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Short Communication

CHANGE IN THE RATE OF SHELL DEPOSITION AND SHELL MICROSTRUCTURE IN RESPONSE TO SHELL BORERS IN THE ABALONE *HALIOTIS RUBRA*

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Gastropod shells consist of two crystal types of calcium carbonate, an outer, prismatic calcite layer and an inner nacreous layer made of aragonite. In cross-section, the nacre of the nacreous layer appears to have a regular brick-like microstructure composed of thin laminae of aragonite crystals, separated by very thin sheets of protein (Lutz and Rhoads, 1980; Nakahara, 1983). In abalone (Genus, *Haliotis*) and other gastropods, thin layers of non-lamellar pigmented material occur within the nacre and have been termed alternatively, fine lines, growth rings or growth lines (Shepherd *et al.*, 1995). It has been suggested that these pigmented layers are small, prismatic, calcite layers (Shepherd and Avalos-Borja, 1997; Zaremba *et al.*, 1996) but investigations using a Raman laser in *Haliotis rubra* show that they contain aragonite rather than calcite (Hawkes *et al.*, 1996). Day and Fleming (1992) suggest that the occurrence of pigmented layers is correlated with regular

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exogenous cues such as reproduction or temperature changes and indeed in some species, pigmented layers in the shell can be used to age abalone (review: Shepherd and Triantafillos, 1997). However, McShane and Smith (1992) suggest that pigmented layers can occur irregularly and therefore may be unreliable indicators of age.

Boring predators and epibionts often damage the shells of molluscs. In abalone, spionid polychaete worms bore holes into the shell and live within the shell matrix (Shepherd and Huchette, 1997). Shepherd and Huchette (1997) found that these worms can infest entire populations, severely weakening the shells of some individuals which can lead to mortality. Given the potential consequences of boring attacks, do abalone show any response these attacks? Shepherd and Huchette (1997) found that the incidence of pigmented layers was much greater in *Haliotis scalaris* with polychaete infestations than those without. They suggested that either the formation of pigmented layers was induced by boring attacks, or abalone with bored shells grew more slowly so that the periodically formed pigmented layers were closer together. Their results did not allow discrimination between the two hypotheses. Gabriel (1981) found that nacre has very little resistance to simulated borer attacks when compared to calcite, but that nacre provides more structural resistance against impacts or bending. Shepherd and Huchette (1997) suggested that pigmented layers might be a way of increasing the resistance of a shell to attacks by borers without compromising the mechanical strength of the shell.

An alternative response to boring organisms would be to increase the rate at which shell material is deposited thereby increasing the thickness of shell. For example, Schleyer (1991) found that the oyster, *Striostrea margaritace*, had thicker shells in areas where *Polydorid* polychaete infestations were greater. Therefore, we were interested in whether the black lipped abalone, *Haliotis rubra*, (a) produced pigmented layers, (b) increased its shell thickness or (c) both, in response to the presence of boring polychaetes. To test this we inserted a chemical mark in the shells of live abalone and then released them in the field for six months. Once the abalone were recaptured, we sectioned the shells, scored the sections for polychaete infestation and examined the structure of the shell ventral to the chemical mark using scanning electron microscopy (SEM).

As part of a larger study, live *Haliotis rubra* were collected using S. C. U. B. A. from Portland in southern Australia on February 7, 1996. The shells of the abalone were marked with manganese using the technique reported by Hawkes *et al.* (1996) in holding tanks supplied by Portland Ocean Trading

Pty. The abalone were then tagged and returned to the field. A number of these were then recaptured approximately six months later on August 20, 1996.

The shells were removed from the recaptured animals and allowed to dry. Later, the shells were cut along the anterior-posterior axis and a small section (10 × 5 mm) of the spire was removed from each shell using a lapidary saw. These sections were embedded in resin (RF Services 61-283) and polished using a series of fine grit papers and finally a silica colloid on a polishing wheel. The sections were then dried in a 50°C oven for four hours. We prepared the sections for scanning electron microscopy by etching them with 1% (v/vol) Hydrochloric acid for thirty seconds in an ultrasonicator. Care was taken to ensure the sections were facing upwards whilst being etched to allow CO_2 bubbles produced in the reaction to escape. After etching, the sections were placed immediately into distilled water and ultrasonicated to remove any proteinaceous debris. We placed the samples in a 50°C oven for four hours and coated them with 100 angstroms of gold using a sputter coater. The shell sections were examined using a scanning electron microscope (20 kV with a tungsten filament). Each section was photographed under the SEM and the number of pigmented layers and thickness of the shell determined from these micrographs.

The sections were then re-polished to remove the thin coating of gold and expose the manganese mark for cathodoluminescence microscopy. The manganese mark was detected using the techniques previously described in Hawkes *et al.* (1996). Abalone shell grows from the inside, therefore the shell ventral to the manganese mark is the material laid down between tagging and re-capture. The mark appears as a thin, continuous band of yellow in aragonite shell. The thickness of shell deposited after the mark was determined using an ocular micrometer attached to the cathodoluminescence microscope. The shell sections were also scored for their degree of boring by polychaetes. Shells were classed as either "infested" if there were visible holes in the section of shell ventral to the manganese mark or "clear" if there were no holes visible at all under the mark. Only shell sections that had very little evidence of boring preceding (dorsal to) the manganese mark were included in this study. This was done so as to reduce the chances of any individual having a prior history of boring which may have affected subsequent shell growth. We were concerned that any boring activity that occurred in areas of shell other than spire might affect shell deposition in the spire. However, it appeared that whenever boring occurred elsewhere on the shell, it also occurred at the spire. The number of pigmented layers that had

formed after the manganese mark was then estimated using the measurement data from the cathodoluminescence examination and the SEM micrographs.

There was no significant difference in the thickness of shell prior to the manganese between the bored and clear groups ($f_{1,21} = 1.207$, $p = 0.284$, $n = 23$). Nor was there a difference in the length of shells between the bored and clear groups ($F_{1,21} = 0.081$, $p = 0.779$, $n = 23$). The presence of borers was correlated with an increase in the number of pigmented layers and the thickness of the shell deposited in the six month period after marking. Shells with boring increased in thickness, on average about 450% more than those shells where no boring occurred between marking and recapture ($f_{1,21} = 4.847$, $p = 0.039$, $n = 23$; Fig. 1). No pigmented layers were seen in shells with no boring after (ventral to) the manganese mark. Shells with boring detected after the mark had deposited, on average, about two pigmented layers and this value was significantly different from zero (one sample t-test: $t = 4.082$, $p = 0.0014$, d.f. $= 9$). Among individuals that were bored, the thickness of shell deposited after the manganese mark increased with the number of pigmented layers present after the mark ($r = 0.667$, $n = 9$). However, the pigmented layers themselves do not account for the differences in the thickness of shells observed between the bored and non-bored groups. The pigmented layers account for, on average, 180 μm of the difference between the two groups whilst the average difference in thickness was about 500 μm.

Our results suggest that the abalone, *Haliotis rubra*, is able to detect and react to the presence of shell boring organisms. The rate of shell deposition

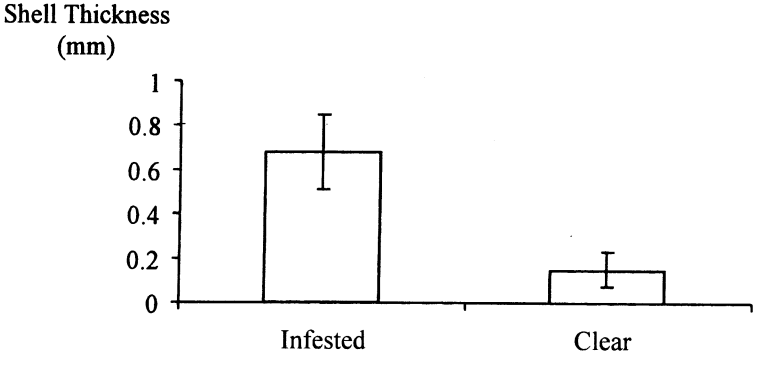

FIGURE 1 Bars represent the mean ± (S. E.) increase in thickness of shells of *Haliotis rubra* over a six month period. Shells were classed as "infested" if any bore holes were observed under the manganese mark in the shell and "clear" if there were no bore holes observed under the manganese mark.

and incidence of pigmented layers greatly increased in shells where borers were present. Individuals with evidence of boring had increased the thickness of their shells, on average, four and a half times more than "clear" individuals. The thickness and length of the shells prior to the mark was not significantly different between the bored and clear groups. Presumably then, the bored and clear groups of abalone did not differ in the rates of shell deposition or layer formation prior to capture and marking. This suggests that the rate at which shell is deposited by *H. rubra* is highly plastic and can vary four fold over a six-month period. Wilbur and Saleuddin (1983) estimate that, in molluscs, one quarter to one third of the total energy of growth is required for shell deposition. It is likely that the four-fold increase in shell secretion rates observed here may result in a decrease in somatic growth or fecundity, although this remains to be determined. Kojima and Imajina (1982) found that abalone heavily infested by boring polychaetes had lower body weights for their size than less infested ones. Similarly, Kent (1979) found that *Mytilis edulis* with heavy infestations of *Polydorid spp.* had much lower condition indexes and fecundity than those mussels that were not infested. Kent suggested that this difference in condition and fecundity was due to extra metabolic demands placed on the mussels by Polydorids inducing shell secretion. Similarly, Wargo and Ford (1993) found that Polydorid infestations reduced the ability of the oyster, *Crassotrea virginica* to accumulate nutrient reserves.

In addition to increasing the thickness of their shells, abalone with evidence of boring produced, on average, more than two pigmented layers during the six months between marking and recapture. Interestingly, the incidence of pigmented layers increased with the thickness of shell that was secreted after the manganese mark. It is possible that these pigmented layers may facilitate the rapid deposition of shell. Pigmented layers appear to have high protein contents (Shepherd and Avalos-Borja, 1997). Zaremba *et al.* (1996) found that shell deposition was preceded by the laying down of an organic protein sheet. Although highly speculative, it may be that pigmented layers perform a similar function in the thickening of shells. That pigmented layers do affect shell deposition in some way seems certain as Shepherd and Avalos-Borja (1997) found that the thickness of the nacre laminae changed with the occurrence of pigmented layers. The increased incidence of pigmented layers observed here may therefore be just a consequence of rapid shell growth. Regardless of their function (if any), it appears that the use of pigmented layers to age abalone is complicated by the increased incidence of the layers associated with shell boring.

Alternatively, the increased incidence of pigmented layers could be a deliberate strategy to increase the resistance of the shell to boring or cracking. Although aragonite provides mechanical strength, it offers little resistance to boring organisms (Gabriel, 1981). Gabriel (1981) found that shell with a high protein content resisted simulated boring attacks better than shells containing less protein. It is possible that the suggested higher protein content of pigmented layers may make them more effective than nacre in resisting attack by boring organisms. Therefore, pigmented layers may be a way of increasing the overall resistance of a shell to boring without compromising its mechanical strength.

If the pigmented layers resist attack by boring polychaetes, they must contain a very different protein to those found in prismatic calcite and lamellar nacre tablets. Zottoli and Carriker (1974) found that the chemicals secreted by the polychaete, *Polydora websteri*, dissolve the protein between the calcium carbonate crystals of both prismatic calcite and lamellar aragonite. Further work is needed to test whether these chemicals are equally effective in breaking down the dark protein of pigmented layers. Regardless of the role of pigmented layers, it appears that abalone can change their shell microstructure in accordance with the activity of boring organisms. Furthermore, abalone appear to be quite sensitive in detecting the presence of symbionts within their shells; some abalone increased their rate of shell deposition and produced pigmented layers in response to only one or two boring polychaete worms present under the manganese layer (pers. obs.). The way that abalone detect these symbionts is unknown, but presumably the response occurs only if the polychaetes penetrate through the shell into the extra-pallial space.

In summary, abalone appear to be able to regulate the rate at which they deposit shell in response to attacks by boring organisms. They also change the physical properties of their shells incorporating pigmented layers into their shells which may provide greater resistance to borers.

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