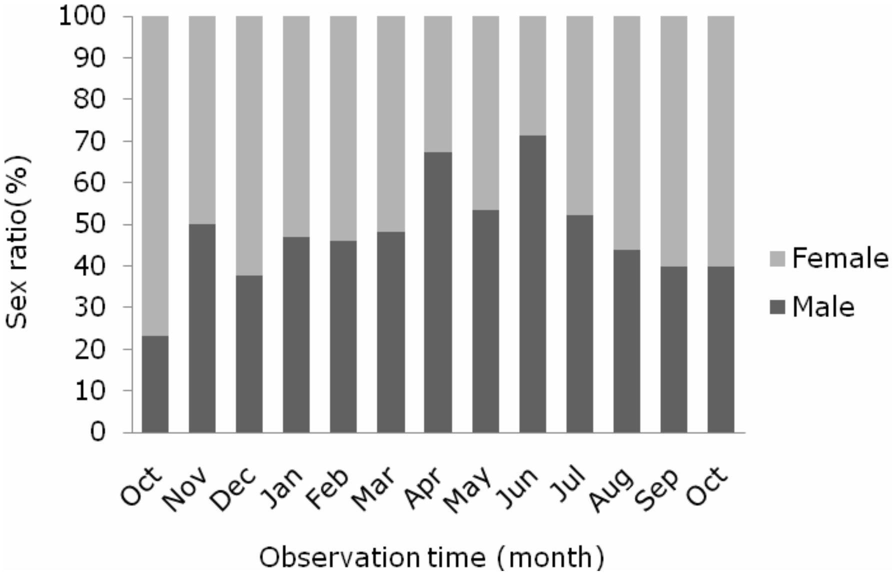

Figure 2. Sex ratio of windowpane oyster (*P. placenta*).

Gonad maturity level. Temporally, the level of development in both male and female gonads was relatively concurrent. Maturity was not found at the beginning of observations in October, November, and December 2017, as demonstrated by the unknown sex of the oysters. Gonad maturity stage I was dominantly found in January and February 2018, after a short time, the gonad maturity level underwent a change to stage II in February, which was dominantly in March. The gonad maturity occurred in April and May, although a large distribution was found from stage I up to IV.

Furthermore, maturity was observed at stage IV and dominantly reached stage V in May, June, and July. The gonads at maturity level V continued from August to October, both males and females reached maturity in April, and spawning occurred from the end of May to October (Figure 3).

Gonadosomatic index. GSI values ranged from 1.57 to 15.7 for male *P. placenta* and from 8.55 to 24.44 for females. The highest value was detected in February, 24.44, and the lowest was observed in January, 8.55. The highest GSI of the male was at 15.70 and was detected in May, while the lowest was in January, at 1.57. The mean GSI values were highest during February-May, and decreased during June-October (Figure 4).

GSI values obtained every month in 2018 showed a relatively similar value overall, the analysis was conducted only in 2018 because, in October-December 2017, no data were found. The results in Tables 2 and 3 displayed different GSI, which was indicated by the value p (a) = 0.05. The Mann-Whitney test at α = 0.05 overall showed that the male and females had the same pattern. At the beginning of the study in January-April 2018, the value was smaller than 0.05, indicating a significant difference. Different results were shown in May-October 2018, where the importance of α was more significant than 0.05, which indicates that the GSI was the same every month. The relatively different GSI values were due to the gonad development phase. The similar pattern observed at the end of the study (May-October) was due to the *P. placenta* being in the spawning phase, hence, the weight of each was relatively similar.

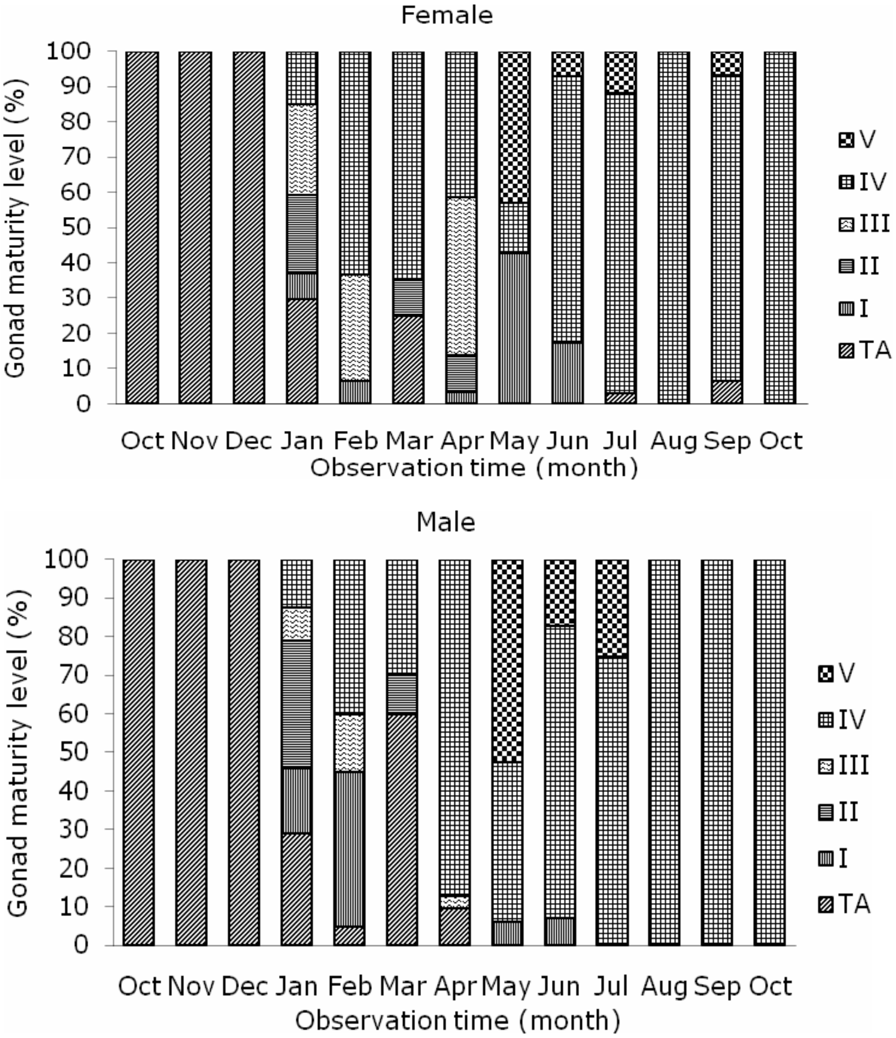

Figure 3. Percentage of the gonad maturity level of female and male *P. placenta* (I-V = level of gonad maturity, TA = without gonad).

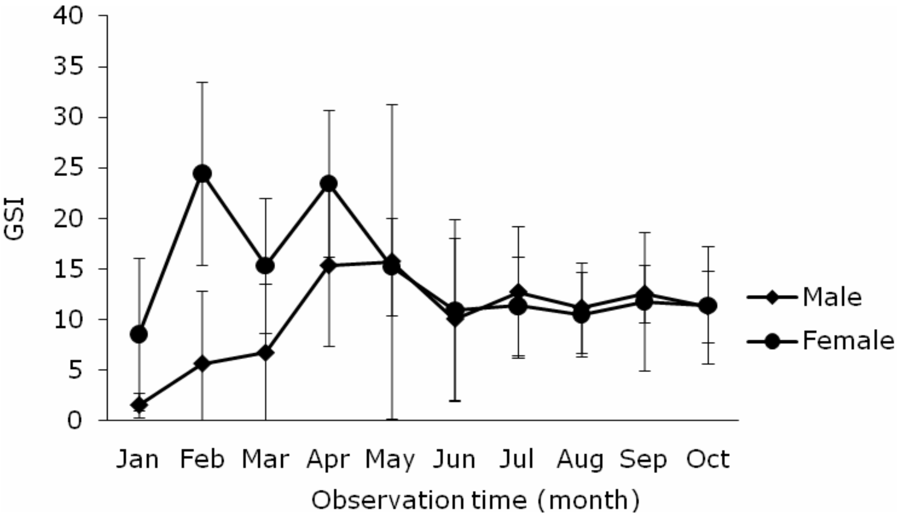

Figure 4. Gonadosomatic index of windowpane oyster (*P. placenta*) in Kulisusu Bay.

Table 2

Analysis result of Mann-Whitney U Test on GSI of male (*P. placenta*) each month

Month	*Jan*	*Feb*	*Mar*	*Apr*	*May*	*June*	*July*	*Aug*	*Sep*
Jan									
Feb	0.015								
Mar	0.075	0.792*							
Apr	0.000	0.000	0.001						
May	0.000	0.003	0.064*	0.239*					
Jun	0.000	0.003	0.087*	0.010	0.774*				
Jul	0.000	0.000	0.006	0.221*	0.403*	0.099*			
Aug	0.000	0.000	0.026	0.049	0.584*	0.424*	0.352*		
Sep	0.003	0.039	0.130	0.754*	0.688*	0.239*	0.702*	0.393*	
Oct	0.000	0.006	0.060	0.196*	0.742*	0.419*	0.723*	0.757*	0.589*

Table 3

Analysis result of Mann-Whitney U Test on GSI of female windowpane oyster (*P. placenta*) each month

Month	*Jan*	*Feb*	*Mar*	*Apr*	*May*	*June*	*July*	*Aug*	*Sep*
Jan									
Feb	0.000								
Mar	0.020	0.001							
Apr	0.000	0.518*	0.001						
May	0.056	0.005	0.724*	0.004					
June	0.704*	0.000	0.036	0.000	0.115*				
July	0.088*	0.000	0.023	0.000	0.065*	0.643*			
Aug	0.154*	0.000	0.006	0.000	0.043	0.994*	0.393*		
Sep	0.201*	0.000	0.124*	0.000	0.220*	0.659*	0.762*	0.915*	
Oct	0.137*	0.000	0.071*	0.000	0.148*	0.738*	0.778*	0.784*	1.000*

Fecundity. The fecundity ranged from 126,973 to 1,621,217 eggs, the linear regression analysis results showed a strong relationship between width of *P. placenta* and fecundity with a coefficient of determination (R^2) of 86.59%. The pattern of relationships can be explained through the equation F = 415462SW-3E+06. The highest fecundity was found to be 1,621,217 eggs at 10.3 cm in width in each oyster, while the lowest was 126,973 eggs at 6.7 cm in width (Figure 5).

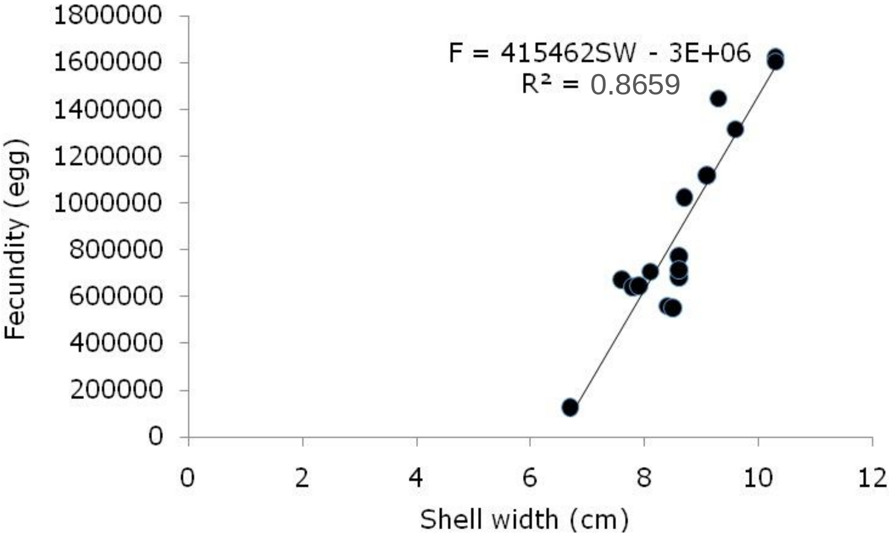

Figure 5. Relationship between fecundity and width and weight of *P. placenta* in the waters of Kulisusu Bay.

***The size at first sexual maturity**.* The size of female *P. placenta* at first sexual maturity was found to be 6.7 cm with a gonadal maturity of 17.8%, while the size of males at their first sexual maturity was 7.2 cm with a gonadal maturity of 15.2% (Figure 6).

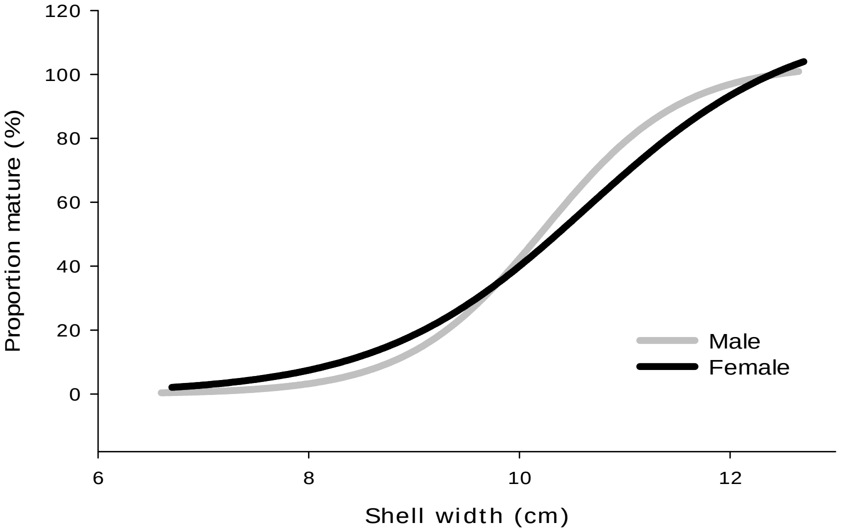

Figure 6. The size of gonad for windowpane (*P. placenta*) in the waters of Kulisusu Bay at their first sexual maturity.

***Water quality parameters**.* Observation and monitoring of water quality in the waters of Kulisusu Bay (Figure 7) showed variations based on the time of observation. Water temperature ranged from 28 to 31^{o}C, chlorophyll level was about 0.15-8.35 mg L^{-1}, and the organic material ranged from 2.39 to 4.35 mg L^{-1}. The concentration of organic matter did not differ significantly in each month.

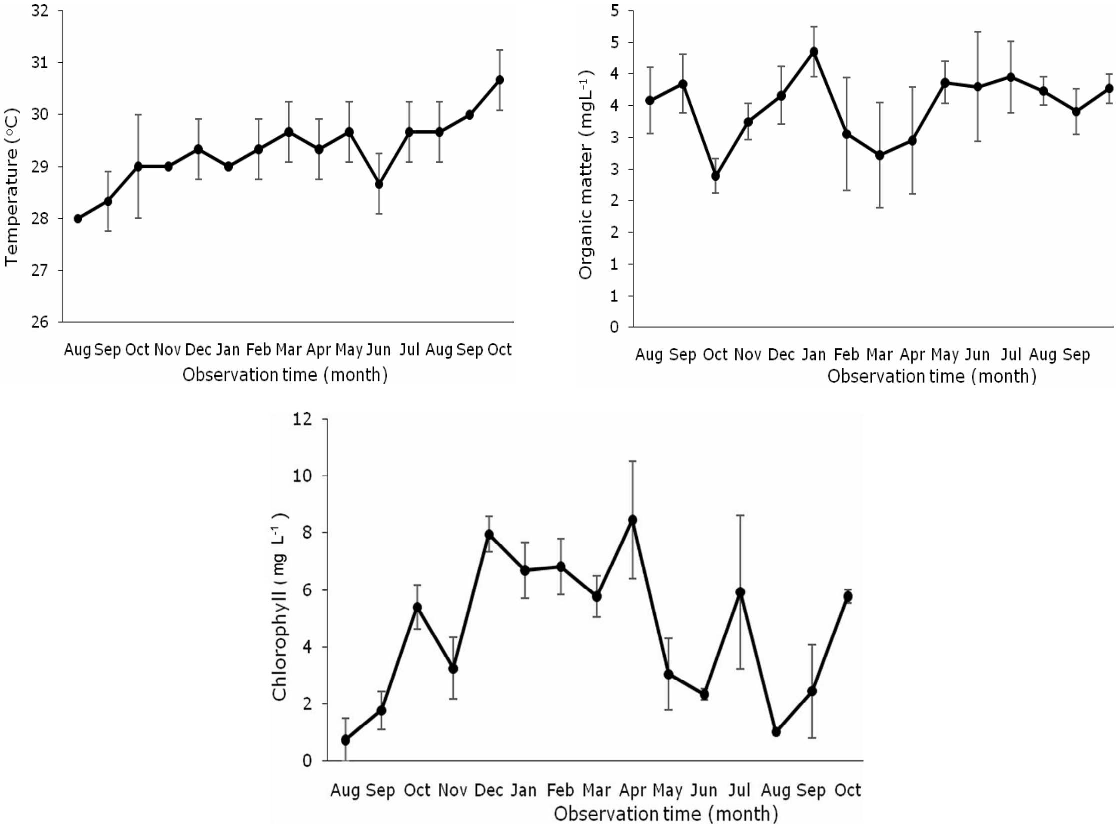

Figure 7. Parameters of water quality.

Discussion. The sex ratio of *P. placenta* in the waters of the Kulisusu Bay during the study period showed that the number of females was greater than the male with values of 645 and 639 individuals respectively, amounting to an unbalanced ratio of 1.01:0.99. The sex ratio of the *Acanthocardia tuberculata* in the north Alboran Sea was 1:1 (Tirado et al 2017) and studies of the bivalves, *Venus verrucosa* in Malaga as well as the Aegean Sea and *Callista chione* in Malaga, also revealed a balanced sex ratio (Sastry 1979; Galinou-Mitsoudi et al 1997; Tirado et al 2002, 2003). The imbalance sex ratio in this study is probably influenced by the excessive *P. placenta* catch that was carried out continuously by the community. Furthermore, Rochmady et al (2012) and Anti et al (2014) who examined *Anodontia edentula* and *P. placenta* respectively stated that the occurrence of excessive exploitation caused an unbalanced sex ratio composition. The level of exploitation is a very important factor in determining the bivalve population, both the number of males and females, in the water. Besides, the rapid and continuous utilization/collection of bivalves regardless to its management can put pressure on the aquatic environment and the population. The population size of bivalves is also affected by environmental changes (Widyastuti 2011). The number of male and female ratios in nature can be influenced by the level of exploitation (Bahtiar 2012). In addition, the large number of males in the waters is presumably due to the adaptations made by these organisms (Bahtiar 2017). Morton (2010) suggested that under normal circumstances, unbalanced sex ratios are a reproductive strategy in certain environmental conditions. In some Bivalvia species, the sex ratio of male to female relatively varies but generally, it tends to be balanced (Rochmady et al 2012). This finding is also supported by Natan (2008) as demonstrated in Table 4.

Based on the results of the percentages, bivalves were in the maturity phase and the spawning or post-spawning phases in May, June, and July (Figure 3). In this phase, the bivalves are ready for spawning and will return to the initial preparation or development phase for the maturation of the remaining gonads in August to December. This is marked by the discovery of gonad maturity stage IV in May to December.

Table 4

Comparison of maturity phase of windowpane oyster (*P. placenta*) with other species of bivalve

Species	Location	*Spawning month*												Research
		J	*F*	*M*	*A*	*M*	*J*	*J*	*A*	*S*	*O*	*N*	*D*	
Anadontia edentula	Water of Ambon Bay, Indonesia	x	x	x	x	x	x	x	x	x	x	x	x	Natan (2008)
Cerastoderma glaucum	Berre Lagoon, Mediterranean Coast, France	x	x	x	x	x	x	x	x	x	x	x	x	Tarnowska et al (2012)
Cerastoderma edule	Ria de Vigo, Galicia, Northwest Spain			x	x	x	x			x	x			Martinez-Castro & Vazquez (2012)
Pharella acutidens	Water of Dumai, Riau	x	x	x	x	x	x	x	x	x	x	x	x	Efriyeldi et al (2012)
Anadara granosa	Strait Lada, Sunda Strait							x	x	x	x	x	x	Komala (2012)
Cerastoderma glaucum	Lake Qarun, El-Fayoum, Egypt		x	x	x	x		x				x		Kandeel et al (2013)
Batissa violacea	Pohara River, Southeast Sulawesi, Indonesia	x	x			xx		x	x	x		x		Bahtiar (2012)
Acanthocardia tuberculata	Nortwest Spain	x	xx			x	x							Tirado et al (2017)
Placuna placenta	Water of Kulisusu Bay, Southeast Sulawesi, Indonesia	x	x	x	x	x	x	x	x	x	x	x		This research

Variation of *P. placenta* reproduction as the gonads mature and during the spawning phase is triggered by changes in water quality. The peak of gonad maturity in April is marked by the increasing rainfall, which causes changes in some water quality, such as the increase in the amount of food indicated by the high level of organic matter and chlorophyll (Figure 7). Previous studies on bivalves discovered that the spawning phase of *Amusium balloti* in Shark Bay, Australia, occurred between December and January (Heald & Caputi 1981). The spawning period of *Amusium* spp. in general, is likely to occur from November to February. This phase in bivalves usually occurs as seasonal or annual cycles and in other types of mollusks (Webber & Giese 1969), seasonal spawning phase occurs due to environmental conditions and genetic influences of some populations (Chung & Ryou 2000; Chung et al 2002). Besides, the increase in gonad maturity and spawning of *P. placenta* were followed by changes/increases in temperature. This is in accordance with the opinions of Seed & Suchanek (1992), Gosling (2003), and Van Woesik et al (2006) who reported that the period, frequency, and duration of reproduction are influenced by several external factors, including temperature, salinity, food and light. High chlorophyll levels in the months of gonad maturity and increased water temperature as well as sediment of organic matter during the gonad maturity phase showed a correlation with a peak of maturity and spawning phases.

Tarnowska et al (2012), Efriyeldi et al (2012), and Natan (2008) on *Cerastoderma glaucum* and *A. edentula* showed that these species can spawn throughout the year. Furthermore, Bahtiar (2012) examined *Batissa violacea* in the waters of the Pohara River and reported that the peak of spawning was from August to November and then return to the initial development namely gonad maturity stage II for the maturation of the remaining gonads. Similar results were found in *C. glaucum*, *Anadara granosa*, and *Cerastoderma edule* (Komala 2012; Martinez-Castro & Vazquez 2012; Kandeel et al 2013). The different peaks of the development are strongly influenced by temperature, salinity of sea water, and availability of food material sources in the water one of which is organic material. This is supported by Freites et al (2010) which found that in general, dominant factors playing an important role in reproduction, including gonad development, in *Anadara notabilis* are organic matter and temperature, while other environmental factors do not have significant effects. Furthermore, Martinez-Castro & Vazquez (2012) and Anti et al (2014 examined *C. edule* and *P. placenta* respectively. They discovered that of these two factors, organic matter had a greater influence than temperature. This is because organic matter is directly related to the availability of food, hence, it boosts body growth, including gonad development.

According to Natan (2008), in young bivalves, the energy obtained is used for growth, while in larger and mature types, only a little energy is used for growth and more energy is used for gonad development as well as spawning. Limited food hampers the gonad recovery process after the end of spawning (Kandeel et al 2013). Bahtiar (2012) also added that gonad maturity and the beginning of spawning are usually triggered by an increase in organic material, which is a source of food for bivalves.

The results obtained during the study period showed that the GSI values of males and females ranged from 1.57 to 15.7 and from 8.55 to 24.44, respectively. In addition, both genders showed a relatively large-sized pattern when they underwent the spawning and post-spawning phases in the development of gonad maturity. The highest peak value of GSI occurred only in May, indicating that May was the month of the spawning season.

Based on the results of the Mann-Whitney test ($\alpha = 0.05$), the GSI values of males in September showed significant differences compared to other studies (Table 2 and Table 3), while the GSI values of females in May did not indicate significant differences from those in other months. This shows that during the study period, the peak of the gonad maturation and spawning phase of *P. placenta* was presumably achieved in May.

GSI values became different as the level of gonad maturity changed and spawning reached its peak, hence, the results can be used to determine the spawning season based on the fecundity and egg size, the greater the fecundity and egg diameter, the greater the GSI value. According to Natan (2008) the GSI values of *A. edentula* ranged from 0.98 to 6.25. In addition, it was also found that the GSI value of the female bivalve was greater than that of the male (Table 5).

Table 5

Comparison of GSI of bivalves in the waters

Species	*Location*	*GSI value (%)*	*Research*
Anadontia edentula	Water of Ambon Bay, Indonesia	0.98-6.25	Natan (2008)
Anadara granosa	Lada Bay, Sunda Strait	0.12-1.14	Komala (2012)
Pharella acutidens	Water of Dumai, Riau	6.66-11.88	Efriyeldi et al (2012)
Cerastoderma glaucum	Lake Qarun, El-Fayoum, Egypt	3.81-10.21	Kandeel et al (2013)
Batissa violacea	Pohara River, Southeast Sulawesi, Indonesia	0.13-8.83	Bahtiar (2012)
Meretrix lyrata	Sarawak, Malaysia	1.14-3.00	Hamli et al (2015)
Acanthocardia tuberculata	Northwest Spain	0.11-4.11	Tirado et al (2017)
Batissa violacea	Laeya River, Southeast Sulawesi	5.61-14.89	Pratiwi et al (2019)
Placuna placenta	Water of Kulisusu Strait, Southeast Sulawesi, Indonesia	1.57-24.44	This research

Efriyeldi et al (2012) revealed that the species of sharp razor clam (*Pharella acutidens*) had an almost similar range of GSI values of 6.66-11.88. The values fluctuated in parallel between males and females every month, although there was a significant decrease in April and May. Very low GSI values were reported by Hamli et al (2015) who examined lyrate hard clam or *Meretrix lyrata*, with GSI values ranging from 1 to 3. In this study, a significant association was found between a low concentration of chlorophyll-*a* in the river and a decrease in the value of the gonad maturity index. This is also supported by Jahangir et al (2014) who discovered that aside from temperature, the concentration of chlorophyll-*a* also influenced the development of gonads of *Anadara antiquata*. In addition, according to Delgado et al (2016), sand substrate (sand bed) is one of the factors that affects gonad development, although this effect is not as strong as those of other aquatic parameters, such as temperature, organic matter, and chlorophyll-*a*.

The analysis results on the relationship between fecundity and shell width showed a significant relationship. This was indicated by the coefficient of determination (R^2), which was close to 1 (Figure 5), indicating a very strong relationship between fecundity and the shell width. In other words, the increase in fecundity was always followed by a rise in the width or body weight (Natan 2008; Bahtiar 2012). However, Maani et al (2016) in a study on *A. antiquata* stated that increased fecundity was not always followed by a rise in the width or body weight.

Based on the calculation of the size at first sexual maturity, the sizes of male and female *P. placenta* were 7.2 cm and 6.7 cm respectively. These results indicate that at the first sexual maturity, female *P. placenta* reached gonad maturity faster than the male. This is in accordance with a study conducted by Darrigan et al (1999) on bivalves, which discovered that the spawning phase started with a size around 5-6 mm. Previous studies in the waters of North America (Brousseau 1979) and Europe (Winther & Gray 1985) also showed that the maximum length of bivalves was between 10 and 17 cm (Cowles 2007; Cohen 2011). Furthermore, soft-shell clams of 2 cm in size are usually not mature, as often demonstrated by indistinguishable gonads (Brousseau 1978, 1987). The first reproduction usually occurs in clams with length sizes of 2-5 cm (Strasser 1998), which are usually between 1 and 4 years old depending on environmental conditions (Strasser 1998). Newell & Hidu (1986) stated that the size of an individual is more important than age in relation to the size at first sexual maturity. According to Chung (2007), the common Orient clam *Meretrix lusoria* can produce mature gonads at the size of 26.3-30.0 mm in width. Based on the results, male clams with size of 30.1-35.0 produced matured gonads amounting to 21.7%.

There was no clear annual reproduction cycle of *P. placenta* found in this study, hence, it was difficult to correlate specific gametogenesis with environmental parameters, such as temperature, salinity, and food availability. However, in the study of *Cerastoderma gluacum in* Lake Qarun, Egypt, the size at first maturity of gonads was affected by temperature (Kandeel et al 2013). Temperature also affects the initial recruitment of bivalve populations, which can accelerate gonad maturation (Natan 2008; Kandeel et al 2013; Barber 2017).

Conclusions. The results indicate that the sex ratio of windowpane oyster (*P. placenta*) had differences but were not significant. The percentage of gonadosomatic index in the adult phase occurred in May, June, and July, and then after that, it returned to the preparation phase in August-December. Meanwhile, the Mann-Whitney test (with α = 0.05) on the GSI showed that the males in September had significant differences compared to other months, while females in May did not exhibit significant differences. This implies that during the study period, the peak of maturation for the gonads and spawning phase of *P. placenta* was achieved in May*.* The fecundity ranged from 126,973 to 1,621,217 eggs, and the sizes of male and female at first sexual maturity were 7.2 cm and 6.7 cm respectively.

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Conflict of interest. The authors declare that there is no conflict of interest.

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