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# **Evidence of a 2-day periodicity of striae formation in the tropical scallop *Comptopallium radula* using calcein marking**

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## Abstract

The periodicity of striae formation in the tropical scallop *Comptopallium radula* (Indo-West Pacific Ocean) was investigated with an in situ marking technique, using the calcein fluorochrome. To minimize scallop stress caused by excessive handling, in situ benthic chambers were used for marking experiments. Once marked, scallops (shell height range: 38.4–75.8 mm) remained on site in a large benthic enclosure and were collected at regular time intervals to count new striae formed after marking, over a period of 3 months. A 3-hour exposure period with calcein ( $150 \text{ mg L}^{-1}$ ) was sufficiently long to create a detectable mark in nearly all shells. It was however impossible to count the striae in 48.2 % of the shells (mainly large specimens) because of a very small growth after marking. Lack of significant mortality during the experiments indicated that tested calcein concentrations were not lethal. A decrease in shell growth rate was observed after marking but the respective impacts of calcein toxicity and changes in environmental conditions could not be discriminated. Our results suggest that in situ calcein marking inside benthic chambers is suitable for shell growth studies of scallops, provided the latter are not too old. After marking, the juvenile *C. radula* formed an average of one stria every 2.1 days in summer. Reports of 2-day periodicity in biological rhythms are rare. Striae formation in *C. radula* may be controlled by an endogenous oscillator, synchronized by an environmental cue acting as a zeitgeber, such as seawater temperature or sea level pressure, both of which exhibit 2-day variations in the Pacific Ocean. As in many other scallop species, *C. radula* forms striae periodically under natural conditions, but this study shows that in pectinid juveniles, this periodicity can deviate from a daily cycle. These results suggest that *C. radula* shells have tremendous potential for recording environmental conditions during periods ranging from months to a few years and with a resolution of 2 days.

## Introduction

New Caledonia is a group of islands situated in the South Pacific Ocean, 1500 km from Australia. The main island is surrounded by a barrier reef enclosing a 23 400 km<sup>2</sup> lagoon. Mining is the main local economic resource as New Caledonia is the 3<sup>rd</sup> largest nickel ore producer in the world and also produces other metals such as cobalt and chromium (Labrosse et al. 2000). Intensive open-cast mining on hilltops enhances terrigenous and associated metal inputs to the lagoon. Recent studies dealing with bioaccumulation of trace metals in tissues of various marine organisms (Bustamante et al. 2000; Breau 2003) in the lagoon have been completed, however there is still a need for a high-resolution trace metal contamination proxy.

Many studies have already focused on the potential of marine carbonate skeletons, especially bivalve shells such as cockles or mussels, to record environmental trace metal contamination (e.g., Price and Pearce 1997; Richardson et al. 2001). The study of such a potential in scallop shells (Mollusca; Bivalvia; Pectinidae) has been few studied even though periodically formed striae (see Richardson (2001) for terminology), that allow accurate dating of each part of the shell, have been described (Chauvaud et al. 1998; Lorrain 2002). Precise dating of the skeletal section of interest is a prerequisite to any study of shell records. Hence, before conducting a study on environmental tracers in the shell of a given species, it is necessary to know the age of each shell sub-sample and the species growth rate.

*Comptopallium radula* (Linne, 1758) is a large (maximal shell height = 110 mm) sedentary scallop living under branching corals, especially *Acropora* sp., or on coralline fragments beds (Dijkstra 1984). In the New Caledonia lagoons, this species is frequently found in shallow muddy to sandy bays that are subject to significant metal inputs. As in many other scallops species (e.g., *Pecten* and *Argopecten* genera), the fine

microscopic concentric striae wrinkling the surface of its shell can be used to estimate growth rates (Fig. 1).

Since Clark (1968), many studies have focused on the rhythm of striae formation in scallops. Most results have suggested a daily periodicity in  $\text{CaCO}_3$  deposition (Clark 1968; Wrenn 1972; Clark 1975; Antoine 1978; Broom and Mason 1978; Helm and Malouf 1983; Hurley et al. 1987; Joll 1988; Parsons et al. 1993; Chauvaud 1998). However, different growth periodicities have been observed in other bivalves. For example, in the Northern Quahog *Mercenaria mercenaria*, six different periodicities were identified (Pannella and MacClintock 1968; Kennish and Olsson 1975): annual (350-380 days), monthly (29 days), tidal (14 days), bi-daily (2 days), daily (1 day), and subdaily (< 1 day). Therefore, the cadence of calcification must be evaluated before a new scallop species can be used as an "environmental sentinel".

Different methods have been used to assess the rhythmicity of striae formation in scallops, e.g., successive measurements (Clark 1968; Broom and Mason 1978), mark-and-recovery experiments (Joll 1988), or chemical marking (Clark 1975; Hurley et al. 1987; Parsons et al. 1993). Chemical techniques using fluorochromes (tetracyclines, alizarin red, xylenol orange, and calcein) have also been used for growth studies on fish (MacFarlane and Beamish 1987; Wilson et al. 1987; Monaghan 1993), gastropods (Pirker and Schiel 1993; Day et al. 1995; Moran 2000) and many other marine organisms (Rowley and MacKinnon 1995). Incorporation of fluorochromes into calcifying otoliths or shells produces an internal fluorescent mark and any subsequent growth can then be estimated readily. Compared to other chemicals (e.g., tetracycline), calcein appeared to induce little toxicity (Rowley and MacKinnon 1995). Despite recent experiments successfully using calcein as a growth marker in the brown mussel *Perna*

*perna* (Kaehler and MacQuaid 1999), this dye has received relatively little attention, especially with respect to bivalve growth studies.

The objectives of the present study were: (1) to investigate the potential of calcein to study growth pattern in scallop shells and (2) to assess the periodicity of striae formation in *C. radula*.

## Methods

### Scallop collection and shell marking

A preliminary study, using mark-and-recovery experiments (Lefort 1994), failed to identify the periodicity of striae formation in *C. radula* (Thébault, unpublished results) as almost all individuals stopped growing after tagging. As it is essential to minimize stress on the scallops during the experiments, we opted for an in situ shell marking experiment, using calcein.

Scallops ( $n = 141$ ) were collected by diving on 23/12/2002 (austral summer) near Nouméa, in the southwest lagoon of New Caledonia. Shell height of these specimens, measured a posteriori (see section 2.2), ranged from 38.4 to 75.8 mm. The individuals were randomly assigned to 6 batches in order to test the effects of calcein concentration and incubation time (Table 1). Coloured plastic tags were fixed on all shells with cyanocrylate glue (1 colour per batch). During these operations, scallops were exposed to air for less than 10 minutes. They were then placed in a large benthic enclosure (2 m  $\times$  1 m) near a natural bed of *C. radula*. Branching corals were positioned in the cage to mimic the biotope of the species.

After allowing for a stress recovery period of 1 month, calcein marking was conducted. Each batch was carefully removed from the enclosure and placed in a benthic chamber made of a clear acrylic hemisphere fastened to a 0.2 m<sup>2</sup> PVC base inserted into the

sediment and partly filled with ca. 7 L of air to prevent anoxia (Fig. 2). A gusher galley pump connected to a 12 V underwater power supply ensured a  $5 \text{ L min}^{-1}$  closed-circuit flow to homogenize the enclosed water (ca. 50 L). Calcein (Sigma-Aldrich Pty. Ltd., Australia - # C0875) stock solutions of 15, 30 and 60 g calcein  $\text{L}^{-1}$  were prepared in 1 L of Milli-Q water, with 105 g sodium bicarbonate added (to adjust the pH to 8.2). Calcein was then injected into the benthic chamber with a syringe through a clamp (Fig. 2). After the marking period was finished (3 or 6 h, depending on the marking experiment), the scallops were carefully returned to the enclosure. Subsequently, 3 scallops were randomly selected and removed from each of the 6 batches after 13, 20, 27, 34, 48, 62, 69 and 91 days.

Bottom-water temperature was measured during the experiment hourly with an EBRO Ebi-85 probe fixed to a bottom mooring. Salinity was recorded weekly using a Sea-Bird SBE 19 CTD profiler. Chlorophyll *a* concentrations were measured on bottom-water samples collected weekly with a 5 L Niskin bottle.

### **Sample preparation and observation**

After sampling, the marked scallops were killed immediately and the soft parts discarded. Shells were air-dried, their final height was measured along the maximal growth axis (umbo-rim axis) with a vernier calliper (accuracy = 0.1 mm) and they were stored in the dark to prevent any fluorescence decrease (Wilson et al. 1987). Shells were sliced using a diamond-bladed rock saw (Fig. 1a) at the Aquatic Animals Sclerochronology Laboratory (Plouzané, France). Shell pieces were cleaned in 90 % acetic acid for 45-60 seconds, rinsed with water, air-dried and embedded in a polyester resin (Sody 33 - polymerisation time: 6 h at 30 °C - Escil, Chassieu, France) before being sagittally sectioned along the maximal growth axis using an Isomet low speed saw equipped with a diamond wafering blade (Buehler, Lake Bluff, IL, USA). The 600  $\mu\text{m}$

thick sections were polished with 0.33  $\mu\text{m}$  calcined alumina powder (Escil, Chassieu, France). The shell slides were observed under a Leitz DM RBE fluorescence microscope (Leica Mikroskopie & Systeme GmbH, Wetzlar, Germany) equipped with an Osram 50-W high-pressure Hg lamp and with an I2/3 filter block: excitation filter BP450-490, dichroic mirror RKP510 and emission filter LP515.

A marking quality index (MQI) was defined according to fluorescence intensity and length of the mark: 0 = no mark, 1 = detectable but faint or not surfacing mark, 2 = bright mark, 3 = long and very bright mark. The shell height at calcein marking, or initial shell height, was calculated as the difference between the final shell height and the shell increase since marking (Fig. 3). For each shell, the number of striae formed within this interval was counted by 3 different readers and then the mean length of the  $n$  increments ( $= a/n$ ) preceding the calcein mark was compared with the mean length of the  $n$  increments between this mark and the ventral edge ( $= b/n$ ) with  $n_{\text{max}} = 10$  (Fig. 3).

### Statistical analyses

ANOVAs were performed to check: (1) the differences in mean shell height of scallops for the 6 experiments and for the 8 post-marking sample periods and (2) if successful calcein markings were related to shell height. The influence of calcein concentration and immersion time on the marking quality index was tested using a two-way ANOVA. An ANCOVA was used to test whether there were significant differences between the slopes of the least-square linear regressions (number of new striae vs. number of days of growth) calculated for each of the 3 readers. Homogeneity of variances (for ANOVA) and residual variances (for ANCOVA) was tested with Bartlett's test ( $\alpha = 0.01$ ). All statistical analyses were performed according to Scherrer (1984).



## Results

### Calcein marking experiments

Only 2 scallops out of 141 died during the 13 weeks following the calcein marking. The dead scallops (first recovered on 19/02/2003 and the second recovered on 24/04/2003) were from groups marked with a calcein concentration of  $300 \text{ mg L}^{-1}$  during 3 h and  $150 \text{ mg L}^{-1}$  during 6 h, respectively. Moreover, 23 scallops lost their coloured plastic tag in the benthic enclosure. Mean initial shell height did not differ significantly for the 6 experiments (Bartlett's test:  $B_C = 3.12$ ,  $df = 5$ ; ANOVA:  $F = 0.26$ ,  $df = 5$  and  $110$ ,  $p = 0.93$ ) and for the 8 post-marking samples (Bartlett's test:  $B_C = 2.73$ ,  $df = 7$ ; ANOVA:  $F = 1.18$ ,  $df = 7$  and  $131$ ,  $p = 0.32$ ). The two-way ANOVA indicated no significant effect of calcein concentration and immersion time on the MQI (Table 2). However, an inverse relationship was found between MQI and initial shell height (i.e., age; Fig. 4, *open diamonds*).

The counting of striae formed after the staining experiment was impossible for 48.2 % of the shells because of either the absence of a calcein mark (7 shells) or an insignificant growth after marking (presence of a disturbance mark resulting from the coalescence of striae around the calcein mark in 60 shells). The mean initial shell height of these 67 scallops (66.3 mm) was significantly larger (Bartlett's test:  $B_C = 0.48$ ,  $df = 1$ ; ANOVA:  $F = 124.93$ ,  $df = 1$  and  $137$ ,  $p < 0.01$ ) than the rest of the experimental population (mean = 53.5 mm). Moreover, an inverse relationship was found between net shell accretion after marking (i.e., distance between the calcein mark and the ventral margin) and initial shell height of the scallops (Fig. 4, *solid circles*). For each of the 72 remaining scallops, the mean length of the growth increments (i.e., mean inter-striae distance) after the marking experiment was smaller than before the calcein mark ( $\Delta < 0$

for all scallops; Fig. 5). For all 72 individuals, the average of the mean length of the increments decreased from 258 to 128.6  $\mu\text{m}$  (mean decrease of 50.2 %). There was no significant relationship between the initial shell height and the decrease in mean length of the increments (linear regression analysis:  $r^2 = 0.02$ ,  $p = 0.33$ ).

During the experiments, bottom-water temperature ranged from 25.4 to 29.3 °C, salinity from 35.01 to 35.74 and chlorophyll *a* concentration from 0.45 to 1.30  $\mu\text{g L}^{-1}$ . The mean temperature in the month preceding the calcein marking was 27.8 °C vs. 26.8 °C in the following month. Monthly averaged chlorophyll *a* concentrations also decreased from 1.42 to 0.59  $\mu\text{g L}^{-1}$  after the marking experiment. No significant difference in salinity was found before and after the marking experiment.

### Periodicity of striae formation

A green calcein mark was detectable on 72 scallops (Fig. 6). For each of the 3 readers, the number of new striae formed after the staining experiment was plotted against the number of days (Fig. 7a-c). The linear regression analysis performed for each set of data indicated a highly significant relationship for each reader ( $p < 0.01$ ). The slopes ranged from 0.47 to 0.52 ( $p < 0.01$ ) and intercepts were not significantly different from 0 ( $p > 0.05$ ), indicating that *C. radula* formed 1 stria every 1.9-2.1 days, depending on the reader. Furthermore, an ANCOVA showed no significant difference between the 3 slopes (Bartlett's test:  $B_C = 1.55$ ,  $df = 2$ ; ANCOVA:  $F = 1.40$ ,  $df = 2$  and 169,  $p = 0.25$ ). Inter-reader differences were assessed by calculating the relative standard deviation (RSD) for each of these 72 scallops: RSD ranged from 0 to 38.9 % (mean = 10.6 %).

When the results of the 3 different readers were pooled, a highly significant linear relationship ( $r^2 = 0.91$ ,  $p < 0.01$ ) was observed between the average number of new striae and the number of days of growth (Fig. 7d). The slope was highly significant ( $p <$

0.01) whereas the intercept was not ( $p = 0.23$ ). On average, *C. radula* formed 1 stria every  $2.1 \pm 0.2$  days (95 % confidence interval). Inter-individual variability was quite important, especially for the 1<sup>st</sup> post-marking sample (13 days, RSD = 42 %). For the 7 other times, RSD values ranged from 13 to 22 %.

As striae production has sometimes been related to the increment of shell growth (i.e., relationship between the number of striae formed per day and the shell growth rate; Owen et al. 2002), we also investigated the relationship between striae number and the shell increment (net accretion) deposited between the calcein mark and the ventral margin, at each of the 8 sampling dates (Fig. 8). Details on the linear regression analysis performed at each of these dates are given in Table 3. There was a large variation in the strength and significance of each of these 8 relationships,  $r^2$  ranging from 0.15 to 0.88, and  $p$  from  $< 0.01$  to 0.40. When scallops which have experienced an important stress after marking were removed from the linear regression analyses (i.e., 12 specimens in the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> post-marking samples with net accretion  $< 0.05$  mm; *open circles* on Fig. 8), then the  $p$ -value for these 3 samples were 0.30, 0.10, and 0.16, respectively. Depending on the significance level ( $\alpha = 0.01$  or 0.05), these 8 relationships may be considered as significant or not. Large variations were also observed in slopes and intercepts of the 8 relationships. Intercepts were significantly different from 0 ( $p < 0.01$ , except for the 4<sup>th</sup> post-marking sample:  $p > 0.05$ ). Finally, it appears that only 19 scallops out of 72 (26.4 %) formed more striae than expected under the hypothesis of a 2-day periodicity of striae formation (Fig. 8, *solid diamonds*).

## Discussion

### Effects of calcein concentration and immersion time

The marking quality in *C. radula* shell was independent of the calcein concentrations and immersion times tested in this study. A calcein concentration of 150 mg L<sup>-1</sup> was sufficient to produce an internal fluorescent mark. This result is in good agreement with previous fluorochrome studies demonstrating that calcein solutions of 100 to 150 mg L<sup>-1</sup> were sufficient to mark otoliths (Wilson et al. 1987), gastropod shells (Moran 2000) or bivalve shells (Kaehler and MacQuaid 1999).

Minimum immersion times tested in previous studies varied from 2 h (Wilson et al. 1987) to 17 h (Fujikura et al. 2003). Immersion time is likely to depend on the shell growth rate of each species because the transfer of ions or calcein through the mantle epithelium is quicker in rapidly growing organisms (Day et al. 1995). A 3-hour incubation period in the benthic chamber was sufficient to create a detectable mark on *C. radula* because of its rapid growth rate (reaching ca. 60-70 mm for the first year, Thébault, unpublished data).

### Calcein toxicity

Important lethal effects of calcein marking have been observed in the larvae and juveniles of walleyes, *Stizostedion vitreum* (Brooks et al. 1994). In *C. radula*, natural mortality has been estimated at 0.47 (Lefort 1994). Therefore, the losses observed in the enclosure (1.4 % over 3 months) may be attributed to natural mortality rather than to a lethal effect of calcein. Previous studies have indicated that calcein induces very little toxicity, with no effect on growth rate (Rowley and MacKinnon 1995), but the absence of lethal effects does not imply absence of toxicity here. On the other hand, high calcein

concentrations ( $> 250 \text{ mg L}^{-1}$ ) have been demonstrated to induce behavioural stress in juvenile striped bass, *Morone saxatilis* (Bumgardner and King 1996). In *C. radula*, the 50.2 % mean decrease in increment length observed after the staining experiment could be related to calcein toxicity. It is also possible that the temperature and chlorophyll *a* concentration decreases in the month following the calcein marking influenced the shell growth rate. Other investigators have already demonstrated that scallop growth is controlled by many environmental parameters including temperature and food availability (Broom and Mason 1978; Wallace and Reisnes 1985; Wilson 1987; Chauvaud et al. 1998; Laing 2000).

### **Relationship between marking success and initial shell height**

Our results suggest an age-dependant effect on the success of calcein marking because the MQI was lower in large (i.e., old) scallops than in small ones. Moreover, the larger the scallops were, the smaller the growth after marking was. This inverse relationship reflects a decrease in the rate of shell accretion throughout the life span, an ontogenetic change widely recognized in the life history of many organisms that grow by accretion. This feature may be partly related to reproductive status. *C. radula* is known to spawn year round with an initial sexual maturity size of ca. 60 mm (Lefort and Clavier 1994). Hence it is likely that the largest scallops included in the staining experiments could have allocated a part of their energy to reproduction at the expense of shell growth. Low growth rates could reduce the transfer of calcein through the mantle epithelium: a 3 or 6 hour immersion in calcein was probably insufficient to produce an internal fluorescent mark in the larger scallops.

The marking technique used in this study appears as a satisfactory method for the investigation of striae formation in scallop shells. However, if the range of initial shell height is large, one can only expect half the scallops to recover and grow satisfactorily

after marking. Consequently, only small juvenile scallops should be used in such studies, in order to increase the marking success.

### **Periodicity of striae formation**

Our results show that juvenile *C. radula* formed 1 stria every 2.1 days (2-day periodicity or bi-daily pattern). However, the number of new striae formed after marking was variable for scallops collected on the same date. Visual identification and counting of scallop striae has an important subjective component that may lead to significant inter-reader discrepancies. For *C. radula*, relative standard deviations calculated for each shell analysis were low and the average RSD of 10.6 % indicated that inter-reader discrepancy was not very important in our study and that all 3 readers counted approximately the same number of striae on each shell.

However, all 3 readers sometimes encountered troubles in deciding whether a structure observed after the calcein mark was a stria or not, because of either an odd morphology or a strangely small spacing with the immediately preceding or following structure (see for example the shell area inside the *dashed line* on Fig. 6). Such structures may be striae, calcification anomalies, or exogenous elements such as sand grains or fouling organisms. These ambiguities partly account for the low variability in the number of new striae formed after marking for scallops collected on the same date, and are mainly due to the method of counting. Actually, accurate identification and counting of scallop striae is much more difficult on a sagittal section of a shell (side view of each stria) than on the external surface of the valves when each stria is observed as a whole. Striae splitting into two "sub-striae" were observed on some *C. radula* shells (see for example the area inside the *dashed line* on Fig. 1b). Such anomalies cannot be detected on a sagittal section when they are easily identified on the external surface of the valves. It is obvious that the observation of shells on a sagittal section is not the best method for an

accurate counting of scallop striae. It is however the only way to proceed when using calcein marking as this dye cannot be observed on the external surface of the valves. This may explain why 19 scallops out of 72 seemed to have more striae (average = +2.2 striae) than expected under the hypothesis of a bi-daily periodicity of striae formation. A large number of shells also presented less striae than expected under this assumption. It is likely that low growth rates observed just after the staining experiment resulted in the coalescence of striae, thus lowering the number of individualized (i.e., countable) striae. This initial disturbance could be responsible for a decrease in the intercept value of the regression line, without having a significant effect on the slope (i.e., on the periodicity of striae formation).

Finally, the low variability observed around the striae/days regression line can be explained and cannot be used as evidence against the periodic formation of *C. radula* striae. Such variability has also been observed in some other studies dealing with the periodicity of scallop striae formation (Antoine, 1978; Parsons et al. 1993).

In their study dealing with the scallop *Pecten maximus*, Owen et al. (2002) suggested that striae production is related to the increment of shell growth and not to any form of periodicity, thus confirming the results of Gruffydd (1981) on the same species. They finally concluded that it was probably fortuitous that in some experiments (e.g., Clark 1968) a daily periodicity of striae production occurred in some pectinid species. In order to check whether the assumption of Owen et al. (2002) could be true in *C. radula*, we investigated the relationship between the number of striae and the increment of shell growth deposited after the calcein mark (Fig. 8 and Table 3). Some relationships were strong and significant. However, this is not an evidence of the truthfulness of their hypothesis: if striae were formed periodically, scallops strongly stressed by calcein marking may have formed less individualized (i.e., countable) striae and added less new

shell material than scallops whose stress recovery was quicker. Their hypothesis could be checked if slopes were approximately the same from a post-marking sample to the next one, which was not true in our study (e.g., slope of 0.90 and 3.56 striae mm<sup>-1</sup> for the 3<sup>rd</sup> and 4<sup>th</sup> post-marking samples, respectively, whereas these two sampling dates were separated by 7 days only). Moreover, their assumption implies that intercepts of the regression lines should be close to 0. This was not the case in our study. Finally, careful scrutiny of Fig. 8 indicates that a large number of scallops have formed as many striae as scallops which have deposited far less new shell material. Consequently, our results do not support the hypothesis of Owen et al. (2002).

The 2-day periodicity of striae formation in *C. radula* has also been confirmed by an independent geochemical method (Thébault et al. in prep.). Since the pioneer works of Epstein et al. (1953), the oxygen isotopic composition ( $\delta^{18}\text{O}$ ) of marine biogenic carbonates is indicative of the temperature and oxygen isotopic composition of the ambient seawater from which it is precipitated. Calcite samples drilled along the maximal growth axis of the shell of 6 juvenile *C. radula* specimens were analysed for their oxygen isotopic composition. A very good agreement was found between seawater temperature and  $\delta^{18}\text{O}_{\text{shell calcite}}$  when dates were affiliated to each calcite sample by backdating from the harvest date and considering a 2-day periodicity of striae formation. This excellent relationship has led to the establishment of a new paleotemperature equation predicting seawater temperature with a mean absolute accuracy of 1.0 °C (Thébault et al. in prep.), thus providing another evidence of the bi-daily periodicity of striae formation: if striae were not produced bi-daily, there would be large differences between predicted temperature and measured temperature.



## Origin of the 2-day periodicity

In the plant and animal kingdoms, rhythmic activities are generally assumed to be controlled by endogenous oscillators, synchronized by environmental cues acting as zeitgebers (Aschoff et al. 1982). In marine bivalves, diurnal, tidal and seasonal cycles are thought to be responsible for growth rhythms as a result of changes in environmental factors (Rosenberg and Jones 1975). However, although the bivalve shell ultimately reflects these cycles, this signal transfer is neither passive nor immediate, and may involve a "rhythmic" tissue in the organism (Thompson 1975). If the organism maintains its growth rhythm in the presence or absence of these environmental cycles, it is said to have a "biological clock" (Thompson 1975).

Reports of 2-day long, or bi-daily, biological rhythms in nature are unusual (Pannella and MacClintock 1968). *M. mercenaria* is the only species in which a 2-day periodicity of growth pattern formation has been observed (Pannella and MacClintock 1968; Kennish and Olsson 1975). Bi-daily variations of meteorological and oceanographic parameters, including sea surface temperature and swell height and direction, have been observed in the Pacific Ocean (Kenyon 1996). For some of these parameters, e.g., sea level pressure and wind velocity, the amplitudes of the 2-day variations were larger than that of the diurnal signals. A traveling nearly 2-day wave ( $T = 2.1$  days) has also been observed in the atmosphere (Craig et al. 1980; Salby 1981; Hagan et al. 1993). Spectral analysis of the seawater temperature data in Nouméa identified a 2.1 day periodicity (Thébault, unpublished data). However, the amplitude of this bi-daily pattern is weak compared to the diurnal and semi-diurnal signals. Thus, it is unlikely that seawater temperature is the zeitgeber controlling the periodicity of striae formation in *C. radula* through an endogenous oscillator. Another environmental factor such as sea level pressure or wind velocity may act as this zeitgeber. Sea level pressure has already been

identified to control activity rhythms of crabs (Palmer 1975). At this time, further data acquisition is required in order to analyze the spectra of these parameters in New Caledonia.

### ***Comptopallium radula* in the pectinid family**

A daily periodicity of striae formation has been suggested for many scallop species. In the genus *Pecten*, such a periodicity has been demonstrated to exist in juveniles of *P. diegensis* (Clark 1968) and *P. vogdesi* (Clark 1975) as well as in juveniles and adults of *P. maximus* (Antoine 1978; Chauvaud 1998). In the *Argopecten* genus, daily formation of striae has been suggested for *A. irradians* (Wrenn 1972; Clark 1975; Helm and Malouf 1983), *A. gibbus* and *A. circularis* (Clark 1975). The same periodicity has also been identified in other genera, e.g., juveniles of *Amusium balloti* (Joll 1988), juveniles of *Chlamys opercularis* (Broom and Mason 1978) and larvae and post-larvae of *Placopecten magellanicus* (Hurley et al. 1987; Parsons et al. 1993).

However, deviations from this daily rhythm have sometimes been observed when scallops were maintained under experimental or semi-natural conditions out of their benthic habitat (Clark 1975; Broom and Mason 1978; Gruffydd 1981; Helm and Malouf 1983; Owen et al. 2002). Results of these studies, conducted on three different pectinid genera (i.e., *Pecten*, *Argopecten*, and *Chlamys*), might indicate inter-taxon differences. But they might have also been distorted because of severe experimental stress on scallops. For this reason, results from studies performed in unrealistic growing conditions are difficult to compare with results from studies carried out on wild populations in their natural habitat and may not be used to disprove the assumption of a periodic formation of striae by scallops. It is obvious that our treatment was also stressful as animals were subjected to calcein marking inside benthic chambers and then held in enclosures. However, marking was performed in situ, for short periods of time,

without removing scallops from seawater, and benthic enclosure was probably large enough to limit cage effects. All these precautions were taken to limit stress as much as possible.

Our results demonstrated that under these experimental conditions, *C. radula* striae are formed periodically, and are in good agreement with other studies dealing with scallop shell growth. However, our study shows that a daily periodicity of striae formation is not a characteristic trait of pectinidae. Clark (1975) has discussed that great differences in the response of organisms to environmental variables exist, and data acquired from one set of organisms cannot necessarily be applied to another, even if they are closely related.

### **Implications for further studies**

Many analytical techniques now exist for high-resolution chemical analysis of selected areas of biogenic carbonates, e.g., LA-ICP-MS (Richardson et al. 2001) and TXRF (Mages et al. 2004). A quantitative description of the periodicity of striae formation in *C. radula* makes possible the accurate affiliation of a formation date to each stria by backdating from the collection date, as long as striae are counted on the external surface of the valve (more accurate counting). Thus it will now be possible, using chemical analyses of the shells and our growth studies, to reconstruct the temporal evolution of contaminant trace metals present in *C. radula* shells, over periods ranging from several months to years, in the lagoon.

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**Table 1** Description of the six marking experiments conducted on scallops in benthic chambers.

Experiment	Concentration (mg L <sup>-1</sup> )	Immersion time (h)	Number of scallops
A	150	3	23
B	300	3	24
C	600	3	23
D	150	6	24
E	300	6	24
F	600	6	23

**Table 2** Average marking quality index for each of the six experiments. The two-way variance analysis indicated the absence of significant effect of calcein concentration or immersion time on MQI.

Experiment	MQI		Two-way analysis of variance				
	Mean	s.d.	Effect	Sum of square	df	F-ratio	p-value
A	1.50	0.82	Calcein concentration	0.486	2	0.39	0.678
B	1.37	0.68					
C	1.78	1.04					
D	1.74	0.65	Immersion time	0.697	1	1.12	0.293
E	1.75	0.74					
F	1.60	0.63					

**Table 3** Coefficients and parameters of the linear regression analyses performed for the 8 post-marking samples between the number of new striae and the shell increment deposited after the calcein mark (see Fig. 8).

Post-marking sample	Linear regression analysis (new striae vs. net accretion)				
	n	slope	intercept	r <sup>2</sup>	p-value
1	17	4.45	3.53	0.52	< 0.01
2	14	1.76	6.97	0.33	0.03
3	7	0.90	10.78	0.15	0.40
4	4	3.56	9.50	0.77	0.12
5	7	1.99	15.72	0.83	< 0.01
6	8	2.47	19.18	0.61	0.02
7	10	2.55	20.73	0.46	0.03
8	5	1.92	30.98	0.88	0.02

## Figure legends

**Fig. 1 a** Photograph of the left valve of the shell. *Dashed line* delimits the shell piece embedded in resin. *Arrow* indicates the maximal growth axis along which shell pieces were sagittally sectioned. **b** Image (scanning electron microscopy) of striae taken along the maximal growth axis and showing 1 stria splitting into 2 "sub-striae" (*dashed line*).

**Fig. 2** Diagram of a benthic chamber designed for calcein marking.

**Fig. 3** Drawing of a sagittally sectioned left valve of a shell. The variation in growth rate was assessed by measuring the mean length of the  $n$  increments (i.e., inter-striae distance) preceding ( $= a/n$ ) and following ( $= b/n$ ) the calcein mark. Here,  $n = 10$ .

**Fig. 4** Mean MQI (*open diamonds*) and mean net accretion (i.e., distance between the calcein mark and the ventral margin; *solid circles*) for each of 7 size classes of *C. radula* (initial shell height of 139 scallops).

**Fig. 5** Difference ( $\Delta$ ) between the mean length of increments before and after the calcein mark, plotted vs. initial shell height. A decrease of the mean length of increments was observed after the calcein marking for all scallops ( $\Delta < 0$ ) but the initial shell height did not influence the importance of this decrease.

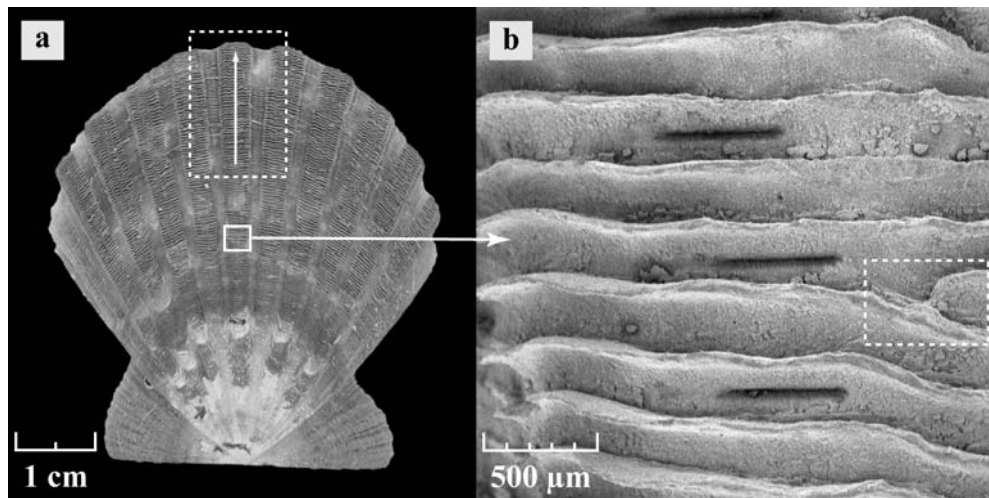
**Fig. 6** Photograph of sagittally sectioned shell viewed with I2/3 block filter. This shell (initial shell height = 55.2 mm) was marked for 3 hours in a  $600 \text{ mg L}^{-1}$  calcein solution and collected 20 days later. The bright green calcein mark (*a*) is readily identifiable and allows for exact counting of new striae (indicated by *arrows*). On this shell, 10 new striae were formed beyond calcein marking, suggesting a 2-day periodicity of striae formation. *Dashed line* delimits an area where the exact number of actual striae (2, 3, or 4) is difficult to assess.

**Fig. 7** Relationship between the number of new striae formed after the calcein marking and the number of days of growth between the calcein marking and the post-marking collection. **a-c** Data collected by each of the 3 readers. **d** Pooled data indicating that *C. radula* formed 1 stria every 2.1 days.

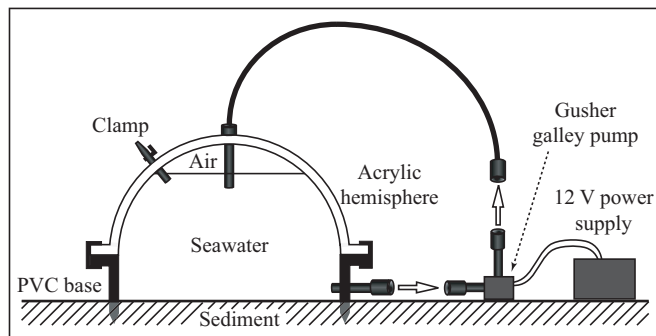
**Fig. 8** Relationship between the number of striae and the shell increment (net accretion) deposited between the calcein mark and the ventral margin, at each of the 8 sampling dates. *Dashed lines* indicate the number of striae that should have been counted under the hypothesis of a 2-day periodicity of striae

formation. *Solid diamonds* represent scallops that have formed more striae than expected under this assumption. *Open circles* represent scallops whose net accretion after marking was  $< 0.05$  mm.

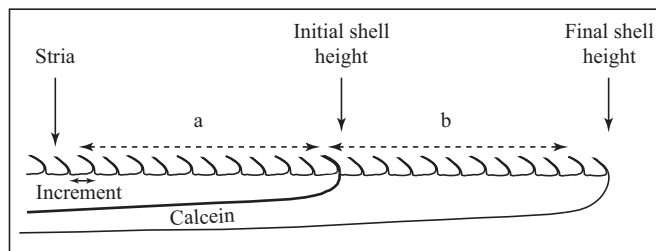
**Figure 1**



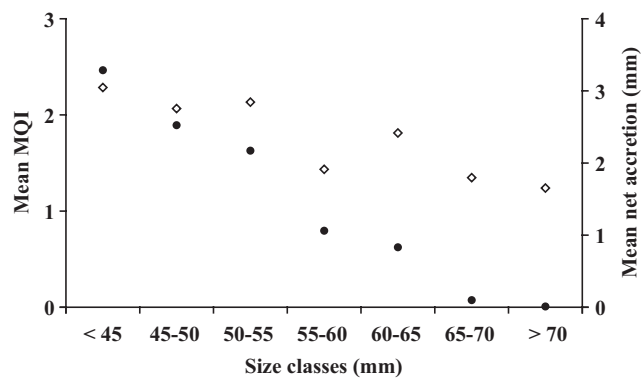
**Figure 2**



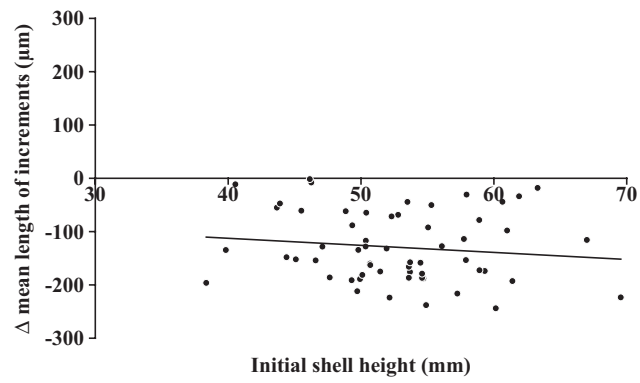
**Figure 3**



**Figure 4**

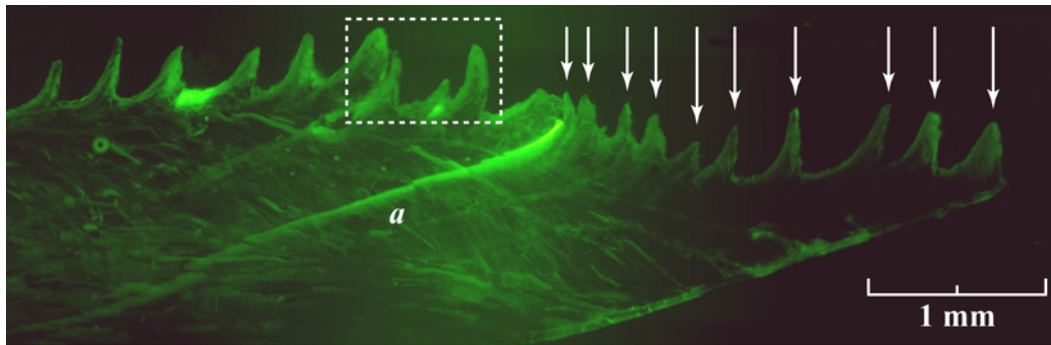


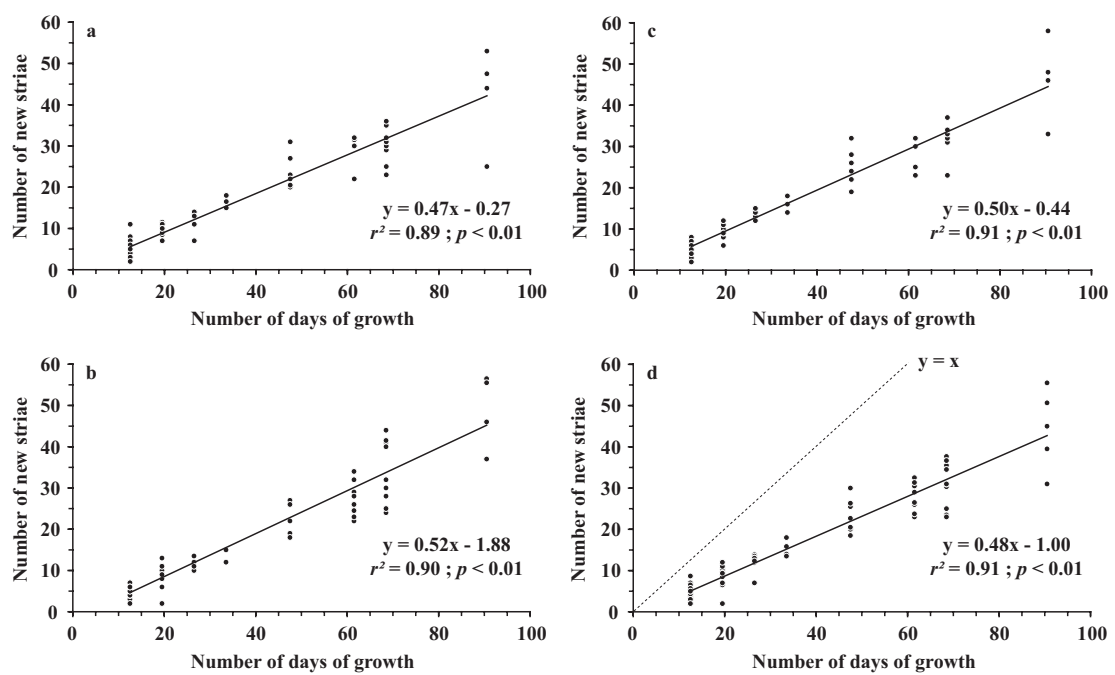
**Figure 5**





**Figure 6**



**Figure 7**

**Figure 8**

