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Toxicity and Accumulation of Mercury in Three Species of Crabs with Different Osmoregulatory Capacities

A. Bianchini,* R. Gilles

Laboratory of Animal Physiology, University of Liège, 22 Quai Van Beneden, B-4020 Liège, Belgium

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Synergism between mercury and salinity has been shown in invertebrates (for review: McLusky et al. 1986). Two authors have tried to correlate salinity effects with a higher or lower accumulation of mercury. Zauke (1977), demonstrated lower mercury levels in several benthic invertebrates from limnic regions of the Elbe estuary when compared to those from marine regions. On the other hand, Kendall (1978) did not report any significant difference in mercury concentrations in benthic macroinvertebrates throughout a salinity gradient in two estuaries from Georgia. In species hyperosmoregulating in diluted media, it could, however, be considered that the high water turnover would favor mercury accumulation In this context, one could also expect a relationship between environmental salinity and mercury toxicity in different euryhaline species depending on their osmoregulatory capacities. We have tested this hypothesis analyzing the toxic effects and accumulation of mercury in three euryhaline crabs presenting different osmoregulatory capacities: ***Eriocheir sinensis (strong*** hyperosmoregulator), ***Carcinus maenas*** (weak hyperosmoregulator) and Cancer ***pagurus*** (osmoconformer) (Schoffeniels and Gilles 1970; Gilles 1974; Wanson et al. 1983).

MATERIAL AND METHODS

Eriocheir sinensis (adult males; 55 - 75 g), were captured in freshwater lakes near Emden (Germany) and maintained in tanks with running tap water at the laboratory. *Carcinus maenas* (adult males; 45 - 65 *g),* were purchased from town markets and maintained in tanks with seawater continuously filtered and aerated. Experiments on *Cancer pagurus* (young males; 45 - 55 g) were performed at the Marine Biology Laboratory of the "College de France" (Concarneau, France). They were maintained in tanks with seawater continuously filtered and aerated.

*Present address: Laboratbrio de Zoofisiologia, Departamento de Ciencias Fisiologicas, Fundacao Universidade do Rio Grande, C.P. 474, 96201-900 Rio Grande/RS, Brazil
Correspondence to: A. Bianchini

All crabs used were in stage C of the intermolt cycle and were fed only during the acclimation period. They were acclimated for at least ten days at different salinities according to their osmoregulatory capacities. *E. sinensis* was acclimated to seawater (SW), twice diluted seawater (SW/2), or freshwater (FW). *C. maenas* was acclimated to SW or one third seawater (SW/3) while *C. pagurus* was acclimated to SW or SW/2. Crabs were exposed in aquaria containing 20 L of experimental medium contaminated with 1 ppm of mercury as $HgCl_2$ (Merck). Experimental media were renewed daily. All experiments were performed at 15 ± 3°C.

The study of mercury toxicity was performed with crabs (n = 15-30; Table 1) acclimated to different salinities. Accumulated mortality curves were established and the LT_{50} determined and statistically compared following Rodriguez (1991).

For the analyses of *in vivo* accumulation of mercury, 5 crabs were exposed to mercury in each medium at each time of exposure. The time of exposure changed with the species and the salinity tested, in view of the different LT_{50} of each species. *E. sinensis* were exposed to mercury for 5, 24 and 48 hr in SW, however they were exposed for 5, 24 or 32 hr in FW. *C. maenas* were exposed for 5, 24 or 48 hr in both SW and SW/3. *C. pagurus were* exposed for 8, 16 or 28 hr in both SW and SW/2. After the crabs were exposed to mercury, hemolymph was then collected, weighed, and frozen at -20°C until mercury determination. Crabs were then killed and the axons and muscle of the pereiopods, exoskeleton, digestive and urinary tracts, heart, hepatopancreas, and gills were dissected, dried, weighed and frozen at -20°C until mercury determination The latter were separated into their anterior and posterior regions due to their structural, biochemical, and functional differences (Gilles and Pequeux 1986; Gilles et al. 1988; Pequeux 1995 for review).

The ***in vitro*** study of mercury accumulation was performed on pieces of dorsal exoskeleton isolated from ***E. sinensis*** acclimated to SW or FW and *C.* ***pagurus*** acclimated to SW or SW/2 not previously contaminated. These pieces were incubated in aquaria containing 2 L of one of the acclimation media (SW, SW/2 or FW) contaminated with 1 ppm of mercury. Some fragments were picked up at different times of exposure to mercury, rinsed, dried, weighed and frozen at -20°C until mercury determination.

Mercury determinations were achieved on tissues previously 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. Data were analyzed by Kruskal-Wallis ANOVA ($\alpha = 0.05$) due to lack of homogeneity of variances.

The unidirectional movement of mercury through the isolated gills of ***E. sinensis*** were estimated using the radiotracer $^{203}Hg^{2+}$ (0.20 µCi.mg^{-1} Hg^{2+}. Gills were isolated and perfused using the method described by Gilles et al. (1988). They were perfused and/or

incubated with the following solutions: (a) "FW saline" (NaC1 240 mM; KC1 5 mM, $MgCl_2$ 5 mM; $CaCl_2$ 12.5 mM, H_3BO_3 8.8 mM; pH 7.6 with Tris-base); (b) "SW saline" (NaCl 480 mM, KC1 10 mM, $MgCl_2$ 10 mM, $CaCl_2$ 25 mM; H_3BO_3 8.8 mM; pH 7.6 with Tris-base) and (c) freshwater. Mercury (1 ppm) was present in both incubation and perfusion solutions. Sample radioactivity was measured by gamma scintillation (Packard). Data were analyzed by ANOVA and Tukey test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Mercury (1 ppm) was lethal for all species studied and the LT_{50} depends on the species and salinity tested (Table 1). In *Eriocheir sinensis,* it was more toxic in FW than in SW/2 or SW. However, its toxicity was similar in SW/2 and SW. In *Carcinus maenas,* mercury was more toxic in SW/3 than in SW. On the contrary, for *Cancer pagurus,* no correlation between mercury toxicity and salinity could be observed (Table 1). These results can be interpreted considering a relationship between mercury accumulation and osmoregulatory capacity. *E. sinensis* and *C. maenas* in FW and in SW/3, respectively, are hyperosmoregulating, maintaining their hemolymph osmolarity higher than that of the external medium (Schoffeniels and Gilles 1970; Gilles 1974). In these conditions, water influx through integument and the urinary flow are largely increased (Kirschner 1991 for review). This increased water turnover could induce an increase in mercury inward diffusion which could in turn account for faster and larger accumulation and therefore for higher toxicity. The fact that toxicity is similar for *C. pagurus* or *E. sinensis* in SW and SW/2 is in agreement with this hypothesis. In these species the blood is indeed isosmotic with the external medium at any salinity between SW and SW/2 (Gilles 1974; Wanson et al. 1983). In these conditions, there should thus be no

Table 1. LT_{50} (hr) for crabs exposed to 1 ppm of mercury as $HgCl_2$ at different salinities. Values in brackets represent the number of crabs employed.

Species	Media	LT_{50}	95% confidence limits
E.sinensis	SW (20)	96.4	86.0 - 107.8 (a)
	SW/2 (10)	99.8	81.7 - 118.9 (a)
	FW (15)	39.3	37.5 - 40.7 (b)
C. maenas	SW (20)	70.2	63.8 - 76.6 (a)
	SW/3 (30)	38.8	36.1 - 41.4 (b)
C. pagurus	SW (15)	46.7	37.3 - 53.4 (a)
	SW/2 (15)	44.1	40.6 - 47.2 (a)

For the same species, equal letters represent LT_{50} not significantly different at different salinities.

change in water fluxes and consequently no modification in mercury accumulation and toxicity. Also in agreement with this hypothesis is the fact that accumulation of mercury is larger in the exoskeleton and the gills (anterior and posterior) of *E. sinensis* and *C. maenas* when hyperosmoregulating in diluted media (FW or SW/3); a phenomenon which is not seen in *C. pagurus* which remains osmoconforming at the dilution tested (SW/2) (Figs. 1 and 2). Diffrential accumulation of mercury with acclimation salinity in *E. sinensis* and *C. maenas* is, however, not seen in the internal tissues tested for contamination: digestive and urinary tracts, hepatopancreas, muscle, axons, hemolymph, and heart. Here, there is no clearcut difference in accumulation, which thus appears independent of the medium salinity (Figs. 1 and 2). This might indicate that the entrance of mercury is not primarily related to the water influx. In this view, the effect of salinity on mercury accumulation in exoskeleton and gills must be due to some other factor. Our measurements of mercury flux in isolated gills are in agreement with this idea. They indeed show that the unidirectional fluxes, IN as well as OUT, remain independent of both the salinity of acclimation medium and the establishment of a large osmotic gradient across the epithelium (Fig. 3). This last result further indicates that the movement of mercury across the gills is not at all related to the water movement. The nature of mercury movement is actually unknown In the anterior gills, the influx is much larger than the efflux, leading to a large net inward movement which might be related to some active process. More data would be needed to assess this hypothesis.

The fact that the mercury fluxes are independent of the osmotic gradients leads to the problems of (1) the cause of a much larger accumulation in gills and exoskeleton of both *E. sinensis* and *C. maenas* when in diluted media (FW or SW/3), (2) the cause of the higher toxicity of the metal in both species when acclimated to these media.

As far as the larger ***in vivo*** accumulation is concerned, our results show that accumulation is also larger ***in vitro,*** on dissected, isolated pieces of exoskeleton placed in freshwater rather than in seawater (Pig. 4). This can be explained considering that mercury can be adsorbed on the external integuments, this phenomenon being sensitive to a salting out process in which some salt(s) concentrated in seawater displace(s) it from sites it can occupy in freshwater.

As far as the higher toxicity is concerned, one can consider that the larger accumulation on the gills disrupt their respiratory and/or osmoregulatory functions, thus leading to an impairment in the maintenance of the blood ion levels in diluted media. Effects of mercury on respiration in crab gills have already been described (Hunter 1949; Depledge 1984) and it has been shown that it affects the osmoregulatory capacities of ***E. sinensis*** as well as *C.* ***maenas*** when in diluted media (Bjerregaard and Vislie 1985; Bianchini 1990; Pequeux et al. 1995).

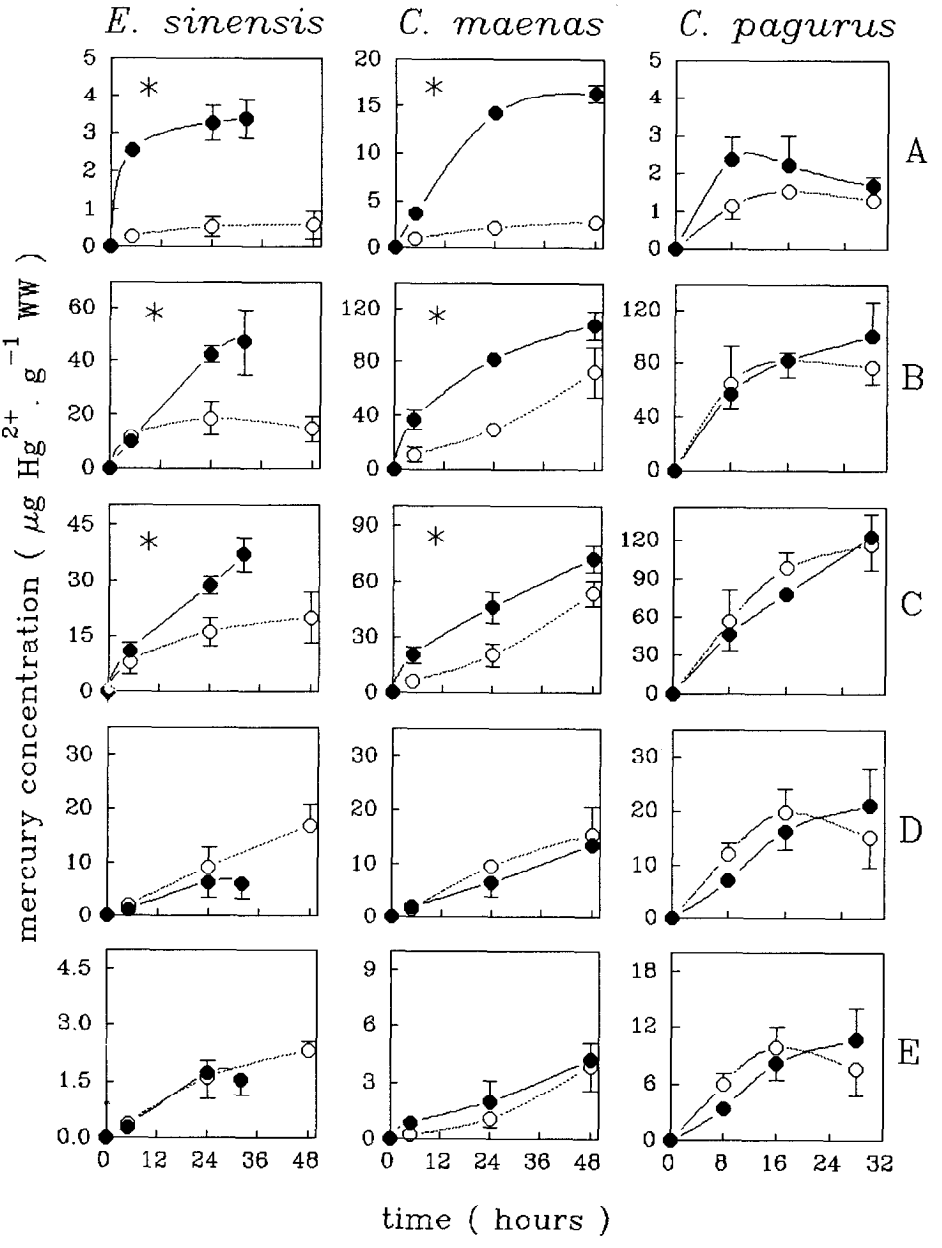

Figure 1. " *In vivo* " mercury accumulation in exoskeleton (A), anterior gills (B), posterior gills (C), urinary tract (D), and digestive tract (E) of *Eriocheir sinensis, Carcinus maenas,* and *Cancer pagurus* after water contamination with 1 ppm of mercury as $HgCl_2$. Data are means ã SD (n=5). (0 seawater - 1 freshwater for *E. sinensis,* SW/3 for *C. maenas,* and SW/2 for *C. pagurus).* * Significant effect of salinity ($P<0.05$)

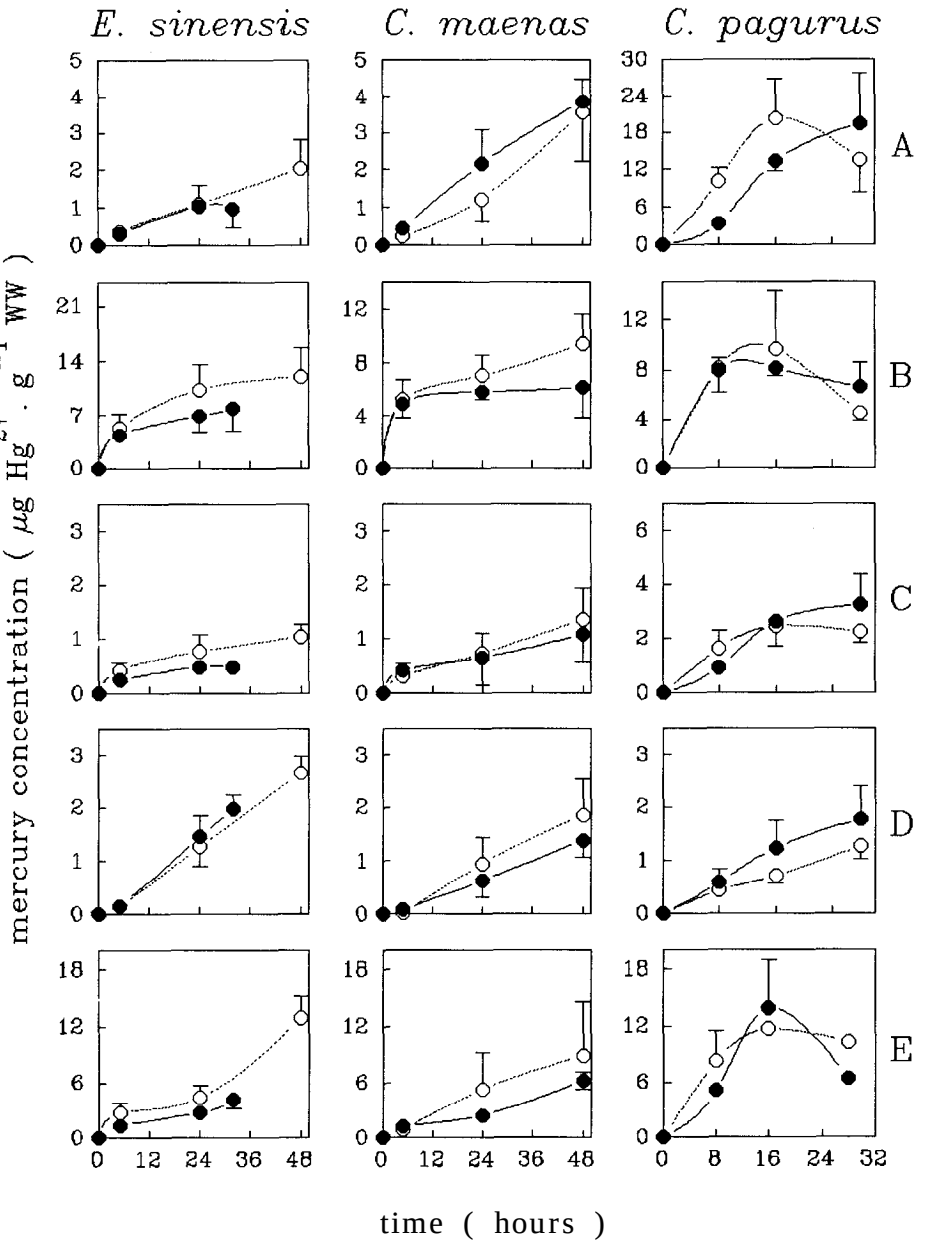

Figure 2. " In vivo " mercury accumulation in hepatopancreas (A), axons (B), muscle (C), hemolymph (D), and heart (E) of *Eriocheir sinensis, Carcinus maenas.* and *Cancer pagurus* after water contamination with 1 ppm of mercury as $HgCl_2$. Data are means ã SD (n=5). (O seawater - ●freshwater for *E. sinensis,* SW/3 for *C. maenas,* and SW/2 for C. pagurus).

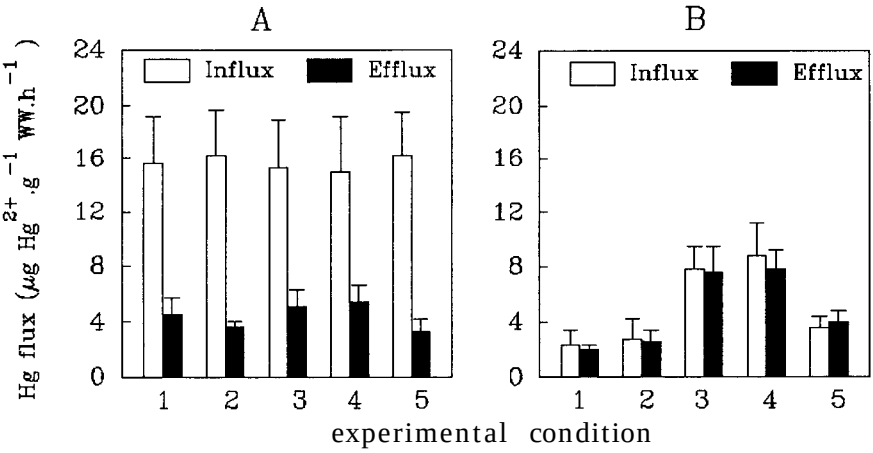

Figure 3. Unidirectional fluxes of mercury through anterior (A) and posterior (B) isolated, perfused gills of *Eriocheir sinensis* acclimated to freshwater (1; 2 or 3) or seawater (4 or 5), in different experimental conditions : (1) perfused by seawater saline and incubated in freshwater; (2) and (5) perfused by and incubated in freshwater saline; (3) and (4) perfused by and incubated in seawater saline. Perfusion and incubation media contain 1 ppm of mercury as $HgCl_2$. Data are means ã SD (n = 5). In A, influxes or effluxes are not different at different experimental conditions (P>0.05). In B. for the same condition, influx and efflux are not significantly different (P>0.05).

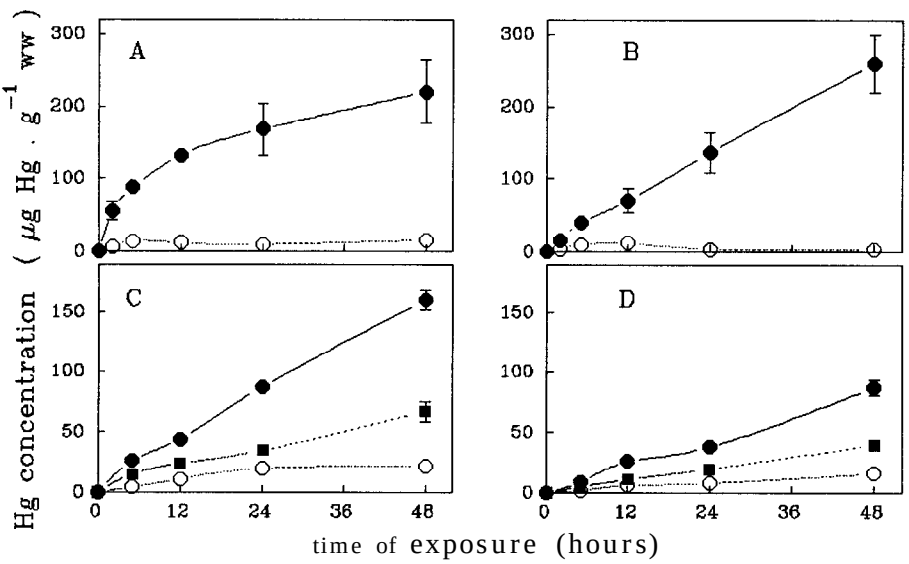

Figure 4. *"In vitro"* mercury accumulation in pieces of exoskeleton isolated from *Eriocheir sinensis* acclimated to freshwater (A) or to seawater (B) and from *Cancer pagurus* acclimated to seawater(C) or to twice diluted seawater (D) in different incubation media: ● freshwater: O seawater: ■ twice diluted seawater. Incubation medium was contaminated with 1 ppm of mercury as $HgCl_2$. Data are means±SD (n=5). In all four cases, salinity had a significant effect (P<0.05) on the mercury accumulation.

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