NOTE

Effect of feeding on cadmium bioaccumulation in the echinoid *Paracentrotus lividus* (Echinodermata)

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ABSTRACT: Echinoids *Paracentrotus lividus* (Lamarck, 1816) were contaminated under laboratory conditions with Cd (as $CdCl_2$) either from sea water or from both sea water and food (*Posidonia oceanica* leaves) simultaneously. The experimental results showed that Cd bioaccumulation in the digestive tract wall and Aristotle's lantern of echinoids contaminated via both sea water and food was significantly less (as much as 50%) than when echinoids accumulated Cd from sea water only. The opposite trend was observed for the coelomic fluid of these echinoids. These observations suggest that food containing high Cd concentrations (1) reduces the efficiency of the digestive tract wall and Aristotle's lantern in taking up Cd from sea water, and (2) could enhance metal depurative activity of the echinoid coelom.

KEY WORDS: Cadmium · Bioaccumulation · Metabolism · *Paracentrotus lividus*

Several recent metal flux studies have focused on examining the relative importance of sea water and food as routes for metal bioaccumulation by marine organisms (see e.g. Guary et al. 1982, Nakamura et al. 1986, Bouquegneau & Joiris 1988, Bremer et al. 1990, Nolan et al. 1992). This aspect has generally been assessed by comparing results from experiments in which the contamination from sea water and food were studied separately. Only a few studies have taken into account mixed-source contamination, i.e. uptake via both sea water and food (Fowler et al. 1981, Borchardt 1983, Carvalho & Fowler 1994). Unfortunately, the latter generally do not include parallel investigations employing separate exposure modes. Based on results from a recent field study on Cd fluxes through the echinoid *Paracentrotus lividus* (Warnau et al. 1995), a comparison between the different exposure modes is however of particular interest.

Warnau et al. (1995) reported that the different body compartments of echinoids exposed to Cd via both sea water and food together contained lower Cd concentrations than corresponding compartments of echinoids exposed to Cd via sea water only. These authors suggested that high metal concentrations in the food could trigger a net decrease of Cd uptake from sea water, presumably through an increase of both excretion and mucus production by the echinoids. However, since stable Cd was the only contaminant for both the sea water and food pathways, the experimental procedure of Warnau et al. (1995) did not allow distinction between contaminant source terms (water vs food) once Cd was incorporated by the echinoids. These authors were consequently not able to test their hypothesis that echinoids actually accumulated less Cd from sea water.

The present work further investigates the effect of feeding on the uptake of Cd by *Paracentrotus lividus*. The methodology followed here was adapted from Warnau et al. (1995) in order to study similar experimental conditions while improving data comparability. For this reason the different exposure modes were studied simultaneously in the same experimental enclosure. In addition, both stable and radioactive Cd were used as contaminants: food was contaminated with stable Cd only, while sea water was contaminated with both stable and radioactive Cd. The use of radio-analytical techniques thereby allowed tracing separately the uptake of Cd directly from sea water.

Materials and methods. *Organisms and laboratory conditioning:* Individual echinoids, *Paracentrotus lividus* (Lamarck, 1816), and shoots of the phanerogam *Posidonia oceanica* (L.) Delile were collected during May 1994 by SCUBA diving between 5 and 10 m depth in the *P. oceanica* meadow near 'La Pointe des Douaniers', Cap d'Ail, France. The echinoids and phanerogams were subsequently acclimatized to labo-

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ratory conditions (closed circuit aquaria, constantly aerated; salinity: 38‰; 16.5 ± 0.5°C; light/dark cycle: 12 h/12 h) for 10 d prior to experimentation.

Metal, radiotracer, and radioanalysis: Experimental contaminations involved the use of stable Cd (as $CdCl_2$; Merck, synthesis quality) and a radiotracer, ^{109}Cd (as $^{109}CdCl_2$, $T_{1/2}$ = 462.6 d; Amersham, carrier free). Stock solutions of the metal and radiotracer were prepared in 0.1 M HCl. Radioactivities were determined using a high-resolution γ-spectrometry system consisting of a coaxial germanium N type detector (EGNC 33-195-R, Intertechnique) connected to a multichannel analyser and a personal computer with spectral analysis software (Intergamma, Intertechnique). ^{109}Cd γ-emissions were detected at 88.034 keV. The radioactivity of the samples was quantified by comparison with known standards of appropriate geometry and corrected for background and physical decay.

Experimental procedure: One polyvinylchloride aquarium (capacity 4 l) was divided into 2 sections with a 5 mm mesh polystyrene grid which allowed sea water to flow through both parts. Sea water was contaminated with 20 μg stable Cd l^{-1} (250 μl stock solution added l^{-1}), spiked with 4.3 kBq ^{109}Cd l^{-1} (7.5 μl stock solution added l^{-1}), and allowed to equilibrate for about 2 h before use. The pH of the radio-labelled sea water remained unchanged at 8.06. One group of 3 echinoids was then placed in each section. Echinoid ambital diameter was 45 ± 3 mm (mean ± SD) for Group I individuals and 47 ± 2 mm for Group II individuals.

All echinoids were fed ad libitum on leaves of *Posidonia oceanica*, the echinoids' main food source in these seagrass meadows (Nedelec & Verlaque 1984). (All *P. oceanica* leaves used in the present experiment bore epiphytes.) Leaves which Group I echinoids ingested were previously contaminated with 20 μg stable Cd l^{-1} for 15 d. The stable Cd concentrations within contaminated leaves were not measured; however, based on identical experimental contamination procedures employed in similar experiments (Warnau et al. 1995, G. Ledent, M. Warnau, A. Temara & Ph. Dubois et al. unpubl.), it was assumed that the leaves contained Cd concentrations of ca 9 μg g^{-1} wet wt, which is about 30 times higher than that of uncontaminated leaves. Group II echinoids were fed uncontaminated leaves. These leaves were previously maintained for 15 d under the same conditions as were contaminated leaves, but without the stable Cd additions. The experimental conditions for the 2 treatments are shown in Table 1.

The sea water media (with both stable and radioactive Cd) and leaf rations (of both groups) were renewed daily in the evening (echinoids feed mainly during the night; Dance 1987, pers. obs.) in order to

Table 1. *Paracentrotus lividus*. Experimental conditions for Cd contamination experiments. Group I: echinoids exposed to Cd via both sea water and food (*Posidonia oceanica* leaves); Group II: echinoids exposed to Cd via sea water only

	Group I	Group II
Sea water	20 μg stable Cd l^{-1} 4.3 kBq ^{109}Cd l^{-1}	
Food	Leaves contaminated for 15 d with 20 μg stable Cd l^{-1} (no ^{109}Cd)	Leaves maintained for 15 d in sea water containing neither stable Cd nor ^{109}Cd

(1) maintain the stable Cd concentration and ^{109}Cd activity in sea water as constant as possible, and (2) reduce possible ^{109}Cd uptake by *Posidonia oceanica* leaves as much as possible. Radioactivity of ^{109}Cd in the water and leaves of the 2 groups were checked daily before and after each renewal in order to determine the time-integrated activity in the water and possible radiolabelling of leaves, respectively.

During the exposure time (10 d), ^{109}Cd radioactivity in each living echinoid was whole-body counted every second day. At the end of the experiment, all echinoids were sacrificed and dissected. The body wall, Aristotle's lantern, digestive tract wall (after removal of the gut contents), gonads, and coelomic fluid were separated, weighed (wet wt), and their ^{109}Cd content measured. Counting times were adapted to obtain counting rates with relative propagated errors ≤5%. Counting times were 5 min for whole-body radioanalysis and ranged from 5 to 60 min for dissected tissues and organs, sea water, and seagrass leaves.

Data analysis: Uptake of ^{109}Cd was expressed by the change in the concentration factor (CF) over time. CF was calculated by dividing radioactivity in echinoids or body compartments (Bq g^{-1} wet wt) by time-integrated radioactivity in sea water (Bq g^{-1}). Whole-body uptake of ^{109}Cd by echinoids contaminated either from sea water alone or from both sea water and food were fitted using simple linear regressions. Linearity was tested by the linearity test for regression with replication. Difference between slopes of the regression equations was tested by the bilateral t-test (Zar 1984). Differences between the CF in each echinoid body compartment after 10 d of exposure were tested by the unilateral t-test. One-way analysis of variance (ANOVA) was used to test (1) variations with time of ^{109}Cd activity measured in the different rations of *Posidonia oceanica* leaves after a 24 h period in the aquarium, and (2) differences between activity measured in *P. oceanica* leaves from the 2 sections of the aquarium (Zar 1984).

Results. After a 24 h period in the aquarium as echinoid food, *Posidonia oceanica* leaves slightly accumulated [109]Cd, regardless of whether or not they were previously contaminated with stable Cd (data not shown). ANOVA did not show any significant differences ($\alpha = 0.05$) in [109]Cd labelling between *P. oceanica* leaves that were previously contaminated (Group I) and those that were not (Group II). This indicated that all leaves (contaminated or not) took up Cd similarly; hence, stable Cd uptake by leaves previously contaminated had not reached a steady-state equilibrium. In addition, ANOVA did not show any significant differences ($\alpha = 0.05$) in [109]Cd labelling between the successive *P. oceanica* leaf rations used in a same section of the experimental aquarium. Food may therefore have contributed slightly to the total [109]Cd activity measured in echinoids during the experiments; this contribution was however identical for the 2 echinoid groups considered.

Whole-body [109]Cd uptake kinetics in echinoids for the 2 investigated treatments (Group I: Cd exposure via both food and sea water; Group II: Cd exposure via sea water only) were both accurately fitted by simple linear regressions (p linearity < 0.05) (Fig. 1). Slopes of the 2 regression equations were not significantly different (bilateral *t*-test, $\alpha = 0.05$).

After 10 d of exposure, the echinoids from the 2 groups were sacrificed and dissected. The [109]Cd concentration factors (CF) in their body compartments are given in Table 2. Statistical comparisons of the CF in digestive tract wall, Aristotle's lantern, and coelomic fluid indicated significant differences in [109]Cd accumulation between echinoids from Groups I and II (unilateral *t*-test, $\alpha = 0.05$). The [109]Cd CF in digestive tract wall and Aristotle's lantern was 30 to 50% lower in echinoids which had ingested contaminated leaves, while the [109]Cd CF in the coelomic fluid in those echinoids was enhanced by a factor of 2.

Discussion. The [109]Cd concentration factor (CF) was significantly lower (as much as 50%) in the digestive tract wall and Aristotle's lantern of *Paracentrotus lividus* exposed to the metal in both sea water and food
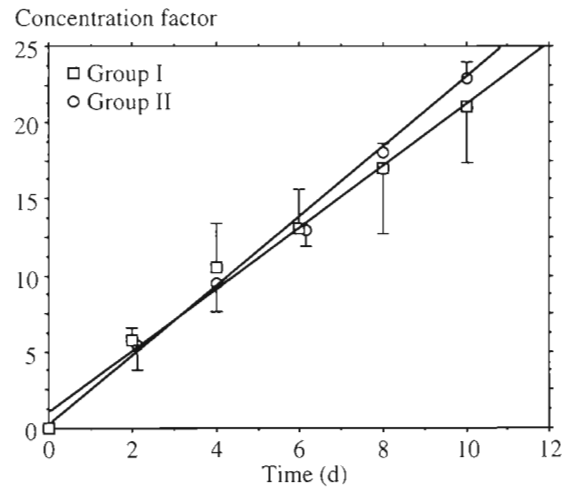

Concentration factor

Fig. 1. *Paracentrotus lividus*. Whole-body uptake kinetics of [109]Cd (mean concentration factor ± SD; n = 3) in echinoids exposed to Cd via sea water (20 µg stable Cd l⁻¹ and 4.3 kBq [109]Cd l⁻¹) and fed on *Posidonia oceanica* leaves previously contaminated for 15 d with 20 µg stable Cd l⁻¹ (Group I) or fed on uncontaminated leaves (Group II). Equations of the linear regressions are (1) Group I: CF = 1.11 + 2.02 t ($R^2 = 0.89$, p ≤ 0.0001) and (2) Group II: CF = 0.36 + 2.31 t ($R^2 = 0.93$, p ≤ 0.0001); where t = time (d)

than when sea water was the sole source of elevated Cd exposure. The body wall and gonads showed a similar decreasing trend, though the differences were not statistically significant. This was probably a result of the short duration of the experiment (10 d) since Warnau et al. (1995) actually observed a significant decrease of Cd uptake in these compartments after 18 d of exposure. In contrast, the [109]Cd CF in coelomic fluid from echinoids contaminated from both sea water and food was twice that of echinoids exposed to elevated Cd in sea water only. The [109]Cd CF decrease was not observed when whole-body uptake was considered. This was due to the fact that the 3 body compartments which did show significant variation in [109]Cd CF are either too small (negligible contribution of digestive tract wall and Aristotle's lantern to total echinoid

Table 2. *Paracentrotus lividus*. Concentration factor (mean CF ± SD; n = 3) of [109]Cd in echinoid body compartments after 10 d of exposure to 20 µg stable Cd l⁻¹ and 4.3 kBq [109]Cd l⁻¹ via sea water and to a diet (*Posidonia oceanica* leaves) previously contaminated for 15 d with 20 µg stable Cd l⁻¹ (Group I) or not contaminated with stable Cd (Group II). p: probability of the unilateral *t*-test

Compartments	% of total echinoid wet wt	CF Group I	CF Group II	p	Feeding effect on CF
Body wall	48 ± 8.7	39 ± 16	47 ± 6.1	0.24	None
Aristotle's lantern	4.4 ± 0.8	9.3 ± 1.5	12 ± 1.9	0.04	Decrease
Digestive tract wall	2.5 ± 0.9	43 ± 21	90 ± 13	0.02	Decrease
Gonads	1.1 ± 0.8	7.1 ± 4.0	10 ± 4.6	0.25	None
Coelomic fluid	44 ± 6.9	1.3 ± 0.3	0.7 ± 0.3	0.04	Increase

wet wt) or do not concentrate sufficient [109]Cd (very low CF in coelomic fluid) to influence significantly the whole-body uptake kinetics.

Our experimental results are in accordance with those of Warnau et al. (1995) who first suggested a suppressive effect of feeding on Cd uptake by *Paracentrotus lividus*. Moreover, our findings demonstrate that the net decrease of Cd uptake observed in echinoids exposed to Cd via both sea water and food is a direct result of lower Cd uptake from sea water. Indeed, the experiment was designed in such a way that sea water constituted the sole significant source of [109]Cd. According to Warnau et al. (1995), a decrease of Cd uptake from sea water could be due to enhanced echinoid excretion which would increase metal elimination, and to increased mucus production which would decrease metal bioavailability. These combined processes could be triggered by the elevated Cd concentration present in the contaminated *Posidonia oceanica* leaves upon which echinoids were feeding. No evidence against this hypothesis was found in the present study. Nevertheless, the occurrence of a significantly higher [109]Cd CF in the coelomic fluid (a body compartment that was not previously investigated) of echinoids accumulating Cd from both sea water and food suggests that the coelomic fluid and/or its cellular components (coelomocytes) could also be involved in the physiological response to contamination. This is in accordance with Pelletier & Larocque (1987) who have previously suggested coelomocyte participation in a Hg detoxification process occurring in the asteroid *Leptasterias polaris* exposed to contaminated food.

Studies of heavy metal fluxes through marine organisms are an important tool in ecotoxicology. Indeed, they allow the assessment of the susceptibility of an organism to accumulate a given metal, to be impacted by this metal, or to constitute a source of contamination for higher trophic levels (see e.g. Bouquegneau & Joiris 1988, Depledge & Rainbow 1990, Besten et al. 1991). Flux studies are also a preliminary requirement to characterize bioindicator species to be selected for environmental monitoring and quality assessments (Phillips 1976, Depledge & Rainbow 1990). The usefulness of such experiments necessitates obtaining key kinetics parameters such as uptake and retention rates, as well as the maximum contaminant concentration incorporated. In the present work it was shown that, in the case of Cd contamination of the echinoid *Paracentrotus lividus*, the ingestion of Cd-contaminated food might interact with metal absorption from sea water and its subsequent metabolism. In our study, such an interaction resulted in the decrease of the Cd CF in different body compartments. This observation indicates that mixed-source exposures should not be neglected in metal flux studies, in particular when studying acute contamination that generally involves the use of relatively high contaminant concentrations.

Although the stable Cd concentrations used in these experiments were high (20 µg l^{-1}), they may be representative of extremely contaminated marine areas. For example, Ward (1984) reported Cd concentrations of 11.5 ± 19.6 µg l^{-1} in sea water flowing above the *Posidonia australis* meadows of First Creek (Spencer Gulf, South Australia) which is situated near the lead smelter at Port Pirie. During the same period, Cd concentrations up to 100 µg g^{-1} wet wt were measured in *P. australis* leaves (Ward 1987). In such a Cd contaminated environment, a feeding effect which interferes with Cd uptake from sea water might represent an adaptative advantage for the echinoids. Indeed, ingestion of *Posidonia oceanica* (i.e. the main echinoid food in the ecosystem considered) would presumably enhance excretion, mucus production, and coelom depurative activity. This would result in a reduction of direct Cd absorption from sea water which, like for many benthic invertebrates, is the main route for Cd bioaccumulation in *Paracentrotus lividus* (Bouquegneau & Joiris 1988, Depledge & Rainbow 1990, Warnau et al. 1995). Further research is needed to determine if the reduction of Cd absorption from sea water demonstrated in this study could be triggered by other food types (e.g. algae, detritus) which *P. lividus* ingests in other habitats.

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