

## SHORT COMMUNICATION

While they were sleeping: dormant egg predation by *Triops*ALINE WATERKEYN<sup>1,2\*</sup>, JOOST VANOVERBEKE<sup>1</sup>, NISSE VAN POTTENBERGH<sup>1</sup> AND LUC BRENDONCK<sup>1</sup><sup>1</sup>LABORATORY OF AQUATIC ECOLOGY AND EVOLUTIONARY BIOLOGY, KATHOLIEKE UNIVERSITEIT LEUVEN, CHARLES DEBERIOTSTRAAT 32, 3000 LEUVEN, BELGIUM AND <sup>2</sup>RESEARCH CENTER FOR MEDITERRANEAN WETLANDS TOUR DU VALAT, LE SAMBUC, 13200 ARLES, FRANCE

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Dormant egg banks provide a means for many species to survive adverse conditions. This study describes a short-term experiment evaluating predation by the notostracan *Triops cancriformis* on dormant eggs buried in sediment, i.e. *Daphnia* ephippia and *Triops* dormant eggs (cannibalism). Significant predation was recorded on both dormant stages, with no apparent selection. These results indicate that *Triops* could eradicate the dormant eggs in surface sediments and thereby impact the benthic-pelagic coupling in aquatic systems.

KEYWORDS: predation; Notostraca; *Daphnia*; dormant egg bank; cannibalism

Dormant egg banks are crucial for the persistence of many invertebrates in temporally variable aquatic environments (Hairston, 1996). The production of dormant stages allows them to temporarily escape unfavorable physical (such as drought or extreme temperatures) or biological (such as deteriorating food conditions or predation) conditions. Dormant stages are stored in the sediment where they can remain viable for decades or longer (Hairston *et al.*, 1995). Once suitable conditions are restored, a variable proportion of the egg bank will hatch to repopulate the water column. Because of this benthic-pelagic coupling, dormant egg banks form an essential component of many invertebrate (mainly zooplankton) communities (Brendonck and De Meester, 2003; Gyllström and Hansson, 2004).

Predation in aquatic ecosystems has been intensively studied; however, predation on dormant eggs is often overlooked (Cáceres and Hairston, 1998). Moreover, from the few studies that have investigated egg predation, even fewer have actually demonstrated it. Cáceres

and Hairston (Cáceres and Hairston, 1998) could not prove that turbellarians, gastropods, chironomids or zebra mussels were capable of ingesting *Daphnia* ephippia. Neither Dumont *et al.* (Dumont *et al.*, 2002) nor De Roeck *et al.* (De Roeck *et al.*, 2005) found evidence that turbellarians were capable of consuming anostracan eggs and suggested that the thick shell and specific shape of dormant eggs act as efficient anti-predation mechanisms. Nevertheless, Viitasalo and Viitasalo (Viitasalo and Viitasalo, 2004) and Viitasalo (Viitasalo, 2007) demonstrated that amphipods and mysids could predate on *Bosmina* ephippia in marine environments. Also, in freshwater environments, amphipods were shown to consume copepod eggs (Parker *et al.*, 1996) and *Daphnia* ephippia (Cáceres and Hairston, 1998).

Recently, the importance of benthic omnivorous notostracans (tadpole shrimp) as a keystone group in temporary waters has been suggested (Yee *et al.*, 2005; Waterkeyn *et al.*, 2010, 2011) and the number of studies documenting their status as significant predators is

growing. Notostracans are not predatory in their early life stages (feeding on detritus, bacteria, phytoplankton, filamentous algae and plant material), but their diet becomes more animal based (cladocerans, copepods, anostracans, dipterans, mayflies, annelids and amphibian eggs/larvae) as they reach a carapace length of 10 mm (Pont and Vaquer 1986; Boix *et al.*, 2006). There are also some anecdotal observations that notostracans may be able to predate on the dormant component of prey populations (Waterkeyn *et al.*, 2011).

Since notostracans can reach very high densities (Boix *et al.*, 2002), dormant egg bank predation by these organisms could strongly influence benthic-pelagic coupling. In this study, we investigate whether *Triops cancriformis* is capable of consuming *Daphnia magna* ephippia which are buried in sediment. Additionally, since cannibalism seems to be an important factor regulating notostracan populations (Boix *et al.*, 2006), we also considered predation on their own eggs.

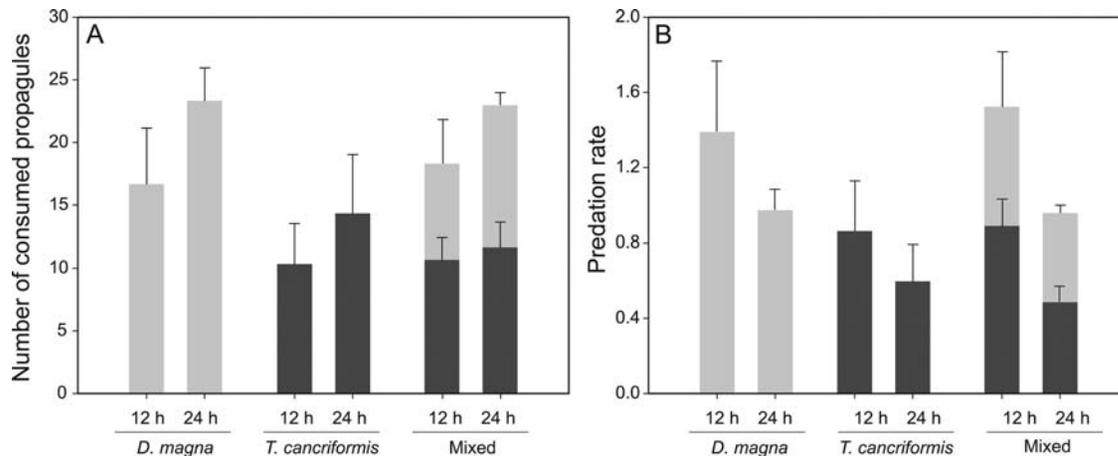
Three types of artificially assembled dormant egg banks, containing either *D. magna* ephippia, *T. cancriformis* dormant eggs or a combination of both, were exposed to *T. cancriformis* during 12 or 24 h. The organisms and sediment originated from Mediterranean temporary ponds in the Camargue (Tour du Valat) where *T. cancriformis* often co-occurs with one of its major prey items, *D. magna*, the dominant zooplankton species. Thirty *D. magna* ephippia (1.1–1.6 mm; Boersma *et al.*, 2000), 30 *T. cancriformis* dormant eggs (350–400  $\mu\text{m}$ ; Defaye *et al.*, 1998) or a combination of both (15 of each) were buried at different depths in a 1.5-cm layer of fine sediment (sieved with a 120- $\mu\text{m}$  mesh to remove dormant stages) on the bottom of 1-L aquaria (surface 120  $\text{cm}^2$ ). This corresponds with egg bank densities of 2500 per  $\text{m}^2$ , which fits within the range of natural densities (Brendonck and De Meester, 2003). Afterwards, the sediment was carefully inundated with EPA medium with  $1 \text{ g L}^{-1}$  salt (optimal condition for Camargue *T. cancriformis*) to avoid resuspension. Afterwards, one starved (for 24 h) adult female *T. cancriformis* (mean  $\pm$  SD carapax length:  $1.5 \pm 0.25 \text{ cm}$ ) without eggs in the brood pouches was added for a period of 12 or 24 h. Each treatment was replicated three times. Additionally, three control aquaria (without *Triops*) per egg bank type were set up during 24 h. All aquaria were kept at  $20^\circ\text{C}$  and a 16 h light/8 h dark regime. At the end of the exposure time, *Triops* was removed, the content of the aquaria was filtered through a 120- $\mu\text{m}$  sieve and the residue was screened for remaining dormant stages under a stereo microscope. Control treatments were used to calculate a correction factor for egg recovery by assessing the percentages of eggs and ephippia that could not be recovered in the absence of *Triops*; 10% for *D. magna*,

3% for *T. cancriformis*. Hatching is assumed not to have occurred during the experiment. Previous experiments with Camargue sediment showed that almost no zooplankters hatched within 1 day, particularly neither *T. cancriformis* nor *D. magna*.

The number of propagules consumed was calculated as the number of presented propagules minus the number of remaining ones (after correction). Predation rates (number of propagules consumed per hour) were calculated for the dormant stages of both species. A two-way ANOVA was used to determine whether total predation rates (sum of both dormant stages in mixed treatments) differed significantly between egg bank types and exposure times (STATISTICA 9.0). Within the mixed egg bank treatment, a paired *t*-test was used to detect potential preferences for propagule type for both exposure times.

*Triops cancriformis* consumed both *D. magna* ephippia and conspecific dormant eggs. Predation rates differed significantly between the different dormant egg bank types ( $F = 8.25$ ;  $P = 0.004$ ) and predation exposure times ( $F = 13.67$ ;  $P = 0.002$ ), while the interaction between both factors had no significant effect ( $P > 0.05$ ). *Triops cancriformis* consumed an average of ( $\pm$  SD)  $23.3 (\pm 2.6)$  *D. magna* ephippia over 24 h, with the predation rate being highest during the first 12 h ( $1.4 \pm 0.4 \text{ h}^{-1}$ ) (Fig. 1). Conspecific dormant eggs were consumed at a significantly slower rate ( $0.9 \pm 0.3 \text{ h}^{-1}$  during the first 12 h) (Tukey HSD:  $P = 0.015$ ), with a total of  $14.3 (\pm 4.7)$  dormant eggs consumed in 24 h. When mixed species egg banks were offered, total predation rates ( $1.5 \pm 0.3 \text{ h}^{-1}$  during the first 12 h) were comparable with those on *D. magna* egg banks (Tukey HSD:  $P = 0.894$ ), but were significantly higher than when only conspecific eggs were present in the sediment (Tukey HSD:  $P = 0.006$ ). *Triops cancriformis* showed no preference for either propagule type in mixed egg banks ( $F = 1.52$ ;  $P = 0.271$ ) over both exposure times (interaction:  $P > 0.05$ ).

Predation rates during the last 12 h were lower than during the first 12 h of the experiment, probably as a result of a dilution effect (i.e. fewer propagules remaining). When calculating the proportion of consumed propagules over the first 12 h period (number of consumed propagules/number of inoculated propagules) and the second 12 h period (number of consumed propagules/mean number of remaining propagules after 12 h) we found that they are comparable during any 12 h period and dormant egg bank type, with the proportion of consumed propagules being  $\sim 52\%$  per 12 h period for *D. magna* (with the exception of the first 12 h period in the mixed egg bank treatment: 26%) and 31% per 12 h period for *T. cancriformis*. We can thus conclude that the



**Fig. 1.** Consumption of *D. magna* ephippia (light grey) and *T. cancriformis* dormant eggs (dark grey) in the three egg banks types for 12 and 24 h exposure times. (A) Mean and standard deviation of the total number of consumed propagules and (B) mean and standard deviation of the predation rate (number of consumed propagules h<sup>-1</sup>).



**Fig. 2.** Pictures of crushed and chewed *D. magna* ephippia by *T. cancriformis*.

predation rates are density dependent. For *Daphnia* ephippia, predation rates ranged between 33 propagules per day for initial densities of 2500 propagules per square metre sediment (extrapolation based on the first 12 h period) and 13 propagules per day for densities of 1110 propagules per square metre (based on the second 12 h period). For *Triops* resting eggs, predation rates ranged between 21 and 10 propagules per day for initial densities between 2500 (first 12 h) and 1640 propagules per square metre (second 12 h), respectively.

Although measured predation rates were not significantly correlated with carapace length of *T. cancriformis* (for *Daphnia* ephippia:  $r = 0.220$ ;  $P = 0.492$ ; for *Triops* eggs:  $r = 0.452$ ;  $P = 0.140$ ), we observed that the two smallest individuals had some trouble ingesting *D. magna* ephippia completely and only crushed and chewed off the edges to consume the eggs inside (Fig. 2). In aquaria with larger *T. cancriformis* no remains of ephippia were found.

This study shows that adult *T. cancriformis* were able to consume relatively large numbers of both *Daphnia*

ephippia and conspecific dormant eggs buried in the sediment. Several other studies investigating dormant egg predation found no evidence of ingestion by turbellarians, gastropods, chironomids or zebra mussels (Cáceres and Hairston, 1998; Dumont *et al.*, 2002; De Roeck *et al.*, 2005). In addition to a limited number of studies where egg predation was confirmed (mysids and amphipods), this study clearly demonstrates significant predation by notostracans on dormant eggs. Moreover, notostracans appear to be much more voracious predators of cladoceran ephippia than other predators studied (Table I). The predation rate of *T. cancriformis* on *Daphnia* ephippia buried in sediment was twice as high compared with floating ephippia presented at much higher densities, which may be explained by its mainly benthic life style (Table I).

Khalaf (Khalaf, 1978) already found pieces of *Triops* dormant eggs during gut analyses, but this is the first direct observation of cannibalistic egg predation in notostracans. Although no selectivity was apparent for one of both dormant propagule types in mixed egg

Table I: Predation rates of invertebrate predators on cladoceran ephippia with information on the methods used (densities and sediment depths)

Predator	Prey ephippia	Predation rate (day <sup>-1</sup> )	Egg bank size (m <sup>-2</sup> )	Sediment depth (cm)	Reference
<i>Mysis mixta</i> , <i>M. relicta</i>	<i>Bosmina longispina</i>	7.2	16 000	2.0	Viitasalo and Viitasalo (Viitasalo and Viitasalo, 2004)
<i>Gammarus fasciatus</i>	<i>Daphnia galeata/pulicaria</i>	0.8	1020	1.0	Cáceres and Hairston (Cáceres and Hairston, 1998)
<i>Monoporeia affinis</i>	<i>Bosmina longispina</i>	2.3	7800	1.0	Viitasalo (Viitasalo, 2007)
<i>Triops cancriformis</i>	<i>Daphnia magna</i>	33	2500	1.5	This study
<i>Triops cancriformis</i>	<i>Daphnia magna</i>	14	6700	Floating	Waterkeyn (Waterkeyn <i>et al.</i> , 2011)

banks, significantly more *D. magna* ephippia were eaten than *Triops* dormant eggs when offered in single species egg banks. This might suggest that the smaller round *Triops* dormant eggs are more difficult to find than the larger *D. magna* ephippia. The smooth round *Triops* eggs may also be more difficult to handle and to chew on. It is also possible satiation played a role. If *T. cancriformis* do not ingest the entire ephippia, but rather chew them into small pieces to access the eggs inside (Fig. 2), they might get less food from such sloppy feeding than from the “cleaner” ingestion of the same number of their own eggs.

Besides *Daphnia* ephippia and conspecific eggs, *T. cancriformis* is also able to ingest the round strongly ridged dormant eggs of *Chirocephalus diaphanus* (250–430 μm) (Van Pottelbergh, pers. obs.). Although Dumont *et al.* (Dumont *et al.*, 2002) suggested that their ornamentation and thick shell offered protection against predation, this does not seem to apply for predation by *Triops*. Considering the known size range of dormant stages ingested by *Triops* (250 μm–1.6 mm) and considering their different shapes and intensity of ornamentation, we can assume that *T. cancriformis* is able to predate on virtually any type of invertebrate dormant stage. Zooplankton egg bank sizes often range between 10<sup>3</sup> and 10<sup>6</sup> eggs per square metre (Hairston, 1996; Brendonck and De Meester, 2003). Considering the high predation rates under experimental conditions and the sometimes high densities of *T. cancriformis* in temporary waterbodies, we can expect that a large adult *Triops* population could rapidly eradicate most dormant eggs from at least the upper 1.5-cm layer of the sediment. When extrapolating our data we could, for example, estimate that, at maximum densities of 300 individuals per square metre (Pont and Vaquer, 1986) and maximum measured predation rates (33 per day), it would take between 1 and 100 days to exhaust the upper layer of the egg bank, depending on its size (10<sup>3</sup>–10<sup>6</sup> eggs per square metre).

There are, however, a few factors that might have influenced our measured predation rates. As the

sediment was filtered through a 120-μm mesh, dormant eggs of copepods or rotifers might still have been present in the artificial dormant egg banks. It is therefore possible that these dormant stages (or their hatchlings) were also used by *Triops* as a supplementary food source, which may have influenced the predation rates measured, but in a similar way in all treatments. The recorded dilution effect likely also affected the accuracy of the estimated predation rates. Also, since the diet of notostracans includes much more than only dormant eggs, actual predation rate in the field may be lower. Our data nevertheless suggest that consumption of ephippia may be partly independent of the presence of other food items, as we did not see a difference in the proportion of consumed ephippia (52% of available ephippia over a 12 h period) in the presence or absence of *T. cancriformis* eggs. Further experiments are nonetheless needed to quantify predation pressure on dormant egg banks when both the active and dormant components of the prey population are present. Additionally, it would be interesting to investigate the effect of *Triops* on the hatching patterns of dormant eggs, since high predation pressure might drive them to hatch earlier (before *Triops* becomes predatory) or in other proportions.

To conclude, we argue that *Triops* has the potential to exert a strong predation pressure on the upper layers of the dormant egg bank (i.e. “active” part of the egg bank). This suggests that Notostracans can not only be considered top predators of the active (Waterkeyn *et al.*, 2011) but also of the dormant community component in temporary waters. Considering their often high population densities, egg bank predation by tadpole shrimp may reduce the efficiency of the benthic-pelagic coupling, thereby affecting ecological and evolutionary dynamics of species with dormant egg banks (Brendonck and De Meester, 2003). Particularly in temporally variable environments, predation cost may reduce the resilience and jeopardize population persistence by reducing the buffering capacity of the egg bank.

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