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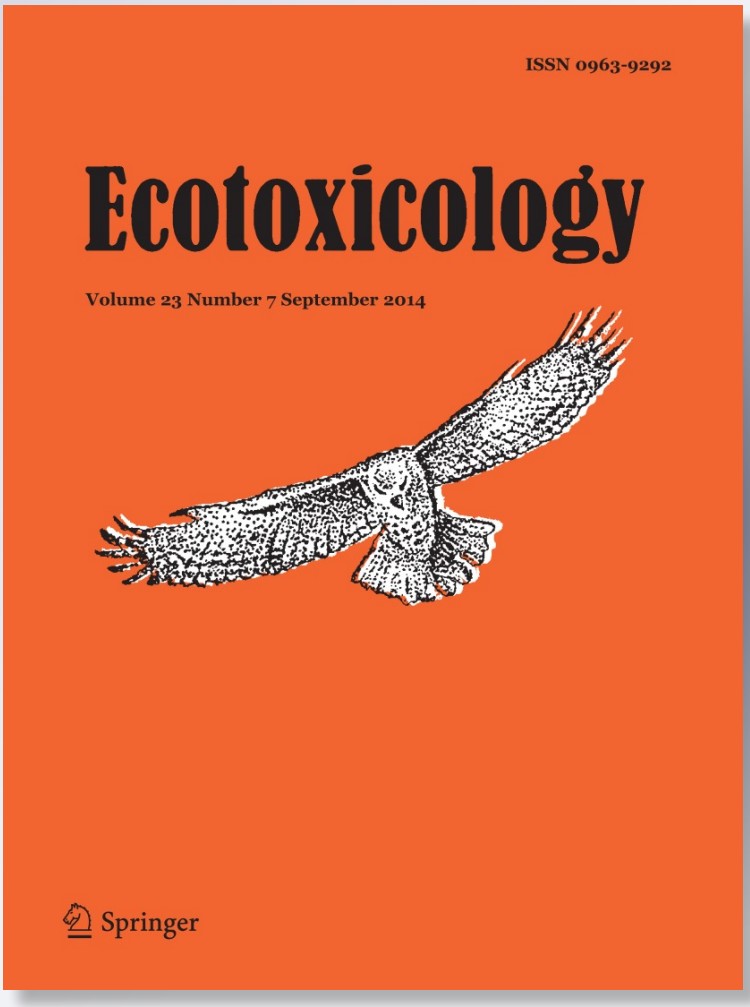

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Predator–prey imbalances due to a pesticide: density and applicability timing as determining factors for experimental assessments

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Abstract Predator–prey relationships are determining factors in sustaining community structure but xenobiotics, including pesticides, have the potential to alter them, causing imbalances at the ecosystem level. Although invertebrate predation on zooplankton is of high importance in shallow lakes, there is still little information regarding disturbances on this trophic interaction. This work assessed the potential effects of a chlorpyrifos-based pesticide (CLP) on the interaction between prawns *Macrobrachium borellii* and cladocerans *Ceriodaphnia dubia*, taking into account prey densities, specific time of exposure and contamination level. The analysis was focused on the specific sensitivity of both species and, especially, on the predation rate of *M. borellii* on *C. dubia*. The latter was evaluated through different treatments that combined predator and/or prey exposure to the insecticide, before (lapse of 12 h) or during the interaction. Under low prey density, when prawns were previously exposed to the insecticide, their consumption rate was lower than that of controls. Conversely, when cladocerans or both species were previously exposed, the prawns' feeding rate was higher. Under high prey density, there were no substantial differences among treatments. Comparatively, cladocerans were significantly more consumed when the exposure of both species was performed before rather than during the interaction. From the results obtained, it can be assumed that the trophic interaction under study is sensitive to CLP and that individual density and specific time of exposure are important variables to be considered in similar studies in order to obtain realistic results.

Keywords *Ceriodaphnia dubia* · *Macrobrachium borellii* · Aquatic environments · Pollution · Biological interactions

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Introduction

In continental aquatic systems, the association of xenobiotics—including pesticides—to multiple natural stressors (hydrological pulses, physicochemical changes, predation, parasitism, etc.) may cause diverse damages to the inhabitant species (Relyea and Hoverman 2006). In order to better comprehend these effects, accurate ecotoxicological tests are required including not only single-species assessments but also possible disruptions in their interactions with the surrounding environment (Rinderhagen et al. 2000; Brooks et al. 2009).

There are many important biological interactions, including competition (Loureiro et al. 2013), host–parasite relationships (Buser et al. 2012) and predation (Lampert and Sommer 1997) which structure biotic communities. Among them, predation is possibly one of the most important ones, especially as a driver of alternative stable states (Pace et al. 1999; Persson et al. 2007). Therefore, any perturbation or imbalance on predator–prey relationship might have negative consequences to the whole ecosystem (Brooks et al. 2009).

So far, it has been suggested that predator identity (vertebrates, invertebrates) and the specific sensitivity of each interacting individual are determining factors in the mentioned imbalances (Relyea and Hoverman 2006).

However, specific times of exposure and population density have not been widely considered in current studies. Such attributes are important because insecticide input is not always continuous—but rather intermittent and fitful—(Jergentz et al. 2004) and because the relative density of the individuals affects encounter probability, independently of environmental conditions (Guerritsen and Strickler 1997).

The lack of information in this line of research as well as the disparities observed in previous reports regarding disruptions in predator–prey interactions (Coors and DeMeester 2008; Scherer et al. 2012) suggest the need for further research to better understand the real effect of pollutants on the biota.

In order to contribute to this topic, the present work aims to analyze possible alterations in the *Macrobrachium borellii* (Nobili, 1896) × *Ceriodaphnia dubia* Richard, 1894 system due to the presence of the insecticide chlorpyrifos, taking into account prey density, specific time of exposure and contamination level. A series of experiments were performed in order to: (a) determine the sensitivity of *C. dubia* to the insecticide; (b) analyse the sublethal exposure on predation rates of *M. borelli* on *C. dubia* when the exposure occurs before the interaction, and (c) analyse the sublethal exposure on predation rates of *M. borelli* on *C. dubia* when the exposure occurs during the interaction.

Macrobrachium borellii and *C. dubia* were selected because they constitute abundant, representative components in many shallow lakes of the alluvial valley of the Paraná River (the second largest hydrographical system in South America) (Collins et al. 2007; José de Paggi and Paggi 2007). Unlike most northern waterbodies, these shallow lakes are characterized by having complex assemblages of invertebrate predators which prey on zooplankton and constitute the main risk factor to such organisms, particularly in littoral areas (Neill 1981; Collins et al. 2007; José de Paggi and Paggi 2007; Gonzales Sagrario and Balseiro 2010). Moreover, the importance of studying zooplankton–invertebrate interactions resides in the fact that, although individually small, they play major roles in the transfer of energy from autothrophs to the tops of the food webs (Dodson and Hanazato 1995).

The insecticide chlorpyrifos was selected because it has gained popularity around the world because of its wide spectrum in the control of pests. In Argentina, it is used in the main herbaceous crops (wheat, barley, corn, sorghum and soybean) to control bedbugs, isocas and other associated insects, even though it has the highest EIQ (Environmental Impact Quotient, Kovach et al. 1992) compared with other pesticides that are also widely used in Argentina for the same purpose (CONICET 2009).

Our working hypothesis is that sublethal concentrations of chlorpyrifos can cause alterations in the predator–prey system *M. borellii* × *C. dubia*, and that the observed alterations will vary according to density and specific time of exposure (before vs. during the interaction).

Materials and methods

Test species

Ceriodaphnia dubia (Cladocera: Daphniidae) was collected using a planktonic net (200 μm) from shallow lakes of the alluvial plain of the Paraná river. After taxonomic identification, a stock culture was initiated in a controlled laboratory with egg-carrying females. The culture was maintained in glass containers with dechlorinated and aerated tap water (control water, CW) under constant 16:8 h (Light:Dark) photoperiod and temperature (21 ± 2 °C) conditions. The incident intensity of light was 2,200 (±244) lx and the physico-chemical characteristics of CW were: dissolved oxygen: 6.4 (±0.8) mg l^{-1}; pH: 8.39 (±0.24); conductivity: 245.33 (±28.18) μS cm^{-1}. During the rearing period, the organisms were daily fed with a *Chlorella vulgaris* concentrate (final algal density: 2.8×10^5 cel. ml^{-1}).

Macrobrachium borellii (Decapoda: Palaemonidae) was collected using a hand net (1 mm mesh size) from the vegetation of shallow lakes belonging to the Paraná River system and carried to the same laboratory in which *C. dubia* was maintained. Prawns were reared in a glass aquarium (6 l) with dechlorinated and aerated tap water and fed ad libitum with a pelletized diet prepared in the laboratory (36 % proteins and 10 % lipids) (Collins and Petriella 1996).

Chlorpyrifos

The insecticide used in the experiments was Clorpi® (Ciagro, Buenos Aires Argentina), containing 48 % chlorpyrifos (0,0-diethyl phosphorotioate of 0-3,5,6–trichloro-2piridyl) and 52 % of non-specified adjuvants. This product was diluted in distilled water in order to make a stock solution of 50 μg l^{-1} chlorpyrifos, which was used to prepare the final tested concentrations immediately before the experiments. The stock solution was maintained at 4 °C in a dark bottle to avoid photodegradation and was renewed once a month. Due to its lipophilic nature (log $K_{ow} = 4.7$) (Murty and Ramani 1992), the actual concentration of the stock solution was required. Thus, total chlorpyrifos concentration was measured by gas chromatography fitted with a standard electron capture and flame photometric detectors, GC-ECD (GC VARIAN 3400) according to USEPA method 508, with minor modifications. An average of 41.5 μg l^{-1} E-chlorp (ethyl-chlorpyrifos) was detected in

water which means that 83 % of the parental product was recovered. Otherwise, no M-chlorp (methyl-chlorpyrifos, metabolite) was detected in any replicates indicating, as expected, no degradation process during the experimental period (Varó et al. 2000). In this sense, it was demonstrated that the degradation time (DT50) surpassed 80 days in distilled water (ATSDR 1997).

Chemical analyses were not performed in the final test concentrations because they were below the detection limit of the analytical techniques; thus, confidence in the chlorpyrifos detections might not be high. Nevertheless, several precautions were taken to assure confidence in the prepared toxicant concentrations prepared, i.e. volumes were measured with volumetric flasks and automatic precision pipettes (Boeco, Germany) by a unique operator in the laboratory, under temperature and light-controlled conditions.

Acute toxicity test

Because of the need to obtain accurate information about the lethal and sublethal concentrations of the used insecticide to *C. dubia*, the first step was to develop an acute (48 h) toxicity test. It was performed according to the standard static bioassay procedures outlined by the United States Environmental Protection Agency (USEPA 2002). Prior to the assay, ovigerous females from the stock culture were isolated in 100 ml glass containers with CW. After egg hatching, females were removed and 30 neonates (<24 h) were randomly placed in groups of five in 100 ml glass containers with 60 ml of each test concentration or the blank control (CW). Six different concentrations were used, ranging from 0.0012 to 0.04 μg l^{-1} chlorpyrifos, and six replicates per control and treatments were performed as a total of 210 individuals were used for the assay.

Temperature (21 ± 2 °C) and photoperiod (16L: 8D) were the same as described for the stock culture conditions. Dissolved oxygen concentrations varied between 8 and 6 mg l^{-1}, and pH varied between 7.6 and 8 during the course of the experiment. Animals were not fed during toxicity trials and they were considered to be dead when they ceased to move and no longer responded to mechanical stimulation. The LC_{50} values and their 95 % confidence limits for 24 and 48 h were estimated with the standard method of Probit Analysis (Finney, 1971).

Feeding experiments

Two experimental phases were performed for the feeding experiments (summarized in Table 1): Phase 1 consisted in the exposure of predator (P+C−), prey (P−C+) or both species (P+C+) to different sublethal concentrations of chlorpyrifos 12 h before the interaction. After exposure, both species were placed together in a new container (500 ml) with CW without the insecticide to evaluate the feeding rate of the predator (*M. borelli*) at 5, 12 and 24 h. The negative control consisted of an interaction between both species without previous exposure to chlorpyrifos (P−C−).

Table 1 Summary of the experimental design to perform the interaction experiments between *M. borelli* and *C. dubia*

Phase 1: previous exposure[a]		
	M. borelli[b]	*C. dubia*
P−C−	−	−
P+C−	+	−
P–C+	−	+
P+C+(b)	+	+
Phase 2: exposure during the interactions[a]		
	M. borelli[b]	*C. dubia*
P−C−	−	−
P+C+(d)	+	+

Table shows the proceeding to each working phase: Phase 1 corresponds to the previous exposure of organisms (12 h before the interaction) and Phase 2 corresponds to simultaneous exposure of the animals with the insecticide during the interaction. This design was fully replicated at two *C. dubia* densities (10 and 30 individuals per container) and two chlorpyrifos concentrations (0.002 and 0.01 μg l^{-1}). In all cases, both controls and treatments were replicated five times. + indicate that individuals were exposed to the pesticide (0.002 or 0.01 μg l^{-1}). – indicate that organisms were not exposed

[a] In both phases, the feeding rate of *M. borelli* was evaluated at 5, 12 and 24 h

[b] Only one animal per container

Phase 2 consisted in the exposure of both predator and prey to the toxicant, but in which chlorpyrifos was added during the interaction (i.e. at the same time in which predator and preys were placed together in the same container)—P+C+(d). The negative control in this case was similar to that in Phase 1 (P−C−). As in Phase 1, the feeding rate of the predator (*M. borelli*) was evaluated at 5, 12 and 24 h.

Final concentrations of chlorpyrifos used in this phase of the work were determined according to Kenaga's considerations (1982) and taking into account the results from the acute toxicity test with *C. dubia*. As a result, 0.01 and 0.002 μg l^{-1} chlorpyrifos were selected, which correspond to the LC_{50} and 20 % of the LC_{50} for *C. dubia* after 48 h of exposure, respectively.

For both concentrations and densities, one predator and two prey densities were used. Prey densities were: 20 and 60 ind. l^{-1} which correspond to 10 and 30 cladoceran

(adults, body length: 568.3 (±121.53) μm) in each experimental container (500 ml). Both were in agreement with microcrustacean densities typical of floodplain lakes of the Paraná river (José de Paggi and Paggi 2007, 2008).

Prior to each feeding experiment, predators were placed individually and maintained in plastic beakers (5 l) for 48 h to prevent the presence of food in the digestive system and to reach similar level of starvation for all predators.

In cases in which cladocerans were exposed to chlorpyrifos, they were arranged in groups of 10 individuals and the corresponding concentration was added 12 h before the experiment.

We employed five replicates for all treatment combinations.

As a first step, and for the analysis of Phase 1, the consumption of prey was expressed in relative terms (dividing the consumption in each treatment by the respective control). This way, a relative consumption index was obtained. Using those data, a three-way repeated measures ANOVA (time * concentration * treatment) was performed to determine whether time was important in the consumption of prey and if it interacted with the other factors. This analysis also allowed testing the effect of pesticide concentrations and treatments.

When necessary, an additional one-way ANOVA for each time (5, 12, and 24 h) was performed to test differences within treatments. In this last case, the original dataset was used.

In Phase 2, a two-way repeated measures ANOVA (time * treatment) was performed to determine whether time was important in the consumption of prey. For post hoc comparisons, the Bonferroni (Dunn–Bonferroni) test, based on the Student's t distribution and the Bonferroni inequality, was performed in order to determine specific statistical differences between treatments. All procedures were carried out with SPSS ver 17.0.

Before all the above mentioned analyses, the normal distribution of data (Kolmogorov–Smirnov's test), homoscedasticity (Levene's test) and sphericity (Mauchly's test) were verified. Statistical differences were considered significant at $p < 0.05$. The significance values and the confidence intervals were adjusted through Bonferroni's sequential method that controls the probability of committing Type I errors (Sokal and Rohlf 1995).

Results

Acute toxicity test with *C. dubia*

The mortality of *C. dubia* varied between 3.3 and 100 % within the range of the tested concentrations. Figure 1 shows the total mortality registered in each case. According to this result, the 24 h—LC_{50} was 0.009 (0.007–0.011) μg l^{-1} chlorpyrifos. After 12 h, the exposure time chosen for the predator–prey experiments, no mortality occurred at this concentration. The main response observed in the cladocerans exposed to 0.01 μg l^{-1}, the highest concentration used in the predator–prey experiments, for 12 h was a slow and, in some cases, erratic swimming behaviour.

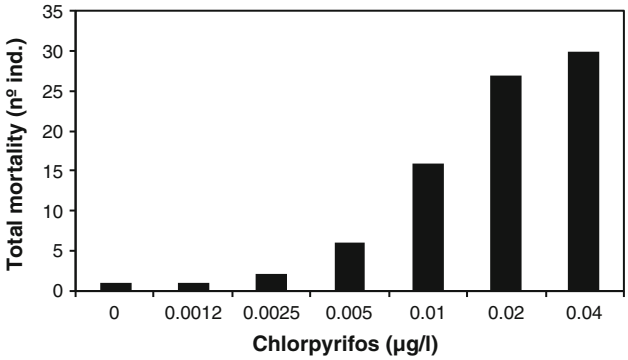

Fig. 1 Mortality of *C. dubia* exposed chlorpyrifos. Each bar represents the total number of dead animals. Thirty animals were exposed to each concentration, including the control (0 μg l^{-1})

Influence of time, treatments and pesticide concentration in the consumption of prey

The actual cumulative consumptions of prey during the time of each treatment in Phase 1 (previous exposure) is shown in Fig. 2.

The three way ANOVA revealed that the influence of time was significant in the consumption of prey; however, this factor did not interact with the other ones: treatments and pesticide concentrations (Table 2).

Also, under low prey density there were statistical differences among treatments (i.e. species exposed to chlorpyrifos: P−C−; P+C−; P−C+ and P+C+), indicating that consumption depended on which species were exposed to the insecticide. Within each treatment, there were no statistical differences between the pesticide concentrations used (Table 2). When experiments were carried out with high prey density (30 *C. dubia*), statistical differences were found neither between treatments nor between chlorpyrifos concentrations (Table 2).

Cumulative consumption depending on the exposed species

Since consumption from both chlorpyrifos concentrations did not differ within treatments, data were pooled in order to test statistical differences among treatments (Fig. 3a, b). In the experiments with 10 *C. dubia*, there were significant statistical differences on the consumption rate at all time

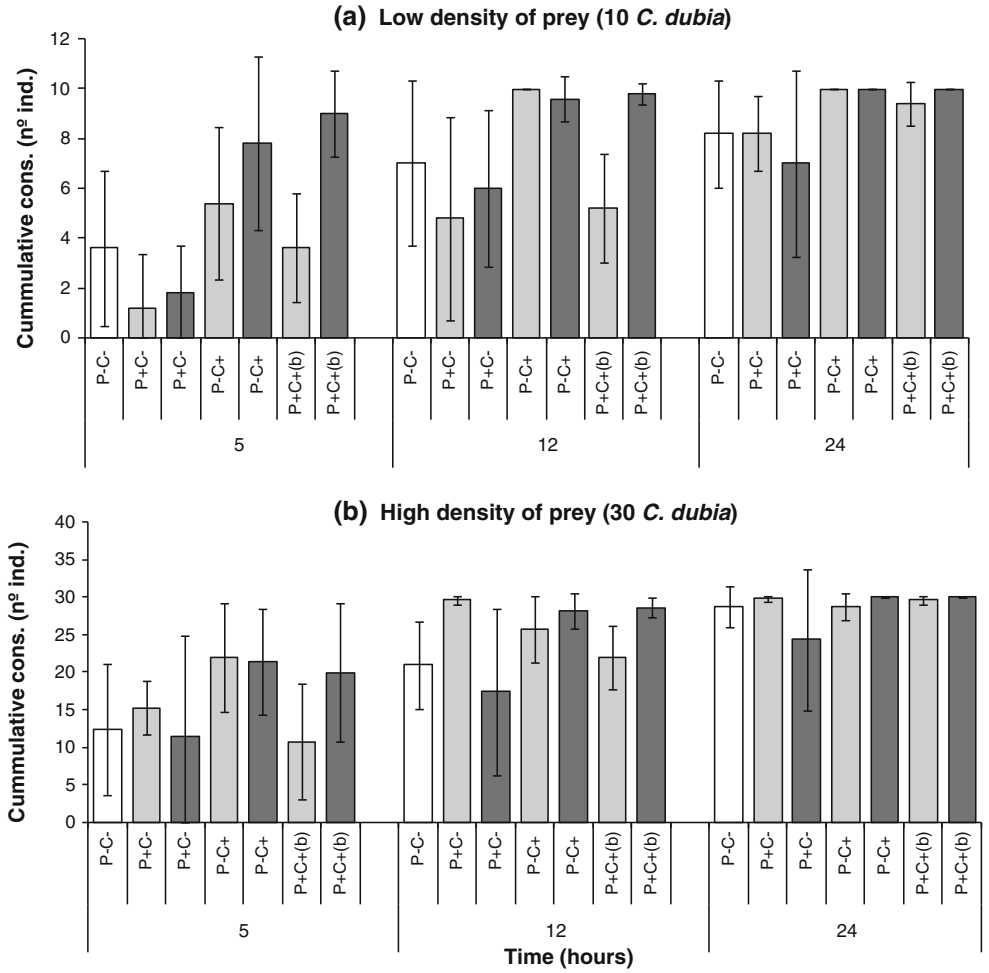

Fig. 2 Cumulative consumption on *C. dubia* throughout time. *Each bar* represents each experimental treatment during Phase 1 (previous expositions): P–C−; P+C−; P–C+ and P+C+(b). *White bars* are the controls (P−C−, without pesticide), *light gray* represents low concentration of pesticide (0.002 μg l^{-1} chlorpyrifos) and *dark gray* represents high concentration of pesticide (0.01 μg l^{-1} chlorpyrifos). *Error bars* represent the ±SD. **a** Consumption rates of *M.borellii* at low prey density; **b** Consumption rates of *M. borellii* at high prey density. Note that the figure shows the actual values, including the cummulative consumption of controls. The statistical analysis were performed using a relative consumption index, which was obtained by dividing the consumption in each treatment by the respective control

points observed: at 5 h (ANOVA df = 3, $F = 11.02$, $p < 0.001$), at 12 h (ANOVA df = 3, $F = 6.58$, $p = 0.002$) and at 24 h (ANOVA df = 3, $F = 3.81$, $p = 0.022$) (Fig. 3).

Cumulative consumption depending on the time of exposure

Phase 2 allowed comparing the effect of chlorpyrifos when it was added before the interaction (P+C+(b)) and during the trophic interaction (P+C+(d)). Since low concentrations (0.002 μg l^{-1}) did not show statistical differences, only the results from the highest concentration (0.01 μg l^{-1}) was considered for the analysis (Fig. 4).

The influence of time was significant in the consumption of prey; however, this factor did not interact with treatments (Table 3).

In the experiments with low prey density (10 *C. dubia*), there were statistical differences among the treatments (RM ANOVA, $F = 14.97$, $p = 0.001$). In all observation times, the exposure to chlorpyrifos before the interaction caused higher consumption than the control and the exposure during the interaction registered less consumption than the exposure before the interaction ($p < 0.05$, in all cases) (Fig. 4a).

With 30 *C. dubia,* treatments with a previous exposure, P+C+(b), and with an exposure during the interaction, P+C+(d), showed a similar trend as the one registered in the experiments with low prey density: higher consumption

Table 2 Results of the three-way RM ANOVA for Phase 1 of the experiment

	df	MS	*F*	*p*
(a) 10 *C. dubia*				
Treatment	2	12.372	4.118	0.020
Concentration	1	8.338	2.775	0.100
Time	2	13.635	4.539	0.014
Treat × conc. × time	6	1.108	0.369	0.896
Conc. × time	2	5.442	1.811	0.171
Treat × time	4	5.24	1.744	0.150
Error	72	3		
(b) 30 *C. dubia*				
Treatment	2	2.221	1.613	0.206
Concentration	1	0.436	0.316	0.576
Time	2	9.897	7.185	0.001
Treat × conc. × time	6	0.438	0.318	0.925
Conc. × time	2	0.652	0.473	0.625
Treat × time	4	1.407	1.022	0.402
Error	72	1.377		

The effects of time, pesticide concentration, treatment and their interactions on consumption rate of *M. borellii*. Treatment refers to the species exposed to chlorpyrifos: P−C−; P+C−; P−C+ and P+C+(b). *df* degree of freedom, *MS* mean square, *p* significance value

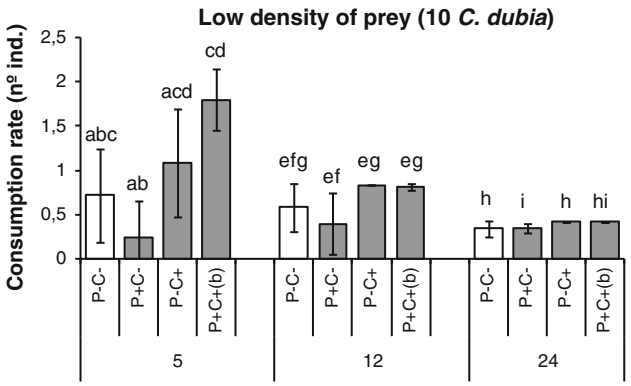

Fig. 3 Results of Phase 1 (pre-exposure experiment): Consumption rates of *M. borellii* at low prey density within each treatment: P–C−; P+C−; P−C+ and P+C+(b). Differences among treatments (Tukey test) within each hour are identified with *lowercase letters*: *different letters* indicate significant difference between these treatments ($p < 0.05$). *White bars* are the controls (P−C−, without pesticide), *grey bars* represent the responses of animals exposed to chlorpyrifos (for this analysis, results of both concentrations used, 0.01 and 0.002 μg l^{-1}, were pooled for a better understanding of the toxic effects). *Error bars* represent the ±SD

in P+C+(b) than in P+C+(d) (Fig. 4b). However, there were statistical differences only between the control and the animals previously exposed (P+C+(b)) ($p = 0.035$).

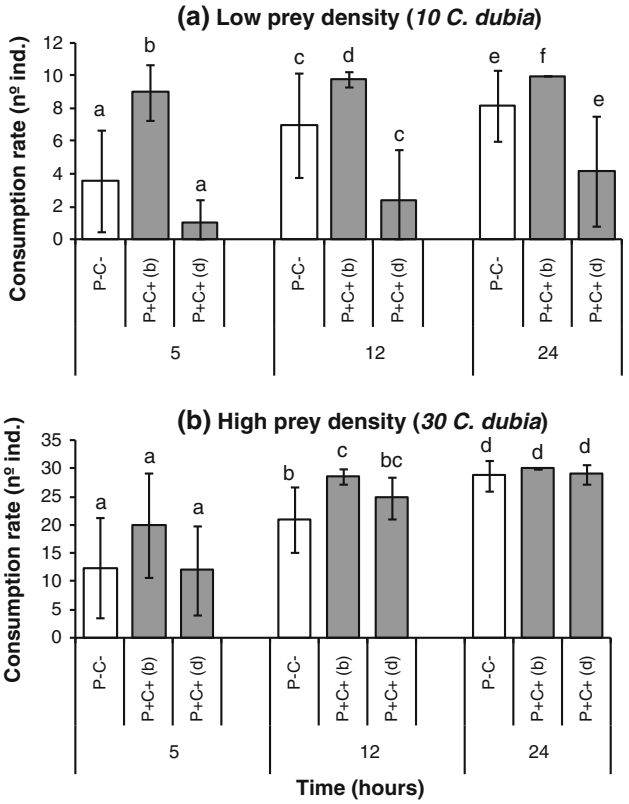

Fig. 4 Consumption rates of *M. borellii* in controls (i.e. when the organisms were not exposed, P−C−), when both species were previously exposed to 0.01 μg l^{-1} chlorpyrifos (P+C+(b)) and when both were exposed to chlorpyrifos during the trophic interaction (P+C+(d)). Differences among treatments (Tukey test) within each hour are identified with *lowercase letters*: *different letters* indicate significant difference between treatments ($p < 0.05$). *Error bars* represent the ±SD. **a** Consumption rates of *M. borellii* at low prey density; **b** Consumption rates of *M. borellii* at high prey density

Discussion

Since predator–prey is an extremely complex interaction, any disruption due to pollutants including pesticides will depend on several interacting variables. In fact, specific sensitivity is one of the most important attributes since it can govern the direction and magnitude of each imbalance (Relyea and Hoverman 2006). Thus, an accurate understanding of the effects of chlorpyrifos on the predator–prey interaction *M. borellii—C. dubia* requires, as a first step, the analysis of the acute sensitivity of chlorpyrifos in each species.

The LC_{50} to *C. dubia* (0.01 μg l^{-1}) is consistent with field studies in which 100 % of mortality was registered in the same species when it was exposed during 48 h to stream samples from the Sacramento-San Joaquín river delta with a concentration of 0.125 μg l^{-1} chlorpyrifos (Werner et al. 2000). Similar studies found LC_{50} values

Table 3 Results of the two-way RM ANOVA for Phase 2 of the experiment

	df	MS	*F*	*p*
(a) 10 *C. dubia*				
Treatment	2	187.467	32.322	<0.001
Time	2	33.067	5.701	0.007
Treat × time	4	4.833	0.833	0.513
Error	36	5.8		
(b) 30 *C. dubia*				
Treatment	2	108.156	3.427	0.043
Time	2	864.422	27.394	<0.001
Treat × time	4	21.689	0.687	0.605
Error	36	31.556		

The effects of treatment, time and their interactions on consumption rate of *C. dubia*. Treatment refers to the species exposed to chlorpyrifos: control (P−C−), both species exposed to chlorpyrifos before the interaction (P+C+(b)) and both species exposed during the interaction (P+C+(d)). *df* degree of freedom, *MS* mean square, *p* significance value

between 0.058 and 0.079 μg l^{-1} in *C. dubia* (Bailey et al. 1997) which, in spite of being slightly higher than the ones obtained in this work, are lower than the ones obtained in the most common species used in ecotoxicity studies, *Daphnia magna* (LC_{50} = 0.6 (±0.04) μg l^{-1}) (Moore et al. 1998), suggesting that *C. dubia* is particularly sensitive to chlorpyrifos. Although this work did not evaluate the sensitivity of *M. borelli* to the insecticide, a recent research report the acute toxicity (96 h-LC_{50}) caused by the same insecticide (Clorpi®) on this species was 2.74 μg l^{-1} while the highest concentration tested at which no significant deleterious effect was observed (NOEC) was 1.92 μg l^{-1} (Negro et al. unpublished). In addition, studies with other freshwater macrocrustaceans can be considered for comparison. For example, the prawn *Palaemonetes argentinus*, a frequent inhabitant of the Paraná alluvial plain and competitor of *M. borelli*, registered acute values of 2.98 and 0.49 μg l^{-1} at 24 and 96 h, respectively (Montagna and Collins 2007a). Among other macrocrustaceans linked by habitat relationships such as the amphipod *Hyalella curvispina* and the crab *Trichodactilus borellianus*, sensitivity values vary between 0.06 and 242.32 μg l^{-1} (Montagna and Collins 2007b; Mugni et al. 2012; Anguiano et al. 2012). In situ studies in polluted environments reported a mortality of *M. borelli* of 100 % when exposed to river water with 64 μg l^{-1} chlorpyrifos in suspended particulates (Jergentz et al. 2005). This information and the comparison with the data obtained from *C. dubia* confirm that *M. borelli* is indeed less sensitive, suggesting that the effects of this insecticide on their interactions could probably depend on such differences in sensitivity.

Results from the interaction experiments confirmed the high sensitivity of the cladoceran when compared with the prawn and also demonstrate that chlorpyrifos can alter the *C. dubia–M. borelli* interaction. As hypothesized, the alterations observed varied according to density and specific time of exposure.

In phase 1, three interesting trends could be detected from the experiments with 10 *C. dubia* (density = 20 ind. l^{-1}): When only *M. borelli* was exposed to the insecticide (P+C−), the consumption rate was less than that of the control group and also quite less than in the experiments where both species (P+C+) or only cladocerans (P−C+) were exposed.

This result suggests a direct toxic effect (though perhaps reversible, as later hypothesized) of the pesticide to the prawn, which reduces prey consumption. This is consistent with what was reported in a previous work where the exposure of the giant freshwater prawn, *M. rosenbergii* (postlarval stage), to chlorpyrifos concentrations between 0.15 and 5 μg l^{-1} significantly inhibited its feeding behaviour (Satapornvanit et al. 2009). Although the physiological mechanisms of feeding inhibition have not been completely assessed yet, it has been demonstrated that organophosphates like chlorpyrifos are effective acetylcholinesterase inhibitors, which result in an accumulation of the neurotransmitter acetylcholine at the nerve ending and can cause physiological and behavioural alterations not only in vertebrates (Baldwin et al. 2009) but also in different invertebrates, including prawns (Montagna and Collins 2007a; SismeiroVivas et al. 2007; Xuereb et al. 2009). Since the concentrations employed in this work were lower than in previous studies and the exposure time lasted only 12 h, it would be speculative to assume a neurotoxic effect. However, it is highly probable that the exposure to the insecticide caused disorientation in the prawns, which made them unable to develop accurate attack, capture or feeding abilities.

Conversely, when only *C. dubia* was exposed, the prawn feeding rate was higher than in the remaining treatments, indicating that the insecticide affected the escape ability of cladocerans. This is in agreement with studies in which planktonic organisms exposed to pesticides were clearly more vulnerable than non-exposed conspecifics (Hanazato 2001; Gutierrez et al. 2011, 2012). Possible explanations of such vulnerability can be the alterations in their perception ability of the mechanical or chemical signals of the predator or in the subsequent reactions to predator's stimulus (Gutierrez et al. 2011).

When both species were exposed (P+C+), the feeding rate of prawns increased notoriously, more than in the remaining treatments. These results are in contrast with those obtained by Langer-Jaesrich et al. (2010), who

studied the *Danio rerio–Chironomus riparius* interaction. These authors found a compensation effect, i.e. when both species were exposed, the feeding behaviour did not differ from that of the control group, without chlorpyrifos. In this case, it is probable that the high sensitivity of *C. dubia* and the need for energy recovering for a detoxification process developed an imbalance of a higher consumption of cladocerans.

On the other hand, high prey consumption in treatments with chlorpyrifos also indicates that, at least to *M. borelli*, the cladoceran *C. dubia* is palatable despite being polluted. This differs from a study with vertebrate organisms, which registered low palatability of prey when they were submitted to organic pollutants (Junges et al. 2012). Although palatability is an important factor to be considered in ecotoxicity tests with predator prey interactions, further studies are necessary to verify the above mentioned hypothesis. This is important because the starvation of animals, which in this kind of ecotoxicity tests is necessary to ensure the equitability in the physiological state of animals, may trigger a forced consumption on a species that in nature is hardly consumed. In this sense, it is probable that *M. borelli* would prefer other species instead of *C. dubia* in environments with high trophic offer.

When experiments were carried out with the high prey density (30 *C. dubia* = 60 ind. l^{-1}), there were practically no statistical differences among treatments (with one exception). This result suggests that the high availability and abundance of preys can 'mask' the toxic effect of certain pollutants. The importance of considering individual density in similar studies reside in the possibility of obtaining realistic results since in nature, especially in subtropical climates, environmental fluctuations as well as hydraulic pulses can modify population densities (José de Paggi and Paggi 2007; 2008).

In the second phase of the work, when organisms were pre-exposed to chlorpyrifos, cladocerans were significantly more consumed than when chlorpyrifos was added during the interaction. As previously mentioned, it is probable that the early exposure of cladocerans during 12 h was enough to alter the physiological mechanism that turns them more susceptible to prawns. A well-recognized effect of neurotoxic pollutants, such as chlorpyrifos, is the induction of hyperactivity (Xuereb et al. 2009). In this sense, hyperactivity—or the excessive alert stage—is negative to preys because it enhances the encounter rate of both individuals (Guerritsen and Strickler 1997). On the other hand, it is likely that the low sensitivity of prawns to the insecticide and the short exposure time allowed them to recover and feed easily on cladocerans. The lower consumption of cladocerans when chlorpyrifos was exposed during the interaction agrees with similar studies in which predators and preys were simultaneously exposed to a toxicant. Ingerman et al. (2002) reported that the pesticide methoxychlor (MTX) impaired the feeding behaviour of dragonfly naiads on tubifex worms; Agostinho et al. (2012) found that the feeding rate of *Echinogammarus meridionalis* on nauplii of the brine shrimp *Artemia franciscana* (Kellog) was impaired by copper; and Weis et al. (2003) found that the consumption rate of larval mummichogs *Fundulus heteroclitus* previously exposed to water samples from different polluted sites was negatively correlated with many environmental contaminants. These authors highlighted that the reduction in the feeding rate of predators during exposure can be attributed to different causes, such as disorientation, miscues in the captures, poor aims or chase abandonment.

Finally, considering that chlorpyrifos values measured in water from different water bodies of Argentina average 0.07 μg l^{-1} (Jergentz et al. 2005), it can be assumed that the interaction between the species here studied might be affected. However, further studies are needed to investigate the nature of the mechanisms responsible for the adverse effects of pesticides on this and other predator–prey interactions.

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