Thesis for the Degree of Master of Engineering

Growth and reproduction of the two-spot swimming crab *Charybdis bimaculata* (Miers: 1886) in the coastal area of the Goheung, Korea

by

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Pukyong National University

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(고흥 연안에 서식하는 두점박이민꽃게 *Charybdis bimaculata*의 성장과 생식)

Advisor: Prof. Chul-Woong Oh

by
Sung Mok Hong

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요약

본 연구는 2020년 2월부터 2021년 2월까지 매월 남해 고흥 연안에 서식하는 두점박이민꽃게 (*Charybdis bimaculata*) 개체군을 채집 하여 월별 출현 개체의 체장빈도분포를 이용하여 성장을 추정하였고, 생식유형, 포란수, 생식 산출력, 군성숙 체장 등 생식생물학적 특성을 조사하였다.

연구기간동안 채집된 게는 총 6,927마리였으며, 이 중 수컷과 암컷의 개체수는 각각 2,799 (40.41%), 4,128 (59.59%)로 암컷의 수가 월등히 더 많았다. 생식소 숙도 지수(Gonadosomatic index: GSI)는 7월에 최대값을 보였으며 12월에 최소값을 보였다. 포란 암컷은 4월에서 9월 그리고 11월에 관찰되었으며, 두점박이민꽃게의 산란시기는 GSI와 포란율이 높은 시기인 6 ~ 8월로 나타났다.

두점박이민꽃게의 포란수는 12,668 ~ 37,967개로, 난 단계별 두흉갑폭 (CW)에 따른 포란수 (EN)와의 관계식은 미발안난 $\ln EN = 1.59 \ln CW + 4.92$ $(\mathrm{n} = 39, r^2 = 0.3141, P < 0.001)$, 발안난 $\ln EN = 1.19 \ln CW + 6.11$ $(\mathrm{n} = 17, r^2 = 0.2355, P < 0.05)$로 나타났다. 두 난 단계의 회귀직선의 기울기에 있어서는 유의한 차이가 없었지만 (ANCOVA, *F* = 1.96, *df* = 1, 52, *P* > 0.05) 절편에는 유의한 차이를 보였다 (ANCOVA, *F* = 21.27, *df* = 1, 53, *P* < 0.001). 난 단계별 배갑면적(AA)에 따른 포란수와의 관계식은 미발안난 $\ln EN = 0.57 \ln AA + 7.38$ $(n = 39, r^2 = 0.2213, P < 0.01)$, 발안난 $\ln EN = 0.51 \ln AA + 7.55$ $(n = 17, r^2 = 0.1759, P > 0.05)$ 로 나타났다. 군성숙체장(CL50)은 20.93 ㎜ CW였으며, 생식 산출력 (reproductive output: RO)은 0.098이었다.

두흉갑폭(CW)와 전중(BW)의 관계는 수컷 $BW = 0.000048CW^{3.3536}$, 암컷은 $BW = 0.000046CW^{3.354}$ 이었다. 평균 두흉갑폭은 수컷은 22.24 ± 0.18 ㎜, 암컷은 20.62 ± 0.14 ㎜이었다. ELEFAN 프로그램을 이용하여 추정한 Von Bertalanffy 성장식의 매개변수는, 이론적 최대갑폭(CW_∞), 성장계수(K), 성장비교지수 (φ')는 각각 수컷 43.65 ㎜, 0.95 yr^{-1}, 3.26 이며 암컷은 40.25 mm, 0.95 yr^{-1}, 3.11로 추정되었다.

1. Introduction

Located in the central part of the southern Sea, Goheung coast is habitat for a variety of fishery resources because abundant nutrients are introduced from land and high primary productivity is maintained under the influence of the southern sea of Korea (NFRDI, 2002). During the study period (February 2020 to February 2021), 25 species of crustaceans were collected, of which the two-spot swimming crab *Charybdis bimaculata* appeared throughout the year and dominated in February, June and July 2020 (Table 1).

The carapace of the two-spot swimming crab is close to a wider hexagonal shape. The species has 6 relatively blunt spines on the front, of which, two middle spines are the most protruding and are located slightly lower than those on both sides. There are a total of 6 spines on the anterior and lateral edges of the carapace. Of these, the rearmost spines are the largest except for the back of the eyes. The dorsal area is slightly convex, with relatively distinct and short hair. There are spots on both branchial regions.

The abdomen of the male is composed of 5 segments with 3~5 abdominal segments attached, and the female consists of 7 segments. When the two-spot swimming crab is alive, the back of the carapace is light purple and the spots are dark purple.

Study on the two-spot swimming crab have been conducted in several countries. In Japan, ecological surveys focus on the assessment of growth, reproduction and resource stock (Doi *et al.*, 2008; Narita *et al.*, 2008). In China, the population genetic structure and larval dispersal strategy (Han *et al.*, 2015) and the characterization of the complete mitochondrial genome sequence (liu *et al.*, 2018) have been studied. In Korea, the distribution of larvae (Lee, 2018) and a study on the mitochondrial genome was conducted in the same manner as in China (Kim *et al.*, 2018).

In Korea, two-spot swimming crabs inhabit in the western and southern seas. Though they have been proven to be useful as biological resources, studies of the ecology and reproductive characteristic of *C. bimaculata* are little.

The purposes of this study are to 1) estimate their growth, and 2) reveal the reproductive traits. This study could provide the foundation for the resource protection and management of the two-spot swimming crabs.

Table 1. Crustaceans collected in Goheung during the study period (February 2020 to February 2021).

	2020											2021		Total
Speicies	F	M	A	M	J	J	A	S	O	M	D	J	F	
Alpheus japonicus	480	86	77	216	4	10	9	44	123	405	241	340	24	2059
Alpheus rapax	66	12	36	100	11	5	3	18	6	29	5	8		299
Arcania undecimspinosa			2	2	2		1	1	16	94		15		133
Batepenaeopsis tenella	880	422	12	26	3	4	763	1953	24	3130	3753	1157	292	12419
Charybdis bimaculata	12826	867	276	1400	585	600	64	41	55	714	153	34	252	6927
Charybdis japonica			1		7	11	33		6	8	1			67
Crangon hakodatei	619	4044	322	2264			3	5	418	2096	8685	1818	1028	21302
Diogenes edwardsii			8		3	27	13	13	6	1	1	2		74
Entricoplax vestita			4			2		1	2	3	2	1		15
Eriocheir sinensis												2		2
Eucrate crenata				2		2		2	8	16	3	34		67
Heikeopsis japonica			3	4	34	56	7	2	7	25	1	19		158
Heptacarpus futilirostris													1	1
Latreutes planirostris	9	54	11	26		30	1		3	15	137	412	80	778
Leptochela gracilis	2	95		10				1			105	327	3	543
Lysmata vittata	1		18	3					2	1	43	52	1	121
Metapenaeus joyneri	437		3	2				12	51	55	681			1241
Oratosquilla oratoria	70	419	680	349	103	46	31	162	481	435	106	11	9	2902
Palaemon carinicauda	1	7					4			7		183		202
Palaemon modestus	1		15		1		3	1				127	40	188
Parthenope validus						2								2
Pinnaxodes major			1						1			5		7
Portunus trituberculatus						7	4	27	12	1	28	1		80
Trachysalambria curvirostris	182	497	41	415	543	115	43	436	23334	27	356	12		5001
Tritodynamia rathbuni			1	1					1		5	19		27
Total	4574	6503	1511	4820	1296	977	982	2719	3556	7062	14306	4579	1730	54615

2. Materials and Methods

2-1. Sample collection

The study was conducted monthly from February 2020 to February 2021 in the Boseong Bay in Goheung (Fig. 1), using a beam trawl (mesh: 18 mm, beam: 8 m, total length: 22 m). YSI is used to measure water temperature and salinity every month during the study period. Collected samples are preserved in 10% neutralized formaldehyde.

2-2. Sex ratio

Sex was classified according to the shape characteristics of the stomach (Kim, 1973). The sex ratio of the sample was expressed as the proportion of females. The carapace width (CW), the distance between lateral spines, was measured to the nearest 0.01 mm using Vernier calipers. A microscope (Zeiss Stemi SV-6) and ZEN 3.1 program were used to measure the abdominal area.

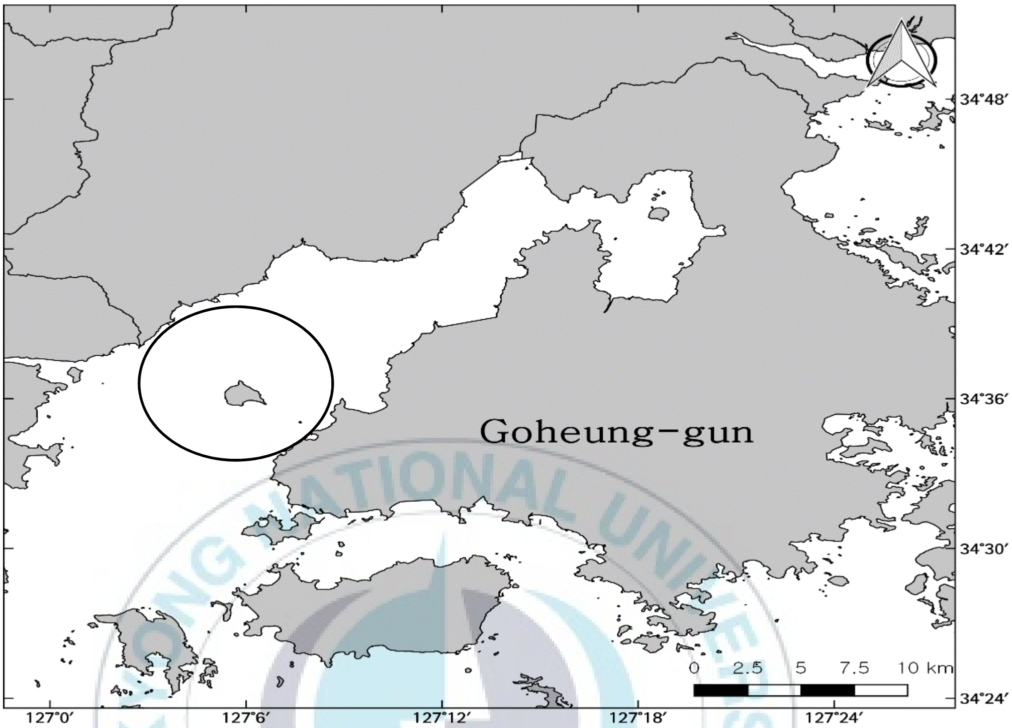


Fig 1. Map showing sampling area of *Charybdis bimaculata* in Goheung.

2-3. Length-frequency distribution

Population structure was revealed through length-frequency distribution of both sexes. Length-frequency distributions of all individuals were constructed using 5 mm intervals of CW.

2-4. Morphometric relationship

Morphometric relationship was examined between 1) carapace width (CW) and body weight (BW) 2) CW and abdominal area (AA). The CW and AA were measured as mentioned in the sex ratio part, and BW was measured using a digital balance to the nearest 0.01 g. A linear regression was analyzed to reveal the relationship between 1) CW and BW 2) CW and AA using natural log transformed data. The equation is:

$$\ln BW = a + b \ln CW$$

$$\ln CW = a + b \ln AA$$

where, *a* and *b* was intercept and slope, respectively.

2-5. Ovarian examination

An ovarian observation was conducted to investigate the female reproductive cycle. The maturity of ovary was expressed in five stages according to Yeon (1997) (I) immature; (II) development; (III) maturity; (IV) spent; (V) recovering. In order to measure the dry weight, the body and ovarian of each individual were dried for 48 h at 60°C and measuring to the nearest 0.0001 g using a digital electronic balance. The gonadosomatic index (GSI) was determined through the following equation;

$$\mathrm{GSI} = \frac{Ovarian\ dry\ weight}{Body\ dry\ weight} \times 100$$

2-6. Reproductive output (RO)

The egg stage was recorded as two stages: (1) non-eyed and (2) eyed egg. In order to count the number of eggs, the eggs in the abdomen are carefully separated using fine forceps. Afterwards, all of the eggs were counted immediately to prevent loss. In order to measure the dry weight, female and egg were dried for 48 h at 60°C and measuring to the nearest 0.0001 g using a digital electronic balance. Reproductive output (RO) was determined as follows:

$$\mathrm{RO} = \frac{Total\ mass\ of\ egg\ batch}{Mass\ of\ female}$$

2-7. Relationship between the number of eggs (EN) and carapace width (CW) & Abdominal area (AA)

The relationships between 1) the number of eggs (EN) and carapace width (CW) and 2) EN and abdominal area (AA) were estimated by ovigerous females carrying non-eyed and eyed eggs. Linear regression analysis using natural log transformed data of CW and EN and AA was used to determine the relationship between body size and the number of eggs by the following equation:

$$\ln EN = a + b \ln CW$$

$$\ln EN = a + b \ln AA$$

Where a was intercept, b was slope. Slope and intercept of linear regression were compared to investigate brood loss.

2-8. Size at sexual maturity (CW_{50})

The proportion of sexually mature female was measured based on individuals with gonadal levels of 3 or higher. The proportion of mature females according to the carapace width was fitted to the logistic equation:

$$P = 1/(1 + e^{-(a+bCW)})$$

As parameters a and b, correlation analysis was performed after linearization the variables P and CW. Size at sexual maturity (CW_{50})which is defined as the proportion of 0.5 of crabs in the reproductive state, was calculated from ratio between the constants a and b:

$$CW_{50} = -\frac{a}{b}$$

2-9. Growth

The length-frequency distributions were constructed using 5 mm length intervals of carapace width. Growth was described using the modified Von Bertalanffy growth function (VBGF) (Pauly and Gaschüz, 1979):

$$CW_t = CW_\infty[1 - e^{[-K(t-t_0)-\left(\frac{CK}{2\pi}\right)sin2\pi(t-t_s)]}]$$

Where CW_∞ is the asymptotic carapace width, K is the intrinsic growth rate, t_0 is the age at which the length of crabs is 0, C is the amplitude of seasonal growth oscillation, t_s is the age at the beginning of growth oscillation, and WP $= t_s + 0.5$ is the time of year when growth is slowest.

Growth curves were estimated from the length-frequency distributions using the ELEFAN program in FISAT II program (Gayanilo *et al*., 2005), a non-parametric method to fit the modified VBGF through modes. The R_n value gives an estimator of the goodness of fit. ELEFAN estimates the growth parameters (L_∞, K, C and WP) without standard errors. t_0 estimates cannot be obtained solely from the length-frequency data (Pauly, 1987), so ELEFAN routines alone allow their calculation. Thus t_0 was estimated using the relation described (Lopes Veiga, 1979).

$$t_0 = \frac{1}{K}(\ln\frac{CW_\infty - CW_n}{CW_\infty})$$

Growth performance of *C. bimaculata* was using a growth performance index

(φ') (Pauly and Munro, 1984):

$$\varphi' = 2log_{10}CW_{\infty} + log_{10}K$$

The growth performance index is preferred for growth comparison rather than comparison of CW_{∞} and K individually, because these two parameters are correlated. The growth performance index is more robust than either CW_{∞} or K individually as it.

2-10. Statistical analysis

Chi-square test was performed to determine whether the observed ratio of both sexes differed from the expected 1:1 ratio. The differences in the length-frequency distribution between male and female were determined by the Kolmogorov-Smirnov two-sample test. The difference in CW between sexes was tested by Mann-Whitney *U*-test. Linear regression analysis with natural log transformed data was used for investigate the relationship between carapace width – body weight on each sex and abdominal area – fecundity and carapace width –fecundity. Analysis of covariance (ANCOVA) was used to determine the difference in intercept and slope of regressions on relation between; 1) carapace width and body weight of both sexes and 2) carapace width and the number of eggs of non-eyed and eyed eggs. The monthly mean variation in the GSI was tested by one-way analysis of variance (ANOVA). MINITAB (v. 18) and SPSS (v. 25) were used for all statistical analysis and mean value was presented with 95% confidence limit.

3. Results

3-1 Sex ratio

A total of 6,927 specimens (4128 females and 2799 males) of *C. bimaculata* was collected during the sampling period. Proportion of females was significantly greater than male ($\chi^2 = 58.83, df = 12, P < 0.001$) (Fig. 2).

3-2 Length-frequency distribution

The mean CW was 22.24 ± 6.25 mm (range 4.78 – 38.56 mm) in males and 20.62 ± 5.11 mm (range 7.53 – 34.99 mm) in females. There was a significant difference in mean CW between males and females (Mann Whitney *U*-test, $P < 0.001$). A statistical analysis showed a significant difference in the length-frequency distribution of the both sexes (Kolmogorov-Smirnov two sample test, $P < 0.001$) (Fig. 3).

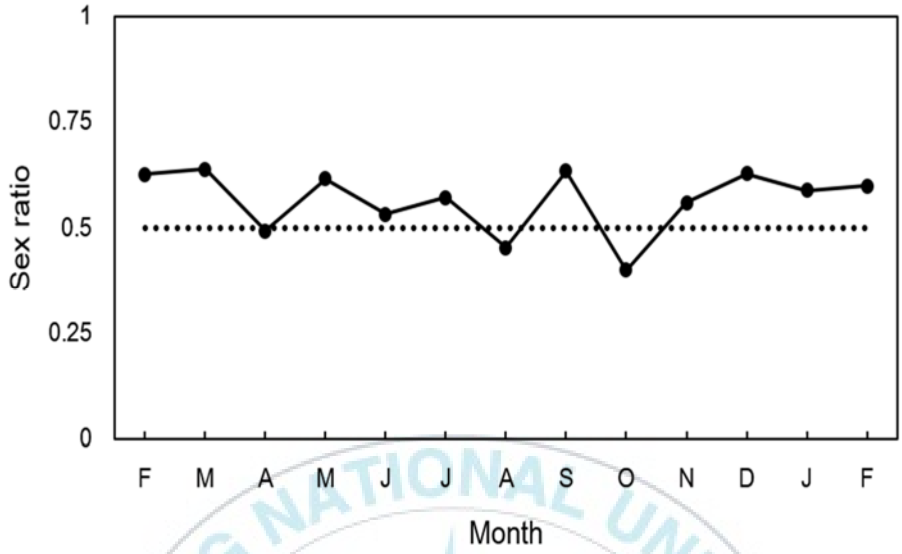


Fig 2. Variations in the female/male ratio in monthly samples of *Charybdis bimaculata* (Miers, 1886)

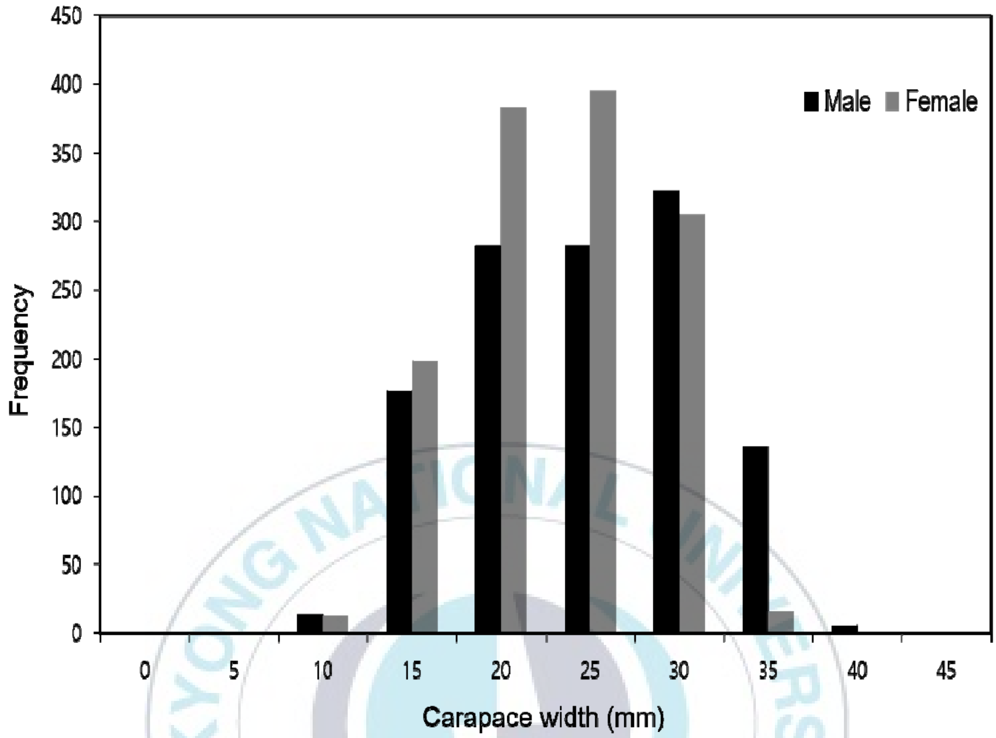


Fig 3. Length-frequency distribution of males and females of *Charybdis bimaculata* (Miers, 1886).

3-3 Morphometric relationship

The mean body weight (BW) was 2.20 ± 1.90 g (range 0.03 – 10.39 g) in males and 1.52 ± 1.13 g (range 0.03 - 6.23 g) in females. The mean abdominal area (AA) of males was $30.13 \pm 15.05\ \mathrm{mm}^2$ varied from 3.75 – 81.39 mm^2 and females $43.58 \pm 47.91\ \mathrm{mm}^2$ varied form range 5.21 – 198.94 mm^2.

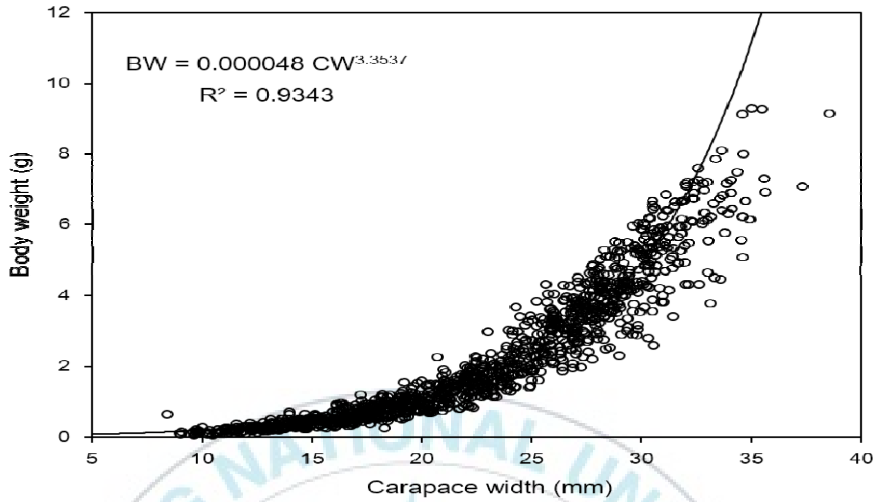
The relationships between 1) carapace width (CW) and BW 2) CW and AA of males and females were analyzed by linear regression using log transformed data (Table 1,2).

We found a significant difference in the slope of the linear regression between ln CW and ln BW for the both sexes (ANCOVA: F =2158.76, df = 1, 2528, P < 0.001) (Fig. 4). ANCOVA revealed significant difference in the ln AA as functions of ln CW (F = 359.51, df = 1,530, P < 0.001) (Fig. 5).

Table 2. Linear regression of carapace width (CW) and body weight (BW) of male and female, mean ± standard deviation of carapace width and body weight of *Charybdis bimaculata* (Miers, 1886).

Sex	Linear regression	CW	BW
Male	$\ln BW = 3.3537\ (\pm 0.0248) \ln CW - 9.9316$ $(n = 1220, r^2 = 0.9376, P < 0.001)$	22.24 ± 6.25	2.20 ± 1.90
Female	$\ln BW = 3.3540\ (\pm 0.0247) \ln CW - 9.9721$ $(n = 1312, r^2 = 0.9339, P < 0.001)$	20.62 ± 5.11	1.52 ± 1.13

a.



b.

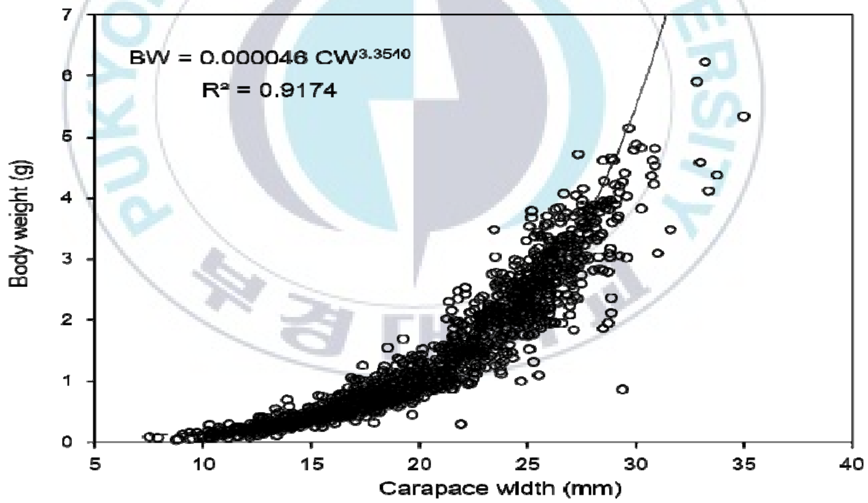
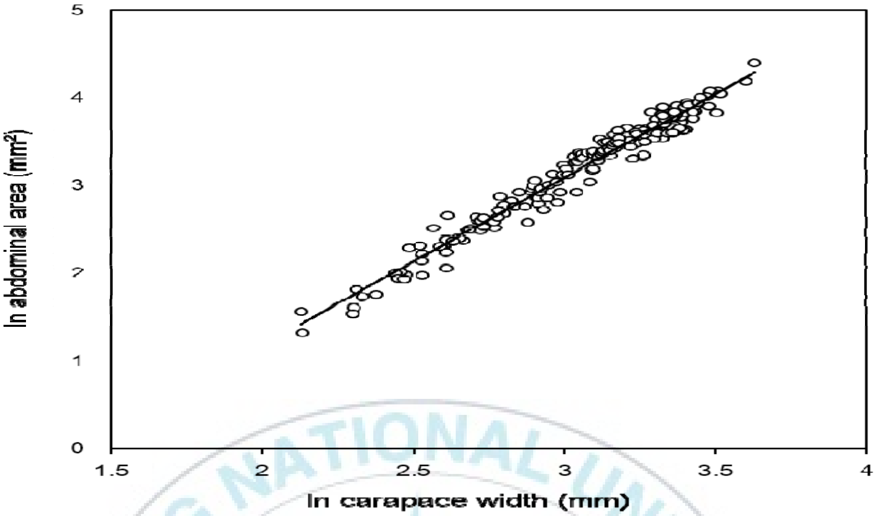


Fig 4. Relationship between carapace width (CW) and body weight (BW) of *Charybdis bimaculata* (Miers, 1886). a: males, b: females.

Table 3. Relationship between Carapace width (CW) and Abdominal area (AA) with natural log transformation *Charybdis bimaculata* (Miers, 1886), mean ± standard deviation of carapace width and abdominal area.

Sex	Linear regression	CW (mm)	AA (mm^2)
Male	$\ln AA = 1.9180\ (\pm 0.0244) \ln CW - 2.6651$ $(n = 200, r^2 = 0.9698, P < 0.001)$	22.24 ± 6.245	30.13 ± 15.05
Female	$\ln AA = 3.0501\ (\pm 0.0484) \ln CW - 5.2454$ $(n = 334, r^2 = 0.9229, P < 0.001)$	20.62 ± 5.11	43.58 ± 47.91

a.



b.

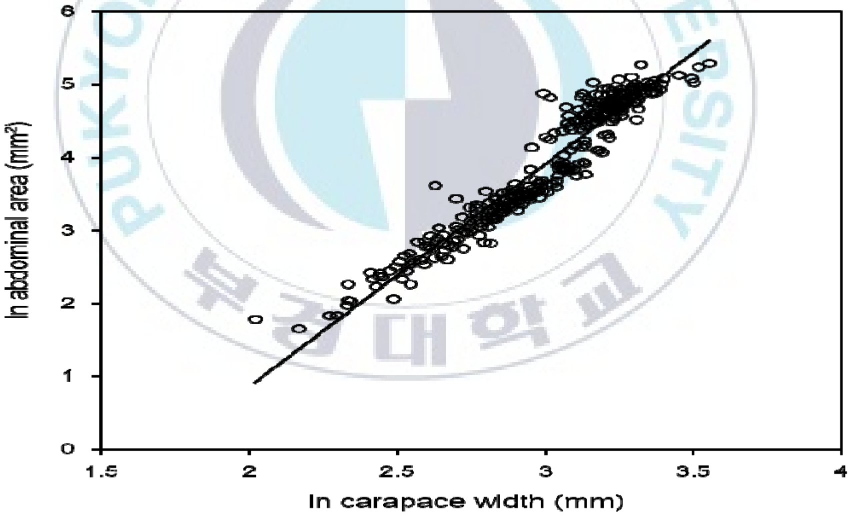


Fig 5. Relationship between carapace width (CW) and abdominal area (AA) with natural log transformation of *Charybdis bimaculata* (Miers, 1886). a: males, b: females.

3-4 Breeding period

The breeding period was determined by the ovarian stages of females and the gonadosomatic index (GSI). *C. bimaculata* carrying eggs was observed from April to October and November (Fig. 6).

The GSI started to increase in April, reached a peak in June (7.55), and decreased to its lowest value in December (4.62). There was a significant difference in the monthly GSI among months $(F = 3.97, df = 12, P < 0.001)$ (Fig. 7).

There was a significant difference in the GSI value with non-eyed and eyed egg stages (Student *t*-test, $t = 2.36, df = 25, P < 0.05$) (Fig. 8). This indicated that *C.bimaculata* is consecutive breeders.

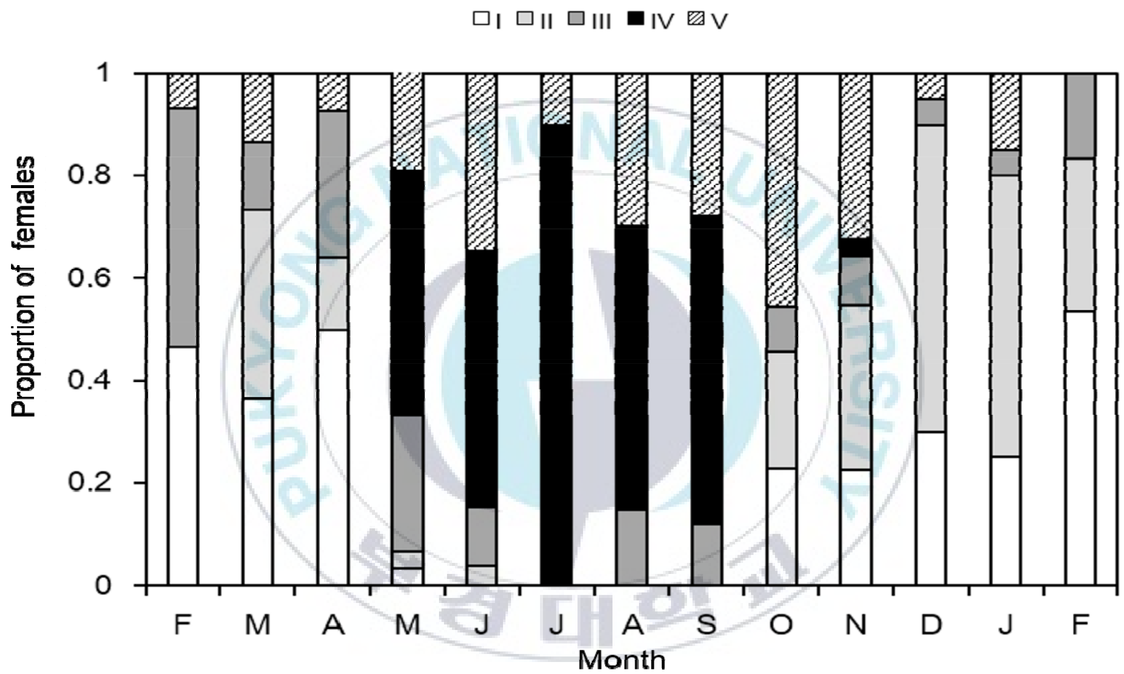
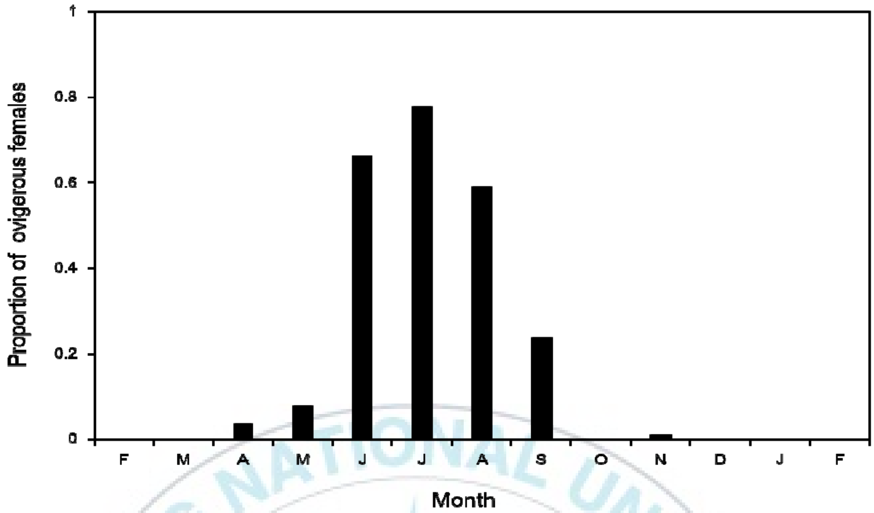


Fig 6. Variations in proportion of female *Charybdis bimaculata* (Miers, 1886), according to the five ovarian stages, in monthly samples taken from February 2020 to February 2021.

a.



b.

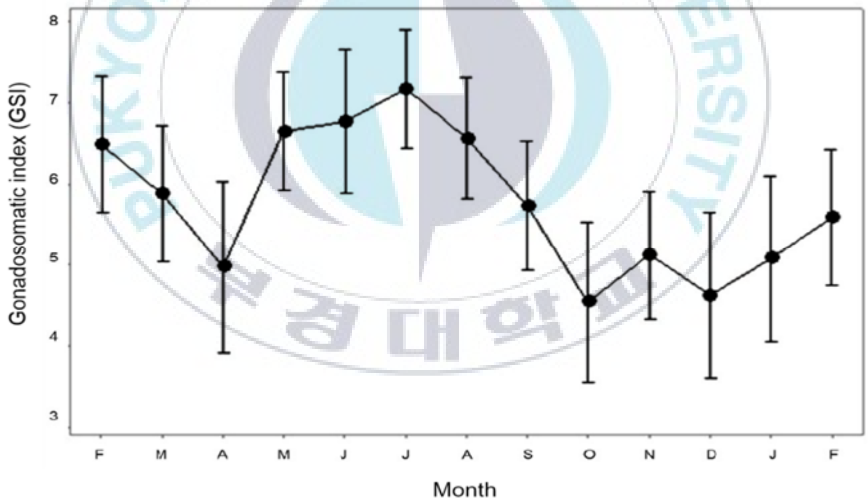


Fig 7. Monthly change of *Chyarybdis bimaculata* from February 2020 to February 2021. a: in the proportion of ovigerous females; b: in gonadosomatic index.

3-5 Fecundity and reproductive output

Total 56 ovigerous females, 39 females with mean carapace width 25.79 ± 2.22 mm (range 21.53 – 30.63 mm) were carried 24783.77 ± 5951.33 non-eyed eggs (range 12668 – 32841) and 17 females with mean carapace width 25.62 ± 2.67 mm (range 21.06 – 30.70 mm) were carried 22123.47 ± 5463.24 eyed eggs (range 15603 – 37967).

The relationships between 1) the number of eggs (EN) and carapace width 2) EN and abdominal area of both sexes were analyzed by linear regression using natural log transformed data (Table 4).

There was no significant difference in the slopes of the linear regression between ln CW and ln EN for the both sexes (ANCOVA, $F = 1.96$, $df = 1, 52$, $P > 0.05$), but a significant difference in the intercept of the linear regression for the both sexes (ANCOVA, $F = 21.27$, $df = 1, 53$, $P < 0.001$) (Fig 9).

Reproductive output ($\pm$ standard deviation) was 0.098 ± 0.029 (n = 56).

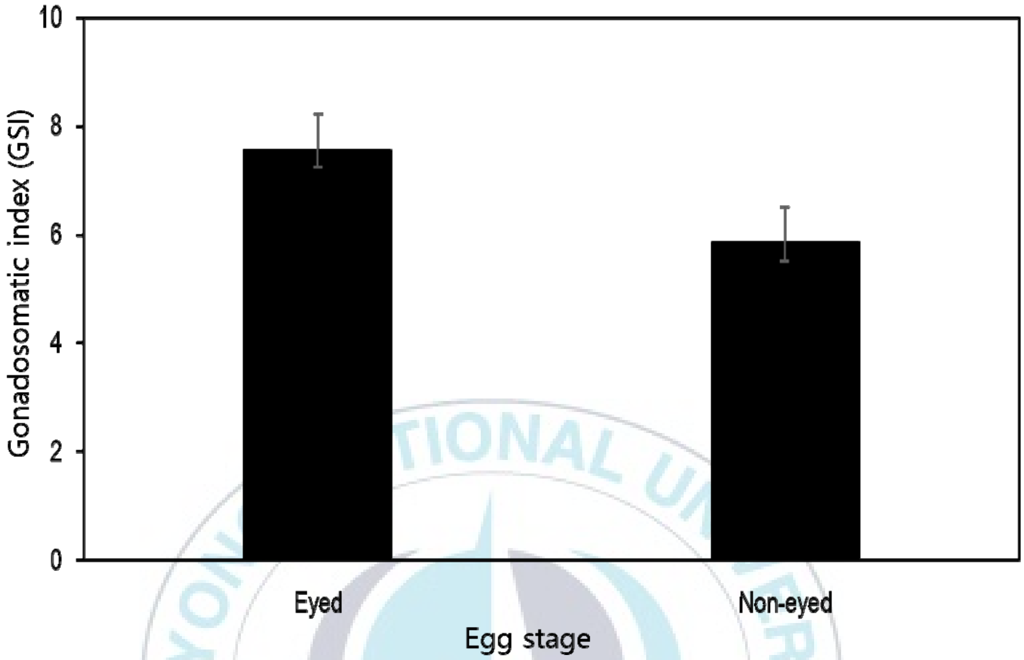
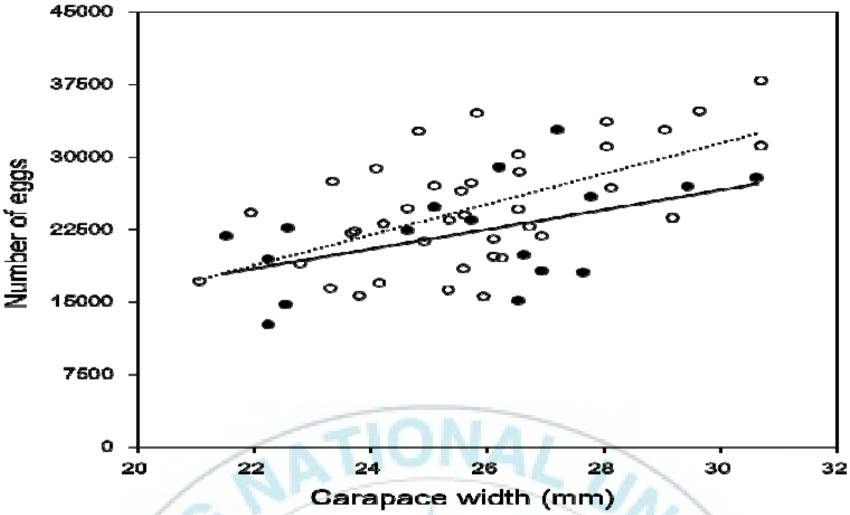


Fig 8. Mean ± standard error of gonadosomatic index (GSI) values for each egg stage of *Charybdis bimaculata* (Miers, 1886).

a.



b.

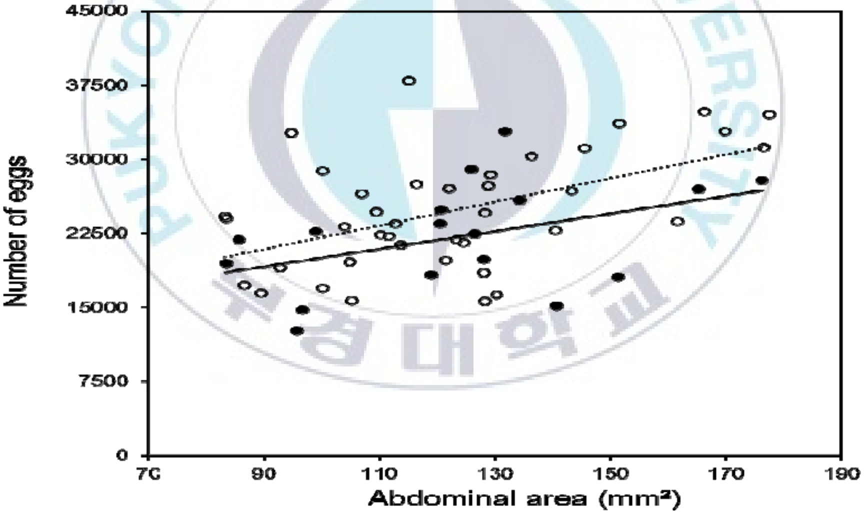


Fig 9. Linear regression of a: ln number of eggs (EN) and ln carapace width (CW) b: ln EN and ln abdominal area of ovigerous females of *Charybdis bimaculata* (Miers, 1886) with non-eyed (○) and eyed (●) eggs.

Table 4. Relationships between 1) ln the number of egg (EN) and ln carapace width (CW) 2) ln EN and ln abdominal area (AA) of *Charybdis bimaculata* (Miers, 1886), mean ± standard deviation.

Egg stages	Relationship	Linear regression
Non-eyed	EN versus CW	$\ln EN = 1.5916\ (\pm 0.3867) \ln CW + 4.9222$ $(\mathrm{n} = 39, r^2 = 0.3141, P < 0.001)$
	EN versus AA	$\ln EN = 0.5657\ (\pm 0.1744) \ln AA + 7.3813$ $(\mathrm{n} = 39, r^2 = 0.3141, P < 0.01)$
Eyed	EN versus CW	$\ln EN = 1.1941\ (\pm 0.5554) \ln CW + 6.1071$ $(\mathrm{n} = 17, r^2 = 0.2356, P < 0.05)$
	EN versus AA	$\ln EN = 0.5052\ (\pm 0.2823) \ln CW + 7.5513$ $(\mathrm{n} = 17, r^2 = 0.1760, P > 0.05)$

3-6. Size at sexual maturity (CW_{50})

Total 391 of the females *C. bimaculata* used in the analysis ranged from 8.5 mm to 35.5 mm. The size at sexual maturity (CW_{50}) of females was estimated as 20.93 mm. The proportion of sexually mature females by size was calculated by fitting a logistic equation with width as followed (Fig. 10):

$$P = \frac{1}{1 + e^{(17.51 - 0.84CW)}}$$

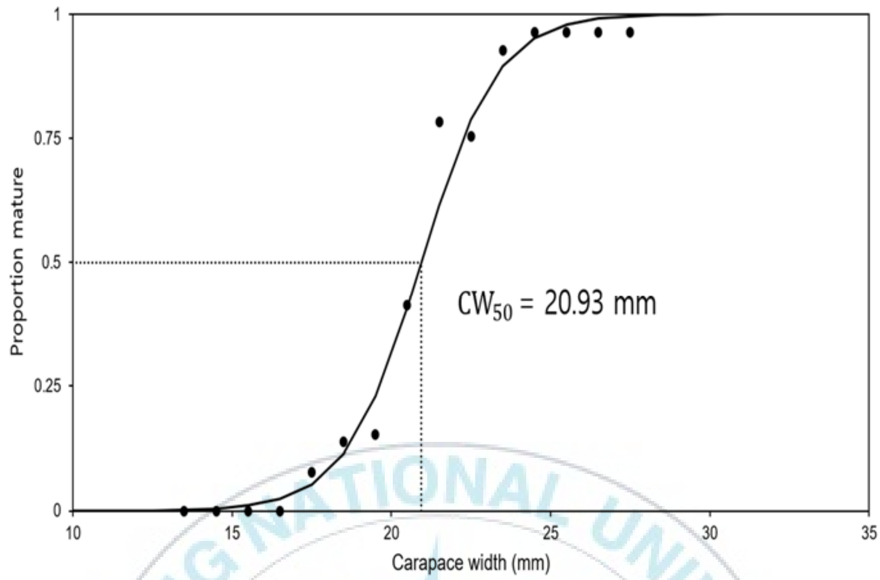


Fig 10. A logistic function fitting proportion of mature females to carapace width of *Charybdis bimaculata* (Miers, 1886). CW_{50}, which corresponds to the CW for 50% of mature females, is 20.93 mm.

3-7. Growth

The length-frequency distribution showed that there was an appreciable modal shift in the length of the cohorts with time (Fig. 11). The Von Bertalanffy growth parameter was determined by ELEFAN for both sexes. Asymptotic length (CW_∞) was higher in males (43.65 mm) than females (40.25 mm). Also, growth coefficient (*K*) was higher in males (0.95 yr^{-1}) than females (0.8 yr^{-1}). The Von Bertalanffy growth curve showed a seasonality growth rate (*C*) of 13% for males, 10% for females. The slowest growth season (WP) is August in males and September in females (Table5).

The Von Bertalanffy growth function is follow (Fig. 12):

$$\text{Male: } 43.65[1-e^{[1-e^{[-0.95(t+0.22)-(\frac{0.13\times0.95}{2\pi})sin2\pi(t+0.17)]}}]$$

$$\text{Female: } 40.25[1-e^{[-0.8(t+0.34)-(\frac{0.1\times0.8}{2\pi})sin2\pi(t+0.25)]}]$$

The growth performance index (φ') were 3.26 in males and 3.11 in females.

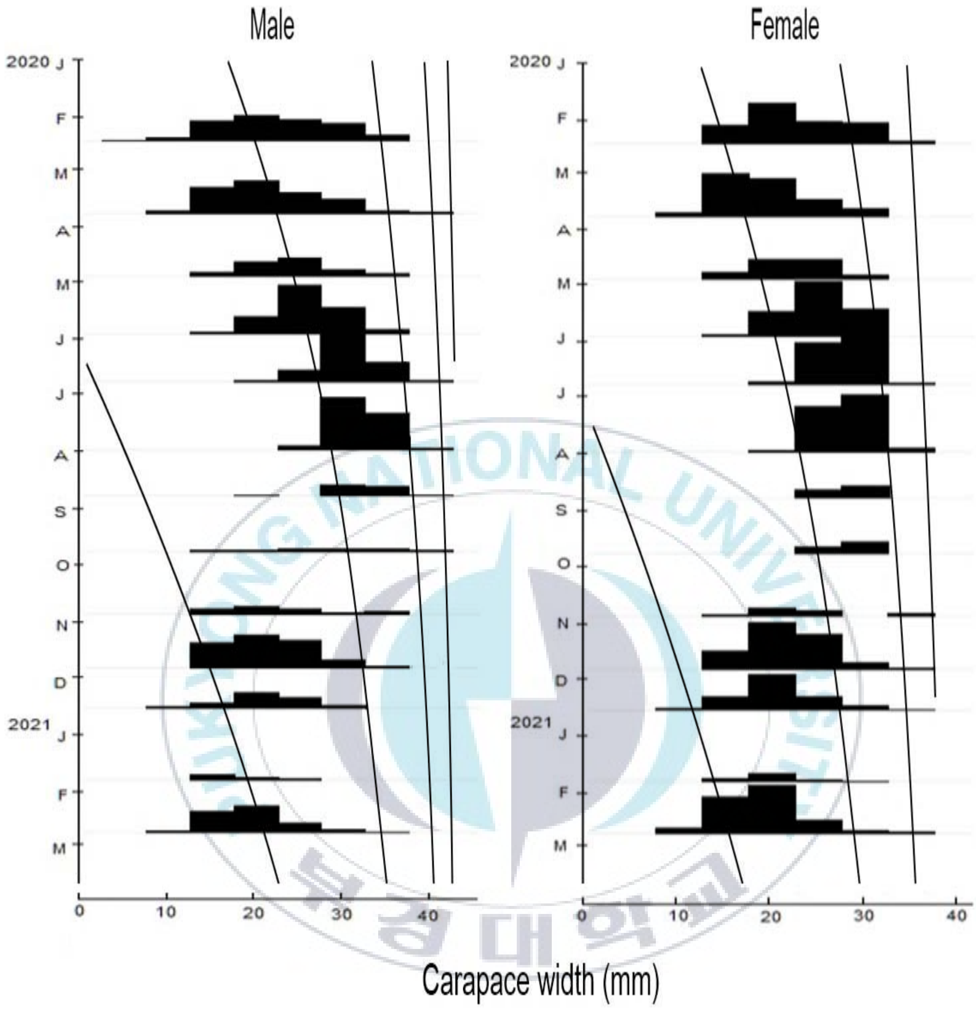


Fig 11. Length-frequency distribution of males (a) and females (b) of *Charybdis bimaculata* with seasonal Von Bertalanffy growth curves superimposed.

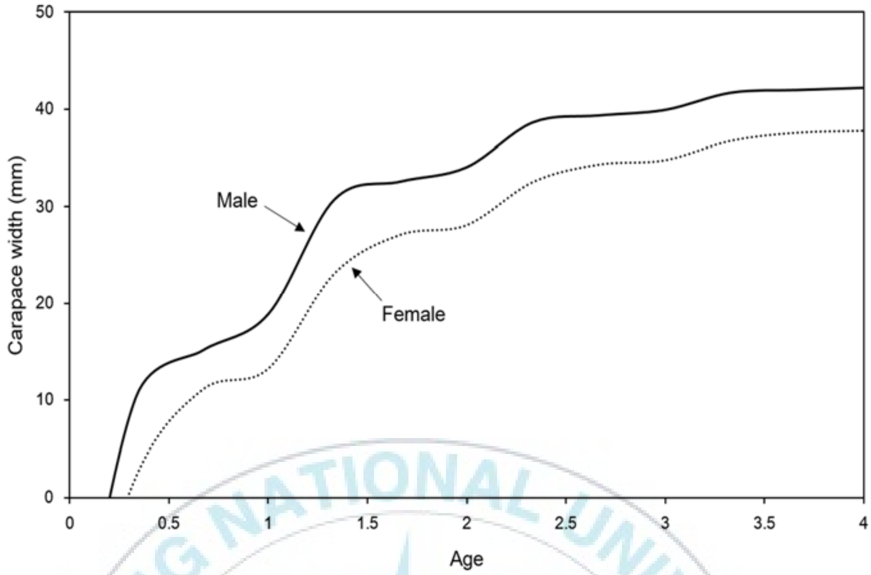


Fig 12. Average growth curves for male and females *Charybdis bimaculata* (Miers, 1886) based on Von Bertalanffy growth equations.

Table 5. Parameter estimation of the ELEFAN analysis of length-frequency distribution for both sexes: CW_{∞}: asymptotic length (mm); K: growth coefficient (yr^{-1}); C: amplitude of growth oscillation; WP: winter point; $\varphi^{'}$: growth performance index; R_n: score function.

Parameter	Males	Females
CW_{∞}	43.65	40.25
K	0.95	0.8
C	0.13	0.1
WP	0.67	0.75
φ'	3.26	3.11
R_n	0.45	0.40

4. Discussion

4-1. Sex ratio

The sex ratio of the two-spot swimming crabs was significantly greater in females than in males. Females of *C. bimaculata* are dominated throughout the year except in July and September. In Tokyo bay, the sex ratio of *C. bimaculata* showed a significant difference as in this study (Doi *et al*., 2008). In the *Charybdis* species, such as *C. hellerii, C. feriata, C. miles, C. natator,* the sex ratio is generally 1:1 in nature (Dineen *et al*., 2001; Dineshbabu, 2011; YU *et al*., 2004; Sallam and Gab-Alla, 2010).

Differences in sex ratio arise for a variety of reasons. In the Seto Inland Sea, about 500 km southwest from Tokyo bay, sex ratio of this species were either male-biased (Anonymous, 1974). In this case, the sex bias was greater during reproductive period, so it is considered to be a reproduction-associated migration. There is information about differential migration between the sexes for reproduction among Portunidae (de Lestang *et al.,* 2003; Keunecke *et al.,* 2012; Severino-Rodrigues *et al.*, 2013). Sex ratio deviations in crab populations usually involve sexual differences in survival rate, growth rate, and sex reversal (Wenner, 1972).

4-2. Growth

There was a significant difference in length-frequency distribution of both sexes, and males were predominance in the larger size classes. These results are identical to study of the same species in japan (Doi *et al*., 2008). Size difference between sexes has been observed in some *Charybdis* species (Sallam and Gab-All, 2010; Sumpton, 1990; Balasubramanian and Suseelan, 2001) as well as other brachyuran crabs, such as *Carcinus aestuarii*, *Johngarthia Lagostoma* (Glamuzina *et al*., 2017; Hartnoll *et al.,* 2009).

This is common in crabs and females may have reduced somatic growth compared to males because they concentrate their energetic budget for gonad development (Hartnoll, 1985). Moreover, males may reach larger sizes, since larger males have greater chances of obtaining females for copulation and win more intra-specific fights (Hartnoll, 1985; Pinheiro and Fransozo, 1998).

There was a significant difference between sexes in the slope of relationships between carapace width versus body weight of *C. bimaculata*. Slope in males was significantly higher than in that females and interpreted as a faster body weight increases in males than in females. This is the same for *C. natator, C. feriata, C. japonica* (Sallam and Gab-Alla, 2010; Dineshbabu, 2011; Kolpakov and Kolpakov, 2011). This sexual difference can be explained by a reproductive behavior. Males exhibit a mate guarding behavior of the females before and after copulation and avoiding male competition and providing protection to the recent post-molt females (Pinheiro and Fransozo, 2002). These behavior are considered reproductive strategies that increase mating rate, and stronger adult males take advantage by developing this behavior (Jivoff, 1997). In contrast, females allocate a large portion of energy to their reproductive activities.

Slopes of the linear regression between carapace width and abdominal area showed significant difference for both sexes. Slope in females was significantly higher than that in males. In brachyuran crab, abdomen in females was considered as secondary sexual characters because of their functions in reproduction (Hartnoll, 1974). The abdomen in mature females consist an incubation chamber for the eggs.

Seasonal Von Bertalanffy growth test revealed that CW_{∞}, K was higher in the male *C. bimaculata*. The higher coefficient (K) for males indicated that they rapidly reach their asymptotic length (CW_{∞}) than females.

The growth performance index (φ') can be used for averaging growth parameters obtained from the Von Bertalanffy growth function of a particular species (Sparre and Venema, 1992) and is a useful tool for comparing growth under a variety of environmental conditions (Pauly, 1991). The growth performance index (φ') is different among the brachyuran crabs (Table 6). The growth performance index of *C. bimaculata, C. feriata* and *Callinectes sapidus* and *Uca sindensis* is higher in male than female. However, the growth performance index of *P. trituberculatus* is lower in male than female. These growth differences between males and females are related to the metabolic cost of reproductive activity and coincided with the breeding season and maturation cycle.

Table 6. Comparison of the growth performance index (φ') for a variety of brachyuran crab.

Genus	Species	φ' Male	φ' Female	Reference
Charybdis	*C. feriata*	2.46	2.45	Dash *et al.*, 2014
	C. bimaculata	3.26	3.11	Present study
Uca	*U. sindensis*	1.98	1.94	Lavajoo *et al.*, 2014
Portunus	*P. trituberculatus*	2.48	2.51	Oh, 2011
Callinectes	*C. sapidus*	4.66	4.55	Sumer *et al.*, 2013

4-3. Reproduction

Reproduction is the main mechanism to maintain species proliferation and continuity, and in brachyuran crabs, is extremely diversified, ultimately shaped to maximize egg production and offspring survivorship (Hartnoll and Gould, 1988).

Breeding period can be estimated from ovarian development and appearance of ovigerous female and larvae. In this study, GSI value of *C. bimaculata* was higher in the period between April and June, and ovigerous females appeared from April to September and November. Lee (2018) reported the appearance of *C. bimaculata* larvae from September to October. These results demonstrate that the main spawning period of *C. bimaculata* is June-August. In Japan, the main spawning period of *C. bimaculata* fell approximately into Summer (Narita *et al.*, 2008; Doi *et al.*, 2008).

GSI value with eyed egg stages was significantly higher than with non-eyed egg stages. This suggests that the ovaries are maturing prior to continuing reproductive activity. Brachyuran crabs of intertidal zones along tropical and subtropical estuarine reproduce over long periods or even continually during the year (Sastry, 1983). Similar patterns have been reported for brachyuran crabs such as *Panopeus americanus* (Vergamini and Mantelatto, 2008), *Paguristes totugae* (Mantelatto *et al.*, 2002), *Porcellana sayana* (Baeza *et al.*, 2013). This reproductive pattern can be understood as a strategy of continuous effort to establish and maintain a stable population.

Fecundity is an important parameter that permits the estimation of the reproductive potential and future stock size of a given species or population (Hattori and Pinheiro, 2001). It is important components of fitness, which is dependent on lifetime reproductive performance (Sastry, 1983).

A significant relationships were observed between 1) the number of eggs and carapace width and between 2) the number of eggs and abdominal area in non-eyed eggs. ANCOVA revealed significant difference in the intercept between the carapace width and the number of eggs in the two egg stages. This result indicates that brood loss occurred. Egg loss is caused by mechanical stress, such as oxygen provision, and burrowing behavior (Brante *et al.*, 2003; Kuris, 1991). Factors such as increasing egg volume, environment (temperature and salinity), photoperiod, food availability (quality and quantity) and parasitism also cause egg loss (Oh and Hartnoll, 1999; Pinheiro and Fransozo, 2002; Giese and Kanatani, 1987; Flores and Negreiros-Fransozo, 1998; Emmerson, 1994).

Reproductive output (RO) is a life-history trait reflecting varying numbers and size of larvae (Hartnoll, 1985). RO expressed as the biomass of the reproductive products per unit biomass of the female (Pianka, 1972; Thessalou-Legaki and Kiortsis, 1977). RO of *C. bimaculata* is highest among *Charybdis* species and lower than *P. trituberculatus* (Table 7). In most brachyuran crabs, brood weight exhibits an isometric or nearly isometric constraint to about 10%

of female body weight and limitations on space available for yolk accumulation in the body cavity appeared to be the main constraint on brood size (Hines, 1982). The differences in RO between the other species, give rise to varied brood sizes, and subsequently lead to changes in annual egg production. The RO can also vary with age and nutrient levels in the diet and between broods for a species with multiple breeding patterns within a single productive period, or as a function of the primiparous or multiparous female condition (Oh, 2010).

Size at sexual maturity is a crucial indicator of the reproductive capacity of populations, and may distinct considerably even in closely related species (Robertson and Butler, 2003). In present study, the estimated proportion of 0.5 of females in the reproductive condition was 20.93 mm CW. Similarly, Doi (2008) reported the female size at sexual maturity between 21.26 – 24.41 mm CW.

Table 7. Comparison of reproductive output for a variety of brachyuran crab.

Genus	Species	Reproductive output (mean)	Reference
Charybdis	*C. hellerii*	0.084	Bolañs *et al.*, 2012
	C. japonica	0.052	Fowler and McLay, 2013
	C. bimaculata	0.098	Present study
Portunus	*P. trituberculatus*	0.34	Oh, 2010

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2 년 동안 동고동락을 함께 해온 해양 생태학방 친구들에게도 감사의 말 전합니다. 선배이자 가장 의지한 재훈이, 힘든 시간 든든하게 옆을 지켜준 하경이, 항상 긍정적이고 웃음 짓게 해준 소연이, 어려운 일도 묵묵히 따라준 동주, 활기찬 실험실 분위기를 이끄는 정민이, 모든 실험실 일에 가장 먼저 도움을 주는 민수, 누구보다 마음이 따뜻한 정원이 그리고 우리 실험실의 마스코트이자 정신적 지주 윤정이 누나 모두가 있어 행복하고 즐거운 시간이 된 것 같습니다. 그리고 논문 작성에 큰 도움을 주신 호진 선배님, 한주 선배님을 비롯한 여러 선후배님들에게도 감사합니다.

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