A universal method for quantifying and comparing the residual variability of element concentrations in biological tissues using 25 elements in the mussel *Mytilus edulis* as a model*

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Abstract

The concentrations of elements in biological tissue may be influenced by a large number of environmental and physiological factors. Even when all known sources of variability have been either eliminated or taken into account, a very high degree of unexplained residual variability may persist between individual organisms within the same population. In the present study, a simple statistical method is described which permits the calculation of the residual variabilities of element concentrations, even from complex multivariate data, and allows statistical comparison between elements to be made (either within a single tissue, between different tissues or between different species). The method is quite general and could also be used for studying residual variability in any other natural phenomena (e.g. enzyme concentrations, water temperature). The case of 25 element concentrations in the whole soft tissue of the mussel *Mytilus edulis* collected from Bellevue, Newfoundland, Canada in June 1988 was used as a model. It was clearly shown that some elements (e.g. the alkali earth elements and B, Mg and Cu) had extremely low residual variability while other elements (e.g. Ce, Zn, Ba, La, U, Pb, Ag, Y, Sr and Ca) showed unusually high degrees of residual variability. Al also showed very high variability but this appeared to be due to the presence of undisgested sediment in the gut rather than to residual variability. Important sources of non-residual variability included sex, size and growth rate. The method described in this paper could be used as an initial screening test for pinpointing intrapopulation genetic differences in element metabolism between individual organisms.

Introduction

Marine organisms can concentrate trace elements in their tissue from the surrounding seawater. Generally speaking, the concentration of a given element in the tissue of a marine organism is more or less proportional to the amount of that element in seawater. Thus, organisms which can accumulate high levels of trace elements (e.g. marine bivalves) have been used as biological indicators of toxic trace metal pollution (Phillips 1980). For example, the blue mussel *Mytilus edulis* is commonly used as an indicator not only of heavy metal pollution but also of pollution associated with oil, polycyclic aromatic hydrocarbons (PAHs), halogenated hydrocarbons, radioactive wastes, etc. (International Mussel Watch 1980, Bayne et al. 1985).

Whilst the level of trace elements in seawater is clearly an important factor in determining the trace element concentration of the individual organism, many other factors also play a role. These extrinsic factors include size, growth rate, age, sex, allometric growth ratios, seasonal variation, reproductive condition, height in the water column, microhabitat, salinity and interaction with other pollutants in the environment (Phillips 1980). Fortunately, it is generally possible to eliminate variability due to extrinsic factors by rigorous collecting techniques. However, even when extrinsic factors are completely eliminated by the collecting technique, a considerable amount of residual variability may remain even within a small single site, i.e., variability which cannot be explained by, or associated with, any known physiological or ecological parameter (International Mussel Watch 1980, Boyd and Phillips 1981, Lobel et al. 1982). Residual variability could result from unknown environmental/physiological factors which influence trace element concentrations or from genetic differences between individual organisms.

In previous studies, it was shown that the Zn concentrations of the whole soft tissues of the filter-feeding mussel *Mytilus edulis* showed an unusually high degree of residual variability (Lobel and Wright 1982a). This was mainly due to kidney Zn concentrations which ranged from 100 to 8000 ppm at an unpolluted site and from 1500 to 24000 ppm (2.4% Zn%) at a polluted site (Lobel 1986, 1987a, b, c). Most of the variability of Zn in the kidney appeared to be due to variability in the amount of Zn stored...
in the insoluble fraction, (i.e., lipofuschin granules). The role of kidney lipofuschin granules in mediating the metabolism of many metals in *Mytilus* spp. is well known (George and Pirie 1980, George et al. 1982, George 1983, Roesijadi et al. 1984). However, Zn variability has also been linked with a unique very low molecular weight (ca 1 000) Zn-binding ligand in the cytosol of *M. edulis* kidney (Lobel and Marshall 1988).

The findings with Zn naturally led to questions concerning the residual variability of other elements in *Mytilus edulis* as well as in other species. In the present study, a method is described for the determination and statistical evaluation of the residual variability of element concentrations in biological tissue using the concentrations of 25 elements (both major and minor) in *M. edulis* as a model. This method permits comparison of the residual variability of different elements either within a given tissue or between tissues or between species. It can also be used for other types of residual variability.

**Materials and methods**

**Collection and analysis of mussels**

Mussels *Mytilus edulis* were collected from Bellevue, Newfoundland in June 1988 and brought back to the Ocean Sciences Centre where they were kept for 3 d in clean seawater to permit gut evacuation. An extremely rigorous collection procedure was used to reduce environmental differences between individual mussels to a minimum. All mussels were collected at the same time from a very small subtidal site, (i.e., within a few m²) on the flat bottom of a lagoon. As these mussels tend to grow in a monolayer, it is believed that all mussels were receiving essentially the same diet and exposure to seawater, with only trivial differences between individuals.

Soft tissues were then separated from the shell and the sex (SE) of each mussel determined. Soft tissues were dried at 90°C and soft tissue weights (SO) determined. Measurements were also taken of shell weight (SH), length (LE), height (HE) and width (WI). Allometric growth ratios were calculated as follows: flesh condition (FL; mg soft tissue per g shell), width : height (WH), width : length (WL) and length : height (LH). Ranges for SO were 0.27 to 5.08 g and for LE were 36 to 95 mm. All size and allometric measurements were made after the method of Seed (1968). Mussels were prepared for elemental analysis by inductively coupled plasma mass spectrometry (ICP-MS) by first digesting the tissues in boiling nitric acid in Teflon beakers. After dissolution of the mussel tissues, hydrogen peroxide was added dropwise (with violent frothing) to the hot nitric acid digest to break down any recalcitrant lipid material. The digest was then evaporated to dryness and re-dissolved in an appropriate amount of 1% nitric acid. After nitric acid/peroxide digestion, a small amount, (i.e., mg) of sediment remained in the bottom of some beakers probably nonbiological material from the gut of the mussel. The amount of insoluble material remaining in each beaker was subjectively rated on a scale from 1 to 10 which was termed the “index of acid insoluble material” (IN).

**Logarithmic transformation**

It has previously been noted that the frequency distributions of element concentrations in both biological and geological materials tend to show significant positive skewness which some authors have termed “log-normal” (Duval et al. 1971, Esmen and Hammad 1977, Giesy and Wiener 1977, Lobel et al. 1982, Talbot and Simpson 1983, Wright et al. 1985). Generally speaking, a log transformation will considerably reduce this positive skewness and often make the distributions normal. For example, in the present study (Table 1), the vast majority of frequency distributions of element concentrations showed some degree of positive skewness. Conversion of all data to logs considerably reduced this positive skewness rendering the majority of elements either normal or nearly normal. Thus, the (log-transformed) data subjected to the statistical procedures of this study were generally much closer to being normally distributed than the raw data. This is an important consideration since analysis of variance (ANOVA) assumes that data tested is more or less normally distributed. Log-transformations are also useful for reducing the inequality of variance often seen in data of this kind. Such transformations are also an important tool for looking at the relationships between element concentrations and other variables, particularly size and allometric growth ratios (e.g. Boyden 1977). For this reason, all procedures in this paper requiring ANOVA, multiple regressions or Students’ *t*-tests have been done using log-transformed data. However, simple arithmetic means and coefficients of variation (CV) have been calculated from the raw data.

**Index of “relative” variability**

A good index of “relative” variability should have the following properties: (a) it should have no units, being equally valid whether one is talking about elephants or mice and, (b) it should be amenable to statistical manipulation so that valid statistical comparisons can be made between different samples. The coefficient of variation (CV = SD/Mean × 100%) is a well known unitless index of “relative” variation. However, the CV is not easily used in statistical procedures and is most commonly employed as a descriptive statistic rather than an analytical one. For analytical purposes, a much more useful unitless index of “relative” variation is the variance of the log-transformed data (VL). Both CV and VL measure “relative” variation and are independent of the units used, however, only VL can be subjected to statistical analysis using a wide variety of common statistical procedures (Lewontin 1966, Zar 1984).

**Index of “residual” variability**

The VL values (Table 1) reflect the total “relative” variability of the element concentrations including both the ex-