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# Cadmium accumulation in *Littorina littorea*, *Mytilus edulis* and *Carcinus maenas*: the influence of salinity and calcium ion concentrations

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**Abstract** Accumulation of waterborne cadmium in *Littorina littorea*, *Mytilus edulis* and *Carcinus maenas* (collected in 1988 and 1989 around the island of Funen, Denmark) was investigated in a matrix of salinities (10 to 30‰) and calcium concentrations (2.9 to 8.9 m*M* $Ca^{++}$). Cadmium accumulation rates in soft parts of *L. littorina*, soft parts and shells of *M. edulis* and whole bodies and exoskeletons of *C. maenas* decreased with increasing salinity. Changes in the calcium concentrations accounted for 72% of the 'salinity effect' on cadmium accumulation rates in *L. littorina*, whereas calcium concentrations had little or no effect on cadmium accumulation in *M. edulis*. Cadmium accumulation in the whole body of *C. maenas* was affected equally by calcium concentrations and total salinity, whereas accumulation in the exoskeleton was mainly affected by changes in total salinity. Individual variability in cadmium accumulation in the organs of *C. maenas* was greater than the variation attributable either to changes in ambient calcium concentrations or total salinity. An appreciable amount of the inter-individual variability in the cadmium accumulation in all three species was correlated with wet:dry weight ratios of the tissues and size of the organisms.

## Introduction

The susceptibility of many marine and estuarine invertebrates to the toxic effects of some trace metals increases as the salinity of seawater decreases (McLusky et al. 1986). Increased toxicity at lower salinities has been explained by higher metal uptake rates (including cadmium) in most species investigated.

Organisms living at low salinities generally contain higher concentrations of cadmium than organisms living at higher salinities (Phillips 1976, 1977), but the physiological mechanisms by which cadmium uptake and accumulation is enhanced at low salinities have not been elucidated.

Cadmium is present in full strength seawater mainly as an uncharged, labile $CdCl_2$ complex (Zirino and Yamamoto 1972; Long and Angino 1977; Mantoura et al. 1978). As salinity (and thereby chloride concentration) decreases, the concentration of free cadmium ions ($Cd^{++}$) increases. As $Cd^{++}$ is considered the only species of cadmium available for uptake in organisms (Sunda et al. 1978), its higher bioavailability at lower salinities might explain the effect of salinity on cadmium uptake.

Calcium has also been shown to influence uptake and/or toxicity of cadmium in crustaceans (Wright 1977a, b; Wright and Frain 1981; Bjerregaard and Vislie 1985), fish (Wicklund and Runn 1988) and algae (Heuillet et al. 1988). In the shore crab *Carcinus maenas* (L.), the effect of salinity on cadmium uptake rates could be explained partly by the presence of lower calcium concentrations in low salinity seawater (Wright 1977b).

In the mussel *Mytilus edulis*, George et al. (1978) and Carpene and George (1981) concluded that cadmium uptake was determined by the total osmolality of the ambient medium and not by the presence of particular ions. Roesijadi and Unger (1993) have, however, demonstrated some effect of calcium channel blockers on cadmium uptake in the excised gills of *Crassostrea virginica*.

The present study was initiated to investigate the effects of changing calcium concentration and total salinity on cadmium uptake in three estuarine, benthic invertebrate species (periwinkles *Littorina littorea*, mussels *Mytilus edulis*, and crabs *Carcinus maenas*).

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## Materials and methods

### Experimental individuals

Periwinkles (*Littorina littorea*), mussels (*Mytilus edulis*) and crabs (*Carcinus maenas*) were collected in 1988 and 1989 at Kerteminde

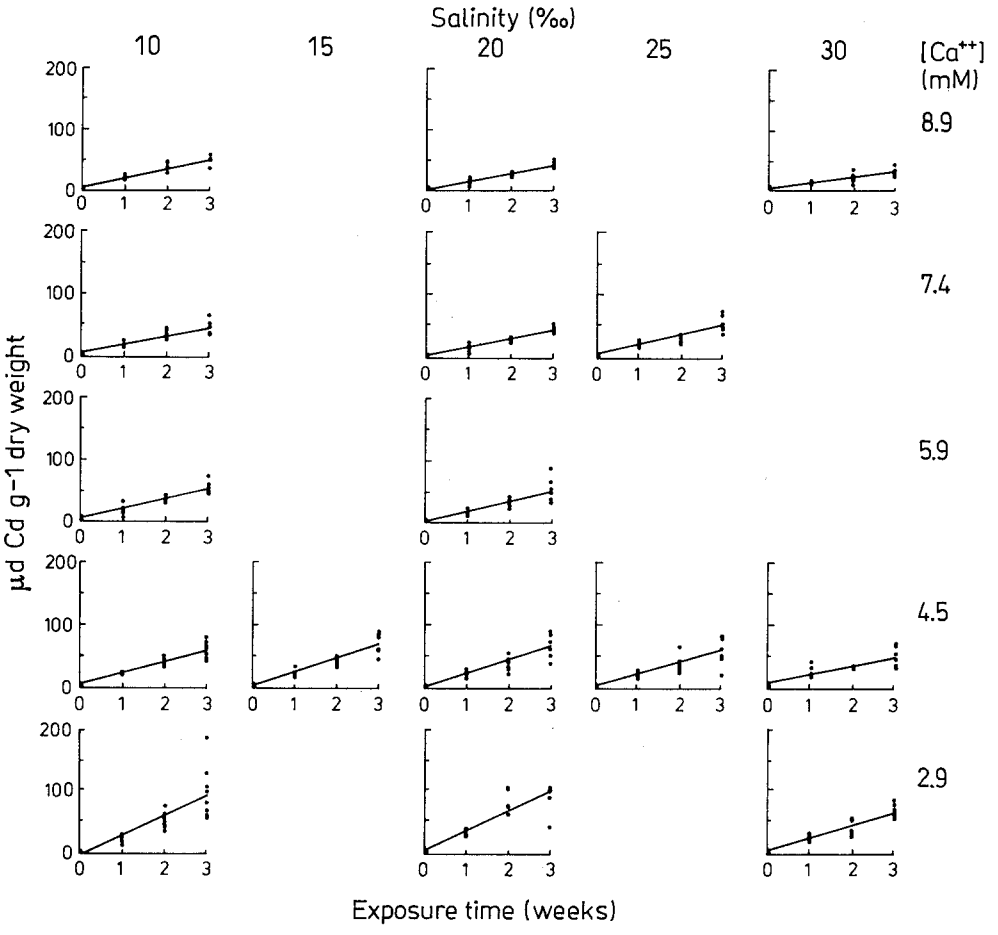


**Fig. 1** *Littorina littorea*. Cadmium concentrations in the soft parts of periwinkles exposed to 100 µg Cd $l^{-1}$ at the salinities and calcium concentrations indicated. For each combination of salinity and calcium concentration, individual values and linear regression shown. All individual regressions statistically significant with $P<0.0001$

Fjord, Lillebælt and Odense Fjord, respectively; the three localities are situated around the island of Funen, Denmark. The periwinkles were collected from an ambient water temperature of 8 °C, and the mussels and crabs were collected from water temperatures in the range 13 to 17 °C. The salinity of the waters at the collection sites varied from 13 to 28‰, depending on prevailing winds and currents.

### Acclimation procedures

The periwinkles were acclimated to 15.5 °C and 20‰ salinity in the laboratory for 2 wk, and thereafter to the experimental salinities and calcium concentrations (Fig. 1) for 2 d. The mussels were acclimated to the experimental salinities and calcium concentrations for 7 (Expt 2) and 5 (Expt 3) d prior to cadmium exposure. Crabs were acclimated for 5 d prior to cadmium exposure.

### Exposure procedures

Artificial seawater was made by dissolving Merck p.a. (pro analysis) chemicals in deionised water. 30‰ seawater was made by adding NaCl, KCl, $CaCl_2 \cdot 2H_2O$, $NaHCO_3$, $MgSO_4 \cdot 7H_2O$ and $MgCl_2 \cdot 6H_2O$ to give a final concentration of: 408 m*M* $Na^+$, 475 m*M* $Cl^-$, 8.7 m*M* $K^+$, 8.9 m*M* $Ca^{2+}$, 46.5 m*M* $Mg^{2+}$, 24.2 m*M* $SO_4^{2-}$ and 2 m*M* $HCO_3^-$. To obtain lower salinities this 30‰ seawater was diluted with deionised water.

The invertebrates were exposed to cadmium at 10, 15, 20, 25 and 30‰ and corresponding (2.9, 4.5, 5.9, 7.4 and 8.9 m*M* $Ca^{++}$, respectively) or modified calcium concentrations as shown in Figs. 1 to 4. Calcium concentrations were adjusted by addition of $CaCl_2 \cdot H_2O$; addition of calcium chloride changed the osmolality less than 6%. Stable cadmium was added as $CdCl_2$ (Merck p.a.). $^{109}Cd$ was obtained from New England Nuclear. Experimental individuals were exposed to cadmium in 10-litre polystyrene aquaria. They were not fed during experiments. No sediment was placed in the aquaria and the water was aerated. Temperature was 15.5 ± 0.5 °C. Cadmium concentrations in the water were monitored during the experiments. Generally, cadmium concentrations decreased to between 50 and 90% of the nominal value between water changes due to accumulation in the organisms.

### Chemical analysis

Concentrations of stable cadmium were determined by atomic absorption spectrophotometry and $^{109}Cd$ in the tissues of crabs from Expt 4 was measured by liquid scintillation counting, as described by Bjerregaard (1988). Concentrations of protein and cations in the haemolymph of *Carcinus maenas* were measured as described by Bjerregaard and Vislie (1985). Whole body $^{109}Cd$ contents were determined with a Bicron well-type NaI(Tl) crystal with a diameter and depth of 7.6 cm. The results were not corrected for self absorption. The activities measured by the two methods were corrected for counting efficiencies and the data are presented as disintegrations per minute (dpm).

### *Littorina littorea* Experiment

Sixteen groups of 24 periwinkles were exposed to 100 g Cd $l^{-1}$ at the salinities and calcium concentrations shown in Fig. 1. Mesh was fitted into each aquarium to prevent the periwinkles leaving the water phase. Water was exchanged once per week. After 7, 14 and 21 d exposure, eight individuals were taken from each group, and the cadmium contents in the soft parts determined. Body wet weights, dry weights of soft parts and wet to dry weight ratios of soft parts were recorded. Cadmium concentrations in the soft parts of eight unexposed periwinkles were also determined. The experiment was carried out in March.

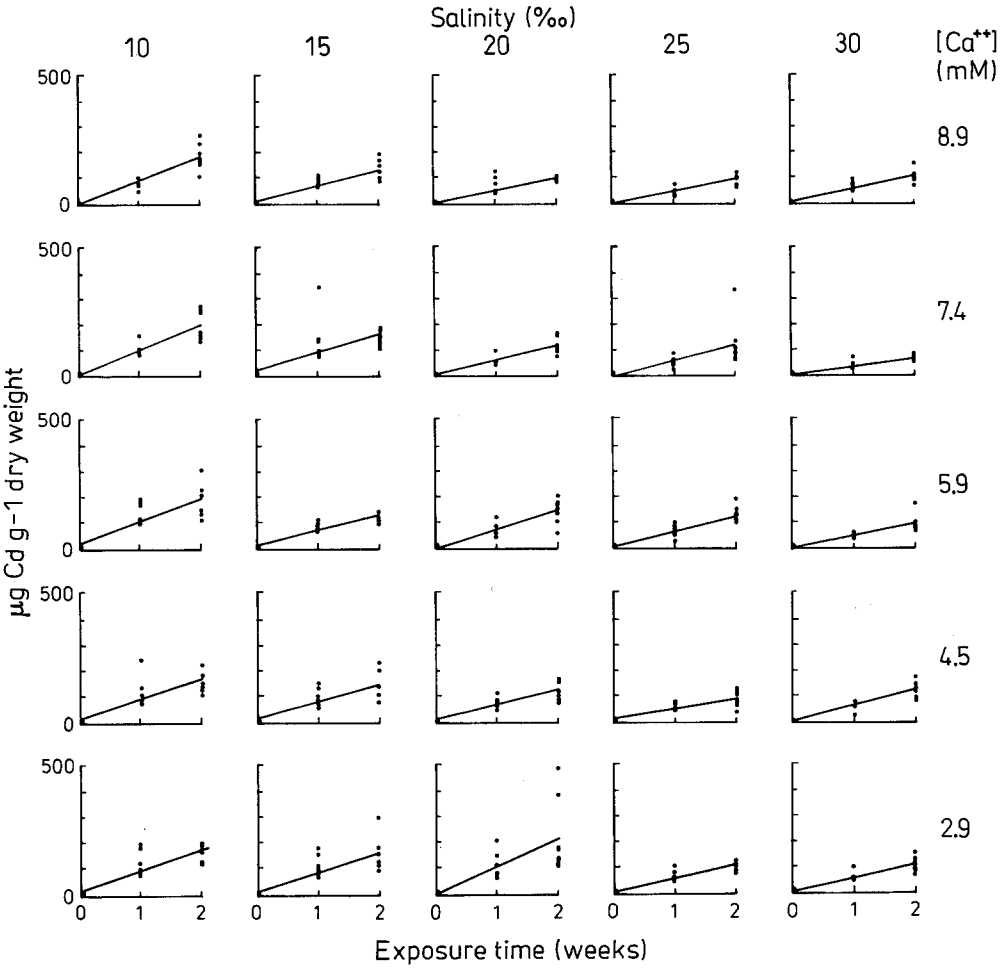


**Fig. 2** *Mytilus edulis*. Cadmium concentrations in the soft parts of mussels exposed to 100 µg Cd $l^{-1}$ at the salinities and calcium concentrations indicated. For each combination of salinity and calcium concentration, individual values and linear regression shown. All individual regressions statistically significant with $P<0.013$

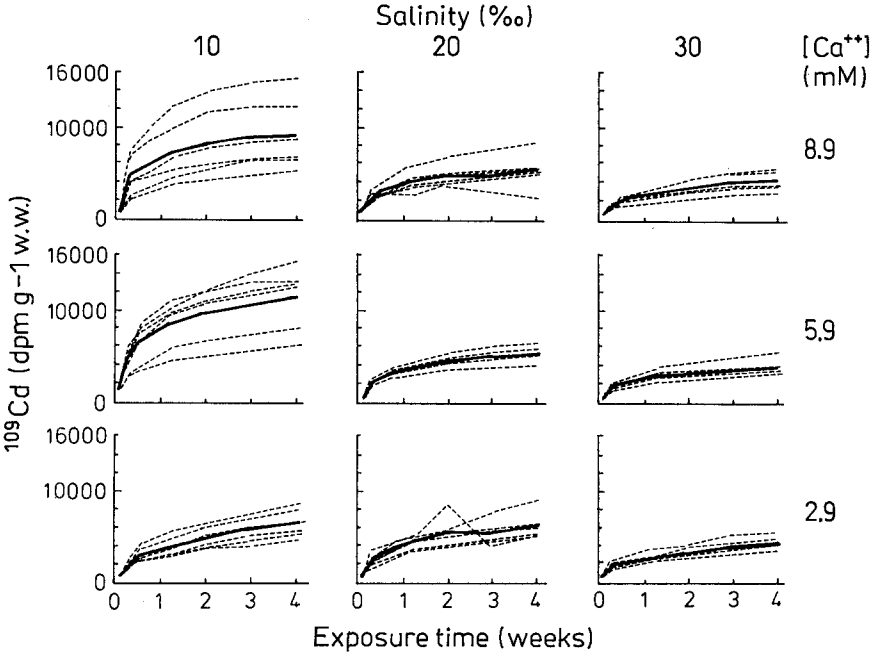


**Fig. 3** *Mytilus edulis*. Uptake of $^{109}Cd$ in whole mussels (soft parts+shell) exposed to 100 dpm $ml^{-1}$ at the salinities and calcium concentrations indicated. For each combination of salinity and calcium concentration, individual mussels represented by dashed lines and mean by the continuous line

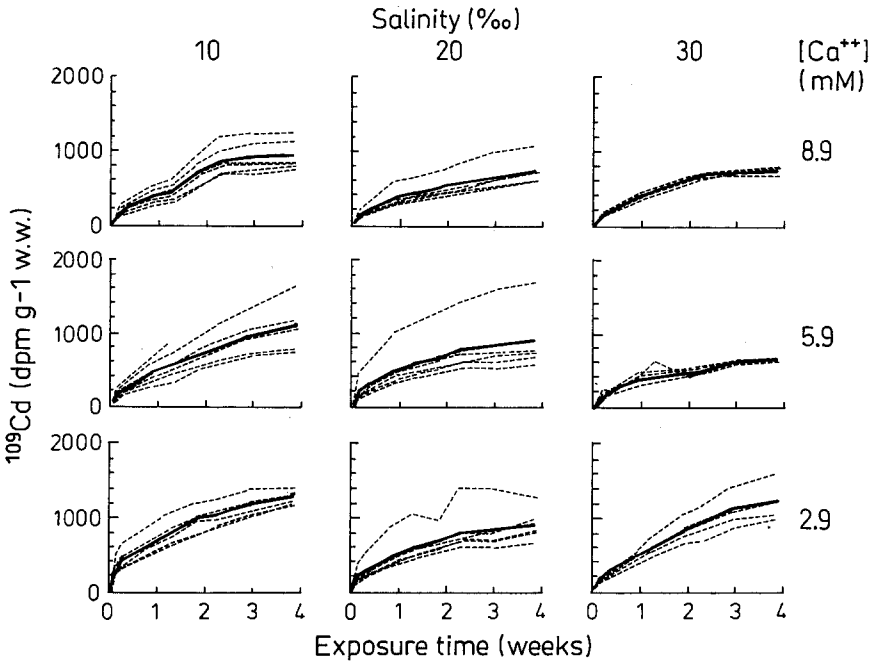


**Fig. 4** *Carcinus maenas*. Uptake of $^{109}Cd$ in crabs exposed to 100 dpm $ml^{-1}$ at the salinities and calcium concentrations indicated. For each combination of salinity and calcium concentration, individual crabs represented by dashed lines and mean by the continuous line

**Table 1** *Littorina littorea*, *Mytilus edulis* and *Carcinus maenas*. Summary of experimental conditions in the four experiments. Numbers of organisms surviving the exposure given in parentheses

| Species | *n* | Body wet wt (mean±SD) (g) | Shell length (mean±SD) (mm) |
|---|---|---|---|
| *Littorina littorea* | 384 (367) | 2.4±0.6 | |
| *Mytilus edulis* (Expt 1) | 400 (400) | 3.2±0.9 | 33±3 |
| *Mytilus edulis* (Expt 2) | 54 (54) | 3.6±0.8 | 33±2 |
| *Carcinus maenas* | 54 (51) | 23±9 | |

*Mytilus edulis* Expt 1

Twenty five groups of 16 mussels were exposed to 100 g Cd $l^{-1}$ at the salinities and calcium concentrations indicated in Fig. 2. The degree of shell opening was assessed semiquantitatively during acclimation and exposure periods. Subsamples of eight specimens were removed from each group on Days 7 and 14, and cadmium concentrations in the soft parts were measured. Body wet weights, shell lengths, dry weights of the soft parts and wet to dry weight ratios of the soft parts were recorded. Cadmium concentrations in eight unexposed mussels were determined. The exposure was initiated in late April.

*Mytilus edulis* Expt 2

Nine groups of six mussels were exposed to 100 dpm $^{109}Cd$+ 0.13 ng stable Cd $ml^{-1}$ at salinities and calcium concentrations indicated in Fig. 3. The activity of the water was monitored daily, and $^{109}Cd$ was added to replace $^{109}Cd$ removed from the water phase in each aquarium. The $^{109}Cd$ contents of the mussels were determined by whole body counting on Days 1, 4, 9, 14, 21 and 29 of the exposure. After 29 d the mussels were dissected and the $^{109}Cd$ contents of soft parts and shells were determined. Body wet weight, shell length, dry weight of soft parts and wet to dry weight ratio of the soft parts were recorded. The exposure was initiated in late September.

*Carcinus maenas* Expt

Nine groups of six crabs were exposed to $^{109}Cd$ with an experimental protocol identical to that used in the *Mytilus edulis* Expt 2. Whole body counts were performed following 1, 2, 6, 9, 13, 16, 20 and 27 d of exposure. After 27 d, midgut gland and gills were removed intact together with samples of carapace, hypodermis, haemolymph and muscle. Their $^{109}Cd$ content was determined. Concentrations of sodium, potassium, calcium, magnesium and protein in the haemolymph and wet to dry ratios of the tissues (except muscle) were measured. The proportion that gills and midgut gland constituted of the whole body weight was found. The exposure was initiated in early September.

Data treatment

Cadmium accumulation rates were assessed in each of the groups in the *Littorina littorea* Expt and *Mytilus edulis* Expt 1 by linear regression of cadmium concentrations against exposure time. For individuals within these two experiments, multiple regression analyses were carried out with cadmium concentration as the dependent variable and exposure time, salinity and calcium concentration as independent variables. $^{109}Cd$ activities in whole bodies (during the exposure) and individual tissues of *M. edulis* (Expt 2) and *Carcinus maenas* were used as dependent variables in multiple regressions on salinity and calcium concentration. In an attempt to account for some of the unexplained variability in the multiple regressions on time, salinity and calcium concentrations (*L. littorea* and *M. edulis* Expt 1) or salinity and calcium concentrations (*M. edulis* Expt 2 and *C. maenas*), all of the variables recorded in each experiment were used in multiple regressions. Variables were included only if their inclusion significantly ($P< 0.05$) improved the multiple correlation coefficient and the *P*-value of each individual parameter in the regression was less than 0.05.

## Results

*Littorina littorea* Expt

Cadmium accumulated linearly with time in the soft parts of the periwinkles at all calcium concentrations and salin-

**Table 2** *Littorina littorea*, *Mytilus edulis* and *Carcinus maenas*. Cadmium accumulation. Results from multiple regression analysis. Cadmium concentrations used as dependent variable and time, salinity and calcium concentration as independent variables in the *L. littorina* Expt and *M. edulis* Expt 1. In *M. edulis* Expt 2 and *C. maenas* Expt, salinity and calcium concentration used as independent variables. For the individual variables the standardised regression coefficient ($\beta$) is given. If $P>0.05$ for the individual parameter, $\beta$ is given in parentheses. * $P<0.0001$

| Species | Days | | Salinity | | Calcium | | Total regression | |
|---|---|---|---|---|---|---|---|---|
| Tissue | | | | | | | | |
| Day | $\beta$ | $P$ | $\beta$ | $P$ | $\beta$ | $P$ | $R$ | $P$ |
| *L. littorina* | | | | | | | | |
| Soft parts | 0.68 | * | −0.12 | * | −0.40 | * | 0.799 | * |
| *M. edulis* (Expt 1) | | | | | | | | |
| Soft parts | 0.49 | * | −0.46 | * | −0.13 | 0.0004 | 0.684 | * |
| *M. edulis* (Expt 2) | | | | | | | | |
| Whole body | | | | | | | | |
| Day 1 | | | −0.46 | 0.003 | 0.26 | 0.036 | 0.525 | 0.0003 |
| Day 2 | | | −0.59 | * | 0.25 | 0.022 | 0.645 | * |
| Day 4 | | | −0.63 | * | (0.18) | 0.100 | 0.655 | * |
| Day 9 | | | −0.63 | * | (0.18) | 0.099 | 0.656 | * |
| Day 14 | | | −0.64 | * | (0.14) | 0.191 | 0.658 | * |
| Day 21 | | | −0.65 | * | (0.15) | 0.171 | 0.667 | * |
| Day 29 | | | −0.66 | * | (0.09) | 0.38 | 0.669 | * |
| Soft parts | | | −0.60 | * | (0.18) | 0.11 | 0.630 | * |
| Shell | | | −0.76 | * | (−0.15) | 0.11 | 0.774 | * |
| *C. maenas* | | | | | | | | |
| Whole body | | | | | | | | |
| Day 1 | | | −0.35 | 0.0051 | −0.37 | 0.0038 | 0.509 | 0.0005 |
| Day 2 | | | −0.38 | 0.0023 | −0.37 | 0.0026 | 0.533 | 0.0002 |
| Day 6 | | | −0.28 | 0.0287 | −0.38 | 0.0035 | 0.470 | 0.0017 |
| Day 9 | | | (−0.20) | 0.1068 | −0.48 | 0.0002 | 0.517 | 0.0004 |
| Day 13 | | | −0.32 | 0.0090 | −0.41 | 0.0012 | 0.522 | 0.0003 |
| Day 16 | | | −0.36 | 0.0044 | −0.36 | 0.0041 | 0.510 | 0.0005 |
| Day 20 | | | −0.31 | 0.0132 | −0.39 | 0.0025 | 0.497 | 0.0007 |
| Dday 27 | | | −0.32 | 0.0084 | −0.44 | 0.0005 | 0.544 | 0.0001 |
| Carapace | | | −0.62 | * | (−0.21) | 0.057 | 0.651 | * |
| Midgut gland | | | (−0.19) | 0.16 | (−0.04) | 0.77 | 0.199 | 0.36 |
| Gills | | | (−0.21) | 0.11 | (−0.22) | 0.10 | 0.308 | 0.08 |
| Muscle | | | (−0.13) | 0.36 | (−0.10) | 0.47 | 0.160 | 0.51 |
| Hypodermis | | | (−0.26) | 0.063 | (−0.07) | 0.60 | 0.27 | 0.15 |
| Haemolymph | | | (0.10) | 0.48 | (−0.22) | 0.12 | 0.24 | 0.23 |

ities tested (Fig. 1). Accumulation rates in the various groups varied between 1.2 and 4.6 µg Cd $g^{-1}$ dry wt $d^{-1}$, and accumulation rates decreased with increasing calcium concentrations as well as with increasing total salinities (Table 2). Multiple regression analysis indicated that cadmium accumulation rates decreased by 38% when salinity increased from 10 to 30‰ (Fig. 5a). 76% of this decrease was accounted for by changes in calcium concentration (Fig. 5c) and 24% by change in total salinity (Fig. 5b). The dependency on exposure time, salinity and calcium concentration explained 64% of the total variability in the cadmium concentrations in *L. littorina* (Table 2). Inclusion of dry weight, wet to dry weight ratio of the soft parts, the total body weight and the percentage that the dry soft parts constitute of the total body weight significantly ($P<0.0001$) increased the correlation coefficient of the multiple regression (Table 3). 70% of the total variability is explained by the regression shown in Table 3.

*Mytilus edulis* Expt 1

No significant difference in valve gape was observed among experimental groups. Cadmium accumulated in the soft parts of the mussels linearly with time at all salinities and calcium concentrations tested (Fig. 2). The rates of cadmium accumulation in the different groups varied between 5.0 and 15.6 µg Cd $g^{-1}$ dry wt $d^{-1}$. Accumulation rates decreased with increasing salinities and calcium concentrations (Table 2). When salinity was increased from 10 to 30‰, cadmium accumulation rates decreased by 54% (Fig. 5a). Seventy eight and 22% of this decrease could be accounted for by changes in salinity (Fig. 5b) and ambient calcium concentration (Fig. 5c), respectively. The dependency on exposure time, salinity and calcium concentration explained 47% of the total variability in the cadmium concentrations in the soft parts of the mussels. Inclusion of the dry weight of the soft parts and the wet to dry weight ratio of the soft parts significantly ($P< 0.001$) improved the multiple regression which now explained 56% of the total variability (Table 3).

*Mytilus edulis* Expt 2

Generally, $^{109}Cd$ was rapidly accumulated in the mussels during the first 2 to 4 d of exposure. During the following weeks, however, $^{109}Cd$ accumulated at slower rates (Fig. 3). After 29 d exposure, the mussels had concentrated $^{109}Cd$ to levels 20 to 160 times above the ambient level and

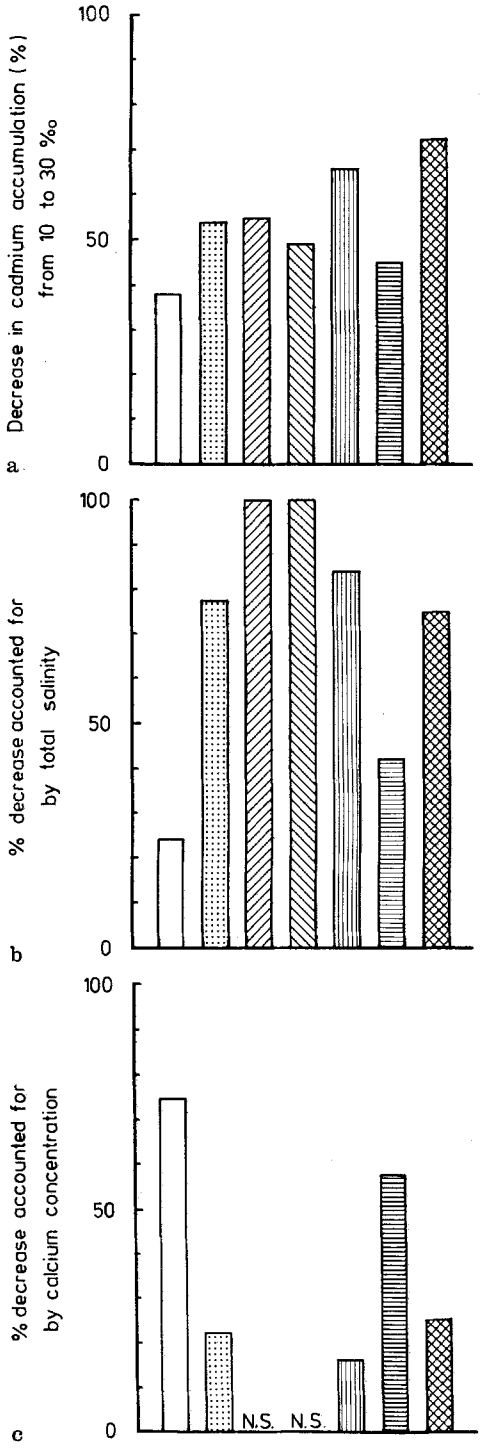


**Fig. 5** *Littorina littorea*, *Mytilus edulis* and *Carcinus maenas*. Effects on accumulation of cadmium when salinity changes from 10 to 30‰. (□ *L. littorea* soft parts; ⊡ *M. edulis* soft parts, Expt 1; ▨ *M. edulis* whole body, Expt 2; ▧ *M. edulis* soft parts, Expt 2; ▥ *M. edulis* shell, Expt 2; ▤ *C. maenas* whole body; ▩ *C. maenas* carapace; *N.S.* not significant)

most of the total body burden was distributed in the soft parts. Only a minor proportion was found in the shell (Table 4). Increasing the salinity from 10 to 30‰ reduced accumulation of $^{109}$Cd by 55, 49 and 66% in whole body, soft parts and shell, respectively (Fig. 5). Most of this decrease could be accounted for by the effect of salinity whereas calcium concentrations played only a minor or no significant role (Table 2 and Fig. 5bc). After 29 d, changes in salinity and calcium concentration accounted for 40 and 60% of the variability in the $^{109}$Cd concentrations in soft parts and shells, respectively (Table 2). Including the body wet weight and wet to dry weight ratio of the soft parts significantly augmented the correlation coefficient of the multiple regression for the soft parts (Table 3). Likewise, including shell length and body wet weight improved the correlation coefficient of the multiple regression for the shell (Table 3).

### *Carcinus maenus* Expt

During 27 d exposure the crabs accumulated $^{109}$Cd to levels 6 to 18 times those in the ambient seawater (Fig. 4). Increasing the salinity from 10 to 30‰ reduced accumulation of $^{109}$Cd in the whole body and carapace by 45 and 72%, respectively (Fig. 5a). For the whole body, the effect of calcium concentration accounted for 58% (Fig. 5c) of the decrease while the total salinity accounted for 42% (Fig. 5b). In the regression for the concentration of $^{109}$Cd in the carapace the contribution from the calcium concentration was very close to being statistically significant (Table 2), and the regression on salinity alone gives a significantly ($P$=0.038) poorer correlation coefficient of the multiple regression (0.618) than the regression on both salinity and calcium concentrations ($R$=0.651). The effect of calcium, however, only accounts for 25% (Fig. 5c) of the decrease in uptake of $^{109}$Cd when the salinity increases.

In midgut gland, gills, muscles, hypodermis and haemolymph salinity and calcium concentrations do not affect the accumulation of $^{109}$Cd (Table 2); concentrations of $^{109}$Cd are given in Table 4. Including variables such as body size, wet to dry weights of the tissues, haemolymph parameters, and tissue proportions with the regressions on salinity and calcium concentrations results in multiple regressions that explain an appreciable amount of the large individual differences between accumulation of $^{109}$Cd in the tissues of the crabs (Table 3). Wet:dry weight ratios in midgut gland, gills and hypodermis of individual crabs were all positively correlated ($r > 0.72$, $p < 0.0001$), and these three parameters were all negatively correlated with the protein concentration in the haemolymph ($r > -0.59$, $p < 0.0001$).

The concentration of protein (57±20 mg ml$^{-1}$) in the haemolymph was not consistently affected by changes in ambient salinity and calcium concentration. Sodium, magnesium and potassium concentrations (given as m$M$) in the haemolymph increased with increasing ambient salinity (given as ‰) ([$Na^+$]=236+27 S, $r^2$=0.13, $p$=0.012; [$Mg^{++}$]=5.8+0.26 S, $r^2$=0.29, $p$=0.0001 and [$K^+$]=4,8+0.13 S, $r^2$=0.21, $p$=0.0004), whereas the calcium concentration in the medium had no consistent effect. Calcium concentrations in the haemolymph increased with increasing ambient calcium concentration ([Ca]haem=5.3+0.50 [Ca]ext, $r^2$=0.21, $p$=0.0013).

**Table 3** *Littorina littorea*, *Mytilus edulis* and *Carcinus maenas*. Cadmium accumulation. Results from multiple regression analysis. In addition to time and ambient salinity and calcium concentrations, variables from the individual specimens included as independent variables to attempt explain the variability in cadmium uptake. * $P<0.0001$. Compare with Table 2

| Species Tissue | Variables | Individual variable | | Total regression | |
|---|---|---|---|---|---|
| | | $\beta$ | $P$ | $R$ | $P$ |
| *L. littorea* | | | | | |
| Soft parts | Days | 0.64 | * | 0.839 | * |
| | Salinity | –0.08 | 0.0050 | | |
| | $[Ca^{++}]$ | –0.43 | * | | |
| | Wet wt:dry wt ratio of soft parts | 0.18 | * | | |
| | Body weight (wet wt) | –0.46 | 0.0007 | | |
| | Soft parts (dry wt) as % of body wt | –0.35 | 0.0033 | | |
| | Dry wt of soft parts | 0.38 | 0.0255 | | |
| *M. edulis* (Expt 1) | | | | | |
| Soft parts | Days | 0.48 | * | 0.748 | * |
| | Salinity | –0.46 | * | | |
| | $[Ca^{++}]$ | –0.14 | * | | |
| | Dry wt of soft parts | –0.22 | * | | |
| | Wet wt:dry wt ratio of soft parts | 0.16 | * | | |
| *M. edulis* (Expt. 2) | | | | | |
| Soft parts | Salinity | –0.51 | * | 0.735 | * |
| | $[Ca^{++}]$ | (0.04) | 0.69 | | |
| | Wet wt:dry wt ratio of soft parts | 0.36 | 0.0015 | | |
| | Body wet wt | –0.32 | 0.0042 | | |
| Shell | Salinity | –0.78 | * | 0.871 | * |
| | $[Ca^{++}]$ | –0.24 | 0.0026 | | |
| | Body wet wt | –0.60 | * | | |
| | Shell length | 0.29 | 0.0241 | | |
| *C. maenas* | | | | | |
| Whole body | Salinity | –0.59 | 0.0003 | 0.703 | * |
| | $[Ca^{++}]$ | (–0.04) | 0.79 | | |
| | $[Ca^{++}]$ in haemolymph | –0.60 | 0.0103 | | |
| | Wet wt:dry wt ratio of gills | –0.44 | 0.0108 | | |
| | Midgut gland dry wt as % of body | 0.41 | 0.0244 | | |
| | $[K^{+}]$ in haemolymph | 0.47 | 0.0419 | | |
| | Wet wt: dry wt ratio of hypodermis | 0.33 | 0.0484 | | |
| Carapace | Salinity | –0.53 | * | 0.732 | * |
| | $[Ca^{++}]$ | (–0.13) | 0.21 | | |
| | Midgut gland dry wt as % of body wet wt | 0.36 | 0.0042 | | |
| | Wet wt:dry wt ratio of hypodermis | 0.35 | 0.0048 | | |
| Midgut gland | Salinity | (–0.10) | 0.32 | 0.771 | * |
| | $[Ca^{++}]$ | –0.25 | 0.0141 | | |
| | Midgut gland dry wt as % of body wet wt | –0.70 | * | | |
| | Wet wt:dry wt ratio of midgut gland | 0.36 | 0.0057 | | |
| | Protein in haemolymph | 0.29 | 0.0255 | | |
| Gills | Salinity | (–0.07) | 0.54 | 0.638 | * |
| | $[Ca^{++}]$ | (–0.16) | 0.18 | | |
| | Body wet wt | 0.48 | 0.0001 | | |
| | Midgut gland dry wt as % of body wet wt | 0.27 | 0.0246 | | |
| Muscle | Salinity | (0.05) | 0.67 | 0.648 | * |
| | $[Ca^{++}]$ | (0.02) | 0.87 | | |
| | Wet wt:dry wt as % of body wet wt | 0.71 | * | | |
| | Midgut gland dry wt as % of body wet wt | 0.62 | * | | |
| Hypodermis | Salinity | –0.10 | 0.41 | 0.605 | * |
| | $[Ca^{++}]$ | (–0.13) | 0.25 | | |
| | Wet wt:dry wt ratio of hypodermis | 0.57 | * | | |
| Haemolymph | Salinity | (–0.20) | 0.10 | 0.651 | * |
| | $[Ca^{++}]$ | (–0.08) | 0.46 | | |
| | $[K^{+}]$ in haemolymph | 0.54 | * | | |
| | Body wet wt | –0.39 | 0.0013 | | |

**Table 4** *Mytilus edulis* and *Carcinus maenas*. Distribution of $^{109}Cd$ in the tissues of mussels and crabs exposed to 100 dpm $^{109}Cd$ $ml^{-1}$ for 29 and 27 d, *M. edulis* Expt 2 and *C. maenas* Expt, respectively

| Species Tissue | dpm $^{109}Cd$ $g^{-1}$ dry wt | $^{109}Cd$ content | % of body burden |
|---|---|---|---|
| *M. edulis* (Expt 2) | | | |
| Soft parts | 240 000±132 000 | 16 400±7 300 | 80.6±5.6 |
| Shell | 3 000± 1 300 | 3 900±1 600 | 19.4±5.6 |
| *C. maenas* | | | |
| Carapace | 2 210± 1 200 | 11 000±8 300[a] | 48.0±9.8 |
| Midgut gland | 9 100± 8 000 | 1 800±1 100 | 11.2±5.5 |
| Gills | 38 000± 18 000 | 5 000±4 200 | 24.8±8.2 |
| Muscle | 1 700± 1 500 | 2 900±2 800[a] | 12.8±6.3 |
| Hypodermis | 6 400± 5 000 | 550± 550[a] | 2.5±1.6 |
| Haemolymph[b] | 35± 22 | 140± 80[a] | 0.9±0.6 |

[a] Tissue proportions taken from Depledge and Bjerregaard (1994)
[b] Given as dpm $ml^{-1}$

## Discussion

Accumulation of cadmium decreased with increasing salinity, but the mechanisms underlying this 'salinity effect' appear to differ among the species investigated. The calcium concentration of the seawater plays a major role in the 'salinity effect' on cadmium accumulation in *Littorina littorea*, and to a certain extent in *Carcinus maenas*, but appears to be of no, or minor importance in *Mytilus edulis*.

The exact mechanism by which cadmium and other divalent trace metals are transported over the external surface of aquatic organisms is unknown (for review see Simkiss and Taylor 1989). Carpene and George (1981) envisage cadmium uptake in marine organisms as a cascade of passive processes whereby cadmium moves along a gradient of increasing binding affinities from the labile chlorocomplexes in the water to sulfhydryl groups at the cellular level. The ionic composition of the ambient medium could thus affect accumulation of cadmium in the organism at four different levels: (1) By affecting cadmium's speciation and thereby bioavailability in the ambient medium; (2) by competition with regard to uptake sites from specific ions (in this case $Ca^{++}$) in the medium; (3) by affecting general permeability properties of external surfaces; or (4) by changing the cadmium binding capacity of the tissues.

With regard to speciation, dissolved cadmium is present in full strength seawater mainly as labile cadmium-chloro complexes, with only a minor part of the total dissolved cadmium present as $Cd^{++}$ (Zirino and Yamamoto 1972; Long and Angino 1977; Mantoura et al. 1978). In the shrimp *Palaemonetes pugio* the toxicity – and probably uptake – of cadmium seem to correlate with the concentration of free cadmium ions ($Cd^{++}$) rather than total dissolved cadmium (Sunda et al. 1978). Similarly, the concentration of free $Cu^{++}$ rather than total copper determines uptake and toxicity of copper in phytoplankton (Sunda and Guillard 1976), oysters *Crassostrea virginica* (Zamuda and Sunda 1982) and crab larvae *Rhithropanopeus harrisii* (Sanders et al. 1983). As salinity decreases, the proportion of the total cadmium present as $Cd^{++}$ increases; these theoretical predictions of $[Cd^{++}]$ from stability constants for the different cadmium complexes (Zirino and Yamamoto 1972; Long and Angino 1977; Mantoura et al. 1978) have largely been confirmed by measurements with cadmium selective electrodes (Sunda et al. 1978). At 10 and 30‰ salinity, 13.4 and 4.3%, respectively, of the total dissolved cadmium is present as $Cd^{++}$ (Sunda et al. 1978). Hence, the concentration of bioavailable $Cd^{++}$ should decrease by ca. 68% when salinity increases from 10 to 30‰. In *Mytilus edulis*, for which calcium does not seem to play a major role in the 'salinity effect', the cadmium accumulation rate is 50 to 60% lower at 30 than at 10‰, and in this organism the reduced accumulation rate could plausibly be explained by the lower concentration of bioavailable $Cd^{++}$ at 30‰.

Interactions between calcium, on the one hand, and uptake and toxicity of cadmium, on the other, have been established in freshwater gammarids (Wright 1980; Wright and Frain 1981; McCahon and Pascoe 1988), in fish (Pärt et al. 1985; Wicklund and Runn 1988; Verbost et al. 1989), in a marine alga (Heuillet et al. 1988) and in *Carcinus maenas* (Wright 1977b). $Ca^{++}$ and $Cd^{++}$ have similar charges and ionic radii, and it has been hypothesised that cadmium is transported over membranes via the calcium transporting system. Transport of calcium in gills of freshwater fish takes place by passive diffusion of calcium over the apical membrane of the epithelial cells followed by active pumping by high-affinity Ca-ATPases over the basolateral membrane (Flik et al. 1985). Studies on rainbow trout gills indicate that cadmium may be taken up via the calcium-transporting system, although no competition between $Ca^{++}$ and $Cd^{++}$ for the passive diffusion through the Ca-channels of the apical membrane could be demonstrated (Pärt et al. 1985; Verbost et al. 1989).

The ability of many euryhaline organisms to maintain body weight and proper osmolalities in the body fluids with changing salinities is lost if calcium is not present in the surroundings (McCutcheon and Lucke 1928; Pantin 1931; Ellis 1933; Fletcher 1974). The apparent water permeability of crabs *Carcinus maenas* decreases with decreasing salinity (Smith 1970) and calcium reduces the permeability of *C. maenas* to sodium (Lucu 1973). Calcium protects rainbow trout exposed to acidification from losing sodium and chloride ions via the gills by affecting transcellular (by stimulating $Na^+K^+$ATP-ases) as well as paracellular (by affecting tight junctions) permeability (for review see McDonald 1983). The effect of calcium on the general permeability of the body surface may influence the uptake of cadmium ions from solution. The possible role of calcium as a modulator of general permeability might explain why calcium concentration affects cadmium uptake in *Littorina littorina* and *C. maenas*, but not in *Mytilus edulis*. *M. mytilus* is an osmoconformer (Conklin and Krogh 1938), while both *L. littorina* (Todd 1962, cited in Robertson 1964; Rumsey 1973) and *C. maenas* (Webb 1940; Robertson 1960; Theede 1969) maintain the osmolality of their haemolymph higher than ambient osmolality at low salin-

ity. As osmoconformers generally have higher permeabilities to water than osmoregulators (Rudy 1967), and as calcium plays a role in the regulation of permeability, it is also possible that calcium may affect cadmium uptake in osmoregulators by modulating the permeability of the general surface. Data on water permeability in *M. edulis* and *L. littorina* are not available, but it would be very interesting to determine whether the four-fold higher cadmium accumulation rate in mussels than in periwinkles and crabs is partly explained by a higher permeability of the former. Other factors such as surface area exposed to contaminated water and higher flow of water over the surfaces might, however, also account for the higher accumulation rates in *M. edulis*.

At present, there is no evidence to support the hypothesis that changes in salinity or calcium concentrations affect the capacity of the tissues to bind cadmium.

### *Littorina littorea*

Cadmium is accumulated in the soft part of *Littorina littorea* linearly with time (Bjerregaard 1988; Marigomez 1989) and almost proportionally with ambient cadmium concentration (Marigomez 1989). The cadmium accumulation rates found in the present study are within the range reported earlier (Amiard et al. 1987; Langston and Zhou 1987; Bjerregaard 1988; Marigomez 1989).

### *Mytilus edulis*

The increase in accumulation of cadmium from solution at lower salinities has been well established in laboratory experiments (Phillips 1976; Jackim et al. 1977; George et al. 1978; Fischer 1986). The magnitude of the 'salinity effect' on cadmium accumulation in these studies seems to depend on the size of mussels, temperature, feeding regime, cadmium concentrations and salinities tested. The effects of salinity on cadmium accumulation found in the present study are within the ranges reported earlier. The difference in accumulation kinetics for cadmium between *Mytilus edulis* Expts 1 and 2 is probably explained by the rapid, initial adsorption of cadmium to the shell in Expt 2 (resulting in saturation kinetics), whereas soft parts (Expt 1) accumulate cadmium linearly.

### *Carcinus maenas*

Accumulation of cadmium in haemolymph (Wright 1977a; Wright and Brewer 1979), gills and carapace (Wright 1977a) is highest at low salinities, while accumulation in muscle and midgut gland (Wright 1977a) is not affected by salinity. Part of the 'salinity effect' is due to calcium (Wright 1977b). The present results generally confirm the earlier findings. However, with regard to accumulation of cadmium in the internal tissues, variability among individuals exceeded influences from salinity and calcium concentration. The large inter-individual variability in cadmium accumulation is linked to the physiological condition of the individual crabs (Bjerregaard 1990, 1991; Depledge and Bjerregaard 1990). The results reflects the fact that an appreciable part of the inter-individual variability in the accumulation of cadmium in the different tissues can be explained when physiological parameters such as wet:dry weight ratios and tissue volumes are included in multiple regressions.

*Carcinus maenas* exposed to cadmium concentrations of the order of 1 mg Cd $l^{-1}$ accumulate cadmium in the tissues in the order gills midgut gland>carapace>muscle (Wright 1977a; Jennings and Rainbow 1979; Bjerregaard 1982, 1990). In the present study cadmium was accumulated from 0.13 µg Cd $l^{-1}$ to four-fold higher concentrations in gills than in midgut gland, and this difference can probably be attributed to a concentration dependency in the handling of cadmium, where the transfer of cadmium from gills to internal tissues may approach saturation as the ambient cadmium concentration increases towards 1 mg Cd $l^{-1}$. Transfer of cadmium through perfused gills of rainbow trout increased by a factor of 100 when the ambient concentration of cadmium increased from 0.6 to 5.6 µg Cd $l^{-1}$ (Pärt and Svanberg 1981), and in vivo rates of accumulation in the gills and Cd-influx over the gills of rainbow trout increased 6.3- and 18-fold, respectively, when the ambient cadmium concentration was raised from 11 to 112 µg Cd $l^{-1}$ (Verbost et al. 1989). For higher ambient cadmium concentrations, a larger proportion of the cadmium reaching the gill seems to transverse the gills into the body, whereas lower exposure concentrations favour accumulation in the gills. The ratio between the total $^{109}Cd$ content in the gills and the midgut gland in the present experiment depended on the size of the individual crabs ($Cd_{gills}/Cd_{midgut} 0.89 \times$ body weight − 11.4; $r^2=0.43$, $p=0.0001$). This indicates that the transfer of cadmium from the gills to the internal tissues is smaller in large crabs than in small ones and this may be explained by the decrease in haemolymph circulation times with size in crabs.

Utilisation of multiple regression analysis in order to identify 'important' parameters in multivariate data sets should be done with very great care unless firm cause-effects relationships have been established (Wilkinson 1979; Freedman 1983). With this caveat in mind, the data from *Littorina littorina* and *Mytilus edulis* consistently indicate that smaller individuals have higher cadmium accumulation rates than larger ones, and individuals with high water contents in their tissues have higher cadmium accumulation rates. This does make sense, since smaller organisms have higher surface to volume ratios and higher weight specific metabolic rates than larger ones. Also, if two individuals with low and high tissue water contents accumulate cadmium to the same level in vivo, cadmium concentrations will appear higher in the individual with the higher water content when the data are expressed on a dry weight basis. For *Carcinus maenas*, the correlations between many of the physiological parameters (wet:dry weight ratios, tissue volumes, protein concentration in the haemolymph, etc.) appear to introduce a certain element

of chance concerning the parameters that are included in the multiple regressions on the accumulation of cadmium in the individual tissues (Table 3). For example, the inclusion of wet:dry weight ratio of the hypodermis in the regression on the accumulation of cadmium in muscles probably reflects a common dependency on the overall physiological condition of the crab rather than a direct causal relationship between these two parameters. These results stress the importance of developing non-destructive techniques that allow determination of the physiological condition of crabs used in experiments.

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