Dynamical assessment of the accretionary record in the shell of the mussel *Semimytilus algosus* from a rocky shore in Chile

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**Abstract**

Patterns of tidal microgrowth bands present in acetate peels of shell sections of the Chilean mussel *Semimytilus algosus* from three tidal levels on a wave-exposed shore were analyzed for the presence of recurring periodicities using numerical tools derived from signal nonlinear analysis. Both chaotic and coherent structure were found within the microgrowth pattern record, with mussels from the middle and lower tidal zones displaying a coherent structure to the patterns, whilst those from the upper tidal level displayed a chaotic banding pattern with a strong tidal component. It is suggested that because mussels in the upper tidal zone are subject to a wider suite of environmental conditions they are influenced by the spring-neap lunar tidal cycle to a greater extent than those growing in the mid to low tidal zone and display deterministic chaotic growth. This allows the mussels to grow continuously in the highly variable conditions that prevail in the upper tidal zone without continuous interruptions to shell deposition. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

**Keywords:** *Semimytilus algosus*; Microgrowth increments; Recurrence quantification analysis; Chaos

1. Introduction

Since the early 1970s the record of internal growth lines and bands contained in the calcified parts of a range of marine invertebrate phyla has been shown to be valuable in understanding ontological changes in growth, particularly in those organisms that possess a shell such as the bivalve molluscs (Pannella and MacClintock, 1968; Rosenberg and Runcorn, 1975; Lutz and Rhoads, 1980; Richardson, 1993). The shell growth record comprises a pattern of microgrowth bands left by the accretionary system during shell construction when the organism is immersed and actively feeding. It has been suggested that the observed patterns of bands result from the sequential alternation of aerobic and anaerobic metabolic pathways that occur for example in intertidal bivalves during tidal immersion and emersion.
During shell formation in bivalves aerobic pathways promote the addition of inorganic material around the growing margin of the shell (Lutz and Rhoads, 1980; Richardson et al., 1981). Whereas during anaerobic metabolism partial dissolution of recently deposited shell occurs in order to buffer the acid environment within the extrapallial fluid involved in shell formation. The consequence of these episodes of aerobic–anaerobic metabolism results structurally in the formation of a wide growth increment and a narrow microgrowth band (Lutz, 1976; Lutz and Rhoads, 1980).

Due to the intimate relationship between the physiological state of the bivalve and the prevailing environmental conditions the microgrowth incremental record has been proposed as a tool for the extraction of environmental information from the marine environment (see Rhoads and Lutz, 1980; Richardson, 1993 and references therein). Moreover, due to the interdependence of the many different physiological compartments of the body (i.e. how energy supply is shared between the different bodily functions) their influence on shell growth could potentially be ascertained from the incremental record of growth (Pannella and MacClintock, 1968; Richardson, 1989). In this way, a global categorization of the manner in which the physiology of an organism is working has been proposed by describing the rhythmicity displayed in the shell in terms of an endogenous–exogenous continuum (Palmer, 1973; Newell, 1979; Rhoads and Lutz, 1980).

In this paper we adopt an alternative, but complementary, approach to the traditional analysis of the molluscan growth record. We describe and analyze the internal microgrowth pattern record in the shell of the Chilean intertidal mussel, *Semimytilus algosus*, and classify the ontogenetic microgrowth pattern signal in the shell by means of techniques for data exploration of patterns in high dimensional space. A comparative approach was adopted, searching initially for order and pattern in the series of microgrowth increments in the shell and then investigating differences in the banding patterns in mussel shells from different tidal levels.

2. Materials and methods

Clumps of mussels *S. algosus*, (length 10–30 mm) were collected on March 30th 1998 from the high, mid and low intertidal zone (1.2, 0.8 and 0.4 m above chart datum (CD) respectively) from an intertidal rocky shore at Playa Blanca, Bahía Coronel (37°02' S; 73°10' W), Chile. Mussels were boiled, the flesh removed and the shells dried at 60°C. Ten shells from each tidal level were embedded in Metaset resin (Buehler Ltd. UK) and then cut radially along the maximum growing axis from the umbo to the margin. The cut surface of each shell was ground smooth, polished with diamond paste and then etched for 30 min in 1% Decal (a formic acid based decalcifier) (Richardson, 1989). Acetate peel replicas were prepared of the dry etched surfaces of nine shells from the high and low tidal levels and ten from mid tide level and mounted on glass slides and viewed in the light microscope. The width of each individual growth increment between adjacent bands was measured using a calibrated eyepiece graticule in sequences of up to 300 microgrowth bands from the shell margin towards the umbo. In this way the pattern of growth increments deposited by adjacent bands was measured using a calibrated eyepiece graticule in sequences of up to 300 microgrowth bands from the shell margin towards the umbo. In this way the pattern of growth increments deposited by individual mussels from similar and different tidal levels was obtained for comparison with the prevailing tidal regime and estimated periods of tidal/daily immersion and emersion.

The microgrowth band measurements from each of the 28 mussels examined from the three tidal levels together with the estimated immersion times were analyzed using methods developed under Chaos Theory (Webber and Zbilut, 1994; Abraham and Gilgen, 1995) in order to investigate whether any patterns or rhythms were contained within the data. The analysis allows a description in a simple iconographic manner of the increment widths and immersion period data signals that behave in a linear or nonlinear fashion. It is possible to recognize structure within what appears to be noisy signals usually classified as ‘random’ by traditional parametric standard techniques (like fast Fourier transformations).

Recurrence plots (RP) were constructed, embedding the unidimensional microgrowth band and immersion period data records in a higher
dimensional abstract space using the time delays method (Eckmann et al., 1987). This dimensional expansion allows an estimation of the number of variables involved in the generation of the observed data (see Takens Theorem in Stark et al., 1996), and also describes the way they are related, e.g. whether the data are in a linear or a chaotic system. An embedding dimension of ten was used for every data set of microgrowth increments measured. When a data series is expanded into a higher dimensional space, the original sequence is fractioned into small pieces, producing a particular array of vectors in this abstract space, where similar sequences share a common neighborhood. To represent this repetition of states, a dot is placed in a delay map every time a couple of vectors are close to each other as defined by a radius $r$ in higher space. In this way, a dot represents a recurrent state of the system under scrutiny (Webber and Zbilut, 1994, 1996, 1997; Zbilut et al., 1997; Schreiber, 1998; Zbilut et al., 1998). Recurrence plots explore graphically the existence of hidden patterns in short sequences, qualifying recurrences of states that are not necessarily periodic. These plots are symmetrical over the main diagonal that roughly represents time so any half of the plot is equal and can be used to interpret the output (Webber and Zbilut, 1997).

A natural extension of RP is recurrence quantification analysis (RQA), which quantifies repeatable patterns in the data and the manner in which they are organized into higher dimensional space, giving six output variables, every one characterizing a different aspect of the system under study (Webber and Zbilut, 1996, 1997). One advantage of RQA is that dynamical indices can be calculated from different data sets and statistically compared by traditional parametric techniques. The variables derived from RQA are (1) the percentage of recurrences ($%R$), which reveal the periodicity of the measurements, (2) the percentage of determinism ($%D$), which estimates the recurrent points forming short strips of two or more points parallel to the main diagonal line in the recurrence plots, as opposed to random scattered dispersion, describing then, organized structure in the signal, (3) entropy, measured by the Shannon index, that quantifies the pieces of information that are required to describe the signal, given a comparative idea of the complexity shown by different data sets, and (4) the mean distance, (DIS) between embedded vectors in multidimensional space. For comparative purposes, $%R$, $%D$, entropy and mean distance were estimated.

RQA was performed on the growth increment records in each mussel from each tidal level to compare the consistency and differences in the patterns of shell construction. The growth pattern sequences were compared using one way ANOVA (Underwood, 1997). The temporal evolution of $%R$ was also explored for both the growth increments and the estimated immersion times in each mussel from each tidal level. A tree diagram was constructed (Legendre and Legendre, 1983) to classify all the microgrowth pattern measurements based on the dynamical variables derived from RQA. The unweighted-paired grouping method of agglomeration was run using a correlation matrix of computed $r$-Pearson correlation index.

Global Lyapunov exponents were also calculated for every series of band width measurements, characterizing time divergence of nearly initial points in higher dimensional space which gives a measure of the ‘order’ exhibited in time by the accretionary records (Eckmann et al., 1986; Collet, 1992).

3. Results

The appearance of the microgrowth pattern record in $S. algosus$ is shown in Fig. 1. Fig. 1a shows the inner record left in the growing margin of the shell during the last activity of the accretionary system. Fig. 1b magnifies another section of a shell to illustrate the resolution under which measurements of the microgrowth increments that were measured.

In Fig. 2, the appearance of the microgrowth pattern record in $S. algosus$ from three tidal levels is shown. The patterns show no obvious similarities or differences. Even when the coefficients of variation (not presented here, Abades, in preparation) for every series of band widths were computed, no clear discrimination could be observed between the microgrowth increments in the mus-
Fig. 1. Acetate peel replicas of a sectioned shell of *Semimytilus algosus*. A series of growing bands (gb) and microgrowth increments (mi) deposited at the shell are shown. Note that they are clearly seen at the inner shell layer but not in the periostracum. (a) Shows the growing margin of the shell and (b) shows the resolution of the microgrowth increments that were measured.
sel shells from the different tidal levels. However, when recurrence plots were constructed (Fig. 3) a higher dimensional patterned structure emerged forming a distribution of points parallel to the main diagonal, a pattern that is usually depicted by low-dimensional chaotic systems (Webber and Zbilut, 1997). Estimated global Lyapunov exponents agree well with these observations (Table 1), having positive values close to zero. When the quantified dynamical variables were estimated through RQA, consistency was found between the microgrowth pattern records (i.e. percentage recurrences) in the mussels from the low and middle tidal levels, but significant differences were recorded in the mussels from the high tidal level (one way ANOVA, 2 d.f., $F = 21.08$, $P < 0.01$) (Fig. 4a). Similar results were obtained using percentage determinism (Fig. 4b; one way ANOVA, 2 d.f., $F = 6.08$, $P = 0.007$) and entropy measurements (Fig. 4c; one way ANOVA, 2 d.f., $F = 4.72$, $P = 0.018$).

Fig. 5 summarizes into a tree diagram (i.e. UPGMA, r-Pearson) the dynamical variables derived from the RQA. It can be clearly seen that the microgrowth pattern records of the mussels from the upper tidal level together with one mid tide level mussel form a separate cluster from the remaining mussels from the mid shore and those from the lower shore level. A comparison of the temporal changes in the microgrowth pattern record at each tidal level with the corresponding immersion time periods (Fig. 6) shows a clear

Fig. 2. Appearance of a series of measurements from the accretionary record of *Semimytilus algosus* and immersion time profiles. Each column represents an intertidal height. The first row of figures are tidal immersion periods profiles for the time concomitant with the process of increment deposition. The second and third rows of figures are records of microgrowth increment measurements. In this example there is no apparent temporal coherence between the immersion periods and the variation in width of the growth increments. Note that the oldest band deposited is labelled zero and located at the right of each of the x axes.
Fig. 3. Recurrence plot to illustrate the kind of picture obtained when records of microgrowth patterns are time delayed and embedded in higher dimensional space to recover system functioning information. The map is a collection of points symmetrical to the main diagonal. Note the organization of points in strips running parallel to the main diagonal. This is suggestive of a non-linear (perhaps chaotic) ensemble of the state variables that forms the accretionary system that originated from the observed microgrowth increments. All the accretionary series depicted the same underlying organization, but not necessarily the same spatial pattern shown here.

correspondence between the microgrowth increments and immersion time in the mussels from high tide (Fig. 6a), which is not apparent in the shells from the other two lower tidal levels (Fig. 6b and c).

4. Discussion

Descriptions of growth patterns can be used to study the degree of correlation between the environment and the physiological state of the organism (see review in Rhoads and Lutz, 1980). As a result this approach has been broadly adapted by many workers during the last two decades (Richardson, 1993). Studies of the effects of environmental factors on shell growth have been largely based on experimental investigations that have demonstrated close agreement between the microgrowth increment patterns and the expected tidal amplitude changes (Richardson et al., 1979, 1980). This evidence for tidally induced growth patterns in bivalve shells has come from species growing under macrotidal conditions, tidal regimes characterized by large tidal amplitudes between high and low water and during the spring-neap lunar cycle. In this paper we have provided quantitative evidence for periodicities in the growth patterns in mussels growing in a microtidal regime (tidal amplitude < 2 m). In the study it was impossible to validate the periodicity of the microgrowth increments using marked transplanted mussels, an approach that has been

Table 1

<table>
<thead>
<tr>
<th>Tidal position</th>
<th>Mean value ($10^{-3}$)</th>
<th>S.E. ($10^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High intertidal</td>
<td>51</td>
<td>11</td>
</tr>
<tr>
<td>Middle intertidal</td>
<td>50</td>
<td>11</td>
</tr>
<tr>
<td>Low intertidal</td>
<td>60</td>
<td>10</td>
</tr>
</tbody>
</table>

*a All mean values are positive and close to zero, which has been suggested as an analytical value characteristic of chaotic trajectories, complementing the qualitative findings of recurrence plots.
undertaken successfully elsewhere (Richardson, 1989). *S. algosus* is a highly mobile mussel occupying rocky intertidal Pacific wave-exposed platforms and in these situations it would have been extremely difficult to retain marked mussels in cages secured to the shore. Statistical analysis of the microgrowth patterns has allowed the identification of recurrent periodicities within the growth record of *S. algosus* although considerable variation in the periodicities amongst mussels from the same tidal level was observed.

Examination of the percentage recurrences within the microgrowth increment record (Fig. 4a) showed good discrimination between the patterns displayed by the mussels inhabiting the high tidal level and those from the middle-low tidal zone. Measurement of the nonlinear dynamical variables, percentage of determinism and entropy suggest that the rate of shell deposition in mussels from the high shore level is different from that observed in mussels growing at mid and low tide shore levels (Fig. 4). If the skeletal record were to be used to understand the complex interaction between the physiological condition of the mussels and their fluctuating environment as has been

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**Fig. 4.** (a) Mean percentage recurrences derived from RQA for accretionary series. Intertidal positions differ in such a way that the middle and low shore mussels present a lower recurrence structure than animals from the high intertidal level. Differences are significant (see text). In (b) the Mean determinism (percentage) derived from the quantification of recurrences forms strips of three or more points. As in (a) differences are significant (see text), high intertidal animals having a dynamically more organized structure than the middle and low animals. (c) Entropy, measured by the Shannon Index of information. The figure shows that the accretionary system in high shore mussels needs more information than the middle and low shore mussels to describe the series of observations, i.e. they are more complex.

**Fig. 5.** Cluster of accretionary series, using as classification variables, those derived from RQA: percentage recurrences, percentage determinism, entropy, mean distance and maxline. b, low intertidal animal; m, middle intertidal animal and a, high intertidal animal. It is clear the appearance of a separate cluster containing mostly ‘a’ accretionary series.
Fig. 6. Correspondence of recurrences in time, between a one series of microgrowth increments and the corresponding series of recurrences derived from tidal immersion periods (daily basis) at every intertidal height. There is a clear synchrony in high shore (a) that is lost at middle (b) and low (c) intertidal.

proposed (Rhoads and Lutz, 1980), then the accretionary system of *S. algosus* would not be suitable as it behaves chaotically in a highly variable and unpredictable manner. Mathematical analysis of the growth record indicates divergences of trajectories in multidimensional space, as measured by an index such as global or local Lyapunov exponents (see Table 1). Negative values are characteristic of stable equilibrium trajectories, positive values and those close to zero are indicative of low dimensional deterministic chaos whilst highly positive values can not discriminate between high dimensional chaos from 'random walk' (Eckmann et al., 1986; Collet, 1992). Biologically the current mathematical analysis implies strong differences in the way individual mussels respond, even when they are growing at the same tidal level under apparently similar growing conditions. Our field observations of *S. algosus* on wave-swept Chilean shores indicate that it is a mobile mussel moving often daily amongst the dense beds of the dominant mussel species *Perumytilus purpuratus*. Unlike many bivalve species that remain anchored to the substrate or within the sediment and display pronounced tidal growth patterns (Richardson, 1993) *S. algosus*’s mobility within a small tidal range (< 2 in) may account for the lack of any clearly defined spring-neap lunar tidal pattern in the shells of all but those mussels that exist in the upper shore levels.

Ecologically, it is convenient to have a system that behaves in this way, because organisms that inherently possess deterministic chaos will be more predisposed to reacting to the rich variety of environmental responses that they will be exposed to in their natural habitat. This promotes flexibility and adaptability through a diverse repertoire of responses when the organism is faced with unexpected environmental changes (May, 1976; Hoyert, 1992). From the standpoint of shell growth it ensures that the growth process, normally a continuous one, does not collapse when stressful conditions prevail. If a system behaves in a strictly deterministic way then any unexpected event will be reflected as a ‘perturbation’ in the growth process. The growth of mussels occupying wave-swept shores would continually be interrupted by the force of the breaking waves and their swash unless they possessed a growth process that was largely independent of these perturbations. The correspondence between periodic tidal events and the shell microgrowth patterns in the shells of *S. algosus* from the upper shore level suggests that variability in the growth increments could
be explained by tidal movements. By contrast no temporal connection is apparent between the microgrowth increments and the tidal pattern of immersion imposed on the mussels in the middle and low intertidal levels. This suggests that *S. algosus* has the freedom to respond to extreme conditions, similar to those experienced by mussels and other bivalve species from coastal waters of high latitudes, where the tidal amplitude is large and the organism is periodically emersed or immersed for long periods of the tidal cycle. We suggest that under such macrotidal regimes, when a mussel is emersed for several hours an effective anaerobic pathway becomes operative, the process becomes reversed under immersed conditions. On exposed rocky shores like Playa Blanca (Chile) where a microtidal regime (a small tidal amplitude is in operation), local conditions like windy days and splash, etc. would maintain the mussels under moist and damp conditions and anaerobic metabolism would probably not be usually necessary. Observations of *S. algosus* in their natural environment suggest that during emersion at low tide a fugitive wave is enough to induce the animals to open their shell valves when they would be otherwise closed to avoid desiccation. Thus periods of anaerobiosis during low water will be punctuated by short aerobic events. If the mussels did not possess deterministic chaos behaviour then the continual switch between aerobic and anaerobic conditions would continually result in disrupted shell deposition rather than continuous shell growth.

The present evidence from the shell microgrowth patterns suggests that along the tidal gradient, mussels growing in the upper intertidal zone experience somewhat different growing conditions to those growing lower down on the shore, in the middle-low intertidal zone. The growth of the upper shore mussels is in response to the chaotic environmental conditions imposed by the rise and fall of the tides whereas the growth of the mid-lower shore mussels appears to reflect individual variations in shell deposition. Despite the unstable mechanisms governing shell accretion the morphology of the shell of *S. algosus* is similar at the different tidal levels.

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References


