Spawning and seed production of the green snail (*Turbo marmoratus* L.) in Indonesia

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Abstract

Of five adult green snails, *Turbo marmoratus* used for spawning (shell opening diameters between 10.25 to 14.35 cm), two males and 1 female successfully spawned. The female produced 1,825,750 eggs, each measuring 0.24 ± 0.02 mm in diameter. Hatching rate was estimated to be 66.7%.

Eggs hatched into planktonic trochophore 14 hours after fertilisation. They metamorphosed into benthic juveniles 60 hours later and began feeding on sessile diatoms.

In the laboratory, the juveniles were fed with cultured sessile diatoms of the genus *Navicula* in 2.25-tonne rectangular fibreglass tanks. Juveniles measuring 0.49 ± 0.05 mm in shell diameter grew into young juveniles of 7.07 ± 2.03 mm within a 28-week period.

Introduction

Green snail (*Turbo marmoratus* L.), locally known as Batulaga, Matabulan or Burgos is the biggest marine gastropod species of the genus *Turbo*, family Turbinidae (Gastropoda: Archaeogastropoda: Trochidea) (Eisenberg 1981; Abbott and Dance 1986; Wilson 1993). The maximum shell diameter encountered is 25 cm, weighing more than 2 kg (Kubo 1991; Yamaguchi 1993).

T. marmoratus inhabits similar habitats as other marine snail species such as topshell (*Trochus niloticus*), *Tectus pyramis* and turbinid snails (*Turbo argyrostomus* and *Turbo chrysostomus*): reef flats with constant clear flowing water, down to depths of 20 m. The animal is active at night (nocturnal) and prefers dead coral beds where micro- and macroalgae grow abundantly. Natural distribution of the green snail includes the Indo-Pacific region from the western Indian Ocean (Kenya, Seychelles, Chagos, Andaman and Nicobar Islands); Southeast Asian waters (Malaysia, Indonesia, Thailand and the Philippines) to Fiji in the South Pacific. In the western Pacific, its distribution extends to 29° north latitude in the Ryukyu Islands. As a result of its introduction in 1960, the green snail is currently found in French Polynesia (Yamaguchi 1993).

Like the topshell (*T. niloticus*), the green snail is harvested for its valuable shell, which has a very high mother of pearl content; its meat is a protein source and is eaten by the fishers. The pearly shell of green snail is used in the ornamental, handicraft, paint and cosmetic industries. According to the Food and Agriculture Organization (FAO) of the United Nations, world production of green snail shell was estimated at 800 tonnes and 1000 tonnes for 1986 and 1987, respectively (Yamaguchi 1993). It is believed that green snail harvesting in Indonesia started early this century. Indonesian Central Bureau of Statistic stated that green snail exports from Indonesia between 1970 and 1981 ranged from 44.25 tonnes to 144.60 tonnes with two main export harbours: Ujung Pandang (now Makassar) and Ambon (Usher 1984). In the Moluccas Province of Indonesia, green snail production between 1985 and 1989 ranged from 1.6 tonnes to 16.6 tonnes (Arifin and Setyono 1992). In the Ambon market, green snail shells sold for IDR 60,000 (about USD 7.5) per kilogram.

As a commercial commodity, the shell does not require special handling at the post-harvest stage and can be readily stock-piled without deterioration to its quality. This advantage leads to intensive harvesting, which in turn endangers the natural population. In order to preserve endangered

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marine resources, the government of Indonesia prohibited the harvesting of several animal species, including green snail, through a ministerial decree (No. 12/Kpts-II/1987). However, in anticipation of its potential for aquaculture, the Indonesian government issued another decree (No. 07/Kpts/DJ-VI/1988) allowing utilisation of protected animals if they are obtained through culture activity. Unfortunately, this decree does not attain its objective since the aquaculture technique for green snail in Indonesia is not available.

In order to fill this gap, research on green snail aquaculture was conducted at the Division of Marine Resources, R&D Center for Oceanology, Indonesian Institute of Sciences (LIPI) in Ambon. This paper describes the results of the first attempt at induced spawning and larval rearing of green snail in the LIPI laboratory in Ambon, Indonesia.

Materials and methods

Broodstocks were collected by SCUBA diving at coastal reefs off the Lontor village, Banda Island, Central Moluccas in October 1996. The broodstocks were brought to Ambon by ship in a tank containing seawater with moderate aeration.

Induced spawning was initiated during the new moon (27 and 28 October 1996). Prior to induction, the selected spawners were cleaned free of epiflora, epifauna and dirt on the morning of spawning induction. They were then rinsed and placed in a 40-litre plastic bin filled with sufficient filtered seawater to immerse all animals. Strong aeration was then supplied to the water in the bin for approximately eight hours. At 6.00 pm, the broodstocks were transferred into a 2.25-tonne rectangular fibreglass tank filled with UV-treated and filtered (2 μm) seawater. Spawning usually occurred during the night.

After spawning, some eggs were collected and observed under the microscope to ensure that fertilisation had taken place, which is indicated by the first cell division that normally occurs within five minutes after fertilisation. Fertilised eggs were then filtered through 60 µm mesh size sieve and rinsed with clean filtered seawater; their numbers were estimated. The eggs were then divided and transferred into one 2.25-tonne rectangular and two 2.0-tonne cylindro-conical fibreglass tanks.

Soon after the eggs hatched into free swimming trochophore larvae, the density of the larvae in each tank was estimated. The larvae together with its medium were poured into similar size tanks containing cultured sessile microalgae, *Navicula* spp. Cultured algae consisted of the mixture of three microalgae species belonging to the genus *Navicula* which had been isolated from Ambon Bay (Makatipu et al. 1996). The growth of the algae was enhanced by adding "Okinawa" culture medium (Dwiono et al. 1995).

In the laboratory, the juveniles were fed on the sessile microalgae. In order to increase the surface area for algal growth, substrates in the form of dead corals and empty shells were added to provide additional surfaces for the growth of diatom species. Faeces and other sediments were siphoned out daily and fresh filtered seawater was added to replace the lost water. Total seawater changes were done only when there were phytoplankton contaminations (which may compete with sessile diatoms in nutrient uptake) or the quantity of remaining benthic microalgae was insufficient and juveniles had to be moved to another tank containing newly cultured microalgae. Growth rates were estimated fortnightly by measuring 50 eggs or juvenile shells selected randomly for measurements.

Results

No spawning activity was observed during the first night although the animals were active and moved around the tank bottom. In the morning, spawning induction was repeated by providing similar aeration 'stimuli' to the animals throughout the day. In the evening they were transferred into a spawning tank containing freshly prepared UV-treated filtered seawater. After this second treatment, the broodstocks were observed to be more active compared to the previous night, and the first spawning was observed in one female and two males that evening (Table 1).

The first male initiated spawning by releasing white clouds (sperm) directly into the water at 9.30 pm. The sperm were expulsed through its siphon by alternate contraction-relaxation movement of the soft part of the body. Sperm were intermittently released every 5 to 10 minutes. At 10.30 pm, two-thirds of the seawater in the spawning tank was drained to reduce the high sperm concentration in the water, and fresh UV-treated filtered seawater was added as replacement. The change of water induced the first male to intensify its spawning; it also induced the second male to spawn followed by the spawning of the female.

The spawning episode in the female was very short (~ 30 minutes) but intense. No further spawning was observed after 11.10 pm; the female remained inactive at the bottom of the tank. Consequently, at 0.30 am (29 October), observation was stopped as no further indication of

Table 1. **Induced spawning of the green snail (*Turbo marmoratus* L.) under laboratory conditions at the Experimental Mariculture Laboratory (28 October 1996).**

No.	Shell diameter (cm)	Sex	Remark
1	14.35	Male	Spawned from 21.30 pm to 00.30 am
2	12.40	Unknown	No spawning
3	12.00	Female	Spawned from 10.40 pm to 11.10 pm
4	11.35	Male	Spawned from 10.39 pm to 00.30 am
5	10.25	Unknown	No spawning

spawning activities was observed, and all spawners were transferred to the recovery tank.

On release, the eggs were round and dark green in colour, measuring 0.24 ± 0.02 mm in diameter. The egg underwent the first cell division within 5 minutes after fertilisation. Four, eight and 16 cells stages were achieved 10 minutes, 20 minutes and 45 minutes after fertilisation, respectively. After the 16-cells stage the eggs were collected with a 60 µm mesh size sieve, rinsed with filtered seawater, and their numbers estimated. The total number of eggs spawned from the female was estimated at 1,825,750. The eggs were transferred into three hatching tanks.

Fourteen hours after fertilisation, the eggs hatched into free-swimming trochophore larvae. Estimation from the three hatching tanks gave an average hatching rate of 66.7%. Approximately 22 hours later (36 hours after fertilisation), most of the larvae had their first shell torsion and metamorphosed into veliger larvae. At 60 hours old, these larvae reached the pediveliger stage with distinctive velum and foot. Pediveliger with fully developed foot tested and moved on the substrate before finally metamorphosing into benthic living juveniles without their velum. The juveniles fed by scraping sessile benthic microalgae that grew on substrate and on the tank's surfaces.

The growth rate of the green snail juveniles are presented in Table 2. The table shows that the instantaneous growth rate of an animal was not constant, but increased with the age and size of the animal. Up to the 14th week, the instantaneous

Table 2. **Early development and growth of green snail (*Turbo marmoratus* L.) in the laboratory.**

Age (weeks)	Stage	Shell diameter (mm)				Instantaneous growth rate
		Min	Max	Mean	SD	
0	Eggs	0.21	0.27	0.24	0.02	–
2	Juvenile	0.40	0.55	0.49	0.05	–
4	Juvenile	0.55	0.85	0.70	0.07	0.10
6	Juvenile	0.82	1.18	0.95	0.09	0.12
8	Juvenile	1.00	1.88	1.21	0.16	0.13
10	Juvenile	1.39	1.64	1.48	0.13	0.13
12	Juvenile	1.27	1.91	1.77	0.16	0.14
14	Juvenile	1.54	3.36	2.10	0.34	0.16
16	Juvenile	1.73	3.54	2.54	0.42	0.22
18	Juvenile	2.00	4.27	3.05	0.65	0.25
20	Juvenile	2.18	5.64	3.34	0.76	0.14
24	Juvenile	2.43	6.37	4.89	1.01	0.39
26	Young snail	2.17	9.64	5.75	1.64	0.44
28	Young snail	3.56	11.31	6.62	1.82	0.43
30	Young snail	4.69	12.62	7.07	2.03	0.22

Values are obtained from random measurements of 50 individuals and presented for minimum (Min), maximum (Max), means (Mean) and standard deviation (SD). Instantaneous growth rate is the average shell growth between two successive measurements.

growth rate ranged from 0.10 mm to 0.16 mm per week. It varied from 0.22 mm to 0.25 mm per week afterwards.

Between the 18th and 20th and the 28th and 30th weeks, the instantaneous growth rate decreased due to the depletion of diatoms (*Navicula* spp.) in the tank. The growth rate picked up again after the juveniles were transferred to another tank containing dense cultured microalgae; the instantaneous growth rate increased up to 0.39–0.44 mm per week.

However, the abundant cultured food in the tank was quickly depleted within a short time (less than eight weeks) and the instantaneous growth rate dropped. This short period to depletion showed that the consumption rate of green snail juveniles was already too high and it was predicted that the consumption rate of the juveniles in the tank would exceed the growth rate of cultured *Navicula* spp. Therefore, another rearing method has to be considered.

Discussion and conclusion

The green snail (*T. marmoratus*) is dioecious (male and female sexes are separate). In nature, the proportion of male and female individuals is equal (Komatsu et al. 1995). The gonad (whitish in males and dark green in females) is located at the posterior extremity of the soft body part, close to digestive glands. Natural spawning of green snails in subtropical areas occurs during warmer months from June to September in the Northern Hemisphere (Murakoshi et al. 1993; Yamaguchi 1993), but in tropical waters, the spawning may occur several times throughout the year.

Research on reproductive biology or spawning of green snails has never been conducted previously in tropical water. The present paper outlines the first attempt at green snail spawning in Indonesia.

The spawning trial in October 1996, produced a total of 1,875,750 eggs from a single female. Subsequent spawning trials conducted on the same female and four males resulted in 583,300 eggs and 1,575,000 eggs in April and June 1997, respectively (unpublished data). Murakoshi et al. (1993) in Okinawa reported that female individuals measuring from 16 to 20 cm in diameter produced from 850,000 to 6,000,000 eggs.

In the present study, spawning occurred during no or new moon periods starting after dusk. Male spawned first, followed by the female. This spawning behaviour is similar to topshells that spawned from 9.00 pm to 12.00 pm (Pradina et al. 1996). However, work at Yaeyama islands (24°–25° North), concluded that the correlation between moon cycles and green snail spawning was not clear (Komatsu et al. 1995).

In Okinawa, green snail eggs hatched into trochophore about 22 hours after fertilisation at water temperatures ranging from 21–23 °C, while in warmer water (~ 25 °C) the hatching period shortened to only 12 hours (Yamaguchi 1993). In the present study, the hatching period was 14 hours with 66.7% hatching rate at 26 °C. Hatching rates of green snail eggs in Okinawa varied from one spawning to another and ranged from 14.6–100% (Murakoshi et al. 1993).

Pediveliger larvae require suitable substrate for settlement, i.e. solid substrate covered with abundant sessile diatoms. This requirement was observed during the study, where one of the three fibreglass tanks used for larval rearing was not sufficiently covered by sessile diatoms. In this tank, the pediveliger were still swimming in the water column, while in the other two tanks the pediveligers were already settled on the third and fourth day. In order to induce the larvae to settle, the pediveliger larvae were transferred to another tank containing more abundant sessile diatom. Three hours after the transfer, the density of swimming pediveliger larvae decreased and three hours later no swimming pediveliger were observed in the water column. This phenomenon was different from observations conducted on topshells (*Trochus niloticus*), where pediveligers required less abundant sessile diatoms in the rearing tank compared to green snail pediveliger (pers. obs.).

Mean shell growth of the green snail recorded in the present study were 0.70 ± 0.07 mm, 1.21 ± 0.16 mm, 1.77 ± 0.16 mm, 2.54 ± 0.42 mm and 3.34 ± 0.76 mm for 1 month, 2 month, 3 month, 4 month and 5 month old juveniles, respectively. This growth rate was slightly lower than that reported for early juveniles in Okinawa reared in experimental aquaria. The green snail juveniles in Okinawa reached mean sizes of 0.5 mm, 2.0 mm and 4.0 mm after 1 month, 3 month and 4.5 month of rearing, respectively (Yamaguchi 1993). In another study, with mass rearing using a running water rearing system, juveniles measuring 0.9 ± 0.1 mm after 1.5 month and juveniles with sizes ranging from 1.3 mm to 4.1 mm were obtained after 3 to 4 month (Murakoshi et al. 1993). These results suggested that the growth of juveniles in the present study was lower than those obtained in experimental aquarium (small scale) in Okinawa, but was higher than those obtained from mass culture.

The difference in growth rates reported in the above studies may be a function of food availability. In small-scale rearing, food supply was easier and more controlled, while in mass culture food supply, juveniles handling and rearing were more complicated.

When the shell diameter was about 7 mm (30 weeks old), the grazing rate of juveniles was very high and almost exceeded the capacity of the laboratory to supply sessile diatoms. In Japan, laboratory rearing was limited until 3 to 4 months when juveniles reached 1.3–4.1 mm shell diameter. These juveniles were then transferred into concrete tanks built in intertidal areas for further on-growing. The success of this rearing technique was unknown since growth and survival data were not available.

Rearing in concrete tanks or cages built in intertidal areas is a possible solution to the problem of food limitation in the laboratory for herbivorous benthic grazing gastropods. The grow-out of other gastropods belonging to superfamily Trochoidea (*Trochus niloticus* and *Turbo chrysostomus*) in cages built in intertidal areas were feasible (Dwiono et al. 1995, 1997, 1998). This ocean nursery technique resulted in a higher growth rate compared to those reared in the laboratory. The minimum size (shell diameter) used in the ocean nursery was 10 mm for *T. niloticus*, while for slow-growing gastropods, such as *T. chrysostomus*, the minimum size was 9.0 mm. Assuming that green snail (*T. marmoratus* L.) juveniles have a similar feeding behaviour to those two gastropods, an ocean nursery for green snail may be expected to yield similar encouraging results.

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