Journal of Biological Research-Thessaloniki **16**: 304 – 307, 2011
J. Biol. Res.-Thessalon. is available online at http://www.jbr.gr
Indexed in: WoS (Web of Science, ISI Thomson), SCOPUS, CAS (Chemical Abstracts Service) and DOAJ (Directory of Open Access Journals)

— SHORT COMMUNICATION —

Encapsulated development of the marine gastropod *Tonna galea* (Linnaeus, 1758) in captivity

Chrisa K. DOXA[1,2*], Aspasia STERIOTI[1,3],
Maroudio KENTOURI[2] and Pascal DIVANACH[1,3]

[1] *Cretaquarium*, *HCMR*, *P.O. Box 2214*, *71003 Iraklio*, *Crete*, *Greece*
[2] *Biology Department*, *University of Crete*, *P.O. Box 1470*, *71110 Iraklio*, *Crete*, *Greece*
[3] *Institute of Aquaculture*, *HCMR*, *P.O. Box 2214*, *71003 Iraklio*, *Crete*, *Greece*

Received: 3 December 2010 Accepted after revision: 1 April 2011

In the present study the reproductive biology of *Tonna galea* (Linnaeus, 1758) was studied for the first time. In mid-September 2006 one individual was found laying a pale pink egg rosette of 39.5 cm length. Number of embryos, stages of development, shape and dimensions were studied in relation to time and measured on microphotographs of randomly sampled capsules. Each oval or spherical shaped capsule of 3.61 mm total length contained 101 developing embryos. The embryo diameter ranged from 297.5 μm of the unsegmented egg to 489 μm of the free veliger. At 21˚C eclosion occurred 34 days after capsule deposition, at a free-swimming veliger stage. The duration of each developmental stage, from one cell to veliger, is reported. Results are discussed in relation to possible culture and use for ecological purposes.

Key words: *Tonna galea*, reproduction, encapsulated development.

INTRODUCTION

Tonna galea is one of the largest Mediterranean gastropods that inhabits sandy/muddy bottoms and seagrass beds, at depths from a few meters to 120 m (Katsanevakis *et al.*, 2008). It has a cosmopolitan distribution and it is widely distributed in Hellenic seas. It is a protected species in the Mediterranean Sea according to Annex II of the Bern convention (Council of Europe, 1979) and the Protocol of the Barcelona convention (Annex II) (European Community, 1999); however, it is still exploited (Katsanevakis *et al.*, 2008). Available information concerning its biology are given in Katsanevakis *et al.* (2008); nevertheless, the life history of *Tonna galea* is little known since it has been seldom studied and many important aspects of its biology still remain unknown.

Prosobranchs are generally gonochoristic species and fertilization is usually internal. Many marine prosobranchs enclose their embryos during a part or the whole period of development in structures such as egg capsules (Fretter, 1984; Pechenik, 1986). In many gastropod species where premature development occurs inside the egg capsule, the female deposits both embryonic and nurse eggs in the capsules in order to provide the embryos with food (Fretter, 1984; Pechenik, 1986). Benthic marine invertebrates present two distinct patterns of development. In the first pattern, development is direct with early juveniles hatching from the egg capsules. In the second pattern, the young hatch from the capsules as swimming veliger larvae that drift in the open water, and undergo metamorphosis before becoming juveniles (Christiansen & Fenchel, 1979). According to Natarajan (1957) and Scheltema (1971), *T. dolium*, *T. galea* and *T. maculosa* follow the second pattern of development that includes the existence of the pelagic veliger larvae. The development of *T. galea* embryos has never been described before and will be the case study.

*Corresponding author: tel.: +30 2810 337705, fax: +30 2810 337782, e-mail: chrisadoxa@cretaquarium.gr, bio983@edu.biology.uoc.gr

MATERIALS AND METHODS

One individual of *Tonna galea* was collected in August 2006 from Northern Crete, Greece, transferred at Cretaquarium and maintained for 4 weeks in a 150 L aquarium. In early September it was transferred to an exposition tank inhabited by 4 fish species (*Dactylopterus volitans*, *Solea solea*, *Scorpaena scrofa* and *Scyliorhinus canicula*), another marine gastropod, *Charonia variegata* and another individual of *T. galea*. In mid-September it was found laying an egg rosette on the gravel bottom of the aquarium (Fig. 1). The rosette, along with the female brood, was transferred in a black cylindrical tank of 500 L capacity and maintained under constant temperature and pH ($21 \pm 0.5^{\circ}$C and 7.7 ± 0.02, respectively), conditions similar to those of the exposition tank. Water renewal was approximately 30% h^{-1} and oxygen saturation always remained above 90%. The female moved away from the rosette just after its deposition on the gravel in the exposition tank and during the experimental period in the cylindrical tank it never approached it.

The egg rosette was photographed for further observation. Five randomly sampled capsules were collected every day during the first 5 days and every 3 days from day 8 to day 34. Then, they were opened and eggs, embryos or larvae were counted under a stereoscope. About 10% of that was photographed alive (stereoscope ×45) for further measurements and comparisons. Stages of development, shape and dimensions of these individuals were studied and measured on microphotographs. Total Length (TL) was measured for unshelled individuals (from uncleavaged egg to trochophore) and Shell Length (SL) for shelled ones (early and free swimming veliger) (Soledad-Romero *et al.*, 2004; Kingsley-Smith *et al.*, 2005).

FIG. 1. *Tonna galea* laying an egg rosette.

RESULTS

The egg rosette of 39.5 cm length had a pale pink color and consisted of 3171 capsules embedded in a gelatinous layer. Each oval, or spherical shaped capsule (Fig. 2) that measured 3.61 ± 0.23 mm (mean ± SD, n = 20) contained 101.2 ± 4.9 developing embryos therefore the total number of embryos was ~320,905. The uncleavaged eggs were spherical and pale pink and measured 297.5 ± 4.9 μm TL. The eggs were arranged in a semilunar mass (Fig. 2), surrounded by a white nutritive substance which the embryos utilized during the development. At hatching the shell length of the free veliger was 489 ± 10.7 μm. At $21 \pm 0.5^{\circ}$C eclosion took place 34 days after capsule deposition, at a free swimming veliger stage.

All developmental stages from one celled embryo to free veliger are illustrated in Figure 3A-F and their relation to time is shown in Table 1. The ontogeny proceeded slowly and the stage of the unsegmented egg lasted up to 14 hrs (Fig. 3A). The formation of the blastula was completed 5 days after deposition and the blastula-gastrula stage lasted 6 days. Trochophore larvae (Fig. 3D) started their circular motion inside the egg-capsule on day 11. The transformation of trochophore larvae into veliger ones started 21 days after deposition and was completed 13 days later (day 34). The larvae hatched from the capsules as free swimming veligers that enter the plankton to drift in the open water. The pelagic veligers presented a semi-transparent dextral protoconch with brown pigmentation that lacked sculpture (Fig. 3E). A ciliated

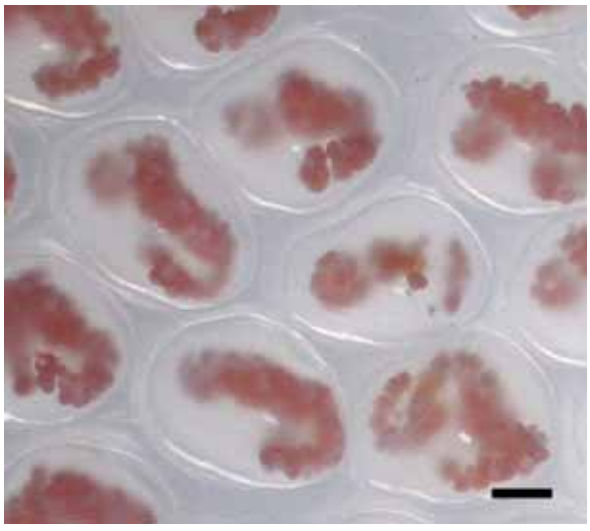

FIG. 2. Part of gelatinous egg rosette consisted in egg capsules containing developing embryos. Scale bar is equal to 1 mm.

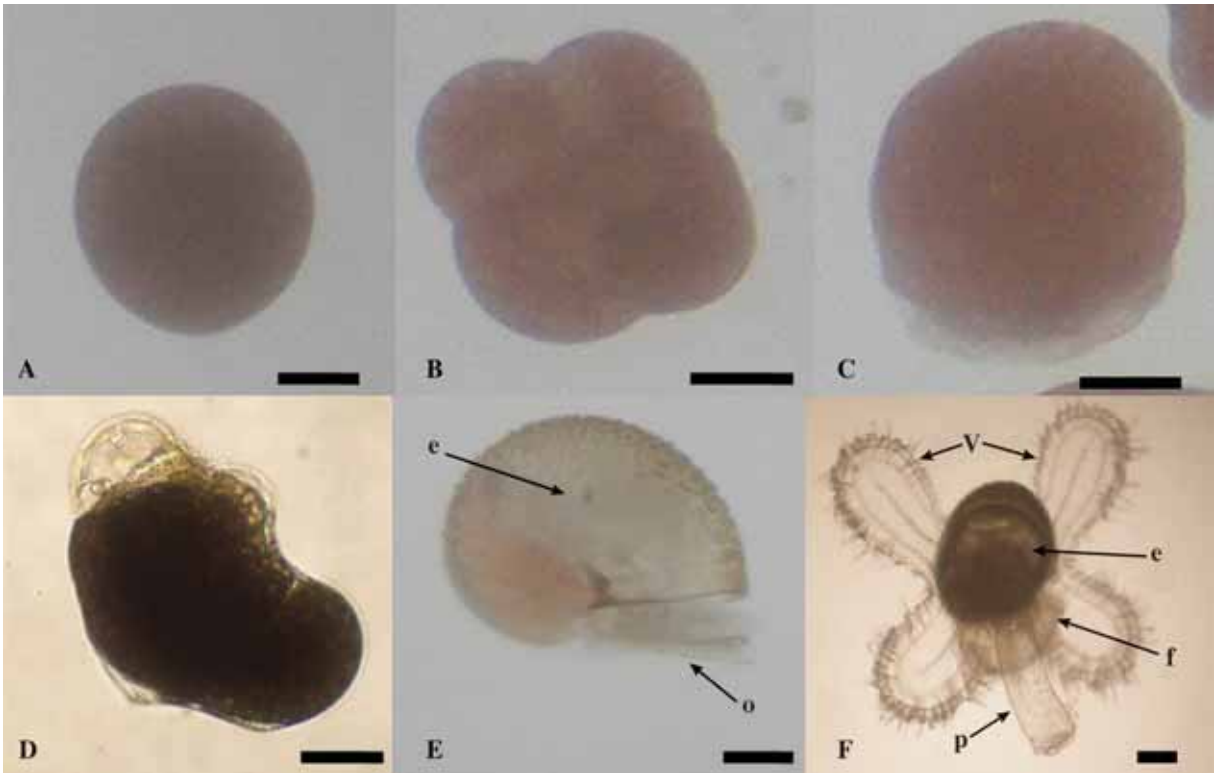

FIG. 3. Developmental stages of *Tonna galea*: A) 1-cell stage, B) 4-cells stage, C) Gastrula, D) Trochophore, E) Intracapsular veliger, F) Free veliger. e: Eye, f: Foot, o: Operculum, p: Proboscis, V: Velum. Scale bars are equal to 0.1 mm.

TABLE 1. Developmental stages and dimensions (TL, for unshelled stages and SL, for shelled stages) of *Tonna galea* in relation to time and behavior (h = hours, D = Days). Dimension values represent mean ± SD (n = 20)

Stage	Time after capsule deposition	Duration of Stage	Dimensions (μm)	Behavior
1 cell	14h	14h	297.5 ± 3.1	No movement
2 cells	21h	7h	316.2 ± 21.4	No movement
4 cells	26h	5h	349 ± 21.4	No movement
Blastula-Gastrula	5D	6D	353.2 ± 13.4	No movement
Trochophore	11D	10D	441.6 ± 14.7	Moving inside the capsule
Early veliger	21D	13D	442.1 ± 15.2	Moving inside the capsule
Hatching (free veliger)	34D		489 ± 10.7	Free swimming

four-lobed velum without pigmentation, the two black eyes, the foot and a transparent operculum were easily visible (Fig. 3F). At this stage a relatively large proboscis is seen, placed ventrally at the base of the velum (Fig. 3F).

DISCUSSION

The Tonnidae or tun shells are large gastropods found in tropical and warm temperate waters throughout the world. Members of this family are restricted to the continental shelf and occur mostly on sandy bottoms (Scheltema, 1971). Little is known regarding the life history of most species. Thorson (1940, pages 192-193) has figured the egg mass and early larva of *Tonna maculosa*, Knudsen (1950, pages 97-98, Fig. 8) shows an egg mass that he attributed to *T. costatum*, and Natarajan (1957) studied the egg masses and larval development of *T. dolium*. These flat gelatinous masses (also cited in website http://www.george-shells.com/live1/to_galea1sm_aug99.html), are similar to the egg

rosette described in the present study. The "egg spaces" of *T. costatum* contain about 100 eggs while each egg-mass may have from 350 to 660 thousand embryos, while egg capsules of *T. dolium* contain 29-32 eggs. The egg capsules described in the present work contained similar number of developing embryos (101.2 ± 4.9) as *T. costatum*. The diameter of both the eggs of *T. costatum* (Knudsen, 1950) and *T. galea* (present study) are similar, 300 μm and 297.5 ± 3.1 μm, respectively, while the shell length of the veliger larvae of *T. galea* (489 ± 10.7 μm) is higher than that of *T. maculosa* (380 μm) and *T. dolium* (217-234 μm).

The protoconch of *T. galea* veliger larvae described in the present study differ from Scheltema (1971) who illustrated an early intermediate stage larva of *T. galea* with long, straight, fairly stout, spirally arranged periostracal spines. In the present study, the veliger of *T. galea* was experimentally described from an egg mass deposited by an identified wild female while Scheltema (1971) used fisheries originated samples, therefore either this was a misidentification or a description of a later stage. The veligers were equipped with a ciliated four-lobed velum that lacked pigmentation, while the eyes and the foot with the transparent operculum on it were visible. At this stage a relatively large proboscis could be seen, a feature that is also described for *T. dolium* by Natarajan (1957). The velum, foot and protoconch rise among the most conspicuous gastropods larval organs which can be of outstanding taxonomic value in prosobranchs (Soliman, 1991). The larvae remained in the capsule for 34 days feeding on yolk. After hatching, the velum of *T. galea* had not been absorbed, leading to the conclusion that a pelagic planktonotrophic phase of unknown duration occurs. Scheltema (1971) estimated the duration of the pelagic stage in ten gastropod species having teleplanic larvae, two of them were *T. galea* with 242 days and *T. maculosa* with more than 198 days.

The most important ecological reason for breeding endangered and/or protected species is the possibility of restoration of the natural resources, wherever this is deemed necessary, by reintroduction of the species into the ecosystem. The knowledge of the reproductive behavior of a species and its unique habits is of great importance, as controlling reproduction and producing good quality offspring is the first and possibly most important step towards effectively rearing a species. The present study enriched the knowledge of the poorly known marine gastropod *T. galea* and paves the way for future possible breeding of the species in captivity for potential ecological restoration.

REFERENCES

Christiansen FB, Fenchel TM, 1979. Evolution of marine invertebrate reproductive patterns. *Theoretical Population Biology*, 16: 267-282.

Council of Europe, 1979. CETS No.:104. Convention on the Conservation of European Wildlife and Natural Habitats. Bern, 19.IX.1979. *European Treaty Series*, 104, 1-26.

European Community (1999). Council Decision of 22 October 1999 on concluding the Protocol concerning specially protected areas and biological diversity in the Mediterranean, and on accepting the annexes to that Protocol (Barcelona Convention). In: *Official Journal of the European Communities*, 14-12-1999, L 322, 1-17.

Fretter V, 1984. Prosobranchs Reproduction. In: Tompa AS, Verdonk NH, Van Den Biggelaar JAM, eds. *The Mollusca vol. 7*. Academic Press, New York: 1-45.

Katsanevakis S, Lefkaditou E, Galinou-Mitsoudi S, Koutsoubas D, Zenetos A, 2008. Molluscan species of minor commercial interest in Hellenic seas: Distribution, exploitation and conservation status. *Mediterranean Marine Science*, 9: 77-118.

Kingsley-Smith PR, Richardson CA, Seed R, 2005. Growth and development of the veliger larvae and juveniles of *Polinices pulchellus* (Gastropoda: Naticidae). *Journal of the Marine Biological Association of the United Kingdom*, 85: 171-174.

Knudsen J, 1950. Egg capsules and development of some marine prosobranchs from tropical West Africa. *Atlantide Report*, 1: 85-130.

Natarajan AV, 1957. Studies on the egg masses and larval development of some prosobranchs from the Gulf of Mannar and the Palk Bay. *Proceedings of the Indian Academy of Sciences, Section B*, 46: 170-228.

Pechenik JA, 1986. The encapsulation of eggs and embryos by molluscs: an overview. *American Malacological Bulletin*, 4: 165-172.

Scheltema RS, 1971. Larval dispersal as a means of genetic exchange between geographically separated populations of shallow water benthic marine gastropods. *Biological Bulletin*, 140: 284-322.

Soledad-Romero M, Gallardo CS, Bellolio G, 2004. Egg laying and embryonic-larval development in the snail *Thais (Stramonita) chocolata* (Duclos, 1832) with observations on its evolutionary relationships within the Muricidae. *Marine Biology*, 145: 681-692.

Soliman GN, 1991. A comparative review of the spawning, development and metamorphosis of prosobranch and opisthobranch gastropods with special reference to those from the northwestern red sea. *Malacologia*, 32: 257-271.

Thorson G, 1940. Studies on the egg masses and larval development of gastropoda from the Iranian Gulf. In: Jessen K, Spärck R, eds. *Danish Scientific Investigations in Iran, part 2*. Einar Munksqaard, Copenhagen: 159-238.