Mussel-watch
Mytilus
Quantitative indicator
Cadmium
Mercury

Surveillance
Moule
Indicateur quantitatif
Cadmium
Mercure

A review of the use of *Mytilus* spp. as quantitative indicators of cadmium and mercury contamination in coastal waters

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Received 25/4/88, in revised form 31/10/88, accepted 8/11/88.

ABSTRACT

The use of marine mussels to monitor cadmium and mercury contamination in coastal waters is reassessed on the basis of the current knowledge of metal metabolism in *Mytilus* spp. Sources and amplitude of variation of metal concentrations in the soft tissues of the mussel are described. Methods (sampling strategies and normalizations) for optimizing the use of *Mytilus* spp. as quantitative indicators of metal contamination are given. Some directions for further research are suggested.

Oceanologica Acta, 1989. **12**, 4, 417-432.

RÉSUMÉ

Revue bibliographique sur l'utilisation du genre *Mytilus* comme indicateur quantitatif de la contamination par le cadmium et le mercure dans les eaux côtières

Les connaissances actuelles sur le métabolisme des métaux chez la moule (*Mytilus*) permettent une réévaluation de l'utilisation de cet organisme comme indicateur quantitatif de la contamination des eaux côtières par le cadmium et le mercure. L'origine et l'amplitude des variations des concentrations en métaux dans les tissus mous de la moule sont examinées en détail. Des méthodes d'optimisation (stratégies de prélèvement et modèles correctifs) de cet outil sont proposées. Quelques recommandations sont faites en matière de recherches futures.

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INTRODUCTION

Monitoring the coastal environment has arisen from the need to protect human health and living marine resources. The simplest solution proposed to date has been to measure and compare the concentration levels of pollutants in space and in time and, from the data collected, to identify natural and anthropogenic contamination levels and pinpoint variations. This means of assessing contamination has been widely used for metals in the different compartments of the coastal environment.

During the last ten years, there has been no lack of literature describing the various strategies available for establishing baselines or a "follow-up" following trends of metals in water, sediments and organisms. It is not the purpose of the present work to enumerate these, although it is worth recalling the fact that the main interest of the use of quantitative biological indicators (sentinel organisms) with regard to water or sediment is their capacity to give information on the bioavailability of the polluant under study. This information is needed since the ultimate purpose is the protection of ecosystems and human beings.

In the light of the above, what are the ideal attributes of a good polluant sentinel organism? They have been catalogued by a number of authors (Butler *et al.*, 1971; Haug *et al.*, 1974; Phillips, 1977; Cunningham, 1979; Bryan *et al.*, 1980), and may be summarized as follows:

– The sentinel organism should accumulate the contaminant.

– It should be sedentary in order to be an authentic representative of the study area.

– It should have a sufficiently long life to permit sampling of more than one year-class.

– It should be large enough to provide sufficient tissue for chemical analysis.

– It should tolerate a wide salinity range.

– It should bioaccumulate sufficiently to allow direct measurement without pre-concentration.

– A correlation should exist between the level of contaminants in the organism and in the surrounding water. This correlation should be similar for all individuals in all study areas under all biotic or abiotic conditions.

– The effects on the organism of variations of salinity and temperature should be known.

No single species combines all these qualities and a compromise must be found. It was in this spirit that Goldberg (1975) suggested the idea of the "Mussel Watch" as the first stage in a worldwide monitoring programme for the marine environment. This proposal has given rise to numerous works as well as to two international conferences. The first was under the auspices of the United States National Academy of Sciences and took place in Barcelona in 1978; the second, organized as part of a programme of studies on Chemical Changes in Coastal Zones (SCOPE/PNUE) convened researchers in Honolulu in 1983.

One outcome of these meetings, in conjunction with the important synthesis established by Phillips (1980) and Phillips and Segar (1986) as well as the results of the American "Mussel Watch" (USMW) (Goldberg *et al.*, 1978; 1983; Farrington *et al.*, 1983) was to establish the superiority of genus *Mytilus* over organisms considered:

– This genus is widespread in sub-arctic and temperate regions.

– The animals are sessile and undergo only minor displacements during their lifetime.

– In relation to their environment they accumulate metals with a concentration factor of about 10^3 to 10^5.

– The species are relatively resistant to pollution.

– They can be transplanted.

– They are euryhaline.

– They are used for human consumption and are therefore a potential source of contaminants for humans.

Despite these advantages, the mussel is not a panacea where monitoring is concerned. The principal drawback cited is that "... mussel monitoring has so far failed to uncover contaminant "hot spots" that were not known or suspected before" (White, 1982). In fact, metal concentrations in natural mussel populations vary widely even in uncontaminated environments. According to Goldberg *et al.* (1978) only variations in concentrations above an order of magnitude level may be considered as significant in showing exploitable trends in monitoring programmes. Given these conditions, the use of the mussel as a quantitative indicator is effectively restricted to the detection of "hot spots". It is almost impossible for it to reveal less obviously contaminated areas. The following alternative is suggested: refining this tool or using other approaches, for instance, multispecific studies, transplantations, the use of artificial bioconcentrators (polyethurane foam, for example), biological relationships between contents and effects, *etc.*

It seems, however, that the use of the mussel as an indicator has not yet been fully exploited. The results of research carried out over the last few years in this area have not been applied systematically to the various monitoring programmes around the world.

In order to optimize the use of the mussel as an indicator species it is first necessary to specify clearly the objectives of this technique. These are: to establish concentration levels or to follow their trends and where possible to identify the sources of the contamination. It is the purpose of this paper to demonstrate that *Mytilus* spp. can meet these objectives for cadmium and mercury.

The current knowledge on metal metabolism and the origin and extent of the variations of concentrations in metals in the genus *Mytilus* are presented; this will permits us to describe how to optimize the use of this genus as a quantitative biological indicator in the coastal environment.

METAL METABOLISM IN THE GENUS *MYTILUS*

Bioconcentration

Bioconcentration was first brought to light at the beginning of the present century, but the work of Vinogradov in 1953 showed that it could be applied generally to all marine organisms and to most divalent metals. Goldberg (1962) and Brooks and Rumsby (1965) were among the authors who later proposed a biogeochemical interpretation of this phenomenon. It is only recently that bioconcentration has been studied in metabolic terms. The first elements studied in this way were those for which a physiological role was apparent, essentially Ca and Si. The biomineralization of Ca was studied in invertebrates, where for the first time the existence of intercellular spherocrystals was clearly shown [*see* the reviews of Simkiss (1976) and Moore (1981) in this context]. The presence of Si in the frustules of diatoms has also long been established. In addition to these two elements, many others have been shown to have a physiological role: Cu is associated with active haemocyanine in crustaceans and certain mollusks; Mo, Mn, Mg and Zn have an enzyme activation function. Fe, Cr and Ni are also essential to various biological functions (Bowen, 1966).

Following the work of Williams (1981), Simkiss *et al.* (1982) suggested a general approach to the phenomenon of bioconcentration of divalent cations. Whereas inorganic ions are hydrophilic the plasmatic membranes are hydrophobic. This is why the majority of ions are only capable of penetrating the cytoplasm with the aid of a carrier-mediated substance. Once inside the cell, the ion must be picked up by another ligand in order to avoid its diffusion back outside. These ligands constitute a system of "kinetic trapping" whose efficiency depends on the strength of the binding. In other words, the accumulation of certain ions is regulated by the synthesis or degradation of chelating molecules (amino acids, metalloproteins, glutathione, *etc.*). Increase in the specificity of the binding is favoured by a series of steady states; the trend towards more stable complexation acts as the basis for metal metabolism within the circulating liquids and the cell. The most stable binding, the final "kinetic sink", may be specific for a metal in the case of a physiological process (*e. g.* metallo-enzymes) or more general in the case of a detoxification system (*e. g.* metallothioneins).

Absorption and transport mechanisms

Metal penetration in bivalves may take place in several ways: diffusion of ions or complexes, mediated transport and/or endocytosis of particulate metal and pinocytosis of organo-metallic aggregates (Fig. 1). These mechanisms were mentioned in the case of Zn in *Mytilus edulis* by George and Pirie (1980) and discussed by Martoja and Martoja (1982).

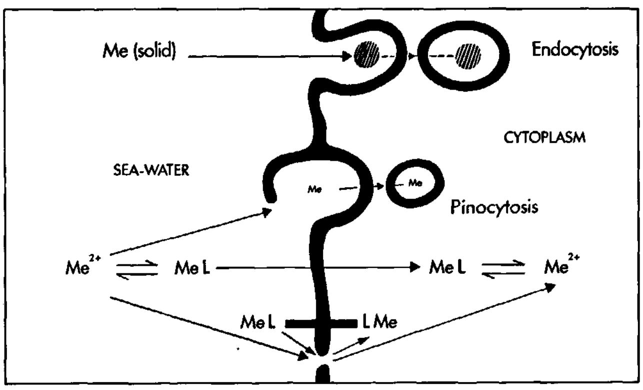

Figure 1
Absorption of divalent metals in bivalves. (L) ligand; (Me) metal. See text for detailed explanation.
Absorption des métaux divalents chez les bivalves. (L), ligant; (Me), métal. *Voir* le texte pour des explications détaillées.

Uptake may take place at the gill or digestive gland or on the surface of the mantle; all these means of absorption are possible for the same metal, and their relative importance is a function of the speciation of the metal in the environment. It may be noted at this point that the coexistence of these mechanisms and the variability of the chemical speciation are what render the notions of bioavailablity so complex.

Experimentation has shown that after passage across the membrane, the metals in the haemolymph become associated with the free proteins and the haemocytes. The occurrence of dissolved metal is probably the result of its absorption as dissolved species with or without mediators. Its presence in particulate form results from the phagocytosis of vesicles formed during the absorption of particulate metal and/or pinocytosis of protein aggregates rich in divalent cations (George and Pirie, 1980).

In the absence of any regulation of the rate of uptake, the metal absorption is directly related to the abundance of a particular species in the environment. If this particular species is in equilibrium with the total metal concentration in the environment, the first order model of Simkiss *et al.* (1982) can be used:

$d[M]/dt \propto [M]$ transport $\propto [M]$ total.

This means that the metal concentration in the haemolymph of the bivalve depends on the concentration of the metal in the environment (in the case of regulated absorption the first order kinetic model is inapplicable).

In addition to this absorption model, the other mechanisms governing the level of metal concentrations in the bivalve may be identified as storage and excretion.

Storage and excretion mechanisms

Molluscs accumulate metals principally in two organs, the hepatopancreas and the kidney, although certain elements may be enriched the cells of other tissues.

Granules or lysosomal deposits make up the intracellular area of metal deposition (George *et al.*, 1978*a*; George *et al.*, 1980; Mason and Nott, 1981; George *et al.*, 1982; Bouquegneau and Martoja, 1982). The case of granules has been studied in particular by Martoja and Martoja (1982) who considered them as accumulation sites for metabolic wastes, formed by the precipitation of metals as phosphate, carbonate, sulphide, or even, in mammals, selenide, as a result or different physiological processes.

It is not unusual for bioaccumulations to result from some metabolic malfunction or an alteration in the environment, and in this case they induce detoxification mechanisms which most frequently utilize normal metabolic pathways. The granules and the lysosomes in which the metals are trapped therefore constitute detoxification sites as well as storage places. In this way the metals are isolated from the cytoplasm and rendered chemically inert with regard to cellular functions. This explains the mussel's great resistance to doses of metal which would be fatal to organisms with no such storage mechanism.

There is no doubt that the kidney is the most important organ of excretion in the mollusc. The metals found there in the lysosomes or the spherocrystals are excreted by exocytosis of the vacuole contents, by elimination from the renal podocytes or else by diapedesis of whole granules (*in toto*) in the urinary tract (Coombs, 1980). Excretion of certain metals may also occur through the tegument, with faeces, by byssus production (George *et al.*, 1976), through the shell, or by way of gamete emission during spawning. Some of these excretory mechanisms are episodic; therefore they could amplify the temporal variations of metal concentrations in bivalves.

Absorption and excretion kinetics for cadmium and mercury

Cadmium

Fowler and Benayoun (1974) studying the kinetics of Cd^{109} in *M. galloprovincialis* show that the rate of absorption of Cd is in direct proportion to its concentration in seawater, and that it is affected neither by temperature nor by the presence of different Zn concentrations. Fowler and Benayoun (1974) also show that equilibrium between the animal and its environment is reached only after two months of exposure. Moreover, there does not seem to be any equilibrium between Cd^{109} and stable Cd already accumulated in the mussel. It can be added that excretion is a very slow and variable process (biological half-life between 300 and 1,200 days) which is perhaps affected by the animal's diet.

More recently, Borchardt (1983 and 1985) has provided an answer to the question of whether water or food is the preferred vector for the absorption of Cd. Using Cd^{115m} to mark an experimental solution and Cd^{109} to mark the food, he showed that food intake was responsible for only 1% of the animal's Cd content, while on the other hand the absorption of Cd in solution was in linear correlation with the amount of food ingested. Biological half-life varied in these experiments from 96 to 190 days in inverse proportion to the quantity of available food. Modelization tended to show that in long-term studies the highest intake of Cd from food sources must be expected near maintenance of the food concentration. Accumulation through food is closely linked to assimilation of carbon (Borchardt, 1985). This preferred absorption *via* water has had been suggested by Janssen and Scholz (1979). According to Scholz (1980), it takes place mainly through the grills.

Other studies confirm and/or complete present knowledge:

The linear accumulation of Cd with time has been shown by several authors (George *et al.*, 1978 *b*; Scholz, 1980; Gutierrez-Galindo, 1980; Köhler and Riisgard, 1982; Poulsen *et al.*, 1982). Thus *Mytilus* proves to have an excellent capacity to accumulate Cd on a large scale covering the whole range of Cd concentrations in even the most contaminated coastal waters. George and Coombs (1977) and Scholz (1980) confirm that there indeed exists a linear relation between absorption and concentrations in the environment, with an experimental concentration factor of about 150 and a relative slowness of excretion (18 times slower than absorption). Cd is principally eliminated *via* the kidney. The need for detoxification is a result of the low rate of elimination. It is effected by a mechanism of immobilization, which most probably implies the formation of metallothioneins and their association in the lysosomes (George and Pirie, 1979).

In addition, George and Coombs (1977) have pointed out a latency period preceding Cd absorption which disappears in the presence of chelating agents. These results are confirmed by Gutierrez-Galindo (1980) and suggest the existence of an absorption mechanism of Cd activated by organic substance(s).

To sum up, it is well established that the absorption of Cd in the genus *Mytilus* is proportional to exposure time and to the concentration of metal in the environment. The kinetics are slow. Biological half-life as calculated from laboratory experiments is extremely variable (14 to 1,254 days). Absorption from solution is preferred and the gills are the principal entry organ. Storage is effected in granule form, especially in the kidney from which a large part of excretion takes place.

Mercury

There is less documentation on the accumulation of Hg in the mussel than for Cd. Miettinen *et al.* (1972) have calculated the biological half-life of CH_3Hg in *M. galloprovincialis* at 1,000 days. For the same species, Fowler *et al.* (1978) has shown differences in kinetics according to the chemical form of Hg: CH_3Hg is accumulated to a higher level than the inorganic forms. Absorption seems to be related to mussel size, with the youngest accumulating more than the older specimens. Similar observations have been made on a natural population (Cossa and Rondeau, 1985). Excretion appears not to be influenced by age but rather by nutritional factors (Fowler *et al.*, 1978).

In an experimental study on Se−Hg interaction in *M. edulis*, Pelletier (1986) shows that the rate of accumulation of Hg is not modified by Se, which leads him to conclude that this bivalve does not possess the regulatory mechanisms as described for mammals by Martoja and Martoja (1978). In addition, there is no evidence for the biomethylation of Hg in the mussel. The different chemical forms of Hg present in the mussel appear to represent different sources of contamination (Riisgard *et al.*, 1985).

Among studies conducted in the field, those of Davies and Pirie (1978) and of Eganhouse and Young (1978) based on transplantations of uncontaminated mussels to an environment suspected of being contaminated show that concentrations in the transplanted mussels increase. The accumulation is proportional to the time of exposure and the concentration in the water (Davies and Pirie, 1978). According to Eganhouse and Young (1978), the concentrations in Hg in transplanted *M. californianus* reach the levels found in the indigenous mussels after one month. After three months in two contaminated locations the accumulation in Hg is quite obvious. Growth of the transplanted animals had the effect of diluting the Hg concentration but the absorption of the metal continued to increase during the experiment. After this period of contamination the animals are purged in the laboratory. Depending on the mussels' origins, biological half-life varies from 53 to 293 days.

ORIGINS AND RANGE OF VARIATIONS OF METAL CONCENTRATIONS IN THE GENUS *MYTILUS*

In this section, the origin and range of variations in concentrations in metals (especially in Cd and Hg) found in the genus *Mytilus* in coastal environments are described.

It is well known that metal concentration levels in organisms are not only the result of their bioavailability in the environment. Environmental factor such as tem-

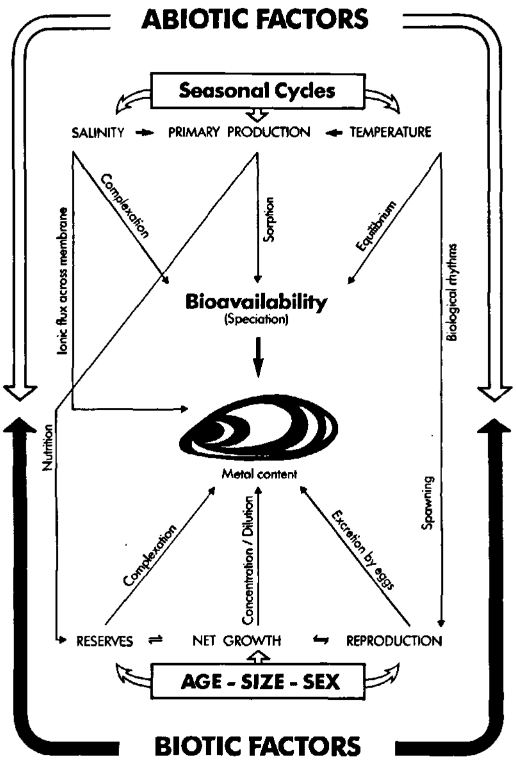

Figure 2

Schematic representation of major factors governing metal concentration levels in the mussel.

Schéma présentant les facteurs principaux réglant la concentration en métal chez la moule.

perature and salinity, and biotic ones such as age and physiological condition are acting. These influences must be identified and measured so that they may be taken into account during sampling, validation of results, or in the interpretation of monitoring data.

Numerous papers report possible sources of variation in the accumulation of metals in the mussel. Figure 2 illustrates the principal factors which act and interact in the establishment of concentration levels in this bivalve. Biotic factors may be considered in two categories: growth (age, size, soft tissue weight) and reproduction (sex and gametogenesis). Environmental factors essentially revolve around seasonal cycles (temperature, salinity, primary production), although other parameters may be involved (metallic interaction, position in the intertidal, *etc.*). These will be reviewed in the following section.

Effects of somatic physiology

The facts

The effects of growth on the accumulation of metal in molluscs were discovered through the relationship which often exists between the concentration in metal and the size or age of the animal. The works of Boyden (1974 and 1977) were a landmark in this field. In most of the cases studied by Boyden involving various metals and different species of molluscs, the regression coefficients between metal contents and size were divided into two groups: those around 0.77 and those in the neighbourhood of 1.00. In the first instance (*e.g.* Zn in *M. edulis*), the concentration of metal is higher in small specimens; in the second case (*e.g.* Cd in *M. edulis*) the concentrations are unrelated to size. Boyden (1977) observed, however, that a certain number of metal-to-size relationships presented a particular characteristic: some showed a curve in the log-log relation (*e.g.* Cu in *Ostrea edulis*) while others exhi-

Table 1

Regression coefficient (*b*), correlation coefficient (*r*) and number of determinations (*n*) in the log-log relationships between Cd content and dry tissue weight in *Mytilus edulis* from different locations and sampling seasons.

Coefficient de régression (b), coefficient de corrélation (r) et nombre de déterminations (n) de la relation entre le log du contenu en Cd et le log du poids des tissus mous de moules (Mytilus edulis) *prélevées en divers endroits et saisons.*

Site	Date (month-year)	*b*	*r*	*n*	Reference
Pointe Mitis (Gulf of St. Lawrence, Canada)	07-78	0.84±0.07	0.96	43	Pouliot, 1983
»	08-78	0.88±0.09	0.94	41	»
»	08-78	1.17±0.09	0.97	40	»
»	09-78	1.37±0.10	0.97	39	»
»	10-78	1.23±0.06	0.99	39	»
»	11-78	0.94±0.05	0.99	38	»
»	12-78	0.97±0.06	0.98	40	»
»	01-79	0.97±0.10	0.95	39	»
»	03-79	0.86±0.09	0.95	39	»
»	03-79	1.06±0.06	0.98	41	»
»	04-79	0.71±0.11	0.88	43	Pouliot, unpublished
»	04-79	0.98±0.13	0.91	42	»
»	05-79	1.17±0.11	0.95	42	»
»	05-79	1.15±0.09	0.97	43	»
»	06-79	1.10±0.11	0.96	34	Pouliot, 1983
»	06-79	0.91±0.08	0.96	42	»
»	07-79	0.96±0.10	0.95	39	»
Bedford Basin (Nova Scotia, Canada)	08-79	0.96±0.07	0.98	43	Cossa and Pouliot, unpublished
Saanich Inlet (British-Columbia, Canada)	07-78	1.14±0.12	0.97	29	Cossa, Piuze and Wong, unpublished
Sète (France)	10-78	0.97±0.06	0.97	46	Pouliot, unpublished
Villefranche (France)	11-78	1.45±0.24	0.91	29	»
	03-79	0.99±0.11	0.89	24	»
England	–	1.01 (min 0.97; max 1.08)			Boyden, 1977
Kiel Bight (RFA)	Autumn 80	1.02	0.98	105	Fisher, 1983
»	Winter 80	0.79	0.96	56	»
»	»	1.16	0.93	54	»
»	»	0.83	0.96	78	»

bited slopes which varied according to the level of contamination (*e.g.* Cd and Zn in *Patella vulgata*).

By multiplication of the sampling in time and space, the general rule was found to be one of large variability in the slopes showing allometric metal-to-size relationships (Cossa *et al.*, 1980; Cossa and Rondeau, 1985). This variability is linked to geographical location, sampling season and mussel size range. A principal cause could be differences in the physiology of the mussels (filtration, assimilation efficiency) during their lifetime and their gametogenetic cycle as governed by environmental conditions (temperature, available food, *etc.*).

Table 1 recapitulates all the results for metal content in relation to size gathered for Cd in *M. edulis*. The slope values compiled have a seasonal and geographical variation of 0.71 to 1.45, that is to say, going from a situation in which there is a reduction in Cd concentrations with the increase in mussel size, to the opposite situation in which the accumulation is greatest in the oldest individuals. This last case is most frequently found in *M. californianus* (Gordon *et al.*, pers. comm.), in which an exponential relationship may be observed between Cd contents and organism size (causing a break in the log-log slope). This situation indicates an irreversible accumulation of metal in the oldest specimens coupled with their very low rate of growth.

In some cases we observed the existence of a break in the slope (log Cd/log size) in *M. edulis* (Cossa *et al.*, 1979); these changes of slope are produced when the animal reaches the size at which it attains sexual maturity. The slowing of somatic growth when the animal attains adulthood may provide an explanation for these observations.

Pouliot (1983), working on mussels collected from an unique site at different times of the year, has shown that the distinction between mature and immature mussels from the point of view of the Cd/size is in several cases justified but is not permanent. In addition, the break in the slope, when statistically significant, usually means a greater accumulation in the older organisms, although the opposite case was occasionally observed (Pouliot, 1983).

Although several indices suggest linking the existence and the variations in the Cd/size ratio to the growth of the animal or, more broadly, to its metabolism (Fagerström, 1977), the changes in regression coefficient can also result from the variation of Cd concentration in the waters and from the rate at which each mussel size-class is able to re-equilibrate itself with the surrounding environment. During experiments in large enclosures (68 m^3) immersed in the Saanich Inlet (1978 CEPEX Programme, Cossa, Piuze and Wong, unpublished), mussels measuring 1.5 to 6 cm were used. A first batch was immersed near the collection site in a bag contaminated with a Cd concentration varying from 1.0 $\mu g.L^{-1}$ at the beginning to 0.6 $\mu g.L^{-1}$ at the end of the two-week experiment. A control batch was placed in a mesh basket attached to the outside of the bag in the waters of the Saanich Inlet, whose Cd concentration remained below 0.04 $\mu g.L^{-1}$ throughout the whole experiment. The slope of the metal/size

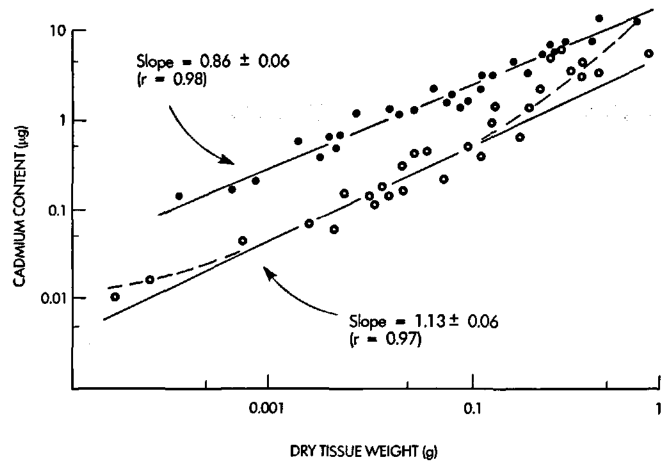

Figure 3

Relationship between Cd content and size in Mytilus edulis *in a mesocosm experiment in Saanich inlet (CEPEX,* 1978). (○) *Reference mussel (concentration* < 40 *ng Cd.* L^{-1}); (●) *Contaminated mussel (concentration* 0.6-1.0 µg *Cd.* L^{-1}). *Cossa, Piuze and Wong, unpublished.*

Relation entre le contenu en Cd et la taille de *Mytilus edulis*. Résultats obtenus lors d'expériences en mésocosme dans la baie de Saanich, en Colombie britanique (CEPEX, 1978). (○) moules témoins ([Cd] < 40 ng.L^{-1}); (●) moules contaminées ([Cd] 0,6 à 1,0 µg.L^{-1}). Cossa, Piuze et Wong, résultats non publiés.

relationship for the control mussels was 1.13 and for the contaminated mussels, 0.86 (Fig. 3). There was a difference in increase by a factor of 10 for Cd values between the mussels exposed to Cd and the control for the youngest specimens, whereas this increase barely reached a factor of 2 for the larger ones. The short length of the experiment suggests that equilibrium between the contaminated mussels and their new environment had not yet been reach. This leads one to think that, because of their greater metabolic activity, the younger mussels tended to reestablish their equilibrium in the environment more quickly than the older ones.

Descriptive model of Cd/size relationships

Metabolic activity and the variation in Cd values in the environment do seem to affect the shape of the curves linking Cd values and mussel size. It was elsewhere proposed (NAS, 1980) a model describing the relationship between metal concentrations and tissue weight for bivalves. Figure 4 shows a generalized form. Within the extreme limits shown, any and all intermediate situations are possible, but the U-shape is the one most frequently observed, as it illustrates the two breaks in the slope. These breaks are also encountered when expressing different physiological parameters with mussel size; for example, O_2 consumption (R) as a function of tissue weight (W) : $R = -aW^b$ where $b=0.89$ for small mussels and $b=0.66$ for the larger ones (Hamburger *et al.*, 1983), or filtration rates (F) : $F = aW^b$ where $b=0.60-0.74$ for the small specimens and 0.38 for the larger ones (Widdows, 1978). Jørgensen (1976) observed that as tissue weight increased, water transport *via* the gills was reduced more quickly than was the general metabolic system. Widdows (1978) has pointed out that allometric relationship curves can be affected by the temperature and the available food, and are thus marked by seasonal cycles. The similarity between allometric and metal vs age relationships are examined later on in the text.

Mechanisms

The shape of the Cd/size curves results from a combination of several factors which act separately or simultaneously. Following the conclusions of Williamson (1980) who studied the accumulation of Cd in the snail (*Cepaea hortensis*), it can be suggested that the model proposed on Figure 4 reflects the competition between the opposite effects of age and growth on the accumulation of Cd: the metal accumulates with age whereas the weight gain of the individual has the effect of reducing the metal concentration in the tissues. The results in Figure 5 show that the existence of such a process in *M. edulis* is not speculation. The stepped curve observed underlying the linear relation could effectively correspond, in a given set range of soft tissue weights, to the presence of individuals of the same age class whose soft tissue weights differ considerably. In this way, while the Cd concentration would increase with age, it would diminish with the weight within each age class; this would explain the exponential curves in the contents/weight ratio or the "U-shape" (parabolic) of the concentration weight ratio described in Figure 4.

To the inverse effect of age and soft tissue weight is added the effect of changes in the bioavailability of Cd in the environment, as the results of Figure 3 show.

In order to demonstrate these mechanisms clearly we will restate this phenomenon in the light of situations

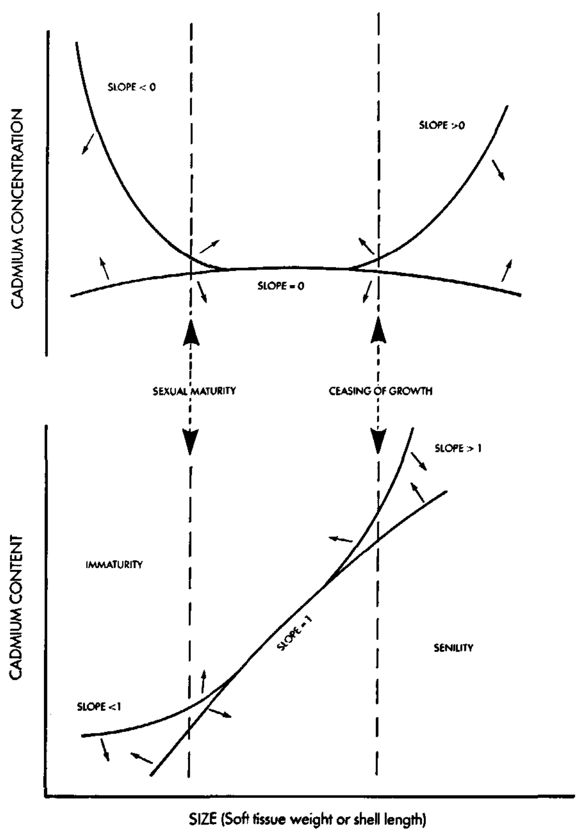

Figure 4
General model for the Cd–size relationship in Mytilus *spp.*
Modèle schématique de la relation Cd-taille chez *Mytilus* spp.

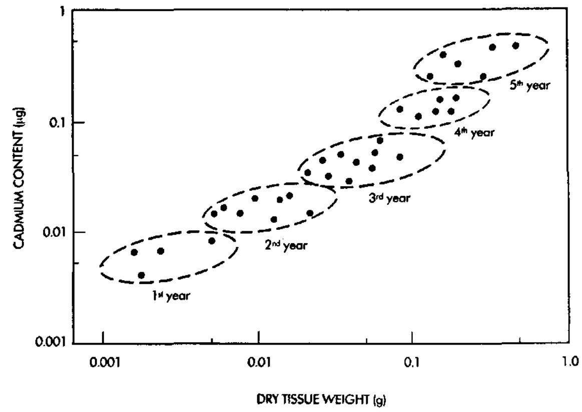

Figure 5
Inverse effect of size and age on Cd content in Mytilus edulis *(Pointe Mitis, Gulf of St. Lawrence). Cossa and Pouliot, unpublished.*
Effet inverse de la taille et de l'âge sur le contenu en Cd chez *Mytilus edulis* (Pointe Mitis, golfe du Saint-Laurent). Cossa et Pouliot, résultats non publiés.

encountered in the case of Cd in *M. edulis.* In short-term bioaccumulation experiments, the uptake of metal is higher in the smaller individuals than in the larger ones. This fact has been observed on several different occasions (Fowler and Benayoun, 1976; Fowler *et al.*, 1978; Fig. 3). The result is a negative slope for metal/-size concentration relationships (or less than one) in the case of contents/size. This explanation is also valid for observations carried out on natural populations (Cossa *et al.*, 1980).

Conversely, higher metabolism in juvenile individuals can cause a reduction in metal concentrations in soft tissues, because the tissues grow more quickly than the metal can be absorbed. Phillips (1976*b*) suggests that this mechanism explains seasonal variations recorded in Cd concentrations in *M. edulis.* Strong and Luoma (1981) have built a generalization around this hypothesis based on their results for Cu and Ag in molluscs: "In nearly all species, growth rates in younger individuals exceed growth rates in older individuals. Thus, dilution of metal concentrations by tissue growth should have a greater effect in smaller animals than in larger animals from the same population, causing a positive slope in metal concentration-body size correlations." It may be added that such a slope may also be interpreted in terms of net accumulation of a certain amount of metal throughout the life of the organism.

In a situation where the metal concentration remains constant no matter what the size of the animal, it may be supposed that absorption is equal to excretion; the uptake depends on the surrounding levels. The rate of excretion is linked to the equilibrium of the metal with the internal ligands and the animal's physiology (*cf.* Simkiss *et al.*, 1982).

In contaminated environments, if molluscs are in a steady state with their environment, positive slopes are more often observed (reflecting net accumulation in old individuals), whereas in environments with low concentration levels the relationship is not significant. These observations made by Bryan (1976) and Boyden (1977) tend to show that the metal/size relationship depends on the level of exposure to the metal.

Mathematical modelling

After the publication of the bio-energetics-based model for contaminant accumulation by Norstrom *et al.* (1976), Fagerström (1977) proposes a general interpretation of the interdependence of the trace substance content and the size of the living organisms. A parabolic equation describes the ratio between the trace substances, especially the metals, and the weight of the animals, molluscs in particular:

$$Y_i = \alpha (W_i)^{\beta}. \quad (1)$$

Where W_i represents the weight of the individual i and Y is the metal content of the individual i; α and β are the particular parameters of each experiment. This equation approaches that of allometric growth.

$$M_i = a(W_i)^b. \quad (2)$$

Where M_i is any measurement of energy flow; a and b are adjustment parameters. This resemblance can lead to confusion between β and b, which are, *a priori*, distinct, as one refers to a static, and the other to a kinetic model.

Fagerström has studied animals having different weights in an experiment where the energy flow is in a steady state. If the animal's food is contaminated by substance Y to a constant concentration level, the absorption of Y is proportionate to the metabolic rate of each animal and varies according to its size:

$$I_i^0 = (W_i)^b. \quad (3)$$

The superscript ^{0} shows temporal independence. Substance accumulation rate is shown by the equation:

$$\dot{X}_i(t) = I_i^0 - \lambda_i(t) X_i(t) \quad (4)$$

which may be otherwise expressed as:

$$X_i(t) = I_i^0 e^{-L_i(t)} \int_0^t e^{L_i(t)} dt \quad (5)$$

with $X_i(0) = 0$.

Here $\lambda_i(t)$, the "turnover" is defined as the average probability of a molecule leaving the animal per time unit; $L_i(t)$ is the primitive of $\lambda_i(t)$.

The form of such a function is knwon and has been verified in a number of cases. Following an initial accumulation phase, tissues attain a constant level and equilibrium is reached.

The "turnover", however, will generally vary over a period of time during the accumulation phase. There is no simple analytic solution to equation (5), and aside from solution (3), there is no real evidence of relation to weight. When body weight reaches steady state, the contents of the substance in the animal reach a stationary level. At equilibrium, λ_i is independent of time (λ_i^0), which indicates that a stage exists in the elimination rate.

According to Reichle and Van Hook (1970) as mentioned by Fagerström, the substance is considered "biologically indeterminant" if its turnover at equilibrium procedes at a rhythm proportional to the animal's metabolism. In this case:

$\lambda_i^0 = M_i/W_i$ and therefore according to equation (2):

$$\lambda_i^0 = (W_i)^{b-1}. \quad (6)$$

At the equilibrium $\dot{X}_i(t)$ tends to zero, equation (4) thus becomes:

$$X_i^0 = \frac{I_i^0}{\lambda_i^0}. \quad (7)$$

Then, replacing I_i^0 by its value in equation (1) and λ_i^0 by its value in (6):

$$X_i = (W_i)^1 \quad (8)$$

dividing by W_i then $C_i^0 = (W_i)^0 = \text{constant}$ with C_i^0 the concentration of the substance in the animal in a steady state.

In the case of a "biological indeterminant" substance, where the turnover is proportional to the rate of metabolism, β is equal to 1 in equation (1). It has been seem that in certain experiments this equation parameter indeed describes the metal/size correlation. In reality, however, parameter β may have values both higher or lower than the unit. This means that the metals in question are not "biologically indeterminant" since their turnover rate is influenced more by the specific cellular activity with which the metal is insolved than by the general metabolic rate.

To this must be added the fact that nothing is less certain than the existence of a steady state of equilibrium of the substance in the animal at any given moment, due to the considerable variability of the physiological activity of the animal and the bioavailability of the metal in the environment over a period of time. Therefore the metal/size relationships established in the environment, although highly significant, are "coincidental" in the sense that the parameter calculated must be interpreted as parameter β in the equation for allometric growth.

In the case of Hg the encountered situation is better explained by a model taking into account also the food transfer (Thomann, 1981).

Effect of sex and reproduction

The possible effects of the reproductive cycle have often been mentioned as an explanation for the variations in metal content in the mussel. The fact that during spawning up to 40% of soft tissue weight is lost shows the importance of gametogenesis in the physiology of the genus *Mytilus*.

What is the metal content of gonad tissue? Is it different from that of somatic tissue? Is there somatic variation? Is there metal content difference between the sexes? The answers to these questions should allow the establishment of a strategy to minimize the effect of gametogenesis while studying mussels during monitoring programmes. However data on Cd and Hg are scarce; informations on the effects of sex and reproduction have to be found from studies on other metals.

Schulz-Baldes (1974) observed that the Pb level in *M. edulis* changes after spawning. Walting and Walting (1976) have clearly shown differences in concentrations of Zn, Mn and Cu in *Choromytilus meridonalis* between mature males and females, with the latter having the highest levels. Orren *et al.* (1980), however, in work on the same species, only observed this difference at a certain time of the year. Latouche and Mix (1982) in the case of Mn and Zn in *M. edulis* observe higher concentrations in the gonad tissue of the female specimens. In contrast, Cd is more concentrated in the females' somatic tissue. According to these authors, this could be the result of energy flow between the somatic and gonad tissues during different gametogenetic stages, at least where metals essential to enzyme activities are concerned. Another finding by Latouche and Mix (1982) indicates that Zn, Mn, Ni, Cu and Cd are more abundant in somatic than in gonad tissue. The gonad tissue mass during the reproductive cycle would entail an increase in metal concentration of those tissues, at the expense of total concentration in the whole animal.

These concentration differences between gonad and somatic tissues were confirmed in the case of Zn in *M. edulis* in a study by Lobel and Wright (1982). Gabbott and Bayne (1973) showed that the acceleration of gametogenesis was accompanied by an increase in energy requirements, and therefore resulted in an increase in the speed of filtration rates and food assimilation. Such potentially influential metabolic changes have been treated in the preceding sections.

However, gametogenesis involves specific metabolic systems which are capable of influencing metal metabolism in a particular way. Widdows (1978) showed that ammonia excretion is at its lowest in autumn and the beginning of winter during the period of sexual inactivity and that it increases to its maximum level in springtime and summer when mature gametes are present in the mussel's mantle. This reveals a change of principal energy source from glycogen in the autumn to proteins in spring. Since metals in molluscs are preferentially associated with proteins, it is likely that metallic flux is influenced by these seasonal metabolic changes. Is the summer increase in nitrogenous excretion with its increase in protein catabolism partly responsible for reduction in metal concentration during the summer season?

Effects of temperature and salinity on the accumulation of cadmium and mercury

The use of radio-isotopes in laboratory accumulation experiment on *M. galloprovincialis* has shown that temperature does not seem to have an influence on the accumulation of Cd but does induce a slight increase in Hg uptake (Fowler and Benayoun, 1974; Fowler *et al.*, 1978). In contrast Fischer (1986) has shown that a rise in temperature accelerates the building of Cd in soft tissues while their growth is reduced. However, it was noticed that the relation between Cd body burden and shell weight is independent of temperature between 7 and 25°C.

Phillips (1976*a*) has shown that Cd is better absorbed at lower than at high salinities, and this observation is confirmed by Jackim *et al.* (1977).

George *et al.* (1978*b*) also observed that mussels accumulate more Cd in dilute seawater than in 100% seawater. They concluded that this increase in uptake of cadmium is due to the effect of osmolarity of the surrounding medium.

In a more recent experiment, Fischer (1986) has pointed out that there is no change in Cd concentration in soft tissues of mussels grown in salinities between 10 and 35 on condition that keeping constant the molar ratio of Cd and seawater salts. This observation could be the result of variation of the Cd bioavailability along salinity gradients.

Action of other metals on the accumulation of cadmium and mercury

The absorption of Cd seems unaffected by the presence of other metals (Phillips, 1976*a*), whereas the results of Jackim *et al.* (1977) show influence by Zn: the absorption of Cd by *M. edulis* is lowered in the presence of increasing does of Zn. Similar results have been obtained by Elliott *et al.* (1986). Phillips (1980) remarks that the effect noticed by Jackim *et al.* only occurs when levels of Zn are extremely high (up to several hundreds of $\mu g . L^{-1}$). The results of Fowler and Benayoun (1974) do not show evidence of any influence of Zn on the uptake of Cd by *M. galloprovincialis.*

Ritz *et al.* (1982) and Eliott *et al.* (1985) show that intermetallic interaction is not simply a question of concentration but may be linked to exposure rhythm. Indeed, mussels when continuously exposed to a constant concentration of Cd, accumulate this metal at the same rate whether or not it is accompanied by other metals in the environment. On the other hand, the rate of accumulation is greatly reduced when the exposure is discontinuous and cyclical. The same type of results have been obtained by Coleman *et al.* (1986) who show that cadmium accumulation in *M. edulis planulatus* may vary with the manner with which the metal dose is administered. These authors conclude that the relationship between metal levels in mussel tissues and average metal levels in the surrounding water is not necessarily a simple one. If there is no doubt that mussel is a good indicator of metal contamination in the environment, Coleman *et al.* (1986) raise the question of the extent of the capacity of the bivalve to integrate the contamination.

Concerning interaction between Hg and other metals, Fowler and Benayoun (1976) have found no significant influence of this metal on the uptake of Se in *M. galloprovincialis.* On the contrary, Pelletier (1986) shows that in *M. edulis* the accumulation of Se is doubled when Hg is spiked in the experimental medium. This effect is visible with inorganic Hg and is even more noticeable with organic Hg. However, in

Pelletier's experiment the uptake rate of Hg is not at all modified by the presence of Se. In addition, attempts to detect a protective effect of Se on preventing toxicity from methylmercury, as observed for marine mammals, remain unsuccessful in the mussel (Pelletier, 1988).

Influence of position in the intertidal

De Wolf (1975) observed that the concentrations of Hg in *M. edulis* and *M. galloprovincialis* were in general, for the same site, higher for individuals in the intertidal zone than for continually immersed animals. Our results on *M. edulis* in the Gulf of St. Lawrence do not give any clear evidence that the location of the mussel in the intertidal has any influence on its Hg concentration level (Bourget and Cossa, 1976).

Coleman (1980) noticed that mussels subjected to periods of emersion accumulated less Cd than those which were continuously immersed, with differences of accumulation which did not depend simply upon immersion times.

Distinct metal concentrations may be generated by the effects of stress from immersion, differences in growth rates or bioavailability of the metal according ot the mussel's location in the intertidal area. In these circumstances, the above differences would be geographically related.

OPTIMIZATION OF THE USE OF THE GENUS *MYTILUS* AS AN INDICATOR ORGANISM OF METAL CONTAMINATION IN THE COASTAL ENVIRONMENT

In the preceding section we have reviewed the principal factors likely to influence metal concentrations in the mussel; in the following sections we shall establish methods allowing to minimize the effects of these factors in order to ensure that the levels of metal in the mussel reflect as faithfully as possible its bioavailability in the environment. This constitutes the optimization of the monitoring tool.

The factors to be considered will be those of size, influence of sampling season, intertidal level of the mussel samples, the presence of food in the digestive tract, temperature and the surrounding salinity levels. Some considerations will follow concerning the statistical aspects of data treatment and the possible developments of "mussel watching" through the use of particular tissues.

Minimizing the size factor

Several strategies may be used to minimize the effects of the mussels' size:

1) Analyzing individuals selected in the narrowest possible range of shell length.

2) Analyzing individuals representing the whole range of size collected from the sampling site under consideration; *i. e.* conducting individual analyses in order to establish, for each sampling site and/or season, relationships between size and metal contents.

3) Analyzing a homogenate of mussels of all sizes presented at the sampling site.

4) Making an *a posteriori* correction by normalizing the metal contents in relation to a given size based on a preestablished model.

Goldberg *et al.* (1978 and 1983) have observed that a range of size from 50 to 80 mm can introduce a variation in concentration of a factor of two. It is therefore necessary to restrain the size range collected in order to arrive at a better resolution. The group of experts assembled at Barcelona in 1978 recommended selecting a size-range of 5 to 10 mm and to center it on shell lengths averaging 60 mm (NAS, 1980). These recommendations are only indicative and derive from a mean situation of mussel size throughout the world. More important than the length of the shell determining this parameter is the age. In temperate climates, mussels measuring 60 mm long are may be two years old, whereas at the same size in subarctic regions they will be at least three time as old. Limiting the range of size of the mussels collected can nevertheless allow optimization of studies of temporal trends in a single site, even if this procedure does not solve the problem of interpreting geographical trends.

We have quantified the variability of concentrations in Cd in the same bed of *M. edulis* (Pointe Mitis, Gulf of St. Lawrence) according to size class. The data used combine 18 series of 30 to 40 mussels from 1 to 6 cm collected at regular periods for one and one-half years (Pouliot and Cossa, unpublished data). Variance analysis shows that when results are expressed terms of Cd contents, 16% of the variation is due to the collecting season, 74% to the size and the remainder to uncontrolled causes. The relative amplitude of the variations differ widely according to the size-class (Table 2). Mussels of average length (3 to 4 or 4 to 5 cm) present the least variability.

Table 2

Coefficient of variation (%) of Cd content and concentration for different size ranges of *Mytilus edulis* from Pointe Mitis (Gulf of St. Lawrence).

Coefficients de variation (%) du contenu et de la concentration en Cd pour différentes classes de taille de moules (Mytilus edulis) *provenant de Pointe Mitis (golfe du Saint-Laurent).*

	Size class				
	1-2 cm	2-3 cm	3-4 cm	4-5 cm	1-5 cm
Content	88	57	47	46	126
Concentration	125	55	41	43	99

The second solution requires the collection of individuals of all sizes. This procedure, however, is difficult to carry out in practice because of the large number of analyses required and the possible changes in the size distribution in the mussel bed with time and location. The third solution may represent an economic choice, Phillips and Segar (1986) discuss the interest of bulking. Space-bulking involves the sampling of animals from several locations and the amalgamation of their tissues into a single sample; time-bulking is also proposed. The advantage of these techniques is to smooth out the effects of short-term variations in space or time.

Table 3

Regression coefficient (*b*), correlation coefficient (*r*) and number of determinations (*n*) for the log-log relationship between Cd content and shell weight and Cd content and intervalve volume of *Mytilus edulis* from Pointe Mitis (Gulf of St. Lawrence).

Coefficient de régression (b), coefficient de corrélation (r) et nombre de déterminations (n) de la relation entre le log du contenu en Cd et le log du poids de la coquille ou le volume intervalvaire chez Mytilus edulis *en provenance de Pointe Mitis (golfe du Saint-Laurent).*

Date	Cd/Shell weight			Cd/Intervalve volume		
(month-year)	*b*	*r*	*n*	*b*	*r*	*n*
07-78	0.70±0.06	0.96	43	0.76±0.11	0.93	32
08-78	0.65±0.09	0.91	41	0.60±0.10	0.89	34
08-78	0.91±0.08	0.96	40	1.06±0.11	0.96	33
09-78	1.14±0.07	0.98	39	1.38±0.15	0.96	31
10-78	1.01±0.07	0.98	39	1.06±0.09	0.97	33
11-78	0.82±0.06	0.98	38	0.88±0.07	0.98	32
12-78	0.94±0.07	0.97	40	0.95±0.07	0.98	34
01-79	0.87±0.11	0.93	39	1.06±0.11	0.96	32
03-79	0.83±0.08	0.96	39	0.92±0.11	0.95	30
03-79	1.02±0.08	0.97	41	1.11±0.09	0.97	36
04-79	0.75±0.12	0.87	43	–	–	–
04-79	0.93±0.10	0.94	42	–	–	–
05-79	0.86±0.07	0.96	42	–	–	–
05-79	1.01±0.08	0.96	43	–	–	–
06-79	1.10±0.10	0.97	34	–	–	–
06-79	0.91±0.07	0.97	42	–	–	–
07-79	1.27±0.08	0.98	39	–	–	–

However, pooling mussels has the consequence of losing information on individual variability and excludes certain statistical treatments.

The *a posteriori* normalization of metal concentrations at a constant size can be recommended. This consists either of normalization at constant weight or normalization based on multiple regression involving several explanatory parameters. Applied to our data on Cd in *M. edulis* from Pointe Mitis (Table 2), normalization in relation to shell length reduces the variation coefficient to 35%, whereas a double normalization (shell length and tissue weight) allows variability reduction to 29%. The results of these treatments constitute clear improvement in the utilization of the unnormalized data presented in Table 2. A similar normalization procedure was used in the case of Hg in the same species (Cossa and Rondeau, 1985). This last approach is, however, open to criticism since it refers to a model which, as we have seen, is not stable due to the spatio-temporal variations of the metal/size regression coefficients. However, we have estimated that for a 20 mm interval in length the absence of normalization was capable of generating a greater error than that made from variations in regression coefficients of the models used.

An alternative to metal concentration correction for the size factor has been proposed by Fischer (1983). In order to obtain a model as independent as possible of the physiological condition of the mussels, Fischer proposed expressing the Cd contents not per unit of dry soft tissue weight but per unit of shell weight. The reason for this is that whereas the relation of Cd content to soft tissue weight gives an exponential curve, the regression line between Cd content and shell weight is perfectly linear. Since the shell weight is a better indicator of mussel age than tissue weight and since the slope obtained is superior to 1, these results show the net accumulation of Cd during the lifetime of the animal. We have applied this model to Pouliot's data (1983). The results are shown in Table 3. Contrary to Fischer's results, the regression coefficients between the Cd contents and the shell weight (or the intervalve volume, both being good indicators of mussel age) present statistically significant variations from single to double in both cases. Expressing Cd contents as a function of shell weight and therefore age leads to eliminating the effects of variations in soft tissue weight; but there still remains another source of variation in the metal/shell weight curves: the changes in metal bioavailability in the environment. At Pointe Mitis where Pouliot sampled mussels (1983), there was a noticeable seasonal hydrological variability (Cossa *et al.*, 1988), thus seasonal variations in Cd bioavailability are likely to occur at this station. This situation probably occurs frequently in coastal areas where physical conditions are variable (water circulation influenced by the proximity of an estuary, upwelling, convergence, unstable stratification, *etc.*), and where cyclic changes in primary productivity, due to successive phytoplanktonic bloom, cause Cd concentration to vary rather rapidly in the euphotic zone. To sum up, the index proposed by Fischer is applicable only in environments which are stable relatively to metal concentrations.

Choice of collecting season

In recent years, numerous studies have appeared which increase the body of knowledge about the structure of seasonal variations of metal concentrations in the genus *Mytilus* (Karbe *et al.*, 1977; Majori *et al.*, 1978; Orren *et al.*, 1980; Ouellette, 1981; Farrington *et al.*, 1983; Meeus-Verdinne *et al.*, 1983; Pouliot, 1983; Cossa and Rondeau, 1985; Amiard *et al.*, 1986). The ratio between maximum and minimum concentrations encountered during an annual cycle is generally below three. Mussels from marine regions show lower variations than those from estuarine environments.

All the studies cited above are in agreement concerning the existence of a spring or winter maximum and a summer or autumn minimum. For several authors, this pattern is the result of changes in soft tissue due to the gametogenetic cycle. Ouellette (1981) and Cossa and Rondeau (1985), however, conclude that variations in metal levels are in large part related to the bioavailability of these elements in the environment.

Different strategies may be used to minimize the influence of seasonal cycles: 1) collect samples several times a year at fixed periods; 2) collect samples during the period of greatest stability of metal concentrations. This corresponds to the moment when changes in soft tissue weight and environmental conditions are at their minimum: neither during spawning nor, in the case of estuarine environments, flood. The collection period should be determined for each site by a special study which includes metal concentration results for over a minimum period of one and a half annual cycles.

Another way to minimize the effect of seasonal changes (3) consists of partially correcting metal contents for soft tissue weight. This correction is made possible by using the multiple regression functions between the metal content on the one hand and the age and weight of the soft tissues on the other; in other words, using the models described in the previous section.

Sampling position on the shoreline

We have mentioned above the possible causes for the differences in metal content in mussels from the same site collected at different intertidal and subtidal levels. In the absence of other information, the recommendation of the Barcelona symposium (NAS, 1980) remains valid: "... (Samples for a large-scale monotoring program) should be collected at the mid-tide zone halfway between marks for spring low and high waters." The vertical position of sampling should be carefully annotated for each location, and preferably also photographed, so that further sampling could be reproducibly undertaken if necessary.

Depuration

The digestive tract of the mussels may be cleared out by maintaining the animals in filtered seawater for a certain length of time (24 to 36 hours). The contents of the gut have a greater or lesser influence on the total metal contents of the undepurated mussel depending on the element. According to Ouellette (1978) the gut content/total content ratio is low for Cd, Cu and Zn ($< 10\%$) whereas it is very high for Al and Fe ($\approx 100\%$), Mn (67%) and Cr (41%). The results of Latouche and Mix (1982) confirm a significant reduction in Mn concentration after purging whereas Cd and Zn concentrations remained unchanged following this treatment.

The procedures for correct cleaning of material used for measuring trace elements in the marine environment should be adopted for depuration, in order to prevent the decontamination treatment from turning into one of contamination.

Temperature and salinity

To interpret the results of monitoring by biological indicator organisms it is important to have hydrological data for the collecting sites. Temporal series are, of course, of greater help than sporadic measurements for characterizing the environment. Such studies are to be encouraged in order to obtain the greatest benefit from monitoring data. In addition, Cd/shell index, as proposed by Fischer (1986) seems relatively independent of temperature between 7 and 25°C and salinity between 15 and 30; this index can be used as a normalization procedure.

Statistical considerations

In monitoring programmes, analysis of the animals one by one is a typical strategy. Gordon *et al.* (1980) have tried to establish the optimum number of individuals to sample for each metal in order to make the best use of *M. californianus* as sentinel organism. Three to seven individual analyses are necessary to detect differences of 80% between two averages for Cd, Cr, Cu, Fe, Ni, Pb and Zn; six to thirty individuals are necessary for differences of 40%. The number of specimens is, however, not the same from one site to another, and even from year to year at the same site. Under these circumstances physiological or hydrological differences become supplementary information of the first order of importance, as they ensure the reliability of trend estimations.

The analysis of a homogeneous sample consisting of many individuals has the advantage of giving a good estimation of the average concentration of the population with a limited number of measurements. However, using a mussel homogenate excludes a certain number of statistical treatments including regression which requires a minimum number of data. In addition, the variance necessary for statistical comparisons is no longer available. It should be remembered that the homogenate variance can only be used if the number of individuals in the homogenate is the same.

Finally, the interpretation of monitoring data requires statistical tests which are most frequently based on the normality of metal concentration frequency distribution. Lobel and Wright (1983) have shown that Zn concentration in *M. edulis* shows a positive asymetrical distribution; several individuals may be considered as "super-accumulators". Under these circumstances if a small number of individuals are collected, it is wise to conduct individual analyses so as to detect any exceptionally high levels.

Use of organs or particular tissues

Soft tissues

The use of particular tissues in bivalves in monitoring programmes can in certain cases offer advantages over using the totality of soft tissues. Certain organs, such as the digestive gland and the kidney, concentrate metal more than others: their analysis can provide a solution to problems of detection limit. The principal interest, however, resides in the fact that different tissues do not have the same reaction to changes in metal bioavailability in the environment (Eganhouse and Young, 1978). To this end, the study of temporal variation of metals in various organs may provide information about: 1) the time necessary for each tissue to respond to changes in the supply of metal in the environment; 2) predominant site of absorption; and 3) the importance of spawning on metal concentrations in total mussel tissue (NAS, 1980).

In *M. edulis* in the Bay of Bourgneuf (France), Amiard *et al.* (1986) have observed a rather uniform distribution of Cd, Pb and Zn among the viscera, the gills and the rest of the body. Seasonal variations in the metals in these three tissues present the same pattern, with a maximum in winter and a minimum in summer. Other tissue studies generally concern accumulation experiments which indicate that the gill and viscera tissues by which uptake occurs accumulate more metals than the others (Schulz-Baldes, 1974; Eganhouse and

Young, 1978; Scholz, 1980). Elimination rates are consistently different depending on the tissues, being fastest in the gills and the digestive tract as shown by Guary and Fowler (1981) for Am and Pu.

During a study carried out over a period of two years at Pointe Mitis in the Gulf of St. Lawrence, Cd levels were studied in different organs: gills, digestive system, mantle, posterior adductor muscles, foot and mantle-edge (Cossa, unpublished). Cd concentration levels in the different organs show the following order: gills > digestive system > mantle > mantle edge > posterior adductor muscle > foot. On the basis of coefficients of variation (CV = standard deviation/average), the mantle presents the greatest concentration variations (44%), followed by the gills and the foot (35%), the posterior adductor muscle and the digestive system (30%); the mantle edge shows the lowest, with only 26%. The overall CV in the total soft tissues is 34%. These seasonal variations are shown in Figure 6. It may be noted that the pattern is characterized by an autumn minimum and a spring maximum, especially as regards the gills and the mantle. The digestive gland presented more uncertain variations perhaps as a result of the use of undepurated animals.

This *in situ* work on Cd in *M. edulis* has on a practical level led to the following conclusions: 1) because of the low variability of their concentrations, the mantle edge and the posterior adductor muscle may be considered as the most adequate tissues for the purposes of monitoring; for routine utilization, however, problems of dissection and microanalysis may be encountered; 2) the use of the mantle is advised against because of wide variability of concentrations during the seasonal cycle; 3) analysis of the gills could prove to be very useful, especially if interest centers on the very short-term changes in Cd bioavailability in the environment.

The shell

The use of the shell of *M. edulis* for measuring contamination was suggested by Sturesson (1978). This author has shown that there exists a proportionality between Cd levels in the water and in the shell (periostracum, aragonite and calcite). The concentration factor between water and aragonite is about 100 in experimental conditions (52 days; 25 to 200 µg Cd.L^{-1}; 18°C). Cd accumulation in the periostracum is greater than in the calcareous part. Koide *et al.* (1982) have established a list of the advantages of using the shell rather

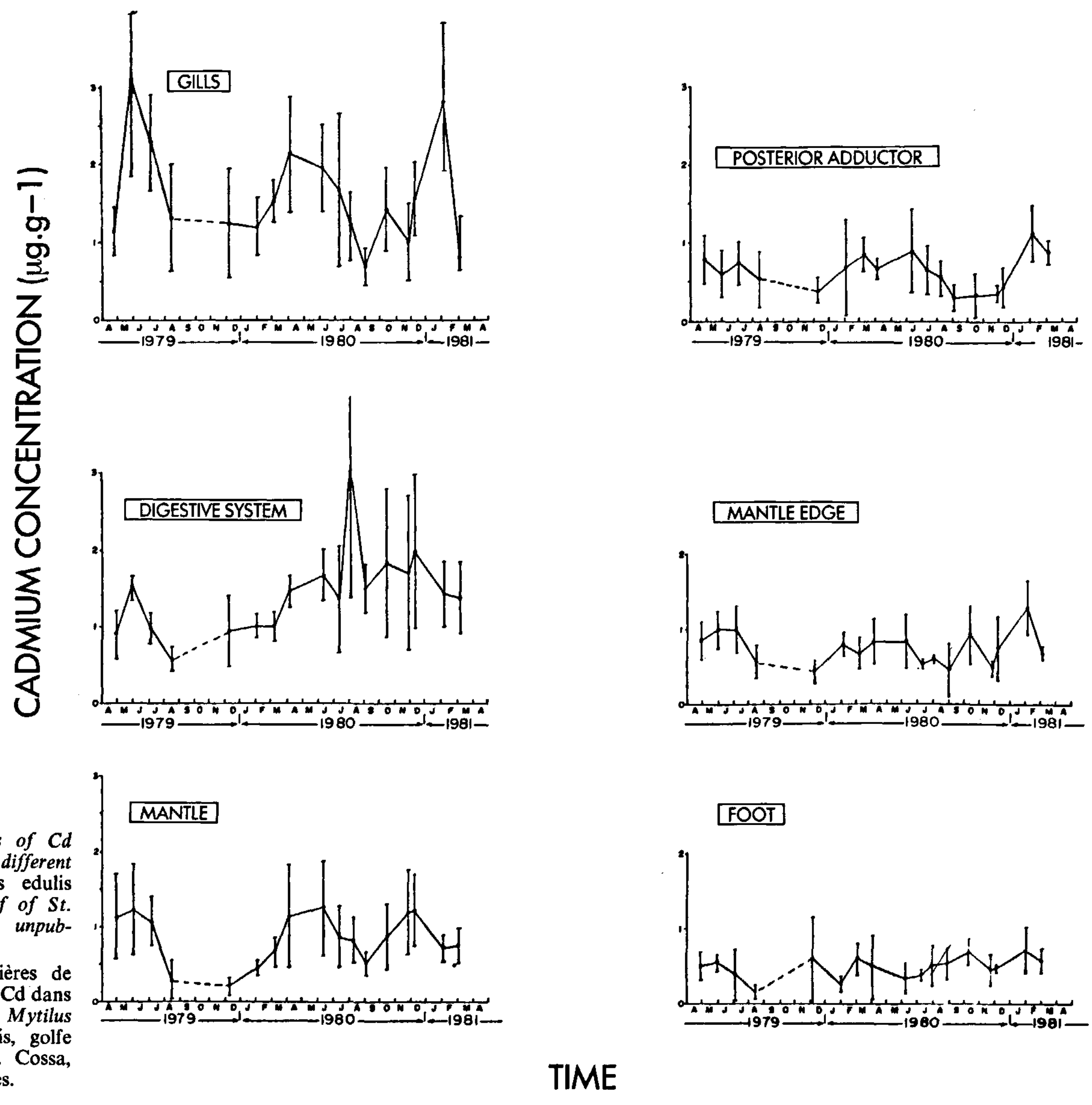

Figure 6

Seasonal variations of Cd concentration in different organs of Mytilus edulis *(Pointe Mitis, Gulf of St. Lawrence). Cossa, unpublished.*

Variations saisonnières de la concentration en Cd dans différents tissus de *Mytilus edulis* (Pointe Mitis, golfe du Saint Laurent). Cossa, résultats non publiés.

than the flesh. Among the metals analyzed by the authors (Cd, Cu, Zn, Pb, Ag, Ni and Pu) only Pb and Pu presented significant correlation between concentrations in the soft tissues and the shell. Al-Dabbas *et al.* (1984) have recently demonstrated the usefulness of *Mytilus* periostracum for Cu contamination monitoring. According to Borchardt (1983 and 1985) Cd accumulation *via* the shell seems to be free from effects of metabolic changes, perhaps as a result of absorption without any excretion. This quality in the shell present may be a great avantage over the use of soft tissues: the "memory" of the shell should go back to its formation and the loss during the animal's lifetime may be slight. However, the observations made by Guary (1980) on uptake and loss of transuranic elements by the shell of *M. galloprovincialis* suggest that further work is needed before drawing a firm conclusion on this subject.

Sub-cellular level

A study at the sub-cellular level rather than the level of the organs could prove interesting. For example, the metal in the haemolymph, which rapidly establishes equilibrium with the environment, could be used as a tracer of current bioavailability of metal in the environment. On the other hand, metal immobilized in the intercellulal granules (a compartment with a slow turnover) can serve as a control for concentration levels in the environment over a longer period of time. The results of this type of research (Paquet, 1983) are still too few in number to allow recommendations for monitoring. Research in this direction has the possible disadvantage of running counter to the need for simplicity in the accomplishment of monitoring strategies, as, for instance, in the case of the high complexity involved in cellular fractioning. At all events, it is necessary to optimize and diversify the information drawn from indicator organisms by defining in advance the specific objective(s) of the particular monitoring operation: concentration levels, location of "hot spots", geographical or temporal trends, *etc.*

CONCLUSION

The examination of the variations of metal concentrations in the genus *Mytilus* has allowed to evaluate the capacities and the limits of the use of the mussel as a sentinel organism of Cd and Hg contamination. We have described optimization methods for this tool. From a practical point of view, there should exist an adequation between the amount of analytical efforts (*i. e.* costs) and the specific goals of each monitoring programme. For temporal trend analyses at a particular station, the analysis of the whole soft tissue of a few mussels or of a pooled sample of similar shell length

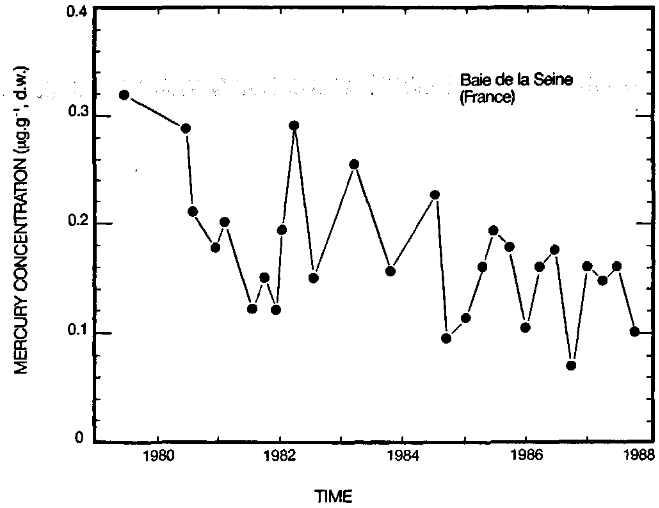

Figure 7

Temporal variations of mercury concentrations in soft tissue of Mytilus edulis *(baie de la Seine, France). Réseau National d'Observation (Claisse, pers. comm.).*

Variations temporelles de la concentration en mercure des tissus mous de *Mytilus edulis* (baie de la Seine, France). Données du Réseau National d'Observation (Claisse, communication personnelle).

collected at different periods during the year should be an acceptable approach. Figure 7 illustrates this point in the case of the French Mussel-Watch (Réseau National d'Observation). In order to determine geographical patterns, normalization techniques, as set out in this review, are definitively required. An example is provided by the treatment of mercury data from the St. Lawrence Mussel-Watch (Cossa and Rondeau, 1985). In this study, the use of normalized mercury contents (for both shell length and soft tissue weight) made the interpretation of the geographical distribution pattern in relation to estuarine and Atlantic influence possible. It remains however to establish the relationship between the concentrations in the sea water and those in the bivalve, especially in so far as good management of the shore environment is concerned. Some attemps have been made (Talbot, 1985; Cossa, 1989).

It is true that the genus *Mytilus* is widely used for monitoring but it is also true that most of the current practices fall well below the possibilities that this animal affords. Research should continue on the best way of optimizing and specializing information from mussels. The transfer of research results to monitoring programmes should be kept in mind as a part of the quality control programmes.

Acknowledgements

Thanks are due to Drs. S. W. Fowler and J.-C. Guary for their comments on the manuscript.

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