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First Sexual Maturity, Spawning Frequency and Deposition of the Egg Capsules of the Female Purple Shell *Rapana venosa* in the Slag Deposit Area, Gwangyang Bay, Korea

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한국 광양만, 슬러그 적재장내에 서식하는 암컷 피뿔고둥 *Rapana venosa*의 군성숙도, 산란빈도 및 난낭 산출

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ABSTRACT : First sexual maturity, sex ratio, spawning frequency, deposition of the egg capsules and fecundity of the female *Rapana venosa*(Valenciennes) inhabited in the artificially closed slag deposit area, Gwangyang Bay were investigated by histological and visual observations for natural living resource management. The rate of individuals reaching the first sexual maturity was 51.6% in females measuring 7.1~8.0 cm in shell height, and 100% in those > 10.1 cm. The total number of egg capsules per individual and the mean number of eggs in an egg capsule were 192~382 and 500, respectively. However, the number of eggs per individual and sizes of egg capsules under lower salinity and deficient food conditions in the closed slag deposit area were smaller than those under the optimum salinity and sufficient food conditions in the open regions. Fecundities of the species were approximately from 96,000 to 191,000 eggs /individual with two to four broods(spawning frequencies) during the spawning season. The duration of development in egg capsules was 18~19 days at about 18~20℃. *R. venosa* is a species whose embryos hatch as veliger larvae, not juvenile snail. The sex ratio of female : male was not significantly different from 1 : 1(χ^2= 0.23, p>0.05).

Key words : *Rapana venosa*, First sexual maturity, Fertility, Egg capsule, Sex ratio.

요 약 : 자연 생물자원 관리를 위해, 광양만내 인공적으로 폐쇄된 슬라그 적재장내에 서식하는 암컷 피뿔고둥 *Rapana venosa*(Valenciennes)를 대상으로 조직학적, 육안적 관찰에 의해 군성숙도, 성비, 산란빈도, 난낭 산출 및 포란수를 조사하였다. 암컷 개체들의 군성숙도(%)는 각고 7.1~8.0 cm의 경우 51.6%이었고, 9.1 cm 이상인 개체들의 경우는 100%이었다. 개체당 총난낭수 및 난낭내의 평균 난수는 각각 192~382개와 500개이었는데, 저염분이나 먹이가 부족한 환경조건을 갖추고 있는 폐쇄된 슬러그 적재장내에 서식하는 피뿔고둥들은 최적 염분농도 및 충분한 먹이조건을 갖추고 있는 개방된 지역의 것보다 개체당 총난낭수 및 난낭내 평균 난수가 적었으며, 난낭의 크기도 작았다. 본 종의 포란수는 대략 개체당 96,000~191,000이었고, 산란기 중 적어도 2~4회의 산란빈도를 갖는다. 난낭내에서의 발생기간은 약 18~20℃에서 18~19일이었다. 피뿔고둥의 배는 어린 고둥이 아닌, 피면자유생으로 부화하였다. 암컷 : 수컷의 성비는 1 : 1로 유의한 차를 보이지 않았다 (χ^2 = 0.23, p>0.05).

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INTRODUCTION

The purple shell, *Rapana venosa*(Gastropoda: Muricidae), one of the most important edible gastropods in Asia(Yoo, 1976; Kwon et al., 1993), is found along the coasts of Korea, China and Japan, especially, 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 natural resources organism that should be managed by a resonable fishing regime. About seven years ago, the slag deposit area in Gwangyang Steel Works was artificially constructed by marine reclamation works in Gwangyang Bay, Thereafter, the mean salinity(psu) in the slag deposit area gradually decreased from 21.1 psu in 1996 to 11.8 psu in 2001.

In 2001, the environmental, ecological survey on benthic fauna carried out in the slag deposit area. In this survey, especially a number of specimens of the purple shell *R. venosa* were collected by the fishing net in this area. In general, however, it is well-known that *R. venosa* inhabits in higher salinities in the seawater or brackish water. Exceptionally, some characteristics were found that especially, external morphological features of the egg capsules attached to the shells of the living purple shell in the special closed environmental area(low salinity) remarkably varied with those in the open seawater area with normal salinity. Therefore, comparative studies on morphological, reproductive characteristics of this species under open or closed environmental conditions, such as high or low salinities and sufficient food organisms or not, will be very important informations for natual living resources management.

So far, there have been some previous studies on *R. venosa*: on reproductive aspects, including reproductive cycle(Chung et al., 1993; Chung & Kim, 1997), spawning and egg capsule (Habe, 1960; Amio, 1963; Harding & Mann, 1999), on ecological aspects, including distribution(Habe, 1960; Zolotarev, 1996), habitat and age estimation(Harding & Mann, 1999), on classification(Habe, 1969; Wu, 1988), on biochemical aspects(Yoon et al., 1986; Yoo et al., 1991) and on morphology(Lee & Kim, 1988).

However, there is still uncertainty in many aspects of reproductive biology of the purple shell in artificial slag deposit area surrounded by brackish water with low salinity. Little information is available on first sexual maturity, fecundity in the egg capsules per individual, and morphological variations of egg capsules(due to artificially closed environmental factors such as brackish water with low salinity and deficient nutrients associated with restricted food organisms) in the slag deposit area. Data on first sexual maturity, fecundity associated with egg capsules, sex ratio and reproductive strategy are very useful informations for natural living resource management and reproductive potential of *R. venosa*. Therefore, the main aim of the present study is to compare first sexual maturity, sex ratio, fecundity in egg capsules per individual of *R. venosa* inhabiting the closed slag deposit area in Gwangyang Bay, Korea.

MATERIALS AND METHODS

1. Sampling

Specimens of the purple shell, *R. venosa* were collected by the fishing net in the slag deposit area artificially constructed in Gwangyang Bay, south coast of Korea, for four months from July to October, 2001(Fig. 1). Purple shells ranging from 3.2 cm to 12.8 cm in shell height were used for the present study. After the purple shells were transported alive to the laboratory, shell heights and total body weights were immediately measured.

2. First Sexual Maturity by Histological and Visual Observations

For light microscopic examination of histological preparations, gonad tissues were removed from shells and preserved in

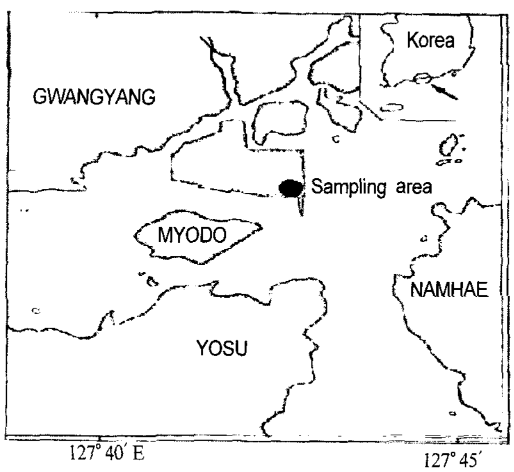

Fig. 1. Map showing the sampling area.

Bouins fixative for 24 h and then washed with running tap water for 24 h. Tissues were then dehydrated in alcohol and embedded in paraffin molds. Embedded tissues were sectioned at 5~7 μm thickness using a rotary microtome. Sections were mounted on glass slides and stained with Hansen's hematoxylin −0.5 % eosin, and examined using a light microscope.

A total of 160 female purple shells(3.2 to 12.8 cm in shell height) were used for the study on first sexual maturity by histological method. The percentages of first sexual maturity were checked to assess shell heights that reached maturity or participated in reproduction during the peak spawning period (July). The size equivalent to 50% of first sexual maturity was estimated to be first sexual maturity(biological minimum size) of the population.

3. Egg Capsule and Fecundity

To investigate the number of egg capsules per individual and the number of eggs in an egg capsule in the laboratory, a total of thirty adult females of 11.2~14.8 cm in shell height were used for observation of spawning behavior in an FRP aquarium (80 cm×60 cm×60 cm) bedded with sand and small gravels. An established filtration and aeration apparatus were employed. Purple shells used for this experiment were those collected in July. Length and width of egg capsules of the adult purple shells(>7.1 cm in shell hight)were measured and their egg developmental processes were checked under a light microscope.

The purple shells were reared at salinity of 12 psu and water temperatures of 21.4~24.8℃. The rearing seawater in the rearing FRP aquarium was changed every 3 days. Sufficient amount of bivalves(*Ruditapes philippinarum, Meretrix lusoria*) were supplied as food during the rearing period.

4. Sex Ratio

Sex ratios in a total of 278 sexually mature individuals(> 7.1 cm in shell height) were investigated from July to October, 2001. Sex of individuals was determined by visual observation of the presence/absence of the penis. Female individuals do not have any genital organ(penis) near the tentacles. To confirm a clear sexuality for each individual, both sexes were confirmed by light microscopic examination of histological preparations of the gonads. A Chi square test on goodness-of-fit was used to test the hypothesis of equal representation of each sex.

RESULTS

1. Morphology and Internal Structures of Reproductive Organs

The purple shell *R. venosa* is dioecious, and the ovary is located on the surface of the digestive gland in the spiral posterior region of the shell. The ovary is composed of numerous oogenic follicles. With gonad maturation, the external color of the ovary becomes pale yellow. Female individuals do not have the genital organ(penis) near the tentacles and can therefore be usually identified from males(Fig. 2).

2. First Sexual Maturity

During the breeding season, a total of 160 individuals(3.2~12.8 cm 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 1. spawning of *R. venosa* was occurred between July and August. In the case of some individuals whose gonad developmental stage being in the late active stage in July, it is supposed that they can reach maturity except for individuals in the early active stage during the breeding season. First sexual maturity was 0% in female purple

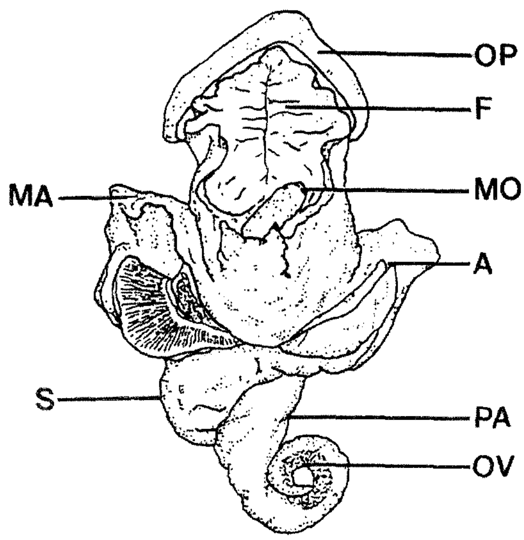

Fig. 2. Anatomy of the female purple shell, *Rapana venosa*, removed from its shell. Abbreviations: A, anus; DG, digesitve gland; F, foot; MA, mantlel; MO, mouth; OP, operculum; OV, ovary; PA, posterior appendage; S, stomach; SC, stomachal caecum.

Table 1. The shell height and first sexual maturity of the female purple shell, *Rapana venosa* from July to October, 2001

Shell height (cm)	Number of individuals by gonadal stage*						
	EA	LA	RI	PS	RE	Total	Mature (%)
3.2~ 4.0	5					5	0.0
4.1~ 5.0	7					7	0.0
5.1~ 6.0	11		2			13	15.4
6.1~ 7.0	7	2	1			10	30.0
7.1~ 8.0	7	4	9	3		23	69.6
8.1~ 9.0	3	3	10	6		22	86.4
9.1~10.0		8	12	7	2	29	100.0
10.1~11.0		4	8	9	2	23	100.0
11.1~12.0		2	9	5	2	18	100.0
12.1~12.8			5	4	1	10	100.0
Total						160	

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

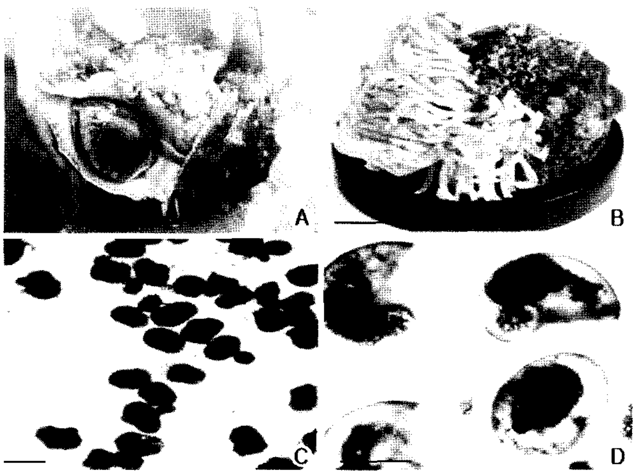

Fig. 3. Photomicrographs of copulation behavior and egg development in the egg capsules attaching to the purple shell(A-D). A, copulation behavior of female and male individuals, scale bar = 2 cm; B, egg capsules attaching to purple shell, scale bar = 2 cm; C, fertilized eggs and early developing trochophore larvae, scale bar = 0.3 mm; D, just before hathing veligers, scale bar = 0.2 mm.

shells of 3.2 to 5.0 cm high if they were at the early active stage during the breeding season. The percentages of first sexual maturity of female snails of 5.1 to 6.0 cm and 6.1 to 7.0 cm in shell height were 15.4% and 30.0%, respectively; most of the individuals were still in the early active stage. Percentage of first maturity in 7.1 to 8.0 cm in shell height were over 50%, in which all snails those at the late active, ripe and partially spawned stages were included. From those over 9.1 cm in shell height, it was 100%.

3. Sex Ratio

Of total 278 purple shells, 135 were females and 143 males(Table 2). There was no significant difference in the prevalence of each sex(not different from a 1:1 sex ratio(χ^2= 0.23, p> 0.05), and monthly comparisons showed no significant differences.

Table 2. Monthly variations in sex ratio of *Rapana venosa*

Month	No. of females	No. of males	Total numbers	Sex Ratio (F/(F+M))	χ^2 (Chi squared)*
Jul. 2001	74	63	137	0.54	0.88
Oct. 2001	61	80	141	0.43	2.56
Total	135	143	278	0.49	0.23

The critical value for χ^2 goodness-of-fit test of equal numbers of females and males(1 df) at 95% significance is 3.84.

4. Copulatory Behavior and Spawning

In the experimental aquaria in the laboratory, copulatory interactions between females and males were observed in 23~25 July 2001(Fig. 3A). During this time, it was confirmed from histological examinations 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 this rearing aquarium(Fig. 3B). This observation indicates that copulation occurs at least 15~17 days earlier than the spawning in female individuals during the peak spawning season.

5. Spawning Behavior and Broods in Aquarium

The number of the eggs, egg capsules and spawning intervals of *R. venosa* were counted under a light microscope(Table 3). Three out of thirty individuals successfully spawned in the experimental aquarium kept in the laboratory from late July to mid August 2001: a total of 192 egg capsules were spawned by No. 1 adult female at intervals of 2 days(2 broods); 306 capsules by No. 2 at 1~2 days(3 broods); 382 capsules by No. 3 at 1~3 days(4 broods).

The average time required for a spawning of this species was 4.5 hours(3~5.5 hours) from the beginning of spawning. According to the observation in the laboratory, most spawning occurred from night to early morning.

6. Fecundity, Egg Size, Hatching Shell Length and Mode of Development

The total number of egg capsules per individual and the mean number of eggs in an egg capsule of *R. venosa* were 192 to 382(Table 3) and 500, respectively. Thus, fecundities were estimated to be 96,000 to 191,000 eggs per individual. The egg size and hatching shell length of *R. venosa* in Korea were 240 μm and 0.41 mm × 0.30 mm(Fig. 3D), respectively(Table 4). The duration of development from fertilized eggs to veliger larvae in egg capsules just before hatching of *R. venosa,* were 18 to 19 days at about 21.4~24.8℃ under laboratory conditions (Figs. 3C and 3D). The mode of development of this species shows a planktotrophic veliger larval pattern because the embryos hatch as planktotrophic veliger larvae.

DISCUSSION

1. First Sexual Maturity with the Gonad Developmental Stage

All specimens of 3.1~5.0 cm high were in the early active stage although individuals were collected during breeding

Table 3. Spawning frequency and the number of egg capsules of *Rapana venosa* observed from July 21 to August 15, 2001

Individual No.	Shell height(cm)	Spawning date	Spawning hours	Water temp.(℃)	No. of egg capsules	Spawning fequency (No. of broods)	Interval (day)
1	11.4	Jul. 21, 2001	23:34-04:30	22.5	106		
		Jul. 23, 2001	05:48-09:50	22.8	86		
					(192)	2	2
2	13.0	Aug. 7, 2001	22:14-03:40	21.6	114		
		Aug. 8, 2001	01:14-06:46	21.8	98		
		Aug. 10, 2001	04:24-08:16	22.3	94		
					(306)	3	1~2
3	14.2	Aug. 9, 2001	22:10-03:09	22.9	112		
		Aug. 10, 2001	19:18-23:50	23.0	96		
		Aug. 12, 2001	16:06-20:43	23.6	90		
		Aug. 15, 2001	05:04-08:07	23.8	84		
					(382)	4	1~3

Table 4. Comparisons of the sizes of eggs and egg capsules, and larval shell of the family Muricidae

Shell length at hatching(mm)	Egg size (mm)	Number of eggs in an egg capsule	Egg capsule size(mm)	Duration of development(days)	Shell length of larva at haching(mm)	Source
Hatch as veligers:						
Rapana venosa	0.26	790~1300	30×2.5	13	0.41×0.29	Amio, 1963
R. venosa	0.24	984~1241	30×2.6	17	0.41×0.30	Chung et al., 1993
R. venosa	0.24	420~590	20×2.0	18~19	0.41×0.30	Present study
R. bulbosa	0.28				0.42	Thorson, 1940
Thais clavigerina	0.19	160~220	3.0×0.85	14	0.30×0.32	Amio, 1963
T. tissoti	0.19	75		11	0.32	Middelfart, 1996
T. floridana	0.11				0.13	D'Asaro, 1966

seasons, and our gonad histology indicates that none of them could fully develop: only small numbers of oogonia and previtellogenic oocytes were present in the oogenic follicle. The size of the oocyte indicates that they could not have reached maturity until late August when spawning was ended. Accordingly, the percentage of first sexual maturity of female snails ranging from 3.1 to 5.0 cm in shell height is 0%. However, individuals 7.1 to 8.0 cm in shell height belonged to one of the early active, late active, ripe and partially spawned stages during the breeding season. Sixteen individuals in the late active, ripe, and partially spawned stage underwent gonadal development, whereas 15 individuals in the early active stage did not. It was observed that among snails of 7.1～8.0 cm high and in the late active stage more than 50 % reached the first sexual maturity. However, all snails in the late active, ripe, or partially spawned stages, reached it if they were larger 9.1 cm. This means that larger individuals can reach maturity earlier than smaller ones. The results suggest that because catching purple shells <7.1 cm can potentially cause a drastic reduction in recruitment, a prohibitory measure should be taken for adequate natural resources management.

2. Morphology of Egg Capsule

The surface of the exit part of the egg capsule of *R. venosa* is flattened and has a chitinous capsular form. The egg capsules in the genus *Rapana* have a curved sickle shape, with a long cylindrical stem, and an albumenous substance being contained in the egg capsules. These findings are consistent with Amio's observation from *R. venosa* in Japan(Amio, 1963). However, the sizes(20×2.0 mm) of the egg capsules of *R. venosa* under lower salinity and deficient food conditions in the closed slag deposit area were smaller than those of the Japanese purple shell(30×2.5 mm) and those of the Korean shell(30×2.6 mm) in the open western coastal waters of Korea(with higher salinity)(Chung et al., 1993). Rawlings(1999) described that the morphology of egg capsules varies within the marine neogastropods, showing differences in shape, size, surface texture within families (D'Asaro, 1970, 1988, 1991, 1993, 1997; Bandel, 1973). Some species in a genus(Perron and Corpuz, 1982; Middelfart, 1996) and sometimes even individuals of the same species population(Rawlings, 1990, 1994, 1995, 1999) show distinct characteristics. These morphological differences in neogastropods may be derived by environmental factors such as physical stresses or geographical latitude and salinity.

3. Number of Eggs in an Egg Capsule

In the present study, *R. venosa* in Korea spawned 192～382 egg capsules per individual, and the mean number of eggs per egg capsule was 500. Thus, the fecundity(the total number of eggs) from each individual ranged from 96,000 to 191,000 eggs. Chung et al.(1993) reported that the Korean purple shell spawned 984 to 1,241 eggs(average 1,096 eggs) in an egg capsule, and fecundity of the Korean purple shell ranged approximately from 320,000 to 450,000. Amio(1963) described that the number of eggs in each egg capsule of *R. venosa* in Japan ranged from 790 to 1,300. Juging from these results, these results indicate a close similarity in reproductive output between the Korean and Japanese purple shells under the optimum salinity and sufficient food conditions with higher salinities, but fecundity of this species under lower salinity and deficient food conditions were smaller than those of Chung's(1993) and Amio's reports(1963). Therefore, fecundity of the Korean purple shell may be closely related with external factors such as salinity and deficient food organism, etc.

4. Sizes of the Eggs, Egg Capsules and Hatching Shell Length

The egg sizes of *R. venosa* collected in the closed slag deposit area, Gwangyang Bay(240 μm) and Piung-do(240 μm) in Korea were slightly smaller than those of *R. venosa*(260 μm) in Japan, and shell lengths of veliger larvae at hatching of the Korean shell(in the slag deposit area and Piung-do) and the Japanese purple shell were 0.41×0.30 mm(Fig. 3D) and 0.41×0.29 mm, respectively. The sizes of eggs and shell length of veliger larvae at hatching of *R. venosa* in the slag deposit area, Korea were similar to the Japanese purple shell but, in general, the egg sizes and hatching shell lengths of veliger larvae of *R. venosa* were larger than those of *Thais clavigera, T. tissoti*, and *floridana*(Table 4).

Spight(1976) and Middelfart(1996) described that egg size in the egg capsule and shell length of larvae at hatching are very similar for genera within Muricidae(Table 4). The factors affecting egg size and hatching shell length of *R. venosa* have not been studied yet.

In the present study, sizes of the egg capsules of *R. venosa* collected in the closed slag deposit area were 20 mm×2.0 mm in length. According to some authors(Chung et al., 1993; Amio, 1963), those of *R. venosa* in Korea and Japan under the optimum salinity and sufficient food conditions were 30 mm× 2.6 mm and 30 mm×2.5 mm in length, respectively. Accordingly, the sizes of the Korean purple shells were similar to the Japanese ones. Judging from these results, it is assumed that the sizes of the egg capsules under the worst environmental conditions, such as lower salinities and restricted deficient food organisms, will be smaller than those under the optimum environmental conditions because reproductive potential are affected by external environmental factors.

5. Duration of Development in the Egg Capsule

We observed that the durations of development of *R. venosa* (which were collected in the slag deposit area and Piung-do) from fertilized eggs to hatching took from 18~19 days at 11.8 psu and 15~17 days at 30 psu at 21.4~24.8°C in the aquarium at the laboratory, respectively. However, Amio(1963) described that the Japanese purple shell(*R. venosa*) took 12 days to develop from trochophore larvae to veliger larvae(i.e., about 13 days after fertilization). In general, the duration of development in egg capsules of the Korean purple shells at two regions occurred at retard than Amio's report(1963)(Table 4). It seems that the duration of development in egg capsules from deposition of an egg mass to hatched larvae varies with both internal and external factors as proposed by Amio(1963). Middelfart(1996) described that duration of development of *Thais tissoti* was 11 days. Accordingly, it is assumed that durations of development of T. tissoti are shorter than *Rapana venosa*(Table 4). The typical prosobranch larva is the planktotrophic veliger which spends days to weeks as a plankton depending on species (Fretter, 1984). It seems that the embryonic pattern of *R. venosa* is the planktotrophic veliger larvae(Middelfart, 1994; 1996).

6. No Evidence of Nurse Eggs for Larval Food

According to our microscopic observations, approximately 80% of eggs in a capsule were normally fertilized, and these embyos hatched as veliger larvae. The remaining 20% failed to develop, but they did not serve as nurse eggs for the normally developing eggs. Spight(1976) has classified sea snails into two groups based on hatching modes:(1) species whose embryo hatch as planktotrophic veliger larvae; and(2) species whose embryos hatch as crawling young snails(juveniles). He(Spight, 1976) also differentiated developing embryos into those that consume nurse eggs and those do not within egg capsules in the process of embryonic development. Of muricidae species, *Murex quadrifrons* (Knudsen, 1950), *M. senegalensis*(Knudsen, 1950), *M. torrefactus* (Cernohorsky, 1965), and *Thai emarginata*(Le Boeuf, 1971; Middelfart, 1994) have been reported to hatch as young snails. These species consume 5.9~91.4 nurse eggs by each embryo in the egg capsule during development(Spight, 1976), and the size of newly hatched young snails are usually large(>1.15 mm). In *Pisania maculosa*, a species whose embryos do not consume nurse eggs, 90% of the eggs did not fertilize, 8% became nurse eggs, and only 2% developed normally (Staiger, 1950). If the encapsulated embryos should share a common nutrient of albumen, developmentally retarded eggs are likely to be fed by surviving embryos(Staiger, 1951). It has been suggested that in case hatching size of veliger is <0.5 mm, embryos of *R. venosa* do not use nurse eggs, whereas the young snails of >1 mm may use nurse eggs for development. Shell size of the veliger larvae at hatching of *R. venosa* was relatively smaller(<0.41 × 0.30 mm) at hatching, indicating that purple shell hatches as veligers without utilization of nurse eggs during development. In view of our present findings and those observed in other prosobranch gastropods(Spight, 1976), an important postulate can be made: species that hatch as large(>1 mm) veliger or juvenile snails consume nurse eggs during deveolpment in the egg capsules, whereas those with smaller ones(<0.5 mm) do not.

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