Korean J. Malacol. 30(2): 139-153 2014
http://dx.doi.org/10.9710/kjm.2014.30.2.139

Depositions of Egg Capsules by Female Shell Heights and Comparisons of Sizes at 50% of Group Sexual Maturities of the Female Rapa Whelk *Rapana venosa* in Three Different Salinity Concentration Regions

Il Ho Lee, Ee-Yung Chung[1], Pal Won Son[2] and Ki-Young Lee[1]

Department of Fisheries Science, Graduate School, Kunsan national University, Gunsan 573-701, Korea
[1]Department of Marine Biotechnology, Kunsan National University, Gunsan 573-701, Korea
[2]Korea marine Eccology Institute, 1001 First Incentum, Busan 612-020, Korea

ABSTRACT

We investigated fecundities in egg capsules and sizes at 50% of group sexual maturities in female *Rapana venosa* in three different salinity concentration regions (S-1, S-2, and S-3). In three different salinities, egg capsule heights, the number of egg capsules and the number of eggs and embryos were remarkably increased with the increase of female shell heights (or ages) and also increased with the increase of salinity concentrations. Heights of egg capsules, the number of egg capsules and fecundities (the number of eggs and embryos) were the maximum at S-1 (Gwangyang Bay (average 31.5 psu)) and the minimum at S-3 (the upper reaches of Seomjin River (average 15.5 psu)). Total numbers of fecundities of *R. venosa* individual^{-1}year^{-1} were about $1x10^6$ at S-1 region, about $8x10^5$ at S-2 region, and about $2x10^5$ at S-3 region. Rates (50%) of individuals reaching first sexual maturities in three different salinity regions (S-1, S-2 and S-3) were over 50% in females measuring 7.1-8.0 cm in shell height (considered to be two years old), and 100% in those > 10.1 cm (considered to be five years old). Biological minimum sizes (RM_{50}) in females in three different regions are 72.0 mm SH at S-1 region, 70.9 mm SH at S-2 region, and 74.6 mm SH at S-3 region, respectively. Exceptionally, smaller individuals (considered to be one year old) were participated in reproduction.

Key words: *Rapana venosa*, egg capsule, fecundity, size at group sexual maturity

INTRODUCTION

The rapa whelk, *Rapana venosa* (Gasropoda: Muricidae) is one of the most important edible gastropods in East Asia (Yoo, 1976; Kwon *et al*., 1993), and it is distributed in Korea, China and Japan. More specifically, in Korea, it is mainly found in silty sand of the intertidal and subtidal zones. However, the standing stock of this species has gradually been decreasing due to extensive loss of habitats from reclamation projects and reckless over-harvesting. Therefore, it has been noted as a target organism that should be managed by a resonable fishing regimen.

To clarify the effect of morphological and physiological changes of a marine gastropod according to some differences in salinity concentrations, first sexual maturity, spawning frequencies (intervals), deposition of the egg capsules in female *R. venosa* inhabited in three different regions (Coastal seawater in Gwangyang Bay and the upper and lower reaches of the Seomjin River) should be investigated by histological and visual observations. For that reason, recently, the environmental, ecological surveys on

Received: June 15, 2014; Revised: June 20, 2014; Accepted: June 23, 2014
Corresponding author : Ki-Young Lee
Tel: +82 (63) 469-1832 e-mail: leekiy@kunsan.ac.kr
1225-3480/24524

environmental conditions of benthic fauna have been carried out around Gwangyang Bay and the upper and lower reaches of the Seomjin River. In this survey, especially, a number of specimens of the rapa whelk *R. venosa* were collected by the fishing net in this area. In general, however, it is well-known that *R. venosa* inhabits in high salinity in the seawater or lower salinities in the brackish waters. Exceptionally, some characteristics were found that especially, external morphological features of the egg capsules attached to the shell of living rapa whelks in the special environmental areas (lower salinities) remarkably varied with those in the open seawater area with normal high salinity. Therefore, comparative studies on morphological, reproductive characteristics of this species under open or relatively semi-closed environmental conditions, such as high or low salinities. In particular, in this case, sufficient food organisms in the seawater or brackish water are very important for reproduction and resource proliferation of *R. venosa*. To date, there have been some previous studies of *R. venosa* on reproductive aspects, including reproductive cycle (Chung *et al*., 1993; Chung and Kim, 1997: Chung *et al*., 2002), reproductive patterns (Middelfart, 1994, 1996; spawning and egge capsules (Knudsen, 1950; Habe, 1960, 1969; Amio, 1963; Spight, 1976; D'Asaro, 1966, 1970, 1988, 1991, 1993a, 1993b; Le Boeuf, 1971; Rawling, 1990, 1994, 1995; Harding & Mann, 1999; Harding *et al*., 2007), on classification (Habe, 1969; Wu, 1988), larval development (Thorson, 1940; Staiger, 1950, 1951; on biochemical aspects (Yoon *et al*.,1986; Yoo *et al*., 1991) and on morphology (Lee and Kim, 1988). However, there are still uncertainty in many aspects of reproductive biology by brackish water with low salinity. Little information is available on first sexual maturity or biological minimum size, fecundity in the egg capsules per individual, and morphological variations of egg capsules (due to semi-closed environmemtal factors such as brackish water with low salinity and deficient nutrients associated with restricted food organisms) in three different salinity concentration areas. In particular, data on sizes at 50% of group sexual maturities, fecundity in the egg capsules and reproductive strategy are very useful information for natural living resource management and reproductive potential of *R. venosa*. Therefore, the main aim of the present study is to investigate the average egg capsule height, number of egg capsules, and number of eggs and larvae in the egg capsules per individual and the sizes at 50% of group sexual maturities of *R. venosa* in three different salinity concentration regions in Gwangwang Bay and the lower and upper reaches Seomjin River, Korea.

MATERIAL AND METHODS

1. Investigations of Environmental Seawater Temperature and Salinity

Seawater temperatures and salinity concentrations were quoted monthly in the coastal zone of the Gwangyang Bay (S-1), the lower reaches of the Seomjin River (S-2), and upper reaches of the Seomjin River (S-3), Hadong, Gyeongsangnam-do, Korea, from January to December, 2009. To get some data for seawater temperatures and salinities in the Gwangyang Bay and the brackish water temperatures in the upper and lower reaches of the Seomjin River, unpublished data of seawater temperatures and salinities in Gwangyang Bay measured daily at 10:00 am at Gwangyang Iron Company, Gwangyang, Jeollanam-do, Korea were used for this study. And also unpublished data of brackish water temperatures and salinities in the upper and lower reaches of Seomjin Rivers, which is provided by water Environment Information System of Yeongsan River Basin Environmental Office, Jeollanam-do, Korea, were used for this study.

2. Sampling Methodology

Specimens of the rapa whelk, *R. venosa* were collected during the spawning period (May to July in 2009) by the fishing net in the coastal zone in Gwangyang Bay (S-1), the lower reaches of the Seomjin River (S-2), and the upper reaches of the Seomjin River (S-3) in Hadong, Geongsangnam-do, Korea (Fig. 1). The rapa whelks ranging from 53.1 mm to 108.2 mm in shell height in Gwangyang Bay (S-1), from 52.3 mm to 101.5 mm SH in the lower reaches of

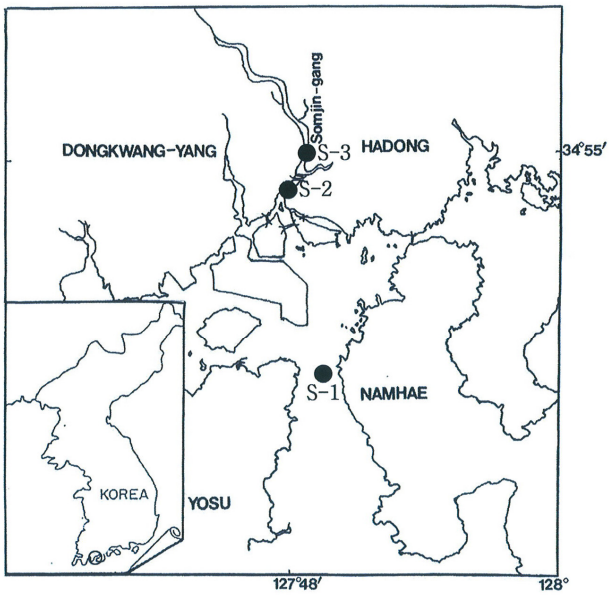

Fig. 1. Map showing the sampling areas (S-1, S-2, S-3).

the Seomjin River (S-2), and from 49.2 mm to 97.1 mm in the upper reaches of the Seomjin River (S-3) were used for this study. After rapa whelks were transported alive to the laboratory in Marine Biology Research & Education Center, Kunsan National University, shell height and total body weights of female individuals at three regions (S-1, S-2, S-3) were immediately measured, and female rapa whelks which were fixed with 10% neutral formalin were used for this experiment

3. Observations of Deposition of egg capsules and calculations of fecundities in the egg capsules, spawning frequency, and the number of broods during the spawning period

To investigate the number of egg capsules per individual and the number of eggs and embryos in an egg capsule in the laboratory, in three different regions of salinity concentrations (S-1, S-2 and S-3) a total of 75 adult females of 72.3-141.5 mm in shell height were used for observation of spawning behavior in the FRP aquaria filled with experiment waters of three different salinity concentrations (high salinity sea water of average 31.5 psu in S-1 region, brackish water salinity of average 26.5 psu in S-2 region, and brackish water salinity of average 15.5 psu in S-3 region). Each FRP aquarium (80 cm x 60 cm x 60 cm) was bedded with sand and small gravels. An established filtration and aeration apparatus was employed. Rapa whelks used for this experiment were those collected in May to July 2009. Height and width of egg capsules of the adult rapa whelks were measured and their egg developmental processes were checked under a light microscope.

Rapa whelks were reared at three kinds of salinity concentrations (psu) and water temperatures of 18.5-23.8°C. Seawater and brackish waters in the rearing FRP aquarium were changed every 3 days. Sufficient amounts of bivalves (*Ruditapes philippinarum*, *Neretrix petechialis* and *Mactra veneriformis*) were supplied as food for *R. venosa* during the rearing period.

4. Production of Histological Preparations of Gonadal Tissues of Rapana venosa in Three Different Salinity Regions

For light microscopic examination of histological preparations, gonad tissues were removed from shell and preserved in Bouins fixative for 24h and then washed with running tap water for 24h. Tissues were then dehydrated in alcohol and embedded in paraffin molds. Embedded tissues were sectioned at 5-7μm thickness using a rotary microtome. Sections were mounted on glass slides and stained with Hansen's hematoxylin-0.5% eosin, and examined using the light microscope. A total of 501 female rapa whelks (32.3 to 130.8 mm in shell height) in three different salinity regions were used for the study in first sexual maturity by histological method. The percentages of first sexual maturity or participated in reproduction during the peak spawning period.

5. Size at First Sexual Maturity by Light Microscopical Observation

For determination of the size at 50% of first sexual maturity, in three different salinity concentration regions a total of 492 females (S-1: 181, S-2: 169, S-3: 142), ovarian histological preparations (32.3 to 130.8 mm in shell height) were examined the size at 50% first sexual maturity (= biological minimum size) by

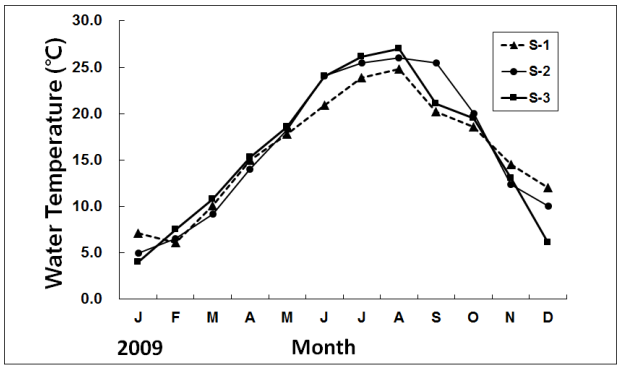

Fig. 2. Monthly changes in water temperature in three regions from January to December 2009.

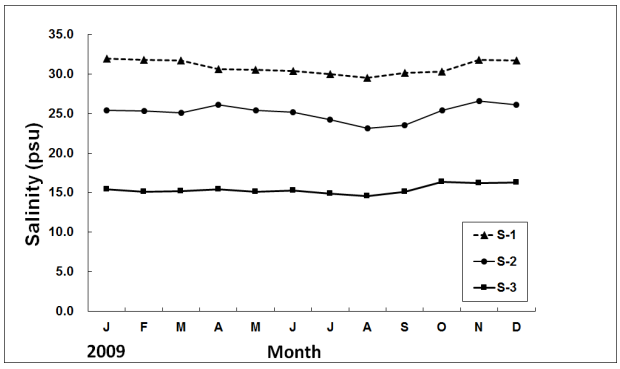

Fig. 3. Monthly changes in salinity in three different regions from January to December 2009.

histological observations from January to December 2009. The percentage (%) of first sexual maturity = No. of mature individuals x 100 / No. of total individuals investigated.

6. Size at the Rate (50%) of Sexual Maturity (RM_{50}) = Biological minimum size

To calculate the size at the rate (50%) of sexual maturity in three different salinity concentration regions (S-1, S-2, S-3) after fitting the rate of sexual maturity to an exponential equation, the size equivalent to the size at 50% of sexual maturity was estimated to be the sexually mature length of the population (Chung and Ryou, 2000). The exponential equation of the rate of sexual maturity is as follows: $RM = 100/1 + \exp^{(a-bx)}$, where, RM: rate of sexual maturity; a, b: constants, x: shell length. The size equivalent to 50% of first sexual maturity was estimated to be biological minimum size of the populations.

Results

1. Monthly changes in environmental seawater temperatures and salinities in the habitats of the rapa whelk at three different regions

Environmental seawater temperatures and salinities were measured in the habitats of *R. venosa* at three different regions (S-1, S-2, S-3) from February to October, 2009.

1) Seawater temperatures

As shown in Fig. 2, during the study period, the range of seawater temperatures at S-1 were 6.1-24.8°C (year average 15.9°C), those of S-2 were 5.0-26.0°C (year average 16.4°C), And the range of seawater temperatures at S-3 were 4.0-27.0°C (year average 16.1°C)

2) Salinity concentrations

As shown in Fig. 3, during the study period, the range of salinity concentrations were measured in the habitats of the rapa whelks at three different regions (S-1, S-2, S-3) from February to October, 2009. During the study period, the range of salinity in the seawater at S-1 were 29.5-31.9 psu (year average 30.9 psu), those at S-2 were 23.1-26.6 psu (year average 25.1 psu). And the range of salinity at S-3 was 14.6-16.4 psu. (year average 15.4 psu). In particular, salinity in July temporaily decreased because of heavy rain.

2. Female shell height, egg capsule height, number of egg capsule, number of eggs and larvae in three different salinity regions.

As shown in Table 1, the range of female shell heights in three regions were 53.1–108.2 mm (average 85.6 ± 18.5 mm) at S-1, 52.3–101.5 mm (average 81.1 ± 14.7 mm) at S-2, and 49.2–97.1 mm (average 74.0 ± 16.1 mm) at S-3, respectively. Over all, the size of female shell height at S-1 (Gwangyang Bay) was the largest (Fig. 4A), that of female shell height at S-2 (the lower reaches) was middle size (Fig. 4B), and that at S-3 (the upper reaches of Seomjin River) was the

Table 1. Mean ± SD of each variable by waters (minimum value–maximum value)

waters	Shell height (mm)	Egg capsule height (mm)	Number of egg capsules	Number of larvae
S-3	74.0 ± 16.1	14.0 ± 1.0	80 ± 11	530 ± 112
(14.6-16.4 psu)	(49.2-97.1)	(12.6-14.8)	(58-92)	(385-688)
S-2	81.1 ± 14.7	2.84 ± 0.23	95 ± 14	1,490 ± 124
(23.1-26.6 psu)	(52.3-101.5)	(23.0-31.0)	(82-112)	(1,273-1,610)
S-1	85.6 ± 18.5	30.2 ± 2.7	102 ± 13	1,624 ± 137
(29.5-32.0 psu)	(53.1-108.2)	(28.6-31.1)	(88-117)	(1,392-1,991)

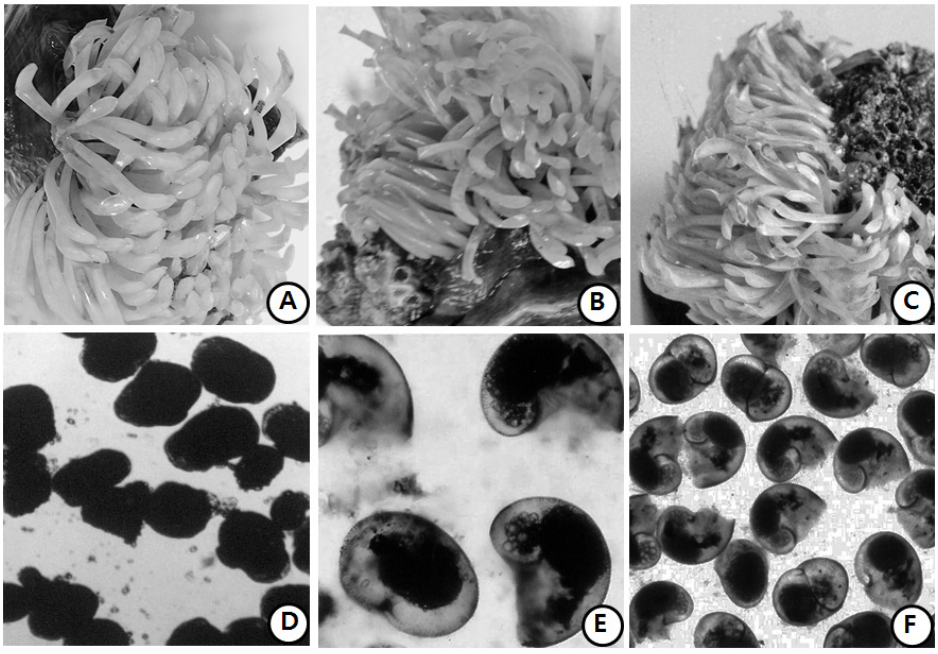

Fig. 4. Photographs showing egg capsules and developing eggsand embryos. **A**: a number of egg capsules at S-1; **B**: a number of egg capsules at S-2; **C**: a number of egg capsules at S-3: **D**: developing eggs and trochophore larva: **E**, **F**: embryos.

smallest. In three sampling areas during the spawning period of this species between June and July, the smallest rapa whelk (49.2 mm SH) laid egg capsules with lower egg capsule heights of 1.26 mm (Fig. 4C) at S-3 (lower salinity average 15.5 psu), while the largest rapa whelk (108.2 mm SH) laid egg capsules with high egg capsule heights of 3.02 mm ± 0.27 (Fig. 4A) at S-1 (high salinity average 31.5 psu). In the egg capsule, egg development and trochopore larvae are found (Fig. 4D), and various larval developmental phases of embryos (veligers) are found (Fig. 4E and 4F).

Of three sampling areas, during the spawning period the largest number of the egg capsules in an egg mass laid from an individual ranged from 88–117 (102 ± 13) at S-1 (Gwangyang Bay), and 82–112 (95 ± 14) at S-2 (the lower reaches of Seomjin River), and ranged from 58–92 (80 ± 11) at S-3. Therefore, the number of egg capsules laid from a female individual at S-1 showed larger than S-2 and S-3 habitats (Table 1).

Fecundity (number of eggs and embryos) of the rapa whelks ranged from 530 ± 112 eggs and embryos (Figs. 4C, D, E) in a low egg capsule height of 14.0 ± 1.0 mm at S-3 to 1,624 ± 137 eggs and embryos (Figs. 4D, E, F) in high egg capsule height of 30.2 ± 2.7 mm at S-1.

3. Fecundities of the rapa whelk Rapana venosa individual^{-1}year^{-1}

The spawning season of this species is from May to early August in Korea and can be subdivided into the spawning periods (early spawning month and late spawning months). To calculate fecundity of this species in three different regions of salinity concentrations, we calculated the number of egg capsules and embryos in an egg capsule by their shell heights and ages (from two years (average 73.27 mm SH) to five years (average 107.00 mm SH).

As shown in Table 2, comparisons of differences in the average fecundity in an egg capsule per individual for a year in female *Rapana venosa* in three different regions (S-1, S-2, S-3) of salinity concentrations are as follow:

1) S-1

Of three regions, average number of fecundities individual^{-1}year^{-1} were 993,888 ± 4,374 in the coastal sea water in Gwangyang Bay (average 31.5 psu). Therefore, the largest fecundity of this species for a year was shown in the highest salinity region (S-1). Accordingly, reproductive potential in the egg capsules of the rapa whelk was the highest in the high salinity region.

2) S-2

Of three regions, S-2 is located in the lower reaches of Seomjin River and their salinity ranged from 24.5-27.0 psu (average 26.5 psu). The average number of fecundities individual^{-1}year^{-1} were 849,300 ± 6,115 in the brackish water in the lower reaches of Seomjin River. Accordingly, reproductive potential in the egg capsules of the rapa whelk in the brackish water salinity concentration region was smaller than that in Gwangyang Bay.

3) S-3

Of three regions, S-3 is located in the upper reaches of Seomjin River and their salinity ranged from 14.5-17.5 psu (average 15.5 psu). The average number of fecundities individual^{-1}year^{-1} were 237,440 ± 6,634 in the low salinity water in the upper reaches of Seomjin River showed the smallest. On the whole, total fecundities of the rapa whelk increased with the increase of salinity concentrations and female shell height.

4. First sexual maturities in female *R. venosa* at three different salinity regions

1) *R. venosa* in Gwangyang Bay (S-1: about 31.0-32.0 psu)

During the breeding season, a total of 181 individuals (32.3-130.8 mm in shell height) were histologically examined to check whether they reached maturity and participated in reproduction. The rate of shells of different size that reached the first sexual maturity is summarized in Table 3, spawning of *R. venosa* was occurred between July and August. In case of some individuals whose gonad developmental stage being in the late active stage in July, it is supposed

Table 2. Depositions of the egg capsules and total fecundities in an egg capsule in female Rapa venosa in three different regions of salinity concentrations

Three different regions of salinity concentrations	S-1 (Average 31.5 psu)	S-2 (Average 26.5 psu)	S-3 (Average 15.5 psu)
Average number of egg capsules / egg mass / individual	102 ± 13	95 ± 14	80 ± 11
Average number of eggs and embryos / egg capsule / egg mass	1,624 ± 137	1,490 ± 124	530 ± 112
Average number of broods / spawning period	3.0 ± 0.3	3.0 ± 0.1	2.8 ± 0.2
Average number of spawning months within the spawning season	2	2	2
Average number of fecundities / individual / year	993,888 ± 4,374	849,300 ± 6.115	237,440 ± 6,634

Table 3. The shell height and first sexual maturity in female *Rapana venosa* inhabited at S-1 (high salinity: 31.5 psu) in Kwanyang Bay, from early May to August, 2009

	Number of individuals by gonadal stage*						
Shell height (mm)	EA	LA	RI	PS	RE	Total	Mature %)
32.3-40.0	4					4	0.0
41.0-50.0	7					7	0.0
50.1-60.0	9	1	1			11	18.2
60.1-70.0	7	2	1			10	30.0
70.1-80.0	13	3	11	3		30	56.7
80.1-90.0	4	6	16	5		31	87.1
90.1-100.0		4	14	8		26	100.0
100.1-110.0		3	9	7	2	21	100.0
110.1-120.0		2	8	6	2	18	100.0
120.1-130.0		1	6	5	1	13	100.0
130.1-130.8		1	4	3	2	10	100.0
Total						181	

* Gonadal stage: EA, early active stage; LA, late active stage; RI, ripe stage; PS, partially spawned stage; RE, recovery stage.

that they can reach maturity except for individuals in the early active stage during the breeding season. First sexual maturity in female rapa whelk of 32.3 to 50.0 mm high was 0% if they were at the early active stage during the breeding season. The percentages of first sexual maturity of female snails of 50.1 to 60.0 mm and 60.1 to 70.0 mm in shell height were 18.2% and 30.0%, respectively; most of the individuals were still in the early active stage. Percentage of first maturity in 70.1 to 80.0 mm in shell height were over 50%, in which all snails those at the late active, rips and partially spawned stages were included. From those over 90.1mm in shell height, it was 100%.

The rate of individuals reaching first sexual maturity in Gwangyang Bay were over 50% in females measuring 70.1-80.0 mm in shell height, and 100% in those > 100.1 mm. Therefore, over 50% of first sexual maturity were 70.1-80.0 mm in shell height (considered to be two years old), and 100% of first sexual maturity was 100.1-110.0 mm in shell height (considered to be five years old).

2) *R. venosa* in lower reaches of the Seomjin River (S-2: 23.1-26.6 psu)

First sexual maturity in female *R. venosa* of 34.2 to 50.0 mm high was 0%. The percentages of first sexual maturity of female snails of 50.1 to 60.0 mm and 60.1 to 70.0 mm in shell height were 13.3% and 23.1%, respectively. Percentage of first maturity in 70.1 to 80.0 mm in shell height were over 50%. From those over 90.1 mm in shell height, it was 100%. The rate of individuals reaching first sexual maturity in lower reaches of the Seomjin River (S-2) were over 50% in females measuring 70.1-80.0 mm in shell height, and 100% in those > 100.1 mm SH (Table 4)

3) *R. venosa* in upper reaches of the Seomjin River (S-3: 14.6-16.4 psu)

As shown in Table 5, First sexual maturity in female *R. venosa* of 36.1 to 50.0 mm high was 0%. The percentages of first sexual maturity of female snails of 50.1 to 60.0 mm and 60.1 to 70.0 mm in shell height were 9.1% and 25.0%, respectively. Percentage of first maturity in 70.1 to 80.0 mm in shell height were 50%, in which all snails those at the late active, rips and partially spawned stages were included. From those over 90.1 mm in shell height, it was 100%. The rate of individuals reaching first sexual maturity in the upper reaches of the Seomjin River (S-3) were over 50% in females measuring 70.1-80.0 mm in shell height, and 100% in those > 90.1 mm SH.

5. Biological minimum size (= Size at the rate (50%) of group sexual maturity

Table 4. The shell height and first sexual maturity in female *R. venosa* inhabited at S-2 (brackish water salinity: 26.5 psu), from July to October, 2009

Shell height (mm)	Number of individuals by gonadal stage*						
	EA	LA	RI	PS	RE	Total	Mature (%)
34.2-40.0	7					7	0.0
40.1-50.0	8					8	0.0
50.1-60.0	13	1	1			15	13.3
60.1-70.0	7	2	4			13	23.1
70.1-80.0	7	4	10	3		24	54.2
80.1-90.0	3	3	10	6		22	86.4
90.1-100.0		8	12	7	2	29	100.0
100.1-110.0		4	8	9	2	23	100.0
110.1-120.0		2	9	5	2	18	100.0
120.1-129.8			5	4	1	10	100.0
Total						169	

* Gonadal stage: EA, early active stage; LA, late active stage; RI, ripe stage; PS, partially spawned stage; RE, recovery stage.

Table 5. The shell height and first sexual maturity in female *R. venosa* inhabited at S-3 (low salinity: 15.5 psu), from July to October, 009

Shell height (mm)	Num ber of individuals by gonadal stage*						
	EA	LA	RI	PS	RE	Total	Mature (%)
36.1-40.0	4					4	0.0
40.1-50.0	9					9	0.0
50.1-60.0	10	1				11	9.1
60.1-70.0	15	3	2			20	25.0
70.1-80.0	12	4	6	2		24	50.0
80.1-90.0	4	3	10	4		21	81.0
90.1-100.0		7	15	5	2	29	100.0
100.1-110.0		3	6	2	2	13	100.0
110.1-116.7		2	6	2	1	11	100.0
Total						142	

* Gonadal stage: EA, early active stage; LA, late active stage; RI, ripe stage; PS, partially spawned stage; RE, recovery stage.

1) Biological minimum size in Gwangyang Bay (S-1)

To understand the biological minimum sizes in female individuals, the sizes at 50% of group sexual maturities (RM_{50}) of *R. venosa* were investigated. In general, we regard the size at the rate (50%) of group sexual maturity as biological minimum size. Sometimes its size (RM_{50}) provided an important information for fishing prohibitory measure as basic data. As shown in Fig. 5, rapa whelk of 32.3-130.8 mm SH of group sexual maturity of *R. venosa* (sizes at the rate (50%) of group sexual maturity, RM_{50}) that were fitted to an exponential equation were 72.0 mm SH in females. Therefore, biological minimum size (RM_{50}) in females is 72.0 mm SH in S-1 region . The size (72.0 mm SH) at 50% of first sexual maturity in females was considered to be two years old.

2) Biological minimum size in the lower reaches of Seomjin River (S-2)

As shown in Fig. 6 , shell heights of group sexually maturity of *R. venosa* (sizes at the rate (50%) of group sexual maturity, RM_{50}) that were fitted to an

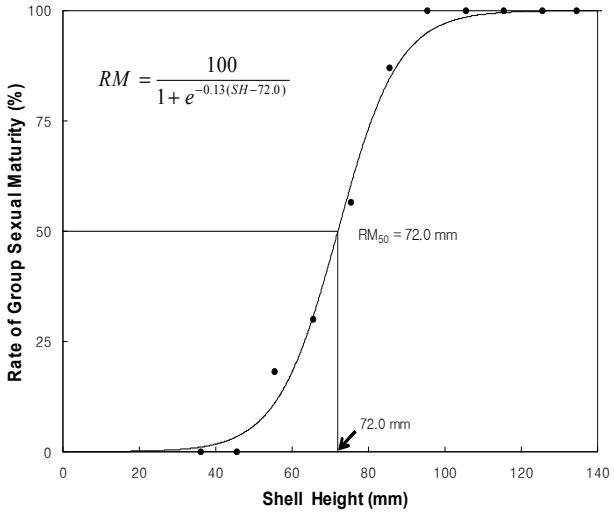

Fig. 5. Relationship between the rate of Group sexual maturities (%) and shell height (mm) in female *Rapana venosa*. RM_{50} at S-1 region.

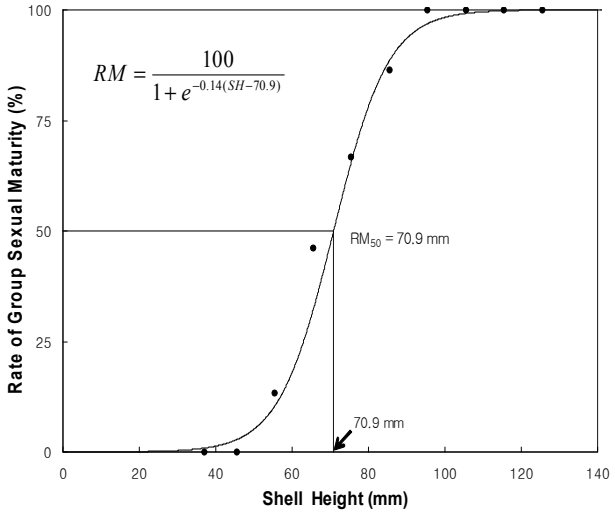

Fig. 6. Relationship between the rate of Group sexual maturities (%) and shell height (mm) in female *Rapana venosa*. RM_{50} at S-2 region.

exponential equation were 70.9 mm in females. Therefore, biological minimum size (RM_{50}) in females is 70.9 mm SH in S-2 region. The size (70.9 mm SH) at 50% of first sexual maturity in female *R. venosa* was considered to be two years old.

3) Biological minimum size in the upper reaches of Seomjin River (S-3)

As shown in Fig. 7 , shell heights of group sexually

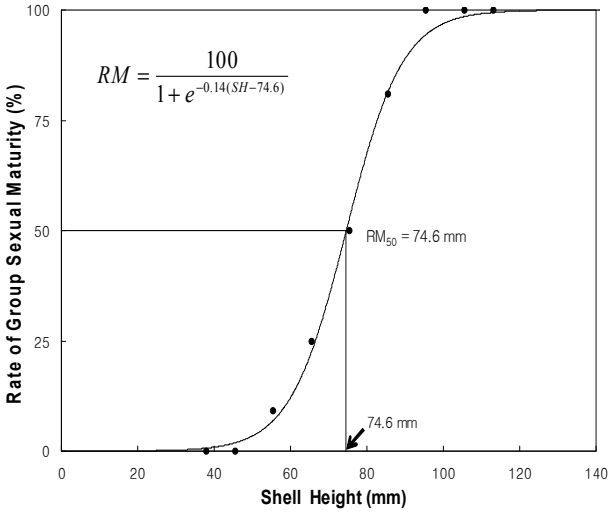

Fig. 7. Relationship between the rate of Group sexual maturities (%) and shell height (mm) in female *Rapana venosa*. RM_{50} at S-3 region.

maturity of *R. venosa* (sizes at the rate (50%) of group sexual maturity, RM_{50}) that was fitted to an exponential equation is 74.6 mm in females in S-3 region. The size (74.6 mm SH) at 50% of first sexual maturity in females was considered to be two years old.

6. Differnces in the Number of Broods and Average Total Fecundity During the breeding season of *R. venosa* according to shell heights or age in three different salinity regions

1) Indoor rearing experiment at the salinity concentration of 31.5 ± 0.5 psu and reared at 18-20℃ in the aquarium

During the period from May 25 to July 30, 5 of 20 individuals of rapa whelks (72.3-128.4 mm SH), which is reared in the coastal seawater, spawned total 18 egg masses (1,885 egg capsules) with one to three broods (spawning frequency or interval) during the spawning period. Two to five masses (average 3.6) per individual were produced, In general, the number of the spawning round per individual was increased with the increase of shell height per individual. On the whole, the deposition of egg capsules (the spawning time) mainly occurred at night and dawn as seen in Table. 6.

Table 6. Spawning frequency and the number of egg capsules of *Rapana venosa* at salinity concentration of 31.5 ± 0.5 psu observed from May to July 2009

Ind. No.	Shell height (mm)	Spawning data	Spawning hours	Water temp. (℃)	No. of egg capsules	Spawning (Number of broods)	Interval (days)
1	72.3	May 25, 2009	20:27-23:10	18.7	116		
		May 27, 2009	24:38-04:16	18.5	114		
					(230)	2	2
2	102.1	May 28, 2009	20:26-24:10	18.4	116		
		May 29, 2009	23:18-02:16	19.3	108		
		May 31, 2009	24:17-04:32	19.1	87		
					(311)	3	1-2
3	112.4	June 16, 2009	20:20-24:32	19.8	115		
		June 17, 2009	23:21-04:12	20.2	104		
		June 20, 2009	24:42-04:24	20.3	98		
		June 21, 2009	02:18-05:22	21.2	83		
					(400)	4	1-3
4	126.7	July 17, 2009	01:20-04:32	20.3	122		
		July 20, 2009	23:28-04:26	21.4	113		
		July 21, 2009	03:46-06:28	21.5	92		
		July 22, 2009	22:32-02:16	22.2	87	2	2
					(414)	4	1-3
5	128.4	July 25, 2009	20:12-24:08	22.5	126		
		July 26, 2009	02:22-05:45	23.7	110		
		July 27, 2009	22:32-03:18	23.4	104		
		July 29, 2009	03:14-05:28	24.1	98		
		July 30, 2009	05:16-09:36	24.2	92		
					(530)	5	1-2

2) Indoor rearing experiment at the salinity concentration of 25.5 ± 0.5 psu and reared at 18-20℃ in the aquarium

During the period from May 25 to July 27, 5 of 20 individuals of rapa whelks (75.1-141.3 mm SH), which is reared in the brackish water, spawned total 16 masses (1,688 egg capsules) with one to three broods (spawning frequencies or interval) during the spawning season. In general, the number of spawning round per individual was increased with the increase of shell height per individual. As shown in Table 7, on the whole, the deposition of egg capsules mainly occurred at night and dawn in the aquarium at the laboratory.

3) Indoor rearing experiment at the salinity concentration of 15.5 ± 0.5 psu and reared at 18-20℃ in an aquarium

During the period from May 15 to July 27, 5 of 20 individuals of rapa whelks (74.1-100.2 mm SH), which is reared in the brackish water, spawned total 13 masses (741 egg capsules) with one to three broods (spawning frequencies or interval) during the spawning season.

In general, the number of spawning round per individual was increased with the increase of shell height per individual. As shown in Table 8, on the whole, the deposition of egg capsules mainly occurred at night and dawn.

In general, the deposition of egg capsules, the spawning round, spawning frequency, the number of broods per individual showed similar patterns between three different regions of salinity concentrations. The average time required for a spawning of this species was 4.5 hours (3.0-5.5 hours) from the beginning of spawning. According to the observation in the laboratory, most spawning occurred from night to dawn morning in three different salinity concentrations in aquaria at the laboratory.

Table 7. Spawning frequency and the number of egg capsules of *Rapana venosa* at salinity concentration of 25. 5± 0.5 psu observed from May to July 2009

Ind. No.	Shell height (mm)	Spawning data	Spawning hours	Water temp. (℃)	No. of egg capsules	Spawning frequency (No. of broods)	Interval (day)
1	75.1	May 25 2009	20:32-23:11	18.2	116		
		May 27 2009	02:42-06:20	18.7	106		
		May 31 2009	01:20-04:46	20.4	102		
					(324)	3	1-4
2	93.2	June 16 2009	21:18-04:22	19.1	117		
		June 17 2009	20:32-24:34	20.3	105		
		June 19 2009	21:30-24:15	20.2	103		
		June 21 2009	02:42-07:10	20.5	102		
					(427)	4	1-2
3	103.4	June 25 2009	21:42-02:30	21.1	116		
		June 27 2009	02:14-04:38	21.7	101		
		June 28 2009	03:22-06:10	22.7	97		
					(314)	3	1-2
4	110.2	July 5 2009	20:43-24:15	22.4	103		
		July 7 2009	24:32-03:28	22.7	112		
					(215)	2	2
5	141.3	July 22 2009	21:15-23:20	22.3	118		
		July 23 2009	20:16-23:40	23.3	105		
		July 26 2009	19:12-22:36	23.7	98		
		July 27 2009	03:08-05:15	23.1	87		
					(408)	4	1-3

Table 8. Spawning frequency and the number of egg capsules of *Rapana venosa* at salinity concentration of 15.5 ± 0.5 psu observed from May to July 2009

Ind. No.	Shell height (mm)	Spawning data	Spawning hours	Water temp. (℃)	No. of egg capsules	Spawning frequency (No. of broods)	Interval (day)
1	74.1	May 15, 2009	21:35-23:12	18.5	65		
		May 17, 2009	03:15-07:22	19.3	54		2
					(119)		
2	80.5	June 13, 2009	24:12-02:15	19.5	61		
		June 15, 2009	20:24-22:22	20.2	51		
		June 18, 2009	21:24-24:36	20.6	48		
					(160)		2-3
3	90.2	June 20, 2009	21:19-01:15	21.4	63		
		June 21, 2009	24:08-03:21	21.9	58		
		June 23, 2009	04:44-07:32	22.5	52		
					(173)	3	1-2
4	94.2	July 18, 2009	23:21-02:21	22.4	62		
		July 20, 2009	03:42-06:51	22.4	57		
					(119)	2	2
5	100.2	July 22, 2009	22:15-02:55	22.6	61		
		July 24, 2009	20:21-23:45	23.3	58		
		July 27, 2009	21:11-24:17	23.7	51		
					(170)	4	2-3

7. Copulatory Behavior and Spawning

In experimental aquaria at the laboratory, copulatory interactions between females and males were observed in 25-27 May 2009. During this time, it was confirmed through histological examination that most of the ovaries were filled with a number of late vitellogenic oocytes or mature oocytes in the follicle representing the late active or ripe stages. During the peak spawning period (July and early August), however, spawning in female individuals occurred 15-17 days later after copulation in the rearing aquarium. This observation indicates that copulation individuals during the peak spawning period in female individuals during the peak spawning season.

Discussion

Recently, some researchers (Zolotarev, 1996; Mann and Harding, 2000; Mann *et al*., 2004; Harding *et al*., 2007) reported that rapa whelks are native to marine habitats off Korean peninsula and Japan, And they were introduced to the Black Sea immediately after World War II and have since spread throughout the Mediterranean, Awgean, and Adratic seas, and these large gastropods were settled in the world.

Harding (2007) reported that the rapa whelk in Chesapeake Bay is a marine gastropod that reaches large adult sizes, but is reproductively active early (from one year old) in life. However, rapa whelks in Chesapeake Bay are long lived (in excess of 15 years) and may reach adult sizes greater than 160 mm shell length. However, recently, it is hard to find female adult sizes greater than 145 mm SH (it is considered to be eight year of age) in Korea (Choi, 2008).

According to the results investigated on depositions of egg capsules and total fecundities (eggs and embryos) in egg capsules per individual of female rapa whelks during the year in three different salinity concentrations (S-1, S-2, S-3), egg capsule heights, the number of egg capsules and the number of eggs and embryos were remarkably increased with the increase of female shell heights (or ages) and also increased with the increase of salinity concentrations. Accordingly, heights (mm) of egg capsules, the number of egg capsules and the number of eggs and embryos were the maximum at S-1 (Gwangyang Bay (average 31.5 psu)) and the smallest at S-3 (the upper reaches of Seomjin River (average 15.5 psu)). Thus, the effects of salinity concentrations on investigation items showed remarkable differences in the results of the present study.

In this study, according to the results of the indoor experiment, the Korean rapa whelks are reproductively capable from one year (average 61.20 mm SH) because rapa whelks produced relatively small number of egg capsules in egg masses in three different salinity concentrations in aquaria. Therefore, the results of this experiment are inconsistent with those of previous studies reported by Harding (2007).

Regarding total fecundities in egg capsules in egg masses, Harding (2007) reported that small (< 80 mm SL) rapa whelks have relatively lower annual fecudities (1×10^5 embryos female^{-1}year^{-1}) than large (> 80 mm SL) rapa whelks that may produce between 1×10^6 and 4×10^6 embryos female^{-1}year^{-1}.

In this study, small rapa whelks (< 80 mm SH, considered to be two years old), they have relatively small fecudities (4×10^5 embryos to 5×10^5 embryos female^{-1}year^{-1}) in each egg capsule, Accordingly, we can confirm that annual total fecundities of the Korean rapa whelks are slightly smaller than annual total fecundities (1×10^6 embryos female^{-1}year^{-1}) reported by Harding (2007).

However, in case of slightly large rapa whelks (> 80 mm SH, over three years of age), the annual fecundities of the Korean rapa whelks are smaller than the results (1×10^6 embryos female^{-1}year^{-1} to 4×10^6 of embryos female^{-1}year^{-1}) reported by Harding (2007). In general, the fecundities of rapa whelks in Chesapeake Bay are larger than those in Gwangyang Bay and Seomjin River in Korea

Regarding the egg capsule height of the rapa whelk *R. venosa*, egg capsule height and the number of embryos per egg capsule are positively correlated with female size. Amio (1963) described egg capsules from Japan 30 mm high containing 790-1,300 embryos per capsule. Chukhchin (1984) collected rapa whelk egg capsule with heights ranging from 6-24 mm containing 200-1999 embryos per egg capsule in the Black Sea

from parents of unknown size. Chung *et al.* (1993) reported that egg capsules with heights of 25-27 mm containing an average of 1,096 embryos per egg capsule laid by the Korean rapa whelks 120-140 mm shell height. D'Asaro (1991) described larger egg capsules of *R. venosa* from southern Japan as 31-36 mm in height.

Exceptionally, Harding (2007) reported that the smallest rapa whelks in Chesapeake Bay were observed in his study (45 mm SL) laid egg capsules with height of approximately 7 mm, whereas the largest rapa whelk (163 mm SL) laid egg capsules with height 30-33 mm.

In this study, the largest rapa whelks (145 mm SH) in Korea laid egg capsules with height 30-31 mm. However, the number of egg capsules and egg capsule heights of rapa whelks vary with salinity concentrations in habitats. In particular, from the studies of reproductive potentials of rapa whelks by three regions (Gwangyang Bay (S-1), the lower reaches of Seomjin River (S-2) and the upper reaches of Seomjin River (S-3)) for six years, we could get several important information associated with growth of shell heights relationships between the number of egg capsules and salinity concentrations, and relationships between the number of embryos in the egg capsules and salinity concentrations. On the whole, reproductive potentials (fecundities) of rapa whelks varied with salinity concentrations in three different regions. Therefore, in case of rapa whelks, salinities in habitats affected reproductive potential of this gastropod.

The number of embryos observed in egg capsules produced by Chespeake Bay rapa whelks ranged from 123 (7.4 mm high egg capsule) to 3,673 (33.5 mm high egg capsule).

Based on these observations, Chespeake Bay rapa whelk females appear to produce egg capsules of comparable size to those produced by rapa whelks in their native range (Korea and Japan), whereas Black Sea rapa whelks seem to produce egg capsules that are smaller than those from either Asia or Chespeake Bay.

When there was variation in the number of embryos per egg capsule, the first egg masses in the egg-laying season had more embryos per egg capsule than egg capsules produced latter in the season. This pattern was observed in some of the smallest whelks (64-71.3 mm SL as well as the largest whelk (163 mm SL).

There may be an adaptive advantage in producing more embryos early in the season for both larval survival to settlement and larval growth post settlement. Under culture conditions, rapa whelks that settle by August 15, often attain sizes > 25 mm SL before water temperature fall below 10°C in October to November, and theses juvenile whelks are also reproductively active the summer after they hatch and settle (Harding, unpublished data). In general, the number of eggs within the egg capsule varied with the time of egg capsule deposition.

Small rapa whelks laid between 100 and 1,000 egg capsule per season, whereas large whelks may lay more than 1,000 egg capsules per year with as many as 2,379 egg capsules per female observed in 2001. Egg capsule production at these levels translates into annual fecundity/ embryos /female/ year, estimates on the order of 1×10^5 for smaller whelks and in excess of 1×10^6 for larger rapa whelks).

In three different salinity concentration regions, total fecundities of rapa whelks individual^{-1}year^{-1} were 993,888 ± 4,374 at S-1 region, 849,300 ± 6,115 at S-2 region and 237,440 ± 6,634 at S-3 region. Therefore, reproductive potential in the egg capsules of the rapa whelk was the highest in the high salinity region.

reproductive potential in the egg capsules of the rapa whelk in the brackish water salinity concentration region was smaller than that in Gwangyang Bay.

Biological minimum size (RM_{50}) in females were 72.0 mm SH in S-1 region, 70.9 mm SH at S-2 and 74.6 mm SH st S-3. These sizes at 50% of first sexual maturity in females were considered to be two years old.

In this study, exceptionally, we found that relatively small number of rapa whelks (concidered to be one year old: 61.20 mm SH (Choi, 2008)) spawned small number of egg capsules in the aquarium. However, the percentage (%) of spawned individuals was relatively small percentage. In this study, although rapa whelks

can be participated in spawning from one year old around the Gwangyang Bay and the lower or upper reaches of Seomjin River, 50% of first sexual maturity can be reached from two years old (approximately 73.27 mm SH (Choi, 2008)). Rapa whelk fecundity (number of embryos female^{-1}yr^{-1}) increases with female size. Egg capsule production was observed in rapa whelks as small as 45 mm SL that had hatched or been released from egg masses

Acknowledgement

The authors are grateful to Prof. Dr. Dong ki Ryou of Kunsan National University for helpful comments on the manuscript. And also the authors thank to Dr. Jung Jun Park of National Fisheries Research and Development Institute for helpful comment on calculations of natural resource data. This paper was supported by research funds (2013) of Kunsan National University. The authors thank to Coastal Research Center, Kunsan National University for supporting the research.

REFERENCES

Amio, M. (1963) A compatative embrology of marine gastropods, with ecological emphasis. *Journal of Shimonoseki College Fisheries*, **12**: 229-253.

Bandel, K. (1973) Spawning and development of some Columbellidae from the Caribbean sea of Columbia from the South America. *Veliger*, **16**: 271-282.

Cernohorsky, W.O. (1965) The radula, egg capsules and young of *Murex* (*Chicoreus*) *torrefactus* Sowerby (Mollusca: Gastropoda). *Veliger*, **8**: 231-233.

Choi, J.D and Ryu, D.K. 2009) Age and Growth of purple whelk, *Rapana venosa* (Gastropoda: Muricidae) in the west sea of Korea. *The Korean Journal of Malacology*, **25**: 189-196.

Chung, E.Y, Kim, S.Y, Kim, Y.G. (1993) *Rapana venosa* (Gastropoda: Muricidae), with species reference to the reproductive cycle, depositions of egg capsules and hatching of larvae. *The Korean Journal of Malacology*, **9**: 1-15.

Chung, E.Y, Kim S.Y. (1997) Cytological studies on testicular maturation and cyclic changes in the epithelial cells of the seminal vesicle of the male purple shells *Rapana venosa* (Gastropoda: Muricidae). *Malacological Review*, **30**: 25-38.

Chung, E.Y., Kim, S.Y., Park, K.H. Park, G.M. (2002) Sexual maturation, spawning and deposition of the egg capsules of the female purple shell, *Rapana venosa* (Gastropoda: Muricidae). *Malacologia*, **44**: 241-257.

D'Asaro, C.N. (1966) The egg capsules, embryogenesis and early organogenesis of a common oyster predator, *Thais haemostoma floridana* (Gastripoda: Prosobranchia). *Bulletin of Marine Science*, **16**: 884-914.

D'Asaro, C.N. (1970) Egg capsules of prosobranch mollusks from south Florida and the Bahamas and notes on spawning in the laboratory. *Bulletin of Marine Science*, **20**: 414-440.

D'Asaro, C.N. (1988) Micromorphology of neogastropod egg capsules. *Nautilus*, **102**: 134-148.

D'Asaro, C.N. (1991) Gunnar thorsons world-wide collection of prosobranch egg capsules: Muricidae. *Ophelia*, **35**: 1-101.

D'Asaro, C.N. (1993a) Gunnar thorsons world-wide collection of prosobranch egg capsules: Melongenidae. *Ophelia*, **46**: 83-125.

D'Asaro, C.N. (1993b) Gunnar thorsons world-wide collection of prosobranch egg capsules: Nassariidae. *Ophelia*, **48**: 149-215.

Habe, T. (1960) Egg masses and egg capsules of some japanese marine prosobran- chiate gastropod. *Bulletin of National Biological Station Asamusi*, **10**: 121-126.

Habe, T. (1969) A nomenclatorial note on *Rapana venosa*. *Venus*, **28**: 109-111.

Harding, J.M, MANN, R. (1999) Observations on the biology of the veined rapa whelk, *Rapana venosa* in the chespeake bay. *Journal of Shellfish Research*, **18**: 9-17.

Harding, J.M., Mann, R. and Kilduff, C.W. (2007) The effects of female size on fecundity in a large marine gastropod *Rapana venosa* (Muricidae). *Journal of Shellfish Research*, **26**: 33-42.

Knudsen, J. (1950) Egg capsules and development of some marine prosobranchs from tropical west africa. *Atlantide Report*, **1**: 85-130.

Kwon, O.K,, Park, G.M, and Lee, J.S. (1993) Coloured Shells of Korea. Academy Publication Co. Seoul, 285 pp.

Lee, J..J, and Kim, S.H. (1988) Morphological study on the osphradium of *Rapana venosa*. *The Korean Journal of Malacology*, **4**: 1-16.

Le Boeuf, R. (1971) Thais emarginata: Description of the veliger and egg capsule. *Veliger*, **14**: 205-211.

Middelfart, P. (1994) Reproductive patterns in Muricidae. *Phuket Marine Biological Center Special Publication*, **13**: 83-88.

Middelfart, P. (1996) Egg capsules and early development of ten muficid gastropods from Thailand Water. *Phuket Marine Biological Center Special Publication*, **16**: 103-130.

Mann, R. and Harding, J.M. (2000) Invasion of a Mid Atlantic estury by the oriental gastropod *Rapana venosa* Valenciennes, 1846. *Biological Invasion*, **2**: 7-22.

Perron, F.E, Corpuz, G.C. (1982) Cost of parenrtal cate in the gastropod conus Ponnaceus: Age specific changes and physical constraints. *Oecologia*, **55**: 319-324.

Rawling, T.A. (1990) Associations between egg capsule morphology and predation among populations of the marine gastropod. nucella emarginata. *Biological Bulletin*, **179**: 312-325.

Rawling, T.A. (1994) Encapsulation of eggs by marine gastropods: Effect of variation in capsules from on the vulnerability of embryos to predation. *Evolution*, **84**: 1301-1313.

Rawling, T.A. (1995) Adaptations to physical stresses in the intertidal zone: The egg capsules of neogastropod mollusca. *American Zoology*, **39**: 230-243.

Spight, T.M. (1976) Ecology of hatching size for marine snails. *Oecologia,* **24**: 283-294.

Staiger, H. (1950) Zur determination der Nhreier bei prosobranchiern. *Revision Suis Zoology*, **57**: 496-530.

Staiger, H. (1951) Cytologische und morphologiche untersuchungen zur determination der Neherier bei prosobranchiern. *Zeitschrift für Zellforsch Mikosk anat*, **35**: 469-549.

Thorson, G. (1940) Studies on the egg masses and larval development of gastropods from the lranian gulf. *Danish Scientific Inverstigations in Iran*, **2**: 159-238.

Wu, Y. (1988) Distribution and shell height-weight relation of Rawling *Rapana venosa* Valenciennes in the Laizhou Bay. *Marine Science/Haiyanh kexue,* **6**: 39-40.

Yoo, J.S. (1976) Korean shells in Colour. Ilgisa Seoul. 196 pp.

Yoo, I.S, Soh, C.T, Lee, I.S, Kim, J.J. (1991) Heavy metals in water, sediments and mollusca along coast line close to the estuaries of Gum-gang (river) and Mangyeong-gang. *The Korean Journal of Malacology*, **7**: 87-93.

Yoon, H.D. (1986) Lipid composition of purple shell and abalone. *Bulletin of Korean Fisheries Society*, **19**: 446-452.

Zolotarev, V. (1996) The Black Sea ecosystem change related to the introduction of new molluska species. *Marine Ecolology*, **17**: 227-236.