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Pathological lesions in larvae hatched from ovigerous females of *Chasmagnathus granulata* (Decapoda, Brachyura) exposed to cadmium

E. M. Rodríguez and D. Medesani

Laboratorio de Fisiología Animal Comparada. Dpto. Cs. Biológicas, FCEyN, University of Buenos Aires, Ineuci-Conicet. Pab. II, Ciudad Universitaria, 1428 Buenos Aires (Argentina)
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Abstract. Morphological abnormalities were noted in larvae hatched from ovigerous females of the estuarine crab *Chasmagnathus granulata*, exposed to 1 and 15 mg/l of cadmium during the egg incubation period. The highest concentration produced a significant incidence of hydropsy, atrophy of dorsal spine, pleon and pereiopods as well as enhancement of pigmentation. Some possible causes of these results are discussed and compared with the effects caused by other pollutants on the same species.
Key words. Cadmium; crab; larvae; teratogenic effects.

Chasmagnathus granulata is the most typical crab species living along the coastline of Samborombón Bay, an outer part of the Río de La Plata estuary (Argentina). Both adult and larvae stages play a significant role as food for many fish species in the local trophic web. Cadmium has been reported as a heavy metal whose concentration exceeds frequently the permissible levels in the estuary, reaching concentration peaks as high as 13 ug/l (ref. 1). The aim of this work was to examine the nature and extent of teratogenic effects of cadmium on one of the most sensitive stages of the crab life cycle.

Materials and methods

Ovigerous females were collected at Faro San Antonio beach, the southern limit of Samborombón Bay (36° 18′ S, 56° 48′ W), in November 1992 (first assay) and March 1993 (second assay). The egg clutches of collected females corresponded to an immature stage of egg development, as judged by their intense purple colour[2].

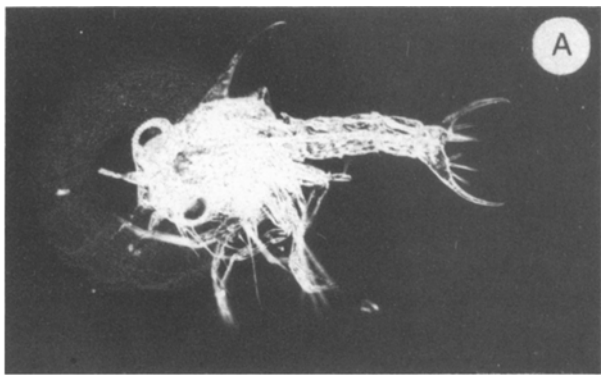
Toxicological bioassays were carried out following the procedure recommended by the American Public Health Association[3] and by Ward and Parrish[4]. Stock solution of $CdCl_2.2\frac{1}{2}H_2O$ (May and Baker Ltd., Dagenham, U.K.) was prepared in distilled water (cadmium concentration: 10 g/l). A first assay was carried out to assess the effect of 1 mg Cd^{++}/l on incubation and hatching processes, and a second one in a 15 mg Cd^{++}/l solution.

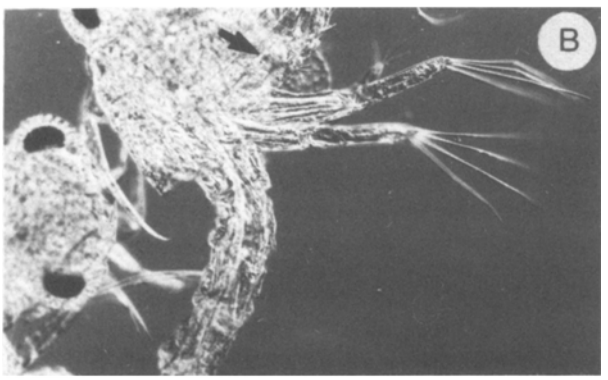
Sea water was prepared by dissolving artificial sea water salts (HW Marinemix, Germany) in dechlorinated tap water (hardness: 80 mg/l as $CaCO_3$), to give a salinity of 30‰, pH 7.4. A total of ten ovigerous females were assigned to each treatment, placing each female in an individual 1 liter beaker under continuous aeration. A water temperature of 21 ± 1 °C and a photoperiod of 12L:12D (fluorescent light) were maintained throughout the experiments. All Cd^{++} test solutions and control sea water were renewed twice a week and no food was given during the assays. All females were checked daily in order to detect hatched eggs or loss of clutches, removing the post-hatching females and those that eventually had died. Once the eggs hatched, a sample of 10 ml was taken with a 25 ml pipet, shaking the beaker beforehand to get a homogeneous larval distribution, according to previous work[5]. Carapace width of each post-hatching female was measured. Each larval sample was fixed in 5% formalin, for later analysis.

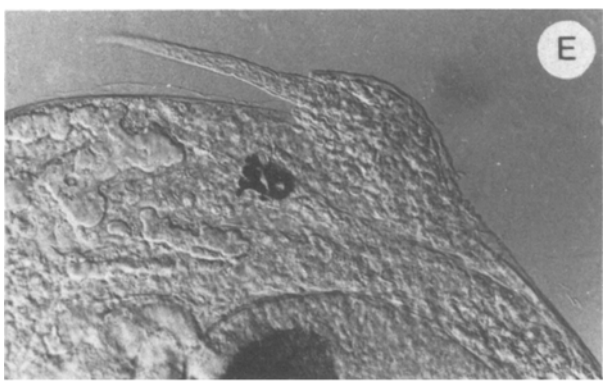
Fifty larvae of each sample were examined under a stereomicroscope (25 × magnification) to assess and quantify the morphological abnormalities that appeared after exposure of embryos to cadmium. The Fisher test[6] was used to analyze the effect of cadmium on the proportion of females whose eggs hatched. Analysis of covariance was used to test the effect on the number of hatched larvae per female, taking the carapace width as covariable. Since no homogeneity of variances was found, the Kruskal-Wallis non-parametric test was applied to assess whether cadmium concentrations affected the incubation period. A two-way analysis of variance was employed for the proportion of abnormal larvae (angular transformation), considering concentration and type of abnormality as independent factors. The Duncan multiple range test was used for comparing the resulting mean values[7].

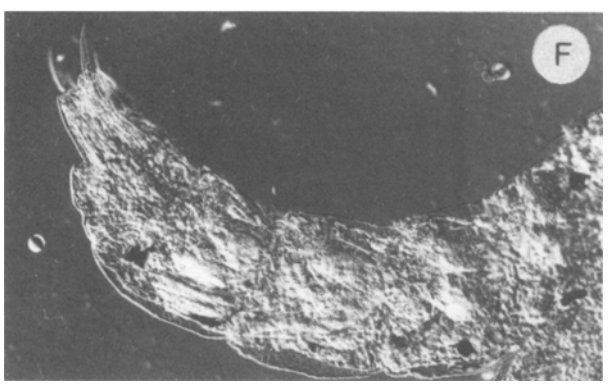
Results

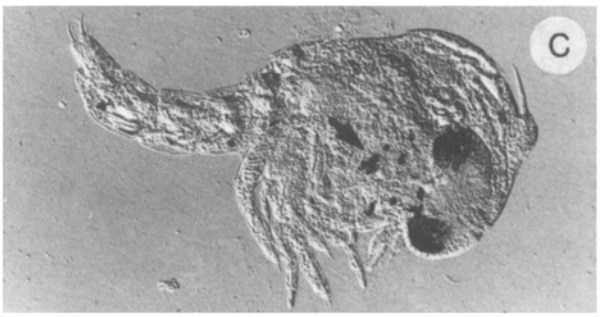
No significant differences were found between treatments with respect to the proportion of females whose eggs finally hatched ($p > 0.05$, ranging from 0.6 to 0.9), nor in number of hatched larvae per female ($p > 0.05$, global mean and standard error: 19703 ± 3081. However, a significantly lower ($p < 0.05$) incubation period

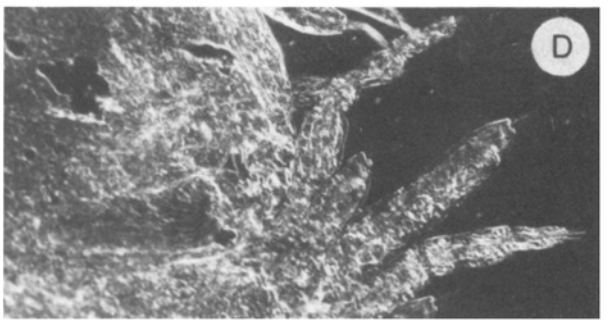


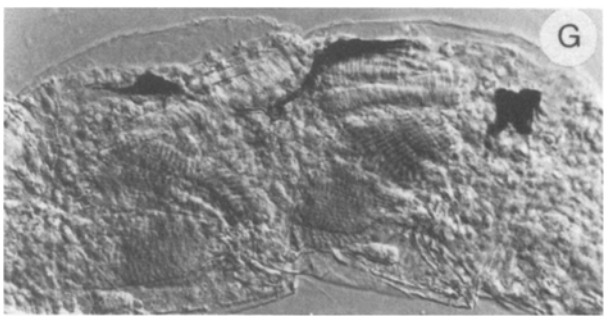


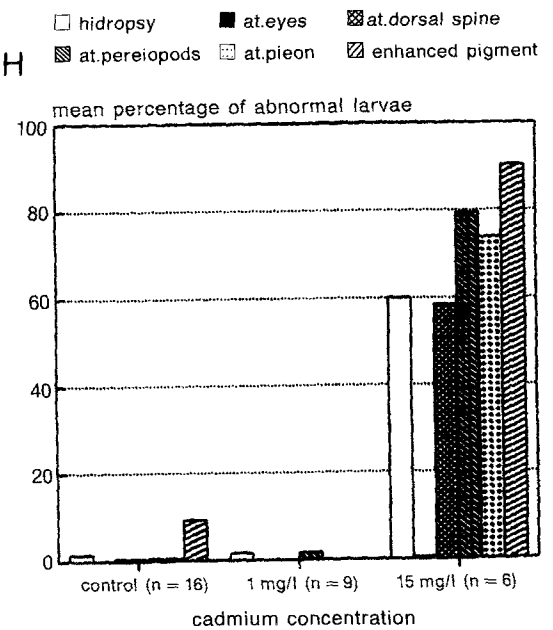












A ×80. Control larvae (general view). Note normal dorsal and caudal spines. *B* ×125. Control larvae. Detail of pereiopods and presence of melanophores (arrow). *C* ×100. General view. Note hydropsy and hypertrophied melanophores (arrow) in cephalothorax. *D* ×312.5. Atrophied pereiopods (note reduced setae). *E* ×400. Atrophied dorsal spine. *F* ×250. Atrophied pleon (note reduced caudal spines). *G* ×500. Hypertrophied melanophores in pleon. *H* Mean proportion of abnormal larvae for each abnormality noted after egg exposure to cadmium. n = number of analyzed samples (hatched clutches); at. = atrophied. Control data were pooled from both assays.

was noted at 15 mg/l of cadmium, with mean values and standard errors of 11.6 ± 1.4, 11.1 ± 1.0 and 5.8 ± 0.4 days for control, 1 mg/l and 15 mg/l respectively.

Recognized morphological abnormalities caused by cadmium, as compared to normal structures, are shown in figures A to E. They included: hydropsy, especially in the cephalothoracic region of larvae (fig. C) and atrophy of the following structures: pereiopods (reduced size and reduction in setae number, fig. D), dorsal spine (reduced size and lack of normal shape, fig. E) and pleon (reduced size, especially in telson and caudal spines, fig. F). Hypertrophied melanophores occured in both cephalothorax and pleon (figs C, D and G). Atrophy of eyes was not observed, in contrast to previous results concerning teratogenic effect of other pollutants on the same species. See figures A and B for comparison with normal (control) larvae.

Statistical comparison of abnormalities between treatments did not show significant differences ($p > 0.05$) between 1 mg/l Cd^{++} and sea water controls, while 15 mg/l caused a significantly higher incidence of abnormalities, except for atrophy of eyes which was not observed in any experimental conditions. Enhancement of pigmentation had the highest incidence of observed abnormalities. Mean percentages for each one can be seen in figure H.

Discussion

Exposure of ovigerous females to cadmium did not seem to affect the number of hatched larvae and did not cause the abortion of egg clutches during the incubation period, in contrast to the effects of the pesticides parathion and 2,4-D on the same species[5]. Instead, at a high cadmium concentration (15 mg/l), a significantly lower egg incubation period was noted. This was unexpected, since a delay of development by zinc and mercury was previously reported for pulmonate molluscans and a delay of development was caused by cadmium in amphibians during gastrulation[9], and a retardation of limb regeneration in crustacean adults was shown to be caused by cadmium an mercury[10]. The present results on *C. granulata*, such as the reduction in size of pereiopods and dorsal and caudal spines, could be interpreted as being indicative of an incomplete development. However, similar abnormalities were observed after parathion and 2,4-D treatment on the same species without a sigificant decrease in incubation period[5].

These results, and those on the above mentioned pesticides indicate that while some abnormalities were produced by both types of pollutants (hydropsy and atrophy of exoeskeletal structures, e.g. pereiopods, pleon and spines) others were not, i.e., both pesticides produced significant eye atrophy, while only cadmium caused an enhancement of pigmentation.

Some direct and/or indirect effect of cadmium on embryonic chromatophores must occur during development to cause the observed hyperpigmentation. According to observations recently made in our laboratory on *C. granulata* (unpubl. data), the melanophores located on the ventral side of pleonites and at the base of dorsal spine are predominant as development progresses. But the abnormal pigmentation was observed in melanophores dispersed throughout the larvae surface, such as the dorsal side of pleonites and lateral sides of larval cephalotorax (see figs C to G). This result suggests a direct effect of cadmium to promote hyperthrophic (and perhaps hyperplastic) processes on melanophores; melanin has the capacity to bind cations[11], and thus could play a role in cadmium detoxification. Among crustaceans, a black pigmentation in ventral sternum and gills of the crab *Scylla serrata* after chronic exposure to cadmium was reported[12].

It can be concluded that some teratogenic effects in *C. granulata* are less specific than others, since they are caused by pollutants as different as an insecticide, a herbicide and a heavy metal, while other effects seem to be specific for a certain pollutant. Although it will be necessary to extend these studies to other pollutants to achieve more generalized conclusions useful for water quality monitoring, the present results represent a stimulus for further studies of the teratogenic effects of aquatic pollutants on crustacean larvae.

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