

# Exposure to sublethal chromium and endosulfan alter the diel vertical migration (DVM) in freshwater zooplankton crustaceans

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Accepted: 3 August 2011 / Published online: 14 August 2011  
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**Abstract** Among zooplankton behaviors, diel migrations constitute one of the most effective predator avoidance strategy and confer metabolic and demographic advantages. We aim to examine whether sublethal concentrations of two widespread pollutants (a pesticide with endosulfan and chromium as potassium dichromate) alter the depth selection, vertical migration and grouping of five freshwater species: *Argyrodiaptomus falcifer*, *Notodiaptomus conifer*, *Pseudosida variabilis*, *Ceriodaphnia dubia* and *Daphnia magna*. In a series of experimental assays, performed with 150 cm length transparent tubes, we analyzed the ascents and descents movements through periods of 24 h. Among controls, the copepods showed a tendency to remain closest to the surface, however, *N. conifer* registered a downward movement of 18.14 cm between 06:00 and 12:00. The cladoceran *P. variabilis* occupied the deeper position (85 cm), *C. dubia* showed a tendency to hike to the surface at 06:00 (57.7 cm) descending to lower levels at 18:00. *D. magna* showed a constant movement of ascent between 00:00 and 18:00, making an average travel of 29.4 cm. When subjected to pollutants, these behaviors were altered. It is hypothesized that a reduction in swimming activity and disorientation would be the main cause of such alterations. The high sensitivity of this endpoint suggests it to be adequate as a complement in future standard toxicity tests.

**Keywords** DVM · Predator-avoidance strategy · Chromium · Endosulfan · Cladocera · Copepoda

## Introduction

Destabilization of the structure and dynamic of natural aquatic ecosystems e.g., simplification of trophic webs is one of the main consequences of pollution from inadequate agricultural and industrial practices. These alterations are the result of direct processes that cause the disappearance of the most sensitive species, and indirect ones such as disruptions of biotic interactions and enhancement of the species vulnerability to natural stressors (i.e., climate changes, seasonal successions, predators) (Hanazato 1998).

Since pesticides and heavy metals are the main pollutants derived from the above mentioned activities, several ecotoxicological contributions have evaluated their direct and indirect effects on zooplankton. The advantage of studying this community resides on their high sensitivity and their central role in the food webs. In general, the commonly observed responses are altered reproduction (Wang et al. 2009), delayed development (Brown et al. 2002; Gutierrez et al. 2010), reduction on feeding rate (Sharp and Stearns 1997; Bengtsson et al. 2004) and alteration in swimming and phototactic activities (Michelsa et al. 2000; Lopes et al. 2004). However, despite its ecological significance, to the best of our knowledge no studies have been published so far analyzing the influence of pesticides and heavy metals on fundamental behavioral features in the ecosystem dynamic, such as depth selection and diel vertical migration (DVM).

There is a consensus in the scientific literature that these behaviors are the result from active habitat choice (Lampert 2005). They constitute effective predator-avoidance

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strategies that confer significant metabolic and demographic advantages to this key community while at the same time they promote the coexistence of different species and maintain the local biodiversity (De Meester et al. 1999; Lampert 2003). Many works have been carried out in order to explain their adaptive significance and to describe the main basic patterns at different environmental conditions (Hutchinson 1967; Zaret and Suffern 1976). At present it is known that these behaviors vary from system to system and between species (De Meester et al. 1999; Tolrian and Dodson 1999) and necessarily imply energetic costs that have to be balanced between all biological traits through the individual life history (Dini and Carpenter 1991; Lampert et al. 2003).

It has also been reported that altered depth selection or DVM due to natural stressors (light intensity, predators, hormonal changes), could potentially leave zooplankton species more vulnerable to environmental changes or predation by fish or invertebrates (Gliwicz 1986; Dodson 1990; Watt and Young 1994; Bentkowski et al. 2010). Therefore, it is reasonable to think that the impact of anthropogenic stressors such as heavy metals and pesticides, which are novel to the organisms, may represent an additional threat, because their physiological mechanisms to compensate any toxicological effect are not naturally adapted (Dawidowicz et al. 1990; Loose and Dawidowicz 1994; Preston et al. 1999). An enhancement of the probability of disappearance or extinction from local areas and alterations of the community structure and ecosystem dynamic would be the main consequences in a middle and long term.

In this study, the authors aim to examine whether sublethal concentrations of two widespread pollutants of different chemical nature (a pesticide and a heavy metal) adversely alter the depth selection, vertical migration, and grouping of five different freshwater zooplankton species. Three of them (*Notodiptomus conifer*, *Argyrodaptomus falcifer* and *Pseudosida variabilis*) are highly representative of the Neotropical region and no work have been done at the present in order to study their behavioral features. The cladocerans *Daphnia magna* and *Ceriodaphnia dubia* were employed because of their high suitability to ecotoxicological test and future comparisons. The selection of different species may also allow us to reach a higher generalization level about the sensitivity of these three endpoints.

We selected a commercial insecticide with the organochlorine endosulfan as active ingredient because it is one of the most widely used insecticides in agricultural activities (Jergentz et al. 2004). It has been characterized as generalist, highly toxic and persistent in the environment by the USEPA (1980). Chromium (as potassium dichromate) was chosen because of its persistence in waterbodies associated

with industrialized areas and also because of the high toxicity it represents to different zooplankton organisms (Gagneten et al. 2007; Gagneten and Paggi 2009).

The present study intends to become the first report on chromium and endosulfan alteration on such ecologically relevant behavioral features as depth selection and DVM, while at the same time trying to demonstrate the potential importance of zooplankton behavior on ecotoxicological laboratory and field studies as well as on biomonitoring assessments.

## Materials and methods

### Species selection and culture conditions

The selected zooplankton species were two copepods (*Argyrodaptomus falcifer* and *Notodiptomus conifer*) and three cladocerans (*Pseudosida variabilis*, *Ceriodaphnia dubia* and *Daphnia magna*) with different behavioral patterns and life cycles. With the exception of *D. magna*, all species inhabit the water bodies of the alluvial plain of Paraná River (Argentina) where they coexist with both vertebrate and invertebrate predators. These species were collected with a planktonic net (200  $\mu\text{m}$ ) and maintained in outdoor tanks culture (100 l) for more than 2 years.

*D. magna* culture belong to our laboratory's own monoclonal stock culture which is permanently controlled and maintained under constant environmental conditions (temperature:  $21 \pm 2^\circ\text{C}$ ; photoperiod: 12 h light:12 h darkness).

Two days before the experiment, all species were acclimated to the control medium, temperature ( $21 \pm 2^\circ\text{C}$ ) and photoperiod (12 h light:12 h darkness). The control medium consisted on filtered (12  $\mu\text{m}$ ) and oxygenated pond water whose physico-chemical characteristics were measured (in  $\text{mg l}^{-1}$ ) according to the Standard Methods for the Examination of Water and Wastewater (2000): nitrates  $<0.1$ ; nitrites: 0.01; ammonium: 0.29; chlorides: 3.5; sulphates: 8.3; total alkalinity: 77  $\text{CaCO}_3$ ; bicarbonates: 94; sodium: 7.7; magnesium: 6.8; calcium: 12.9; potassium: 1.8; chemical demand of oxygen: 10; biological demand of oxygen: 0.08. Dissolved oxygen was 6.4 ( $\pm 0.8$ ) ppm; pH: 8.39 ( $\pm 0.24$ ); conductivity: 245.33 ( $\pm 28.18$ )  $\mu\text{S cm}^{-1}$ . In all cases the organisms were fed ad libitum with a *Chlorella* sp. concentrate (algal density:  $2.8 \times 10^5$  cel  $\text{ml}^{-1}$ ).

### Toxicant preparation

The stock solutions of the metallic salt (potassium dichromate:  $\text{K}_2\text{Cr}_2\text{O}_7$ ) were prepared by dissolving it in distilled water. The insecticide employed was Zebra

**Table 1** The table shows the 48 h EC<sub>50</sub> values (in  $\mu\text{l l}^{-1}$ ) of chromium and endosulfan to each species and the sublethal concentrations used in the DVM experiments. The confidence limits of each EC<sub>50</sub> values are in parenthesis

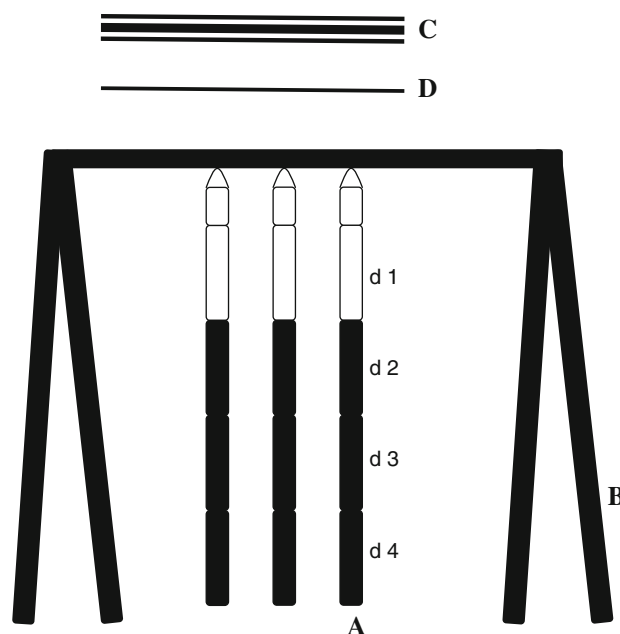
	EC <sub>50</sub> 48h	Concentrations used	
		Low	High
Chromium (μl l <sup>-1</sup> )			
<i>P. variabilis</i>	52.47 (18.6–141.4)	3.9	15.7
<i>C. dubia</i>	100.02 (10.3–168.1)	7.5	30
<i>D. magna</i>	46.58 (27.7–73.06)	3.5	14
<i>N. conifer</i>	170.76 (125.6–243.2)	8.5	34
<i>A. falcifer</i>	680.15 (333–1334.9)	15	60
Endosulfan (μl l <sup>-1</sup> )			
<i>P. variabilis</i>	1.04 (0.6–1.8)	0.08	0.3
<i>C. dubia</i>	0.67 (0.4–1.08)	0.05	0.2
<i>D. magna</i>	11.21 (27.7–73.06)	0.05	0.2
<i>N. conifer</i>	2.8 (1.7–3.7)	0.2	0.8
<i>A. falcifer</i>	11.23 (6.3–27.7)	0.85	3.4

Ciagro<sup>TM</sup> (Ciagro, S.A. Buenos Aires, Argentina), containing 35% of organochlorine endosulfan as active ingredient (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methane-2,4,3,benzodioxanthiopin-3-oxide). This product, as a liquid formulation, was diluted with distilled water to prepare a stock solution. The stock solution of this endosulfan formulation was prepared as in metals and, for the final test solutions, only the amount of the active ingredient was taken into account.

Two concentrations (one high and one low) of chromium and endosulfan were used in the experiments (see Table 1). Each one was abbreviated as Cr1–Cr2 and endosulfan1–endosulfan2, respectively. The “high” concentrations tested were in a range of 20–30% of the acute values, according Kenaga’s considerations (Kenaga 1982). Each specific value was established taking as a reference preliminary assays and was different depending on the sensitivity of the species. The “low” concentrations were calculated dividing by four the selected high concentrations. In both cases, the toxicants were prepared prior to each test by dissolving the stock solutions in the same medium for culture and controls.

### Experimental design

Vertical migration assays were performed inside transparent plastic tubes (Fig. 1) (total length: 150 cm; diameter: 7.2 cm) filled with 2 l of culture medium (treatment or control). The tubes were externally marked every 30 cm, determining 4 depth levels (from 0 to 30, from 30 to 60 and from 60 to 120 cm) suspended from a 2 m-high iron support built *ad hoc*. The contour of each tube was covered with opaque black plastic (0.075 mm thick) so that the



**Fig. 1** Experimental design to quantify the diel vertical migration of zooplankton crustaceans. **a** transparent plastic tubes (*d1*, *d2*, *d3*, *d4* are the depth levels) **b** 2 m-high iron support, where the tubes were suspended, **c** light source generated from fluorescent tubes, **d** diffusive plaque. The contour of *d2*, *d3* and *d4* levels was covered with an opaque black plastic so that the light entered only from the surface. The DVM was quantified as a function of the number of individuals present at each one of the levels marked on the tubes every 6 h (00:00; 06:00; 12:00 and 18:00)

white cold light entered only from the surface. The presence of an acrylic diffusive plaque allowed the light source generated from the fluorescent tubes to illuminate diffusively and uniformly imitating the above light of a natural aquatic environment. The incident intensity of the tubes was 3593.3 ( $\pm 77$ ) Lux at the top; 2200 ( $\pm 244$ ) Lux at the middle and 1200 ( $\pm 154$ ) Lux at the bottom.

In order to estimate the organisms DVM, thirty individuals from the same specie were placed in each water column. The number of individuals present at each levels was quantified every 6 h (00:00; 06:00; 12:00 and 18:00). Controls were replicated four times and chromium and endosulfan treatments were replicated three times. During nocturnal hours (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.

In this experimental stage, several indicators were calculated which allowed to recognize each normal behavior and compare it with the one manifested in the presence of the toxicants.

1. In order to quantify possible significant differences between daily movements to each species and

treatments (control, chromium and endosulfan), three factor ANOVA was employed (factor 1: treatment, factor 2: depth level and factor 3: time of day), with repeated measures in the last two factors. In order to ascertain which treatments were different, a Dunnet's post test was employed. Finally, to evaluate the effects of the interactions between the factors, paired comparisons between each treatment at each depth level and time were employed. In this case, the critical values (*sig.*) and the confidence intervals were adjusted through Bonferroni's correction. In all cases, differences of  $P < 0.05$  were considered significant. The dependent variable was the number of individuals present, transformed in  $\text{Log}_n (x + 1)$ . Prior to each analysis, normality (Komogorov-Simov's test), homoscedasticity (Levene's test) and sphericity (Mauchly test) of the data obtained were verified.

- In order to locate the organisms in the water column, for each replica, mean depth ( $D$ ) was calculated (Cruz Pizarro 1978; Dodson 1988) according to the following equation:

$$D = \sum Nidi / \sum di$$

where  $Ni$  is the number of individuals,  $di$  is the depth level. Then, in order to analyze differences in  $D$  between control, chromium and endosulfan treatments at every moment of the day, two-factor ANOVA was employed with repeated measures in one factor, for each species separately. Normal distribution of data (Komogorov-Simorov test), homoscedasticity (Levene test) and sphericity (Mauchly test) were previously verified. Differences were considered significant at values of  $P < 0.05$ .

- Index  $Pi$  was used to know the level of aggregation of individuals in the water column (Lloyd, 1967). This index was calculated using the following equation:

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

where  $\sigma$  is the variance;  $x$  is the number of individuals in the column. From the indices thus obtained for each replica, two-factor ANOVA was employed with repeated measures in one factor (time). In order to recognize which treatment was different from control, a Dunnet's post test was employed. To evaluate the effect of the interactions between treatments (control, chromium and endosulfan) and time of day (00:00, 06:00, 12:00 or 18:00 h) multiple comparisons were employed, in this case with critic levels (*sig.*) and confidence intervals adjusted with a Bonferroni's correction.

Previously to the mentioned analysis, the normal distribution of data (Komogorov-Simorov's test), homoscedasticity (Levene's test) and sphericity

(Mauchly's test) were verified. Differences were considered significant at values of  $P < 0.05$ .

## Results

### DVM-normal pattern

Figure 2 a and b shows the migratory movements of each species during 24 h in response to treatments and controls, both with chromium and endosulfan.

The studied species showed different migration patterns: Although, *N. conifer* registered a downward movement average of 18.14 cm between 06:00 and 12:00, both copepods species showed a tendency to remain in a position closest to the surface throughout the day. In comparison, *N. conifer* occupied a higher average level of depth (48.8 cm) than *A. falcifer* (47.2 cm) in the water column.

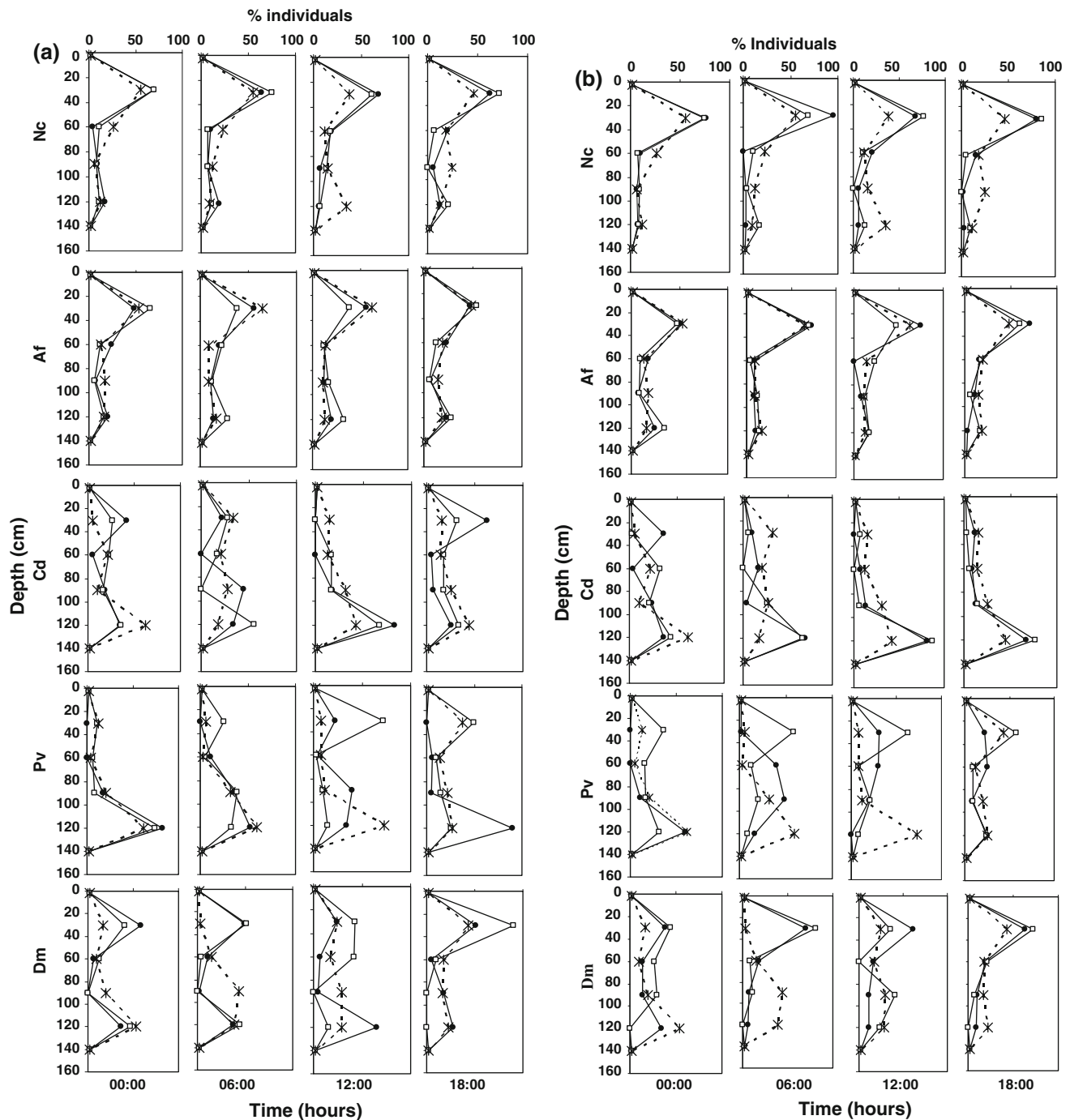
Among the cladocerans, *P. variabilis* occupied a deeper middle position (85 cm) than the rest of the species over time and crowded only to intermediate levels (56.7 cm) at 12:00. *C. dubia* showed a tendency to hike to the surface at the end of the period of darkness, 1 h before the light switch (06:00, upper level max.: 57.7 cm), then descending to lower levels between 12:00 and 18:00. The peak level was less than 82.2 cm at 00:00. *D. magna* showed a constant movement of ascent between 00:00 and 18:00, making an average travel of 29.4 cm over 24 h.

### Effects of toxics

In general, the five species under toxic treatments (chromium and endosulfan) showed alterations of the aforementioned diel vertical migration patterns (ANOVA *N. conifer*  $F = 1.71$ ,  $P = 0.021$ ; *A. falcifer*  $F = 1.761$ ,  $P = 0.017$ ; *C. dubia*  $F = 1.58$ ,  $P = 0.048$ , *P. variabilis*  $F = 2.39$ ,  $P = 0.026$ ; *D. magna*  $F = 2.94$ ,  $P = 0.008$ ) (Fig. 2 a and b).

### Mean depth

*N. conifer* copepods subjected to toxics, maintained a more stable position in the water column during the 4 h under observation (Fig. 3), i.e., unlike the control individuals, they did not decrease at midday, registering an average depth level significantly lower (Table 2). Multiple comparisons showed that for chromium such differences were observed at 12:00 between the control and Cr 1 (Bonferroni-adjusted test,  $P = 0.019$ ) and between the control and Cr 2 (Bonferroni-adjusted test,  $P = 0.008$ ). For the treatment with endosulfan differences were also evident at 12:00 (Bonferroni-adjusted test,  $P = 0.026$ ) and 18:00



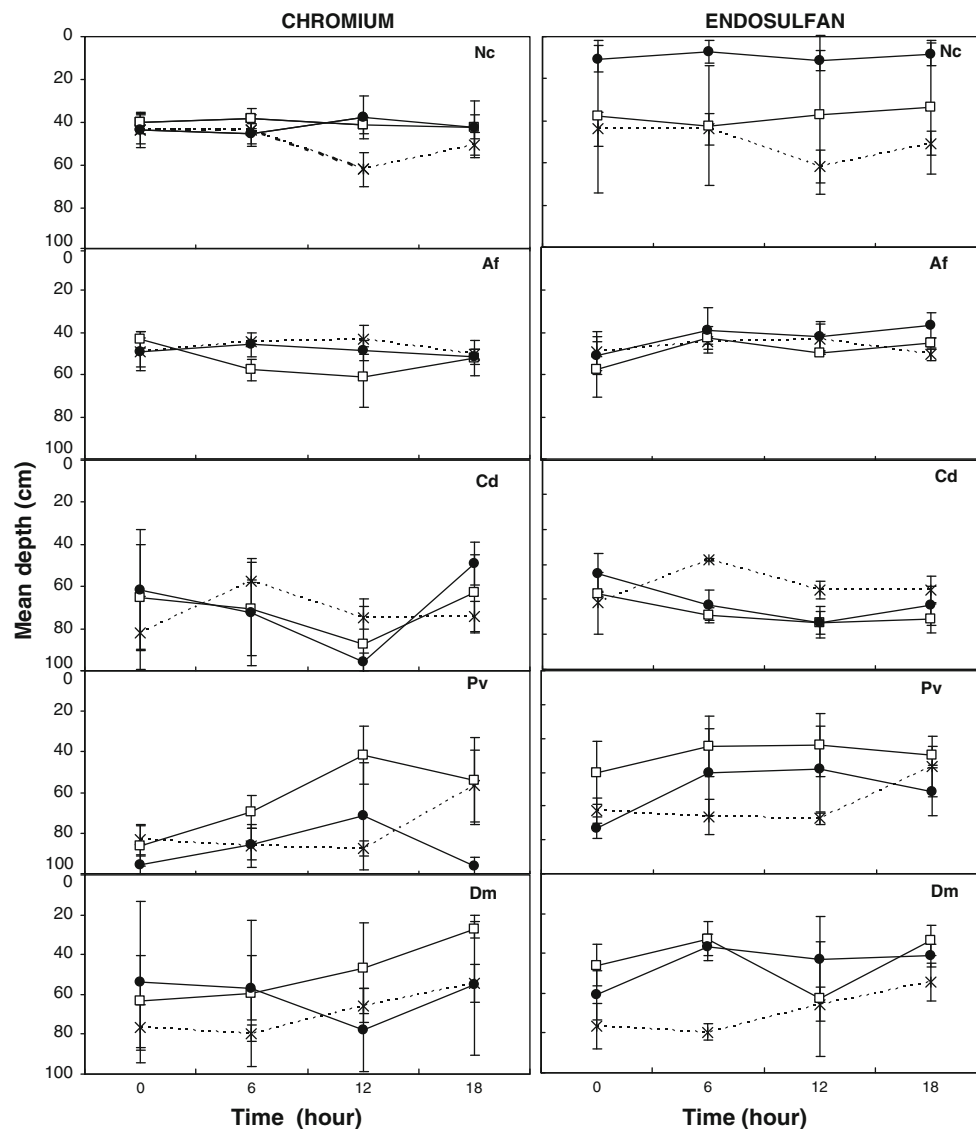
**Fig. 2** a and b Diel vertical migration of each species (*Nc*: *Notodiaptomus conifer*, *Af*: *Argyrodiaptomus falcifer*, *Cd*: *Ceriodaphnia dubia*, *Pv*: *Pseudosida variabilis*, *Dm*: *Daphnia magna*) during 24 h to controls (pointed lines) and the treatments (continuous lines) with chromium (a) and endosulfan (b). In both cases, white

symbols represent the “low” concentration and black symbols represent the “high” concentrations of the toxicants. Plots represent the averages of four replicates to the controls and three replicates to the treatments

(Bonferroni-adjusted test,  $P = 0.012$ ) between the control and endosulfan 1 and 06:00 (Bonferroni-adjusted test, 0.042), 12:00 (Bonferroni-adjusted test,  $P = 0.013$ ) and 18:00 (Bonferroni-adjusted test,  $P = 0.017$ ) between the control and endosulfan 2.

*A. falcifer* from the control group showed no extensive migratory movements over time. However, in the presence of chromium and endosulfan, these specimens developed vertical movements by significantly changing the depth, in comparison with the movements recorded in the control





**Fig. 3** Mean depth (cm) of each species at 00:00, 06:00, 12:00 and 18:00 h. Nc: *N. conifer*, Af: *A. falcifer*, Cd: *C. dubia*, Pv: *P. variabilis*, Dm: *D. magna*. Left panels show the comparison between controls (pointed lines) and treatments with chromium (continuous lines). Right

panels show the comparison between controls (pointed lines) and treatments with endosulfan (continuous lines). In both cases, white symbols represent the “low” concentration and black symbols represent the “high” concentrations of the toxicants

(Table 2) (Fig. 3). With regard to chromium, such differences were observed at 06:00 between the control and Cr 1 (Bonferroni-adjusted test,  $P = 0.013$ ) and for endosulfan, differences were observed at 18:00 between the control and endosulfan 2 (Bonferroni-adjusted test,  $P = 0.005$ ).

No significant differences were registered to *C. dubia* mean depth between the control and treatment with chromium but there were differences between the control and treatments with endosulfan (Table 2). In this case, there was an increase in the average depth during most of the day and a marked rise at 00:00 (Fig. 3). Significant differences were recorded at 06:00 for endosulfan 1 (Bonferroni-adjusted test,  $P < 0.001$ ) and endosulfan 2

(Bonferroni-adjusted test,  $P = 0.001$ ) and at 12:00 to endosulfan 1 (Bonferroni-adjusted test,  $P = 0.023$ ) and endosulfan 2 (Bonferroni-adjusted test,  $P = 0.021$ ).

*P. variabilis* showed large-scale changes in the mean depth when submitted to treatments with chromium and endosulfan (Table 2; Fig. 3). In both cases, we observed an inverse pattern to that in the control. With regard to chromium, there were significant differences between the control and Cr 1 at 12:00 (Bonferroni-adjusted test,  $P = 0.023$ ) and between the control and Cr 2 at 18:00 (Bonferroni-adjusted test,  $P = 0.033$ ). With respect to endosulfan, there were significant differences between the control at 12:00 and endosulfan 1 (Bonferroni-adjusted test,  $P = 0.035$ ).

**Table 2** Results obtained from the comparison between controls and the treatments with chromium and endosulfan to the endpoints Mean depth and Grouping (two-factor ANOVA, with repeated measures)

	Mean depth		Grouping	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
<i>N. conifer</i>				
Control				
Chromium	6.038	0.03*	0.093	0.014*
Endosulfan	30.506	<0.001*	7.07	0.026*
<i>A. falcifer</i>				
Control				
Chromium	3.79	0.017*	7.45	0.022*
Endosulfan	6.21	0.028*	2.85	0.125
<i>C. dubia</i>				
Control				
Chromium	0.06	0.94	0.29	0.75
Endosulfan	30.962	<0.001*	0.17	0.84
<i>P. variabilis</i>				
Control				
Chromium	10.64	0.008*	0.49	0.63
Endosulfan	21.28	0.001*	6.59	0.025*
<i>D. magna</i>				
Control				
Chromium	1.86	0.225	1.52	0.75
Endosulfan	45.97	<0.001*	1.94	0.212

\* Value statistically different from the control

*D. magna* did not differ in average depth between the control and treatment with chromium but between the control and treatments with endosulfan (Table 2; Fig. 3). In the presence of this poison, the organisms tended to decrease the depth, closer to the surface. There were significant differences between the control and endosulfan 1 at 00:00 (Bonferroni-adjusted test,  $P = 0.021$ ), and between the control and both treatments with endosulfan (Bonferroni-adjusted test,  $P < 0.001$ ) at 06:00 and at 18:00 between the control and endosulfan 1 (Bonferroni-adjusted test,  $P = 0.02$ ).

In general, considering the five species studied, 64.3% of the significant differences between controls and both treatments were observed during daylight hours (12:00 and 18:00), and only one case showed differences at 00:00 h (7%).

### Grouping

The aggregation index for each species and control treatments is shown in Fig. 4.

The *N. conifer* copepod was the only species with a tendency to cluster after being subjected to toxics (Fig. 4). When subjected to chromium and endosulfan, this grouping was significantly different from the control (Table 2).

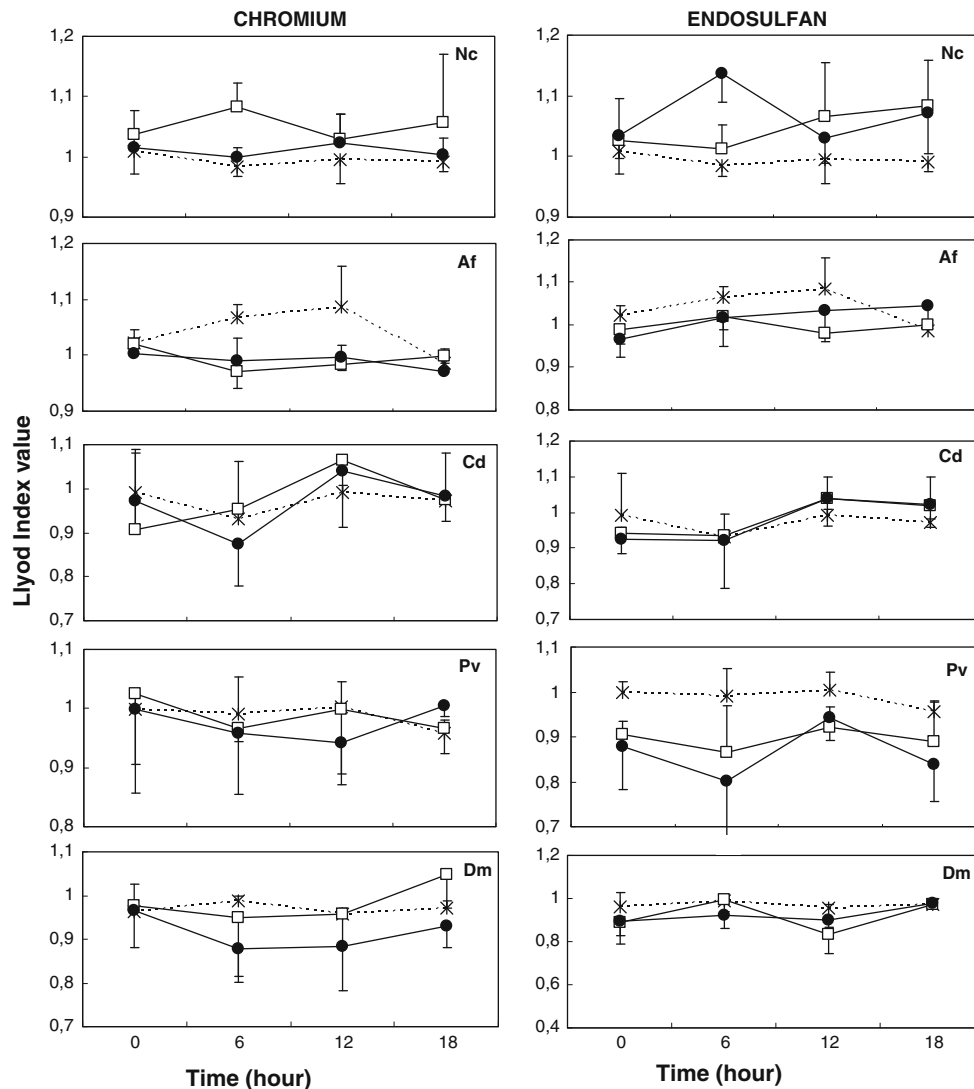
The rest of the species tended to disperse when subjected to toxics (Fig. 4). *A. falcifer* showed significant differences between the control and treatment with

chromium but not between the control and endosulfan treatments (Table 2). *C. dubia* and *D. magna* showed no significant differences between the control and treatment with chromium or between the control and endosulfan treatments (Table 2). *P. variabilis* did not show differences in the degree of crowding between the control and treatment with chromium but between the control and endosulfan treatments (Table 2).

The analysis of the interaction between factors: treatment and time of day allowed us to recognize that 100% of the significant differences between controls and treatments were carried out at 06:00.

### Discussion

Zooplankton migrations have been categorized as the biggest animal migrations, in terms of biomass in our planet (Hays 2003). However, as shown in this work, that phenomenon not necessarily implies a fixed behavioral pattern. Extensive laboratory studies enumerate diverse environmental factors as the proximate causes of this phenomenon (Forward 1988). Other author emphasise the analysis on the ultimate evolutionary causes such as predators avoidance (Gliwicz 1986; Watt and Young 1994; De Robertis et al. 2000). Although, there is a consensus that the interaction of both causes favours the differences among specific migration patterns (Enright and Hamner 1967; Landry 1978;



**Fig. 4** Grouping index (Lloyd index) to each species at 00:00, 06:00, 12:00 and 18:00 h. Nc: *N. conifer*, Af: *A. falcifer*, Cd: *C. dubia*, Pv: *P. variabilis*, Dm: *D. magna*. Left panels show the comparison between controls (pointed lines) and treatments with chromium (continuous

lines). Right panels show the comparison between controls (pointed lines) and treatments with endosulfan (continuous lines). In both cases, white symbols represent the “low” concentration and black symbols represent the “high” concentrations of the toxicants

Vitasalo et al. 1998), unfortunately, works that effectively demonstrate it are scarce or nule. Notwithstanding, given the natural characteristics of this aforementioned causes, it is possible to hypothesize that each particular migration pattern offers fitness advantages to the species, mainly in freshwater systems, favoring their successful development and perpetuation in nature (Kiorboe 2011).

As expected, and in agreement with the hypothesis proposed by Dawidowicz et al. (1990) and Loose and Dawidowicz (1994), when the organisms were subjected to the anthropogenic stressors -in this case, chromium and endosulfan- they manifested changes in their natural behavior in at least one situation.

Under natural conditions (control), the cladocera ctenopoda *P. variabilis* manifested a simple pattern, most

of the time staying in the low depth level of the experimental water column, with a relative high grouping index and making brief evening excursions. Considering that it is a littoral species, the contact with the bottom of the tube seems protective in the absence of vegetation (phenomenon known as thigmotaxis). On the other hand, this pattern would protect them from nocturnally migrating predators (Ohman 1988). When this species was subjected to both toxicants its migration pattern was not only altered, but reversed. Interestingly, this change was also opposite in a greater magnitude to the responses of the same species exposed to fish infochemicals (Gutiérrez et al. submitted). Disaggregation and reversion of a pattern, which probably have important defensive advantages to this specie, may cause serious consequences, mainly by increasing



predation risk and reducing the energy balance efficiency (Christensen et al. 2005).

Among copepods, *A. falcifer* from the control remained in the top level during the whole daily cycle. It is important to mention that its relative big size ( $1.75 \text{ mm} \pm 0.14$ ) and swimming speed give this species an intrinsic advantage against predators. At the same time, the maintenance in higher levels would favor the use of phytoplankton that normally stays in the photic zone. However, in the presence of chromium and endosulfan they made significant vertical movements which were reflected in the variations of the mean depth. It is probable that disorientation was the main reason for such movements and the indirect consequences in the long time would be an important reduction in the resources optimization.

In opposing to *A. falcifer*, *N. conifer* from the control group, made migratory movements, being kept dispersed during the day and grouped at night. The adaptive advantages of selecting different depths and grouping levels throughout the day have been well studied from an eco-physiological point of view (Young and Watt 1993; Lampert et al. 2003; Lampert 2005). In general, despite the fact that the main cost of grouping resides in a higher intra-specific competitiveness and lesser food availability, higher local densities have been identified as effective anti-predator strategies (Johnsen and Jacobsen 1987). Chromium and endosulfan caused *N. conifer* the impossibility of making diel migratory movements. In both cases they remained in a stable position and unlike the other species, with a significant tendency to gather in crowds. It has been demonstrated that in the presence of stress situations, maintenance costs have the highest priority in the energy allocation budget (Forbes and Callow 1996). Therefore, it is reasonable to think that if facing toxics implies additional costs, the “option” not to descend in the water column might be associated with the minor energetic input available to this movement.

The cladoceran anomopods *C. dubia* and *D. magna* were kept in a middle level of the water column: *C. dubia*, made excursions toward the surface at 06:00 (before dawn) and *D. magna* made a slow but continuous ascent between 06:00 and 18:00. This pattern, reverse DVM, would probable be related, in terms of ultimate causes, with the avoidance from invertebrate predators and with a more efficient phytoplankton and energy utilization (McLaren 1963; Kerfoot 1980). Surprisingly, both *D. magna* and *C. dubia* were not severely affected by chromium, but endosulfan caused an ascent toward higher levels at light hours (in some cases the animals were observed closer to the water surface).

One of the most noticeable general pattern was that the effects of toxics appeared more pronounced during the day than during night. A possible explanation would be that

metabolic and nervous activities are higher in the day than at night (as a consequence of increasing temperatures, feeding activities or a more alert stage due to the presence of visual predators), which could activate toxicant mechanisms of actions *sensu* Castañé et al. (2003). However, even though further investigations are necessary, it is reasonable to assume that there would be an ethological constraint at night that makes the depth selection more constant, even under stress situations.

Even though endosulfan is an important hormonal disruptor and neurotoxic compound (Palma et al. 2009) and chromium can alter numerous metabolics functions (Eisler 1986), the mechanisms through which the DVM is altered require more clarifications. Nevertheless, in line with other authors, our results allow us to hypothesize, that reduced swimming activity and disorientation would be the main causes of such alteration, preventing the species to reach their optimum fitness and maintain the populational dynamic (Goodrich and Lech 1990; Christensen et al. 2005). In a broader perspective, DVM alterations may enhance the probability of disappearance or extinction of the species which can alter the community structure and ecosystem dynamic in the middle and long term (Forbes and Callow 1996).

Finally, the present work constitutes the first report of altered DVM, depth selection and grouping pattern of zooplankton species as a consequence of chromium and endosulfan sublethal concentrations. Although most toxicological assessments employed standard toxicity tests (survival and reproduction) due to their simplicity, it was shown that they often underestimate the real risk they represent (Heugens et al. 2001). Therefore, due to its ecological importance, sensitivity and, a priori, of general application to various species, the DVM would be an adequate complement to evaluate the toxicological potential of different chemicals under more realistic conditions.

**Acknowledgment** This research was supported with grants from the Universidad Nacional del Litoral, Santa Fe, Argentina (Project CAI+D 2009 N° PI 69-351).

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