

Predatory and toxic effects of the turbellarian (*Stenostomum cf leucops*) on the population dynamics of *Euchlanis dilatata*, *Plationus patulus* (Rotifera) and *Moina macrocopa* (Cladocera)

S. Nandini · S. S. S. Sarma · Henri J. Dumont

Published online: 7 October 2010
© Springer Science+Business Media B.V. 2010

Abstract Catenulid turbellarians, common in shallow, tropical ponds, affect their rotifer prey via the production of toxins. There is, however, no quantitative information on their effect on the demography of their prey. Here, we test the impact of *Stenostomum cf leucops* on the population dynamics of the rotifers *Euchlanis dilatata* and *Plationus patulus*, and the cladoceran *Moina macrocopa*. Experiments were initiated with rotifers at 0.5 ind. ml⁻¹ and the cladoceran at 0.2 ind. ml⁻¹; growth patterns were compared in the absence and presence of worms (2 *Stenostomum* ind. per 50 ml). Results revealed that brachionids were most adversely affected: there was a lower growth rate of the rotifers in the presence of worms ($P < 0.01$, repeated measures ANOVA), although at the densities applied, the predator did

not wipe out its prey. These littoral predators may therefore regulate rotifer prey in natural conditions. In *Moina*, the population evolved differently; initially, we found no difference between control and treatment, but after about 10 days, the population collapsed, irrespective of a direct or indirect contact with the predator. This delayed effect deserves more study, as it could represent flatworm toxin accumulation by the cladoceran.

Keywords Flatworms · Population growth · *Plationus patulus* · *Euchlanis dilatata* · *Moina macrocopa* · Egg ratio

Introduction

Rotifers are subject to predation by vertebrates and invertebrates. In deep water bodies, fish larvae, juveniles and insect larvae exert the bulk of the predation pressure, while in shallow waters, the impact of amphibians and micro-invertebrates such as copepods, cladocerans, other rotifers or protozoans is usually higher (O'Brien, 1987; Gilbert, 1998). Since the life cycle of micro-invertebrate predators is often similar to that of their rotifer prey, they show increased consumption rates (functional response) as well as population numbers (numerical response) with increasing availability of rotifer prey (Murdoch & Bence, 1987).

Guest editors: N. Walz, R. Adrian, J.J. Gilbert, M.T. Monaghan, G. Weithoff & H. Zimmermann-Timm / Rotifera XII: New aspects in rotifer evolution, genetics, reproduction, ecology and biogeography

S. Nandini (✉) · S. S. S. Sarma
Laboratorio de Zoología Acuática, División de Investigación y Posgrado, Edificio UMF, Universidad Nacional Autónoma de México, Campus Iztacala, AP 314, CP 54090 Tlalnepantla, Estado de Mexico, Mexico
e-mail: nandini@servidor.unam.mx

H. J. Dumont
Department of Biology, University of Gent,
Ledeganckstraat, 35, 9000 Ghent, Belgium

Many pelagic rotifer taxa show defenses to avoid predation. These include the presence of large and small spines in *Plationus macracanthus* and *Brachionus budapestinensis*, respectively (Garza-Mouriño et al., 2005), darting behaviour in *Filinia longiseta* and *Hexarthra mira* (Iyer & Rao, 1996), small body size in *Anuraeopsis fissa* (Sarma & Nandini, 2007), epizoic behaviour in *Brachionus rubens* (Iyer & Rao, 1995) and colonial life or production of toxins as in *Sinantherina semibullata* (Felix et al., 1995). Little information is available on the defense strategies of littoral rotifers. Serrania-Soto & Sarma (2009) have shown that *Lecane stokesii* shows spine elongation in the presence of infochemicals from *Asplanchnopus multiceps* but the efficacy of this behaviour as a defense mechanism is yet to be proven. High population growth rates or retreat into the resting egg stage could also be an effective method to withstand predation pressure and could be the strategy that zooplankton species adopt to survive such stress (Nielsen et al., 2000).

While certain organisms that feed on rotifers, such as fish larvae, the predatory rotifer *Asplanchna* or notonectid insect larvae, have been well studied, little information is available on the effect of other invertebrate predators. These include turbellarians, frequently found in high densities (80 ind. l⁻¹ and up to 27,000 ind. m⁻²; Kolasa, 2001) in shallow water and in the benthos of lakes and ponds. It has been suggested that turbellarian predation could structure zooplankton communities (Dumont & Carels, 1987; Blaustein & Dumont, 1990).

There are about 400 turbellarian species, several of them with a wide distribution. The genus *Stenostomum* (Family Catenulidae) has 50 species, 25 of which are found in South America (Noreña et al., 2005). They feed on detritus, bacteria and protozoans, and they consume rotifers as well. They produce infochemicals, in response to which protozoans such as *Euplotes* develop defensive ‘wings’ (Fyda et al., 2005; Altwegg et al., 2006). That they also hunt for littoral rotifers was claimed more than 70 years ago (Nutting & Waters, 1935) but after that, little work on their impact on zooplankton populations was conducted. While *Stenostomum predatorium* bites parts of their prey, other *Stenostomum* sp. feed by suction (Kolasa, 2001; Kratina et al., 2009).

We found that our field-collected *Stenostomum* cf. *leucops* measured 2.0–3.0 mm but could measure up

to 5 mm when extended. In laboratory cultures, they had their guts filled with rotifers and their eggs (10–15 per worm). They fed on live and dead individuals by sucking them with their protrusible pharynx. Here, we present information on the impact of *S. leucops* on the population dynamics of *Plationus patulus*, *Euchlanis dilatata* and the cladoceran *Moina macrocopa*. The rotifer species are part of the aufwuchs community where the worms are also found; *M. macrocopa*, on the other hand, is found in both the plankton of deep water and in shallow ponds. We analysed the direct and indirect effects of *Stenostomum* sp. on its prey by studying the feeding behaviour and the population dynamics patterns.

Methods

We isolated *Stenostomum* cf. *leucops* (hereafter *Stenostomum*) from a shallow, eutrophic pond in the State of Veracruz, Mexico. Cultures were established from a single worm and fed a mixture of *Chlorella vulgaris*, *Scenedesmus acutus*, *Lecane bulla*, *Lecane quadridentata*, *Plationus patulus* and *Euchlanis dilatata*. Prey were provided ad libitum, and their adequacy was confirmed by the fact that prey were present at densities between 1 and 2 ind. ml⁻¹ when cultures were changed. The culture volume was 300 ml in 500-ml glass vessels at 23 ± 2°C, under diffuse fluorescent illumination. The worms reproduced asexually by paratomy; most were at the bottom of the culture vessel, often attached to the glass; a few were observed swimming. We maintained the cultures with around 200 ind. l⁻¹. The medium was changed once a week, and fresh algae and rotifers were added. We selected uniformly sized worms which showed an active swimming movement for all the experiments.

For culturing prey, we cultured *Chlorella vulgaris* in transparent bottles using Bold’s basal medium (Borowitzka & Borowitzka, 1988). Algae in the log-phase of growth were harvested, centrifuged and resuspended in distilled water, from which, using a Neubauer haemocytometer we counted the number of cells and obtained an algal density of 1 × 10⁶ cells ml⁻¹ as the food level appropriate for mass culture of the brachionid rotifers. Zooplankton cultures were maintained at 23 ± 2°C, in diffuse fluorescent light. For routine maintenance of all

zooplankton and *Stenostomum* sp. as well as the experiments, we used moderately hard water (EPA medium: Weber, 1993), prepared by dissolving 96 mg NaHCO₃, 60 mg CaSO₄, 60 mg MgSO₄ and 4 mg KCl in 1 l of distilled water.

Plationus patulus, *Euchlanis dilatata* and *Moina macrocopa* were used as prey. All cultures were established from a single female and were maintained under laboratory conditions for more than 6 months prior to experimentation. All the prey taxa were isolated from Lake Xochimilco (Mexico City). Cultures were maintained separately in 1-l glass beakers. They were filtered every alternate day with a 50- μ m mesh and transferred to a fresh medium with algae at the above mentioned concentration.

Population growth studies

The experiments were conducted in 100-ml transparent plastic vessels with 50 ml of medium in each. For experiments with the rotifers we used two treatments, controls (without *Stenostomum* sp.) and treatments in which *Stenostomum* sp. were introduced at a density of 0.08 ind. ml⁻¹. *Plationus patulus* and *Euchlanis dilatata* were introduced at a density of 1 ind. ml⁻¹. The experimental design consisted of 16 test jars: 2 prey species \times 2 treatments (with and without *Stenostomum* sp.) \times 4 replicates.

Following initiation of the experiment, we daily counted rotifers and eggs (loose and attached) in each replicate in the case of *P. patulus*. Next, all prey and predators were transferred to fresh medium in a new vessel. The predator population was culled to maintain it at a constant 0.08 ind. ml⁻¹. *Euchlanis dilatata* laid their eggs on the walls of the beaker which were difficult to dislodge, therefore we only changed the medium and not the test vessel daily. In the case of *Moina macrocopa*, we set up a control and two treatments, one in which worms were introduced directly into the test vessels and one in which worms were separated from the cladocerans by a mesh (they were fed 20 individuals of *B. patulus* in the mesh per day). The cladoceran *Moina macrocopa* was introduced at a concentration of 0.2 ind. ml⁻¹ and was counted and transferred to fresh medium daily. The experiments were terminated after day 15 when prey populations began to decline. Based on the data collected, we derived the rate of population increase (r) using the exponential growth equation (Krebs,

1985): $r = (\ln N_t - \ln N_0)/t$, where N_0 is the initial population density, N_t population density at time t and t time in days. For each replicate, we derived five values during the exponential phase of the population growth, by selecting different time periods, the mean of which gave the r per replicate. Population growth data were analysed by means of repeated measures ANOVA and population growth rates (r) compared by one way ANOVA (Sigma-Plot 11).

Results

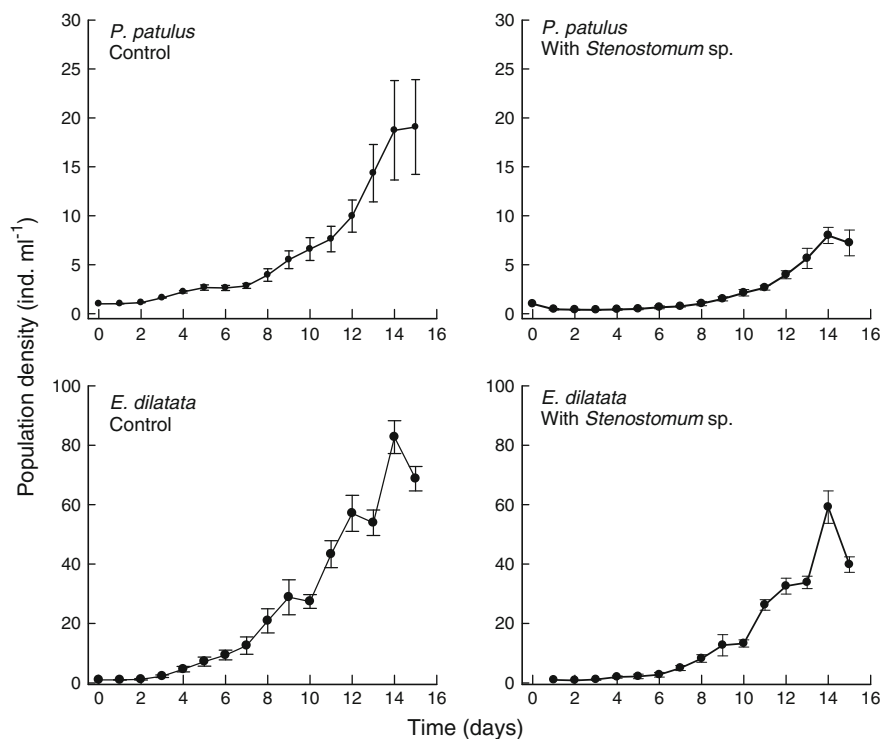
The population growth curves of *Plationus patulus* and *Euchlanis dilatata* in the presence and absence of *Stenostomum* sp. are shown in Fig. 1. Peak densities of *P. patulus* were three times higher in the absence of the catenulid worm, and the lag phase was also prolonged. This resulted in significant differences in growth pattern with or without the presence of worms (repeated measures ANOVA, $P < 0.01$). In *E. dilatata*, we also observed significantly lower population growth in the presence of worms (repeated measures ANOVA, $P < 0.01$), although the percent difference in the lag-phase and peak population density in the presence and absence of worms was less than in *P. patulus*.

The egg ratio of *P. patulus* in the absence and presence of *Stenostomum* sp. is shown in Fig. 2. In the controls, egg-bearing *P. patulus* were abundant even at densities higher than 20 ind. ml⁻¹; in the presence of *Stenostomum* sp., no egg bearing females were found at densities higher than 10 ind. ml⁻¹. Egg ratios of *E. dilatata* were not calculated since several eggs were attached to the vessel and it was difficult to identify and count them.

Growth patterns of *Moina macrocopa* in direct or indirect contact with *Stenostomum* and in the controls are shown in Fig. 3. Population growth trends were similar in all three cases for the first 10 days; after that, the cladoceran population collapsed, irrespective of a direct or indirect contact with the worms.

Population growth rates of the rotifers and *M. macrocopa* in the presence and absence of *Stenostomum* sp. are shown in Figs. 4 and 5, respectively. The flatworm had a greater adverse impact on *P. patulus* than on *E. dilatata* and was able to lower the growth rate significantly ($P < 0.002$ and $P < 0.03$, respectively, One-way ANOVA, Table 1). On the

Fig. 1 Population growth curves of *Plationus patulus* and *Euchlanis dilatata* in the absence (controls) and presence of *Stenostomum* sp. Shown are mean \pm SE values based on four replicate recordings



other hand, considering only the exponential phase of growth of the population, no significant differences in the growth rates of *M. macrocopa* were observed ($P > 0.05$, One-way ANOVA). Nevertheless, after about 10–12 days of direct or indirect contact with the worms, the cladoceran population crashed.

Discussion

Population growth patterns of rotifers in nature are influenced, simultaneously, by several abiotic and biotic factors (Dumont, 1977; Wallace et al., 2006). Among the role of biotic factors, predation by micro-invertebrates has been well analysed (Kerfoot & Sih, 1987). Predators such as *Asplanchna* have a devastating effect due to two reasons: (i) they consume large numbers of prey (Sarma & Nandini, 2007) and (ii) they have short life cycles which allow a rapid increase in population numbers (Dumont & Sarma, 1995). We did observe some details of the consumption process of rotifer prey by *Stenostomum* sp. in this study and found that they sucked out prey from the bottom of the feeding vials using their eversible pharynx and occasionally captured free swimming

animals. Others have documented their feeding on large numbers of protozoans in a short period of time (Kratina et al., 2009) and thus, it came as no surprise that *Stenostomum* sp. significantly reduced population densities of both *Plationus patulus* and *Euchlanis dilatata* after few days of exposure. That the worms did not drive their rotifer prey to extinction within the duration of our experiment, we ascribe to (1) the relatively short experimental time and (2) the low density of the worms. In shallow water with low oxygen and in the absence of vertebrates, higher densities of worms can be found (Kolasa, 2001) and in such cases, we expect them to structure their prey community.

The feeding behaviour of a few turbellarians has been studied in detail. Dumont & Carels (1987) have shown that *Mesostoma ehrenbergi* attacks its prey individually and sucks out its body contents. Other species, like *Stenostomum predatorium*, bite parts of their prey at each feeding attempt (Kolasa, 2001) while we observed *Stenostomum leucops* in this study to suck in prey using the pharynx. There have been few studies on the feeding behaviour of this flatworm; we observed that they capture and ingest whole rotifers, although not as voraciously as *Asplanchna*.

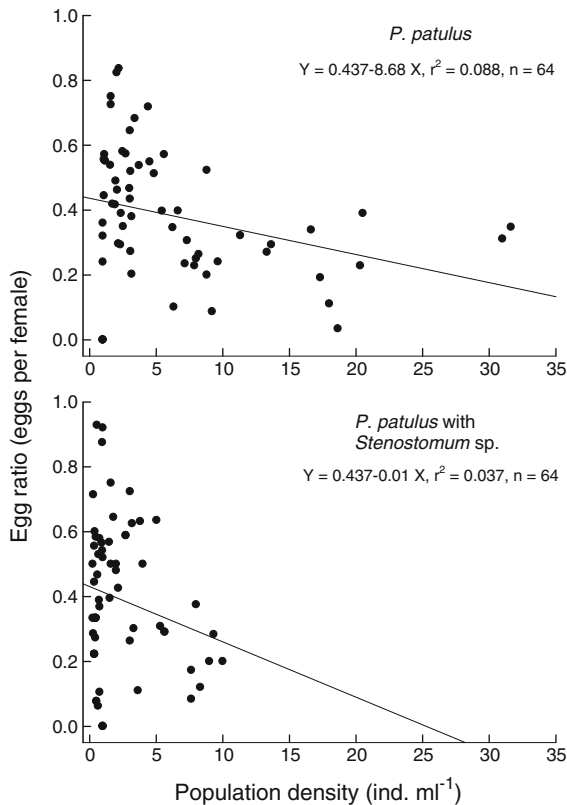


Fig. 2 Egg ratio of *Plationus patulus* in the absence (controls) and presence of *Stenostomum* sp. Shown are all values from four replicate recordings

They do not ‘chase’ their prey, but swim around and capture the ones that are in the appropriate zone. We analysed the stomach contents of several *S. leucops* and found more animal than algal matter in them. They do suction dead animals but also feed on live prey. In our test vessels, around 70% of the predator population was attached to the bottom or sides and 30% swimming freely in the column.

A different resistance to the sucking force of *Stenostomum* sp. could explain the differences in vulnerability of the two rotifer taxa studied. A larger proportion of the *Euchlanis dilatata* settled at the bottom or sides of the experimental containers, yet their population was relatively less affected by the worms than that of *P. patulus*, possibly reflecting the fact that the secretions of the pedal glands of *E. dilatata* are stronger than those of *P. patulus* (Koste, 1978). An alternative explanation is that the two rotifers differ in their tolerance to toxins produced by the flatworms. The release of such

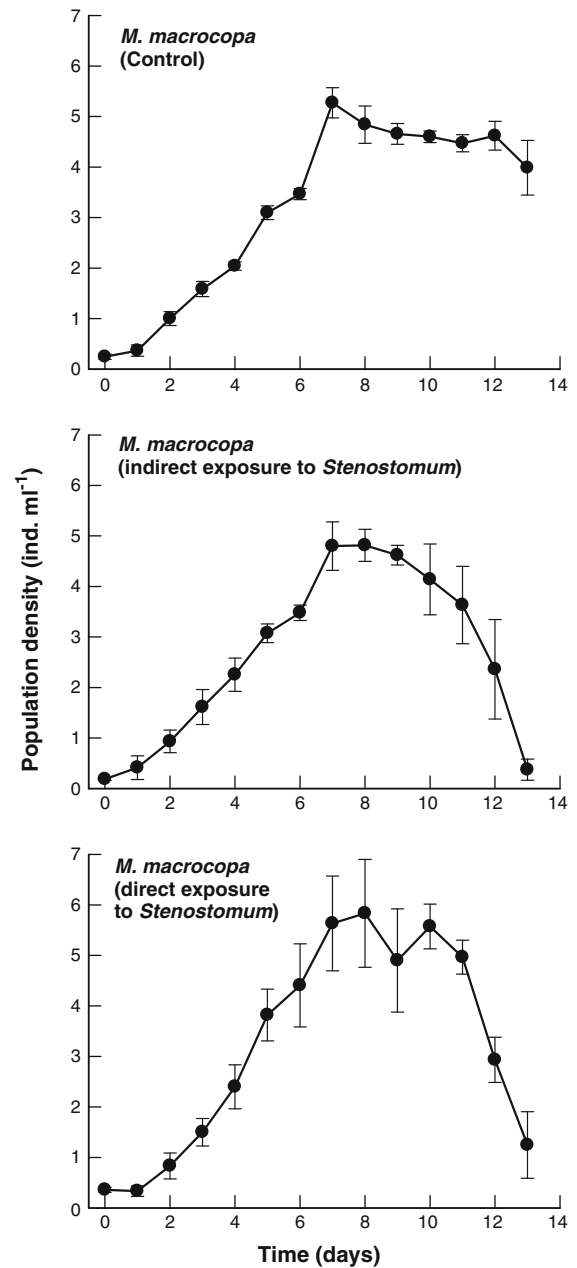


Fig. 3 Population growth curves of *Moina macrocopa* in the absence (controls), direct presence of *Stenostomum* sp. and indirect contact with *Stenostomum* sp. Shown are mean \pm SE values based on four replicate recordings

toxins by catenulids (they have been known in typhloplanids where they are used to sedate prey before consumption: Dumont & Carels, 1987) was suggested by the behaviour of the cladoceran *Moina* (see below) and by the rotifer egg ratios.

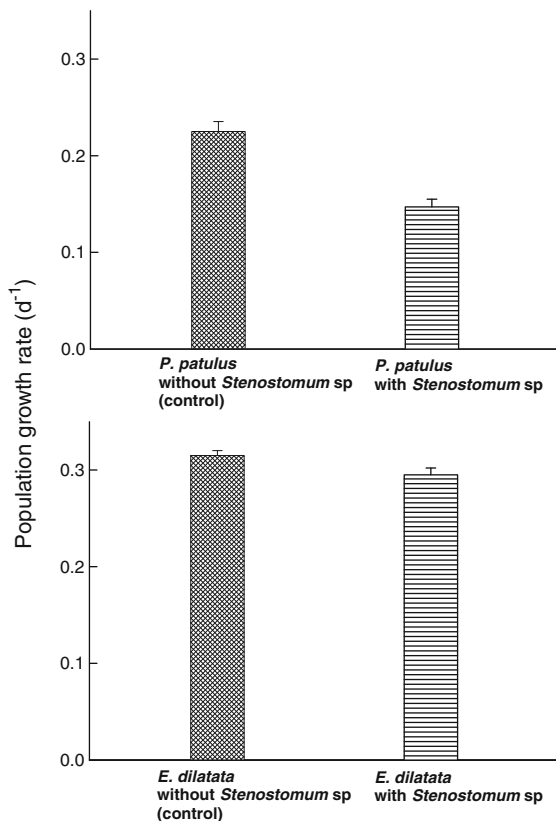


Fig. 4 Population growth rates of *Platyonus patulus* and *Euchlanis dilatata* in the absence (controls) and presence of *Stenostomum* sp. Shown are mean \pm SE values based on four replicate recordings

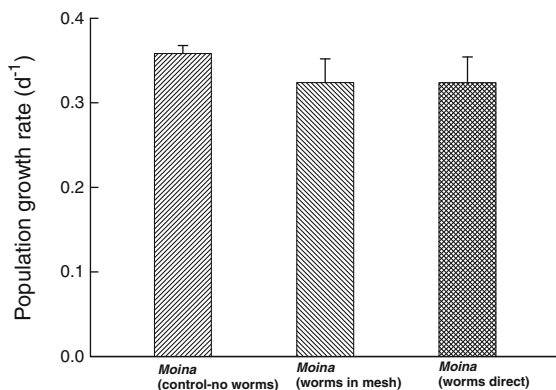


Fig. 5 Population growth rates of *Moina macrocopa* in the absence (controls), direct presence of *Stenostomum* sp. and indirect contact with *Stenostomum* sp. Shown are mean \pm SE values based on four replicate recordings

An aspect of toxin ecology appeared in our experiments with *Moina macrocopa*: whether prey were in direct or indirect contact with the predator,

Table 1 One-way analysis of variance on the rates of population growth of the rotifers *P. patulus* and *E. dilatata* and the cladoceran *Moina macrocopa* cultured in the presence and absence of *Stenostomum* sp.

Source	DF	SS	MS	F	P
<i>P. patulus</i>					
Between groups	1	0.0104	0.0104	36.621	0.002
Residual	5	0.00142	0.000285		
<i>E. dilatata</i>					
Between groups	1	0.00120	0.00120	8.066	0.03
Residual	6	0.000893	0.000149		
<i>M. macrocopa</i>					
Between groups	2	0.00283	0.00141	0.872	0.459
Residual	7	0.0114	0.00162		

Levels of significance are shown as *P* values

DF degrees of freedom, *SS* sum of squares, *MS* mean square, *F* Fischer's ratio, *P* probability level

there was a delayed but precipitous decline after about 10 days. This suggests that the cladocerans may have accumulated toxin in their bodies until a lethal threshold was reached. However, this would require the flatworm toxin to remain active for a period of time longer than was suggested by Dumont and Carels (1987).

Egg ratio analysis is a useful tool in analyzing stress-related effects on rotifer populations (Sarma et al., 2005). In this study, we analysed the effect of *Stenostomum* sp. on the egg ratio of *P. patulus* and found that it declined significantly in the presence of the turbellarian. We consider that this inhibited egg production or decrease in population density is also consistent with the release of a toxin by the worms. It is also possible that the decline was due to the worms feeding on egg-bearing females. This has not been quantified in our study. Further studies will be needed to confirm the nature of these toxins and their ecological effects.

Acknowledgments We thank Dr. J. Kolasa for identifying the flatworm. The first two authors thank National Autonomous University of Mexico (DGAPA-PASPA) for financial support.

References

- Altwegg, R., M. Eng, S. Caspersen & B. R. Anholt, 2006. Functional response and prey defence level in an experimental predator-prey system. *Evolutionary Ecology Research* 8: 115–128.

- Blaustein, L. & H. J. Dumont, 1990. Typhloplanid flatworms (*Mesostoma* and related genera): mechanisms of predation and evidence that they structure aquatic invertebrate communities. *Hydrobiologia* 198: 61–77.
- Borowitzka, M. A. & L. J. Borowitzka, 1988. *Micro-Algal Biotechnology*. Cambridge University Press, Cambridge: 480 pp.
- Dumont, H. J., 1977. Biotic factors in the population dynamics of rotifers. *Archiv für Hydrobiologie Beiheft* 8: 98–122.
- Dumont, H. J. & I. Carels, 1987. Flatworm predator (*Mesostoma cf. lingua*) releases a toxin to catch planktonic prey (*Daphnia magna*). *Limnology and Oceanography* 32: 699–702.
- Dumont, H. J. & S. S. S. Sarma, 1995. Demography and population growth of *Asplanchna girodi* (Rotifera) as a function of prey (*Anuraeopsis fissa*) density. *Hydrobiologia* 306: 97–107.
- Felix, A., M. E. Stevens & R. L. Wallace, 1995. Unpalatability of a colonial rotifer, *Sinantherina socialis*, to small zooplanktivorous fishes. *Invertebrate Biology* 114: 139–144.
- Fyda, J., A. Warren & J. Wolinska, 2005. An investigation of predator-induced defense responses in ciliated protozoa. *Journal of Natural History* 39: 1431–1442.
- Garza-Mouriño, G., M. Silva-Briano, S. Nandini, S. S. S. Sarma & M. E. Castellanos-Páez, 2005. Morphological and morphometrical variations of selected rotifer species in response to predation: a seasonal study of selected brachionid species from Lake Xochimilco (Mexico). *Hydrobiologia* 546: 169–179.
- Gilbert, J. J., 1998. Kairomone-induced morphological defenses in rotifers. In Tollrian, R. & C. D. Harvell (eds), *The Ecology and Evolution of Inducible Defenses*. Princeton University Press, Princeton: 127–141.
- Iyer, N. & T. R. Rao, 1995. The epizoic mode of life in *Brachionus rubens* Ehrenberg as a deterrent against predation by *Asplanchna intermedia* Hudson. *Hydrobiologia* 313–314: 377–380.
- Iyer, N. & T. R. Rao, 1996. Responses of the predatory rotifer *Asplanchna intermedia* to prey species differing in vulnerability: laboratory and field studies. *Freshwater Biology* 36: 521–533.
- Kerfoot, W. C. & A. Sih (eds), 1987. *Predation: Direct and Indirect Impacts on Aquatic Communities*. University Press of New England, Hanover, NH.
- Kolasa, J., 2001. Flatworms: Turbellaria and Nemertea. In Thorp, J. H. & A. P. Covich (eds), *Ecology and Classification of North American Freshwater Invertebrates*. Academic Press, New York: 145–172.
- Koste, W., 1978. *Rotatoria. Die Rädertiere Mitteleuropas. Ein Bestimmungswerk begründet von Max Voigt, Vol. 1, Textband: 673 pp; Vol. 2, Tafelband: 234 pp. Borträger, Stuttgart.*
- Kratina, P., M. Vos, A. Bateman & B. R. Anholt, 2009. Functional responses modified by predator density. *Oecologia* 159: 425–433.
- Krebs, C. J., 1985. *Ecology: The Experimental Analysis of Distribution and Abundance*, 3rd ed. Harper and Row, New York.
- Murdoch, W. W. & J. Bence, 1987. General predators and unstable prey populations. In Kerfoot, W. C. & A. Sih (eds), *Predation: Direct and Indirect Impacts on Aquatic Communities*. University Press of New England, Hanover, NH: 17–30.
- Nielsen, D. L., F. J. Smith, T. J. Hillman & R. J. Shiel, 2000. Impact of water regime and fish predation on zooplankton resting egg production and emergence. *Journal of Plankton Research* 22: 433–446.
- Noreña, C., C. Damborenea & F. Brusa, 2005. A taxonomic revision of South American species of the genus *Stenostomum* O. Schmidt (Platyhelminthes: Catenulida) based on morphological characteristics. *Zoological Journal of the Linnaean Society* 144: 37–58.
- Nuttycomb, J. W. & A. J. Waters, 1935. Feeding habits and pharyngeal structure in *Stenostomum*. *Biological Bulletin* 69: 439–446.
- O'Brien, W. J., 1987. Planktivory by freshwater fish: thrust and parry in the pelagia. In Kerfoot, W. C. & A. Sih (eds), *Predation: Direct and Indirect Impacts on Aquatic Communities*. University Press of New England, Hanover, NH: 3–16.
- Sarma, S. S. S. & S. Nandini, 2007. Small prey size offers immunity to predation: a case study on two species of *Asplanchna* and three brachionid prey (Rotifera). *Hydrobiologia* 593: 67–76.
- Sarma, S. S. S., R. D. Gulati & S. Nandini, 2005. Factors affecting egg-ratio in planktonic rotifers. *Hydrobiologia* 546: 361–373.
- Serrania-Soto, C. & S. S. S. Sarma, 2009. Morphometric changes in *Lecane stokesii* (Pell, 1890) (Rotifera: Lecanidae) induced by allelochemicals from the predator *Asplanchnopus multiceps* (Schränk, 1793). *Allelopathy Journal* 23: 215–222.
- Wallace, R. L., T. W. Snell, C. Ricci & T. Nogrady, 2006. *Rotifera Part 1: Biology, Ecology and Systematics. Guides to the Identification of the Microinvertebrates of the Continental Waters of the World*. Kenobi Productions Gent/Backhuys, The Netherlands.
- Weber, C. I., 1993 *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, 4th ed. United States Environmental Protection Agency, Cincinnati, OH, EPA/600/4-90/027F, xv + 293 pp.