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Heavy Metals in the Sea-Skater *Halobates robustus* from the Galápagos Islands: Concentrations in Nature and Uptake Experiments, with Special Reference to Cadmium

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Abstract

Samples of *Halobates robustus* Barber (Heteroptera: Gerridae) from the Galápagos Islands were analysed by optical emission spectrometry. The levels (in $\mu\text{g g}^{-1}$ dry weight) of Zn (134), Cu (155), Pb (<1), Cd (7), and Cr (3) were not significantly different among insects of different sexes or developmental stages. The low natural levels of Cd in *H. robustus* from the relatively unpolluted environment of the Galápagos Islands are compared to the high concentrations of Cd in *Halobates* spp. from relatively polluted regions. Since the measured levels of Cd in their natural zooplankton food rarely exceed $10 \mu\text{g g}^{-1}$, and very little of the Cd is found in the soft tissues, the high Cd concentrations (100 to $200 \mu\text{g g}^{-1}$) in some sea-skater species have evidently been derived by drinking from the surface microlayer of the seawater.

Introduction

Cadmium (Cd), when ingested, is toxic to man and other animals (Friberg *et al.*, 1971). Because of its toxicity, Cd is one of the heavy metals which has been most extensively investigated in the marine environment. Cd concentration rarely exceeds $0.1 \mu\text{g l}^{-1}$ in the open ocean, and is generally less than 10 ng l^{-1} in nutrient-depleted surface waters (Boyle *et al.*, 1976; Bruland *et al.*, 1978). However, in some geographic locations, especially nearshore waters near industrialized areas, the Cd content of seawater can be considerably elevated, presumably by anthropogenic pollution (Westernhagen *et al.*, 1974). In such places, Cd concentration of the surface film, like other heavy metals, may be several hundred or thousand times greater than that of the underlying water (Duce *et al.*, 1972; Weisel, 1978). The Cd in the surface film might be expected to affect pleustonic organisms which live at the sea surface (see Cheng, 1975); such effects might be toxic reactions or, at least, elevated Cd concentrations in the bodies of the organisms. High levels of Cd (100 to $200 \mu\text{g g}^{-1}$) have indeed been found in some sea-skaters (hemipterous insects of the genus *Halobates*) living in the Gulf of California (Cheng *et al.*, 1976), where-

as plankton samples from the same area contained only 10 to $20 \mu\text{g g}^{-1}$ Cd (Martin and Broenkow, 1975). Similar high Cd levels were also found in Atlantic sea-skaters (Bull *et al.*, 1977). Presumably in these sea-skaters Cd was derived from the surface film, although input from Cd-enriched prey was also possible, as *Halobates* spp. live exclusively at the sea-air interface and are fluid-sucking predators (Cheng, 1974).

The first purpose of the present study was to determine natural levels of Cd and other heavy metals in the bodies of sea-skaters (*Halobates robustus*) which abound on the relatively unpolluted seawater around the Galápagos Islands, a habitat far from sources of anthropogenic pollution. As expected, heavy-metal concentrations were low, making *H. robustus* a favorable, uncontaminated starting material for uptake experiments. Our second purpose was, therefore, to study how *H. robustus* concentrates heavy metals, especially Cd, added either to seawater or to prey organisms.

Since we would like to know whether the presence of other heavy metals would suppress or enhance Cd uptake by the sea-skaters, our third purpose was to find out the effect of added Zn, Cu, Pb and Cr on the Cd uptake of *Halobates robustus*.

Materials and Methods

Specimens of *Halobates robustus* Barber were collected from three different locations at the Galápagos Islands: at Punta Cormorant (on Isla Floreana), and at Punta Espinosa and Bahía Cartago (both on Isla Isabella) during Expedition Sea-skater II on board the R.V. "Alpha Helix". Collections were made between 28 January and 8 February, 1978. The insects were caught by using a clean rigid plastic net (a vegetable strainer) with 1-mm nylon mesh, and were kept in a polyethylene bucket while in the field. Some of the insects were frozen soon after capture to serve as baseline samples, while the rest were transferred to a 100 l glass aquarium half-filled with clean, filtered seawater, where they were kept until used for uptake experiments. Experimental individuals were then transferred to polyethylene 2 l Nalgene beakers containing 500 ml of seawater and were allowed to acclimatize overnight: fewer than 30 insects were put in each beaker. During the experimental period (1 to 4 days), they were fed every morning either with control *Drosophila melanogaster* (a flightless mutant) or with similar flies which had been reared on Cd-enriched medium (see below). At the end of each experiment, the *H. robustus* were strained from the seawater with a clean plastic strainer (10 cm diameter, 1 mm mesh) and rinsed in distilled water for a few seconds. Excess water was blotted from the underside of the strainer with absorbent paper (Kimwipe), and the insects were transferred into pre-cleaned Beckman scintillation vials (20 ml). Each sample vial, with the lid screwed on, was stored in a freezer at -20°C . At the end of the expedition, all the samples were allowed to thaw; the lids were removed; and the insects were dried overnight in an oven at 60°C . The dried samples were transported back to the University of California, Los Angeles (UCLA), for heavy-metal analyses carried out with an optical emission spectrometer system (OES; see Cheng et al., 1976). For the sample size used, the sensitivities of the OES system (i.e., lower limits of detection in $\mu\text{g g}^{-1}$ dry weight) are as follows: Zn, 5.0; Cu, 0.2; Pb, 1.0; Cd, 3.0; Cr, 0.2.

The control *Drosophila melanogaster* used in these experiments were of a vestigial-winged strain, and were reared on a standard cornmeal molasses medium. Flies of the same genetic stock were raised on a high-Cd medium prepared by adding CdCl_2 to the same medium (before autoclaving) to give a final Cd concentra-

tion of about $30 \mu\text{g g}^{-1}$. In preliminary experiments, it had been found that raising the concentration of Cd to $100 \mu\text{g g}^{-1}$ inhibited normal development of *D. melanogaster*, whereas $50 \mu\text{g g}^{-1}$ or less produced no evident adverse effects and the flies emerged normally (Cheng, 1979). Control and experimental flies completed their life cycles and emerged at about the same time. Such first-generation adult flies, grown with or without added Cd, were transferred to fresh media (with or without Cd, respectively), and the adults were discarded after eggs had been laid. The second-generation flies which eventually emerged were used in the experiments on board ship. They had an average Cd concentration of $100 \mu\text{g g}^{-1}$ (dry weight). The control flies were Cd-free.

For feeding experiments, two or three live fruit flies per specimen of *Halobates robustus* were introduced into beakers containing the sea-skaters, which had been starved overnight. Feeding normally commenced immediately after the flies were introduced. Each sea-skater usually took one or more fruit flies, feeding being completed within about 30 min. Once satiated, they began to preen, and apparently took no further interest in the excess flies, which were removed about 2 h later by means of a small scoop made of plastic screening material.

In order to compare rates of uptake of Cd from food with and without the presence of additional Cd in the seawater, we also carried out similar experiments on seawater supplemented with Cd to give a final concentration of 0.5 mg l^{-1} . In another series of experiments we added a metal mixture containing 0.5 mg l^{-1} each of Zn, Cu, Pb, Cd, and Cr to the seawater. (The beakers used for these experiments were first preconditioned with the heavy-metal solution for 24 h)

All the experiments were carried out in the air-conditioned laboratory of the R.V. "Alpha Helix", where the temperature was kept at approximately 21°C . For the present purposes, the Cd content of ordinary seawater is negligible: less than $0.001 \mu\text{g g}^{-1}$ is present in non-polluted open-ocean waters.

We carried out three main sets of Cd uptake experiments with somewhat different durations and different numbers of *Halobates robustus*. In the first two uptake experiments (48 h and 96 h duration, respectively), we subjected the sea-skaters to 4 different conditions: (1) control food and control seawater; (2) control food and Cd-enriched seawater; (3) control food and heavy-metal-enriched seawater; (4) Cd-enriched food

and control seawater. In the third experiment (48 h) we used only two groups of sea-skaters, both kept on control seawater and fed with Cd-enriched food.

Since we could not analyse the insects individually using the OES system (even the largest adult females have a dry weight less than 4 mg), we pooled the specimens, using 5 to 10 sea-skaters per sample. For each 24 h period we usually obtained only one datum per sample. Nevertheless, for most samples there was good agreement among the Cd concentrations so determined.

Results

Baseline samples of *Halobates robustus* were obtained from each of the three collection sites. The samples from Floreana and Punta Espinosa were subdivided into subsamples respectively containing males, females, mixed adults and nymphs; those from Cartago Bay were not divided. Since we are still unable to rear *Halobates* spp. in the laboratory, all of these experimental studies had to be carried out and completed in the short period available during the Galápagos expedition. We did not observe any differences between the behavior and activity of insects used for uptake studies and those kept in aquaria on deck, nor did we observe any behavioral differences between insects kept on clean seawater and those kept on seawater to which Cd and other heavy metals had been added.

Although the mean concentration of each heavy metal differed from locality to locality, for Cu, Cd and Cr, the differences (one-way ANOVA, Texas Instrument TI-59 program) were not significant (at 90% level), but the values for Zn were significantly different between localities. However, when all the values were combined by sexes and developmental stages (males, females, mixed adults, mixed adults and nymphs), among the various stages there were no significant differences (at 99% level) between the means of any of these heavy metals (see Table 1). We could thus regard the overall mean content of each of the metals as the baseline value.

The results of our various uptake experiments are shown in Table 2. During the 4-day experimental period, Cd levels in sea-skaters kept on control seawater and fed control food remained more or less constant (around 6 $\mu\text{g g}^{-1}$). This value is not significantly different from that of the baseline samples measured (7 $\mu\text{g g}^{-1}$). In sea-skaters supplied with additional Cd, either in sea-

Table 1. *Halobates robustus*. Mean heavy-metal concentrations ($\mu\text{g g}^{-1}$) and standard deviations (in parentheses) of different stages in baseline samples. nd: no data

Stage	Zn	Cu	Pb	Cd	Cr
Males (n=5)	115.4 (22.8)	129.4 (23.8)	<1 (-)	6 (4)	1 (1)
Females (n=6)	145.7 (29.6)	162.3 (24.7)	<1 (-)	8 (5)	3 (3)
Nymphs (n=5)	109.8 (31.3)	134.6 (31.8)	<1 (-)	7 (3)	2 (2)
Males + females (n=11)	136.9 (29.3)	162.1 (29.3)	<1 (-)	8 (2)	3 nd
Mixed adults + nymphs (n=6)	153.8 (30.6)	173.2 (37.0)	<1 (-)	6 (3)	4 (4)
Total ^a (n=33)	134.2 (31.4)	155.0 (32.1)	<1 (-)	7 (3)	3 (2)

^a Means of all stages combined, calculated from raw data.

Table 2. *Halobates robustus*. Cd concentrations ($\mu\text{g g}^{-1}$) of sea-skaters subjected to various treatments. Numbers of pooled samples (each with 5 to 10 *H. robustus* individuals) analysed differed between treatments and duration of experiment

Treatment	Cd concentrations at various times after start of treatment			
	24 h	48 h	72 h	96 h
Control food and control seawater	6	5	6	6
Control food and Cd-enriched seawater	14	18	14	17
Control food and seawater enriched with Zn, Cu, Pb, Cd and Cr	25	17	18	17
Cd-enriched food and control seawater	10	6	8	20
	10	5		
	14	14		

water or in food, there was an increase in Cd concentration. The mean Cd concentration (17 $\mu\text{g g}^{-1}$) of sea-skaters kept on Cd-enriched seawater was much higher than that (11 $\mu\text{g g}^{-1}$) of sea-skaters given Cd in their food. This suggests that uptake of Cd from surface seawater may be the more important route.

In *Halobates robustus* kept for 4 days on seawater supplemented with a mixture of Zn, Cu, Pb, Cd and Cr (0.5 $\mu\text{g l}^{-1}$ each), the concentration of each of these met-

als was much higher than in insects kept on seawater with only added Cd. However, the levels of each heavy metal (except for Cr) fell rapidly and, by the fourth day, in the two groups the levels of all of these metals were similar (see Fig. 1). The levels of Cu found in the insects measured after 24 h and subsequently were all inexplicably lower than those in the control and baseline individuals.

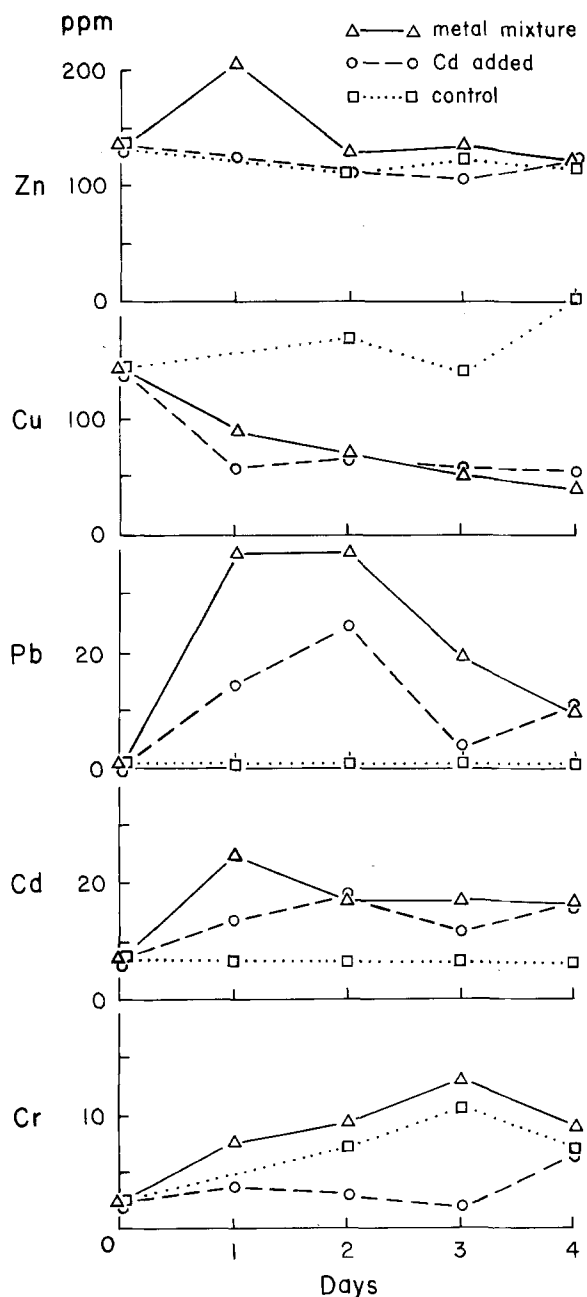


Fig. 1. *Halobates robustus*. Concentrations ($\mu\text{g g}^{-1}$ dry weight) of Zn, Cu, Pb, Cd, and Cr in sea-skaters kept on seawater or with additional metal added over a period of 4 days. Squares: control seawater; circles: Cd-enriched seawater; triangles: metal-mixture-enriched seawater

It is conceivable that during the first day the Cu values may have risen and quickly fallen again due to the induced production and excretion of chelating agents such as metallothioneins (Clubb et al., 1975; Webb, 1975), but this possibility remains to be tested. It is also possible that the metals in seawater were lost to the vessel wall and were thus less available to the sea-skaters. Although Pb was not detected in our baseline samples of *H. robustus* (see Table 1), it was found in the individuals kept on Cd-enriched seawater, having probably been introduced as a contaminant of the CdCl_2 used. The results of the experiment with metal mixtures suggest that *H. robustus* can probably regulate heavy-metal concentrations within its body. The initial increase of Zn, Cd and Pb (see Fig. 1) observed may have been due to a lag-time in activating possible excretory or detoxifying mechanisms.

Discussion

The tolerance of aquatic or marine organisms to Cd varies considerably according to species, salinity, calcium concentration, and other factors (Olson and Harrel, 1973; Westernhagen et al., 1974). The Cd contents of most planktonic marine organisms are unknown because of the difficulties in collecting samples of single planktonic species relatively free from other organisms. In certain euphausiid and copepod samples, Martin and Knauer (1973) found Cd values generally below $5 \mu\text{g g}^{-1}$; the highest Cd level that Martin and Broenkow (1975) determined in zooplankton was $21 \mu\text{g g}^{-1}$. In *Meganyctiphanes norvegica*, a shrimp from the surface water off Monaco, Fowler (1977) found that the Cd content was less than $1 \mu\text{g g}^{-1}$ for whole shrimp, although it was higher in faecal pellets ($9.6 \mu\text{g g}^{-1}$) and moulted cuticles ($2.1 \mu\text{g g}^{-1}$). This suggests that much of the Cd taken up by such animals is either excreted or accumulated in the cuticle, very little remaining in the soft tissue. Similarly, Cheng et al. (1977) found that more than 98% of the polonium in the beach flies *Fucellia* spp. was located in the cuticles and less than 2% in the soft tissue. Thus, these organisms appear to have an effective mechanism for excluding heavy metals from their soft tissues, perhaps by chemical binding of metals to the cuticle. However, in a recent study on the uptake of Cd from Cd-enriched seawater by the crab *Carcinus maenas*, 59 to 80% of

the absorbed Cd was found in the exoskeleton, probably accumulated by passive adsorption, but when Cd was supplied in the food source, only 22% was found in the exoskeleton (Jennings and Rainbow, 1979). Similarly, when *Drosophila melanogaster* was reared in a medium with added Cd, almost all the Cd taken up was found in the soft tissues and haemolymph, less than 5% being found in the exoskeleton (Cheng, 1979). It appears likely from these studies that incorporation of heavy metals into an organism may be considerably influenced by the uptake route.

The Cd concentration of the *Drosophila melanogaster* used as food in our uptake experiments was about $100 \mu\text{g g}^{-1}$. Since each fly has a dry weight of about $300 \mu\text{g}$, and almost all of the Cd is in the soft tissues and haemolymph (Cheng, 1979), the amount of available Cd in each fruit fly was about 33 ng . Also, since about 30 to 40% of the ingested Cd can be lost, possibly by excretion, within 3 h after uptake (Schulz-Baldes and Cheng, 1979), we can reasonably account for the increase in Cd concentration ($5 \mu\text{g g}^{-1}$) of *Halobates robustus* fed with just one or two Cd-enriched fruit flies. We cannot, however, account for the increase of Cd concentration in *H. robustus* solely by its drinking Cd-enriched seawater, unless we assume either (a) that it drinks more than $60 \mu\text{l}$ per day, i.e., 12 times the body volume of a female, or (b) that the Cd added to the seawater ($487 \mu\text{g l}^{-1}$ or about 0.5 mg l^{-1}) is considerably concentrated at the surface. Previous experiments (Schulz-Baldes and Cheng, 1979) indicated that an adult female *H. robustus* may drink $2 \mu\text{l}$, or up to 29% of her body weight per day; thus, in order that she should increase her Cd content by $11 \mu\text{g g}^{-1}$, the enrichment factor of the surface water that she drinks would have to be more than 33. This is not an unreasonably large factor: physico-chemical analyses have indicated that the concentration of Cd may be enriched up to 100 or more times in aqueous surface films (Duce et al., 1972; Weisel, 1978; Elzerman and Armstrong, 1979).

Although we had no time to carry out extended or duplicated experiments for these uptake studies, we were able to show that *Halobates robustus* could take up Cd from food as well as from seawater, but that relatively more Cd was taken up from seawater than from food. Since these insects are fluid-feeders and suck only the haemolymph or fluidized soft tissues of their prey (Cheng, 1974), heavy metals incorporated in the cuticles of their prey are not available to

them. Marine zooplankton, the natural food of these seaskaters, does not generally contain high levels of Cd, the few published data indicating Cd concentrations below $10 \mu\text{g g}^{-1}$ and rarely exceeding $20 \mu\text{g g}^{-1}$ (Robertson et al., 1972; Martin and Knauer, 1973; Martin and Broenkow, 1975). Since much of the Cd found in marine zooplankton is evidently located in the exoskeleton rather than in the soft tissues (Fowler, 1977), the relatively large amounts of Cd that we have found in oceanic *Halobates* spp. (Cheng et al., 1976; Bull et al., 1977) may have been derived chiefly from seawater. Ultimately, we hope that we may be able to relate the levels of Cd in oceanic sea-skaters to the Cd concentrations in the ocean surface waters where they occur.

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