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Radula tooth turnover in the chiton *Acanthopleura hirtosa* (Blainville, 1825) (Mollusca: Polyplacophora)

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Abstract

The rate of radula production in the chiton *Acanthopleura hirtosa* (Blainville, 1825) was determined by following structural irregularities induced in the radula using a cold-shock treatment of 4°C for 48 h. In animals treated in this manner and subsequently maintained in an artificial intertidal habitat to ensure, as far as possible, a natural feeding regimen, the rate of radular production was calculated as 0.36 rows per day. This indicates that *A. hirtosa* renews its total radular length (mean 78 rows, SD = 6.5, n = 23) approximately once every 6.5 months, a relatively slow rate when compared with that of other molluscs.

Additional keywords: biomineralisation, cold-shocking, mollusc.

Introduction

The chiton radula consists of a ribbon like series of transverse tooth rows that are produced in a 'conveyor belt' manner (Lowenstam and Weiner 1989) from odontoblasts at the posterior end of the radula sheath (Kaas *et al.* 1998). Depending on the species, each transverse row consists of between 13 and 17 individual teeth. However, the main teeth that are involved in feeding are the prominent, glossy-black second lateral teeth, usually termed the major laterals. These teeth undergo a process of maturation along the length of the radula that involves the incorporation of iron and calcium based minerals onto an organic matrix (Kirschvink and Lowenstam 1979; Kim *et al.* 1989). This imparts to the teeth a unique combination of hardness and resilience, suitable for grazing on hard substrata (Webb *et al.* 1989). The anterior most teeth are worn by abrasion as the animals graze (Bullock 1989) and, as such, replacement appears to be in a state of dynamic equilibrium, where the rate of teeth being shed anteriorly is balanced by their production posteriorly (Runham 1963).

While a number of studies have been conducted in relation to the turnover rate of the molluscan radulae (Runham 1962, 1963; Runham and Isarankura 1966; Isarankura and Runham 1968; Padilla *et al.* 1996;), these have concentrated on gastropods, with a particular emphasis on pulmonates. Isarankura and Runham (1968) have developed a number of methods for studying the turnover rate, ranging from direct counts of teeth found in the faeces of animals, to marking the radula by surgical methods. These investigators also discovered that radula abnormalities occurring in *Helix aspersa* Möller, 1774, due to cold winter conditions, could be induced experimentally by a 'cold-shocking' technique. This method has since been found to be simple and reliable and facilitates the testing of a large number of individuals (Padilla *et al.* 1996).

The ongoing interest in the biomineralisation process in chiton teeth (see, for example, Webb *et al.* 1989; Lee *et al.* 1998, 2000) has led to the need to estimate the rate of tooth turnover, so that the demand for mineral precursors can be ascertained and some estimate of the flux of these elements through the chiton body established. The species used in the

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present study was *Acanthopleura hirtosa* (Blainville, 1825), which is an abundant member of the Western Australian molluscan community. It occurs in the upper intertidal region of coastlines from Albany to Shark Bay (Wells and Bryce 1986) and has proved to be an ideal model for studies of the biomineralisation process (Evans *et al.* 1990, 1992; Macey and Brooker 1996; Macey *et al.* 1996).

Materials and methods

Adult specimens (mean length $37.6 \pm 4.8 \text{ mm}$, n = 36) of *A. hirtosa* were collected from various sites in the Perth metropolitan area (Lat. 32° S, Long. 116° E) between February and March 2001. To reduce the likelihood of any damage to the specimens during collection, chitons were removed from their home scars by driving a screwdriver into the substratum near the mantle of each specimen. Each animal, along with a portion of the substratum to which it was attached, was levered free of the rock surface, placed in fresh seawater and transported back to the laboratory as quickly as possible.

To minimise the chances of the study altering the natural rate of radula production, all experimental animals were kept in an artificial environment, or 'mesocosm'. This consisted of a large open air tank containing limestone rocks collected from the intertidal region normally occupied by *A. hirtosa*. The rocks were positioned in the tank such that they partially protruded above the water line, providing the chitons with a habitat approximating their natural intertidal environment. In order to keep the exposed rocks moist and to simulate surf spray, the tank was continuously supplied with fresh seawater that was delivered via an overhead reticulation system. No organisms were removed from the rocks, which contained various species of algae, anemones, limpets, barnacles, crabs, polychaetes and other small invertebrate fauna. A number of juvenile and small adult chitons of various other species were also present.

In order to test the viability of the mesocosm as a chiton habitat, it was initially seeded with 21 specimens of A. hirtosa collected from the wild in early February and left to reside for a period of 2 months. Ten of these animals were included in the cold-shocking procedure, along with a further 15 collected from the wild in late March. Differentiation of the freshly collected specimens from the control animals was achieved by using a Dremel (Racine, Wisconsin, USA) engraving tool to score lines on either the fourth or fifth valve, respectively. All 25 animals were then subjected to a derivation of the cold-shocking technique of Isarankura and Runham (1968). A constriction was produced in the developing radula membrane by plunging the chitons into seawater prelowered to a temperature of 4°C and maintaining them at that temperature for 48 h. Directly following the cold-shocking procedure, while the animals were in a relaxed and extended state, their lengths were recorded. Two specimens were dissected and their radulae removed in order to provide Day 0 data, whereas all remaining specimens were transferred to the mesocosm, where they attached to the substratum. On subsequent alternate days, at the same time each day (± 2 h), a single specimen was selected at random for dissection. Prior to removal of the radula, chitons were anaesthetised by placing them in a shallow container of fresh tap water, which was then chilled at -1° C for 1 h. Radulae were then dissected out, cleaned using 2.5% sodium hypochlorite and the radula length and total number of transverse rows recorded. Radula constriction was observed and photographed using an Olympus DP10 digital camera, attached to an Olympus SZH10 stereo dissecting microscope, and the number of teeth prior to the position of the constriction was counted and recorded. In order to verify that the constriction anomaly was not a natural construct of the radular ribbon, five chitons were dissected straight from the wild for comparison.

Results

No constrictions were found in the radulae of animals dissected fresh from the field, indicating that the observed deformations were attributable to the cold-shocking procedure rather than being a natural construct of the radula. Only a single animal died in the mesocosm during the initial 2-month viability study. This occurred within a few days of transfer, suggesting that the combined shock of collection and transfer was the cause of death. A further two animals died following the 4°C cold-shocking procedure and transfer to the mesocosm. Animals that survived were assumed to be feeding in a normal manner due to the maintenance of limited algal coverage on the substratum and the deposition of a large number of faecal pellets in the mesocosm. Indeed, following the experimental period,



Fig. 1. Constriction (arrow) produced in the radula by placing animals in seawater precooled to a temperature of 4°C and maintaining them at that temperature for 48 h. Scale bar: 1 mm.

some of the smaller chiton species from the mesocosm were found to have spawned, indicating that these chitons, at least, were capable of reproducing while in the system. Preliminary cold-shocking trials at a temperature of -1° C produced 90% mortality, suggesting that this lower temperature is beyond the tolerance limit of this species.

The cold-shocking technique produced an obvious constriction of the radula membrane that could be tracked along the radula in all experimental animals throughout the experimental period (Fig. 1). Whereas some individual variation was seen in tooth turnover rate towards the end of the experimental period, the overall results were very consistent and allowed the calculation of an average rate of tooth turnover of 0.36 rows per day (SD = 0.06, n = 20; Fig. 2). Initial determination of the position of the constriction with regard to tooth row number was made difficult by the very low turnover rate, which meant that fractions of tooth rows had to be estimated. However, there does appears to be an initial lag phase, because the mean turnover rate for the first 8 days is only 0.27 rows per day (SD = 0.04, n = 4), which could be attributable to recovery from the cold-shocking procedure. Data obtained from animals in the study indicate that the average total number of transverse tooth rows for *A. hirtosa* is 78 (SD = 6.5, n = 23). Combining the data for tooth turnover rate and total row number gives a total radula replacement time of approximately 203 days (~6.5 months).



Fig. 2. Number of tooth rows formed after cold shock treatment.

Discussion

This paper reports, for the first time, the use of a cold-shocking method for measuring tooth turnover rate in temperate intertidal chitons, revealing a radula replacement rate of 0.36 rows per day in A. hirtosa. A previous study of radula production, using a variety of techniques including cold-shocking, found that radula turnover rates of 15 prosobranch and pulmonate species varied from 1 to 6.4 tooth rows per day (Isarankura and Runham 1968). In a subsequent study of two littorinid species, the cold-shocking technique revealed a calculated turnover rate of approximately 2.95 rows per day (Padilla et al. 1996). Isrankura and Runham (1968) found that a number of factors affect tooth turnover rate in gastropods, none more profound than that of ambient temperature, which increased radula production two to threefold in response to incremental rises of 10°C. The Perth region experiences a 'Mediterranean' climate, with average maximum and minimum temperatures of 29 and 18°C, respectively, in summer and 18 and 9°C, respectively, in winter. Local nearshore water temperatures range from 15 to 24°C, from winter to summer, respectively (Pearce et al. 1999). The present study was conducted during the months of late summer to early autumn, with the mesocosm at a relatively high water temperature of 22°C, with a variation of less than 1°C overnight. Hence, the production rate of 0.36 rows per day, although low in comparison with the gastropod species of previous studies, is likely to reflect a relatively high turnover rate for A. hirtosa. It would be interesting to repeat the study during the winter months to determine whether the radula turnover rate is reduced at a lower temperature. Isarankura and Runham (1968) found that active herbivores have higher rates of radula production than more sluggish or carnivorous forms, indicating that turnover is adapted to the feeding activity of the animal. Although it is difficult to compare the level of feeding intensity between gastropods and A. hirtosa, it can be argued that because A. hirtosa is an intertidal species that has an intermittent, semidiurnal feeding regimen, it is not an active herbivore for much of each 24 h period. As such, this could account, in part, for its comparatively low turnover rate.

There are several other factors that could affect the wide variation exhibited in the turnover rates of the different molluscs studied, including the physical size of the teeth, their structural design, their resistance to wear and the hardness of the animal's food source. The initial size of the mature teeth, prior to their use in feeding, determines how quickly they will wear away, with smaller teeth becoming functionally ineffective and in need of replacement sooner than larger teeth. Unfortunately, the lack of any data on tooth size in previous studies precludes direct comparison with the chiton teeth in the present study. However, the main teeth used by chitons in feeding, the second laterals, are usually large prominent teeth. In *A. hirtosa*, the cusps of these teeth measure 254 μ m high and 247 μ m wide (SD = 4.7, 3.9, respectively; *n* = 8). Hence, it may well be that the possession of large cusps affords the chiton a slower rate of tooth wear.

The majority of gastropods in the studies of both Isrankura and Runham (1968) and Padilla et al. (1966) were herbivores feeding off seaweeds, soft algae or lettuce. The only species that has a natural feeding strategy similar to that of A. hirtosa is Patella vulgata. Both species are hard substrate grazers, and although P. vulgata was not given a natural substrate to feed off, at approximately 1.5 rows per day it returned one of the lowest radula turnover rates for the gastropods (Isrankura and Runham 1968). This is somewhat contradictory to what would be expected, because it could be assumed that the abrasive, high impact nature of chiton and limpet feeding would actually necessitate a high turnover rate. However, another factor that both these species have in common is the incorporation of hard minerals into their tooth design, which affords them greater resistance to wear. The main working teeth of A. hirtosa are hardened with iron oxides, such as magnetite and lepidocrocite (Kim et al. 1989; Lee et al. 1998), whereas P. vulgata incorporates iron and silicon into its teeth (Lowenstam 1962, 1971). As such, it could be suggested that radula turnover in chitons and limpets is slower than that found in other molluscan groups due to the reduced mechanical wear of the teeth afforded by their extensive mineralisation. A further point to consider is that mineralisation is a physiologically expensive process, which necessitates rationalisation of tooth production rates with respect to the total energy allocation of radula manufacture.

The only other published study on radula production rates in the polyplacophora was that performed by Nesson (1969), where the rate of radula replacement for *Mopalia muscosa* (Gould, 1846) was determined by labelling the teeth with ⁵⁹Fe. In that study, the tooth turnover rate was calculated to be approximately 0.6 rows per day, nearly double that of *A*. *hirtosa*. However, this is still considerably slower than the rates calculated for the gastropod species, suggesting that the polyplacophora, as a group, may have slower radula production rates than other mollusc groups. Whether this reduced rate can be attributed to their mineralisation process remains to be seen and further research into radula production may provide insights into the tooth hardness–turnover rate association.

The difference in the rates observed between *A. hirtosa* and *M. muscosa* may lie in the differing tooth morphology and feeding regimens of these two species. The working face of the major lateral teeth in chitons varies in structure from a broad chisel-like form (as in the chitonidae) to one to three pronounced cusps (in most other chiton families) (Nesson and Lowenstam 1985). The cusp structure seen in *A. hirtosa* is of the broad unicuspid type (Macey *et al.* 1996), whereas that of *M. muscosa* is of a tricuspid design (Nesson 1969). While no direct comparison has been made between these two species, comparisons of the structural organisation of the cusps of chiton teeth have been made between *A. hirtosa* and *Plaxiphora albida* (de Blainville, 1825), which also has tricuspid teeth (Macey *et al.* 1996). The two cusp designs are used differently with respect to feeding, with the major laterals of

P. albida used in a rake-like manner, whereas those of *A. hirtosa* have a more excavational capacity (Macey *et al.* 1996). From this feeding strategy, it would be expected that the teeth of *A. hirtosa* would be worn away more quickly. The fact that this does not occur may well be attributable to differences in tooth structure at the microcrystalline level.

A number of factors may be responsible for the slight variation seen in rates of tooth row formation for individual specimens of A. hirtosa. Isarankura and Runham (1968) found a close correlation between radula replacement and the age of very young specimens of the gastropod Achatina fulica (Bowdich, 1822), whereas in older specimens the rate appears to be more closely related to the size of individuals. Although only adult chitons were used in the present study, there was a difference of 16 mm in length between the largest and smallest specimens, so size cannot be ruled out as a factor. Perhaps of greater significance to the individual variation is the effect of temperature on metabolism, because any fluctuations may have slowed or increased radula production over the course of the study. While the ambient temperature of the artificial mesocosm was relatively constant for the experimentation period, and day to day variations would be expected to have a compound effect that should be similar for all chitons in the experiment, the location of individual chitons within the mesocosm may have caused some variation. Because some of the rocks were submerged and others exposed, some chitons may have adopted positions that were sheltered from the elements, whereas others may have been subjected to a more variable range of conditions with regard to temperature and water availability.

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