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The Larval Development Stages of the Japanese Snail, *Rapana thomasiana*, Gross 1861, in the Egg Capsule

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Abstract: In the present study, the larval development stages in the egg capsule of the Japanese snail (*Rapana thomasiana*, Gross 1861), distributed in the Black Sea were investigated. At the moment of capsule release, the diameter of the spherical egg was 151.5 ± 2.61 µm. After the capsule was released, the eggs gradually lost their spherical structure. After the completion of the early larval development stage in the egg capsule, the larvae hatched from the capsule as a veliger on day 20. The larvae were pelagic during the following 5 days and settled to the bottom day 25. The length and width of the settled larvae reached 426.8 ± 2.84 µm and 305.6 ± 2.2 µm with a growth rate of 181.2% and 101.7%, respectively.

Key Words: Japanese snail, *Rapana thomasiana*, Gross 1861, Larval development, Black Sea

Deniz Salyangozunun, *Rapana thomasiana* Gross 1861, Yumurta Kapsülü İçerisindeki Larval Gelişim Evreleri

Özet: Bu çalışmada, Karadeniz'de yayılış gösteren deniz salyangozunun (*Rapana thomasiana*) yumurta kapsülü içerisindeki larval gelişim dönemlerinin saptanması amaçlanmıştır. Yumurtlama anında küresel olan yumurta çapı 151,5 ± 2,61 µm olarak ölçülmüştür. Larvalar ilk gelişimlerini kapsül içerisinde tamamlamış ve 20. günde veliger larva olarak kapsülden dışarı çıkmışlardır. Kapsülü terk eden pelajik larvalar, 25. günde türe özgü larval şekil alarak bentik bölgeye yerleşmişlerdir. Bu dönemde larvaların eni ve boyu, sırasıyla % 181,2 ve % 101,7 oranındaki artışla 426,8 ± 2,84 µm X 305,6 ± 2,2 µm olarak belirlenmiştir.

Anahtar Sözcükler: Deniz Salyangozu, *Rapana thomasiana*, Gross 1861, Larval Gelişim, Karadeniz

Introduction

Rapana thomasiana (Gross 1861) originated in the Japanese Sea, although it has also become an economically important species in other seas after its introduction. The species known as the Japanese snail or sea snail was first described as *R. benzoar* and *R. thomasiana*, which are now used as synonyms (Bilecik, 1990; Demirsoy, 1999).

The history of *R. thomasiana* in the Black Sea is not old (the first being recorded in 1946). It is believed that the gastropod was introduced into the Black Sea by a ship carrying its eggs attached to the hull. *Rapana* adapted well to its new environment, reproduced and became widespread, except in low salinity areas of the Black and Azov Seas. It exerts a mayor influence on local malacofauna. In the 1950s it depleted the Gudauta oyster bank in the Caucasus and began to feed on the mussels living near the southern shores of the Crimea and near the Bulgarian coast. In the 1970s it penetrated the Sea of Marmara. At first, the only factor limiting *Rapana* population growth was the local souvenir industry. Only in the 1980s was it discovered that there was a demand for *Rapana* meat on the international market. Initially, massive commercial catches of the *Rapana* were undertaken only off Turkish shores. Later, the industry moved to the Bulgarian coast and began to affect the *Rapana* population there. According to the fishery statistics of Turkey, 2000 t of *Rapana* were landed annually between 1995 and 1998 (DİE, 1997; Düzgüneş et al., 1988).

R. thomasiana is a member of the neogastropods. In general, mesogastropods facilitate sperm transfer through copulation, have internal fertilisation, and deposit eggs either in gelatinous masses or in more substantial egg capsules produced by the pallial oviduct. Developmental stages are provided with a nutrient

source, which may be albumen or, in some neogastropods, special nurse eggs. In a few cases eggs and developing stages are retained in the oviduct or a special brood pouch in a ovoviviparous condition. Neogastropods deposit their eggs in well-defined capsules that are formed in the oviduct. Eggs pass first into the albumen gland. The eggs that will appear in the capsule, plus the surrounding coats of albumens, pass into the capsule gland. The lumen of the capsule gland is filled with a mucoid-protein solution. The eggs and the albumen occupy the central part of the lumen. Ciliary action rotates the capsule mass and gives preliminary shape to the capsule. The capsule may be fitted with a mucous plug while in the capsule gland. The capsule, which is still very flexible, moves down the oviduct and is released through the genital aperture. After leaving the oviduct, the egg capsule (which quickly hardens on contact with seawater) is generally transferred to the foot for deposition. In neogastropods the foot has a special pedal gland for this function. The pedal gland receives the capsule from the genital opening, rotates it for some time, and then applies it to the substrate. In neogastropods, development (including torsion) may take place partially or completely in egg capsules. A developed veliger larva has most adult organs including a shell and foot equipped with an operculum. The operculum allows the soft tissues to be protected when retracted into the shell. The distinguishing feature of the veliger is the lobed velum (Figure 1). The morphological structure associated with both feeding and swimming in gastropod larvae is the velum (bilobed or in some cases multilobed). In planktotrophic larvae, the velum is normally a pair of large flatlobes of tissue edged with two opposed bands of compoundcilia, extending from behind the head. The anterior band ofcilia, the preoral band, and the posterior, postoral band flank a "food groove" lined with shorter cilia. Velar lobes have heavily ciliated tracts that are responsible for the propulsive force, although some species swim by flapping the velum. After development is over, larvae are released from egg capsules either at the veliger stage or as juveniles and go on living in the benthic zone (Barnes, 1987; Karleskint, 1998).

Due to the importance of this non-native species in the Black Sea, the objectives of the present study were to understand the reproduction characteristics of *R. thomasiana*, and to investigate the larval development stages.

Materials and Methods

Egg capsules of *R. thomasiana* were collected from the Akliman region of Sinop, which lies on the north coast of Turkey, in August 2000 (Fig. 2). The egg capsules were harvested over 1 day by diving from 20 m. The one female which was releasing to the capsule was found by direct observation underwater, and the egg capsules belonging to the female were sampled from substrate, assuming day 0. The collected capsules were placed in an aquarium 80 x 40 x 40 cm and were supplied with unfiltered and aerated seawater at 18‰ salinity at a constant water temperature of 22 °C. The seawater of the aquarium was replaced with fresh seawater 3 times a week. Sampling eggs from a capsule was performed by cutting the top of the capsule, and the eggs or larvae were poured onto a slide. The lengths and widths of the developing eggs and larvae were measured under a microscope and photographed (Nikon, Lobophop-2A AFX-BX microphotograph apparatus). When the larvae hatched from the egg, the capsules settled on the bottom completing the metamorphosis, and the trial was finished.

Results

The characteristics of the newly released fertilised eggs (day 0) were identified (Fig. 3). Their yellowish appearance gives the egg capsule its most noticeable characteristic, although their colour will alter according to development stages.

Larval activity in the capsule was not observed during the first development stage (Fig. 4).

The first moving larva was observed on day 10 (Fig. 5). In this period, larvae were able to move using their ciliates (CL in Fig. 5) in the capsule and their colour became gradually darker depending on the occurrence of larval shell.

A larva which actively moves in the capsule by using its velum was photographed before torsion (Fig. 6). Because torsion did not occur in this period, the appearance of the larva was not similar to the typical shape of the given species, and the larva was quite long.

The typical shape of the larva was observed after it completed torsion (Fig. 7). A larva moves by using its velum in the capsule. This could be seen by simple visual observation of the capsule's physical appearance, which

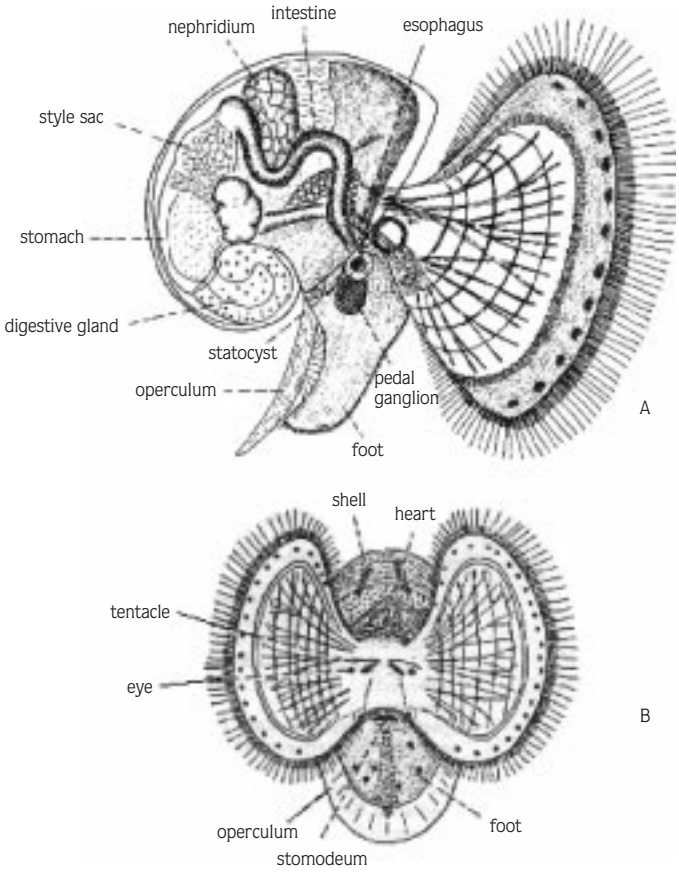

Figure 1. A veliger larva of a gastropoda (*Crepidula*) (Strathmann and Leise, 1979), A: Lateral view, B: Frontal view (Photographs: original x100).

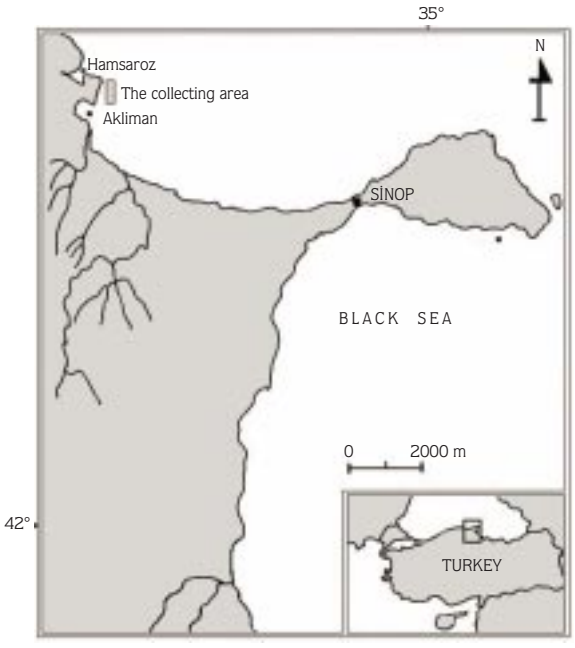

Figure 2. The collecting area of the egg capsules.

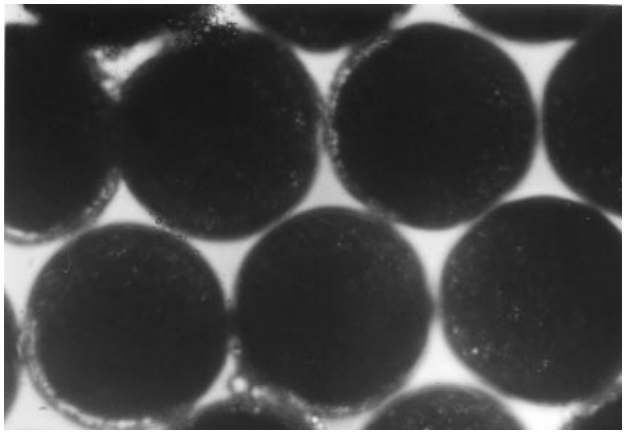

Figure 3. The newly fertilised eggs: Day 0 (original x100).

was darker than on day 0. Larvae escaped from the egg capsule after this period (day 20). The hatched larvae which were able to move in water with their velums were generally dependent on the bottom. When they were disturbed or were catching food organisms living in the

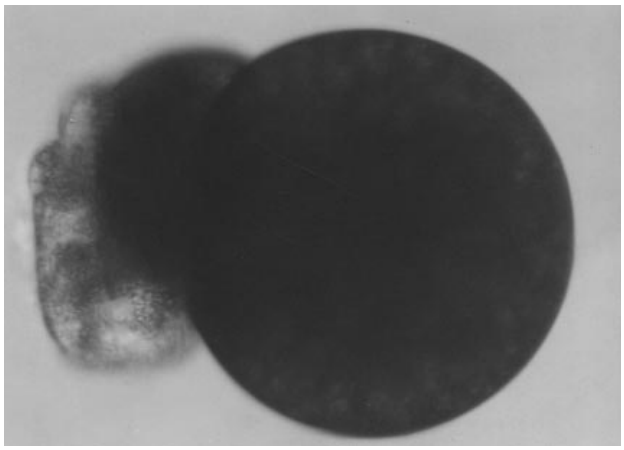

Figure 4. The larva: Day 5 (original x100).

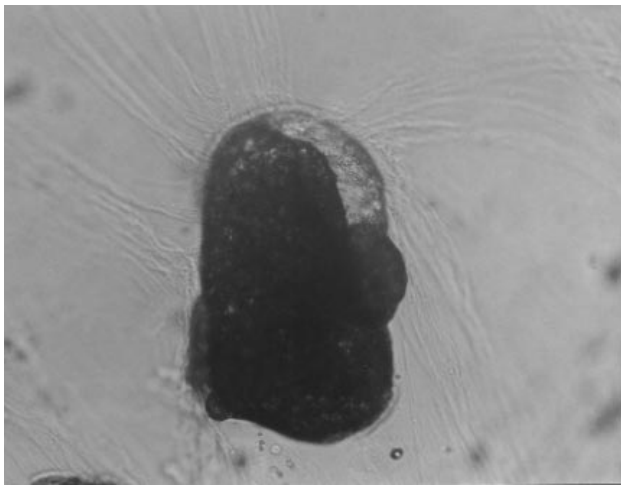

Figure 5. The larva: Day 10 (original x100).

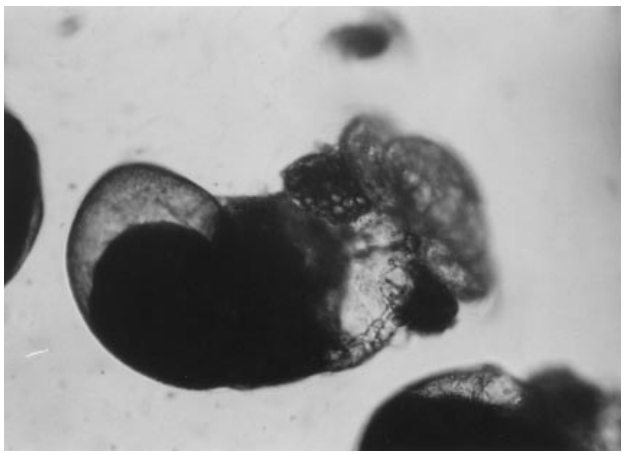

Figure 6. The larva: Day 15 (original x100).

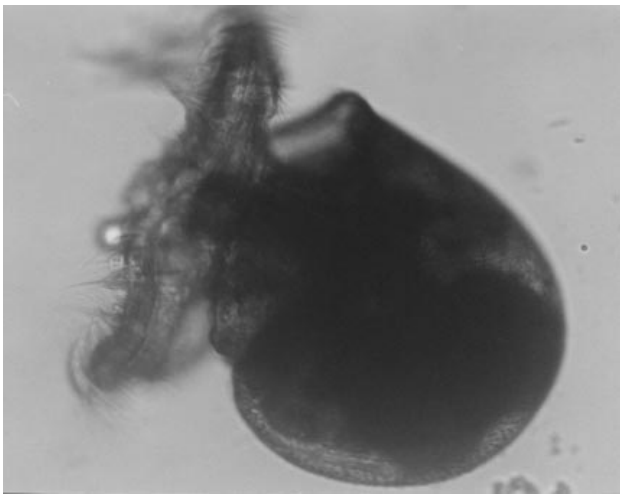

Figure 7. The larva: Day 20 (original x100).

pelagic, the larvae could alternate between periods of swimming fairly rapidly upwards and slowly sinking through the water column.

The larvae which have completed metamorphosis settled to the bottom by day 25 (Fig. 8). The velum was completely resorbed at the end of this stage and larvae started to live in this form on the bottom.

Variations in the dimensions, growth rates and shapes of the larvae during their development were also determined (Figs. 9-11).

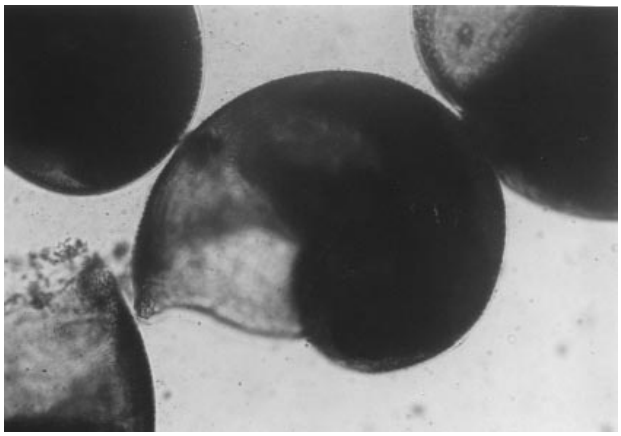

Figure 8. The larva: Day 25 (original x100).

Discussion

In the present study, the larval development stages of the Japanese snail (*R. thomasiana*) in egg capsules from the Black Sea were investigated.

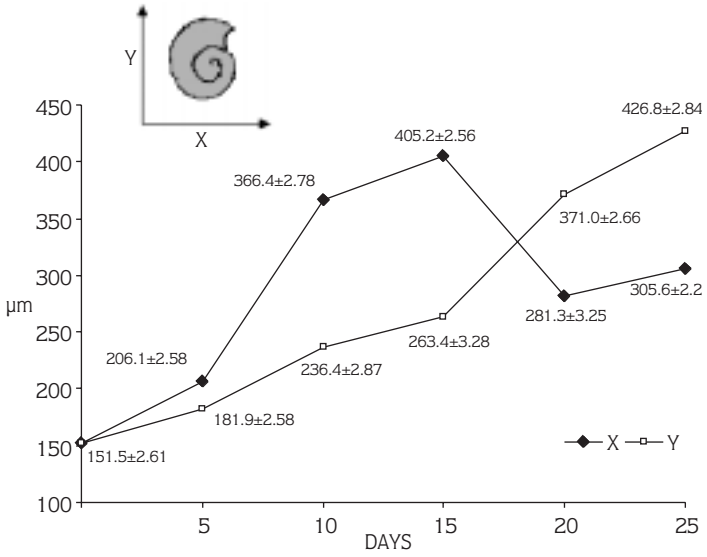

Figure 9. Variations in the dimensions of the larvae during development.

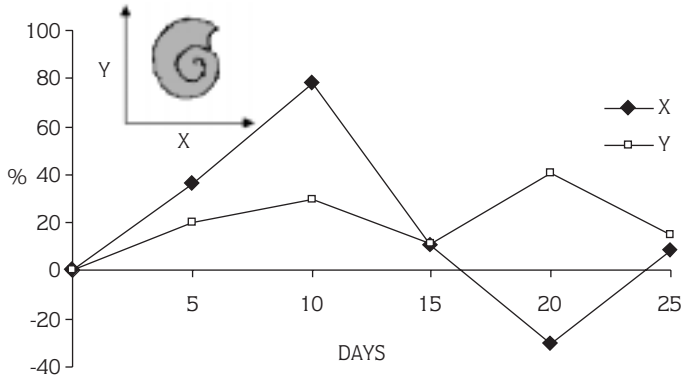

Figure 10. Variations in the growth rates of the larvae during development.

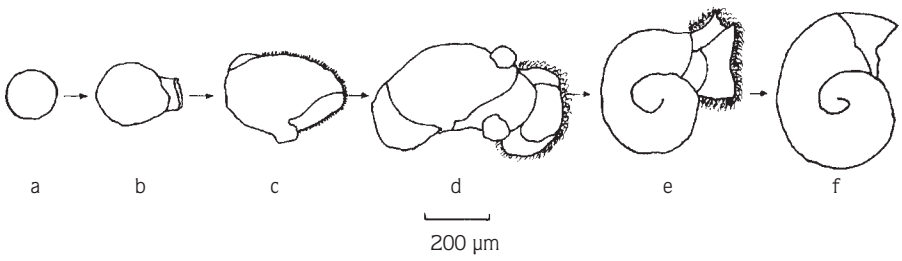

Figure 11. Variations in the shape of the larvae during development (a, b, c, d, e: larval forms in the egg capsule; e: pelagic form; f: benthic form, original).

The larvae hatched out 20 days after completing the larval development in the capsule. This finding is in agreement with the literature. Hatching time could vary between 2 months and 2 days depending on the species reported (Barnes, 1987; Pechenik, 1995; Karleskint, 1998). In *R. thomasiana*, Mann and Harding (2000) showed that egg case incubation time ranged from 14 to 45 days and Wei et al. (1999) demonstrated that veliger

larvae hatched after 20-26 days. Similarly, Ramesh (1999) also reported that veliger larvae hatched out after 24-26 days.

Veliger larvae of *R. thomasiana* display considerable variation in time from when they hatch to settlement, which could range from 23 to 70 days in experimental conditions, when food was given (Mann and Harding, 2000; Wei et al., 1999; Ramesh, 1999; Harding and Mann, 2001). In the present study, hatched larvae metamorphosed and settled to the bottom after spending 5 days pelagically. In the pelagic stage, no food was supplied to the larvae. This difference in the pelagic stage might be caused by inadequate food, which can also affect other physical characteristics of Gastropod larva (Chaparro et al., 2002).

The yellow colour of the egg capsules, which is caused by the colour of the eggs at the beginning, becomes blackish as the time of hatching approaches, because of the formation of the larval shell. Since dark capsule groups mean adult larvae, dark egg capsules, which can be distinguished from others easily underwater, can be used to obtain adult larvae from natural habitat.

In the course of the development stage, the feeding of the larvae is done in the egg capsule. This property is considered to facilitate obtaining larva. When the growth rate during the development stage is examined (Figs. 10 and 11), despite the fast rate of increase since the beginning, a sudden fall on the 12th day attracts attention. The reason for is the twisting of the ellipsoidal larvae (Fig. 6), which is called torsion (Barnes, 1987; Pechenik, 1995; Giese and Pearse, 1977; Karleskint, 1998).

After this stage, the larvae are shaped according to species and continue to develop proportionally (Fig. 7).

R. thomasiana is an economical species which is harvested from the Black Sea by fishing in Turkey. That overfishing has reduced the population of *R. thomasiana* over the course of time should always be considered. This decrease can easily be understood from the statistics. According to the fishery statistics of Turkey, 10,000 t of total production in 1988 decreased to 2000 t in 1997 (DİE, 1998; DPT, 1999; Zaitsev and Mamayev, 1997). Although *R. thomasiana* has a negative effect on bivalves since it is a carnivore species, it is considered to be important in terms of ecological variety. That overfishing harms biological variety and causes economical losses should not been neglected when the sustainable use of the biological sources and their contribution to economy are considered. The purpose of the sustainable use of biological sources is to provide continuity of production without damaging nature. Should the supporting of stocks and culture of *R. thomasiana* be required, the information about the biology of the species and the possibility of culture will be needed.

In the present study, as the first step of obtaining larva is concerned, the possibility of the use of *R. thomasiana* in aquaculture has been investigated and successfully carried out.

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