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Can large branchiopods shape microcrustacean communities in Mediterranean temporary wetlands?

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Abstract. It was recently suggested that large branchiopods may play a keystone role in temporary aquatic habitats. Using a microcosm experiment manipulating microcrustacean communities of Mediterranean temporary wetlands (Camargue, Southern France), we tested the following hypotheses: (i) large branchiopods (the notostracan *Triops cancriformis* and the anostracan *Chirocephalus diaphanus*) can limit microcrustacean densities through both competition and predation; (ii) notostracans create high suspended-matter concentrations through bioturbation, which can negatively impact microcrustaceans; and (iii) the outcome of these biotic interactions is more detrimental at high salinities. We found a strong predatory impact of *T. cancriformis* on active microcrustacean populations, but also on dormant populations through the consumption of resting eggs. They also preyed on anostracans and their conspecifics and can indirectly have a negative effect on microcrustaceans through bioturbation, probably by impeding filtering capacities. The presence of *C. diaphanus* also limited most microcrustacean groups, probably through competition and/or predation. We did not find a significant effect of the tested salinity range ($0.5–2.5\,g\,L^{-1}$) on the biotic interactions. Our study shows that large branchiopods can shape microcrustacean communities under a wide range of environmental conditions and confirms their potential for a keystone role, especially one of notostracans as top predators.

Additional keywords: Anostraca, bioturbation, interference competition, predation, *Triops*.

Introduction

Predation and competition are recognised as strong community-structuring forces (Sih *et al.* 1985), often mediated by keystone species. The presence or absence of such keystone species may therefore severely alter the characteristics of a community (Power *et al.* 1996). In permanent aquatic environments, this regulatory role is often attributed to fish (Zaret 1980). As a result of periodic drying, fish are usually excluded from temporary aquatic habitats. This does not mean, however, that these habitats are 'enemy free' (Brendonck *et al.* 2002). On the contrary, they often exhibit high predation pressures (Boix *et al.* 2006). A whole range of predators have been suggested to play an important role in temporary freshwater systems, such as turbellarians, notonectids, diving beetles, dragonfly larvae, *Chaoborus* spp. larvae, notostracans, and amphibian larvae (e.g. Brendonck *et al.* 2002; De Roeck *et al.* 2005; Boix *et al.* 2006). By contrast, competition has often been underestimated in temporary aquatic systems, mostly because frequent disturbance as a result of drying was thought to truncate competitive interactions. However, the limited research on competitive interactions in temporary waters has confirmed the importance of competition in structuring of zooplankton communities (Bengtsson 1993; Jocqué *et al.* 2010).

Large branchiopods have long been recognised as being a distinctive group of temporary waters. Their role as a keystone group, shaping invertebrate communities in temporary wetlands, has only been suggested more recently (Yee *et al.* 2005; Jocqué *et al.* 2010; Waterkeyn *et al.* 2010). The predatory role of notostracans (tadpole shrimps) has long been underestimated, mostly because of controversy about their diet (Dumont and Negrea 2002). Additionally, their role in modifying wetland environments by disturbing sediments leading to increased turbidity of the water column has largely been neglected. Similarly, the influence of anostracans (fairy shrimps) on zooplankton in temporary wetlands, through competition (both resource and interference competition) and predation, remains largely unknown (Sánchez and Angeler 2007; Jocqué *et al.* 2010). The high degree of environmental variability among wetlands often compromises the detection of such structuring roles (Sánchez and Angeler 2007). Therefore, experimental studies based on manipulative designs are more appropriate to test for the keystone role of large

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branchiopods in temporary wetland food webs (Sánchez and Angeler 2007).

Water quality plays an important role shaping communities in temporary wetlands, directly by selecting for tolerant species, and indirectly by influencing biotic interactions. Salinity is one of the factors that play an important role in determining diversity and community structure in wetlands (Brendonck and Williams 2000; Brock *et al.* 2005; Brucet *et al.* 2010), particularly in the Mediterranean coastal temporary wetlands of the Camargue (Waterkeyn *et al.* 2008, 2009). Coping with osmotic stress can trade off with other traits, such as the performance of organisms in their interactions with other species, and can therefore change the outcome of competition (e.g. Sarma *et al.* 2002) or predation rates (e.g. Chandramohan *et al.* 2008). However, the impact of salinity on the structuring role of large branchiopods on the microcrustacean communities of Mediterranean temporary wetlands remains unknown. Using a microcosm experiment, we tested the following hypotheses: (1) large branchiopods can effectively limit microcrustacean densities through both competition and predation; (2) notostracans create high suspended matter concentrations through bioturbation, which can in turn negatively impact microcrustaceans; and (3) the outcome of these biotic interactions is more detrimental at high salinities.

Materials and methods

Biotic interactions across a salinity gradient

This experiment was carried out in December 2008 in the Research Centre of the Tour du Valat (43°30′N, 4°40′E). At three different salinities ($0.5\,\mathrm{g\,L^{-1}}$, $1.0\,\mathrm{g\,L^{-1}}$, and $2.5\,\mathrm{g\,L^{-1}}$), microcrustacean communities were exposed in 10-L aquaria to six different treatments: (a) control, (b) *Chirocephalus diaphanus*, (c) *Triops cancriformis*, (d) sediment, (e) *T. cancriformis* + sediment, and (f) *T. cancriformis* + *C. diaphanus* + sediment. Each treatment was replicated four times for a total of 72 aquaria. The experiment lasted for 11 days.

The aquaria for treatments (d), (e), and (f) were inoculated with a 0.5-cm layer of sterilised sediment collected from a freshwater temporary wetland on the estate of Tour du Valat. The sediment was put in a drying oven at 200°C for three days to kill any viable resting stages. The organisms used in this experiment naturally co-occur in Camargue temporary wetlands (Waterkeyn *et al.* 2008, 2009). Both the experimental microcrustacean community and the branchiopods were collected from two sources to reach the required densities: (i) from samples taken in three temporary wetlands on the estate of Tour du Valat that were inundated for four weeks, and (ii) from outdoor mesocosm samples in which pooled sediment from 15 temporary wetlands was hatched for six weeks. Organisms from both sources were thoroughly mixed. A pooled experimental microcrustacean community was created in three 400-L tanks with an average (±s.d.) 50 (±7) individuals $\mathrm{L^{-1}}$ (30 ± 5 cladocerans, largely dominated by *Daphnia magna*, 8 ± 2 calanoid and 2 ± 1 cyclopoid copepods, and 10 ± 3 ostracods). The three salinities were created gradually over 12 h using sea-salt from local salt works in the Camargue (Salin de Giraud).

All aquaria were inoculated with 12 L of the experimental microcrustacean community, from which 2 L was taken out the next day, before starting the large branchiopod treatments. This 2-L sample was filtered through a 64-μm net and preserved in 70% ethanol to serve as reference for the starting condition, thus accounting for variation in microcrustacean densities during the inoculation process. Afterwards, for each of the large branchiopod treatments, ten adult *T. cancriformis* (average ± s.d. size: 23 ± 9 mm from head to base of furca) and/or ten adult *C. diaphanus* (average ± s.d. size: 32 ± 8 mm from head to base of furca) were added (one individual per L). The aquaria were given an inoculum of phytoplankton from the outdoor mesocosms (from which the experimental microcrustacean community was taken), combined with a daily addition of 2 mL of *Scenedesmus obliquus* (2×10^8 cells $\mathrm{mL^{-1}}$) and 2 mL of yeast (1 g of dried baker's yeast in 100 mL of distilled water) during the first four days. Afterwards, feeding was stopped to ensure limiting food conditions enabling the detection of competition. During the experiment, temperature was kept between 13°C and 16°C and lights were turned on between 0830 hours and 1830 hours. The chosen treatments reflect natural conditions. The salinities reflect the natural gradient in which microcrustaceans co-occur with large branchiopods (the latter being less tolerant), whereas their densities reflect rather high, but natural densities found in some Camargue ponds in the field (A. Waterkeyn, unpubl. data; Pont and Vaquer 1986).

The aquaria were sampled 1, 4, 8, and 11 days after the start of the exposure to large branchiopods, except for the *T. cancriformis* and sediment treatments, which were stopped after 8 days because by that time most of the microcrustaceans were already eaten in the *T. cancriformis* treatments. Salinity ($\mathrm{g\,L^{-1}}$) was measured with a WTW conductivity meter 330i (Wissenschaftlich-Technische Werkstätten, Welheim, Germany). On the first three sampling events, a 1-L microcrustacean sample was taken after mixing the water to homogenize the community. The sample was filtered through a 64-μm net and preserved in 70% ethanol. During the last sampling event, the whole content of the aquaria was filtered through a 64-μm net. The filtered water was used to measure total suspended solids (TSS in $\mathrm{mg\,L^{-1}}$) using a portable Hach spectrophotometer DR2800 (Hach Co., Loveland, CO, USA) and chlorophyll *a* concentration using a methanol extraction method (Talling and Driver 1963). All microcrustacean samples were processed under a stereo microscope to count and identify all individuals according to Alonso (1996). Cladocerans were identified to species and copepods to order. Ostracoda were not identified to a lower taxonomic level.

We tested the effect of treatment and salinity on the density of cladocerans, ostracods and cyclopoid and calanoid copepods using a repeated-measures two-way factorial ANCOVA, correcting for differences in inoculation densities at the start of the experiment using the starting condition as a covariate (STATISTICA 7.0, StatSoft Inc., Tulsa, OK, USA). The data satisfied model assumptions. When the interaction term between salinity and treatment was not significant, main effects ANCOVA was performed. Afterwards, pairwise comparisons between treatments were conducted using *post-hoc* Tukey's HSD tests. The control treatment (a) was compared with treatment (b) to test whether Anostraca had a negative effect on microcrustacean densities through competition and/or predation, with treatment (c) to study the negative impact of predation by Notostraca, and with treatment (d) to study a potential impact

Table 1. Results of Tukey's HSD *post-hoc* tests for relevant pairwise treatment comparisons

Significant *P*-values are indicated; n.s. indicates when *P*-values were not significant. All significant effects were negative

Pairwise comparisons	Effect tested	Time	Cladocera	Ostracoda	Calanoida	Cyclopoida
(a) *v.* (b)	Competition + predation by *C. diaphanus*	Day 1	n.s.	n.s.	n.s.	n.s.
		Day 4	n.s.	n.s.	n.s.	n.s.
		Day 8	n.s.	n.s.	n.s.	<0.001
		Day 11	n.s.	0.048	n.s.	0.003
(a) *v.* (c)	Predation by *T. cancriformis*	Day 1	<0.001	<0.001	n.s.	0.001
		Day 4	<0.001	<0.001	0.007	<0.001
		Day 8	<0.001	<0.001	0.004	<0.001
(a) *v.* (d)	Sediment presence	Day 1	n.s.	n.s.	n.s.	n.s.
		Day 4	n.s.	<0.001	n.s.	0.002
		Day 8	n.s.	<0.001	n.s.	<0.001
(c) *v.* (e)	Bioturbation by *T. cancriformis*	Day 1	n.s.	n.s.	0.050	n.s.
		Day 4	n.s.	n.s.	n.s.	n.s.
		Day 8	n.s.	n.s.	n.s.	n.s.
(d) *v.* (f)	Predation + bioturbation + competition by *C. diaphanus* and *T. cancriformis*	Day 1	<0.001	<0.001	0.002	<0.001
		Day 4	<0.001	n.s.	<0.001	n.s.
		Day 8	<0.001	0.013	<0.001	n.s.

of presence of sediment. Treatment (c) was compared with treatment (e) to assess the additional negative impact of bioturbation by Notostraca on top of predation. Also, the sediment control treatment (d) was compared with combination treatment (f) to study the negative impact of all measured biotic interactions together.

Predation by Triops cancriformis

For six hours, we monitored the predation activity of *T. cancriformis* on *D. magna* adults and ephippia. Twelve 200-mL jars were filled with 64-μm-filtered pool water at room temperature (∼20°C). One *T. cancriformis* (average ± s.d. size: 24 ± 5 mm from head to base of furca) was added to each jar after being starved for 24 h. Four of the jars were inoculated with 20 adult *D. magna*, four others with 20 *D. magna* resting eggs and the last four were used as controls for *D. magna* survival in the absence of predators. Every hour, the remaining adults and resting eggs were counted, and eaten ones were replaced. Mean predation rates were calculated and compared with predation rates of other temporary wetland predators from the literature.

Bioturbation by Triops cancriformis

Twelve 10-L aquaria were inoculated with a 1-cm layer of sterilized sediment collected from a freshwater temporary wetland on the estate of Tour du Valat and subsequently filled with 10 L of non-chlorinated tap water at room temperature (∼20°C). The next day 0, 1, 2, or 4 *T. cancriformis* (average ± s.d. size: 29 ± 6 mm from head to base of furca) were released in the aquaria. The four treatments were replicated three times. After 48 h, total suspended solids were measured in each aquarium using a Hach spectrophotometer DR2800 (Hach Co., Loveland, CO, USA). The difference in total suspended solids of the water in the aquaria with different densities of *T. cancriformis* was tested using one-way ANOVA after checking that the data satisfied the assumptions (STATISTICA 7.0, StatSoft Inc., Tulsa, OK, USA).

Results

Biotic interactions across a salinity gradient

The results show, over all salinities, a strong effect of predation by *T. cancriformis*, with a significant negative effect on cladoceran, cyclopoid copepod, and ostracod densities after 24 h (Table 1). By that time, cladoceran and cyclopoid copepod populations were reduced to almost zero (average ± s.d. initial abundances of both groups: 300 ± 54 and 24 ± 13 per aquarium, respectively). For the ostracods, 90% of the initial abundance was consumed, whereas only 5% of the calanoid copepods were eaten in the first 24 h (average ± s.d. initial abundances of both groups: 96 ± 28 and 79 ± 22 per aquarium, respectively). For the calanoid copepods, a significant effect was revealed after four days of exposure (Table 1; Fig. 1).

On day 1, the calanoid copepod abundance was significantly lower in the *T. cancriformis* + sediment treatment than in the *T. cancriformis* treatment without sediment (Table 1; Fig. 1). Total suspended solids was positively related to the number of surviving *T. cancriformis* in the aquaria ($F_{1,22} = 6.650$, $P = 0.017$, $r^2 = 0.232$). The presence of only sediment had a significant negative effect on the abundances of cyclopoid copepods and ostracods (Table 1; Fig. 1). *C. diaphanus* had a negative effect on cyclopoid copepods starting from day 8, and on ostracods at day 11. A negative non-significant trend was also found for the Cladocera (Fig. 1).

Salinity had no significant effect on the microcrustacean abundances and also did not interact with the different biological treatments, except for the ostracods. On days 4 and 8, ostracod abundances were lower in the intermediate salinity treatment ($1.0\ \mathrm{g\,L^{-1}}$) than in 0.5 and $2.5\ \mathrm{g\,L^{-1}}$ ($F_{2,53} = 9.860$, $P = 0.0002$), but only in the control (a) and *C. diaphanus* (b) treatments,

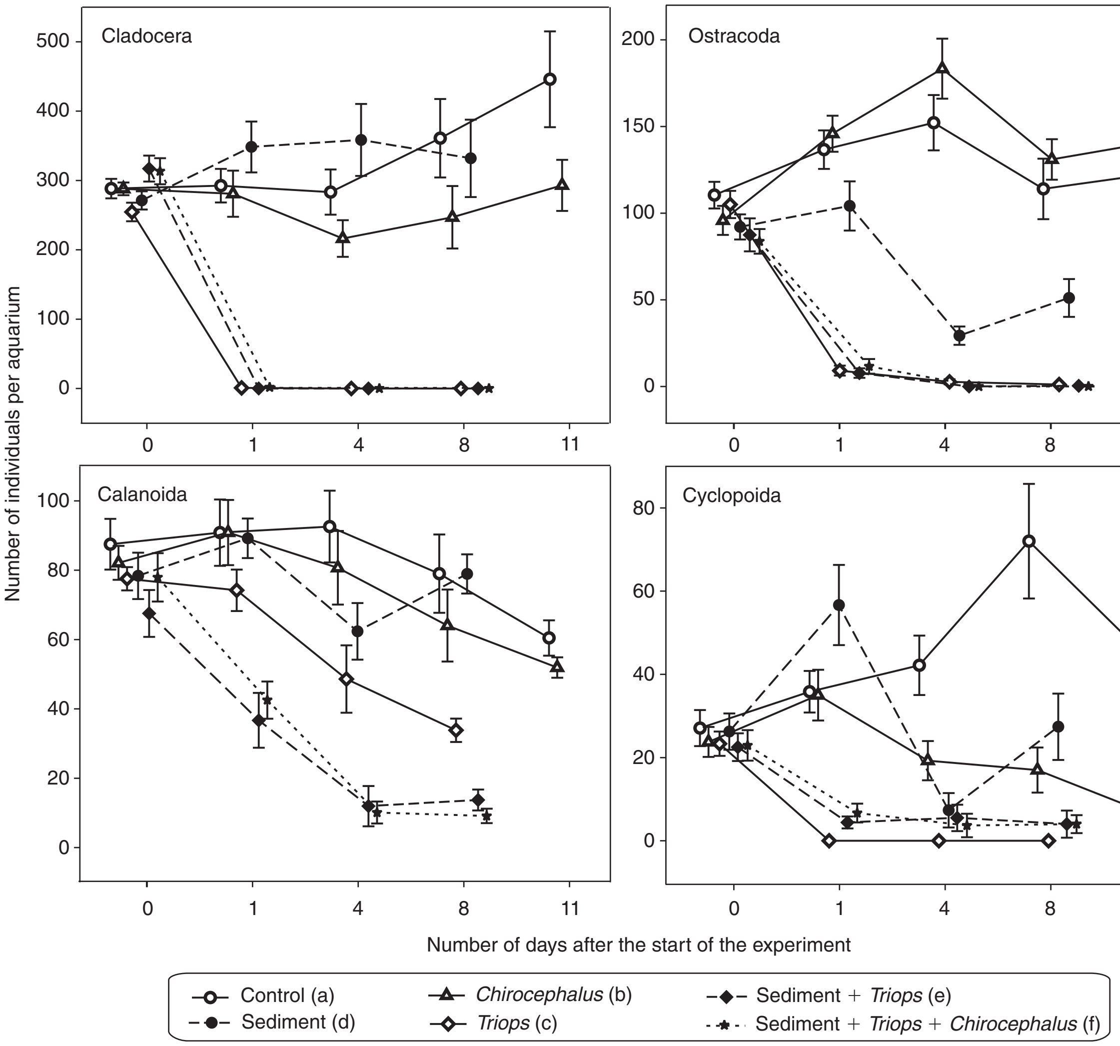

Fig. 1. Mean and standard errors of microcrustacean abundances (cladocerans, ostracods, calanoid, and cyclopoid copepods) during eleven days of exposure to the control and large branchiopod treatments (over all salinities).

resulting in a significant interaction term ($F_{10,53} = 2.965$, $P = 0.005$). At the end of the experiment, no effect of salinity or of large branchiopod treatments was found on chlorophyll *a* concentrations ($F_{2,64} = 0.727$, $P = 0.488$ and $F_{5,64} = 1.047$, $P = 0.400$, respectively).

As soon as most of the microcrustaceans were consumed, *T. cancriformis* started eating each other. On day 8 an average (±s.d.) of 5.4 (±1.4) out of 10 *T. cancriformis* had disappeared (Fig. 2). These numbers did not include the individuals that were found dead. *T. cancriformis* also ate *C. diaphanus* in the combination treatment (f). In the *C. diaphanus* treatment (b), very few specimens died naturally during the experiment (average ± s.d.: 0.44 ± 0.65 per aquarium), whereas an average (±s.d.) of 7.9 (±1.9) individuals disappeared in the aquaria containing *T. cancriformis* (f). Neither predation by *T. cancriformis* on conspecifics nor on *C. diaphanus* were influenced by salinity of the medium ($F_{2,33} = 0.512$, $P = 0.604$ and $F_{2,6} = 2.018$, $P = 0.189$, respectively).

Predation by Triops cancriformis

Triops cancriformis individuals ate an average (±s.d.) of 9.8 (±5.0) *D. magna* per hour. Most prey items were eaten during the first hour (average ± s.d.: 16.3 ± 3.9). Subsequently, their consumption stabilised to an average (±s.d.) of 8.6 (±4.2) *D. magna* per hour (Fig. 3). In the controls, none of the adult *D. magna* died. Over the total period of six hours, *T. cancriformis* ate on average (±s.d.) 3.6 (±1.9) *D. magna* ephippia.

Bioturbation by Triops cancriformis

After 48 h, the amount of total suspended solids was significantly higher in the aquaria with *Triops cancriformis* than in the aquaria without *T. cancriformis* ($F_{3,8} = 49.12$, $P < 0.001$). The amount of suspended solids increased gradually with *T. cancriformis* density (Fig. 4), although the differences were not statistically significant.

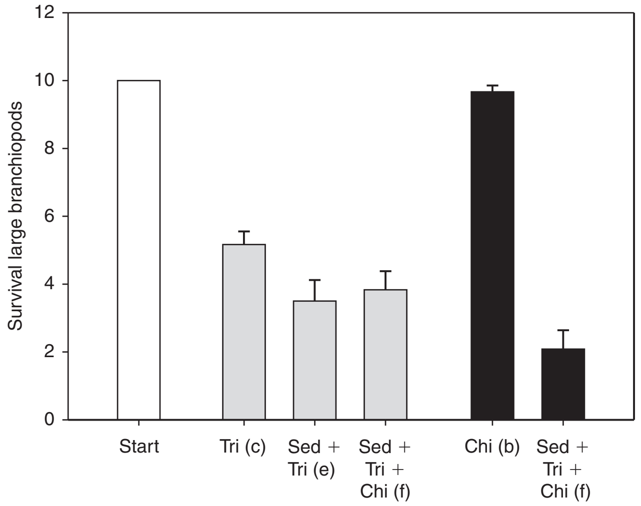

Fig. 2. Mean and standard errors of the survival of large branchiopods during the experiment (white bar, number of large branchiopods per aquarium at the start of the experiment; grey bar, number of surviving *T. cancriformis*; black bar, number of surviving *C. diaphanus* after 8 days). Treatment abbreviations: Sed, sediment; Tri, *T. cancriformis*; Chi, *C. diaphanus*.

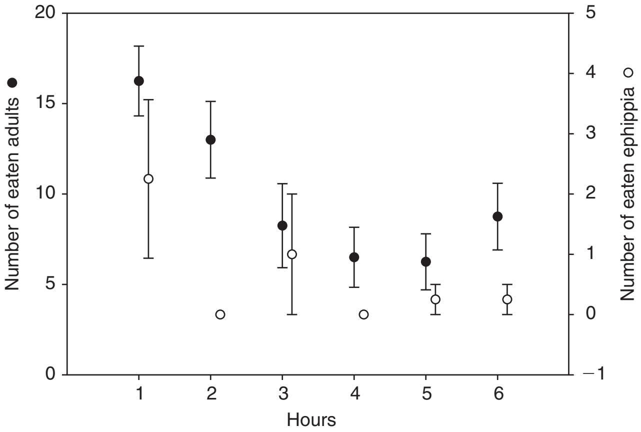

Fig. 3. Mean and standard error of number of *D. magna* adults (full symbols) and ephippia (open symbols) eaten during six hours of exposure to *T. cancriformis*.

Discussion

The results of this study confirm that large branchiopods can strongly influence microcrustacean communities in temporary wetlands. Large branchiopods negatively influenced microcrustacean densities in several ways, the strongest impact being predation by notostracans.

Predation by Notostraca

In our microcosm experiment, most of the microcrustaceans were eaten within the first 24 h. Only the large calanoid copepods survived longer, probably because of their faster swimming, and hence better escape potentials, than other microcrustaceans (Browman *et al.* 1989). Although according to Dumont and Negrea (2002), the predatory nature of *T. cancriformis* is not yet widely accepted, several other studies report that the diet of adult *T. cancriformis* is mostly composed of small and large prey species. Their diet is diverse and can include detritus, bacteria, phytoplankton, filamentous algae, plant material, cladocerans, copepods, ostracods, anostracans, dipterans, mayflies, annelids and even amphibian eggs and larvae (Pont and Vaquer 1986; Walton 2001).

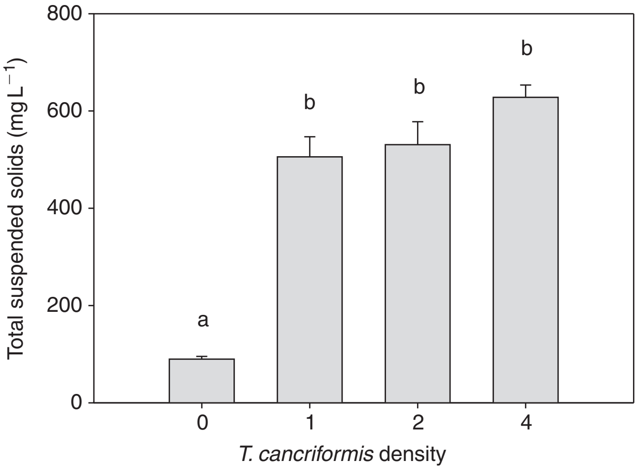

Fig. 4. Mean and standard error of total suspended solids in 10-L aquaria containing variable densities of *T. cancriformis*. Bars marked with different characters refer to significant differences among pairwise comparisons (Tukey's HSD *post-hoc* tests: $P < 0.05$).

Although fish are absent, it is already widely accepted that temporary waters are not an 'enemy free' environment (Brendonck *et al.* 2002). Generally, flying insects, such as odonates and notonectids, are considered to be the top predators in temporary wetlands (Brendonck *et al.* 2002). In this study, we confirm that in wetlands where notostracans occur, they can act as top predators, as proposed by Pont and Vaquer (1986) for rice fields. Comparing literature concerning average predation rates on large cladocerans by different invertebrate predators present in temporary wetlands (using similar methods, i.e. laboratory microcosms with high prey densities), we found that the only predators with higher predation rates than notostracans (10 *Daphnia* spp. h^{-1}) were the dragonfly larvae *Ischnura elegans* and the backswimmer *Notonecta hoffmani* (see Accessory Publication). As in most predation rate studies, our predation rates may be somewhat overestimated compared to field conditions, owing to the artificial conditions of the experiment. First of all, prey densities in our predation experiment (100 *Daphnia magna* L^{-1}) were three times higher than in natural Camargue ponds (32 ± 30 zooplankters L^{-1} (50% being *Daphnia* spp.): A. Waterkeyn, unpubl. data), and encounter rates were high because of the lack of escape possibilities for prey from the experimental microcosms. Although this could seem a methodological limitation, temporary wetlands can undergo substantial ecosystem contraction during draw down, leading to much higher prey concentrations (e.g. in Camargue ponds: 222 ± 300 zooplankters L^{-1} (45% being *Daphnia* spp.)). Also, predator densities were not exaggerated as natural densities of up to 300 individuals per m^2 can be found on Camargue rice fields (Pont and Vaquer 1986). It is therefore likely that the effects detected in our microcosms reflect realistic field conditions.

Triops cancriformis was able to consume *D. magna* ephippia by crushing and chewing the edges of ephippia, consuming the eggs inside. Although predation on resting eggs can have a tremendous impact on the resting egg bank dynamics, and consequently on the persistence of microcrustacean communities (Brendonck and De Meester 2003), very few studies have investigated this possibility. In marine environments, Viitasalo (2007) demonstrated predation of *Bosmina* sp. ephippia by amphipods and mysids. In freshwater environments, Cáceres and Hairston (1998) found that only amphipods were capable of ingesting *Daphnia* spp. ephippia, contrary to turbellarians, gastropods, chironomids, and zebra mussels. De Roeck *et al.* (2005) found no evidence for predation of anostracan resting eggs by turbellarians. Because the time between egg laying and hatching is often quite long for microcrustaceans (months or even years: Hairston *et al.* 1995), predation risk during that time can be high. However, Dumont *et al.* (2002) hypothesised that predation defence mechanisms probably reduce predation risk. The strong ridges on *C. diaphanus* resting eggs were suggested as such a mechanism. Compared to predation rates on adult *Daphnia*, much lower numbers of ephippia were eaten during our predation experiment. However, we have to take into account that the ephippia used in our experiment were floating, probably making them less accessible to *T. cancriformis*. Floating ephippia are often encountered in the field, but predation rates may be higher when ephippia are on the bottom of the pond, considering the predominantly benthic lifestyle of *T. cancriformis*.

Triops cancriformis also ate other large branchiopods, such as *C. diaphanus*, and also their conspecifics. The fact that cannibalism occurs in notostracans was already suggested by Thiéry (1988). Analysing gut contents of *T. cancriformis* from the Spanish Espolla pond, Boix *et al.* (2006) occasionally encountered notostracan exuviae or metanauplii. As cannibalism is often density-related, intraspecific predation pressure is probably only high at high densities, as was the case in our aquaria (one adult *T. cancriformis* per L). On the other hand, we expect that when anostracans and notostracans co-occur in naturally communities, any effects of anostracans may be swamped by notostracans.

Bioturbation by Notostraca

As already suggested by Yee *et al.* (2005), notostracans may play a dual role in temporary wetlands. Besides strongly reducing microcrustacean populations via predation, *T. cancriformis* can indirectly affect microcrustaceans through bioturbation. Bioturbating invertebrates are generally considered typical examples of ecosystem engineers (Mermillod-Blondin and Rosenberg 2006), which may be the case for *T. cancriformis* (and notostracans in general) in temporary wetlands. These mainly benthic organisms constantly stir the pond sediment in search for food or to deposit resting eggs (Pont and Vaquer 1986). In our experiments, the amount of suspended solids strongly increased in the presence of increasing numbers of *T. cancriformis* (up to 600 $\mathrm{mg\,L^{-1}}$ total suspended solids). This was also experimentally demonstrated for the benthic clam shrimp *Caenestheriella belfragei*, causing serious problems in fish culture pools by clogging drainage filters and reducing fish production (Luzier and Summerfelt 1997). Some *Triops* species are considered pests in rice fields in at least seven countries on four continents (California, Swaziland, Spain, France, Italy, India, and Japan) because of their sediment-disturbing behaviour, uprooting seedlings and limiting light penetration (Grigarick *et al.* 1961). On the other hand, it is possible that the created turbidity has a positive feedback effect on the presence of *T. cancriformis* itself as it might reduce predation by birds, which is suspected to strongly limit *T. cancriformis* populations in Camargue wetlands (Waterkeyn *et al.* 2009).

In our biotic interaction experiment, the strong *T. cancriformis* predation effect made it difficult to detect additional effects of bioturbation. We did find a significant negative effect of bioturbation on calanoid copepods, before notostracan predation reduced their populations. Butler (1996) demonstrated that high amounts of suspended matter can decrease the ingestion rate of calanoid copepods. High turbidities are also often found to negatively affect cladocerans, by limiting their grazing efficiency, limiting light-dependent phytoplankton growth or reducing food quality (Kirk 1991). However, it is likely that, owing to the rapid removal of all cladocerans by *T. cancriformis* in our experiment, we did not find evidence for the additional effect of bioturbation on this microcrustacean group. To test more specifically for the unique effect of bioturbation by notostracans, more experiments are needed. Also, more research is needed to further quantify the negative and positive impacts of notostracans as ecosystem engineers. Sediment presence on its own (without bioturbation) was also found to negatively affect ostracod and cyclopoid copepod densities. However, this might be the result of an unintentional sampling bias because of a less-thorough homogenization of the microcrustacean community in the aquaria containing sediment to avoid resuspending the sediment.

Influence of Anostraca

Sánchez and Angeler (2007) did not succeed in demonstrating a clear community-structuring effect of the anostracan *Branchinecta orientalis* using a field survey approach, likely a result of the high degree of environmental variability between wetlands. Using a more manipulative approach, even for a short duration (11 days), we showed a negative effect of *C. diaphanus* on the populations of cyclopoid copepods and ostracods. The same negative, but non-significant, trend was found for cladoceran populations. Anostracans are generally considered highly efficient filter-feeders, able to out-compete filter-feeding zooplankters (Jocqué *et al.* 2010). Moreover, it has been demonstrated that anostracans can also physically harass (i.e. interference competition) or even prey upon small microcrustaceans (Jawahar *et al.* 1996; Sarma and Nardini 2002). We suspect that all three processes (competition for food, interference competition and predation) played a role in reducing microcrustacean densities in our experiment.

Salinity-mediated biotic interactions

Coping with an environmental stressor can often trade off with other traits, such as the performance of an organism in its interactions with other species. However, the hypothesis that osmotic stress can, on the one hand, make microcrustaceans less able to cope with predation or competition and/or, on the other hand, make large branchiopods less able to prey upon or to outcompete microcrustaceans, could not be confirmed in this study. Some studies have found such salinity-mediated changes in biotic interactions. For example, Sarma *et al.* (2002) found that salinity influenced the outcome of competition between two rotifer species, whereas Chandramohan *et al.* (2008) showed that salinity influenced predation rates of a water bug. In our experiment, the measured biotic interactions, and especially predation, may have been too strong to detect subtle changes in sensitivity to predation, competition, or bioturbation caused by salinity stress. Also, it is possible that the chosen salinity range was not broad enough, with maximal salinity levels of 2.5 g L^{-1}. This level may not be stressful for Camargue crustaceans, which are probably locally adapted to the naturally high salinities of this region (Waterkeyn *et al.* 2008). Higher values were, however, not chosen because large branchiopods are not tolerant to higher salinities (no hatching at 5.0 g L^{-1}: Waterkeyn *et al.* 2010). On the other hand, the finding that the biotic effects exerted by large branchiopods are similar under a range of salinity conditions is in line with the argument that temporary wetland inhabitants are generally resilient to environmental stress (Wiggins *et al.* 1980). Being able to optimise foraging under a gradient of stressful conditions would underpin their ‘r-strategic’ behaviour.

Can large branchiopods shape microcrustacean communities?

Our results indicate that large branchiopods can exert a strong top-down (predation) and lateral (competition) pressure on microcrustacean communities, and can also change the habitat physically by creating high turbidities. This confirms their potential for playing a keystone role in temporary waters. Based on the results of a mesocosm study investigating the effect of different salinity and hydroregimes on hatching crustacean communities from temporary wetlands, Waterkeyn (2009) and Waterkeyn *et al.* (2010) hypothesised that large branchiopods, and especially *Triops cancriformis*, can promote a shift from a clear-water microcrustacean-rich state to a turbid microcrustacean-poor state with low macrophyte development. This hypothesis is supported by the present study. This would suggest that a turbid ecosystem state can have a purely biological cause in temporary habitats, whereas in shallow lakes it is often the result of abiotic alterations, maintained by hysteretic effects and altered community structure (Scheffer *et al.* 1993). Although these contrasting states are considered ‘stable’ in shallow lakes (Scheffer *et al.* 1993), our findings also suggest that a turbid state can be transitional in temporary habitats (in ponds with a hydroperiod longer than the life span of notostracans). The use of *Triops* species as biological control agent of mosquito larvae in temporary water bodies has often been considered because of their ability to develop synchronously with their prey (Su and Mulla 2002). However, we want to stress that it is likely that non-target species will also suffer from their introduction.

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Table. Literature review on experimentally determined average predation rates (number of prey eaten per predator per hour) of invertebrate predators on Cladocera. When several prey densities or sizes were tested, we selected those closest to the ones used in the present study (density: 20 prey in 200 mL; size: 1.0 - 2 mm).

Taxon	**Invertebrate predator**	**Cladoceran prey**	**Predation rate**	**Reference**
Notostraca	*Triops cancriformis*	*Daphnia magna*	10	This study
	Lepidurus arcticus	*Daphnia pulex*	10	Christoffersen 2001
Anostraca	*Chirocephalus diaphanus*	*Ceriodaphnia dubia*	2	Sarma and Nandini 2002
Odonata	*Ischnura elegans*	*Daphnia magna*	12	Van de Meutter *et al*. 2005
	Sympetrum sanguineum	*Daphnia cucullata*	5	Wojtal *et al*. 2007
Hempitera	*Notonecta hoffmani*	*Daphnia pulex*	27	Scott and Murdoch 1983
	Anisops wakefieldi	*Ceriodaphnia dubia*	6.7	Gilbert and Burns 1999
	Arctocorisa carinata	*Daphnia magna*	0.5	Ranta and Espo 1989
	Callicorixa producta	*Daphnia magna*	0.2	Ranta and Espo 1989
Diptera	*Chaoborus obscuripes*	*Daphnia magna*	2	Van de Meutter *et al*. 2005
	Chaoborus obscuripes	*Daphnia pulex*	1.4	Jeschke and Tollrian 2000
Coleoptera	*Acilius semisulcatus*	*Daphnia pulex*	1	Arts *et al*. 1981
	Potamonectes griseostriatus	*Daphnia magna*	0.2	Ranta and Espo 1989
Turbellaria	*Mesostoma ehrenbergii*	*Ceriodaphnia dubia*	0.3	Trochine *et al*. 2008
	Mesostoma sp.	*Daphnia laevis*	0.2	Rocha *et al*. 1990
	Mesostoma lingua	*Daphnia pulex*	0.2	Schwartz and Hebert 1986
	Mesostoma lingua	*Daphnia magna*	0.1	Dumont and Schorreels 1990
	Dugesia tigrina	*Daphnia magna*	0.1	McKee *et al*. 1997
Acari	*Piona exigua*	*Daphnia carinata*	0.5	Butler and Burns 1993

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