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Allometry of Heavy Metal Bioconcentration in the Echinoid *Paracentrotus lividus*

M. Warnau[1], G. Ledent[1], A. Temara[1], V. Alva[1], M. Jangoux[1,2], Ph. Dubois[1]

[1] Laboratoire de Biologie Marine (CP 160-15), Université Libre de Bruxelles, 50 av. F.D. Roosevelt, B-1050 Bruxelles, Belgium
[2] Laboratoire de Biologie Marine, Université de Mons-Hainaut, 19 av. Maistriau, B-7000 Mons, Belgium

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Abstract. The relationships between concentration and size in *Paracentrotus lividus* were studied for seven metals (Zn, Pb, Cd, Fe, Cr, Cu, and Ti). In the calcified body compartment, Zn concentrations showed inverse power relationships, while concentrations of Pb, Cd, and Cr increased as power functions of echinoid size; Fe, Cu, and Ti concentrations did not vary significantly with echinoid size. In the non-calcified body compartment of the echinoid, the concentrations of Zn, Pb, Fe, Cu, and Ti showed inverse power relationships, Cd concentrations increased linearly, and Cr concentrations did not show any significant relationship with echinoid body size. Most relationships were rather weak, except in the cases of Pb and Fe in the non-calcified compartment and Cd in the calcified compartment. The allometric relationships were affected by seasonal factor in the non-calcified compartment only. The results indicate that biomonitoring programs using *P. lividus* as an indicator of metal contamination must take into account both echinoid size and season as source of metal concentration variations.

Contamination by pollutants is often surveyed and monitored through the use of bioindicator species. An effective characterization of the contamination status of an ecosystem must rely on several of these species (Gray 1989). As a consequence, there is a continuous need for new bioindicator species in order to encompass the most significant ecosystems.

The echinoid *Paracentrotus lividus* lives in numerous ecosystems from rocky shores to seagrass beds all around the Mediterranean and on northeast Atlantic coasts (Hayward and Ryland 1990). It is a key species in several of these communities (*e.g.*, in the Mediterranean *Posidonia oceanica* meadows), including communities where usual bioindicators (such as mussels) do not occur. The different life stages of *P. lividus* are recognized or potential bioindicators of heavy metal contamination. The gametes, embryos, and larvae are sensitive to numerous pollutants, including heavy metals, and are widely used in studies involved in toxicological testing and environmental monitoring of pollutants (Kobayashi 1984; Pagano *et al.* 1986; Dinnel *et al.* 1988; Walter *et al.* 1989; Warnau and Pagano 1994). The short duration together with the planktonic character of these life stages make them useful short-term indicators. In contrast, sedentary post-metamorphic *P. lividus* are potential long-term indicators. *Paracentrotus lividus* are abundant, of sufficient size, and easily collected by seashore fishing or SCUBA diving, and they concentrate heavy metals in a dose dependent-way, which differs depending on whether the body compartment considered is calcified (body wall, Aristotle lantern) or non-calcified (digestive wall, gonads) (Miramand *et al.* 1982; Augier *et al.* 1989; Warnau *et al.* 1995). However, several aspects have to be investigated before post-metamorphic *P. lividus* can be used as bioindicators of heavy metal contamination. One is the relationship between echinoid age and the concentrations of metals in its tissues. This information is important to track a possible age-related accumulation in the echinoid, a fact which would bias the indicative value of *P. lividus* if it is not taken into account. Indeed, age-related bioconcentration of heavy metals appears to be a general feature among marine organisms (see Newman and Heagler 1991) but has never been addressed in any echinoderm. Furthermore, this phenomenon is influenced by physiological factors showing seasonal patterns (such as reproduction) (Cossa *et al.* 1980; Strong and Luoma 1981; Newman and Heagler 1991), making it necessary to take into account the season parameter.

As in many invertebrate species, age determination is very difficult in echinoderms (*e.g.* Ebert 1986; Ebert *et al.* 1993). Studies devoted to age-related bioconcentration of heavy metals generally bypass this practical problem through the use of age-related parameters such as body size or body weight (Cossa *et al.* 1980; Strong and Luoma 1981; Newman and Heagler 1991). For the echinoid *Paracentrotus lividus,* ambital diameter is a valuable age-related parameter. The size (ambital diameter)-age relationship in *P. lividus* follows the Von Bertalanffy model (Alain 1978). This indicates, among other things, that the ambital diameter of the echinoid increases monotonically with age. Consequently, the occurrence of a monotonic increase or decrease of metal concentration with echinoid size implies the existence of a similarly monotonically increasing or decreasing relationship between metal concentration and echinoid age.

Correspondence to: M. Warnau

The goal of the present paper is to establish if there are relationships between size (age) and metal concentrations (Zn, Pb, Cd, Fe, Cr, Cu, and Ti) in *Paracentrotus lividus*, and if these possible relationships vary between calcified and non-calcified compartments of the echinoid and/or according to seasons of the year.

Materials and Methods

Collection and Sample Preparation

Paracentrotus lividus (Lamarck, 1816) over the population size range were collected by SCUBA diving in the Mediterranean *Posidonia oceanica* meadow off Lacco Ameno (Ischia Island, Bay of Naples, Italy). Echinoids were collected in December 1991 (n = 74) (winter collection) and in May 1992 (n = 45) (spring collection). As they were in the post-spawning phase (lack of gonads), possible variabilities of metal concentrations due to sex or sexual maturation stage were avoided.

The echinoids were maintained for a maximum of 5 h in sea water from the collection site until further processing. They were then measured (ambital diameter) and dissected. Two body compartments were separated: (1) a calcified compartment (body wall and Aristotle lantern) and (2) a non-calcified compartment (gut—after removal of its contents—and haemal digestive annexes). The samples were dried at 100°C for 48 h, weighed (dry weight—DW), and stored in hermetically sealed polyethylene containers until analysis.

Metal Analysis

A known amount of each sample (usually 0.5 g DW) was digested with 2 ml of 65% HNO_3 (Merck, *p.a.*) per g DW. The acid digestion was carried out successively at 20, 40, 60, and 80°C for 24, 24, 12, and 12 h, respectively. Digests were diluted 12 times with milli-Q water (Millipore) and filtered on Whatman GF/A glass microfiber filters. The concentrations of Zn, Pb, Cd, Fe, Cr, Cu, and Ti were measured by atomic emission spectrometry, using a Jobin-Yvon 38+ ICPS and taking into account matrix corrections.

Data Analysis

Data analyses were computed taking into account all the individual measurements. Metal measurements lower than the detection limit were replaced by half the value of the detection limit (Black 1991). Detection limits for Zn, Pb, Cd, Fe, Cr, Cu, and Ti were, respectively, 0.002, 0.014, 0.001, 0.004, 0.001, 0.002, and 0.001 mg of metal per liter of digested sample.

Allometric relationships were fitted by simple linear regression between metal concentrations (in mg/Kg DW) and echinoid body size (ambital diameter, in cm). Semi-logarithmic, bi-logarithmic, and inverse transformations of the data were systematically performed to test biologically relevant non-linear relationships (exponential, power, and hyperbolic functions, respectively) (Scherrer 1984; Zar 1984). Examinations of residuals were systematically performed and used to test homoscedasticity of the non-transformed and transformed data (Zar 1984).

The best fitting regression lines about a given metal and a given echinoid compartment were compared between the 2 collections when the same regression models applied to data from the 2 collections. These comparisons were performed by a two-tailed Student t-test which compared regression slopes and, if relevant, elevations (Zar 1984).

Mean concentrations of metals that did not show significant allometric relationships were calculated over the whole size range of echinoids collected. Mean concentrations of a given metal in a given echinoid compartment were compared by a one-tailed Student t-test between the 2 collections when this metal showed no significant allometric relationship in either of the 2 collections. The level of significance was always set at $\alpha = 0.05$.

Results

Allometric Relationships

The calculation of determination coefficients, together with examination of the residuals, showed that the most accurate regression fittings were generally provided by the power model (Tables 1, 2; Figures 1, 2). In one case (Cd in the non-calcified compartment), the linear model provided the most accurate regression fitting (Table 1, Figure 1). The power relationships describing variation of Cd and Cr concentrations with size in the calcified compartment are almost straight lines (Table 2, Figure 2). However, examination of residuals indicated that these allometric relationships had to be described by a power model to respect homoscedasticity requirements.

In the non-calcified compartment, the concentrations of all metals, except for Cr, showed highly significant relationships with size in at least 1 of the 2 collections (Table 1, Figure 1). Cd did not show significant size dependence in the collection of May 1992. Zinc, Pb, Fe, Cu, and Ti data were best fitted by the power regression model; concentrations of these metals were inversely correlated with echinoid size. Cadmium data in December 1991 were best fitted by linear regressions; Cd concentrations were positively correlated with echinoid size. Although most allometric relationships were significant, their determination coefficients were generally low, except for Pb (both collections) and Fe (May 1992 collection) (Table 1).

For metals that did not show a significant allometric relationship in the non-calcified compartment, a mean concentration and associated standard deviation were calculated over the whole size-range of the echinoid population. Mean concentrations of Cr were 1.39 ± 0.95 and 0.88 ± 0.70 mg/Kg DW in the collections of December 1991 and May 1992, respectively. Mean concentration of Cd in echinoids collected in May 1992 was 2.89 ± 0.94 mg/Kg DW.

In the calcified compartment, the concentrations of Zn, Pb, Cd, and Cr were significantly correlated with size, except for Zn concentrations in the collection of May 1992 (Table 2, Figure 2). Lead, Cd, and Cr data were best fitted by the power regression model; concentrations of these metals were directly correlated with echinoid size. Zinc data were also best fitted by the power regression model, but Zn concentrations were inversely correlated with echinoid size. In general, significant allometric relationships were characterized by low determination coefficients. Allometric relationships of Cd showed, however, determination coefficients higher than 0.6 in both collections (Table 2).

The mean concentrations and associated standard deviations of metals which did not present a significant allometric relationship in the calcified compartment were calculated. These were 12.1 ± 7.30 and 9.50 ± 3.61 mg/Kg DW for Fe in December 1991 and May 1992, respectively, 0.69 ± 0.52 and 0.60 ± 0.15 mg/Kg DW for Cu, and 0.16 ± 0.11 and 0.16 ± 0.11

Table 1. Statistics of the best fitting regressions between metal concentrations (mg/Kg DW) and size (ambital diameter, cm) in the non-calcified compartment of the echinoid *Paracentrotus lividus*. N: number of analyzed individuals showing metal concentrations lower than detection limit; P: power model (metal concentration = a Diameter^b); L: linear model (metal concentration = a + b Diameter); se: standard error; R^2: determination coefficient; p: probability of the regression slope; n.s.: no significant relationship found (α = 0.05)

A. Collection from Ischia, December 1991. Diameter range: 1.20–6.50 cm, n = 74

Metal	N	Concentration range (mg/Kg DW)	Regression model	Regression parameters a	b(se)	R^2	p
Zn	1	25–407	P	146	−0.442 (0.157)	0.10	0.006
Pb	6	1.2–14	P	11.0	−1.051 (0.122)	0.51	0.0001
Cd	0	0.55–11	L	1.26	0.497 (0.166)	0.11	0.004
Fe	2	34–804	P	620	−0.835 (0.181)	0.23	0.0001
Cr	3	0.34–6.4	n.s.				
Cu	0	3.7–31	P	23.3	−0.530 (0.122)	0.21	0.0001
Ti	1	0.05–17	P	9.18	−1.150 (0.266)	0.21	0.0001

B. Collection from Ischia, May 1992. Diameter range: 1.20–5.00 cm, n = 45

Metal	N	Concentration range (mg/Kg DW)	Regression model	Regression parameters a	b(se)	R^2	p
Zn	0	34–152	P	136	−0.534 (0.170)	0.19	0.003
Pb	7	0.28–6.5	P	15.4	−1.912 (0.297)	0.50	0.0001
Cd	0	1.2–5.0	n.s.				
Fe	0	52–757	P	1003	−1.901 (0.210)	0.66	0.0001
Cr	3	0.21–5.0	n.s.				
Cu	0	7.8–33	P	33.5	−0.678 (0.168)	0.28	0.0002
Ti	7	0.02–27	P	8.44	−2.146 (0.809)	0.14	0.01

mg/Kg DW for Ti. Mean concentration of Zn in May 1992 was 5.67 ± 1.15 mg/Kg DW.

Seasonal Variation of Allometric Relationships

The regression lines revealed that, for a given metal, allometric relationships might differ between seasons (collections) due to (1) the nonsignificance of some regressions (Cd in the non-calcified compartment, and Zn in the calcified compartment), and (2) differences in the parameters of the describing model. In order to assess seasonal differences between relationships following the same model (considering a given metal and a given compartment), the two-tailed Student t-test was applied to the fitted regression lines.

In the non-calcified compartment (Table 3A, Figure 1), regression lines for Zn data did not differ significantly between the winter and spring collections. On the contrary, the regression lines for Pb, Fe, Cu, and Ti data differed significantly between collections. Differences affected elevations for Cu, while they affected slopes for Pb, Fe, and Ti.

In the calcified compartment (Table 3B, Figure 2), the regression lines for Pb, Cd, and Cr data did not differ significantly between the 2 collections considered.

Concerning metals which showed no significant allometric relationships in both collections, a one-tailed Student t-test showed that the mean Cr concentration in the non-calcified compartment and the mean Fe concentration in the calcified compartment were significantly higher (p = 0.003 and 0.03, respectively) in the winter collection. Mean Cu and Ti concentrations in the calcified compartment did not differ significantly (p = 0.26 and 0.88, respectively) between the 2 collections.

Discussion

The present study shows that bioconcentration of several heavy metals by *Paracentrotus lividus* varies significantly with echinoid body size. However, the occurrence of metal concentration-size relationships is not a general feature in *P. lividus*. Among the metals investigated, Cr never showed size-related bioconcentration in the non-calcified compartment, nor did Fe, Cu, and Ti in the calcified compartment. Moreover, strong concentration-size relationships ($R^2 \geqslant 0.50$) were only found for some metals (*viz.* Pb and Fe in the non-calcified compartment and Cd in the calcified compartment).

Both the occurrence and the direction (direct vs inverse relation) of significant relationships between metal concentration and size differed strongly according to the body compartment. The concentrations of Fe, Cu, and Ti showed inverse power relations with echinoid size in the non-calcified compartment, whereas no significant relationship occurred for these metals in the calcified compartment. The contrary was true for Cd and Cr (concentration-size relationship for Cd in the winter collection was significant in the non-calcified compartment but very weak $-R^2 = 0.11$; it will not be considered in what follows). Lead concentrations showed a much stronger relationship with size in the non-calcified compartment than in the calcified compartment, and the 2 relationships were in opposite directions (inverse in the non-calcified compartment and direct in the calci-

Table 2. Statistics of the best fitting regressions between metal concentrations (mg/Kg DW) and size (ambital diameter, cm) in the calcified compartment of the echinoid *Paracentrotus lividus*. N: number of analyzed individuals showing metal concentrations lower than detection limit; P: power model (metal concentration = a Diameter^b); se: standard error; R^2: determination coefficient; p: probability of the regression slope; n.s.: no significant relationship found (α = 0.05)

A. Collection from Ischia, December 1991. Diameter range: 1.20–6.50 cm, n = 74

Metal	N	Concentration range (mg/Kg DW)	Regression model	Regression parameters			
				a	b(se)	R^2	p
Zn	0	2.1–23	P	12.5	−0.559 (0.112)	0.26	0.0001
Pb	6	0.21–3.7	P	1.49	0.291 (0.074)	0.18	0.0002
Cd	0	0.09–0.80	P	0.09	0.914 (0.077)	0.66	0.0001
Fe	2	3.1–41	n.s.				
Cr	3	0.02–0.43	P	0.08	0.798 (0.120)	0.38	0.0001
Cu	0	0.36–4.1	n.s.				
Ti	1	0.01–0.49	n.s.				

B. Collection from Ischia, May 1992. Diameter range: 1.20–5.00 cm, n = 45

Metal	N	Concentration range (mg/Kg DW)	Regression model	Regression parameters			
				a	b(se)	R^2	p
Zn	0	3.9–8.8	n.s.				
Pb	1	0.27–4.2	P	1.21	0.448 (0.144)	0.19	0.003
Cd	0	0.08–0.41	P	0.09	0.880 (0.110)	0.62	0.0001
Fe	0	4.9–19	n.s.				
Cr	3	0.06–0.42	P	0.08	0.799 (0.161)	0.38	0.0001
Cu	0	0.29–1.0	n.s.				
Ti	5	0.01–0.53	n.s.				

fied compartment). It is thus highly recommended that these 2 compartments be treated separately.

In the non-calcified compartment, the concentrations of Zn, Pb, Fe, Cu, and Ti showed inverse power relationships with echinoid size in both winter and spring collections. High concentrations of these elements were measured in individuals of ambital diameter up to 2.5-3.0 cm. Echinoids of this size range are experiencing important ecological and physiological changes (progressive change in the diet and acquisition of sexual maturity) (Alain 1978; Nedelec and Verlaque 1984; Pearse and Cameron 1991). Zinc, Fe, and Cu are essential elements for animal metabolism and their concentrations are subjected to cellular regulations (Harlow *et al.* 1989; Sorensen 1991; Roesijadi 1992). The decrease of these metal concentrations could be related to a change in metal regulation due to physiological changes occurring during that life stage of *P. lividus*. Lead and Ti have no known biological functions and are not subjected to biological regulation (Beeby 1991; Sorensen 1991). However, their concentrations showed allometric relationships similar to those presented by Zn, Fe, and Cu. Size-dependent variations of Pb and Ti concentrations could also be related to the progressive change of feeding mode of *P. lividus* during its post-metamorphic growth.

In the calcified compartment, Pb, Cd, and Cr showed significant allometric relationships in the 2 collections. Concentrations of those metals increased with echinoid size, indicating that these elements could be trapped in the calcified compartment. This trapping occurs presumably in the mineralized fraction (*i.e.*, the high-magnesium calcite endoskeleton). Indeed, Pb and Cd are able to substitute for Ca cations and are well known to have high affinity for calcium-containing skeletons (Beeby 1991; Sorensen 1991). In particular, it was shown that the Pb load in the skeleton of *P. lividus* can account for up to 87% of the total body wall burden (Warnau *et al.* 1993). Moreover, *P. lividus* skeleton effectively accumulates Cd when echinoids are exposed experimentally to Cd in sea water, and

Table 3. Comparisons (two-tailed Student t-test) between the regression lines describing the relationships between metal concentrations and size in the echinoids *Paracentrotus lividus* collected in Ischia in December 1991 and May 1992. p(t): significance levels of the t statistics calculated by the two-tailed Student t-test; n.s.: no significant difference (α = 0.05)

A. Non-calcified compartment

Metal	Data transformation[a]	p(t) slope	p(t) elevation
Zn	log-log	n.s.	n.s.
Pb	log-log	<0.001	—
Fe	log-log	<0.002	—
Cu	log-log	n.s.	<0.02
Ti	log-log	<0.01	—

B. Calcified compartment

Metal	Data transformation	p(t) slope	p(t) elevation
Pb	log-log	n.s.	n.s.
Cd	log-log	n.s.	n.s.
Cr	log-log	n.s.	n.s.

[a] Comparison tests are performed on regression lines (*i.e.*, using transformed data when appropriate)

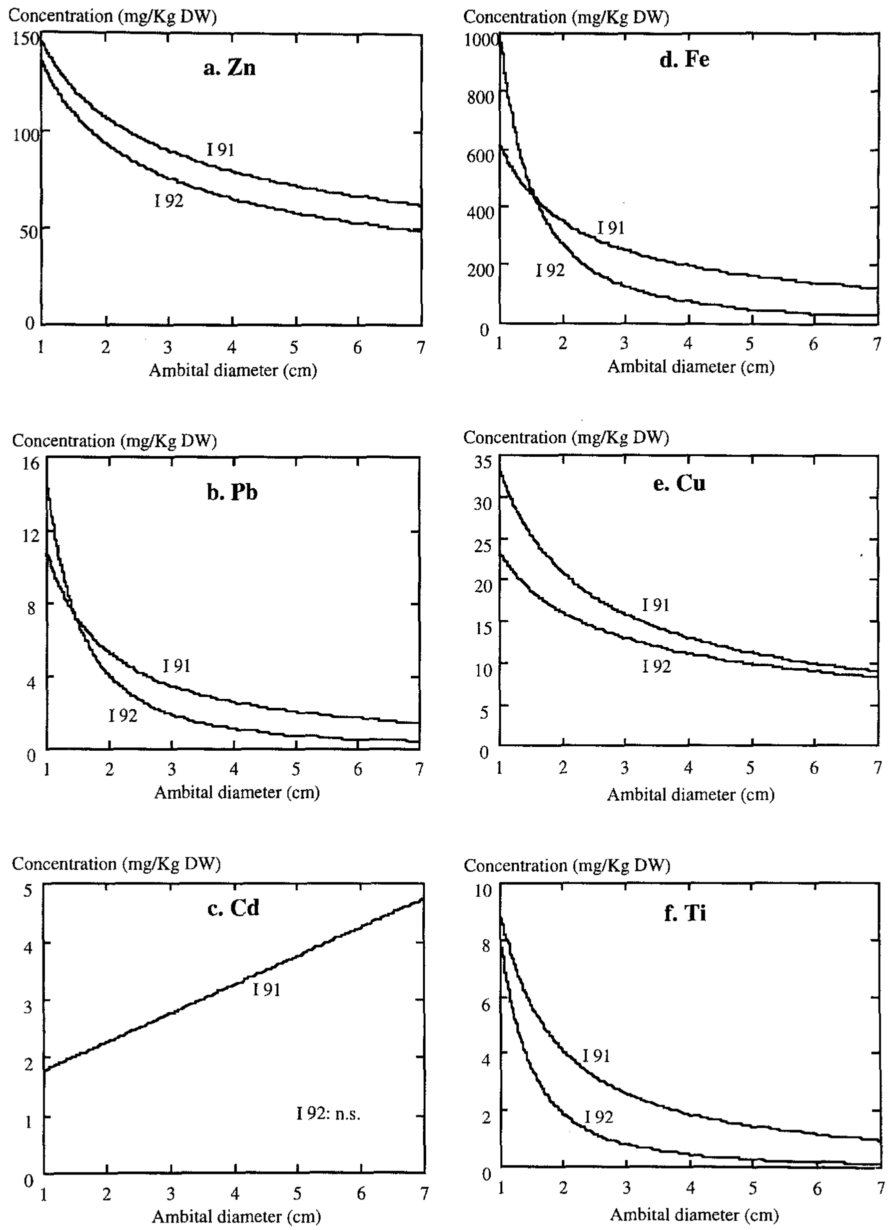

Fig. 1. Regressions between metal concentrations (mg/Kg DW) in the non-calcified compartment and size (ambital diameter, cm) of *Paracentrotus lividus* collected in December 1991 (I 91) and in May 1992 (I 92). Although the size ranges of the echinoids from the 2 collections are not identical, regressions are plotted here on the basis of the wider size range observed. n.s.: no significant relationship found

and no subsequent Cd elimination is detected when echinoids are returned to an uncontaminated environment (Warnau *et al.* 1995).

Seasonal variations in concentration-size relationships were recorded for several metals in the non-calcified compartment (*viz.* Pb, Fe, Cu, and Ti), while only Zn showed variations from that point of view in the calcified compartment (the spring collection provided no significant relation, while the winter collection did). Variations in the non-calcified compartment could be due partly to seasonal changes in the physiology of echinoids, including weight variations of the digestive tract (Lawrence *et al.* 1965; Bishop and Watts 1992). These variations could alternatively indicate that metal concentration-size relationships in the non-calcified compartment of *P. lividus* are dependent upon environmental factors (*i.e.*, ecotoxicological quality of the environment). Several authors have shown that the echinoid digestive wall is sensitive to metal contamination in the environment as it accumulates various metals rapidly and efficiently (*e.g.*, Miramand *et al.* 1982; Warnau *et al.* 1995).

That almost no seasonal variations of the allometric relationships were recorded in the calcified compartment could be due to the lower efficiency of this compartment in accumulating metals (Miramand *et al.* 1982; Warnau *et al.* 1995) together with the characteristics of the skeleton. The latter is massive (up to 90% of the dry weight of the body wall), shows a quite low winter growth, and is apparently a static structure in which metals are probably permanently trapped (Dubois and Chen 1989).

The occurrence of relationships between metal concentrations and size in *P. lividus* implies that biomonitoring programs using this species as an indicator of metal contamination must take into account the body size parameter. The use of the non-calcified compartment (*i.e.*, the digestive wall) is more advisable because of the high sensitivity of this compartment to metal contamination in the environment compared with the low sensitivity of the calcified compartment (Miramand *et al.* 1982; Warnau *et al.* 1995). In the non-calcified compartment of *P. lividus*, all significant concentration-size relationships were in-

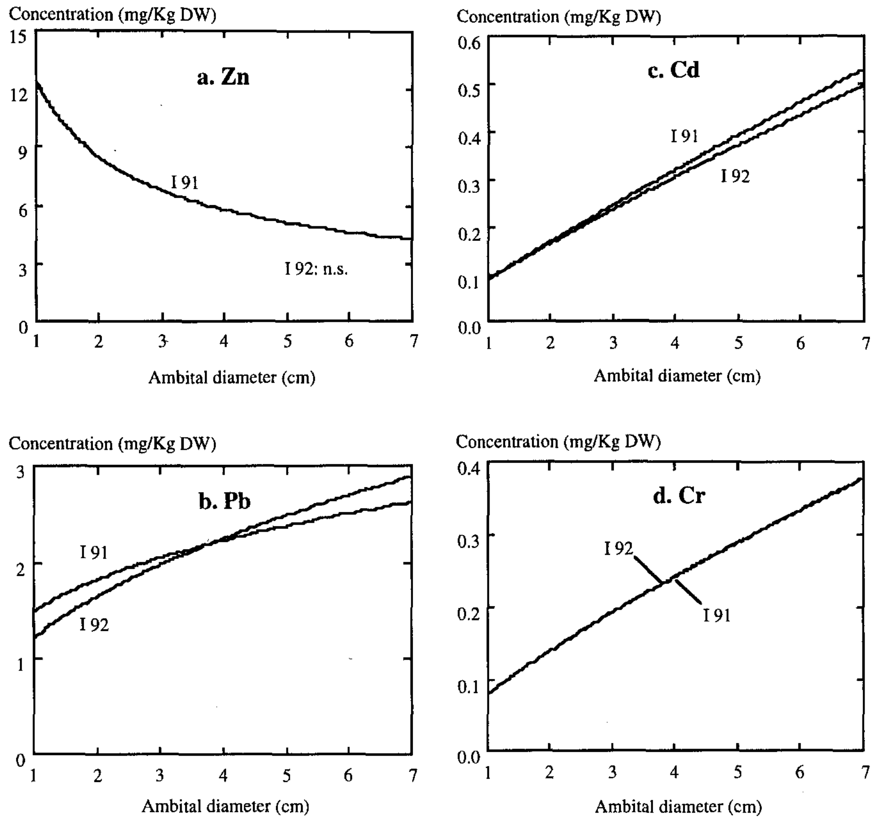

Fig. 2. Regressions between metal concentrations (mg/Kg DW) in the calcified compartment and size (ambital diameter, cm) of *Paracentrotus lividus* collected in December 1991 (I 91) and in May 1992 (I 92). Although the size ranges of the echinoids from the 2 collections are not identical, regressions are plotted here on the basis of the wider size range observed. n.s.: no significant relationship found

verse power functions. Thus, the size (age) effect is mainly effective in the small size-classes (typically, echinoid diameter ≤3 cm). For larger individuals, small variations of size resulted in small variations of metal concentrations. In consequence, if practical reasons prevent collection over the population size range, echinoids whose diameter exceeds 3 cm should be used for monitoring these metals in order to minimize size-related variations of concentrations. Although metal concentrations measured in the large individuals were generally lower than those in small echinoids and those currently observed in usual bioindicator species (*e.g.*, the mussel, *Mytilus edulis;* Phillips 1976), they were high enough to be measured accurately. Furthermore, since season has been shown to affect relationships between metal concentration and size, care should be taken when comparing data from collections achieved over the population size range at different seasons.

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