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Is the Digestive Tract an Important Access Route for Mercury in the Chinese Crab *Eriocheir sinensis* (Crustacea, Decapoda)?

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In crustaceans, when direct contamination is applied, metal accumulation and toxicity change as a function of medium salinity (Rainbow and Kwan 1995; Bianchini and Gilles 1996; Rainbow 1997). Despite that, crustaceans gills are usually the most contaminated organs when mercury is considered (Luoma 1976; Andersen and Baatrup 1988; Brown et al. 1988; Bianchini and Gilles 1996). Further, it has been demonstrated that important fluxes of mercury occur across the gills of the euryhaline Chinese crab *Eriocheir sinensis* acclimated either to freshwater or seawater (Bianchini and Gilles 1996). Thus, several data indicate that gills could be an important access route for mercury. Nevertheless, we cannot disregard a possible contribution of other access routes, specially the digestive tract. In fact, it has been reported that food play a major role as metal source in insects (Hare 1992) and crustaceans (Weeks and Rainbow 1993). Further, it has been demonstrated that gills and gut could be important transfer routes for mercury in insect nymphs (Saouter et al. 1991; Odin et al. 1995, 1996). We have, thus, analyzed the contribution of the digestive tract for the *in vivo* mercury accumulation in different tissues of the Chinese crab *Eriocheir sinensis.*

MATERIALS AND METHODS

Adult male Chinese crabs *Eriocheir sinensis* (55-75g) were captured in freshwater lakes near Emden (Germany) and maintained in tanks with either running tap water or seawater, continuously filtered and aerated, for at least ten days. All crabs were in stage C of the intermoult cycle and were fed during the acclimation period with pellets for trout.

Acclimated crabs were divided into the control and experimental groups. The crabs of the last group had their oral cavity completely blocked with a cianoacrylate glue. All crabs were then maintained in aquaria containing 20 L of the experimental medium previously contaminated with 1 ppm of mercury as $HgCl_2$ (Merck), for 32 hr (freshwater) or 48 hr (seawater). Times of exposure were selected considering the LT_{50} for mercury chloride when *E. sinensis* was exposed either to freshwater or seawater (Bianchini and Gilles 1996). Experimental media were renewed daily. Experiments were performed at 15±3°C. After different exposure times, hemolymph from 5 crabs was collected and weighed. Crabs were

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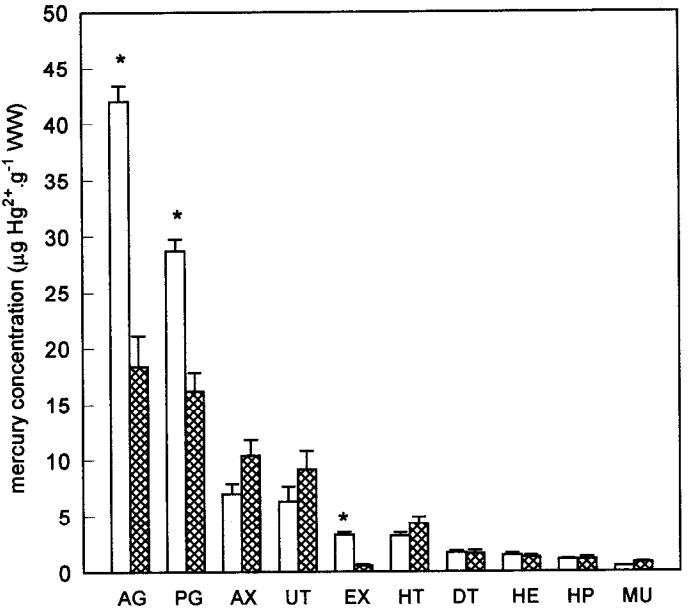

Figure 1. *In vivo* mercury accumulation in anterior gills (AG), posterior gills (PG), axon (AX), urinary tract (UT), exoskeleton (EX), heart (I-IT), digestive tract (DT), hemolymph (HE), hepatopancreas (HP), and muscle (MU) of freshwater (open bars) and seawater (crossed bars) acclimated Chinese crabs, *Eriocheir sinensis,* after 24 hr of water contamination with 1 ppm of mercury as $HgCl_2$. Data are means ± SE (n=5). * Indicates means significantly different between acclimation media.

then sacrificed and the axons and muscle of the pereiopods, exoskeleton, digestive and urinary tracts, heart, hepatopancreas, and gills (anterior and posterior) were dissected, dried, and weighed. Samples were frozen at -20°C until mercury determination. Tissue mineralization and mercury determination were performed as previously described (Bianchini and Gilles 1996). Briefly, tissues were mineralized with sulfuric acid and the mercury concentration measured by atomic absorption spectrophotometry (Perkin-Elmer 50 A, detection limit = 0.05 µg; range = 0.05 to 0.70 µg; recovery = 97%). For calibration, different volumes of a standard solution of $HgCl_2$ (1 ppm) were added to mineralized tissues from non-contaminated crabs. Results were submitted to ANOVA followed by Tukey's multiple range test (α=0.05).

RESULTS AND DISCUSSION

After 24 hr of exposure, mercury accumulation in gills (anterior and posterior) and exoskeleton of freshwater acclimated *E. sinensis was* higher than that observed in seawater acclimated crabs. Further, in both freshwater and seawater acclimated crabs, gills were the most contaminated tissue (Fig. 1). These facts were observed even though the crab oral cavity was blocked (Figs. 2 and 3). This larger

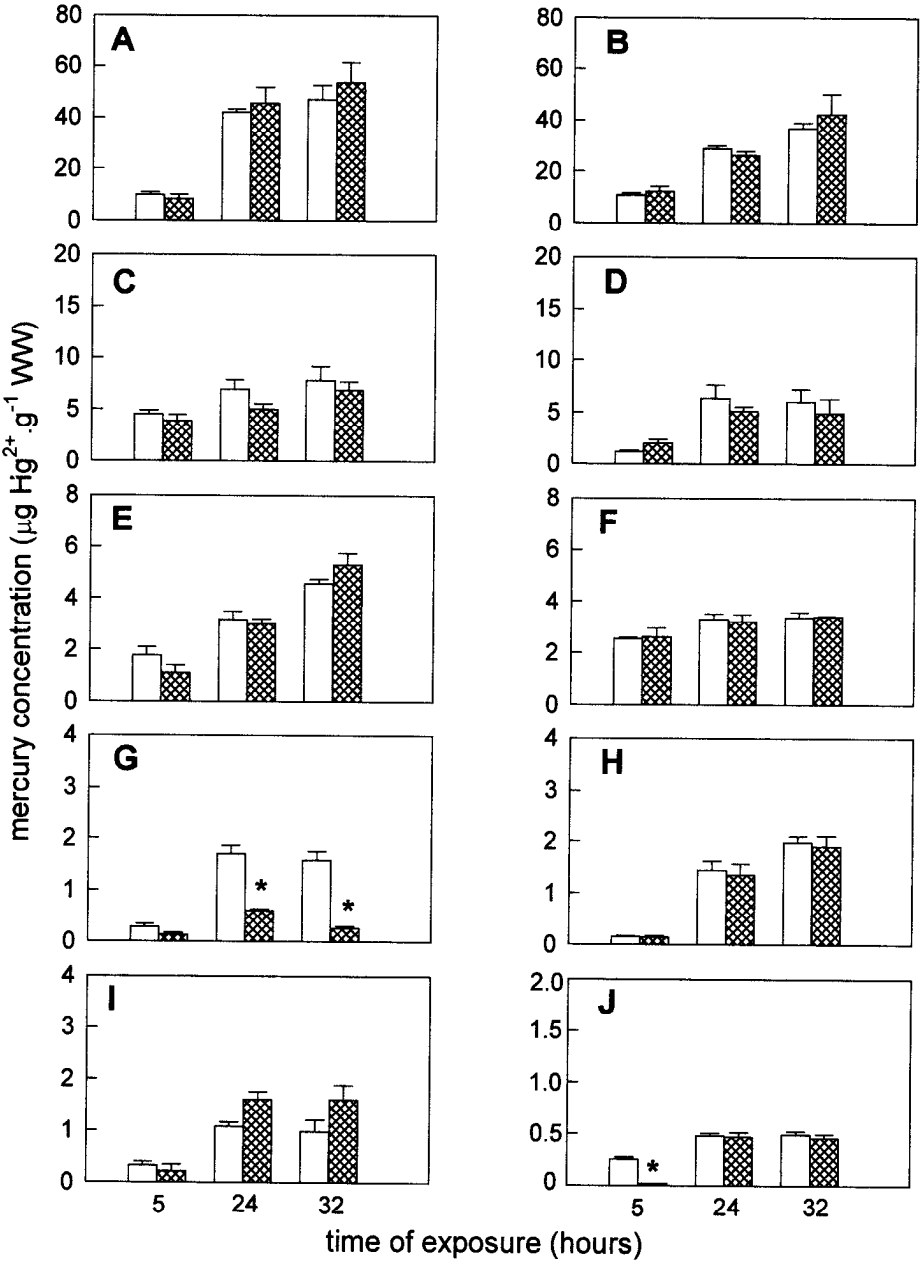

Figure 2. *In vivo* mercury accumulation in anterior gills (A), posterior gills (B), axon (C), urinary tract (D), heart (E), exoskeleton (F), digestive tract (G), hemolymph (H), hepatopancreas (I), and muscle (J) of control (open bars) and experimental (crossed bars) Chinese crabs, *Eriocheir sinensis*, acclimated to freshwater and contaminated with 1 ppm of mercury as $HgCl_2$. Data are means ± SE (n=5). * Indicates mean significantly different from the control condition.

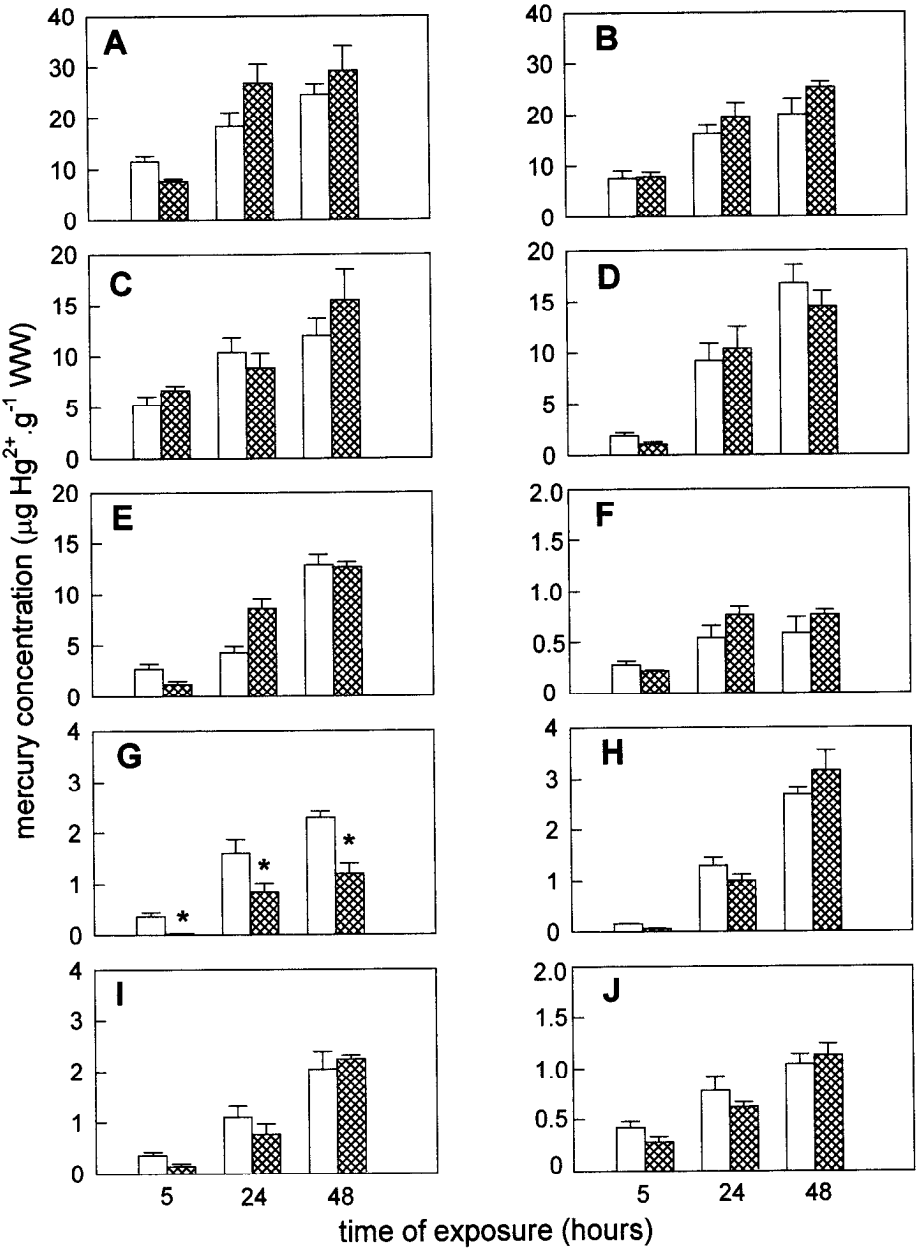

Figure 3. *In vivo* mercury accumulation in anterior gills (A), posterior gills (B), axon (C), urinary tract (D), heart (E), exoskeleton (F), digestive tract (G), hemolymph (H), hepatopancreas (I), and muscle (J) of control (open bars) and experimental (crossed bars) Chinese crabs, *Eriocheir sinensis*, acclimated to seawater and contaminated with 1 ppm of mercury as $HgCl_2$. Data are means ± SE (n=5). * Indicates mean significantly different from the control condition.

accumulation of metal in low salinities can be related both to a higher metal adsorption on the chitinous exoskeleton (Bianchini and Gilles 1996) or the binding to transporting proteins from the membranes, like pumps and channels. In *E. sinensis,* Bianchini and Gilles (1996) showed that mercury can be adsorbed on the external integuments and that this phenomenon was sensitive to a salting out process in which some salt(s) concentrated in seawater displace(s) it from sites it can occupy in freshwater. Concerning gill ion channels and pumps, in *E. sinensis* they are more active at lower salinities (Péqueux 1995). In fact, the higher metal toxicity to *E. sinensis* exposed to low salinities has been attributed to an osmoregulatory imbalance due to an inhibitory effect of mercury on these transport mechanisms (Péqueux et al. 1996).

Regarding the effect of oral cavity blockage, mercury accumulation was similar for both control and experimental crabs, except in the digestive tract. This fact was observed in both freshwater (Fig. 2) and seawater (Fig. 3) acclimated crabs. A reduction of more than 50% in tissue mercury was observed. These results demonstrate that approximately 50% of the mercury charge in the digestive tract could be due to a mercury uptake which takes place at this system The fact that the total blockage of the oral cavity only significantly changed the mercury charge in the digestive tract, demonstrates that contamination of internal tissues and organs was due to mercury uptake via gills. According to Bianchini and Gilles (1996), a net influx of mercury takes place only through anterior gills.

It is also important to note that the effect of oral cavity blockage was similar in freshwater and seawater acclimated crabs (Figs. 2 and 3), suggesting a similar mercury balance. This was expected, since the crabs fasted during experiments, and the water intake in *E. sinensis* does not seem to change with the salinity increase. This latter assumption is based on the fact that, in seawater, this crab is isosmotic in relation to the medium (Schoffeniels and Gilles 1970). Further, despite a differential accumulation of metal in gills from Chinese crab as a function of medium salinity, the rate of mercury uptake by gills is not dependent upon that parameter. It has been demonstrated that the application of an osmotic gradient did not affect the metal net flux across the isolated anterior or posterior gill of this species (Bianchini and Gilles 1996). A similar result, for other metals, was described for the amphipod crustacean *Orchestia gammarellus.* In this species, the uptake of cadmium and zinc did not increase in low salinities (Rainbow and Kwan 1995).

Our results show that, during direct mercury contamination, the digestive tract does not play an important role in the metal accumulation in the Chinese crab *E. sinensis,* either in freshwater or seawater. These results, and those reported by Bianchini and Gilles (1996), suggest that the anterior (respiratory) gills really appear to be the most important access route for mercury in this species. This access route could not only be important for non essential metals uptake, but it could play an important role in the trace metal uptake, as described for the amphipods *O. gammarellus* and *O. mediterranea.* In this case, food was the major source of zinc for both species, but *O. mediterranea was* unable to satisfy its

copper requirements from food source, achieving it only through absorption from solution (Weeks and Rainbow 1993).

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