

## Reproductive Biology of *Charybdis hellerii* in Lasongko and Kendari Bays, Southeast Sulawesi, Indonesia

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### ABSTRACT

Limited Data have been found on the reproductive biology of *Charybdis hellerii* (A. Milne-Edwards, 1867) in Indonesia. To fill this gap, the current study aimed to analyze various aspects of the reproductive biology of *C. hellerii*, male and female, in Lasongko and Kendari Bays. Using traps, samples of crab species were monthly collected (from March-November 2019) with the help of blue swimming crab fishermen. Results showed that the total sex ratio of *C. hellerii* in Lasongko and Kendari Bays were 1: 0.48 and 1: 0.57, respectively. However, the temporally sex ratio of the crabs varied. In Lasongko Bay, male gonads of *C. hellerii* were majorly immature, while those of the females and ovigerous females were mature. Meanwhile, in Kendari Bay, all three types were predominantly immature, and they were all temporally varied. The first sizes sexual maturity of the male and female *C. hellerii* in Lasongko Bay were 60.04 mmCW and 53.12 mmCW, respectively, while those of Kendari Bay were 60.23 mm CW and 53.24 mmCW, respectively. The spawning season of this crab species tends to occur throughout the year. Therefore, the data obtained in this study can be used for the management of *C. hellerii* in Lasongko and Kendari Bays and Southeast Sulawesi waters.

### INTRODUCTION

The *Charybdis hellerii* species was first restricted to inhabit the Western Indo Pacific region (Wee & Ng, 1995; Spiridonov, 1999; Cabi, 2020); however, since these crabs are globally invasive (Watanabe *et al.*, 2015), they are widely distributed in various continents, including: Asia, Africa, America, Australia and Europe (Cabi, 2020). These crabs have been detected in the intertidal zone at a depth of 51 m (Stephenson *et al.*, 1957; Lamaitre, 1995; Wee & Ng, 1995; Cabi, 2020). Furthermore, their habitat

contains mudflats substrate (Stephenson *et al.*, 1957; Wee & Ng, 1995; Spiridonov, 1999), rock fragments, coral reefs, rock piles (Lamaitre, 1995; Wee & Ng, 1995; Cabi 2020), seagrass beds and algae, in addition to mangrove roots in the sand (Spiridonov 1999; Dineen *et al.*, 2001; Ferry *et al.*, 2017), ).

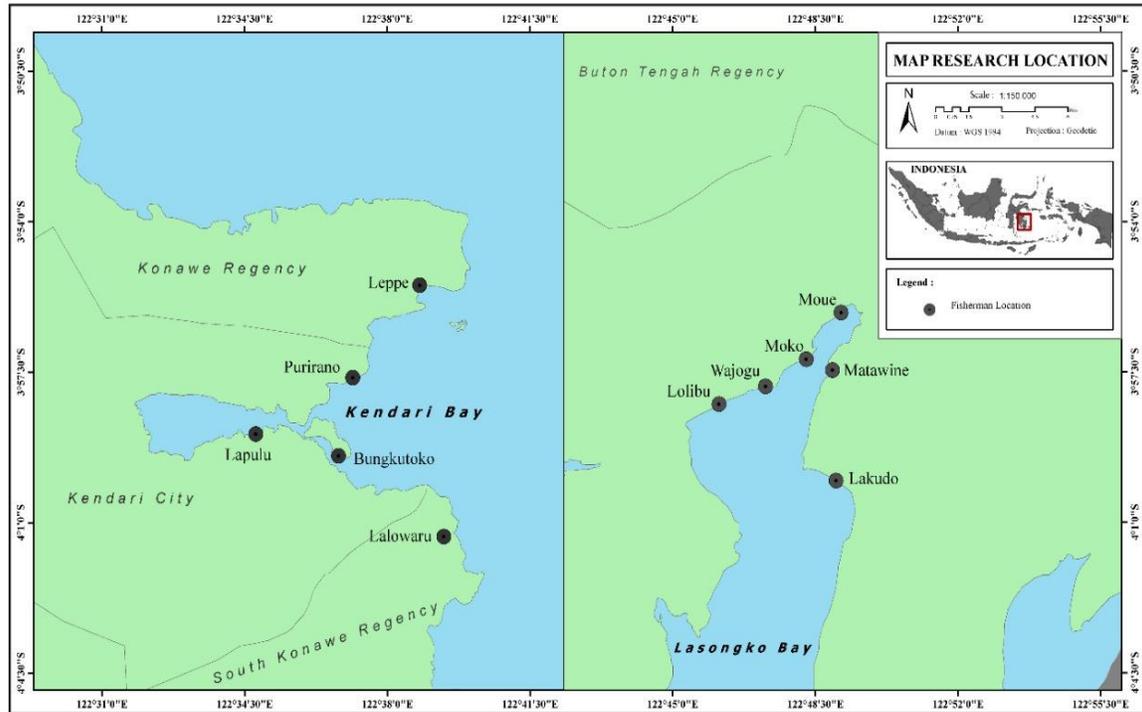
Most studies on *C. hellerii* are generally associated with invasive species; namely, Lemaitre (1995), Dineen *et al.* (2001), Mantelatto and Garcia (2001), Ozcan *et al.* (2010), Bolanos *et al.* (2012), Sant'Anna *et al.* (2012), Watanabe *et al.* (2015), Ferry *et al.* (2017), Evans *et al.* (2018) and Searles *et al.* (2019). Furthermore, research on the reproductive biology of *C. hellerii* as an invasive species was carried out in the studies of Mantelatto and Garcia (2001), Bolanos *et al.* (2012), Sant'Anna *et al.* (2012) and Ferry *et al.* (2017). Nevertheless, research on the reproductive biology of *C. hellerii* in the Western Indo Pacific region is still limited, and the only found study was conducted on the Persian Gulf coast (Bahremand *et al.*, 2018). Notably, no research has been conducted on the Indonesian waters.

Information on the aspects of reproductive biology is essential for crab management policies, such as determining the fishing season, size and phase that can be caught (Kamrani *et al.*, 2010; Ikhwanuddin *et al.*, 2012; Zairion *et al.*, 2015; Hamid *et al.*, 2016a, b, 2017; Hamid & Wardiatno, 2018a) as well as its stock assessment (Abdelhak *et al.*, 2020). Data on the aspects of reproductive biology include sex ratio, gonad development, ovigerous female, first size sexual maturity and spawning season (Kamrani *et al.*, 2010; Ikhwanuddin *et al.*, 2012; Hamid *et al.*, 2015, 2016a,b, 2017; Zairion *et al.*, 2015; Tureli & Yesilyurt, 2017; Hamid & Wardiatno, 2018a).

The waters of Lasongko and Kendari Bays are potential fishing grounds in Southeast Sulawesi for the blue swimming crab, *Portunus pelagicus* (Hamid & Wardiatno, 2018b; Hamid *et al.*, 2020). All *C. hellerii* found in this study are bycatches of blue swimming crab fisheries, which use traps and gillnets. However, the aspects of their reproductive biology are not known till now. This study was focused to determine aspects of the reproductive biology of male and female of *C. hellerii* in Lasongko and Kendari Bays. The studied aspects included sex ratio, gonad development, first size sexual maturity and spawning season.

## MATERIALS AND METHODS

This study was conducted in Lasongko and Kendari Bays, Southeast Sulawesi at eleven locations of the blue swimming crab fishermen sites (Fig.1). It was conducted from March till November 2019, and crab sampling was performed by blue swimming crab fishermen through the use of traps.



**Fig. 1.** Map of research and locations of the landing of blue swimming crab fisherman (black circles) in Lasongko and Kendari Bays, Southeast Sulawesi, Indonesia  
Modified by (Hamid & Kamri, 2019)

A total number of 600 individuals of *C. hellerii*, with males and females in addition to ovigerous females, were subjected to analysis in the present study. Crab carapace width was measured using digital calipers (Vernier Caliper 0-150 mm x 0.05) with an accuracy of 0.05 mm. The carapace of each crab was opened to detect their gonad development; for *C. hellerii* female, four stages were determined. Those stages were identified based on the morphological changes, the color and the distribution of ovaries occupying hepatic (Kamrani *et al.*, 2010; Ikhwanuddin *et al.*, 2012; Hamid *et al.*, 2016a). On the other hand, three stages were detected for the gonad development of *C. hellerii* male, based on the morphological changes, the color, and the distribution of testes and vas deferens (de Lestang *et al.*, 2003; Hamid *et al.*, 2016a).

The sex ratio of *C. hellerii* was analyzed with respect to the sampling period and the total number of crabs at each study location. Furthermore, sex ratio was determined by dividing the number of females by the number of males, using the following equation: sex ratio :  $\frac{\sum \text{Female}}{\sum \text{Male}}$  (Hamid & Wardiatno, 2018a). Statistical test ( $\chi^2$ ), using the equation of  $p = 0.05$  (Steel & Torrie, 1980), was performed to state the balance of the ratio of males to females (sex ratio 1: 1).

The progress of gonadal development of *C. hellerii* was descriptively analyzed in accordance to sex, sampling period and changes in the color of the ovigerous female

eggs. Spawning season is remarkably dependent on the presence of ovigerous female of *C. hellerii* (Karmani *et al.*, 2010; Hamid *et al.*, 2015, 2016a, 2018).

The carapace width of the first size sexual maturity was analyzed using an explorer curve of 12.0 and determined using the successive logistic model equation as follows:  $P = \frac{1}{1 + \exp[-r(L - L_m)]}$  (King, 2017); where P is the progress of the crab's gonad maturity; r is the slope of the curve; L is mean carapace width (mm), and L<sub>m</sub> is carapace width at 50% sexual maturity (mm). The width of the class used for analyzing the frequency distribution of carapace width was 3 mm.

## RESULTS

### 1. Carapace Width and Sex Ratio

The carapace widths of males *C. hellerii* in the Lasongko Bay ranged between 42.26 and 78.89 mm, while those of the females ranged between 42.78 and 68.65 mm. Furthermore, the carapace widths of the Kendari Bay ranged between 36.08 and 89.84 mm and 36.81&66.70 mm, for males and females, respectively. On the other hand, the carapace widths of the ovigerous females of *C. hellerii* in Lasongko Bay ranged between 45.28 and 63.88 mm, and in the Kendari Bay, the carapace widths ranged from 38.34 - 65.01 mm.

The sex ratio of *C. hellerii* determined in this study varied; the total of which was 1: 0.48 in the Lasongko Bay, while in the Kendari Bay, the ratio was 1: 0.57. Both figures were significantly different (p <0.05) from a sex ratio of 1:1 because they were unbalanced. The sex ratio of *C. hellerii* from each sampling in the Lasongko Bay ranged from 1:0.27 to 1:0.94, while that of the Kendari Bay ranged from 1: 0.17 to 1: 0.91 (Table 1).

**Table 1.** The monthly number and sex ratio of *C. hellerii* in Lasongko and Kendari Bays, Southeast Sulawesi, Indonesia

Sampling time	Lasongko Bay			Kendari Bay		
	Male	Female	Sex ratio (M : F)	Male	Female	Sex ratio (M : F)
March	-	-		26	5	1 : 0.19
April	0	0		34	29	1 : 0.85
May	26	19	1 : 0.70	46	28	1 : 0.61
June	23	3	1 : 0.14	11	3	1 : 0.27
July	33	9	1 : 0.27	6	1	1 : 0.17
August	13	8	1 : 0.62	20	5	1 : 0.25
September	34	32	1 : 0.94	0	1	-
October	44	15	1 : 0.34	22	20	1 : 0.91
November	26	9	1 : 0.35	36	22	1 : 0.61
Total	190	95	1 : 0.48*	201	114	1 : 0.57*

Note: \*significantly different (p<0.05) 0 = not found - = no sampling was done

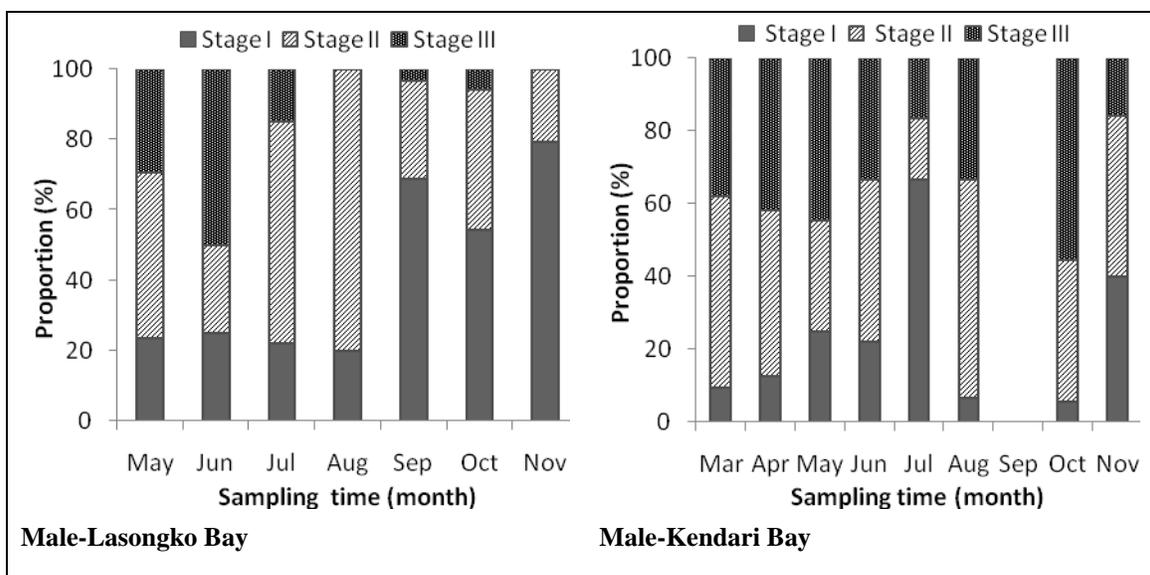
## 2. Gonad Development

The gonad development of *C. hellerii* males in the Lasongko Bay was mostly immature, while the majority of the females and ovigerous crabs had mature gonads. In the Kendari Bay, all three (males, females & ovigerous females) were dominated by immature gonads (Table 2). The gonad developments (between sampling periods) of males and females of *C. hellerii* in Lasongko and Kendari Bays were different (Fig. 2).

**Table 2.** Gonad development conditions of male, female, and ovigerous female of *C. hellerii* in Lasongko and Kendari Bays, Southeast Sulawesi, Indonesia

Sex	Undeveloped (%)	Immature (%)	Mature (%)	Total (ind.)
Lasongko Bay				
Male	18.13	71.50	10.36	193
Female	13.83	34.04	52.13	94
Ovigerous	3.39	38.98	57.63	59
Kendari Bay				
Male	23.00	48.50	28.50	200
Female	11.54	48.08	40.38	104
Ovigerous	4.55	65.91	29.55	44

The gonad development of *C. hellerii* males in Lasongko and Kendari Bays (between sampling periods) was mostly of stages I and II (immature). Furthermore, the highest proportion of stage III (mature) crabs in Lasongko Bay was found in June, while that of Kendari Bay occurred in June (Fig. 2). The gonad development of *C. hellerii* females in Lasongko Bay (between sampling periods) was mostly dominated in stage III and IV (mature), whereas that of the Kendari Bay tended to balance between stages I and II (immature) and stages III and IV (mature).



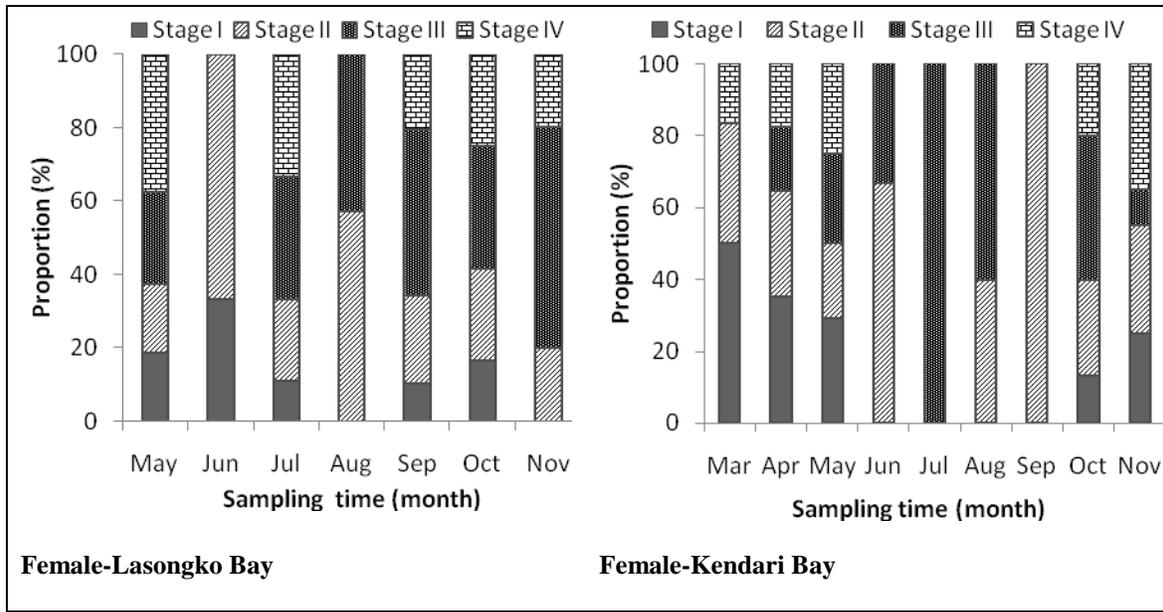


Fig. 2. The proportion of gonads stage of male and female of *C. hellerii* in each sampling time in Lasongko and Kendari Bays, Southeast Sulawesi, Indonesia

The gonad development of yellow female ovigerous of *C. hellerii* in Lasongko and Kendari Bays ranged from stages I to IV, while the other three egg colors in Lasongko Bay were found in stages III and IV. Consequently, the gonad development of the orange colored ovigerous female of *C. hellerii* in Kendari Bay consisted of stages II, III and IV, while those in brown and dark gray consisted of stages I, II and IV (Fig. 3).

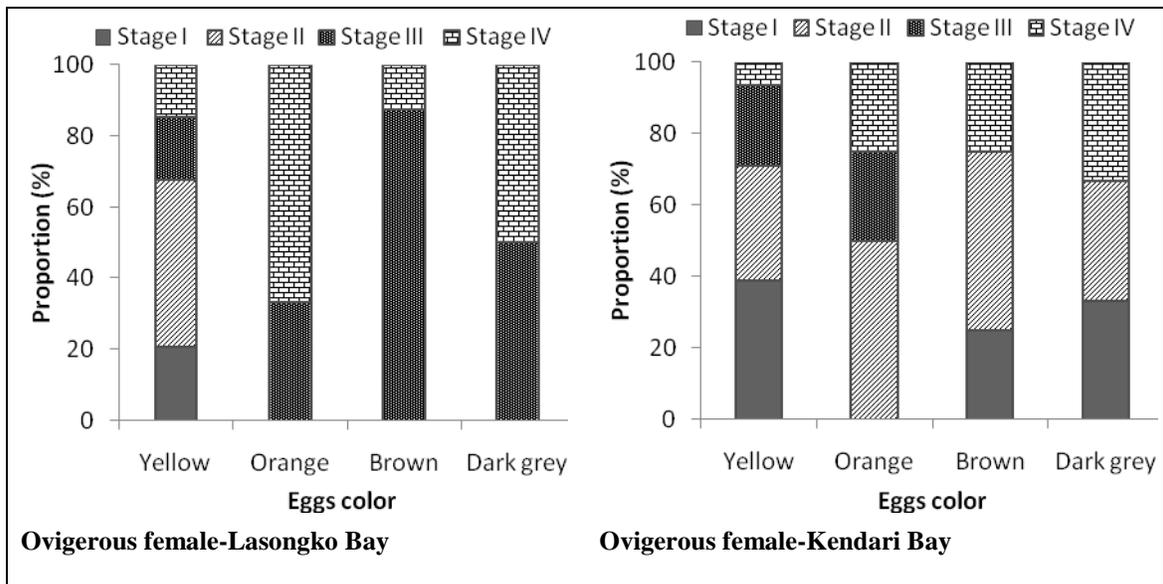


Fig. 3. The proportional gonad stages of female ovigerous *C. hellerii* based on egg color in Lasongko and Kendari Bays, Southeast Sulawesi, Indonesia

### 3. Ovigerous Female and Spawning Season

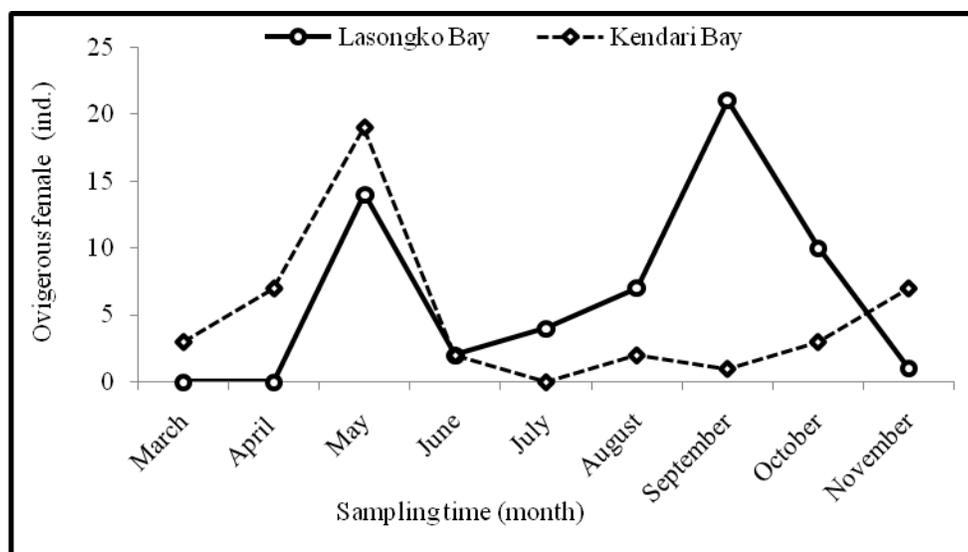
The number of ovigerous females of *C. hellerii* in Lasongko Bay was 59 (62.11%), while their number in Kendari Bay was 44 (38.60%). Ovigerous females of *C. hellerii* were found almost every month. However in July, none was found in Kendari Bay. In addition in April, female and ovigerous female crabs were not found in Lasongko Bay (Table 3 & Fig. 4).

**Table 3.** Number and proportion of ovigerous female (OF) to female of *C. hellerii* in each sampling time in Lasongko and Kendari Bays, Southeast Sulawesi, Indonesia

Sampling time	Lasongko Bay			Kendari Bay		
	Female number (ind.)	OF number (ind.)	OF Proportion (%)	Female number (ind.)	OF number (ind.)	OF Proportion (%)
March	-	-	-	5	3	60.00
April	0	0	0	29	7	24.14
May	19	14	71.43	28	19	67.86
June	3	2	66.67	3	2	66.67
July	9	4	44.44	1	0	0
August	8	7	87.50	5	2	40.00
September	32	21	65.63	1	1	100.00
October	15	10	66.67	20	3	15.00
November	9	1	11.11	22	7	31.82
Total	95	59	62.11	114	44	38.60

Note: 0 = not found    - = no sampling was done

The spawning season of *C. hellerii* in Lasongko and Kendari Bays due to the presence of ovigerous females tends to exist around the year. However, the peak spawning season of *C. hellerii* in Lasongko Bay occurs during May and September, while that of Kendari Bay only occurs in May (Fig. 4).



**Fig. 4.** The presence of *C. hellerii* ovigerous female in each month in Lasongko and Kendari Bays, Southeast Sulawesi, Indonesia

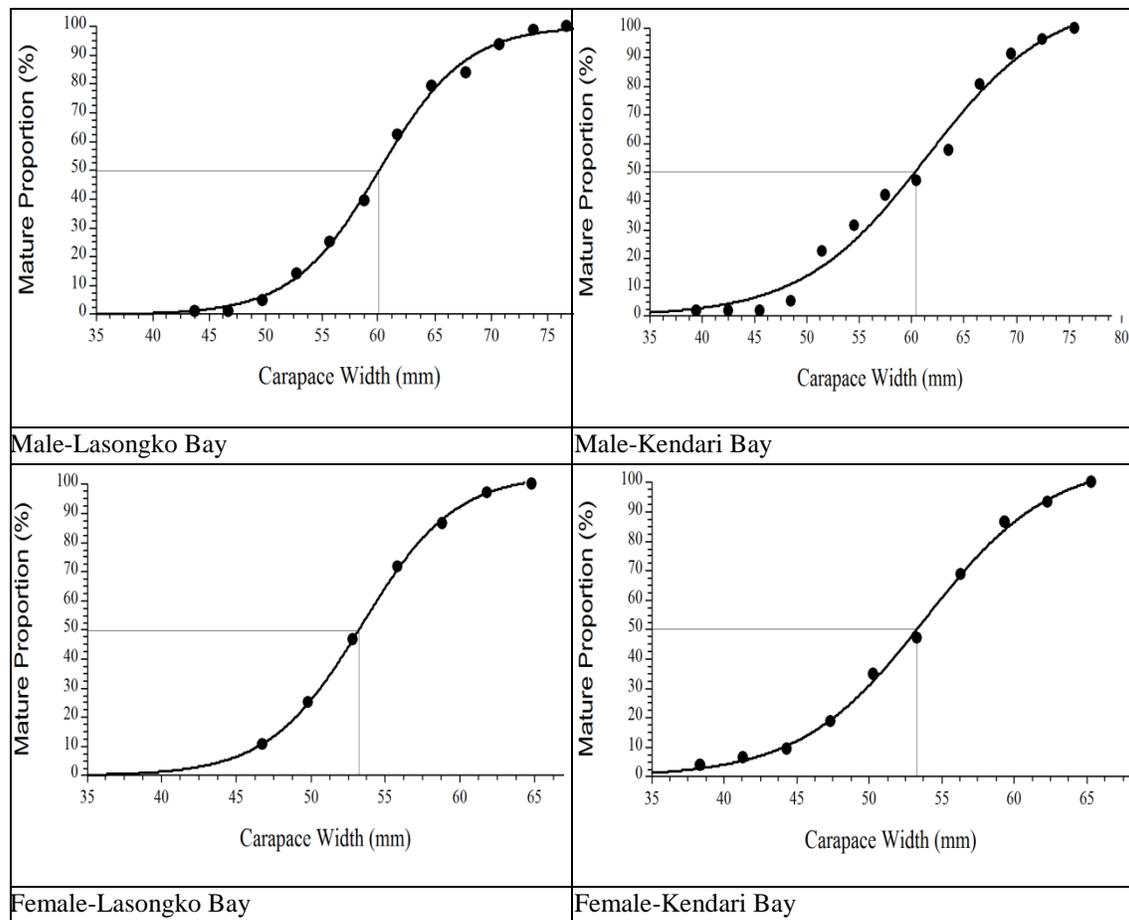
**4. First size sexual maturity**

The first size at 50% of sexual maturity (CW50%) in *C. hellerii* male in Lasongko Bay was 60.04 mm CW, while in the female, it was 53.12 mm CW. On the other hand, in Kendari Bay, the sizes were 60.23 mm CW and 53.24 mm CW, respectively (Table 4 & Fig. 5).

**Table 4.** Initial sizes of sexual and gonad maturity of male and female in Lasongko and Kendari Bays Southeast Sulawesi, Indonesia

Sex	First size sexual maturity-CW50% (mm)	
	Lasongko Bay	Kendari Bay
Male	60.04	60.23
Female	53.12	53.24
<b>CW-size minimum gonad maturity (mm)</b>		
Male	42.26	37.98
Female	36.81	45.43

The smallest identified carapace size of *C. hellerii* ovigerous females in Lasongko Bay was 45.28 mmCW, while that of Kendari Bay was 38.34 mmCW. Furthermore, the smallest carapace width of *C. hellerii* mature gonad is shown in Table (4).



**Fig.5.** Logistic curve estimating first size at 50% sexual maturity of *C. hellerii* male and female in Lasongko and Kendari Bays, Southeast Sulawesi, Indonesia

## DISCUSSION

The total sex ratio of *C. hellerii* in Lasongko Bay was smaller than that of Kendari Bay (Table 1). Furthermore, the detected sex ratio of *C. hellerii* in some waters varied, and the females were more than the males (Table 5). The sex ratio of *C. hellerii* obtained in this study are greater than those of **Sant'Anna et al. (2012)**, **Bolanos et al. (2012)** and **Ferry et al. (2017)**, whereas it is smaller than the sex ratio determined in the study of **Bahreman et al. (2018)** as presented in Table (5) In comparison, the obtained sex ratio of *C. hellerii* is lower than those detected with respect to *C. natator* (**Sumpton, 1998**; **Vidhya, 2016**) and *C. bimaculata* (**Doi et al., 2008**), but higher than the sex ratio of *C. feriata* (**Nieves et al., 2015**) and *C. anisodon* (**Hamid & Wardiatno, 2018a**, Table 5).

**Table 5.** Sex ratio and spawning season of *C. hellerii* and other species at several water locations

Location	Species	Sex ratio	Spawning season	Source
São Vicente, Brazil	<i>C. hellerii</i>	1: 0.32	Year-round	Sant'Anna et al. (2012)
Caribia sea, Venezuela	<i>C. hellerii</i>	1 : 0.46	Year-round	Bolanos et al. (2012)
Martinique, France	<i>C. hellerii</i>	1: 0.42	-	Ferry et al. (2017)
Persian Gulf, Iran	<i>C. hellerii</i>	1: 0.74	Year-round	Bahreman et al. (2018)
Lasongko Bay, Indonesia	<i>C. hellerii</i>	1 : 0.48	Year-round	This study (2021)
Kendari Bay, Indonesia	<i>C. hellerii</i>	1 : 0.57	Year-round	
Moreton Bay, Queensland	<i>C. natator</i>	1: 0.55	Year-round	Sumpton (1998)
Mannar Bay, India	<i>C. natator</i>	1 : 0.62	Year-round	Vidhya (2016)
Tokyo Bay, Japan	<i>C. bimaculata</i>	1: 2.07	Not year-round	Doi et al. (2008)
Northeastern New Zealand	<i>C. japonica</i>	1: 0.65	Not year-round	Wong (2013)
Karnataka, India	<i>C. feriata</i>	1: 1	Year-round	Dineshbabu (2011)
San Miguel Bay, Philippines	<i>C. feriata</i>	1: 0.50	Year-round	Nieves et al.(2015)
Lasongko Bay, Indonesia	<i>C. anisodon</i>	1 : 0.38	Year-round	Hamid and Wardiatno (2018a)

The sex ratio of *C. hellerii* in this study varied with the sampling periods. This is identical to the results obtained in the Caribbean Sea, Venezuela (**Bolanos et al., 2012**), on the Persian Gulf coast, Iran (**Bahreman et al., 2018**), and also in crabs such as *C. anisodon* (**Hamid & Wardiatno, 2018a**). Additionally, the present finding is similar to that detected with respect to *C. natator* in India (**Kannathasan & Rajendran, 2011**). The variation in the sex ratio may be attributed to the tendency of crabs to spawn and forage at Portunidea, the differences in sampling procedures and variations in seasons (**Kannathasan & Rajendran 2011**; **Hamid et al., 2016a**; **Hamid & Wardiatno, 2018a**). The differences in longevity and growth rates can also cause the aforementioned phenomenon (**Doi et al., 2008**).

The pattern of gonad development in the *C. hellerii* of Lasongko and Kendari Bays was identified from the sex, sampling period, and the change in eggs' color of ovigerous females which tend to be different. The gonad development of *C. hellerii* male in Lasongko and Kendari Bays was dominated by immature gonads, however the percentage did not match. Consequently, the gonad development in Lasongko Bay was higher than that in Kendari Bay (Table 2). The gonad development of female and ovigerous female of *C. hellerii* in Lasongko Bay was dominated by mature gonads, while in Kendari Bay, immature gonads were dominant (Table 2).

The gonad development of *C. hellerii* male in Lasongko and Kendari Bays, depending on the sampling period, was generally dominated by immature gonads, while the proportions between the two were different. The proportion of immature male of *C. hellerii* in Lasongko Bay ranged from 71.00- 100%, while in Kendari Bay, it ranged from 56.00- 84.00%. In addition, the proportion of male crabs in Lasongko Bay with matured gonads was lower than that detected in Kendari Bay (Fig. 2). The gonad development of *C. hellerii* female in Lasongko Bay, according to the sampling period, was dominated by mature gonads, and ranged from 58.33- 80.00%. While, that of Kendari Bay was dominated by immature gonads, with proportions ranging from 55.00-100% (Table 2). The gonad development of male and female of *C. hellerii* were variant, which is similar to the results found in the study of **Bahremand *et al.* (2018)** on the Persian Gulf coast, Iran. Moreover, the current result coincides with those of **de Lestang *et al.* (2003)**, **Ikhwanuddin *et al.* (2012)** and **Hamid *et al.* (2016a)** who deduced the same finding in their works on crabs; namely, *P. pelagicus* and that of **Wong (2013)** conducted on *C. japonica*.

The proportion of *C. hellerii* ovigerous female in Lasongko and Kendari Bays with immature gonads were low, recording values of 3.39% and 4.55% (Table 2). This indicates that most ovigerous females of *C. hellerii* found in this study had embryonic and gonadal developments though they did not occur simultaneously. The embryonic development of ovigerous female of *C. hellerii* is characterized by an egg color change; from yellow to dark gray during the incubation period (**Hamid *et al.*, 2015, 2016c**). Consequently, the proportion of (gonad development) ovigerous female of *C. hellerii* in Kendari Bay in stage IV increased when the color of the crab's eggs changed from yellow to dark gray (Fig. 3).

The development of gonads and embryos recorded in ovigerous females of *C. hellerii* indicates that these crabs spawned partially. Sperm that was not used by ovigerous female of *C. hellerii* was stored in the seminal receptacle to support subsequent gonad development (**Dineen *et al.*, 2001; Sant'Anna *et al.*, 2012**). It is worthy to mention that, this is a strategy used to increase the success of larval production (**Dineen *et al.*, 2001; Sant'Anna *et al.*, 2012; Hamid *et al.*, 2015**).

The spawning season of *C. hellerii* in Lasongko and Kendari Bays occurs throughout the year, however that of ovigerous females in Lasongko Bay was not

found in July. Furthermore, in July, the mature gonads of the females (stage III, Fig. 2) could be found. In addition, 7 ovigerous females of *C. hellerii* in Kendari Bay were found in July 2018 (**Hamid, data not published**). In previous studies (**; Bolanos et al., 2012; Sant'Anna et al., 2012; Bahremand et al., 2018**), it was reported that *C. hellerii* spawned throughout the year (Table 5).

The initial sizes of sexual maturity (CW50%) in male and female of *C. hellerii* in Lasongko and Kendari Bays were relatively similar, and both showed that male sizes were larger than those of the females (Table 4 & Fig. 5). The size of CW 50% female of *C. hellerii* in this study relatively concurs with those detected on the Persian Gulf coast, Iran, which was 54.55 mm CW (**Bahremand et al., 2018**). The smallest recorded size (sexual maturity) of *C. hellerii* ovigerous female is smaller than those of **Stephenson et al. (1957)** in Australia which were 47 mm and matured in the laboratory at 77 mm CW in 12 months (**Dineen et al., 2001**).

The smallest size of *C. hellerii* ovigerous female found in Lasongko Bay was bigger than that found in Kendari Bay. In comparison, it is bigger than the sizes detected on the Persian Gulf coast, Iran, which was 38 mm CW (**Bahremand et al., 2018**). Therefore, the smallest size of mature gonads in the *C. hellerii* of Lasongko Bay was greater than that found in Kendari Bay because the female crabs in Kendari Bay were larger than those found in Lasongko Bay (Table 4).

## CONCLUSION

The total sex ratio in Lasongko and Kendari Bays were 1:0.48 and 1:0.57, respectively, and the temporally sex ratio of *C. hellerii* varied. The gonad development of *C. hellerii* male in Lasongko Bay were mostly immature, while females and ovigerous females were dominated by mature gonads. Meanwhile, in Kendari Bay, all the three sexes tend to be dominated by immature gonads, and the gonad development of male and female of *C. hellerii* temporarily varied. The first sizes of sexual maturity in the male and female of *C. hellerii* in Lasongko Bay were 60.04 mmCW and 53.12 mm CW, respectively, while those of Kendari Bay were 60.23 mmCW and 53.24 mmCW, respectively. The spawning season of *C.hellerii* tends to occur throughout the year. Consequently, the data obtained in this study can be used for fishing management, specifically for determining the size of crabs that can be caught and the fishing season of *C.hellerii* in Lasongko and Kendari Bays and the Southeast Sulawesi waters.

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