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Experimental cadmium contamination of *Asterias rubens* (Echinodermata)

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ABSTRACT: Asteroids *Asterias rubens* (Linneaus, 1758) were exposed in the field to various concentrations of waterborne or dietary Cd (from contaminated mussels). Cadmium uptake, and subsequent loss, kinetics were studied for 45 d for each. Dietary (110 µg Cd g^{-1} dry wt of the prey) and waterborne (5 and 20 µg Cd l^{-1}) Cd was significantly accumulated in the 3 measured body compartments: pyloric caeca, body wall, and skeleton. Waterborne Cd (1 µg Cd l^{-1}) was also significantly accumulated in the body wall but not in the pyloric caeca or the skeleton. Dietary Cd accumulation occurred in all body compartments. In the pyloric caeca, a steady state equilibrium was reached within 20 d, the concentration at steady state ranging from 3 to 9 µg Cd g^{-1} dry wt according to the exposure mode. The body wall and the skeleton accumulated Cd linearly during the exposure period at a rate of 0.07 to 0.25 µg Cd g^{-1} dry wt d^{-1} according to the exposure mode. Cadmium loss kinetics were fitted by inverse exponential functions to all body compartments, except to the skeleton where the loss was generally nonsignificant. Results indicate that there is a Cd flux through the asteroid body from the digestive system to the body wall where Cd is slowly incorporated to the skeleton. Concentration factors calculated for the pyloric caeca fit the range of values previously reported from laboratory experiments and thus validate them in field conditions. Thus *A. rubens* may be considered a valuable bioindicator of Cd contamination.

KEY WORDS: *Asterias rubens* · Field contamination · Cadmium · Uptake kinetics · Loss kinetics

INTRODUCTION

Although international commissions have regulated dumping operations in the areas of jurisdiction of contracting countries, heavy metals are still recognized as threatening to continental shelf ecosystems (Newman & McIntosh 1991, NSTF 1993). This is partly due to their conservative nature that keeps them in food webs and sediments. Cadmium is considered as one of the most toxic metals to aquatic biota (Sorensen 1991), and its uptake by marine filter-feeders is well documented (Borchardt 1983, Bebianno & Langston 1991). Surprisingly, little attention has been paid to a main consumer of filter-feeders, the top predator asteroids. Yet, these often form abundant populations that are present in numerous ecosystems (Hayward & Ryland 1990), indicating that the fluxes of metals through these animals could be significant. Furthermore, several asteroids are key species in their communities (Menge 1982). Metal impacts on them will thus affect the whole community.

Cadmium uptake kinetics have previously been studied in *Asterias rubens* (the main NE Atlantic predator asteroid) exposed in aquaria or mesocosms to high concentrations (from 25 to 400 µg Cd l^{-1}) (Bjerregaard 1988, Besten et al. 1989, 1990). However, modelling of the kinetics was not achieved and therefore steady-state concentrations could not be determined. Cadmium bioaccumulation by the same species exposed to low concentrations (0.0125 to 12.5 µg Cd l^{-1}) was measured in the laboratory for 14 d but no kinetics were established (Bjerregaard 1988). Also, elimination kinetics of Cd by asteroid body compartments have not been described, though these data are crucial in assessing the fate of the bioaccumulated metal. The aims

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of the present study are modeling the routes of Cd uptake and elimination in the top predator *A. rubens* exposed in the field and to assess the fate of the contaminant when natural conditions are restored.

MATERIALS AND METHODS

Individuals of *Asterias rubens* (Linneaus, 1758) (size: 6 to 9 cm from the tip of the longest arm to the opposite interradius) were collected in the Eastern Scheldt (Scharendijke, The Netherlands) by SCUBA divers. They were experimentally contaminated in the field from May to August 1994. Two exposure modes of *A. rubens* to Cd were investigated: (1) exposure via sea water and (2) exposure via food.

Contamination from sea water. Contaminations were performed in the field in Plexiglas containers (capacity: 30 l) anchored in the sediment at a depth of 9 m (Warnau et al. 1995). Cadmium was injected into the containers as $Cd(NO_3)_2$ (Titrisol, certified quality, Merck). Asteroids were fed mussels (*Mytilus edulis* L.) ad libitum. Water, Cd content, and mussels were renewed twice a day. Three sets of asteroids (n = 27 each) were exposed for 45 d to 3 different Cd concentrations in sea water (nominal concentrations: 1, 5, and 20 µg Cd l^{-1}). Actual Cd concentrations in the containers were assessed by collecting a small volume (5 ml) of water from 3 control containers (with 15 asteroids each) 2, 8, and 12 h after Cd injection. Water was also sampled in the field to assess background Cd concentration. The water samples were filtered (0.45 µm, glass microfiber filters, Whatman) and acidified (acidifying solution: 65% nitric acid, Suprapur quality, Merck; pH of the final solution < 2) immediately after they had been brought to the surface. Cadmium concentrations in these water samples were measured by electrothermal atomic absorption spectrometry with a Varian SpectrAA-300 Zeeman spectrometer equipped with a GTA-96 graphite furnace.

Contamination through food. The chosen prey was the mussel *Mytilus edulis*. A total of 2000 mussels were collected in the Eastern Scheldt (Wemeldinge, The Netherlands) and exposed for 2 mo in a closed-circuit aquarium (250 l) to Cd (as $Cd(NO_3)_2$) dissolved in sea water at a nominal concentration of 20 µg Cd l^{-1}. Cadmium concentrations in sea water were measured daily by atomic absorption spectrometry (GBC 906 AA spectrometer) and adjusted when required. Contamination of the asteroids was then performed in the field at 9 m depth, in a polyethylene meshed cage. Over 45 d, 27 asteroids were fed the previously contaminated mussels ad libitum. These were replaced by freshly contaminated mussels every 15 d. Mussels were regularly collected in the cages and Cd concentration in their soft tissues was measured.

Decontamination. After Cd exposure by seawater or diet, tested asteroids were placed in polyethylene meshed cages and fed noncontaminated mussels for 45 d.

Controls. Asteroids from the same population were sampled regularly 100 m away from the experimental site to take into account individual fluctuations of Cd concentrations under noncontaminating conditions ('field controls'). In order to assess possible effects of the experimental device, 27 asteroids were placed for 45 d under a Plexiglas container ('container controls') or 90 d in a polyethylene meshed cage ('cage controls') and submitted to the same treatment and sampling schedule as the experimental asteroids except for Cd exposure.

Sample preparation and metal analysis. Samples of 3 asteroids each were collected at regular time intervals in every experimental and control group. Each sampled asteroid was dissected just after collection. The pyloric caeca and the body wall were kept for analysis. As the major part of the experiment was carried out during the postspawning period, gonads were not considered. Processing of the samples and isolation of skeleton were performed as described by Warnau et al. (1995). The concentrations of Cd in the body compartments of the asteroids were measured by atomic absorption spectrometry (GBC 906 AA spectrometer) after acid digestion of the samples. The reproducibility of the methodology was checked using an internal standard (homogenized powder of asteroid body wall). Cadmium concentrations of 3 samples of the internal standard were analysed with each sample set. The variations of these measurements (n = 15) were low (always within ±2 SD of the first set mean). A certified material (*Mytilus edulis*, CRM no. 278, Griepink & Muntau 1988) was analysed along with the experimental samples to check the accuracy of the methodology [certified value: 76 ± 2 µg g^{-1} dry wt (mean ± SD); measured values: 76 to 85 µg g^{-1} dry wt, n = 5]. Detection limit (mean + 3 SD of the blank) was 12 µg Cd l^{-1}. Measurements below the detection limit were replaced by half of this value for data analysis (Black 1991).

Data analysis. The variations of Cd concentration in the soft tissues of the contaminated mussels was analysed by simple linear regression to describe Cd uptake and to test the significance of Cd loss when noncontaminating conditions had been restored.

Uptake kinetics of the asteroid body compartments were described using either a simple linear or a nonlinear model. Regressions using both models were systematically performed; statistics of the best fit (highest R^2) are presented. When the linear model showed the best fit, linearity was tested by the linearity test for regression with replication (Zar 1984). Significance of the slopes of the linear regression equations was tested by 1-way analysis of variance (ANOVA). Comparisons

between slopes of the linear regression equations were tested either by the bilateral *t*-test (comparison between 2 regression slopes) or by analysis of covariance (ANCOVA) followed by Tukey's multiple comparison test (multiple comparisons between more than 2 regression slopes) (Zar 1984). The nonlinear model was: $C_t - C_0 = (C_s - C_0)(1 - e^{-kt})$; where C_0, C_t, and C_s are the Cd concentrations at the times 0, t, and steady-state; k is the uptake rate constant (d^{-1}) (adapted from Whicker & Schultz 1982). Parameters of the equations (C_0, C_s, k) were calculated by fitting the model by iterations. Standard errors of the parameters were computed by estimating the Hessian matrix after iterations had terminated (Systat 5.2.1 software, Systat Inc.). Significance of the nonlinear regression equations was determined by comparing the calculated F-value and the distribution of Fisher-Snedecor (Statable Software). Significance of the difference between calculated C_s at different contaminating concentrations was tested by ANOVA using means and standard errors and by Tukey's multiple comparison test (Zar 1984).

Cadmium loss kinetics were described by assuming that the residual concentration at the end of the noncontaminating period could depart significantly from 0. Hence, the nonlinear regression model: $C_t = C_D + Ae^{-t/l}$ was used, where C_D is the Cd concentration at the end of the noncontaminating period, A is the difference between concentration at the beginning and at the end of the noncontaminating period, l is a loss rate constant (d) (Kock & Kramer 1994). The parameters of the equations (C_D, A, l) were calculated by fitting the model by iteration (Systat 5.2.1 software, Systat Inc.). The level of significance was always set at $\alpha = 0.05$.

RESULTS

Cadmium contamination of *Mytilus edulis*

For the contamination experiment through food, mussels were preliminarily contaminated in an aquarium by exposure to $Cd(NO_3)_2$ (20 µg Cd l^{-1} for 80 d). Mussels accumulated Cd linearly (linearity test: $p > 0.25$) at a rate of 1.53 ± 0.16 µg Cd g^{-1} dry wt d^{-1}. Three sets of contaminated mussels were successively provided to the asteroids. Cadmium concentration in the soft tissues of the mussels of the 2 first sets provided to the asteroids during the first 30 d of the contamination period was 109 ± 29 µg Cd g^{-1} dry wt. This concentration was found to remain unchanged in clean sea water (slope of the linear regression not significantly different from zero). Cadmium concentration in the last set of mussels did decrease significantly, but Cd loss was limited (23% maximum) and did not affect Cd uptake by the asteroids.

Table 1. *Asterias rubens*. Cd concentrations (µg Cd g^{-1} dry wt, means ± SD; n = 45) in the body compartments of asteroids in the different controls

	Pyloric caeca	Body wall	Skeleton
Field controls	0.45 ± 0.25	0.34 ± 0.23	0.31 ± 0.11
Container controls	0.34 ± 0.17	0.39 ± 0.32	0.34 ± 0.17
Cage controls	0.56 ± 0.34	0.43 ± 0.23	0.39 ± 0.22

Cadmium contamination of *Asterias rubens*

Uptake and loss kinetics of Cd were followed in the pyloric caeca, the body wall and the skeleton of asteroids exposed to waterborne or dietary Cd (Fig 1A–C). Regression models were calculated for each body compartment according to the exposure conditions. Different controls were performed to test possible fluctuations of Cd concentrations in the field or possible effects of the experimental devices. None of these controls revealed significant Cd variations in any asteroid compartments (slopes of the regression lines not significantly different from zero). Mean Cd concentrations in the controls are given in Table 1.

Actual concentrations of dissolved Cd in control containers used for contamination through water were measured regularly over a 12 h period. These measurements correspond to concentrations during the first 12 h of the exposure period and are a reasonable minimal estimate of the prevailing conditions during the whole period of exposure. After a rapid drop, the concentration in each container remained stable at about 0.5, 3, and 9 µg Cd l^{-1} (nominal concentration: 1, 5 and 20 µg Cd l^{-1}, respectively).

Cadmium uptake

Exposure to 5 and 20 µg dissolved Cd l^{-1} and to dietary Cd revealed significant uptake kinetics in the pyloric caeca. Kinetics were better described by the nonlinear model, and showed a saturation phase within 20 d (Table 2). Concentrations at saturation were compared by ANOVA and Tukey's multiple comparison test. They increased significantly with the contaminating concentration and were higher for contamination through food (Tables 2 & 3). Exposure to 1 µg dissolved Cd l^{-1} for 45 d did not result in a significant uptake of Cd in the pyloric caeca as tested by regression analyses ($p = 0.17$) (Table 2).

Exposure to 5 or 20 µg dissolved Cd l^{-1} or to dietary Cd revealed significant uptake kinetics in the body wall as well as in the skeleton. Kinetics were described accurately by linear regressions in both compartments (Table 2). Uptake rates (slopes) varied significantly as

tested by ANCOVA. They increased significantly with the contaminating concentration as tested by Tukey's multiple comparison test, and were higher for contamination through food (Table 3). Comparisons between the uptake rate of the skeleton and that of the body wall at a same contaminating concentration (bilateral *t*-test) revealed that they were nonsignificantly different regardless of exposure mode, except at 1 µg Cd l^{-1} Indeed, exposure to 1 µg dissolved Cd l^{-1} for 45 d did not result in a significant Cd uptake in the skeleton as tested by regression analyses ($p = 0.57$) (Table 2). On the contrary, uptake kinetics were significant at 1 µg Cd l^{-1} in the body wall and the hypothesis of linearity was rejected at this concentration. These kinetics showed a saturation phase (equilibrium reached within 30 d).

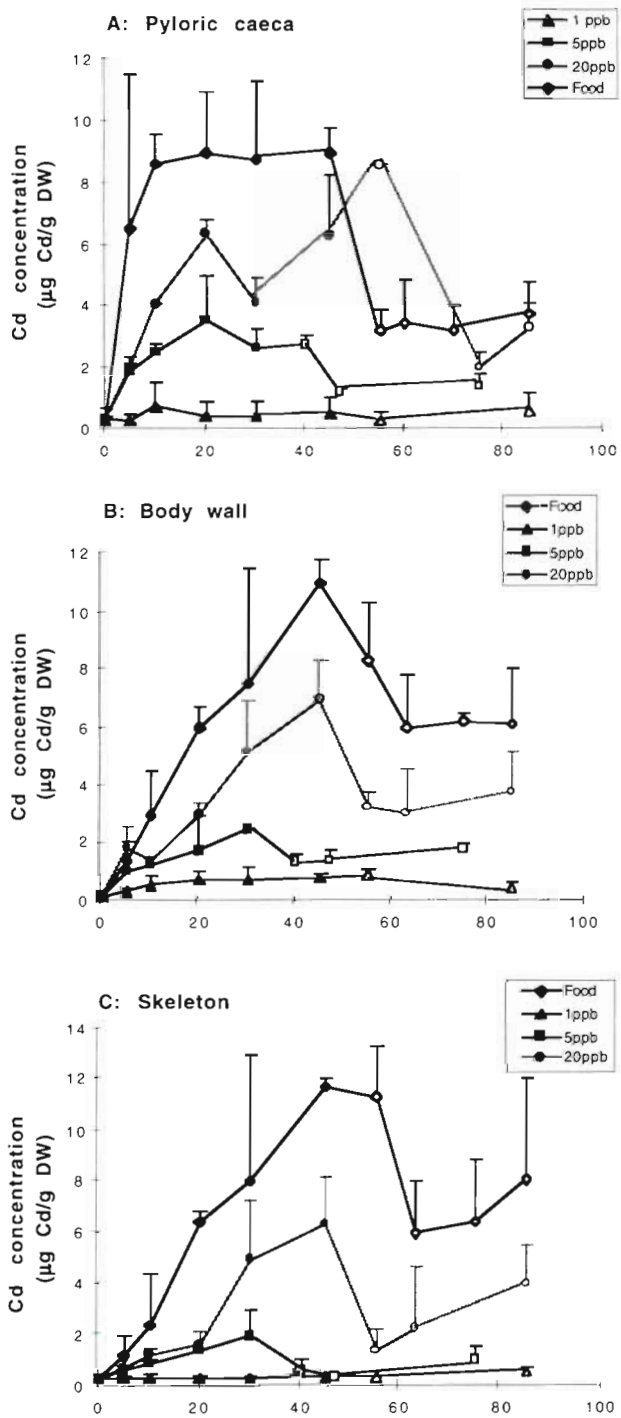

Fig. 1 *Asterias rubens*. Uptake and loss kinetics of Cd (mean + SD when larger than dots) in 3 body compartments (pyloric caeca, body wall, skeleton) of asteroids exposed to Cd through food, or through sea water at concentrations of 1, 5, or 20 µg Cd l^{-1} Solid and open symbols: uptake and loss kinetics, respectively

Cadmium loss

At the end of the Cd exposure, asteroids were transferred to uncontaminated regimens and the fate of Cd in the asteroids was studied (Fig. 1) Parameters of loss kinetics were calculated (Table 4). Cadmium concentrations in the body wall decreased rapidly, regardless of previous exposure mode. In contrast, loss of Cd by the skeleton was nonsignificant (regression analyses) regardless of previous exposure mode except in asteroids contaminated by waterborne Cd at the concentration of 20 µg Cd l^{-1}. Moreover, although mean Cd concentration did not increase significantly in the skeleton during exposure of asteroids to 1 µg Cd l^{-1}, the mean concentration at the end of the noncontaminating period increased significantly by 34%. At the end of the experiments, 83, 48, 72, and 77% of the total Cd load of the body wall was located in the skeleton of asteroids exposed to 1, 5, 20 µg dissolved Cd l^{-1} or to dietary Cd respectively. By comparison, about 55% of the total body wall Cd load was located in the skeleton of control asteroids.

In the pyloric caeca of asteroids previously contaminated through food, Cd concentration decreased rapidly. On the other hand, the pyloric caeca of asteroids previously contaminated through water did not lose Cd during the first 10 d of the noncontaminating period. Afterwards, Cd loss was significant and rapid. Parameters of loss kinetics in the pyloric caeca of asteroids contaminated through water (Table 4) were hence calculated from the 10th day of the noncontaminating period.

DISCUSSION

Effective concentrations of dissolved Cd in the containers settled down after 8 h at about 0.5, 3, and 9 µg Cd l^{-1}, according to the nominal concentration. The differences from the nominal concentration could be

Table 2. *Asterias rubens*. Parameters of the regression models [linear (L): $C_t = a + bt$; nonlinear (NL): $C_t - C_0 = (C_s - C_0)(1 - e^{-kt})$] describing Cd uptake kinetics in the body compartments of asteroids exposed to waterborne or dietary Cd. *a*: intercept of the linear regressions (µg Cd g^{-1} dry wt) *b*: slope of the linear regressions (= uptake rate, µg Cd g^{-1} dry wt d^{-1}); C_t: Cd concentration (µg Cd g^{-1} dry wt) at time *t* (d); C_0: initial Cd concentration; C_s: Cd concentration at saturation; *k*: uptake rate constant (d^{-1}). Parameters are given as mean ± SE. p: probability of the regression; p(linearity): probability of the linearity test performed on the linear regressions. R^2: determination coefficient. ns: neither regression model is significant

	Regression model	p(linearity)	*a*	*b*	C_0	C_s	*k*	R^2	p
Pyloric caeca									
Waterborne Cd									
1 µg Cd l^{-1}	ns								0.17
5 µg Cd l^{-1}	NL	0.03			0.34 ± 0.23	3.06 ± 0.37	0.18 ± 0.06	0.80	<0.001
20 µg Cd l^{-1}	NL	0.01			0.27 ± 0.34	5.82 ± 0.52	0.11[a]	0.84	<0.001
Dietary Cd	NL	0.04			0.30 ± 0.33	9.03 ± 1.08	0.30[a]	0.76	<0.001
Body wall									
Waterborne Cd									
1 µg Cd l^{-1}	NL	0.04			0.17 ± 0.07	0.80 ± 0.13	0.09 ± 0.05	0.63	<0.001
5 µg Cd l^{-1}	L	0.79	0.43	0.07 ± 0.01				0.68	<0.001
20 µg Cd l^{-1}	L	0.49	0.39	0.15 ± 0.01				0.87	<0.001
Dietary Cd	L	0.98	0.38	0.25 ± 0.04				0.75	<0.001
Skeleton									
Waterborne Cd									
1 µg Cd l^{-1}	ns								0.57
5 µg Cd l^{-1}	L	0.99	0.34	0.06 ± 0.01				0.61	<0.001
20 µg Cd l^{-1}	L	0.40	−0.09	0.14 ± 0.03				0.79	<0.001
Dietary Cd	L	0.38	0.17	0.27 ± 0.05				0.77	<0.001

[a]Singular Hessian standard errors not computable

Table 3. *Asterias rubens*. Comparisons between significant kinetics of Cd uptake by compartments of asteroids submitted to different exposure modes (dissolved Cd: 5, 20 µg l^{-1}, dietary Cd: D). Comparisons between calculated concentrations at saturation (C_s in Table 2; for pyloric caeca, by ANOVA) or between slopes of the regression lines (*b* in Table 2; for body wall and skeleton, by ANCOVA), using Tukey's multiple comparison test. p: Fisher's (*F*) or Tukey's (*q*) probability parameters

Body compartment	F_{calc}	$p_{F_{calc}}$	Comparison	q_{calc}	$p_{q_{calc}}$
Body wall	11.81	<0.001	5 vs 20	5.56	<0.001
			5 vs D	5.48	<0.001
			20 vs D	3.96	0.02
Skeleton	12.86	<0.001	5 vs 20	4.54	<0.001
			5 vs D	5.92	<0.001
			20 vs D	4.11	0.02
Pyloric caeca	47.51	<0.001	5 vs 20	5.87	<0.001
			5 vs D	12.7	<0.001
			20 vs D	6.83	<0.001

attributed to interactions between Cd ions and some particulate and/or dissolved chemical species. Indeed, the placing of the container was followed by a resuspension of bottom muddy sediments and therefore of its interstitial waters. These waters are known to be anoxic and rich in sulfide (Fenchel & Riedl 1970). Hence Cd could have been rapidly adsorbed on resuspended particles and precipitated as sulfide species. The concentrations of nitrate injected with Cd were very low (maximum: 0.36 µmol l^{-1}) and were unlikely to have had any effect on primary production and hence on the generation of suspended particules. Indeed, background nitrate concentrations in the southern bight of the North Sea exceed 20 µmol l^{-1} (NSTF 1993). After the initial drop, Cd concentrations remained stable. As Cd was renewed during the contamination experiment, adsorption and precipitation sites or reactives were progressively reduced and the effective concentrations in the containers became closer to the nominal concentrations. Hence, the measured concentrations of dissolved Cd probably correspond to the minimal bioavailable Cd concentrations in the containers. The lowest effective concentration of dissolved Cd (0.5 µg Cd l^{-1}) corresponds to highly polluted sea water, while the 2 other concentrations are encountered only very rarely in nature (Bryan 1984).

Mussels used in the present study accumulated Cd linearly during the preliminary contamination period up to a concentration of 110 µg Cd g^{-1} dry wt. On return to uncontaminated sea water, Cd loss was either

Table 4. *Asterias rubens*. Parameters of the regression model ($C_t = C_D + Ae^{-lt}$) describing Cd loss kinetics in the body compartments of asteroids exposed to waterborne or dietary Cd and percentage loss of Cd at the end of the noncontaminating period. C_t: Cd concentration (µg Cd g^{-1} dry wt) at the time t. C_D: Cd concentration at the end of the noncontaminating period; A: difference between concentration at $t = 0$ (initial concentration, i.e. concentration at the end of the contamination period) and the end of the noncontaminating period. l: loss rate constant (d). p: probability of the regression; ns: not significant. R^2: determination coefficient. t: time (d)

	Initial concentration	C_D	A	l	R^2	p	Variation (%)
Pyloric caeca							
Waterborne Cd							
1 µg Cd l^{-1}	0.50 ± 0.07					ns	
5 µg Cd l^{-1}	2.62 ± 0.64	1.2 ± 0.63	1.64 ± 0.69	1.15[a]	0.79	<0.001	−56
20 µg Cd l^{-1}	6.30 ± 1.95	2.35 ± 0.45	5.13 ± 0.78	0.30[a]	0.77	<0.001	−79
Dietary Cd	9.01 ± 0.70	3.37 ± 0.25	5.69 ± 0.55	0.10[a]	0.90	<0.001	−63
Body wall							
Waterborne Cd							
1 µg Cd l^{-1}	0.77 ± 0.21					ns	−51[b]
5 µg Cd l^{-1}	2.45 ± 0.08	1.25 ± 0.09	1.20 ± 0.17	5.26[a]	0.83	<0.01	−49
20 µg Cd l^{-1}	6.96 ± 1.14	3.34 ± 0.38	3.61 ± 0.76	0.47[a]	0.69	<0.01	−52
Dietary Cd	11.01 ± 0.52	5.01 ± 0.43	6.27 ± 0.84	12.5[a]	0.81	<0.01	−57
Skeleton							
Waterborne Cd							
1 µg Cd l^{-1}	0.38 ± 0.05					ns	+34
5 µg Cd l^{-1}	2.01 ± 1.02					0.45	
20 µg Cd l^{-1}	6.39 ± 1.85	2.71 ± 0.61	3.70 ± 1.23	0.42[a]	0.49	<0.05	−58
Dietary Cd	11.29 ± 1.83					ns	

[a]Singular Hessian standard errors not computable. [b]Significant decrease, but inadequate model

insignificant or limited. Continuous linear accumulation of Cd as well as low Cd loss over short periods are classically reported for mussels (Borchardt 1983, Bebianno & Langston 1991, Besten et al. 1991). Concentrations around 100 µg Cd g^{-1} dry wt in the soft tissues of *Mytilus edulis* are rare in natural populations. However, concentrations exceeding this value have been reported in heavily contaminated areas (see Bryan 1984 for a review).

The experimental conditions of the present study were thus representative of acute contamination in nature. They had the advantage of allowing the tracking of Cd in the field. Furthermore, the concentration factor of Cd in an aquarium was demonstrated to be stable in *Asterias rubens* for ambient concentrations up to 3 µg Cd l^{-1} (Bjerregaard 1988). The results obtained with 2 of the effective Cd concentrations (0.5 and 3 µg Cd l^{-1}) used in the present study are thus probably representative of Cd processing by asteroids living in contaminating conditions.

Significant kinetics of Cd uptake in the pyloric caeca are best described by saturation models regardless of exposure mode. The steady-state concentration increased with the contaminating concentration. Concentration factors in the pyloric caeca calculated in the present study have been compared to values calculated by previous authors (Bjerregaard 1988, Besten et al. 1990) after Cd exposure in laboratory conditions (Table 5). They fit the range of values previously reported, therefore validating them in field conditions. In asteroids exposed to dietary Cd in the present study, mean concentration at saturation was 9 µg Cd g^{-1} dry wt. Similar concentrations at saturation have also been reported in pyloric caeca of *Asterias rubens* exposed to

Table 5. *Asterias rubens*. Concentration factors in the pyloric caeca of asteroids exposed to waterborne Cd in laboratory (Bjerregaard 1988, Besten et al. 1990) or in field conditions (present study)

Nominal contaminating concentration	Concentration factor	Reference
1 µg Cd l^{-1}	800	Bjerregaard (1988)[a]
3 µg Cd l^{-1}	700	Bjerregaard (1988)[a]
5 µg Cd l^{-1}	612	Present study
20 µg Cd l^{-1}	291	Present study
20 µg Cd l^{-1}	300	Bjerregaard (1988)[a]
50 µg Cd l^{-1}	250	Bjerregaard (1988)[a]
50 µg Cd l^{-1}	200	Besten et al. (1990)[a]

[a]Inferred from graphical data

50 µg Cd l^{-1} (Besten et al. 1990) and 200 µg Cd l^{-1} (Bjerregaard 1988). A mean concentration of about 9 to 10 µg Cd g^{-1} dry wt would therefore be the maximal value possibly found in the pyloric caeca of asteroid populations exposed to a single Cd source. This would indicate a regulation of Cd concentration in pyloric caeca. According to Bjerregaard (1988), this behaviour is in sharp contrast with the large Cd uptake by many other marine invertebrates which may concentrate Cd up to several hundreds of µg g^{-1} dry wt. It is noteworthy, however, that under natural noncontaminating conditions, Cd bioaccumulation by *A. rubens* is not thoroughly regulated as Cd concentration in its body compartments increases as a power function of size (Temara et al. 1996).

According to Besten et al. (1990), the metallothionein-like proteins synthesized in the pyloric caeca of asteroids which have accumulated such high concentrations (9 to 10 µg Cd g^{-1} dry wt) do not reach their maximal saturation level. Moreover, Bjerregaard (1988) showed that concurrent exposure of *Asterias rubens* to selenite increases Cd uptake rates 5-fold without any saturation phase being reached. Hence it seems that, although they could accumulate more Cd, asteroids are endowed with an efficient protective mechanism that keeps the concentration of this metal relatively low. This has already been pointed out by several authors studying asteroid contamination by Cd or Hg (Pelletier & Laroque 1987, Bjerregaard 1988, Besten et al. 1989). However, it is noteworthy that Cd uptake by asteroids is proportional to the ambient concentration from 0.025 to 2.5 µg Cd l^{-1}, the concentration factor decreasing only at higher concentrations (Bjerregaard 1988). This makes *A. rubens* a valuable species in the study of Cd contamination. It provides a striking example of an organism controlling its internal Cd concentration under extreme conditions while reflecting environmental conditions in the usual range of Cd pollution. *A. rubens* may therefore be used as a suitable bioindicator of Cd contamination as well as a model for a better understanding of Cd exclusion mechanisms. This exclusion could hypothetically be related to a translocation process from the pyloric caeca to other body compartments, to an excretory process that would increase metal elimination, or to other protective processes that would decrease metal bioavailability (e.g. increased mucus production). Warnau et al. (1995) showed that another echinoderm species, the echinoid *Paracentrotus lividus*, is protected against waterborne Cd by such mechanisms.

Cadmium translocation from the pyloric caeca to the body wall obviously occurred in asteroids contaminated through food. This translocation was still ongoing when the saturation phase had been reached in the pyloric caeca, as evidenced by the linear uptake kinetics shown by the body wall. Cadmium ingested with food is therefore rapidly distributed through the organism. Mercury is also rapidly translocated from digestive system to body wall in asteroids (Maheu & Pelletier 1994). Interestingly, Hg and Cd often share the same cellular ligants (Sorensen 1991). As saturation phases in the body wall are only observed after exposure to very high concentrations (4 wk, 200 µg Cd l^{-1}; Bjerregaard 1988), this body compartment can be considered as a virtually unlimited accumulation site for Cd in *Asterias rubens*.

In asteroids exposed to 1 µg Cd l^{-1}, the accumulation kinetics were significant and showed a saturation phase in the body wall, while no significant uptake was found in the skeleton under the same conditions. However, at the end of the 90 d, a significant increase was observed in the skeleton, indicating that inclusion in this compartment is a slow process. Similarly, no significant accumulation was measured in the pyloric caeca at this contaminating concentration (nominal concentration: 1 µg Cd l^{-1}, measured concentration: 0.5 µg Cd l^{-1}). However, 0.5 µg l^{-1} of dissolved Cd is a high concentration hardly found in natural ecosystems, even at a polluted site, e.g. the polluted Western Scheldt (NSTF 1993). But Besten et al. (1991) showed that asteroids fed mussels from the polluted Western Scheldt in clean sea water accumulate Cd in concentrations up to 8 µg g^{-1} dry wt in their pyloric caeca. As the pyloric caeca uptake of waterborne Cd was nonsignificant at low concentration up to 45 d, Cd is accumulated in the pyloric caeca of asteroids living in polluted sites only because Cd had been previously bioconcentrated by its prey.

Loss kinetics studied in the present work were significant for all the studied body compartments except the skeleton. Thus, the retention time of Cd in the skeleton seems to be longer than in the other body compartments, even if asteroids exposed to 20 µg Cd l^{-1} did lose Cd during the noncontaminating period. Furthermore, asteroids exposed to the lowest studied concentration (1 µg Cd l^{-1}) accumulated Cd in their skeleton after noncontaminating conditions had been restored. After Cd exposure, the percentage of Cd in body wall that was present in skeleton was either close to, or higher than in controls. It could thus be hypothesized that after the rapid Cd translocation from the digestive system to the body wall, Cd is slowly incorporated in the skeleton. Once in the skeleton, it is trapped for a large proportion and for a long time. While internal organs and the soft part of the body wall would be protected by such a detoxification mechanism, biomineralization could be affected. Indeed, D'Andrea (1994) showed deleterious effects of Cd on skeletal regeneration in another echinoderm species, the ophiuroid *Microphiophilis gracillima*.

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