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HORIZONTAL DYNAMICS OF ZOOPLANKTON IN SUBTROPICAL

LAKE BLANCA (URUGUAY) HOSTING

MULTIPLE ZOOPLANKTON PREDATORS AND AQUATIC PLANT REFUGES

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1   **ABSTRACT**

2   In the subtropics, the effects of macrophytes on trophic interactions are more complex than in

3   temperate lakes. Fish, particularly the smallest species and individuals, aggregate in high

4   numbers in the vegetation, and a strong predation pressure on zooplankton by shrimps and

5   invertebrates, as *Chaoborus*, can occur in these systems. We studied seasonal and diel changes in

6   zooplankton and their potential predators (both fish and invertebrates) and physical and chemical

7   characteristics among open water and vegetated habitats (emergent and submerged plants), in the

8   subtropical lake Blanca (34°54´S; 54°50´W), a shallow system with an extensive and complex

9   littoral area and high abundance of vertebrate and invertebrate predators on zooplankton. We

10   found differential horizontal distribution of some zooplankton species under the scenario of high

11   abundance of small omnivorous-planktivorous fish and *Chaoborus*, especially in the seasons

12   with intermediate catch per unit effort of fish. We found indications of a diel horizontal

13   migration (DHM) opposite than described for temperate systems, as the two main cladocerans

14   *Bosmina longirostris* and *Diaphanosoma birgei* were found in higher densities in the submerged

15   plant beds during night, in spring and autumn respectively. Although we need experiments to

16   prove DHM, *Chaoborus* seemed to be the main trigger of the apparent DHM, followed by small

17   omnivorous fish. During summer no spatial differences were found likely because of high

18   densities of fish in all habitats. In absence of piscivorous fish, the distribution of fish *Jenynsia*

19   *multidentata* seemed to be conditioned by food availability and by predation risk of waterfowl.

20   The refuge capacity of aquatic plants for zooplankton in subtropical systems seems weak and

21   with consequent weak or no cascading effects on water transparency, as under very high fish and

22   invertebrate densities (summer) the refuge for zooplankton was lost.

23

INTRODUCTION

The spatial distribution of animals is regarded as a compromise between several costs and benefits, especially the avoidance of predation and the search for optimal feeding and environmental conditions. In freshwater systems, aquatic plants play a relevant role in affecting the spatial distribution of several organisms and shaping predator-prey interactions (Jeppesen et al., 1997a). Submerged macrophytes can often provide daytime refuge for *Daphnia* and other cladocerans from fish predation in shallow temperate lakes (diel horizontal migration (DHM), Timms & Moss, 1984; Burks et al., 2002), although this pattern has not been observed in oligotrophic and mesotrophic lakes (Lauridsen et al., 1999). However, many juvenile fish often use the littoral zone as daytime refuge against predatory fish, which compromise the refuge capacity of macrophytes for zooplankton (Carpenter & Lodge, 1986). Also, invertebrate predators in the pelagic can affect zooplankton populations and DHM, but evidence is scarce yet (Burks et al., 2001; R. Burks et al., unpublished). It has been suggested that the effects of co-existing fish and pelagic invertebrate predators can be additive and increase the likelihood of large zooplankton seeking refuge in the littoral zone (Burks et al., 2002). The net effect of multiple predators, however, seems to depend on the direction of the response of the prey to each predator alone, and their relative risk for that prey. The response of the prey to combined predators would thus be similar to its response to the most risky predator in the pair (Relyea, 2003; Wojtal et al., 2003). Wicklum (1999) suggested that vertebrate and invertebrate predation act alternately because invertebrate predators are themselves subject to fish predation. As a consequence, the impact of invertebrate predation increases as fish predation declines (Mumm, 1997).

In the tropics and subtropics, the effects of macrophytes on trophic interactions are more complex than in temperate lakes, not least because all plant life forms (emergent, submerged, floating-leaved and large free-floating species) can be very abundant (Talling & Lemoalle,

3

1   1998), and the predation pressure on zooplankton seems stronger (Jeppesen et al., 2006). Fish are

2   usually present in very high numbers (Scasso et al., 2001), and particularly the smallest species

3   and individuals, aggregate in the vegetation (Meschiatti et al., 2000, Meerhoff et al., 2003).

4   Besides, the zooplanktivorous phantom midge *Chaoborus* seems to be more abundant in tropical

5   lakes (Lewis, 1996). This might reflect that tropical lakes become anoxic in the bottom more

6   quickly than temperate lakes, providing *Chaoborus* with a fish predation refuge for longer

7   periods (Lewis, 1996), which, in turn, permits a stronger predation pressure on zooplankton

8   (Arcifa et al., 1992). Also shrimps can represent important predators on zooplankton in warm

9   regions (Collins, 1999; Collins & Paggi, 1998), probably due to the dominance of small fish

10 species with consequent lower risk of predation on shrimps, as seen for *Neomysis integer* Leach

11 in north temperate brackish lakes with fish communities dominated by small sticklebacks

12 (*Gasterosteus* spp.) (Søndergaard et al., 1997).

13 Our objectives were to describe the seasonal and diel variation in the horizontal distribution of

14 zooplankton under the scenario of combined vertebrate and invertebrate predators and relate it to

15 variations in predator abundance and the changes promoted by the aquatic plants, in a subtropical

16 shallow lake with a very well-developed littoral zone hosting different plant life-forms and high

17 abundance of small omnivorous fish and invertebrate predators (Mazzeo et al., 2003). Based on

18 knowledge from eutrophic temperate lakes we hypothesised that predation-sensitive zooplankton

19 would seek refuge during the day in those vegetated habitats with less potential predators, and

20 move to open water at night to decrease predation risk. However, an alternative scenario is that

21 the plants are not feasible refuges for zooplankton due to a potential high density of small fish in

22 the plant beds.

23

24 **STUDY AREA**

1　Lake Blanca (34°54′S; 54°50′W) is a shallow (total area=40.5 ha, Z_{max}=3.6m; Mazzeo et al.,

2　2003) lake used for water supply in the main tourist area of Uruguay. It is a warm polymictic

3　(temperature range: 11.3-26.3ºC) and eutrophic lake (in-lake annual mean TP, TN (Valderrama,

4　1981) and chlorophyll a (Nusch, 1980) concentrations: 93.6, 1010, and 31.9 µg l^{-1}, respectively).

5　A drastic reduction of the lake volume and a consequent loss of piscivorous fish occurred during

6　1998-1999. Since then, turbid and clear water states have alternated in the system, and the fish

7　community has been dominated by small (13mm<SL<86mm) *Jenynsia multidentata* Jenyns

8　(Anablepidae) (Mazzeo et al., 2003). This is an omnivorous-planktivorous fish (Escalante, 1983;

9　Goyenola et al., unpub. data), with a mean whole lake density=13 ind m^{-2} (density in littoral

10　areas≥100 ind m^{-2}, Mazzeo et al., 2003). The littoral zone of Lake Blanca is rather complex and

11　dynamic, from the shore to the open water area there is an emergent plant zone (*Schoenoplectus*

12　*californicus* L. and floating islands dominated by *Typha latifolia* L.), followed by submerged

13　plants (*Egeria densa* Planch. and *Ceratophyllum demersum* Planch.), and finally by another zone

14　of emergent plants (only *S. californicus*) (Fig. 1).

15

16　**MATERIALS AND METHODS**

17　The study was carried out seasonally from southern hemisphere winter 2003 to autumn 2004.

18　The percent volume infested of the submerged plants (PVI, according to Canfield et al., 1984)

19　and the distribution of the emergent plants were estimated from more than 100 sites

20　homogeneously distributed over seven transects that were perpendicular to the maximum length

21　of the lake and covered all the system. Each season, we mapped the vegetation according to

22　these observations and recorded the positions using a GPS. From the map, we randomly selected

23　five sampling points per strata: emergent plants (EP), submerged plants (SP: PVI ≥25%), and the

24　pelagic or open water area (OW: PVI=0%). Water samples for physical, chemical and plankton

25　analyses were collected with a vertical tube along the whole water column, at midday and

1   midnight. Dissolved oxygen (DO), conductivity, pH, and transparency were registered *in situ*,

2   using Horiba sensors and a Secchi minidisk. The euphotic zone depth (Z_{eu}, 1% of incident light)

3   was determined from the photosynthetic active radiation (Licor Model Li-250). In each habitat

4   we took a 20-litre depth-integrated sample with the tube sampler for zooplankton and *Chaoborus*

5   (filtered through a 50-μm mesh size net). Counts were made according to Paggi & de Paggi

6   (1974). Fish and shrimps were sampled with two minnow traps (40x40x60 cm, 5-cm opening)

7   per station, one close to the surface and one close to the sediment. These catches integrated the

8   periods between sunrise and sunset (day), and sunset and sunrise (night), respectively (capture

9   per unit effort (CPUE) = total number of individuals collected in the two traps per hour).

10   *Data analysis*

11   We used repeated measures 2-way ANOVA (rmANOVA) to detect significant differences within

12   seasons, considering two main factors: a) sampling zones (SP, EP, OW) and b) time as repeated

13   measures (day, night) (Underwood, 1997). In case of relevance, also 1-way ANOVA were

14   performed for specific times (day or night). Non-parametric correlations (Spearman, rs) were

15   performed between relevant variables. When DHM by zooplankton species was detected in the

16   ANOVAs, we tested the overlap between the prey and the potential predators distributions under

17   two scenarios: the "direct" (i.e. day prey-day predator and night prey-night predator

18   distributions) and the "reverse" (i.e. day prey-night predator), using Spearman correlations. In

19   this scenario, the prey is allowed to move while the predator is fixed (in its night location), thus

20   enhancing the detection of migration patterns by the prey (modified from Bezerra-Neto & Pinto-

21   Coelho, 2002).

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2 **RESULTS**

3 *Effects of the aquatic plants on the physico-chemical variables*

4 The mean area (annual averages) covered by the emergent plants (EP) was 55% and 46% for

5 submerged plants (SP), including some areas where both EP and SP were present (Fig. 1). We

6 did not observe any clear seasonal pattern in coverage of the two plant types (authors, unpub.

7 results). The SP sites had lower DO concentrations than OW and EP in spring (9.35, 10.41 and

8 10.12 mg l^{-1}, respectively), summer (5.28, 8.12 and 8.06 mg l^{-1}) and autumn (8.37, 11.82 and

9 10.51 mgl^{-1}). DO was always higher during day (Table 1). In SP, the sub-saturation dropped to

10 44% in summer during night. Intermediate pH values occurred in OW during day, while the

11 same habitat had the highest pH during night (Table 1). The euphotic zone comprised the entire

12 water column. Water transparency was most of the year higher within the submerged plant sites,

13 especially in summer and autumn (Table 1). In summer, the water transparency was 46% higher

14 in SP (35 cm Secchi minidisk) than in OW (21 cm Secchi minidisk), while in autumn the

15 transparency was 34 and 14 cm (Secchi minidisk) in SP and OW respectively.

16 *Structure of main communities*

17 The zooplankton community was numerically dominated by small-sized specimens (mostly

18 nauplii and rotifers) all year round, except in winter when medium-sized filtrators dominated

19 (small cladocerans and calanoid copepods) (Fig. 2). The small *Bosmina longirostris* Müller and

20 *Diaphanosoma birgei* Korinec were the most abundant cladocerans, but the calanoid copepod

21 *Notodiaptomus incompositus* Brian was also common. The maximum total abundance occurred

22 in autumn (2252 ind l^{-1}) and the minimum in spring (856 ind l^{-1}) (whole system averages).

23 The phantom midge *Chaoborus sp* occurred all year round, but was found only during night. Its

24 abundance varied considerably through seasons, with minimum in winter (100 ind m^{-3}) and

25 maximum during spring and summer (*ca.* 1000 ind m^{-3}) (whole system averages, Fig. 2). In

1   terms of numbers, the nekton community was dominated by the fish *J. multidentata* in autumn

2   and summer, when we found the highest CPUE (Fig. 2). The also small omnivorous fish

3   *Cnesterodon decemmaculatus* Jenyns (Poecilidae) occurred in low abundances (<1.5% of total

4   fish density and biomass). The shrimp *Palaemonetes argentinus* Nobili dominated in terms of

5   abundance in winter and spring (Fig. 2).

6   The interseasonal variation in the total zooplankton abundance was negatively correlated with

7   the CPUE of *P. argentinus* and *J. multidentata* (rs= -0.49, p<0.001; rs= -0.29, p<0.05,

8   respectively), while there was no significant correlation with the abundance of the invertebrate

9   predator *Chaoborus*.

10   *Use of the space of zooplankton and potential predators*

11   The diel use of the different habitats by most zooplankton species varied strongly among

12   seasons. *Bosmina longirostris* appeared mainly in OW in winter (2-way ANOVA $F_{(2,12)}$= 9.9,

13   p<0.05), and in OW and EP in autumn (2-way ANOVA $F_{(2,12)}$= 31.1, p<0.001), during both day

14   and night. We found diel differences only in spring, when *Bosmina*, from an homogeneous

15   distribution during day, became associated with SP and disappeared from OW at night (1-way

16   ANOVA $F_{(2,12)}$= 4.62, p<0.05) (Fig. 3A$_2$). *Diaphanosoma birgei* was collected mostly in OW in

17   winter (2-way ANOVA $F_{(2,12)}$= 136.2, p<0.001), while it was more equally distributed among

18   habitats in spring and summer. In autumn, *Diaphanosoma* had the lowest density within the SP

19   during day (1-way ANOVA $F_{(2,12)}$= 5.21, p<0.05), but increased strongly its density in that

20   habitat at night (from 2 to 67 ind l^{-1}, Fig. 3B$_4$). *Chydorus sphaericus* Müller was associated with

21   SP in winter (2-way ANOVA $F_{(2,12)}$= 29.4, p<0.001) and spring ($F_{(2,12)}$= 37.6, p<0.001). We

22   found higher abundances of *Moina micrura* Kurz in OW in winter (2-way ANOVA $F_{(2,12)}$= 4.9

23   p<0.05), and a homogenous spatial distribution during the rest of the year. Cyclopoids had higher

24   abundances within the SP stands during all seasons (2-way ANOVA $F_{(2,117)}$= 12.9, p<0.001),

25   while the calanoid *N. incompositus* was homogeneously distributed among the three habitats.

1   Nauplii showed different density patterns with seasons, being higher within vegetation (both

2   plant types) in winter and summer (2-way ANOVA $F_{(2,12)}$= 5.9, p<0.01; $F_{(2,12)}$= 6.7, p<0.01,

3   respectively).

4   *Chaoborus* was found only during night. It was more abundant in OW in winter and in spring (1-

5   way ANOVA $F_{(2,12)}$= 16.4, p<0.01; $F_{(2,12)}$= 6.45, p<0.01, respectively) (Fig. $3C_{1,2}$). In summer

6   and autumn it was mainly associated with EP and OW (1-way ANOVA $F_{(2,12)}$= 4.3, p<0.05;

7    $F_{(2,12)}$=3.5, p<0.05, respectively) (Fig. $3C_{3,4}$). *Jenynsia multidentata* tended to occur mainly

8   within the emergent vegetation. In winter, CPUE was higher within SP (though not

9   significantly), while in spring it was collected primarily in EP sites (2-way ANOVA $F_{(2,12)}$=

10   12.7, p<0.001), especially at night (Fig. $3D_2$). In summer, the CPUE was higher at night (2-way

11   ANOVA $F_{(1,12)}$= 9.2, p<0.05), and the sequence was OW≥EP>SP during day and EP>SP>OW

12   during night (significant interaction Sites x Time $F_{(2,12)}$= 5.2, p<0.05) (Fig. $3D_3$). The spatial

13   distribution in autumn did not show a significant pattern (Fig. $3D_4$).

14   The third potential predator, the shrimp *P. argentinus*, was collected in winter mostly from OW

15   and during night (Fig. $3E_1$), though not significantly. In spring and summer, the highest CPUE

16   occurred during night (2-way ANOVA $F_{(1,12)}$= 14.93, p<0.01 and $F_{(1,12)}$= 9.6, p<0.01

17   respectively). For the summer, the sequence of the densities were SP≥OW≥EP during day and

18   OW>SP>EP at night (significant interaction Sites x Time $F_{(2,12)}$= 4.3, p<0.05). In autumn, no

19   statistical differences were found in the habitat preference of shrimps. Only in summer there was

20   an inverse spatial correlation between the CPUE of adult *J. multidentata* and *P. argentinus* (rs= -

21   0.56, p<0.01, during night) (Figs. $3D_3$ & $3E_3$).

22   *DHM analysis*

23   We focus here on *B. longirostris* and *D. birgei*, as they were the only species showing significant

24   diel variations between habitats in their densities (in spring and autumn, respectively). In spring

25   the density and distribution of *B. longirostris* during day was positively, though not significantly,

1 correlated with the distribution of *Chaoborus* during night. Contrary, the "direct" (day/day,

2 night/night) correlation of both species during night was negative and significant ($rs = -0.59$,

3 $p < 0.05$) (Figs. $3B_2$ & $3C_2$). In the case of *J. multidentata*, both species were positively but not

4 significantly correlated in the "direct" comparison, while their "reverse" correlation (day

5 prey/night predator) was negative and also not significant (Figs. $3B_2$ & $3D_2$). Besides, the

6 distribution of *B. longirostris* was negatively correlated with DO concentrations in night ($rs = -$

7 0.72, $p < 0.01$). In autumn, the day distribution of *D. birgei* was significant and positively

8 correlated with the distribution of *Chaoborus* during night ($rs = 0.81$, $p < 0.001$), while the "direct"

9 correlation of both species in the night was negative but not significant ($rs = -0.33$, n.s.). The

10 "direct" correlations between *Diaphanosoma* and *J. multidentata* were positive and significant at

11 night ($rs = 0.79$, $p < 0.001$), while the "reverse" correlation was negative but not significant. We

12 did not detect any statistically significant relationship between the distribution of *D. birgei* and

13 the main physico-chemical variables (DO, pH, T). In these intra-seasonal analyses, no significant

14 correlation was found between the distribution of these cladocerans and the abundance (CPUE)

15 of shrimps.

16

17 **DISCUSSION**

18 Predators are thought to be the main factor controlling the spatial distribution, abundance and

19 body size of zooplankton in shallow lakes (e.g. Scheffer, 1998; Burks et al., 2002). In Lake

20 Blanca, the small size of the dominant cladocerans and the dominance by copepods and rotifers

21 likely reflect the extremely high abundance of the potential predators, particularly the

22 omnivorous-planktivorous fish *Jenynsia multidentata*. The most important finding of our study

23 was the lack of fixed patterns in the horizontal distribution of the main zooplankton groups

24 among seasons, particularly of cladocerans. Cyclopoid copepods were associated mainly with

1   submerged plants (day and night) as seen in other studies (Jeppesen et al., 1997b), while

2   calanoids showed a homogeneous distribution.

3   However, in contrast to most studies from eutrophic shallow lakes in the temperate zone (Burks

4   et al., 2002), the most abundant cladocerans *B. longirostris* and *D. birgei* preferred the vegetated

5   areas (especially the submerged plants) during night and not as expected during day (in seasons

6   when distribution differed significantly). The significantly higher water transparency in sites

7   with plants indicates zooplankton would probably find lower quantity of food resources in these

8   habitats than in open water, which suggests that moving towards the plants was a predator-

9   avoidance behaviour.

10 The occurrence of DHM (and its reverse direction) in Lake Blanca seemed controlled by two

11 factors related to the potential predators: the temporal variability in fish CPUE, and in the

12 abundance and the habitat use of *Chaoborus*. However, despite the very high fish CPUE,

13 *Chaoborus*, occurring in high densities compared with those of temperate lakes (Liljendahl-

14 Nurminen et al., 2002), seemed to play a more relevant role than fish. During winter, with the

15 lowest densities of fish and *Chaoborus*, *B. longirostris* and *D. birgei* had the highest abundances

16 and we did not find evidence of DHM as these cladocerans were mostly found in open waters.

17 The reverse DHM occurred during the seasons with intermediate CPUE of fish (spring and

18 autumn). From the "direct" and "reverse" correlation analyses, we can argue that if *Bosmina* did

19 not migrate, from a relatively homogeneous diurnal distribution towards SP at night (in spring),

20 it would encounter with *Chaoborus* in open waters, while with fish in the emergent plants.

21 In the case of *D. birgei* the DHM pattern seemed more clear than in the case of *B. longirostris*,

22 day and night densities were balanced in the former. By migrating to SP at night (in autumn), *D.*

23 *birgei* decreased the encounter rate with *Chaoborus* (which by then mostly appeared among EP),

24 even though the overlapping with *J. multidentata* would increase. Probably, the costs of

25 migrating to the submerged plant beds at night are high, but still lower than of migrating to the

pelagic zone where the absence of physical structures would increase the predation efficiency by *Chaoborus* and fish. In summer, *J. multidentata*'s density increased enormously after the main reproductive season. Together with a remarkable decrease in cladoceran abundance, the spatial distribution of *B. longirostris* or *D. birgei* became homogeneous, with no indications of DHM. Clearly, no habitat seemed to offer a significant refuge for zooplankters under this enhanced-predation scenario.

Although we think DHM is the most likely explanation for the observed distributions, we cannot prove it as the sampling method used allows no mass balances to be performed. The pattern, particularly of *B. longirostris*, could also be due to, or enhanced by, differential vertical migration (stronger in the submerged plant sites) or differential predation pressure in the different habitats or their combination. *In situ* and *ex situ* experiments would provide further insights into this subject.

In their review, Burks et al. (2002) did not report examples of diel horizontal migration in the scenario of both pelagic fish and active-at-night invertebrate predators (e.g. *Chaoborus*). Jeppesen et al. (1997a) suggested that DHM induced by invertebrates would probably be more important in lakes with low fish densities, and that the direction of this migration would depend on the habitat choice of the predacious invertebrates (whether pelagic or plant-associated). Likewise Lauridsen et al. (1997) suggested that the reverse DHM by *Daphnia* in the fishless Central Long Lake (USA) could be induced by pelagic nocturnal invertebrates, as *Chaoborus*. R. Burks et al. (unpublished), on the contrary, found direct DHM triggered by *Chaoborus* in a Danish lake with very low abundance of fish, as earlier observed by Kvam & Kleiven (1995) in a Norwegian lake. The advantage of DHM as an anti-predation mechanism depends on both the predators and the refuge capacity of the aquatic plants. Our results can be interpreted as a variation of the additive interaction between vertebrate and invertebrate predators, proposed by Burks et al. (2002). We did not find evidence that the occurrence of invertebrates can lead to

1   higher densities of large-bodied zooplankton, by serving as alternative food for fish, as found by

2   Sagrario & Balseiro (2003) in mesocosm experiments with some of the same predatory species

3   that appeared in Lake Blanca. The physico-chemical differences among sites played a minor role

4   in shaping these spatial patterns. Although we found a negative relationship between the spatial

5   distribution of *B. longirostris* and the oxygen concentration in spring, the differences of DO

6   among sites were low.

7   Also the predators showed temporal variability in habitat choice, on a diel and seasonal scale.

8   Several authors reported that *Chaoborus* displays vertical migration to avoid fish predation,

9   spending day in the sediments and migrating upwards at night (Davidowicz et al., 1990; Voss &

10 Mumm, 1999). The same behaviour was evidenced in Lake Blanca as *Chaoborus* was only

11 caught during night, a likely less risky time to occupy the water column when visual predatory

12 fish are present.

13 The habitat selection by *J. multidentata*, as top predator, probably depended on the search for

14 food and shelter from the piscivorous waterfowl. The emergent plant zone is likely a safer place

15 than the submerged plants, as bird attacks can be avoided (e.g. from yellow-billed tern *Sterna*

16 *superciliaris* Vieillot, olivaceous cormorant *Phalacrocorax olivaceus* Browning present in the

17 lake). The preference of *J. multidentata* for the emergent plants also by night, when predation

18 risk by birds is absent, suggests this fish species also find a comparatively good availability of

19 resources in this habitat. The differences in physical and chemical factors among sites seemed to

20 play a minor role in the horizontal distribution of fish, especially considering that both fish

21 species, *J. multidentata* and *C. decemmaculatus*, have a broad tolerance to different and

22 sometimes extreme environmental conditions (Ortubay et al., 2002).The habitat selection of fish

23 and shrimps seemed to be mutually dependent, as we observed a clear spatial segregation

24 between fish and shrimps. Contrary to earlier findings (Collins 1999), there is no strong evidence

1   that the shrimps were important predators on zooplankton in this lake (G. Goyenola et al.

2   unpublished).

3   *Final remarks*

4   Studies on the behavioural response of preys to combinations of predators are still scarce (Relyea

5   2003). Based on experimental studies, Van de Meutter *et al.* (2005) proposed that the effects of

6   particular predators on the spatial distribution of a prey (*Daphnia* in that case) can vary strongly

7   in strength and direction, depending on the presence and activity of more than one predator.

8   These authors also suggested that interactions and facilitation among predators can be relevant,

9   and can change with time and environmental characteristics, as we found in Lake Blanca.

10   According to our field evidence, the horizontal distribution of zooplankton was apparently

11   conditioned by complex interactions with the potential predators and the architecture and spatial

12   distribution of the aquatic plants, and indirectly probably also by the activity of piscivorous

13   birds. We propose a conceptual model of the main interactions (predation *versus* refuge) in this

14   lake, to be tested in experiments or under other field conditions (Fig. 4). We suggest that aquatic

15   plants in subtropical shallow lakes offer refuge for zooplankton only at intermediate fish and

16   invertebrate predator densities, and that refuge-mediated positive effects on water transparency

17   therefore might be weak or rare.

18

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1 **Table 1**. Summary of 2-way rmANOVA statistics. Effects of sites, time (day, night) and their

2 interaction, on temperature (T), dissolved oxygen (DO), and pH, along seasons. The differences

3 among sites for water transparency (mini Secchi Disk) correspond to midday (1-way ANOVA).

4 Sites: OW= open water, SP= submerged plants, EP= emergent plants, ordered according to *post*

5 *hoc* tests. The F values and the respective degrees of freedom are indicated in all cases.

6 Significance level: p>0.1 ns, p<0.05*, p<0.01**, p<0.001***. We indicate the p-value in the

7 marginally significant cases (0.05<p<0.1[+]).

		SITES				TIME	SITE x TIME
		DAY		NIGHT			
T	win	SP=OW=EP	$2.34_{(2,12)}$ ns	SP=OW=EP	$0.35_{(2,12)}$ ns	$30.2_{(1,12)}$ ***	$1.85_{(2,12)}$ ns
	spr	SP=OW=EP	$0.90_{(2,12)}$ ns	SP=OW=EP	$1.16_{(2,12)}$ ns	$1.16_{(2,12)}$ ns	$0.05_{(2,12)}$ ns
	sum	SP=EP>OW	$9.72_{(2,12)}$ ***	SP=OW=EP	$2.47_{(2,12)}$ ns	$2.47_{(2,12)}$ ns	$1.62_{(2,12)}$ ns
	aut	SP=OW>EP	$3.08_{(2,12)}$ p=0.08[+]	SP=OW>EP	$3.37_{(2,12)}$ p=0.07[+]	$11.7_{(1,12)}$ **	$3.46_{(2,12)}$ ns
DO	win	OW=SP=EP	$0.60_{(2,12)}$ ns	OW=EP=SP	$1.38_{(2,12)}$ ns	$34.7_{(1,12)}$ ***	$0.90_{(2,12)}$ ns
	spr	OW=SP=EP	$0.15_{(2,12)}$ ns	OW=EP>SP	$4.95_{(1,12)}$ **	$66.3_{(1,12)}$ ***	$1.82_{(2,12)}$ ns
	sum	OW=EP>SP	$6.87_{(2,12)}$ **	OW=EP>SP	$45.6_{(2,12)}$ ***	$41.8_{(1,12)}$ ***	$9.6_{(2,11)}$ **
	aut	OW=EP>SP	$25.9_{(2,12)}$ ***	OW>EP>SP	$5.14_{(2,12)}$ **	$101.9_{(1,12)}$ **	$3.9_{(2,11)}$ p=0.05[+]
pH	win	SP=OW=EP	$0.2_{(2,12)}$ ns	EP=OW=SP	$0.9_{(2,12)}$ ns	$93.6_{(1,12)}$ ***	$0.8_{(2,12)}$ ns
	spr	SP=OW=EP	$1.0_{(2,12)}$ ns	OW=EP=SP	$1.9_{(2,12)}$ ns	$5.3_{(1,11)}$ *	$9.2_{(2,11)}$ **
	sum	EP=OW=SP	$0.5_{(2,12)}$ ns	OW=EP>SP	$5.3_{(2,12)}$ *	$0.6_{(2,12)}$ ns	$2.6_{(2,12)}$ ns
	aut	EP=SP=OW	$1.0_{(2,12)}$ ns	OW=EP>SP	$9.8_{(2,12)}$ **	$33.7_{(1,12)}$ ***	$7.7_{(2,12)}$ **
mSD	win	EP=SP=OW	$1.8_{(2,12)}$ ns				
	spr	OW=EP=SP	$0.3_{(2,12)}$ ns				
	sum	SP>EP=OW	$10.25_{(2,12)}$ **				
	aut	SP>EP=OW	$3.1_{(2,12)}$ p=0.08[+]				

FIGURE LEGENDS

Figure 1. Bathymetric map of Lake Blanca (upper panel), and spatial distribution of aquatic plants: area covered by emergent *Schoenoplectus californicus* (intermediate panel), and % PVI of submerged plants *Egeria densa* and *Ceratophyllum demersum* (lower panel). Distances are marked on the x and y axes (in metres). Data correspond to winter 2003.
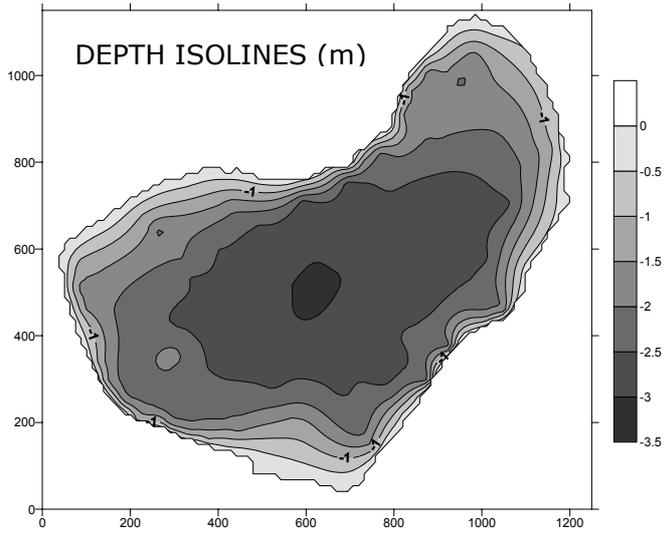
Figure 2. Temporal variation of: Main zooplankton functional groups (according to diet and size): microfiltrators= rotifers + nauplii, mesofiltrators= calanoid copepods + cladocerans (upper panel); Potential vertebrates and invertebrate predators on zooplankton (nekton= fish *J. multidentata* + shrimps *P. argentinus*) (lower panel). Data represent whole system averages (error bars= 1 SE).
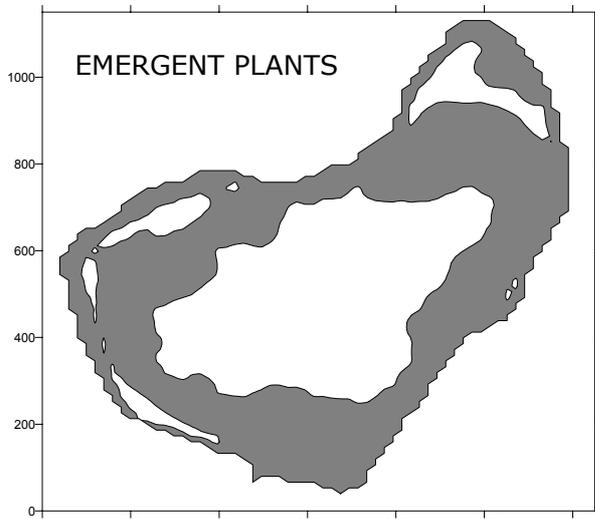
Figure 3. Diel habitat choice in all seasons of *B. longirostris* (A), *D. birgei* (B), and main potential predators: *Chaoborus* (C), *J. multidentata* (D) and *P. argentinus* (E). From left to right, panels correspond to winter (1), spring (2), summer (3), and autumn (4). Symbols: □= open water, ■= emergent plants, ▨= submerged plants. The cases of DHM are bold-framed. Note the different scales in the figures (error bars= 1 SE).
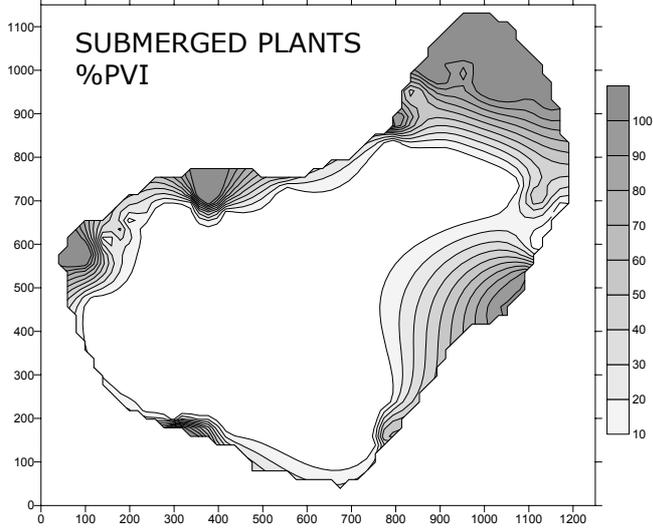
Figure 4. Theoretical model of the main interactions (predation and refuge) among biotic and abiotic components that can influence the spatial distribution of zooplankton and nekton in Lake Blanca. Complete lines correspond to findings of this study while dotted lines to literature data.
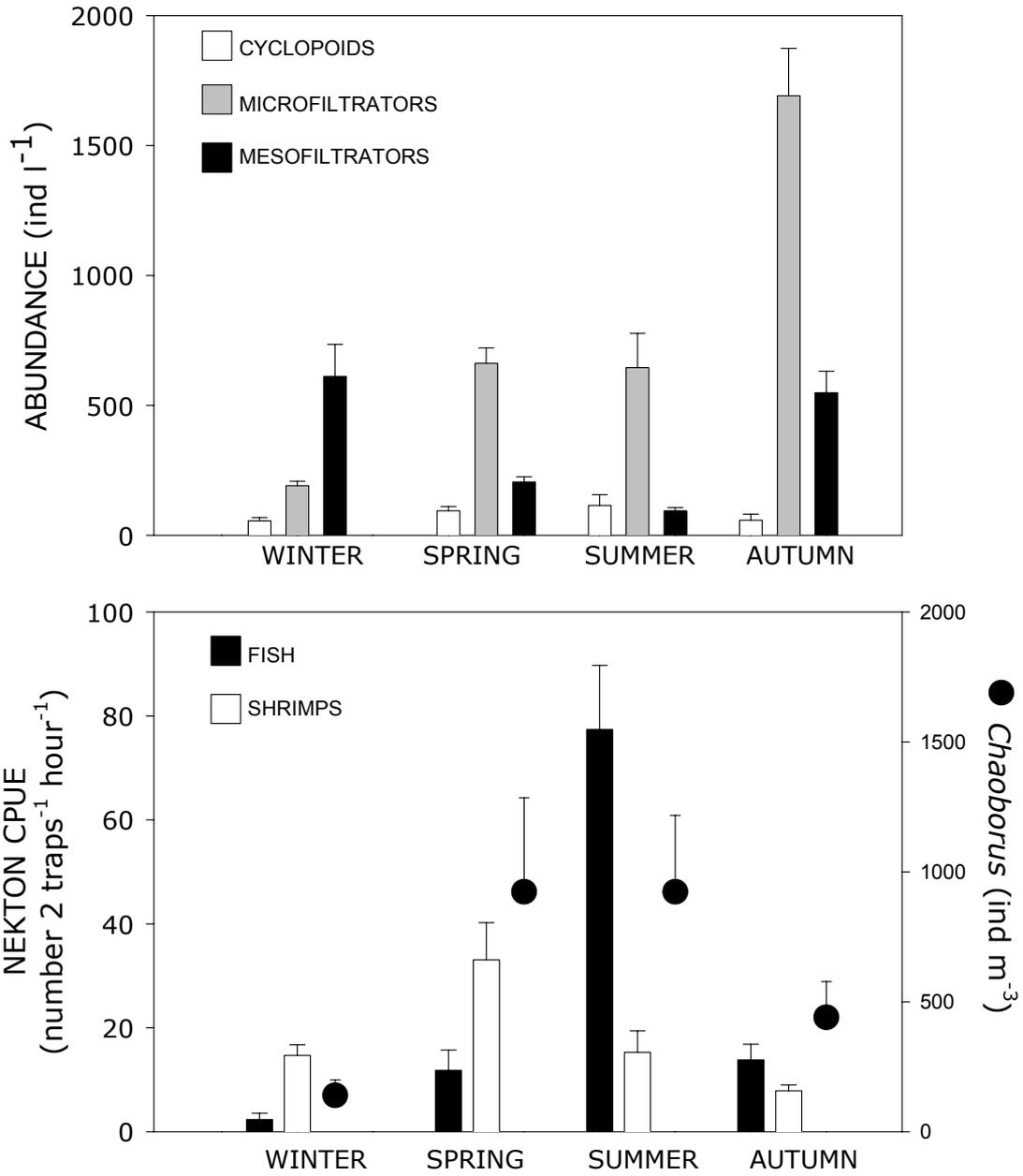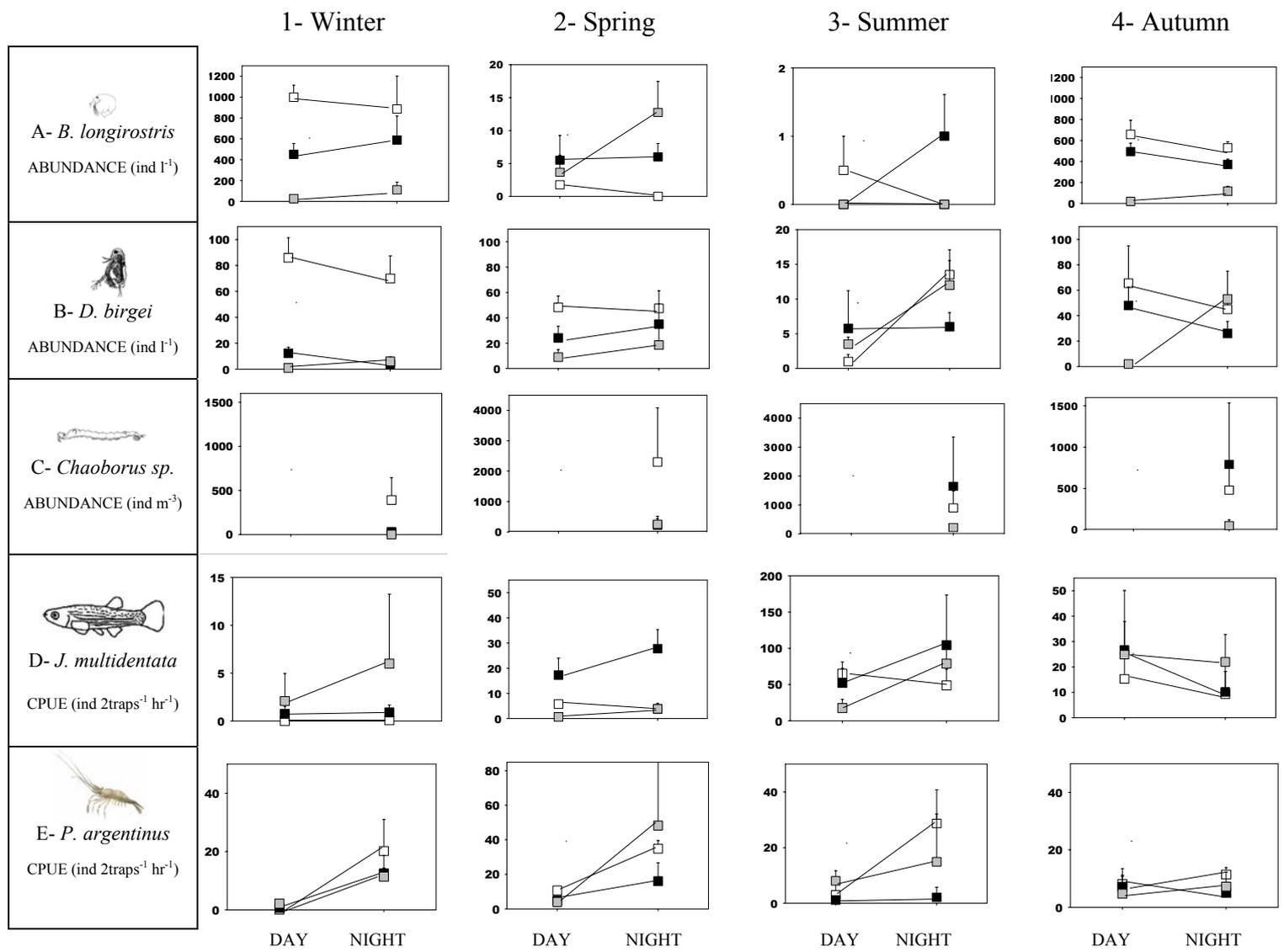
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2

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4
5
6   Fig. 1
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1

2
3Fig. 2

23

1

	1- Winter	2- Spring	3- Summer	4- Autumn

A- *B. longirostris*
ABUNDANCE (ind l⁻¹)

B- *D. birgei*
ABUNDANCE (ind l⁻¹)

C- *Chaoborus sp.*
ABUNDANCE (ind m⁻³)

D- *J. multidentata*
CPUE (ind 2traps⁻¹ hr⁻¹)

E- *P. argentinus*
CPUE (ind 2traps⁻¹ hr⁻¹)

DAY NIGHT   DAY NIGHT   DAY NIGHT   DAY NIGHT

2   Fig. 3
3
4

Fig. 4

Piscivorous birds

Sediment

Shrimps

predation

refuge

refuge

predation

J. multidentata

predation

Chaoborus

predation

Zooplankton

refuge

Aquatic plant beds

predation

refuge