

Macrophytes as refuge or risky area for zooplankton: a balance set by littoral predacious macroinvertebrates

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SUMMARY

1. Zooplankton use macrophytes as day-time refuge areas when trying to escape from pelagic predators. But macrophytes can also host a diverse and abundant macroinvertebrate assemblage and zooplankton are also likely to face predacious macroinvertebrates once they enter the littoral zone. This study aimed to elucidate the role of macroinvertebrates in determining the refuge capacity of macrophytes.
2. We conducted a field enclosure experiment using plastic bags and complementary laboratory feeding trials to test how macroinvertebrates counteract the benefits to zooplankton of the macrophyte refuge. The field experiment consisted of three treatments with different macroinvertebrate assemblages: without predators (WP), low abundance and diversity (LAD) and high abundance and diversity of predators (HAD – which represents lake conditions).
3. Populations of *Diaphanosoma brachyurum*, *Bosmina huaronensis* and *Moina micrura* (Cladocera) and of both male and female *Notodiaptomus incompositus* (Copepoda, Calanoida) declined (by nearly 80%) in the presence of HAD in comparison to WP and LAD treatments.
4. Feeding trials revealed that *Buenoa* sp. (backswimmer), adults of *Palaemonetes argentinus* (grass shrimp) and *Cyanallagma interruptum* (damselfly) had a significant negative impact on cladocerans (*D. brachyurum*, *B. huaronensis*) and the calanoid copepod population (males, females and copepodites). These predators showed a strong predation effect ranging from 75% to 100% reductions of zooplankton populations.
5. The refuge effect offered by macrophytes to zooplankton depends on and is balanced by the predacious macroinvertebrate assemblage that plants host. The risk of confronting littoral predators is high and macroinvertebrate presence can turn the macrophytes into risky areas for zooplankton.

Keywords: macroinvertebrates, macrophytes, predation, refuge, zooplankton

Introduction

Macrophytes are key organisms in shallow lakes as they modify trophic interactions and other aspects of lake functioning. Their establishment and dominance

is correlated with a clear water phase (Timms & Moss, 1984; van den Berg *et al.*, 1998), promoted by mechanisms including reductions in sediment resuspension, wave and wind action (Barko & James, 1998; Horppila & Nurminen, 2003), and the provision of

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refuges for zooplankton that promote higher grazing rates on phytoplankton (Schriver *et al.*, 1995; Søndergaard & Moss, 1998; Scheffer, 1999).

Several studies have reported the migration of zooplankton to the edge of macrophyte stands during daytime (diel horizontal migration, DHM) to seek refuge from planktivorous fish (Schriver *et al.*, 1995; Lauridsen & Buenk, 1996; Lauridsen *et al.*, 1998). Large zooplankton species, like *Daphnia*, and even smaller ones, like *Ceriodaphnia*, display horizontal migrations (Lauridsen & Buenk, 1996; Stansfield *et al.*, 1997; Moss, Kornijów & Measy, 1998). The effectiveness of macrophytes as refuge areas depends on various factors including plant architecture, size, shape and density of plant patches, and also on the predators that these plants host (Jeppesen *et al.*, 1998). Indeed, macrophytes provide habitat for predacious macroinvertebrates, including odonates, notonectids, dytiscid beetles and water mites, which could exert a strong predation impact on zooplankton populations (Balseiro, 1992; Hirvonen, 1999; Hampton, Gilbert & Burns, 2000). In shallow lakes, therefore, zooplankton must face planktivorous fish and predacious invertebrates in the pelagic zone, and benthic, epiphytic and epineustonic invertebrate predators in the littoral zone. As a consequence, the benefit of escaping from pelagic predators must be balanced with the cost of confronting predators in the littoral zone.

Direct predation on zooplankton by epiphytic and benthic invertebrates is a potentially large, and overlooked, cost of DHM (Burks *et al.*, 2002). Most studies supporting the effectiveness of DHM as an anti-predator behaviour have focused on northern temperate lakes (Burks *et al.*, 2002), mainly in Europe. In contrast, knowledge about the effectiveness of macrophytes as refuge areas for zooplankton from tropical and subtropical as well as temperate areas from the southern hemisphere is limited (Burks *et al.*, 2002; González Sagrario, 2004). Moreover, the lack of a DHM pattern for cladoceran species has been associated with the presence of predacious macroinvertebrates in the littoral zone of some New Zealand and North America lakes (Smiley & Tessier, 1998; Lauridsen *et al.*, 1999). Still, the role of macroinvertebrates and how they can alter macrophyte refuge provision are not well understood or documented. Our objective in this study is to assess the cost associated with the use of macrophyte stands when these host a rich assemblage of macroinvertebrates and to determine

whether predacious invertebrates counteract the refuge offered by macrophytes.

Methods

Study site

Los Padres Lake, located in Buenos Aires Province of Argentina (Pampa Plain, at 37°55' and 38°02'S and 57°34' and 57°33'W) is a small, shallow, eutrophic lake (area = 2 km²; mean depth = 1.8 m) with a polymictic thermal regime and alkaline water (pH = 8.6). The transparency is variable with turbid and clear periods varying from year to year (Secchi disk depth ranged from 40 to 90 cm during 1998–2002). Values for chlorophyll-*a* ranged from 20 to 90 µg L⁻¹ and for total phosphorus from 100 up to 400 µg L⁻¹ during 1999–2001 (M. González Sagrario, unpubl. data).

In the littoral zone of Los Padres Lake, the bulrush *Schoenoplectus californicus* (Meyer) Steud. constitutes an outer ring around the whole lake, while in the inner part and near emergent plants, different species of submerged macrophytes dominate, such as *Potamogeton pectinatus* Ruiz and Pavón and *Ceratophyllum demersum* L. Plant coverage varies inter-annually, and in some years submerged plants are absent. Vegetated areas host small- to medium-sized fish species, birds and a rich macroinvertebrate assemblage.

In open waters, the zooplankton community is mainly composed of small- to medium-sized cladocerans including *Bosmina* (*Neobosmina*) *huaronensis* Delachaux, *Ceriodaphnia dubia* Richard, *Moina micrura* Kurz, and *Diaphanosoma brachyurum* (Lievin). Sporadically the large cladoceran species *Daphnia* (*Ctenodaphnia*) *spinulata* Birabén also occurs. Two species of calanoid copepods are present – *Notodiaptomus incompositus* (Brian) and *Boeckella bergi* Richard, with *N. incompositus* being the dominant species. *Acanthocyclops robustus* (Sars) dominates the cyclopoid copepod assemblage (González Sagrario, 2004).

In littoral areas, macrophytes host a very rich and diverse macroinvertebrate assemblage, comprised the predacious odonates [Coenagrionidae – *Cyanallagma interruptum* (Selys) and *C. bonariense* (Ris)- and Aeshnidae – *Aeshna* sp.], hemipterans [*Notonecta* sp. and *Belostoma elegans* (Mayr)], hydrophilid larvae (*Berosus* sp.), flatworms (*Mesostoma ehrenbergii* Focke), water mites (mainly Unionicolidae, *Koenikea curvipes* Rosso de Ferradás, and also *Arrenurus oxyurus* Rosso de

Ferradás, *Eylais multiespina* Ribaga, *Hydrachna* sp. and *Limnesia* sp.) and the grass shrimp *Palaemonetes argentinus* (Nobili) (González Sagrario, 2004). Macroinvertebrates show a strong association with macrophyte stands, occupying the inner parts and/or edges of vegetated patches during day and night, with potential zooplankton predators reaching very high abundances (e.g. sum of *C. bonariense* and *C. interruptum*: 150–200 ind. m⁻², *P. argentinus*: 20–30 ind. m⁻²) (González Sagrario, 2004).

Experiment 1: Effect of littoral macroinvertebrate diversity and abundance on zooplankton populations

We performed a field enclosure experiment in the littoral zone of the lake using plastic bags filled with zooplankton in lake water in February 2003. The experimental design consisted of three treatments: (i) without predacious macroinvertebrates (without predator; WP); (ii) low abundance and diversity treatment (LAD), with seven larvae of the damselfly *C. interruptum* (Coenagrionidae, Zygoptera) in the two final instars and (iii) high abundance and diversity treatment (HAD), containing eight early and eight late instars of *C. interruptum* larvae, two adults and three zoeae of the grass shrimp *P. argentinus* (Palaemonidae, Decapoda), one juvenile of *B. elegans* (Belostomalidae, Hemiptera), three adults of *Buenoa* sp. (Notonectidae; Hemiptera) and one larva of the dytiscid beetle *Megadytes* sp. (Dytiscidae; Coleoptera). Each treatment had six replicates. The diversity and abundance of HAD resembled the field assemblage in macrophyte patches examined the previous year (mean of inner and edge abundances for each taxon in a study of macroinvertebrate distribution – González Sagrario, 2004). We chose *C. interruptum* as the unique predator in the LAD treatment because it was the most abundant potential predator in littoral areas during the previous summer.

Each enclosure (transparent plastic bags, length 80 cm, diameter 38 cm, area 0.12 m², thickness 100 µm) hung freely from four wooden rafts anchored in the sediment in open water near the littoral zone. Each bag was sealed at the bottom, with 35–40 cm immersed in the water column and 40–45 cm projecting from the water surface. A plastic mesh covering the upper part excluded bird interference. To fill the enclosures, we first collected lake water at an open water station 100 m from the shore, sampling the

whole water column with a Schindler-Patalas trap (6 L) and storing it in two buckets. Then, after mixing the water 10 times in each bucket, we took 1 L with a graduated beaker to fill each enclosure. After pouring 1 L into each bag, the remaining water in the buckets was discharged and fresh water was collected. This procedure lasted until each enclosure had a final volume of 13 L. This methodology followed that of Hampton *et al.* (2000) to achieve a homogeneous zooplankton distribution amongst replicates and a representative zooplankton in terms of abundance and assemblage.

Macroinvertebrate collection occurred in Las Nutrias Lake (close to our lake), because in Los Padres Lake no macrophyte stands appeared in summer 2003 and macroinvertebrate abundances were very low. Las Nutrias Lake is a small shallow lake with macroinvertebrate and fish assemblages similar to those of Los Padres Lake. Macroinvertebrate sampling took place 2 days before the set up of the experiment. Once caught, we transported animals to the laboratory and placed them in plastic containers (length 20 cm; width 10 cm), in groups of four to five individuals, containing lake water with natural zooplankton densities (food) from Los Padres Lake. After 24 h, we placed the macroinvertebrates in filtered lake water (mesh 65 µm) free of zooplankton for another 24 h. The sequence of 24 h of feeding and starvation aimed to standardise macroinvertebrate feeding conditions (Hirvonen, 1999).

After filling the plastic enclosures with lake water, we distributed macroinvertebrates into the enclosures according to the experimental design. Lengths of all macroinvertebrates used are shown in Table 1. All the enclosures had several stems of *P. pectinatus* (with a per cent volume infested by plants of 10–15%) to allow macroinvertebrate perching.

Zooplankton sampling occurred at the beginning (after filling the bags) on February 20, and at the end of the experiment, on February 24. We took a 1 L sample after very gently mixing the entire water column inside the bags four times. Then, we filtered the sample using a 65 µm mesh and fixed it with acid Lugol's solution. We counted all zooplankton in each sample under a dissecting microscope using a 5 mL Bogorov chamber. We identified cladocerans to species and enumerated calanoid copepods as female, male, sum of V and IV copepodites and sum of III and II and I copepodites.

Table 1 Body length of predacious macroinvertebrates in Experiment 1 in Lake Los Padres

	Head capsule width (mm) Mean \pm SD	Cephalothorax length (mm) Mean \pm SD	Body length (mm) Mean \pm SD
<i>Cyanallagma interruptum</i> early instar	1.28 \pm 0.39	–	5.74 \pm 2.30
<i>C. interruptum</i> final instar	2.78 \pm 0.32	–	12.72 \pm 1.47
<i>Megadytes</i> sp.	2.58 \pm 0.60	–	26.25 \pm 6.50
<i>Belostoma elegans</i>	–	–	9.81 \pm 2.10
<i>Buenoa</i> sp.	–	–	6.11 \pm 0.25
<i>Palaemonetes argentinus</i> adults	–	3.60 \pm 0.40	15.80 \pm 1.50
<i>P. argentinus</i> zoeae	–	1.40 \pm 0.30	6.50 \pm 0.60

Caudal appendices and uropods of *Cyanallagma interruptum* and *Palaemonetes argentinus*, respectively, were excluded from body length measures.

–, not determined.

Experiment 2: Direct prey–predator interactions between zooplankton and macroinvertebrates

To evaluate specific prey–predator relationships we used feeding trials. Experiments lasted 48 h and took place in the laboratory under ambient temperature and normal light conditions (photoperiod of 14 : 10 h of light : dark, with a maximum light (photosynthetically active radiation) of 150 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ according to a Licor Li-250 radiometer (LI-COR Inc., Lincoln, NE, U.S.A.). Each macroinvertebrate predator used in the field experiment was represented in its own treatment in the laboratory: (i) *Cyanallagma* early instars; (ii) *Cyanallagma* late instars; (iii) *Palaemonetes* adults; (iv) *Palaemonetes* zoeae; (v) *Buenoa*; (vi) *Belostoma*; (vii) *Megadytes* and (viii) control (without predacious macroinvertebrates). Each feeding trial hosted one predator. Treatments had five replicates, except *Megadytes* ($n = 3$) and *Buenoa* ($n = 4$). Forty-eight hours before experiment initiation, we standardised predator food conditions by exposing animals to 24 h of zooplankton presence and 24 h of starva-

tion, as in experiment 1. Macroinvertebrate lengths are shown in Table 2.

To set up the experiment we filled each 1 L plastic beaker with 600 mL of filtered lake water (free of zooplankton, mesh size = 65 μm), and then we added an aliquot of a 'zooplankton concentrate' to ensure that final abundance resembled lake densities. To determine natural lake zooplankton abundances, we sampled with a Schindler-Patalas trap the whole water column at three stations ($n = 3$) and counted the zooplankton. To make the 'zooplankton concentrate' we filtered lake water and counted the density in the concentrate to determine the aliquot to be taken. This procedure achieved homogeneous and natural zooplankton abundance in the feeding trials. We allowed zooplankton to acclimate to the trial condition for 30 min; after that, we added the predator and let it acclimate for 30 min. After acclimation, we considered the experiment to have begun. One stem of the submerged macrophyte *Bacopa* sp. served as substrate for macroinvertebrates if needed.

Table 2 Body length of predacious macroinvertebrates in Experiment 2 (feeding trials)

	Head capsule width (mm) Mean \pm SD	Cephalothorax length (mm) Mean \pm SD	Body length (mm) Mean \pm SD
<i>Cyanallagma interruptum</i> early instar	1.80 \pm 0.00	–	8.61 \pm 1.57
<i>C. interruptum</i> final instar	3.21 \pm 0.08	–	17.07 \pm 0.71
<i>Megadytes</i> sp.	3.75 \pm 2.33	–	41.55 \pm 26.09
<i>Belostoma elegans</i>	–	–	15.84 \pm 5.13
<i>Buenoa</i> sp.	–	–	8.04 \pm 1.70
<i>Palaemonetes argentinus</i> adults	–	4.09 \pm 0.31	16.2 \pm 1.36
<i>P. argentinus</i> zoeae	–	1.60 \pm 0.21	6.89 \pm 0.81

Caudal appendices and uropods of *Cyanallagma interruptum* and *Palaemonetes argentinus*, respectively, were excluded from body length measures.

–, not determined.

To determine initial zooplankton abundance we sampled an aliquot of 100 mL. At the end of the experiment (48 h later) we filtered the whole water volume through a mesh of 65 μm and fixed with acid Lugol's solution. We counted all samples without subsampling.

Statistical analyses

We tested differences in initial conditions in experiments 1 and 2 using one way-ANOVA, with predator treatment as the single factor. Then, we used repeated measures ANOVA to determine differences amongst the experimental treatments. To accomplish data normality and homoscedasticity we applied the appropriate transformation according to data characteristics. Within each experiment, we performed the Dunn-Šidák formula to adjust the acceptable P -value to allow for non-independence of zooplankton taxa across analyses (experiment-wise $P = 0.05$). For experiments 1 and 2, the adjusted α was 0.0073 and 0.00851, respectively. For *post hoc* comparisons we carried out the Student-Newman-Keuls (SNK) test (Underwood, 1997). We tested differences only for the final condition. Significance level was fixed at $P = 0.05$. Again, we applied the Dunn-Šidák correction for non-independence of zooplankton taxa and for multiple comparisons of means, resulting in an adjusted α of 0.00244 and 0.00106 for experiments 1 and 2, respectively.

For the laboratory experiment, we estimated relative strengths of trophic interactions by determining the net predation effect (PE) of each predator treatment on each prey group. We followed Paine's formula (Paine, 1992) to calculate per capita net PE:

$$\text{PE} = [(N_t - N_c)/N_c]/D_p$$

where N_t , the abundance of prey in the treatment; N_c , the abundance of prey in the control and D_p , the abundance of the predator (which, in this case, is 1). Also, we classified PE according to Johnson *et al.* (1996), as weak ($|\text{PE}| < 25\%$), moderate ($25\% \leq |\text{PE}| \leq 50\%$) or strong ($|\text{PE}| > 50\%$).

Finally, we analysed the degree of selectivity of each predator using Chesson's selectivity index (α) (Chesson, 1983):

$$\hat{\alpha}_i = \frac{r_i/n_i}{\sum_{j=1}^m r_j/n_j}, \quad i = 1, \dots, m$$

where r_i , the amount of i th ingested prey by the predator and n_i , the abundance of the i th prey at the beginning of the experiment. We estimated r_i as the difference between initial and final prey abundances. This index varies between 0 and 1 and assumes a preference for prey i when the value exceeds 0.5. An interesting advantage of α is that results are unaffected by the relative abundance of the food item. Comparisons amongst indexes were analysed with an ANOVA after square-root transformation.

Results

Predation impact of low and high diversity assemblages of macroinvertebrates on zooplankton populations (Experiment 1)

Initial zooplankton abundance did not differ among treatments, neither for cladocerans or calanoid copepods (one way-ANOVA: *D. brachyurum*: $F_{2,15} = 0.05$, $P = 0.95$; *M. micrura*: $F_{2,15} = 0.82$, $P = 0.46$; *B. huaronensis*: $F_{2,15} = 1.1$, $P = 0.36$; *N. incompositus*: females: $F_{2,15} = 0.09$, $P = 0.91$, males: $F_{2,15} = 1.16$, $P = 0.34$, V + IV copepodites: $F_{2,15} = 0.17$, $P = 0.40$, III + II + I copepodites: $F_{2,15} = 0.97$, $P = 0.84$). At the beginning of the experiment, *D. brachyurum* was the most abundant species (80 ind. L^{-1}), followed by *N. incompositus*, with adults equally distributed among sexes (30–35 ind. L^{-1}). The least abundant species were *M. micrura* and *B. huaronensis* (8–10 ind. L^{-1}). At the end of the experiment, the presence of a highly diverse and abundant assemblage of predators (HAD treatment) reduced all cladoceran and adult copepod abundances (Figs 1 & 2). Large- and medium-sized cladoceran species showed the same predation trend and only proved vulnerable to the HAD assemblage. However, small cladoceran species were vulnerable to both HAD and LAD assemblages. The larger cladoceran species *D. brachyurum* declined in HAD (Fig. 1) (SNK test: $P = 0.0002$ for both comparisons), but not in LAD and WP (where abundances did not differ, SNK test: $P = 0.50$). *Diaphanosoma brachyurum* survival was only 15% in HAD while in LAD it was 76% of that in WP treatment. *Moina micrura*, a medium-size species, also decreased in the HAD treatment and its final abundance was lower than in WP and marginally lower than in LAD (Fig. 1; SNK test: HAD–WP: $P = 0.0005$, HAD–LAD: $P = 0.003$). In HAD, only 13.51% survived, while 67% of *M. micrura* survived

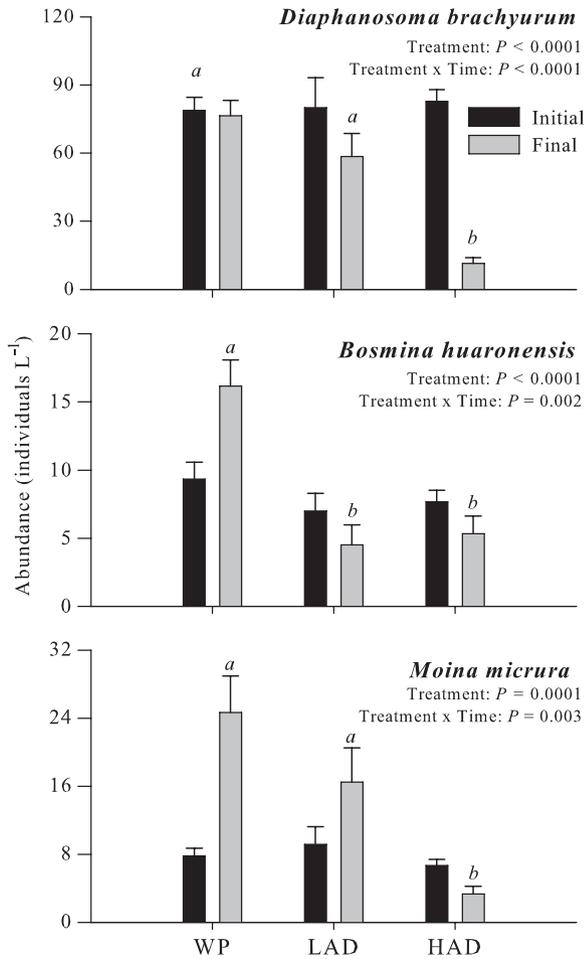


Fig. 1 Mean initial and final Cladocera species abundances (ind. L⁻¹) in the field enclosures in Lake Los Padres (Experiment 1). WP, without predators treatment; LAD, low abundance and diversity treatment; HAD, high abundance and diversity treatment. Blank and grey bars represent initial and final abundances. Error bars represent standard errors. Letters indicate significant differences among treatments at the end of the experiment (experiment-wise $P = 0.05$). P -values correspond to repeated measure-ANOVA results (d.f. for all tests: 2, 30; adjusted $\alpha = 0.0073$).

in LAD, compared to the WP treatment. In contrast, the smallest cladoceran, *B. huaronensis*, declined in the presence of both assemblages of predators (Fig. 1). In both LAD and HAD treatments, *Bosmina* abundance was lower than in WP (SNK: $P = 0.0004$ for LAD and $P = 0.0003$ for HAD), being 28% to 33% of the WP treatment.

Only larger individuals of the calanoid copepod *N. incompositus* declined in the HAD treatment. The HAD treatment suppressed both large females and males (Fig. 2, males SNK test: HAD-WP: $P = 0.001$;

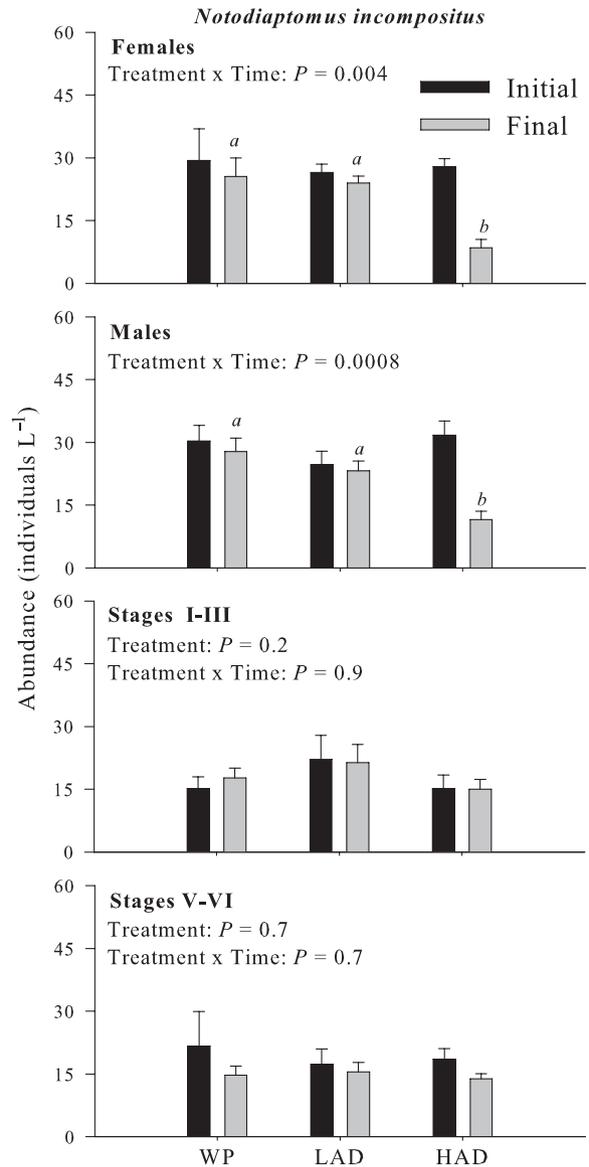


Fig. 2 Mean initial and final abundances (ind. L⁻¹) of *Notodiaptomus incompositus* in the field enclosures in Lake Los Padres (Experiment 1). WP, without predators treatment; LAD, low abundance and diversity treatment; HAD, high abundance and diversity treatment. Blank and grey bars represent initial and final abundances. Error bars represent standard errors. Letters indicate significant differences among treatments at the end of the experiment (experiment-wise $P = 0.05$). P -values correspond to repeated measure-ANOVA results (d.f. for all tests: 2, 30; adjusted $\alpha = 0.0073$).

females SNK test HAD-WP: $P = 0.004$), but the LAD treatment had no effect on these fractions of the population (males SNK test: LAD-WP: $P = 0.43$ and females SNK test LAD-WP: $P = 0.93$). Male and female survival in the HAD treatment was 33–41%

of the WP treatment while in LAD survival ranged between 84–93%. Neither treatment, LAD or HAD, had an effect on the different copepodite stages (V + IV or III + II + I) (Fig. 2).

Direct prey–predator interactions between zooplankton and macroinvertebrates (Experiment 2)

Bosmina huaronensis and *D. brachyurum* were the most abundant potential prey (613 and 143 ind. L⁻¹, respectively) in feeding trials. Cyclopoid copepods (103 ind. L⁻¹) and *N. incompositus* (25 females, 14 males and 115 copepodites L⁻¹) were also abundant. Potential prey distribution was homogeneous among treatments and reflected lake zooplankton structure and abundance (one-way ANOVA: *D. brachyurum*: $F_{7,29} = 0.37$, $P = 0.91$; *B. huaronensis*: $F_{7,29} = 1.17$, $P = 0.35$; *N. incompositus*: females: $F_{7,29} = 0.63$, $P = 0.73$, males: $F_{7,29} = 2.3$, $P = 0.06$, calanoid copepodites: $F_{7,29} = 0.28$, $P = 0.96$; Cyclopoida copepods: $F_{7,29} = 1.35$, $P = 0.26$).

Bosmina huaronensis and *D. brachyurum* were preferentially selected by several predators in the feeding trials (Table 3, Fig. 3). At the end of the experiment the abundance of both species had declined in treatments with *Buenoa*, *Cyanallagma* late instar and *P. argentinus* adults, while no suppression occurred in the control and treatments with *Belostoma*, *Megadytes*, *P. argentinus* zoeae or *Cyanallagma* early instar (SNK test in all cases: $P \geq 0.8$) (Fig. 3). Adults of *P. argentinus* were responsible for most of the decline in *D. brachyurum* (0.3% survival) (SNK test for all comparisons: $P \leq 0.00016$). *Buenoa* and *Cyanallagma* late instar had the next biggest impacts, registering a

marginally significant decrease and causing 88% and 70% of *Diaphanosoma* mortality, respectively (SNK test: *Buenoa*–control: $P = 0.001$, *Cyanallagma*–control: $P = 0.002$, adjusted $\alpha = 0.001$). *Buenoa*, *Cyanallagma* late instar and *P. argentinus* adults reduced *B. huaronensis* abundance, but with no differences among them (SNK test: *P. argentinus*–*Buenoa*: $P = 0.08$; *P. argentinus*–*Cyanallagma*: $P = 0.12$, *Cyanallagma*–*Buenoa*: $P = 0.8$) (Fig. 3). Instead, their impact significantly (SNK test: $P \leq 0.00016$ for *P. argentinus*) and marginally significantly differed with respect to the remaining treatments (SNK test: $P \leq 0.003$ for *Buenoa* and *Cyanallagma*) (Fig. 3). *Buenoa*, *Cyanallagma* late instar and *P. argentinus* adults showed strong predation impacts (>64%) on *Diaphanosoma* and *Bosmina* and for both prey, adults of *P. argentinus* accounted for the major PE, reducing the abundance by 100% relative to the control (Fig. 3).

The different size fractions of the calanoid population (*N. incompositus*) showed different vulnerability to predators, which was particularly high for *P. argentinus*. Males, females and calanoid copepodites all seemed to decline in the presence of *P. argentinus* adults (4%, 1.3% and 25.6% survival, respectively), accounting for the highest PE on these prey (84–99%) (Table 4; SNK test in all cases: $P \leq 0.0008$ for all comparisons) (Fig. 3). In contrast, the late instar of *Cyanallagma* only marginally suppressed calanoid females and copepodites (SNK test: females: *Cyanallagma*–control: $P = 0.004$, copepodites: *Cyanallagma*–control: $P = 0.001$). *Megadytes*, *Belostoma*, *Buenoa*, *Cyanallagma* early instar and the zoeae of *P. argentinus* had no effect on any of the size classes of this calanoid population (SNK test in all cases: $P \geq 0.06$) (Fig. 3).

Cyclopoid copepods were one of the more abundant prey in the feeding trials. Nevertheless, the overall mean was 103 and 104 ind. L⁻¹ for the initial and final conditions. Cyclopoids were the only prey that exhibited no effect of predation by any of the potential predators in the experiment (Table 3, Fig. 3).

Among all the predators, adults of *P. argentinus* caused the strongest predation impact on prey (75–100%), followed by *Cyanallagma* and *Buenoa* (Fig. 3). Despite the strong PE observed for these predators, none of them showed a clear preference for a specific prey according to Chesson's index (Table 4). Not a single selectivity index exceeded a value of 0.5, the threshold for assuming prey preference.

Table 3 *P*-values from repeated measure-ANOVA comparing mean prey abundance amongst predator treatments and time (Experiment 2)

Zooplankton prey	Treatment	Treatment × time
<i>Diaphanosoma brachyurum</i>	<0.00001	<0.00001
<i>Bosmina huaronensis</i>	<0.00001	<0.00001
<i>Notodiaptomus incompositus</i> females	0.003	0.006
<i>Notodiaptomus incompositus</i> males	0.0002	0.008
Calanoid copepodites (I–V)	0.00004	0.00001
Cyclopoid (adults and copepodites)	0.7	0.03

Significance level fixed at 0.05. After correction for non-independence of taxa, α equals 0.00851. d.f.: 7, 56.

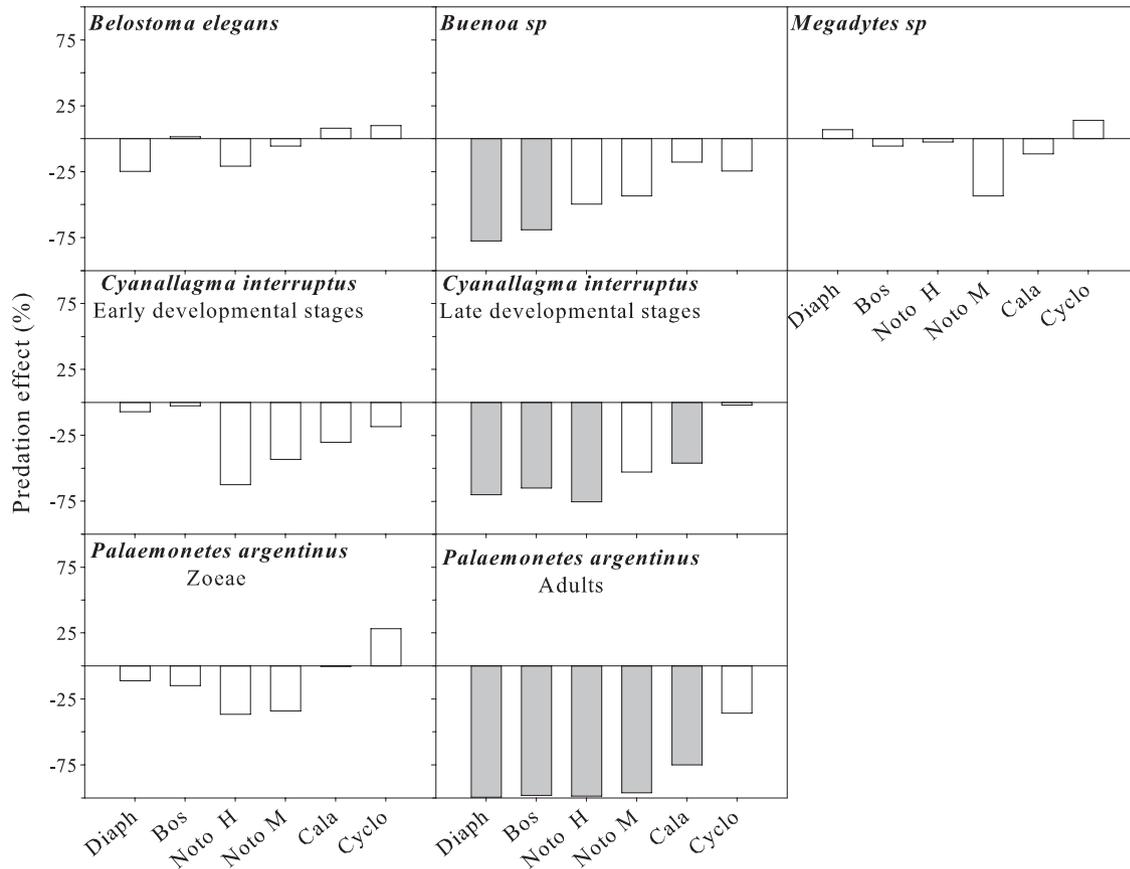


Fig. 3 Per capita net predation effect (PE) (%) on each zooplankton prey item caused by each potential macroinvertebrate predator in the feeding trial Experiment. PE was considered as weak ($|PE| < 25\%$), moderate ($25\% \leq |PE| \leq 50\%$) or strong ($|PE| > 50\%$) according to Johnson *et al.* (1996). Grey bars show predation effects associated with significant results of repeated measure-ANOVA in feeding trials (Experiment 2). Diaph., *Diaphanosoma brachyurum*; Bos., *Bosmina huaronensis*; Noto H and M, *Notodiptomus incompositus* female and male, respectively; Cala, sum of all calanoid copepodites; Cyclo., sum of cyclopoid adults and copepodites.

Table 4 Chesson's selectivity index of each predator for the various zooplankton prey offered in the feeding trials (Experiment 2)

Zooplankton prey	<i>Cyanallagma</i> late instar	<i>Cyanallagma</i> early instar	<i>Palaemonetes</i> adults	<i>Palaemonetes</i> zoeae	<i>Buenoa</i>	<i>Belostoma</i>	<i>Megadytes</i>
<i>Bosmina huaronensis</i>	0.141	0.023	0.189	0.044	0.197	0.027	0.0
<i>Diaphanosoma brachyurum</i>	0.170	0.098	0.192	0.054	0.194	0.376	0.017
<i>Notodiptomus incompositus</i> female	0.280	0.341	0.190	0.331	0.081	0.326	0.104
<i>N. incompositus</i> males	0.137	0.257	0.182	0.313	0.163	0.043	0.344
Calanoid copepodites	0.209	0.183	0.148	0.083	0.122	0.118	0.401
Cyclopoida	0.063	0.097	0.099	0.174	0.043	0.111	0.133

Values that exceed 0.5 indicate a preference for a specific prey.

Moreover, values were far below the critical 0.5 point and no difference amongst indexes occurred (one-way ANOVA: $P > 0.05$ in all cases, except for *P. argentinus*, which exhibited the lowest index for cyclopoid copepodites).

Discussion

Both the laboratory and field experiments demonstrated low survival of zooplankton in macrophyte stands containing littoral predacious macroinverte-

brates. These pelagic prey were vulnerable to several littoral predators, although the impact of predators differed among zooplankton species. Cladocerans suffered strong per capita net predation by adults of *P. argentinus*, *Buena* sp. and late instar *C. interruptum*, while calanoid copepods exhibited strong impacts by the adults of *P. argentinus*. On the other hand, cyclopoid copepods did not show any negative effect. In both experiments macroinvertebrate predation affected all zooplankton size fractions, implying that size is not a refuge advantage when macrophytes are used as daytime locations. Small and large cladocerans (*Bosmina*, *Moina* and *Diaphanosoma*) declined in both the feeding trials and the field experiment. Our results confirm the high predation risk imposed by littoral predacious macroinvertebrates on zooplankton in macrophyte stands and, more important, indicate that macroinvertebrates can counteract the benefits of a macrophyte refuge effect for zooplankton against fish. In particular, the field experiment revealed the risky nature of macrophyte stands for zooplankton when these stands host a rich and abundant macroinvertebrate assemblage.

A high abundance and diversity of predators (HAD) induced a zooplankton decline (small and large cladocerans and adult calanoid copepods) close to 80%. In contrast, the LAD treatment produced almost no effect on zooplankton except for the small cladoceran *B. huaronensis*. LAD contained only one kind of predator, late instar *C. interruptum* (Zygoptera), identified in feeding trials as causing a strong PE. The difference in predation impact between HAD and LAD treatments is related to macroinvertebrate assemblage and abundance. The HAD treatment included a more complex macroinvertebrate assemblage containing predators that use different strategies to capture prey (tactile, vision, etc.) and occupied different places in the water column. For example, *C. interruptum* always remains on a perch and is a 'sit and wait' strategist, *Buena* sp., *Megadytes* sp. and *B. elegans* are associated with near-surface waters (Herwig & Schindler, 1996; Gilbert & Hampton, 2001) while the grass shrimp *P. argentinus* migrates vertically (González Sagrario, 2004), although in our experiment this movement was reduced by the bags. The large difference in the survival of all cladoceran species and adults of *N. incompositus* between HAD and LAD treatments reflects how difficult it is for zooplankton to evade one

predator without being confronted by another. A diversity of predators brings with it a variety of voraciousness, selectivity and prey capture mechanisms that would be likely to have synergic effects and increase zooplankton predation risk. Moreover, the role of littoral macroinvertebrates as zooplankton predators could be compared to that exerted by pelagic macroinvertebrates in shallow or deep lakes. In fact, we found predation impacts and reductions in lake refuges that were similar to those observed for pelagic macroinvertebrates such as *Leptodora* or *Mysis* (Spencer *et al.*, 1999; Alajärvi & Horppila, 2004).

The mortality observed under experimental conditions can be extrapolated to natural lake conditions because both the zooplankton and the macroinvertebrate assemblage reflect natural composition and abundances. Moreover, the macroinvertebrate assemblage included species that show intra and inter-specific interactions (agonistic behaviour, predation, cannibalism), that can reduce predator activity or impact (Johnson, 1991; Corbet, 1999). Our aim was to make the macroinvertebrate effect on the zooplankton community as realistic as possible (*Megadytes* can consume *Cyanallagma* and *Buena*, and *Cyanallagma* showed cannibalistic behaviour; M. González Sagrario, pers. obs.). However, our results are probably indicative of the maximum impact that the macroinvertebrate assemblage can exert on the planktonic calanoids and cladocerans in littoral areas, because no escape was possible in the enclosures or feeding trials. As a consequence, the results display what happens when zooplankton are confronted by predacious macroinvertebrates, and explains why in nature zooplankton may actively avoid submerged macrophytes. In our experiment, the cyclopoids, which share vegetated areas with predacious macroinvertebrates, did not show any predation impact, indicating that they may have obtained protection due to the presence of more vulnerable prey such as pelagic cladocerans and/or calanoid copepods, as pointed out by Jeffries (1988) and Arnér *et al.* (1998).

The direct predation of macroinvertebrates on zooplankton has been documented in detail before (e.g. Gilbert & Burns, 1999; Hirvonen, 1999; Hampton *et al.*, 2000). Nevertheless, the manner in which this process constrains DHM has been overlooked (Burks *et al.*, 2002), although in some cases the absence of a DHM pattern has been linked to the predacious macroinvertebrate assemblage in the littoral area

(Smiley & Tessier, 1998; Lauridsen *et al.*, 1999). A comparison of warm and temperate lakes showed that DHM occurred only in cold temperate lakes (Danish lakes) (Meerhoff *et al.*, 2007a,b). Moreover, the lack of DHM in warm subtropical lakes (Uruguay) was related to the littoral fish and predacious macroinvertebrate assemblage (Meerhoff *et al.*, 2007a,b). Our lake could be considered to be warm temperate, with a similar macroinvertebrate assemblage and abundance to that of subtropical lakes (Uruguayan lakes: *c.* 200 ind. m⁻², Los Padres: 364 ind. m⁻²), but lower fish abundance (Uruguayan lakes: 67 ind. m⁻², Los Padres: 30 ind. m⁻²). A previous study showed a lack of DHM by zooplankton species in Los Padres Lake (González Sagrario, 2004), and now our experimental results support the hypothesis that the lack of DHM (during both day and night) is a consequence of the high predation risk imposed by the rich macroinvertebrate assemblage in the macrophyte stands. Danish lakes (temperate) have 52 times the abundance of predacious macroinvertebrate abundance compared to our lake, even though in our system the macroinvertebrates imposed a higher predation risk. As a consequence, the nature of the macroinvertebrate assemblage (omnivorous and voracious, see Table 4 and Experiment 2), the diversity of prey capture strategies and the habitat selected by them turn out to be more important than density when the refuge effect is considered. Small littoral fish can prey on early macroinvertebrate stages (González Sagrario & Balseiro, 2003) but the combination of a littoral fish assemblage composed of small gape limited fish and large macroinvertebrates seems to result in ineffective fish control over the whole macroinvertebrate assemblage.

Macrophytes are key organisms in lake functioning. The clear state dominated by macrophytes is the result of a series of synergistic mechanisms including higher water stability, reduction of resuspension and wave action, and nutrient competition set by the macrophyte stands (Barko & James, 1998; van Donk & van de Bund, 2002; Horppila & Nurminen, 2003). According to Scheffer (1999), zooplankton contributes to the clear state, exerting higher clearance rates on phytoplankton thanks to the refuge provided by the vegetation. Our results imply that lakes with a highly abundant and diverse assemblage of littoral macroinvertebrates would have less control on phytoplankton due to the lack of refuge provided by the macrophyte

vegetation. In terms of lake functioning, a predacious macroinvertebrate assemblage seems likely to favour a less clear lake than would a detritivorous assemblage.

In conclusion, a rich assemblage of predacious macroinvertebrates that use different prey capture techniques (e.g. sit and wait, diving, hunters) can heavily depress zooplankton, switching the role of macrophyte stands from being a good refuge against visual fish predation to a risky habitat imposed by the diversity of predation strategies. In turn, this reduces the possible top-down control of phytoplankton by grazer zooplankton. The balance between cost and benefits of seeking refuge in macrophyte stands will depend upon the pressure faced by different zooplankton species from fish in the open water and the diversity of predacious macroinvertebrates in the littoral zone.

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