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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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ABSTRACT: This study assess 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 one year period and belonging to a large range of size. All metals were

selectively distributed among the different considered body compartments, and their

concentrations were influenced by the size of the specimens or the sampling-season. The

present work shows the importance to consider the body compartment, the sampling

period and the size as important factors in studies aiming to use 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 kidney accounted for

85 % of the Zn load. Those tissues are therefore particularly recommended for use in

biomonitoring programs.

Key words: Heavy metals; Trace elements; Bioaccumulation; Biomonitoring; Seasonal

variation; Pectinid

2

#### 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 & Miramand 2004, 2005). Therefore, it has been suggested that the variegated scallop could be 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 to describe the detoxification processes leading to the bioaccumulation of metals in the storage organs and those aiming to determinate 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 & 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 (eg. Lukyanova & Evtushenko 1989, Roméo & Gnassia-barelli 1995, Widdows et al. 1995). Gonads are likely to display important variations of their weight according to the reproductive cycle, which might affect its 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 one 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. MATERIAL 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, forbidden seashore fishing in this area generally allowed good sampling of the different size classes.

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 & Audouin 1956).

After collection, the scallops were depurated for 48 hours in filtrated seawater (close circuit, constant aeration, 34 p.s.u., 12h/12h 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 contains of the soft parts.

## 2.2. Trace element analyses

Tissue samples were dried for several days at 80°C to constant weight. Whenever it was possible, two aliquots (100 to 300 mg) of each homogenised dry sample were digested with 4 ml of 65% ultrapure HNO<sub>3</sub> and 0.3 ml of ultrapure 70 % HClO<sub>4</sub> at 80°C until the solution was clear. Then acids were evaporated and residues were dissolved in 10 ml 0.3N 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<sup>-1</sup> dry wt): 0.004 (Cd), 0.5 (Cu), 1.5 (Zn). Metal concentrations in tissues and proportion in each body compartment are given relatively to the dry weight (µg.g<sup>-1</sup> dry wt and % of the metal loads, respectively).

#### 2.3. Data analyses

Statistical analyses of the data were performed by 1-, 2-, or 3-way analysis of variance (ANOVA) followed by the 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, 3 & 4, respectively. Among tissues, metals greatly varied from 2 (i.e. Cd and Cu) to 3 orders of magnitude (i.e. Zn). Statistical analyses were therefore used to discriminate the relative contribution 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 3-way

ANOVA (Table 5). These analyses showed that among the three factors of variation considered (body compartment, period of sampling and size), the body compartment was the predominant factor for the three metals, accounting for 79.3 to 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 metal concentrations were not synchronous in the different body compartment. Therefore, 2-ways ANOVA were used to discriminate the influence of the samplingperiod and the size of the scallops in each of the body compartment (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 to 84.8 % of the variation of Cu and for 33.9 to 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, gonads, 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 investigated tissues, the digestive gland clearly appeared to be the main storage compartment of Cd and Cu (Table 7), while this organ only accounted for 10 to 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 to 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 considered sampling period except for Cu in the digestive gland (p = 0.124) (Tables 6 & 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 gonads, for which the lowest concentrations corresponds 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 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 gonads and the muscle, and for Zn in the muscle and the remaining tissues (Table 6, Figures 1, 2 & 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 gonads decreased significantly with size (Figures 2 & 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 to 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).

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 & Miramand 2005), which indicated that the digestive gland and kidneys efficiently concentrated heavy metals. Such storage capacity is to be related with 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 & Gould 1988). However, to our best knowledge, the residence time and transfer processes of metals in and between the different tissues are still to be determined.

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<sup>-1</sup> dwt whatever the size or the sampling period (Table 4). Consequently to 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 gonads in the scallops sampled in June, which corresponds to the beginning of the spawning period of Chlamys varia in the Bay of Biscay (Letaconnoux & 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 & 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 & Oregioni 1976), the variations related to the reproductive cycle (Latouche & Mix 1981) or the seasonal changes during the year (Páez-Osuna & 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 & Phillips 1981, Latouche & Mix 1981, Khristoforova & 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 molluses 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 of the considered body compartment for all metals. However, trends of size variations might be related to the physiology of the metal in the scallop.

First, Cd is primarily accumulated through the digestive pathway as it 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 the size for the gills (Figure 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 would dependent of exogenous factors influencing the uptake of Cd and its subsequent redistribution (Bustamante & Miramand 2005). Similarly 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 later 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 to 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. Consequently 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). This phenomenon has been previously reported in bivalves (e.g. Boyden & Phillips 1981, Lobel et al. 1982). Later on, 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 in which 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 to use 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 to use the variegated scallop as a biomonitor species. Indeed, the variations in metal concentrations could be higher between different body compartments than between seasons. The same also appears true for the variations in concentrations between sizes for some elements. Therefore, particular attention should be taken to compare metal concentrations only between the same compartments from scallop collected at the same period of the year. Among these compartment, the digestive gland, and the kidneys are proposed for consideration in biomonitoring programs, these compartments showing the highest metal concentrations.

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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  $\pm$  SD).

Period	Height	n x N			% soft part w	eight (% water)		
	(mm)		Digestive gland	Kidneys	Gills	Gonad	Adductor muscle	Remaining tissues
November 95	$18.0 \pm 4.2$	1 x 5	20.8 (80.7)	-	_	-	36.4 (82.4)	42.9 (89.9)
	$27.5 \pm 2.1$	3 x 10	$14.5 \pm 1.0 \ (83.4 \pm 0.2)$	$2.6 \pm 0.2 \ (84.4 \pm 3.1)$	$1.8 \pm 0.4 \ (93.6 \pm 0.5)$	$10.0 \pm 0.6 \ (84.2 \pm 2.9)$	$42.1 \pm 1.8 \ (82.9 \pm 0.6)$	$29.0 \pm 2.9 \ (87.5 \pm 0.9)$
	$33.2 \pm 1.6$	3 x 10	$13.1 \pm 0.8 \ (84.9 \pm 1.9)$	$1.9 \pm 0.4 \ (84.5 \pm 4.1)$	$2.3 \pm 0.1 \ (92.8 \pm 0.5)$	$11.4 \pm 0.3 \ (88.9 \pm 1.6)$	$42.9 \pm 2.4 \ (81.8 \pm 0.3)$	$28.5 \pm 3.2 \ (87.9 \pm 0.4)$
	$39.4 \pm 1.8$	3 x 10	$14.9 \pm 0.5 \ (83.2 \pm 1.1)$	$2.2 \pm 0.3 \ (83.7 \pm 2.9)$	$2.4 \pm 0.2 \ (92.5 \pm 0.2)$	$10.5 \pm 0.7 \ (89.5 \pm 1.8)$	$43.4 \pm 1.9 \ (81.4 \pm 0.5)$	$26.6 \pm 1.7 \ (88.0 \pm 0.2)$
	$44.9 \pm 1.9$	3 x 10	$14.7 \pm 1.9 \ (81.4 \pm 4.3)$	$1.6 \pm 0.3 \ (83.8 \pm 2.2)$	$2.2 \pm 0.4 \ (92.8 \pm 1.0)$	$8.9 \pm 1.5 \ (90.9 \pm 0.1)$	$47.1 \pm 3.4 \ (80.6 \pm 1.4)$	$25.5 \pm 1.1 \ (88.4 \pm 0.7)$
	$50.8 \pm 1.6$	2 x 7	$14.3 \pm 1.1 \ (82.2 \pm 2.8)$	$2.1 \pm 0.5 \; (84.1 \pm 2.4)$	$2.3 \pm 0.2 \ (92.6 \pm 0.8)$	$9.1 \pm 0.6 \ (89.2 \pm 1.4)$	$45.2 \pm 2.4 \ (81.2 \pm 0.8)$	$26.1 \pm 1.5 \ (88.2 \pm 0.4)$
February 96	$8.5 \pm 1.5$	2 x 30	$15.9 \pm 5.1 \ (73.0 \pm 3.9)$	-	-	-	$11.3 \pm 2.5 \ (81.4 \pm 1.8)$	$71.8 \pm 3.9 \ (70.0 \pm 11.6)$
	$12.4 \pm 2.3$	1 x 45	19.3 (74.2)	-	-	-	23.6 (76.4)	57.0 (80.6)
	$22.9 \pm 1.6$	2 x 10	$18.9 \pm 0.0 \ (80.3 \pm 0.9)$	-	-	-	$26.3 \pm 1.4 \ (80.7 \pm 0.0)$	$54.8 \pm 1.4 \ (85.1 \pm 0.7)$
	$28.3 \pm 1.5$	3 x 10	$12.3 \pm 0.5 \ (85.7 \pm 0.8)$	$4.9 \pm 0.2 \ (92.5 \pm 0.9)$	$11.6 \pm 0.2 \ (93.1 \pm 0.6)$	$1.6 \pm 0.6 \ (90.6 \pm 3.3)$	$41.0 \pm 0.4 \ (83.2 \pm 0.3)$	$28.6 \pm 0.8 \; (88.7 \pm 0.7)$
	$32.3 \pm 1.7$	3 x 10	$11.4 \pm 0.7 \ (84.8 \pm 1.2)$	$4.6 \pm 0.5 \ (92.7 \pm 0.9)$	$10.7 \pm 0.7 \ (93.1 \pm 0.8)$	$1.9 \pm 0.2 \ (90.7 \pm 1.2)$	$43.3 \pm 1.3 \ (81.9 \pm 0.9)$	$28.2 \pm 0.6 \ (88.6 \pm 0.6)$
	$38.9 \pm 2.0$	3 x 10	$12.6 \pm 0.2 \ (83.9 \pm 0.9)$	$4.1 \pm 0.5 \ (92.4 \pm 0.4)$	$9.7 \pm 0.4 \ (94.1 \pm 0.3)$	$2.4 \pm 0.1 \ (90.4 \pm 0.8)$	$43.8 \pm 2.0 \ (81.5 \pm 0.9)$	$27.4 \pm 1.3 \ (89.1 \pm 0.1)$
	$44.8 \pm 2.1$	3 x 10	$13.5 \pm 1.1 \ (81.3 \pm 1.3)$	$3.1 \pm 0.1 \ (92.2 \pm 1.4)$	$8.2 \pm 0.5 \ (94.0 \pm 0.2)$	$2.4 \pm 0.4 \ (90.4 \pm 1.3)$	$46.1 \pm 1.2 \ (79.0 \pm 1.2)$	$26.6 \pm 1.0 \ (89.0 \pm 0.2)$
	$49.7 \pm 2.4$	2 x 10	$13.1 \pm 0.1 \ (83.4 \pm 0.5)$	$2.1 \pm 0.2 \ (90.2 \pm 0.1)$	$8.1 \pm 1.1 \ (93.9 \pm 0.3)$	$2.1 \pm 0.0  (90.2 \pm 0.1)$	$48.9 \pm 0.4 \ (78.3 \pm 0.7)$	$25.7 \pm 0.7 \ (88.3 \pm 0.7)$
June 96	$15.2 \pm 2.0$	3 x 30						
	$28.5 \pm 2.1$	3 x 9	$17.9 \pm 1.3 \ (83.2 \pm 0.3)$	$5.0 \pm 0.9 \ (93.7 \pm 1.0)$	$4.0 \pm 0.4 \ (97.2 \pm 0.1)$	$14.8 \pm 1.5 \ (84.2 \pm 1.0)$	$37.1 \pm 0.5 \ (83.4 \pm 0.3)$	$21.3 \pm 1.2 \ (88.9 \pm 0.2)$
	$32.9 \pm 1.2$	3 x 10	$17.5 \pm 0.8 \ (83.3 \pm 0.6)$	$5.0 \pm 0.2 \ (92.0 \pm 0.5$	$4.8 \pm 0.6 \ (96.7 \pm 0.0)$	$17.4 \pm 3.9 \ (83.0 \pm 1.3)$	$33.3 \pm 2.9 \ (83.6 \pm 0.2)$	$22.1 \pm 2.1 \ (91.2 \pm 0.2)$
	$39.5 \pm 1.3$	3 x 10	$15.0 \pm 0.2 \ (84.8 \pm 0.5)$	$4.5 \pm 0.4 \ (89.5 \pm 1.0)$	$6.0 \pm 1.2 \ (95.9 \pm 1.3)$	$17.4 \pm 0.8 \ (83.9 \pm 1.2)$	$32.9 \pm 2.9 \ (84.2 \pm 0.7)$	$24.3 \pm 1.8 \ (90.4 \pm 1.0)$
	$45.2 \pm 1.4$	3 x 10	$14.2 \pm 0.9 \ (85.2 \pm 0.6)$	$4.2 \pm 0.8 \ (89.4 \pm 2.2)$	$6.1 \pm 0.5 \ (95.5 \pm 0.5)$	$19.3 \pm 0.9 \ (83.6 \pm 1.6)$	$30.8 \pm 1.1 \ (84.6 \pm 0.5)$	$25.5 \pm 0.3 \ (89.7 \pm 0.8)$
	$49.5\pm1.2$	3 x 7	$14.5 \pm 1.2 \ (83.9 \pm 0.9)$	$3.2 \pm 0.7 \ (88.3 \pm 1.1)$	$9.6 \pm 0.2 \ (93.3 \pm 0.5)$	$22.7 \pm 4.6 \ (81.9 \pm 2.8)$	$26.4 \pm 3.3 \ (84.5 \pm 0.2)$	$23.7 \pm 1.5 \; (88.8 \pm 0.4)$
September 96	$9.2 \pm 1.5$	1 x 20	16.2 (78.7)	-	-	-	17.9 (83.2)	65.8 (82.9)
	$20.0 \pm 1.4$	1 x 7	17.6 (75.2)	-	-	-	34.8 (77.9)	47.6 (82.9)
	$26.7 \pm 1.9$	3 x 10	$18.0 \pm 0.9 \ (72.8 \pm 0.5)$	$2.7 \pm 0.3 \ (78.3 \pm 0.7)$	$9.3 \pm 0.3 \ (88.3 \pm 0.5)$	$2.0 \pm 0.7 \ (83.2 \pm 4.2)$	$39.1 \pm 1.1 \ (77.1 \pm 0.5)$	$28.8 \pm 0.8 \; (81.7 \pm 0.2)$
	$33.2 \pm 1.8$	2 x 7	$18.5 \pm 0.5 \; (74.1 \pm 0.3)$	$2.6 \pm 0.1 \ (87.2 \pm 2.4)$	$3.5 \pm 0.5 \ (96.1 \pm 0.5)$	$1.7 \pm 0.5 \ (87.6 \pm 2.8)$	$50.3 \pm 0.2 \ (79.1 \pm 0.6)$	$23.4 \pm 1.8 \ (87.7 \pm 0.6)$
	$39.6 \pm 1.6$	3 x 10	$18.3 \pm 0.7 \ (72.0 \pm 0.4)$	$2.6 \pm 0.2 \ (84.2 \pm 1.5)$	$3.6 \pm 0.5 \ (95.4 \pm 0.8)$	$2.0 \pm 0.9 \ (85.5 \pm 2.3)$	$52.1 \pm 1.4 (77.4 \pm 0.1)$	$21.3 \pm 0.4 \ (86.8 \pm 0.3)$
	$45.1 \pm 1.4$	3 x 10	$18.1 \pm 1.0 \ (72.7 \pm 2.3)$	$2.5 \pm 0.4 \ (84.9 \pm 1.3)$	$4.5 \pm 0.2 \ (94.8 \pm 0.4)$	$2.1 \pm 0.1 \ (86.7 \pm 1.1)$	$52.1 \pm 0.4 \ (76.4 \pm 0.3)$	$20.7 \pm 0.4 \ (86.2 \pm 1.3)$
	$50.9 \pm 2.3$	3 x 7	$20.0 \pm 1.3 \ (72.8 \pm 1.2)$	$2.6 \pm 0.1 \ (88.2 \pm 0.4)$	$5.9 \pm 0.6 \ (94.2 \pm 0.5)$	$2.3 \pm 0.4 \ (89.1 \pm 1.3)$	$49.6 \pm 1.7 \ (76.9 \pm 0.5)$	$19.6 \pm 0.7 \; (87.2 \pm 0.3)$

Table 2. Cd concentrations (mean  $\pm$  SD,  $\mu$ g.g<sup>-1</sup> dwt) 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	$22.3 \pm 0.6$	$32.7 \pm 5.0$	$39.6 \pm 2.5$	50.7 ± 5.4
	February 96	$34.0 \pm 2.2$	25.6	$23.7 \pm 1.1$	$30.5 \pm 3.9$	$28.8 \pm 2.9$	$35.4 \pm 4.8$	$37.9 \pm 4.7$	$43.3 \pm 0.5$
	June 96	-	$16.4 \pm 1.0$	-	$24.6 \pm 2.2$	$23.7 \pm 2.6$	$31.1 \pm 5.8$	$47.5 \pm 11.5$	$41.7 \pm 8.1$
	September 96	13.2	-	10.8	$9.3 \pm 0.3$	$15.4 \pm 1.9$	$19.4 \pm 1.1$	$23.5 \pm 3.6$	$26.9 \pm 2.7$
Kidneys	November 95	-	-	-	$35.3 \pm 5.8$	$46.1 \pm 1.7$	$47.0 \pm 14.2$	$52.4 \pm 10.0$	$60.9 \pm 3.6$
	February 96	-	-	-	$31.8 \pm 1.2$	$31.7 \pm 4.1$	$34.3 \pm 0.7$	$34.6 \pm 5.8$	$47.5 \pm 15.1$
	June 96	-	-	-	$30.3 \pm 3.0$	$32.7 \pm 1.4$	$29.8 \pm 11.9$	$45.3 \pm 5.8$	$48.8 \pm 3.9$
	September 96	-	-	-	$32.5\pm1.6$	$26.4 \pm 5.3$	$30.9 \pm 1.8$	$35.6 \pm 4.7$	$34.6 \pm 5.4$
Gill	November 95	-	-	-	$3.29 \pm 2.65$	$2.91 \pm 0.06$	$3.65 \pm 1.37$	$4.43 \pm 0.84$	$4.91 \pm 0.61$
	February 96	-	-	-	$3.25\pm0.29$	$3.14 \pm 0.26$	$3.60 \pm 0.37$	$3.98 \pm 0.26$	$4.33 \pm 0.57$
	June 96	-	-	-	$3.25 \pm 0.24$	$3.03 \pm 0.35$	$3.53 \pm 0.83$	$4.36 \pm 0.36$	$4.46 \pm 0.14$
	September 96	-	-	-	$1.63 \pm 0.17$	$2.72 \pm 0.07$	$2.96 \pm 0.27$	$3.49 \pm 0.44$	$3.67 \pm 0.41$
Gonad	November 95	-	-	-	$2.44 \pm 0.30$	$1.89 \pm 0.08$	$2.29 \pm 0.22$	$2.38 \pm 0.20$	$2.30 \pm 0.18$
	February 96	-	-	-	$3.62 \pm 0.21$	$2.42 \pm 0.49$	$1.67 \pm 0.35$	$2.11 \pm 0.35$	$2.10 \pm 0.21$
	June 96	-	-	-	$1.12 \pm 0.21$	$1.16 \pm 0.37$	$1.46 \pm 0.35$	$1.54 \pm 0.28$	$0.64 \pm 0.21$
	September 96	-	-	-	$1.31 \pm 0.43$	$4.67\pm1.55$	$4.17\pm1.36$	$3.94 \pm 0.18$	$3.43 \pm 0.75$
Adductor muscle	November 95	-	-	0.70	$0.57 \pm 0.11$	$0.55 \pm 0.09$	$0.55 \pm 0.10$	$0.55 \pm 0.13$	$0.54 \pm 0.04$
	February 96	$0.39 \pm 0.18$	0.64	$0.41 \pm 0.05$	$0.50 \pm 0.03$	$0.51 \pm 0.04$	$0.50 \pm 0.07$	$0.59 \pm 0.05$	$0.46 \pm 0.04$
	June 96	-	$0.18 \pm 0.01$	-	$0.48 \pm 0.06$	$0.48 \pm 0.11$	$0.55 \pm 0.19$	$0.64 \pm 0.13$	$0.45 \pm 0.06$
	September 96	0.33	-	0.35	$0.33 \pm 0.18$	$0.34 \pm 0.03$	$0.36 \pm 0.03$	$0.38 \pm 0.04$	$0.38 \pm 0.05$
Whole soft parts	November 95	-	=	5.90	$5.04 \pm 0.24$	$4.94 \pm 0.21$	$7.13 \pm 1.41$	$7.95 \pm 1.27$	$9.47 \pm 0.88$
	February 96	$8.15\pm1.71$	7.72	$7.57 \pm 0.08$	$6.38 \pm 0.33$	$5.75 \pm 0.29$	$6.98 \pm 0.85$	$7.38 \pm 0.91$	$8.16 \pm 0.06$
	June 96	-	$5.33 \pm 0.11$	-	$6.71 \pm 0.34$	$6.72 \pm 0.31$	$7.17 \pm 1.50$	$10.00 \pm 1.66$	$8.74 \pm 1.32$
	September 96	3.93	-	3.48	$3.15 \pm 0.33$	$4.19 \pm 0.27$	$5.05 \pm 0.13$	$5.86 \pm 0.50$	$7.09 \pm 0.85$

Table 3. Cu concentrations (mean  $\pm$  SD,  $\mu$ g.g<sup>-1</sup> dwt) 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	$68.0 \pm 3.6$	$77.0 \pm 9.5$	$134 \pm 9.5$	$175 \pm 18.3$
	February 96	$74.3 \pm 30.7$	39.4	$52.1 \pm 3.1$	$46.7 \pm 5.0$	$49.7 \pm 4.6$	$64.7 \pm 6.1$	$81.7 \pm 8.1$	$97.5 \pm 17.7$
	June 96	-	$29.6 \pm 0.2$	-	$45.0\pm2.7$	$48.6 \pm 5.6$	$89.6 \pm 26.1$	$129 \pm 16.7$	$112\pm19.2$
	September 96	97.6	-	58.0	$55.7 \pm 10.9$	$65.4 \pm 4.7$	$71.4 \pm 2.1$	$96.6 \pm 15.4$	$103 \pm 8.7$
Kidneys	November 95	-	-	-	$230 \pm 32.9$	$288 \pm 12.7$	$246 \pm 39.1$	$271 \pm 14.1$	$280 \pm 18.5$
	February 96	-	-	-	$147 \pm 35.4$	$143 \pm 21.0$	$132 \pm 8.7$	$147 \pm 25.0$	$134 \pm 9.9$
	June 96	-	-	-	$176\pm21.3$	$184 \pm 18.3$	$168 \pm 81.2$	$184\pm13.9$	$188 \pm 23.3$
	September 96	-	-	-	$502 \pm 27.3$	$367 \pm 21.7$	$292 \pm 11.6$	$295 \pm 16.7$	$255 \pm 15.7$
Gill	November 95	-	-	-	$15.3 \pm 3.1$	$14.0 \pm 1.7$	$10.3 \pm 1.5$	$11.7 \pm 2.1$	$11.2 \pm 1.6$
	February 96	-	-	-	$10.0\pm0.1$	$10.3 \pm 0.6$	$8.3 \pm 0.6$	$8.0 \pm 1.0$	$8.5 \pm 0.7$
	June 96	-	-	-	$10.6 \pm 0.7$	$8.7 \pm 0.8$	$9.3 \pm 0.8$	$8.7 \pm 0.9$	$6.8 \pm 0.5$
	September 96	-	-	-	$13.3 \pm 0.1$	$24.7 \pm 3.0$	$18.5\pm1.3$	$15.9 \pm 1.6$	$14.1 \pm 0.7$
Gonad	November 95	-	-	-	$44.7 \pm 5.5$	$33.0 \pm 4.0$	$33.0 \pm 7.2$	$15.9 \pm 1.6$ $40.0 \pm 3.5$	$38.0 \pm 1.2$
	February 96	-	-	-	$9.2 \pm 0.4$	$15.7 \pm 6.4$	$21.0 \pm 3.6$	$24.0 \pm 3.6$	$31.5 \pm 7.8$
	June 96	-	-	-	$8.9 \pm 0.7$	$8.9 \pm 1.4$	$17.6 \pm 7.1$	$18.4 \pm 5.0$	$12.3 \pm 2.3$
	September 96	-	-	-	$52.5 \pm 14.7$	$59.9 \pm 18.9$	$50.2 \pm 14.6$	$50.7 \pm 3.1$	$56.5 \pm 2.9$
Adductor muscle	November 95	-	-	5.0	$4.3 \pm 1.2$	$4.0 \pm 0.1$	$3.3 \pm 0.6$	$4.0 \pm 1.0$	$4.3\pm1.1$
	February 96	$13.0 \pm 5.4$	7.3	$2.9 \pm 0.9$	$2.6 \pm 0.2$	$2.3\pm0.2$	$2.0\pm0.1$	$1.9\pm0.2$	$1.7 \pm 0.0$
	June 96	-	$2.9 \pm 0.1$	-	$3.3 \pm 0.6$	$3.3 \pm 0.2$	$3.7 \pm 0.3$	$4.1 \pm 0.6$	$2.6\pm0.2$
	September 96	27.4	-	3.0	$2.1 \pm 0.6$	$5.2 \pm 0.8$	$3.8 \pm 0.4$	$3.5 \pm 0.1$	$2.8 \pm 0.0$
Whole soft parts	November 95	-	-	21.0	$20.6 \pm 1.7$	$21.4 \pm 0.9$	$22.5 \pm 3.0$	$30.2 \pm 2.9$	$30.0 \pm 4.2$
	February 96	$29.0 \pm 13.4$	22.4	$24.3 \pm 1.4$	$17.0 \pm 2.1$	$16.5\pm0.5$	$17.3\pm1.1$	$19.2\pm1.1$	$19.2 \pm 2.9$
	June 96	-	$19.7 \pm 0.7$	-	$20.8 \pm 0.6$	$21.8 \pm 0.5$	$27.1 \pm 4.3$	$32.8 \pm 2.1$	$27.5 \pm 3.3$
	September 96	63.5	-	39.3	$31.6 \pm 1.3$	$29.9 \pm 1.7$	$27.3 \pm 0.2$	$33.0 \pm 3.2$	$31.0 \pm 2.9$

Table 4. Zn concentrations (mean  $\pm$  SD,  $\mu g.g^{-1}$  dwt) 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	$346 \pm 23$	344 ± 44	245 ± 29
	February 96	$1416\pm254$	1437	$1314 \pm 416$	$196 \pm 6$	$196 \pm 21$	$157\pm25$	$129 \pm 2$	$179 \pm 2$
	June 96	-	$511 \pm 80$	-	$171\pm15$	$184\pm25$	$184 \pm 10$	$163 \pm 11$	$142 \pm 4$
	September 96	630	-	377	$221 \pm 61$	$96 \pm 6$	$89 \pm 10$	$88 \pm 11$	$92 \pm 12$
Kidneys	November 95	-	-	-	$27286 \pm 5058$	$38168 \pm 4603$	$34831 \pm 2451$	$34212 \pm 1885$	$36019 \pm 5630$
	February 96	-	-	-	$21482 \pm 2694$	$21018\pm3568$	$31372 \pm 3171$	$22806 \pm 2304$	$26058 \pm 4973$
	June 96	-	-	-	$34932 \pm 3340$	$38796 \pm 2730$	$33575 \pm 15478$	$42648 \pm 4351$	$37693 \pm 582$
	September 96	-	-	-	$23271 \pm 1194$	$26215 \pm 2764$	$31857\pm1916$	$32309 \pm 5036$	$28683 \pm 1479$
Gill	November 95	-	-	-	$631 \pm 296$	$569 \pm 105$	$313\pm20$	$383 \pm 27$	$254 \pm 49$
	February 96	-	-	-	$462 \pm 211$	$406 \pm 97$	$298 \pm 62$	$233 \pm 119$	$230 \pm 103$
	June 96	-	-	-	$500 \pm 140$	$351 \pm 97$	$416 \pm 69$	$308 \pm 87$	$149 \pm 37$
	September 96	-	-	-	$177\pm38$	$179 \pm 47$	$146\pm18$	$131\pm26$	$112\pm10$
Gonad	November 95	-	-	-	$1035 \pm 263$	$799 \pm 93$	$542 \pm 62$	$477 \pm 103$	$324 \pm 86$
	February 96	-	-	-	$390 \pm 24$	$409 \pm 16$	$375 \pm 81$	$249 \pm 15$	$224 \pm 82$
	June 96	-	-	-	$131 \pm 10$	$114 \pm 32$	$153 \pm 39$	$136 \pm 41$	$82 \pm 27$
	September 96	-	-	-	$452\pm181$	$286 \pm 106$	$178 \pm 29$	$179 \pm 60$	$112 \pm 9$
Adductor muscle	November 95	-	-	179	$201 \pm 107$	$175 \pm 25$	$176 \pm 73$	$175 \pm 24$	$174 \pm 18$
	February 96	$216\pm133$	195	$241 \pm 24$	$112 \pm 7$	$94 \pm 18$	$97 \pm 8$	$108 \pm 14$	$93 \pm 6$
	June 96	-	$96 \pm 7$	-	$131 \pm 41$	$108 \pm 9$	$115 \pm 19$	$129 \pm 19$	$84 \pm 2$
	September 96	326	-	59	$45 \pm 11$	$70 \pm 6$	$64 \pm 6$	$61 \pm 8$	$56\pm2$
Whole soft parts	November 95	-	-	1140	$1063 \pm 188$	$1062 \pm 132$	$1003 \pm 97$	$799 \pm 48$	$772 \pm 13$
	February 96	$1846 \pm 572$	1414	$1830 \pm 94$	$1213\pm75$	$1113 \pm 86$	$1428 \pm 35$	$845 \pm 97$	$686 \pm 90$
	June 96	-	$1177\pm131$	-	$1864 \pm 163$	$2064 \pm 197$	$1630 \pm 599$	$1906 \pm 211$	$1309 \pm 250$
	September 96	812	_	567	$761 \pm 36$	$762 \pm 54$	$889 \pm 54$	$876 \pm 107$	$822 \pm 63$

Table 5. Variability (%) in metal concentrations in the tissues of the variegated scallop *Chlamys varia* explained by the different factors considered (period of sampling and size of the scallops) and their interactions. Highest proportions of variation are shown in bold. ns: not significant ( $P_{ANOVA}$ <0.05)

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
$Compartment \times Period$	3.6	9.4	2.5
$Compartment \times Size$	3.1	2.2	0.6
$Period \times Size$	0.4	0.8	Ns
$Compartment \times Period \times Size$	1.0	3.1	0.7
Residual	2.6	1.4	1.8

Table 6. Variability (%) in Cd, Cu and Zn concentrations in the tissues of the variegated scallop *Chlamys varia* explained by the different factors considered (period of sampling and size of the scallops) and their interactions. Highest proportions of variation are shown in bold. ns: not significant  $(P_{ANOVA}>0.05)$ 

	Factors	Digestive gland	Kidneys	Gills	Gonads	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
	$Period \times Size$	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
	$Period \times Size$	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
	$Period \times Size$	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

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

Metal	Digestive gland	Kidney	Gonads	Gills	Adductor muscle	Remaining tissues
Cd	$65.3 \pm 6.7$	$18.5 \pm 5.0$	$1.4 \pm 0.3$	$4.2 \pm 1.7$	$3.3 \pm 1.0$	$7.3 \pm 2.3$
Cu	$47.6 \pm 10.9$	$28.8 \pm 9.5$	$4.7 \pm 3.6$	$3.8 \pm 2.1$	$5.9 \pm 3.1$	$9.1 \pm 3.6$
Zn	$3.0\pm1.9$	$84.7 \pm 9.4$	$1.0\pm0.7$	$2.5\pm2.0$	$4.4 \pm 2.6$	$4.5\pm3.8$

Table 8. Seasonal variations of metal concentrations in the tissues of the variegated scallop *Chlamys varia*: results of the multiple comparison tests of Tukey performed after ANOVA. Mean metal concentrations are ranking 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.

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
Gonads	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

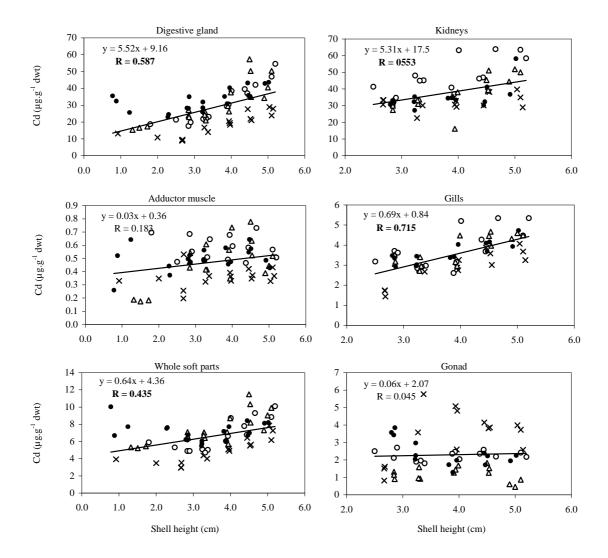


Fig. 1 Variations of Cd concentrations with size in the tissues of the variegated scallop *Chlamys varia* from the French Atlantic coast.  $\circ$ : November 1995;  $\bullet$ : February 1996;  $\Delta$ : June 1996;  $\times$ : September 1996. Significant correlation coefficients are shown in bold.

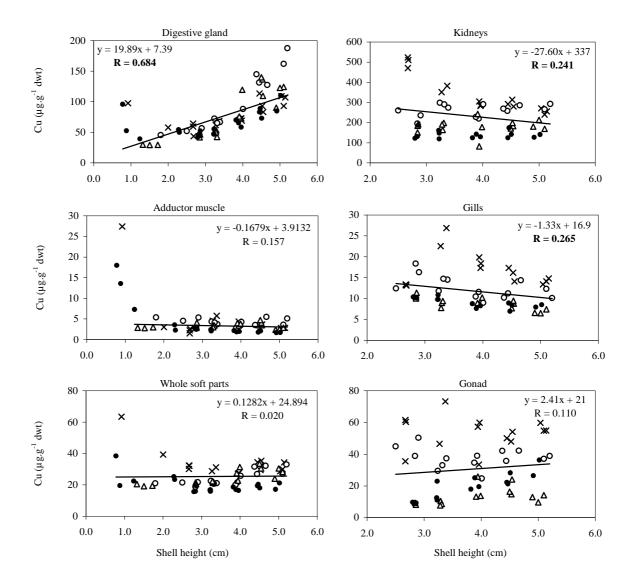


Fig. 2. Variations of Cu concentrations with size in the tissues of the variegated scallop *Chlamys varia* from the French Atlantic coast.  $\circ$ : November 1995;  $\bullet$ : February 1996;  $\Delta$ : June 1996;  $\times$ : September 1996. Significant correlation coefficients are shown in bold.

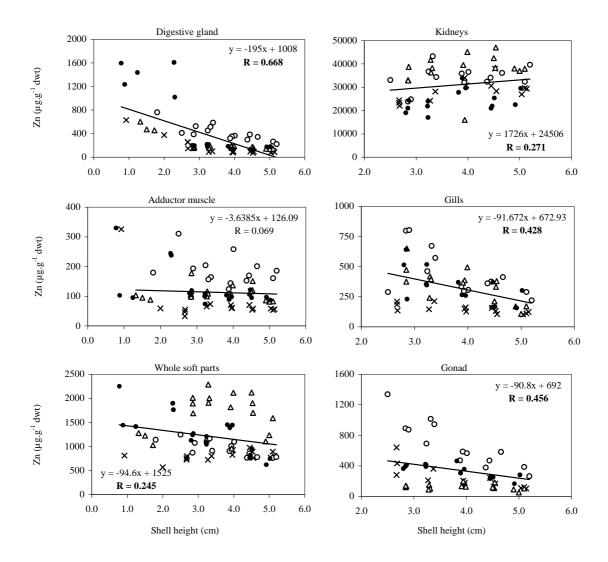


Fig. 3. Variations of Zn concentrations with size in the tissues of the variegated scallop *Chlamys varia* from the French Atlantic coast.  $\circ$ : November 1995; •: February 1996;  $\Delta$ : June 1996; ×: September 1996. Significant correlation coefficients are shown in bold.