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Cost of reproduction. Changes in metabolism and endosulfan lethality caused by reproductive behavior in *Hyalella curvispina* (Crustacea: Amphipoda)

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ABSTRACT

Biocides are periodically applied in agricultural activities, reaching aquatic systems and acting upon the biota. Amphipods are widely used in toxicity tests because of their sensitivity to a wide range of pollutants. In this work, we report the differential lethality of a widely used pesticide, endosulfan, on the amphipod *Hyalella curvispina* at two life stages and in three different adult groups, males and females separated by sex and both sexes grouped together. In addition, oxygen consumption of adult groups was determined as a way to estimate the role of behavioral activities and exposure to endosulfan in metabolism shifts. There were no differences between the LC_{50} of juveniles and the adults when they were separated by sex ($p > 0.05$). Nevertheless, the LC_{50} of adults without sexual differentiation was significantly lower than the LC_{50} of juveniles and adults separated by sex ($p < 0.05$). The oxygen consumption rate was higher when adults were grouped without sexual differentiation in the control group. The exposure to low concentrations of endosulfan causes an increase in oxygen consumption in all the treatments. The sexual behavior increased the metabolism and the sensitivity to endosulfan. In future evaluations, adults grouped without sexual differentiation, which were the most sensitive group, should be included in order to mimic the environmental conditions. Using only juveniles or adults separated by sex in toxicity tests may inaccurately estimate the lethality of biocides, especially in species with constant reproductive activities.

1. Introduction

Several biocides are applied in agricultural activities to minimize crop damages produced by pests. These compounds eventually reach aquatic environments, imposing a risk to the biota inhabiting there. Endosulfan (6,7,8,9,10,10-hexacloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,3,4-benzodioxathiepin-3-oxide) is a broad spectrum cyclodiene insecticide extensively used in crop areas throughout the world. This pesticide is used for the control of boring, chewing and sucking insects and cyclamen mites (Hose and Van den Brink, 2004; Wan et al., 2005). In Argentina, its application has grown due to increased soybean acreage. The widespread and indiscriminate use of this pesticide causes the pollution of aquatic ecosystems through drift and runoff (Ernst et al., 1991; Miglioranza et al., 2004; Jergentz et al., 2005; Silva et al., 2005).

Endosulfan is a quite persistent pesticide. In water, its half-life can range from several months to years, depending on environmental conditions (Leonard et al., 2001; Wan et al., 2005). It is a neurotoxic pesticide, which acts on organisms by blocking the chloride channels at the gamma-aminobutyric acid (GABA) receptor in the central nervous system, leading to neural excitation and eventually killing the organism (Murray et al., 1993). It is known to be a very highly toxic compound to fish and aquatic invertebrates, as *Daphnia magna*, *Hyalella azteca*, *Oncorhynchus mykiss*, *Oncorhynchus kisutch*, *Prochilodus lineatus*, *Cichlasoma dimerus*, *Jenynsia multidentata*, among others (Hose and Van den Brink, 2004; Wan et al., 2005; Ballesteros et al., 2007; Bacchetta et al., 2011a,b).

Hyalella curvispina (Shoemaker, 1942) is a freshwater amphipod extensively distributed in the south of the neotropical region. It is representative of zoobenthic and epiphitic communities from Punta Arenas, Chile and Islas Malvinas, Argentina in the south to Cangallo, Peru and Rio de Janeiro, Brazil in the north (García et al., 2010). As a phytobenthos and detritus feeder, this species plays a key role in nutrient cycling and is also an important link in food webs as a prey of several invertebrates, fish, amphibians and birds (Giorgi and Tiraboschi, 1999; Galassi et al., 2006, Casset et al., 2001; Peralta and Grosso, 2009; Saigo et al., 2009).

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Amphipods, mainly *H. azteca*, are generally used in laboratory tests and in situ bioassays to evaluate rural and urban pollution, because of their easy maintenance and high abundance in aquatic environments (Wang et al., 2004; Wan et al., 2005; You et al., 2004; Smith et al., 2007; Ding et al., 2011). However, there is a global trend to evaluate the impact of pollutants with native species, as they are adapted to particular aquatic system conditions and may provide more realistic results than those obtained from exotic species (Jergentz et al., 2004; Giusto et al., 2012; Mugni et al., 2012a,b; Paracampo et al., 2012).

The standard methods recommend lethal toxicity tests to assess pollutant effects, and the median lethal concentration as a reference value for comparisons among species or toxic agents. Nevertheless, there are many factors that may influence the sensitivity of biological organisms to toxicants, which in turn may modify the median lethal concentration values, such life stage, sex and reproduction cycle (Wirth et al., 2001; Key et al., 2003; Li et al., 2006). Standardized toxicity tests recommend the use of juveniles for two main reasons. First, it is a way to avoid sex-dependent factors. Second, available data suggest that these organisms are usually more vulnerable to toxic agents during juvenile stages than mature ones, and on this basis, the use of juveniles will result in the most protective water quality criteria (Muysen and Janssen, 2007).

Pesticides are mainly applied during spring and summer. Although the reproduction of *H. curvispina* occurs throughout the entire year, there is a population peak in those seasons, when water temperature is higher, suggesting an important increase in reproduction activities (Casset et al., 2001). These activities may cause changes in metabolism, as the individuals have to spend energy searching for mate, courting and copulation. The exposure to toxicants may also produce shifts in metabolism, as observed in other crustaceans (Chinni et al., 2000; Williner and Collins, 2003; Montagna and Collins, 2008; Negro et al., 2012).

The effects of sexual behavior in toxicity tests, despite their importance, are not well documented. If reproduction activities increase metabolism, lethal effects could be observed at lower concentrations, causing differences in LC_{50} when adult individuals without sexual differentiation are exposed to toxic agents. Also, endosulfan effects in *Hyalella* metabolism are not well documented and to the best of our knowledge, there are few records of the physiological and reproductive effects of pesticides on amphipods (Dutra et al., 2008, 2009). The aims of this work are to determine the differences in sensitivity to acute endosulfan exposition between juveniles and adults of both sexes with and without reproductive activity and to evaluate the effects of endosulfan on the specific metabolic rate of *H. curvispina*.

2. Materials and methods

Amphipods were collected in the Paraná River floodplain (latitude 31° 30′S; longitude 60° 41′W, Santa Fe, Argentina) during early spring (September–October), using a hand net below the aquatic vegetation (area: 0.9 m^2; mesh size of 1 mm). About 2000 individuals were cultured in the hatchery of the Instituto Nacional de Limnología (CONICET-UNL) for at least 1 month, in 10,000 l aquaria with phytoplankton, zooplankton and aquatic plants (*Eichornia crassipes*, *Pistia stratiotes*, *Myriophyllum aquaticum* and *Salvinia molesta*) and exposed to environmental light and temperature conditions (12:12 light/darkness; 14.55 ± 4.32 °C (Mean ± SD)). Before the tests, healthy individuals, which showed normal swimming movements, were separated according to size into juveniles (3.2–5 mm length) or adults (5.1–10 mm. length) (García et al., 2010). Adults were separated by sex according to the development of the second right gnathopod, through observation in a stereoscopic microscope. Immature individuals (juveniles) could not be separated by sex because of the absence of sexual dimorphism (Lopretto, 1983, 1995). Once the adults and juveniles were separated, they were acclimated in plastic containers with dechlorinated tap water (the same water used as dilution media, see below) and aquatic plants (*M. aquaticum*). Fish muscle pieces were given to the amphipods during the first day and removed during the second day. Assays started on day three, after two days of acclimation.

The assays, both oxygen consumption and toxicity tests, were made under static conditions in a 20 ± 1 °C incubator with a light/dark photoperiod of 12/12 h (UV light). The dilution media was tap water with the following physicochemical parameters (mean ± standard deviation) measured at initial time: dissolved oxygen (mg l^{-1}): 7.77 ± 0.57; pH 7.8 ± 0.5; conductivity (μS cm^{-1}): 900 ± 62; temperature (°C): 20 ± 0.5; salinity (percent) 0.05 ± 0.00; ammonia (mg NH_3–N l^{-1}): 0.65 ± 0.09 and hardness (mg $CaCO_3$ l^{-1}): 320 ± 35.9.

Acute toxicity tests were conducted according to standard methods (APHA, 1998). The groups used in these tests were juveniles (J) and three adult groups; males and females together (50 percent from each sex) (M–F), males only (M) and females only (F). Animals were placed in 400 ml clear plastic (biaxially oriented polystyrene) containers with ten individuals per tank. Two control tests were conducted; one with dechlorinated water without pesticides and another one with acetone used as the reference solvent. The concentration used in solvent control was similar to the commercial product volume used in the higher concentration of the toxicity tests (0.5 μl acetone l^{-1}). Three replicates were made for every treatment including controls (30 individuals per concentration). The assayed product was Zebra®, a commercial product containing 35 percent of endosulfan. The endosulfan concentrations used, based on range finding test previously performed, were 0, 1.75, 3.5, 7, 14, 28 and 56 μg endosulfan l^{-1} in the M–F group and 0, 3.5, 7, 14, 28, 56 and 112 μg endosulfan l^{-1} in the M, F and J groups. The different endosulfan concentrations were prepared using dilution series of a nominal stock solution of 35 mg endosulfan l^{-1} (Table 1). Water for the control group, acetone and pesticide solutions were renewed daily since we previously observed that the concentration of endosulfan decreased about ten–twenty percent, (our laboratory). Thirty liters of every used solution were prepared and subsamples for every replicate and for chemicals analysis were taken from that solution. The containers were not aerated because the medium was replaced daily with water with dissolved oxygen values of 7.77 ± 0.57 mg l^{-1}. Pelletized food, according to a formula developed for crustacean growth, was supplied in the evening after 48 h of exposure (Lordi and Collins, 2004). The leftovers were removed with the media early in the morning of the next day. Mortality and precopulatory guardian behavior (amplexus) were recorded before media exchange and dead animals were removed. Median lethal concentrations at 96 h and 95 percent confidence limits were estimated by Probit. The differences in median lethal concentrations among different groups were considered significant when the higher LC_{50}/lower LC_{50} ratio exceeds the corresponding critical value established by the American Public Health Association (Rodriguez and Amin, 1991). The differences in the survival as a function of time for each group were analyzed using the Kruskal Wallis test followed by Mann Whitney test ($p < 0.05$).

Oxygen consumption tests were conducted in 250 ml glass DBO bottles. Ten individuals were placed in each bottle and kept capped for 24 h. Three concentrations of pesticides, related to toxicity test survival curves, and a control group of tap water were tested. The concentrations used were 6, 37.6 and 60.8 μg endosulfan l^{-1} (C_1, C_2 and C_3 respectively), prepared as described above. Based on toxicity tests, we expected almost no mortality for 6, a high survival in 37.6 and some death for the exposed to 60.8 μg endosulfan l^{-1}. The differential lethality may have an impact on oxygen consumption. The groups tested were males and females together (50 percent from each sex) (M–F), males only (M) and females only (F). Ten replicates were made for each concentration and group. Dissolved oxygen (DO) was measured following the Winkler method (Grasshoff, 1983) Initial DO concentrations of every endosulfan treatment were measured at the beginning of the tests, with ten replicates per concentration. No food was supplied during these tests. A set of ten bottles of endosulfan solutions without animals was tested for every concentration, to determine the normal dissolved oxygen decrease without *Hyalella*. Final DO was measured after 24 h. Any observed amplexus were recorded before oxygen fixation. Juveniles were not tested because preliminary tests showed that the oxygen consumption was not different from normal

Table 1
Nominal and measured endosulfan concentrations in toxicity and oxygen consumption tests (96 h and 24 h of exposure respectively) with *Hyalella curvispina*.

Endosulfan concentrations (μg l^{-1})			
Acute toxicity tests		Oxygen consumption tests	
Nominal	Measured	Nominal	Measured
0	< D.L.	0	< D.L.
1.75	< D.L.	7	6.0
3.5	< Q.L.	35	37.6 ± 3.4
7	6.0	70	60.8 ± 7.4
14	11.7 ± 2.2		
28	25.6 ± 4.8		
56	49.1 ± 8.3		
112	102.6 ± 20.3		
Detection limit: 2 μg endosulfan l^{-1}			
Quantification limit: 6 μg endosulfan l^{-1}			

D.L. = Detection limit and Q.L. = Quantification limit.

dissolved oxygen decrease. The wet weight of every group was determined after dissolved oxygen measurements.

Oxygen consumption rates (OCR) were calculated as the ratio of the consumed oxygen (the difference among the initial and the final dissolved oxygen) and the wet weight (mg O_2 g^{-1}). The differences in OCR caused by pesticide exposure (differences between the different exposure concentrations of endosulfan inside each group) and the differences in OCR of each group (differences between the different groups at the same pesticide concentrations) were compared with one way ANOVA and Tukey post test ($p < 0.05$), after normality and variance homogeneity were checked (untransformed data). The relationship between oxygen consumption rates and weight were evaluated by regression lines and their R^2 and p values were calculated according to Zar (1996).

Total endosulfan concentration in the water samples were analyzed by solid phase microextraction (SPME) and concentrations were measured by GC-ECD (GC Hewlett Packard 5890 Series II) with nitrogen as a carrier gas (1 ml/min) (ASTM D 6520-06 method; 2006). Nominal and measured concentrations used in toxicity and oxygen consumption tests are expressed as a sum of endosulfan isomers.

3. Results

3.1. Acute toxicity tests

Nominal and effective concentrations of endosulfan are listed in Table 1. The two lowest concentrations of endosulfan in acute toxicity tests (1.75 and 3.5 μg l^{-1}) were not analyzed because they were below the detection or quantification limits of the techniques.

Mortality was less than ten percent in both types of controls. The LC_{50} of M–F was significantly lower than the LC_{50} of M, F and J categories ($p < 0.05$). There were no differences between median lethal concentrations of adults when they were separated by sex ($p > 0.05$). The LC_{50} of F was lower than the LC_{50} of J ($p < 0.05$) (Fig. 1). Survival was time dependent, since there was an increase in mortality as time passed. The kinetics analysis showed differences in homogeneous groups at 24 and 96 h observations (Fig. 2, Table 2). In all cases, the 95 percent confidence intervals were lower than 30 percent of the mean, as recommended by APHA (1998).

In M–F acute toxicity tests, amplexus was observed in the control group and in animals exposed to 1.75; 3.5; 6 and 11.7 μg endosulfan l^{-1}. No amplexus was observed in animals exposed to 25.6 and 49.1 μg endosulfan l^{-1} (Table 3).

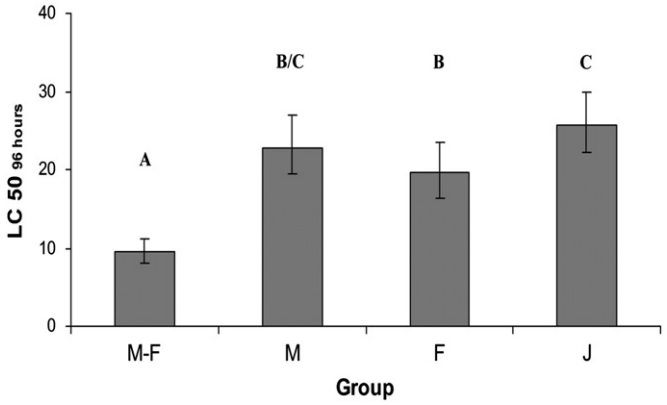

Fig. 1. Median lethal concentration (with lower and upper 95 percent confidence limits) at 96 h exposition of endosulfan (LC_{50}, μg endosulfan l^{-1}) of different groups of *Hyalella curvispina*. M–F: males and females grouped together (50 percent from each sex); M: Males; F: Females and J: juveniles. Homogeneous groups are identified by the same letter.

3.2. Oxygen consumption

The individuals of the control group and of the group exposed to 6.0 μg endosulfan l^{-1} showed normal movements, swimming freely in the water column. Amplexus was observed in both groups. Mortality was not recorded because looking for and removing dead animals involved mechanical stimulus of the medium that would cause interference with the levels of dissolved oxygen. Individuals exposed to 37.6 μg endosulfan l^{-1} showed slower and shorter movements. In the case of individuals exposed to 60.8 μg endosulfan l^{-1} almost no movements were observed. These individuals remained in the bottom of the container most of the time. No copulatory amplexus was observed at the two highest concentrations.

In the control group the oxygen consumption rate (OCR) of M–F was higher than the OCR of F and of M. In the case of amphipods exposed to 6.0 μg endosulfan l^{-1}, the M–F group had a higher OCR than M but there were no significant differences with F. There were no differences in OCR of any group in the amphipods exopsed to 37.6 μg endosulfan l^{-1} and 60.8 μg endosulfan l^{-1} (Fig. 3). Several amplexus were observed, but only in the control and in the exposed to 6 μg endosulfan l^{-1} groups (Table 4).

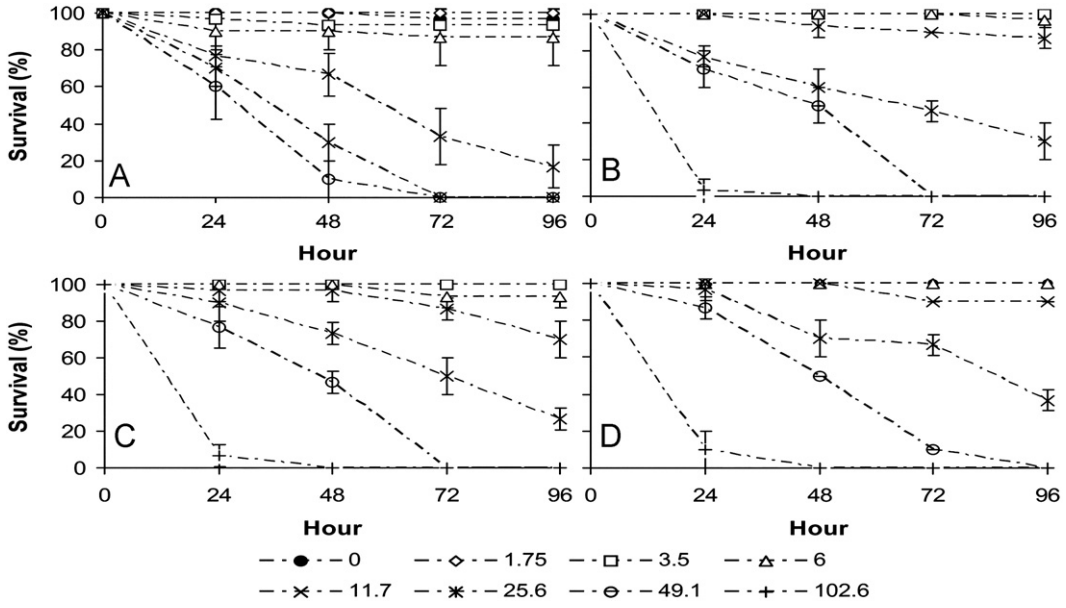

Fig. 2. Survival (percent) of *Hyalella curvispina* exposed to different endosulfan concentrations (μg endosulfan l^{-1}). (A) males and females grouped together; (B) males; (C) females and (D) juveniles.

The relationship between oxygen consumption rate and weight shows differences among groups and concentrations. There was no relation between weight and OCR under control conditions and exposure to 6 μg endosulfan l^{-1} for any of the assayed groups. Regressions become significant when M–F group was exposed to 37.6 μg endosulfan l^{-1} and when M group was exposed to 37.6 and 60.8 μg endosulfan l^{-1}. In the case of F, regressions among weight and OCR were not significant for both control and endosulfan exposure conditions (Fig. 4, Table 5).

Table 2
Survival of different groups of *Hyalella curvispina* exposed to endosulfan. Differences in survival homogeneous groups between 24 and 96 h. Homogeneous groups ($p > 0.05$) are identified by the same letter.

Homogeneous groups				
Concentration	Males–Females		Males	
	24 h	96 h	24 h	96 h
0	A	A	A	A
1.75	A	A	A	A
3.5	A B	A	A	A
7	A B	A	A	A
14	B	B	A	B
28	B C	B	B	C
56	C	B	B	D
112			C	D
	Females		Juveniles	
	24 h	96 h	24 h	96 h
0	A	A	A	A
1.75	A	A	A	A
3.5	A	A	A	A
7	A	A	A	A
14	A B	B	A B	B
28	A B	C	A B	C
56	B	D	B	D
112	C	D	C	D

Table 3
Amplexus observed per replica in males and females of *Hyalella curvispina* grouped together from 96 h endosulfan toxicity tests (mean value ± standard deviation). n=total number of individuals per concentration (ten individuals per replicate; 50 percent from each sex; three replicates per concentration).

Amplexus observed				
Concentration	Acute toxicity tests (n=30)			
	24 h	48 h	72 h	96 h
0	0.66 ± 0.47	1.33 ± 0.47	0.66 ± 0.47	0
1.75	2 ± 0.81	1.66 ± 0.47	1.66 ± 0.47	0.66 ± 0.47
3.5	0.66 ± 0.47	0.66 ± 0.94	1 ± 0.81	0
6	1	1.33 ± 0.47	0.66 ± 0.47	0
11.7	0.33 ± 0.47	0.33 ± 0.47	0	0
25.6	0	0	0	0

4. Discussion

Adults grouped without sexual differentiation had a lower LC_{50} than groups separated by sex. These differences could be related to sexual behavior. Some amphipods of the Neotropical region, as *H. curvispina* and *H. pampeana*, among others are animals with an intense year round sexual activity, especially during spring and summer. Their reproductive behavior is characterized by several stages such as mate searching, copulatory amplexus, ovulation and fertilization (Calow, 1979; Lopretto, 1983). These activities increase their energy demands and consequently their metabolism, as observed in OCR assays. Higher metabolism might cause an increased pesticide uptake, which in turn could increase mortality at lower concentrations. On the other hand, when adults grouped without sexual differentiation went into sexual behavior, their oxygen consumption rate increases. Thus, a direct relation between oxygen consumption and energy expenditure is proposed by several authors (Pillai and Diwan, 2002; Montagna and Collins, 2008; Negro et al., 2012). The increased activity associated with reproductive behavior might increase the metabolism and deplete energy reserves, which in turn would lead to increased mortality. The higher tolerance to endosulfan when adults were separated by sex might be related with the reduced metabolic activity as a result of the absence of sexual activities.

Table 4
Amplexus observed per replica in males and females grouped together from 24 h oxygen consumption tests of *Hyalella curvispina* exposed to different concentrations of endosulfan (mean value ± standard deviation). n=total number of individuals per concentration (ten individuals per replicate; 50 percent from each sex; ten replicates per concentration).

Amplexus observed	
Concentration	Oxygen consumption tests (n=100)
	24 h
0	0.7 ± 0.48
6	1 ± 0.47
37.6	0
60.8	0

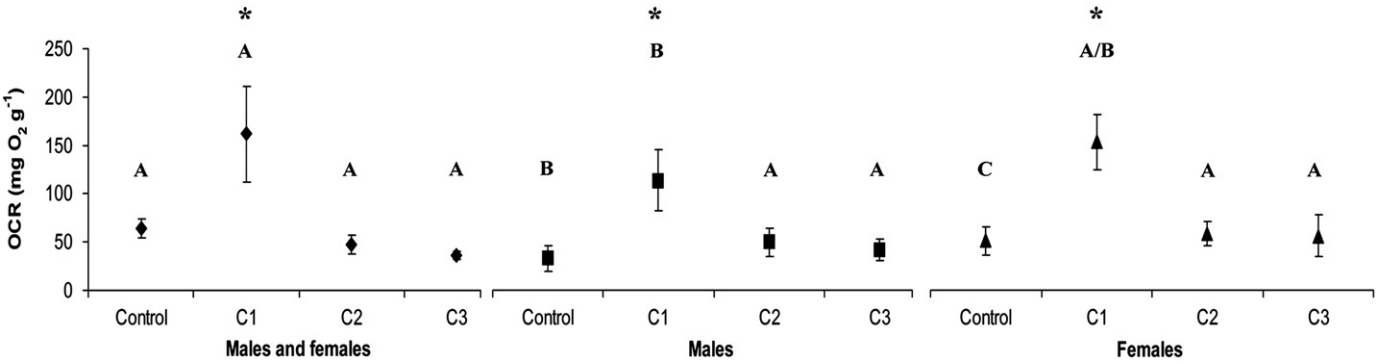

Fig. 3. Mean values (± SD) of oxygen consumption rate from control and exposed groups (n=10 replicates per concentration and group). * indicates significant differences within each group. Letters indicates the differences between the groups in each concentration (different letters indicates significant differences between the groups) ($p < 0.05$) Control=0 μg endosulfan l^{-1}; C_1=6 μg endosulfan l^{-1} C_2=37.6 μg endosulfan l^{-1} and C_3=60.8 μg endosulfan L^{-1}.

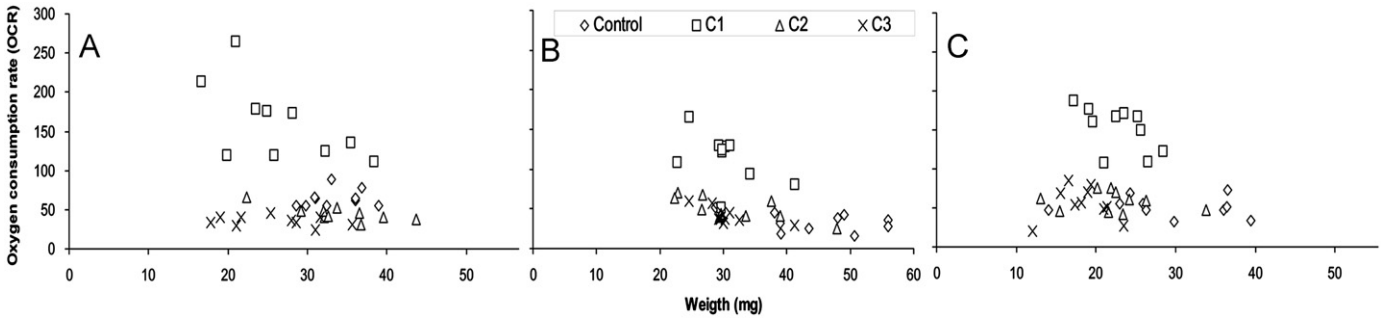

Fig. 4. Oxygen consumption rate of *Hyalella curvispina* exposed to endosulfan as a function of weight (24 h tests). (A) males and females grouped together, (B) males and (C) females.

Table 5
Linear regressions between oxygen consumption rate and weight of *Hyalella curvispina* exposed to endosulfan (24 h tests).

Concentration (μg endosulfan l^{-1})	Equation	R^2	p
Males–Females			
0	$y=0.5835x+44.914$	0.179	0.62
6	$y=-4.2511x+274.9$	0.6	0.067
37.6	$y=-1.2471x+87.448$	0.773	0.009*
60.8	$y=-0.3093x+43.73$	0.299	0.401
Males			
0	$y=-0.5141x+56.27$	0.394	0.26
6	$y=-2.9531x+202.89$	0.467	0.173
37.6	$y=-1.3549x+92.935$	0.737	0.015*
60.8	$y=-16968x+93686$	0.688	0.028*
Females			
0	$y=-0.2207x+57.415$	0.138	0.704
6	$y=-4.358+251.77$	0.553	0.097
37.6	$y=-0.368x+66.575$	0.162	0.654
60.8	$y=-0.1168x+5829$	0.18	0.961

* Statistically significant regression ($p<0.05$).

The exposure to the two highest concentrations of endosulfan tested reduced swimming activities and sexual behavior, as only one amplexus was observed in both concentrations. The animals reduced their movements and were mainly founded in the bottom. Behavioral changes in swimming and reproduction activities, which are pointed out as sensitive biomarkers of stressful conditions, were also observed in *Gammarus lawrencianus* exposed to cadmium and in fish exposed to microcystin-RR (Wallace and Estephan, 2004; Cazenave et al., 2006, 2008). These authors pointed out that swimming activity was reduced and energy resources might be used in detoxification processes. The inhibition of sexual behavior at biocide exposure was also observed in *H. castroi*, *H. curvispina* and *H. pleoacuta* when exposed to carbofuran and in *H. azteca* exposed to lindane (Blockwell et al., 1998; Dutra et al., 2008, 2009).

Juveniles were as resistant as adults separated by sex, since LC_{50} values were similar. This life stage, although recommended by standard methods, may not be the most sensitive to pesticides. This was also observed when *H. curvispina* was exposed to cadmium, where juveniles were less susceptible than adults, and in shrimp, where larvae of *Palaemonetes pugio* were more resistant to endosulfan than adults (Key et al., 2003; García et al., 2010).

Looking at the exposure to 6 μg endosulfan l^{-1}, it causes an increase in oxygen consumption rate in all groups. This may be related with the hyper-excitability caused by this pesticide at low concentrations, as endosulfan acts upon biota by blocking the chloride channels at the gamma-aminobutyric acid (GABA) receptor in the central nervous system, leading to neural excitation (Murray et al., 1993; Montagna and Collins, 2008; Da Cunha et al., 2011). At high endosulfan concentrations, OCR decreases and was not different from control conditions, as observed in fish and crabs (Rao et al., 1981; Negro et al., 2012).

In crustaceans, and also in mammals, there is a negative relation between the oxygen consumption rate and weight. In our case, the regression lines between those variables were not significant in many cases. That might be related with the fact that in the case of a population the net oxygen consumption rate is not just the sum of the basal oxygen consumption rates of every individual, but there are also several intraspecific interactions (such as reproductive activities) that might cause shifts in the oxygen consumption rates. The randomness of reproduction activities, which are not directly related with weight, might cause data dispersion and prevent fitting to the linear regression model. In the two higher endosulfan concentrations, where swimming and reproduction activities were almost suppressed and individuals spent the time at the bottom of the bottle, there were some significant regressions between the OCR and the weight, as observed in experiences where animals were kept isolated (Bridges and Brand, 1980; Schmidt-Nielsen, 1997; Montagna and Collins, 2008).

Non target aquatic organisms inhabiting streams contiguous to farm ditches, as *H. curvispina*, may be at risk from the adverse effects of endosulfan, in view of its environmental residues concentrations detected in past years, which ranged from 0.01 to 74 μg endosulfan l^{-1} (Ernst et al., 1991; Silva et al., 2005; Mugni et al., 2011). The environmental exposure to endosulfan affects both juveniles and adults. Based on these data, the median lethal concentration obtained with juveniles alone, as recommended by the standardized methods, may not be the most protective quality criteria. In future evaluations, adults grouped without sexual differentiation should be included in order to mimic the environmental conditions. Moreover, since endosulfan isomers are moderately persistent in the aquatic environments and they might continue to act several days after their applications, other parameters besides lethality, like growth and reproduction success should be evaluated (Paracampo et al., 2012; Mugni et al., 2012b).

Reproduction activities increase metabolism and may modify the sensitivity to biocides. These differences in lethality become especially important in species with an extended reproductive period as *H. curvispina* and other species from the Hyalellidae family. Also, amphipod populations may be sublethally affected by endosulfan in concentrations that may be present in aquatic environments, as observed in oxygen consumption tests.

5. Conclusions

Sexual behavior influences the sensitivity of *H. curvispina* to endosulfan. The occurrence of this behavior should be taken into account when comparing the toxicity effects of biocides to

different life stages. The increased metabolism caused by sexual behavior could increase the sensitivity to pesticides. The evaluation of biocide effects on reproductive behavior and subsequent reproductive success of the population needs to be researched further.

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