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Statistical Assessment of a Sampling Pattern for Evaluation of Changes in Mercury and Zinc Concentrations in *Patella coerulea*

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The validity and representativeness of sampling, whether in temporal or in geographic terms, are always a problematical aspect of the evaluation of metal pollution in coastal seawater. Numerous field studies have underscored the great variety in metal levels in a given site, even in the very short term (Tagrull et al. 1983). Grid-mapping, too, has made it clear that concentrations can vary by a factor of ten over a few hundred metres (Bryon and Hummerstone 1977).

The presence or otherwise of currents, emissary watercourses or estuaries, changes in the weather and navigation patterns are all factors capable of inducing rapid changes in seawater metal concentrations, both directly and through modulation of the rate of uptake or release by sediments (Boudou 1982).

Another feature is that in the absence of pollution naturally occurring metal levels in the marine environment are very low : mercury 10-100 ng/l (Fitzgerald 1979), cadmium 0.1-1 µg/l (Coombs 1979), and zinc 5-50 µg/l (Morris 1974). At levels such as these, each factor in the operating protocol is likely to influence the results (Olafson 1978).

Attempts have thus been made to use part of the biotope as a local pollution indicator owing to its ability to accumulate pollutant residues : metals, organochloric pesticides, aromatic hydrocarbons, etc...

Since the "Minamata" discovery, the phenomenon of bio-magnification of trace elements through the food chains has been subtantiated experimentally (Phillips et al 1980, Boudou and Ribeyre 1981). Despite widely varying results in function of the type of pollutant investigated and the protocol applied, it is generally true that concentrations of metals (especially mercury) are highly in the biotope than in seawater.

The choice of sedentary test species disposes of the variation factor associated with those that move from place to place. Because they are ubiquitous and easy to collect, molluscs have very often been used for this purpose (Lee et al 1972, Schulz 1973, Chow et al. 1976). This is particularly true of the mussel and the oyster because they are eaten by Man.

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Table 1. Mean and (range) *Patella* flesh and shell mercury and zinc values in function of date of sampling.

Date of sampling	N°	Part analysed	Weight (g)	Mercury (µg/g)	Zinc (µg/g)
10.12	12	flesh	0.9(0.40-2.20)	1.5(0.75-3.25)	53(3.2-165)
		shell	1.5(0.65-3.50)	1.5	24
16.12	7	flesh	0.4(0.30-0.55)	1.9(1.52-2.27)	107(34-138)
		shell	0.7(0.60-0.95)	0.9(0.17-1.45)	47(13-97)
22.12	10	flesh	0.4(0.20-0.80)	0.5(0.17-0.78)	120(43-254)
		shell	0.6(0.25-1.25)	0.1(0.01-0.29)	28(<1-75)
30.12	17	flesh	0.5(0.30-0.80)	0.2(<0.04-0.62)	28(<1-84)
		shell	1.0(0.60-1.95)	0.1(<0.04-0.86)	22(<1-117)
23.1	12	flesh	0.7(0.40-1.00)	0.2(0.08-0.38)	28(1-92)
		shell	1.0(0.55-2.35)	0.1(0.04-0.30)	9(<1-32)
26.1	6	flesh	0.5(0.30-0.75)	0.4(0.06-0.57)	24(11-33)
		shell	1.0(0.60-1.65)	0.1(<0.04-0.17)	3(<1-25)
02.2	19	flesh	0.7(0.30-1.10)	0.1(<0.04-0.18)	22(<1-66)
20.2	24	flesh	0.4(0.15-0.95)	0.1(0.07-0.29)	72(3.4-333)
05.3	24	flesh	0.4(0.08-1.00)	1.8(0.15-9.50)	36(<1-159)
10.3	24	flesh	0.4(0.10-1.50)	0.5(0.13-1.80)	16(<1-47.5)

Table 2. Analysis of variance of *Patella* weight and flesh and shell mercury and zinc concentrations in function of sampling date.

Variable	Source of variation	Sum of squares	Degrees of freedom	Variance	F
Weight	Date of sampling	3.94	9	0.44	6.1**
	Residual	10.50	145	0.07	
	Total	14.44	154		
Log[Hg] Flesh	Date of sampling	151.4	9	16.8	34 **
	Residual	68.6	145	0.5	
	Total	220	154		
Log[Hg] Shell	Date of sampling	96.8	5	19.4	32 **
	Residual	32.9	58	0.6	
	Total	129.7	63		
Log[Zn] Flesh	Date of sampling	93.0	9	10.3	6 **
	Residual	248.4	145	1.7	
	Total	341.4	154		
Log[Zn] Shell	Date of sampling	36.4	5	7.3	3 *
	Residual	139.4	58	2.4	
	Total	175.8	63		

* Significant to the 5 % threshold
** Significant to the 1 % threshold

The limpet (*Patella)* is a primary herbivore consumer and would seen an equally elective test organism. Its very localised feeding habits (grazing the algae colonising the rocks where it lives) are less prone to sudden changes than those of the filtering molluscs. Indeed, it has already been employed in campaigns evaluating pollution by hydrocarbons (Dicks 1973) and metals (Boyden 1974, Bryan and Hummerstone 1977, Deutsch et al. 1980, Lobel 1982).

To be able to compare several areas in this way, however, or follow their progress over the course of time, it is essential to work out a representative sampling plan. This paper reports an investigation of this aspect of the question with regard to two metals : mercury, which is toxic and plays no part in any natural metabolic process, and zinc, a cation required in weak concentrations by certain enzyme systems (Parisi and Vallee 1969, Tal 1969).

MATERIALS AND METHODS

Limpets (*Patella coerulea*) were randomly collected at low tide over a distance of 15 metres along the Cap de Nice, Alpes-Maritimes, France. The flesh and shell were separated, weighed and mineralised in a hot (80°C) 2:1 nitroperchloric mixture for 48 h. Mercury was measured by cold vapour atomic absorption in a Perkin Elmer Colman Mas 50, zinc by anodic redissolution (ESA 2011). Ten spaced campaigns between 10 december and 10 March supplied 155 specimens.

RESULTS AND DISCUSSION

Six sets of means and ranges for the three parameters studied (wet weight, mercury content and zinc content) and for each sampling date are set out in Table 1. Analysis of variance in function of sampling date gave the results shown in Table 2. These overall analyses are based on log concentrations to be more in keeping with the assumed stability of the variance (Lellouch and Lazar 1974).

The flesh and shell between-concentration coefficients and the partial correlations between mercury and zinc concentration and wet weight for the last three series are shown in Table 3.

Table 4 illustrates the results of analysis of variance of the amount of mercury fixed in function of mollusc size class and the date of sampling. This study was run on the last three campaigns. Twenty of the twenty-four limpets collected on each occasion were divided into two size classes : class 1, 0.10-0.39 g and class 2, 0.40-0.69 g. The complete blocks method was employed to allow for the disuniformity induced by the time factor in the study of the weight factor.

Lastly, Table 5 presents the mercury and zinc levels in the sea-water at the sampling site during the course of the study.

There was a marked and rapid variation in mollusc mean weight over the four month period (F test significant to 1 %, Table 2). It is clear from Table 1 that there fluctuations do not solely correspond to natural biological variations, since the mean shell weight also changed very quickly, e.g. 50 % decrease between 10 and 16 december.

Table 3. Coefficient of correlation between concentrations in flesh and in shell, and partial correlations between mercury and zinc concentration and flesh weight.

Date of sampling	Variable 1	Variable 2	N° of observations	Coefficient of correlation	
10.12.80 to 26.01.81	Hg flesh	Hg shell	64	0,77**	Partial coefficients
	Zn flesh	Zn shell	64	0,34	
20.02.81	Hg flesh	Zn flesh	24	0,30	
	Hg flesh	weight	24	-0,68**	Partial coefficients
	Zn flesh	weight	24	-0,08	
05.03.81	Hg flesh	Zn flesh	24	0,25	
	Hg flesh	weight	24	-0,57**	Partial coefficients
	Zn flesh	weight	24	-0,18	
10.03.81	Hg flesh	Zn flesh	24	-0,35	
	Hg flesh	weight	24	-0,62**	Partial coefficients
	Zn flesh	weight	24	-0,33	

**Significant to the 1 % threshold

Table 4. Analysis of variance of amount of mercury taken un by *Patella* in function of size class and date of sampling.

Origin of fluctuation	Degree of freedom	Variance	F	Significance level
Date	2	21,1	107,46	> 1 %
Weight	1	0,24	1,22	
Interaction	2	0,87	4,23	> 5 %
Residual	54	0,20		
Total	59			

Table 5. Seawater mercury and zinc levels during the ten sampling campaigns.

Date	9.12	16.12	22.12	23.1	28.1	2.2	11.2	20.2	26.2	5.3	10.3
Hg ng/l	<50	<50	<50	<50	80	70	<50	50	<50	<50	50
Zn ng/g	<100	100	100	<100	200	600	<100	100	<100	100	1100

This probably reflects uneven sampling, since the gatherer tends to concentrate his attention on heavily populated rocks that do not necessarily contain representatives of all size classes. It would thus be desirable in campaigns of this kind to make a grid map of the area studied, however small (in our case, about 15 metres of rocky coasts).

Flesh metal values varied greatly from one mollusc to another. Even if only samples of comparable weight (0.4 g flesh) are examined, mercury concentrations vary by a factor of 19 and zinc by a factor of 7.5 in the same campaign (Table 1). Similar findings have been reported by Lobel (1982) for the uptake of zinc by mussels and iron by limpets, and by Renzoni et al (1975) for limpet mercury concentrations. For our campaigns, therefore, a minimum of around twenty individuals was essential to be able to calculate means with a 5 % confidence interval (Table 3). The same conclusion was reached by Gordon (1980) with regard to metal pollution levels in *Mytilus californianus*.

Marked and rapid variations were also noted in function of time as shown by an F value with a significance of better than 1 % for both metals (Table 2).

This rapid uptake (itself in agreement with most of the "in vitro" findings for molluscs) was matched by comparably speedy decontamination. Campaigns 2 and 3 suggest that mercury has a resident half-life of the order of 3 days, while a lifetime of 4 days can be deduced for zinc from campaigns 3 and 4.

It should be noted that seawater mercury and zinc values, on the other hand, were both very low and very stable throughout the sampling period, since the area had been chosen to provide an uncontaminated benchmark. In his study of *Mytilus*, Simpson (1979) observed substantial changes in metal levels (primarily referred to concentrations) that were mainly attributable to weight fluctuations linked to the reproduction cycle. The conclusion to be drawn, is that at least 2-3 samplings per month over a period of several months are needed to obtain a reliable estimate of limpet metal levels in an uncontaminated area.

Two points of interest emerge from the correlations between the factors compared in this study. There is a close correlation (significant at the 1 % threshold, Table 3) between flesh and shell mercury, and hence the flesh value can be regarded as representative of the entire mollusc. The correlation between flesh mercury and total mollusc weight is equally significant (Table 3). The literature data on this point are discordant. Renzoni et al (1973) were unable to establish a mercury : weight correlation owing to a great variability of between-individual levels. By contrast, Boyden (1974) observed an inverse relation (-0.23) between zinc, copper and lead values and limpet weight, as well as a correlation (1.0) between cadmium and weight. In our experiment, there was an inverse correlation (mean -0.6) between mercury and weight. This marked decrease in mercury level when weight increases points to the existence of a background concentration in the limpet, irrespective of its weight. Further evidence in this direction is provided by the

analysis of variance : the factor "weight" is not significant (Table 4).

It would thus appear that correct evaluation of mercury contamination in the limpet must rest on the total amount taken up and not on its concentration, which is the usual parameter. The same opinion has already been expressed by Breittmayer et al (1985) with respect to *Mytilus*.

Our findings do not disclose a significant zinc : weight correlation. This is not necessarily in conflict with Boyden (1974), since our concentrations (5-100 µg/g), and a correlation may have been masked by their dispersion.

Three substantial conclusions can be drawn from this study : strict grid-mapping of the site investigated is essential ; prior consideration must be given to grading by size to ensure significant results ; account must be taken of mollusc weight, since its changes may have an appreciable influence on the fluctuation of some metal values.

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