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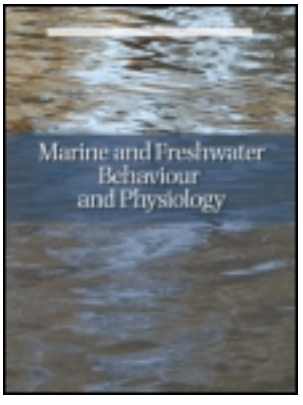

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Behavioural responses of two cladocerans and two copepods exposed to fish kairomones

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In natural predator–prey interactions, chemical communication is one of the most advantageous strategies for prey organisms because they can anticipate possible harm by means of phenotypic changes. This study compares the changes in the behaviour of four freshwater zooplankton species in the presence and absence of infochemicals from the same predator. The studied organisms are two copepods and two cladocerans living in highly variable freshwater environments. The analysis is focused on two predator defensive behaviours: a pre-encounter and a post-encounter response. First, we analysed the diel vertical migration (DVM) of the organisms inside 150 cm long transparent plastic tubes. Second, we used a novel hydraulic apparatus to quantify their ability to escape from a potential predator. The results revealed that the species have different behavioural patterns in the absence of infochemical. The differences were mainly in the way DVM developed and reflect their life histories and adaptive strategies relative to their natural environment. When faced with kairomones, the escape ability of the organisms was enhanced in all cases and DVM changed, although not always in agreement with the expected patterns. The interaction between each species and the multiple environmental components is discussed.

Keywords: Behaviour; Chemical communication; Cladocera; Copepoda

Introduction

Behaviour is one of the most important factors in the distribution and evolutionary success of zooplankton species due to the advantages it can bestow on both individuals and entire populations (Gliwicz 1994; Vos et al. 2002). Genetic makeup, physiological condition and life history shape natural behaviours (De Meester 1993; De Meester and Pijanowska 1996). However, the physical, chemical and biological conditions of the environment can modify them by favouring different phenotypes (Kerfoot 1980; De Meester et al. 1999; Tollrian and Dodson 1999; Hanazato 2001). Among the biological conditions, the threat of being eaten leads to the development of defensive mechanisms (Gliwicz 1994; De Meester et al. 1999). It has been demonstrated that these mechanisms can favour coexistence among organisms

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(Jamieson 2005; Aránguiz-Acuña et al. 2010) and help to structure the diversity of ecosystems (Ohman 1988; Agrawal 2001).

Danger detection can take different forms, but chemical communication has been reported as one of the most advantageous strategies because prey can anticipate harm by means of phenotypic changes (Lass and Spaak 2003). It has been extensively reported for cladocerans, copepods and rotifers that such changes tend to be manifested only if they produce benefits that overcome the energetic costs of production (Gliwicz 1994). From a theoretical perspective (Kerfoot et al. 1980), it is possible to assume that the cost/benefit balance differs between systems and is more complex in highly variable environments (such as shallow lakes or littoral areas) than in more stable environments (great lakes or marine areas) (Castro et al. 2007; Jensen et al. 2010).

Here, we report the results of an investigation that contributes to knowledge of the interactions that take place in such variable environments. The phenotypic changes in the behaviour of four zooplankton microcrustaceans, coexisting in these systems, were compared in the presence and absence of infochemicals from the same predator.

The analysis is focused on two ethological responses: a pre-encounter (DVM, diel vertical migration) and a post-encounter response (escape ability at three mechanical capture speeds). For this purpose, two copepod species (*Argyrodiaptomus falcifer* and *Notodiaptomus conifer*) and two cladoceran species (*Ceriodaphnia dubia* and *Pseudosida variabilis*) were selected. They were all frequent shallow water bodies in the alluvial plain of the Paraná river. These systems are highly variable, and the presence of small fish plays a key role as selection pressure (José de Paggi 1995) even more than other factors such as thermal and oxygen stratification, typical of greater lakes (Esteves 1988). Diurnal vertical migration (DVM) was selected as the main preventive strategy. It involves a considerable energy cost by zooplankton organisms and is one of the main spatial and temporal defence mechanisms in the water column (Dodson 1988; Lampert 1993; Young and Watt 1993; Van Gool and Ringelberg 2002). On the other hand, escape behaviour is the most effective and immediate post-encounter strategy (Viitasalo et al. 1998; KiØrboe and Visser 1999; Titelman 2001). In both cases, the mechanisms involved are quite variable and bring about important consequences at population level (Gerritsen and Strickler 1977).

Two hypotheses were tested: (a) in the absence of fish infochemicals (kairomones), the selected species have different behavioural patterns and (b) the presence of the infochemicals enhances their escape ability and changes their migration movements according to the ''normal'' pattern proposed by Hutchinson (1967) and Lampert (1989). These authors suggested that the presence of vertebrate predators would result in nocturnal ascent and diurnal descent.

Materials and methods

Test organisms and treatments

The four selected species were collected with a plankton net (200 μm) and cultured in the laboratory over several months under constant photoperiod (12 light:12 darkness) and temperature ($21 \pm 2^{\circ}C$) conditions in glass containers. The mean body sizes of the species are summarized in Table 1. We employed filtered (53 μm) and aerated pond water, aged for at least 24 h as culture medium. This medium was

Table 1. Average size of the studied species.

		Length (mm)	
Species	*N*	Mean	±SD
A. falcifer	28	1.752	0.142
N. conifer	40	1.503	0.051
P variabilis[a]	30	1.461	0.104
C. dubia	30	1.216	0.120

Note: The table shows the number of measured individuals (*N*), mean size in mm (mean) and the standard deviation (±SD). Average size of copepods was measured from the tip of prosome to the end of caudal rami, and that of cladocerans was measured from the top of the head to the basis of the carapace.
[a]Juveniles.

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also used as the control treatment and its physicochemical characteristics were measured according to APHA et al. (1998): nitrates: $<0.1\,mg\,L^{-1}$; nitrites: $0.01\,mg\,L^{-1}$; ammonium: $0.29\,mgNH_3\,L^{-1}$; chlorides: $3.5\,mg\,L^{-1}$; sulphates: $8.3\,mg\,L^{-1}$; total alkalinity: $77\,mg\,CaCO_3\,L^{-1}$; bicarbonates: $94\,mg\,L^{-1}$; sodium: $7.7\,mg\,L^{-1}$; magnesium: $6.8\,mg\,L^{-1}$; calcium: $12.9\,mg\,L^{-1}$; potassium: $1.8\,mg\,L^{-1}$; DQO: $10\,mg\,L^{-1}$; and DBO5: $0.08\,mg\,L^{-1}$. Dissolved oxygen was 6.4 (±0.8) ppm; pH: 8.39 (±0.24); conductivity: 245.33 (±28.18) $\mu S\,cm^{-1}$.

During the period of culture, the organisms were fed daily for *ad libitum* consumption with a *Chlorella* sp. concentrate (algal density: $2.8 \times 105\,cells\,mL^{-1}$). Among the different species of zooplanktivorous fish, *Cnesterodon decemmaculatus* was selected due to its abundance in shallow water bodies associated with the alluvial plain of the Paraná river. Numerous investigators have studied the ecological characteristics of this fish, both at individual and population levels (Oliveros 1980; Escalante 1983; Oliveros and Rossi 1991; Barros 2004). The treatment with fish kairomone (kairomone water, KW), the infochemical, was obtained by placing 20 adults (length: $3.6 \pm 0.6\,cm$) into a fish-tank (6 L) with the same culture medium as for copepods and cladocerans. The fish remained at least 24 h in this medium before the assays and they were not fed during this period in order to avoid altering the quality of the medium chemistry.

Escape assays

In the case of copepods (*A. falcifer* and *N. conifer*), only adult males were used, whereas in the case of *Cladocera Anomopoda* (*C. dubia*), adult females were used. In the case of *Cladocera Ctenopoda* (*P. variabilis*) planktonic juveniles were employed because they have a more constant swimming activity than adults. To obtain quantitative information on the ability of the organisms to perceive and react to the approach of a possible predator (considered as escape response), a novel hydraulic mechanism based on early Szlauer (1964) experiments was designed. The mechanism consisted in placing a transparent tube (length: 20 cm and diameter: 5 mm) moved by

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a piston among the organisms swimming in a bigger glass container (capacity: 18 mL). The piston, located in a polyvinyl chloride cylinder, was elevated by the entry of running water through a plastic hose. When water was released, the piston descent provoked the descent of the capture glass tube. The three capture speeds (0.27, 2.87 and 15 cms^{-1}) were regulated by a manual valve placed at the extreme of the water outlet.

These capture speeds were selected taking into account the range used by Szlauer (1964) and described here as low capture speed (LCS), mean capture speed (MCS) and rapid capture speed (RCS). The escape ability of the organisms was calculated as the difference between the number of exposed organisms and the number of captured ones. In each experiment, "catching attempts" were repeated 100 times, except for *P. variabilis*, for which catching experiments were repeated 50 times. Thirty individuals per replicate were exposed and this number was maintained throughout the experiments.

To prevent fatigue or habituation from influencing the results, each catching attempt was carried out with different groups of organisms. Nevertheless, a preliminary experiment with 50 identically successive catches at MCS was undertaken and no significant correlation between the success of catches and the abundance of captured copepods was found (*N. conifer*: $r^2 = 0.0046$ and *A. falcifer*: $r^2 = 0.0192$) or cladocerans (*C. dubia*: $r^2 = 0.0042$ and *P. variabilis* $r^2 = 0.001$).

Since the dependent variable (number of captured individuals) produced an asymmetrical distribution (mean values of kurtosis, dispersion and asymmetry coefficients being, respectively, −15, 0.66 and 0.69 for LCS; 1.07, 1 and 0.53 for MCS; and 0.23, 1.5 and 0.53 for RCS), the significance of the effect of infochemicals on escape success was tested using deviance analysis (ANODEV) (McCullagh and Nelder 1989). Differences were considered significant at $p < 0.05$.

Vertical migration

Vertical migration assays were performed inside transparent plastic tubes (total length: 150 cm and diameter: 7.2 cm) filled with 2 L of the treatment (KW) or control. The tubes were externally marked every 30 cm, determining four depth levels (0–30 cm, 30–60 cm, 60–90 cm and 90–120 cm) suspended from a 2-m high iron support built *ad hoc*. The contour of each tube was covered with an opaque black plastic sheet (0.075 mm thick) so that the white cold light entered only from the surface. The presence of a diffusive plaque made of white acrylic allowed the light source generated from the fluorescent tubes to diffusively and uniformly illuminate, imitating the above light of a natural aquatic environment. The incident intensity of the tubes was 3593.3 (±77) lux at the top; 2200 (±244) lux at the middle and 1200 (±154) lux at the bottom. To estimate the DVM of the organisms, the number of individuals present at each level was quantified for every 6 h (00:00; 06:00; 12:00 and 18:00). Each treatment was replicated thrice (control and KW). At night (00:00 and 06:00), the organisms were quantified using a red-light lantern so as not to alter their sensitivity. In every case, quantifications were performed as quickly as possible so as to reduce stress probability and counting errors. At this experimental stage, several indicators were calculated which allowed a comparison of the behaviour of the organisms with the one considered "normal" in the presence of vertebrate predators: nocturnal ascent and diurnal descent (Lampert 1993); degree of crowding

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(Jacobsen and Johnsen 1988; Pijanowska and Kowalczewski 1997) and differences in migration distance between nocturnal and diurnal hours (Tollrian and Harvell 1999). To quantify significant differences between daily movements, we employed a two-way repeated measure (RM) analysis of variance (ANOVA). The analyses were carried out separately for each treatment (control and KW) as a means to simplify the statistical design. The dependent variable was the number of individuals present in each section of the water column, transformed in $\log_n(x+1)$. The within-subject factors of the RM ANOVA were time of the day (four levels) and depth (four levels) while between-subject factors were treatments (control and FW). Prior to each analysis, normality (Komogorov–Smirnov test), homoscedasticity (Levene test) and sphericity (Mauchly test) of the data obtained were verified. To locate the organisms in the water column for each replicate, mean depth (D) was assessed (Hoffman 1975; Cruz Pizarro 1978; Dodson 1988) by calculating the number of individuals per depth level according to the equation:

$$D = \Sigma N_i d_i / \Sigma N_i$$

where N_i is the number of individuals and d_i the depth level. Then, to analyse the differences in index D between control and KW at every moment of the day, a two-way RM ANOVA was employed separately for each species. In this case, the within-subject factors of the RM ANOVA were time of the day (four levels) and between-subject factors were treatments (control and FW). Normal distribution of the data (Komogorov–Smirnov test), homoscedasticity (Levene test) and sphericity (Mauchly test) were previously verified.

Index P_i was used to determine the level of aggregation of individuals in the water column (Llyod 1967). This index was calculated using the equation:

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

where σ is the simple variance and x the number of individuals in the column. From the indices thus obtained for each replicate, a two-way RM ANOVA was employed. The within-subject factors of the RM ANOVA were time of the day (four levels) and between-subject factors were treatments (Control and FW). This analysis allowed for analysing the aggregation differences between control and KW throughout the day, previously verifying normal distribution of the data (Komogorov–Smirnov test), homoscedasticity (Levene test) and sphericity (Mauchly test). Finally, to compare the migration distances (maximum depth reached − minimum depth) for each species between control and KW, the t-test for independent samples was employed (with a reliability interval of 95%). Homogeneity of variances was previously verified by Levene's test. The data were previously transformed to $\log_n(x)$. Differences were considered significant at values of $p < 0.05$ in all cases.

Results

Escape

The escape ability of organisms was quantified as a function of the number of captured individuals per capture event (number of events per treatment = 100, except for *P. variabilis*, where $n = 50$). The average number of captured individuals varied according to the species and increased in all cases with capture speed

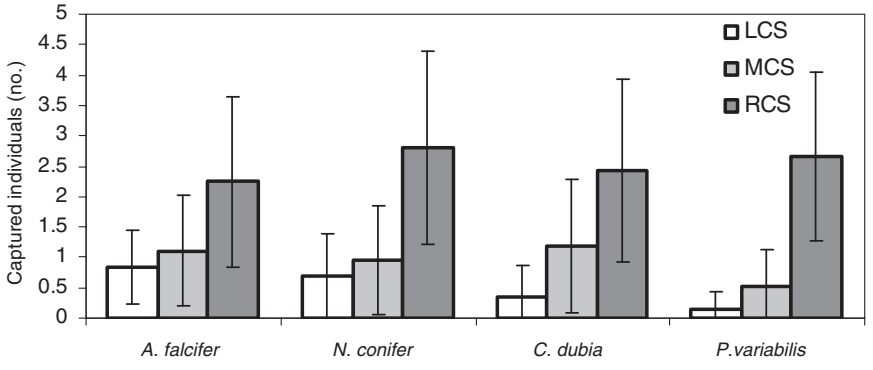

Figure 1. Average number of captured individuals in escape assays for each species and capture speeds: LCS, low capture speed; MCS, mean capture speed and RCS, rapid capture speed. Error bars represent ±SD.

(*N. conifer*: $r = 0.83$, $p < 0.05$; *A. falcifer*: $r = 0.88$, $p < 0.05$; *C. dubia*: $r = 0.99$, $p < 0.05$; and *P. variabilis*: $r = 0.86$, $p < 0.05$) (Figure 1). *Pseudosida variabilis* was the least captured species both at LCS and MCS, whereas *A. falcifer* was the least captured species at RCS.

The differences between the control and KW averages for each capture speed are shown in Figure 2. The four species under study manifested different responses to the presence of the fish infochemical in both senses (positive or negative) and also in magnitude (bar length). Based on these averages, copepod responses were positive for the three capture speeds, i.e. the organisms increased their escape efficiency with respect to the control, being statistically significant for the rapid speed (*A. falcifer*: $p = 0.037$, Wald Chi-Square$_{1,198,0.05} = 4.329$ and *N. conifer*: $p = 0.028$, Wald Chi-Square$_{1,198,0.05} = 4.576$, ANODEV). Cladoceran *C. dubia* increased its escape efficiency at the two highest speeds (MCS: $p = 0.003$, Wald Chi-Square$_{1,198,0.05} = 9.075$ and RCS: $p < 0.001$, Wald Chi-Square$_{1,198,0.05} = 14.523$, ANODEV), whereas at LCS trended towards the opposite behaviour but was not significantly different from that of the control ($p > 0.05$, ANODEV). *Pseudosida variabilis* also increased the escape efficiency at the most RCS ($p = 0.022$, Wald Chi-Square$_{1,98,0.05} = 4.459$, ANODEV) but showed no significantly different responses from those of the control at lower speeds ($p > 0.05$, ANODEV).

Vertical migration

The daily migration pattern for each species under analysis is shown in Figure 3. Copepods *A. falcifer* from the control treatment did not show a significant migration movement during the day ($p = 0.113$; df = 3; and $F = 3.061$; RM ANOVA). They stayed at the top level, which was significantly different from the rest of the depth levels ($p < 0.001$; df = 3; and $F = 42.572$; RM ANOVA). When these organisms were exposed to KW, no significant differences were observed between the four daily observations ($p = 0.455$; df = 3; and $F = 1$; RM ANOVA). However, significant differences were registered at the four depth levels ($p = 0.013$; df = 3; and $F = 8.688$, RM ANOVA), confirming the slight migratory movements evident in Figure 3.

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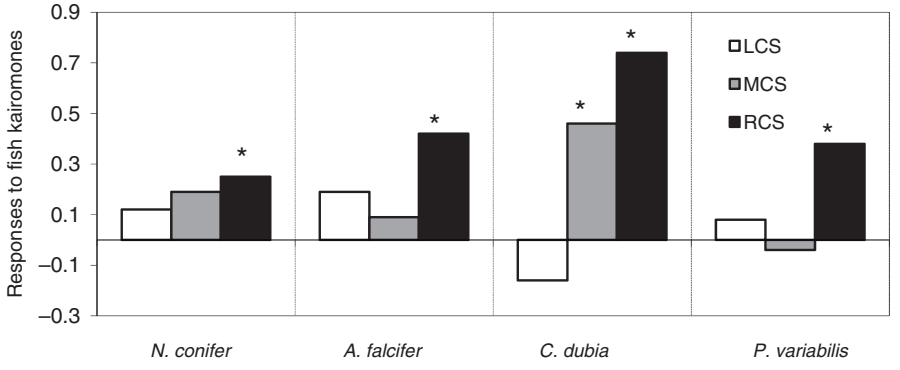

Figure 2. Differential responses to kairomones at the three capture speeds (LCS, MCS and RCS). Positive values indicate that the organisms had the highest ability to escape than the control. On the contrary, negative values indicate that the organisms were more captured than those of the control. The length of each bar represents the magnitude of this response. Asterisks indicate statistically significant differences from the control (ANODEV; $p < 0.05$).

In both the cases, no interaction was registered among factors: time of the day and depth level ($p > 0.05$; RM ANOVA).

The control *N. conifer* copepods tended to migration movements between daytime (12:00 and 18:00) and night-time (00:00 and 06:00) (Figure 3) but the trends were not significant ($p = 0.248$; df = 3; and $F = 1.796$; RM ANOVA). In contrast, significant differences were registered between the four depth levels ($p = 0.011$; df = 3; and $F = 0.944$; RM ANOVA). On the other hand, the organisms exposed to KW showed significant differences during migration both at depth levels ($p = 0.01$; df = 3; $F = 24.505$; RM ANOVA) and during the different day hours ($p = 0.027$; df = 3; and $F = 6.322$; RM ANOVA), moving during night-time towards higher levels. No interaction was registered among factors: time of the day and depth level ($p > 0.05$; RM ANOVA).

Cladocerans *C. dubia* significantly performed different migration movements during the daytime both for the control ($p = 0.29$; df = 3; $F = 6.197$, RM ANOVA) and for KW ($p = 0.25$; df = 3; and $F = 6.\ 584$; RM ANOVA). Similarly, significant differences were registered in the depth levels for both treatments (control: $p = 0.003$; df = 3; $F = 15.957$ and KW: $p = 0.002$; df = 3; $F = 4.944$; RM ANOVA). In the control group, these cladocerans stayed at the deepest levels (depth levels: 3 and 4) during most of the day (00:00, 12:00 and 18:00), slightly migrating towards higher levels by dawn, until reaching equal distribution at the different depths at 06:00. Organisms subject to KW showed more substantial upward movements at 06:00 and 18:00 but, as with the control group, stayed at lower levels at 00:00 and 12:00. In both the cases, no interaction was registered among factors: time of the day and depth level ($p > 0.05$; RM ANOVA).

Control *P. variabilis* performed minimum migration movements during daytime, staying most of the time at lower levels, appearing to make brief excursions towards higher levels at 18:00. Those movements were not significant during the day ($p = 0.578$; df = 3; and $F = 0.714$, RM ANOVA), but were significant at depth levels ($p = 0.005$; df = 3; and $F = 12.934$; RM ANOVA). When *P. variabilis* was subject to the KW treatment, it did not ascend at any time ($p = 0.5$; df = 3; and $F = 0.886$; RM

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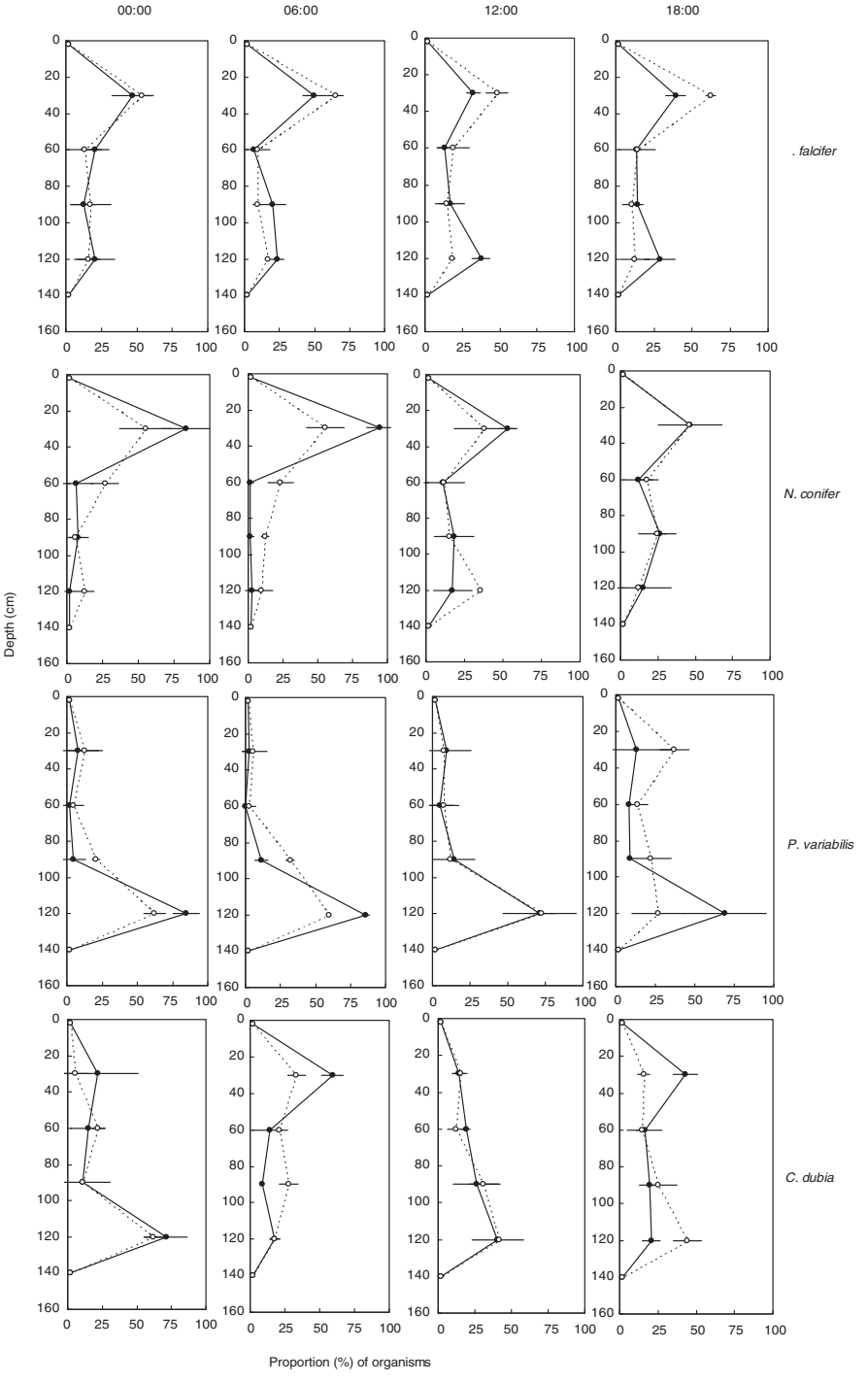

Figure 3. Depth distribution of *A. falcifer*, *N. conifer*, *P. variabilis* and *C. dubia* at 00:00, 06:00, 12:00 and 18:00 h. Pointed lines (- - - -) represent the control groups and continue lines (——)the KW groups. Horizontal error bars represent ±SD (no of replicate = 3 and no of individuals per tube = 30).

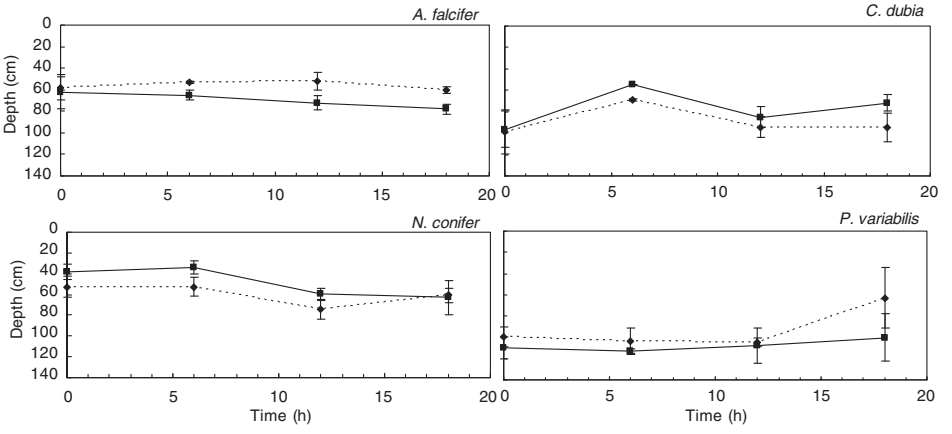

Figure 4. Depth (average and ±SD) to each species at each daily observations (00:00, 06:00, 12:00 and 18:00 h). Pointed lines (- - - -) represent the control groups and continue lines (——) the KW groups. Error bars represent ±SD (no of replicate = 3 and no of individuals per tube = 30).

ANOVA) staying at the deepest level of the water column at all times observed ($p = 0.003$; df = 3; and $F = 16.814$, RM ANOVA).

Differences at mean depth between control and KW (Figure 4) were significant for the four species under study (*A. falcifer*: $p = 0.019$, $F_{22,1,0.05} = 14.671$; *N. conifer*: $p = 0.001$, $F_{22,1,0.05} = 10.204$; *C. dubia*: $p = 0.03$, $F_{22,1,0.05} = 8.601$ and *P. variabilis*: $p = 0.018$, $F_{22,1,0.05} = 5.009$, RM ANOVA). Among copepods, *A. falcifer* exposed to KW, kept a lower position with respect to the control group, especially at daytime (12:00 and 18:00), whereas *N. conifer* showed a significant upward movement mainly at night-time. Among cladocerans, *C. dubia*, in KW showed a response similar to *N. conifer*, keeping a higher position with respect to the control most of the time. *Pseudosida variabilis* was the species staying at the greatest depth in both the treatments, even though when subject to KW, it reflected an even greater descent during daytime, especially at 18:00.

Copepods registered significant differences in the Lloyd indexes between control and KW (*A. falcifer*: $p = 0.031$, $F_{1,22,0.05} = 10.58$ and *N. conifer*: $p = 0.016$, $F_{1,22,0.05} = 16.316$) (Figure 5). However, they exhibited different responses: *N. conifer* showed a greater crowding in KW, the main differences being observed during night-time when the organisms stayed more crowded at the higher levels (Figure 5). *Argyrodiaptomus falcifer* acquired a greater dispersion when subject to fish water. In the case of cladocerans (Figure 5), no significant differences were registered between both the treatments (*C. dubia*: $p = 0.358$, $F_{1,22,0.05} = 1.079$ and *P. variabilis*: $p = 0.151$, $F_{1,22,0.05} = 3.141$). *Ceriodaphnia dubia* was clearly dispersed in every case, whereas *P. variabilis* was noticeably crowded at lower levels both in control and KW (though greater in the latter case).

Figure 6 shows the migration distance of every species analysed. The three planktonic species showed a trend to greater migration distance when exposed to the fish exudates than when not exposed (control), but the differences were not statistically significant: *A. falcifer* ($p = 0.387$, df = 4 and $t = 0.939$), *N. conifer* ($p = 0.299$, df = 4 and $t = 1.421$), *C. dubia* ($p = 0.182$, df = 4 and $t = 2.601$). On the

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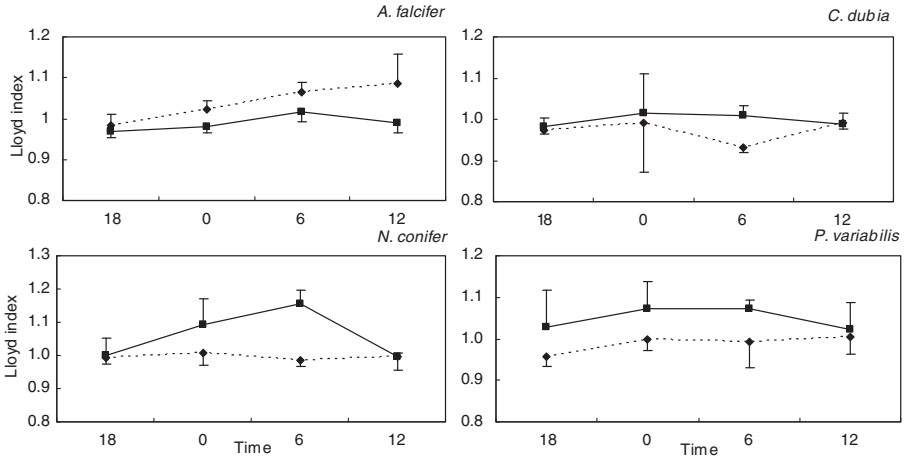

Figure 5. Aggregation index (Lloyd index). The figure shows the values (average and ±SD) of the aggregation index to each species at each daily observations (00:00, 06:00, 12:00 and 18:00 h). High values indicate higher aggregation level. Pointed lines (- - - - -) represent the control groups and continue lines (——) the KW groups. Error bars represent ±SD (no of replicate = 3; no of individuals per tube = 30).

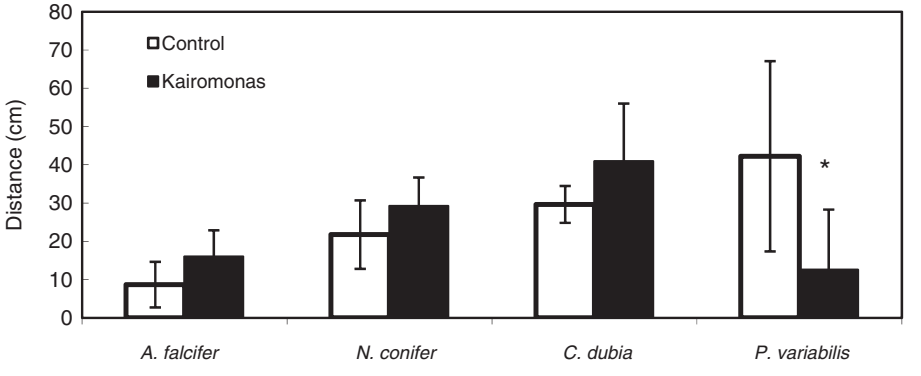

Figure 6. Migration distances of each species to each treatment (control and KW). The asterisk indicates significant different values ($p < 0.05$) from the control group (two-tailed *t*-test). Error bars represent ±SD (no of replicate = 3 and no of individuals per tube = 30).

other hand, *P. variablis* did not migrate in the presence of infochemicals but stayed at a greater, constant depth during daytime which was significant with respect to the control ($p = 0.03$, df = 4 and $t = 9.202$).

Discussion

As expected, the four species under study exhibited different behavioural patterns in both escape ability and migration movements. The cladoceran *P. variabilis* was the least captured species at LCS and MCS, but in the water column it exhibited a more

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passive behaviour during daytime, staying at the lowest level and making brief excursions at dusk (18:00 h). Field work reveals that this cladoceran is a species particularly associated with densely vegetated environments, coexisting with numerous tactile invertebrate predators such as *Chaoborus*, noctonectids, damselflies, dragonflies, shrimps and bellostomatids (Neil 1990 in José de Paggi 1995; González Sagrario and Balseiro 2010). In this sense, contact with the bottom would provide a certain protection in the absence of plants (phenomenon known as ''thigmotaxis''). Very likely, its great sensitivity to subtle mechanical stimuli and the permanence at deep levels with a high degree of swarming reflect its possible strategies in the presence of non-visual predators (Ohman 1988).

Argyrodiaptomus falcifer was the most captured at LCS but the least captured at RCS when compared to the other species. Due to its relatively large size and limnetic swimming, it is reasonable to assume that this strategy is a response to predation by selective visual fish (Zaret and Suffern 1976), whose capture mechanisms are more active and rapid (Lauder 1980; Lazzaro 1987). Differing from *P. variabilis*, this copepod stayed at higher levels during the night-time, being more dispersed during the daytime, in the same way as *N. conifer*, even though at a lower mean depth. This behaviour probably takes advantage of resources.

The cladoceran *C. dubia* exhibited the most complex migration pattern, similar to the ''twilight DVM'', occasionally maintaining a homogeneous distribution and making excursions to the surface at dusk. What can be inferred that the interpretation of these movements is limited because a given phenotype is the result of so many natural factors, but the movements show the importance of habitat selection as a proactive process to maximize fitness (Gliwicz et al. 2006).

Considering the second hypothesis, it can be concluded that at least in one situation, kairomones enhanced the escape ability of the organisms (in terms of greater speed or by refining their sensitivity). These results are in agreement with those from other studies which have reported the important role of chemical communication in rapid responses such as escape (De Meester and Pijanowska 1996; Brewer et al. 1999; Pijanowska et al. 2006), food encounter (Poulet and Marsot 1978; Buskey 1984) and mate finding (Fleminger 1967; Katona 1973; Griffith and Frost 1976). In general, *C. dubia* was the species with the highest escape ability, especially when compared with copepods, which usually exhibited greater swimming speed. These results indicate that predation sensitivity depends on perceptive abilities rather than on movement performance or speed, especially in complex-structure habitats. These interpretations have also been suggested by Titelman (2001) who found that nauplii of *Acartia tonsa* and *Temora longicornis* exhibited different sensitivity to the same predator, despite having the same escape speed. Additionally, numerous authors have reported that cladocerans constitute the main item in the diet of *C. decemmaculatus* in natural environments (Oliveros 1980; Escalante 1983). It could be assumed that *C. dubia* has evolutionally developed a high sensitivity to infochemicals, acquiring an early alert state and an advantage over other less preferred organisms.

In vertical migration assays, organisms manifested phenotypical changes in their behaviour; however, the organisms did not always respond to the expected pattern. Notably, *A. falcifer* stayed more dispersed but at lower mean depths than the control. The density decrease could be a temporary safety strategy in the presence of visually oriented predators while at the same time, an effective gain for fitness, as observed by Gliwicz et al. (2006) for certain daphnids. Even though high local

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densities have been identified as anti-predator strategies, especially against visually oriented predators, numerous authors have considered them as maladaptive strategies since fish would be able to perform intensive search in patches of crowded organisms, thus optimizing the exploitation of these resources (McNara and Houston 1985). Additionally, according to the model of patch optimal selection (McNaught and Hasler 1961), fish are able to remember the nutritional value of each place they have gone through and not return to them after having sufficiently exploited the resources. On the contrary, a greater crowding with respect to controls was observed in the rest of the smaller species under study (*N. conifer*, *C. dubia* and *P. variabilis*). Considering that the main crowding cost lies in the reduced availability of food, and that these costs are higher for big, active organisms, with greater energy requirements, it is reasonable to think that the species mentioned could acquire different defensive strategies.

Another interesting response was the significant permanence at lower levels of the coastal cladoceran *P. variabilis*, enhanced by the presence of kairomones. These results agree with those reported by other authors both in field (Pijanowska and Dawidowicz 1987) and experimental works (Loose and Dawidowicz 1994), which allow reconsidering the ''stay down'' model developed by Vos et al. (2002) as an anti-predator strategy. Such behaviour would allow fitness to increase as it would reduce the energy cost implied in migration. At the same time, it would provide important population benefits by maintaining the optimum time of the first reproduction and litter size. These results allow us to sustain the hypothesis mentioned above and suggest its consideration within the theoretical framework both for fieldwork and laboratory studies. Finally, the phenotypic differences found experimentally and the comparison with classic theoretical models make us question why organisms did not acquire identical directional strategies in all cases (towards a normal migration pattern, a higher degree of crowding and positive escape) in the presence of kairomones from the same predator. This study sustains the hypothesis that the differential ethological characteristics described above would be the result of multiple environmental components interacting with evolutionally fixed genetic features (Landry 1978; Viitasalo et al. 1998). The great variability in the physicochemical and biological conditions of the systems, where the studied organisms live, would require more complex responses than those manifested in more stable or predictable environments. However, the cost/benefit balance of each particular strategy permits achievement of similar fitness, thus favouring the successful development and coexistence of the different species.

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