

# Effect of selenium on cadmium uptake in selected benthic invertebrates

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**ABSTRACT:** Effect of selenium on uptake of cadmium in the benthic marine invertebrates *Asterias rubens*, *Mytilus edulis*, *Littorina littorea*, and *Arenicola marina* was investigated in a series of laboratory experiments. *A. rubens* exposed to 200  $\mu\text{g Cd l}^{-1}$  alone took up cadmium in body wall, pyloric caeca, and tube feet at initial rates of 1 to 2  $\mu\text{g Cd g}^{-1}$  dry wt  $\text{d}^{-1}$ ; steady state concentrations of 10 to 30  $\mu\text{g Cd g}^{-1}$  were reached after 1 to 2 wk. Concurrent exposure to 141  $\mu\text{g Se-SeO}_3^{-1}$  increased cadmium uptake rates to 5 to 10  $\mu\text{g Cd g}^{-1}$   $\text{d}^{-1}$ ; uptake proceeded linearly with time over 4 wk. Exposure to 200  $\mu\text{g Cd l}^{-1}$  did not affect uptake of selenium from 141  $\mu\text{g Se-SeO}_3^{-1}$ . Uptake of cadmium (from 200  $\mu\text{g Cd l}^{-1}$ ) in tube feet and body wall was augmented by 17  $\mu\text{g Se-SeO}_3^{-1}$ , whereas higher selenite concentrations were required to augment cadmium uptake in pyloric caeca. In specimens exposed to radioactive cadmium for 2 wk, selenite concentrations at and above 75  $\mu\text{g Se-SeO}_3^{-1}$  augmented cadmium uptake. *A. rubens* exhibited concentration factors (expressed on dry weight basis) for cadmium of ca 800 for ambient cadmium concentrations up to 3  $\mu\text{g Cd l}^{-1}$ ; between 3 and 400  $\mu\text{g Cd l}^{-1}$ , concentration factors decreased from 800 to 100. When exposed to 200  $\mu\text{g Cd l}^{-1}$  alone, *M. edulis* took up cadmium at initial rates of 9.3, 36, 7.7, 6.4, 24, and 0.8  $\mu\text{g Cd g}^{-1}$  dry wt  $\text{d}^{-1}$  in mantle, digestive gland, foot, adductor muscle, gills, and shell, respectively. *L. littorea* took up cadmium at 11 and 0.6  $\mu\text{g Cd g}^{-1}$  dry wt  $\text{d}^{-1}$  in soft parts and shell, and *A. marina* took up cadmium at 6.1 and 10.4  $\mu\text{g Cd g}^{-1}$  dry wt  $\text{d}^{-1}$  in body wall and gut and 0.23  $\mu\text{g Cd ml}^{-1}$   $\text{d}^{-1}$  in blood. Uptake proceeded linearly with time over 4 wk exposure in mantle, digestive gland, foot, and adductor muscle of *M. edulis*, soft parts of *L. littorina*, and gut, body wall, and blood of *A. marina*. In the other tissues uptake rates declined after 1 to 2 wk of exposure. Exposure to an equimolar concentration (141  $\mu\text{g Se-SeO}_3^{-1}$ ) of selenite did not affect cadmium uptake in these 3 species. Exposure to a 5-fold concentration of selenite (703  $\mu\text{g Se-SeO}_3^{-1}$ ) augmented cadmium uptake in gills, mantle, and foot of *M. edulis*.

## INTRODUCTION

Since the discovery that selenium protects rat testes against the toxic effects of cadmium (Kar et al. 1960), interactions between selenium and cadmium and mercury in mammals have been studied intensively (reviewed by Magos & Webb 1980). Although the interaction between selenium and mercury in aquatic organisms has been investigated in some detail (reviewed by Pelletier 1985), the effect of selenium on cadmium uptake and toxicity in aquatic organisms is poorly understood.

Positive correlations between cadmium and selenium concentrations in black marlin *Makaira indica* (Mackay et al. 1975) and several species of marine birds (Norheim 1987) have been found. Selenium augments uptake of cadmium in gills and haemolymph of the shore crab *Carcinus maenas* (Bjerregaard 1982, 1985, 1988) and counteracts the

toxic effects of cadmium in the freshwater snail *Lymnaea stagnalis* (Puymbroeck et al. 1982).

Selenium is present in unpolluted seawater in ng  $\text{l}^{-1}$  quantities (Measures & Burton 1980, Measures et al. 1980, Cutter & Bruland 1984, Apte et al. 1986). Selenium concentrations in fresh and coastal waters may be augmented from anthropogenic sources such as drainage from irrigated and selenite-fertilized soils (Phillips 1987), leaching from fly-ash deposits (Ahsanullah & Brand 1985), and discharges from refineries (Phillips 1987). Fly-ash leachates and effluents from refineries may contain up to 2400  $\mu\text{g Se l}^{-1}$  (Ahsanullah & Brand 1985) and 150  $\mu\text{g Se l}^{-1}$  (Phillips 1987), which may locally augment the selenium concentration of the seawater to  $\mu\text{g Se l}^{-1}$ . It is not known if discharges of selenium compounds in coastal and estuarine environments affect uptake of metals by organisms living in these habitats.

This study examines the effect of selenite on cad-

mium uptake in sea stars *Asterias rubens*, mussels *Mytilus edulis*, periwinkles *Littorina littorea*, and lugworms *Arenicola marina*.

## MATERIALS AND METHODS

**Experimental animals.** Sea stars *Asterias rubens* and periwinkles *Littorina littorea* were obtained from Lillebælt, Denmark. For Expt 2, sea stars were caught in seines, while those used in the remaining experiments were collected from the shore. Sea stars used in December were collected in October and kept in flowing seawater tanks at the Marine Biological Station, Bøgebjerggård, until they were brought to the laboratory. Lugworms *Arenicola marina* and mussels *Mytilus edulis* were collected in Denmark at Bregvær, NE Funen, and Kertinge Nor, E Funen, respectively.

**Exposure procedures.** Animals were acclimated in the laboratory for 1 to 5 d prior to experiment and then

exposed to cadmium as  $\text{CdCl}_2$  and selenium as  $\text{Na}_2\text{SeO}_3$ . Details of exposure conditions in each experiment are given in Tables 1 and 2. Cadmium and (in some of the experiments) selenite concentrations in the aquaria were monitored, and unless otherwise stated concentrations varied by less than 10% between water changes. The water in the exposure aquaria was aerated and no sediment was placed in the aquaria. The animals were not fed during the experiments. Store Bælt seawater with background cadmium and selenite concentrations of ca  $25 \text{ ng Cd l}^{-1}$  (Magnusson & Rasmussen 1982) and less than  $10 \text{ ng Se-SeO}_3^- \text{ l}^{-1}$  (Bjerregaard 1982) was used in the experiments.

**Selenium analysis.** Selenium concentrations in the tissues were determined by gas chromatography with a modified version of the technique described by Shimoishi (1976). The 5-nitropiaselenol produced (Shimoishi 1976) was extracted into toluene and 2  $\mu\text{l}$  samples were manually injected into a Hewlett Packard 5830 A Gas Chromatograph equipped with an

Table 1. Summary of experimental conditions in which animals were exposed to  $200 \mu\text{g Cd l}^{-1}$  and various selenite concentrations

Organism Experiment no.	$\mu\text{g Se-SeO}_3^-$ added $\text{l}^{-1}$	Initial no. of animals	Days of sampling and no. of animals (n) sampled	Type and volume of aquaria	Salinity (S‰) Temperature (°C)	Size of animals (g wet wt) Date																																																																																		
<i>Asterias rubens</i> Expt 1	0	25	5, 10, 14,	60 l glass aquaria	22–23 15.5	20–50 13 Jun 1986																																																																																		
	141	25	18, 28 (5)				<i>Asterias rubens</i> Expt 2	0	5	21 (4)	8 l poly- styrene aquaria	21–23 15.5	20–50 30 Jul 1986	2.2	5	21 (3)	4.4	5	21 (2)	8.8	5	21 (4)	17.6	5	21 (4)	35	5	21 (0)	70	5	21 (2)	141	5	21 (5)	281	5	21 (5)	562	5	21 (0)	1124	5	21 (0)	2248	5	21 (0)	4496	5	21 (0)	<i>Asterias rubens</i> Expt 3	141 <sup>a</sup>	16	7 (5), 17 (5)	30 l glass aquaria	23–24 15.5	20–50 16 Dec 1986	141	16	28 (6)	<i>Mytilus edulis</i> Expt 7	0	21	7 (5), 14 (8)	8 l poly- styrene aquaria	22–24 15.5	4.7–5.7 <sup>c</sup> 2 Jun 1985	141	21	28 (8)	703	21		<i>Littorina littorea</i> Expt 8	0	40	7, 14, 21,	8 l poly- styrene aquaria <sup>b</sup>	21–23 15.5	41–128 <sup>d</sup> 30 Jul 1986	141	40	27 (10)	<i>Arenicola marina</i> Expt 9	0	20	5, 12, 19,	8 l poly- styrene aquaria	19–23 10.0
<i>Asterias rubens</i> Expt 2	0	5	21 (4)	8 l poly- styrene aquaria	21–23 15.5	20–50 30 Jul 1986																																																																																		
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<i>Arenicola marina</i> Expt 9	0	20	5, 12, 19,	8 l poly- styrene aquaria	19–23 10.0	10–18 10 Oct 1985																																																																																		
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<sup>a</sup> No cadmium added  
<sup>b</sup> Equipped with a mesh to prevent the periwinkles from leaving the water phase  
<sup>c</sup> Shell length (cm)  
<sup>d</sup> Dry wt (mg) of soft parts

Table 2. *Asterias rubens*. Summary of experimental conditions in Expts 4, 5, and 6. Each of the experimental groups initially consisted of 7 small sea stars (1 to 9 g wet wt) placed in 8 l polystyrene aquaria containing ca 600 000 dpm  $^{109}\text{Cd}$ . Water was not changed during the 14 d exposure period, and  $^{109}\text{Cd}$  in the water was determined on Days 0, 7 and 14. Total radioactivity after exposure in the 5 to 7 sea stars from each aquarium is given. Temperature was 15.5 °C and salinity was 20.5‰

Experiment no. Date	Exposure ( $\mu\text{g l}^{-1}$ )		[ $^{109}\text{Cd}$ ] (dpm ml $^{-1}$ )			Weight <sup>a</sup> and no. (n) of animals sampled	$^{109}\text{Cd}$ in animals (dpm)	Loss from water accounted for by uptake in animals (% of $^{109}\text{Cd}$ )
	Cd	Se-SeO $_3^{2-}$	Day					
			0	7	14			
Expt 4 1 Jul 1987	0	0	63	60	48	2.5 ± 1.6 (6)	168 405	105
	0	141	65	37	25	2.4 ± 1.8 (5)	270 370	84
	0.0125	0	76	64	46	3.4 ± 1.7 (7)	136 310	57
	0.0125	141	66	33	22	3.6 ± 3.0 (7)	340 695	97
	0.025	0	71	59	46	3.1 ± 1.1 (7)	180 945	90
	0.025	141	70	34	26	2.9 ± 1.7 (7)	377 485	107
	0.05	0	76	65	52	3.3 ± 2.0 (7)	139 195	73
	0.05	141	71	33	24	3.4 ± 2.0 (7)	376 210	100
	0.1	0	79	68	60	3.9 ± 2.2 (7)	108 820	72
	0.1	141	74	36	20	2.8 ± 1.5 (7)	397 585	92
	0.2	0	74	62	46	2.7 ± 1.6 (7)	114 150	51
	0.2	141	74	46	29	3.1 ± 1.2 (7)	395 595	110
	0.4	0	71	64	62	3.1 ± 2.2 (7)	145 400	83
	0.4	141	75	28	22	2.3 ± 1.4 (7)	444 870	105
	0.8	0	78	65	54	3.0 ± 1.9 (7)	166 360	87
	0.8	141	73	33	23	2.4 ± 1.0 (7)	445 640	111
	1.6	0	75	71	56	2.5 ± 1.3 (7)	119 850	79
	1.6	141	78	25	20	2.4 ± 1.7 (7)	429 455	93
	3.2	0	79	67	53	3.1 ± 1.6 (7)	154 975	75
	3.2	141	75	28	19	2.3 ± 1.1 (7)	425 111	95
	6.3	0	75	70	55	3.2 ± 2.2 (7)	81 120	51
	6.3	141	73	23	16	3.0 ± 1.1 (7)	281 440	62
	12.5	0	74	70	62	2.7 ± 1.2 (7)	49 765	52
	12.5	141	74	42	20	3.1 ± 1.3 (7)	466 060	108
	25	0	74	71	62	2.7 ± 0.9 (7)	66 430	69
	25	141	74	52	43	3.2 ± 1.9 (7)	153 900	62
	50	0	79	75	63	2.8 ± 1.7 (7)	65 220	51
	50	141	76	60	46	2.7 ± 1.3 (7)	220 710	92
100	0	78	73	67	3.2 ± 1.9 (7)	43 315	50	
100	141	76	67	57	2.9 ± 2.0 (7)	151 795	100	
200	0	79	79	72	2.4 ± 1.2 (7)	34 520	62	
200	141	78	69	54	3.5 ± 2.1 (7)	94 650	50	
400	0	78	75	71	3.5 ± 2.0 (6)	31 010	55	
400	141	80	72	57	3.7 ± 2.1 (7)	91 570	50	
Expt 5 1 Aug 1987	b	0	74	50	35	5.4 ± 2.4 (7)	189 725	61
	b	0.25	78	55	37	5.8 ± 2.3 (7)	184 859	56
	b	0.50	76	52	37	5.7 ± 3.2 (7)	168 155	54
	b	1	74	56	41	4.9 ± 2.3 (7)	142 290	54
	b	2	78	54	43	4.2 ± 2.2 (7)	164 815	59
	b	4	78	56	39	5.3 ± 1.6 (7)	164 695	53
	b	8	77	52	34	5.9 ± 2.4 (7)	190 870	56
	b	16	72	55	40	5.0 ± 1.9 (7)	186 220	73
	b	32	78	49	33	5.5 ± 1.5 (7)	225 705	63
Expt 6 22 Sep 1987	b	64	75	53	37	5.2 ± 2.0 (7)	213 670	70
	b	0	65	55	49	3.4 ± 1.8 (7)	94 905	91
	b	25	64	61	53	2.9 ± 1.3 (7)	100 610	125
	b	50	69	50	44	3.2 ± 1.1 (7)	155 290	72
	b	75	67	56	47	3.6 ± 0.8 (5)	147 890	92
	b	100	69	54	42	4.0 ± 1.3 (6)	220 185	101
	b	125	65	47	30	3.4 ± 1.4 (7)	196 220	70
	b	150	69	32	21	3.0 ± 1.8 (7)	325 615	85
b	200	63	15	16 <sup>c</sup>	2.4 ± 0.8 (5)	285 920	76	

<sup>a</sup> Mean ± SD of wet weight. <sup>b</sup> No stable cadmium added. <sup>c</sup> This group analysed after 9 d

electron capture detector and a 2000 mm × 2 mm glass column packed with 10% SE-30 and acid-washed dimethyldichlorosilan-treated chromosorb W 100/120. Oven temperature was 200°C. The selenium concentration in an NBS oyster standard with a certified selenium concentration of  $2.1 \pm 0.5 \mu\text{g Se g}^{-1}$  was found to be 2.05 and  $2.17 \mu\text{g Se g}^{-1}$  in duplicate measurements. Determinations of selenium concentrations in 6 replicate samples of pyloric caeca from selenite exposed sea stars resulted in a standard deviation of 4.2%. Selenite concentrations in the seawater were determined with a Perkin-Elmer MHS-20 hydride generating system.

**Cadmium analysis.** Tissue samples were freeze dried, weighed and dissolved in 2 to 3 ml concentrated nitric acid at 120°C to obtain a clear yellow solution. The samples were evaporated almost to dryness, then 200  $\mu\text{l}$  30%  $\text{H}_2\text{O}_2$  was cautiously added. Thereafter the samples were evaporated to dryness and redissolved in 10.0 ml 0.2% nitric acid. The cadmium concentration in the solution resulting from this procedure was determined on a Perkin-Elmer 2380 atomic absorption spectrophotometer. Air-acetylene flame and deuterium background correction were used. An NBS oyster standard with a certified cadmium concentration of  $3.5 \pm 0.4 \mu\text{g Cd g}^{-1}$  dry wt was found to contain 3.1 and  $3.2 \mu\text{g Cd g}^{-1}$  dry wt (duplicate determinations).

**Radioactivity measurements.** Radioactive cadmium ( $^{109}\text{Cd}$ ) was obtained from New England Nuclear and radioactivity was measured with a Searle Mark III Liquid Scintillation Counter. Tissue samples were dissolved in Lumasolve prior to determination of  $^{109}\text{Cd}$  content. Radioactivity is given as disintegrations per minute (dpm).

**Statistical treatment of data.** Two-tailed student's *t*-tests and regression analysis were used in statistical evaluation of the data (Sokal & Rohlf 1969).

**Experiments.** The 4 experimental species were exposed to  $200 \mu\text{g Cd l}^{-1}$  ( $1.78 \mu\text{M}$ ) alone and  $200 \mu\text{g Cd l}^{-1}$  plus an equimolar selenite concentration ( $141 \mu\text{g Se-SeO}_3^- \text{l}^{-1}$ ) for ca 1 mo (Expts 1, 7, 8 and 9; Table 1). An additional group of mussels was exposed to  $200 \mu\text{g Cd l}^{-1}$  and a 5-fold molar selenite concentration (Expt 7; Table 1).

Marked effects of selenite on cadmium uptake were noted in *Asterias rubens*, and additional experiments (Expts 2 to 6) were carried out with this species. In Expt 2, the effect of varying selenite concentrations on cadmium uptake (from  $200 \mu\text{g Cd l}^{-1}$ ) was assessed and in Expt 3 it was investigated whether cadmium affected selenium uptake (Table 1). In Expt 4 the effect of selenite on cadmium uptake was investigated at cadmium concentrations ranging from background levels to  $400 \mu\text{g Cd l}^{-1}$  and in Expts 5 and 6 the effects of different selenite concentrations on uptake of  $^{109}\text{Cd}$

from background concentrations were investigated (Table 2). Background levels of cadmium and selenium in *A. rubens* were determined.

## RESULTS

### Experiment 1

Background levels of selenium and cadmium in *Asterias rubens* from Lillebælt are shown in Table 3.

Sea stars exposed to  $200 \mu\text{g Cd l}^{-1}$  took up cadmium in tube feet and pyloric caeca at a rate of ca  $1.2 \mu\text{g Cd g}^{-1}$  dry wt  $\text{d}^{-1}$  during the first 10 d, when steady state

Table 3. *Asterias rubens*. Background selenium and cadmium concentrations ( $\mu\text{g g}^{-1}$  dry wt  $\pm$  SD) in 40 to 50 g sea stars from Lillebælt

	Pyloric caeca	Body wall	Tube feet	<i>n</i>
$\mu\text{g Se g}^{-1}$	$3.4 \pm 0.7$	$0.85 \pm 0.03$	$1.62 \pm 0.23$	7
$\mu\text{g Cd g}^{-1}$	$0.18 \pm 0.06$	$0.48 \pm 0.21$	$0.26 \pm 0.07$	5

concentrations of 10 to  $12 \mu\text{g Cd g}^{-1}$  were reached (Fig. 1a). Uptake in the body wall proceeded at a slightly higher rate and a steady state concentration of 20 to  $25 \mu\text{g Cd g}^{-1}$  was reached after 2 wk (Fig. 1a).

Sea stars exposed to  $200 \mu\text{g Cd} + 141 \mu\text{g Se-SeO}_3^- \text{l}^{-1}$  took up cadmium in body wall and pyloric caeca at a rate of  $5 \mu\text{g Cd g}^{-1} \text{d}^{-1}$  and in tube feet at a rate of  $10 \mu\text{g Cd g}^{-1} \text{d}^{-1}$  (Fig. 1b). Uptake proceeded linearly over 4 wk, and no trend toward a steady state level was observed. Differences between cadmium concentrations in selenite and non-selenite exposed groups were statistically significant ( $p < 0.01$ ) for all tissues and sampling times.

Selenium was taken up in sea stars exposed to selenite and cadmium at rates of 2.2, 2.9, and  $5.2 \mu\text{g Se g}^{-1}$  dry wt  $\text{d}^{-1}$  in body wall, pyloric caeca, and tube feet, respectively (Fig. 1c). No trend toward steady state levels was observed during 4 wk.

Cd:Se molar ratios in the tissues of sea stars exposed to selenite + cadmium increased for the first 2 wk, reaching steady state levels of ca 1.1, 1.2, and 1.8 in pyloric caeca, tube feet, and body wall, respectively (Fig. 1d).

### Experiment 2

All of the sea stars exposed to selenite concentrations of 562 to  $4496 \mu\text{g Se-SeO}_3^- \text{l}^{-1}$  died during the experiment. This may be due to the toxicity of selenite, but the overall mortality in this experiment was consider-

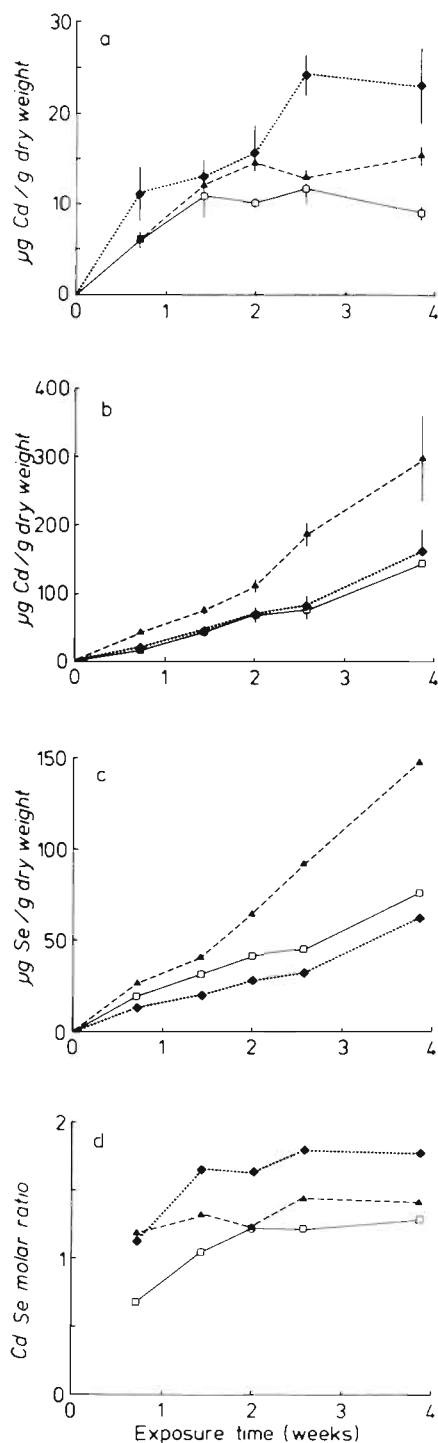


Fig. 1. *Asterias rubens*. (a) Cadmium concentrations in tissues of sea stars exposed to  $200 \mu\text{g Cd l}^{-1}$  (b) Cadmium concentrations in tissues of sea stars exposed to  $200 \mu\text{g Cd} + 141 \mu\text{g Se-SeO}_3^{2-} \text{ l}^{-1}$ . (c) Selenium concentrations in tissues of sea stars exposed to  $200 \mu\text{g Cd} + 141 \mu\text{g Se-SeO}_3^{2-} \text{ l}^{-1}$  (d) Cd:Se molar ratios in tissues of sea stars exposed to  $200 \mu\text{g Cd} + 141 \mu\text{g Se-SeO}_3^{2-} \text{ l}^{-1}$ . (▲) Tube feet; (□) pyloric caeca; (◆) body wall. Mean  $\pm$  SEM shown in (a) and (b). Selenium was determined in samples pooled from individuals. Numbers of animals can be seen in Table 1

able (Table 1), probably a result of damage from the seines in which the sea stars were caught. In spite of the mortality in this experiment, cadmium uptake in individuals exposed to  $200 \mu\text{g Cd} + 141 \mu\text{g Se-SeO}_3^{2-}$  was comparable to uptake after 3 wk in Expt 1.

Cadmium uptake in body wall, tube feet and pyloric caeca was augmented by 17.6, 17.6, and  $70 \mu\text{g Se-SeO}_3^{2-} \text{ l}^{-1}$ , respectively (Fig. 2a), while lower selenite

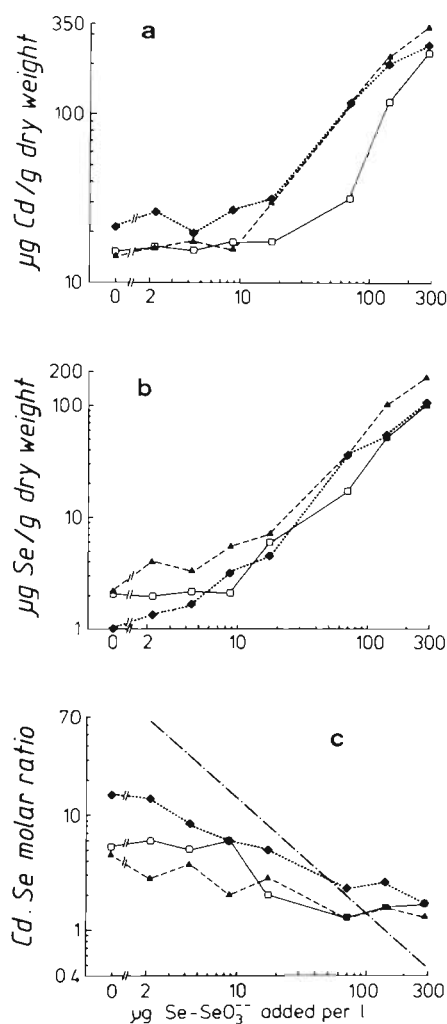


Fig. 2. *Asterias rubens*. (a) Cadmium concentrations, (b) selenium concentrations and (c) Cd:Se molar ratios in tissues of sea stars exposed to  $200 \mu\text{g Cd l}^{-1}$  and different selenite concentrations for 21 d. Results from analysis of samples pooled from individuals in each group are shown. (—) in (c): Cd:Se molar ratio in seawater. Symbols as in Fig. 1

concentration had no or only marginal effects on cadmium uptake over 3 wk.

Exposure to all of the selenite concentrations seemed to augment selenium concentrations in tube feet and body wall (Fig. 2b), whereas selenium concentrations in pyloric caeca were augmented only by exposure to

selenite concentrations above  $10 \mu\text{g Se-SeO}_3^- \text{ l}^{-1}$  (Fig. 2b). Selenium concentrations in the pyloric caeca of sea stars exposed to  $200 \mu\text{g Cd l}^{-1}$  for 2 wk seemed somewhat low compared with unexposed individuals (Fig. 2b; Table 3).

Cd:Se ratios in the tissues decreased as the ambient selenite concentration increased (Fig. 2c). The changes in Cd:Se molar ratios in the tissues as the selenite concentration increased were much smaller than the changes in the water phase (Fig. 2c).

### Experiment 3

Exposure to  $200 \mu\text{g Cd l}^{-1}$  did not consistently affect the uptake of selenium from  $141 \mu\text{g Se-SeO}_3^- \text{ l}^{-1}$  (Fig. 3a, b). At Day 17, the selenium concentration in the body wall of the 2 groups showed a statistically significant difference ( $p = 0.039$ ), but for the other tissues and sampling days no differences were observed. After 1 wk selenium was taken up linearly with time at rates of 1.4, 1.7, and  $2.0 \mu\text{g Se g}^{-1} \text{ dry wt d}^{-1}$  in body wall, pyloric caeca and tube feet, respectively (Fig. 3a, b). In the selenium + cadmium exposed group, cadmium was taken up linearly with time at rates of 2.9, 3.5, and  $7.0 \mu\text{g Cd g}^{-1} \text{ dry wt d}^{-1}$  (Fig. 3c). During the experiment Cd:Se ratios approached 1 and 1.2 in pyloric caeca and body wall, respectively, while the Cd:Se molar ratio in tube feet increased from 1.3 to 2.2 during the 4 wk (Fig. 3d).

Cadmium and selenium uptake was lower in this winter experiment compared with the 2 summer experiments (Expts 1 and 2).

### Experiments 4, 5 and 6

Generally, 50 to 100 % of the decrease in the  $^{109}\text{Cd}$  concentration in the water phase could be accounted for by uptake by the sea stars (Table 2).

In Expt 6, sea stars exposed to  $200 \mu\text{g Se-SeO}_3^- \text{ l}^{-1}$  were in such poor condition after 9 d exposure that the remaining 5 specimens were analysed for  $^{109}\text{Cd}$  on that day.

In the groups exposed to radioactive cadmium alone the concentration factors for  $^{109}\text{Cd}$  in whole sea stars were  $1127 \pm 541$  (Expt 4; Fig. 4),  $829 \pm 328$  (Expt 5; Fig. 5), and  $809 \pm 380$  (Expt 6; Fig. 5). Sea stars exposed to stable cadmium concentrations between 0.0125 and  $3.2 \mu\text{g Cd l}^{-1}$  had concentration factors in the range 600 to 900, and although the concentration factors in 3 of these groups were significantly lower than in the control group of the experiment, no decreasing trend was seen for this range of stable cadmium concentrations (Fig. 4). Concentration factors

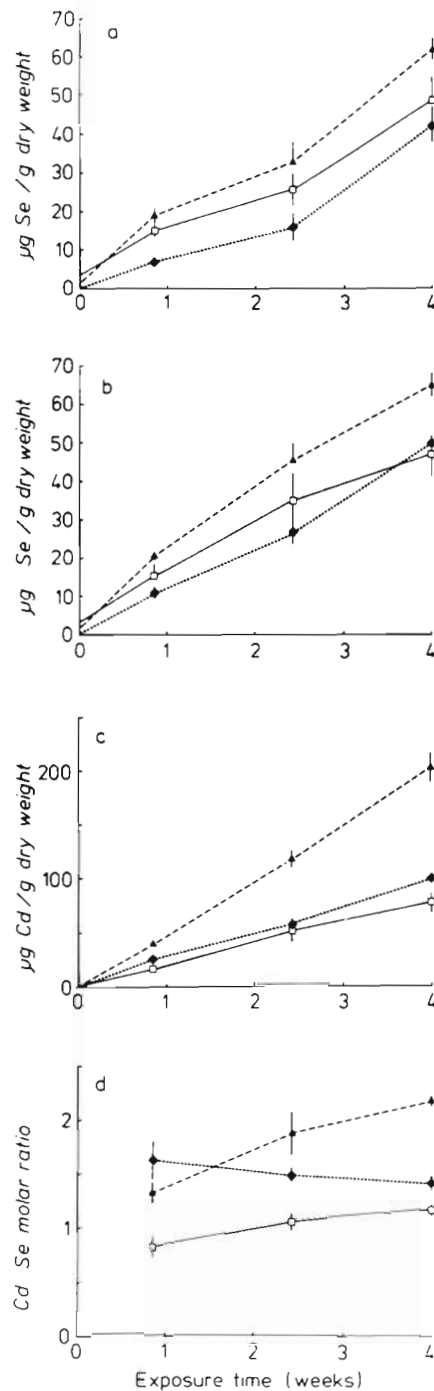


Fig. 3. *Asterias rubens*: (a) Selenium concentrations in tissues of sea stars exposed to  $141 \mu\text{g Se-SeO}_3^- \text{ l}^{-1}$  (b) Selenium concentrations in tissues of sea stars exposed to  $141 \mu\text{g Se-SeO}_3^- + 200 \mu\text{g Cd l}^{-1}$  (c) Cadmium concentrations in tissues of sea stars exposed to  $141 \mu\text{g Se-SeO}_3^- + 200 \mu\text{g Cd l}^{-1}$  (d) Cd:Se molar ratios in tissues of sea stars exposed to  $141 \mu\text{g Se-SeO}_3^- + 200 \mu\text{g Cd l}^{-1}$ . Symbols as in Fig. 1

for sea stars exposed to higher stable cadmium concentrations decreased from 710 at  $3.2 \mu\text{g Cd l}^{-1}$  to ca 130 at 200 and  $400 \mu\text{g Cd l}^{-1}$  (Fig. 4).

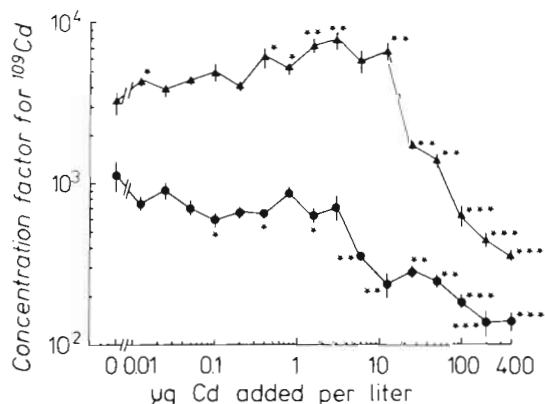


Fig. 4. *Asterias rubens*. Concentration factor (dpm g<sup>-1</sup> dry wt tissue ÷ dpm ml<sup>-1</sup> seawater) for <sup>109</sup>Cd in sea stars after exposure to different concentrations of stable cadmium for 14 d. With (▲) and without (●) addition of 141 µg Se-SeO<sub>3</sub><sup>-</sup> l<sup>-1</sup>. Means ± SEM are shown. Numbers of individuals are given in Table 2. \*, \*\* and \*\*\* indicate that the difference from the control group in each series is statistically significant at the 0.05, 0.01 and 0.001 level, respectively

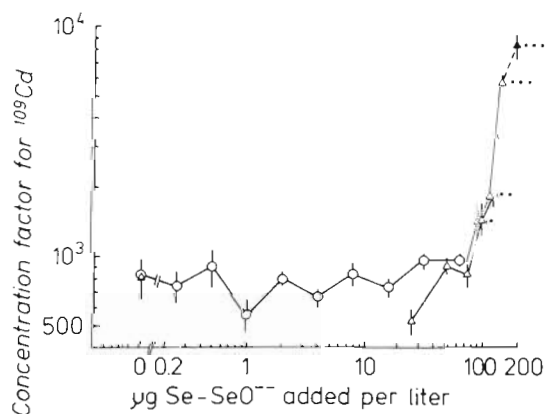


Fig. 5. *Asterias rubens*. Concentration factor (dpm g<sup>-1</sup> dry wt tissue ÷ dpm ml<sup>-1</sup> seawater) for <sup>109</sup>Cd in sea stars after exposure to different selenite concentrations for 14 d. (○) Expt 5; (Δ) Expt 6 (▲: exposed for 9 d only). Means ± SEM are shown. Numbers of individuals are given in Table 2. \*, \*\* and \*\*\* indicate that the difference from the non-selenite exposed group is statistically significant at the 0.05, 0.01 and 0.001 level, respectively

Exposure to 0.25 to 75 µg Se-SeO<sub>3</sub><sup>-</sup> l<sup>-1</sup> for 2 wk did not affect <sup>109</sup>Cd-concentration factors in the sea stars (Fig. 5), while selenium concentrations at and above 100 µg Se-SeO<sub>3</sub><sup>-</sup> l<sup>-1</sup> significantly augmented <sup>109</sup>Cd-concentration factors (Fig. 5).

In the groups exposed to 141 µg Se-SeO<sub>3</sub><sup>-</sup> l<sup>-1</sup>, the concentration factor for <sup>109</sup>Cd increased from 3300 to 7800 with from 0 to 3.2 µg Cd added l<sup>-1</sup> (Fig. 4). In this interval of stable cadmium concentrations the correlation between concentration factors and cadmium concentrations was highly significant (log concentration factor = 3.78 + 0.148 log µg Cd added l<sup>-1</sup>, r<sup>2</sup> = 0.4179, n = 68; p < 0.001). For cadmium concentrations above

12.5 µg Cd l<sup>-1</sup>, concentration factors for <sup>109</sup>Cd decreased considerably, reaching concentration factors of 350 at 400 µg Cd l<sup>-1</sup>.

### Experiment 7

Uptake of cadmium by *Mytilus edulis* reduced cadmium concentrations in the seawater from 200 µg Cd l<sup>-1</sup> to between 80 and 120 µg Cd l<sup>-1</sup> in the period between successive water changes.

*Mytilus edulis* exposed to 200 µg Cd l<sup>-1</sup> alone for 4 wk took up cadmium in mantle, digestive gland, foot and adductor muscle linearly with time at rates of 9.3, 36, 7.7, and 6.4 µg Cd g<sup>-1</sup> dry wt d<sup>-1</sup>, respectively (Fig. 6). In gills and shell, cadmium was taken up at an

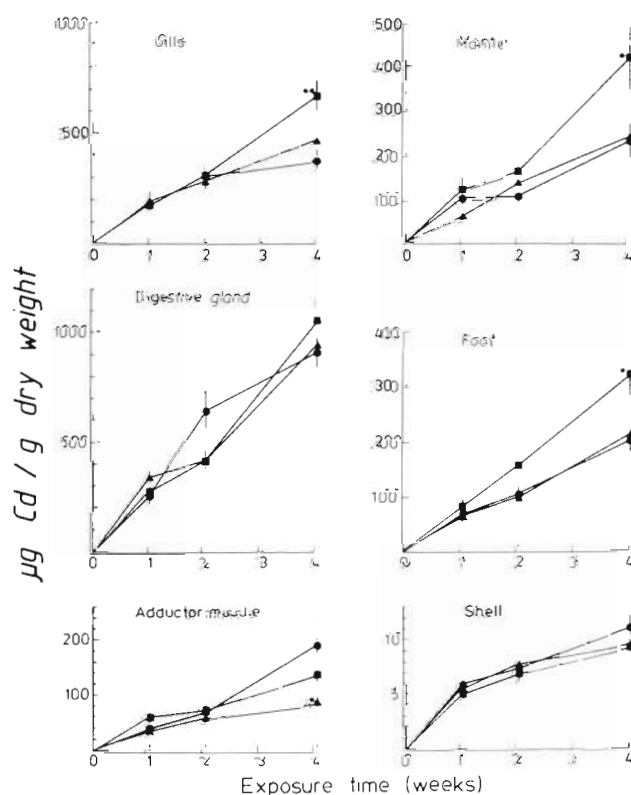


Fig. 6. *Mytilus edulis*. Concentrations of cadmium in tissues of mussels exposed to (●) 200 µg Cd l<sup>-1</sup>, (▲) 200 µg Cd + 141 µg Se-SeO<sub>3</sub><sup>-</sup> l<sup>-1</sup>, and (■) 200 µg Cd + 703 µg Se-SeO<sub>3</sub><sup>-</sup> l<sup>-1</sup>. Means ± SEM are shown. Numbers of individuals are given in Table 1. \* and \*\* indicate that difference between the selenite and non-selenite exposed group is statistically significant at the 0.05 and 0.01 level, respectively

initial rate of 24 and 0.8 µg Cd g<sup>-1</sup> dry wt d<sup>-1</sup> for 1 to 2 wk after which time uptake rates decreased. Concurrent exposure to 141 µg Se-SeO<sub>3</sub><sup>-</sup> l<sup>-1</sup> only affected cadmium uptake in the adductor muscle which contained less cadmium after 4 wk than the group exposed to cadmium alone (Fig. 6). Concurrent exposure to 703 µg Se-SeO<sub>3</sub><sup>-</sup> l<sup>-1</sup> did not affect cadmium uptake in

digestive gland, shell, and adductor muscle, whereas mantle and foot took up cadmium linearly with time at rates 1.6 and 1.5 times higher than in the group exposed to cadmium alone (Fig. 6). In gills, the initial cadmium uptake rate was not affected by  $703 \mu\text{g Se-SeO}_3^{2-} \text{ l}^{-1}$ , but uptake proceeded linearly over 4 wk, leading to increased cadmium concentration relative to the group exposed to cadmium alone (Fig. 6).

### Experiment 8

Exposure to  $141 \mu\text{g Se-SeO}_3^{2-} \text{ l}^{-1}$  did not affect uptake of cadmium from  $200 \mu\text{g Cd l}^{-1}$  in *Littorina littorea* (Fig. 7). Uptake in soft parts proceeded linearly

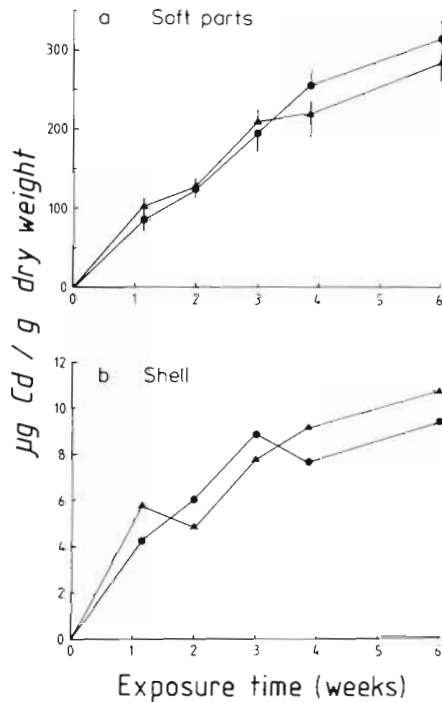


Fig. 7. *Littorina littorea*. Concentrations of cadmium in (a) soft parts and (b) shells of periwinkles exposed to (●)  $200 \mu\text{g Cd l}^{-1}$ , (▲)  $200 \mu\text{g Cd} + 141 \mu\text{g Se-SeO}_3^{2-} \text{ l}^{-1}$ . For soft parts means  $\pm$  SEM for 10 individuals are shown. For shells one determination on a pooled sample from the 10 individuals was carried out

with time at a rate of  $11 \mu\text{g Cd g}^{-1} \text{ dry wt d}^{-1}$  for 3 to 4 wk; thereafter the uptake rate levelled off (Fig. 7a). The uptake rate in the shell was  $0.6 \mu\text{g Cd g}^{-1} \text{ dry wt d}^{-1}$  for 1 wk; thereafter the uptake rate levelled off (Fig. 7b).

### Experiment 9

Exposure to  $141 \mu\text{g Se-SeO}_3^{2-} \text{ l}^{-1}$  did not affect uptake of cadmium from  $200 \mu\text{g Cd l}^{-1}$  in *Arenicola*

*marina* (Fig. 8). Cadmium was taken up in the body wall linearly with time over 4 wk at a rate of  $6.1 \mu\text{g Cd g}^{-1} \text{ dry wt d}^{-1}$  (Fig. 8a). In the gut, the uptake rate over the first 3 wk was  $10.4 \mu\text{g Cd g}^{-1} \text{ dry wt d}^{-1}$ ; a trend toward an increased uptake rate late in the experiment

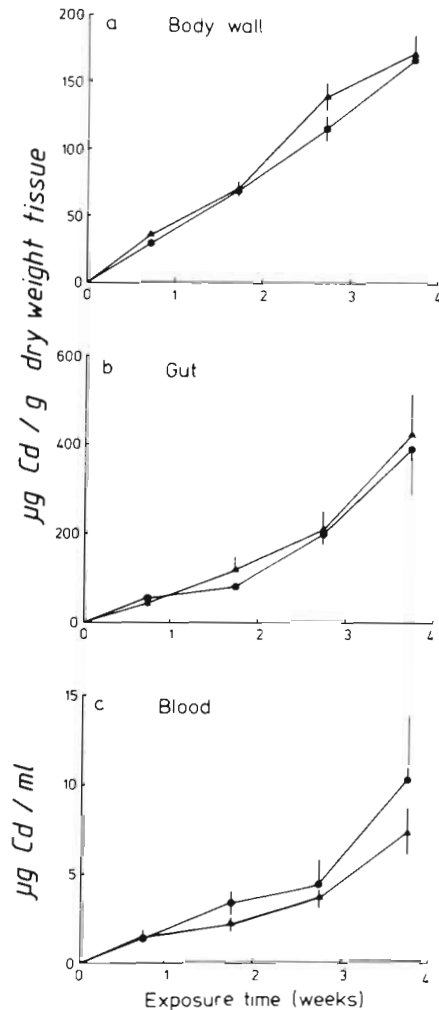


Fig. 8. *Arenicola marina*. Concentrations of cadmium in (a) body wall, (b) gut, and (c) blood of lugworms exposed to (●)  $200 \mu\text{g Cd l}^{-1}$  and (▲)  $200 \mu\text{g Cd} + 141 \mu\text{g Se-SeO}_3^{2-} \text{ l}^{-1}$ . Means  $\pm$  SEM are shown. Numbers of individuals are given in Table 1

was observed (Fig. 8b). The same trend was seen in the blood where the uptake rate during the first 3 wk was  $0.23 \mu\text{g Cd ml}^{-1} \text{ d}^{-1}$  (Fig. 8c).

## DISCUSSION

### Cadmium uptake

The rate at which cadmium is taken up by *Mytilus edulis* increases with decreasing salinity (George et al.



1977, Jackim et al. 1977, Fischer 1986) and increasing amounts of algae filtered (Janssen & Scholz 1979, Köhler & Riisgaard 1982, Borchardt 1983). Although temperature does not affect cadmium accumulation in *Mytilus* spp. (Fowler & Benayoun 1974, Jackim et al. 1977), uptake rates may depend on season (Jackim et al. 1977). Concentration factors for cadmium decrease with increasing cadmium concentrations (Amiard et al. 1986, Riisgaard et al. 1987).

Cadmium uptake rates reported for *Mytilus edulis*

exposed to 100 and 200  $\mu\text{g Cd l}^{-1}$  in the laboratory show large variations (Table 4), and the present results are within the range reported earlier. The cadmium uptake rate in *Littorina littorea* reported by Langston & Zhou (1987) appears very low compared with that reported by Amiard et al. (1987) and the results of the present work (Table 5). Langston & Zhou (1987), however, exposed the periwinkles in tidal tanks, and as the degree of accumulation is related to the immersion time (Langston & Zhou 1987), the difference in uptake

Table 4. *Mytilus edulis*. Cadmium uptake rates in soft parts of mussels exposed to 100 and 200  $\mu\text{g Cd l}^{-1}$ . Uptake rates have been calculated in the initial exposure period, where cadmium is taken up linearly with time. For semi-static exposure the intervals between water changes are given

Tissue	Cadmium uptake rate ( $\frac{\mu\text{g Cd g}^{-1} \text{ dry wt d}^{-1}}{\mu\text{g Cd ml}^{-1}}$ )	Exposure concentration ( $\mu\text{g Cd ml}^{-1}$ )	Shell length (mm)	Salinity (‰)	Temperature (°C)	Exposure system	Time of year	Fed	Source
Gills	46	200 <sup>a</sup>	–	35	15.5	Semi-static	Winter	No	Amiard et al. (1987)
Viscera	36					1 d			
Rest	24								
Gills	87	100 <sup>a</sup>							
Viscera	44								
Rest	29								
Total	94	100	15–18	29	18	Flow	Jun	Yes	Poulsen et al. (1982)
Total	21 (33)	200	50–70	27	5	Semi-static	Jun	No (yes)	Köhler & Riisgaard (1982)
Mantle	24 (37)					3 d			
Muscle	9 (12)								
Rest	28 (42)								
Total	74 (166)	100	60 (42)	25	10	Semi-static	Oct	No (yes)	Janssen & Scholz (1979)
Midgut gland	158 (252)					2 d			
Gills	81 (216)								
Mantle	28 (126)								
Foot	25 (46)								
Add. muscle	49 (88)								
Kidney	79 (172)								
Total	128	200	30	17	–	Flow	Jun	No	Riisgaard et al. (1987)
Total	231	100	27						
Total	740	100	<sup>b</sup>	17	10	Semi-static	–	No	George et al. (1977)
Total	514			23		3 d			
Total	407			28/33					
Total	38	200	50–70	33	13	Semi-static	–	No	George & Coombs (1977)
Mantle	16					4 d			
Viscera	48								
Gills	24								
Kidney	203								
Muscle	7								
Mantle	47	200	47–59	22–24	15.5	Semi-static	Jun	No	Present results
Digestive	180					3–4 d			
Gills	120								
Foot	39								
Add. muscle	32								

<sup>a</sup> Calculated from regression line

<sup>b</sup> Mature mussels

Table 5. *Littorina littorea*. Cadmium uptake rates in soft parts of periwinkles exposed to cadmium in seawater. Uptake rates have been calculated in the initial exposure period, where cadmium is taken up linearly with time. For semi-static exposure systems the intervals between water changes are given

Cadmium uptake rate ( $\frac{\mu\text{g Cd g}^{-1} \text{ dry wt d}^{-1}}{\mu\text{g Cd ml}^{-1}}$ )	Exposure concentration ( $\mu\text{g Cd ml}^{-1}$ )	Soft part dry wt (mg)	Salinity (‰)	Temperature (°C)	Exposure system	Time of year	Fed	Source
8	400	110 ± 20	33	15.4	Flow, tidal tanks	Winter	No	Langston & Zhou (1987)
32	200 <sup>a</sup>	—	35	15.5	Semi-static 1 d	Winter	No	Amiard et al. (1987)
24	400 <sup>a</sup>	—	—	—	—	—	—	—
55	200	41–128	21–23	15.5	Semi-static 3–4 d	Aug	No	Present results

<sup>a</sup> Calculated from regression line

rates can probably be explained by the different exposure procedures. Salinity differences may explain why uptake rates in the present study exceed those of Amiard et al. (1987). Studies on uptake kinetics for cadmium in *Arenicola marina* and *Asterias rubens* have not previously been reported. Cadmium uptake kinetics in sea stars are apparently different from those of the 3 other species investigated here. Where the soft tissues of mussels, periwinkles and lugworms concentrate cadmium to several hundreds of  $\mu\text{g Cd g}^{-1}$  dry wt, sea stars reach steady state levels of no more than 10 to 25  $\mu\text{g Cd g}^{-1}$  dry wt. Shore crabs *Carcinus maenas* exposed to 200  $\mu\text{g Cd l}^{-1}$  under similar conditions reach cadmium concentrations of 50, 10, 30 and 12  $\mu\text{g Cd g}^{-1}$  dry wt in gills, carapace, hypodermis, and hepatopancreas after 4 wk (Bjerregaard 1988), and therefore the cadmium uptake rates for *A. rubens* cannot be considered to be exceptionally low. Finding that starfish *Leptasterias polaris* fed mercury-contaminated mussels do not accumulate mercury to very high levels, Pelletier & Larocque (1987) suggested that sea stars possess an efficient detoxification/elimination mechanism for mercury. Such a mechanism might also operate in *A. rubens* with cadmium.

Although the concentration factor for cadmium in sea stars decreases at the highest cadmium concentrations tested, it is noteworthy that uptake in the sea stars is proportional to the ambient cadmium concentration from 25 to 2500  $\text{ng Cd l}^{-1}$ .

Accumulation of cadmium in the blood of *Arenicola marina* exposed to 200  $\mu\text{g Cd l}^{-1}$  differs from the conditions in *Carcinus maenas*, which regulates haemolymph cadmium concentration below that of the surrounding medium if the cadmium concentration is lower than 4  $\text{mg Cd l}^{-1}$  (Wright 1977, Wright & Brewer 1979, Bjerregaard & Vislie 1985, Bjerregaard 1988).

#### Effect of selenium on cadmium uptake

Whereas selenium has no or only marginal effects on cadmium uptake in *Mytilus edulis*, *Arenicola marina*, and *Littorina littorea* the augmenting effect of selenium on cadmium uptake reported for *Carcinus maenas* (Bjerregaard 1982, 1985, 1988) was also seen in *Asterias rubens*. When exposed to cadmium alone, shore crabs (Bjerregaard 1988) and sea stars take up cadmium in their soft tissues 5 to 20 times more slowly than do mussels, periwinkles and lugworms, and it is interesting to note that, in the few species investigated, selenium only augments cadmium uptake in the species with the lowest cadmium uptake rate.

The mechanisms by which selenium augments cadmium uptake are not fully understood. Concurrent administration of selenite to cadmium-treated rats greatly increases the binding of cadmium in the blood plasma (Gasiewicz & Smith 1976, 1978, Nishiyama et al. 1987). Gasiewicz & Smith (1976, 1978) suggest that the effect of selenite on cadmium binding depends on the metabolic conversion of Se(IV) to Se(-II), which may form a protein-stabilized CdSe-complex with a molar ratio Cd:Se close to 1. A similar mechanism might be operating in the tissues of sea stars, but the Cd:Se ratio in the tissues shows some deviation from unity. However, in the pyloric caeca where surface adsorption can probably be excluded, the Cd:Se molar ratio approaches unity during 4 wk exposure.

Results indicate that selenite concentrations of 200 and 500  $\mu\text{g Se-SeO}_3^- \text{ l}^{-1}$  are lethal to small and medium sized sea stars, respectively, over 2 to 3 wk exposure. For small sea stars it takes selenite concentrations of ca 15% of this concentration to augment cadmium uptake in whole organisms. It is possible that these selenite concentrations adversely affect the total

metabolism of the sea star in a way that will inhibit normal elimination of cadmium from the organism. In medium sized sea stars and shore crabs *Carcinus maenas* cadmium uptake is affected by selenite concentrations 50 and 200 (Bjerregaard 1988) times, respectively, below the lethal concentrations, so a non-specific mechanism hardly explains all of the interactions between cadmium and selenium in marine invertebrates.

Exposure to 50  $\mu\text{g Se-SeO}_3^{--} \text{ l}^{-1}$  does not affect uptake of mercury from 30  $\mu\text{g Hg l}^{-1}$  in *Mytilus edulis* (Pelletier 1986), while 1000  $\mu\text{g Se-SeO}_3^{--} \text{ l}^{-1}$  augments mercury uptake from 100  $\mu\text{g Hg l}^{-1}$  in the clam *Anadara granosa* (Chandy & Patel 1985). A similar effect of the selenite concentration may be present in the interaction between selenium and cadmium in bivalves, since 703 but not 141  $\mu\text{g l}^{-1}$  augments cadmium uptake in some tissues of *M. edulis*.

### Ecological impacts

Whereas the behaviour of selenium in open ocean water has been well established during recent years (Measures & Burton 1980, Measures et al. 1980, Cutter & Bruland 1984), the behaviour in coastal waters seems more complex (Apte et al. 1986). In coastal waters and surface ocean waters selenite constitutes only a minor fraction of the total selenium concentration and values from <2 to 16 ng Se-SeO<sub>3</sub><sup>--</sup> l<sup>-1</sup> have been reported (Measures & Burton 1980, Apte et al. 1986). In coastal areas with anthropogenic input of selenium,  $\mu\text{g l}^{-1}$  quantities of selenium may be found locally (Ahsanullah & Brand 1985, Phillips 1987). As selenite concentrations of ca 10  $\mu\text{g Se-SeO}_3^{--}$  augment cadmium uptake in sea stars in short-term experiments, cadmium concentrations in organisms living their whole lives in selenite polluted areas may be affected.

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