

Ecological significance of deep-layer sloughing in the eulittoral zone coralline alga, *Sponites yendoi* (Foslie) Chamberlain (Corallinaceae, Rhodophyta) in South Africa.

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Abstract

The crustose coralline alga *Qongites yendni* (Foslie) Chamberlain (Corallinaceae, Rhodophyta) dominates the lower eulittoral zone along the South Coast of South Africa, together with the limpet *Patella cochlear* Born. This coralline alga was previously shown to undergo a deep-layer sloughing twice a year, as well as continuous epithallial sloughing. The hypothesis that deep-layer sloughing could perform an antifouling function was tested by determining the percentage cover of fleshy algae developing in a limpet exclusion experiment on live and killed coralline. Substantial fleshy algal cover developed in both limpet removal treatments, negating any possible antifouling function of deep-layer sloughing. A measurable decrease in the mean thickness of the coralline population was noted during periods of sloughing. A thicker crustose coralline, *Leptophytum ferox* (Foslie) Chamberlain & Keats, grew more slowly and was much more heavily burrowed and more weakly attached than *S. yendoi*. These results are consistent with a hypothesis that deep-layer sloughing contributes to the alga's relatively thin thallus, thereby conferring the advantages of faster growth and stronger attachment. However, further studies are still needed to evaluate these hypotheses further.

1. Introduction

Encrusting coralline algae are important components of benthic marine communities within the euphotic zone throughout the marine realm (Adey & McIntyre, 1973; Dethier et al., 1991; Keats, 1986; Paine, 1984; Steneck et al., 1991). Some crustose coralline algae regularly slough an outer layer of cells (Masaki et al., 1981, 1984; Johnson & Mann, 1986; Keats et al., 1993). There are two types of cell sloughing in coralline algae: epithallial sloughing and deep-layer sloughing (Keats et al., 1993). Epithallial sloughing is probably common among corallines (Johnson & Mann, 1986; Masaki et al., 1981, 1984), but deep-layer sloughing has only been described for the South African littoral zone crustose coralline alga, *Spongites yendoi* (Foslie) Chamberlain (Keats et al., 1993).

Spongites yendoi is probably the most abundant coralline alga in the eulittoral zone along the southern coasts of South Africa (Chamberlain, 1993; Keats et al., 1993). It dominates the lower shore together with the gardening Limpet *Patella cochlear* Born. And has been

shown to undergo deep-layer sloughing twice a year (Keats et al., 1993). The sloughing of outer cell layers by corallines, and other marine algae, has generally been viewed as an antifouling mechanism (Masaki et al., 1981, 1984; Johnson & Mann, 1986; Moss, 1982). However, Keats et al. (1993) noted that it would be an energy-wasting process for deep-layer sloughing to occur as an antifouling mechanism, since up to 50% of the thallus is lost with each sloughing event, and sloughing events occur twice a year.

Possible reasons for deep-layer sloughing were mentioned by Keats et al. (1993), but not evaluated experimentally, including:

- antifouling (hypothesis 1)
- shedding of old conceptacles (hypothesis 2)
- shedding a grazer-damaged surface layer (hypothesis 3)
- remaining thin to reduce the probability of mortality resulting from the undercutting of a thick thallus by burrowing invertebrates (hypothesis 4)

A further hypothesis, not mentioned previously, is that sloughing allows the crust to remain thin because this allows for higher lateral growth rates (hypothesis 5). It is important to realise that none of these hypotheses are mutually exclusive, and all may contribute to the ecological advantage of deep-layer sloughing. While hypotheses 2 and 3 have not been experimentally tested, Keats et al. (1993) showed that the shedding of old conceptacles and of a grazer damaged surface layer were certainly consequences of deep-layer sloughing. The purpose of the research reported here is to evaluate experimentally hypotheses 1, 4 and 5.

2. Materials and methods

This study was conducted at Holbaapunt, in the southwestern Cape Province, South Africa (Fig. 1). The distribution pattern of encrusting coralline algae at this site was described by Keats et al. (1993). The hypotheses outlined below were evaluated by a combination of field experiments and sampling.

Hypothesis 1: Deep layer sloughing effectively reduces or prevents the growth of epiphytes. A prediction of this hypothesis is that if the dominant herbivore is removed from experimental plots, the percentage cover of fleshy macroalgae will be greater on experimentally killed than live *S. yendoi*. This will be so because the killed coralline will not be able to undergo sloughing. Fifteen 0.1 m² plots were established in the lower eulittoral zone, and five plots were assigned to each of one of three treatments: (1) unmanipulated; (2) live corallines with herbivores removed; or, (3) killed corallines with herbivores removed. Corallines were killed by applying heat with a blow torch until calcified material was oxidised, and then the plot was scoured with a wire brush. This size plot was chosen because previous unpublished observations showed it to be logically manageable and effective at limpet exclusion.

To exclude limpets, the plots for both experimental treatment were surrounded by a copper-based antifouling paint applied with a paint brush. All plots were marked using tagged screws embedded in the substrate. Percentage cover was estimated using transparent perspex 0.1 m² quadrats with 8 evenly spaced 1.5 mm holes. A two-way

analysis of variance was performed on the removal data using the Minitab statistical package. Variation about the mean is expressed as standard error in all tables and figures.



Fig. 1. Map showing the location of the study area at Holbaaiapunt, southwestern Cape Province, South Africa.

Hypothesis 2: Deep layer sloughing enables *S. yendoi* to remain thin and this reduces the probability of mortality resulting from the undercutting of a thick thallus by invertebrates which burrow down from the surface. It follows from the nature of deep-layer sloughing that thallus thickness will increase up to the time of a major sloughing event, after which there will be a decrease in thickness. This hypothesis predicts that there will be a lower frequency of burrowing by invertebrates in *S. yendoi* than in a thicker coralline from the same habitat. Attachment strength will therefore be greater in *S. yendoi* than in a thicker coralline. Thickness measurements were made by random monthly sampling of 10 patches of *S. yendoi* from the lower eulittoral zone. Specimens were returned to the laboratory, preserved in 10% formalin, and later transferred to the laboratory, preserved in 10% formalin, and later transferred to 70% ethanol with 5% glycerol. Rock chips with *S. yendoi* were oven dried at 60°C and fractured using diagonal cutters, and examined under a dissecting microscope equipped with an ocular micrometer.

To determine attachment strength, separate pieces of substrate covered by *S. yendoi* and the thicker *Leptophytum ferox* (Foslie) Chamberlain & Keats (1994) were chipped off and returned to the laboratory. A paper clip was glued to the surface of the thallus using Pratley Quickset epoxy glue, and the force required to pull the thallus from the substratum was recorded by pulling with a spring balance until the thallus pulled from the rock or the paper clip broke. To determine the degree of burrowing and undercutting, thalli were removed from the substrate as described above. The undersurface of the thallus was then examined under a dissecting microscope equipped with an ocular

micrometer having 100 scale markers. A scale marker crossing a point that was burrowed or undercut was taken as 1% undercutting.

Hypothesis 5: Sloughing allows the thallus to remain thin, and this allows for higher lateral growth rates. This hypothesis predicts the seasonal pattern of thallus thickness mentioned above. It also predicts that the growth rate of *S. yendoi* will be significantly greater than the growth rate of a thicker coralline (*L. ferox*) from the same habitat. To mark plants for growth measurements, holes were drilled into the substrate, away from the margins of the thalli. Two numbered tagged screws were inserted into plastic wall anchors embedded in these holes, and the distance of the thallus margin from one of the tagged screws was recorded using plastic callipers. This measurement was repeated monthly for 5–10 thalli, and growth was measured as the advance of a thallus margin from the original point. These data were extracted from a larger data set used by Keats et al. (in prep.).

3. Results

The results of the herbivore removal experiment are inconsistent with the hypothesis that deep-layer sloughing effectively reduces or prevents the growth of epiphytes (Figs. 2 and 3). While there was a considerable increase in the cover of fleshy algae in the limpet exclusion plots, there was no significant difference in the cover of fleshy algae on live versus killed corallines (Fig. 2, Tables 1 and 2). A severe storm in late July caused a heavy mortality of fleshy algae in both experimental treatments, but again there was no significant difference in the cover of fleshy algae on live *versus* killed corallines (Fig. 2).

Soon after herbivore removal, the exclusion plots were colonised by pseudofilamentous diatoms. *Gelidium micropterum* Kuetzing, which occupied the former territories of the excluded gardening limpet, *P. cochlear*, increased considerably in stature in the exclusion plots with live corallines. It was almost absent from the plots with killed corallines because it was also killed during the treatment. *Gelidium micropterum* was the only fleshy alga with significant cover in the control plots. Within a month, the removal plots were dominated by *Ulva* sp.

The results of thallus thickness measurements are consistent with the predictions of hypotheses 4 and 5 (Fig. 4), although August 1991 shows an anomalous pattern. *Leptophytum ferox* sampled from the same shore level was approximately twice as thick as *S. yendoi*, and showed about 75% burrowing (Table 3). In contrast, *S. yendoi* showed almost no burrowing (Table 3). This is consistent with the hypothesis that remaining thin reduces undercutting. *S. yendoi* also showed a significantly higher lateral growth rate than *L. ferox*, suggesting that remaining thin also promotes a higher lateral growth rate (Table 3). The attachment strength of *S. yendoi* was significantly higher than that of *L. ferox* (Table 3).

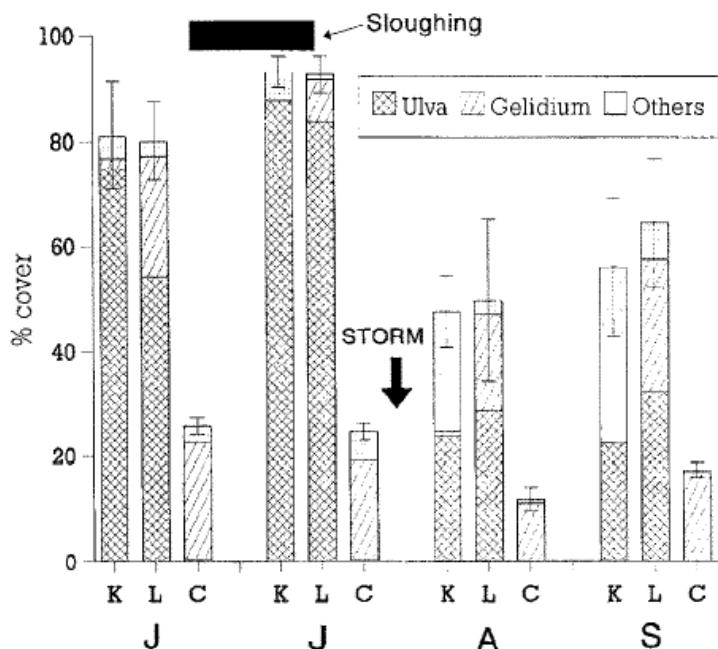


Fig. 2. Monthly fleshly algal cover in the three experimental treatments during the limpet removal experiment at Holbaapunt, June to September. K = killed coralline; L = live coralline; C = unmanipulated control plots.

4. Discussion

In contrast with previous studies of cell sloughing in coralline algae (Masaki et al., 1981, 1984; Johnson & Mann, 1986), we show no significant effect of sloughing on the percentage cover of fleshy algae in herbivore exclusion experiments. This is so despite the fact that *S. yendoi* shows a continuous loss of epithelial cells, in addition to which a major episode of deep-layer sloughing occurred during the study (Keats, unpubl. obs.). There are two possible reasons for this; either sloughing did not occur underneath the cover of epiphytes, or the epiphytes recovered rapidly following sloughing. If the former case is true, then it would imply a trigger mechanism for deep layer sloughing which is absent under a cover of fleshy algae, or in the absence of grazers. *Ulva* spp., which dominated the herbivore exclusion plots, are well known as rapidly growing opportunistic species (Littler & Littler, 1980), so the latter could also be correct. Unfortunately, we are unable to distinguish between these possibilities on the basis of available data. Steneck (1986) noted that, while sloughing of epithelial cells by *Lithophyllum yessoense* inhibited the recruitment of *Laminaria japonica* onto the surface of the coralline (Masaki et al., 1981, 1984), *L. yessoense* is heavily grazed by limpets and chitons in the natural environment. Thus, grazing may be the main factor keeping the surface of *L. yessoense* relatively free of epiphytes. Johnson & Mann (1986) showed that epithelial sloughing by *Phymatolithon* spp. could reduce surface fouling, but not prevent it altogether.

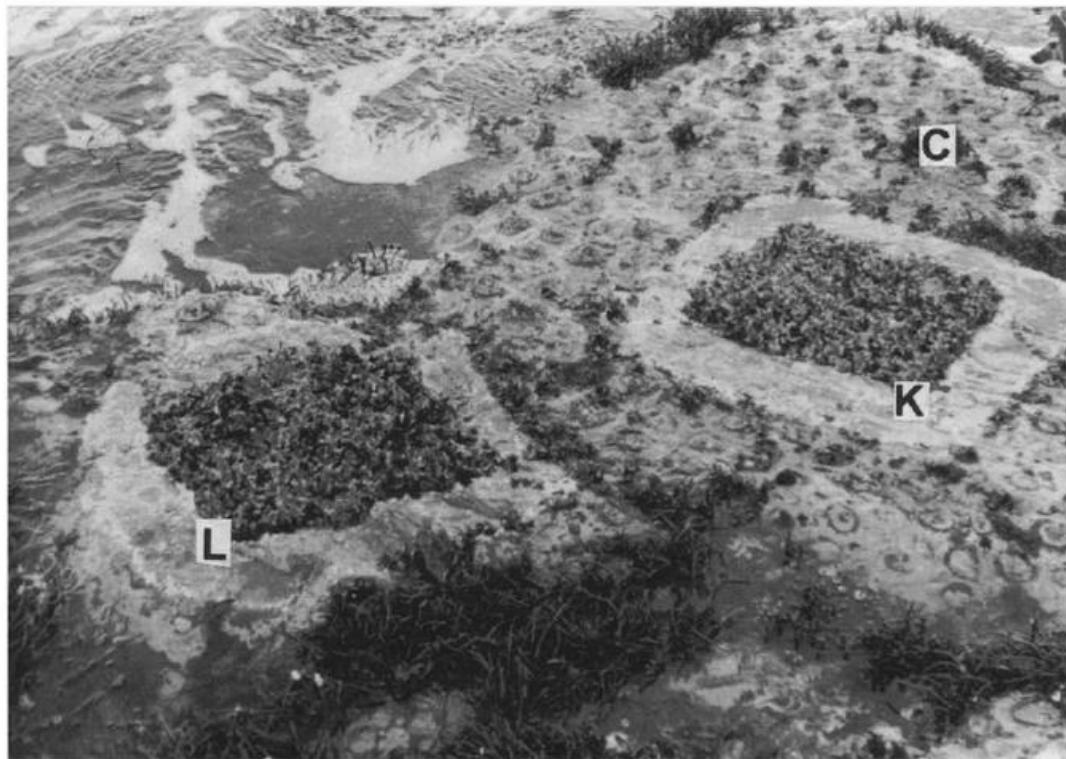


Fig. 3. Photograph of a representative series of experimental plots. C = unmanipulated control; L = live coralline; K = killed coralline.

Table 1
Analysis of variance for percentage cover in limpet exclusion experiment

Source of variation	df	MS	F-ratio	p-value
Date	3	3422	10.28	<0.001
Treatment	2	16097	48.34	<0.001
Date \times treatment	6	308	0.92	0.49
Error	48	333		
Total	59			

Table 2
Comparison of mean percentage cover of fleshy algae for limpet exclusion experiment data

Factor	Level	Mean % cover	Group
Date:	June	62	AB
	July	69	B
	August	35	A C
	September	45	A C
Treatment:	Live	67	X
	Killed	71	X
	Control	20	Y

Means with the same letter are not significantly different at $p < 0.05$ according to multiple comparison confidence interval.

The combined effect of deep-layer sloughing and continuous epithallial shedding cannot prevent the fouling of the surface of *S. yendoi* in the absence of grazing, and sloughing may have a weak effect against surface fouling in other corallines (Steneck 1986, cf. Johnson & Mann, 1986). It therefore seems likely that both deep-layer and epithallial sloughing serve functions other than antifouling, and that antifouling effects (if any) may be secondary.

Herbivory is well documented as an ecological interaction keeping the surface of coralline algae relatively free of fleshy epiphytes (Brock, 1979; Breitburg, 1984; Keats, 1986; Paine, 1980; Steneck, 1982, 1986; Vince, 1974). The results of our limpet-removal experiment shows that grazing by *P. cochlear* is clearly the main factor preventing the surface of *S. yendoi* from being overgrown by fleshy algae in the lower eulittoral zone.

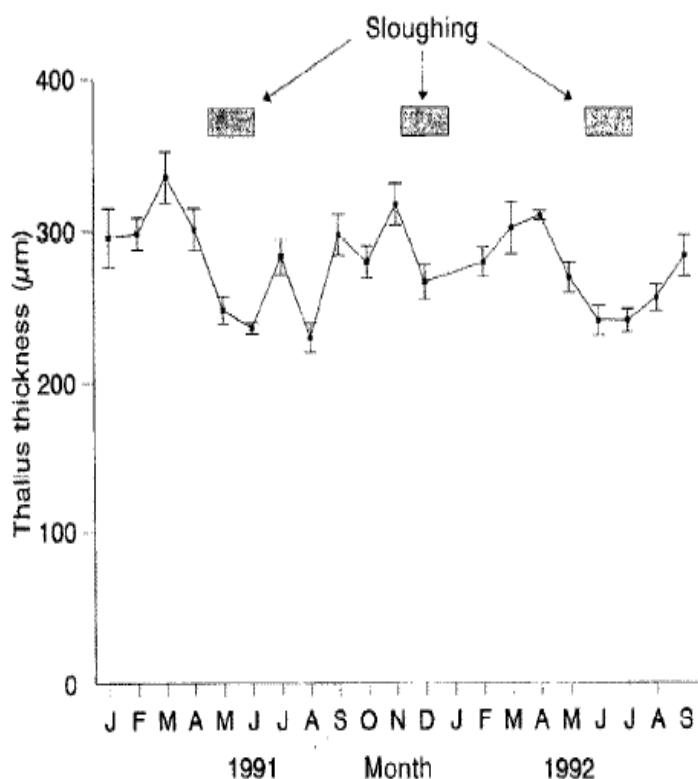


Fig. 4. Monthly thallus thickness measurements for *S. yendoi* at Holbaapunt.

The data suggest that deep-layer sloughing may help the thallus of *S. yendoi* remain thin, and this appears to have two advantages. The thin thallus of *S. yendoi* shows very little effect of burrowing invertebrates, and therefore remains firmly attached to the substrate. This contrasts with the thicker *L. ferox* which is heavily burrowed, and consequently shows a much weaker attachment to the substrate. On the Washington coast of North America, a thick coralline that is competitively dominant in terms of overgrowth competition (Paine, 1986), *Pseudolithophyllum muricatum* (Foslie) Steneck & Paine is heavily burrowed by invertebrates, which reduces its attachment strength (Steneck & Paine, 1986).

Table 3

Comparison of thallus thickness, growth, percentage burrowing and attachment strength of *S. yendoi* with those of a thicker coralline, *Lithothamnion ferox*

	<i>S. yendoi</i>	<i>L. ferox</i>
Thallus thickness	243 μm	487 μm
Standard error	9.7	19.0
Paired sample <i>t</i> -test		$p < 0.0001$
Growth over 6 months ^a	2.8 mm	1.3 mm
Standard error	0.36	0.35
Paired sample <i>t</i> -test		$p = 0.0052$
Percentage burrowing	1.9	75.0
Standard error ^b	0.10 \pm 0.034	1.06 \pm 0.038
Paired sample <i>t</i> -test		$p < 0.0001$
Removal force	7.1 kg	0.7 kg
Standard error	1.2	0.36
Paired sample <i>t</i> -test		$p < 0.0001$

^a Three items of negative growth data for *L. ferox* set to zero to be conservative in comparisons.

^b Mean of arcsin transformed data \pm SE.

The thin thallus of *S. yendoi* shows a more rapid rate of marginal growth than that of the thicker *L. ferox*. Thus, remaining thin apparently gives the advantage of firmer attachment and more rapid growth. This pattern agrees with the suggestion of Steneck (1986) that thinner thalli grow faster, and that thicker thalli are more susceptible to invasion by boring organisms. This may help to explain the greater abundance of *S. yendoi* in the lower eulittoral zone (pets. obs.).

While we have clearly demonstrated that neither deep-layer sloughing nor the shedding of epithelial cells is an effective antifouling mechanism in *S. yendoi*, the link between thallus thickness, invertebrate burrowing and growth rates is largely circumstantial. Studies of growth rates in thick and thin thalli of *S. yendoi*, and determining if there is a relationship between thickness and burrowing frequency within *S. yendoi* would provide a further evaluation of this relationship.

Keats et al. (1993) showed that *S. yendoi* can slough up to 50% of thallus thickness twice each year. This study showed that this sloughing does reduce thallus thickness seasonally, although by a smaller amount than expected. Steneck et al. (1991) showed that limpet grazing can also reduce thallus thickness in encrusting corallines. *Spongites yendoi* is usually associated with the limpet *P. cochlear* (Chamberlain, 1993), and coralline algae contribute substantially to the diet of this limpet (Branch & Griffiths, 1988).

Keats et al., (1993) showed that the surface of *S. yendoi* is usually heavily grazed by *P. cochlear* and this could also account for the thinning of the thallus. We have recently found a thick population of *S. yendoi*, with plants up to 5 mm thick, which was undergoing sloughing (Keats & Maneveldt, unpublished). The degree to which grazing and sloughing contribute to thallus "thinness" in *S. yendoi* thus needs to be determined in order to contribute to our understanding of factors influencing the ecology of littoral zone corallines in South Africa.

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