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Toxicity of mercury during the embryonic development of *Chasmagnathus granulatus* (Brachyura, Varunidae) ☆

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Abstract

Ovigerous females of the estuarine crab *Chasmagnathus granulatus* were exposed to mercury (0.1 mg/L) during the entire, early, or late embryonic development. A delay in the egg incubation period and some morphological abnormalities were detected in larvae hatched from exposed females. Particularly, hypopigmentation of body chromatophores was the abnormality that showed the highest incidence, this incidence being greater when ovigerous females were exposed to mercury either during the totality or just the first half of the egg incubation period. In contrast, the effect of mercury on the morphology and pigmentation of eyes was greater when the exposure comprised the totality or just the second half of the incubation period. These results correlate with the timing of both body pigment synthesis and eye formation during embryonic development. Although these abnormalities have been observed in the same species with other heavy metals, such as zinc and copper, the responsiveness during the early and late embryonic development was different with mercury.

Keywords: Mercury; Crustacean larvae; Abnormalities; Eye; Pigmentation

1. Introduction

Pollution by mercury is widespread in the aquatic environment, with a clear tendency toward an increase (Nriagu and Pacyna, 1988; Botton, 2000; Gonzalez et al., 2002). It is one of the most common, persistent, and toxic pollutants in the aquatic environment (see, e.g., González et al., 2002, for a review). Crustaceans have been reported to accumulate heavy metals from the environment, and many species have been used as bioindicators (Pérez, 1999; Chou et al., 2002). Particularly, many toxic effects have been reported in larvae, postlarvae, and embryos (MacRae and Pandey, 1991; Selvakumar et al., 1996; Rodríguez and Medesani, 1994; López Greco et al., 2002, among others). The great affinity that mercury, as other heavy metals, has to sulfur groups is well known, changing the structure and function of many proteins (Viarengo and Nott, 1993). On the other hand, mercury is a nonessential cation that tends, as a consequence, to progressively accumulate in exposed organisms (Rainbow, 1988). Embryonic development, perhaps the most complex stage during the life cycle of an organism, can be affected by mercury through the disruption of several processes: e.g., the inhibition of metabolic pathways and the assembling of microtubules and microfilaments, leading to failure in the energetic balance, cellular division and differentiation, and cellular migration, among other events (Itow et al., 1998).

☆ All assays were conducted in accordance with the guidelines for human protection and animal welfare of our institution (University of Buenos Aires, Faculty of Exact and Natural Sciences) as well as by other competent organizations (American Public Health Association, American Water Works Association, Water Pollution Control Federation).

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The Río de la Plata is one of the largest South American estuaries, an area in which both commercial and noncommercial fisheries are active. In the external zone of this estuary lives the crab *Chasmagnathus granulatus*, an ubiquitous species distributed from Rio de Janeiro (22°S, Brazil) to the Gulf of San Matías (41°S, Argentina). Adult and juvenile crabs live in the meso- and supralittoral zones of the coast, forming very dense populations. This species reproduces during spring and summer; toward the end of the egg incubation period, the ovigerous females migrate offshore to allow the hatching of larvae and subsequent larvae development (López Greco and Rodríguez, 1999). As a relevant component of the benthic community, this species can play a major role in transferring pollutants to higher trophic levels (Oberdorster et al., 1999). Both the larvae and the adults of several fish species predate on larval and adult crabs, respectively (Menni, 1983; Sánchez et al., 1991).

Levels of mercury above the permissible threshold for the protection of aquatic life (0.01 μg/L) have been reported in the Río de la Plata estuary (Comisión Administradora del Río de La Plata, 1990). Several studies of the toxicity of pollutants on embryonic development and larvae hatching involving heavy metals other than mercury have been carried out previously on *C. granulatus* (Rodríguez and Pisanó, 1993; Rodríguez and Medesani, 1994; Zapata et al., 2001; Lavolpe et al., 2004). This study was aimed at evaluating the toxicity of mercury on developing embryos and hatched larvae of *C. granulatus*, addressing the effect of exposures on the entire period of embryonic development, as well as on only early or late embryonic development.

2. Materials and methods

Ovigerous females of *C. granulatus* were randomly collected at the southern edge of Samborombon Bay, an unpolluted area located at the mouth of the Río de la Plata estuary, Argentina (Comisión Administradora del Río de la Plata, 1990). Once in the laboratory, an egg sample was taken from each ovigerous female and inspected under a stereomicroscope to determine the degree of embryonic development. Only those females carrying early embryos, i.e., those with plenty of vitellin with no embryonic tissue observable (Bas and Spivak, 2000), were selected for the experiments.

For the toxicological bioassays, each female was isolated in a glass jar containing 500 mL of saline water prepared by diluting salts for artificial sea water (Marine Mix, Germany) in chlorine-free tap water (final salinity, 30‰, pH 7.4), following a methodology previously used for this species (Rodríguez and Pisanó, 1993; Rodríguez and Medesani, 1994; Lavolpe et al., 2004). Assays were performed at 20 ± 1 °C, with a 14 h light:10 h dark photoperiod and continuous aeration.

Analytical-grade mercury chloride was used (Merck, Damstadt, Germany). A stock solution was prepared in deionized water, from which small aliquots were added to the saline water in the aquaria, in order to obtain the desired mercury concentration. The nominal concentration assayed was 0.1 mg/L, a concentration chosen according to the results of preliminary range-finding tests. Up to 20 ovigerous females were assigned to each of the following experimental groups:

C–C: females maintained in clean (only saline) water during the entire egg incubation period.

Hg–Hg: females exposed to mercury during the entire egg incubation period. A higher number of females was assigned to this group due to a possible enhanced mortality compared to that in the other groups.

C–Hg: females maintained in clean water for 7 days and then exposed to mercury until larval hatching.

Hg–C: females exposed to mercury for 7 days and then kept in clean water until larval hatching.

All solutions were replaced twice a week. Females were not fed during the assays. They were checked daily for mortality, egg loss, or hatching larvae. Following previous methodology (Rodríguez and Pisanó, 1993), two 10-mL samples were taken from each pool of hatched larvae and fixed in 5% formalin, with a shaking to obtain a homogeneous larvae distribution. The number of hatched larvae was estimated by calculating the mean of the two samples in relation to the total volume of water in the jar. To determine the proportion of abnormalities in each spawning, a random subsample of 50 larvae was examined under a stereomicroscope; the presence of each abnormality detected was recorded as in previous works (summarized by Lavolpe et al., 2004). The number of hatched larvae, the incubation time, and the proportion of each abnormality (after angular transformation) were evaluated by means of a one-way ANOVA. Comparisons between each concentration and the controls were carried out by the least-significant-difference method. The confidence level was always 95% (Sokal and Rohlf, 1979).

3. Results

Practically no mortality or egg losses were noticed in the experimental groups, i.e., no significant differences ($P > 0.05$) with respect to the control (C–C group) were found in any case. Instead, mercury produced a significant delay ($P < 0.05$) in the egg incubation period during both the early and the late exposure (Hg–C and C–Hg groups, respectively) compared to controls. No statistical differences ($P > 0.05$) were found in the number of hatched larvae, although a tendency to a