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Toxicity of Cadmium, Lead, and Zinc to Larval Stages of *Lithodes santolla* (Decapoda, Anomura)

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Ushuaia Bay (54°48' S, 68°19' W, Beagle Channel, Tierra del Fuego, Argentina) has been receiving an increase in pollution since 1986, mainly from urban effluents and industrial wastes. Besides, fuel marine station and intensive maritime traffic (oil tank, tourist and commercial ships) in the local port, are also source of heavy metal in the area. Values up to 109.62 μg/L for zinc; 31.86 μg/L for lead and less than 1 μg/L for cadmium have been reported in water samples from Ushuaia Bay (Amin et al. 1997). The Southern King Crab *Lithodes santolla* is the most important shellfish commercially exploited in the Beagle Channel, but nowadays its fisheries are restricted into Ushuaia Bay by a protective program on this species. Larval development involves three larval stages, during a period comprising 21 to 23 days, in experimental conditions, and one postlarval, glaucothoe stage (Campodónico 1971), being the shallow waters mentioned as a possible recruitment area (Vinuesa and Lovrich 1992).

Heavy metals have been described as very dangerous pollutants for several aquatic species. Particularly on crustaceans, these compounds could alter different processes, such as molting and reproduction (Weis et al. 1992; Fingerman et al. 1996). First larvae stage of crabs has shown to be more sensitive to heavy metals such as cadmium and copper than other stages of the life cycle, while molting of juvenile crabs was inhibited by cadmium (López Greco et al. 2001). Moreover, a reduced number of hatching larvae, as well as abnormal hatched larvae (hydropsy, small size and atrophy), has been reported for eggs of *L. santolla* exposed to cadmium an lead (Amin et al. 1998).

This study was aimed at evaluating both the acute and chronic lethal toxicity of cadmium, lead and zinc on larvae of *L. santolla*, in order to provide useful information for environmental monitoring and further sublethal studies.

MATERIALS AND METHODS

Semi-static toxicological bioassays were conducted according to the standard procedures stated by the American Public Health Association et al. (1995), with the particularities stated described below. Larvae were obtained from ovigerous females collected in the Beagle Channel during the spring months. They were

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kept in the laboratory until zoeae hatching at a water temperature of 7.5 ± 0.5°C, 28 mg/L salinity and 12L:12D (fluorescent light). Only actively swimming larvae were selected and any that eventually deformed or became damaged were discarded. No food was given to larvae during the assays, since no effects of starvation on their survival was previously noted (Comoglio et al. 1993). Filtered marine water (5 μm minimum pore size) was used as dilution water in all experimental groups. Metal stock solutions, from which small aliquots were added to dilution water, were prepared from the respective metal salts: $ZnCl_2$; $CdCl_2.5H_2O$ or $PbNO_3$ and distilled water. Glass jars filled with 150 ml of test solution were used and daily renewed for every experimental group. The same routine was conducted for both acute and chronic bioassays.

For the acute assays, first day zoeae were selected from a pool of seven ovigerous females. Groups of ten zoeae each were randomly distributed to each beaker. Ranged finding test was conducted in order to determine the final concentrations used. Besides other concentrations according to previous experimental test were included to accurate the final results. The concentration series used was (in mg/L): 0.76; 1.53; 3.06; 6.12; 12.25 for cadmium, 1; 1.5; 2.1; 2.42; 2.51 for lead and 0.25; 2.5; 4.45; 7.74; 14.2; 25 for zinc. Each series was run in triplicate, including a dilution water control (no heavy metal added). The exposure period comprised 96 hr. Cessation of zoea heart beat was used as death criterion. Dead larvae from all recipients were daily recorded and surviving animals were then transferred to a fresh test solution. Probit analysis (Finney 1971) was employed to estimate the LC50 value and its 95% confidence limits, with Abbot's correction for mortality in controls. To compare LC50 values, differences were considered to be statistically significant when the *higher LC50/lower LC50* ratio exceeded the corresponding critical value established by the American Public Health Association et al. (1995).

To evaluate chronic toxicity, groups of ten larvae were exposed from hatching up to zoea III stage. The nominal concentrations used for each metals were (in mg/L): 0.01; 0.1 for cadmium, 0.001; 0.01 for lead and 0.005; 0.05 for zinc. A dilution water control group was always included in each series, which was run in triplicate. Date of molting and death was daily recorded in every group. TL50 was calculated by Probit analysis and compared treatments against control as well. Time for molting was compared among experimental groups by means of one-way ANOVA, followed by planned comparisons. The proportion of molting was always estimated on the initial number of larvae and it was compared between each concentration and control by means of the Fisher's test (Sokal and Rohlf 1981).

RESULTS AND DISCUSSION

Values of LC50 and the adjusted linear regression to Probit analysis are listed in Table 1. Mortality in controls was always lower than 10 % and no molting was observed either in concentrations nor controls during the 96 hr of exposure.

Table 1. LC50 values, confidence limits, slopes and correlation coefficients from Probit analysis for Zoea I of *Lithodes santolla* acutely exposed to heavy metals.

Heavy metal	Time (hr)	LC50 (mg/L)	95% Confidence limits	Slope	R^2
Cd^{++}	24	14.49	10.98 - 27.86	3.23	0.91
	48	4.37	3.63 - 5.18	4.58	0.93
	72	2.44	2.02 - 2.78	8.24	0.99
	96	2.07	1.76 - 2.40	7.25	0.79
Pb^{++}	24	>2.1	--------	-----	-----
	48	2.04	1.96 - 2.11	19.90	0.86
	72	1.91	1.84 - 1.99	14.76	0.91
	96	1.66	1.53 - 1.76	10.71	0.57
Zn^{++}	24	> 25	--------	-----	-----
	48	25.87	18.28 - 50.79	1.81	0.79
	72	4.07	3.59 - 4.61	6.57	0.74
	96	2.54	2.28 - 2.83	20.49	0.76

No significant ($p>0.05$) differences between 72hr and 96hr-LC50 indicates an asymptotic trend in the acute toxicity and were only found in cadmium assay. The statistical comparison of the 96hr-LC50 values among the assayed heavy metals (Zn-Pb; Cd-Pb; Zn-Cd) indicated significant differences ($p<0.05$) in all cases, resulting in the following relative scale of acute lethal toxicity: $Pb^{++} > Cd^{++} > Zn^{++}$.

Cadmium 96hr-LC50 for *L. santolla* showed to be, in general terms, one order of magnitude higher than comparable values of lethal toxicity estimated for other crustacean larvae (Martin et al. 1981; Greenwood and Fielder 1983; Ramachandran et al. 1997, López Greco et al. 2001). In a lesser extent, this comparison is also pertinent to zinc, a physiological cation. This relatively high resistance of the Southern King crab larvae could be associated with their big size, compared to other decapods larvae and their lecitotrophia could also be involved, since it allows them to avoid intake of pollutants by the digestive pathway. However, lead was the most toxic heavy metal among the three acutely assayed on *L. santolla* larvae, in spite of the relatively lower toxicity of lead with respect to cadmium and zinc found in other species (Martin et al. 1981; Itow et al. 1998). This somewhat high lead toxicity to a representative species such as *L. santolla* larvae has a high ecotoxicological relevance, taking into account the high lead concentrations detected in their natural environment and preliminary "in situ" assay has demonstrated a bioaccumulation of lead in coastal organisms (Amin et al. 2000).

Table 2 shows the TL50 values for each concentration of the heavy metals assayed. For chronic assays no differences were observed among replicates for

each treatment, so that data were combined for further analysis. Except for cadmium 0.1 mg/L, all larvae had molted to zoea III stage or died after 16 days of exposure. According to the statistical comparisons made, two groups can be distinguished (also indicated in Table 2), one of them represented by the highest concentrations of cadmium and zinc, that caused an early mortality, and the second one made up of the remaining concentrations used, having significant ($p<0.05$) higher TL50 values than the former group and therefore producing the same lethal effect at higher times of exposure.

Table 2. TL 50 values and molting time (± standard error) of zoea I and II to the next stages, to Cadmium, Lead and Zinc.

Heavy metal	mg/L	TL50 and 95% confidence limits (days)	Molting time (days) zoea I-II	zoea II-III
Cadmium	Ctrl	--------	5.19 ± 0.17 (26)	12.67 ± 0.13 (21)
	0.01	14.40 (13.00 - 16.60)	5.80 ± 0.35 (20)	12.69 ± 0.17 (13)
	0.1	8.20 (7.76 - 8.63)*	5.00 ± 0.00 (2)	--------
Lead	Ctrl	--------	5.11 ± 0.14 (27)	11.67 ± 0.11 (18)
	0.001	13.31 (11.99 - 15.27)	5.67 ± 0.11 (18)*	12.23±0.12 (13)*
	0.01	12.99 (11.73 -14.83)	5.57 ± 0.22 (23)	11.91 ± 0.09 (11)
Zinc	Ctrl	--------	5.50 ± 0.11 (28)	12.30 ± 0.13 (20)
	0.005	13.07 (11.81-14.93)	6.28 ± 0.49 (18)	13.0 ± 0.30 (13)*
	0.05	7.98 (7.40-8.55)*	8.00 ± 0.98 (8)*	13.33 ± 0.33 (3)*

Number of molted larvae is indicated between brackets. Asterisks indicate significant differences ($p<0.05$) with respect to the lowest concentration (for TL50) or controls (for molting time).

Time course for molting is showed in Figure 1 and Table II. The highest cadmium concentration significantly ($p<0.05$) reduced the percentage of molting from zoea I to zoea II stage and in this concentration no zoeae reached to zoeae III, while lowest cadmium concentration used (0.01 mg/L) yielded a percentage of molting from zoea II to zoea III significantly ($p<0.05$) lower than that of control. As for lead, the lowest concentration used (0.001 mg/L) significantly ($p<0.05$) lowered the molting to zoea II, while the highest one (0.01 mg/L) produced a significant ($p<0.05$) lower percentage of molting to zoea III. Concerning zinc, both concentrations employed (0.005 and 0.05 mg/L) caused a significant ($p<0.05$) reduction of the first molting event, while molting to zoea III was only significantly ($p<0.05$) reduced at the highest zinc concentration.

Time for molting of zoea I and II to the next stages (Table 2), showed a tendency to delay molting at most of the concentrations tested. Lead caused a significant ($p<0.05$) delay at the lowest concentration used, for both molting events. Zinc caused the strongest effect in this respect, since a significant ($p<0.05$) delay at

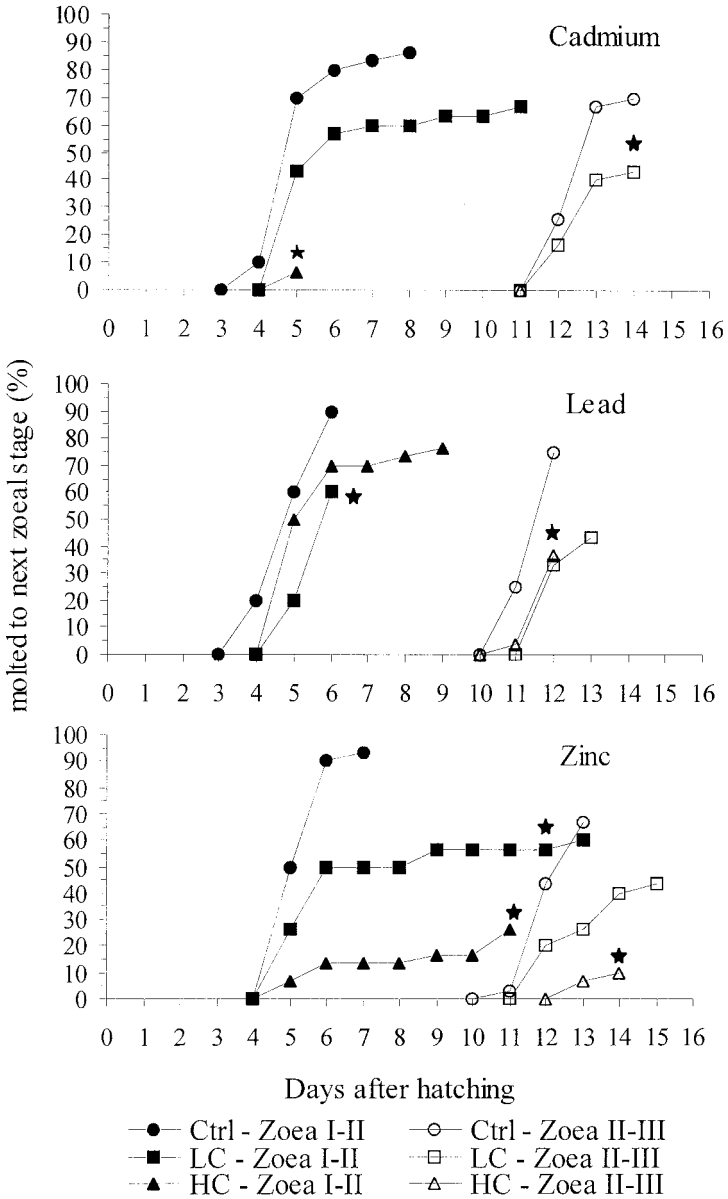

Figure 1. Time course for molting of *L. santolla* larvae. Ctrl: dilution water control group. LC and HC: lowest and highest concentrations tested, respectively. Asterisks indicate significant difference ($p<0.05$) with respect to Control (Ctrl).

both concentrations was observed for zoea II molting to zoea III, and only at the highest concentration for molting from zoea I to zoea II. No differences were detected for cadmium regarding molting time. In spite of these results it is necessary to take into account for a fit analysis the high mortality registered in the highest concentrations of zinc and cadmium.

Molting and growth of crustaceans can be affected by several heavy metals, in terms of decreasing growth as well as molting frequency (Weis et al. 1992; Fingerman, et al. 1996). Molting percentage was reduced and molting time delayed in *C. granulata* juvenile crabs exposed to 10 μg/L of cadmium (López Greco et al. 2001). For this latter species, some evidences of an interference of that heavy metal with the endocrine control of molting have been also reported (Rodríguez Moreno et al. 1998). The results obtained in *L. santolla* exposed to cadmium, indicate a clear reduction in the percentage of molting to zoea III, but for those larvae that could molt, no delay in time of molting was evident.

Lead treatments have not shown significance differences in terms of LT50 related to control. The concentration 0.001 mg/L produced a higher delayed in time for molting to both zoea II and III, as well as lower molting percentage to zoea II than the highest lead concentration used. Such differences, although statistically significant, can not allow us to state definitive conclusions about the dose-response relationship for a broad range of lead concentrations. Nevertheless, a lower biological availability as lead concentration increases, due to ionic complexation or other physico-chemical processes, seems unlikely taking into account the results of the acute assay, where mortality increases as lead concentration increases; EC50 values at 1 mg/L of lead were also estimated for other marine species (Martin et al. 1981). More probably, higher concentrations of lead could be really effective in inducing the synthesis of metallothioneins and/or accumulation of lead in carapace than lower ones (no threshold reached); these depuration mechanisms significantly reduce the free lead concentration inside the organism. Particularly, crustacean carapace is a relevant deposit site for lead (du Preez et al. 1993), allowing the elimination of accumulated lead with molting.

Zinc has been reported as less toxic than cadmium on molting and regeneration of fiddler crabs, having inhibitory effect at concentrations ranging from 1 to 5 mg/L (Weis et al. 1992). However, zinc has shown to be at least as toxic as cadmium for *L. santolla* larvae chronically exposed, in terms of mortality and molting success. At 0.005 mg/L of zinc a significant reduction of molting to zoea II was observed, while no significant reduction was detected at 0.01 mg/L of cadmium compared with each one control. Moreover, zinc produced a significant delay in molting to both zoea II and III, while cadmium did not. Compared to lead, zinc has also shown a higher chronic toxicity in terms of LT50 values and also on molting success. Therefore, the lowest zinc toxicity observed for the acute lethal toxicity assays could not be extrapolated to larvae molting, at least involving molting up to zoea III stage. As a result, concerning larvae development, zinc was shown to have the same or even a higher toxicity than the other heavy metal assayed.

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REFERENCES

American Public Health Association, American Water Works Association, Water Pollution Control Federation (1995) Standard Methods for the Examination of Water and Wastewater, 19th ed. American Public Health Association, Washington, DC.

Amin OA, Andrade S, Ferrer L, Comoglio LI, Marcovecchio J, San Román N (1997) Use of macroalgae as bioindicator of heavy metals concentration in coastal zone of Beagle Channel, Tierra del Fuego, Argentina. Proc Int Symp Cold Region Devel. (ISCORD 97), 337-340

Amin OA, Rodríguez EM, Hernando M, Comoglio LI, Lopez LS, Medesani DA (1998) Effects of lead and cadmium on hatching of the Southern King Crab Lithodes santolla (Decapoda, Anomura). Invert Reprod Dev 33: 81-85

Amin OA, Comoglio LI, Ferrer L, Marcovecchio JE (2000) Variación en la concentración de plomo, zinc y cadmio en el mejillón *Mytilus edulis* transplantado dentro de la bahía Ushuaia, Tierra del Fuego. Proc Jornadas Nacionales de Ciencias del Mar, 11-14 de Septiembre de 2000, Puerto Madryn, Chubut, Argentina. p. 31

Campodónico I (1971) Desarrollo larval de la centolla *Lithodes antarctica* Jacquinot en condiciones de laboratorio. Anales del Instituto de la Patagonia 2:181-190

Comoglio LI, Vinuesa J, Amin OA (1993) Effects of early starvation periods on zoeal development of *Lithodes santolla*. World Aquacult '93, May 26-28, Torremolinos, España, 124-125

duPreez HH, Steenkamp VE, Schoonbee HJ (1993) Bioaccumulation of zinc and lead in selected tissues and organs of the freshwater crab, *Potamonautes warreni*. Sci Tot Environ 1993, 469-478

Fingerman M, Devi M, Reddy PS, Katyayani R (1996) Impact of heavy metal exposure on the nervous system and endocrine-mediated processes in crustaceans Zool Stud 35: 1-8

Finney DJ (1971) Probit analysis. 3rd edition. Cambridge University Press, Cambridge.

Greenwood JG, Fielder DR (1983) Acute toxicity of zinc and cadmium to zoeae of three species of portunid crabs (Crustacea:Brachyura). Comp Biochem Physiol 75C:141-144

Itow T, Loveland RE, Botton ML (1998) Developmental abnormalitites in horseshoe crab embryos caused by exposure to heavy metals. Arch Environ Contam Toxicol 35:33-40

López Greco LS, Sánchez MV, Nicoloso GL, Medesani DA, Rodríguez EM (2001) Toxicity of cadmium and copper on larval and juvenile stages of the estuarine crab *Chasmagnathus granulata* (Brachyura, Grapsidae). Arch Environ Contam Toxicol 41:333-338

Martin M, Osborn KE, Billig P, Gliekstein N (1981) Toxicities of ten metals to *Crassostrea gigas* and *Mytilus edulis* embryos and *Cancer magister* larvae. Mar Pollut Bull 12:305-308

Ramachandran S, Patel TR, Colbo MH (1997) Effect of copper and cadmium on three Malasyan tropical estuarine invertebrate larvae. Ecotoxicol Environ Saf

36:183-188
Rodríguez Moreno PA, Medesani DA, Rodríguez EM (1998) Effects of cadmium on molting of the estuarine crab *Chasmagnathus granulata*. Proceedings of the Fourth International Crustacean Congress, Amsterdam, p. 177
Sokal RR, Rohlf FJ (1981) Biometría. Principios y métodos estadísticos en la investigación biológica. Blume Ed, Madrid.
Vinuesa JH, Lovrich GA (1992) Biología y pesca de la centolla en el Canal Beagle, Tierra del Fuego, Argentina. Recomendaciones para su manejo. Contribución científica, Centro Austral de Investigaciones Científicas N°15: 26 pp.
Weis JS, Cristini A, Ranga Rao K (1992) Effects of pollutants on molting and regeneration in Crustacea. American Zool 32: 495-500.