

Preliminary investigation on the use of artificial substrates to favor *Tritia mutabilis* (Linnaeus, 1758) spawning in Central Adriatic Sea: a possible contribution to stock maintenance

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*Tritia mutabilis, formerly classified as Nassarius mutabilis, represents an important but declining resource for small scale fishery in the central Adriatic Sea, likely due to overfishing. A preliminary investigation on the use of artificial substrates to favour its spawning was carried out in a coastal area off the Abruzzi region, central Italy. Between May and June 2015, five groups of six pyramid structures made of a steel frame covered by plastic net, with a 0.5 m² surface available for deposition, were placed on sandy bottoms at about 0.5 nautical miles from the coast and at sea depths ranging from 5.5 m to 7.5 m. A density of *T. mutabilis* egg capsules > 1 per 100 cm² was observed in two of the pyramid groups. The number of embryos present in each capsule ranged from 8 to 22 (mean number: 14). At the end of the study, almost all the capsules presented an apical opening and were empty, thus indicating successful hatching and larval release. This preliminary investigation shows that the natural deposition of *T. mutabilis* egg capsules can be facilitated by placing artificial substrates on sandy bottoms and it is conceivable that their use would contribute to maintain sustainable stocks of this species.*

Key words: *Tritia mutabilis*, *Nassarius mutabilis*, artificial substrates, spawning, Adriatic Sea

INTRODUCTION

The changeable nassa *Nassarius mutabilis* (Gastropoda, Nassariidae) (Fig. 1a), recently classified as *Tritia mutabilis* (GALINDO *et al.*, 2016; MARSHALL & GOFAS, 2016), is a common nassarid found in sandy or muddy bottoms throughout the Mediterranean coastal waters, at depths ranging between 2 and 15 m (PICCINETTI & PICCINETTI MANFRIN, 1998; GRATI *et al.*, 2010; POLIDORI *et al.*, 2015). It has separate sexes and reproduction occurs between late winter and spring (CESPUGLIO *et al.*, 1999). Fertilization is internal and

spheroidal embryos are deposited within benthic, transparent egg capsules attached to hard substrates (SODERI, 1975; D'ASARO, 1993; POLIDORI *et al.*, 2015). Planktotrophic larvae emerge from the capsules and require approximately two months to complete metamorphosis and settlement. The life cycle is completed within two to three years (CESPUGLIO *et al.*, 1999; RIEDL, 1991).

Small-scale *T. mutabilis* fishery is a common practice and an important resource along the central and northern Adriatic Sea Italian coast (PICCINETTI & PICCINETTI MANFRIN, 1998; BALDUCCI *et al.*, 2006; GRATI *et al.*, 2010; POLIDORI *et al.*,



Fig. 1. a) Photographs of *Tritia mutabilis* specimens, b) Pyramid used as artificial substrate for *T. mutabilis* egg capsule deposition, c) *T. mutabilis* egg capsules laid on a pre-cut plastic net square on the side wall of a pyramid artificial substrate, d) egg capsule detached from the artificial substrate and observed at the stereomicroscope. Numerous embryos can be seen inside the capsule, e) hatched egg capsule presenting an apical opening

2015). The consumption of this species is popular among the local population and, in winter time, it represents an important source of income for small scale fishery in the Adriatic Sea, accounting for 19.6% of the total catch and 13.0% of the total revenues in the year 2011 (IREPA, 2012). Fishing activity is conducted in the period between early fall and late spring using small cone-shaped basket traps baited with dead fish (GRATI *et al.*, 2010), and it is regulated in terms of fishing periods, maximum daily catch amount, minimum landing size (20 mm shell height), and sanitary classification of the catching sea areas in accordance with Regulation 854/2004 / EC (EUROPEAN COMMISSION, 2004). Despite such a detailed regulatory framework, *T. mutabilis* resource has undergone a constant decrease in

the last decade, probably due to an excessive fishing effort (PICCINETTI & PICCINETTI MANFRIN, 1998; BALDUCCI *et al.*, 2006; FABI *et al.*, 2006; GRATI *et al.*, 2010; POLIDORI *et al.*, 2015). As a matter of fact, the annual catch of *T. mutabilis* passed from 1.579 tons in 2010 to 2.220 tons in 2015 (<http://www.ismea.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/9709#MenuV>).

A further threat for this species could be represented by predation on encapsulated larvae by crabs or hermit crabs, as reported for the gastropod *Ilyanassa obsoleta* (BRENCHLEY, 1982), and by the negative effects on the reproduction caused by the imposex phenomenon, recently reported by LAHBIB *et al.* (2013) in a *T. mutabilis* population from the Lagoon of Bizerta (northern Tunisia). Imposex is associated with water pollution due to antifouling paints (FENT, 1996) and consists on a superimposition of male sex organs onto female gastropods, which causes an alteration of the gametogenesis, with cessation of oocyte maturation and development of semen tubules with early stages of spermatogenesis (GIBBS *et al.*, 1988).

To achieve an enhancement of *T. mutabilis* stocks in central and northern Adriatic Sea, POLIDORI *et al.* (2015) recently proposed a revision of the current management measures, suggesting a shortening of the fishing season and a revision of the minimum landing size. Another possible approach to contribute to maintenance and enhancement of *T. mutabilis* stocks could be the adoption of measures to support the reproduction of the species in the area. *T. mutabilis* attaches its egg capsules to solid substrates (SODERI, 1975; POLIDORI *et al.*, 2015), which are not frequent on the fine sandy bottoms that characterize most of the central Adriatic Sea coast of Italy. In these areas, *T. mutabilis* egg capsules are commonly found attached to the sidewalls of the basket traps and to other submerged gear used for small-scale fishing, laid on sandy bottoms during the spawning season. Moreover, an attempt to provide artificial substrates to favor egg capsules deposition was recently carried out with encouraging results by fishermen in the Marche region, central Italy (http://www.blumarineservice.it/images/download/brochure_sea_

snail_it.pdf). These observations prompted us to conduct a preliminary experimental investigation to evaluate the use of artificial substrates designed to optimize *T. mutabilis* egg capsules deposition in sandy sea bottoms.

MATERIAL AND METHODS

The study was carried out in a Central Adriatic Sea area facing the Abruzzi region, nearby the port of Ortona (Fig. 2). The artificial substrates used for egg capsule deposition were pyramid structures with a square base, measuring 50 cm in width and 40 cm in height, and made of a steel frame ($\varnothing = 8$ mm) covered by net (Fig. 1b). The pyramid base was covered with a high strength nylon net with a mesh size of 20 mm, while the four side walls were covered with a plastic net with a mesh size of 3 mm. The lateral surface available for deposition in each structure was about 0.5 m². On each pyramid, squares with 50 mm side were pre-cut in the plastic net to make them easily removable from the structure, thus allowing the enumeration of the embryos present in the capsules. A 1 kg weight was placed in the center of the pyramid base to provide stability and avoid overturning. The structures were realized by Blu Marine Service Soc. Coop. (San Benedetto del Tronto, AP, Italy), using non-toxic

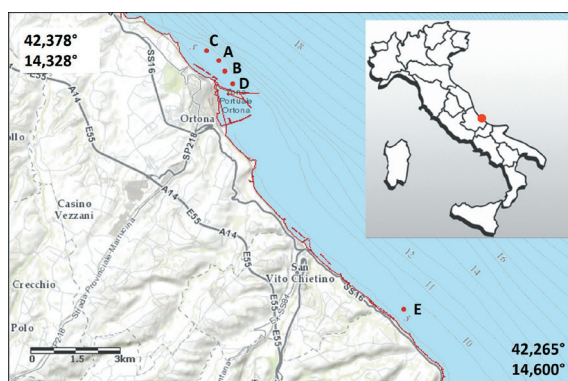


Fig. 2. Map of the study area in the Central Adriatic Sea, nearby the port of Ortona, Abruzzi region, Italy. The dots indicate the position of the five groups of artificial substrates deployed for the study

materials.

A total of 30 pyramids, assembled in five groups (from A to E) constituted by six units, were lowered into the sea by professional fisherman between 12 and 13 May 2015, at a distance from the coast ranging from 0.32 to 0.64 nautical miles, at a sea depth ranging from 5.5 m to 7.5 m (Fig. 2). The pyramid groups were kept in position on the sandy seabed with anchors and identified on the surface by a signal. A monitoring visit was carried out after about 15 days and the substrates were definitively removed from the sea and examined after about one month

Table 1. Presence of *Tritia mutabilis* egg capsules on pyramid artificial substrates placed on sandy bottoms in the central Adriatic Sea. ND = Not determined

Artificial substrate group	Depth (m)	Observation date	Mean pyramid surface covered by egg capsules (%)	Mean number of egg capsules per 100 cm ²	Mean number of embryos per capsule	Proportion of hatched capsules (%)
A	6.3	26 May 2015	<1	<1	ND	ND
		15 June 2015	<1	<1	0	100
B	5.6	26 May 2015	<1	<1	ND	ND
		15 June 2015	<1	<1	0	100
C	5.5	26 May 2015	31.2	957 ± 156	15.0 ± 1.1	0
		15 June 2015	40.0	1,167 ± 114	12.8 ± 1.8	87
D	5.5	29 May 2015	<1	<1	ND	ND
		15 June 2015	<1	<1	0	100
E	7.5	29 May 2015	3.0	60 ± 38.5	14.3 ± 2.8	0
		16 June 2015	<1	<1	0	100

(Table 1). Water temperature and salinity were measured at the beginning and at the end of the study, as well as at the monitoring visit, using a multi-parametric probe HI9828 (Hanna Instruments, Ronchi di Campanile, Padua, Italy). During the visit and at the end of the study the presence of egg capsules was assessed and the side walls of the pyramids were photographed using a digital camera with a graduated reference. *T. mutabilis* egg capsules were identified through microscope observation, on the basis of their morphology (SODERI, 1975; D'ASARO, 1993). In particular, they were distinguished from the capsules of *Nassarius reticulatus* (D'ASARO, 1993; ZUPO & PATTI, 2009) by the presence of complex lateral ridges and spines and by the relatively low number of embryos per capsule. The percentage of available pyramid surface covered with egg capsules was estimated by analyzing the calibrated digital images using the ImageJ software (Research Services Branch, National Institute of Mental Health, Bethesda, Maryland, USA). The pre-cut squares were removed from the side wall plastic nets and transferred to the laboratory in refrigerated water containers to estimate the density of the egg capsules, expressed as their number per 100 cm². Egg capsules were removed with a scalpel and observed with a stereomicroscope Wild M3 (Leica Microsystems, Milan, Italy) to determine the number of embryos present. The percentage of hatching egg capsules was also estimated. The relation between egg capsule size (height) and number of embryos per capsule was evaluated by linear regression analysis using the statistical software PAST 3.14 (HAMMER *et al.*, 2001)

RESULTS

During the study period, the water temperature at the surface ranged from 19 °C at the beginning of the study to 25 °C at its end, and that at the bottom from 19 °C to 22 °C. Salinity ranged from 31.8 and 34.2 gL⁻¹ at the surface and from 32.4 to 34.3 gL⁻¹ at the bottom. At the monitoring visit, carried out between 26 and 29 May 2015, a density of *T. mutabilis* egg capsules >1 per 100 cm² was observed in two of the five

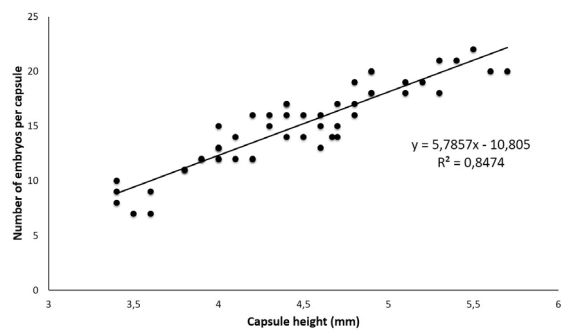


Fig. 3. Correlation between number of embryos and egg capsule size in *Tritia mutabilis*

groups of pyramid substrates (Table 1). The mean numbers of capsules per 100 cm² of substrate surface were 957 and 60 for the group C and group E pyramids, respectively. Only a few egg capsules were observed on the side walls of the other pyramid groups, preferentially located on the corners. Numerous cuttlefish (*Sepia officinalis*) and black goby (*Gobius niger*) eggs were also found on the side walls of all the pyramids.

T. mutabilis egg capsules (Fig. 1c) appeared as sculptured, almond-shaped structures with a mean height of 4.7 ± 0.7 mm, a mean basal length of 3.4 ± 0.1 mm, and complex lateral ridges (D'ASARO, 1993). They stick firmly to the plastic net, had a rubbery texture, rather resistant to compression and cuts, and could be removed only by using a sharp scalpel blade. At the cut surface, the capsule membrane was smooth and transparent, allowing the observation and enumeration of the embryos (Fig. 1d). None of the observed egg capsules was hatched. The number of embryos present in each capsule was significantly related with its size ($p < 0.001$, $r^2 = 0,8474$, $n = 54$, Fig. 3), and ranged from a minimum of 7 to a maximum of 22 embryos for the largest capsules (mean number: 14). The embryos usually presented a uniform light brown color and a spherical or trapezoidal shape, but sometime elongated, rod-shaped forms were observed. In some capsules, the embryos showed a disc-shaped swelling with a light grey color at one end, probably indicating a different development stage.

The artificial substrates were removed on 15 and 16 June 2015, at the end of the study, and an egg capsule density > 1 per 100 cm² was

observed only in group C pyramids (Table 1). The mean number of capsules was 1,167 per 100 cm², with a 22% increase with respect to the previous monitoring visit. Most egg capsules (87%) presented an apical opening and were empty (Fig. 1e), thus indicating successful egg capsule hatching and larval release. Most of the egg capsules observed on the group E pyramids at the first monitoring visit were no longer present, and the few remaining along the pyramid corners were empty, as well as the few scattered capsules observed on the other pyramid groups.

DISCUSSION

The results of this preliminary investigation indicate that the natural deposition of *T. mutabilis* egg capsules can be facilitated by placing artificial substrates on sandy bottoms, even though the number of egg capsules found on three of the five groups of pyramid substrates deployed for the study was very low. For the other two groups of pyramids (C and E) the surface covered with egg capsules corresponded respectively to about 40% and 3% of the total available surface. These figures are lower than those previously observed by fishermen in the coastal waters of the Marche region, about 100 km north of our study area, using artificial substrates similar to the pyramids employed in this study (http://www.blumarineservice.it/images/download/brochure_sea_snail_it.pdf). That attempt was conducted leaving the substrates at sea between January and June 2013, and abundant amounts of egg capsules on the pyramid surfaces were reported. This suggests that the low yield obtained in our study was likely due to a late placement of the artificial substrates at sea, due to technical problems with their availability. Indeed, the substrates were placed at sea in mid-May, when the water temperature at both the surface and the bottom was already 18-19 °C. In the Nassariidae family, egg capsule deposition is usually timed according to seasonal changes of temperature and salinity (SCHELTEMA, 1961; BARROSO & MOREIRA, 1998; CHAN & MORTON, 2005). For *Nassarius obsoletus*, an optimal temperature of 16°C for egg capsule laying has been reported

(SCHELTEMA, 1961), while BARROSO & MOREIRA (1998) observed that the release of gametes by *N. reticulatus* in Portuguese coastal waters was associated with the increase of water temperature above 11°C, at the end of the winter. In our study area, the water temperature usually increases rapidly from 10-11 °C at the beginning of March to 15-16 °C around middle April, and *T. mutabilis* spawning probably begins in that period. Accordingly, the local fisherman reported the presence of *T. mutabilis* egg capsules on submerged fishing gear mainly in April. Therefore, it is likely that our experimental study has been carried out when the spawning season was close to its end, as also suggested by the observation that almost all the egg capsules still present on the pyramids at the end of the study, in mid-June, were hatched. On the other hand, the deposition of egg capsules on our devices and the increase in their number on the group C pyramids observed between the monitoring visit and the end of the study indicate that spawning individuals were present until the end of May, which probably represents the limit of *T. mutabilis* spawning season in the study area.

Despite the socio-economic importance of the species and the detailed fishery regulatory framework, the reproductive biology of *T. mutabilis* has not been thoroughly described so far (SODERI, 1975; RIEDL, 1991; D'ASARO, 1993; PICCINETTI & PICCINETTI MANFRIN, 1998; POLIDORI *et al.*, 2015). In this respect, this study may contribute to a better knowledge of the spawning process of the species in the Adriatic Sea, because we observed a mean number of embryos per capsule (14, with a range varying from 7 to 22 embryos) that was higher than that (6-7 embryos for capsule) recorded in a study conducted in the laboratory (SODERI, 1975) and comparable with the number reported for specimens from the Tyrrhenian Sea (D'ASARO, 1993). A significant positive correlation between the number of embryos and the dimension of the capsule was also observed, and the egg capsule hatching time at 19 °C could be estimated around 15 days, since most of the recorded egg capsules hatched during the interval between the monitoring visit and the end of the study.

CONCLUSIONS

The devices deployed in this study, pyramids made by a steel frame covered by a plastic net, proved to be an adequate artificial spawning substrate for *T. mutabilis*. Moreover, they are easy and economical to build, and the fishermen who participated in the project judged them easy to use. Indeed, they are relatively light and their use requires a limited workload, since they can be placed at sea at the beginning of *T. mutabilis* reproductive period and removed only at its end, or otherwise transferred to other areas selected for restocking before egg capsule hatching. In the central Adriatic Sea, the artificial spawning substrates should be kept at sea from mid-March to the end of May and their appropriate use could increase the spawning possibilities for *T. mutabilis* in sandy bottom areas where suitable natural substrates are scarce, thus contributing

to maintain sustainable stocks of this species. Finally, the observation that other marine organisms, in particular cuttlefish and black goby, laid their eggs on the pyramids, indicates that these artificial substrates can also facilitate the reproduction of other species of interest to small-scale fishing, with the further added value of favoring biodiversity.

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Preliminarna istraživanja o uporabi umjetnih supstrata koji bi pogodovali mrijestu puža *Tritia mutabilis* (Linnaeus, 1758) na središnjem Jadranu: mogući doprinos održavanju stocka

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SAŽETAK

Puž *Tritia mutabilis*, nekad klasificiran kao *Nassarius mutabilis*, predstavlja važan, ali opadajući resurs priobalnog ribolova u središnjem Jadranu, vjerojatno zbog pretjeranog izlova. Na obalnom području pokrajine Abruzzo (središnja Italija) provedena su preliminarna istraživanja o korištenju umjetnih supstrata koji pogoduju mrijestu *T. mutabilis*. U razdoblju od svibnja do lipnja 2015. godine, na pješčana dna udaljena oko 0,5 nautičkih milja od obale, postavljeno je pet grupa od šest piramidalnih konstrukcija izrađenih od čeličnog okvira prekrivenog plastičnom mrežom površine od 0,5 m² na dubini od 5,5 do 7,5 metara.

Opažena je gustoća kapsule jajašaca *T. mutabilis* od > 1 po 100 cm² u dvije piramidalne skupine. Broj embrija prisutnih u svakoj kapsuli kretao se od 8 do 22 (srednji broj: 14).

Na kraju istraživanja, gotovo sve kapsule imale su apeksni otvor i bile su prazne, što ukazuje na uspješno lučenje i otpuštanje larve.

Ovo preliminarno istraživanje pokazuje da se prirodno taloženje jajčanih kapsula *T. mutabilis* može olakšati stavljanjem umjetnih supstrata na pješčana dna, te je vjerojatno da bi njihova uporaba doprinijela održavanju zaliha ove vrste.

Ključne riječi: *Tritia mutabilis*, *Nassarius mutabilis*, umjetni supstrati, mriješćenje, Jadransko more