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Relationship Between Body Zinc Concentration and Allometric Growth Measurements in the Mussel *Mytilus edulis* *

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Abstract

The frequency distribution of shell lengths in the mussels *Mytilus edulis* (L.) taken from a site in the Tyne Estuary (UK) in summer, 1980, typically displayed a bimodal shape, with a small peak in the 8 to 20 mm size range and a much broader peak in the 22 to 50 mm region. A collection of mussels was made consisting of two groups, one from each of the above size peaks. The group of larger mussels had a significantly higher mean zinc concentration than the group of smaller mussels, mainly because a few large individuals had very high zinc concentrations. The ratios of shell width : height, width : length and length : height were also significantly higher in larger mussels. However, the ratio of flesh dry wt : shell dry wt (flesh condition) was lower in larger individuals. The whole soft tissue zinc concentration was positively correlated with width : height and width : length, but negatively correlated with flesh wt : shell wt. It was concluded that allometric ratios may provide an attractive alternative to simple size characteristics as a basis for trace metal determinations, particularly in a comparison of metal levels in mussel populations from widely differing habitats where absolute size is a poor indicator of age or growth rate.

Introduction

Bivalve molluscs are now widely used as indicators of trace metal pollution, although individual variability within and between sample batches can still cause problems of interpretation. In monitoring zinc in mussels from the Tyne and Blyth estuaries in northeast England, considerable variation was found in zinc concentration even with a mussel collection made at the same time and from the same site (Lobel and Wright, in press). There is no one factor which can account for such high variances, and it seems likely that several factors contribute to the observed differences between mussels from apparently identical environments.

It has been noted by various workers that the metal concentration in mussels may depend on the size of the mussels collected. The term "size" usually refers to "soft tissue weight" or occasionally "shell length" rather than to some other parameter of body size, e.g. total weight, shell volume, etc. Boyden (1974, 1977) examined 6 metals in *Mytilus edulis* and found that in 5 cases (zinc, iron, manganese, nickel and lead, but not cadmium), the metal concentration was negatively correlated with the soft parts dry weight. Similarly, a negative association between several trace metals and body size was found in *Choromytilus meridionalis* by Watling and Watling (1976). Cadmium concentration in *M. edulis* was found to bear a negative relationship to body size (Phillips, 1976). He also found that weight-dependency for copper and lead in *M. edulis* did not occur consistently and was mainly found in the winter, apparently coincident with gametogenesis. A zinc concentration : body wt relationship was rarely observed by Phillips (1976), although a possible drawback may have been the relatively small sample size used by him, i.e., 10 mussels per collection.

Somewhat contradictory results have been reported by Harris *et al.* (1979) for *Mytilus edulis planatus.* They noted significant *positive* correlation between zinc (also iron and cadmium) and shell length. Negative correlation was found between manganese and shell length. The freshwater mussel *Anadonta anatina* displayed a variety of weight-metal relationships with different metals, depending on location (Manly and George, 1977). Both positive and negative correlations were observed, but no significant relationship was noted for zinc. Davies and Pirie (1978) measured the mercury concentration of every 5 mm size class from 21 to 74 mm in *M. edulis* taken from the Clyde estuary (UK). For most size classes, a tendency towards a negative correlation was observed between metal concentration and wet weight. However, all classes above 55 mm had a higher

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mean metal concentration than all classes below 55 mm. This gave the overall regression line a positive slope. Cossa *et al.* (1980) showed significant negative correlation between dry weight and 6 metals (zinc, iron, manganese, nickel, cadmium and copper). The value of the regression slope *b* was seasonally dependent as well as location-dependent. Cossa *et al.* (1980) studied gonad condition and concluded that seasonal variations of the regression slope was not caused by gametogenic weight changes; they surmised that these variations must be due to other seasonal changes in the physiology and biochemistry of the mussels. This contrasts with Phillips' (1976) results; he places considerable emphasis on the role of gametogenesis in determining metal/weight associations. Cossa *et al.* also noted that age, as measured by shell thickness (Goldberg *et al.* 1978), did not influence the metal concentration *per se,* only insofar as weight differences occurred.

It is obvious then that there is no clear understanding of the mechanisms relating body size to metal concentrations, and the current confusion in the literature still confounds attempts to rationalize such data.

The popularity of determining metal-weight relationships stems from the fact that it is necessary to measure body weight, in any case, in order to calculate metal concentrations. Hence, weight data are always available. Few authors have studied other parameters such as shell thickness, true chronological age or total weight because such data are either difficult or time-consuming to collect. However, the value of "flesh weight" as an indicator of physiological condition is severely restricted. Knowing the flesh weight of a mussel about which nothing else is known tells us very little about the state, condition or age of that mussel. As Seed (1976) pointed out, the size and growth rate of individual mussels is highly dependent on the environment in which the mussel is found.

Although flesh weight *per se* does not appear to be a very useful parameter in metal investigations, a study of flesh weight combined with shell weight, shell length, shell width, shell height and/or various allometric ratios of these measures may be more profitable. The current investigation was therefore designed to assess the usefulness of allometric ratios as a basis for zinc determinations and to discover which of these parameters correlated with zinc levels in specimens of *Mytilus edulis* taken from a relatively homogeneous environment. The site chosen for the investigation was the Black Middens mussel bed on the north side of the Tyne estuary. This is by far the largest mussel bed on the northeast coast of England. The location is named after the black carpet of mussels coating the rocks for a stretch of about one-third of a mile (0.5 km).

Materials and Methods

Mytilus edulis (L.) used for this study came from the Black Middens site described in an earlier paper (Lobel and Wright, in press). The mussels used for zinc determinations were collected on June 16, 1980 as a single "clump" taken from an area of horizontal rockface less than 1 m². Collected specimens were purged in filtered sea water from a nearby site for 48 h and both soft parts and shell from each individual were dried at 105 °C and weighed to within 0.1 mg on a Stanton Unimatic analytical balance. Measurements were also taken of shell length, width and height. Seed (1968) defines the length of a mussel as the maximum measure along the anterior-posterior axis; the height is the maximum dorsoventral axis; the width is the maximum lateral axis.

Oxidation of soft tissues was achieved using a 30 : 1 mixture of nitric and perchloric acid photocatalyzed by an ordinary 100 W tungsten bulb placed about 6" from the digestion vial (Lobel, 1978). Digests from individual specimens were then further diluted for analysis by atomic absorption spectroscopy. All zinc concentrations are expressed as micromoles of zinc per dry gram of whole soft tissue (μmol g^{-1}). A zinc concentration of 1 μmol g^{-1} is equivalent to 65.4 ppm.

Results

The *Mytilus edulis* population of the Black Middens mussel bed had been under intense study throughout the years 1977–1980 with regard to individual size characteristics. Frequency distribution of shell lengths had been plotted for different collections taken at different seasons during the year. Fig. 1 shows the frequency distribution taken on May 4, 1980. This was typical of a summer distribution, having a small peak in the 8 to 20 mm region (termed Peak A), while a much broader peak appears in the 22 to 50 mm region (termed Peak B).

Ninety-eight individuals from a June 1980 collection were selected for zinc analysis. Of these, 29 were especially selected to coincide with Peak A and had shell lengths between 15 and 18 mm. The remaining 69 mussels were chosen to correspond to the much broader peak in the frequency histogram (Peak B) and had lengths between 25 and 51 mm. Frequency distribution of zinc concentrations

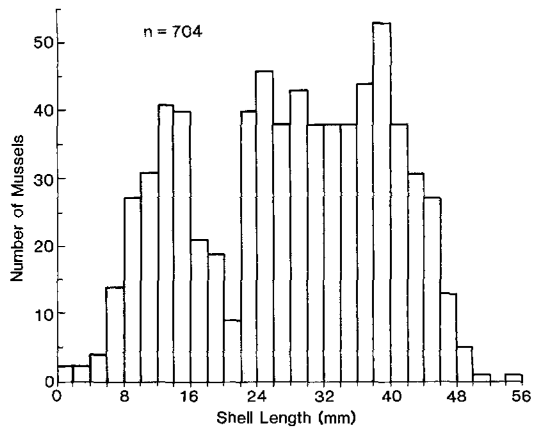

Fig. 1. *Mytilus edulis.* Frequency distribution of shell lengths of mussels collected from site in Black Middens, Tyne River, May 4, 1980

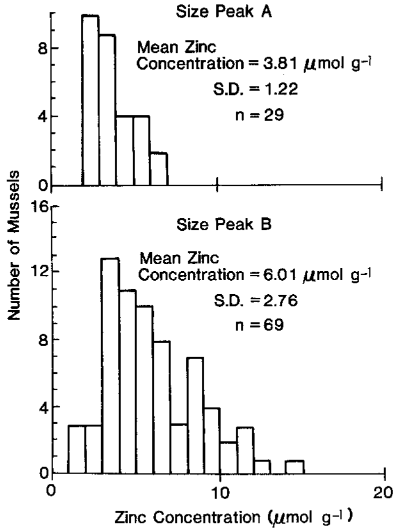

Fig. 2. *Mytilus edulis.* Frequency distribution of zinc concentrations in mussels collected as a single "clump" from Black Middens, Tyne River, June 16, 1980

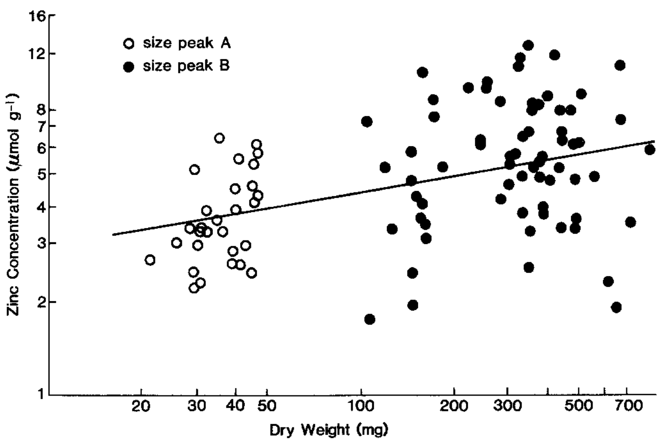

Fig. 3. *Mytilus edulis.* Double-log plot showing relationship between whole soft tissue zinc concentration and whole soft tissue dry weight in 98 mussels taken as a single "clump" from Black Middens, Tyne River, June 16, 1980

in these two groups is shown in Fig. 2, from which it is clear that the Peak B mussels have a significantly ($p < 0.001$) higher mean zinc concentration than the Peak A mussels. In fact, there seems to be little difference in the lower limits of the distributions, i.e., the frequency distribution appears similar for both groups in the region 2 to 7 μmol g^{-1}. Hence, while some Peak B mussels seem to have developed high zinc concentrations with increased age, other Peak B mussels retained low zinc concentration. Fig. 3 shows a double-log plot of zinc concentration against dry weight for both Peak A and Peak B mussels. This indicates for the whole group of 98 individuals a positive association between metal concentration and size.

The size and allometric characteristics of the two size groups from the June 1980 collection are given in Table 1. As expected, Size Peak A mussels have a significantly lower flesh weight, shell weight, shell length, width and height than Size Peak B mussels. These parameters are all clearly size-related. The allometric functions require further explanation, however, and are based on Seed's (1968) paper describing the factors influencing shell shape and size in *Mytilus edulis* on the northeast coast of England. Seed noted that older mussels became progressively wider in relation to their length and height. Shell weight and volume also continued to increase when increase in length and height had almost completely ceased. This was apparently accomplished by the mantle edge folding inwards so that shell accretion became directed toward the midline. This phenomenon was viewed as being a function of growth rate rather than of age *per se* or size *per se*. As each mussel reached its maximum potential size in a given habitat, shell growth ceased in both lengthwise and heightwise directions. However, owing to the high degree of shell erosion found in most mussels, the mussels were forced to continue to increase shell thickness as a means of shell reparation. Hence, older individuals characteristically tended to have thick eroded shells with a rounded look. As cessation of linear growth was also found to correspond to a cessation

Table 1. *Mytilus edulis.* Comparison between Size Peak A and Size Peak B mussels collected from Black Middens site on June 16, 1980. Mean values, with standard errors in parentheses

Parameter	Size Peak A	Size Peak B	Significance (p) of difference
No. of mussels	29	69	–
Zinc concentration (μmol g^{-1})	3.81	6.01	< 0.001
Weight measurements (mg)			
Whole soft tissue	36.8 (1.3)	348.1 (19.5)	< 0.001
Total: flesh plus shell	238.4 (9.3)	4 660 (330)	< 0.001
Flesh condition, FC (mg flesh g^{-1} shell)	181.6 (3.9)	92.7 (3.59)	< 0.001
Shell measurements (mm)			
Length	15.8 (0.20)	36.9 (0.90)	< 0.001
Width	6.37 (0.10)	18.1 (0.54)	< 0.001
Height	9.32 (0.11)	19.5 (0.39)	< 0.001
Width:Height	0.683 (0.0081)	0.921 (0.017)	< 0.001
Width:Length	0.404 (0.0050)	0.487 (0.0066)	< 0.001
Length:Height	1.69 (0.013)	1.88 (0.018)	< 0.001

Table 2. *Mytilus edulis.* Association between whole soft tissue zinc concentration (μmol g^{-1}) and various "size factors" measured in 98 mussels collected from Black Middens, June 16, 1980. Regressions calculated according to equation: log zinc conc = log $a + b$ (log size factor)

Size factor	Correlation with zinc		Intercept log a	Slope b (SE)
	Coefficient (r)	Significance (p)		
Flesh weight (mg)	0.434	<0.001	0.2674	0.1874 (0.03971)
Total weight (mg)	0.461	<0.001	0.1904	0.1527 (0.0300)
Flesh condition	-0.481	<0.001	1.750	−0.5245 (0.09749)
Length (mm)	0.442	<0.001	−0.02389	0.4868 (0.1018)
Width (mm)	0.462	<0.001	0.2202	0.4154 (0.08134)
Height (mm)	0.441	<0.001	0.01059	0.5642 (0.1171)
Width : Height	0.416	<0.001	0.7637	1.042 (0.2286)
Width : Length	0.404	<0.001	1.176	1.4587 (0.3371)
	Size Peaks A and B combined ($n = 98$)			

of flesh weight growth (except for seasonal changes), one would expect to see the ratio of flesh weight to shell weight diminish with age as linear growth ceases or slows. A convenient index for this is:

$$\text{flesh condition (FC)} = \text{mg flesh per 1000 mg shell} = \frac{\text{flesh wt of mussel (mg)}}{\text{shell wt of mussel (g)}}$$

It can be seen from Table 1 that the FC value for Size Peak A is twice as high as that for Peak B mussels. This is as predicted. Seed (1976) also predicts an increase in the ratios of width : height (W : H) and width : length (W : L) with increasing age and slowing linear growth rate. Both of these phenomena are confirmed in Table 1 and it is apparent that Seed's model is a useful one for the present population. Judging by the spread of values, it would appear that the FC value and width : height ratio have the widest relative ranges and might form the most useful basis for metal concentration studies.

Using the log/log format introduced by Boyden (1974, 1977), correlation and regression coefficients were calculated for the relationship between zinc concentration and these various size/growth parameters (Table 2). A positive correlation is seen between zinc concentration and the various size factors (flesh weight, total weight, length, width and height) as well as the W : H and W : L ratios. The FC value, which is known to decrease with size, shows negative correlation with the zinc level. Interestingly, if Size Peak B is considered alone, none of the above correlations is valid. However, if only Size Peak A mussels are considered, several of the associations are still significant at $p = 0.05$ or better, including the associations between zinc level and dry flesh weight, total weight, length, width and FC. This, in spite of a very small size spread in the latter group. This may mean that the mean zinc concentration reaches a plateau in the larger size group and that the zinc is more associated with growth than with age or size *per se*.

Discussion

Frequency distributions of shell lengths of *Mytilus edulis* collected throughout 1980 (Table 3) suggested that Size Peak A mussels from June 1980 were about 1 yr old (16 mm length). Group B mussels on the other hand, represented a far more amorphous group, comprising several age classes (probably a composite of several smaller peaks), whose mean values were not determined. Examination of newly formed rings near the shell margin on Peak B mussels suggested that some of these mussels may attain an age of 10 yr or more, though it was not possible to decipher exact ages. These older mussels appear to have very slow growth, since the newly formed rings (presumably "annual" rings) were spaced very closely together indicating growth of not more than 1 mm per year. Hence, the overall picture of mussel growth is clear. Within any selected environment, the smallest mussels in a given population represent the youngest individuals with the fastest growth rates. As the mussels get older and larger, the growth rate slows down. The slowing of the growth rate causes morphological changes in the mussel, i.e., an increase in the relative magnitude of shell width and shell weight. Thus, older mussels have increased ratios of W : H, W : L and an increased ratio of shell weight to flesh weight. It has been shown that all these changes are accompanied by a rise in mean zinc level. It can therefore be concluded that *within a selected* environment (mussels from a single clump), the zinc level is positively associated with both age and size and that the zinc level is negatively correlated with growth rate. A further paper (Lobel and Wright, in preparation)

Table 3. *Mytilus edulis.* Average shell lengths of young mussels (Size Peak A) taken from shell-length frequency distributions during 1980. A new peak, first observed in September, represents newly settled mussels only a few months old. np: no peak

Date	Average length of Size Peak A (mm)	Average length of *new* size peak (mm)
May 4	13	np
June 16	16	np
Sept. 10	19	7
Dec. 8	23	9

will show that these rules do not necessarily apply where collections are made from different but nearby habitats. As noted here (Fig. 2) and in an earlier paper (Lobel and Wright, in press), the higher mean zinc concentration of the older Peak B mussels was owing to the presence of a comparatively small number of individuals with a high zinc content. Bearing in mind that this group may include individuals of from 2 to 10 yr or more in age, it might be construed that the small number of high zinc individuals may coincide with the few older individuals present in the population. The lack of significant correlation between body zinc concentration and *any* of the size or allometric parameters from Group B mussels when considered alone argues against this possibility. The mussels with very high zinc levels from Size Peak B appear to represent a variety of age classes and to have a variety of shell sizes and shell shapes. The same is true for the mussels from Size Peak B with very low zinc contents. The significant correlations observed between soft tissue zinc concentration and the various size/age parameters are only seen when both Size Peak A and Size Peak B mussels are combined together and treated as one collection.

In metal monitoring studies, the construction of a metal/body weight relationship facilitates the normalization of metal concentrations to a standard weight in order that different geographic locations may be compared. This could easily be done for the mussels studied here, provided that a suitable size range of individuals was used. However, it must be emphasized that such standardization could only really be applied to a single population. Seed (1968, 1969, 1976) discusses a number of factors affecting the growth rate and size potential of mussels such as shore level, food supply, exposure to light, population density, wave action, strength of currents, temperature and salinity. According to Seed (1968), mussels from the upper shore tend to be older than lower shore mussels. However, this may not be reflected in size, and the lower shore mussels in receiving a better food supply may in fact be larger than the upper shore individuals. On the other hand, it might be possible to find populations of tiny mussels from a wave-swept shore which may be several years old. Therefore, a good deal must be known about the mussel population as a whole before age/size relationships can be confidently assigned. Serious problems of compatibility may arise when surveys take in a variety of habitats, since mussels of standard flesh weight from different sites may differ considerably in both age and growth rate.

The important conclusion to be drawn from the current work is that allometric ratios such as W : H, W : L and FC provide viable alternatives to simple size measurements as possible bases for normalization procedures. Being independent of size, they simply define a population in terms of growth rate. For instance, very young mussels from all populations have a W : H ratio of about 0.6 to 0.7, while very old slow growing mussels from all populations have a W : H ratio of about 1.0 or 1.1, even though the actual flesh weights of these latter mussels may easily vary by an order of magnitude depending on habitat. In view of the great difficulties and uncertainties involved in determining mussel age using growth rings, these allometric ratios provide an attractive alternative to methods of normalization currently in use. The implications this has for a monitoring programme involving several different populations will be discussed in a later paper.

Although allometric ratios have not been used in this context before, Phillips (1976) and Simpson (1979) both found strong negative correlations between zinc concentration and flesh weight in *Mytilus edulis* when mussels of *standard shell length* were used in studies of seasonal variation. This useful technique is analogous to the method used here employing the FC index, and permits the observation of seasonal variation in flesh weight simultaneously with the variation in zinc content and zinc concentration.

It remains to be seen whether the methods suggested here can be adapted for use with other metals or other animals.

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