

Observations of *Cyclope neritea* Linnaeus 1758 Breeding Behaviour in Artificial Habitat

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Cyclope neritea Linnaeus 1758 is a popular Mediterranean Sea gastropod mollusk. A scavenger associated with shellfish farming, and used for population genetics studies and monitoring of environmental pollution. The aim is to evaluate *C. neritea*'s propagation under long-term artificial condition. *C. neritea* is maintained in 34 ppt substitute sea-water, along with copepods, *Tigriopus californicus* Baker 1912 at 25 °C and 12/12 hr light/dark cycle. It is an oviparous marine gastropod laying scattered single-egg-containing capsules. The females with retarded egg-capsule deposition eat the egg immediately after deposition. Normal capsules are not attacked. Juveniles supplement their diet with eggs of their kind regardless of the stage of development. Intra- and extra-capsular development and growth were observed. Its transient longevity reasons the work for optimising the conditions for the successful maintenance, and popularising it for the ornamental fish habitats.

Keywords: *Cyclope neritea* Linnaeus 1758, Marine gastropod mollusk, Artificial habitat.

INTRODUCTION

Cyclope neritea Linnaeus 1758, Nassariidae family, has been named initially *Buccinum neriteum* Linnaeus 1758 (Linnaeus, 1758), and later with various other names in Portugal, Spain, and France in 19th and the early 20th centuries. Recently genetic results move the genus *Cyclope* to *Tritia* (Boissin et al., 2020; Galindo et al., 2016), suggesting a new name *Tritia neritea* Linnaeus 1758, contributing for

the confusion of *Cyclope* with *Tritia* sp. (*Tritia reticulata* Linnaeus 1758) that has a free-swimming veliger stage in its development, while *Cyclope* sp. has direct development - crawling juveniles.

Cyclope neritea has a broad patchy distribution along the Mediterranean shorelines, the eastern Atlantic coasts of Morocco, the Iberian Peninsula to the English Channel, and the Black and Azov Seas.

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The northwards “expansion” along the French Atlantic coast has been explained with the climatic changes, and the unintentional recurrent introductions due to the shellfish culture (Pacific cup oyster, *Crassostrea gigas* Thunberg 1793, and the Manila clam, *Tapes philippinarum* A. Adams & Reeve 1850 from the Veneto region, Adriatic Sea), rendering this species as invasive by some authors (Bachelet et al., 2004; Sauriau, 1991). *C. neritea* is found around Milos Island, Greece (Dando et al., 1995; Ruiz-Chancho et al., 2013; Southward et al., 1997; Thiermann et al., 1997), the Gialova lagoon, Ionian Sea, Greece (Koutsoubas et al., 2000), Lake Timsah, Suez Canal, Egypt (Mohammad, 2007), Bizerta Lagoon and Menzel Jemil in Tunisia (Lahbib et al., 2010). Salt- and brackish water lagoons and estuaries, sheltered bays and hydrothermal hyper-saline seeps around the Mediterranean provide habitats for *C. neritea*. It is found in clean sands with organic content <1% (Bachelet et al., 2004; Sauriau, 1991; Simon-Bouhet et al., 2006), where it occurs at tidal levels between -0.8 and +3.1 m depth. In Lake Timsah, Suez Canal, Egypt (Mohammad, 2007) and Gialova lagoon, the Ionian Sea, Greece (Koutsoubas et al., 2000) *C. neritea* prefers muddy and clay substrate. *C. neritea* is found around Milos Island, Greece at 5 - 14 m water depth adjacent to areas influenced by hydrothermal hyper-saline discharges through the sandy sediment (Southward et al., 1997). *C. neritea* is a hardy species, tolerant to a wide range of environmental conditions: salinity (6 - 50 ppt), pH (5.6 - 8.9) and temperature (14 - 39 °C) (Bachelet et al., 2004; Koutsoubas et al., 2000; Mohammad, 2007; Southward et al., 1997; Thiermann et al., 1997).

The *Nassariidae* family originates in the Atlantic - dated to the Lower Cretaceous (120 million years ago). The molecular phylogeny of 218 species of Atlantic and Indo-Pacific region mud whelks has been proposed (Galindo et al., 2016). Strong geographic clustering of haplotypes of 5 mitochondrial clades has been described (Couceiro et al., 2012). Studies of population diversity, origins

and dynamics support the hypothesis for the inter- and intraregional spread of *C. neritea* (Bachelet et al., 2004; Simon-Bouhet et al., 2006). The low molecular diversity and strong genetic structure in the native range (Italian Veneto region, Adriatic Sea) contrast to the high genetic diversity and low genetic structure in the newly colonised areas (Couceiro et al., 2012; Simon-Bouhet et al., 2006).

Cyclope neritea lives predominantly in calm waters. Compared to *Tritia reticulata* Linnaeus 1758, *C. neritea* is more active and faster, and the flow conditions further increase this competitive advantage (Bachelet et al., 2004). *C. neritea* spends most of the day buried in the sand with its proboscis protruded from it. Light- and thermo-periods influence the emerging-burrowing cycles (Bedulli, 1977). It emerges during the night to search for food. During low tide, it remains buried in the substrate and emerges in response to mechanical disturbance, like human activity and trampling on the sandy beaches (Garcia et al., 2015). *C. neritea* crawls with a smooth gliding motion. Burrowing is achieved by discontinuous movements of the shell, and ceases as soon as the shell is covered. *C. neritea* has flattened, disc-shaped foot settling a fairly rapid and shallow burial (Trueman & Brown, 1992). 80% of the *C. neritea* burrow within 4 min. Gradually they emerge and within 14 min 50% of the animals are moving on the sand surface (Southward et al., 1997).

C. neritea is an active scavenger able to locate food (dead fish, salps and bivalves) from a distance. However, it does not respond to the presence of damaged, or death conspecifics until it first makes contact (Garcia et al., 2015). It ingests large quantities of sand grains together with adhering bacteria and diatoms (colourless sulphur bacterium *Achromatium* sp., *Naviculid* diatoms, including *Entomoneis* sp.), characterising it as a deposit feeder (Bachelet et al., 2004; Southward et al., 1997; Thiermann et al., 1997). It is absent from the areas of the brine seeps, dominated by cyanobacterial mats. *C. neritea*, a dominant macrofaunal species at

Milos (Greece), is not found at Santorini (Greece), where the bacteria at the venting sites are dominated by iron bacteria, whereas at Milos large globular sulphur bacteria, *Achromatium volutans*, cover the hydrothermal brine seeps (Dando et al., 1995). In a different study, benthic microalgae largely contribute to the diet (high ^{13}C values) of *C. neritea* (similarly to *Haminoea navicula da Costa 1778*), contrasting to the suspension feeders (the polychaetes, *Hediste diversicolor* O. F. Muller 1776 and *Cirriphormia tentaculata Montagu 1808*) that have a diet based on phytoplankton. Further, *C. neritea* has high ^{15}N values, supplied by dead animal material (Como et al., 2015). Among the benthic consumers, the *Astropecten* sea stars prey on *C. neritea* (Baeta & Ramon, 2013).

Cyclope neritea embryos undergo direct development during 2 to 5 weeks within a single- embryo-containing egg capsules, deposited in clusters on hard substrata (rocks, cockle shells *Cerastoderma edule* Linnaeus 1758, and sea grass *Cymodocea nodosa* Ucria Asch.), hatching as benthic juveniles (Bachelet et al., 2004; Sauriau, 1991; Southward et al., 1997). The lifespan of *C. neritea* is probably similar to this of *T. reticulata* that has a maximal longevity of 280 days (Bachelet et al., 2004), shortened for the parasitised gastropods, reaching earlier mortality peak - 100 to 120 days, as opposed to 140 - 160 days in uninfected snails.

Cyclope neritea tolerates high sulphide (1 mM) and arsenic (3-6 g/L) concentrations (Ruiz- Chanco et al., 2013). A number of environmental pollutants (copper, nonylphenol, polychlorinated biphenyls, aroclor, and organotin compounds - tributyltin) cause imposex (Lahbib et al., 2010; Quintela et al., 2006), which lead to population decline due to sterility and reproduction failure.

C. neritea is present in European clam farms (Couceiro et al., 2012) and natural fishing areas, like Lake Timsah, Egypt (Mohammad, 2007), supplying European fish-food markets. Although *C. neritea* is considered free of parasites, 1% of the animals are parasitised by digenean trematodes:

Schistosomatidae, *Zoogonidae*, *Lepocreadiidae*, *Echinostomatidae*, and *Microphallidae* (Bachelet et al., 2004). *C. neritea* has been used for pollution monitoring of arsenic in Milos Island, Greece (Ruiz-Chanco et al., 2013), trace non-essential heavy metals (Cd, Hg, Pb) of Igneada shores, Black Sea (Bat & Sahin, 2018), petroleum hydrocarbons and heavy metals (Pb, Cd, Co, Mg and Zn) of Tamsah Lake and Suez Canal, Egypt (Sharaf & Shehata, 2015), and marine pollution due to boating traffic (tributyltin) of the Bizerta Lagoon and Menzel Jemil in Tunisia (Lahbib et al., 2010). Further, the anthropological value of *C. neritea* has been demonstrated by the use of its shells as exotic types of embroidery ornaments on wear of ceremonial significance in Late Mesolithic Vlasac of the Danube Gorges region, the central Balkans, suggesting mid-7th millennium BC, Mesolithic foragers' corporeal symbolism, and regional and long- distance acquisition networks (Cristiani, E. & Boric, D. 2012). Similarly, *Cyclope neritea* along *Antalis* and *Columbella spp.* shells have been used for the production and rejuvenation of embroidered garments (Upper Palaeolithic - Neolithic) found in the famous Franchthi Cave excavations in Greece (Perles, 2018).

The aim of the study is to evaluate *Cyclope neritea*'s propagation under long-term artificial condition. Its transient longevity reasons the work for optimising the conditions for the successful maintenance, and popularising it for the ornamental fish habitats.

MATERIALS AND METHODS

Cyclope neritea Linnaeus 1758 snails were obtained from European trader, and maintained in artificial sea-water (34 g Salinity for reefs, Aquavitro, SeaChem Laboratories, Madison, USA in 1L of tap-water), along with copepods, *Tigriopus californicus* Baker 1912. The sea-water was supplemented with Reef Calcium, Reef Complete, Reef Plus, Reef Trace, Reef Phytoplankton, Reef Iodide, Vitality, and Stability (SeaChem, USA), according to the manufacturers

recommendations at 25°C and 12/12hr light/dark cycle and 947 lm, colour 7700 K light lux illumination (EHEIM Classic LED lights, EHEIM GmbH & Co. KG, Germany).

RESULTS AND DISCUSSION

Cyclope neritea Linnaeus 1758 (Fig. 1A) is a small mollusc (~15 mm shell width). The hemispherical (convex above and flattened beneath) shell is smooth and gloss. The flattened spire is formed of 3 indistinct whorls. The aperture is ovate, obliquely emarginated. The outer lip is smooth and slightly margined. The almost circular columella callosity widens the inner lip and extends upon the body whorl. The colouring is variable - yellowish to reddish white, greenish to bluish with darker brown lines, or interrupted bands. Some individuals have an operculum (Fig. 1B). Interestingly, *C. neritea* holds in place the food with the front parts of its foot (Fig. 2A) during eating. Males have a penis visible only during the quick copulation. The female genitalia has an opening in the ventral part of the foot (Fig. 2B).

C. neritea does not have a veliger stage. It is an oviparous marine gastropod mollusk laying scattered adherent on the surface of hard substrates - rocks and shells, egg capsules (Fig. 3), containing a single embryo. The deposition of the egg lasts on average 8 - 10 min, and the separate depositions are spaced by 2 - 7 min intervals. In a representative observation, 4 females (9 adults) deposited 44 eggs during a period of 1 week, where only 23 hatched after 15 days at 25°C.

C. neritea egg capsules (Fig. 4) contain a single egg. The capsules are transparent, convex with whitish oval-shaped 0.8 - 1.0 mm diameter edge attached to the substratum. The white single egg sinks in the capsules cavity. The capsules are covered by transparent mucus. Taken out of the water, the capsule fills with air, which prevents the development of the embryo. Thus, suggesting that the longitudinal fissure allows a contact of the capsule contents with the sea water. The

egg capsules with hazy intra-capsular fluid never develop embryos.

Interestingly, some females eat their eggs immediately after deposition (Fig. 5). The attacked egg capsules unlike the normal ones contain 2 or 4 whitish cells that are most likely a result of the retarded egg deposition and the initiated 1st or 2nd division. The retarded deposition could be a consequence of imposex, and the caused mechanical obstruction due to the presence of a vas deference. Females laying normal, single-cell containing capsules never attack them. Normal, single-egg capsules, laid by other females, are never attacked. Further, growing juveniles supplement their diet, attacking developing eggs of their kind (Fig. 6).

The intra-capsular development of *C. neritea* embryos lasts 11 - 15 days at 25°C. The initial divisions of the ovum occur within the first 24 hr after deposition. The trochophore is visible after the 4th day, and the shell appears after the 5th day (Fig. 7). The developing trochophore moves after the 5th day and the snail starts to crawl after the 7th day (Fig. 8). While the developing embryo is freely moving within the convex capsule, it is positioned either on the left- or right-side before hatching (Fig. 9). The embryonic shell extends, and the juvenile leaves the egg capsule with 1-whorl shell. Juvenile shell stays upright, with the apex on the right, and the syphon on the left side of the 1st whorl. Juveniles grow slowly, starting with 0.5 mm shell length after hatching. They extend their shell length with 0.5 mm every 2 weeks during the first 2 months. Coiling the whorls over the older ones so only 3 whorls remain visible. Although a definitive lifespan cannot be given at the moment, it is probably similar to those of other *Nassariidae* species, like *Tritia reticulata* Linnaeus 1758 that has a maximum longevity of about 9 - 10 months (Bachelet et al., 2004), less than a year.

Cyclope neritea's high juvenile mortality rate and short lifespan make it not easy to propagate in an artificial habitat. The presence of pollutants in the water, and parasite infestation shorten its lifespan,

influencing the population density in the wild. The effect of marine pollutants has been attributed to the induction of imposex - the superimposition of male sexual characteristics in female gastropods. In *C. neritea* females, only the 1st of the 4 stages of imposex is observed, characterised by the development of a bud penis behind the right ocular tentacle. *C. neritea* males present small penis or are aphyallic (Lahbib et al., 2010; Quintela et al., 2006). The presence of imposex results in population decline due to sterility and reproduction failure. In this study, the retarded egg capsule deposition was ascribed to be a consequence of imposex and the induced mechanical obstruction due to the presence of

a vas deference. The observed behaviour of affected females to eat the content of their egg capsules can further augment the population decline.

C. neritea has a low dispersal ability and isolation-by-distance population pattern. The probable reason for their observable presence at some spots around the Mediterranean shorelines is the increased food supply due to mussel farms common aquaculture activities (Simon-Bouhet et al., 2006). Although labeled as invasive (Bachelet et al., 2004; Sauriau, 1991) *C. neritea* propagation is not pathological when compared to other popular European gastropods.

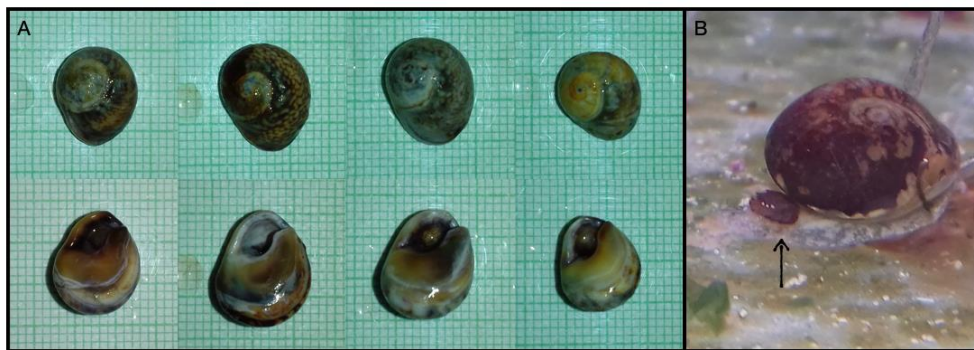


Figure 1: *Cyclope neritea* Linnaeus 1758 shells (A). Some individuals have an operculum (B).



Figure 2: *Cyclope neritea* holds in place the food with the front parts of its foot (A). Males have a penis visible only during the quick copulation, and the female genitalia has an opening in the ventral part of the foot (B).

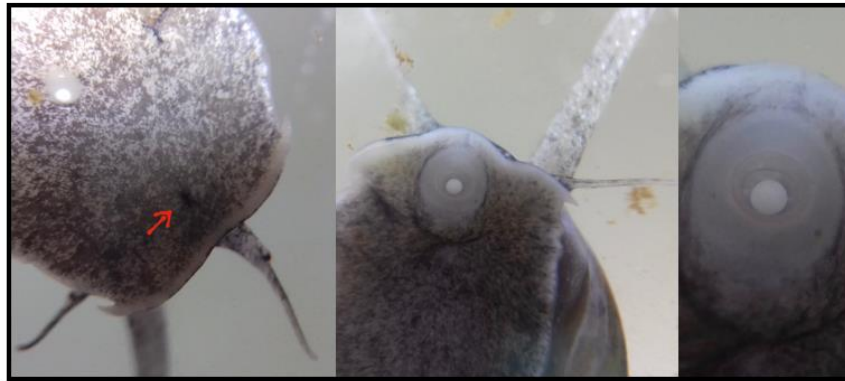


Figure 3: *Cyclope neritea* is an oviparous marine gastropod mollusk.

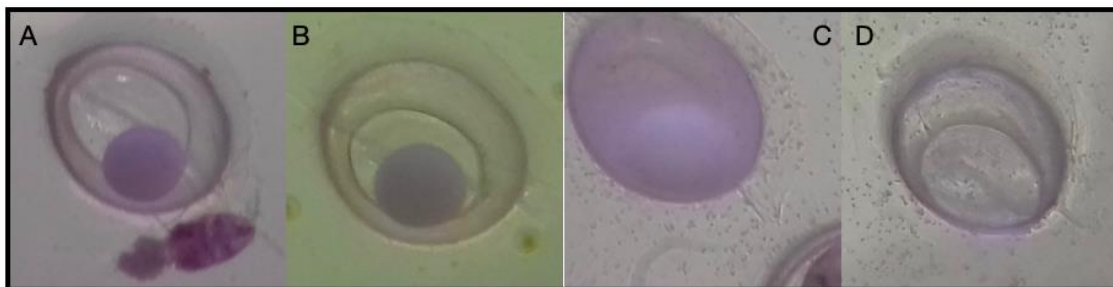


Figure 4: *Cyclope neritea* egg capsules: transparent, 0.8 - 1.0 mm in diameter, covered by transparent mucus, the longitudinal fissure allows a contact of the capsule contents with the sea water. The white single egg sinks in the capsules cavity (A, B). Capsules with hazy intra-capsular fluid never develop embryos (C). Empty egg capsule - the content has been eaten before the development of the shell (D).



Figure 5: Some *Cyclope neritea* females eat their eggs immediately after deposition: the retarded egg deposition, consequence of imposex is the possible reason.

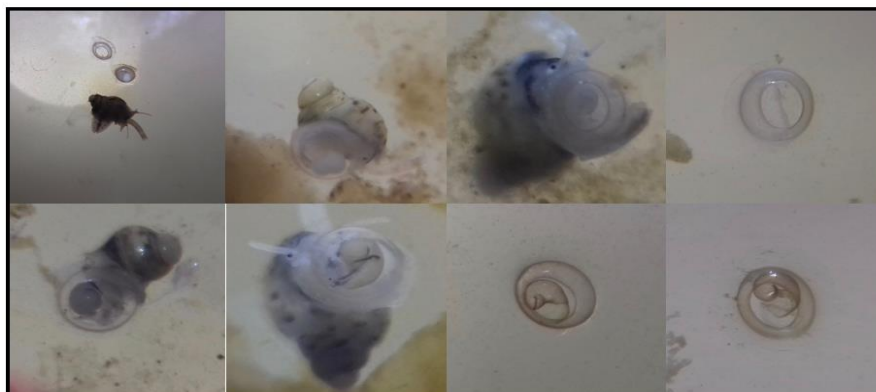


Figure 6: Growing *Cyclope neritea* juveniles supplement their diet, attacking developing conspecific eggs.

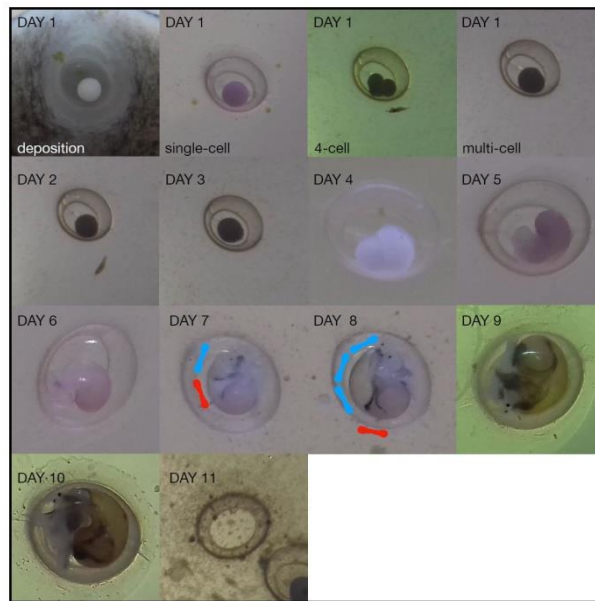


Figure 7: Intra-capsular development of *Cyclope neritea* embryos.

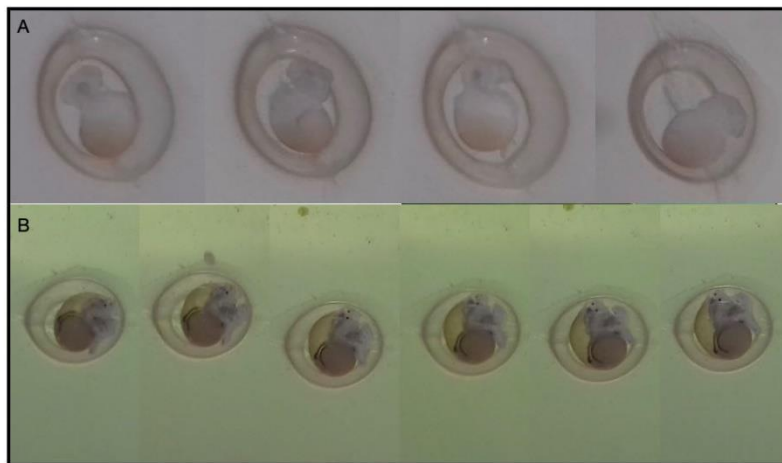


Figure 8: The *Cyclope neritea* trochophore (A) moves after the 5th day and the developing snail (B) starts to crawl after the 7th day.

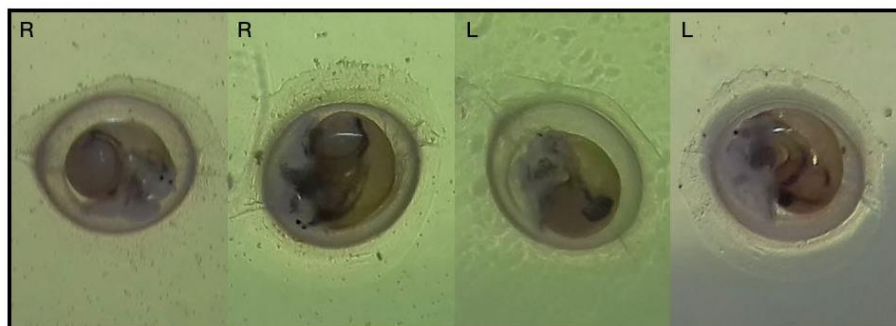


Figure 9: *Cyclope neritea* developing snail is positioned either on the left- or right-side before hatching.

CONCLUSION

Cyclope neritea Linnaeus 1758 can be maintained and reproduce successfully in artificial conditions, and may be popularised in the ornamental fish trade and as a possible laboratory model animal. In order to establish the proper laboratory conditions for long-term maintenance and propagation, optimisation of the conditions for care is necessary to increase juvenile's growth and survival.

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Conflict of Interest:

The author declares no conflict of interest.

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