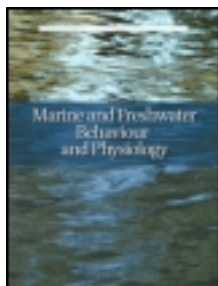


This article was downloaded by: [Universidad Nacional del Litoral], [María Florencia Gutierrez]

On: 01 November 2012, At: 05:41

Publisher: Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



## Marine and Freshwater Behaviour and Physiology

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/gmfw20>

### Behavioural responses of freshwater zooplankton vary according to the different alarm signals of their invertebrate predators

María Florencia Gutierrez<sup>a</sup>, Florencia Rojas Molina<sup>a b</sup> & Débora A. Carvalho<sup>a</sup>

<sup>a</sup> Laboratorio de Plancton, Instituto Nacional de Limnología (CONICET-UNL), Ciudad Universitaria, 3000 Santa Fe, Argentina

<sup>b</sup> Facultad de Ciencias Médicas, Universidad Nacional del Litoral, Ciudad Universitaria, 3000 Santa Fe, Argentina

Version of record first published: 31 Oct 2012.

To cite this article: María Florencia Gutierrez, Florencia Rojas Molina & Débora A. Carvalho (2012): Behavioural responses of freshwater zooplankton vary according to the different alarm signals of their invertebrate predators, *Marine and Freshwater Behaviour and Physiology*, DOI:10.1080/10236244.2012.737697

To link to this article: <http://dx.doi.org/10.1080/10236244.2012.737697>



PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.tandfonline.com/page/terms-and-conditions>

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae, and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings,

demand, or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

## Behavioural responses of freshwater zooplankton vary according to the different alarm signals of their invertebrate predators

María Florencia Gutierrez<sup>a\*</sup>, Florencia Rojas Molina<sup>a,b</sup> and Débora A. Carvalho<sup>a</sup>

<sup>a</sup>Laboratorio de Plancton, Instituto Nacional de Limnología (CONICET-UNL), Ciudad Universitaria, 3000 Santa Fe, Argentina; <sup>b</sup>Facultad de Ciencias Médicas, Universidad Nacional del Litoral, Ciudad Universitaria, 3000 Santa Fe, Argentina

(Received 18 May 2012; final version received 4 October 2012)

Single behavioural interactions between two freshwater planktonic crustaceans and invertebrate predators from different zones of the common environment were studied. The planktonic prey organisms were the cladoceran *Ceriodaphnia dubia* and the copepod *Notodiaptomus conifer*. The three invertebrate predators were the shrimp *Macrobrachium borellii*, the crab *Trichodactylus borellianus* and the larva of the dipteran midge *Chaoborus*. Feeding experiments were first performed to document the effects of exposure time and prey density on predation. All the selected predators fed on *C. dubia* and *N. conifer*, and predation rates were dependent on prey density and exposure time. The ability of microcrustaceans to modify their swimming and avoidance behaviour when faced with cues from each predator was then analysed. The cladoceran was more vulnerable to decapod predation, whereas the prey microcrustaceans were taken equally by the dipteran larvae. The analysis showed that the microcrustaceans detected the presence of at least one invertebrate predator through the predator alarm signals. The cladoceran responded to chemical signals from the three predators but copepods modified their behaviour only in the presence of infochemicals of *M. borellii*. The different outcomes suggest that macrocrustaceans have different vulnerabilities when faced with the same predator and so have evolved specific and different strategies to reduce invertebrate predation.

**Keywords:** predator–prey interactions; infochemicals; behavioural experiments; zooplankton; prey; *Ceriodaphnia*; *Notodiaptomus*; predators; *Chaoborus*; *Trichodactylus*; *Macrobrachium*

### Introduction

Food webs in temperate shallow lakes are extremely complex due to the spatial heterogeneity and high diversity of functional feeding groups that such environments support (Meerhof et al. 2006; Gonzales Sagrario and Balseiro 2010). Current knowledge about the processes that shape them is frequently focused on direct predator consumption of prey (Neill 1981; Lazzaro 1987; Collins and Paggi 1998; Ramcharan et al. 2001). However, there are other top-down and bottom-up indirect

---

\*Corresponding author. Email: fgutierrez@inali.unl.edu.ar, flopigutierrez@hotmail.com

and related effects of predation, typically underestimated but probably even more important than mortality (Hanazato and Yasumo 1989; Gliwicz 1994). The energetic demand of avoiding predators through the detection of 'alarm signals' is one of the most important indirect effects, and can be crucial in determining prey population dynamics (Lass and Spaak 2003; Lind and Cresswell 2005). In this sense, there is considerable evidence that antipredator morphological, behavioural or life history responses can lower reproduction (Burks et al. 2000; Boeing et al. 2005; Castro et al. 2007), reduce feeding activity (Cieri and Stearns 1999) or influence competition efficiency in many zooplanktonic organisms (Folt and Goldman 1981; Jamieson 2005; Aranguiz Acuña et al. 2010). Shifts in energy allocation could also alter the organisms' fitness and, in the long term, may cause the regional disappearance of the most vulnerable ones (Lass and Spaak 2003).

Unlike most northern water bodies, shallow lakes of the alluvial valley of the Paraná River (South America) have complex assemblages of invertebrate predators. They comprise larvae and adults of insects, micro and macrocrustaceans, and invasive bivalves among others (Neill 1981; Collins et al. 2007, José de Paggi and Paggi 2007, González Sagrario and Balseiro 2010; Rojas Molina et al. 2010). Those invertebrates have a central ecological role since they prey on smaller organisms, and also serve as food for higher trophic levels such as fish, birds, mammals and amphibians (Bonetto et al. 1963; Oliva et al. 1981; Lajmanovich and Beltzer 1993; Ferriz et al. 2000; Gori et al. 2003; Port-Carvalho et al. 2004; Kellogg and Dorn 2012). However, information regarding their indirect effects in determining the zooplankton community structure is not yet clear and more information is needed for a better understanding of how all the elements of these ecosystems are interrelated.

A starting point for this line of research is to determine whether zooplanktonic organisms are able to recognize and respond to alarm signals from invertebrate predators in their environment. The aim of this study is to investigate experimentally whether the cladocera *Ceriodaphnia dubia* Richard, 1894 and the copepod *Notodiaptomus conifer* (Sars 1901) modify their behaviour when faced with alarm signals from three numerous invertebrate predators that differ from each other in their ecological zonation. The predators selected were the phantom midge larvae, *Chaoborus*, which is bounded to limnetic areas; the prawn *Macrobrachium borellii* (Nobili 1896) which has an even distribution between limnetic and littoral environments; and the crab *Trichodactylus borellianus* Nobili, 1896 that remains in the littoral-benthic area (Collins et al. 2007).

Information on the trophic spectrum from the selected predators indicates that they are important consumers of zooplankton organisms (Swift and Fedorenko 1975; Neill 1981; Collins and Paggi 1998; Collins et al. 2007). However, to determine whether they feed on *C. dubia* and *N. conifer* in similar proportions, we first carried out a feeding experiment. In this one, the consumption rate of each invertebrate was analysed in relation to the time, density and swimming ability of the prey. Then, through two indoor microcosm assays, we separately analysed the swarming and avoidance behaviour of the two microcrustacean species. These behaviours were selected because they have a short response time and occur only when the predator is present (Ohman 1988; de Meester and Cousing 1997).

Based on previous investigations (Pijanowska and Kowalczewski 1997), we hypothesised that the two microcrustaceans are able to detect the presence of the

three predators by means of alarm signals and respond by swarming and swimming away from the vicinity of the predator.

This study provides insight into the behavioural responses of microcrustaceans when faced with alarm signals from predators and also information about the trophic ecology of three common invertebrates of neotropical freshwater shallow lakes.

## Materials and methods

### *Experimental species*

The zooplanktonic species used in this study (*N. conifer* and *C. dubia*) were collected with a plankton net (200 µm) from shallow lakes of the Middle Paraná floodplain (31°40'17.2"S–60°34'07.4"W). In such environments, both microcrustaceans coexisted with several invertebrate predators suggesting that they have effective defence mechanisms against these predators. The collected individuals were cultured in the laboratory in glass containers with dechlorinated and aerated tap water (control water, CW). Such stock cultures were maintained under constant 16:8 h (light: dark) photoperiod and temperature ( $21 \pm 2^\circ\text{C}$ ) conditions. The 2200 ( $\pm 244$ ) Lux intensity of the incident light was generated by fluorescent tubes. The physicochemical characteristics were as follows: dissolved oxygen: 6.4 ( $\pm 0.8$ ) ppm; pH: 8.39 ( $\pm 0.24$ ); conductivity: 245.33 ( $\pm 28.18$ )  $\mu\text{S cm}^{-1}$ . During the rearing and experimentation periods, the organisms were fed daily *ad libitum* with a *Chlorella vulgaris* concentrate (algal density:  $2.8 \times 10^5$  cells  $\text{mL}^{-1}$ ). The body length of the used organisms was measured under a compound binocular microscope: 568.3 ( $\pm 121.53$ ) µm for cladocerans and 1.5 ( $\pm 0.05$ ) µm for copepods.

Samples of the invertebrate predators (*M. borellii* and *T. borellianus*) were taken using a hand net with a 1 mm mesh size from the vegetation of shallow lakes belonging to the Paraná River system (31°40'17.2"S–60°34'07.4"W). *Chaoborus* instars were collected with a plankton net (200 µm) in outdoor mesocosm tanks (1000 L) near our laboratory, where predators were then transported and transferred to glass containers filled with control water. They were maintained under the same photoperiod and temperature conditions as the zooplankton, with continuous aeration. During their acclimation and maintenance, a laboratory culture of live microcrustaceans was provided as a food supply.

The mean length of predators used in this study was determined from 15 individuals of each species: *M. borellii* ( $6.9 \pm 1.2$  mm); *T. borellianus* ( $2.9 \pm 0.4$  mm) and *Chaoborus* ( $0.87 \pm 0.006$  mm). Each one was photographed and the total length was obtained with the free morphometric software TpsDig2 (Rohlf 1997).

All experiments were conducted under constant temperature ( $21 \pm 2^\circ\text{C}$ ) and in darkness since invertebrate predators lurk at night (Collins et al. 2007).

### *Feeding experiments*

Feeding experiments with *Chaoborus* were carried out in glass beakers with 35 mL of control water. For decapods, 100 mL of the control water was used. Prior to the feeding experiments, the predators were placed individually into test beakers and acclimated for 24 h to ensure a similar level of satiety and food in the digestive system.

For each feeding experiment, one predator and different concentrations of one of the prey organisms were used in five replicate beakers plus five controls (i.e. without predators). For each predator, *C. dubia* and *N. conifer* were offered in four densities similar to the microcrustacean densities reported for floodplain lakes in this system (José de Paggi and Paggi 2007, 2008). These were 30, 70, 145 and 345 ind L<sup>-1</sup>, which were denoted D1, D2, D3 and D4, respectively. D1 was not examined for *Chaoborus*. Before placing the microcrustaceans into the experimental beakers, a suspension of algae (*C. vulgaris*) was added at a final concentration of 1240 cells mL<sup>-1</sup> (which is similar to the reported algal concentrations in the Middle Paraná River floodplain: García de Emiliani 1990; Zalocar de Domitrovic et al. 2007).

In this study, 1, 2, 3, 6 and 24 h after the placement of the prey in each beaker, the predator was removed and transferred to another beaker and the microcrustacean survivors were counted. The predator was then returned to the test container.

### Grouping experiments

In this experiment, the alarm signals were the predator exudates (hereafter, termed kairomones). To obtain the water containing kairomones, four *Chaoborus* larvae, three adult of *T. borellianus* or three adult of *M. borellii*, were placed separately in a glass container (2 L) with control water for 24 h immediately before the trial. Each treatment was denominated *Chaoborus* water (CW), *T. borellianus* water (TW) and *M. borellii* water (MW), respectively. During the conditioning period, the invertebrates were not fed so as to avoid altering the water chemical quality, and after the incubation, the invertebrates were removed.

The effects of kairomone on the distribution of *C. dubia* and *N. conifer* were tested in 380 mL prismatic glass containers (11 × 6.75 × 6.75 cm<sup>3</sup>) which were marked in a grid of 12 fields with 3 mm sides (Figure 1). The grid determined three depth levels with four compartments with equal volumes.

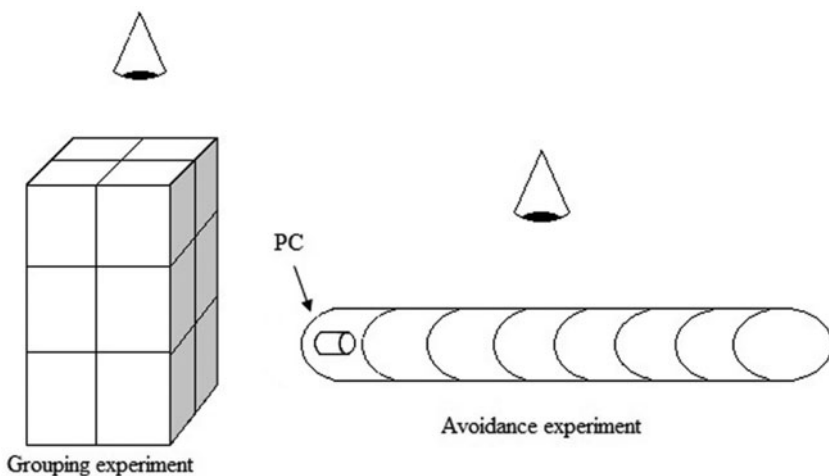


Figure 1. Diagram of the glass containers used in the grouping and avoidance experiments. Schematic eyes indicate the point of view of the observer. PC, predator cage.

Four treatments with five replicates were performed on each prey (control, CW, TW and MW). Each assay was run in 315 mL of conditioned water with 20 microcrustaceans of the same species. One hour after the exposure of the organisms to each treatment (period of adaptation), the number of individuals present in each compartment of the container was quantified at 1, 3 and 24 h.

### *Avoidance experiments*

Avoidance experiments were performed inside horizontal transparent glass tubes (length: 21 cm; diameter: 2.6 cm) filled with 100 mL of control water (Figure 1). The tubes were externally divided every 3 cm, so a total of seven compartments were established. Each predator was located in the first compartment of the tube in a small plastic cage especially designed for this purpose. In this experiment, the alarm signals were chemical (kairomones) and mechanical, but not visual, as the cages were opaque. Twenty microcrustaceans of the same species were introduced in each tube. Each treatment (control, CW, TW and MW) was replicated five times.

To estimate the organism avoidance from the predator location, the number of prey organisms present in each compartment of the tubes was quantified three times after an adaptation period of 1 h: at 1, 3 and 24 h.

In both behavioural experiments, quantification was performed as quickly as possible to reduce the chance of counting swimming animals more than once.

### *Data analysis*

For the feeding experiments, the Spearman's correlation coefficient was calculated to evaluate whether the consumption of prey of each predator is dependent on the prey density and/or exposure time. All replicate data were included in the test. To compare predation rate between the three predators, we used analysis of variance (ANOVA) with repeated measures in one factor (time; RM ANOVA).

Index  $P_i$  was used to establish the level of aggregation of individuals in the water column (Lloyd 1967). This index was calculated with the equation:

$$P_i = \sigma/x^2 - 1/x + 1 \quad (1)$$

where  $\sigma$  is the simple variance and  $x$  the number of individuals in the column.

From the indices thus obtained for each replica, differences among treatments were tested with two-factor ANOVA (factor 1: predator, factor 2: time) with repeated measures in one factor (time) (RM ANOVA). This test allowed analysis of the aggregation differences of the prey between control and each conditioned water (CW, TW and MW) throughout the experiment. The normal distribution of data (Kolmogorov–Smirnov test), homoscedasticity (Levene test) and sphericity (Mauchly test) was all verified prior to the analysis. In this design, it is possible to test the effect on the aggregation of each factor separately, time (h) and conditioned water and their interaction (time  $\times$  conditioned water). To ascertain which treatments were different, a Dunnett's post-test was used. Finally, to evaluate the effects of the interactions between the factors, paired comparisons between each treatment at each depth level and time were employed.

For the avoidance experiments, RM ANOVA were also employed, to evaluate differences in the distance from the prey to the predator between control and

treatment throughout the experiment. Factors, significant values and post-test were the same as for the previous RM ANOVA. The normal distribution of data (Komogorov–Simorov’s test), homoscedasticity (Levene’s test) and sphericity (Mauchly’s test) was verified prior to this analysis. In all cases, differences were considered significant at values of  $p < 0.05$ .

## Results

### Feeding experiments

Survival of microcrustaceans was 100% in all controls throughout the experiments. Decapods (*T. borellianus* and *M. borellii*) consumed more microcrustaceans than *Chaoborus* and, in general, the prawn was the most voracious predator (Figure 2).

Between both prey, decapods consumed more cladocerans than copepods (RM ANOVA, *M. borellii*: D2:  $F = 58.67$ ; D3:  $F = 7.76$ ; D4:  $F = 5.78$ ; *T. borellianus*: D2:  $F = 6.69$ ; D3:  $F = 8.34$ ; D4:  $F = 46.08$ ;  $p < 0.05$  in all cases). However, *Chaoborus* ingested both microcrustaceans at a similar rate (RM ANOVA, D2:  $F = 1.23$ ; D3:  $F = 0.29$ ; D4:  $F = 0.23$ ;  $p > 0.05$  in all cases; Figure 2).

The consumption of cladocerans was positively associated with its density (Table 1) and exposure time (Table 2). Most values for the Spearman’s correlation were remarkably high for decapods.

*Chaoborus* increased their consumption rate of copepods only with increasing prey density (Table 1) whereas decapods, in general, increased their consumption rate with the increase of prey density (especially for *M. borellii*, Table 1) as well as with exposure time (Table 2).

### Grouping experiments

*Ceriodaphnia dubia* showed a similar grouping pattern through time within each treatment (RM ANOVA,  $F = 0.79$ ,  $p = 0.461$ ), but with some differences among treatments (RM ANOVA  $F = 7.21$ ,  $p = 0.003$ ; Figure 3). With CW, cladocerans were more grouped than the control after the third hour of exposure (Dunnett’s test = 0.03 and 0.03,  $p = 0.009$  and  $p = 0.001$ ; for 3 and 24 h, respectively). In TW, they reflected a tendency to remain more clustered than the control, the differences being statistically significant only at 24 h (Dunnett’s test = 0.02,  $p = 0.025$ ; Figure 3). Although cladocerans exposed to MW showed a trend to remain more clustered compared with the control (Figure 3), there were no statistically significant differences between them (Dunnett’s test,  $p > 0.005$ ). Further tests with a larger sample size may reveal such a difference.

In general, all grouping behaviours occurred at the bottom of the container (ratios greater than 50% of the individuals), and levels 1 and 2 maintained low and similar numbers of cladocerans (Figure 4).

In the case of copepods, the grouping index ( $P_i$ ) was different through the exposure time (RM ANOVA,  $F = 3.55$ ,  $p = 0.040$ ) and among treatments (RM ANOVA,  $F = 4.22$ ,  $p = 0.022$ ). Regarding this last case, unlike cladocerans, copepods exposed to the predator’s infochemicals tended to be more dispersed than in the control. However, these differences were statistically significant only for TW (Dunnett’s test = 0.02,  $p = 0.01$ ; Figure 3).



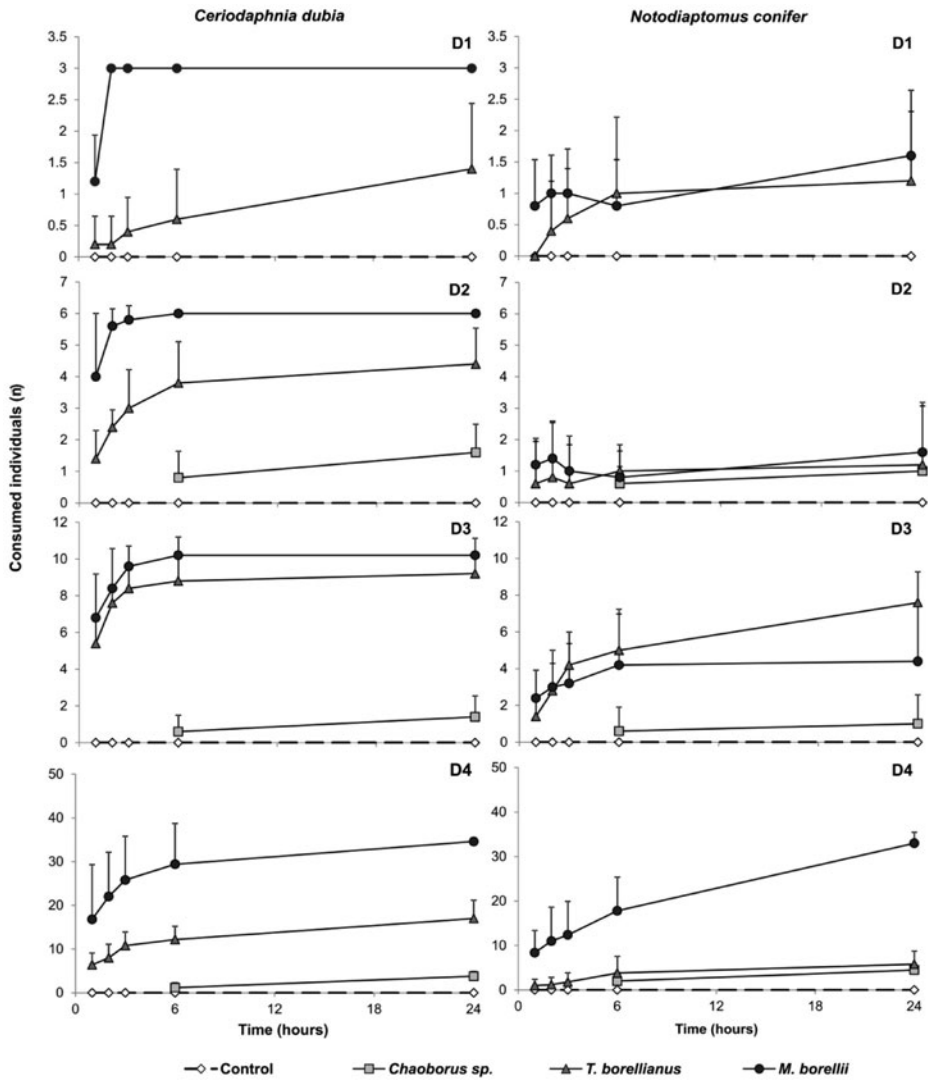


Figure 2. Number of consumed organisms (mean and standard deviation,  $n = 5$ ) at each prey density (D1, D2, D3 and D4) for each invertebrate predator. Left and right panels show the consumption of *C. dubia* and *N. conifer*, respectively.

On the other hand, depth selection by copepods was less predictable than that of cladocerans and it depended on the exposure time and invertebrate water (Figure 4). In some cases, they remained in the first level (e.g. with TW) but in other cases, they stayed at the lower level (e.g. with CW and MW).

**Avoidance experiments**

During the experiments, *C. dubia* was located between 5 and 14cm from the first level of the tube, where the predator remained in the cage (Figure 5). Each observed

Table 1. Spearman's correlation coefficients and critical values between the prey consumption of each predator and prey density at each time of observation.

		1 h	2 h	3 h	6 h	24 h
<i>Ceriodaphnia dubia</i>	<i>Chaoborus</i> sp.	Nm	Nm	Nm	0.1517	0.6190*
	<i>Trichodactylus borellianus</i>	0.7775**	0.8785**	0.9147**	0.9335**	0.9632**
	<i>Macrobrachium borelli</i>	0.6856**	0.8282**	0.9840**	0.9912**	0.9960**
<i>Notodiptomus conifer</i>	<i>Chaoborus</i> sp.	Nm	Nm	Nm	0.3685	0.7622**
	<i>Trichodactylus borellianus</i>	0.4062	0.3242	0.3573	0.5253*	0.6880**
	<i>Macrobrachium borelli</i>	0.6738*	0.6428*	0.8028**	0.8235**	0.7812**

Notes: Asterisks denote significant differences (\* $p < 0.05$ ; \*\* $p < 0.001$ ). Nm, not measured.

Table 2. Spearman's correlation coefficients and critical values between the prey consumption of each predator and time of observation for each analysed density (D1, D2, D3 and D4).

		D1	D2	D3	D4
<i>Ceriodaphnia dubia</i>	<i>Chaoborus</i> sp.	Nm	0.4488	0.4017	0.8033*
	<i>Trichodactylus borellianus</i>	0.4604*	0.7606**	0.3995*	0.7699**
	<i>Macrobrachium borelli</i>	0.7021**	0.5558*	0.4362*	0.6329**
<i>Notodiptomus conifer</i>	<i>Chaoborus</i> sp.	Nm	0.3187	0.4339	0.6220
	<i>Trichodactylus borellianus</i>	0.4300*	0.3122	0.7981**	0.6020*
	<i>Macrobrachium borelli</i>	0.1582	0.5017*	0.5652*	0.6978**

Note: Asterisks denote significant differences (\* $p < 0.05$ ; \*\* $p < 0.001$ ). Nm, not measured.

location of *C. dubia* depended on the predator and exposure time (MR ANOVA  $F = 4.58$ ,  $p = 0.002$ ).

Among the three predators, only the presence of the prawn *M. borellii* caused significant avoidance by cladocerans compared with the control (Dunnett's test = 2.79,  $p < 0.05$ ; Figure 5). Even though we detected no significant differences between the control and the other treatments, cladocerans always remained further away than the control in the presence of *T. borellianus* and tended to gradually move away from the location of *Chaoborus* when it was present, reaching a higher mean distance than the control at 3 h of exposure (Figure 5).

In general, copepods located themselves between 8 and 13 cm from predators. Despite an apparent trend towards a higher distance between the aforementioned organisms in the treatments than in the control, the result was not significant (MR ANOVA  $F = 0.17$ ,  $p = 0.913$ ).

## Discussion

### Feeding experiments

As expected from the results of previous field studies, the three predators observed here fed efficiently on *N. conifer* and *C. dubia* and the prawn *M. borellii* was the most voracious predator (Gonzales Sagrario et al. 2009).

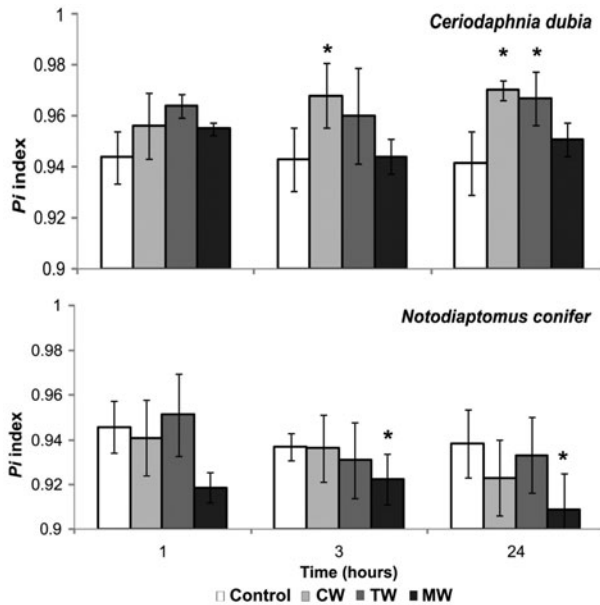


Figure 3. Level of aggregation of prey ( $P_i$  index) in each conditioned water (control, CW, *Chaoborus* water; TW, *T. borellianus* water; and MW, *M. borellii* water) during the experiment. The graph shows mean and standard deviation values for each treatment ( $n = 5$ ). Asterisks denote significant differences.

The feeding experiments also showed that the cladoceran *C. dubia* was the most vulnerable species when faced with to decapods, which might correlate with its weak evasive behaviour and slower swimming speed by comparison with copepods (Li and Li 1979; Berner 1986). Some authors have analysed the biological attributes of copepods that could explain their success, highlighting in this regard an elongated and articulated body, a highly developed sensory system and well-developed neuromuscular coordination all of which endow them with the ability to perform rapid evasive manoeuvres (Dussart and Defaye 2001; Kiørboe 2011).

Unlike that observed for decapods, the predation rate of *Chaoborus* was unexpectedly the same for both zooplankton species in all densities analysed. From an ecophysiological perspective, this result reflects the relative nature of any advantageous attribute, which means that an acquired trait might be considered advantageous on certain occasions but not on others (Harvel 1990; Lass and Spaak 2003). Even though the aforementioned biological traits of copepods may be advantageous against certain predators, they may not be so in the face of other predators or during the post-contact process. Furthermore, it is recorded that once contact with *Chaoborus* is made, copepods are handled much more efficiently than cladocerans of the same size (Swift and Fedorenko 1975). This would also explain why calanoid copepods are selectively eaten by *Chaoborus* under laboratory (Winner and Greber 1980) or natural conditions (Hanazato and Yasuno 1989). In this sense, although the vulnerability of a prey has been intensively analysed in terms of the pre and post-contact stages (Kiørboe and Visser 1999; Titelman 2001), the ability of a predator to manipulate the prey once captured has been underestimated.

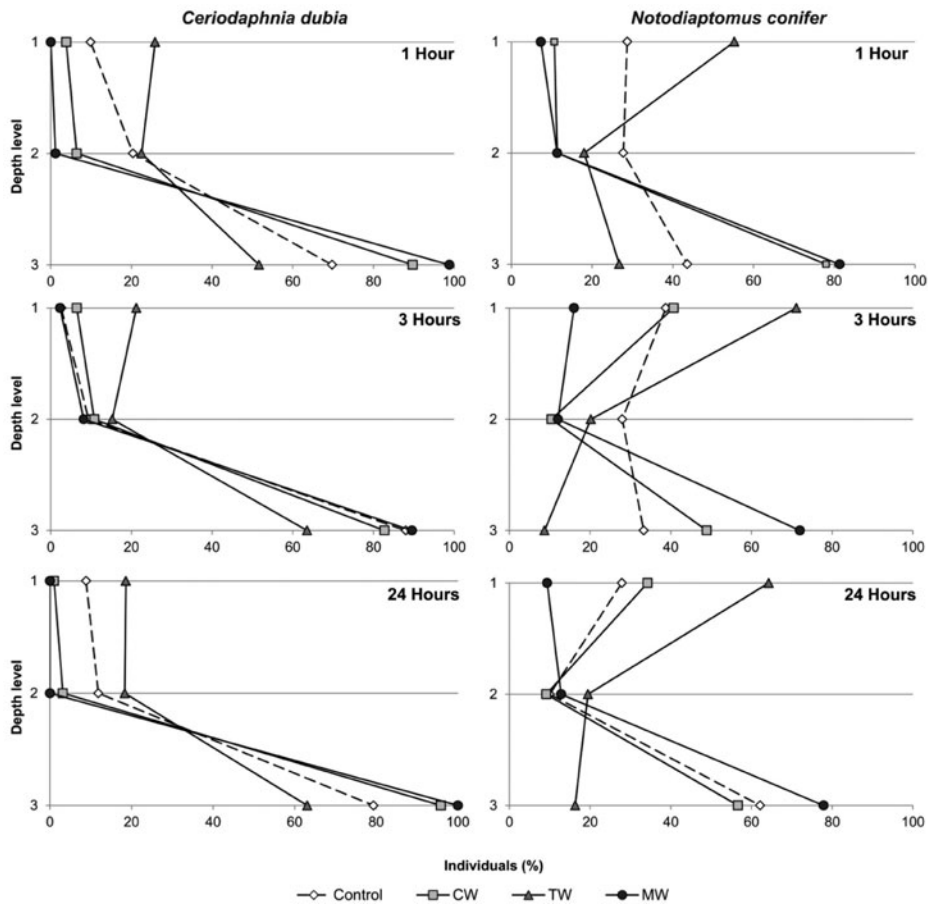


Figure 4. Percentage of prey in each depth level for each treatment (control, CW, *Chaoborus* water; TW, *T. borellianus* water; and MW, *M. borellii* water) over the experiment. Left panels show the values of *C. dubia* and right ones the values of *N. conifer*.

Further studies are therefore needed to understand the relative importance of such trait in predator–prey interactions.

Finally, the feeding experiments demonstrate that in agreement with previous plankton studies, predation rates of *M. borellii*, *T. borellianus* and *Chaoborus* are strongly dependent on prey density (Murdoch et al. 1984; Matveev et al. 1989; Carvalho and Collins 2011). In this sense, it is suggested that a higher number of prey in the same space not only enhances the encounter probability (Titelman 2001) but also reduces their swimming activity and resources intake (Dodson et al. 1997; Carvajal-Salamanca et al. 2008).

### Behavioural experiments

From the behavioural experiments, it can be assumed that the microcystaceans studied here are able to detect the presence of at least one invertebrate predator effectively through their alarm signals.

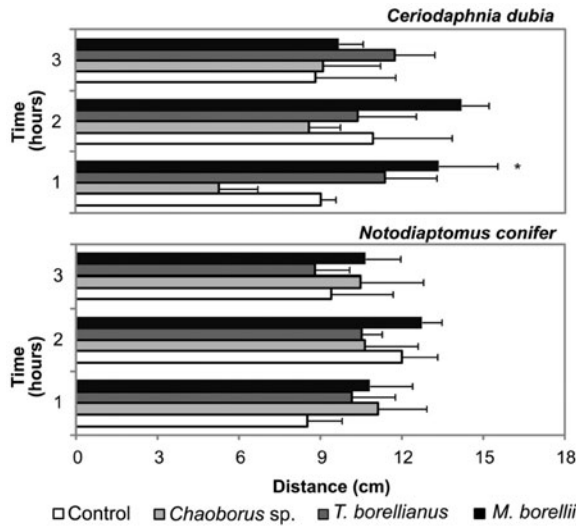


Figure 5. Distance (cm) between each predator and *C. dubia* (upper panel) and *N. conifer* (bottom panel) during the experiments. The graph shows mean and standard deviation values for each treatment ( $n = 5$ ). Asterisks denote significant differences.

*Ceriodaphnia dubia* responded to chemical signals from the three predators in two different ways: by grouping in the presence of infochemicals released by *Chaoborus* and the crab *T. borellianus*, and by swimming away from the ‘dangerous site’ (where the predator remained) in the presence of alarm signals of the prawn *M. borellii*. In the first case, the response triggered by *C. dubia* has also been documented in other cladocerans, and it is suggested that grouping is a good antipredation strategy because a great number of moving organisms, simultaneously visible, produce a confusion effect in predators (Ohman 1988). Additionally, the survival probability of an individual is greater when hidden among a large number of similar-looking conspecifics generating a dilution effect (Pijanowska and Kowalczewski 1997).

The observed responses of the cladocera were statistically significant after a relatively long exposure time (at 3 and 24 h after the start of the experiments). This result is not in agreement with those of de Meester and Cousin (1997), who found that the phototactic response of *C. dubia* begins in less than 30 min. Similarly, Van Gool and Ringelberg (1998) and Forward and Rittschof (1993) registered rapid responses in other zooplankton species. All these studies described, however, responses to kairomones released by fishes. Since this study analyses the responses to smaller invertebrates, the differences here suggest that the ‘reactivity’ of the prey could be related to the nature of the released kairomones. Although the knowledge of how fast an organism is able to react in the presence of an inducing environmental signal constitutes a key aspect of adaptive prey responses, there is only limited research into this issue (Aránguiz-Acuña et al. 2010). However, it has also been suggested that this functional trait is species-specific, constrained by the organisms’ own physiology and dependent on the set of costs and benefits associated with the available options (Harvel 1990). On the other hand, even though the early response of *C. dubia* exposed to kairomones released by *Chaoborus* was not different from that of the control, they progressively tended to swim away from the predator. It is likely that the residence time of the alarm signals is an important factor, and although the

experiment was not continued, we hypothesise that the distance from the predator would be higher with further passage of time. This observation also demonstrates that the latency period is of high importance when making decisions about the proper time of developing a phenotypic response. In this sense, higher reactivity implies more risk of exhibiting an unnecessary and costly dependent state, and the more intense the signal (or its residence time) is, the more certain the organisms will be about the actual risk of being predated (Aránguiz-Acuña et al. 2010).

The copepods *N. conifer* modified their behaviour only in the presence of the infochemicals from the most active and voracious predator, the prawn *M. borellii*. Despite the fact that some studies have shown that some copepods react to mechanical or visual stimuli rather than to chemical ones (Bollens et al. 1994; Lass and Spack 2003), here it has been demonstrated that the chemical signal is of high importance to induce a specific response.

In contrast to the results from cladocerans, copepods remained more dispersed when the infochemicals were present. Although aggregation has been considered a good strategy on some occasions, this would be especially related to visual predators (Pijanowska and Kowalczewski 1997; Gutierrez et al. 2011). Moreover, numerous authors have considered grouping as a maladaptive strategy since predators would be able to perform intensive search in patches of crowded organisms, thus optimising the exploitation of these resources (McNara and Houston 1985). In this case, the density decrease could be a temporary safety strategy in the presence of predators while at the same time, an effective gain for fitness as observed by Gliwicz et al. (2006) for certain daphnids.

In the case of avoidance experiments, copepods did not manifest significant responses in relation to controls, which means that, in spite of the risk of being captured, they did not swim away from predators. This absence of response might be explained by the hypothesis that all biological responses are induced only if real benefits exceed the costs (Aránguiz-Acuña et al. 2010) since in this case, the risk of being predated was more apparent than real. Moreover, a higher distance from the predator would imply that the animals would stay more grouped in the extreme of the experimental tube, being negatively affected by the consequences of being crowded (Carvajal-Salamanca et al. 2008). On the other hand, these results also suggest that avoidance and grouping are not the most advantageous antipredator strategies of copepods against certain predators. Probably, under natural conditions, they must find refuge in other behavioural or life-cycle strategies (Gutierrez et al. 2010).

Finally, the absence of responses to the other two predators (*Chaoborus* and *T. borellii*) does not necessarily imply that the prey are not able to recognise the alarm signals, since the behaviours analysed constitute only a small part of the feasible set of strategies. In this sense and following the conclusions of Lind and Cresswell (2005), further studies will be necessary to establish unambiguous conclusions about the fitness consequences of antipredation behaviours in response to other invertebrate predators.

In brief, the observed differences in the responses of *C. dubia* and *N. conifer* allowed us to recognise that both microcrustaceans have different vulnerabilities and strategies when faced with the same invertebrate predators. As a whole, these results may help to explain the differences in the distribution and some cyclic behaviours (such as diel horizontal or vertical migrations) of these planktonic crustaceans registered in nature (Meerhof et al. 2006; Gonzales Sagrario et al. 2009).

## Acknowledgements

The authors thank to Prof. Juan César Paggi for his critical lecture and constructive contributions and to Mr. Cristian De Bonis for his technical assistance. The authors also wish to thank to Prof. David Macmillan and two anonymous reviewers for their valuable comments and suggestions. This research was granted by the Agencia Nacional Científica y Tdecnológica, FONCYT, PICT 2011 – 2350.

## References

- Aránguiz-Acuña A, Ramos-Jiliberto R, Sarma N, Sarma SSS, Bustamante RO, Toledo V. 2010. Benefits, costs and reactivity of inducible defenses: an experimental test with rotifers. *Freshwat Biol.* 55(10):2114–2122.
- Berner D. 1986. Taxonomy of *Ceriodaphnia* (Crustacea: Cladocera) in US environmental protection agency cultures. Cincinnati (OH): Environmental Protection Agency. EPA/600/S4-86/032.
- Boeing WJ, Wissel B, Ramcharan CW. 2005. Costs and benefits of *Daphnia* defense against *Chaoborus* in nature. *Can J Fish Aquat Sci.* 62(6):1286–1294.
- Bollens SM, Frost BW, Cordell JR. 1994. Chemical, mechanical and visual cues in the vertical migration behaviour of the marine planktonic copepods *Acartia hudsonica*. *J Plankton Res.* 16(5):555–564.
- Bonetto AA, Pignalberi C, Cordiviola E. 1963. Ecología alimentaria del amarillo y moncholo, *Pimelodus clarias* (Bloch) y *Pimelodus albicans* (Valenciennes). *Physis.* 24:87–94.
- Burks RL, Jeppesen E, Lodge DM. 2000. Macrophyte and fish chemicals suppress *Daphnia* growth and alter life-history traits. *Oikos.* 88(1):139–147.
- Carvajal-Salamanca JL, Aránguiz Acuña A, Ramos-Jiliberto R, Zúñiga LR. 2008. Immediate and delayed life-history responses of *Daphnia ambigua* to conspecific cues. *J Plankton Res.* 30(10):1117–1122.
- Carvalho DA, Collins PA. 2011. Ontogenetic predation capacity of *Macrobrachium borellii* (Caridea: Palaemonidae) on prey from littoral-benthic communities. *Nauplius.* 19(1):71–77.
- Castro BB, Marques SM, Gonçalves F. 2007. Habitat selection and diel distribution of the crustacean zooplankton from a shallow Mediterranean lake during the turbid and clear water phases. *Freshwat Biol.* 52(3):421–433.
- Cieri MD, Stearns DE. 1999. Reduction of grazing activity of two estuarine copepods in response to the exudate of a visual predator. *Mar Ecol Prog Ser.* 177:157–163.
- Collins PA, Paggi JC. 1998. Feeding ecology of *Macrobrachium borellii* (Nobili) (Decapoda: Palaemonidae) in the flood valley of the River Paraná, Argentina. *Hydrobiologia.* 362(1–3):21–30.
- Collins PA, Williner V, Giri F. 2007. Macrocrustaceans. In: Iriondo MH, Paggi JC, Parma MJ, editors. *The Middle Paraná River: limnology of a subtropical wetland. Littoral communities.* Chapter 11. Berlin (Germany): Springer-Verlag. p. 277–301.
- de Meester L, Cousing C. 1997. The change in phototactic behaviour of a *Daphnia magna* clone in the presence of fish kairomones: the effect of exposure time. *Hydrobiologia.* 360(1–3):169–175.
- Dodson SI, Ryan S, Tollrian R, Lampert W. 1997. Individual swimming behavior of *Daphnia*: effects of food, light and container size in four clones. *J Plankton Res.* 19(10):1537–1552.
- Dussart BH, Defaye D. 2001. Introduction to the copepoda. *Guides to the identification of the microinvertebrates of the continental waters of the world.* 2nd ed. Leiden (The Netherlands): Backhuys Publishers. Revised and enlarged.
- Ferriz RA, Villar CA, Colautti D, Bonetto C. 2000. Alimentación de *Pterodoras granulosus* (Valenciennes) (Pises, Doradidae) en la baja cuenca del Plata. *Rev Mus Argent Cienc Nat.* 2(2):151–156.

- Folt CL, Goldman CR. 1981. Allelopathy between zooplankton: a mechanism for interference competition. *Science*. 213(4512):1133–1135.
- Forward Jr RB Jr, Rittschof D. 1993. Alteration of photoresponses involved in diel vertical migration of a crab larva by fish mucus and degradation products of mucopolysaccharides. *J Exp Mar Biol Ecol*. 245(2):277–292.
- García de Emiliani MO. 1990. Phytoplankton ecology of the Middle Paraná River. *Acta Limnol Bras*. 3(1):391–417.
- Gliwicz ZM. 1994. Relative significance of direct and indirect effects of predation by planktivorous fish on zooplankton. *Hydrobiologia*. 272(1–3):101–210.
- Gliwicz ZM, Dawidowicz P, Maszczyk P. 2006. Low density anti-predation refuge in *Daphnia* and *Chaoborus*? *Arch Hydrobiol*. 167(1–4):101–114.
- Gonzales Sagrario MA, Balseiro E. 2010. The role of macroinvertebrates and fish in regulating the provision by macrophytes of refugia for zooplankton in a warm temperate shallow lake. *Freshwat Biol*. 55(10):2153–2166.
- Gonzales Sagrario MA, Balseiro E, Ituarte R, Spivak E. 2009. Macrophytes as refuge or risky area for zooplankton: a balance set by littoral predatory macroinvertebrates. *Freshwat Biol*. 54(5):1042–2053.
- Gori M, Carpaneto GM, Ottino P. 2003. Spatial distribution and diet on the Neotropical otter *Lontra longicaudis* in the Ibera Lake (northern Argentina). *Acta Theriol*. 48(4):495–504.
- Gutierrez MF, Gagneten AM, Paggi JC. 2011. Behavioural responses of two cladocerans and two copepods exposed to fish kairomones. *Mar Freshwat Behav Physiol*. 44(5):289–303.
- Gutierrez MF, Paggi JC, Gagneten AM. 2010. Fish infochemicals alter life cycle and growth of a calanoid copepod. *J Plankton Res*. 32(1):47–55.
- Hanazato T, Yasuno M. 1989. Zooplankton community structure driven by vertebrate and invertebrate predators. *Oecologia*. 81(4):450–458.
- Harvel CD. 1990. The ecology and evolution of inducible defenses. *Quart Rev Biol*. 65(3):323–340.
- Jamieson CD. 2005. Coexistence of two similar copepod species, *Eudiaptomus gracilis* and *E. graciloides*: the role of differential predator avoidance. *Hydrobiologia*. 542(1):191–202.
- José de Paggi S, Paggi JC. 2007. Zooplankton. In: Iriondo MH, Paggi JC, Parma MJ, editors. *The Middle Paraná River: limnology of a subtropical wetland. Littoral communities*. Chapter 9. Berlin (Germany): Springer-Verlag. p. 229–249.
- José de Paggi S, Paggi JC. 2008. Hydrological connectivity as a shaping force in the zooplankton community of two lakes in the Paraná River floodplain. *Int Rev Hydrobiol*. 93(6):659–678.
- Kellogg CM, Dorn NJ. 2012. Consumptive effects of fish reduce wetland crayfish recruitment and drive species turnover. *Oecologia*. 168(4):1111–1121.
- Kjørboe T. 2011. What make pelagic copepods so successful? *J Plankton Res*. 33(5):677–685.
- Kjørboe T, Visser AW. 1999. Predator and prey perception distances in zooplankton due to hydromechanical signals. *Mar Ecol Prog Ser*. 143:65–75.
- Lajmanovich RC, Beltzer AH. 1993. Aporte al conocimiento de la biología alimentaria de la pollona negra *Gallinula chloropus* en el Paraná Medio, Argentina. *El Hornero*. 13(4):289–291.
- Lass S, Spaak P. 2003. Chemically induced anti-predator defences in plankton: a review. *Hydrobiologia*. 491(1–3):221–239.
- Lazzaro X. 1987. A review of planktivorous fishes: their evolution, feeding behaviours, selectivities, and impacts. *Hydrobiologia*. 146(2):97–167.
- Li JL, Li WL. 1979. Species-specific factors affecting predator-prey interactions of the copepod *Acanthocyclops vernalis* with its natural prey. *Limnol Oceanogr*. 24(4):613–626.
- Lind J, Cresswell W. 2005. Determining the fitness consequences of antipredation behavior. *Behav Ecol*. 16(5):945–956.
- Lloyd M. 1967. Mean crowding. *J Anim Ecol*. 36(1):1–30.



- Matveev VF, Martinez CC, Frutos SM. 1989. Predatory-prey relationships in subtropical zooplankton: water mite against cladocerans in an Argentine lake. *Oecologia*. 79(4):489–497.
- McNara JM, Houston IA. 1985. Optimal foraging and learning. *J Theor Biol*. 117(2):231–249.
- Meerhof M, Fosalba C, Bruzzone C, Mazzeo N, Noordoven W, Jeppesen E. 2006. An experimental study of habitat choice by *Daphnia*: plants signal danger more than refuge in subtropical lakes. *Freshwat Biol*. 51(7):1320–1330.
- Murdoch WW, Scott MA, Ebsworth P. 1984. Effects of the general predator, Notonecta (Hemiptera) upon a freshwater community. *J Anim Ecol*. 53(3):791–808.
- Neill WE. 1981. Impact of *Chaoborus* predation upon the structure and dynamics of a crustacean zooplankton community. *Oecologia*. 48(2):164–177.
- Ohman MD. 1988. Behavioral responses of zooplankton to predation. *Bull Mar Sci*. 43(3):530–550.
- Oliva A, Ubeda C, Vignes EI, Iriondo A. 1981. Contribución al conocimiento de la ecología alimentaria del bagre amarillo (*Pimelodus maculatus* Lacépède 1803) del río de la Plata (Pisces, Pimelodidae). *Comun Mus Argent Cienc Nat 'Bernardino Rivadavia' Ecol*. 1(4):31–50.
- Pijanowska J, Kowalczewski A. 1997. Predators can induce swarming behaviour and locomotory responses in *Daphnia*. *Freshwat Biol*. 37(3):649–656.
- Port-Carvalho M, Ferrari SF, Magalhães C. 2004. Predation of crabs by Tufted Capuchins (*Cebus apella*) in Eastern Amazonia. *Folia Primatol*. 75(3):154–156.
- Ramcharan CW, Yan ND, McQueen DJ, Pérez-Fuentetaja A, Demers E, Rusak JA. 2001. Complex responses of *Chaoborus* to changes in fish populations. *Arch Hydrobiol Spec Iss Adv Limnol*. 56:81–100.
- Rohlf F. tpsDIG [internet]. 1997. Department of Ecology and Evolution, State University, Stony Brook, New York. Available from: <http://life.bio.sunysb.edu/morph/soft-dataacq.html>
- Rojas Molina F, Paggi JC, Devercelli M. 2010. Zooplanktophagy in the natural diet and selectivity of the invasive mollusk *Limnoperna fortunei*. *Biol Invasions*. 12(6):1647–1659.
- Swift MC, Fedorenko AY. 1975. Some aspects of prey capture by *Chaoborus* larvae. *Limnol Oceanogr*. 20(3):418–425.
- Titelman J. 2001. Swimming and escape behavior of copepod nauplii: implications for predator-prey interactions among copepods. *Mar Ecol Prog Ser*. 213:203–213.
- Van Gool E, Ringelgerg J. 1998. Quantitative effects of fish kairomones and successive light stimuli on downward swimming responses of *Daphnia*. *Aquat Ecol*. 32(4):291–296.
- Winner RW, Greber JS. 1980. Prey selection by *Chaoborus punctipennis* under laboratory conditions. *Hydrobiologia*. 68(3):231–233.
- Zalocar de Domitrovic Y, Devercelli M, García de Emiliani MO. 2007. Phytoplankton. In: Iriondo MH, Paggi JC, Parma MJ, editors. *The Middle Paraná River: limnology of a subtropical wetland*. Chapter 7. Berlin (Germany): Springer-Verlag. p. 175–203.