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Research Article

Observations on *Neritina turrita* (Gmelin 1791) Breeding Behaviour in Laboratory Conditions

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ABSTRACT

Neritina turrita (Gmelin 1791) along with other Neritina, Clithon, Septaria, and other freshwater snails are popular animals in ornamental aquarium trade. The need for laboratory-bred animals, eliminating the potential biohazard risks, for the ornamental aquarium trade and the growing demand for animal model systems for biomedical research reasons the work for optimising a successful breading protocol. The initial results demonstrate N. turrita as tough animals, surviving fluctuations in pH from 5 to 9, and shifts from a fresh-water environment to brackish (2 - 20 ppt), to sea-water (35 ppt) salinities. The females laid over 630 (at salinities 0, 2, 10 ppt and temperatures of 25 - 28°C) white oval 1 by 0.5 mm egg capsules continuously within 2 months after collecting semen from several males. Depositions of egg capsules are set apart 6 +/-3 days, and consist on average of 53 (range 3 to 192) egg capsules. Production of viable veligers was recorded under laboratory conditions.

Keywords: Neritina turrita, Sea-water, Temperatures, Environment

INTRODUCTION

Neritininae are found in the coastal swamps of most tropical regions. The existing species of *Neritina* were divided into 4 subgenera in the 20's by Baker based on differences in shell shape, and in shape of the teeth of the radula. Later, Thiele suggests 5 subgenera, and in the 80's Cunningham Vaught - 11 subgenera. Nowadays, Haynes describes the subgeneric system as confusing, not reflecting the true relations among the existing species. Studying the richest neritinid fauna around the Fiji Islands, she describes species belonging to supposably different genera forming hybrids with each other, suggesting their close relation. Describing them all as different species is likely invalid, let alone allocating them to different subgenera or, even worse, to independent genera (Bandel, 2001).

Neritina turrita (Gmelin, 1791) snails have shells that are oblong-conical, lightly striated, shiny, spire elevated, pointed, yellow with oblique, curved or rippled black stripes. The shells are 25-32 mm in length. The aperture is white and the columellar area is yellow tinted.

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It is found throughout the Indo-Pacific region: including Madagascar, Japan to Indonesia, and the western Pacific Ocean islands. It is not found in India and Australia. In the Mariana Islands, mainly Guam (Micronesia), *N. turrita* is present, along with a total of 25 other species of gastropods known to inhabit fresh and brackish waters, including 18 native species: 13 Neritidae, 4 Thiaridae, and 1 Lymnaeidae; and 7 introduced species - 2 Ampullariidae, 2 Planorbidae, as well as 1 each from the Physidae, Thiaridae and Viviparidae (Kerr, 2013).

Neritina turrita inhabits tidallyinfluenced brackish waters near the mouths of rivers and streams. It is often found near the shore in mud, or on stones. Members of the gastropod families Neritidae and Thiaridae are good travellers, having larvae that disperse on ocean currents, so that they have a broad geographical distributions, and the adults move several kilometres up-stream estuaries a habitat shift with growth. Migrating adults have higher densities in riffles than in pool habitats. Neritids prefer large substrates (Liu & Resh, 1997). In Moorea (French Polynesia) and Mariana Islands - Guam, Palau and Yap (Micronesia) they are found along with other species introduced by man - either as potential food, or due to accidental release from aquaculture or the aquarium trade. On Iriomote Island in the Ryukyu Islands, southern Japan, N. turrita is found together with N. violacea and achieves high densities and is widely distributed in the mangrove swamps and the adjacent seashore (Okuda & Nishihira, 2002). The molluscan community is a key component of the mangrove food chain, preyed by many fish and waterbird species. Anthropogenic disturbances resulting in habitat degradation impact the populations of such amphidromous gastropods.

Neritina turrita snails are herbivorous in the adult stage, developing from planktonic veliger larvae. Its growth is faster in the warmer season (April-October), and slower in the cooler season (Takada, 2016). Fresh-water nerites are observed in massive up-stream migrations, consisting of both adults and juveniles moving together in long lines and/or dense aggregations. Such migrations have been reported in Hawaii (Hau, 2007), Costa Rica, Japan (Kano, 2009), French Polynesia (Liu & Resh, 1997), and Puerto Rico (Pyron & Covich, 2003; Gorbach et al., 2012). The controlling factors for such migrations are unknown, although seasonal factors (like rain and flash-floods), and increased predation in the estuary (by fish and birds) have been suggested (Schneider & Lyons, 1993).

Neritina, and the related genera Clithon and Septaria usually settle on tropical coasts, arriving after a planktonic larval stage at sea. Veligers metamorphose in the estuaries, where the benthic young are exposed to the influence of fresh-water. From the estuary, many species crawl upstream and spend the rest of their adult lives in the fresh-water environment (Bandel, 2001). Female Neritidae capsules (spermatophores) store sperm received from the males, which permits continuous spawning. Neritids lay flat. elliptical egg capsules on hard substrata, including rocks and shells of other snails. Newly laid capsules are white, becoming cream to brown in a few days. Each capsule contains up to 300 eggs that hatch several weeks later. Morphological studies of larvae and their shells are published since the 70's by Robertson, Scheltema, Lauren, and by Bandel at the present time. There is a limited timewindow for neritid larvae to reach salt-water: veligers held in fresh-water die within 6 days. In Hawaii, larvae of Neritina granosa are found year-round in drift collections, in pools and run-off areas during low-flow conditions. Floods cause a decrease in water temperature and an increase in dissolved oxygen, providing optimum conditions for egg capsules to be laid, and hatched larvae to be transported to the ocean. Genetic studies (Hodges & Allendorf, 1998; Myers et al., 2000; Crandall et al., 2010) confirm that larvae from amphidromous lineages are capable of pelagic dispersal across the open ocean that lasts from weeks to months. The growth rate is higher and the life-span is longer in females than males. The populations of amphidromous species are genetically structured at scales similar to fully marine species. As the planktotrophic larvae of amphidromous species must settle in a rare, unstable habitat, they could be selected for the ability to delay metamorphosis, and extend their planktonic life indefinitely (Abdou et al., 2015).

Although popular animals in the ornamental aquarium trade, *Neritina turrita* (Gmelin 1791) are not successfully bred in captivity, and the available animals are wild-caught and present a potential biohazard as well as depleting natural populations. There are no reports in the available literature for their successful breeding in laboratory condition. The aim of the undergoing study in my laboratory is the optimisation of a breeding protocol for *Neritina turrita* for the ornamental aquarium trade, and the growing demand of animal model systems for the biomedical research.

MATERIALS AND METHODS

Neritina turrita adult snails were kept in 2, 10, and 60 L glass vessels. Tap-water was used to achieve a salinity range of 0 (fresh-water), 2, 5, 10, 15, 20 ppt (brackish water), 35 ppt (seawater) in conjunction with 'Salinity for reefs', Aquavitro, SeaChem Laboratories Inc., Madison, USA. The brackish water (2 ppt) pH was adjusted using Discus Buffer (pH 5.8-6.8); Neutral Regulator (pH 7.0); Malawi Victoria Buffer (pH 7.8-8.4); Tanganyika Buffer (pH 9.0-9.4), SeaChem Laboratories Inc., Madison, USA.

The snails were fed with spring and autumn oak (*Quercus robur*) and plum (*Prunus cerasifera*) leaves, sliced courgettes/zucchini (*Cucurbita pepo*), and cucumber (*Cucumis sativus*) peel strips.

Light microscope images were acquired with the help of Prof. N. E. Lazarov, and Mrs D. Brazicova with a Nikon research microscope, equipped with a DXM1200c digital camera in the Department of Anatomy, Histology and Embryology, Medical University - Sofia, Sofia - 1431, Bulgaria.

RESULTS AND DISCUSSION

Neritina turrita (Fig. 1A) tolerated salinities from 0 (fresh-water) to 35 ppt (sea-water). The snails were kept at 0, 2, 5, 10, 15, 20, and 35

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ppt for 4, 4, 1, 4, 1, 4, and 1 week, respectively. No changes in their behaviour (grazing (Fig. 1B), and sleep/wake cycles) were observed. Similarly, the changes of water pH using SeaChem Laboratories buffering salts did not influenced their behaviour. The animals were kept in 2 ppt brackish-water, and pH altered from 5 to 9 for a week at each pH point. Neritina turrita were observed to copulate several times (Fig. 1C), regardless of the salinity (2, and 15 ppt). They laid over 630 egg capsules (Fig. 1D) continuously over a period of 2 months in both fresh (0 ppt) and brackish water (2 and 10 ppt) at temperatures of 25 - 28°C. Each deposition was set apart by 6 ± -3 days, and comprised a mean of 53 (with a range of 3 to 192) egg capsules. Light microscopy evaluation of N. turrita egg capsules and veligers used a Nikon research microscope. The typical for N. turrita egg capsule structure (Fig. 2A) was observed, and the release of early veligers from the eggs when placed in tap-water (Fig.2C).

The family Neritidae is one of the most primitive families in the Gastropoda, and includes members that are diadromous species capable of escaping the instability of freshwater habitats (due to droughts or cyclonic flood events), to colonise new environments through oceanic dispersal. Amphidromy is one of the modalities of diadromy: while the adults grow, feed, and reproduce in rivers and lowsalinity areas of estuaries, larvae drift downstream after hatching to reach the marine salinity necessary for their development, and to complete the dispersive, planktotrophic marine larval stage. Later, they recruit to estuaries, colonising the adult freshwater habitats (Quintero-Galvis & Castro, 2013). Neritina turrita (Gmelin 1791) inhabits intertidal and supra-tidal rocks and mangroves, brackish and fresh waters on temperate to tropical coasts.

The roots and stems of 5 species of mangrove - Avicennia marina, Bruguiera gymnorrhiza, Rhizophora mucronata, Ceriops tagal, and Lumnitzera racemosa, and the surrounding mud provide a substratum for a maroon algal felt termed the bostrychietum.

The bostrychietum is present throughout the tropical world. The pneumatophores of the white mangrove A. marina, support the richest algal flora. Microcoleus chthonoplastes has the widest biogeographical range (Lambert et al., 1989). These algae are common in mangroves the world. While some around are opportunistic epiphytes (Colaconema sp. and Acrochaetium globosum), or topical tropical shallow water algae (Canistocarpus cervicornis) not specific to mangroves, others are associated with mangroves (Bostrychia and Caloglossa). While some are widespread, others are specifically limited to the Indo-Pacific ocean, and other occurs in Florida -USA, Malaysia, Micronesia, New Caledonia, and Saudi Arabia. Low-molecular weight carbohydrates are contained differentially within the species with different geographical distribution: Bostrychia moritziana and B. radicans specimens from Australia, Brazil, Georgia-USA, and Florida-USA, Mexico, Micronesia, Peru, and Venezuela have sorbitol and dulcitol, but populations from North Caroline to Connecticut, USA lack dulcitol (West et al., 2013).

The Rhodophycean algae (Bostrychia, Catenella, and *Murrayella*) Caloglossa, comprise the bostrychietum along with the other classes: Cyanophyceae, Chlorophyceae, Phaeophyceae, and Bacillariophyceae. Freshwater algae are also an associate of the bostrychietum. Bostrychia tenella (supratidal localities) and B. scorpiodes (a salt marsh species) are cosmopolitan species, recorded as a mangrove epiphyte in Brazil, New Zealand, Sumatra and Australia. B. tangatensis is a mangrove epiphyte in Tanzania, Mozambique, southern Africa, Australia and Japan, occurring on the pneumatophores of Avicennia species. There is an algal zonation on roots of Rhizophora in Puerto Rico, where C. ripens establishes on the drier sector of the pneumatophore (Lambert et al., 1987).

Although herbivorous algae-eaters, *Neritina turrita* were fed with spring and autumn oak (*Quercus robur*) and plum (*Prunus cerasifera*) leaves, sliced courgettes/zucchini (*Cucurbita pepo*), and cucumber (*Cucumis sativus*) peel strips, and successfully grew and reproduce on that diet.

Other species of Neritina and related genera occupy similar habitats. For example, Neritina natalensis (Reeve 1855) is a tropical snail found in estuarine habitats in South Africa: from Umgeni river, Natal; as far south as Umzimkulu river and Umlalazi river to Mozambique, and northwards to the Pangani river, Tanganyika, Tanzania, at the head of estuaries (not in rivers). In the Mngazana estuary, Transkei, South Africa N. natalensis have been observed in salinity of 2ppt (Prof. G. Branch, University of Cape Town, South Africa, personal communication). In Kosrae (Micronesia), the mangrove forest of Yela River is dominated by Sonneratia alba and Bruguiera gymnorrhiza at its estuary, mature fresh-water swamp forest (dominated by Terminalia carolinensis and Pandanus spp.) up to 40m asl, and undisturbed upland forest (dominated by *Campnosperma brevipetiolata*) above 40m asl (above sea level). Zonation in the distribution of snails with elevation, or distance from the river mouth has been observed: Neritina canalis and Melanoides tuberculata are found only at the highest stations (temperature ~25°C and conductivity ~106 µS/cm). Neritina pulligera is found at the lower stations (temperature ~27°C and conductivity ~83 µS/cm), along with Clithon castanea. Migrations of other neritids similar to those in Puerto Rico and other places has not been observed (Benstead et al., 2009).

During the current study adults of *Neritina turrita* were found to tolerate increases in salinity from 0 (fresh-water) to 35 ppt (sea-water) for extended periods of time. No changes in their behaviour (grazing and sleep/wake cycles) were observed. Similarly, the changes of water pH from 5 to 9 at 2 ppt brackish-water did not influenced their behaviour.

Neritidae have internal fertilisation, and encapsulate their eggs after fertilisation. A unique organ, located close to the anus and oviduct opening, strengthens the egg capsule. The aragonitic or calcitic spherulites, 5 - 500µm in diameter, are secreted in the digestive

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tract of females. When secreted from the anus, a portion is carried by cilia to the crystal sac, where the mineral particles are sorted and stored. The base half of the egg capsule is flattened and fixed to the substrate, and the upper convex half lifts off when the young escape. The walls are made of tough conchiolin, lined internally by a membrane enclosing an albuminous fluid in which eggs float. Neritina pulligera collected from Kyushu Island, Japan, and maintained in freshwater at 27°C deposited clusters of 4 - 39 large egg capsules mostly on shells, while Clithon corona deposited their egg capsules on the aquarium devices and stones (Kano & Fukumori, 2010). Similarly, Neritina zebra, a common species of muddy bottoms in brackish water environments found in Suriname, and along the cost of Brazil to Cabo Frio, Rio de Janeiro, produces oval 1 - 1.5 mm-long egg capsules containing ~68 eggs (32 - 106) that become darker as development progresses (Barroso & Mattews-Cascon, 2009). Regardless of the salinity (2 and 15 ppt), N. *turrita* were observed to copulate several times (Fig. 1C). Likewise, they laid over 630 egg capsules (Fig. 1D) continuously over a period of 2 months in both fresh and brackish water (2 and 10 ppt). Each deposition was set 6 (2 -11) days apart, consisting on average of 53 (3 -192) egg capsules.

Salinity is critical for hatching: egg capsules of N. zebra release veligers after 21 days at salinity 5 ppt (26-28°C). Despite the presence of well-formed veligers, eggs do not hatch at higher salinity (15 ppt), and development is slower (25 days). Decreasing the salinity to 10 ppt results in the release of the veligers (Barroso & Mattews-Cascon, 2009). In this study, N. zebra veligers could be kept alive only for 2 days after leaving the egg capsules (Dr. C. X. Barroso, Universidade Federal do Ceara, Instituto de Ciencias do Mar, Brazil, personal communication). Unlike N. pulligera (Kano & Fukumori, 2010), and N. zebra (Barroso & Mattews-Cascon, 2009), which prey on their own egg capsules, N. turrita was not observed to harm its own egg capsules.

Neritids in Hawaii (Neritina granosa) and Puerto Rico (N. punctulata and N. virginea) are observed to participate in up-stream migrations in coastal and insular streams and rivers in massive aggregations ($>3000 \text{ ind/m}^2$), moving up to 10 km inland with speeds of 1 -50 m/day (Pyron & Covich, 2003; Blanco & Scatena, 2005; Blanco & Scatena, 2007). Successive flash floods draw them back to the mouth. Up-stream migrations river are frequent - once every 15 days. Migrations typically occur within 5 days after a flood, and end after nearly a week of continuous upstream movement. During non-migratory periods, snails are dispersed and rarely form groups of more than 5 individuals. Neritids prefer large objects for attachment, more turbulent and erosive habitats, and reahc greater densities in riffles and fast-flowing areas. Neritina virginea undergoes significant growth during upstream migrations. The upstream migration is probably a response to downstream, higher predation higher productivity upstream, and the tolerances of physical parameters (salinity and temperature) by adults and juveniles (Pyron & Covich, 2003; Blanco & Scatena, 2005; Blanco & Scatena, 2007). Interestingly, a number of rivers in dry southern Puerto Rico lack populations of N. virginea. They are absent from streams draining limestone that have high concentrations of CaCO₃, greater total suspended solids and SiO₂, elevated conductivity and high concentrations of dissolved ions (Na, Cl, K, Mg, and SO₄) (Blanco & Scatena, 2006).

The settlement, metamorphosis, development and growth of veliger larvae of gastropods have many marine been extensively studied. Water-borne chemical and substrate-associated (algal-surface cues ligands, barnacle-associated substances, and bacterial-biofilm substances) induce settlement and metamorphosis (Pires & Hadfield, 1993; Plaut et al., 1995; Hadfield et al., 2000; Nedved & Hadfield, 2009; Ruiz-Jones & Hadfield, 2011). The morphogenic effects are mediated through the central nervous system (Pires & Hadfield, 1993). K^+ and Cs^+ have

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been shown as a potent metamorphosis inducers, acting by depolarising the sensory cells responding to the natural metamorphosis inducer, and also on the entire nervous system (Hadfield et al., 2000). It should be noted that potassium is toxic to fresh-water molluscs, and KCl, as potash has been suggested as a potential pesticide for treatment of infestation

with zebra (*Dreissena polymorpha* Pallas 1769) and quagga mussels (*Dreissena rostiformis bugensis* Andrusov 1897). Interestingly, sodium concentration affects veliger survival, and supplementation with NaCl increases their survival (Moffitt et al., 2016).

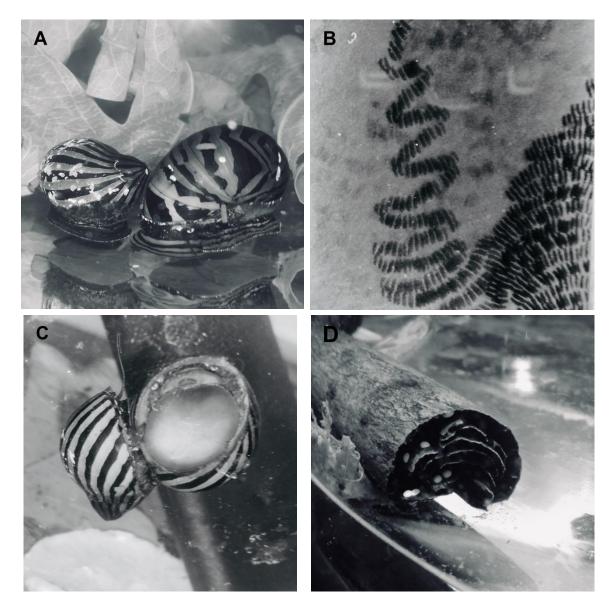


Figure 1: *Neritina turrita* (Gmelin, 1791) were maintained in fresh, brackish (salinity from 2 to 20 ppt) and sea water (35 ppt) at 25 - 28° C (A). The grazing pattern on algae-covered glass surface (B). Copulating pair: the male is on the left, and the protruding penis is visible (C). *N. turrita* laid over 630 egg capsules, continuously within 2 months. Each deposition was set apart 6 +/-3 days, consisting on average of 53 (from 3 to 192) egg capsules (D).

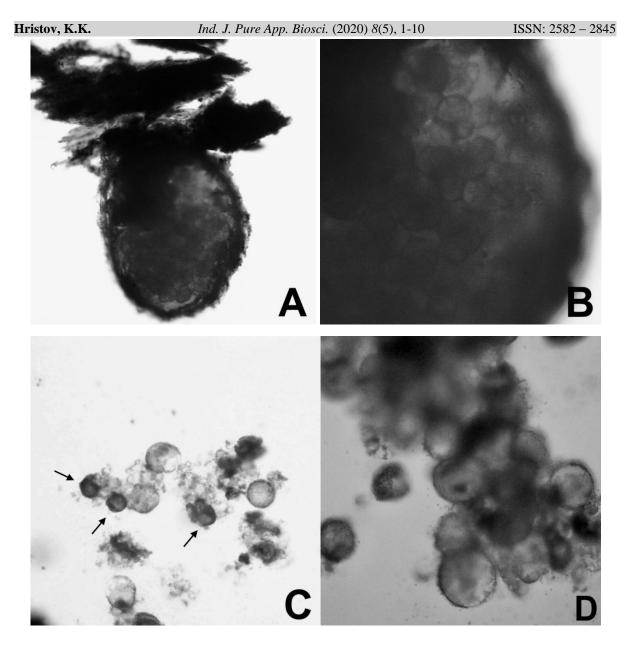


Figure 2: Light microscopy evaluation of *Neritina turrita* (Gmelin, 1791) egg capsules. The distinct for *N. turrita* egg capsule structure (magnification 10x in A, and 20x in B) was observed. Egg mass after its release from the egg capsule (D), and the release of early veligers from the eggs when placed in tap-water (C, magnification 20x).

CONCLUSIONS

Neritina turrita snails are a hardy species, surviving increase in salinity to levels of seawater, and change in acidity from pH 5 to 9. Currently, I am working on two hypotheses aimed at producing a reliable breeding protocol: i) Everything happens in the estuary, where veligers stay in the mud after hatching, but those that don't stay there have the ability to survive at sea, and disperse to new locations. The adult snails are maintained at the same below-sea-water salinity. I am looking for developmental and

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metamorphosis-inducing cues present in the natural estuary habitat that regulate veliger's faith. ii) Similarly to other amphidromous species (like *Caridina multidentata* for which there is an established breeding protocol), veligers spend a short time in fresh-water after hatching and are carried down-river to the estuary, where they face the increased salinity, necessary for the induction of metamorphosis and the maturation of benthic juveniles. Is the increased salinity acting as a cue that grounds the veligers in the mud at the estuary? As noted (Abdou et al., 2015), considering the

high levels of larval mortality, and the effects of diffusion, it seems unlikely that significant numbers of larvae that drift away from their natal archipelago would be able to find suitable fresh-water habitat for settlement. Therefore, local selection for traits that favour self-recruitment is particularly strong for species. Further, massive amphidromous aggregations (>3000 ind/m²) of adults as well as juveniles are observed to migrate up-stream in coastal and insular streams and rivers (Pyron & Covich, 2003; Blanco & Scatena, 2005; Blanco & Scatena, 2007). They come from the estuary, where they breed, lay egg capsules, and grow in their hundreds and thousands. Even the new arrivals, brought into the estuary habitats continuously by the ocean currents have to grow and breed in this environment to achieve the numbers of snails migrating up-river. It will be interesting to determine the reasons why some veligers stay in the estuary and grow to move up-river later, and why some survive at sea and disperse.

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