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In situ assays with tropical cladocerans to evaluate edge-of-field pesticide runoff toxicity

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Abstract

Tropical regions' economy is usually based on agriculture, which involves an intensive use, and even frequent overuse, of pesticides. Nevertheless, not much research has been done on the impact of pesticides on tropical aquatic ecosystems, which are often contaminated by runoff-related pesticide inputs due to unpredictable and torrential rainfalls. This study aimed to: (i) adapt and evaluate a short-term sublethal in situ assay using post-exposure feeding as an endpoint, to the tropical cladoceran species *Diaphanosoma brachyurum* (collected at the Pedra do Cavalo dam in the Paraguaçu River basin, Bahia, Brazil), and, (ii) assess the role of the standard species *Daphnia magna* as an adequate laboratory surrogate. Lethal and sublethal (post-exposure feeding) responses were assessed for the two species. To evaluate these responses under environmentally realistic exposure conditions, a runoff event was simulated in an agricultural area previously contaminated with different deltamethrin concentrations. The resultant runoff water was used to set up microcosms with different dilutions, simulating the entrance of runoff water in an adjacent lentic system. An in situ assay with *D. brachyurum* was performed inside the microcosms, allowing to discriminate the effects due to deltamethrin from those due to other potential stressors associated with the experimental design (e.g. organism handling, load of suspended particles, microcosm design). Water samples were collected from microcosms to conduct a laboratory assay with *D. magna*. The in situ methodologies revealed to be suitable to conduct assays with *D. brachyurum* under tropical conditions, since all exposed organisms were successfully retrieved from the chambers. Furthermore, none of the potential stressors associated with the experimental design influenced the daphnids' performance. The tropical cladoceran species, exposed under more realistic conditions, revealed to be more sensitive than the laboratory standard species: lethal effects were only observed for *D. brachyurum* and sublethal effects were noticed at a lower deltamethrin concentration for this species than for *D. magna*.
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1. Introduction

Most ecotoxicological research has been conducted in ecosystems of temperate zones located in the Northern hemisphere. Being the protection and conservation of bio-diversity one of mankind major goals, as recognised at the Rio Convention, it is a paradox that relative little research has been conducted on the impacts of contaminants on tropical ecosystems, since they harbour most of the global species diversity. In particular, the availability of toxicity tools to perform ecologically relevant toxicity assessments with autochthonous tropical species or with adequate surrogates for these regions is poor (Lacher and Goldstein, 1997; Do Hong et al., 2004).

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Physical, chemical and biological environmental parameters differ greatly between tropical and temperate regions, differently influencing the fate and toxicity of chemicals (Castillo et al., 1997; Lacher and Goldstein, 1997). For example, the degradation rates of pesticides appear to be greater in tropical than in temperate regions, due to higher temperatures and sunlight intensities (Sprague, 1985; Viswanathan and Murti, 1989), and the high contents of biomass in tropical aquatic systems is a factor contributing to a decrease in environmental residues (Calero et al., 1992). Furthermore, main environmental problems in tropical regions differ from those encountered in temperate ones. Tropical regions' economy is usually based on agriculture, which involves an intensive use, and even frequent overuse, of agrochemicals (e.g. pesticides), in order to increase productivity and meet food requirements of growing populations (Abdullah et al., 1997; Lacher and Goldstein, 1997). Also, high amounts of toxic substances are used in the prevention of human diseases and disease vectors (Lacher and Goldstein, 1997). The application methods of pesticides, apart from leading to the contamination of target fields, may lead to the contamination of surface waters as a result of spray drift from aerial spraying and of edge-of-field runoff (Abdullah et al., 1997; Schulz et al., 1998; Liess and Schulz, 1999). Particularly, the contamination of aquatic systems by runoff-related pesticide inputs from agricultural fields, due to unpredictable and torrential rainfalls, is a frequent and severe problem in tropical and subtropical environments. Thus, it is being increasingly recognised that innovative approaches, including the use of sensitive local species as assay organisms, are a major need to assess the effects of contaminants in tropical ecosystems. In this process, apart from the traditional laboratory-based methodologies, in situ toxicity testing should also be considered. In situ exposure methods have several advantages over laboratory ones; they reduce sampling and laboratory-related errors and integrate the natural fluctuations of field conditions (e.g. temperature, sunlight) (Ireland et al., 1996; Pereira et al., 1999; Tucker and Burton, 1999), removing the need for laboratory-to-field extrapolations. Moreover, by combining laboratory and in situ toxicity testing, a more realistic and accurate assessment of ecosystem quality would be expected (Culp et al., 2000).

The present study was integrated in a broader research project that intended to contribute to the development of ecotoxicological tools to perform ecologically relevant assessments of ecosystem quality in tropical aquatic systems, by adapting to tropical conditions and species, belonging to different trophic levels, a suite of laboratory and in situ assays previously developed for temperate regions. This study deals with the research conducted with cladocerans. Two specific objectives were to be attained: (i) to adapt and evaluate a short-term sublethal in situ assay using post-exposure feeding as an endpoint, with the tropical cladoceran species *Diaphanosoma brachyurum* (Liéven), and, (ii) to assess the role of the standard test species *Daphnia magna* Straus as an adequate sensitive lab-

oratory surrogate. The sensitivity and suitability of both assays was evaluated under a realistic exposure scenario of surface water contamination by edge-of-field pesticide runoff. Post-exposure feeding depression was chosen as a rapid, sensitive and biologically significant assay endpoint. Being a measure of energy acquisition by an organism, the feeding activity is linked to fitness components such as growth rate, fecundity and survival (Maltby et al., 2001). Regarding its sensitivity, several authors showed that feeding impairment is a general response to toxicant exposure, which can be detected in a short period of time (Allen et al., 1995; Lee et al., 1997; McWilliam and Baird, 2002).

2. Materials and methods

2.1. Experimental design

To evaluate the suitability of the proposed methodologies for toxicity assessment, a realistic exposure scenario of surface water contamination was simulated in an agricultural area located in the Paraguaçu River basin (Cruz das Almas, Bahía, Brazil: $12°38'23''$ S, $39°52'13''$ W), with a slope of 45% and with no application of pesticides for the last five years. Three 30-m^2 strips of land (20 × 1.5 m), parallel to each other, were isolated and a buffer area of 40-m^2 (20 × 2 m) was left between contiguous strips, to avoid cross-contamination. The total selected area was tilled and two strips of land were sprayed with deltamethrin, (Decis EC25 – emulsifiable concentrate at 25 g a.i./l) (Aventis CropScience, São Paulo, São Paulo, Brazil), one at the recommended (low dose: 10 g a.i./ha) and the other at 20 times the recommended (high dose: 200 g a.i./ha) application dose to control for the grasshopper *Rhammatocerus conspersus* (Bruner). The use of a high dose intended to simulate a worst-case scenario of contamination. To assess the effects of the runoff per se, the third strip of land was not sprayed and was used as control.

To obtain the desired deltamethrin concentrations (low and high doses, respectively), a volume of 0.4 ml and 16 ml of Decis was mixed with reference water, collected at a nearby reference lagoon, in a 20-l portable atomizer. At a time with no wind and an air temperature of 25 °C, a homogeneous spraying of these solutions was carried out in the respective 30-m^2 strips of land. Three hours after spraying, a runoff event simulating a heavy rainfall was provoked on each of the three strips of land, using 150 l of water from the reference lagoon. This quantity of water was based on average precipitation values for the rainy season in the region (5 mm/d). To collect the runoff water, a funnel made of metal, connected to a 20-l polyethylene container, was placed at the lower limit of each strip of land. The runoff water was then used to set up microcosms, which intended to simulate the entrance of water from an agricultural field into an adjacent lentic aquatic system, after a heavy rainfall.

Microcosms consisted of 20-l polypropylene containers filled with 3 l of sediment (∼15-cm layer), collected from

the top 5 to 10 cm of the reference lagoon margins, and with 15-l of the respective treatment water. To discriminate the effects caused by deltamethrin from those due to other potential stressors, eight treatments were set up: (i) an in situ assay control, with chambers directly deployed at the reference lagoon (L), (ii) microcosms with water from the reference lagoon (R), (iii) and (iv) microcosms with 25% (RR25) and 75% (RR75) of runoff water from the strip of land not sprayed with Decis, diluted with 75% and 25% of reference water, to assess the effect of the runoff per se; (v) and (vi) microcosms with 25% (LD25) and 75% (LD75) of runoff water from the strip of land sprayed with the low dose of Decis, diluted with 75% and 25% of reference water, to assess the effects of Decis; and, (vii) and (viii) microcosms with 25% (HD25) and 75% (HD75) of runoff water from the strip of land sprayed with the high dose of Decis, diluted with 75% and 25% of reference water, also to assess the effects of Decis. Six replicate microcosms were prepared for each treatment; one set of three replicates was used to perform the in situ assay and the other set to collect water for the laboratory assay. All microcosms were sunk in the reference lagoon at a depth that levelled the inner and outer water surface, and were left for stabilisation during 6 d, allowing the partial settling of particles.

Deltamethrin real concentrations measurements were considered unnecessary since the objective of this work was to develop in situ methodologies specific for tropical systems, rather than to determine absolute self-standing effective concentrations of deltamethrin to the used assay organisms. Our major concern was to ensure a range of environmentally realistic concentrations of deltamethrin following agricultural runoff, in order to be able to compare relative responses among treatments and assays. Nominal deltamethrin concentrations were 0.05 mg/l (LD25), 0.15 mg/l (LD75), 1.00 mg/l (HD25) and 3.00 mg/l (HD75).

2.2. Assay organisms

Cladoceran species were selected to perform this study because they are one of the most sensitive groups of organisms, they occupy a central position within lentic aquatic food chains and they are often the dominant herbivores in lakes and ponds (Hanazato, 2001). Furthermore, due to their small size, short life cycle and high parthenogenetic reproduction rates, cladoceran species are commonly used to determine toxicity of chemicals and set environmental health standards (Hanazato, 1998a).

For the in situ assay, the local cladoceran *D. brachyurum* was selected for three major reasons. First, it was the cladoceran species most abundant in all the zooplankton community samples collected at the initial stages of this work. Second, *Diaphanosoma* sp. is a genus widely spread in Brazil (Elmoor-Loureiro, 2000). And third, some authors refer large zooplankton species as generally more sensitive to insecticides than smaller ones (Hanazato, 1998b). *Daphnia magna* was chosen to perform the laboratory assay because it is a standard test species commonly used and recom-

mended for lethal and sublethal toxicity assays (OECD, 1998, 2004; Environment Canada, 2000; ASTM, 2001), and also because its body size is closer to *D. brachyurum* than that of *Ceriodaphnia dubia* Sars, the cladoceran species commonly used in North America to conduct toxicity assays (USEPA, 1989, 1993).

Locally occurring *D. brachyurum* was sampled at the Pedra do Cavalo dam (in the Paraguaçu River basin). The collection of organisms followed the procedure described by Lopes et al. (1999). At the laboratory, immature individuals were isolated under a dissecting microscope, transferred to polystyrene multiwell-plates (COSTAR, Corning, NY, USA) and fed with the green algae *Pseudokirchneriella subcapitata* (Koršhikov) Hindak (formerly known as *Selenastrum capricornutum* Printz; 3×10^5 cells/ml), until used for toxicity testing within 24 h of collection.

Daphnia magna was provided by the Institute of Marine Research (Universidade de Coimbra, Coimbra, Portugal). Organisms were reared under controlled conditions of photoperiod (12:12-h light:dark) and temperature (25 ± 1 °C) in ASTM (American Society for Testing and Materials) hardwater medium (ASTM, 2001). Four-days old juveniles from the third brood were used to perform the laboratory toxicity assay.

2.3. In situ assay

In situ assay chambers used to expose *D. brachyurum* were similar to those developed by Pereira et al. (1999). They were made of inexpensive materials and designed to optimise light penetration and ease of deployment. They consisted of 50-ml polypropylene beakers with three 20-mm diameter windows, covered with a 50-μm nylon mesh. Two of these windows were on opposite sides of the beaker body and one on the bottom. A float was glued to the top of the assay chamber to ensure its maintenance at the water surface.

All assay chambers were deployed one day prior to organisms' deployment. Nine chambers were directly deployed in the lagoon (treatment L, 9 replicates) and three chambers in each microcosm (a total of nine sub-replicates per treatment). At the assay-starting day, ten juveniles of *D. brachyurum* were introduced into each assay chamber. After a 24-h exposure period, chambers were removed from microcosms and transported to the laboratory in plastic containers filled with the respective treatment water. In the laboratory, dead organisms were counted and the live ones were used to immediately quantify post-exposure feeding rates. Organisms were considered dead when no movement was observed, for 10 s, after gentle prodding.

At the beginning and end of the in situ assay, pH (Digi-Sense 5938-00 pH/mv/ORP meter, Cole-Parmer, Niles, IL, USA), conductivity (HI 9033 conductivity meter, Hanna Instruments, Singapore), oxygen and temperature (Wissenschaftlich Technische Werkstätten OXI 196, Weilheim, Germany) were measured in the lagoon and in each replicated microcosm.

2.4. Laboratory assay

The laboratory assay started on the same day as the in situ assay. Four-days old *D. magna* juveniles were exposed to the same treatments as those of the in situ assay, plus a laboratory standard control consisting of ASTM hardwater. For this, water samples were collected from the extra microcosms set up for this purpose and immediately conditioned in the dark at approximately 15 °C. In the laboratory, water samples from the three replicates of each treatment were mixed to make a single sample. Ten organisms were introduced per glass beaker filled with 100 ml of treatment water, and three beakers were run for each treatment. The exposure occurred for a 24-h period under controlled conditions of temperature (25 ± 1 °C) and photoperiod (12:12-h light: dark). At the end of the assay, organisms were retrieved and mortality was assessed. Live organisms were used to immediately assess post-exposure feeding rates.

2.5. Feeding quantification

The methodology used to quantify post-exposure feeding was based on that developed by McWilliam and Baird (2002) for *D. magna*. Alive organisms remaining from each replicate were transferred into 15-ml polypropylene plastic tubes filled with 12 ml of 50-µm filtered water from the reference lagoon for *D. brachyurum* and ASTM hardwater for *D. magna*. A concentration of 3×10^5 cells/ml of *P. subcapitata* was added to each tube. For a 4-h period, organisms were allowed to feed (post-exposure feeding) at 25 ± 1 °C, in the dark. Blanks (tubes with algae but without organisms) were run simultaneously to assure that the algal concentration did not change during the assay. After the 4-h period, organisms were removed from the tubes and the amount of ingested algae was estimated by direct fluorescence measured in a Turner Designs 10-AU Fluorometer (Sunnyval, CA, USA). A significant linear relationship was registered between the fluorometric readings and the cell density counted in a Neubauer chamber ($r^2 = 72.8\%$, df = 10, $p < 10^{-3}$).

2.6. Data analysis

Mortality data was compared among treatments using the Mann–Whitney U test. Post-exposure feeding rates (ingested cells/individual/h) were calculated as the difference between the final and the initial number of cells divided by the number of organisms present in the vessel and by the duration of the feeding period (4 h). One-way analysis of variance (ANOVA) were used to compare post-exposure feeding rates among treatments. For the Decis-dosed treatments, post-exposure feeding was expressed as feeding depression (%) relatively to the control (L for *D. brachyurum* and ASTM hardwater for *D. magna*). Feeding depression values were arcsine square root transformed for homogeneity of variances and analysed with one-way ANOVA followed by the Dunnett's test. ANOVA assumptions were verified with the Shapiro–Wilk's test for normality and with the Barttlet's test for homoscedasticity.

3. Results

3.1. Abiotic parameters

The pH values during the assays ranged between 7.0 and 8.5, with small variations between the beginning and the end of each assay. Conductivity values ranged between 184 and 226 µS/cm and also did not change markedly during each assay. The temperature during the in situ assays ranged from 22.3 to 27.1 °C. In all treatments, oxygen values were above the critical level (2 mg/l) for cladocerans (OECD, 1998).

3.2. Effects of potential experimental stressors

In the undosed treatments, the mortality of *D. brachyurum* was above 10% only for treatment RR75 (Table 1). Yet, significant differences in mortality were observed neither between treatments L and R nor between treatments R and RR (RR25 or RR75) (Mann–Whitney: $U < 64.5$; $p > 0.05$). Mortality was never observed for *D. magna* (Table 1). For both the in situ and laboratory assays (with *D. brachyurum* and *D. magna*, respectively), no significant effects on post-exposure feeding rates were observed when organisms were exposed to the undosed treatments (one-way ANOVAs: $F_{3,32} = 2.53$, $p = 0.08$ and $F_{4,10} = 1.36$, $p = 0.32$, respectively) (Table 2).

3.3. Effects of Decis

The addition of Decis caused a significant increase in mortality for *D. brachyurum*, but only at the two highest

Table 1
Mean mortality (%) of *Diaphanosoma brachyurum* and *Daphnia magna* at the end of the 24-h exposure period of the in situ and laboratory assays, respectively

Treatments	*D. brachyurum* (in situ)	*D. magna* (laboratory)
Lagoon (L)	4.4	0
100% Lagoon water (R)	10.0	0
25% Runoff without Decis (RR25)	6.7	0
75% Runoff without Decis (RR75)	16.7	0
25% Runoff with low Decis dose (LD25)	18.9	0
75% Runoff with low Decis dose (LD75)	16.7	0
25% Runoff with high Decis dose (HD25)	72.2	0
75% Runoff with high Decis dose (HD75)	100	0

For treatment abbreviations please refer to Section 2.

Table 2
Mean post-exposure feeding rates (ingested cells/individual/h) (±standard deviation) of *Diaphanosoma brachyurum* and *Daphnia magna* after being exposed for 24 h to the undosed treatments in situ and in the laboratory, respectively

Treatments	*D. brachyurum* (in situ)	*D. magna* (laboratory)
Control (ASTM)	–	6056 (±1293)
Lagoon (L)	3664 (±1001)	6083 (±481)
100% Lagoon water (R)	1644 (±1394)	5958 (±1521)
25% Runoff without Decis (RR25)	3300 (±2208)	8194 (±3110)
75% Runoff without Decis (RR75)	3446 (±2090)	4861 (±1512)

For treatment abbreviations please refer to Section 2.

doses (Mann–Whitney: $U = 81$; $p < 10^{-3}$), while no mortality was observed for *D. magna* after the 24-h exposure to Decis (Table 1). Feeding depression relatively to the control was observed for both tested species when exposed to Decis (Fig. 1). For *D. brachyurum*, a significant feeding depression (93%) was observed at the second highest dose of Decis (HD25; one-way ANOVA: $F_{6,54} = 4.81$, $p < 10^{-3}$); post-exposure feeding at the highest Decis dose (HD75) could not be quantified due to excessive mortality during exposure. For *D. magna*, a significant feeding depression (100%) was only observed at the highest tested dose of Decis (HD75; one-way ANOVA: $F_{8,18} = 4.97$, $p = 0.002$) (Fig. 1).

4. Discussion

4.1. Effects of potential experimental stressors

The described in situ assay methodology and assay chambers revealed to be suitable for conducting in situ assays with the tropical cladoceran *D. brachyurum*. No organisms escaped from the assay chambers and all were recovered, even the dead ones. Furthermore, the entrance

of indigenous organisms into the assay chambers was not registered. This could largely influence assay results since indigenous organisms could interact with the test organisms through competition and/or predation leading ultimately to their death (Pereira et al., 1999, 2000). The low mortality (<10%) registered at the lagoon (control) also corroborates the suitability of these assay chambers for in situ toxicity assays with *D. brachyurum*. Moreover, it shows the suitability of this tropical cladoceran species to perform more ecologically relevant environmental assessments. Similarly, the microcosm design did not cause adverse effects on the assay organisms. The daphnids mortality in the microcosms with water from the lagoon was also low (10%) and no significant differences were observed between post-exposure feeding rates at the lagoon and at the microcosms set up with lagoon water. These results suggest this microcosms design as a valid approach to evaluate the proposed assay under a realistic scenario of pesticide contamination. Furthermore, it constitutes a simple and inexpensive design for this type of toxicity evaluation studies, originating more ecologically relevant assessments than laboratory assays (Culp et al., 2000).

Although the load of suspended particles was visually higher in the microcosms set up with runoff water than in those set up directly with lagoon water, the runoff per se did not induce significant effects on both *D. brachyurum* and *D. magna*, either at the lethal or sublethal levels. However, the slight increase in *D. brachyurum* mortality registered in the microcosms with 75% of runoff water (16.7%), relatively to those set up with lagoon water and 25% runoff (10% and 6.7%, respectively) points to the need for extra caution when interpreting results from studies dealing with pesticide contamination and involving an increase in the load of particles in suspension.

Moreira-Santos et al. (2005a,b) used the same microcosms to perform in situ toxicity assays with algae (local phytoplankton and *P. subcapitata* originated from laboratory cultures) and with the chironomid *Chironomus xanthus* Rempel. For all tested species, the authors observed no
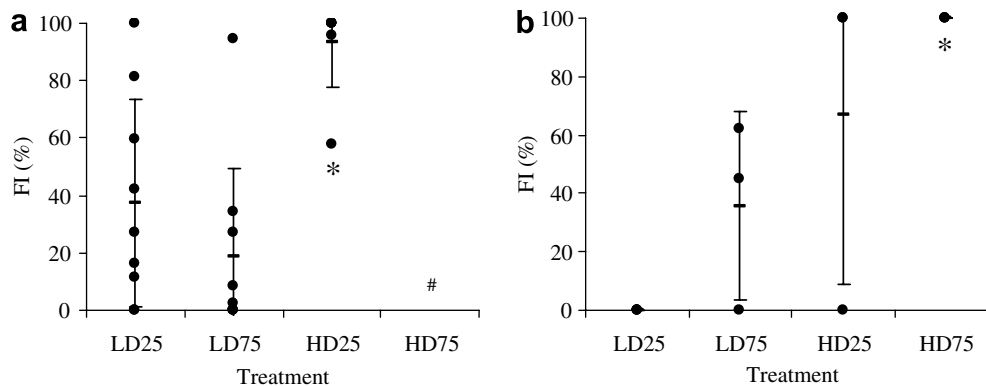

Fig. 1. Feeding depression (%) of *Diaphanosoma brachyurum* (a) and *Daphnia magna* (b) after being exposed for 24 h to the Decis dosed treatments, in situ and in the laboratory, respectively. Error bars indicate ±standard deviation; * indicates statistical significant differences relatively to the control by the Dunnett's test ($p < 0.01$). # the feeding for the HD75 treatment was not performed since all organisms died during the 24-h exposure period.

effects of the microcosms design or runoff per se on the performance of the organisms, for both lethal and sublethal responses. Thus, the intersection of the present results with those of the latter two studies allows concluding that the proposed microcosm methodology is suitable to conduct in situ assays with different groups of organisms under a realistic scenario of surface water contamination (through a runoff event), while controlling for the effects of other potential environmental stressors.

4.2. Effects of Decis

Deltamethrin has been characterised as a pesticide highly toxic for both fish, with 96-h LC50s ranging between 0.4 and 2.0 µg/l, and aquatic invertebrates, with the 48-LC50 for *Daphnia* sp. being 5 µg/l (WHO, 1990). Although all the deltamethrin nominal concentrations used in this study were higher than these LC50 values, significant lethal effects were only observed for *D. brachyurum*, while no mortality was observed for *D. magna*. Such results were partly expected since as a highly hydrophobic compound deltamethrin is known to rapidly adsorb to soil particles (WHO, 1990; Zhu and Selim, 2002). Furthermore, deltamethrin is also known to be rapidly degraded in water (half-life of 8–48 h) into a mixture of inactive and active stereoisomers, which usually pose less to equal risk to organisms relatively to the parent chemical (Maguire, 1992; Belfroid et al., 1998; Erstfeld, 1999). As the microcosms were allowed to stabilise for a period of 6 days, it is very likely that a high proportion of the applied deltamethrin was degraded prior to the start of the assays. The difference in mortality observed between both species suggests a higher sensitivity of *D. brachyurum* relatively to *D. magna*. Yet, because pyrethroids strongly adsorb to soil and sediments and are hardly eluted with water (WHO, 1990), the *D. brachyurum* organisms exposed in the microcosms may have been exposed to an additional, though realistic, load of small suspended particles carrying deltamethrin, that entered into the chambers through the 50-µm mesh. Because dafnids are able to filter such small particles, this could have lead to the observed lethal effects. On the contrary, in the laboratory assay, water collected from the microcosms was filtered (50-µm mesh) and introduced into the assay vessels for a 24-h static exposure period, which precluded an increase in the load of suspended particles to which *D. magna* was exposed.

Impairment of feeding seems to be a general response to toxicant exposure. Allen et al. (1995) exposed *D. magna* to several contaminants (metals and organic compounds) and observed feeding depression to all tested substances. For cladocerans, this response can be detected within 2 h of exposure to the toxicant, thus constituting a rapid and general indicator of stress (Lee et al., 1997; McWilliam and Baird, 2002). Further, feeding depression is more sensitive, protective and ecologically relevant than lethality as an endpoint, since it is often the initial reaction of organisms to stress and can be detected before mortality occurs (Ger-

hardt, 1996). In this study, feeding was also revealed to be more responsive than mortality; deltamethrin induced no mortality to *D. magna*, even though at the highest tested dose of Decis a significant feeding depression was observed. At the sublethal level, *D. brachyurum* also showed a higher sensitivity than *D. magna*, since a significant post-exposure feeding depression was registered at the second highest dose of Decis rather than only at the highest Decis dose. Such decrease in feeding is in agreement with several data in the literature, which reported feeding depression in cladocerans exposed to sublethal concentrations of several pesticides (Day and Kaushik, 1987; Fernández-Casalderrey et al., 1993, 1994; McWilliam and Baird, 2002). In particular, McWilliam and Baird (2002) exposed *D. magna* to two pyrethroids (λ-permethrin and cyhalothrin) and found that both chemicals induced feeding depression in organisms both during exposure to the pesticide and after removing organisms from exposure. Yet, they observed a recovery pattern in feeding rates during the post-exposure period. This fact could explain why *D. brachyurum* feeding depression was only registered at the same dose of Decis (HD25) that also exerted strong lethal effects (72.2%). As deltamethrin is readily metabolised and excreted (WHO, 1990), after being exposed to the two lowest doses of this pyrethroid (LD25 and LD75) the organisms could have rapidly recovered, resulting in unaffected post-exposure feeding rates.

Our findings highlighted the need of incorporating more realistic scenarios in pesticide toxicity assessment studies since the interaction with other stressors (particles in suspension resulting from runoff events) may influence pesticide toxicity. Furthermore, the in situ assay with the local species *D. brachyurum* revealed to be more sensitive to deltamethrin contaminated runoff than the standard laboratory assay with *D. magna*, suggesting that, to avoid toxicity underestimation, the use of local species exposed in situ should also be considered in toxicity assessments in tropical systems.

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