Bull. Environ. Contam. Toxicol. (1996) 56:98-105

Bulletin of Environmental Contamination and Toxicology

Allometric Variations in Heavy Metal Bioconcentration in the Asteroid *Asterias rubens* (Echinodermata)

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Received: 17 December 1994/Accepted: 12 June 1995

Bioindicators are commonly used to assess ecosystem contamination by pollutants (Peakall 1992). It is now well established that several of these bioindicators are necessary to give a satisfactory account of the pollution status of the ecosystems under study (see, e.g., Gray 1989). To qualify a species as a bioindicator, several aspects of its biology must be known (see Phillips 1976). One of these aspects is the relationship between pollutant concentrations in the biota and age (Newman and Heagler 1991). The common European asteroid *Asterias rubens* has numerous characteristics of a bioindicator species for heavy metal contamination, viz., effective metal accumulation and sensitivity, wide geographical and bathymetrical distribution, presence in numerous ecosystems, sedentary behavior, large size of adult individuals, and status as key species in several communities (den Besten 1989, 1990; Everaarts et al. 1990; Hayward and Ryland 1990). Yet, the relationships between age and metal concentrations in this organism still need to be investigated in order to assess the bioindicator value of the species. The present study examines the age-metal concentration relationships for seven heavy metals (Zn, Cu, Fe, Cd, Pb, Cr, and Ti) in *Asterias rubens.*

MATERIALS AND METHODS

One hundred specimens of the asteroid *Asterias rubens* (Linnaeus, 1758) representing the whole size range of the population were collected by SCUBA diving in August 1992 in Scharendijke (Zeeland, The Netherlands). At that period, the gonads are extremely reduced (Jangoux and Vloebergh 1973) and the possible variation of metal concentrations due to sex factor is therefore minimal. All asteroids were maintained in sea water from the sampling location until dissection.

The asteroids were first measured (arm length -R- measured from the tip of the longest arm to the opposite interradius). They were then dissected,

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within the day of sampling, using stainless steel instruments. Two body compartments (the digestive system and the body wall) were separated, finely chopped and dried for 24 hr at 100°C. The dried samples were stored in hermetically sealed polyethylene containers at room temperature until further processing. For analysis, an aliquot of each sample (0.5 g dry weight) was processed according to the method described by Warnau et al. (1995). The concentrations of Zn, Cu, Fe, Cd, Pb, Cr and Ti were measured by atomic emission spectrometry (ICPS-Jobin Yvon 38+). A certified material (*Mytilus edulis*, CRM n°278, Community Bureau of Reference) was analysed along with the experimental samples in order to check the accuracy of the methodology (Table 1). Detection limits (mean + 3 SD of the blank)equaled: 2 µg Zn / L, 14 µg Pb / L, 1 µg Cd / L, 4 µg Fe / L, 1 µg Cr / L, 2 µg Cu / L, 1 µg Ti / L. Measurements below the detection limit were replaced by half of this value.

Table 1. Metal concentrations (µg g^{-1} DW) in standard tissues used in the analyses (values in brackets are uncertified).

Metal	Certified (mean ± SD)	Measured (min and max). n = 5
Zn	76 ± 2	77 - 82
Pb	1.91 ± 0.04	1.91 - 1.95
Cd	0.34 ± 0.02	0.31 - 0.37
Fe	133 ± 4	126 - 161
Cr	0.8 ± 0.08	0.87 - 1.04
Cu	9.60 ± 0.16	9.60 - 10.71
Ti	(2 ± 0.2)	2.8 - 3.8

The rejection of outliers was performed according to the test of discordancy described by Black (1991). The goodness of fit of the frequency distribution of the asteroid sizes to a succession of normal distributions was tested by a quasi-Newton algorithm using the MIX 3.0 software (Ichthus Data Systems). The relationships between size and metal concentrations in the considered compartments of the asteroids were fitted using linear regression analysis (least square method). 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) (Zar 1984). Examinations of the residuals were systematically performed to check homoscedasticity of the transformed and nontransformed data (Zar 1984). The regression models presented are those showing the best fit (highest determination coefficient) while respecting homoscedasticity of the data. Heavy metal concentrations in the two body compartments of *Asterias rubens* were then compared using a student t-test.

RESULTS AND DISCUSSION

The different-sized asteroids were sampled from a single population at Scharendijke (The Netherlands) in August 1992. The size frequency distribution of the studied population fits a succession of four normal distributions (Fig. 1). The parameters of these distributions were used to define the four size classes considered below (S1: arm length -R- $\leq$ 5.3 cm; S2: 5.3 cm < R $\leq$ 7.8 cm; S3: 7.8 cm < R $\leq$ 10.3 cm; S4: 10.3 cm < R).

Size is usually considered as the most suitable approximation of age in asteroids (Guillou 1983). This hypothesis is supported by population dynamics studies which show that (1) asteroids are continuously growing during their life, and (2) negative growth events are limited. The demographic approach of the present study revealed that the size frequency distribution of the *A. rubens* population from Scharendijke may be split up into four normal distributions or cohorts. Considering the reproductive strategy of *A. rubens,* Guillou (1983) ascribed to such cohorts an annual value. This would mean that the Scharendijke population showed a mean life-span of 4 yr with a mean size of 12.3 cm for the 4-yr-old individuals. These values are intermediate between extremes described in the literature (see Guillou and Guillaumin 1984). Assuming that an annual value may effectively be assigned to each of the four components of the size-frequency distribution, a linear relationship does exist between the measured size and the putative age of the asteroid. The regressions between size and heavy metal concentrations calculated in the present study may thus be considered as representative of the relationships between the asteroid age and the heavy metal concentrations in the two investigated body compartments.

Heavy metal concentrations were measured in the body wall and the digestive system of these asteroids and plotted against the organism's arm length. Simple linear regression analysis of these data in transformed and nontransformed formats were performed. These analysis indicate that relationships between metal concentration and size vary according to both the compartment and metal (Table 2).

In the body wall, Cd and Cr show a significant increase in concentration with size following a power and hyperbolic function, respectively. Zn and Cu concentrations show significant relationships with size only in the simple linear model (nontransformed data). However, the requirement of homoscedasticity is not fulfilled for these data. No significant relationship was found between Pb, Fe, and Ti concentrations and size.

In the digestive system, Zn and Cr concentrations show significant positive linear relationships with size. Pb and Ti concentrations show decreasing power relationships, and Cd concentrations an increasing power relationship with size. No significant relationship was found between Cu and Fe concentrations and size.

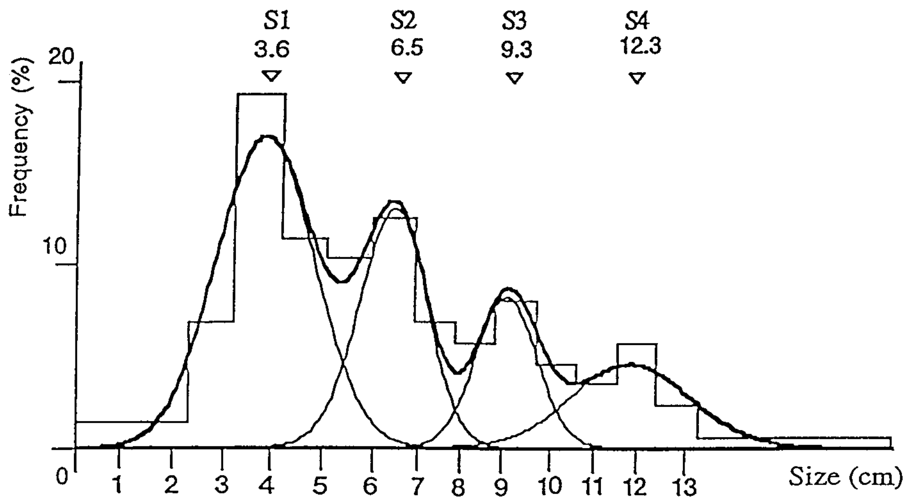

Figure 1. Size-frequency distribution of the *Asterias rubens* population of Scharendijke (August 1992): size-frequency histogram and fitted normal distributions. The bold line is the sum of the fitted normal distributions. Triangles indicate the mean sizes (cm) of the size classes (n = 100).

Table 2. Regression analysis of the relationships between metal concentrations (MC) in the body compartments of *Asterias rubens* and size (arm length -R- in cm). n: number of data used for regression analysis; R^2: determination coefficient; p: probability of the slope; b and a: parameters of the regression models; L: linear model (MC = a+b.R); P: power model (MC =a .R^b); H: hyperbolic model (MC = $\{a+b.R\}^{-1}$); n.s.: no significant relationship found (a = 0.05); *: simple linear model significant (other models nonsignificant) but heteroscedasticity of the data.

	n	MC range ($\mu g\ g^{-1}$ DW)	Regression model	Regression parameters		Model parameters	
				R^2	p	b	a
Body wall							
Zn	90	40-154	L *				
Pb	90	3.9-8.2	n.s.				
Cd	90	0.3-0.8	P	0.17	≤ 0.0001	0.2	0.4
Fe	90	24-156	n.s.				
Cr	90	0.5-3.3	H	0.23	≤ 0.0001	-0.06	1.6
Cu	90	1.5-3.7	L *				
Ti	90	0.2-0.8	n.s.				
Digestive system							
Zn	90	28-195	L	0.05	0.03	1.9	86.8
Pb	86	0.3-3.8	P	0.57	≤ 0.0001	-0.9	4.0
Cd	88	0.1-2.8	P	0.52	≤ 0.0001	1.3	0.05
Fe	90	190-1800	n.s.				
Cr	85	0.5-0.8	L	0.46	≤ 0.0001	0.03	0.06
Cu	90	9-150	n.s.				
Ti	90	0.02-2.6	P	0.40	≤ 0.0001	-1.2	0.4

Mean metal concentrations (and the associated standard deviations) were calculated either for each size-class (metals whose concentrations are significantly related to size) or for the whole size range (metals whose concentrations do not present a significant relationship with size) (Table 3). Metal concentrations were compared class by class between the two body compartments of the asteroid. All pair comparisons were found to be significantly different (t-test; a = 0.05) except for Cd in the first size-class.

Table 3. Mean concentrations (M) ($\mu g.g^{-1}$ dry weight) and standard deviations (SD) of heavy metals in the body wall and the digestive system of *Asterias rubens* individuals of the size-classes defined in Fig. 1.

	Body wall			Digestive system		
	Size class	M ± SD	n	Size class	M ± SD	n
Zn	all	69.1 ± 18.4	90	S1	91.0 ± 26.7	42
				S2	104.6 ± 26.8	22
				S3	115.0 ± 36.9	13
				S4	102.4 ± 20.7	13
Pb	all	5.07 ± 0.8	90	S1	1.78 ± 1.29	38
				S2	0.62 ± 0.23	22
				S3	0.63 ± 0.38	13
				S4	0.51 ± 0.16	13
Cd	S1	0.47 ± 0.12	42	S1	0.44 ± 0.55	40
	S2	0.50 ± 0.11	22	S2	0.81 ± 0.56	22
	S3	0.52 ± 0.11	13	S3	0.94 ± 0.59	13
	S4	0.54 ± 0.10	13	S4	1.36 ± 0.73	13
Fe	all	66.2 ± 27.6	90	all	497 ± 279	90
Cr	S1	0.79 ± 0.33	42	S1	0.24 ± 0.32	37
	S2	0.83 ± 0.30	22	S2	0.32 ± 0.13	22
	S3	1.13 ± 0.68	13	S3	0.45 ± 0.16	13
	S4	1.25 ± 0.69	13	S4	0.44 ± 0.15	13
Cu	all	2.16 ± 0.41	90	all	55.9 ± 28.6	90
Ti	all	0.35 ± 0.1	90	S1	0.19 ± 0.41	42
				S2	0.14 ± 0.37	22
				S3	0.03 ± 0.02	13
				S4	0.03 ± 0.02	13

The increasing relationships between Cr and Cd concentrations and size in both asteroid compartments indicate that these metals are continuously accumulated through life and are not, or only poorly, regulated. This is in sharp contrast with the results of Hornung et al. (1991) who showed highly significant negative relationships between Cr concentrations in the tissues of individuals of *Astropecten bispinosus* and the weight of these individuals.

Pb (and to a less extent Ti) concentrations are inversely related to size in the digestive system of *A. rubens.* The highest Pb concentrations were measured in asteroids up to 4-5 cm. This size corresponds to the life stage when sexual maturity and high growth rate are initiated (Mead 1899; Nauen and Böhm 1979). Therefore, from this life stage, a different allocation of absorbed Pb might occur, viz. in the gonads and/or in the body wall. A better understanding of Pb fluxes through the asteroid body during gametogenesis would clarify the role of gonads in the process leading to the concentration lowering in the digestive system. Accumulation of Pb in the body wall is important in large *A. rubens*, being two orders of magnitude larger in S4 size-class than in S1 size-class asteroids (Table 4). We suggest that, once a high growth rate is initiated (i.e., in asteroids reaching 5 cm), Pb fluxes in the asteroid are speeded up, most of the absorbed Pb being finally driven into the body wall, and trapped in the skeleton (95 % of lead in the body wall is actually in the skeleton; Temara et al. unpubl.). This phenomenon would make the instantaneous concentration of metal in the digestive system lower.

Table 4. Pb loads in different compartments of *Asterias rubens* in prespawning and postspawning stages (Temara et al., unpubl.) (µg metal per organ, means ± standard deviations, n= 40). (-): gonads extremely reduced at that period.

Size class	Period	Body wall	Digestive system	Gonads
S4	prespawning	155 ± 41	2.0 ± 1.1	2.3 ± 0.2
S1	postspawning	2 ± 0.5	0.17 ± 0.01	-
S4	postspawning	161 ± 36	2.1 ± 1.4	-

No significant change in Fe and Cu concentrations with size was detected; this observation probably reflects the physiological regulation of these metals (Roesijadi 1992). On the contrary, Zn concentrations show a positive linear relationship with size. It is noteworthy, however, that this relationship is barely significant ($p = 0.03$) and that the size effect is of very low importance ($R^2 = 0.05$). These observations are thus in good agreement with those obtained by Hornung et al. (1991) who found no significant relationship between the concentrations of Cu, Zn, and Fe and weight in *A. bispinosus.*

All significant relationships between size and metal concentrations (except for Cr in the digestive system) show their maximal variation range in the small size class (S1). This means that allometric effects are minimized in the large size-class (S4). We thus propose that this size-class should be sampled if *A. rubens* is used as a bioindicator of metal pollution. If Cr has to be considered, this size range should be further narrowed.

Acknowledgments. We are grateful to Prof. S. Fowler (IAEA) for comments on the manuscript, and to Prof. M. Hecq (UMH) for granting us access to his ICPS. Research supported by the Impulsion Program in Marine Sciences, financed by the Belgian Government (SSTC MS/11/020), by an IRSIA grant to M. Warnau (ref 910528) and by the NFSR (Belgium;

FRFC. Contract Ref. SUB. 92/N9466B). Ph. Dubois is a Research Associate of the NFSR. Contribution of the CIBIM.

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