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Evaluation of the variegated scallop *Chlamys varia* as a biomonitor of temporal trends of Cd, Cu, and Zn in the field

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Digestive gland and kidneys of scallops suit to biomonitor trace element concentrations.

Abstract

This study assesses the potential of the scallop *Chlamys varia* as a biomonitor of metal contamination in the field. Cd, Cu, and Zn concentrations were determined in the soft tissues and organs of individuals from the French Atlantic coast sampled over a 1 year period and covering a wide range of size. All metals were selectively distributed among the different body compartments considered, and their concentrations were influenced by the size of the specimens or the sampling-season. The present work shows the importance of considering the body compartment, the sampling period and the size in studies aiming at using this scallop as a biomonitor species. Among tissues, the digestive gland and kidneys exhibited the highest metal concentrations whatever the season or the size. The digestive gland contained 65 and 48% of the whole Cd and Cu body burdens, respectively, and kidneys accounted for 85% of the Zn load. Those tissues are therefore particularly recommended for use in biomonitoring programs.

Keywords: Heavy metals; Trace elements; Bioaccumulation; Biomonitoring; Seasonal variation; Pectinid

1. Introduction

The variegated scallop *Chlamys varia* is a common seashell on the rocky shore of the French Atlantic coast. Important shoals of this scallop are encountered in Brittany and in Charente-Maritime where this scallop is targeted by commercial fisheries as well as for leisure activities. This species has been previously reported to bioaccumulate high concentrations of several trace elements including metals, rare earth elements and radionuclides (Martoja et al., 1989; Bustamante, 1998; Bustamante et al., 2002; Bustamante and Miramand, 2004, 2005). Therefore, it has been suggested that the variegated scallop could be a useful biomonitor to survey marine pollution. However, metal concentrations in bivalves may vary with biological and environmental factors such as age (size) and seasons (e.g. Bryan, 1973; Boyden, 1977). Therefore, there is a need to assess the variations of elemental concentrations according to the size and to the different periods of the year before using a species as a biomonitor. On the other hand, whole bivalves are often employed to monitor metals, faking the actual variations of the concentrations due to natural dilution by non-storage organs. In this context, it appears particularly important to make the link between studies aiming at describing the detoxification processes leading to the bioaccumulation of metals in the storage organs and those aiming at determining

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valuable biomonitor species. Indeed, the strong capacity of scallops to bioaccumulate numerous trace elements at high concentrations in their tissues has been related to their very efficient detoxification systems in the digestive gland and kidneys (e.g. George et al., 1980; Ballan-Dufrançais et al., 1985; Stone et al., 1986; Fowler and Gould, 1988; Mauri et al., 1990; Viarengo et al., 1993). Therefore, these tissues have to be considered separately in biomonitoring studies. Furthermore, given the high filtration rates of bivalves, gills would constitute a significant pathway of incorporation of metals via seawater (e.g. Lukyanova and Evtushenko, 1989; Roméo and Gnassia-Barelli, 1995; Widdows et al., 1995). The gonad is likely to display important variations of weight according to the reproductive cycle, which might affect the metal concentrations.

The aim of this study was thus to investigate the variations of Cd, Cu, and Zn concentrations in the tissues and organs of the variegated scallop *Chlamys varia* over a 1 year period in order to assess the potential of this species as a biomonitor. Concentrations and distribution of metals in the different body compartments were determined at different seasons and at different sizes. Statistical analyses were then performed to discriminate the influence of the different factors (i.e. body compartment, sampling period and size) in the variability of the metal concentrations.

2. Materials and methods

2.1. Sampling and sample preparation

Individuals of the variegated scallop *Chlamys varia* (Linné, 1758) were collected at low tide in the infralittoral zone in the Bay of La Rochelle, Atlantic coast of France. This site harbours a stable scallop population. Moreover, as seashore fishing is forbidden in this area, good sampling of the different size classes was available.

Scallop samplings were performed in November 1995 ($n = 139$), February ($n = 265$), June ($n = 228$) and September 1996 ($n = 152$). These periods correspond to the main stages of the reproductive cycle of the variegated scallop in this area (Letaconnoux and Audouin, 1956). After collection, the scallops were depurated for 48 h in filtrated seawater (close circuit, constant aeration, 34 p.s.u., 12 h/12 h light/dark cycle) to eliminate faecal and pseudo-faecal material. Whenever possible, they were then pooled by size classes of 10 individuals (up to 30 for the smallest ones) according to the height of the shell (called size in the rest of this work), and dissected. Scallop tissues were pooled to avoid, as much as possible, individual variations of the metallic concentrations. The characteristics of the pooled samples are given in Table 1.

For each individual, the digestive gland, kidneys, gonad, gills, and adductor muscle were separated from the rest of the soft parts (mantle, foot, heart and intestine), and pooled for each defined size class. However, for scallop below 20 mm, only the digestive gland and the adductor muscle allowed an accurate dissection. Thus, in the case of these small scallops, the remaining tissues also included gills, kidneys and gonad. In both case, remaining tissues were analysed in order to calculate the whole trace element content of the soft parts.

2.2. Trace element analyses

Tissue samples were dried for several days at 80 °C to constant weight. Whenever possible, two aliquots (100–300 mg) of each homogenised dry sample were digested with 4 ml of 65% ultrapure HNO_3 and 0.3 ml of ultrapure 70% $HClO_4$ at 80 °C until the solution was clear. Then acids were evaporated and residues were dissolved in 10 ml of 0.3 N ultrapure nitric acid.

Cadmium, copper and zinc were determined by flame and graphite furnace atomic absorption spectrophotometry with a Varian spectrophotometer Vectra 250 Plus with deuterium background correction. Reference standard materials, dogfish liver DOLT-2 (National Research Council, Canada) and dogfish muscle DORM-2 (NRCC) were treated and analysed in the same way as the samples. The results for standard reference materials displayed recoveries of the elements ranging from 98% to 102% and the detection limits calculated for 100 mg of dry material were (μg g^{-1} dry wt): 0.004 (Cd), 0.5 (Cu), 1.5 (Zn). Metal concentrations in tissues and the proportion in each body compartment are given in dry weight (μg g^{-1} dry wt) and % of the metal loads, respectively.

2.3. Data analyses

Statistical analyses of the data were performed by one-, two-, or three-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test (Zar, 1996). The variability explained by each factor was derived from the sum of squares. The significance for statistical analyses was always set at $\alpha = 0.05$.

3. Results

3.1. Relative contribution of the investigated factors to metal concentration variability

The mean Cd, Cu and Zn concentrations measured in the body compartments of the variegated scallop are presented in Tables 2–4, respectively. Among tissues, metals greatly varied from two (i.e. Cd and Cu) to three orders of magnitude (i.e. Zn). Statistical analyses were therefore used to discriminate the relative contribution

Table 1
Characteristics of the samples analysed: number of pools analysed (n), number of scallop per pool (N), scallop height (mm), fraction of each tissue relatively to the soft part weight (% of the soft part weight); values in brackets are the water fraction (%) of the wet body compartments (all data are means ± SD)

Period	Height (mm)	$n \times N$	% soft part weight (% water)					
			Digestive gland	Kidneys	Gills	Gonad	Adductor muscle	Remaining tissues
November 95	18.0 ± 4.2	1×5	20.8 (80.7)	–	–	–	36.4 (82.4)	42.9 (89.9)
	27.5 ± 2.1	3×10	14.5 ± 1.0	2.6 ± 0.2	1.8 ± 0.4	10.0 ± 0.6	42.1 ± 1.8	29.0 ± 2.9
			(83.4 ± 0.2)	(84.4 ± 3.1)	(93.6 ± 0.5)	(84.2 ± 2.9)	(82.9 ± 0.6)	(87.5 ± 0.9)
	33.2 ± 1.6	3×10	13.1 ± 0.8	1.9 ± 0.4	2.3 ± 0.1	11.4 ± 0.3	42.9 ± 2.4	28.5 ± 3.2
			(84.9 ± 1.9)	(84.5 ± 4.1)	(92.8 ± 0.5)	(88.9 ± 1.6)	(81.8 ± 0.3)	(87.9 ± 0.4)
	39.4 ± 1.8	3×10	14.9 ± 0.5	2.2 ± 0.3	2.4 ± 0.2	10.5 ± 0.7	43.4 ± 1.9	26.6 ± 1.7
			(83.2 ± 1.1)	(83.7 ± 2.9)	(92.5 ± 0.2)	(89.5 ± 1.8)	(81.4 ± 0.5)	(88.0 ± 0.2)
	44.9 ± 1.9	3×10	14.7 ± 1.9	1.6 ± 0.3	2.2 ± 0.4	8.9 ± 1.5	47.1 ± 3.4	25.5 ± 1.1
			(81.4 ± 4.3)	(83.8 ± 2.2)	(92.8 ± 1.0)	(90.9 ± 0.1)	(80.6 ± 1.4)	(88.4 ± 0.7)
	50.8 ± 1.6	2×7	14.3 ± 1.1	2.1 ± 0.5	2.3 ± 0.2	9.1 ± 0.6	45.2 ± 2.4	26.1 ± 1.5
			(82.2 ± 2.8)	(84.1 ± 2.4)	(92.6 ± 0.8)	(89.2 ± 1.4)	(81.2 ± 0.8)	(88.2 ± 0.4)
February 96	8.5 ± 1.5	2×30	15.9 ± 5.1	–	–	–	11.3 ± 2.5	71.8 ± 3.9
			(73.0 ± 3.9)				(81.4 ± 1.8)	(70.0 ± 11.6)
	12.4 ± 2.3	1×45	19.3 (74.2)	–	–	–	23.6 (76.4)	57.0 (80.6)
	22.9 ± 1.6	2×10	18.9 ± 0.0	–	–	–	26.3 ± 1.4	54.8 ± 1.4
			(80.3 ± 0.9)				(80.7 ± 0.0)	(85.1 ± 0.7)
	28.3 ± 1.5	3×10	12.3 ± 0.5	4.9 ± 0.2	11.6 ± 0.2	1.6 ± 0.6	41.0 ± 0.4	28.6 ± 0.8
			(85.7 ± 0.8)	(92.5 ± 0.9)	(93.1 ± 0.6)	(90.6 ± 3.3)	(83.2 ± 0.3)	(88.7 ± 0.7)
	32.3 ± 1.7	3×10	11.4 ± 0.7	4.6 ± 0.5	10.7 ± 0.7	1.9 ± 0.2	43.3 ± 1.3	28.2 ± 0.6
			(84.8 ± 1.2)	(92.7 ± 0.9)	(93.1 ± 0.8)	(90.7 ± 1.2)	(81.9 ± 0.9)	(88.6 ± 0.6)
	38.9 ± 2.0	3×10	12.6 ± 0.2	4.1 ± 0.5	9.7 ± 0.4	2.4 ± 0.1	43.8 ± 2.0	27.4 ± 1.3
			(83.9 ± 0.9)	(92.4 ± 0.4)	(94.1 ± 0.3)	(90.4 ± 0.8)	(81.5 ± 0.9)	(89.1 ± 0.1)
	44.8 ± 2.1	3×10	13.5 ± 1.1	3.1 ± 0.1	8.2 ± 0.5	2.4 ± 0.4	46.1 ± 1.2	26.6 ± 1.0
			(81.3 ± 1.3)	(92.2 ± 1.4)	(94.0 ± 0.2)	(90.4 ± 1.3)	(79.0 ± 1.2)	(89.0 ± 0.2)
	49.7 ± 2.4	2×10	13.1 ± 0.1	2.1 ± 0.2	8.1 ± 1.1	2.1 ± 0.0	48.9 ± 0.4	25.7 ± 0.7
			(83.4 ± 0.5)	(90.2 ± 0.1)	(93.9 ± 0.3)	(90.2 ± 0.1)	(78.3 ± 0.7)	(88.3 ± 0.7)
June 96	15.2 ± 2.0	3×30	–	–	–	–	–	–
	28.5 ± 2.1	3×9	17.9 ± 1.3	5.0 ± 0.9	4.0 ± 0.4	14.8 ± 1.5	37.1 ± 0.5	21.3 ± 1.2
			(83.2 ± 0.3)	(93.7 ± 1.0)	(97.2 ± 0.1)	(84.2 ± 1.0)	(83.4 ± 0.3)	(88.9 ± 0.2)
	32.9 ± 1.2	3×10	17.5 ± 0.8	5.0 ± 0.2	4.8 ± 0.6	17.4 ± 3.9	33.3 ± 2.9	22.1 ± 2.1
			(83.3 ± 0.6)	(92.0 ± 0.5)	(96.7 ± 0.0)	(83.0 ± 1.3)	(83.6 ± 0.2)	(91.2 ± 0.2)
	39.5 ± 1.3	3×10	15.0 ± 0.2	4.5 ± 0.4	6.0 ± 1.2	17.4 ± 0.8	32.9 ± 2.9	24.3 ± 1.8
			(84.8 ± 0.5)	(89.5 ± 1.0)	(95.9 ± 1.3)	(83.9 ± 1.2)	(84.2 ± 0.7)	(90.4 ± 1.0)
	45.2 ± 1.4	3×10	14.2 ± 0.9	4.2 ± 0.8	6.1 ± 0.5	19.3 ± 0.9	30.8 ± 1.1	25.5 ± 0.3
			(85.2 ± 0.6)	(89.4 ± 2.2)	(95.5 ± 0.5)	(83.6 ± 1.6)	(84.6 ± 0.5)	(89.7 ± 0.8)
	49.5 ± 1.2	3×7	14.5 ± 1.2	3.2 ± 0.7	9.6 ± 0.2	22.7 ± 4.6	26.4 ± 3.3	23.7 ± 1.5
			(83.9 ± 0.9)	(88.3 ± 1.1)	(93.3 ± 0.5)	(81.9 ± 2.8)	(84.5 ± 0.2)	(88.8 ± 0.4)
September 96	9.2 ± 1.5	1×20	16.2 (78.7)	–	–	–	17.9 (83.2)	65.8 (82.9)
	20.0 ± 1.4	1×7	17.6 (75.2)	–	–	–	34.8 (77.9)	47.6 (82.9)
	26.7 ± 1.9	3×10	18.0 ± 0.9	2.7 ± 0.3	9.3 ± 0.3	2.0 ± 0.7	39.1 ± 1.1	28.8 ± 0.8
			(72.8 ± 0.5)	(78.3 ± 0.7)	(88.3 ± 0.5)	(83.2 ± 4.2)	(77.1 ± 0.5)	(81.7 ± 0.2)
	33.2 ± 1.8	2×7	18.5 ± 0.5	2.6 ± 0.1	3.5 ± 0.5	1.7 ± 0.5	50.3 ± 0.2	23.4 ± 1.8
			(74.1 ± 0.3)	(87.2 ± 2.4)	(96.1 ± 0.5)	(87.6 ± 2.8)	(79.1 ± 0.6)	(87.7 ± 0.6)
	39.6 ± 1.6	3×10	18.3 ± 0.7	2.6 ± 0.2	3.6 ± 0.5	2.0 ± 0.9	52.1 ± 1.4	21.3 ± 0.4
			(72.0 ± 0.4)	(84.2 ± 1.5)	(95.4 ± 0.8)	(85.5 ± 2.3)	(77.4 ± 0.1)	(86.8 ± 0.3)
	45.1 ± 1.4	3×10	18.1 ± 1.0	2.5 ± 0.4	4.5 ± 0.2	2.1 ± 0.1	52.1 ± 0.4	20.7 ± 0.4
			(72.7 ± 2.3)	(84.9 ± 1.3)	(94.8 ± 0.4)	(86.7 ± 1.1)	(76.4 ± 0.3)	(86.2 ± 1.3)
	50.9 ± 2.3	3×7	20.0 ± 1.3	2.6 ± 0.1	5.9 ± 0.6	2.3 ± 0.4	49.6 ± 1.7	19.6 ± 0.7
			(72.8 ± 1.2)	(88.2 ± 0.4)	(94.2 ± 0.5)	(89.1 ± 1.3)	(76.9 ± 0.5)	(87.2 ± 0.3)

of the different factors in the variability of the metal concentrations. With the exception of the smallest sizes for which pools are lacking, the whole set of data was analysed using three-way ANOVA (Table 5). These analyses showed that among the three factors of variation considered (body compartment, sampling period and size), the body compartment was the predominant factor for the three metals, accounting for 79.3–93.7% of the variation (Table 5). Other factors appeared to be less important except the interaction between body compartment and sampling-period factors, which indicated that seasonal variations of the

Table 2
Cd concentrations (mean ± SD, μg g^{-1} dry wt) in the tissues of the variegated scallop *Chlamys varia* of different size classes

Tissue	Period	<11 mm	11–17 mm	17–24 mm	24–30 mm	30–36 mm	36–41 mm	42–47 mm	47–53 mm
Digestive gland	November 95	–	–	18.8	19.6 ± 1.9	22.3 ± 0.6	32.7 ± 5.0	39.6 ± 2.5	50.7 ± 5.4
	February 96	34.0 ± 2.2	25.6	23.7 ± 1.1	30.5 ± 3.9	28.8 ± 2.9	35.4 ± 4.8	37.9 ± 4.7	43.3 ± 0.5
	June 96	–	16.4 ± 1.0	–	24.6 ± 2.2	23.7 ± 2.6	31.1 ± 5.8	47.5 ± 11.5	41.7 ± 8.1
	September 96	13.2	–	10.8	9.3 ± 0.3	15.4 ± 1.9	19.4 ± 1.1	23.5 ± 3.6	26.9 ± 2.7
Kidneys	November 95	–	–	–	35.3 ± 5.8	46.1 ± 1.7	47.0 ± 14.2	52.4 ± 10.0	60.9 ± 3.6
	February 96	–	–	–	31.8 ± 1.2	31.7 ± 4.1	34.3 ± 0.7	34.6 ± 5.8	47.5 ± 15.1
	June 96	–	–	–	30.3 ± 3.0	32.7 ± 1.4	29.8 ± 11.9	45.3 ± 5.8	48.8 ± 3.9
	September 96	–	–	–	32.5 ± 1.6	26.4 ± 5.3	30.9 ± 1.8	35.6 ± 4.7	34.6 ± 5.4
Gill	November 95	–	–	–	3.29 ± 2.65	2.91 ± 0.06	3.65 ± 1.37	4.43 ± 0.84	4.91 ± 0.61
	February 96	–	–	–	3.25 ± 0.29	3.14 ± 0.26	3.60 ± 0.37	3.98 ± 0.26	4.33 ± 0.57
	June 96	–	–	–	3.25 ± 0.24	3.03 ± 0.35	3.53 ± 0.83	4.36 ± 0.36	4.46 ± 0.14
	September 96	–	–	–	1.63 ± 0.17	2.72 ± 0.07	2.96 ± 0.27	3.49 ± 0.44	3.67 ± 0.41
Gonad	November 95	–	–	–	2.44 ± 0.30	1.89 ± 0.08	2.29 ± 0.22	2.38 ± 0.20	2.30 ± 0.18
	February 96	–	–	–	3.62 ± 0.21	2.42 ± 0.49	1.67 ± 0.35	2.11 ± 0.35	2.10 ± 0.21
	June 96	–	–	–	1.12 ± 0.21	1.16 ± 0.37	1.46 ± 0.35	1.54 ± 0.28	0.64 ± 0.21
	September 96	–	–	–	1.31 ± 0.43	4.67 ± 1.55	4.17 ± 1.36	3.94 ± 0.18	3.43 ± 0.75
Adductor muscle	November 95	–	–	0.70	0.57 ± 0.11	0.55 ± 0.09	0.55 ± 0.10	0.55 ± 0.13	0.54 ± 0.04
	February 96	0.39 ± 0.18	0.64	0.41 ± 0.05	0.50 ± 0.03	0.51 ± 0.04	0.50 ± 0.07	0.59 ± 0.05	0.46 ± 0.04
	June 96	–	0.18 ± 0.01	–	0.48 ± 0.06	0.48 ± 0.11	0.55 ± 0.19	0.64 ± 0.13	0.45 ± 0.06
	September 96	0.33	–	0.35	0.33 ± 0.18	0.34 ± 0.03	0.36 ± 0.03	0.38 ± 0.04	0.38 ± 0.05
Whole soft parts	November 95	–	–	5.90	5.04 ± 0.24	4.94 ± 0.21	7.13 ± 1.41	7.95 ± 1.27	9.47 ± 0.88
	February 96	8.15 ± 1.71	7.72	7.57 ± 0.08	6.38 ± 0.33	5.75 ± 0.29	6.98 ± 0.85	7.38 ± 0.91	8.16 ± 0.06
	June 96	–	5.33 ± 0.11	–	6.71 ± 0.34	6.72 ± 0.31	7.17 ± 1.50	10.00 ± 1.66	8.74 ± 1.32
	September 96	3.93	–	3.48	3.15 ± 0.33	4.19 ± 0.27	5.05 ± 0.13	5.86 ± 0.50	7.09 ± 0.85

Table 3
Cu concentrations (mean ± SD, μg g^{-1} dry wt) in the tissues of the variegated scallop *Chlamys varia* of different size classes

Cu	Period	<11 mm	11–17 mm	17–24 mm	24–30 mm	30–36 mm	36–41 mm	42–47 mm	47–53 mm
Digestive gland	November 95	–	–	45.0	51.7 ± 5.5	68.0 ± 3.6	77.0 ± 9.5	134 ± 9.5	175 ± 18.3
	February 96	74.3 ± 30.7	39.4	52.1 ± 3.1	46.7 ± 5.0	49.7 ± 4.6	64.7 ± 6.1	81.7 ± 8.1	97.5 ± 17.7
	June 96	–	29.6 ± 0.2	–	45.0 ± 2.7	48.6 ± 5.6	89.6 ± 26.1	129 ± 16.7	112 ± 19.2
	September 96	97.6	–	58.0	55.7 ± 10.9	65.4 ± 4.7	71.4 ± 2.1	96.6 ± 15.4	103 ± 8.7
Kidneys	November 95	–	–	–	230 ± 32.9	288 ± 12.7	246 ± 39.1	271 ± 14.1	280 ± 18.5
	February 96	–	–	–	147 ± 35.4	143 ± 21.0	132 ± 8.7	147 ± 25.0	134 ± 9.9
	June 96	–	–	–	176 ± 21.3	184 ± 18.3	168 ± 81.2	184 ± 13.9	188 ± 23.3
	September 96	–	–	–	502 ± 27.3	367 ± 21.7	292 ± 11.6	295 ± 16.7	255 ± 15.7
Gill	November 95	–	–	–	15.3 ± 3.1	14.0 ± 1.7	10.3 ± 1.5	11.7 ± 2.1	11.2 ± 1.6
	February 96	–	–	–	10.0 ± 0.1	10.3 ± 0.6	8.3 ± 0.6	8.0 ± 1.0	8.5 ± 0.7
	June 96	–	–	–	10.6 ± 0.7	8.7 ± 0.8	9.3 ± 0.8	8.7 ± 0.9	6.8 ± 0.5
	September 96	–	–	–	13.3 ± 0.1	24.7 ± 3.0	18.5 ± 1.3	15.9 ± 1.6	14.1 ± 0.7
Gonad	November 95	–	–	–	44.7 ± 5.5	33.0 ± 4.0	33.0 ± 7.2	40.0 ± 3.5	38.0 ± 1.2
	February 96	–	–	–	9.2 ± 0.4	15.7 ± 6.4	21.0 ± 3.6	24.0 ± 3.6	31.5 ± 7.8
	June 96	–	–	–	8.9 ± 0.7	8.9 ± 1.4	17.6 ± 7.1	18.4 ± 5.0	12.3 ± 2.3
	September 96	–	–	–	52.5 ± 14.7	59.9 ± 18.9	50.2 ± 14.6	50.7 ± 3.1	56.5 ± 2.9
Adductor muscle	November 95	–	–	5.0	4.3 ± 1.2	4.0 ± 0.1	3.3 ± 0.6	4.0 ± 1.0	4.3 ± 1.1
	February 96	13.0 ± 5.4	7.3	2.9 ± 0.9	2.6 ± 0.2	2.3 ± 0.2	2.0 ± 0.1	1.9 ± 0.2	1.7 ± 0.0
	June 96	–	2.9 ± 0.1	–	3.3 ± 0.6	3.3 ± 0.2	3.7 ± 0.3	4.1 ± 0.6	2.6 ± 0.2
	September 96	27.4	–	3.0	2.1 ± 0.6	5.2 ± 0.8	3.8 ± 0.4	3.5 ± 0.1	2.8 ± 0.0
Whole soft parts	November 95	–	–	21.0	20.6 ± 1.7	21.4 ± 0.9	22.5 ± 3.0	30.2 ± 2.9	30.0 ± 4.2
	February 96	29.0 ± 13.4	22.4	24.3 ± 1.4	17.0 ± 2.1	16.5 ± 0.5	17.3 ± 1.1	19.2 ± 1.1	19.2 ± 2.9
	June 96	–	19.7 ± 0.7	–	20.8 ± 0.6	21.8 ± 0.5	27.1 ± 4.3	32.8 ± 2.1	27.5 ± 3.3
	September 96	63.5	–	39.3	31.6 ± 1.3	29.9 ± 1.7	27.3 ± 0.2	33.0 ± 3.2	31.0 ± 2.9

Table 4
Zn concentrations (mean ± SD, µg g^{-1} dry wt) in the tissues of the variegated *Chlamys varia* of different size classes

Zinc	Period	<11 mm	11–17 mm	17–24 mm	24–30 mm	30–36 mm	36–41 mm	42–47 mm	47–53 mm
Digestive gland	November 95	–	–	763	443 ± 74	518 ± 68	346 ± 23	344 ± 44	245 ± 29
	February 96	1416 ± 254	1437	1314 ± 416	196 ± 6	196 ± 21	157 ± 25	129 ± 2	179 ± 2
	June 96	–	511 ± 80	–	171 ± 15	184 ± 25	184 ± 10	163 ± 11	142 ± 4
	September 96	630	–	377	221 ± 61	96 ± 6	89 ± 10	88 ± 11	92 ± 12
Kidneys	November 95	–	–	–	27286 ± 5058	38168 ± 4603	34831 ± 2451	34212 ± 1885	36019 ± 5630
	February 96	–	–	–	21482 ± 2694	21018 ± 3568	31372 ± 3171	22806 ± 2304	26058 ± 4973
	June 96	–	–	–	34932 ± 3340	38796 ± 2730	33575 ± 15478	42648 ± 4351	37693 ± 582
	September 96	–	–	–	23271 ± 1194	26215 ± 2764	31857 ± 1916	32309 ± 5036	28683 ± 1479
Gill	November 95	–	–	–	631 ± 296	569 ± 105	313 ± 20	383 ± 27	254 ± 49
	February 96	–	–	–	462 ± 211	406 ± 97	298 ± 62	233 ± 119	230 ± 103
	June 96	–	–	–	500 ± 140	351 ± 97	416 ± 69	308 ± 87	149 ± 37
	September 96	–	–	–	177 ± 38	179 ± 47	146 ± 18	131 ± 26	112 ± 10
Gonad	November 95	–	–	–	1035 ± 263	799 ± 93	542 ± 62	477 ± 103	324 ± 86
	February 96	–	–	–	390 ± 24	409 ± 16	375 ± 81	249 ± 15	224 ± 82
	June 96	–	–	–	131 ± 10	114 ± 32	153 ± 39	136 ± 41	82 ± 27
	September 96	–	–	–	452 ± 181	286 ± 106	178 ± 29	179 ± 60	112 ± 9
Adductor muscle	November 95	–	–	179	201 ± 107	175 ± 25	176 ± 73	175 ± 24	174 ± 18
	February 96	216 ± 133	195	241 ± 24	112 ± 7	94 ± 18	97 ± 8	108 ± 14	93 ± 6
	June 96	–	96 ± 7	–	131 ± 41	108 ± 9	115 ± 19	129 ± 19	84 ± 2
	September 96	326	–	59	45 ± 11	70 ± 6	64 ± 6	61 ± 8	56 ± 2
Whole soft parts	November 95	–	–	1140	1063 ± 188	1062 ± 132	1003 ± 97	799 ± 48	772 ± 13
	February 96	1846 ± 572	1414	1830 ± 94	1213 ± 75	1113 ± 86	1428 ± 35	845 ± 97	686 ± 90
	June 96	–	1177 ± 131	–	1864 ± 163	2064 ± 197	1630 ± 599	1906 ± 211	1309 ± 250
	September 96	812	–	567	761 ± 36	762 ± 54	889 ± 54	876 ± 107	822 ± 63

Table 5
Variability (%) in metal concentrations in the tissues of the variegated scallop *Chlamys varia* explained by the different factors considered (sampling period and size of the scallops) and their interactions

Factors	Cd	Cu	Zn
Compartment	**86.3**	**79.3**	**93.7**
Period	1.2	3.6	0.5
Size	1.7	0.2	0.1
Compartment × period	3.6	9.4	2.5
Compartment × size	3.1	2.2	0.6
Period × size	0.4	0.8	ns
Compartment × period × size	1.0	3.1	0.7
Residual	2.6	1.4	1.8

Highest proportions of variation are shown in bold. ns, not significant ($p_{\mathrm{ANOVA}} < 0.05$).

metal concentrations were not synchronous in the different body compartments. Therefore, two-way ANOVA was used to discriminate the influence of the sampling-period and the size of the scallops in each of the body compartments (Table 6). These analyses revealed that the variability of essential Cu and Zn concentrations in the different tissues was explained by the sampling period, except for the Cu in the digestive gland which variability was mainly related to the size of the scallops. The sampling period accounted for 57.2–84.8% of the variation of Cu and for 33.9–72.3% of the variation of Zn (Table 6). In the case of Cd, size was the predominant factor for digestive gland, gills, and whole soft parts (43.6, 44.9 and 42.8%, respectively). In contrast, most of the variability of Cd concentrations in kidney, gonad, adductor muscles and remaining tissues was related to the sampling period (i.e. from 34.4 to 49.6% of the variation).

3.2. Metal distribution

Among the tissues investigated, the digestive gland clearly appeared to be the main storage compartment of Cd and Cu (Table 7), while this organ only accounted for 10–20% of the soft part weight (Table 1). Zn occurred in larger proportion in the kidneys, which contained up to 85% of the total body burden of the metal despite its very low contribution in the soft part weight (less than 5%). Body burdens of all elements in gills and gonads were generally low (<5%) because these organs also represented a relatively small fraction of the soft part weight (Table 1). Among tissues, the adductor muscle accounting for up to 50% of the soft part weight, always displayed the lowest metal concentrations and only contained a small fraction of the total metal body burdens, i.e. 3.3, 5.9 and 4.4% of the total Cd, Cu and Zn, respectively.

3.3. Seasonal variations of metal concentrations

The mean concentrations of Cd, Cu and Zn in the body compartment varied significantly according to the sampling period considered except for Cu in the digestive gland ($p = 0.124$) (Tables 6 and 8). Multiple comparison tests on the mean concentrations for each sampling period (i.e. all compartments considered together) indicated that the sampling period showing the highest or lowest concentrations differed from one metal to another. Moreover, when considering the different compartments separately, the metal concentrations of several sampling periods were frequently not significantly different from each other (Table 8). However, the lowest Cd concentrations were always measured in September in all the compartments except in the gonad, for which the lowest concentrations correspond to the sexual maturity period (i.e. June). For essential elements, scallops sampled in September had the highest Cu but the lowest Zn concentrations in most tissues. Highest Zn concentrations in most of the tissues were recorded in November.

3.4. Size variations of metal concentrations

Metals were measured in scallops belonging to the whole size range of the population. The size of the

Table 6
Variability (%) in Cd, Cu and Zn concentrations in the tissues of the variegated scallop *Chlamys varia* explained by the different factors considered (sampling period and size of the scallops) and their interactions

	Factors	Digestive gland	Kidneys	Gills	Gonad	Adductor muscle	Remaining tissues	Whole soft parts
Cd	Period	36.7	**34.4**	22.3	**49.6**	**44.0**	**46.3**	33.1
	Size	**43.6**	26.0	**44.9**	ns	ns	12.2	**42.8**
	Period × size	8.4	ns	ns	35.2	ns	17.8	ns
	Residual	11.3	27.4	25.1	13.4	42.3	23.7	15.3
Cu	Period	ns	**69.4**	**64.3**	**80.7**	**45.6**	**84.8**	**57.2**
	Size	**67.2**	4.4	7.6	ns	ns	2.7	22.0
	Period × size	14.4	19.9	21.0	ns	31.0	7.0	11.2
	Residual	7.8	6.3	7.0	10.3	20.9	5.5	9.7
Zn	Period	**72.3**	**48.3**	**33.9**	**57.6**	**66.7**	**62.3**	**69.0**
	Size	12.4	10.4	29.0	19.9	ns	ns	8.0
	Period × size	10.5	ns	ns	13.8	ns	ns	11.3
	Residual	4.8	6.3	26.2	8.7	27.3	24.0	11.7

Highest proportions of variation are shown in bold. ns, not significant ($p_{\mathrm{ANOVA}} > 0.05$).

Table 7
Proportions (%, mean ± SD) of metal loads in the body compartments of the variegated scallop *Chlamys varia* (all locations and periods together)

Metal	Digestive gland	Kidneys	Gonad	Gills	Adductor muscle	Remaining tissues
Cd	65.3 ± 6.7	18.5 ± 5.0	1.4 ± 0.3	4.2 ± 1.7	3.3 ± 1.0	7.3 ± 2.3
Cu	47.6 ± 10.9	28.8 ± 9.5	4.7 ± 3.6	3.8 ± 2.1	5.9 ± 3.1	9.1 ± 3.6
Zn	3.0 ± 1.9	84.7 ± 9.4	1.0 ± 0.7	2.5 ± 2.0	4.4 ± 2.6	4.5 ± 3.8

scallops significantly affected the concentrations of Cd, Cu and Zn in most of the scallop body compartments but size was only the main factor explaining most of the variability for a few tissues, i.e. the digestive gland for Cd and Cu, and the gills for Cd (Table 6). However, the contribution of this factor to the variability of the concentrations was not significant ($p > 0.05$) for Cd and Cu in the gonad and the muscle, and for Zn in the muscle and the remaining tissues (Table 6, Figs. 1–3). When significant, the correlations between Cd concentration and size were always positive. The only positive relationship for essential elements was found for Cu in digestive gland and Zn in kidneys. In contrast, Cu in kidneys and gills and Zn in digestive gland, gills and gonad decreased significantly with size (Figs. 2 and 3).

4. Discussion

Metal concentrations in the variegated scallop *Chlamys varia* appeared to be significantly affected by the three factors considered (body compartment, sampling period and size) in this study. Less than 3% of the variability in Cd, Cu and Zn concentrations is not explained by these three factors. The main factor was clearly the body compartment, which accounted for 79–94% of the variability (Table 5). Sampling period and size have a far lower weight in the variability but significantly influence the metal concentrations according to the tissue. Therefore, the influence of these two factors on the different compartments has been considered specifically (Table 6).

Table 8
Seasonal variations of metal concentrations in the tissues of the variegated scallop *Chlamys varia*: results of the multiple comparison test of Tukey performed after ANOVA

Tissues	Cd	Cu	Zn
Digestive gland	S N J F	F J N S	S J N F
Kidneys	S F J N	F J N S	S F N J
Gills	S F J N	F J N S	S F J N
Gonad	J N F S	F J N S	J S F N
Adductor muscle	S F J N	F J N S	S F J N
Remaining tissues	S F N J	F J N S	S J F N
Whole soft parts	S F N J	F J N S	S N F J

Mean metal concentrations rank from the left to the right by increasing order. Concentrations in underlined seasons are not significantly different ($\alpha_{Tukey} = 0.05$). N, November 95; F, February 96; J, June 96; S, September 96.

Metals were selectively distributed between the body compartments. This observation is consistent with other studies on different scallop species (e.g. Bryan, 1973; Mauri et al., 1990; Viarengo et al., 1993; Bustamante and Miramand, 2005), which indicated that the digestive gland and kidneys efficiently concentrated heavy metals. Such storage capacity is related to their role in the metabolism and to the detoxification processes occurring in both organs. The food is digested and absorbed in the digestive gland, where Cd, Cu and Zn are generally reported to be bound to cytosolic proteins such as metallothionein-like proteins (Mauri et al., 1990; Viarengo et al., 1993; Bustamante, 1998). Once in the organism, heavy metals might be translocated to other organs, particularly to the kidneys because of their excretory function (e.g. Rainbow, 1990, 1993). In this organ, a high proportion of heavy metals are trapped in granules mainly composed of calcium and phosphate (Carmichael et al., 1979; George et al., 1980; Stone et al., 1986; Fowler and Gould, 1988). However, the residence time and transfer processes of metals in and among the different tissues have not been reported.

Despite clear excretion of residual bodies containing granules or residues of the digestion (George et al., 1980), trace element bioaccumulation in the digestive gland and kidneys lead to very high concentrations of heavy metals. This is particularly obvious for Zn in the kidney where the metal varied from 20 to more than 40 mg g^{-1} dry wt whatever the size or the sampling period (Table 4). As a consequence of the very high metal concentrations, the highest Cd, Cu and Zn loads are contained in the digestive gland and kidneys, which are therefore considered as the main accumulator body compartments. Very high concentrations and loads in the digestive gland and relatively low metal concentrations in the gills, suggest that food would be a major pathway of metal uptake in this species. However, further investigation using metal radiotracers should be carried out to help in confirming this hypothesis and to characterise the uptake biokinetics of metals following both food and seawater pathways.

Most of the compartments displayed seasonal variations, which were, however, not synchronous among the different tissues. If we assumed that transfer between body compartments occurs relatively rapidly as reported for other bivalve species using radioisotopes (e.g. Fisher et al., 1996), it is likely that the asynchronous variations of metal concentrations are due to physiological factors rather than to environmental ones. Among physiological factors, sexual maturity related to gametogenesis and gamete emission is one process which leads to the loss of a fraction of the metal load. The lowest concentrations of all metals were measured in the gonad of the scallops sampled in June, corresponding to the

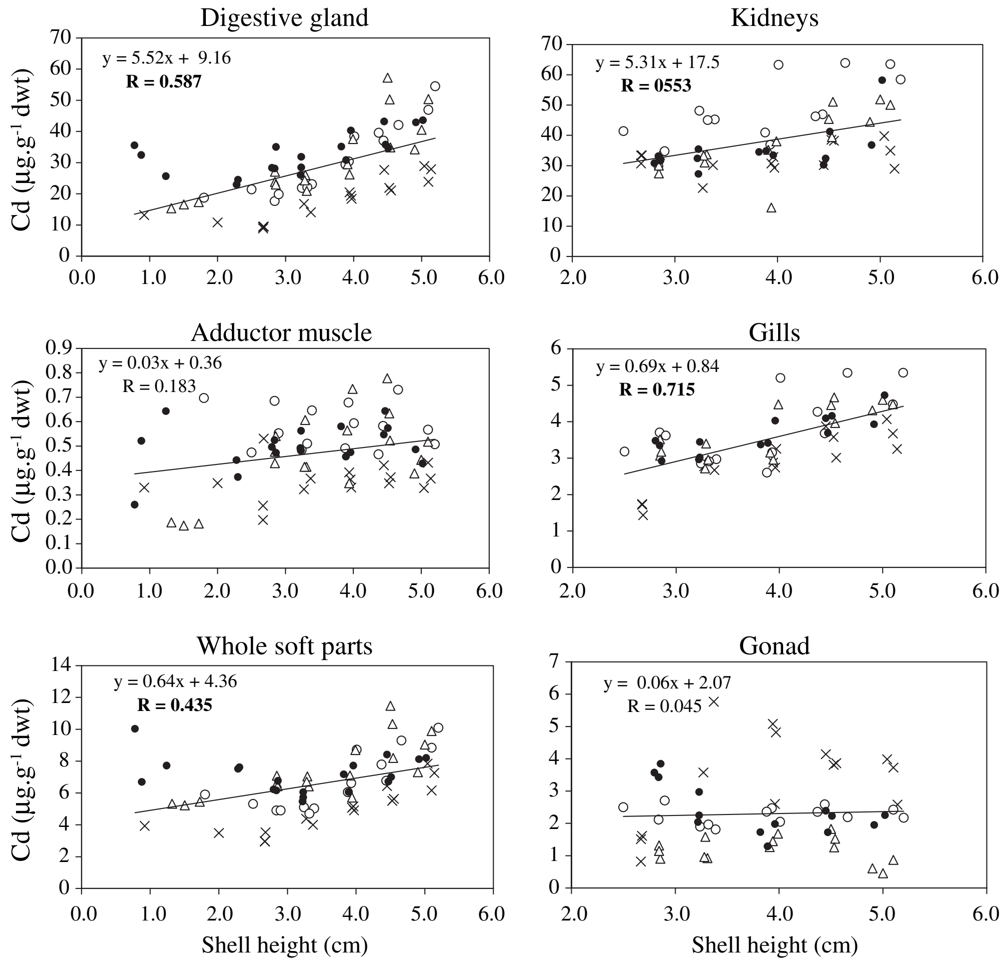

Fig. 1. Variations of Cd concentrations with size in the tissues of the variegated scallop *Chlamys varia* from the French Atlantic coast. ○, November 1995; ●, February 1996; △, June 1996; ×, September 1996. Significant correlation coefficients are shown in bold.

beginning of the spawning period of *Chlamys varia* in the Bay of Biscay (Letaconnoux and Audouin, 1956). In this species, spawning could therefore constitute a way of metal elimination from the organism. Similar observations have been reported for different invertebrates or fish species such as the echinoid *Paracentrotus lividus*, the oyster *Crassostrea iridescens* or the dab *Limanda limanda* for metals and polychlorinated biphenyls (Loizeau and Abarnou, 1994; Páez-Osuna et al., 1995; Warnau et al., 1998). Seasonal variations of metal concentrations might be explained by several factors such as the fluctuations in food supply (Bryan, 1973), the changes in run-off of particulate matter to the sea due to high precipitation (Fowler and Oregioni, 1976), the variations related to the reproductive cycle (Latouche and Mix, 1981) or the seasonal changes during the year (Páez-Osuna and Marmolejo-Rivas, 1990). In other bivalves such as oysters or mussels, seasonal variations of metal concentrations have been related to changes in their tissue weight (Boyden and Phillips, 1981; Latouche and Mix, 1981; Khristoforova and Chernova, 1990). The variegated scallop described a winter–spring maximum and summer pre-spawning minimum of Cd and Zn concentrations, as previously reported for oysters and mussels. However, depletion of Cu at the end of the spawning period could indicate a drastic loss of this metal through gamete emission.

Because they poorly regulate metal concentrations in their tissues, bivalves might reflect the contamination of their environment. However, metal concentrations within these molluscs could vary with age (i.e. length and/or weight) (e.g. Boyden, 1977; Phillips, 1980). When considering the scallop body compartment, metal concentrations varied significantly with size. These size differences were dependent on the body compartment considered for all metals. However, trends of size variations might be related to the physiology of the metals in the scallop.

First, Cd is primarily accumulated through the digestive pathway as is reflected by the large contribution of size in the variability of Cd concentrations in the digestive gland. However, the contribution of the dissolved pathway could not be considered as negligible as the accumulation of Cd is positively correlated with

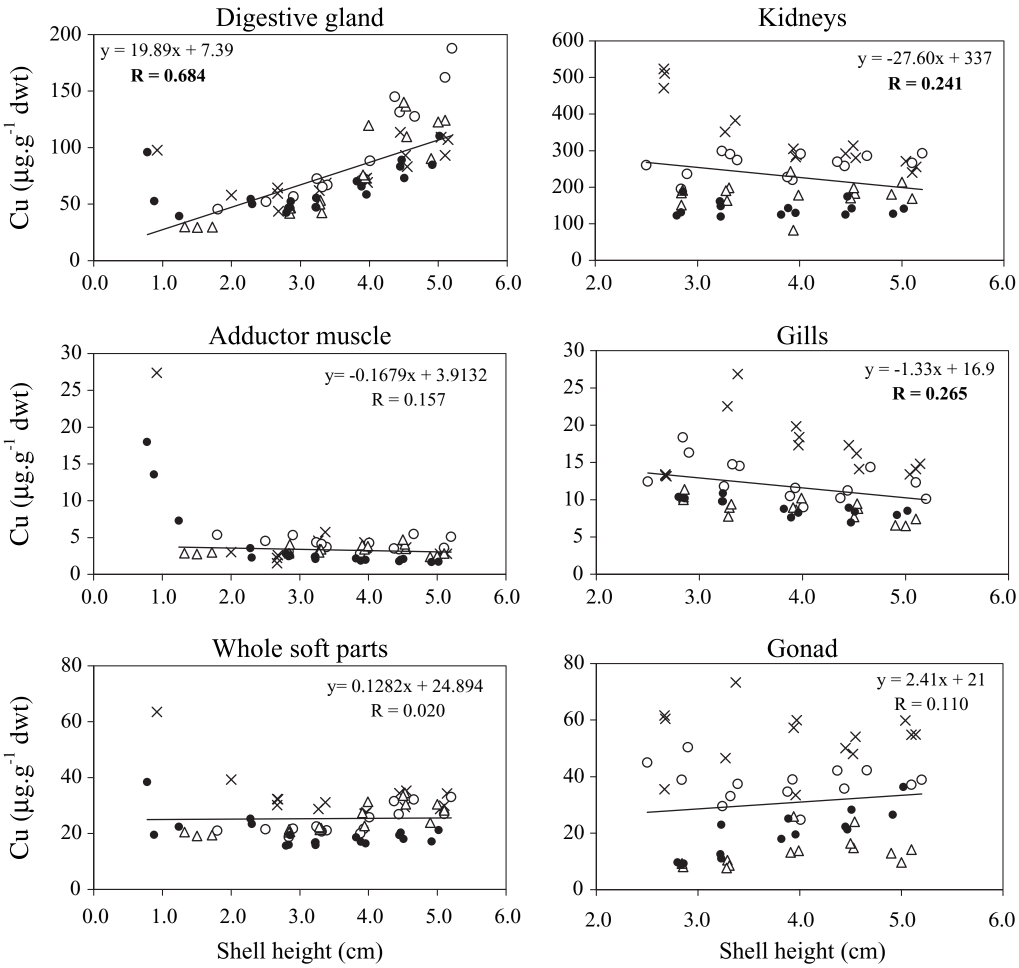

Fig. 2. Variations of Cu concentrations with size in the tissues of the variegated scallop *Chlamys varia* from the French Atlantic coast. ○, November 1995; ●, February 1996; △, June 1996; ×, September 1996. Significant correlation coefficients are shown in bold.

the size for the gills (Fig. 1). Once incorporated, Cd accumulates linearly with size in most tissues except in gonads and muscles. This suggests a slow turn-over of the metal in most of the scallop tissues, which has, however, not been determined. Further studies should therefore aim to determine the residence time of this element in the variegated scallop.

Second, Cd concentrations in gonads and adductor muscle were not affected by size suggesting a metabolic control of this metal in those tissues. As Cd has no known physiological function, such constant levels might result from the detoxification processes occurring in the scallop tissues rather from a real homeostatic process. However, the seasonal variations of Cd concentrations in gonads and adductor muscle show that the capacity of such detoxification processes is dependent on exogenous factors influencing the uptake of Cd and its subsequent redistribution (Bustamante and Miramand, 2005). Similar to Cd, Cu concentrations in gonads and adductor muscle were not affected by size. This also suggests a metabolic control of the metal in those tissues aiming to maintain the Cu concentrations in both tissues relatively constant. Thus, scallops appear to use both exogenous and endogenous Cu, the latter use resulting in a decrease of Cu concentrations in kidneys, gills and remaining tissues due to a remobilization of this metal. Cu accumulation with size in the digestive gland might reflect a low bioavailability of the dissolved Cu, which is therefore mainly incorporated through the food pathway. Moreover, such an accumulation is related to efficient detoxification processes, resulting in the immobilisation of a fraction of the metal. As for Cu, the concentrations of Zn in adductor muscle and in the remaining tissues were not affected by size, also suggesting a metabolic control of Zn in those tissues. Consequent to these homeostatic needs, the concentrations of Zn tended to decrease in most of the tissues except in the kidney. In this organ, long term storage may be due to the precipitation of Zn on phosphate granules, which are slowly excreted (George et al., 1980).

Despite the importance of the sampling season and the size in metal concentration variability, a high degree of unexplained variability occurs in some organs, especially in the adductor muscle (Table 6).

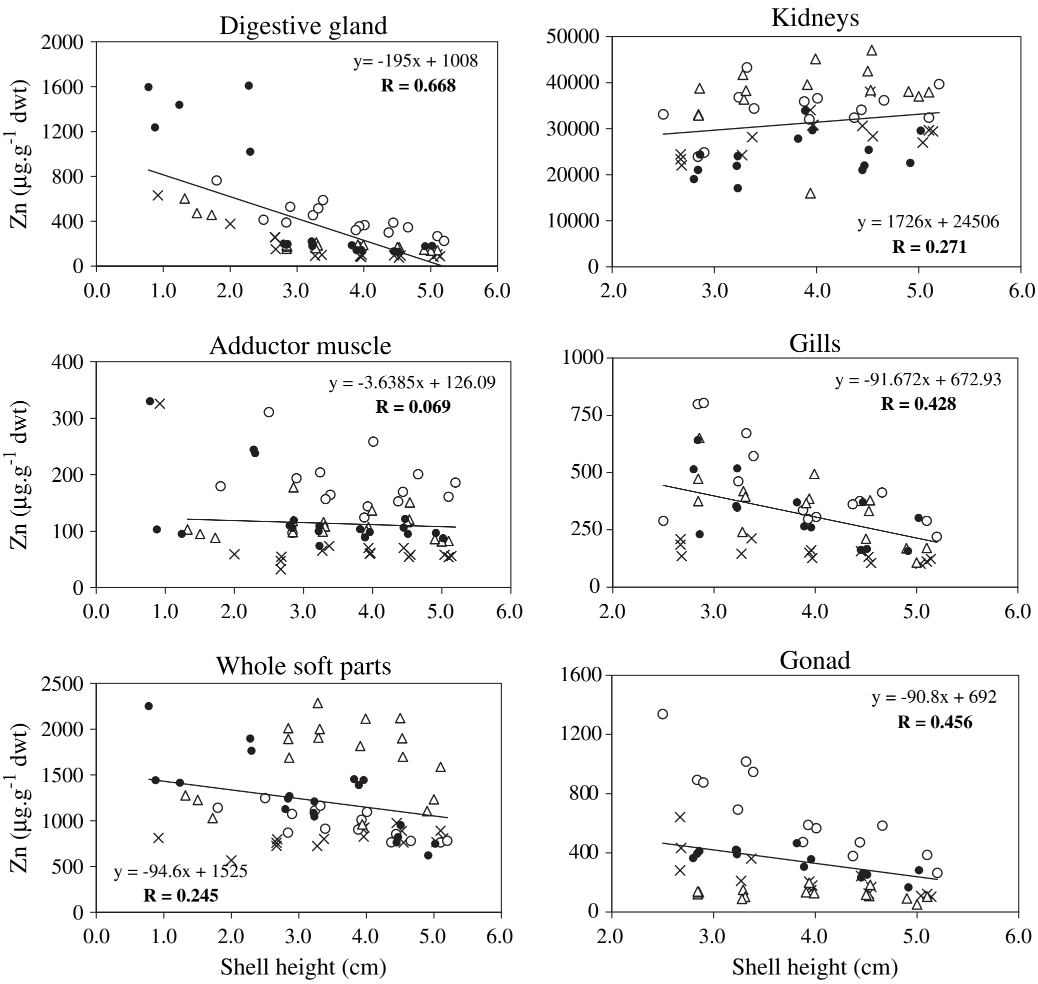

Fig. 3. Variations of Zn concentrations with size in the tissues of the variegated scallop *Chlamys varia* from the French Atlantic coast. ○, November 1995; ●, February 1996; △, June 1996; ×, September 1996. Significant correlation coefficients are shown in bold.

This phenomenon has been previously reported in bivalves (e.g. Boyden and Phillips, 1981; Lobel et al., 1982). Later, Lobel et al. (1991) suggested that elements which are stored largely in an insoluble form (e.g. granules) show a much higher degree of variability than those stored in soluble form (e.g. bound to cytosolic proteins). It would therefore be of particular interest to investigate to what extent the partitioning of metals between insoluble and soluble fractions could influence the residual variability in the tissues of the variegated scallop.

5. Conclusion

There is a great interest in using Pectinidae in biomonitoring studies because of their high bioaccumulation potential compared to other bivalves. However, the present work underlines the need to consider both sampling period and size in separated body compartments when aiming at using the variegated scallop as a biomonitor species. Indeed, the variations in metal concentrations could be higher among different body compartments than among seasons. The same also appears true for the variations in concentrations among sizes for some elements. Therefore, particular attention should be taken to compare metal concentrations only among the same compartments from scallop collected at the same period of the year. Among these compartments, the digestive gland and the kidneys are proposed for consideration in biomonitoring programs; these compartments show the highest metal concentrations.

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