

## Mediterranean triton *Charonia lampas lampas* (Gastropoda: Caenogastropoda): report on captive breeding

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*Two females and a male triton of *Charonia lampas lampas* (Linnaeus, 1758) were collected from March 2010 to September 2012 in S. Raineri peninsula in Messina, (Sicily, Italy). They were reared in a tank at the Aquarium of Messina. Mussels, starfish, and holothurians were provided as feed for the tritons. Spawning occurred in November 2012, lasted for 15 days, yielding a total number of 500 egg capsules, with approximately 2.0-3.0 x 10<sup>3</sup> eggs/capsule. The snail did not eat during the month, in which spawned. Spawning behaviour and larval development of the triton was described.*

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**Key words:** *Charonia lampas lampas*, Gastropod, triton, veliger, reproduction

### INTRODUCTION

The triton *Charonia seguenzae* (ARADAS & BENOIT, 1870), in the past reported as *Charonia variegata* (CLENCH AND TURNER, 1957) or *Charonia tritonis variegata* (BEU, 1970), was recently classified as a separate species present only in the Eastern Mediterranean Sea (BEU, 2010).

Gastropods of this genus are rare and occasionally extinct due to over-fishing, for human consumption and, unfortunately as decorative objects (HOSKING, 1996; KANG AND KIM, 2004; KATSANEVAKIS *et al.*, 2008; MCLEAN, 1999; RUSSO *et al.*, 1990; WANG, 1997). In the Mediterranean Sea, two allopatric species, *Charonia lampas* and *C. seguenzae*, are present respectively distributed

in the Western in the Eastern Mediterranean with probable co-occurrence in Malta (BEU, 1985, 1987, 2010).

The Gastropod *Charonia lampas lampas* (Linnaeus, 1758) is a large Mediterranean Sea and Eastern Atlantic carnivorous mollusk from the Ranellidae family, Tonnoidea superfamily, Caenogastropoda.

Although *C. lampas* is not listed under the IUCN (2012) red-list, species are protected according to Annex II of the Bern convention (COUNCIL OF EUROPE, 1979) and the Protocol of the Barcelona convention (EUROPEAN COMMUNITY, 1999) because it is species at risk of extinction and it would be desirable the creation of captivity breeding programs, in order to allow the

“conservation and/or, where appropriate, captive breeding, repopulation or reintroduction of species into the wild”. Capture, however is rarely followed by release (KATSANEVAKIS *et al.*, 2008).

Protection of *Charonia* populations also requires solid knowledge of the biology and ecology, which can be further improved by studies on the maintenance and breeding in captivity and, despite the ecological importance of this specie, the information on its reproductive biology are very limited in spite of some referring to nutrition as reported by (BIRKELAND & LUCAS, 1990; KANG & KIM, 2004; LAXTON, 1971; PERCHARDE, 1972; RUSSO *et al.*, 1990). Furthermore, a recent study (DOXA *et al.*, 2013 a e b) describe that *C. seguenzae*, shows a high ability for feeding adaptation in captivity, as individuals consumed a large variety of species.

The only information available from the period of reproduction in the Mediterranean Sea is that reported by Lo Bianco (1888) that observed, in an aquarium of the Zoological Station of Naples, some specimens of *C. lampas* coupling two times a year (December and June). He also observed egg deposition and describes them as abundant and enclosed in numerous club-shaped capsules (some hundreds of eggs per capsule). Furthermore, AMOUROUX (1974) has observed in Aquarium spawning to be induced by the quality of food, in particular after feeding a particular type of prey. In 1988 GHISOTTI describes a case of ovodeposition in the Aquarium of Castel Dragone di Camogli (Italy), where only a big female was present.

The first report on the captive reproduction of triton (*Charonia tritonis*), was performed by Berg, 1971 who described the production of eggs and veliger from a female maintained in a culture vessel, obtained from diving in Phuket (Thailand) (NUGRANAD *et al.*, 2000). Recently embryogenesis and early larval development were widely described by LÜ-PING *et al.* (2013).

This report describes the biological behaviour of three specimens of triton (two female and one male) lived in captivity at the Aquarium of Messina (Sicily, Italy). Data on food and feeding, spawning, management and species restock are reported in order to improve the knowledge on *C. lampas*.

## MATERIAL AND METHODS

### Broodstock collection

Two females and a male triton of *Charonia lampas lampas* (Linnaeus, 1758) were collected from the S. Raineri Peninsula (+38° 11' 38.01", +15° 34' 25.69") in the Straits of Messina (Fig. 1), at a depth of about 35 metres on a sandy bottom. All specimens were kept in a box with seawater and artificial aeration and transported to the Municipal Aquarium of Messina. The first female (1-♀) was transported in March 2010; the second female (2-♀) and the male (3-♂) in February 2011 and in September 2012 respectively (Fig. 2). At the laboratory of Aquarium of Messina all specimens were measured (Fig. 3) according to Nateewathana and Aungtonya method (1994) as shown in Tab. 1.

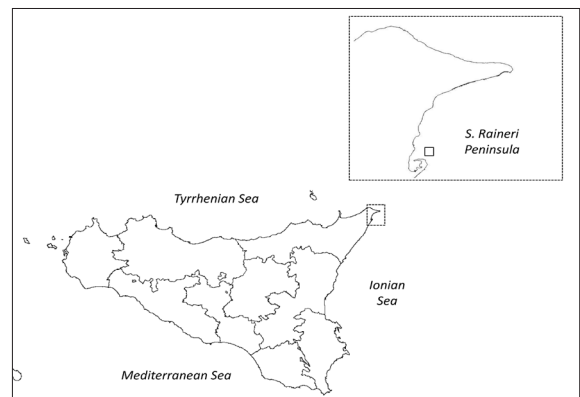


Fig. 1. S. Raineri Peninsula, Messina Italy: the □ indicates the site where the specimens were captured (+38° 11' 38.01", +15° 34' 25.69").

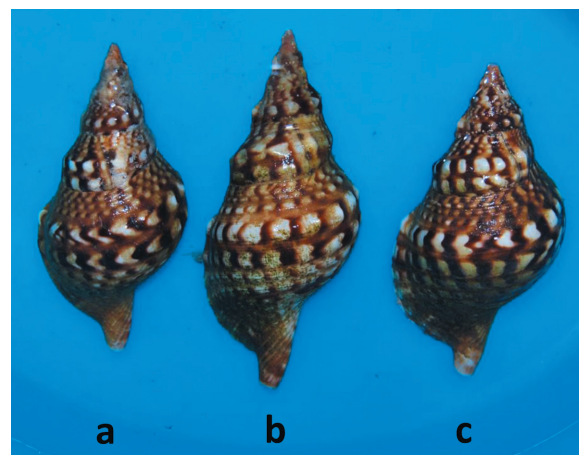
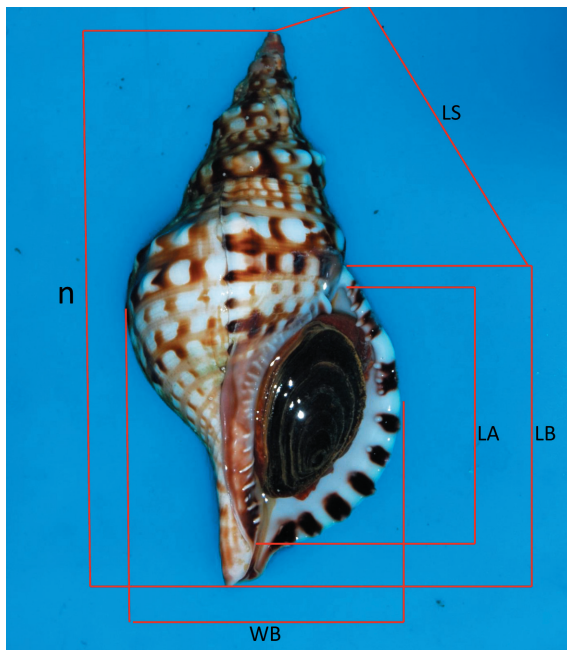


Fig. 2. Specimens of *Charonia lampas lampas* (Linnaeus, 1758): a) male (3-♂); b) female (1-♀); c) female (2-♀)

Table 1 Data on 3 broodstock of *Charonia lampas* at arrival at Aquarium of Messina

Broodstock data	<i>C. lampas</i> 1	<i>C. lampas</i> 2	<i>C. lampas</i> 3
Date arrival in Aquarium	March 2010	February 2011	September 2012
Total shell length (TL) cm	21	19	19
Width of body whorl (WB) cm	10.5	9.5	9
Length of body whorl (LB) cm	12	11.5	11
Length of spire (LS) cm	9.5	8	9
Length of aperture (LA) cm	9.5	8.5	8.5
Total weight in g	580	560	410
Sex	female	female	male
Condition at arrival	Good	Good	Good

Fig. 3. Measurement scheme for *Charonia lampas lampas*

### Culture tanks

All specimens were maintained at the Aquarium (Fig. 4) in an open system tank (tank A) with a capacity of 23000 litres. In February 2012 the females were transferred in a open system tank (tank B) with a capacity of 2200 litres, with a checked temperature of  $15\pm 2^{\circ}\text{C}$ . Parameters of water include pH 8.0, nitrites absent and nitrates concentration of 6 mg/l. On September 2012 the male was also transferred.

### Food and feeding

The tritons were fed with a mix of sea star (*Echinaster* sp.), sea cucumbers (*Holothuria* sp.) and Mediterranean mussels (*Mytilus galloprovincialis*). All specimens of *C. lampas* did not feed during the month in which spawned.

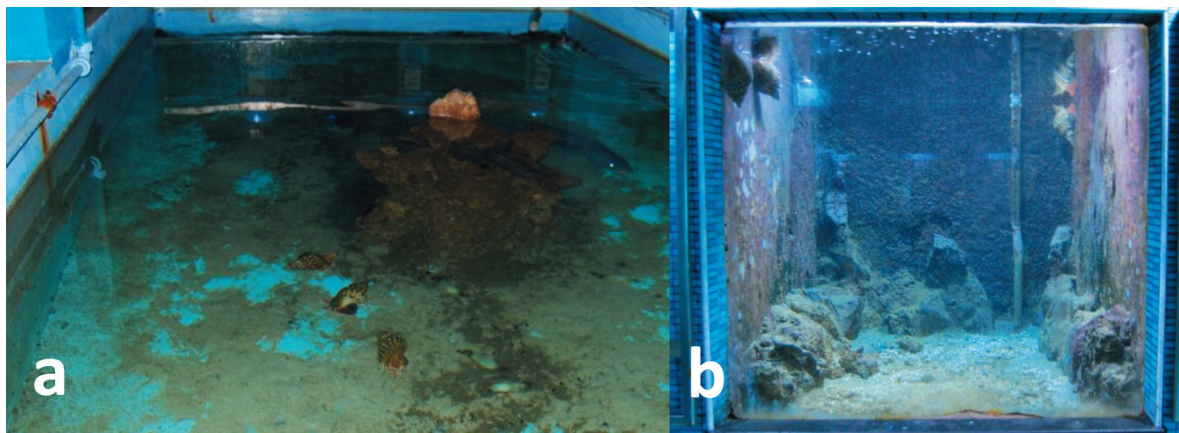


Fig. 4. Aquarium of Messina: a) tank A; b) tank B

### Reproduction and larval culture

Triton's behavior was continuously monitored. Data on mating and spawning behavior were recorded. Egg capsules were counted. After deposition some egg capsules were collected and transferred into the hatchery for measurement and to observe their development.

The incubation and the hatching of veligers were performed in 40-litres fiberglass tank (tank C) provided with natural light, clean sea water and gentle aeration. Thirty percentage of sea water was changed every 4 days. The larvae were fed with cultured unicellular phytoplankton Dupla Rin<sup>®</sup> supplied by Dohse Aquaristik GmbH & Co (Grafenschaft, Germany). It contains *Spirulina*, *Laminaria*, *Chlorella*, protein, essential oligo elements, omega-3 fatty acids, *Cyclopeeze*, astaxantina, beta-glucan, enriched with vitamin A, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>5</sub>, B<sub>6</sub>, C, E.

### Microscope observations

The observations of eggs and veliger were made by using stereomicroscope Leica M205C (Leica, Bensheim, Germany) fitted with a digital camera Leica ICC50 HD (Leica, Bensheim, Germany). The images were recorded and treated using the stereological analysis software Metamorph, Leica Application Suite (Leica, Bensheim, Germany) for counts and measurements of eggs and veliger. Veligers morphology was studied by using a Phenom Scanning Electron Microscopy (SEM) (Phenom-World BV, Eindhoven, The Netherlands).

## RESULTS AND DISCUSSION

The two females were kept in the central basin of the Aquarium (open system tank with a capacity of 23000 L) until 25 February 2012 and subsequently kept in a smaller tank of 2200 litres. On 20 September 2012 the male was introduced into. The weekly food consumption of the three tritons, during relaying period, was estimated at about 300g/snail. Predation occurred mostly in the evening. No difference in food consumption between male and female were observed. However, the feeding of all



Fig. 5. Eggs capsules of *Charonia lampas* on the wall of the tank. Larvae swim inside the capsule and are closed to hatch

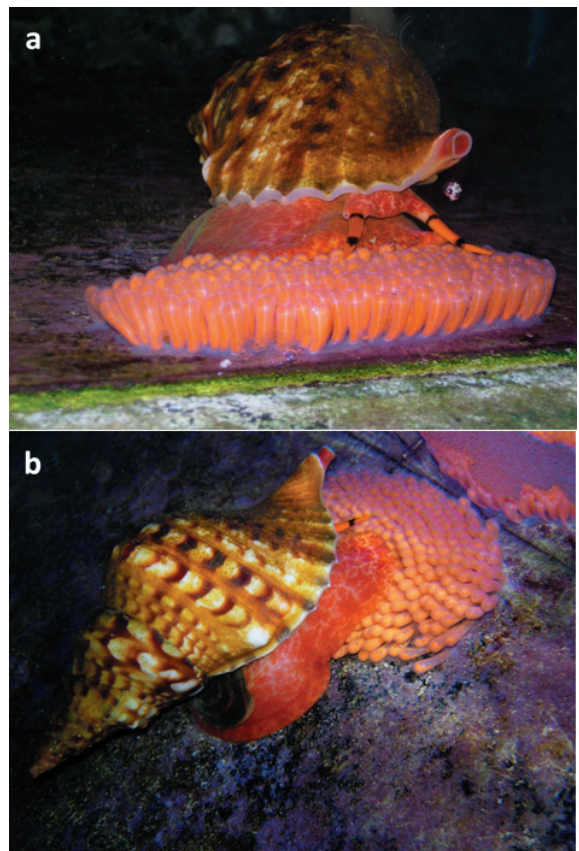


Fig. 6. *Charonia lampas* on egg capsules: a) female (1-♀); b) female (2-♀)

females ceased for months during the egg-laying period. The smaller female (2-♀) began the spawning on 5 November 2012, while the bigger female (1-♀) on 21 November 2012. Spawning lasted for about 20 days. The coupling rituals occurred exclusively at night and lasted for sev-

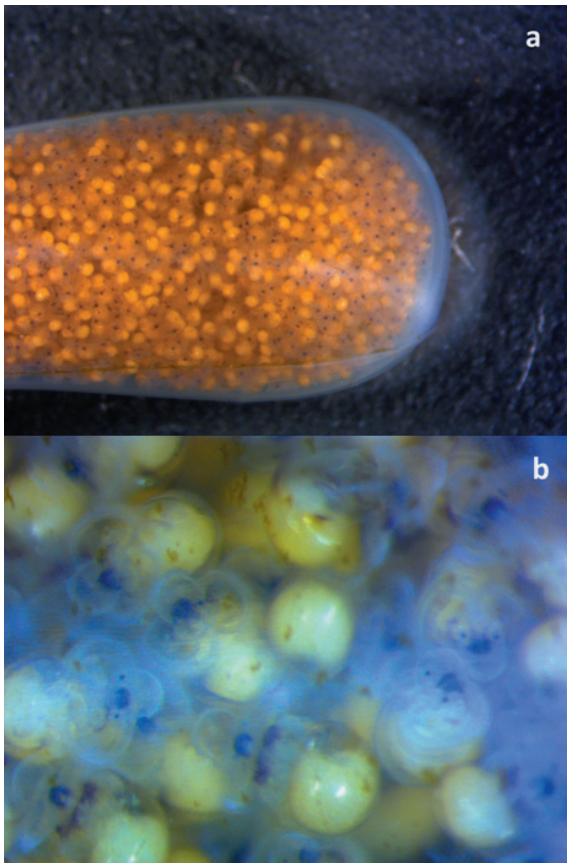


Fig. 7. a) egg capsules with free-swimming veligers; b) particular of free-swimming veligers before hatching

eral hours. The eggs were deposited in long sacular capsules of transparent jelly-like material clustered together (Fig. 5). The capsules were 15-20 mm high and 5-7 mm wide. Each capsule contained 2000-3000 bright orange colored eggs with a diameter of 0.30-0.40 mm. The number of egg capsules was about 200 for the little female (2-♀) and about 400 for the big one (1-♀). An average number of  $1500 \pm 300$  veligers hatched for capsule were observed, as reported by Nugranad *et al.* (2001) for *C. tritonis*. The females showed a similar behavior in taking care of the egg capsules. After eggs laying, both

females remained on the egg capsules without feeding for many days (brooding behavior). The females, in fact, used their proboscis to clean egg capsules (Fig. 6).

Fertilized eggs developed into capsules. The embryo started to move inside the egg after 10 days. The prehatching veligers swam actively inside the capsules while the jelly content of the capsule became more transparent (Fig. 7).

#### Details on female 1

The female 1 produced a total of 414 capsules. On 27 February 2013, the membrane on the apical portion became thinner, then torn off, making a hole through which the veligers hatched out.

In the same day, two egg capsules were transferred into tank C. The following day the release of the hatching veliger was observed. The egg capsules, in tank B, do not have hatched all at once, but gradually until the 17 March 2013.

#### Details on female 2

The female 2 produced a total of 223 capsules. On 20 March 2013, the membrane on the apical portion became thinner as reported for female 1. Also the egg capsules of female 2 have not hatched all at once, but gradually until the 7 April 2013.

At environment temperatures of 14-16 °C and salinities of 32-34 ‰, the veligers developed and hatched from the capsules, between 12 and 20 weeks after oviposition. The details are summarized in Table 2.

Each capsule contained veligers in different stages of development and size, but hatching from a single capsule occurred in the same day. Newly hatched veligers measured  $0.28 \pm 0.02$  mm at the greatest shell diameter, and had a 4-lobed velum expanded for swimming (Fig. 8).

Table 2. Data on females activity

	Starting date deposition	End date deposition	Capsules deposited	Date of first hatching capsules	End date of hatching capsules
Female 1	21/11/2012	12/12/2012	414	27/2/2013	17/3/2013
Female 2	5/11/2012	27/11/2012	223	20/3/2013	7/4/2013

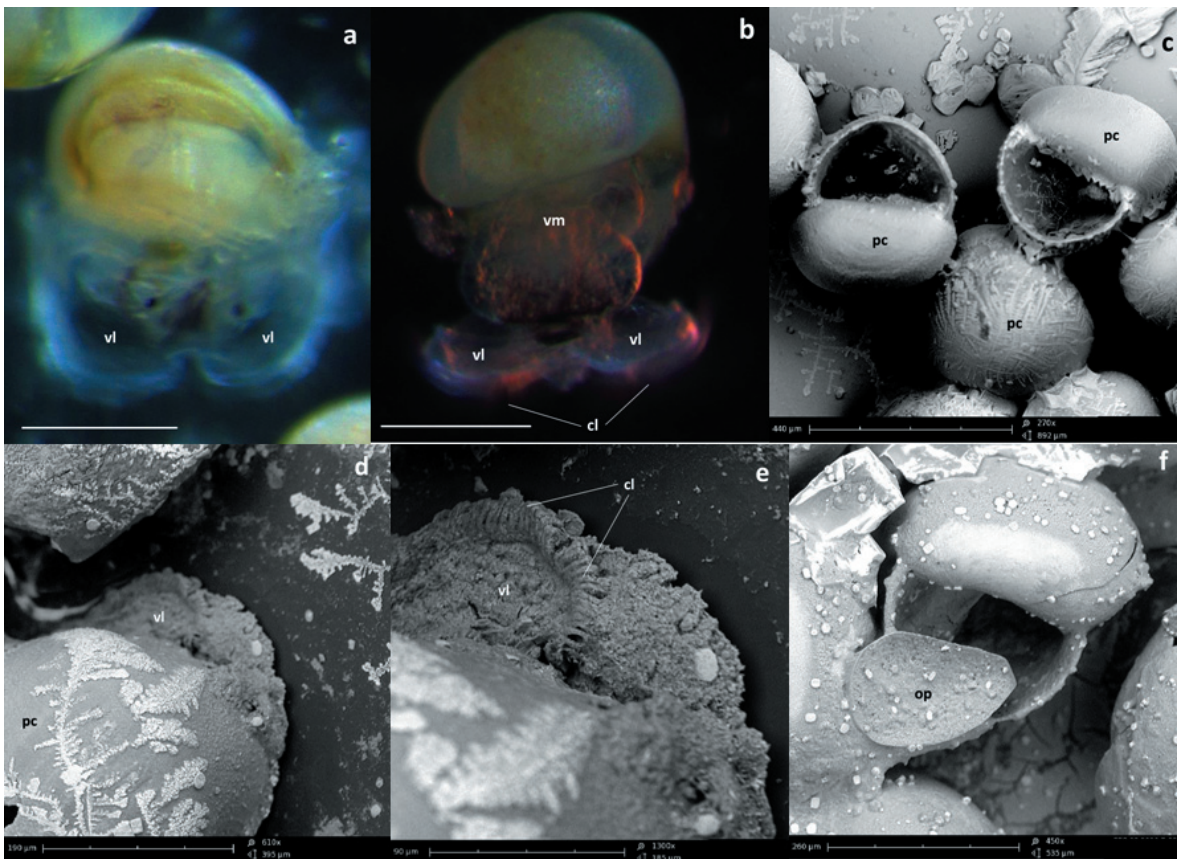


Fig. 8. Morphological characteristics of the free-swimming veliger of *C. lampas*: a) free-swimming veliger in frontal view at ten days after hatching, note one pair of velar lobe; b) lateral view, showing one pair of velar lobes, cilia and some visceral mass; c) veliger before hatching showing the original shell in a completed helix; e-d) detailed part of velum and cilia; f) detailed part of the head of veliger before hatching, showing the discoidal operculum.

Legend: ci, cilia. op, operculum. pc, protoconch. vl, velar lobe. vm, visceral mass. Scale bar = 200 µm in a and b

### Survival

After one month from hatching, the survival of veligers was estimated at 40%. Mortality increased over the extended rearing period probably due to a problem of tropism. We have tried to keep diatomee, and *Artemia salina* and food species of fitoplankton in the tanks with veligers, but settlement was not induced. Shell growth and development of the soft body were obvious, but all veligers remained swimming with no sign of settlement in culture.

### Larval production

The number of veliger larvae produced by each female ranged from about 200 000 to more than 800 000. The capsules were properly developed for the entire period of incubation until hatching, involving the 95% of the total.

### CONCLUSIONS

Adult tritons have been successfully reproduced in captivity in the structures of the Aquarium of Messina. A large number of swimming larvae (veligers) have been obtained, and their development was fine.

The good survival of adult *C. lampas* in our experimental tank confirms its physiological adaptability, and as described NASUTION & ROBERTS (2004) for the species *Buccinum undatum* that stated the possibility of introducing this species into aquaculture practices, the same could be done for our species. In fact the consistent timing of egg laying, exhibited by animals living for a long time in an aquarium, at a constant temperature, indicates that internal clocks (FABIoux *et al.*, 2005) or dependence on

photoperiod (PEARSE *et al.*, 1986; WAYNE, 2001) may synchronize gametogenesis and trigger spawning.

However, despite many trials of isolation of larvae in closed controlled environments, no metamorphosis was observed, as well as the transition to benthic life.

This study demonstrated a great adaptation of this species to captivity, considering that tritons feed regularly and started coupling rituals after about one month.

Further studies will be necessary to improve the techniques for the production of juveniles.

The larvae of this species, in fact, leave the pelagic life after a long period. Consequently, the critical points of the development have a difficult resolution, in fact, the small dimensions and especially the habits of the trophic veliger, are particularly selective and almost unknown.

After consolidated *ex situ* breeding techniques, could be important to refine and implement the breeding techniques of this species in order to constitute “contingent” of young specimens to be used for experiments aimed at re-entry into easily controlled areas such as marine protected areas.

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## Sredozemni morski puž Tritonova truba, *Charonia lampas lampas* (Gastropoda: Caenogastropoda): izvješće o akvarijskom uzgoju

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### SAŽETAK

Dvije ženke i mužjak puža Tritonova truba, *Charonia lampas lampas* (Linnaeus, 1758) su prikupljeni od ožujka 2010. do rujna 2012. na poluotoku S. Raineri, Messina, (Sicilija, Italija). Ovi primjerci su uzgajani u spremniku akvarija u Messini. Puž Tritonova truba je hranjen s školjkama, zvjezdačama i trpovima. Mrijest se dogodio u studenom 2012. godine, trajao je 15 dana tijekom kojih je izneseno 500 jajčanih kapsula s približno  $2.0-3.0 \times 10^3$  jaja po kapsuli. Puževi nisu jeli tijekom mjeseca u kojem se odvijalo mriješćenje. U radu su opisani ponašanje tijekom mrijesta i razvoj ličinke Tritonove trube.

**Ključne riječi:** *Charonia lampas lampas*, Gastropodi, puž Tritonova truba, trepetljikasta ličinka (veliger), razmnožavanje

