METABOLIC RESPONSES OF PLEUSTONIC AND BURROWING FRESHWATER CRABS EXPOSED TO ENDOSULFAN

Carlos Leandro Negro [1], Lidia Eloisa Senkman[1,2] and Pablo Collins[1,2,3]

[a] Instituto Nacional de Limnología (CONICET-UNL), Paraje El Pozo s/n, (3000) Santa Fe, Argentina.
[b] Facultad de Ciencia y Tecnología, UADER, Corrientes y Andrés Pazos, (3100) Paraná, Entre Ríos, Argentina.
[c] Escuela Superior de Sanidad, Facultad de Bioquímica y Ciencias Biológicas, UNL Paraje El Pozo s/n, (3000) Santa Fe, Argentina.

ABSTRACT

This work focused on the effects on metabolism of environmental concentrations of endosulfan in the freshwater crabs *Dilocarcinus pagei* and *Zilchiopsis collastinensis*. The concentrations used were 0, 6 and 62 µg of endosulfan l^{-1}. Oxygen consumption, oxyregulation capacity and critical oxygen concentration were modified by the presence of endosulfan in *D. pagei*. In contrast, *Z. collastinensis* crabs showed a decrease in the ammonia-N excretion rate, but the oxygen consumption was similar between exposed and control crabs. A significant increase in the O:N ratio was evidenced in *Z. collastinensis* exposed to this biocide, demonstrating a shift toward lipid and carbohydrate primary metabolism. *Dilocarcinus pagei* controlled its oxygen consumption through isolation as a defense mechanism, while *Zilchiopsis collastinensis* modified the ammonia excretion. These different defense mechanisms might be related to the dissimilar habitats that each species occupies.

KEYWORDS: Pesticide pollution; metabolism shifts; freshwater crabs; defense mechanisms.

1. INTRODUCTION

Widespread and indiscriminate use of pesticides in agricultural areas provokes the contamination of aquatic ecosystems through drift and runoff. Endosulfan is an insecticide widely used in agroecosystems, mainly in soybean crops [1, 2]. Different concentrations of this biocide are often founded in rivers and lakes [3]. Argentina is a grain-exporting country, characterized by an intensive agricultural activity, mainly in the Pampean region. This area is furrowed by streams and rivers, many of which flow into de Paraná-Del Plata hydrosistem. Despite of controls, different concentrations of endosulfan were founded in water, bottom sediments, suspended particles and water runoff [4, 5]. Although biocides pose a risk to aquatic life, the effects of environmental concentrations on the biota are not well documented.

* Corresponding author

Decapod crustaceans are present in freshwater environments associated with agricultural activities. *Dilocarcinus pagei* and *Zilchiopsis collastinensis* (Decapoda, Trichodactylidae) are two common crab species occurring in ponds and rivers. As other crabs, they have a key role in matter and energy transport between water and terrestrial phases. The straight relationship with the sediment that crabs have promotes a constant exposition to the lipophilic pesticides adsorbed there, increased by the bioturbation caused with walking, feeding and burrowing activities [6-8].

When exposed to stressful situations, as constant biocide exposure, there is a shift in metabolism and in energy substrate. Some pesticides, polycyclic aromatic hydrocarbons and metals not only modify the energy substrate but also produce physiological changes that alter the oxygen consumption [9-11]. The exposure to biocides may also modify the energy expenditure [12]. Modifications in metabolism and energy expenditure caused by biocides may change the normal activities of decapods, e.g., growth, trophic activity and reproduction, which, in turn, could alter their density and affect aquatic food webs [13, 14].

Despite of the importance of crabs, there is little information regarding the effects of endosulfan on metabolic physiology of freshwater crabs, especially in the species of the Trichodactylidae family. The aim of this study was to determine the effects of sublethal concentrations, which regularly occur in the environment, of a widely used insecticide, endosulfan, on the metabolism of *D. pagei* and *Z. collastinensis* juveniles to gain insights into their metabolism and their response to insecticides.

2. MATERIALS AND METHODS

Six-month-old juveniles were obtained from the crab hatchery of the Instituto Nacional de Limnología (CONICET-UNL). They were grown in captivity conditions at 25 ± 1° C. Mean (± SD) carapace width was 10.49 (± 1.46) mm in *Z. collastinensis* and 16.46 (± 3.23) mm in *D. pagei* crabs. Mean (± SD) weight was 0.48 (± 0.19) g for *Z. collastinensis* and 2.78 (± 1.21) g for *D. pagei* crabs. One

hundred and five individuals of each species were selected. They were isolated without been feed for 4 days. The aquaria were cleaned every day. Molted crabs, if any, were removed and were not included in the experiments.

Commercial grade pesticides were employed, which contained 35% of active ingredient, i.e., endosulfan (Zebra Ciagro®; Red Surcos S. A., Argentina). The commercial product, with all the components used in farmland applications, was diluted with distilled water to obtain solutions with different concentrations. The nominal concentrations were 0, 7 and 70 µg of endosulfan l^{-1}(C_0, C_1 and C_2 respectively). Endosulfan concentrations were measured by gas chromatography following the ASTM D 6520-06 method [15]. Oxygen consumption and ammonia-N excretion were measured for each crab. Thirty replicates were used for each treatment and control. At the beginning of each test, crabs were transferred to individual, sealed, respiratory, 500 ml-capacity glass chambers equipped with an oxygen sensor connected to an oxygen meter (HANNA model HI9143) [16, 17]. Temperature was kept at 25 ± 1° C and a photoperiod was set at 12:12 h Light – Dark.

Dissolved oxygen (DO) was measured at each hour. Data of the first four hours were used for detecting rapid responses to toxicant exposure. Data collected up until 22nd hour were used because oxygen consumption brought the DO to concentrations lower than 2 ppm in several replicates, and the hypoxia and the accumulation of metabolic waste products may introduce measurements errors [18]. In 10 animals per concentration, dissolved oxygen was measured until crabs become lethargic as a result of hypoxic stress or dual stresses of hypoxia and endosulfan (Dissolved oxygen levels below 1 ppm). The oxyregulating capacity and the different critical oxygen concentrations (C_c) of control and exposed crabs were calculated using the a/b ratios obtained with that data [19, 20]. Ammonia-N excretion was measured at the beginning of the test and at the 22nd hour. Samples were colorimetrically analyzed following the Nessler method (Test kit model FF-2 of a Hatch spectrophotometer).

The oxygen consumption rate (OCR) and ammonia-N excretion rate were calculated and expressed as mg O_2 g^{-1} h^{-1} and mg NH_3-N g^{-1} h^{-1} respectively. Correlations among oxygen consumption rate and dissolved oxygen were analyzed, as a way of observing modifications in the oxygen consumption behavior [12]. Slopes and *Y*-interceptions were compared among control and treatment groups and between species. Oxygen: nitrogen (O:N) ratios at each concentration were calculated as the ratios of atoms of oxygen consumed to atoms of nitrogen excreted at the 22 hours interval [16]. Energy expenditure of crabs exposed to endosulfan was calculated based in Pillai and Diwan [21]. Oxygen consumption was converted to energy equivalent using an oxycalorific value of 20.11 J ml O_2^{-1}. Oxygen consumption and ammonia-N excretion rates as well as O:N ratio and energy expenditure rate were analyzed. Differences were compared with Kruskal-Wallis and Mann-Whitney U tests ($p < 0.05$). Regression lines, slopes and *Y*-interceptions were compared with the Student *t* test [22].

3. RESULTS

Endosulfan concentrations were similar to nominal concentrations. These environmentally relevant concentrations had lethal effects on some crabs after 22 hours of exposure. Yet, survival was high in both species (Table 1). The oxygen consumption rate increased as the dissolved oxygen decreased in both species. The presence of endosulfan changed this behavior in *D. pagei* crabs. Regression lines between OCR and DO of control and exposed to C_1 crabs had similar slopes ($p > 0.05$) but different *Y*-interception ($p < 0.05$). Regressions line of control and exposed to C_2 crabs had different slopes and *Y*-interception ($p < 0.05$). Slopes and *Y*-interceptions were similar for both ex-

TABLE 1 - Endosulfan concentrations and crab survival

	Endosulfan concentrations (µg l^{-1})		Survival (%)	
	Nominal	Real	*D. pagei*	*Z. collastinensis*
Control	0	0	100	96,66
Concentration 1	7,00	6,00	96,66	80
Concentration 2	70,00	62,00	90	83

TABLE 2 - Slopes values from regression analysis among dissolved oxygen and oxygen consumption rate of crabs exposed to endosulfan

Groups	Slope	*Y*-interception
Dilocarcinus pagei		
C_0	-0,0139	0,1164
C_1	-0,0145	0,1406*
C_2	-0,0036*	0,0529*
Zilchiopsis collastinensis		
C_0	-0,0019	0,1368
C_1	-0,0082	0,1859
C_2	-0,023	0,292

* Slopes and *Y*-interceptions values statistically different from control ($p < 0.05$)

posed to endosulfan and control *Z. collastinensis* crabs ($p > 0.05$) (Table 2).

During the first four hours, OCR was similar in endosulfan concentrations and control group in *Z. collastinensis* ($p > 0.05$). In contrast, OCR of *D. pagei* was modified by the presence of endosulfan. Crabs exposed to C_1 had a significant increasing during the first four hours ($p < 0.001$ for hours 1, 2 and 3; $p < 0.05$ for hour 4) and a decrease during the first hour in crabs exposed to C_2 ($p < 0.01$). OCR at 22 hours was different only for crabs exposed to C_1, which had a higher rate compared with the control ($p < 0.05$). The oxygen consumption rate of *Z. collastinensis* was clearly higher than that of *D. pagei* (Fig. 1).

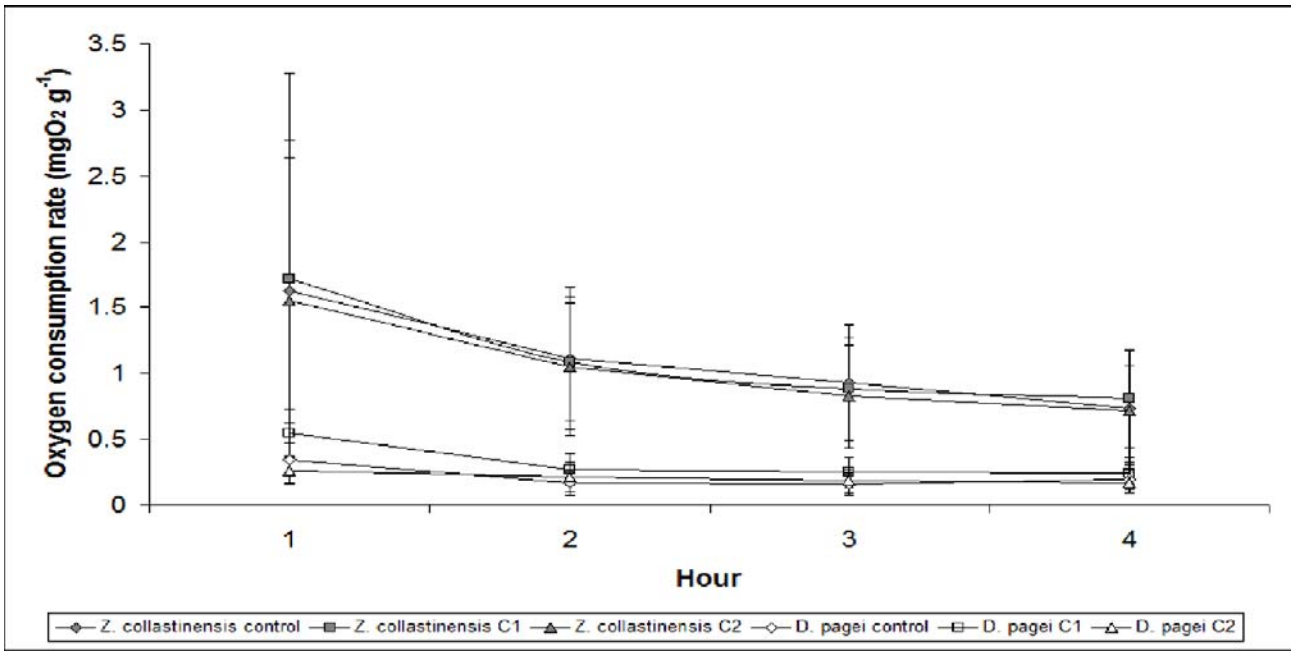

FIGURE 1 - Mean values of oxygen consumption rate from control and exposed to endosulfan crabs.

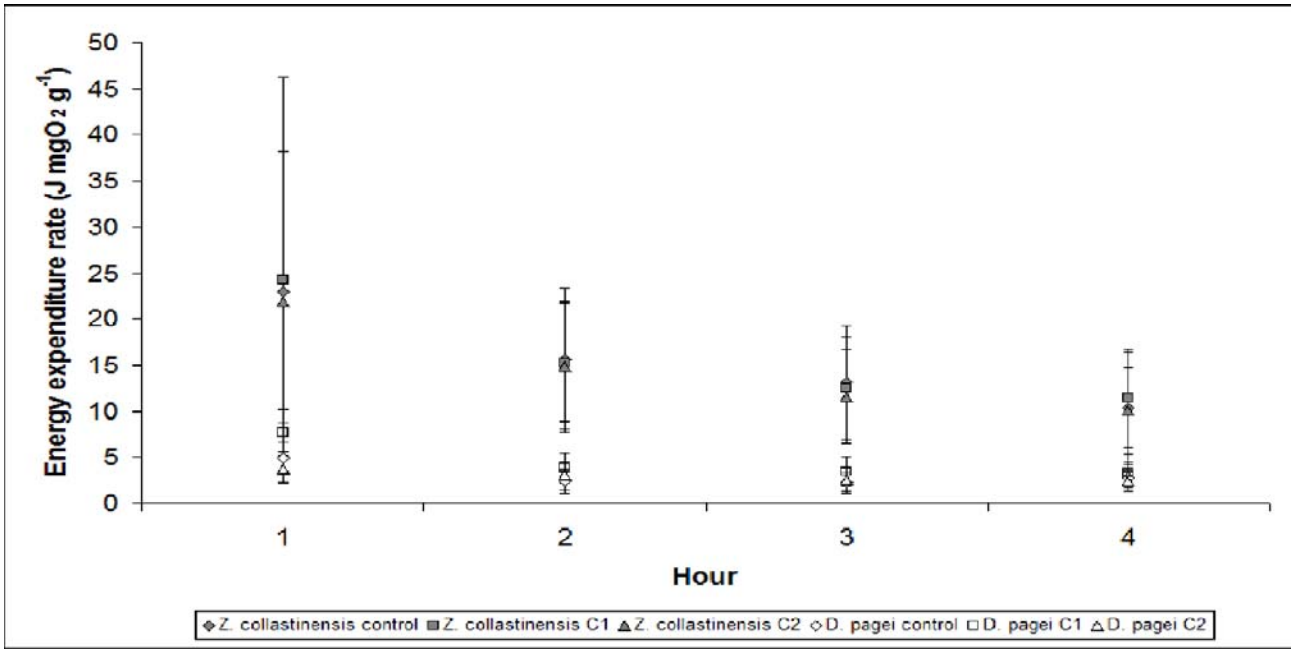

FIGURE 2 - Mean values of energy expenditure rate during the first four hours of control and exposed to endosulfan crabs.

In addition, *Dilocarcinus pagei* exposed to C_1 increased the energy expenditure rate as a response to endosulfan. Differences among control and exposed to 6 µg endosulfan l^{-1} were significant in the first four hours ($p < 0.05$). Moreover, the energy expenditure rate was greater in *Z. collastinensis* ($p < 0.001$) because the individuals of this species were smaller than those of *D. pagei* crabs and had a higher oxygen consumption rate. *Zilchiopsis collastinensis* control and those exposed to endosulfan crabs had similar energy expenditure rates in the first four hours ($p > 0.05$) (Fig 2).

Another effect of endosulfan was the modification in the oxyregulation capacity of *D. pagei*, recognized by the a/b ratio. Compared with control crabs, C_1 caused a reduction in the oxyregulation capacity and an increase in the critical oxygen concentration (C_c) ($p < 0.05$). The oxyregulation capacity and critical oxygen concentration of crabs exposed to C_2 were similar to control crabs ($p > 0.05$). The oxyregulation capacity and critical oxygen concentration were similar in both control and exposed *Z. collastinensis* crabs ($p > 0.05$) (Table 3).

The ammonia-N excretion rate of *Z. collastinensis* was higher than that of *D. pagei* in control and exposed to C_1 crabs ($p < 0.001$). Crabs of both species exposed to C_2 had a similar ammonia-N excretion rate ($p = 0.917$). This rate was similar in both control and exposed to endosulfan *D. pagei* crabs ($p = 0.246$). In contrast, *Z. collastinensis* crabs exposed to C_2 had a lower ammonia-N excretion rate than those exposed to C_1 and control crabs ($p < 0.001$) (Fig 3).

TABLE 3 - Oxyregulation capacity (a/b ratio) and critical oxygen concentration (C_c) from control and exposed crabs

	D. pagei		*Z. collastinensis*	
	a/b ratio	C_c (ppm)	a/b ratio	C_c (ppm)
Control	0.17	1.21	0.35	1.73
6 µg l^{-1}	1.22	3.22*	0.61	2.26
62 µg l^{-1}	0.96	2.85	0.51	2.06

* Differences statistically different from control ($p < 0.05$)

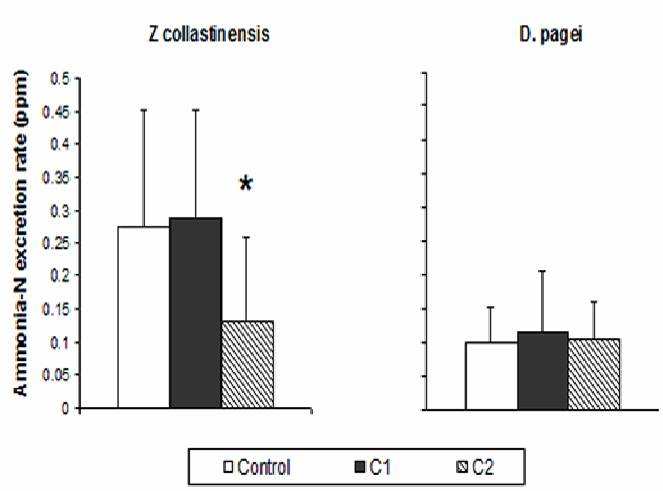

FIGURE 3 - Ammonia-N excretion rate of exposed to endosulfan and control crabs. Mean and standard deviation values after 22 hour of assay. (*) values statistically different from control ($p < 0.001$)

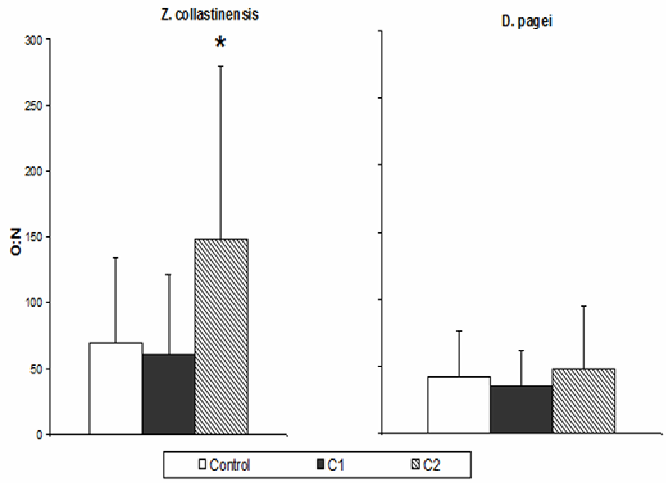

FIGURE 4 - Mean and standard deviation values of the O:N ratio from exposed to endosulfan and control crabs after 22 hours of exposition. (*) values statistically different from control ($p < 0.005$)

The O:N ratio was similar for both species in exposed to C_1 and control crabs ($p = 0.295$; $p = 0.055$ respectively). In crabs exposed to C_2, this ratio was higher in *Z. collastinensis* crabs ($p < 0.001$). The O:N values were modified by the presence of endosulfan in *Z. collastinensis* crabs exposed to C_2 because the differences with control crabs were significant ($p = 0.003$). In *D. pagei* crabs, the O:N relation was similar among exposed and control crabs ($p = 0.79$), demonstrating no effects of endosulfan (Fig 4).

4. DISCUSSION

Environmental pollution by pesticides has been increasingly documented in rivers and lakes and poses a risk to the biota that inhabits those ecosystems. The exposure to endosulfan-containing products caused a shift in metabolism of *D. pagei* and *Z. collastinensis*.

Endosulfan modified the oxygen consumption rate of *D. pagei*. Low concentrations resulted in an increase in the oxygen consumption rate, while a higher concentration caused a decrease of it. Endosulfan is a neurotoxic pesticide, which acts upon biota by blocking the chloride channels at the gamma-aminobutyric acid (GABA) receptor in the central nervous system, leading to neural excitation [23]. Also, it may have relevant sites of toxicological actions related with acetylcholinesterase inhibition [12, 24]. The increase in oxygen consumption rate could be related to those neurotoxic and physiological effects. The reduction in oxygen consumption of crabs exposed to 62 $\mu g\ l^{-1}$ of endosulfan may be related with behavioral responses. Both species have the capacity of closing their gill chamber and isolate from the medium, which allows them to walk in terrestrial environments. When these crabs detect a stressor agent, they close their gill chamber, reducing their oxygen consumption and the intake of compounds present in the water. As the oxygen in the gill chamber water decreases and as the concentrations of excretion compounds increase, they replace this water for new oxygenated one, with the consequent influx of endosulfan. The net oxygen consumption rate is a balance between metabolic decrease caused by isolation and the increase related to acetylcholinesterase inhibition. Low concentrations of endosulfan caused an exiting stimulus in crabs, but the effect was not as strong as the one needed to activate behavioral defense mechanisms such as isolation. Montagna and Collins [12] reported a significant shift in oxygen consumption when individuals of the freshwater crab *Trichodactylus borellianus* were exposed to endosulfan. Similarly, shifts in oxygen consumption, as those observed in *D. pagei* crabs, were documented in the fishes *Labeo rohita* and *Geophagus brasiliensis* exposed to pesticides at different concentrations [25, 26].

In crustaceans, the metabolic pathways involved in nitrogen excretion are catabolism of amino acids and certain amides, degradation of nucleic acids as well as deamination of purine nucleotides and urea. Decapods crustaceans excrete nitrogen mainly as ammonia, and the most of it is excreted through the gill epithelium [27, 28]. The reduction in the ammonia-N excretion observed in *Z. collastinensis* exposed to endosulfan might be related to a reduction in amino acid catabolism, suggesting that the pesticide interfere with the metabolism of this crab. Likewise a reduction in ammonia-N excretion was observed in the fish *Macrognathus aculeatum* when exposed to endosulfan [25].

Oxygen consumption could be combined with nitrogen excretion according to atomic equivalents. Information about the fuel used in metabolism and energy substrate type showed how environmental characteristics affect the metabolism of crabs. A high O:N ratio suggests an increase in lipid or carbohydrate metabolism, and a low O:N indicates an increase in protein metabolism [21]. In general protein consumption indicates as a stressful condition. However, in the present study, *Z. collastinensis* crabs exposed to endosulfan showed a shift toward lipid consumption as energy substrate and a decrease in ammonia-N excretion. Because endosulfan causes histological damages in the hepatopancreas of crustaceans, proteins might be synthesized for cell and tissues production, with a decrease in amino acid catabolism and a reutilization of nitrogenous compounds. Therefore, the effects of sublethal endosulfan concentrations could not be strong enough to turn metabolic pathways to protein use in 22 hours, maintaining essentially lipid and carbohydrate use as fuel. The same behavior was observed in *T. borellianus* exposed to endosulfan [12].

In several shallow lakes of the Paraná-La Plata floodplain hypoxia is common [29]. Aquatic animals are able to regulate their oxygen consumption until the environment reaches a particular dissolved oxygen concentration called critical concentration (C_c). When the oxygen concentration falls below the C_c, animals cannot maintain a stable oxygen uptake and become oxyconformers. Endosulfan caused a shift in the oxyregulating capacity of *D. pagei*, increasing the C_c. Pesticide pollution, as a result of agricultural activities, make crabs, and probably crustaceans in general, more susceptible to environmental hypoxia. If the amount of oxygen needed for maintaining an aerobic metabolism is not achieved, there is a change to anaerobic metabolism, glycolysis of carbohydrates, lactic acid release and disturbance in acid-base balance, with several physiological effects [30]. A reduction in the oxyregulating capacity was reported for the shrimp *Penaeus aztecus* when exposed to naphthalene [20]. Unfortunately, there is a lack of information about oxyregulation capacity and critical dissolved oxygen concentration in native species of the Parana-La Plata hydrosystems.

As other organic compounds, endosulfan is a hydrophobic biocide mainly associated with sediments of marine and freshwater environments. Crabs spend a large amount of time on the bottom, and any disturbance will promote the release of pesticides from sediments to the

immediate water phase. Hence, it is likely that crabs encounter endosulfan in the environment at similar concentrations to those used in the present study. When exposed to certain stressors, in this case endosulfan, *D. pagei* crabs controlled their oxygen consumption by getting isolated and avoiding a metabolism change as a defense mechanism. In contrast, *Z. collastinensis* crabs, which maintained the same oxygen consumption in all the treatments, suffered a shift in their metabolism, which caused several damages and the death of some individuals. This differential outcome could be related to the habitat of each species. *Dilocarcinus pagei* is a pleustonic crab that lives associated with roots of aquatic plants in rivers and ponds, where there is a high availability of dissolved oxygen. A defense mechanism related to oxygen consumption shifts and enzymes production could be developed by this crab because the availability of this gas is usually high. Instead, *Z. collastinensis* lives in burrows of the riverside, submerged in muddy water with low dissolved oxygen. A defense mechanism for this crab could be not related with oxygen consumption shifts because the typical availability of this gas is already low. Therefore, other physiological mechanisms, such as those concerning ammonia excretion shifts, might be employed by this species.

ACKNOWLEDGMENTS

This study was supported by the grant CAI+D PI 69-352 (UNL).

REFERENCES

[1] Lorenzatti, E., Maitre, M. I., Lenardón, A., Lajmanovich, R., Peltzer, P. and Anglada, M. (2004). Pesticide residues in immature soybeans of Argentina croplands. Fres. Environ. Bull. 13 (7) 675-678.

[2] Lorenzatti, E., Altahus, R., Lajmanovich, R. and Peltzer, P. (2004). Residues of endosulfan in soy plants in Argentina croplands. Fres. Environ. Bull. 13 (2) 89-92.

[3] Ernst, W. R., Jonah, P., Doe, K., Julien, G. and Hennigar, P. (1990). Toxicity to aquatic organisms of off-target deposition of endosulfan applied by aircraft. Environ Toxicol. Chem. 10: 103-114.

[4] Jergentz, S., Pessacq, P., Mugni, H., Bonetto, C. and Schulz, R. (2004). Linking in situ bioassays and population of macroinvertebrates to asses agricultural contamination in streams or the Argentine pampa. Ecotoxicol. Environ. Saf. 59: 133-141.

[5] Jergentz, S., Mugni, H., Bonetto, C. and Schulz, R. (2005). Assesment of insecticide contamination in runoff and stream water of small agricultural streams in the main soybean area of Argentina. Chemosphere 61: 817-826.

[6] Collins, P., Williner, V. and Giri, F. (2007). Littoral Communities. Macrocrustaceans. In: Iriondo, M.H., Paggi, J.C. and Parma, M.J. (Eds). The Middle Paraná River: Limnology of a subtropical Wetland. Springer-Verlag, Heidelberg, pp. 277-301.

[7] Menone, M. L., Miglioranza, K. S. B., Iribarne, O., Aizpún de Moreno, J. E. and Moreno, V. (2004). The role of burrowing beds and burrows of the SW Atlantic intertidal crab *Chasmagnathus granulata* in trapping organochlorine pesticides, Mar. Pollut. Bull. 48 (3-4): 240-247.

[8] Menone, M. L., Miglioranza, K. S. B., Botto, F., Iribarne, O., Aizpún de Moreno, J. E. and Moreno, V. (2006). Field accumulative behavior of organochlorine pesticides. The role of crabs and sediment characteristics in coastal environments Mar. Pollut. Bull. 52: 1717-1724.

[9] Silva Santos, M. H., Troca da Cunha, N. and Bianchini, A. (2000). Effects of cooper and zinc on growth, feeding and oxygen consumption of *Farfantepenaeus paulensis* postlarvae (Decapoda: penaeidae). J. Exp. Mar. Biol. Ecol. 247: 233-242.

[10] Vijayavel, K. and Balasubramanian, M. P. (2006). Changes in oxygen consumption and respiratory enzymes as stress indicators in an estuarine edible crab *Scylla serrata* exposed to naphthalene. Chemosphere, 63: 1523-1531.

[11] Collins, P. and Capello, S. (2006). Cypermethrin Toxicity to Aquatic Life: Bioassays for the Freshwater Prawn *Palaemonetes argentinus*, Arch. Environ. Contam. Toxicol. 51: 79-85.

[12] Montagna, M. C. and Collins, P. (2008). Oxygen consumption and ammonia excretion of the freshwater crab *Trichodactylus borellianus* exposed to chlorpyrifos and endosulfan insecticides. Pestic. Biochem. Physiol. 92: 150-155.

[13] Collins, P., Williner, V. and Giri, F. (2006). Trophic relationships in Crustacea: Decapoda of river with a floodplain, in: Elewa, A. (Ed.), Predation in organisms: A distinct fenomenon. Springer-Verlag, Heidelberg.

[14] Collins, P., Williner, V., Montagna, M. (2011). Contamination? Natural and Anthropic Stressors on Freshwater Decapod Crustaceans. In: Velasquez (Ed.), Pollution Control. Management, Technology and Regulations. Nova Science, New York.

[15] APHA (American Public Health Association), AWWA (American Water Works Association), and WEF (Water Environment Federation), 1976. Standard methods for the examination of water and wastewater (20th ed.). Washington DC, USA.

[16] Chinni, S., Khan, R. N. and Rao Yallapragada, P. (2002). Acute Toxicity of Lead on Tolerance, Oxygen Consumption, Ammonia-N Excretion, and Metal Accumulation in *Penaeus indicus* Postlavae. Ecotoxicol. Environ. Saf. 51: 79-84.

[17] Maciak, S. and Konarzewski, M. (2010). Repeatability of standard metabolic rate (SMR) in a small fish, the spined loach (*Cobitis taenia).* Comp. Biochem. Phys. A 157: 136-141.

[18] Eriksen, N. T. (2002). Accuracy and precision of aquatic respirometers with emphasis on monophase oxystats. Fish. Physiol. Biochem. 26: 139-147.

[19] Zou, E., Nanshan, D. and Wei, L (1992). The effects of acute progressive hypoxia on the respiration rate of the Chinese crab *Eriocheir sinensis.* Zool. Res. 14: 327-334.

[20] Zou, E. and Stueben, B. (2006). Acute exposure to naphthalene reduces oxyregulating capacity of the brown shrimp, *Penaeus aztecus*, subjected to progressive hypoxia. Mar. Biol. 149: 1411-1415.

[21] Pillai, B. R. and Diwan A. D. (2002). Effects of acute salinity stress on oxygen consumption and ammonia excretion rates of the marine shrimp Metapenaeus monoceros. J. Crust. Biol. 22: 45-52.

[22] Zar, J. H. (1996). Biostatistical Analysis. Prentice Hall, New York.

[23] Murray, E., Jewell, C., Rabe, C., Segal, S. (1993). Toxicological profile for endosulfan TP-91/16. US Department of Health and Human Services. Washington D C.

[24] Bhavan, P. S. and Geraldine, P. (2001). Biochemical Stress Responses in Tissues of the Prawn *Macrobrachium malcolmsonii* on Exposure to Endosulfan. Pestic. Biochem. Physiol. 70: 27-41.

[25] Rao, D. M. R., Devi, A. P. and Murty, A. S. (1981). Toxicity and Metabolism of Endosulfan and its Effects on Oxygen Consumption and Total Nitrogen Excretion of the Fish *Macrognathus aculeatum*. Pestic. Biochem. Physiol. 15: 282-287.

[26] Barbieri, E. (2009). Effects of 2,4-D herbicide (2,4-dichlorophenoxyacetic acid) on oxygen consumption and ammonium excretion of juveniles of *Geophagus brasiliensis* (Quoy & Gaimard, 1824) (Osteichthyes, Cichlidae). Ecotoxicology 18: 55-60.

[27] Kormanik, G. A. and Cameron, J. N. (1981). Ammonia excretion in animals that breathe water: a review. Mar. Biol. Lett. 2: 11-23.

[28] Pequeux, A. and Guilles, R. (1981). Na^+ fluxes across isolated perfused gills of the chinese crac *Eriocher sinensis*. J. Exp. Biol. 92: 173-186.

[29] Izaguirre, I., O'Farrel, I., Unrein, F., Sinistro, R., Afonso, M. S. and Tell, G. (2004). Algal assemblages across a wetland, from shallow lake to relictual oxbow lakes (Lower Paraná River, South América). Hydrobiologia 511: 25-36.

[30] Schmidt-Nielsen, K. (1997). Animal Physiology, Adaptation and Environment, fifth ed. Cambridge University Press, New York.

Received: August 11, 2011
Revised: August 31, 2011
Accepted: September 14, 2011

CORRESPONDING AUTHOR

Carlos L. Negro
Laboratorio de Macrocrustáceos
Instituto Nacional de Limnología
(CONICET –UNL)
Ciudad Universitaria
Paraje El Pozo s/n.
3000 Santa Fe
ARGENTINA

E-mail: leonegro82@hotmail.com