

Chemical signals and habitat selection by three zooplankters in Andean Patagonian ponds

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SUMMARY

1. Zooplankton may react differently to chemical signals produced by macrophytes in shallow systems. They may be attracted by macrophytes, as the plants may be used as a refuge against predators, or the plants may have a repellent effect (e.g. when the plants are a habitat for numerous invertebrate predators or fish). In fishless Patagonian ponds, the structural complexity provided by macrophytes modulates the rate of predation on zooplankton by the invertebrate predator *Mesostoma ehrenbergii* (Turbellaria).

2. We performed a field study to analyse the coexistence of *M. ehrenbergii* and three of its prey (two copepods, the calanoid *Boeckella gracilis* and the cyclopoid *Acanthocyclops robustus*, and the cladoceran *Ceriodaphnia dubia*) in four ponds. In two of the ponds, we carried out day and night sampling to evaluate the influence of macrophytes on the distribution of these zooplankters.

3. In laboratory experiments, we analysed the response of the zooplankters to the chemical signals produced by macrophytes (the emergent *Juncus palleescens* and the submerged *Myriophyllum quitense*), the predator *M. ehrenbergii* and the 'alarm signal' provided by a homogenate of conspecifics.

4. Our field studies demonstrated the coexistence of *M. ehrenbergii* and the selected prey in different seasons and that *A. robustus* and *C. dubia* choose the vegetated area (a mixed bed of *J. palleescens* and *M. quitense*) over the non-vegetated area. The habitat choice experiments indicated that the presence of *M. ehrenbergii* may directly affect the habitat selection of *B. gracilis*, because this zooplankter swam away from the predator. In addition, *Mesostoma* may indirectly affect the habitat selection of the cyclopoid copepod *A. robustus* and the cladoceran *C. dubia* as both zooplankters exhibited a negative response to the alarm signal produced by crushed conspecifics.

5. The presence of the submerged *M. quitense* did not affect the horizontal movements of any of the zooplankters studied. In contrast, the emergent macrophyte *J. palleescens* elicited a positive response of *B. gracilis*, suggesting that this aquatic plant may act as a predation refuge.

6. Our results suggest that predator avoidance behaviour can occur in fishless environments in response to a tactile invertebrate predator like *Mesostoma*. In addition, the refuge effect of emergent macrophytes, enhancing the survival of pelagic zooplankters, may act as a key factor in stabilizing predator–prey interactions in fishless Patagonian ponds, as has been widely recorded in northern temperate lakes with fish.

Keywords: alarm signal, chemical cues, crushed prey, macrophytes, *Mesostoma ehrenbergii*

Introduction

Predator–prey interactions in aquatic environments are partly mediated by chemical cues released from various sources, including predators and disturbed and/or injured prey (Kats & Dill, 1998; Tollrian & Harvell, 1999; Wisenden, 2000a). The ability of prey to distinguish predators from non-predators has important implications. Failing to recognize a predator increases the probability of being captured during an encounter (Hirsch & Bolles, 1980), and defensive responses to non-predators are likely to reduce secondary production (Black & Dodson, 1990; Cieri & Stearns, 1999), partly because of the loss of foraging and mating opportunities (Lima & Dill, 1990). Recognition of predators through chemical signals occurs in a variety of vertebrate and invertebrate prey (Weldon, 1990; Dodson *et al.*, 1994). Most prey species learn to recognize predators when signals from injured conspecifics are paired with signals from predators (Magurran, 1989; Chivers & Smith, 1994a,b; Chivers, Brown & Smith, 1995). Chemical alarm cues ('alarm signals') are released after a successful predatory attack and indicate imminent and proximate danger to conspecifics of the prey (Chivers & Smith, 1998). In response to these cues, prey adopt species-specific antipredator behaviour, such as reduced activity, search for shelter and area avoidance (Wisenden, 2000b).

Chemical cues have been shown to influence the horizontal migration of zooplankton (Lauridsen & Lodge, 1996; Burks, Jeppesen & Lodge, 2001). Migration of pelagic zooplankton into the vegetated littoral zones of shallow lakes during the day is termed diel horizontal migration (Timms & Moss, 1984; Lauridsen & Buenk, 1996; Lauridsen *et al.*, 1998). Diel horizontal migration occurs in some shallow lakes, but appears particularly prevalent in north temperate rather than in subtropical and Mediterranean lakes (Burks *et al.*, 2002; Castro, Marques & Gonçalves, 2007; Meerhoff *et al.*, 2007). Furthermore, different driving forces, such as light, food concentration, predators and macrophyte characteristics, can affect this behaviour. Prey may react to chemical signals produced by macrophytes, as they can be used as a refuge against predation (Crowder & Cooper, 1979; Dvorak & Best, 1982; Winfield, 1986; Diehl, 1988, 1992), though submerged macrophytes usually have a repellent effect (Hasler & Jones, 1949; Pennak, 1966, 1973; Quade, 1969). Macrophytes are a habitat for many

invertebrate predators of zooplankton (Burks *et al.*, 2002). In addition, exudates from macrophytes (e.g. resorcinol) can reduce growth and reproduction, and increase mortality, in *Daphnia* (Sütfield, Petereit & Nahrstedt, 1996; Burks, Jeppesen & Lodge, 2000). In particular, Meerhoff *et al.* (2006) showed that *Daphnia obtusa* Kurz avoids plants that are common in (sub) tropical lakes, despite cues that indicate a risk of predation from a very common planktivorous fish (*Cnesterodon decemmaculatus* Jenyns) and from crushed conspecifics. The influence of macrophytes on the horizontal distribution of zooplankton, therefore, depends on a trade-off between avoiding macrophytes and the risk of predation (Lauridsen & Lodge, 1996; Lauridsen *et al.*, 1996).

Previous studies of fishless Patagonian ponds (Trochine, Modenutti & Balseiro, 2006) showed that the structural complexity provided by macrophytes modulates the rate of predation by the turbellarian *Mesostoma ehrenbergii* (Focke). The flatworm *M. ehrenbergii* is a common predator in fishless ponds of the North Patagonian Andean Region (Trochine, Modenutti & Balseiro, 2005; Trochine *et al.*, 2006). Within these habitats, adults and juveniles were observed not only in the littoral but also in the open waters. In Patagonian ponds *M. ehrenbergii* can be the top predator and consume prey >1 mm (1.5–2 mm) (Trochine *et al.*, 2005, 2006). Because the feeding strategy of *M. ehrenbergii* includes mucus trapping and external digestion, prey size does not depend upon the predator's gape, as is the case for copepods and many insect predators (Swift, 1992; Diéguez & Balseiro, 1998).

Trochine *et al.* (2006) showed that the rates of predation by *M. ehrenbergii* on two different copepods, the calanoid *Boeckella gracilis* (Daday) and the cyclopoid *Acanthocyclops robustus* (Sars), reached saturation in the treatments run with horizontal spatial heterogeneity (provided by emergent plants). However, *M. ehrenbergii* ate more copepods (*B. gracilis* and *A. robustus*) in the treatments run with vertical spatial heterogeneity (provided by submerged plants) (Trochine *et al.*, 2006). These previous experiments (Trochine *et al.*, 2006) suggested that, in natural habitats, interactions between the extent of macrophyte development and intensity of predation by *M. ehrenbergii* on copepods species are probable. In particular, the survival of *A. robustus* and *B. gracilis* is enhanced when they are associated with emergent macrophyte stands (Trochine *et al.*, 2006). Based on this previous

study, we hypothesized that *Mesostoma* modifies the habitat distribution of these two copepods.

Here, we examined the ability of the two copepods and another common Patagonian zooplankter, the cladoceran *Ceriodaphnia dubia* Richard, to recognize and react to different chemical signals. We performed a field study to analyse the coexistence of *M. ehrenbergii* and these three prey species and the influence of macrophytes stands on the horizontal distribution of the zooplankters. We further conducted experiments to elucidate the response of these zooplankters to the signals produced by macrophytes (one emergent and one submerged), the predator *M. ehrenbergii* and the alarm signal provided by crushed conspecifics. We hypothesized that the three zooplankters would react to the chemical signals provided by macrophytes, *Mesostoma* and injured conspecifics. Our predictions were that all zooplankters would react negatively to the cues provided by the presence of the predator and the homogenate of conspecifics, because these signals are associated with a greater risk of predation. In addition, we expected that the zooplankters would react positively to macrophytes only when faced at the same time with a predator cue.

Methods

Field data collection and analysis

Mesostoma ehrenbergii and its prey. We conducted this study to assess the coexistence and seasonal distribution of *M. ehrenbergii* and the three selected prey (two copepods, the calanoid *B. gracilis* and the cyclopoid *A. robustus* and the cladoceran *C. dubia*) in four temporary fishless ponds in northern Patagonia (41°S), near the city of San Carlos de Bariloche, Río Negro, Argentina.

The ponds were: Fantasma (FA), Mallín Km 12 (M12), Ñirihuau Chica (ÑCh) and Ñirihuau Grande (ÑGr) (Table 1). These ponds are located in a 300 km² area surrounding the eastern basin of Lake Nahuel Huapi. This area covers an east-west gradient of increasing precipitation (800–1400 mm year⁻¹). The hydroperiod of each pond varies between 7 and 9 months, the depths of the ponds are between 0.7 and 2 m and maximum water levels occur in late autumn and winter. Water temperature ranges from 27 °C in December to 0 °C in July and August (winter), when all the ponds may be ice-covered. Ponds ÑCh and ÑGr are located in the forest-steppe ecotone, while ponds M12 and FA are located in forest. The bottom of all the ponds is partially covered with macrophytes, mainly Juncaceae. Other invertebrate predators occurring in the ponds are the copepod *Parabrotteas sarsi* (Daday), Dytiscidae, Hydrophilidae, water mites, *Hydra* and Hirudinea. Fieldwork covered the complete 2004–05 hydroperiod, and the ponds were sampled monthly at mid-day. Zooplankton samples were collected with a small plankton net (200 µm mesh) by making two horizontal tows of 10 m in the littoral and pelagic areas. The tows were pooled into one sample and preserved with 4% formalin solution. In the laboratory, crustacean zooplankton species were identified and counted in a 5 mL Bogorov chamber under an Olympus SZX9 stereomicroscope at 12–50× magnification. *Mesostoma ehrenbergii* abundance was also estimated from this set of samples.

Zooplankton diel distribution: vegetation versus non-vegetated area

Field design. Zooplankton samples were collected on three consecutive days in November 2007 in two

Table 1 Features of the northern Patagonian ponds included in this study

Ponds	Location	Altitude (m a.s.l.)	Z _{max} (cm)	A _{max} (m ²)	Conductivity (µS cm ⁻¹)	Oxygen (%)	TDP (µg L ⁻¹)
FA	41°05'35"S 71°27'01"W	780	200	10.000	92.8	93.0	6.6
M12	41°06'24"S 71°26'10"W	780	70	30.000	85.3	93.3	6.7
ÑCh	41°05'09"S 71°10'59"W	750	70	4.000	74.4	71.9	6.9
ÑGr	41°05'09"S 71°11'03"W	750	150	6.000	86.9	86.5	5.9

FA, Fantasma; M12, Mallín Km 12; ÑCh, Ñirihuau Chica; ÑGr, Ñirihuau Grande; Z_{max}, maximum depth; A_{max}, maximum surface area during the hydroperiod; oxygen %, percentage of oxygen saturation; TDP, total dissolved phosphorus.

ponds (FA and ÑGr). These ponds are subject to differing precipitation and are located in forest and the forest-steppe ecotone, respectively. The bottom of pond FA is mainly covered with Juncaceae, while pond ÑGr has a mixed bed of Juncaceae and *Myriophyllum quitense* Kunth (Andean water milfoil). A detailed description of these ponds and their macrophyte composition is given by Trochine, Balseiro & Modenutti (2008).

We performed two samplings during each day, one at mid-day and the other at midnight (00:00 hours). On each sampling occasion we obtained whole column (from the surface to a few cm above the sediment) zooplankton samples using 1.0 or 0.5 m long tubes (6 cm diameter). Four samples were taken in vegetated areas and four in non-vegetated areas. The samples (5 L each) were taken every 5 m along transects in both areas and filtered through a 55 µm pore size plankton net. The samples were preserved in 4% formalin solution. Physical and chemical parameters were recorded for each day. Light intensity was measured with a PAR irradiance sensor (2100 Series; Biospherical Instruments Inc., San Diego, CA, U.S.A.) and temperature, oxygen concentration and conductivity were measured with a multiprobe (YSI 85; Biospherical Instruments Inc., San Diego, CA, U.S.A.). In the laboratory, crustacean zooplankton and *M. ehrenbergii* individuals were identified and counted, crustacean zooplankton were quantified in 5 mL Bogorov chambers, and crustacean nauplii in a 1 mL Sedgwick-Rafter chamber under a microscope. Total phosphorus (TP) and total nitrogen (TN) were determined in unfiltered pond water. For TP determinations, the samples were digested using persulphate at 125 °C and 1.5 atm for 1 h. Phosphorus concentrations were determined using the ascorbate-reduced molybdenum method and ammonium by the indophenol blue method (APHA, 1992). TN was determined according to Valderrama (1981). Chlorophyll-*a* (Chl-*a*) concentration was determined by filtering 100 mL on GF/F filters (Whatman, Maidstone, U.K.), samples were extracted with hot ethanol (Nusch, 1980) and measured with a 10-AU fluorometer (Turner Designs, Sunnyvale, CA, U.S.A.) calibrated using spectrophotometric determinations.

Experimental design. A set of experiments were conducted in order to test how different zooplankton species (the copepods *B. gracilis* and *A. robustus* and the cladoceran *C. dubia*) reacted to different chemical cues.

For each species, we used five different treatments: without signal (CONTROL), the presence of the invertebrate predator *M. ehrenbergii* (PREDATOR), alarm signal from crushed conspecifics (SIGNAL) (*sensu* Pijanowska, 1997) and two aquatic plants (VEG1 and VEG2). In addition, combined signals (e.g. PREDATOR plus VEG1) were used when a zooplankton species reacted significantly to more than one signal.

The three zooplankters and the predator *M. ehrenbergii* were collected from pond ÑGr using a hand net (200 µm mesh size). Copepods and cladocerans were identified and separated using a stereomicroscope (12×). We used ovigerous *C. dubia* and ovigerous and non-ovigerous *B. gracilis* and *A. robustus* for the experiments. The zooplankters were maintained in containers filled with natural lake water while *M. ehrenbergii* individuals were kept in 3 L aquaria and fed daily with a diet of assorted zooplankton. All the aquaria were maintained in an incubator at 15 °C and a light : dark cycle of 10 : 14 h. We also collected *M. quitense* and *Juncus pallescens* Lam. from the pond and thoroughly rinsed them with distilled water at least 10 times, after which the plants were examined under stereomicroscope to ensure the absence of organisms on their surface. Macrophytes were maintained under the same temperature and light regime as described above until the start of the experiments.

Experiments were run in a special device that consisted of two glass vessels (1 L volume each) with an inverted glass T tube between them (500 mm length × 17 mm diameter) (modified from Meerhoff *et al.*, 2006). The water flow within the set up permitted the generation of a gradient in chemical cues (Meerhoff *et al.*, 2006). Before performing the habitat choice experiments we used dye (methylene blue) to determine the existence of a gradient after 2 h. The experiments showed that the gradient was still strong after 2 h, as the dye reached only 75% of the distance from the vessel to the central tube (where the prey were introduced) in the T tube after this period of time.

To conduct the trials, we used two experimental devices at a time, filled with 2.15 L of filtered pond water (55 µm mesh size). The water for the experiments was kept in a 100 L aquarium with an oxygen supply.

To generate the signal for the PREDATOR treatment, we used six *M. ehrenbergii* adults held in small cages (plastic cylindrical tubes covered with a mesh

1 mm size pore in both ends). We used these cages to allow the prey to be exposed to the presence of the predator, while enabling *Mesostoma* to move along the tube (see Dodson, 1988). *Mesostoma ehrenbergii* individuals were starved for 48 h prior to the experiments, as we were interested in the chemical signal generated by the predator *per se* (see Kats & Dill, 1998) and not the one due to the feeding regime. The alarm signal in the SIGNAL treatment consisted of a homogenate of 20 crushed prey and, in the VEG treatment, the aquatic vegetation types used were *M. quitense* (equivalent to 1.2 g dry weight) and *J. pallescens* (equivalent to 6 g dry weight). The different cues were placed in the corresponding vessels 1 h prior to the beginning of the experiments to build up a chemical gradient. To run the experiments with simultaneous signals, the cues were placed together in one vessel to determine which signal effect prevailed in determining the zooplankton response. In each trial, one prey individual was placed in the middle tube of the experimental device (17 mm diameter). After running the experiment for 1 h, we observed the location of the prey and three possibilities were considered, right vessel, left vessel or in the tube.

The units were rinsed thoroughly with distilled water before starting the experiments. Each treatment was repeated 20 times (the unit received the treatment 10 times on each side to minimize errors), except the controls where we used 10 repetitions. Note that the controls were run to assess if the device *per se* had an effect on the horizontal movement of the zooplankters, and they were not paired with the treatments in the statistical analysis (see below). The experiments were run at 18 °C and controlled light (one 30 W fluorescent tube placed at 60 cm above the experimental devices). The light was measured with a radiometer QSL-2100 (Biospherical Instruments, Inc.) to ensure that there were no differences in light along the experimental device.

Statistical analysis

Data obtained in the zooplankton diel distribution sampling were analysed using repeated measure (RM) ANOVAS, with the sampled sub-areas within each site as the subjects. The analyses were carried out for each day independently, as a means to simplify the statistical design (by reducing the number of factors

and interactions) but also to assess if the distribution patterns observed were consistent over the 3 days. Since this artificial separation was decided merely for statistical convenience, the associated significance level of the family-wise type I error was adjusted using the Dunn-Sidak procedure (Quinn & Keough, 2002), for a total of three comparisons (one per day) for each parameter (Castro *et al.*, 2007). A significance level of 0.017 was therefore used in all analyses. The factors of the (RM) ANOVAS were habitat (macrophytes and open waters) and time of the day (midday and midnight). In addition, two-way ANOVAS were applied to test for differences in the parameter values obtained in the open water and macrophyte area during the three days of sampling in ponds FA and ÑGr. Data for temperature, light, nutrient and chlorophyll concentrations and zooplankton relative abundances were \log_{10} or $\log_{10}(x + 1)$ -transformed to fulfil the requirements of homoscedasticity and normal distribution of residuals.

The data obtained in the experiments were analysed using a chi-squared test, assuming that the number of prey moving to one vessel would equal the other (H_0 : cue vessel = non-cue vessel). For each treatment we considered the difference between the number of times that the targeted prey moved to the side containing the cue and the number of times that they moved to the non cue vessel. When significant differences were obtained, the larger number (cue vessel or non cue vessel) indicated the predominant direction for the prey in that particular treatment.

Results

Field study: *Mesostoma* and the crustacean zooplankton

The turbellarian predator *M. ehrenbergii* was present between October and January although it made up <3% of total zooplankton abundance. High abundances of juvenile *M. ehrenbergii* were recorded in June in ponds FA, ÑCh and ÑGr. Our study showed that common zooplankters, such as the daphniid *C. dubia*, the cyclopoid copepod *A. robustus* and the calanoid copepod *B. gracilis*, co-occur with *M. ehrenbergii* in the ponds studied (Fig. 1). Moreover, the three zooplankters occurred together with *M. ehrenbergii* in ponds FA, M12 and ÑCh at different times during the hydroperiod (Fig. 1). Chydorids and the macrtriticid *Pseudomoina* sp. also co-occurred with

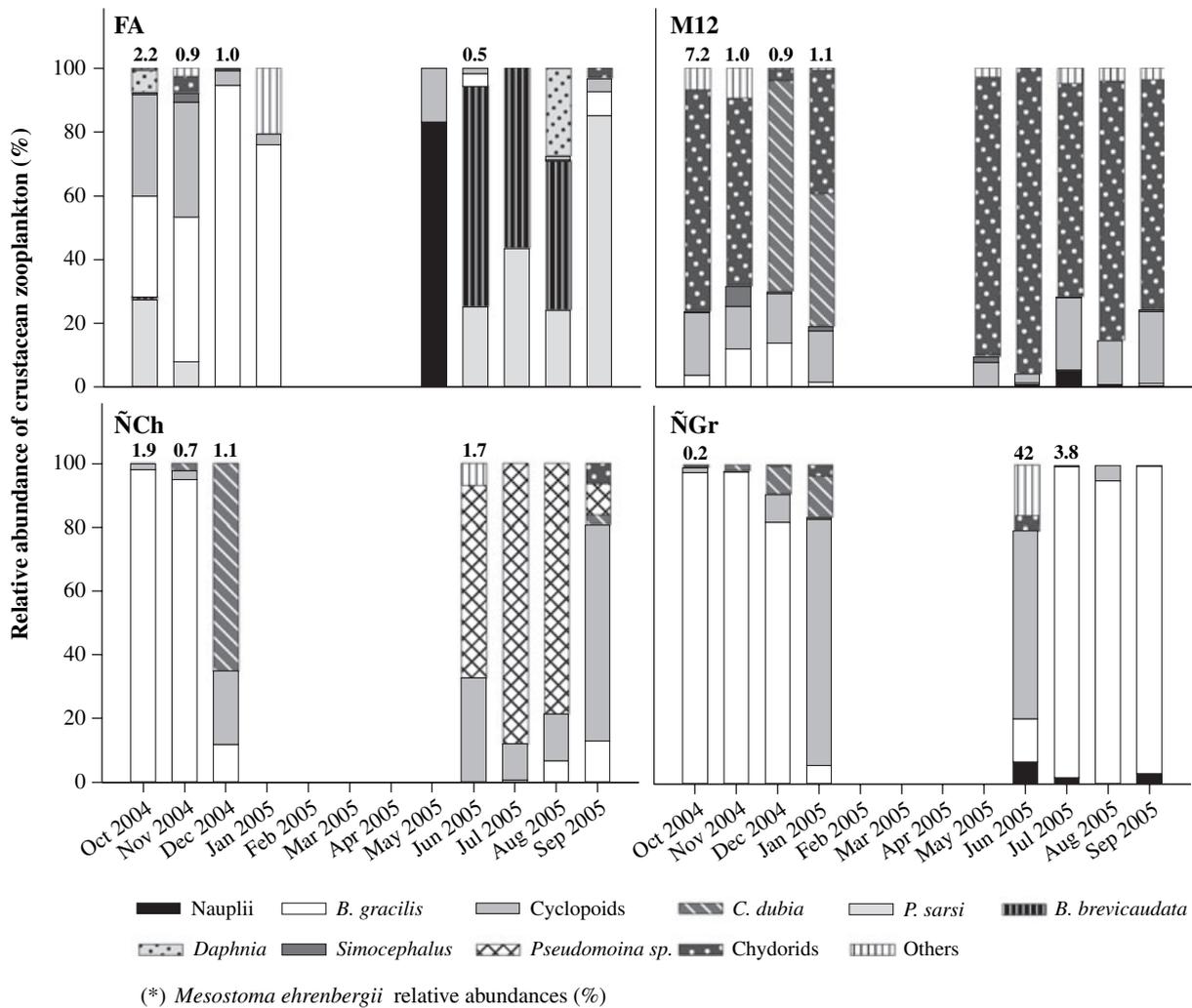


Fig. 1 Relative abundance of zooplankton species in northern Patagonian ponds. Groups shown in these graphs represented >1% of the zooplankton abundance during at least one sample period during the study. FA, Fantasma; M12, Mallín Km 12; ÑCh, Ñirihuau Chica; ÑGr, Ñirihuau Grande; CH, Del Chancho. Lack of data indicates the dry months in each pond.

Mesostoma in ponds M12 and ÑCh, respectively (Fig. 1).

Zooplankton habitat selection in two ponds (FA and ÑGr)

During the three consecutive days of our sampling in spring, the weather remained calm and sunny (average irradiance = 2212 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$). Physical and chemical conditions in ponds FA and ÑGr also remained relatively similar throughout the sampling campaign (Table 2). Chl-*a* was the most variable parameter, exhibiting significant fluctuations between samples taken in the open waters and in the

macrophyte habitats of pond ÑGr (Table 2, $P = 0.017$ ANOVA and Holm-Sidak's *post hoc* test). Significant differences in TN between habitats were also obtained for pond ÑGr (Table 2, $P = 0.023$ ANOVA and Holm-Sidak's *post hoc* test). The daily PAR irradiance (both at the surface and at 30 cm depth) varied between the open waters and macrophyte habitats for ponds FA and ÑGr ($P = 0.003$ and $P < 0.001$, respectively, ANOVA). In addition, macrophyte zones exhibited significantly lower PAR values ($P < 0.05$ Holm-Sidak's *post hoc* test).

The crustacean zooplankton community differed between ponds FA and ÑGr. In pond FA copepod nauplii were the most abundant crustacean

Parameter	Habitat	FA	ÑGr
Water temperature (°C)	–	17.70 ± 1.77	23.6 ± 2.08
Oxygen (mg L ⁻¹)	–	5.29 ± 0.4	8.53 ± 0.12
Conductivity (µS cm ⁻¹)	–	110.43 ± 2.58	89.37 ± 4.67
TP (µg L ⁻¹)	O	51.95 ± 1.67	23.57 ± 0.44
	M	54.74 ± 2.91	33.09 ± 0.83
TN (µg L ⁻¹)	O	682.1 ± 16.56	339.74 ± 8.65
	M	744.06 ± 12.15	410.76 ± 24.87
Chlorophyll- <i>a</i> (µg L ⁻¹)	O	13.04 ± 0.51	1.96 ± 0.36
	M	11.54 ± 1.14	5.71 ± 1.11
PAR irradiance air (µmol photon m ² s ⁻¹)	–	2130 ± 60.28	2293.33 ± 35.28
PAR irradiance under water-surface (µmol photon m ² s ⁻¹)	O	911.67 ± 59.47	976.67 ± 40.96
	M	810 ± 106.93	508.33 ± 104.26
PAR irradiance under water-30 cm depth (µmol photon m ² s ⁻¹)	O	479 ± 39.68	663.33 ± 81.72
	M	127 ± 21.73	46 ± 19.6

TP, total phosphorous; TN, total nitrogen; O, open water; M, macrophytes.

zooplankters (replicates ranged from 12 to 148 ind. L⁻¹), followed by chydorids (0–238 L⁻¹), *A. robustus* (1–213 L⁻¹) and *B. gracilis* copepods (2–40 L⁻¹) and *Daphnia* cf. *commutata* (<8 L⁻¹). The predatory copepod *P. sarsi* and the cladoceran *Simocephalus serrulatus* Koch were present but sparse (<4 L⁻¹). In pond ÑGr, the zooplankton community was dominated mainly by copepod nauplii (0–447 L⁻¹), *B. gracilis* (29–237 L⁻¹), *A. robustus* (0–137 L⁻¹) and *C. dubia* (0–44 L⁻¹) (Fig. 3). Other taxa (*Simocephalus vetulus*, Chydoridae and harpacticoid copepods) were present but very rare (<2 L⁻¹). In both ponds, the predator *M. ehrenbergii* was present at abundances of <4 L⁻¹.

The distribution patterns of the most abundant zooplanktonic taxa and *M. ehrenbergii* in pond FA and ÑGr are presented in Figs 2 & 3, respectively. In pond FA, *M. ehrenbergii* preferred the vegetated area over the open water, but only on the first day of sampling (Fig. 2; Table 3). In addition, there were no consistent differences in the abundance of copepod nauplii among days (Table 3), although a positive selection for the open water over macrophytes (Juncaceae) was observed on day 2 (Table 3). *Boeckella gracilis* copepodites showed a regular distribution pattern in pond FA over the 3 days and nights, the same was observed for the predatory copepod *P. sarsi*. The distribution pattern of adult *B. gracilis* (found only during the third day of sampling), where there was an increase in all habitats at night (significant effect of 'time'), was consistent with a daily vertical migration (Table 3;

Table 2 Average ± SE of physical and chemical parameters measured during the sampling campaign in ponds Fantasma (FA) and Ñirihuau Grande (ÑGr)

Fig. 2). This suggests that the animals were not caught by the sampling device during the day, suggesting that they were in or just above the sediments. A significant effect of time and habitat was observed for *A. robustus* on the three consecutive days of sampling (Table 3). This cyclopoid chose the vegetated area (Juncaceae) over the non-vegetated one, but it was more numerous in both the water column and in macrophytes at night (Table 3; Fig. 2). This is again consistent with a daily vertical migration. The cladoceran *Daphnia* cf. *commutata* selected the open waters during the day and by night (Table 3; Fig. 2). There were no consistent significant differences in the daily spatial distribution of the other crustacean zooplankton species.

In pond ÑGr, the predator *M. ehrenbergii* preferred the vegetated area over the water column on one of the three survey days (day 2) (Table 4). Copepod nauplii, *B. gracilis* adults and copepodites did not exhibit a distinctive distribution pattern, either in the pond or during the 3 days (Table 4; Fig. 3). Data on the distribution pattern of *A. robustus* showed a significant overall effect of habitat and time on days 2 and 3. This is consistent with a daily vertical migration. The distribution of the cladoceran *C. dubia* showed a similar pattern over the three days, the interactions obtained between habitat and time indicating a daytime preference for the vegetated area (a mixed bed of Juncaceae and *M. quitense*) (Table 4; Fig. 3), which is consistent with a daily horizontal migration.

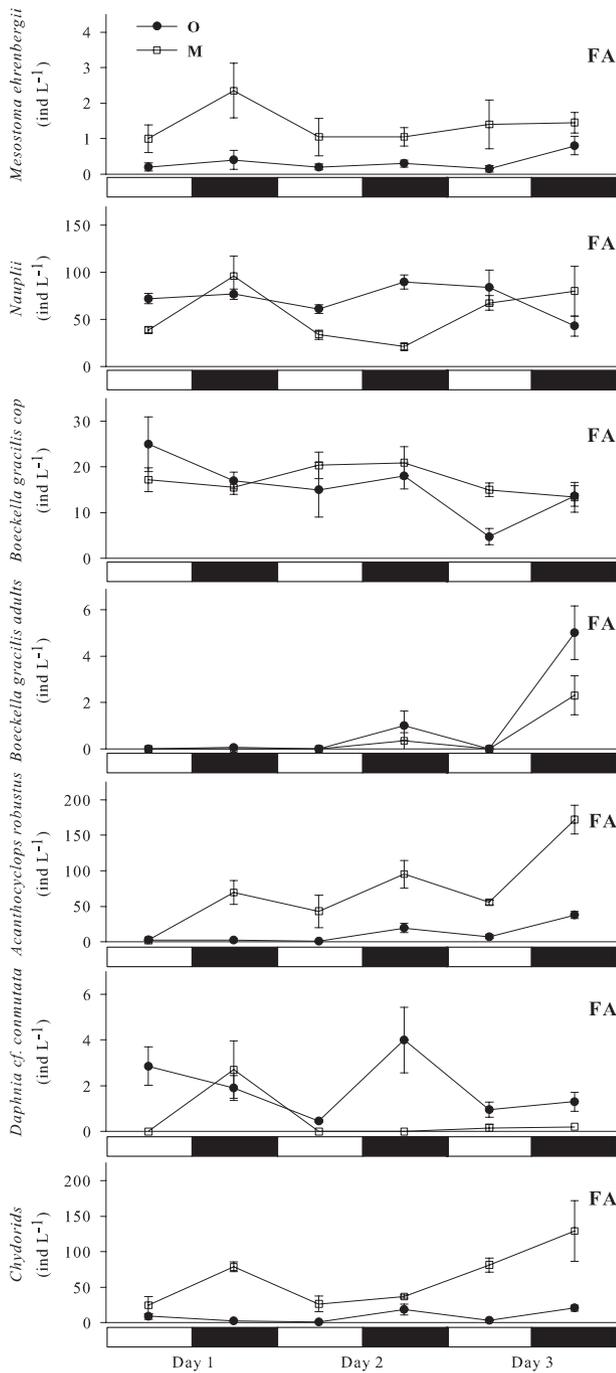


Fig. 2 Diel variation of mean (\pm SE) *Mesostoma* and zooplankton abundances during the campaign in pond Fantasma (FA). The bars on the x-axis represent day (white) and night (black) sampling periods: O, open water; M, macrophytes.

The response of zooplankters to chemical cues

Since the three zooplankters showed no selection in the controls, the results of the experiments suggest that the device *per se* had no effect on their horizontal

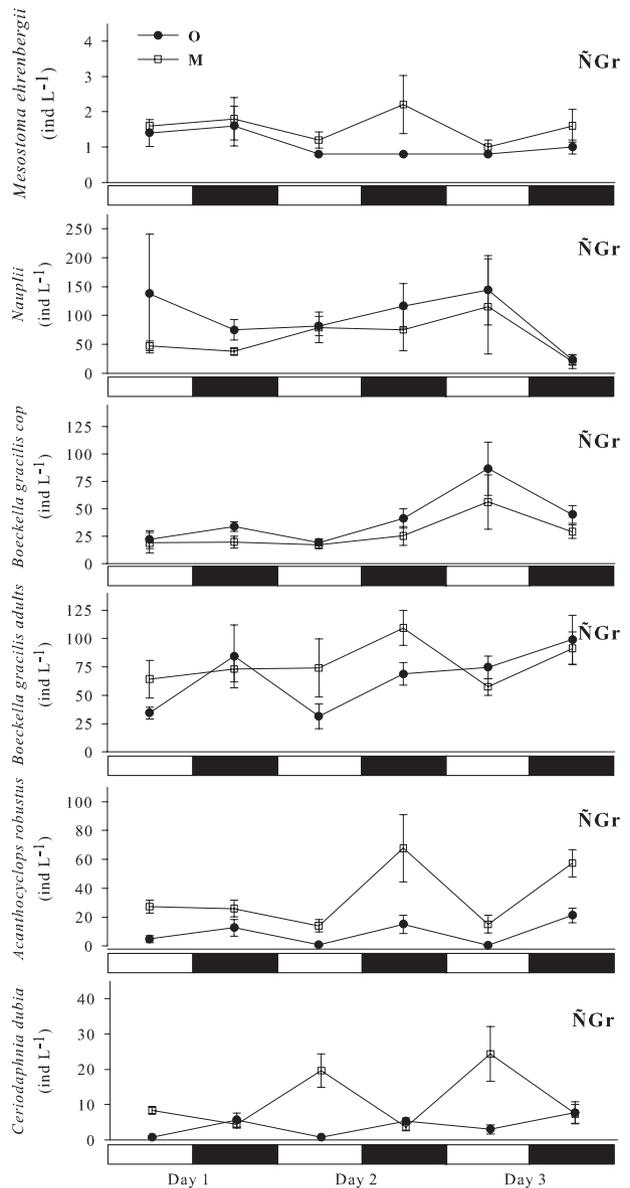


Fig. 3 Diel variation of mean (\pm SE) *Mesostoma* and zooplankton abundances during the campaign in pond Ñirihuau Grande (ÑGr). The bars on the x-axis represent day (white) and night (black) sampling periods: O, open water; M, macrophytes.

movement (Table 5). However, the different chemical signals studied elicited species dependent responses. In particular, the signal provided by the presence of the predator (PREDATOR) affected the behaviour of *B. gracilis* ($\chi^2 = 4.263 P < 0.05$; Table 5), which selected the side opposite to that containing *M. ehrenbergii*. *Acanthocyclops robustus* and *C. dubia*, on the other hand, did not significantly evade or select the vessel that contained the predator. Instead, these two zooplankters responded negatively to the presence of

Table 3 Two-way RM ANOVA values obtained for the comparison of zooplankton abundance between the vegetated area and the water column of pond Fantasma at mid-day and night

RM ANOVA	Day 1			Day 2			Day 3		
	d.f.	MS	P-value	d.f.	MS	P-value	d.f.	MS	P-value
<i>Mesostoma ehrenbergii</i>									
Habitat	1	0.323	0.016	1	0.153	0.047	1	0.174	0.038
Time	1	0.069	0.113	1	0.004	0.345	1	0.053	0.116
Habitat × time	1	0.025	0.305	1	0.000	0.892	1	0.019	0.315
<i>Nauplii</i>									
Habitat	1	0.041	0.102	1	0.795	0.001	1	0.011	0.768
Time	1	0.150	0.024	1	0.002	0.702	1	0.118	0.126
Habitat × time	1	0.110	0.043	1	0.137	0.014	1	0.062	0.244
<i>Boeckella gracilis</i> copepodites									
Habitat	1	0.028	0.307	1	0.050	0.220	1	0.232	0.032
Time	1	0.027	0.220	1	0.023	0.455	1	0.152	0.088
Habitat × time	1	0.009	0.466	1	0.019	0.500	1	0.287	0.031
<i>B. gracilis</i> adults									
Habitat							1	0.091	0.138
Time							1	1.456	0.001
Habitat × time							1	0.091	0.138
<i>Parabroteas sarsi</i>									
Habitat	1	0.000	0.862	1	0.069	0.143	1	0.047	0.236
Time	1	0.059	0.163	1	0.031	0.140	1	0.025	0.375
Habitat × time	1	0.002	0.764	1	0.090	0.027	1	0.039	0.276
<i>Acanthocyclops robustus</i>									
Habitat	1	1.803	0.006	1	3.972	0.002	1	2.522	0.001
Time	1	1.683	0.001	1	2.188	0.003	1	1.538	0.002
Habitat × time	1	1.909	0.001	1	0.210	0.193	1	0.082	0.262
<i>Daphnia cf. commutata</i>									
Habitat	1	0.230	0.021	1	0.653	0.001	1	0.227	0.001
Time	1	0.154	0.129	1	0.237	0.004	1	0.009	0.600
Habitat × time	1	0.381	0.032	1	0.237	0.004	1	0.001	0.832
<i>Simocephalus serrulatus</i>									
Habitat	1	0.006	0.278	1	0.016	0.341	1	0.193	0.014
Time	1	0.000	0.993	1	0.037	0.040	1	0.063	0.016
Habitat × time	1	0.012	0.291	1	0.007	0.288	1	0.019	0.119
<i>Chydorids</i>									
Habitat	1	3.130	0.001	1	1.624	0.039	1	4.156	0.001
Time	1	0.034	0.448	1	1.771	0.020	1	0.708	0.005
Habitat × time	1	1.061	0.004	1	0.228	0.305	1	0.344	0.022

Significant values are indicated in bold (the level of significance used was 0.017 - see text for further explanation).

alarm cues (SIGNAL) in the system ($\chi^2 = 5.556$ $P < 0.05$ and $\chi^2 = 4.264$ $P < 0.05$, respectively; Table 5). Individuals of both species selected the side opposite to that emitting the signal from the homogenate of their conspecifics. In contrast, *B. gracilis* did not respond to the alarm signal ($\chi^2 = 0.842$ $P > 0.05$; Table 5).

When the signal was generated by vegetation, we obtained contrasting responses, depending on taxa. None of the prey studied showed any particular reaction to the presence of the submerged macrophyte *M. quitense* (Table 5). However, the signal provided by

the emergent macrophyte *J. pallescens* elicited a response from *B. gracilis* ($\chi^2 = 5.556$ $P < 0.05$; Table 3), which selected the side containing the Juncaceae.

In this study, we observed that *B. gracilis* reacted to two different signals; negatively to that provided by the invertebrate predator *M. ehrenbergii* and positively to the aquatic plant *J. pallescens*. Therefore, we performed a combined experiment, placing the predator and the vegetation in the same vessel to determine which signal was stronger. However, this combination elicited no clear choice by *M. ehrenbergii*, a similar number of individuals choosing the side that

Table 4 Two-way RM ANOVA values obtained for the comparison of zooplankton abundance between the vegetated area and the water column of pond Nirihuau Grande at midday and at night

RM ANOVA	Day 1			Day 2			Day 3		
	d.f.	MS	P-value	d.f.	MS	P-value	d.f.	MS	P-value
<i>Mesostoma ehrenbergii</i>									
Habitat	1	0.006	0.604	1	0.080	0.008	1	0.019	0.275
Time	1	0.001	0.872	1	0.015	0.481	1	0.019	0.122
Habitat × time	1	0.000	0.886	1	0.015	0.481	1	0.003	0.476
<i>Nauplii</i>									
Habitat	1	0.185	0.301	1	0.120	0.487	1	0.220	0.565
Time	1	0.005	0.776	1	0.003	0.775	1	3.019	0.023
Habitat × time	1	0.012	0.657	1	0.069	0.202	1	0.001	0.965
<i>Boeckella gracilis</i> copepodites									
Habitat	1	0.146	0.244	1	0.086	0.165	1	0.180	0.178
Time	1	0.260	0.291	1	0.189	0.121	1	0.225	0.044
Habitat × time	1	0.015	0.789	1	0.025	0.539	1	0.003	0.761
<i>B. gracilis</i> adults									
Habitat	1	0.047	0.295	1	0.319	0.038	1	0.016	0.387
Time	1	0.177	0.107	1	0.355	0.014	1	0.083	0.149
Habitat × time	1	0.060	0.311	1	0.027	0.379	1	0.010	0.581
<i>Acanthocyclops robustus</i>									
Habitat	1	1.687	0.027	1	2.570	0.005	1	1.989	0.002
Time	1	0.043	0.544	1	2.308	0.002	1	3.499	0.001
Habitat × time	1	0.080	0.414	1	0.021	0.644	1	0.300	0.045
<i>Ceriodaphnia dubia</i>									
Habitat	1	0.516	0.013	1	0.817	0.004	1	0.680	0.059
Time	1	0.096	0.176	1	0.009	0.494	1	0.024	0.492
Habitat × time	1	0.659	0.007	1	1.459	0.001	1	0.591	0.012

Significant values are indicated in bold (the level of significance used was 0.017 - see text for further explanation).

Table 5 Chi-squared values obtained for the different treatments in the choice experiments

Treatments/zooplankters	<i>Boeckella gracilis</i>	<i>Acanthocyclops robustus</i>	<i>Ceriodaphnia dubia</i>
CONTROL	1.000	2.000	0.667
PREDATOR	4.263* (-)	2.579	0.200
SIGNAL	0.842	5.556* (-)	4.264* (-)
VEG1/ <i>Myriophyllum quitense</i>	2.778	0.889	0.889
VEG2/ <i>Juncus pallescens</i>	5.556* (+)	0.222	2.000
PREDATOR and <i>Juncus pallescens</i>	0.050	-	-

(-) Negative response to the treatment; (+) positive response to the treatment.

*Significant differences ($\chi^2 > 3.84$).

contained both the predator and the plant and that without a signal ($\chi^2 = 0.050$ $P > 0.05$; Table 5).

Discussion

Our study indicated that the turbellarian predator *M. ehrenbergii* can modify habitat selection by different zooplankton species in fishless ponds. Overall, in the field samplings performed when *M. ehrenbergii* was present, we observed consistent, either vertical or horizontal, patterns in the spatial distribution of

A. robustus and *C. dubia*, although data on *B. gracilis* abundances did not, as expected, reveal habitat selection by this copepod. Laboratory experiments showed that the presence of *M. ehrenbergii* can indirectly affect habitat selection by the cyclopoid copepod *A. robustus* and the cladoceran *C. dubia*, since both of these zooplankters exhibited a negative response to the alarm signal produced by crushed conspecifics. Their response indicates recognition of a non-specific predation risk (Pijanowska, 1997; Meerhoff *et al.*, 2006). Moreover, the experiments suggested

that *M. ehrenbergii* can directly affect the habitat selection of the calanoid copepod *B. gracilis*, since in our experiment this zooplankton avoided the predator. These apparent contradictions obtained between field and laboratory results are explored below.

The field survey showed that the cyclopoid *A. robustus* preferred the macrophyte stand over the water column, and its greater abundance at night indicates a daily vertical migration. We interpret the responses of *A. robustus* as a potential defence mechanism against predation by *M. ehrenbergii*, which is less effective in structured habitats (Trochine *et al.*, 2006). A similar result was obtained by Burks *et al.* (2001), who found that the presence of macrophytes (*Elodea canadensis* Rich.) protected daphniids against predation by roach (*Rutilus rutilus* L.), which foraged less efficiently among the macrophytes. In addition, the distribution of *C. dubia* in the field (pond ÑGr) indicated that this cladoceran preferred the vegetated area to the water column during the day, probably because of the refuge effect and the higher availability of food.

The distribution of *B. gracilis* in the field (ponds FA and ÑGr) was homogeneous. Possible causes for this are: (i) *Mesostoma* did not positively select either the vegetated area or the water column habitats, resulting in a lack of gradient in the chemical cues of the predator; (ii) the biomass and composition of macrophytes, and the abundances of *Mesostoma* in the field, may have differed from those used in the laboratory experiments (possibly the balance/imbalance of the signals produced by these two factors altered migration by the prey) and (iii) the presence of many predators in the field may have altered the prey's spatial behaviour. Van de Meutter, Stoks & De Meester (2005) tested the spatial behaviour of *Daphnia magna* Straus in a multipredator context and found that the predator effect on the spatial behaviour of the prey can vary considerably in strength and direction, depending on the presence of more than one predator. These authors suggest that interactions and facilitation effects among the predators can be important, and that these may vary. Supporting this idea, previous studies conducted in pond FA revealed effects of predation by *P. sarsi* on the tail spine length of *Daphnia* (Balseiro & Vega, 1994) as well as on the size of the colony of planktonic rotifers (Diéguez & Balseiro, 1998). Another study from another pond nearby showed that *Ceriodaphnia* was neither attacked nor eaten by the predatory water mite *Limnesia*

patagonica Lundblad (Balseiro, 1992). The other predators mentioned in our study, especially Dytiscidae and Hydrophylidae, might have an effect on the zooplankton communities in ponds FA and ÑGr, although their impact has not yet been assessed.

Physical and chemical gradients were identified in pond ÑGr. The vegetated areas exhibited significantly less PAR irradiance and higher values of Chl-*a* and TP. This suggests that the environment of pond ÑGr is patchy and highly heterogeneous. Physical and chemical gradients that provide directional cues presumably increase habitat selection by zooplankton. In addition, the abundance of zooplankton differed markedly between replicate samples, indicating micro-scale patchiness. A study performed by Nurminen, Horppila & Tallberg (2001) in a turbid and eutrophic lake basin in Finland also showed that helophytes encourage the aggregation of large-bodied filterers (cladocerans) within the vegetation. However, in a subsequent investigation by Nurminen & Horppila (2002) in a basin with clear water, the emergent vegetation stand seemed insufficient to provide refuge for zooplankton or induce diel migration, as the highest cladoceran densities were obtained on the fringes of the plant stand.

According to our experimental results, the presence of the submerged *M. quitense* did not affect the horizontal movements of any of the zooplankters studied. This is in contrast to the findings of other choice experiments (Lauridsen & Lodge, 1996) and field studies (Lauridsen & Buenk, 1996; Meerhoff *et al.*, 2003), where zooplankters have been shown to react negatively to the signals provided by the submerged plants. Moreover, these investigations showed preference for the submerged *Myriophyllum exalbescens* L. by *D. magna* in north temperate lakes (Lauridsen & Buenk, 1996; Lauridsen & Lodge, 1996), whereas *Ceratophyllum demersum* L. repelled *D. obtusa* in subtropical lakes in the presence of predator cues (Meerhoff *et al.*, 2006). Further, the results of Meerhoff *et al.* (2007) indicated that the more complex structure of the submerged plants offers better conditions than other plant forms for the free-swimming cladocerans in temperate and subtropical lakes, despite the high density of fish among the plants, especially in the subtropics. In previous laboratory experiments (Trochine *et al.*, 2006), however, we have shown that vertical structured habitats do not provide a refuge from *Mesostoma* predation. Further, Marko *et al.* (2008) recently showed the importance of the chemical

profiles of different species of *Myriophyllum* in determining the ability of plants to compete and defend themselves against herbivores. Marko *et al.* (2008) suggested that higher concentrations of polyphenols may facilitate competitive success in a new environment. These generalized defences can help protect the plants against UV damage, phytoplankton competitors and generalist herbivores. Conversely, a higher carbon content may help protect the plants against damage by specialist herbivores. In this sense, the chemical profile of different species of *Myriophyllum* may have different potential effects on the response of zooplankters towards them (e.g. horizontal migration), which could explain the difference between our results and those obtained previously. Finally, the experiments with the emergent macrophyte *J. palleseus* showed that this aquatic plant affects the habitat selection of *B. gracilis*. This copepod species significantly selected for the side that contained the Juncaceae. This result is consistent with the lower predation risk for *B. gracilis* by *Mesostoma* found in horizontally structured habitats (Trochine *et al.*, 2006).

The antipredator response to predator odours and injury-released chemical cues of conspecifics presumably reduces the probability of being detected and attacked (Wisenden, 2000b). The predator avoidance behaviour exhibited by the zooplankters in the current laboratory studies concurs with this hypothesis, and many other examples of antipredator behaviour can be found in the literature (see Kats & Dill, 1998). Our investigation did not explore instantaneous responses such as reduced activity (and/or motility) and the response of prey to the diet of the predator, although two different responses could clearly be distinguished, one towards the predator and the other towards crushed prey (alarm signal). Our results therefore support our initial hypothesis that cues provided by the presence of *Mesostoma* and crushed prey are associated with a higher predation pressure on zooplankton in fishless Patagonian ponds. Even though *Mesostoma* was present in low abundances in ponds FA and ÑGr in the daily migration surveys, they were sufficiently numerous (<4 L⁻¹) to exert a significant predation pressure on the zooplankton (Trochine *et al.*, 2008). In particular, this previous investigation indicated that the magnitude of *M. ehrenbergii* predation is high (8–12 prey per predator per day) for intermediate-bodied zooplankton species such as *B. gracilis*,

A. robustus and *C. dubia* (Trochine *et al.*, 2008). Thus, we are certain that the three zooplankters used in the experimental study are highly and similarly susceptible to *Mesostoma* predation. In particular, Van de Meutter, Stoks & De Meester (2004) suggested that the antipredator behaviour induced by macroinvertebrate predators may be more predator-specific than that induced by fish. In this sense, the response exhibited by *B. gracilis* in the laboratory experiments may indicate a species-specific antipredator response. This concurs with the finding that *B. gracilis* uses Juncaceae as a refuge (this work) in that horizontally structured habitats lower the risk of predation by *M. ehrenbergii* (Trochine *et al.*, 2006). In contrast, as the response exhibited by the other two species was generic (triggered by injured conspecifics and not by cues from the predator), these zooplankton species may show the same avoidance response to the presence of *Mesostoma* or other potential predators. This, however, differs from the idea proposed by Van de Meutter *et al.* (2004).

Investigations into the effects of an invertebrate predator such as *M. ehrenbergii* on the habitat selection of zooplankters in fishless habitats bring increased insight into the different theories that exist on the responses of prey to predation. Our investigation supplements the investigations carried out in temperate (Kvam & Kleiven, 1995; Lauridsen & Buenk, 1996; Jeppesen *et al.*, 1998; Masson *et al.*, 2001; Burks *et al.*, 2002; Wojtal *et al.*, 2003), subtropical and tropical (Burks *et al.*, 2002; Meerhoff *et al.*, 2006; Iglesias *et al.*, 2007), and Mediterranean lakes (Castro *et al.*, 2007), and it reveals that the physical and chemical characteristics of the environment, and the composition and abundance of macrophytes beds, in addition to the presence of the turbellarian *M. ehrenbergii*, are all factors that combine to determine habitat selection by zooplankters in fishless Andean Patagonian ponds. Our results suggest that the zooplankton from south temperate shallow fishless environments may encounter less predation in macrophytes habitats, as has been documented for north temperate lakes with fish. In addition, the apparent contradictions between field and experimental results, and the important variation in the spatial patterns obtained in the field, underline the importance of combining different research approaches and conducting longer field behavioural studies than the most common method involving snap-shot samplings.

Acknowledgments

Special thanks go to Marcelo Guerrieri for his invaluable help in the field samplings and to Dr Sol Souza. We are grateful to Dr Erik Jeppesen for his comments on and suggestions to an earlier version of this manuscript. Linguistic assistance was provided by A.M. Poulsen. This work was supported by FONCyT PICT 01-13395, CONICET PIP 6507 and UNC B119. C. Trochine has a CONICET fellowship and B. Modenutti and E. Balseiro are CONICET researchers. We thank Alan Hildrew and two anonymous referees for valuable comments and linguistic improvements.

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(Manuscript accepted 14 September 2008)