

## Effects of Cadmium on Somatic and Gonadal Growth of Juvenile Females of the Estuarine Crab *Chasmagnathus granulata* (Brachyura: Grapsidae)

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**Mariela Kogan, Laura S. López Greco, Luis A. Romano and Enrique M. Rodríguez (2000)** Effects of cadmium on somatic and gonadal growth of juvenile females of the estuarine crab *Chasmagnathus granulata* (Brachyura: Grapsidae). *Zoological Studies* 39(4): 344-350. Juvenile female crabs of *Chasmagnathus granulata* (Decapoda: Brachyura) were exposed to 0.1 and 0.5 mg/l of cadmium for at least 50 d before and 15 d after molting. The gonads were dissected and fixed for histopathological studies, while the carapaces were preserved to measure calcium content. A cadmium concentration of 0.5 mg/l was lethal for juvenile crabs. The few surviving crabs at this concentration were unable to molt. Although most crabs exposed to 0.1 mg/l of cadmium survived and molted, they presented a significantly higher incidence of pathologies in the ovaries than did control crabs. These pathologies were hypertrophy and cariorhexis in primary oocytes, as well as reactional atresia in secondary oocytes. Although no significant difference in the proportion of crabs with mature ovaries after molting was noted between the control and cadmium-exposed animals, a trend towards slower maturity was noticed due to the effect of cadmium.

**Key words:** Cadmium, Ovary, Crustaceans, Sexual maturity.

*Chasmagnathus granulata* (Decapoda: Brachyura: Grapsidae) is a semiterrestrial estuarine crab, living along the Atlantic coast of Argentina, Uruguay, and Brazil, from Golfo San Matías (41°S) to Río de Janeiro (22°S) (Boschi 1964). Adult and juvenile crabs of this species live in the meso- and supra-littoral zone of the coast, forming very dense populations. Crabs are fed upon by several fish species of high commercial value.

Adult crabs reproduce during the spring and summer seasons (September to March). In the Río de la Plata estuary (Argentina), ovigerous females migrate to the bottom of the estuary for hatching of zoea. The megalopa stage returns to the coast and molts to the 1st juvenile stage, which acquires sexual maturity after several further molts. The onset of female sexual maturity in *C. granulata* begins with the start of exogenous vitellogenesis in the ovary, before changes in the allometric growth of secondary char-

acters take place (López Greco and Rodríguez 1999). The minimum carapace width for gonadal maturation for juvenile crabs of *C. granulata* was estimated as 18.5 to 19 mm, while the carapace width corresponding to the puberal molt (when changes in the relative growth of some reproductive structures take place) has been reported as 22.7 mm (López et al. 1997).

Cadmium was detected above the permissible levels for the protection of aquatic life (according to the Comisión Administrativa del Río de la Plata 1990) in 82% of water samples from the Río de la Plata estuary (Argentina), with mean values ranging from 2 to 4 µg/l. In Samborombón Bay, cadmium concentration found in superficial sediments was 9.43 ± 4.63 µg/g, while a concentration of 11.91 ± 6.39 µg/g was reported in suspended material (Marcovecchio 1988). An accumulation of cadmium has been described in different tissues of crusta-

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ceans (Rainbow and Dallinger 1993, Zanders and Rojas 1996). Cadmium accumulation in gills and carapace of *Chasmagnathus granulata* has previously been reported from laboratory experiments (Bigi et al. 1996).

Ovaries of adult *C. granulata* crabs have been shown to be very sensitive to exposure to pollutants such as pesticides (Rodríguez et al. 1994). The aim of this work was to evaluate the effect of cadmium on oocytes of juvenile crabs during the onset of sexual maturity. Additionally, the impact of cadmium on the molting of crabs was assessed.

## MATERIALS AND METHODS

Juvenile female crabs were collected at Faro San Antonio beach (36°18'S, 56°47'W), the southern point of Samborombón Bay (Argentina), during 1997. No detectable amounts of heavy metals were found in tissues of crabs sampled at that site (Bigi et al. 1996). Once in the laboratory, animals were maintained for 2 wk at the same environmental conditions which were used later for bioassays: 12L:12D photoperiod (fluorescent light), temperature  $20 \pm 1$  °C, pH  $7.4 \pm 0.1$ , and salinity 12‰, prepared from dechlorinated tap water (total hardness: 80 mg/l as CaCO<sub>3</sub> equivalents) and HW (Marine Mix®, Germany) salts for artificial marine water. Crabs were fed twice a week on commercially available pellets of rabbit food and chicken liver, as in previous studies (Rodríguez et al. 1994, Rodríguez Moreno et al. 1998) during both the 2-wk acclimation period and the experiments.

For the experiments, each juvenile crab was isolated in a 400-ml plastic container filled with 50 ml of 12‰ saline water. Cadmium (as cadmium chloride, Merck) was dissolved in distilled water and added to test containers from a concentrated stock solution (0.5 g/l). A water dilution control was also run, containing only the saline water employed.

The experiment began on 23 Oct. 1997. Fifteen juvenile crabs were randomly assigned to either control or cadmium-exposed (0.1 mg/l, nominal concentration) treatments, while 20 crabs were assigned to a 2nd, higher nominal cadmium concentration (0.5 mg/l). These cadmium concentrations were sublethal concentrations for adult crabs, i.e., fractions of 1/250 and 1/50 of the 96-h-LC<sub>50</sub> value determined for adults of the same species (Bigi et al. 1996). Carapace width of all employed crabs was  $20.90 \pm 0.07$  mm ( $n = 50$ ). Crabs were checked daily for molting or death. Lethal time for 50% of animals (LT<sub>50</sub>) was calculated by means of probit analysis (Finney 1971)

in cadmium concentrations causing significant mortality.

After molting, crabs remained exposed during 15 d to the same test solutions. After that, gonads were dissected and their carapaces were measured and preserved. The experiment ended 15 d after all control crabs had molted.

For dissecting gonads, crabs were cold anesthetized at  $-20$  °C for 15 min. Because of the small size of the gonads, the entire hepatopancreas together with the slightly adhering gonadal tissue, were quickly dissected and fixed in Bouin's solution for 4 h at 20 °C. The tissues were then sequentially passed through 90% ethanol for 20 min, 96% ethanol for 20 min, 96% ethanol plus butyl alcohol (1:1 v/v) for 30 min, and butyl alcohol for 30 min, after which the gonads were embedded in paraffin, and 5- $\mu$ m sections were prepared and stained with hematoxylin-eosin.

A representative cutting of the ovary of each crab, showing both oogonias and oocytes, was systematically analyzed to count pathologies. In addition, the diameter of each oocyte (as the average of the major and minor diameters) was measured by means of a micrometer ocular lens, calibrated with a Leitz Wetzlar plate with 1/100-mm spacing. Mean oocyte diameter was then estimated for each control and cadmium-exposed crab.

After dissecting the gonads, carapaces as well as exuviae, were isolated and dried at 70 °C until constant weight. In both cases, a sample of 400 mm<sup>2</sup> was taken from the dorsal branchial lobe of the carapace. Samples were digested at room temperature in 1 ml of nitric acid (analytical grade) for 1 wk, and then brought to a 10-ml volume with distilled water. Calcium content were measured by means of a flame photometer (Crudo Caamaño S.A.)

Oocyte diameter and the incidence of each pathology observed in oocytes were compared between control and cadmium-exposed crabs by ANOVA, using an appropriate transformation of data when needed. The proportion of crabs having secondary oocytes (vitellogenic ovaries) as well as the proportions of molted and dead crabs were compared by means of a Fisher test. The increment of size with molting was compared by ANCOVA, taking the final size as the variable and the initial size as the co-variable (Sokal and Rohlf 1979).

## RESULTS

Table 1 shows the results concerning the percentages of molted and dead crabs. A significant

high percentage of dead crabs was found in 0.5 mg/l of cadmium, while no significant differences were detected between the 0.1 mg/l of cadmium and control groups.  $LT_{50}$  for that concentration was 39.8 d (95% confidence interval: 35.2-44.5 d). All crabs died before molting. Table 1 also shows the days elapsed from the beginning of the experiment to molting of crabs; no significant differences were detected between control and cadmium-exposed crabs in this respect. The final control crab molted on day 87 of the experiment; 15 d later it was processed and the experiment ended.

Size increment and specific calcium content of carapaces and exuviae are shown in table 2. Although calcium content showed a decrease in all cases due to the effect of cadmium, the differences against control crabs were not statistically significant ( $p > 0.05$ ).

No differences in mean oocyte diameter were detected between the control and crabs exposed to 0.1 mg/l of cadmium ( $p > 0.05$ ), with the corresponding values being  $56.64 \pm 4.08 \mu\text{m}$  ( $n = 11$ ) and  $52.83 \pm 5.29 \mu\text{m}$  ( $n = 8$ ) respectively.

Pathologies observed in the ovaries of molted crabs were hypertrophy and cariorhexis in primary (previtellogenic) oocytes (Fig. 1), while reactional atresia, with invasion of follicular cells, was seen in secondary (vitellogenic) oocytes (Fig. 2). The incidence of each pathology is shown in table 3. A statistically ( $p < 0.05$ ) higher incidence due to the effect of cadmium was detected both for hypertrophy and cariorhexis of primary oocytes, as well as for atresia of secondary oocytes. Figure 3 shows the number of crabs having previtellogenic and vitellogenic ovaries for control and cadmium-exposed groups. A clear tendency for a drop in the proportion of crabs with vitellogenic ovaries was noted in exposed crabs, although no significant differences were found ( $p > 0.05$ )

## DISCUSSION

Growth and reproduction are processes commonly studied in long-term ecotoxicological bioassays with aquatic animals. Fishes are the most often used species in this respect (Weis et al. 1989, Holm et al. 1991). Concerning invertebrates, bivalve mollusks have also been studied. As examples, low levels of cadmium were found in gonads of clams exposed to natural water from different sites, and chromosome and larvae abnormalities were also found (Stiles et al. 1991). McKenney (1986) reported both a delay in the onset of reproduction and retarded growth rates in mysid crustaceans when exposed to the insecticide, fenthion, during the complete life cycle.

A significant lethal effect of cadmium was found in this study for juvenile crabs of *Chasmagnathus granulata* chronically exposed to 0.5 mg/l, with a  $TL_{50}$  of about 40 d. The few surviving crabs at this concentration were unable to molt, as was observed by Rodríguez Moreno et al. (1998) for adults of the same species also exposed to 0.5 mg/l of cadmium during premolt. In that study with adult crabs, an effect of cadmium on ecdysteroid secretion seemed to

**Table 2.** Size increment of molted crabs and specific calcium content of carapaces and exuviae

	Control	0.1 mg/l Cd <sup>++</sup>
Initial CW (mm)	20.78 ± 0.13	21.02 ± 0.19
CW increment (%)	5.87 ± 0.43	5.56 ± 1.06
[Ca <sup>++</sup> ] in exuviae (µg/mm <sup>2</sup> )	89.78 ± 3.96	82.52 ± 2.14
[Ca <sup>++</sup> ] in carapace (µg/mm <sup>2</sup> )	3.80 ± 1.61	3.42 ± 1.05

Means ± standard errors are indicated.

CW: maximum carapace width (mm).

The number of molted crabs is shown in table 1.

**Table 1.** Number ( $n$ ) and percentage (%) of molted and dead crabs at the end of the experiment; percent (%) always refers to the initial number of crabs

Event	Control		0.1 mg/l Cd <sup>++</sup>		0.5 mg/l Cd <sup>++</sup>	
	$n$	%	$n$	%	$n$	%
No. at start	15	100	15	100	20	100
No. molting	14	93.33	11	73.33	0	0*
Time for molting (d)	14	53.14 ± 6.25	11	47.03 ± 5.72	0	—
No. dead before molting	1	6.66	3	20	17	85
No. dead while molting	0	0	1	6.66	0	0*
No. surviving that did not molt	0	0	0	0	3	15

Times (d) for molting (± standard error) are also shown.

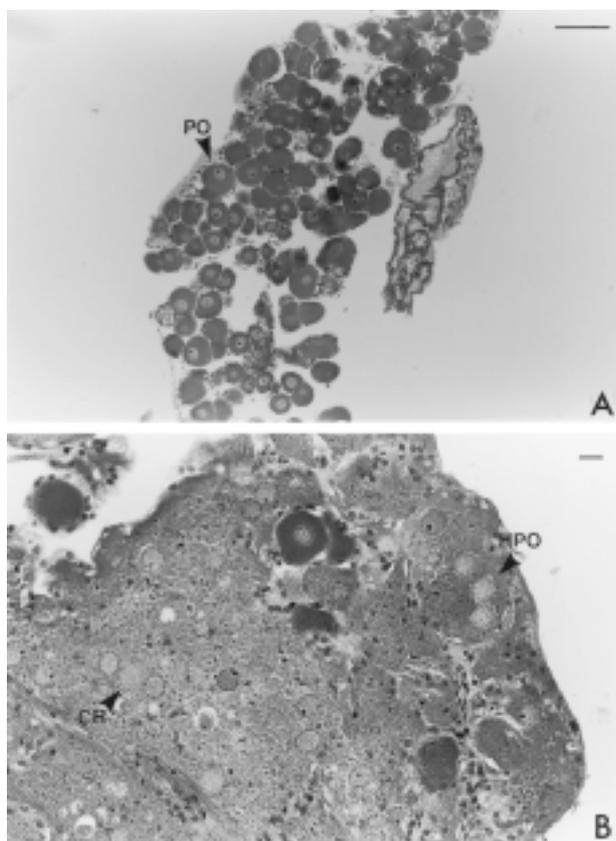
\*indicates significant differences ( $p < 0.05$ ) with respect to the control.

occur, rather than an effect of cadmium on normal calcium deposition during postmolt. The same suggestion was made by Bodar et al. (1990) concerning molting impairment in cadmium-exposed daphnids. The absence of significant differences in calcium content of carapaces after molting between control and juvenile crabs exposed to 0.1 mg/l (this study) is in accordance with the results found in adults by Rodríguez Moreno et al. (1998).

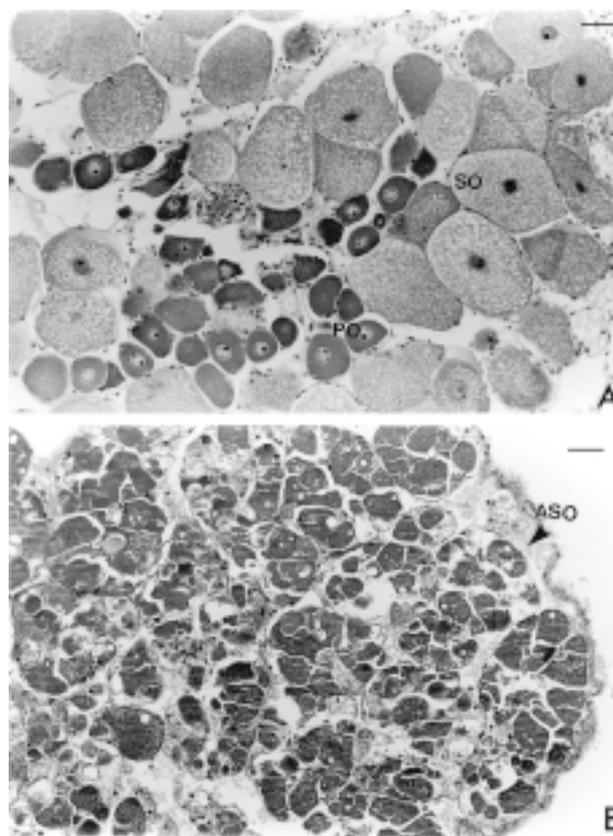
At 0.1 mg/l of cadmium, most juveniles survived and molted, but deleterious effects on gonadal development were noted, i.e., a significant incidence of some pathologies in both primary and secondary oocytes, at a size where juveniles achieve sexual maturity (López et al. 1997). Cariorhexis was also noted in the fish, *Lebistes reticulatus* (Ramana et al. 1992) chronically exposed to the pesticide, dimethoate, as well as smaller size of oogonias and oocytes. Kling (1981) reported total atresia in mature oocytes of *Tilapia leucosticta* exposed to lebaycid. A delay in vitellogenesis and atresia of primary and secondary

oocytes were found in fishes chronically exposed to pesticides (Mani and Saxena 1985). Organophosphate pesticides also caused a drop in the proportion of mature oocytes in fish and histopathological damage to ovaries, consisting of necrosis and fibrosis of connective tissue, and disintegration of cortical alveoli and yolk globules in mature oocytes (Rastogi and Kulshrestha 1990).

The effects of the organophosphate pesticide, parathion, and the herbicide, 2, 4-D, have previously been assayed in adults of *Chasmagnathus granulata* (Rodríguez et al. 1994). The main effects of these pesticides were exerted on mature oocytes, but while the herbicide caused atresia, parathion increased the oocyte size, possibly due to hormonal imbalances. From that study, we concluded that mature oocytes are more sensitive to pesticides than are immature ones, as Kling (1981) also stated for fish. In the current study, we have found a high degree of atresia in secondary, mature oocytes, but also a significant effect of cadmium on previtellogenic



**Fig. 1.** Main pathologies observed in previtellogenic ovaries of molted crabs. A: control; B: exposed to cadmium (0.1 mg/l). PO: primary oocyte (normal), HPO: hypertrophied primary oocyte, CR: cariorhexis in primary oocytes. Horizontal bars: 1 mm.



**Fig. 2.** Main pathologies observed in vitellogenic ovaries of molted crabs. A: control; B: exposed to cadmium (0.1 mg/l). SO: secondary oocyte (normal), PO: primary oocyte, ASO: atretic secondary oocytes. Horizontal bars: 1 mm.

genic, immature oocytes. All these deleterious effects seriously compromise the recruitment of juveniles to the reproductive function, at a cadmium concentration (0.1 mg/l) of 1 order of magnitude below that which causes embryo abnormalities in the same species (Rodríguez and Medesani 1994) and 50 fold lower than the 96h-LC<sub>50</sub> value for *C. granulata* adults (Bigi et al. 1996).

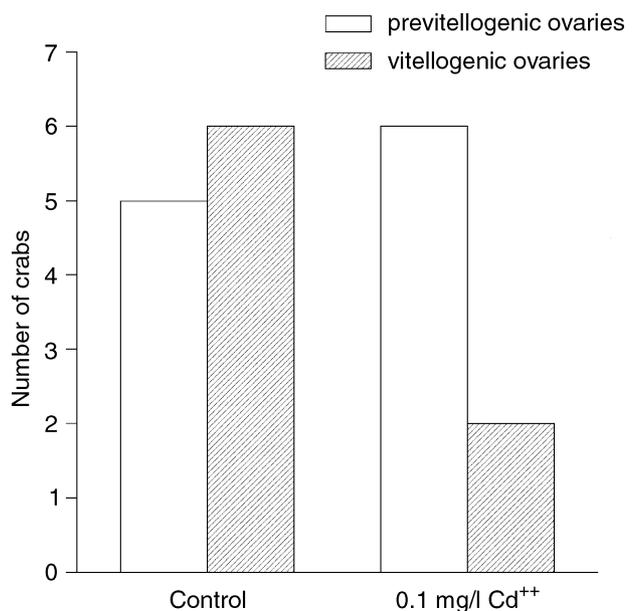
Cadmium also produced a drop in the percentage of crabs with mature ovaries after molting. Although no significant differences were found with the number of crabs used, the percentages ranged from 54.5% in the control to 25% in cadmium-exposed crabs. That, in principle, would indicate a delay in the acquisition of sexual maturity. Ravera (1991) reported a similar delay for pulmonate snails exposed to several heavy metals, with cadmium being the one that also strongly affected fertility. Nevertheless, some contradictory reports about the effect of cadmium on gonadal growth have been made in different species. Thomas (1989) found an acceleration of gonadal growth in fish, as well as did Gould et al. (1988) in mollusks, while Povlsen et al. (1990) and Paksy et al. (1990) have reported inhibition in fish and rats, respectively.

Studies made on decapod crustaceans showed inhibitory effects of heavy metals, like mercury, on gonadal growth (Reddy et al. 1997). Reduction of fecundity in crustaceans exposed to cadmium and other heavy metals was also noticed by Nagabhushanam et al. (1998), and the reproductive impairments caused by heavy metals have been strongly related to imbalances in hormonal secretion and/or synthesis (Fingerman et al. 1996). A recent study made with cadmium on the crab, *Uca pugilator* (our own results), also found inhibition of gonadal growth of adults during the pre-reproductive season, strongly suggesting interference by heavy metals with the secretion of the hormones typically reported as involved in gonadal growth. Concerning juveniles, the eventual effect of cadmium on the secretion of methyl farnesoate, the "juvenile crustacean hormone",

an endogenous gonadotropin for both juveniles and adults (Laufer et al. 1998), should not be disregarded as a way to explain the results obtained in the current study.

Although the cadmium concentration of 0.1 mg/l, while dangerous for gonadal maturation of juveniles, is far below the mean cadmium concentration reported for the Río de la Plata estuary and referred to in the Introduction (Comisión Administrativa del Río de la Plata 1990); however, chronic intake of cadmium from both water and sediments (fed on by crabs as detritus) could be a relevant factor that has not been studied previously.

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**Fig. 3.** Ovarian development in juveniles of *Chasmagnathus granulata* at the end of the experiment.

**Table 3.** Main proportion of each ovarian pathology (on total number of oocytes or nuclei)

Kind of oocyte	Pathology	Treatment	
		Control	0.1 mg/l Cd <sup>++</sup>
Primary	hypertrophy	0.210 ± 0.035 (11)	0.491 ± 0.041* (8)
	cariorhexis	0.015 ± 0.005 (11)	0.057 ± 0.013* (8)
Secondary	atresia	0.195 ± 0.109 (6)	0.768 ± 0.090* (2)

\*indicates significant differences ( $p < 0.05$ ) found with respect to the control. The number of cases (measured crabs) is indicated in parentheses.

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## 鎘處理對幼態雌性粒狀張口蟹生長與性腺發育之影響

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幼態雌性粒狀張口蟹，在蛻皮前 50 天及蛻皮期間 15 天以鎘處理(0.1 及 0.5 mg/l)。其性腺經切除、固定後作組織病理檢查、並定量其背甲之鈣含量。蛻皮前之幼態雌蟹經 0.5 mg/l 鎘處理後，多數致死，僅少數存活並蛻皮。在 0.1 mg/l 鎘處理後，大部分螃蟹可存活並蛻皮，但其性腺比對照組呈現顯著病變，包括初級卵母細胞肥大、破裂及次級卵母細胞萎縮。經 0.1 mg/l 鎘處理後，在蛻皮後其卵巢無明顯病變，但性成熟顯著延緩。

**關鍵詞：**鎘，卵巢，甲殼類，性成熟。

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