# PREDATION ON HARDEST MOLLUSCAN EGGS BY CONFAMILIAL SNAILS (NERITIDAE) AND ITS POTENTIAL SIGNIFICANCE IN EGG-LAYING SITE SELECTION

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## ABSTRACT

Neritid snails (Gastropoda: Neritimorpha) protect their eggs in a hard capsule, of tough conchiolin, reinforced by mineral particles derived from the faeces and stored in a special sac near the anus and oviduct opening. Predation on this arguably hardest of molluscan egg capsule is described and illustrated here; neritids of the freshwater to brackish-water genera *Clithon* and *Vittina*, generally classified as herbivores, feed facultatively on the eggs of various confamilial species after breaking the reinforced capsule lid by means of prolonged radular rasping. Intensive predation pressure by these common inhabitants in Indo-West Pacific coastal streams may have given rise to the remarkable egg-laying behaviour of *Neritina* on the shells of other living snails. Our laboratory examination showed that *Neritina* species deposited clusters of egg capsules more frequently on the living shell than on other substrates, and that the predation rate was significantly lower on this moving 'nursery'. Predation rate was even lower on the small egg capsules of *Clithon* and *Vittina* themselves, which were deposited one by one in the depressions on the rough surfaces of stones.

## INTRODUCTION

Snails of the family Neritidae (Gastropoda: Neritimorpha) produce robust, reinforced egg capsules that are comparable in strength to eggshells of birds and reptiles. Andrews (1933) reported that female neritids have a unique organ for the strengthening of the egg capsule called a reinforcement or crystal sac, located close to the anus and oviduct opening. The crystal sac of the marine intertidal genus *Nerita* Linnaeus, 1758 contains specially made 'crystals' or calcareous spherulites. The spherulites are either aragonitic or calcitic, 5–500 μm in diameter, and are presumably secreted in the digestive gland of females (Andrews, 1933, 1935; Bandel, 1990; Tan & Lee, 2009). When faecal pellets containing the spherulites are released from the anus, a portion is carried by cilia to the crystal sac where the mineral particles are sorted and stored (Andrews, 1937; Fretter, 1946; Houston, 1990). The formation of spherulites is a shared-derived feature or synapomorphy of *Nerita* species alone. In contrast, in other marine to freshwater neritids, it is sand grains, diatom skeletons and other hard material taken in with food that are instead sorted out from faeces and stored in the same sac; this is the case in species of *Neritodryas* Martens, 1869, *Fluvinerita* Pilsbry, 1932, *Theodoxus* Montfort, 1810, *Clithon* Montfort, 1810, *Vitta* Mörch, 1852, *Vittina* Baker, 1923, *Puperita* Gray, 1857, *Neritina* Lamarck, 1816, *Neripteron* Lesson, 1831 and *Septaria* Férussac, 1807 (Andrews, 1935; Bandel, 1982; Knudsen, 1992).

The neritid egg capsule is a flattened spheroid, 0.5–4.5 mm in length, made up of two approximately equal halves sutured together around the equator (Andrews, 1935). One half, the base, is fixed to the substrate and rises up to form part of the side wall of the capsule; the other, the lid, lifts off when the young escape. The walls are of tough conchiolin, lined internally by a membrane enclosing an albuminous fluid in which eggs float (Fretter, 1946). As the capsule passes through the oviduct opening, the contents of the crystal sac are poured onto the thick wall of the lid for further reinforcement (Andrews, 1937; Fretter, 1946; Houston, 1990). Calcified egg capsules are also found in freshwater snails of the family Ampullariidae (Caenogastropoda) and in pulmonate land snails (Heterobranchia), but the thin walls of those capsules provide protection mainly from dehydration rather than physical damage (Bandel, 1990). Sand-coated eggs in some caenogastropods, including the Cerithiidae, Thiaridae, Hydrobiidae and Naticidae, are not encapsulated in a conchiolin case (Andrews, 1935; Amio, 1963; Soliman, 1987). Thus, neritids arguably produce the hardest egg capsules among molluscs.

Such reinforced egg capsules are presumably less susceptible to predation than the relatively soft capsules produced by other gastropods (e.g. Pechenik, 1986; Rawlings, 1990; Turner, Turner & Ray, 2007; Dumont, Roy & Himmelman, 2008). However, there have been only few reports of predation on neritid eggs (e.g. drilling by snails of the family Muricidae in high intertidal rocky pools; Taylor, 1976), and the adaptive significance of the reinforcement has not been established. Here we first demonstrate that limnic neritids of the genera *Clithon* and *Vittina*, generally classified as herbivores, feed facultatively on the eggs of various confamilial species after breaking the reinforced capsule wall by means of intensive radular rasping. We also propose that extensive predation by these common and ubiquitous inhabitants of coastal streams and estuaries in the Indo-West Pacific may have caused the remarkable egg-laying behaviour of *Neritina* species reported previously by many authors (Andrews, 1935; Adegoke, Dessauvagie & Yoloye, 1969; Vermeij, 1969; Maciolek, 1978; Brown, 1980). These frequently deposit their egg capsules on the shells of other living snails, presumably to increase the offspring survival in safe 'nurseries'.

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**Table 1.** Specimens used in the 4-day laboratory observation on the oviposition and egg-eating behaviour of limnic neritid snails.

| Species | *n* | Males | Females | Shell length in mm* | Shell width in mm* |
|---|---|---|---|---|---|
| *Clithon corona* (Linnaeus, 1758) | 9 | 2 | 7 | 23.0 ± 3.7 (17.3–28.4) | 15.3 ± 2.0 (12.4–19.0) |
| *Clithon retropictus* (Martens, 1878) | 1 | 0 | 1 | 19.4 | 13.4 |
| *Vittina variegata* (Lesson, 1831) | 10 | 5 | 5 | 24.6 ± 2.3 (22.8–30.5) | 17.6 ± 1.8 (16.6–22.5) |
| *Neritina pulligera* (Linnaeus, 1767) | 12 | 1 | 11 | 28.4 ± 3.3 (22.6–34.4) | 20.6 ± 1.8 (17.7–23.5) |
| *Neritina iris* (Mousson, 1849) | 5 | 0 | 5 | 24.6 ± 1.2 (23.5–26.2) | 16.9 ± 0.6 (16.6–18.0) |
| *Neritina petitii* (Récluz, 1841) | 3 | 2 | 1 | 23.7 ± 0.5 (23.1–24.0) | 17.8 ± 0.2 (17.5–17.9) |
| *Septaria porcellana* (Linnaeus, 1758) | 10 | 5 | 5 | 23.1 ± 3.8 (17.9–29.1) | 17.4 ± 2.4 (14.2–20.7) |

*Average ± SD (range).

## MATERIAL AND METHODS

A total of 50 neritid snails, including 20 individuals of *Neritina* (*N. pulligera*, *N. iris* and *N. petitii*), 10 *Clithon* (*C. corona* and *C. retropictus*), 10 *Vittina* (*V. variegata*) and 10 *Septaria* (*S. porcellana*), were collected from a ditch in Ibusuki, Kagoshima, Kyushu Island, Japan, and brought back to the laboratory (Table 1). The snails were measured and placed in an aquarium (60 × 28 × 28 cm) filled with freshwater, together with 11 stones of various sizes (3.5–19 cm in maximum diameter) and composition (limestones and sandstones). Egg capsules deposited naturally on the stones and snail shells were carefully removed with a brush and tweezers prior to aquarium observations. The aquarium water was kept filter cleaned at room temperature (*c.* 27°C). The sex of the neritid snails was determined by the presence of either penis or female ridge (see Andrews, 1937; Fretter, 1946). This was achieved by boiling the snails in 80–95°C water for 30–60 s after which the soft part was removed from the shell following all aquarium observations.

Egg capsules laid in the aquarium were identified to species by the combination of the following ways: (1) direct observation of the oviposition; (2) size comparison with capsules from separate aquaria with a single neritid species; and (3) DNA sequencing of the egg. For species identification of several capsules, genomic DNA was obtained from inside eggs using a Qiagen DNeasy kit. Fragments of the mitochondrial COI gene were amplified using a PCR and the 'universal' primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTG G-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAA ATCA-3′) (Folmer *et al.*, 1994). PCRs were carried out in a final volume of 25 µl [2.5 µl genomic DNA template, 17.5 µl $ddH_2O$, 2.5 µl Takara ExTaq buffer, 2 µl dNTPs, 0.2 µl of each primer (20 µm stock) and 0.1 µl Takara ExTaq enzyme]. After an initial denaturation for 3 min at 94°C, the reaction solution was run for 35 cycles with the following parameters: denaturation for 30 s at 94°C, annealing for 40 s at 42°C and followed by extension for 60 s at 72°C. A single strand was directly cycle-sequenced using the amplification primer HCO2198 with a BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems) on an ABI310 automated sequencer at University of Miyazaki. The determined sequences were compared to those obtained from adult snails (Kano, 2009; Y.K. and H.K, unpubl.).

Numbers of intact and predated egg capsules on the glass walls of the aquarium were counted several times a day. After 4 days of culture, the numbers were counted for three more groups of different substrates, i.e. stones, plastic filter devices, shells of the living snails as well as for the glass walls. The surface area available for oviposition was measured for each substrate type in order to assess site-dependent preferences for egg laying and predation. For this measurement, each snail shell was approximated as a hemisphere (with a diameter of average of its shell length and width; Table 1). The flat surface of the hemisphere, which corresponds to the base of a creeping snail, was not considered as a part of the available surface.

Details of predatory behaviour were observed and documented photographically through the glass wall for six species of *Clithon* and *Vittina*. In addition to the three species in the main aquarium for the 4-day culture, *Clithon cyanostoma* (Morelet, 1853), *Clithon faba* (Sowerby, 1836) and *Vittina waigiensis* (Lesson, 1831) from the same ditch in Ibusuki were kept in another aquarium with the egg capsules of various neritids for this observation. All specimens used in this study were preserved in pure ethanol and deposited at the Department of Biological Production and Environmental Science, University of Miyazaki, Japan.

## RESULTS

### *Egg capsules and oviposition*

Thirty-five females of seven neritid species laid a total of 1,485 egg capsules in the 4-day aquarium observation. The capsules were variable in size and shape among species, but relatively uniform within a species (Table 2). This contrasts with the results reported by Andrews (1935) that showed considerable intraspecific size variation of neritid egg capsules, which presumably reflected the variable size of capsule gland of maternal snails (Andrews, 1935; Fretter, 1946). The constant sizes of conspecific capsules observed in this study may be attributed to the rather uniform sizes of females of each species in the samples used (Table 1).

The largest egg capsules in the aquarium were produced by *Septaria porcellana*, and the second largest by *Neritina pulligera* (Fig. 1). Although their size distributions slightly overlapped, the capsules of the former species generally had a more circular outline than those of other neritids in this study. The smaller capsules of *Neritina iris* and *Vittina variegata* could not be clearly distinguished from each other by size alone. Two *Clithon* species produced the smallest, most elongate capsules. The capsule of *Neritina petitii* was not identified, presumably because only one subadult female was included in the aquaculture (Table 1).

The egg capsules of *Neritina* and *Septaria* were characterized by being laid as clusters on smooth and flat surfaces such as the glass walls of the tank and snail shells (Fig. 2), and not individually in small concavities in limestones and sheltered surfaces of the filter devices, as was the case for *Clithon* and *Vittina*. Of the total 1,485 capsules, 688 were laid on the snail shells, 335 were on the glass walls, 238 on the aquarium devices and 224 on the stones (Table 3). Nearly all (681) capsules on the shells belonged to *Neritina*; only seven in the concavities of eroded shell apices were deposited by *Clithon* species. Similarly, all but two capsules on the glass walls were laid by the species of *Neritina* (274) and *Septaria* (59), whereas those on

**Table 2.** Measurements of neritid egg capsules identified in the present study.

| Species | *n* | Length in mm* | Width in mm* |
|---|---|---|---|
| *Clithon corona* | 20 | 1.22 ± 0.09 (1.03–1.34) | 0.73 ± 0.06 (0.60–0.87) |
| *Clithon retropictus* | 30 | 1.15 ± 0.09 (0.95–1.30) | 0.78 ± 0.10 (0.60–1.02) |
| *Vittina variegata* | 16 | 1.64 ± 0.03 (1.59–1.72) | 1.12 ± 0.06 (0.96–1.20) |
| *Neritina pulligera* | 42 | 2.11 ± 0.11 (1.87–2.35) | 1.57 ± 0.11 (1.36–1.78) |
| *Neritina iris* | 9 | 1.49 ± 0.07 (1.35–1.58) | 1.03 ± 0.09 (0.90–1.17) |
| *Septaria porcellana* | 28 | 2.21 ± 0.11 (2.00–2.48) | 1.96 ± 0.99 (1.77–2.14) |

*Average ± SD (range).

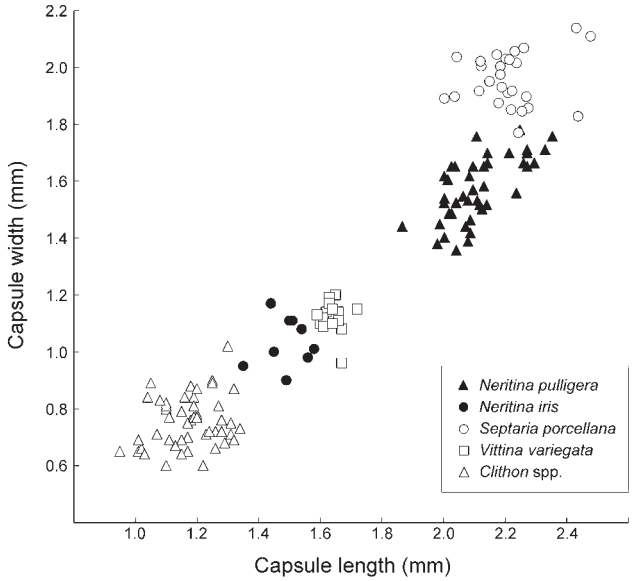


**Figure 1.** Plot of the measurements of neritid egg capsules identified in the present study.

the aquarium devices and stones were nearly exclusively of *Clithon* and *Vittina* snails.

The density of the *Neritina* capsules was 47 times higher on the snail shells (1.89 $cm^{-2}$) than on the walls (0.04 $cm^{-2}$; binomial test, $P < 0.00001$). Shells of all four genera bore these capsules, but congeneric snails carried most of them (658 or 96.6%). Of the 20 individuals of *Neritina* (Table 1), 11 females and three males carried 1–170 capsules on each (Fig. 2G), while six females had none. The density on the congeneric shells was 3.92 $cm^{-2}$ on average and up to 17.12 $cm^{-2}$ on each snail. The three males had fewer capsules (1–25; 1.43 $cm^{-2}$ on average) than females (0–170; 4.29 $cm^{-2}$), but the difference of the density was not significant (Mann–Whitney *U*-test, $P = 0.914$).

The females of *N. pulligera* deposited clusters of 4–39 egg capsules (Fig. 2A) and very quickly attached each capsule produced from the oviduct opening on to the glass wall by means of the female ridge. This action took only in 1–2 s, but there were intervals of *c.* 10 min between the ovipositions, presumably for preparation of the capsule walls in the oviduct. The female snail stopped moving its cephalic tentacles, mouth and foot in the last 1 min of the interval.

### *Predation behaviour*

Egg-eating behaviour by the species of *Vittina* (*V. variegata* and *V. waigiensis*) and *Clithon* (*C. corona*, *C. faba*, *C. retropictus* and *C. cyanostoma*) was frequently observed during the study, mainly at night, whereas none of *Neritina* and *Septaria* fed on the eggs.

Twenty-three predation attempts by 14 snails were observed in detail through the glass walls (Figs. 2A–F, 3). When a snail located an egg capsule, it first tried to make a hole in the reinforced lid of the capsule by repeated rasping with the radula (Fig. 2B). The movement of the mouth and radula was similar to that described for the algal feeding of *Theodoxus* and *Nerita* species by Whitaker (1951) and Fretter (1965). Breaking the capsule wall took 4.9 ± 4.8 min (range: 1.0–20.5 min) in 17 successful predations, while five capsules were abandoned after 5.5 ± 5.3 min (0.8–14.5 min) of fruitless radular rasping (Fig. 3). The larger capsules of *N. pulligera* took longer to open than the smaller ones of *N. iris* (Mann–Whitney *U*-test, $P = 0.018$).

Once the hole was made, the snail sucked the inside eggs (*c.* 150 μm) by using the buccal cavity as a pump (Fig. 2C–F). It sometimes enlarged the hole further by eating the broken edge of the lid with the radula, especially when the eggs could not easily be sucked out. The egg feeding was completed in 3.3 ± 3.0 min (0.9–11.2 min) after the piercing of the lid; none of the eggs inside the capsule remained in all the 17 cases of predation. The clustered egg capsules of *Neritina* were sometimes attacked sequentially by the same snail, which started to rasp the wall of the next target 2.7 ± 1.9 min (0.5–5.0 min, $n = 6$) after finishing the previous capsule. No clear difference in the predatory behaviour was observed among the species of *Clithon* and *Vittina*.

Almost all consumed egg capsules retained a part (up to two-thirds) of the lid, with a broken edge (Fig. 2G). They can therefore easily be distinguished from hatched capsules, where the lid is lifted off when the young escape (see Fretter, 1946: fig. 2A; Bandel, 1982: figs 63, 64). Although several capsules were so intensively grazed that the entire lid was missing, they can still be distinguished from hatched ones by a damaged base (Fig. 2A); the circular rim of the base remains intact for a prolonged period after the hatching in natural field conditions (Andrews, 1935; Adegoke *et al.*, 1969; Bandel, 1982; Tan & Lee, 2009).

Of the 1,485 capsules laid in the 4 days of observation, 333 were opened and eggs inside were consumed by *Clithon* and *Vittina* snails. Among the four substrate categories, the glass walls showed the highest predation rate (34.0%; Table 3). The capsules of *Neritina* species on the walls were much more frequently opened (109 out of 274 capsules) than those of *S. porcellana* (five out of 59; Fisher's exact test, $P < 0.00001$). The second highest rate was recorded on the snail shells, where 21.9% of the capsules were opened (all the 151 predated capsules belonged to *Neritina* species). A significant difference was found between the predation rates of *Neritina* capsules on the glass walls (39.8%) and the shells (22.2%; Fisher's exact test, $P < 0.00001$). The egg capsules on the other two substrate categories, laid mostly by the species of *Clithon* and *Vittina*, were less frequently opened, and the predation rates were 19.7% and 9.4% on the aquarium filter devices and the stones, respectively.

Newly laid white egg capsules were apparently more vulnerable to predation than older, tan-coloured capsules with a presumably harder lid. Nearly half (46.7%) of successful predation on *Neritina* capsules was made within 24 h of deposition on the glass walls.

## DISCUSSION

In the present study, we found the first evidence that the Indo-West Pacific, freshwater to brackish-water neritids of the genera *Clithon* and *Vittina* facultatively feed on the eggs of various confamilial snails, along with the usual algal food


**Figure 2. A.** *Clithon* snails attacking a cluster of egg capsules laid by a female individual of *Neritina iris*, seen through the glass wall of aquarium. The snail on the left, *C. corona*, is trying to make a hole in the reinforced lid of a capsule (arrow) by repeated rasping of the radula. The other snail on the right, *C. cyanostoma*, has finished most eggs inside of another capsule (arrowhead). Seven other egg capsules have already been opened and consumed. **B–F.** *Clithon cyanostoma* feeding on the eggs of *N. iris*, the same specimen in **A. B.** A small hole is made after a few minutes of radular rasping. Seen in the mouth are the outer lateral and marginal teeth of the radula. **C.** Further enlarging the hole. **D, E.** Sucking the inside eggs by using the buccal cavity as a pump. **F.** The radula appears in the mouth by the movement of the odontophore but it does not usually rasp the capsule once the hole is made large enough. After finishing all eggs, the snail headed to another in the same cluster of capsules. **G.** Egg capsules of *Neritina* species, deposited densely on the shell surface of a female *N. pulligera* in the 4-day laboratory observation (larger ones by *N. pulligera* and smaller by *N. iris*). The broken lid of the capsule records the predation of *Clithon* or *Vittina* species on the inside eggs. The predation rate on these capsules (24 out of 170 capsules) is much lower than on the glass wall, suggesting that the back of living snails may act as a safe 'nursery'.

**Table 3.** Substrate selection for oviposition by freshwater neritids and predation rates of egg capsules on different substrate types in the 4-day laboratory observation.

| Substrate | Surface area ($cm^2$) | *n* capsules | Density ($cm^{-2}$) | *n* predated | Predation rate | Id of capsules (in order of frequency) |
|---|---|---|---|---|---|---|
| Snail shells | 361 | 688 | 1.91 | 151 | 21.9% | *Neritina pulligera*, *Neritina iris*, *Clithon* spp.* |
| Glass walls | 6,643 | 335 | 0.05 | 114 | 34.0% | *N. pulligera*, *N. iris*, *Septaria porcellana*, *Clithon* spp.* |
| Aquarium devices | 768 | 238 | 0.31 | 47 | 19.7% | *Clithon* spp., *Vittina variegata*, *N. iris** |
| Stones | 2,212 | 224 | 0.10 | 21 | 9.4% | *Clithon* spp., *V. variegata* |

*Very rare, representing less than *c.* 1% of capsules laid on the respective substrate type.

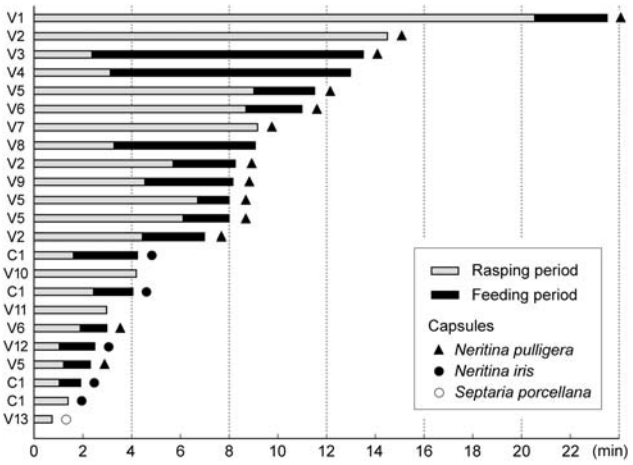


**Figure 3.** Durations of 23 attacks by one *Clithon corona* (C1) and 13 *Vittina variegata* (V1–V13) on the egg capsules of *Neritina pulligera*, *N. iris* and *Septaria porcellana* (four capsules of *Neritina* could not be identified to species). Six snails failed to open the capsule despite 0.75–14.5 min of radular rasping.

items. Neritid snails are considered to be generalist herbivores feeding on diatoms and green algae as well as cyanobacteria to a lesser extent (Russell, 1941; Hughes, 1971; Underwood, 1976; Ohara & Tomiyama, 2000; Kirkegaard, 2006). Exceptions are the species of *Smaragdia* Issel, 1869, which feed exclusively on sea-grass leaves (Kano, Chiba & Kase, 2002; Rueda & Salas, 2007). Although blackfly larvae have been recorded as a food item of the European-North African limnic genus *Theodoxus* (Scott & Kenny, 1998), animal diet (crushed isopod) gave a much lower growth rate for *T. fluviatilis* than diatoms and green algae (Skoog, 1978). Egg predation in general by freshwater gastropods has previously been reported only for the Ampullariidae, Lymnaeidae and Planorbidae (Turner *et al*., 2007).

The neritid predation on confamilial eggs cannot be a result of random grazing on substrates. The egg capsules were frequently attacked by all six species of *Clithon* and *Vittina* in the aquaria, but by none of the *Neritina* and *Septaria*. Each predatory snail spent a long time creating a hole in the stiff wall of the egg capsule by repeated rasping of the radula; once it broke through the wall, it sucked up all eggs inside by the pumping action of the buccal cavity (Fig. 2). The capsule wall itself may possibly constitute a valuable diet, but the eggs seem to be the more attractive food source (see Dumont *et al*., 2008) and a large part of the wall remains after predation in most instances. The observed successive predation attempts on multiple capsules also indicate that egg feeding is an established behaviour of *Clithon* and *Vittina*. In comparison with other neritids, however, these predatory snails do not show any obvious modification of their radular morphology to effectively break the capsule wall (see Baker, 1923).

The egg predation occurs not only in the aquarium, but certainly also in natural environments. As mentioned above, emptied egg capsules retain a part of the lid with a broken edge as a predation scar (Fig. 2). We have seen numerous neritid capsules with a comparable scar in a number of Indo-West Pacific streams, as well as in published photographs (e.g. Walker, 1998: fig. 1.74). These neritid snails are seemingly the most important predators on the robust, reinforced egg capsules of the Neritidae, although predation rates in streams may be lower than in the present laboratory observation where algal food supply was limited and snail density might be unnaturally high. A recent investigation revealed that freshwater slugs of the genus *Strubellia* (Heterobranchia: Acochlidea) also feed on neritid eggs in Melanesian streams (T. Neusser, personal communication). However, acochlid slugs are relatively rare with limited geographic distributions and they seem to be less significant predators than *Clithon* and *Vittina*, which are among the commonest animals in the coastal streams and estuaries of the tropical Indo-West Pacific, ranging from eastern Africa to French Polynesia (Starmühlner, 1976; Holthuis, 1995; Scott & Kenny, 1998; Kano, 2010). The only other case of predation on neritid eggs has been reported for high intertidal muricid snails in the Seychelles, Indian Ocean; they drill the reinforced capsules as well as mollusc and barnacle shells by means of an acid-secreting organ and the radula (Taylor, 1976).

Despite their presumed significance as contemporary predators, these neritid snails could not have been the initial selective force for the development of the reinforced egg capsules in Neritidae, because the acquisition of the crystal sac and reinforced capsule must have predated the occurrences of the two predatory genera. Our preliminary molecular phylogeny of Neritidae suggests somewhat ambiguously that *Clithon* and *Vittina* form a terminal clade in the family and that the egg-feeding behaviour may have evolved only once as a synapomorphy (Y. Kano, unpubl.). The crystal sac is clearly a synapomorphy of the Neritidae and shared by almost all members of the family (Andrews, 1935, 1937; Houston, 1990; Holthuis, 1995). Among neritids, only the sea-grass snails of the genus *Smaragdia* have secondarily lost the crystal sac and mineral reinforcement of the egg capsule (Bandel, 1982; D'Asaro, 1986; Kano & Kase, 2002), presumably in the absence of suitable particles in the diet and lack of requirement for such protection.

The origin of the reinforced capsule may even be earlier than the divergence of the family and perhaps dates back to the middle Mesozoic. A probably homologous, albeit less sophisticated and apparently primitive reinforcement of egg capsules with sand grains and diatom skeletons, has been described for another neritimorph family, Neritiliidae (Andrews, 1935; Kano, Sasaki & Ishikawa, 2001; Kano & Kase, 2002, 2003). Although all species of Phenacolepadidae, the sister taxon of Neritidae, produce smooth egg capsules without the reinforcing particles (e.g. Warén & Bouchet, 2001: fig. 32e), this could also be due to a secondary loss of the reinforcement in the cryptic habitats of these limpets (Kano *et al*., 2002). Predators on early neritimorph egg capsules are unknown, but various carnivorous and omnivorous animals may have driven the evolution of the reinforcement by mineral particles. Gastropods, chitons, crabs, isopods, polychaetes and sea urchins are known to feed on snail eggs in conchiolin capsules (Pechenik, 1986; Rawlings, 1990; Dumont *et al*., 2008). Zatoń, Niedźwiedzki & Pieńkowski (2009) recently discovered presumed gastropod egg capsules from oligohaline waters of Early Jurassic age (198–200 million years ago), which were similar to those of the Recent Neritidae and Phenacolepadidae. The presence or absence of the reinforcement particles was not clearly determined in the fossil capsules.

The predation rate on the capsules was not equal among the prey species. The eggs of *Clithon* and *Vittina* were eaten infrequently by congeneric snails. These snails deposited very small egg capsules (Fig. 1) individually in concavities where it was difficult for the radula of adult snails to reach. Larger capsules produced by the limpet-like snails of the genus *Septaria* were also resistant to the attack of the confamilial snails, although they were all deposited in clusters on the smooth, unprotected surfaces of aquarium glass walls. The lid of the capsule of *Septaria* may possibly be thick enough to prevent breakage by the radular rasping of the predatory snails, especially if hardened over time. Even in the smaller *Neritina* capsules, size difference seems to affect the time required to open the capsule (Fig. 3). This result is comparable with the case of the muricid

gastropod *Nucella emarginata* where thicker capsules are more resistant to predation by isopods (Rawlings, 1990, 1994). We have observed similar substrate preferences in the field populations of *Clithon*, *Vittina* and *Septaria*; the snails of the former two groups tend to lay their capsules in the concavities and depressions of uneven surfaces of stones and driftwood, and the latter limpets select smooth surfaces of large stones and rocks for oviposition.

*Neritina* species have seemingly evolved a more intriguing strategy to protect their egg capsules from the attack of confamilial snails and perhaps also from other predators. The present laboratory observation showed that the distribution of the egg capsules of *Neritina* is strongly biased to the shells of living snails, especially to those of congeneric individuals (Fig. 2G). This capsule attachment behaviour in *Neritina* has attracted the attention of many malacologists (e.g. Andrews, 1935; Adegoke *et al*., 1969; Vermeij, 1969; Maciolek, 1978; Brown, 1980), who interpreted its adaptive significance differently. Vermeij (1969) speculated that the egg capsules impart a granular texture to the external surface of the shell and serve to scatter the shearing force of the current. Hence, they might possibly be beneficial in minimizing effects of strong current in which many species of *Neritina* live (e.g. Starmühlner, 1976; Kano, 2009, 2010). On the other hand, Maciolek (1978) suggested that the snail shells simply provide convenient, suitable hard substrates for capsule attachment where such substrates may be scarce in the habitat.

The attached mode of life on other living shells is a known way of avoiding predators for adult and juvenile gastropods (Vermeij, 1993: 147; Bromley & Heinberg, 2006; Kano, 2009), although protection of eggs by attaching them to other shells has not previously been documented in free-living gastropods. In our laboratory observation, the predation rate of *Clithon* and *Vittina* snails was indeed significantly lower – nearly half – for *Neritina* eggs on the shell than for those on the glass walls. This strategy is effective probably because the predatory snails have more difficulties in opening the egg capsules than the maternal snails do in depositing them on moving shells. Female *Neritina* lay a capsule in only a few seconds, while the predatory snails take at least several minutes to open the capsule lid and to consume the eggs inside (Fig. 3). A similarly intriguing example of egg protection has been reported for a subtidal snail of the genus *Oenopota* (Conidae), where the egg capsule is attached to the body of an ovigerous shrimp, just under shrimp's own eggs (Miglavs, Sneli & Warén, 1993).

Another possible advantage of the egg capsules on the living shells may be protection from desiccation stress. All species of *Neritina* seem to be amphidromous and adult snails inhabit coastal streams in tropical to subtropical regions (Schneider & Lyons, 1993; Kano, 2006, 2009, 2010). Many such streams exhibit rapid changes in flow. During the rainy season they become raging torrents, while in the dry season the water level drops significantly and they may be reduced to a series of stagnant pools (McDowall, 2007). Egg capsules deposited on riverbed rocks and stones may be exposed to the air in the dry season, whereas those on mobile snails should stay wet as long as water exists nearby. Similar protection from predation and desiccation has been suggested for the eggs of the cichlid fish genus *Aequidens* in the tropical streams of South America. Those cichlid eggs are deposited on a mobile leaf, moved and cared for by the parents (Keenleyside & Bietz, 1981).

The deposition of egg capsules on living shells has also been found in a brackish-water neritiliid species, *Neritilia mimotoi*. Their capsules are often laid on the shells of other conspecific individuals, most frequently on the apertural callus on the inner lip of males, as well as on submerged stones and leaves (Kano *et al*., 2001: fig. 8C, D). The callus is the area covered with the mantle tissue over the operculum when the snail creeps. Their capsules might therefore possibly be more secure than those of *Neritina* on the back and spire of the shell. However, neritiliid capsules are minute, containing only a single embryo, and apparently more fragile than the neritid capsules, regardless of their mineral reinforcement (see Andrews, 1935; Kano & Kase, 2003). Predation on neritiliid eggs has never been investigated, and the advantage of the capsule on the callus remains an open question. The very remote phylogenetic relationship of *Neritina* and *Neritilia* (Kano & Kase, 2002; Kano *et al*., 2002) clearly indicates that the two groups have independently evolved the egg-attaching behaviour.

Although *Clithon* and *Vittina* snails seem to be primarily herbivorous, adding protein-rich eggs and embryos to their diet likely enhances their growth rate and reproductive performance (see Dumont *et al*., 2008 and references therein). Because of broadly overlapping use of algal diet and microhabitat, exploitative competition is apparently a major mechanism underlying negative interspecific interactions among limnic neritid species (Starmühlner, 1976; Scott & Kenny, 1998; Kano, 2010). Yet, the egg predation of *Clithon* and *Vittina* may play another important role in the community structure of limnic neritids, as in the case of intraguild egg predation among pulmonate pond snails (Turner *et al*., 2007).

In conclusion, the present study reveals a previously unknown type of predation that exists only in freshwater and brackish biota where predation pressure is generally more relaxed than in the marine environment (Vermeij, 1978, 1993; McDowall, 2007), and that various strategies seem to have evolved to reduce the predation risk in different lineages of the prey snails.

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