INFLUENCE OF THE INTERMOULT CYCLE ON THE METAL BIOACCUMULATION BY THE SHRIMP : *CRANGON CRANGON* L.

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Abstract

At the various intermoult stages (A, B, C, D), the cephalothorax, abdominal muscle, exoskeleton and legs of shrimp *(Crangon, crangon* L.) from the Seine Bay Estuary have been analysed for Hg, Cd, Cu, Fe and Zn. A statistical test of the results of the metal analyses, and the variations of the concentrations during the intermoult cycle are used to estimate to what extent the shrimp is a representative indicator of its environment. The test demontrates the influence of the intermoult-cycle on the cadmium, zinc and copper concentrations in shrimp. These concentrations reach a maximum at the end of the cycle (stages C or D).

Résumé

INFLUENCE DU CYCLE D'INTERMUES SUR LA BIOACCUMULATION DES MÉTAUX PAR LE CREVETTE *CRANGON CRANGON* L.

Aux différents stades d'intermue (A, B, C, D) les teneurs en Hg, Cd, Cu, Fe et Zn ont été déterminées dans le muscle, la cuticule, le céphalothorax et les appendices locomoteurs de la crevette *(Crangon crangon* L.) pêchée dans l'estuaire de la baie de Seine. Une analyse statistique des résultats des dosages métalliques et l'étude de la variation des concentrations en fonction du cycle d'intermue sont utilisées pour estimer la représentativité de la crevette comme indicateur biologique de contamination par les métaux. L'influence du cycle d'intermue sur les concentrations en Cd, Cu et Zn est statistiquement démontrée. Les concentrations de ces trois éléments augmentent à la fin du cycle (stade C ou D).

Introduction.

The influence of the intermoult cycle on the variations of metal concentrations in crustaceans has been studied since 1957 with renewed interest. The most studied elements are life essential : copper, zinc and iron. ZUCKERKANDL (1957, 1960) worked on copper variations in the blood and in the hepatopancreas of *Maia squinado* during its various intermoult stages. The compared metabolism of copper and zinc during the intermoult cycle was described by MARTIN (1975) in the organs of *Cancer irroratus* and by ADAMS *et al.* (1981) in the crayfish. Iron concentrations in moulting decapods were analysed by MARTIN (1973) and by ADAMS *et al.* (1981). The results of these studies are interpreted in terms of metabolisms and intracellular regulation of metals.

The purpose of the present study is to determine the influence of the intermoult cycle on copper, zinc, iron, cadmium and mercury content in the different organs of *Crangon crangon* L. Bioaccumulation studies of marine invertebrates is an usual tool of the Coastal monitoring Programs for estimating the pollution level in marine environments. The internal metal concentrations in these animals may reflect to a certain degree the levels of these metals in the surrounding water and sediments. However, physiological factors playing a part in metal metabolism may interfere with the metal concentrations in the different organs. We show how metal concentrations vary according to the intermoult stages of shrimp samples, all taken from the same stock at the same site and the same time. We intend to evaluate to what extent the shrimp is a representative indicator of his environment.

Materials and methods.

Sampling.

The shrimps from Seine Bay Estuary (fig. 1), live on the surface of muddy sands. Ratier bank lies at a very low depth (— 2 m). The salinity at the river mouth varies considerably at spring-tides : variations of 31.10^{-3} (‰) are common. A sampling of 5 kg shrimp was taken from Ratier Bank on February 24, 1980. The shrimps were kept alive for two days in artificially aerated seawater. From observing one uropode under the microscope, the A, B, C, D_0, D_1 and D_2 intermoult stages were sorted according to the criteria of DRACH & TCHERNIGOVTZEFF (1967). For each stage, several samples of at least 50 g wet weight were chosen, as described in table 1. Eggs were taken from the mature females and the exoskeleton (shell) was removed from the whole body. The abdominal muscle was separated from the cephalothorax, containing the gills, the digestive system and the sex-glands. The pereipodea and the pleopodea were regrouped as « legs ».

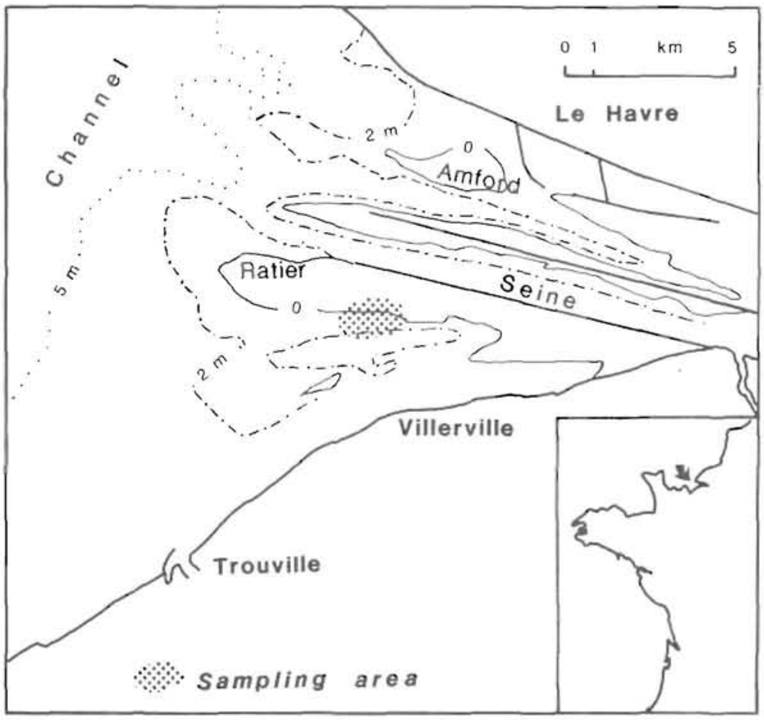

FIG. 1. — *Localization of the Seine Bay Estuary — sampling aera.*
Situation de la baie de Seine — zone d'échantillonnage.

Metal analyses.

The different tissues were dry-frozen, and metal analyses were performed according to the method described by THIBAUD (1983). Mercury was analysed by flameless atomic absorption spectrophotometry after mineralization at 60 °C of samples of 0.2 to 0.5 g of the dry tissues with a nitric — and sulfuric — acid solution (1 + 1). The mercury contained in the mineralized samples was oxidized by a 6 g/l potassium permanganate solution in an ice-bath. After dilution and filtration, Hg^{++} was reduced into Hg° by $SnCl_2$ and its absorption measured at 254 nm.

As for the analyses of the other four elements, samples of 1 to 2 g dry tissues were mineralized in teflon bombs, using 20 ml nitric-acid and 3 ml sulfuric-acid, heated at 140 °C for 3 hours ; 5 ml bidistilled water and 5 ml of hydrogen peroxide were added. The nitric-acid was eliminated by heating at 100 °C for the duration of one night. The mineralization was completed by repeating this treatment twice. After dilution in bidistilled water and filtration, Cu, Zn and Fe were analysed by flame atomic absorption spectrophotometry and Cd by furnace atomic absorption spectrophotometry.

Results and discussion.

For each metal the significance of the factors : sample, organ and stage has been determined from analysis of variance and by comparing the calculated ratio to the theoretical F of the SNEDECOR tables. From table 2, showing the significance of the various factors, it appears that the organ factor plays an important part in all the metal concentrations.

Stage	Sample n°	Sample wet weight (g)	Sex and numbers of specimens ♂	♀ o	♀ ω	Total	Mean length of the shell (mm)
A	1	60	1	3	28	32	60.5
	2	60	1	1	26	28	63.4
	3	58		8	31	39	56.6
	4	62		5	22	27	64.1
	5	69		2	29	31	63.1
B	1	65		1	30	31	61.3
	2	62	1	1	27	29	62.0
	3	62		3	23	26	64.4
	4	63		1	21	22	63.9
	5	86	2	7	41	50	56.8
C	1	63	2	6	23	31	60.1
	2	60		2	27	29	60.7
	3	60		6	23	29	60.3
	4	29		13	4	17	57.3
D_0	1	65	2	14	21	38	58.4
	2	62		19	12	31	59.7
	3	53			20	20	64.3
	4	70	2	44		46	55.2
D_1	1	56	—	—	—	38	56.0
	2	64		26	4	30	
	3	62	2	42		44	
D_2	1	60		36	1	37	
	2	78		47	1	48	

TABL. 1. — *Sampling description (♂ mâles, ♀ o immature females, ♀ ω females with eggs).*
Description de l'échantillonnage (♂ mâles, ♀ o femelles immatures, ♀ ω femelles œuvées).

	Cephalo-thorax	Exoskele-ton	Muscle	Legs	Eggs
Number of samples (n)	9	10	10	6	8
Cadmium (mg/kg dry weight)	1.90 2.55 *	0.58 0.50 *	0.37 0.16 *	0.60	0.29
Copper	112.7 173.0 *	37.70 32.40 *	30.50 24.20 *	51.20	25.70
Zinc	131.6 105.0 *	58.60 28.30 *	69.90 47.70 *	111.80	303.90
Iron	199.1 338.0 *	39.50 45.10 *	24.50 14.20 *	56.0	63.50
Mercury	0.26	0.16	0.46	0.25	0.06

(*) *HOROWITZ & PRESLEY (1977)* in *EISLER 1981.*

TABL. 3. — *Average metal content (mg/kg dry weight) in shrimp all stages included.*
Teneurs moyennes (mg/kg sec) en métaux chez la crevette pour l'ensemble des stades.

Concentration levels.

In the analyses of the whole intermoult cycle the mean metal concentrations for each organ, without any stage distinction, are comparable to those recorded by HOROWITZ & PRESLEY (1977) and reported by EISLER (1981) in their study of the brown shrimp (table 3).

The mean metal amounts calculated in the various organs at the four sampled intermoult stages are shown in figure 2. Without any distinction of stages and organs, the ranges of the mean concentrations in mg/kg dry weight, are distributed as follows : mercury 0.045 to 5.25 ; cadmium 0.14 to 2.35 ; copper 25.7 to 121.5 ; zinc 48.3 to 316.0 ; iron 15.7 to 247.0.

MÉTAL	FACTOR		
	sample	organ	stage
Mercury	—	+ +	—
Cadmium	—	+ +	+
Copper	—	+ +	+
Zinc	—	+ +	+
Iron	—	+ +	—

TABL. 2. — *Significance of sample, organ and stage factors :*
— non significant ; + significant (P < 0.05 or P < 0.025 ; + + highly significant (P < 0.01).

Significativité des facteurs, échantillon, organe et stade.

Of the five analysed elements, copper, zinc and iron present, in all the organs, concentrations which are considerably higher than those of mercury, and cadmium. The high amounts of copper, zinc and iron may be in relation to their many biochemical roles in the living organisms. More than ninety enzymes and proteins contain zinc, which is also a catalyst for many reactions (BRYAN, 1984). Copper is an element of the oxygen-carrier hemocyanin in the crustaceans. Copper and zinc are therefore life-essential elements for the crustaceans (MARTIN, 1978). This could explain why relatively high amounts of these elements are stored in the organisms.

The concentrations of cadmium, copper, zinc and iron are higher in the cephalothorax than in the other organs, on account of the use of the hepatopancreas as a store for copper, zinc and iron during the moult cycle (ADAMS *et al.* 1981). Metallothioneniens, proteins of high cystein percentage, are able to form complexes of Zn, Cd or Cu in many invertebrates. In crabs the presence of copper binding metallothioneins has been shown by OVERNELL (1982) in the hepatopancreas. According to ELKAÏM (1980) a « fossilisation » phenomenon of zinc allowing detoxification is responsible of the retention of Zn at the level of the antennal gland of *Crangon crangon*. The eggs, however, contain a higher zinc concentration (300 mg/kg) than the organs, which is also the case in the shrimp *Palinurus vulgaris* (BRYAN, 1968). A previous cadmium contamination experiment performed on *Crangon crangon* (DETHLEFSEN, 1977/1978) has already shown that the major sites of concentration were gills and hepatopancreas, whereas no significant uptake was found to take place in the abdominal muscle. Contrary to the other elements, mercury amounts are highest in the abdominal muscle, where the relative proportions between fibrillar and sarcoplasmic proteins influence the Hg distribution in the organism (ARIMA & UMEMOTO, 1976). The influence of the organ factor on the metal distribution in shrimp is confirmed by our statistical test which ascribe to the organ factor a highly significant importance on all the metal concentrations.

Intermetallic and interorganic correlations.

The calculation of the linear correlation coefficient (r) shows that significant correlations exist in the cephalothorax between the concentrations of Cd and Fe ($r = 0.993$), Cu and Zn ($r = 0.968$), Hg and Cd ($r = 0.902$), and in the exoskeleton between Cu and Cd concentrations ($r = 0.965$). There is likewise a positive correlation between Cd and Fe levels in the muscle and in the exoskeleton ; the coefficients are respectively : 0.961 and 0.971. Concerning the relations between eggs and muscles, the concentrations are negatively correlated for Hg ($r = -0.921$). A comparable result for copper and zinc ($r = 0.899$) has been obtained in the crab *Cancer irroratus*. A coefficient close to 1 demonstrates the similarity of quantitative behaviour of these metals. According to MARTIN (1975) this would be in connexion with the common relation of copper and zinc in the metabolism of cuproproteins or even in the hemocyanin molecule.

Evolution of metal concentrations during the intermoult cycle.

Figure 2 shows that metal concentrations generally do not remain constant during the whole intermoult cycle. By the statistical analysis the physiological state of the shrimp is shown to exert a significant influence on the bioaccumulation of cadmium, zinc and copper.

The cadmium concentration in the dry matter decreases regularly during the intermoult cycle, except in the cephalothorax, where it rises until stage C and falls suddenly after stage D. Cadmium proved to be more or less competitive with calcium in the calcification process (NRIAGU, 1980). Evidence for a continual loss of calcium from the body during premoult (stage D), as the exoskeleton is resorbed, was found in crayfish (ADAMS *et al.*, 1981). It was suggested that cadmium presumably follows the internal movements of calcium in the organism during the intermoult cycle. Thus, after the moulting Ca progressively moves from the hepatopancreas into exoskeleton to form the new shell and returns to the hepatopancreas during the premoult stage D. Our results does not confirm that Ca and Cd metabolize along the same biochemical patways.

As for zinc, the highest concentrations are reported at stage C. During this important storage period, a greater stability of the metabolism is generally reached, accompanied by sexual activity (MARTIN, 1978). The considerable Zn amounts could be in relation to reproduction, as high levels of zinc are also found in eggs.

Copper concentrations seem to increase during the whole intermoult cycle, except in the muscles, which contain a relatively steady amount about 30 mg/kg dry weight. This concentration is comparable to those obtained in monitoring programs along French coasts : mean value 31.5 ± 10.4 from 30 *Crangon crangon* samples.

The findings of the other metals which, after statistical analysis, did not show any relation between concentration and intermoult stage are the following :

- Iron concentrations in the cephalothorax follow an evolution comparable to the variations of the cadmium concentrations, while the lowest iron values have been observed at stage B in all the other organs ;
- Mercury amounts in the abdominal muscle, in the cephalothorax and in the exoskeleton show a decreasing tendency from stage A to stage D, while they increase in the eggs and in the legs.

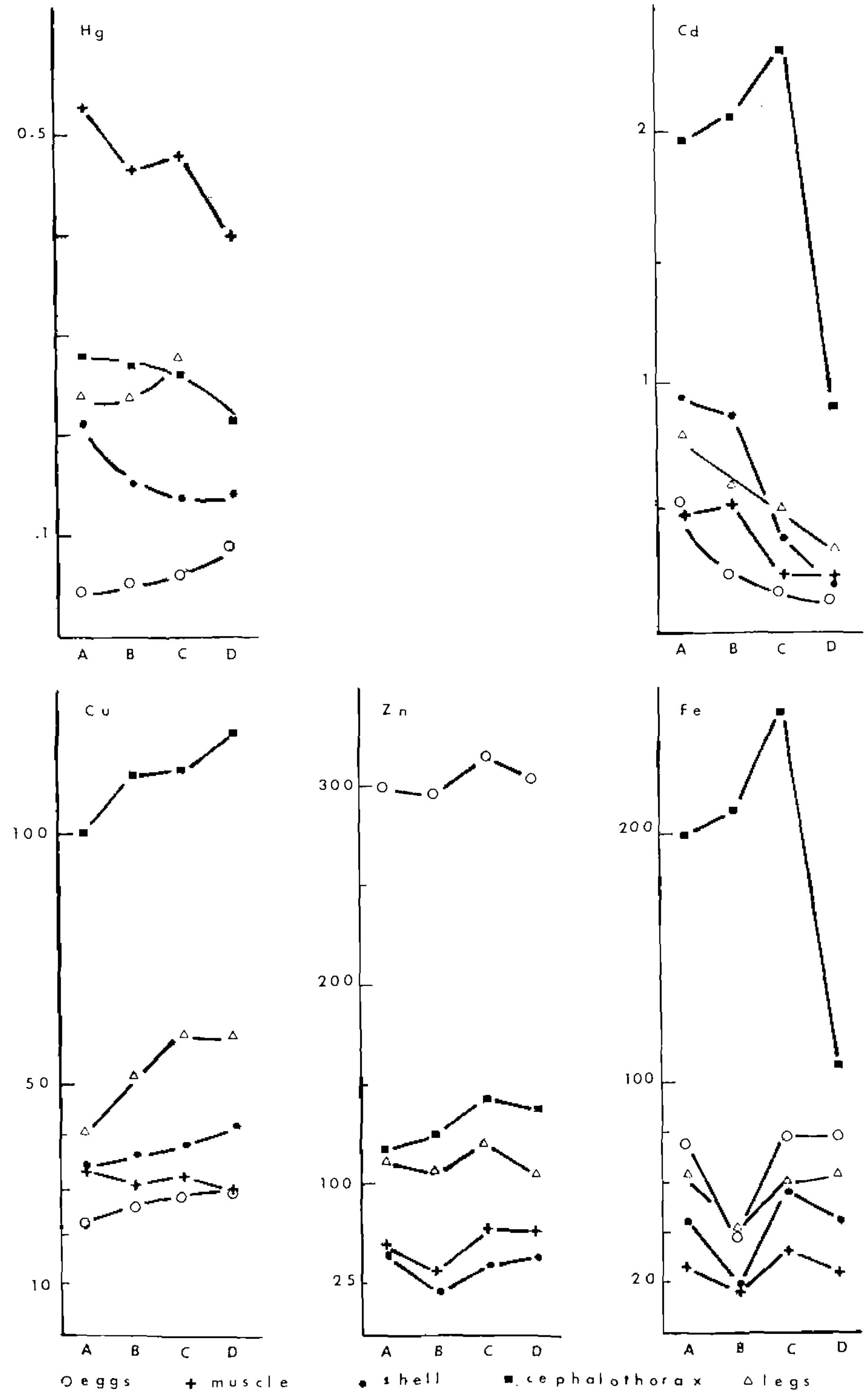

FIG. 2. — *Evolution of metals concentrations, mg/kg dry weight, during the intermoult period (stages A, B, C, D).*

Evolution des concentrations, mg/kg de poids sec, en métaux pendant la période d'intermue (stades A, B, C, D).

Conclusions.

The influence of the intermoult cycle of *Crangon crangon* on the metal bioaccumulation of the considered five elements is settled for Cu, Zn and Cd and presumed for Fe. It proves that these four metal concentrations measured in shrimp are not representative of the respective metal concentrations in the environment. In fact, copper, zinc and iron are regulated and stored mainly in the hepatopancreas until moulting. In the case of cadmium the total amount in the organism is affected by an important decrease observed at stage D in all body parts.

As for zinc, the concentrations vary similarly in all the body parts during the intermoult cycle, the highest Zn level being at stage C. It is therefore conceivable that of two samples of shrimps collected in the same site, the sample containing the highest percentage of shrimps at stage C will show higher values of zinc than the other one. This will reflect the physiological state of the shrimps and not any environmental factor.

Because of the biochemical reactions in which zinc, copper, iron and cadmium appear to be more or less involved during the intermoult cycle, the bioaccumulation of these metals will be largely influenced by the intermoult stage. This restricts the use of shrimp as a Zn, Cu, Fe and Cd contamination indicator in the monitoring programs for the marine environment. On the contrary, the mercury concentrations do not seem to be affected by the intermoult cycle of the shrimps and they probably reflect the environmental concentrations in a better way.

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