

Gonadal Maturation and Main Spawning Period of *Haliotis gigantea* (Gastropoda: Haliotidae)

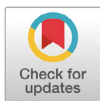
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Conflict of interests

The authors declare no potential conflict of interest.

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Abstract

This study was carried out to obtain information on the developmental biology and the management of biological resources of the abalone *Haliotis gigantea* in Korea. The sex ratio (female:male) in the present study was 1:1.7 and the proportion of females was 36.6% (n=106/290). Their gonadal structures displayed definitive seasonal changes which were similar in pattern to the changes in the gonad index (GI). The GI showed a pattern of definitive seasonal changes in both males and females it was high in the fall and low in the spring. The reproductive cycle could be categorized into the following six stages: inactive, early active, late active, ripe, spent, and degenerative stage. Based on the monthly changes in GI and stages of gonadal development, October to November was determined to be the main spawning period for *H. gigantea* on Jeju Island, Korea.

Keywords: *Haliotis gigantea*, Sex ratio, Reproductive cycle, Main spawning period

INTRODUCTION

Studies on the reproductive cycle, sexual maturation, and spawning of Haliotidae species have been conducted to accumulate data on basic biology, resource management, and culture technology development (Webber & Giese, 1969; Sobhon et al., 1999; Najmudeen & Victor, 2004; Visser-Roux, 2011).

In Korea, two species and two subspecies of the genus *Haliotis*, and two subspecies of the genus *Sulculus* have been reported (Lee et al., 2015). Research on their reproduction was mostly undertaken using *H. discus hannai* (Lee, 1974; Rho & Park, 1975; Kim et al., 2016), whereas studies on *H. gigantea* have rarely been conducted.

H. gigantea is an edible gastropod with a shorter shell height than that of the other Haliotidae found in Korea, and it also shows relatively rapid growth. In Korea, this species is found mainly on the southern coast and on the coast of Jeju Island, which have relatively high water temperature (WT). This gastropod inhabits rocky bottom in intertidal zones 30 m underwater (Lee et al., 2015). This study aimed to obtain the reproductive biological data (sex ratio, gonadal development, reproductive cycle, main spawning season etc.) of *H. gigantea* from Jeju Island, Korea.

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Authors' contributions

Conceptualization: Lee JS, Kim JW.

Data curation: Shin SR, Kim H.

Formal analysis: Kim HJ, Lee DH, Kim H.

Methodology: Lee JS.

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Validation: Sohn YC, Kim JW.

Investigation: Shin SR, Kim HJ, Lee DH, Kim H.

Writing - original draft: Shin SR, Kim JW, Lee JS.

Writing - review & editing: Shin SR, Kim HJ, Lee JS.

Ethics approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

MATERIALS AND METHODS

1. Sampling

H. gigantea specimens were collected by divers from the coast of Gujwa-eup, Jeju Island in Korea (Fig. 1). Specimens were collected monthly from January to December 2014, and a total of 290 specimens were collected for the study. The specimens analyzed had a shell length of 111.2 (± 13.3) mm and a total weight of 136.1 (± 55.8) g. Morphological characteristics are shown in Fig. 2.

2. Environmental conditions

The data of monthly WT in the sampling area were referenced from the Korea Hydrographic and Oceanographic Administration (KHOA, 2014).

3. Histological analysis

Specimens were dissected for light microscopy analysis after their morphometric characteristics (i.e., shell length, total weight, and body weight) had been measured. The hepatopancreas including the gonads was fixed in Bouin's solution for 24 h, rinsed with running water for 36–48 h, and dehydrated through a graded ethyl alcohol series (70%–100%). The specimens were then embedded in Paraplast (Leica, Germany), following which they were sectioned into slices of 4–6 μm thickness

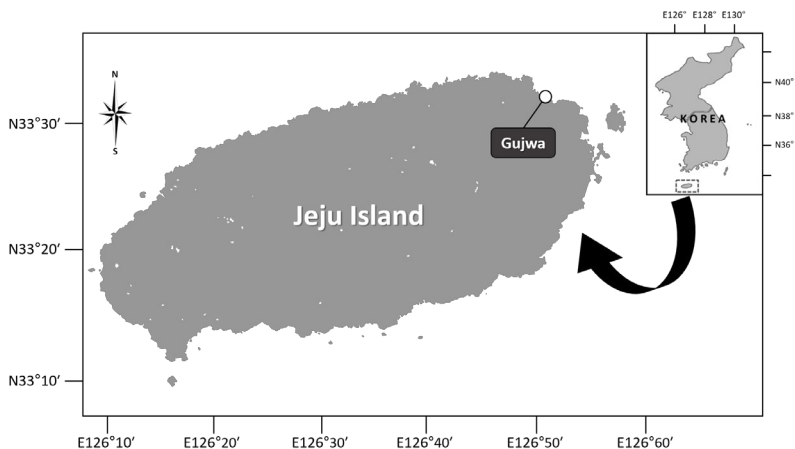


Fig. 1. Sampling area of the abalone *Haliotis gigantea*.

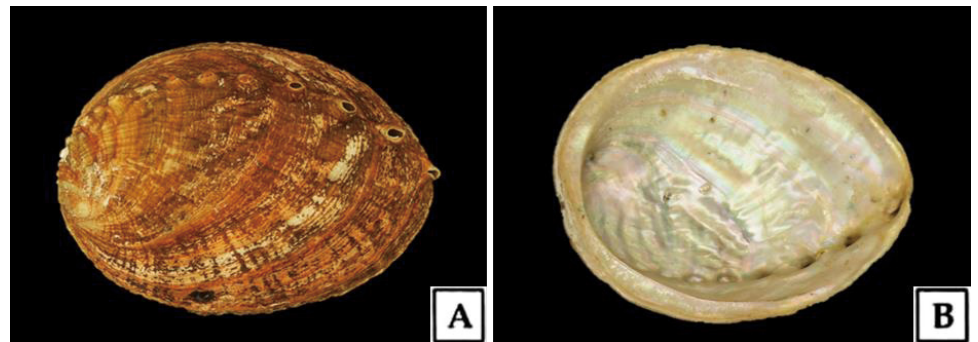


Fig. 2. Morphology of *Haliotis gigantea*. (A) External picture of shell. (B) Internal picture of shell.

using a microtome (RM2235, Leica, Germany). The tissue specimens were then stained with Mayer's hematoxylin–0.5% eosin (H–E) and Masson's trichrome stain. An image analyzer (IMT-VT Image analysis, IMT, USA) was used to quantify the size of the oocytes.

4. Sex ratio

The sex ratio and the proportion of females were computed with the following equations:

$$\text{Sex ratio} = \text{females (n)}:\text{males (n)}; \text{Female (\%)} = [\text{female} / (\text{female} + \text{male})] \times 100$$

5. Gonad index

The gonad index (GI) was corrected, in part, using the method described by Eversole (1997). Gonad development was categorized into the following six stages: inactive (In), early active (Ea), late active (La), ripe (R), spent (Sp) and degenerative stage (D). Samples were quantified by multiplying each individual with a constant (In, 1; Ea, 2; La, 3; R, 4; Sp, 3; D, 2) for each gonadal development stage.

$$\text{GI} = \frac{(\text{N of In} \times 1) + (\text{N of Ea} \times 2) + (\text{N of La} \times 3) + (\text{N of R} \times 4) + (\text{N of Sp} \times 3) + (\text{N of D} \times 2)}{\text{Total N observed monthly}}$$

6. Meat weight index and condition index

The meat weight index (MWI) and condition index (CI) and were calculated with the following equations: $\text{MWI} = [\text{Body weight (g)} / \text{Total weight (g)}] \times 100$; $\text{CI} = [\text{Body weight (g)} / \text{Shell length (mm)}^3] \times 1,000$.

7. Statistical analysis

The sex ratio was analyzed using SPSS 21.0 software (SPSS, USA). The observed female:male ratio for each shell length group was compared with the expected ratio of 1:1 using the chi-square (χ^2) test. Differences were considered statistically significant at a p -value of less than 0.05.

RESULTS

1. Water temperature

The average WT at the sampling area of *H. gigantea* during the sampling period was 17.2°C, with the lowest temperature in January being 12.2°C and the highest in September being 23.1°C.

2. Sex ratio

In the histological analysis of the gonads, simultaneous hermaphroditism was not observed in *H. gigantea*. The average sex ratio (female:male) was 1:1.74, where the female proportion (F/F+M) of the 290 specimens was 36.6%, indicating a distinctly higher proportion of males (Table 1).

3. Gonadal development stages

The gonad of *H. gigantea* comprised many gametogenic follicles and showed distinct seasonal differences, as outlined in the next two subsections.

Table 1. Sex ratio with shell length of the abalone *Haliotis gigantea*

Shell length (mm)	Total	Female	Male	Sex ratio (F:M)	Chi-square	p-value
70.01–80.00	12	5	7	1:1.40	0.333	0.564
80.01–90.00	49	17	32	1:1.88	4.592	0.030
90.01–100.00	77	32	45	1:1.41	2.195	0.138
100.01–110.00	74	24	50	1:2.08	9.135	0.003
110.01–120.00	54	20	34	1:1.70	3.630	0.057
120.01–130.00	16	4	12	1:3.00	4.000	0.046
130.01≤	8	4	4	1:1.00	-	1.000
Total/average	290	106	184	1:1.74	20.979	0.000

1) Female

The developmental stages of ovary showed seasonal differences. At the inactive stage, the ovary was filled with mainly oogonia (diameter 10–15 μm) and previtellogenic oocytes (diameter about 20 μm) (Fig. 3A). During January and February, 100% of females were in the inactive stage, and more than 50% remained in this stage for a relatively long period (from January to July) (Fig. 5).

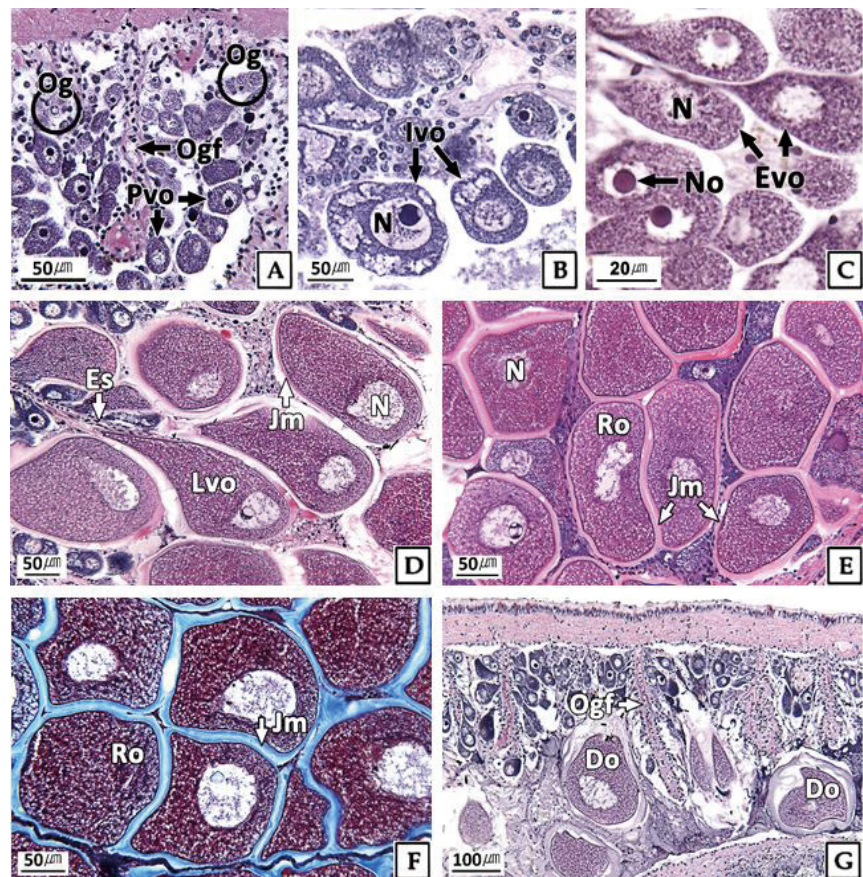


Fig. 3. Photomicrographs of the ovarian development stage in the abalone *Haliotis gigantea*. (A) Inactive stage. (B,C) Early active stage. (D) Late active stage. (E,F) Ripe stage. (G) Spent and degenerative stage. Do, degenerative oocytes; Es, egg stalk; Evo, early active vitellogenic oocytes; Ivo, initial vitellogenic oocytes; Jm, jelly membrane; Lvo, late active vitellogenic oocyte; N, nucleus; No, nucleolus; Og, oogonia; Ogf, oogenic follicle; Pvo, previtellogenic oocytes; Ro, ripe oocytes.

The ovaries at the early active stage were found mainly from March to September, where initial vitellogenic oocytes (diameter about 30 μm) (Fig. 3B) and early active vitellogenic oocytes (diameter about 65 μm) of oval shape were mainly observed (Fig. 3C). A high percentage of females in the late active stage occurred mainly in August and September (Fig. 5). During this period, the ovaries were filled mainly with late active vitellogenic oocytes but did have some early active vitellogenic oocytes. The late active vitellogenic oocytes were rectangular in shape, having a distinct egg stalk connected to the oogenic follicle, which was approximately 160 μm in diameter (Fig. 3D). In October, the percentage of females in the ripe stage was 46%, which was the highest percentage observed during the year. The ovaries were filled mainly with ripe oocytes of rounded polygonal shape with a well-developed jelly membrane and an average diameter of approximately 200 μm (Fig. 3E, F). Ovaries in the spent and degenerative stages were observed mainly from October to December. During this period, the ovarian tissue showed the degradation and absorption of undischarged oocytes after spawning (Fig. 3G).

2) Male

The testicular development stages of *H. gigantea* also showed seasonal changes. At the inactive stage, spermatogonia were observed in parts of testicular tissue, but the proportion of empty space was high owing to the undeveloped germ cells (Fig. 4A). During January and February, 100% of males were in the inactive stage, and more than 50% remained in this stage during the relatively long period from January to July (Fig. 5). Testes at the early active stage were found mainly from March to September, where mostly spermatogonia and spermatocytes and a small number of spermatids were observed (Fig. 4B, C). A high percentage of the males were in the late active stage during August and September, during which the testes were filled mostly with basophilic spermatids but had some spermatocytes present (Fig. 4D). In October, the percentage of males in the ripe stage was 46%, which was the highest percentage observed during the year, and the testis was filled mainly with basophilic sperm (Fig. 4E). Testes at the spent and degenerative stages were observed mainly from October to December, where the testicular tissue showed degradation and absorption of remaining sperm after the spent (Fig. 4F).

4. Monthly changes of gonad index

Similar to the gonad developmental stages, the GI changes in both males and females showed distinct seasonal patterns; namely, an increase in fall, and a decrease in spring and winter. The GI began to increase in March (1.36) and was the highest in October (3.57), and then decreased in November to less than 3.0 (Fig. 6).

5. Monthly changes of meat weight index and condition index

Although the MWI and CI for May and November displayed higher values than those at the other times of the year, they did not display definitive seasonal changes as observed with the GI (Figs. 7 and 8).

6. Reproductive cycle

On the basis of the monthly changes in gonadal development stage and the GI, the reproductive cycle could be categorized into inactive (January to May), early active (May to July), late active (August to September), ripe (October), spent (October to November) and degenerative stage (November to December).

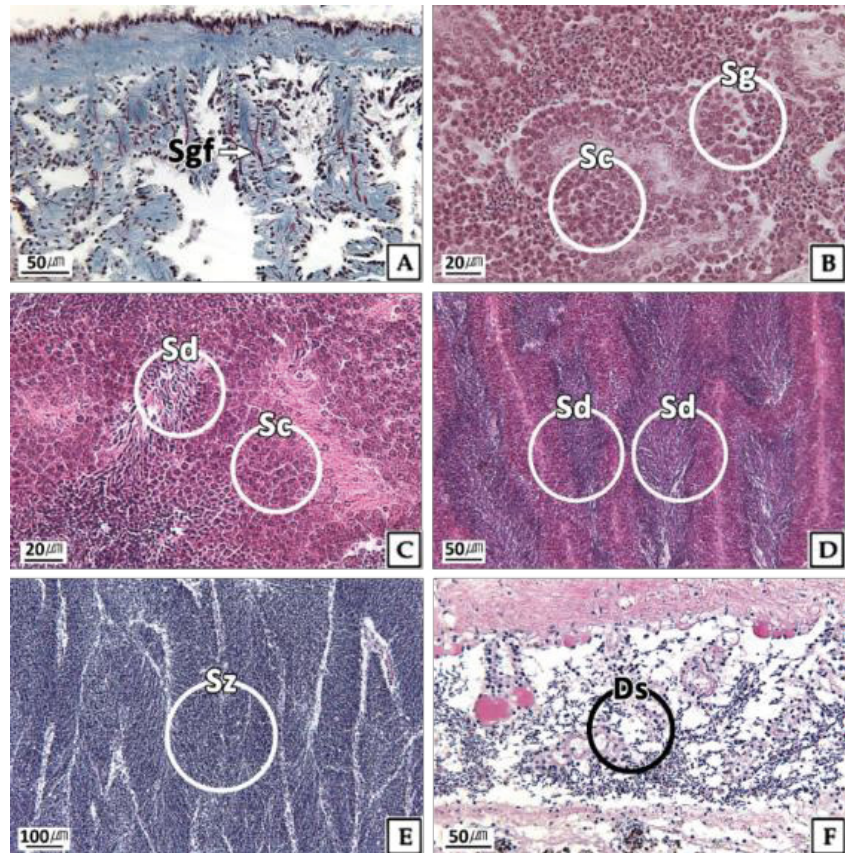


Fig. 4. Photomicrographs of the testicular development stage in the abalone *Haliotis gigantea*. (A) Inactive stage. (B,C) Early active stage. (D) Late active stage. (E) Ripe stage. (F) Spent and degenerative stage. Ds, degenerative sperm; Sc, spermatocytes; Sd, spermatids; Sg, spermatogonia; Sgf, spermatogenic follicle; Sz, sperm.

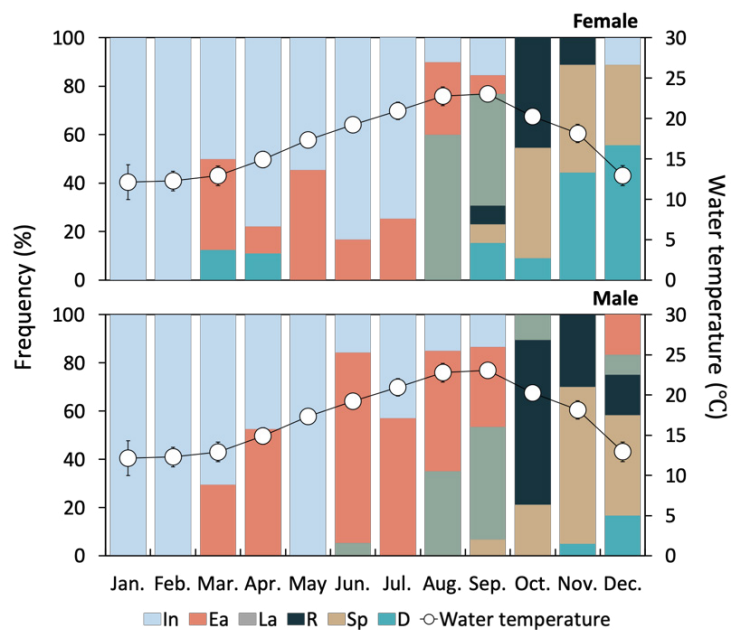


Fig. 5. Monthly variation of the gonadal development stage in the abalone *Haliotis gigantea*. In, inactive stage; Ea, early active stage; La, late active stage; R, ripe stage; Sp, spent stage; D, degenerative stage.

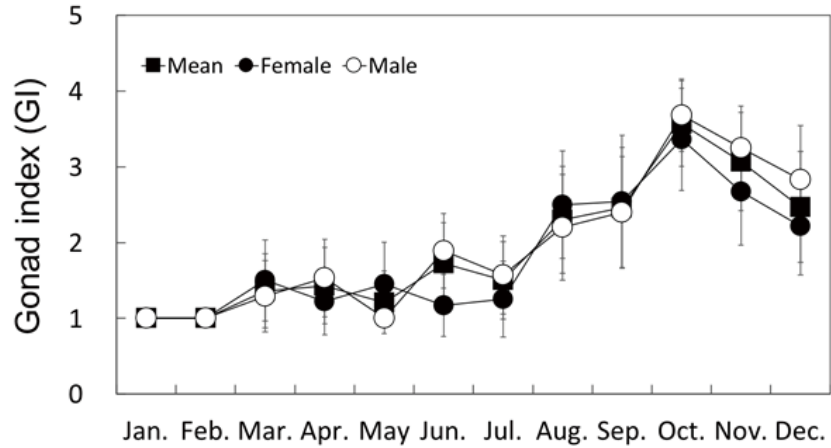


Fig. 6. Monthly variation of gonad index in the abalone *Haliotis gigantea*. Vertical bars indicate standard deviation.

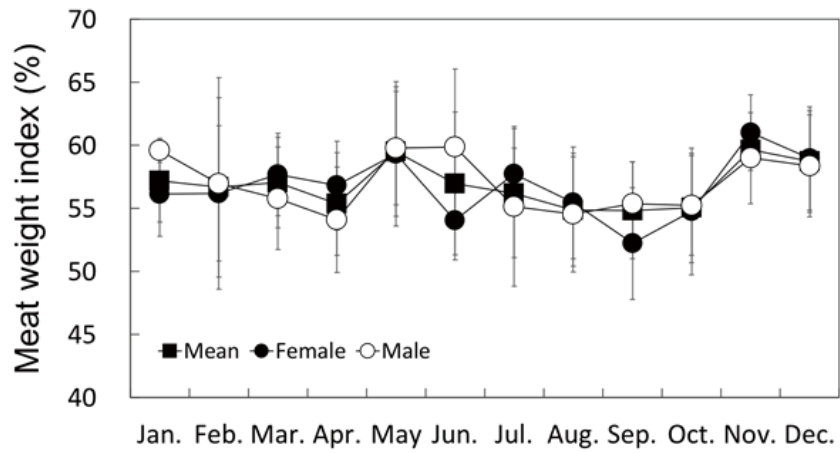


Fig. 7. Monthly variation of meat weight index in the abalone *Haliotis gigantea*. Vertical bars indicate standard deviation.

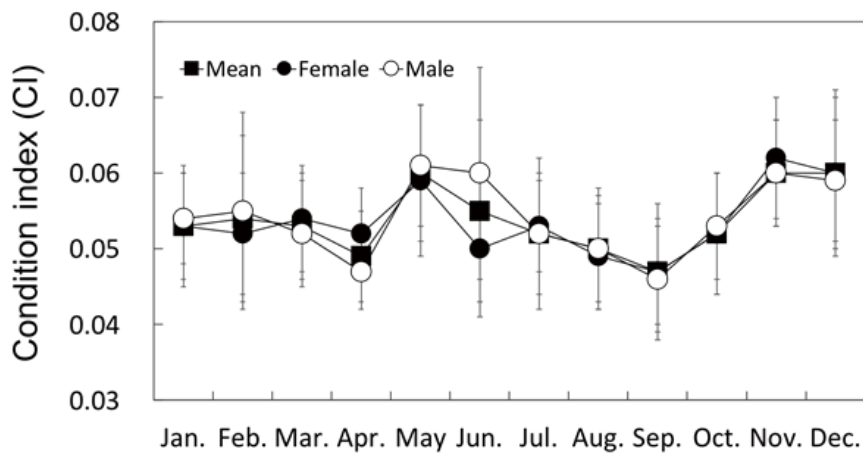


Fig. 8. Monthly variation of condition index in the abalone *Haliotis gigantea*. Vertical bars indicate standard deviation.

DISCUSSION

The sex ratio (female:male) of Haliotidae was reported to be approximately 1:1 in *Haliotis cracheroidii* (Webber & Giese, 1969), *H. rufescens* (Young, 1970), *H. australis* (Wilson & Schiel, 1995), *H. asinina* (Capinpin et al., 1998), and *H. varia* (Najmudeen & Victor, 2004). The sex ratio of *H. discus hannai* collected from 3 regions in Korea by our group was approximately 1:0.9 on average, showing a slightly higher proportion of females, although not significantly different from 1:1 (Kim et al., 2016).

The sex ratio of *H. gigantea* was 1:1.74 (female:male), showing a lower proportion of females (approximately 36.6%, $n=106/290$). In December, which represents the inactive stage of *H. gigantea*, the sex ratio was 1:2.0, and the proportion of females was 33.3%. As abalones can be divided into males and females in the inactive stage after spawning, the low proportion of females was not a consequence of spawning. Further research is needed to investigate why the proportion of females in *H. gigantea* is lower than that in other abalone species.

The morphological expression of sex and changes in the sex ratio in molluscs are affected by genetic factors, age and environmental factors such as the WT and food (Yusa, 2007; Chávez-Villalba et al., 2011; Park et al., 2012). Therefore, there is a need for more detailed research in the future to identify the reason for the lower proportion of *H. gigantea* females.

The diameter of the ripe oocytes differs depending on the species within the same genus in the Haliotidae, as reported for the following species in various countries: 80–140 μm for *H. asinina* in Thailand (Sobhon et al., 1999), in excess of 125 μm for *H. asinina* in Panagatan Cays, Philippines (Capinpin, 1998), 180 ± 20 μm for *H. varis* in the Gulf of Mannar in India (Najmudeen & Victor, 2004), 202 ± 21 μm for *H. discus hannai* in Korea (Kim et al., 2020), 250–300 μm for *H. midae* in South Africa (Visser-Roux, 2011), and 250 μm for *H. rufescens* in California (Giorgi & DeMartini, 1977).

In this study, the diameter of the ripe oocytes of *H. gigantea* was measured to be approximately 200 μm . This suggests that the size variation of ripe oocytes of haliotids could be due to the extent of yolk accumulation. However, there is a need to investigate whether the extent of yolk accumulation is due to a species-specific characteristic or an ecological factor.

The WT is a major environmental factor that determines the sexual maturity of many molluscs (Mackie, 1984; Eversole, 2001; Grubert & Ritar, 2004). In general, the reproductive period of invertebrates that thrive in low-latitude regions is long, whereas that of invertebrates in high-latitude regions is short. These characteristics are due to the effects of different WTs on the reproductive characteristics of the species (Fretter & Graham, 1964).

On the bases of the monthly changes in gonadal development stages and the GI, the reproductive cycle of *H. gigantea* could be categorized distinctly into the inactive (January to May, WT 12.2°C – 17.3°C), early active (May to July, WT 17.3°C – 20.9°C), late active (August to September, WT 22.8°C – 23.1°C), ripe (October, WT 20.3°C), spent (October to November, WT 20.3°C – 18.1°C), and degenerative stages (November to December, WT 18.1°C – 12.9°C). Based on these results, *H. gigantea* has one spawning season per year, in the fall, and its sexual maturation and reproductive cycles are affected by the WT.

From the data of reproductive cycle analysis, the gonadal development stages and GI changes are used to identify the period needed for gonadal maturation and the maturation speed of the species. Of the *H. discus hannai* specimens collected in the same region as *H. gigantea*, over 40% of the population were found to be at the inactive stage, which was from September to February for the females and from December to February for the males (Kim et al., 2016).

However, the periods with over 50% of *H. gigantea* at the inactive stage were from January to

July for the females and from January to May for the males, which were longer than those for *H. discus hannai*. The periods in which there were a high proportion of individuals in the early active stage lasted approximately 3 and 4 months for *H. discus hannai* females and males, respectively, but were again longer (at approximately 6 months) for both the *H. gigantea* females and males. The late active stage lasted 2 months for *H. gigantea*, whereas it was 3 months for *H. discus hannai*. Furthermore, the GI increase gradient of *H. discus hannai* after the inactive and early active stages was relatively gradual compared with that of *H. gigantea*. These results showed that the reproductive characteristics of *H. gigantea* included longer inactive and early active stages, and shorter late active and ripe stages, relative to those of *H. discus hannai*. In addition, such reproductive characteristics could contribute to the difference in the ripe oocyte size.

The monthly change in the CI of bivalves has been shown to be correlated to their reproductive cycle (Park et al., 2003; Gosling, 2004; Marroquin-Mora & Rice, 2008). However, it is difficult to find research studies on the correlation between the CI change and the reproductive cycle in the Haliotidae. In this study, the MWI and CI of *H. gigantea* did not show definitive seasonal changes like those of *H. discus hannai* (Kim et al., 2016).

REFERENCES

- Capinpin EC, Encena II VC, Bayona NC (1998) Studies on the reproductive biology of the donkey's ear abalone, *Haliotis asinina* Linné. *Aquaculture* 166:141-150.
- Chávez-Villalba J, Soyez C, Huvet A, Gueguen Y, Lo C, Le Moullac G (2011) Determination of gender in the pearl oyster *Pinctada margaritifera*. *J Shellfish Res* 30:231-240.
- Eversole AG (1997) Gametogenesis of *Mercenaria mercenaria*, *M. campechiensis* and their hybrids. *Nautilus* 110:107-110.
- Eversole AG (2001) Reproduction in *Mercenaria mercenaria*. In: Kraeuter JN, Castagna M (eds), *Biology of the Hard Clam*. Elsevier, New York, NY, pp 221-260.
- Fretter V, Graham A (1964) Reproduction. In: Wilbur KM, Yonge CM (eds), *Physiology of Mollusca*, vol. 1. Academic Press, New York, NY, pp 127-164.
- Giorgi AE, DeMartini JD (1977) A study of the reproductive biology of the red abalone, *Haliotis rufescens* Swainson, near Mendocino, California. *Calif Fish Game* 63:80-94.
- Gosling E (2004). *Bivalve Molluscs: Biology, Ecology and Culture*. Blackwell Science, Oxford, UK, p 443.
- Grubert MA, Ritar AJ (2004) Temperature effects on the dynamics of gonad and oocyte development in captive wild-caught blacklip (*Haliotis rubra*) and greenlip (*H. laevigata*) abalone. *Invertebr Reprod Dev* 45:185-196.
- Korea Hydrographic and Oceanographic Agency (2014) Real time coastal data; Jeju-do. Available from: http://sms.khoa.go.kr/koofs/kor/observation/obs_past_search_statistic.asp. Accessed at Jan 25, 2020.
- Kim H, Kim BH, Son MH, Jeon MA, Lee YG, Lee JS (2016) Gonadal development and reproductive cycle of cultured abalone, *Haliotis discus hannai* (Gastropoda: Haliotidae) in Korea: Implications for seed production. *J Shellfish Res* 35:653-659.
- Kim H, Kim HJ, Kim YS, Lee JS (2020) Microstructural differentiation of the oocyte in the abalone *Haliotis discus hannai*. *Korean J Fish Aquat Sci* 53:90-97.
- Lee JS, Son MH, Lee JS, Lim HK, Won SH, Lee JH, Kang JC, Kim JW, Kim SY (2015) *Korean Abalone (I): Morphology, Taxonomy and Ecology*. Maple Design, Busan, Korea, p 68.
- Lee TY (1974) Gametogenesis and reproductive cycle of abalones. *Publ Mar Lab Busan Fish Coll*

7:21-50.

- Mackie GL (1984) Bivalves. In: Tompa AS, Verdonk NH, van den Biggelaar JAM (eds), *The Mollusca: Reproduction*, vol. 7. Academic Press, New York, NY, pp 351-418.
- Marroquin-Mora DC, Rice MA (2008) Gonadal cycle of northern quahogs, *Mercenaria mercenaria* (Linne, 1758), from fished and non-fished subpopulations in Narragansett Bay. *J Shellfish Res* 27:643-652.
- Najmudeen TM, Victor ACC (2004) Reproductive biology of the tropical abalone *Haliotis varia* from Gulf of Mannar. *J Mar Biol Ass India* 46:154-161.
- Park JJ, Lee JY, Lee JS, Chang YJ (2003) Gonadal development and gametogenic cycle of the equilateral venus, *Gomphina veneriformis* (Bivalvia: Veneridae). *Korean J Fish Aquat Sci* 36:352-357.
- Park JJ, Kim H, Kang SW, An CM, Lee SH, Gye MC, Lee JS (2012) Sex ratio and sex reversal in two-year-old class of oyster *Crassostrea gigas* (Bivalvia: Ostreidae). *Dev Reprod* 16:385-388.
- Rho S, Park CK (1975) Studies on the propagation of the abalones. II- The spawning season of *Haliotis discus hannai* Ino in the adjacent areas of Yeosu. *Bull Korean Fish Soc* 8:234-241.
- Sobhon P, Apisawetakan S, Chanpoo M, Wanichanon C, Linthong V, Thongkukiatkui A, Jarayabhand P, Kruatrachue M, Upatham SE, Poomthong T (1999) Classification of germ cells, reproductive cycle and maturation of gonads in *Haliotis asinina* Linnaeus. *Sci Asia* 25:3-21.
- Visser-Roux A (2011) Reproduction of the South African abalone, *Haliotis midae*. Ph.D. Dissertation, University of Stellenbosch, Matieland, South Africa. p 88.
- Webber HH, Giese AC (1969) Reproductive cycle and gametogenesis in the black abalone, *Haliotis cracheroidii* (Gastropoda: Prosobranchiata). *Mar Biol* 4:152-159.
- Wilson NHF, Schiel DR (1995) Reproduction on two species of abalone (*Haliotis iris* and *H. australis*) in southern New Zealand. *Mar Freshwater Res* 46:629-637.
- Young JS (1970) The reproductive cycle, gonadal histology and gametogenesis of the red abalone *Haliotis rufescens* (Swainson). *Calf Fish Game* 56:298-309.
- Yusa Y (2007) Causes of variation in sex ratio and modes of sex determination in the Mollusca: An overview. *Am Malacol Bull* 23:89-98.