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Pesticide effects on crabs: How environmental concentrations of endosulfan and chlorpyrifos affect embryos

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The objectives of this work were to evaluate the effects of environmentally relevant chlorpyrifos and endosulfan, concentrations in the incubation period, effective hatching and survival of embryos and neonates of the freshwater burrowing crab, *Zilchiopsis collastinensis* (Decapoda, Trichodactylidae). Both pesticides were prepared from commercial and technical grade products. The exposure to about 100, 200, and 400 ng endosulfan L^{-1}, and 48, 240, and 1,200 ng chlorpyrifos L^{-1} did not cause differences in the incubation period or in effective hatching but decreased survival of neonates, especially in the concentrations prepared from the technical grade product. Even if these concentrations are below the median lethal concentration (LC_{50}) values for embryos, these caused a significant decrease in the survival of neonates, i.e. when crabs are outside the egg and not protected by chorion. The decrease in the neonate population caused by these concentrations, which could be found in the environment, might impact aquatic communities.

Keywords: Agricultural pollution, crustaceans, embryos, neonates.

Introduction

Large-scale application of pesticides may result in the pollution of aquatic ecosystems, especially after rains, when different concentrations of pesticides may be found in rivers and streams near crop areas.[1,2] Different concentrations of chlorpyrifos (O,O-diethyl-O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate)) and endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide), two heavily used insecticides that affect the central nervous system at the nerve impulse level, are generally found in river and lakes.

Chlorpyrifos and endosulfan modes of action affect the central nervous system, especially at the nerve impulse level.[3,4]

Among freshwater fauna, crabs are an important component of aquatic ecosystems. The semi-terrestrial species has a key role in the riparian zone, occupying an intermediate position and contributing to matter and energy exchange between lower and upper links in food webs. Crabs of the *Zilchiopsis* genera are active predators and detritus feeders, and are an important food source for fish, reptiles, birds, and mammals with a central position in both aquatic and terrestrial food webs.[5,6] *Zilchiopsis collastinensis* is a common crab of lotic and lenitic freshwater environments.[7,8] The reproduction of these crabs is characterized by an extended incubation period of about two months, typical of freshwater species, in which the females carry their eggs in the abdomen.[9] Eggs are characterized by a thickened chorion, which prevents the effects caused by the hypo-osmotic environment.[10] As in many freshwater species, the free larval phase is suppressed, and the offspring hatch as juveniles. The hatching is a synchronized event, occurring at the beginning of the summer.[11]

Crabs are quite resistant to pesticides such as chlorpyrifos and endosulfan with LC_{50} values higher than the pesticide concentrations usually found in water. Embryos are very resistant to pesticides with LC_{50} values higher than adults.[12] However, exposure to sub-lethal concentrations, even in an acute exposure system, may cause several sublethal effects such as an increase in incubation period, a decrease in effective hatching, and physiological effects that prevent the survival of exposed neonates. Nevertheless, these observations were made at relatively high

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concentrations and there is a lack of information about the sub-lethal effects caused by lower and environmentally relevant pesticide concentrations. The aims of this study were to recognize the effects of environmentally relevant concentrations of chlorpyrifos and endosulfan on incubation period, effective hatching, and survival of neonates, with pesticide stock made from both commercial and technical grade products.

Materials and methods

Zilchiopsis collastinensis ovigerous females were collected in late spring (November–December) in the Paraná River floodplain (31°30'S, 60°41′W; Santa Fe, Argentina) away from cities and crop areas, where pesticide concentrations were below the detection limits. The crabs were maintained in a laboratory until embryos showed eye pigmentation (circular shape) and a noticeable heartbeat, which facilitates differentiation between living and dead individuals.[12,13] Once in this stage, the eggs were gently removed from the female abdomen with a brush and placed individually in plastic 6-mL plates filled with 5 mL of dechlorinated water (control) or the pesticide concentrations and placed in a rocker shaker at 40 rpm inside a 25 ± 1°C incubator with a 24-h dark cycle. Seventy embryos, pooled from seven females, were used in every concentration and control. The dechlorinated water and the solutions were renewed daily. Exposure occurred until the animals died or entered the first molt cycle after hatching. The embryos and hatched crabs were observed every day before water or solution renewal. After hatching, small plant pieces (*Ceratophyllum demersum*) were offered to juvenile crabs because, in previous works, we have observed that survival increased with the addition of vegetal support.[12]

The pesticides tested included Zebra® (Red Surcos, Argentina), a commercial product containing 35% endosulfan, Clorpi® (Red Surcos, Argentina), a commercial product containing 48% chlorpyrifos, and their respective technical grade products (98.5% pure), received from Red Surcos (Argentina). Stock solutions were prepared every 48 h because these pesticides are stable when prepared in distilled water and refrigerated at 4°C (median degradation time (DT50) may surpass 80 days in distilled water).[14] Exposure solutions were prepared on the day of use, just before solution renewal. The nominal concentrations used were 100, 200, and 400 ng endosulfan L^{-1}, and 48, 240, and 1,200 ng chlorpyrifos L^{-1}, according to the range of concentrations found in aquatic systems. Pesticide concentrations were measured by gas chromatography fitted with standard electron capture and flame photometric detectors, GC-ECD (GC VARIAN 3400, Varian, Walnut Creek, CA, USA), according to the US Environmental Protection Agency (USEPA) method 508,[15] with minor modifications. Pesticide concentrations were measured in duplicate at an initial time on days 7 and 10 following a methodology that is permanently tested.[12] Pesticide concentrations, both nominal and measured in commercial and technical grade products, are listed in Table 1. As we always used the same methodology, we assume that the concentrations were similar for all days. It was suggested that a constant pesticide concentration in the test solution could be kept during exposure by the method of daily renewal.[16]

The effects of endosulfan and chlorpyrifos were observed on embryo survival, embryo hatching time, effective hatching, and survival after hatching. The Kruskal–Wallis tests followed by the Dunn's method were performed to determine the significant effects of each insecticide on embryo hatching time. The effects of pesticides on embryo survival, effective hatching, and neonate survival were determined using the Chi-square method, in which the control group data were used as the expected value and compared with the observed values in different concentrations ($P < 0.05$).[17]

Results and discussion

Exposure to environmentally relevant pesticide concentrations did not cause lethal effects on embryos because their mortality was lower than 10%, and there were no differences between exposed and control crabs. There were no effects on incubation period because there were no differences between

Table 1. Nominal and measured concentrations and mean values (±Standard Deviation) of chlorpyrifos and endosulfan, both from commercial and technical grade products.

	Pesticide Concentrations (ng L^{-1})					
	Chlorpyrifos			*Endosulfan*		
Concentration	*Nominal*	*CCP*	*CTG*	*Nominal*	*ECP*	*ETG*
1	48	50.25 (3.18)	48 (1.41)	100	110.2 (4.52)	108.5 (9.19)
2	240	248.5 (4.95)	242 (3.53)	200	182.8 (20.93)	184 (35.3)
3	1,200	1,123.5 (61.51)	1,158 (16.97)	400	418 (2.82)	418 (18.38)

CCP: chlorpyrifos commercial product; CTG: chlorpyrifos technical grade product; ECP: endosulfan commercial product; ETG: endosulfan technical grade product.

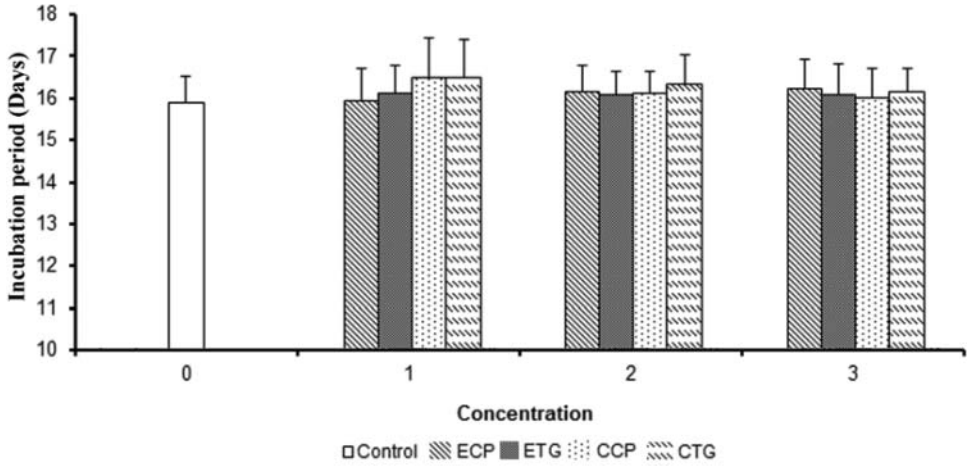

Fig. 1. Incubation time (mean value + standard deviation) for *Z. collastinensis* embryos exposed to three concentrations of endosulfan from commercial product (ECP) and technical grade (ETG), and chlorpyrifos from commercial product (CCP) and technical grade (CTG). Concentration values are listed in Table 1.

control and exposed crabs (Fig. 1). In a previous study we observed that exposure to higher concentrations of endosulfan and chlorpyrifos (concentrations ranging from 629 to 10,254 μg endosulfan L^{-1}, and from 26.2 to 164.3 μg chlorpyrifos L^{-1}) causes a delay in hatching in *Z. collastinensis*.[12] Although the thickened chorion of freshwater crustaceans isolates the embryos, protecting them for the osmotic stress of hyposaline systems, this isolation is not complete because pesticides such as chlorpyrifos may enter the eggs and produce effects on embryos.[13] However, exposure to low pesticide concentrations, as used in this study, and the barrier effect of the thickened chorion, typical of freshwater species, may reduce pesticide entry, preventing this delay. In the case of grass shrimp, *Palaemonetes pugio*, a delay in hatching occurs when exposed to endosulfan, but only at higher concentrations (200 μg L^{-1}), while there were no differences at lower concentrations (from 12.5 to 100 μg L^{-1}), which were also higher than those used in this study and are rarely found in the environment.[18]

Exposure to both pesticides did not affect effective hatching (i.e., crabs that survived and successfully hatched; Fig. 2). When crustaceans are close to hatching, the chorion becomes thinner and is broken by the embryo, which could cause direct contact with the pesticide and a deleterious effect. Exposure to higher concentrations causes reduction in effective hatching.[12] The exposure of *P. pugio* to

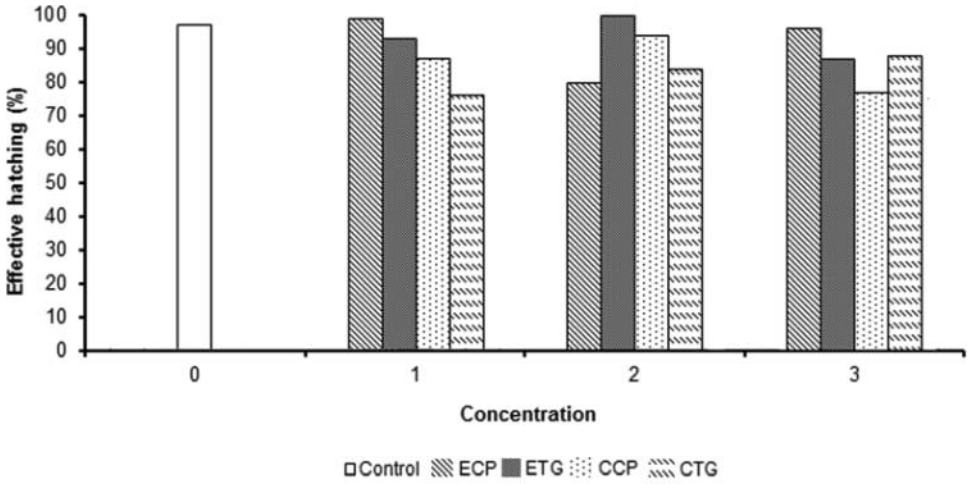

Fig. 2. Effective hatching (%) of *Z. collastinensis* embryos exposed to three concentrations of endosulfan from commercial product (ECP) and technical grade (ETG), and chlorpyrifos from commercial product (CCP) and technical grade (CTG). Concentration values are listed in Table 1.

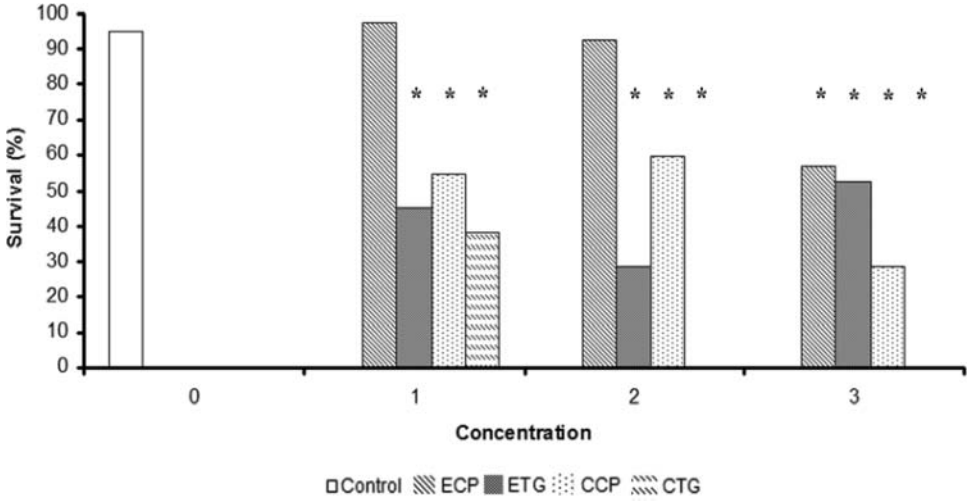

Fig. 3. Neonates survival (%) of *Z. collastinensis* embryos exposed to three concentrations of endosulfan from commercial product (ECP) and technical grade (ETG), and chlorpyrifos from commercial product (CCP) and technical grade (CTG). Concentration values are listed in Table 1. *Significantly different from the control group ($P < 0.05$).

endosulfan and the exposure of *Neohelice granulata* to parathion decreased the number of hatched individuals.[18,19]

In this study, the exposure of *Z. collastinensis* embryos to these concentrations were not sufficiently high to cause a decrease in effective hatching, as we observed when this crab was exposed to higher endosulfan and chlorpyrifos concentrations (concentrations ranging from 629 to 10,254 μg endosulfan L^{-1}, and from 26.2 to 509.4 μg chlorpyrifos L^{-1}). However, exposure to endosulfan and chlorpyrifos caused a decrease in neonate survival. Moreover, in the case of chlorpyrifos prepared from technical grade product, neonate mortality was 100% in individuals exposed to 242 (±3.53) and 1,158 (±16.97) ng L^{-1} (Fig. 3).

Chorion protection appears to be the cause of embryo resistance because there were no effects related to exposure inside the egg, as mentioned above, but the "naked" exposure of neonates causes effects that resulted in significant mortality (Fig. 3). In many of these neonates, several abnormalities were observed in pereiopods and chelipeds. The neonates remain with their pereiopods retracted below the abdomen, resulting in hindrances to movement and eating (Fig. 4).

Pesticide concentrations similar to those used in this work could be found in several aquatic environments all over the world, including the Argentinean Pampa, with endosulfan concentrations ranging from 0.06 to 2.9 μg L^{-1} and chlorpyrifos concentrations ranging from 0.03 to 10.8 μg L^{-1}.[1,2,20–22] The exposure of ovigerous females to these environmental concentrations would not cause short-term lethal effects because this species resists higher concentrations of pesticides.[12] However, the neonates of these crabs might not survive exposure to these concentrations. A generalized reduction in female reproduction fitness most likely reduces the crab population over time.

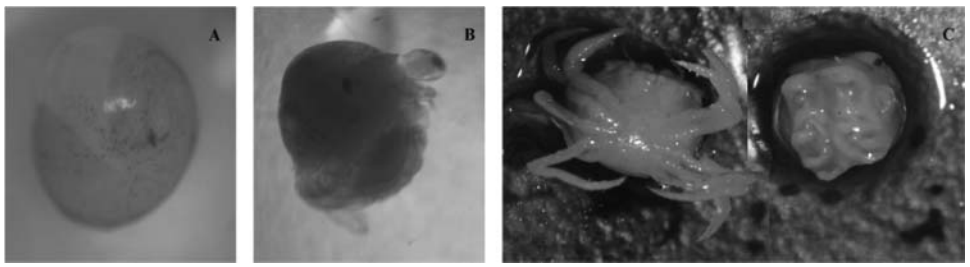

Fig. 4. Neonates of *Zilchiopsis collastinensis*. (A) Individual died during hatching. Observe the absence of dorsal spine or any other kind of structure typical of marine larvae. (B) Neonate exposed to chlorpyrifos, with their pereiopods retracted below the abdomen. Observe the hydropsy in the cephalothoracic region. (C) Neonate from the control group (left), showing regular shape; neonate exposed to endosulfan (right), with their pereiopod underdeveloped and retracted below the abdomen.

Conclusions

The exposure to environmentally relevant concentrations of chlorpyrifos and endosulfan did not cause any effects on embryos, since there was no mortality in them, and the incubation period and effective hatching were not affected. Nevertheless, pesticide effects reduced the neonate survival.

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