A metabolic model for the determination of shell composition in the bivalve mollusc, *Mytilus edulis*

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This research describes compositional variations within the shell of the extant mussel *Mytilus edulis* and proposes that they are produced by metabolic gradients within the shell-secreting mantle. Because we have previously proposed that the same metabolic gradients are responsible for variations in shell form (curvature), we establish here a model for molluscan shell growth integrating, for the first time, shell form and composition with mantle metabolism. The electron microprobe was used to measure the distribution of Mg, S, and Ca in the outer calcitic shell layer of sectioned, polished, and either Al- or C-coated shell. Mg/Ca and S/Ca ratios in the outer shell are respectively 1.25 and 1.40 times higher along slow-growing, commissure-umbo axes of high shell curvature and high metabolic activity than along rapidly growing axes of low curvature and low metabolic activity. The ratios within the inner surface of the calcitic shell layer decline most rapidly along commissure-umbo axes where mantle metabolic activity also declines rapidly. We reject the null hypothesis, generally at high levels of significance (*t*-tests, *F*-tests, regression analyses, and discriminant analysis, with $p \ll 0.01$) that there is no difference in either Mg or S concentration in sections of the calcitic shell layer that differ in shell curvature and mantle metabolic activity. We conclude that calcium (mineral)-rich portions of shells are energetically less costly to produce than matrix or minor element-rich portions, in agreement with the proposal that natural selection favors mineral-rich shells because they are more efficient to produce than matrix-rich shells. Among-specimen differences are also highly significant (mixed model ANOVA). This confirms our assertion that paleontologists need to describe variations in skeletal composition among populations and throughout ontogeny as systematically as classical taxonomists describe morphology, if ever the environmental and the genetic influences on skeletal composition are to be distinguished. □ *Bivalves, biomineralization, shell composition, magnesium, sulfur, calcium, metabolism, growth, MYTILUS EDULIS*

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This paper is the third in a series of studies aimed at developing an integrated model of metabolism, shell form and (here, for the first time) shell composition. Simply stated, our model predicts that metabolic gradients within skeletal-depositing tissues such as the mantles of the bivalve mollusc or brachiopod determine accretion rates and shell form: the higher the metabolic gradient across the mantle, the greater the curvature of the shell deposited along the same axis.

The model is counterintuitive only if one insists that the orientation of the marginal mantle along the commissure is the sole determinant of shell form (the '*marginal mantle*' hypothesis which Huxley (1932) began to develop). Mantle growth and metabolism along the 'inner' surface of the shell, away from the margin, are deemed irrelevant to determining shell form.

But the alternative model recognizes that growth actually does occur along the entire inner shell surface in contact with the mantle, albeit over a range in rates. This is exemplified by the fact that growth increments are not simply limited to the margin of the shell. They extend from the outer into the inner shell layers, decreasing in thickness away from the commissure at species-specific rates as well as maintaining species-specific angles to the shell surface. Moreover, the physiological data thus far obtained strongly suggest that mantle metabolism and accretion rates across the entire mantle are responsible for controlling shell form. Rosenberg *et al.* (1988) described gradients in (glucose) metabolic activity in the mantle of the articulate brachiopod, *Terebratalia transversa*, and in the mantle of the bivalve mollusc, *Chlamys hastata*. They found that metabolic activity is higher in the mantle of the bivalve than in that of the brachiopod, confirming the original work of Hammen (1968,

1969, 1971, 1977), Hammen *et al.* (1962) and Hammen & Lum (1966). Rosenberg *et al.* (1988) also found that the rate of glucose metabolism is highest within the mantle lying along the margin of the commissure (marginal mantle), and that it declines within the strip of mantle ('medial mantle') adjacent but just distal to the commissure. The ratio of glucose metabolic activity within the marginal mantle to that within the 'medial' mantle was 3.7/1 in the brachiopod and 1.78/1 in the bivalve. Rosenberg *et al.* (1988) postulated that this difference accounted for the difference in shell curvature between the two taxa: The metabolic gradient was envisioned to have produced a proportional allometric, accretionary gradient within each shell consequently creating inflated valves in the brachiopod and flattened valves in the bivalve. Hence the model was named the *metabolic gradient* model.

Results of a metabolic study in *Mytilus edulis* support the 'metabolic gradient' model. If the 'marginal mantle' hypothesis were correct, one would predict that mantle metabolic activity would be highest where accretion rates are highest, along axes of maximum growth. But, Rosenberg *et al.* (1989) found that glucose activity surprisingly is lowest within marginal mantle along the axis of maximum growth, and is highest within marginal mantle lying closest to the umbo, along the axis of minimal growth (Fig. 1). Furthermore, Rosenberg *et al.* (1989) found that the metabolic gradients between the marginal mantle sectors and the umbo were proportional to shell curvature along the same axis (Fig. 1). These gradients were calculated as ratios of metabolic activity measured in the marginal mantle to a constant value representing 'inner' mantle metabolic activity beneath the umbo. We will publish elsewhere supporting data from two bivalve species that show that metabolic gradients actually measured in contiguous mantle sections between the shell margin and the umbo are proportional to valve inflation along the same axis.

Why the mantle metabolic rate should vary inversely with the rate of accretion is a physiological problem, important regardless of the val-

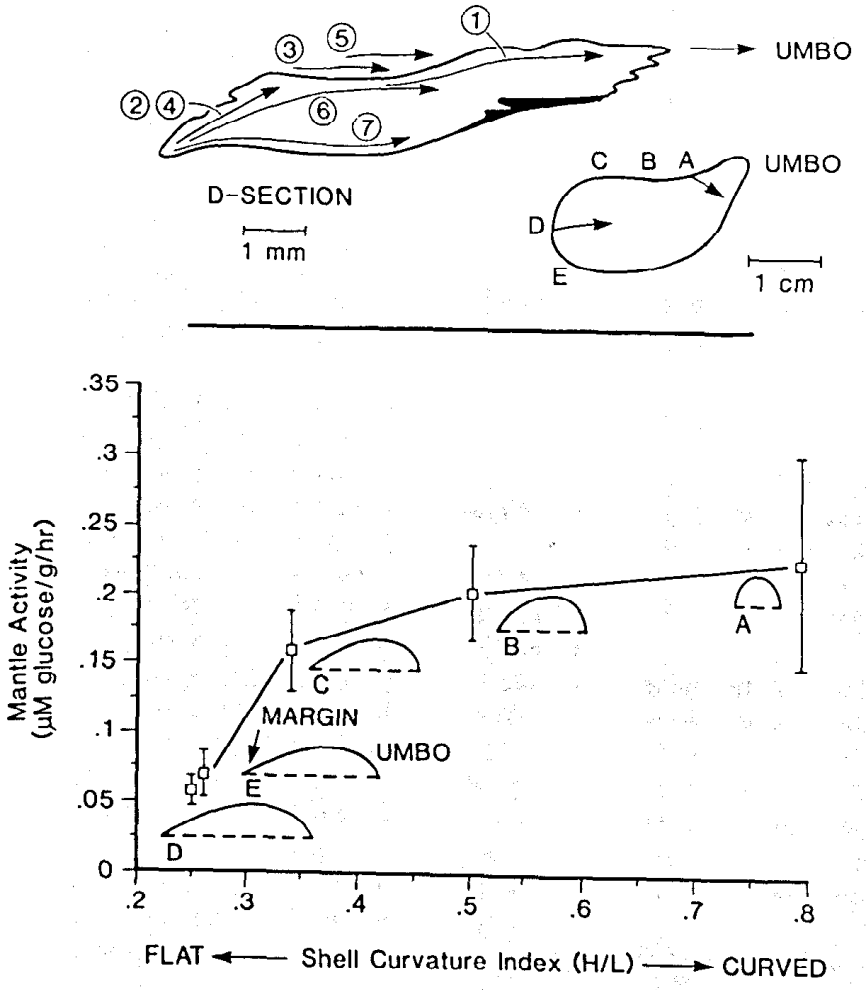

Fig. 1. Top. Location of electron microprobe experiments in sectioned shell of *Mytilus edulis*. A section taken from shell margin at position D towards the umbo is shown for example. Six experiments (1–6) were taken within the calcitic shell layer near the outer surface of the shell. Four additional traverses were also taken along the inner surface of the calcitic layer (7a–d) for a distance of 3 mm from the margin towards the umbo. Inner, aragonitic shell layer (black) begins to appear along the inner shell surface at right. *Bottom.* Glucose metabolic activity (µM glucose/g/hr) within the mantle at five intervals (A–E) along the margin vs shell curvature measured from the margin at each of the five intervals towards the umbo. Standard error bars are shown for metabolic activity. From Rosenberg *et al.* (1989).

idity of our model integrating growth rate, shell form, and metabolic gradients. Although we do not presume to solve the problem here, our research does establish the hypothesis that mantle metabolism is highest where shell is chemically most expensive to produce, and is secondarily related to growth rate. In *Mytilus edulis*, calcium-rich shell lies along the axis of maximum growth, least curvature, and lowest metabolic activity, whereas matrix- and trace element-rich shell lies along the axis of least growth, greatest curvature, and highest metabolic activity.

The inverse relationship between the matrix/mineral ratio in a shell and the efficiency of mantle metabolism was formalized by Palmer (1981, 1983), who noted that shell regeneration was more rapid in gastropods with mineral-rich rather than matrix-rich shells. This prompted Palmer to generalize that marine invertebrates with calcium-rich shells have a selective advantage over taxa with organic-rich shells. In this paper, we will extend Palmer's model to suggest that the metabolic determinants of shell composition among marine taxa are the same as those that determine variations in composition within shells. It is axiomatic that biochemical processes are fundamentally the same in whatever taxa they are found and thus it is reasonable to assume that the metabolic processes that determine variations in shell composition within a single taxon are the same as those that determine variations among different taxa.

In this paper, we test the prediction that the matrix/mineral ratio within the shell will vary directly with metabolic rate of the mantle sector that deposits it, and that gradients in shell composition will vary directly with gradients in mantle metabolism.

Accordingly, we have measured the concentration of Ca, Mg, S, C, and N within the outer calcitic shell layer of *Mytilus edulis* using the electron microprobe. We have asked whether the concentration of minor elements relative to Ca is uniform across the shell, or whether the ratios vary. We have also asked whether the elemental variations that were found could result from variations in the metabolic rate of the underlying mantle.

Our study focuses on the distribution of S and Mg relative to Ca within the shell. S is primarily concentrated in various acid mucopolysaccharides and amino acids within the shell and it is therefore an index of matrix content. Mg may substitute for Ca in the calcite unit cell, and it may also be coordinated directly to the organic (matrix) fraction. Mg has been one of the most intensively studied minor elements in skeletons. Its concentration in marine invertebrate skeletons is generally taken to be sensitive to changes in temperature or salinity, but all proposed correlations between skeletal Mg concentration and the environment have been compromised by disregard for ontogenetic (temporal and spatial) variations within the shell (Rosenberg 1980, 1990a, b). Furthermore, Mg was chosen because it would be interesting to establish a connection between the involvement of Mg^{+2} in cellular metabolism and transport on the one hand and the ion's skeletal concentration on the other, for such relationships would be important in understanding the evolution of skeletal composition. The establishment of such a connection is beyond the scope of this research, but we do contend that the variations within the shell that we do observe are not explained by environmental variations, a cautionary note to those who would account for all variations in skeletal content in environmental terms.

Materials and methods

The specimens of *Mytilus edulis* used in this study were obtained in July, 1988 and were living at mean sea level on rocks along Northwest Island, a few hundred meters offshore of Walla Walla College Marine Lab, Anacortes, Washington. They were sacrificed by inserting a scalpel between the valves in the region of the byssus, and severing the adductor muscle.

Dodd (1964, 1965, 1966) has determined that *M. edulis* shells at this latitude have a thick outer calcitic layer and a thin inner aragonitic layer. We confirmed this with Feigl/Friedman staining (Friedman 1959, 1977) of a few shells not subsequently used for electron microprobe analyses. In all of the shells the boundaries between the different layers can be seen readily using an optical microscope, thus permitting one to avoid the inner aragonitic shell layer during analyses (concentration of elements such as Mg varies with crystallography). All analyses reported here were done on the outer calcitic layer.

Our study of skeletal composition is based on more than 17,000 electron microprobe measurements of nine shells of *Mytilus edulis*. The shells were sectioned from the commissure toward the

umbo. The sections originated within areas A and D along the commissure (Fig. 1). Fig. 1 shows that marginal mantle activity is respectively maximal and minimal at these positions (Rosenberg *et al.* 1989). Likewise, the mantle metabolic gradient and the shell curvature extending from these positions toward the umbo are respectively maximal and minimal (Fig. 1 and Rosenberg *et al.* 1989). The sections were mounted on 1-inch diameter circular slides and ground and polished with successively finer grades of carborundum grit and alumina paste. They were scrubbed with a tooth brush and in a sonic cleaner before each change in grit or polish, and upon completion of polishing.

We have compared results obtained from Al- and C-coated shells. The specimens were coated in a vacuum evaporator with C for the first series of analyses. They were polished a second time to remove the C coating and then they were simultaneously coated with Al. Most analyses were completed with Al-coated specimens because an Al coating is presumed to be more effective than a C coating at preventing burning by the electron beam, and thus is presumed to enhance the precision of microprobe analyses of carbonates (Smith 1986; Jarosewich & Macintyre 1983). Analyses of variance (F-tests, not shown) of the S/Ca ratios measured in Areas A and D of Experiments 1–6 are significant at $p < 0.01$ and $p \leq 0.05$, respectively. Analyses of variance of the S/Ca ratios in Experiments 2–6 (only Al-coated specimens) are not significant at $p \leq 0.05$. That is, there are no significant differences in S/Ca ratios among experiments on Al-coated specimens wherein duration of measurement ranged between 10 and 100 seconds. This suggests that an Al coating will retard burning. Nevertheless, we regard the data obtained from C-coated specimens as useful because S/Ca and Mg/Ca ratios are consistently higher in Area A vs Area D, regardless of type of coating (*t*-tests presented in the Results).

The shells were analyzed in six experiments along the outer, calcitic shell layer and in one interval (four repeated experiments) along the inner surface of the calcitic layer extending from the margin towards the umbo (anteriorly) for a distance of approximately 3 mm (Fig. 1). Each traverse in the A sector was duplicated in the D sector (Fig. 1). Distinctive growth bands and changes in slope along the surface of the shell (e.g. produced by retardation in growth) were traced between areas A and D in order to find comparable intervals within both sectors for duplication. Experiments 1–6 (Fig. 1) traverse the outer surface of the shell that was deposited sequentially (growth increments intersect the outer surface at a high angle) and Experiment 7 (Fig. 1, repeated four times) follows along the inner surface of simultaneously produced shell (growth increments parallel the inner surface). The traverses varied in length, number of analyses, and duration of each measurement (Table 1). The operating conditions of the instrument were varied between traverses to *minimize* replicability. For example, in order to determine whether specimen burning adversely affected precision, the analysis time ranged between 10 and 100 seconds.

The concentrations of Ca, Mg, and S were measured simultaneously, as were Ca, C, and S and Ca, N, and S in repeated traverses. C was measured only in specimens coated with Al. The spectrometers were fixed on peak during each traverse. This eliminated the imprecision associated with repeaking of spectrometers during multi-element analyses. This did increase the number of traverses along each section but multi-

Table 1. Experimental parameters for electron microprobe analysis of the outer calcite shell layer of *Mytilus edulis*.

Experiment*	Coat	Analysis time (sec)	Beam diameter (μm)	Interval (μm)	Elements
1	C	10	10	10	Ca, Mg, S
2	Al	10	10	10	Ca, C, S
3	Al	30	10	20	Ca, C, S
4	Al	10	10	10	Ca, Mg, S
5	Al	10	5	5	Ca, Mg, S
6	Al	100	10	70	Ca, N, S

* As shown in Fig. 1.

ple traverses along a section did facilitate assessing the replicability and precision of the results.

Ca was detected using an LiF crystal and a sealed detector. Mg using an RAP crystal and a P-10 flow proportional detector, and S using a PET crystal and a P-10 flow proportional detector. Both C and N were detected with a LOD crystal and a P-90 flow proportional counter. A crystal of calcite was used for standardizing Ca and C, gypsum for S (and secondarily for Ca), MgO for magnesium, and BN for nitrogen.

All analyses were corrected for drift, dead time, and background. Concentrations, and ratios of concentrations, were determined by first approximation:

$$[X_{shell}] = C_{shell}[X_{std}]\ C_{std}$$

where

$[X_{shell}]$ = weight % of the element in the shell
C_{shell} = spectrometer count rate for the element in the shell
$[X_{std}]$ = weight % of the element in the standard
C_{std} = count rate for the element in the standard

ZAF corrections (atomic number, absorption, and fluorescence) were not made. We have previously determined (Rosenberg & Hughes 1990) that these are small relative to actual (corrected) variations. The results of the present study support this assertion.

Several statistical tests were used to evaluate the data. Mean differences, linear regression, analyses of variance, and discriminant analyses (Davis 1986; Sokol & Rohlf 1981) were evaluated by *t*-tests and *F*-tests (as specified in the Results). Our objectives were to test the null hypothesis that (1) there are no differences in composition (element to Ca ratios) between areas of the shell deposited by different sectors of the mantle, and (2) there are no differences in shell composition associated with type of coating or duration of analyses (i.e. artifacts attributable to specimen burning).

Results

The elements of greatest interest are Mg and S. The average Mg concentration ranges between 0.16 and 0.33 wt%, and the average S concentration between 0.08 and 0.30 wt%. The concentrations of Mg and S relative to Ca consistently differ between sectors A and D. The Mg/Ca and S/Ca ratios reported below compare favorably with the values obtained by Lorens & Bender (1980) in the same species.

Table 2 lists the mean S/Ca ratios in the outer shell layer of sectors A and D (six traverses of each of nine specimens). The mean S/Ca ratio is clearly higher (2-tailed *t*-tests) within sector A than sector D. In seven of the nine specimens, the difference is highly significant ($p \leq 0.01$) in most traverses. In one of the specimens (770), the ratio does appear to be greater in sector A in five cases, but is significantly so in only three of the six traverses. In specimen 772, there is no consistent difference between the S/Ca ratio within the A and D sectors at $p \leq 0.05$.

Table 3 summarizes the three experiments in which Mg/Ca ratios were measured in areas A and D. In all but one of the specimens, the Mg/Ca ratio is higher in the A than in the D sector of the shell in at least two out of the three traverses. The difference is generally greater than 10% and is highly significant (at $p \leq 0.001$, 2-tailed *t*-test). In two specimens (771 and 772), the Mg/Ca ratio was found to be higher in the D than in the A sector in one of the three traverses, but the difference is less than about 4 wt% (albeit highly significant). In one specimen (773) the Mg/Ca ratios in the A and D sectors do not differ at $p \leq 0.05$ in any of the three traverses.

Mixed model analyses of variance of S/Ca and Mg/Ca ratios (Tables 4 and 5, respectively) confirm a highly significant variation among specimens. Tables 4 and 5 exclude data from experiment 1 (specimens coated with C). Differences due to coating type (a mixed model ANOVA including experiment 1) are significant (at $p < 0.01$) for S/Ca but not for Mg/Ca, suggesting that the former are more sensitive to burning.

In Fig. 2 the mean Mg/Ca ratio is graphed against the mean S/Ca ratio for the three experiments in which the two ratios were simultaneously measured in the outer shell layer (a total of N = 54 pairs, 27 within each of the A and D sectors). Plots of the A and D sectors are distinguished. Fig. 2 thus shows three estimates within each of two areas of the same shell as independent values. We wanted to determine if compositional differences between 'metabolic areas' were robust in view of variations either among specimens or within shells (ontogeny). Clearly (Fig. 2), they are.

A discriminant analysis of the data in Fig. 2

Table 2. Mean S/Ca ratios in the outer shell layer of *Mytilus edulis*.

Spec. no.	Exper.	Area A	S.D.	Area D	S.D.	DF	*t*	*p*
764	1	0.0036	0.0010	0.0029	0.0011	588	8.08	****
	2	0.0053	0.0008	0.0024	0.0009	313	34.51	****
	3	0.0060	0.0007	0.0026	0.0010	210	28.76	****
	4	0.0055	0.0010	0.0030	0.0012	246	17.47	****
	5	0.0053	0.0010	0.0018	0.0004	398	45.96	****
	6	0.0054	0.0006	0.0027	0.0010	78	14.64	****
765	1	0.0034	0.0011	0.0023	0.0007	436	12.75	****
	2	0.0048	0.0009	0.0044	0.0016	320	2.73	***
	3	0.0042	0.0007	0.0040	0.0008	269	2.19	**
	4	0.0049	0.0009	0.0040	0.0012	382	8.38	****
	5	0.0052	0.0008	0.0050	0.0021	397	1.26	NS
	6	0.0052	0.0005	0.0046	0.0010	64	3.02	***
766	1	0.0035	0.0016	0.0027	0.0010	573	7.24	****
	2	0.0061	0.0014	0.0038	0.0018	319	12.77	****
	3	0.0047	0.0012	0.0046	0.0017	281	0.56	NS
	4	0.0068	0.0012	0.0036	0.0015	275	19.72	****
	5	0.0055	0.0016	0.0031	0.0008	378	18.18	****
	6	0.0054	0.0016	0.0046	0.0017	72	2.08	*
770	1	0.0033	0.0009	0.0025	0.0006	450	10.82	****
	2	0.0063	0.0016	0.0058	0.0029	328	1.93	NS
	3	0.0051	0.0008	0.0048	0.0022	205	1.29	NS
	4	0.0047	0.0010	0.0048	0.0021	352	−0.57	NS
	5	0.0055	0.0009	0.0046	0.0012	398	8.48	****
	6	0.0050	0.0008	0.0042	0.0020	66	2.14	*
771	1	0.0027	0.0009	0.0020	0.0006	590	11.10	****
	2	0.0055	0.0011	0.0029	0.0017	267	15.04	****
	3	0.0054	0.0013	0.0024	0.0011	231	19.07	****
	4	0.0060	0.0011	0.0032	0.0020	252	14.12	****
	5	0.0060	0.0001	0.0018	0.0004	398	130.13	****
	6	0.0047	0.0012	0.0027	0.0016	75	6.22	****
772	1	0.0031	0.0010	0.0053	0.0016	491	−18.23	****
	2	0.0056	0.0011	0.0059	0.0023	358	−1.57	NS
	3	0.0063	0.0012	0.0053	0.0019	227	4.78	****
	4	0.0060	0.0012	0.0061	0.0020	353	−0.57	NS
	5	0.0058	0.0009	0.0070	0.0027	384	−5.94	****
	6	0.0065	0.0011	0.0061	0.0022	66	0.95	NS
773	1	0.0032	0.0009	0.0029	0.0017	626	2.71	***
	2	0.0045	0.0007	0.0032	0.0009	291	13.90	****
	3	0.0036	0.0005	0.0030	0.0013	198	4.29	****
	4	0.0045	0.0009	0.0037	0.0012	239	6.20	****
	5	0.0043	0.0008	0.0027	0.0006	398	22.84	****
	6	0.0047	0.0009	0.0037	0.0018	65	2.89	***
774	1	0.0031	0.0008	0.0023	0.0008	550	11.70	****
	2	0.0043	0.0010	0.0024	0.0006	330	20.70	****
	3	0.0034	0.0009	0.0027	0.0007	246	6.89	****
	4	0.0048	0.0009	0.0032	0.0010	315	14.99	****
	5	0.0040	0.0008	0.0042	0.0008	378	−2.43	**
	6	0.0041	0.0009	0.0027	0.0006	68	7.78	****
775	1	0.0045	0.0010	0.0038	0.0016	595	6.39	****
	2	0.0061	0.0012	0.0035	0.0012	301	18.86	****
	3	0.0055	0.0005	0.0040	0.0018	212	8.44	****
	4	0.0066	0.0011	0.0034	0.0010	246	23.85	****
	5	0.0052	0.0011	0.0037	0.0017	387	10.36	****
	6	0.0043	0.0009	0.0031	0.0011	71	5.08	****

DF = Degrees of freedom = $n_A + n_D$ = number of analyses in Area A + Area D, respectively.
**** $p < 0.001$; *** $p < 0.01$; ** $p < 0.02$; * $p < 0.05$; NS = not significant.

Table 3. Mean Mg/Ca ratios in the outer shell layer of *Mytilus edulis*.

Spec. no.	Exper.	Area A	S.D.	Area D	S.D.	DF	*t*	*p*
764	1	0.0054	0.0005	0.0043	0.0004	588	29.56	****
	4	0.0050	0.0007	0.0041	0.0004	246	12.75	****
	5	0.0053	0.0007	0.0035	0.0004	398	31.57	****
765	1	0.0050	0.0006	0.0043	0.0004	436	14.63	****
	4	0.0048	0.0009	0.0040	0.0004	382	10.34	****
	5	0.0045	0.0007	0.0038	0.0005	397	11.49	****
766	1	0.0061	0.0007	0.0050	0.0004	573	22.34	****
	4	0.0069	0.0011	0.0041	0.0005	275	25.91	****
	5	0.0053	0.0007	0.0038	0.0004	378	25.28	****
770	1	0.0048	0.0005	0.0044	0.0004	450	9.22	****
	4	0.0054	0.0010	0.0046	0.0010	352	7.53	****
	5	0.0047	0.0005	0.0037	0.0004	398	22.09	****
771	1	0.0056	0.0007	0.0058	0.0009	590	−3.02	***
	4	0.0065	0.0016	0.0041	0.0007	252	14.95	****
	5	0.0042	0.0005	0.0036	0.0004	398	13.28	****
772	1	0.0046	0.0005	0.0049	0.0006	491	−6.02	****
	4	0.0062	0.0011	0.0049	0.0007	353	13.35	****
	5	0.0049	0.0006	0.0047	0.0006	384	3.21	***
773	1	0.0044	0.0045	0.0046	0.0005	626	−0.80	NS
	4	0.0037	0.0036	0.0039	0.0004	239	−0.63	NS
	5	0.0046	0.0004	0.0046	0.0004	398	0.00	NS
774	1	0.0046	0.0005	0.0038	0.0005	550	18.72	****
	4	0.0059	0.0008	0.0040	0.0004	315	26.56	****
	5	0.0044	0.0005	0.0038	0.0004	378	12.82	****
775	1	0.0052	0.0006	0.0037	0.0004	595	36.02	****
	4	0.0064	0.0011	0.0042	0.0005	246	19.82	****
	5	0.0076	0.0014	0.0049	0.0008	387	23.28	****

Symbols as defined at bottom of Table 2.

confirms that the A and D sectors of the outer shell are distinguished compositionally, for an *F*-test with 2 and 51 degrees of freedom is significant at $p \leq 0.025$. A discriminant analysis of the data in Experiments 4 and 5 only (eliminating the effects of coating) is significant at $p \leq 0.005$.

A regression of the Mg/Ca against the S/Ca ratios plotted in Fig. 2 yields a correlation coefficient of 0.453, which is significant at $p < 0.01$ for k = 2 independent variables (*t*-test in which both of the correlated values are measured). The goodness-of-fit is 0.206, which is significant at

Table 4. Mixed model ANOVA on S/Ca × 10^3 (experiments 2–6).

Source	df	Sum SQ	Mean SQ	Denom MS	F	*p**
Area (A)	1	44.94	44.94	AC	15.33	0.004
Experiment (B)	4	1.72	0.43	BC	1.33	NS
Specimen (C)	8	46.85	5.86	ABC	20.36	0.000
AB	4	0.75	0.19	ABC	0.65	NS
AC	8	23.45	2.93	ABC	10.19	0.000
BC	32	10.38	0.32	ABC	1.13	NS
ABC	32	9.20	0.29	–	–	–

* All except BC significant at $p \leq 0.05$ in mixed model ANOVA using experiments 1–6.

Table 5. Mixed model ANOVA on Mg/Ca × 10^3 (experiments 4–5).

Source	df	Sum SQ	Mean SQ	Denom MS	F	*p**
Area (A)	1	13.44	13.44	AC	22.59	0.001
Experiment (B)	1	1.28	1.28	BC	2.00	NS
Specimen (C)	8	8.02	1.00	ABC	4.35	0.026
AB	1	0.40	0.40	ABC	1.74	NS
AC	8	4.76	0.60	ABC	2.58	NS
BC	8	5.13	0.64	ABC	2.78	NS
ABC	8	1.84	0.23	–	–	–

* All except B significant at $p \leq 0.02$ in mixed model ANOVA using experiments 1, 4–5.

$p \leq 0.025$ (*F*-test). The slope of the regression line fitted to the data is 0.299 (significantly different from 0 at $p \leq 0.05$, *F*-test with 1, 52 degrees of freedom). The Y-intercept is at 0.0035 (value of Mg/Ca when S/Ca = 0). Note that the ratios within area A have a correlation coefficient of 0.386 and the regression line a slope of 0.297. The ratios within Area D have a correlation coefficient of 0.149 and a slope of 0.0627. Neither of these correlation coefficients is significant at $p \leq 0.05$, but the correlation coefficient within Area A is close (the correlation coefficient for significance at $p \leq 0.05$ is 0.462). The significant, positive correlation of the ratios over the entire data and the higher correlation coefficient and slope in Area A vs Area D suggest that the two elements occur within the same phase at high but not necessarily low concentrations.

The absolute concentrations of Ca, Mg, and S (data not shown) were also regressed against each other. The electron microprobe is insensitive to phases in which elements are detected, and we chose not to assume that the distributions of any two elements are dependent. The correlation coefficient for S–Mg was 0.448 (significant at $p \leq 0.01$, 52 degrees of freedom, k = 2). The correlation coefficient for Ca–S was 0.071, which is not significant at $p \leq 0.05$ (i.e. there is no apparent correlation between Ca and S content). The correlation coefficient for Ca–Mg is −0.292 (not significant at $p \leq 0.05$). The levels of significance are the same if we eliminate differences due to coating by correlating only the values obtained in Al-coated samples (Experiments 4 and 5).

The positive correlation between Mg–S is expected because both elements tend to be concentrated at the expense of Ca. S is primarily concentrated within the chondroitin sulfate (polysaccharide) and cysteine and methionine (amino acid) fractions of the shell conchiolin. However, some S may be present as sulfate in the mineral fraction. Mg substitutes for Ca in the calcite unit cell, but also may be coordinated to molecules in the organic matrix.

There are several possible reasons why the Ca–S correlation coefficient is close to 0 and why the Ca–Mg correlation coefficient is not more negative (although it is close to significance at $p \leq 0.05$ where $r = -0.330$ for 52 df). Based solely on calcite stoichiometry, Mg is concentrated at the expense of Ca (which would yield a negative correlation coefficient for Ca–Mg). But if Mg is also chelated to the organic matrix, the microprobe analyses would encounter Mg within both the Ca-rich and Ca-poor phases. This would yield a more positive correlation coefficient than predicted from calcite stoichiometry alone.

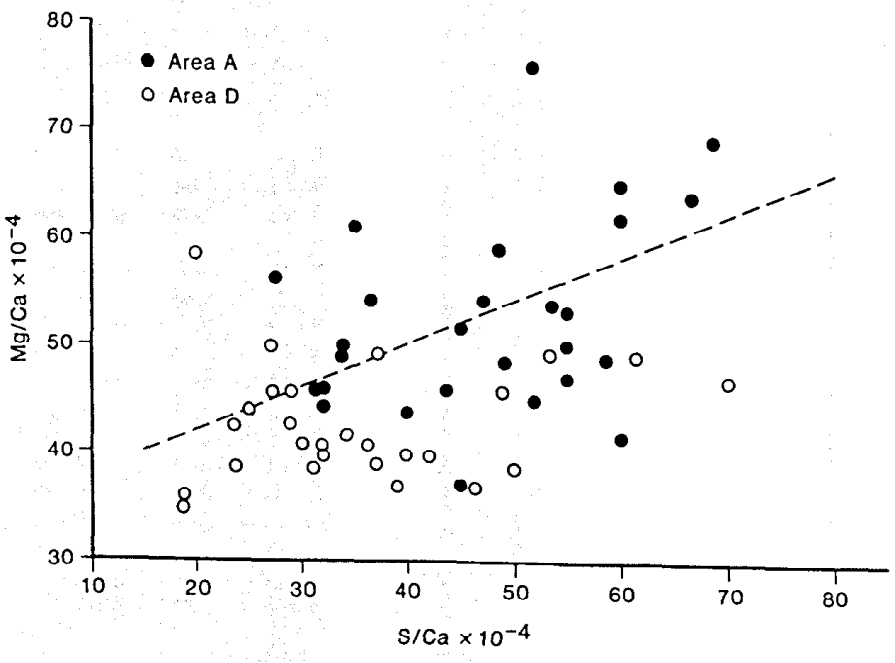

Fig. 2. Mean Mg/Ca and S/Ca ratios in the outer calcitic shell layer of *Mytilus edulis*. Mg/Ca wt% ratios for Area A (●) and Area D (○) are from Table 3. S/Ca ratios are from Table 2 (Experiments 1, 4, 5). Regression line fitted to all of the data has a slope of 0.299, a Y-intercept at Mg/Ca = 0.0035 wt%, and a correlation coefficient, $r = 0.453$ significant at $p \leq 0.01$ (*t*-test). The separate regression lines for the data within Area A and Area D are not shown, but are discussed in the text. The ratios within Area A and Area D constitute two significantly different populations at $p \leq 0.025$ (discriminant analysis *F*-test).

Second, microporosity must be treated as a 'third phase' in microprobe analyses. The concentration of all elements will appear to decrease simultaneously as porosity increases; microporosity introduces a positive correlation between all elements where a negative or no correlation actually exists. Finally, Mg concentration is so low relative to Ca that a small percentage of uncertainty in Ca would mask the Ca–Mg correlation.

There is evidence for higher compositional (Mg and S) gradients from the commissure towards the umbo in Area A than in Area D. The compositional gradient is determined by dividing the element-to-Ca ratio within the calcitic shell layer at the margin by the ratio within the layer along its inner surface (Experiment 7). Table 6 shows that the S/Ca ratio declines 11% from the outer to the inner surface of the calcitic shell layer within Area A, but increases 2% along the same axis within Area D. The Mg/Ca ratio increases 4% in the same direction within Area A, but increases 32% within Area D. The difference is significant at $p \leq 0.05$ for S/Ca and at $p \leq 0.01$ for Mg/Ca (2-tailed *t*-tests).

Finally, we note that we detected no significant differences (at $p \leq 0.05$) in C or N relative to Ca content within the shells. There are several alternative hypotheses for this. First, C is present within both the inorganic (carbonate) and the organic (chonchiolin) phase. The microprobe measures total carbon and is generally insensitive to phase. C constitutes 12 wt% of calcite and anywhere from 30 to 60 wt% of amino acids. It is unlikely that the electron beam was ever positioned solely on conchiolin (organic laminae within the shell are quite thin) and it is conceivable that different proportions of mineral and matrix beneath the electron beam could yield similar weight percentages of C. Second, the precision of N analyses is limited by a very low peak to background level for N (2:1 on BN which contains 56.5 wt% N) on the LOD crystal. Consequently, slight variations in N content (less than 10%) will be difficult to detect without greatly increasing the duration of measurement (100 seconds for all experiments in which N was measured). Third, absorption is probably a more significant factor in precision of C and N analyses than in analyses of heavier elements (for the former produce low energy X-rays that are more readily absorbed by the Al coating). And, fourth, microporosity again must be regarded as a 'third phase' whose volumetric variation can yield apparent changes in concentration of elements within the shell. Nevertheless, we believe that S is a valid index of organic matrix content and that the differences noted (Results) in S/Ca and Mg/Ca ratios between sectors A and D indicate that matrix and trace element content vary regularly within the shell of *Mytilus edulis*.

Table 6. Compositional gradients within the shell of *Mytilus edulis*: ratios of Mg/Ca or S/Ca in the outer to inner surface of calcitic shell.

	Section A		Section D	
Spec. no.	Mg/Ca	S/Ca	Mg/Ca	S/Ca
764	1.16	1.28	1.02	0.62
		1.18		0.60
		1.02		0.93
		1.15		0.72
765	0.79	1.22	0.56	0.89
		1.37		1.07
		1.24		0.96
		1.17		1.00
766	1.23	1.24	0.79	0.72
		1.00		0.55
		1.04		0.94
		0.89		1.92
770	0.69	0.98	0.73	1.33
		1.29		1.02
		1.04		0.93
		1.02		0.96
771	1.00	0.95	0.55	0.58
		0.96		0.47
		0.89		0.44
		0.92		0.58
772	0.90	1.00	0.79	1.17
		1.12		1.18
		1.20		1.27
		1.03		1.15
773	0.80	1.15	0.56	1.03
		1.22		0.76
		1.24		1.00
		1.09		1.11
774	0.91	1.09	0.60	1.60
		0.81		0.92
		0.98		1.23
		0.97		1.23
775	1.18	1.65	0.48	0.81
		1.15		0.97
		1.26		1.00
		1.12		1.48
Mean	0.96	1.11	0.68	0.98
S.D.	0.19	0.16	0.17	0.32

A vs D:
Mg/Ca $t = 3.30$, $p \leq 0.01$ for 16 df (Experiment 7a).
S/Ca $t = 2.18$, $p \leq 0.05$ for 70 df (Experiments 7a-d).

Discussion

Our emerging model of shell growth suggests that metabolic gradients within the mantle of the mussel, *Mytilus edulis*, determine shell chemistry as well as shell form. In this report we reject the null hypothesis, generally at a high level of significance, that there is no difference in either the S/Ca or Mg/Ca ratios between sectors of the shell that also differ in curvature and underlying mantle activity.

Mg/Ca and S/Ca ratios are highest in the area of the shell previously found (Rosenberg *et al.* 1989, 1988) to be secreted by mantle with the highest metabolic activity (outer shell layer at the commissure in Area A vs Area D), and they decline most rapidly in shell along axes where mantle metabolic activity was previously found (Rosenberg *et al.* 1989, 1988) to decline most rapidly (from the commissure toward the umbo in Area A vs Area D). The distribution of S, as an index of matrix content, supports Palmer's (1981, 1983) contention (see Introduction) that matrix-rich shell is metabolically more expensive to produce than mineral-rich shell and helps us understand why metabolically active mantle should underlie a sector of the margin (Area A) that is slowly accreting. In contrast, note that the inner, aragonitic shell layer is organic-rich, yet is deposited by mantle that is relatively inactive. In compensation, the aragonitic layer accretes very slowly. Thus both growth rate and shell composition are functions of metabolic activity.

We recognize that a causative connection between metabolism and shell composition is not established by their covariance alone. Palmer (pers. comm.) acknowledged that matrix-rich shells might only appear to be metabolically more costly than mineral-rich shells (i.e. grow less than mineral-rich shells for a given energy intake), because the amino acids essential to the elaboration of organic matrix may become limiting at different rates. Furthermore, we do not know what fraction of mantle metabolism is devoted to mineral vs shell matrix deposition.

Several studies of the metabolism of *Mytilus edulis* (and a wide range of other taxa) clearly indicate that growth rate is more directly a function of metabolic efficiency than of absolute metabolic rate (Bayne & Newell 1983; Bayne 1987; Diehl *et al.* 1985; Hawkins *et al.* 1989, 1985; Thiesen 1982; Vahl 1973; Widdows & Hawkins 1989; Widdows *et al.* 1984). Most relevant to this study is the work of Bayne (1987), who found that a cohort of small, slow-growing, relatively homozygous specimens of *Mytilus edulis* had higher metabolic rates than did large, fast-growing, relatively heterozygous individuals. Bayne (1987) ascribed the difference to more intense turnover of protein and to reduced metabolic efficiency in the slower growing individuals. The genetic underpinnings of the differences in metabolic efficiency are not relevant here because all cells within the mantle of an individual are genetically identical. But our results are consistent with those of Bayne (1987) in the implication that slow-growing shell in *Mytilus edulis* is metabolically less efficient to produce than rapidly growing shell because it is enriched in matrix or minor elements.

The work of Hawkins *et al.* (1989) may provide a reason for the difference in efficiency. Hawkins *et al.* (1989) partitioned the metabolism of *M. edulis*. They measured protein synthesis, heat loss, and the deposition of protein and non-protein substrates in tissue and shell. They found that the deposition of non-protein substrate accounted for 33% of energy utilization in contrast to 66% for deposition of protein substrates. It is not clear from their study what fraction of the latter value is devoted to deposition of shell protein, but it is noteworthy that the partitioning of energy utilization was found to be constant regardless of metabolizable energy intake. More energy would be available for both protein and non-protein deposition by metabolically active mantle. But the preferential deposition of matrix-rich shell by metabolically active mantle would imply that the greater absolute energy utilization is associated with matrix elaboration and would be consistent with Palmer's (1983, 1981) model of the relative cost of producing matrix- vs mineral-rich shell.

The next question is whether the presence of Mg in the shell is metabolically significant. There are several alternative answers. It is possible that Mg plays no special role in the energetics of shell deposition. Crystallography is an important determinant of skeletal Mg content (Onuma *et al.* 1979), for Mg^{++} fits more readily into the calcite than aragonite unit cell. Mg^{++} also may be coordinated directly to various organic molecules as the matrix is elaborated (hence the strong positive correlation between Mg and S concentration in the shell). However, the hypothesis that the presence of Mg in the skeleton is due to stoichiometric factors alone does not explain why the con-

centration of Mg in the skeleton varies during ontogeny in taxon-specific patterns (Rosenberg 1990a, b), including those suggested for *Mytilus edulis* in the present study. Nor does the hypothesis explain why the Mg content in the shell of *Mytilus edulis* fluctuates about low mean levels in the first place. The high Mg/Ca ratio in seawater favors initial deposition of high-Mg calcite or aragonite (Dodd & Stanton 1981; Mackenzie *et al.* 1983), although the very presence of Mg^{++} in seawater (and in calcifying organic fluids) may actually limit the crystallization of high-Mg phases (Dodd & Stanton 1981). Because organisms rarely deposit Mg-enriched minerals such as dolomite, magnesite, and rhodochrosite except pathologically (Lowenstam & Weiner 1983; LeGeros 1981), it does seem plausible that organisms have evolved the physiological means of limiting the Mg content in their skeletons. And Lorens & Bender (1977) found that *Mytilus edulis* does appear to exclude Mg^{++} from its shell when growing at normal salinity.

A second alternative is that there is no physiological control over Mg content in the shell but there are environmental determinants. That is, physical/chemical factors (such as diffusion rates, unit cell limitations on trace element content) that change with the environment determine Mg content of the shell. Active transport of Mg through the cell membrane of the mantle epithelium, to the extrapallial space, and into the shell is not a factor.

Mg is one of the most intensively studied elements in the skeletons of marine organisms, and a great deal of research has been devoted to using Mg content in both living and fossil organisms as an index of seawater chemistry and temperature (see reviews by Rosenberg 1990a, b; Rosenberg 1980; Dodd & Stanton 1981; Dodd 1967; Chave 1954; Brand & Morrison 1987; Morrison & Brand 1986; Jones 1985; Mackenzie *et al.* 1983; Lowenstam 1961, 1964). The studies of skeletal Mg content are difficult to generalize because the relationships between skeletal composition and the environment have inevitably proven more complex than originally proposed. For example, Clarke & Wheeler (1922) originally proposed that Mg content in skeletons of marine invertebrates is directly proportional to ambient temperature. They predicted that there were 'probable exceptions' and, indeed, subsequent data have proven to be so contradictory that the validity of the generalization for all taxa is very much in doubt (Rosenberg 1980). Dodd (1965) found a positive correlation between ambient temperature and Mg content in the outer calcitic shell layer of *Mytilus californianus* but an ambiguous correlation in *Mytilus edulis*. In one study of the latter species there was evidence of a seasonal Mg variation (higher Mg content in the shell during summer than winter), in a second series of analyses there was a positive correlation between shell content and temperature, but in a third series there was no significant correlation. Dodd (1965) also found that Mg in the outer calcitic shell layer of *Mytilus edulis* decreased steadily with increasing salinity (a nearly twofold decrease in shell Mg as salinity changed from 15 to 33‰). However, environmental variations cannot explain the differences in Mg content in the two sectors of the *Mytilus edulis* shell studied here because the shell that was analyzed in each sector was deposited simultaneously. This was readily determined by matching the analyzed areas with time-markers such as growth bands and surficial slope changes on the shell that could be followed from one area to the other.

Thus, a third alternative needs to be considered. The content of Mg in the shell is a direct function of the organism's physiology. Ca may be concentrated in the skeleton in favor of Mg as an adaptation to protect Mg^{++}-potentiated enzymes (Kretsinger 1977; Lowenstam & Margulis 1980). In this role, Mg levels in the shell could be directly dependent on either the total Mg^{++} in the secreting mantle/extrapallial space or on the surplus Mg^{++} not needed for enzyme protection. However, it is unlikely that a simple, direct relationship exists between Mg^{++} content in the extrapallial fluid and that in the shell. Kitano (1962), Berner (1975), and Kitano *et al.* (1976) found that the concentration of Mg^{++} and other ions and organic compounds in the extrapallial fluid determine skeletal mineralogy and thereby influence skeletal chemistry. Mg^{++} fits into the calcite unit cell more readily than into the aragonite unit cell, so that one would predict that calcitic skeletons would have a higher Mg content than aragonitic skeletons – but even in this instance the data are contradictory and the Mg content of skeletons of different mineralogies may overlap (cf. summaries by Lowenstam (1963, 1964) with that by Morrison & Brand (1986) and see Rosenberg's (1990a) analysis).

In a related physiologic role, Mg^{++} has been identified as an inhibitor of biomineralization.

because it is not an exact substitute for Ca^{++} and thus distorts the unit cell of carbonates and apatites (Simkiss 1983; Boskey & Posner 1974; Carafoli *et al.* 1975; Pak & Diller 1969; Wilbur & Bernhardt 1984). The genetic determination of allometric growth rates and shell form thus might exploit the inhibitory function of Mg; active transport of the ion to calcifying sites would be a means of retarding accretion, while exclusion of the ion from other sites would facilitate accretion.

Finally, as a fourth alternative, cellular energetics may affect Mg content in the shell, but only indirectly. At first glance, some studies suggest that growth rate determines skeletal Mg content. Dodd (1965) found that the wt% of Mg in the last-formed portion of the outer prismatic (calcitic) shell layer of *Mytilus californianus* was strongly size (age and growth rate) dependent. The Mg content was maximum in small and intermediate-sized shells (up to 50 mm long) from one locality, and maximum in intermediate-sized shells (50 mm long) from another. In both populations, Mg content was lowest in the last-formed portion of the largest shells (80 mm long) where accretion was slowest. Zolotarev (1974) similarly found a decrease in Mg in the calcitic layer of *Mytilus yessoensis* from the beak to the posterior margin (i.e. with decreasing growth rate). Furthermore, Zolotarev (1974) observed that the average Mg content was lower in the slow-growing than in the rapidly growing specimen that he studied. If we propose that growth rate is the determining factor of skeletal Mg content then the above trends contradict the results of the present study (where we report that the Mg/Ca ratio is higher along a slow-growing than a rapidly growing axis in *M. edulis*). The trends are consistent if we assume the determining factor is metabolic activity, not growth rate, and if we presume that mantle metabolic activity declines during ontogeny because then, in all cases, skeletal Mg content decreases with decreasing metabolic activity. Thus, we concur with Lorens & Bender (1980) that the skeletal chemistry of *M. edulis* is not related to growth rate, per se.

Moberly (1968) reported that Mg content in the outer shell layer of the scallop, *Aequipecten irradians*, increased with seasonal (post-spawning and post-winter) growth increases, but his data also show a gradual long-term, ontogenetic increase in Mg content which he did not discuss. The seasonal changes are consistent with the metabolic thesis of the present paper, but the ontogenetic trend apparently is not. However, we note that the shell of *A. irradians* is much flatter, and the curvature along different axes more uniform than that of *Mytilus* spp. Thus, our model would predict that the metabolic and shell compositional gradients also would be gentler in *A. irradians*. Although, to our knowledge, neither gradients have been studied thoroughly in the species, it is interesting that the rate of carbonate deposition along the margin of the shell of the scallop, *Argopecten irradians*, does not change with increase in shell size (Wheeler & Sikes 1975). This suggests that the mantle metabolism may be more uniform through ontogeny in *Argopecten irradians* than in *M. edulis*.

In conclusion, whatever the physiologic underpinnings of compositional variations in the shell, we submit that the description of such variations is necessary to fully understand the evolution of biominerals. It is self-evident to us that descriptions of (1) the ontogenetic changes in skeletal composition, (2) the consequent patterns of elemental distribution throughout the skeleton, and (3) the variations in ontogenetic patterns among populations are as requisite to the chemical taxonomy of the skeleton as the thorough description of variation in form is essential to classical taxonomy based on morphology. The nearly 30% variation in Mg/Ca and S/Ca ratios across the shell of *Mytilus edulis* and the highly significant variations in composition among specimens prompt us to ask whether (1) all populations of the bivalve in all habitats present similar variations in elemental distribution, (2) all individuals of the species display such patterns throughout ontogeny, (3) all species of bivalves, and even of other shelled invertebrates such as brachiopods display the similar relationships between skeletal composition, form, and metabolism that *Mytilus edulis* apparently does, and (4) fossil relatives of extant species show comparable patterns of elemental distribution in their skeletons.

We predict that taxon-specific ontogenetic patterns of shell composition and mantle metabolism exist, just as taxon-specific patterns of shell growth and form do. This ultimately would lead to drawing contour maps of metabolic activity throughout the entire mantle to determine their covariance with contour maps of skeletal composition. Needless to say, this promises to be a formidable task considering the limitations of methodology. It takes a long time to simultaneously analyze the distribution of only three

elements in continuous electron microprobe traverses across limited sections of the shell, let alone to repeat traverses of many elements whose ontogenetic distribution throughout the entire shell could have important physiological meaning. The precision of metabolic studies is limited by the minimum amount of tissue required to measure nutrient uptake or to assay protein so that the measurement of metabolic gradients across the mantle becomes an increasing problem with decreasing size of the animal. Yet these daunting tasks are worthwhile because we doubt that the evolution of skeletal composition and 'fossil metabolism', and their responses to environmental and physiological factors can be understood thoroughly until systematic, ontogenetic descriptions of skeletal composition and mantle metabolism are undertaken.

We submit that it will be of evolutionary interest to know what the distribution of elements within skeletons is, even if skeletal composition proves to be so variable that regular, taxon-specific patterns cannot be confirmed. This would imply evolutionary independence of the physiology of form and skeletal composition, a complication unexpected from principles of modern biochemistry that predict a physiological unity underlying all taxa.

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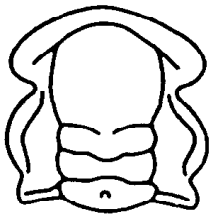

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Growth rate and substrate-related mortality of a benthic brachiopod population

MATTHEW J. COLLINS

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Vital staining and careful examination of potential substrates enabled an accurate census of *Terebratulina retusa* to be made and prompted a study of their population dynamics. Seasonal samples of *T. retusa* from a deep water *Modiolus*-brachiopod assemblage were always dominated by small (<1 mm) individuals as growth rate of the post-larvae, estimated from changes in mean cohort length, was unexpectedly slow. Six months after settlement the animals had barely doubled in length, a rate of increase consistent with laboratory studies, but an order of magnitude less than conventional estimates. As the attainment of a size refuge is the only documented strategy by which articulate brachiopods counter overgrowth or disturbance this observation has profound implications for survival. Mortality of *T. retusa* in the Firth of Lorn, from different substrates, followed an unexpected pattern. Virtually the only substrate on which adult *T. retusa* were recorded was the surface of *M. modiolus* shells, although juvenile *T. retusa* attached to this substrate suffered enhanced levels of mortality. Grazing pressures and spatial competition, believed to be reduced on complex surfaces, may account for the elevated mortality levels of *M. modiolus*-attached post-larvae prior to the apparent size refuge at a length of 2 mm. □ *Brachiopoda*, *TEREBRATULINA*, *growth rate*, *disturbance*, *ecology*, *population structure*.

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It is not surprising that growth rate and mortality of juveniles have received little attention in studies of brachiopod population dynamics. Very small brachiopods are only exceptionally recovered as fossils (Boucot 1981:290), while extant post-larvae are difficult to survey because of their small size, the possession of transparent valves and a propensity for cryptic settlement. However, an understanding of the survival of juveniles is vital to an understanding of the ecology of brachiopods. The relative success of juveniles is a major component of overall survival and growth rate a major component of this success, since articulate brachiopods are able to counter disturbance and overgrowth only by attainment of a size refuge (e.g. Jackson *et al.* 1971; Doherty 1979; Thayer 1981).

In this paper I report on a preliminary study of juvenile *Terebratulina retusa* (L.) from a deep water *Modiolus modiolus* (L.) community off the west coast of Scotland, where *T. retusa* form a significant component of both the living community and death assemblage (Collins 1986a, b). The investigation was initiated when seasonal sampling demonstrated that the population of *T. retusa* was dominated throughout the year by very small brachiopods, an observation inconsistent with prevailing views on growth rate and reproduction (e.g. Curry 1982).

Methods

Seasonal samples of *Terebratulina retusa* from a *M. modiolus* community were collected by grab sampling a deep water (200 m) channel in the Firth or Lorn (west coast of Scotland; site 1 of Curry 1982). Grab samples of 0.1 m^2 were collected on 23/08/83, 29/11/83, 10/02/84 and 24/05/84. The volume of each sample was measured prior to on-deck sieving, and those samples containing less than six litres were rejected (mean sediment penetration <6 cm). Each sample was then washed across a 1 mm mesh sieve to remove fine residues and the residue fixed by the addition of neutralized (seawater) formalin-rose bengal. Since *T. retusa* is pedically attached, animals smaller than 1 mm are retained.

In the laboratory, the residue was fractionated by wet sieving across 4 mm, 2 mm and 1 mm mesh sieves during which process some specimens of *T. retusa* were dislodged. Small dislodged animals