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A symbiotic shell-encrusting bryozoan provides subtidal whelks with chemical defence against rock lobsters

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The subtidal whelk *Burnupena papyracea* co-occurs with a voracious predator, the rock lobster *Jasus lalandii*, in situations where other potential prey are largely eliminated. The survival of *B. papyracea* has been ascribed to a symbiotic bryozoan, *Alcyonidium nodosum*, which characteristically encrusts the shells of this mollusc and deters feeding by *J. lalandii*. Although *B. papyracea* shells encrusted with *A. nodosum* were found to be stronger than non-encrusted shells, this bryozoan-induced physical defence was insufficient to deter predation by *J. lalandii*. However, laboratory bioassays using individual *J. lalandii* suggested a chemical basis for feeding deterrence originating from *A. nodosum* and highlighted the advantages of using ecologically relevant bioassays to monitor chemically mediated interactions between species in the marine environment. The nature of the chemical compound(s) responsible for the acquired chemical defence passively imparted by *A. nodosum* to *B. papyracea* is unknown.

Keywords: *Alcyonidium nodosum,* bryozoan, *Burnupena papyracea,* chemical defence, *Jasus lalandii,* predation, rock lobster, whelk

Introduction

The physical defence provided by the robust external shell of marine prosobranchs usually obviates an additional acquired chemical defence to protect their soft tissues. Not surprisingly, the presence of a chemical defence system sequestered from other marine invertebrates is occasionally observed in prosobranchs where the shell is reduced, e.g. the Antarctic prosobranch *Marseniopsis mollis* (McClintock *et al.* 1994a). An alternative to a sequestered chemical defence is one in which an animal uses the chemical defences of another species to avoid predation without necessarily accumulating defensive compounds in its own tissues. This strategy has recently been suggested for a mutualism between nemertean worms and a *Vibrio* bacterium (Carroll *et al*. 2003) and has been observed in two other forms in marine amphipods; abduction of a chemically defended pteropod by an Antarctic amphipod (McClintock and Janssen 1990), and construction of tubes from chemically defended macrophytes by amphipods (Hay *et al*. 1990).

This study describes the chemical ecology of a marine bryozoan, *Alcyonidium nodosum*, which was prompted by observations made during an ecological study of a predator-prey role reversal in a benthic ecosystem (Barkai and McQuaid 1988). Rock lobsters *Jasus lalandii* are important benthic predators associated with kelp beds on the west coast of South Africa (Griffiths and Seiderer 1980, Barkai and Branch 1988, Mayfield and Branch 2000). They are generalist predators and their diet consists mainly of mussels, sea urchins, sponges, algae, barnacles, small molluscs and crustaceans (Griffiths and Seiderer 1980, Barkai and Branch 1988, Barkai *et al.* 1996). However, one species of whelk, *Burnupena papyracea*, which is common in these benthic ecosystems, is protected from predation by *J. lalandii* by a commensal bryozoan, *A. nodosum*, which covers its shell (Barkai and McQuaid 1988). *A. nodosum* is an encrusting ctenostome bryozoan commonly found living on the shells of *Burnupena* species (O'Donoghue and de Watteville 1944, O'Donoghue 1956, Dempster and Branch 1999) forming, as is usual in this genus (Peck *et al*. 1995), a unilaminar sheet of autozooids, which totally covers the shell and creates an orange or purple cloak over the whelk.

A. nodosum may reduce predation on its host whelk in several ways. Bryozoans have yielded a large number of biologically active natural products consisting mainly of alkaloids or macrocycles, although some terpenes and simple organic molecules have also been isolated (Blunt *et al*. 2005 and previous reviews therein). The only species of *Alcyonidium* that has been examined chemically, *A. gelatinosum*, contained a potent biologically active component, the (2-hydroxyethyl)dimethylsulfoxonium ion (Carlé and Christophersen 1980, 1982). It is therefore reasonable to assume that the defence conferred on *B. papyracea* by *A. nodosum* may have a chemical basis. Alternatively, some

physical factor may also be responsible for the defence of the whelk because predation on bryozoans may trigger the rapid production of defensive spines or chitinous structures (Harvell 1984, 1986, 1990, 1992). Induction of this type of physical defence in *A. nodosum* as a response to damage caused by *J. lalandii* while feeding on *B. papyracea* could result in reduced consumption of the host whelk.

The presence of *A. nodosum* on *B. papyracea* shells could also protect the whelk if bryozoan encrustation significantly increased shell strength. Given a choice between different species of mussels, *J. lalandii* shows a distinct preference for species with weaker shells (Griffiths and Seiderer 1980). The skeletal material of some encrusting bryozoans has been calculated to be of comparable strength to coral, echinoid spines and bivalve shells (Best and Winston 1984). Though a feature of the genus *Alcyonidium* is a lack of calcification of the exoskeleton (Thorpe and Ryland 1979), *A. nodosum* nevertheless forms a firm layer over its host's shell (O'Donoghue and de Watteville 1944). It is therefore possible that the presence of bryozoans on *B. papyracea* may increase the strength of the whelk's shell. Finally, it is also possible that, when covered with a layer of bryozoan, the whelks are less conspicuous as prey items or cannot be handled efficiently by *J. lalandii*, thus decreasing the number of bryozoan encrusted individuals consumed.

In this study we examined whether *A. nodosum* deters feeding by *J. lalandii* through physical or chemical means. Specifically we tested the hypotheses that:

(1) Rock lobsters avoid eating whelks encrusted by live *A. nodosum*
(2) Rock lobsters avoid eating *A. nodosum* and its chemical extracts
(3) *A. nodosum* protects whelks by producing induced physical defences or by strengthening whelk shells.

To support the experiments carried out, *A. nodosum* extracts were tested using standard spectroscopic and toxicity screening as well as analysing bryozoans for the production of volatile, low molecular mass compounds.

Material and Methods

Jasus lalandii *feeding preference experiments*

Bioassays were conducted as choice experiments using mature rock lobsters with live whelks and food pellets. *J. lalandii* were housed individually in separately filtered seawater aquaria under constant temperature (12°C) and a 12h light/dark cycle. After four weeks the rock lobsters were assumed to be laboratory-acclimatised and were submitted to a series of four feeding assays. Each bioassay ran for 14 days, with 14 days between bioassays; it was assumed that this allowed the trials to be regarded as independent. In bioassays involving live prey, the control and test prey items made available to each rock lobster were always of similar size. When no bioassays were being performed, the rock lobsters were fed daily on mussels.

Assays 1A–4A were carried out using six *J. lalandii* (mean carapace length = 110.1mm ± 18.7 SD). These assays were repeated (Assays 1B–4B) in a random sequence (4B, 3B, 1B and 2B), with a second group of seven *J. lalandii* of similar size (mean carapace length = 121.3 ± 19.6mm, t-test $p > 0.05$). For Assays 5 and a further repeat of Assay 2 (2C), a third group of 12 smaller *J. lalandii* (mean carapace length ± SD = 87.2 ± 7.0mm) was used.

B. papyracea and *B. cincta* were collected subtidally using SCUBA from depths of 3–19m in False Bay, near Simonstown (34°14'S, 18°26'E), South Africa. The whelks were transported to Grahamstown in aerated seawater at 15°C and then maintained in recirculating seawater aquaria at 12°C under ambient light conditions and fed on a diet of mussels.

The *A. nodosum* used in the assays was obtained by scraping the shells of *B. papyracea* (859 whelks) collected from depths of 4–9m in Saldanha Bay (33°02'S, 17°58'E), South Africa. Bryozoan material (1 520g wet weight, approximately 1.8g per whelk) was transported and stored frozen. Portions of *A. nodosum* were lyophilised as required with a yield of approximately 0.50g freeze-dried *Alcyonidium* per whelk.

A further portion of *A. nodosum* (1 396g wet weight) obtained from 519 *B. papyracea* (approximately 2.7g per whelk) was freeze-dried (dry weight 259.50g) and extracted into a 1:1 dichloromethane/methanol mixture under nitrogen at –20°C for 24h in the dark. Following filtration, the red/orange organic extract was concentrated *in vacuo* to yield a dark red oil (7.50g; 15mg per whelk) that was stored at –20°C in the dark.

Bioassay 1A, B: encrusted vs scraped B. papyracea

Each rock lobster was offered three *B. papyracea* encrusted with *A. nodosum* and three *B. papyracea* from which the commensal bryozoan had been removed by scraping. Consumed whelks were replaced daily with similarly sized individuals.

Bioassay 2A–C: pellets containing Alcyonidium *vs control food pellets*

Freeze-dried *A. nodosum* was suspended in a gelatinous matrix (Chanas and Pawlik 1995, Pawlik *et al.* 1995) to form pellets prepared daily. Sodium alginate (1.00g), freeze-dried powdered mussel (0.50g) and freeze-dried powdered *A. nodosum* (2.00g) were added to deionised water (30ml) and the mixture was stirred vigorously until homogenous. After centrifuging to remove air bubbles, the resulting paste was loaded into a syringe and extruded into calcium chloride solution (250ml, 0.25 M) until it was hard throughout (90 minutes). The resultant gel was then removed, blotted dry and cut into pellets (radius = 0.35cm, length = 2.00cm; wet weight c. 2.0g; each containing approximately 50mg freeze-dried *A. nodosum*). Pellets were attached to separate, marked mussel shell valves using superglue. Control pellets were prepared using sodium alginate and freeze-dried mussel only. Each rock lobster was offered three fresh test pellets and three fresh control pellets daily. Separate batches of *A. nodosum* were used to prepare pellets in Assays 2B and 2C.

Bioassay 3A, B: encrusted B. papyracea *vs* B. cincta

Each rock lobster was offered three *B. papyracea* encrusted with *A. nodosum* and three specimens of *B. cincta*, a

species that is not encrusted by *A. nodosum*. Consumed whelks were replaced daily.

Bioassay 4A, B: B. papyracea *encrusted with live vs dead* A. nodosum
Each rock lobster was offered three *B. papyracea* encrusted with untreated *A. nodosum* and three *B. papyracea* encrusted with bryozoans that had been treated with liquid nitrogen immediately prior to use. Liquid nitrogen was applied thoroughly to the bryozoans using a cotton wool pad, taking care to avoid contact with the whelk. Treated shells did not differ visibly from controls and were marked by drilling a small (1mm diameter) indentation with a dentist's drill on the inner rim of the opercular aperture. Consumed whelks were replaced daily and uneaten whelks were replaced after a few days.

Bioassay 5: pellets containing Alcyonidium *extract vs control food pellets*
The third group of *J. lalandii* (n = 12) was also used to test the *A. nodosum* extract by modifying the method used for suspending freeze-dried *Alcyonidium*. Sodium alginate (2.00g) was suspended in a diethyl ether solution (10.0ml) of the *A. nodosum* dichloromethane/methanol extract (0.52g). The solvent was removed by rotary evaporation to give a yellow powder. Freeze-dried mussel (1.00g) and deionised water (60.0ml) were added to the treated alginate and the mixture stirred vigorously until homogenous. The resultant paste was then prepared as above to yield 36 treated pellets (wet weight c. 2.0g), each containing approximately 15mg of *A. nodosum* extract. Consequently, the concentration of extract in pellets was approximately equal to that found in *A. nodosum* tissue. Control pellets were prepared using sodium alginate, diethyl ether and freeze-dried mussel only. Each rock lobster was offered three fresh test pellets and three fresh control pellets daily.

Statistical analyses

The data were non-homogenous and not normally distributed, even after transformation. Consequently, analyses were carried out using Yates' corrected χ^2 goodness-of-fit tests performed on the cumulative, pooled totals of control and test prey items consumed by the end of each assay.

Spectroscopic and toxicity screening of the A. nodosum *extract*

The *A. nodosum* extract was screened by ^{1}H nuclear magnetic resonance (NMR) spectroscopy at 400MHz on a Bruker Avance 400 spectrometer in deuterochloroform, deuterium oxide and deuteromethanol respectively (spectra calibrated to residual protonated solvent). The extract was also subjected to a standard toxicity assay against *Artemia salina* larvae (Solis *et al.* 1993).

Analysis for volatile chemicals

Bryozoans can produce volatile chemicals (Christophersen and Carlé 1978, Blackman *et al*. 1992) and this was examined by solid phase microextraction (SPME) of the headspace above *A. nodosum* samples onto a fibre coated with polydimethylsiloxane (100mm-film thickness). Subsequent desorption, separation and examination of volatile components was performed by gas chromatography on a Hewlett Packard HP 6890 GC system fitted with a 50% phenyl-50% methylsiloxane DB17 column (30m x 0.25mm I.D., stationary phase thickness 0.25mm, J & W Scientific, California) and a flame ionisation detector (FID).

The SPME fibre and DB17 column were both conditioned for one hour before each set of experiments (front inlet temperature and oven temperature: 250°C). Two control extractions testing the extraction vial/septum and seawater from the aquaria in which *A. nodosum* were being maintained were performed and analysed prior to extracts of *A. nodosum*. Live bryozoans from two *B. papyracea* removed from whelks were immediately placed in a glass vial fitted with a silicone septum. The vial was placed in a water bath (approximately 60°C) and the headspace extracted for 15 minutes. The extracts were loaded onto the column by thermal desorption from the SPME fibre in the front inlet of the GC (temperature: 250°C; splitless injection mode; fibre exposed for 5 minutes) and eluted using the following oven parameters: 120°C for 10 minutes, then ramped at 10°C min^{-1} to 250°C, held at 250°C for 5 minutes. Bryozoan extraction was performed three times (different colonies sampled each time) in every set of experiments and the full set of five experiments (two independent controls and three replicated tests) was repeated three times.

Scanning electron microscopy of A. nodosum

The possibility of an inducible defence mechanism being used by the bryozoans on *B. papyracea* shells was investigated. The surfaces of *A. nodosum* colonies on whelks exposed to *J. lalandii* (i.e. by sharing the same aquaria for one week) were compared with the surfaces of bryozoan on untreated whelks using scanning electron microscopy (n = 6 for each treatment). Samples of bryozoan colonies were removed from each shell and fixed overnight in 5% glutaraldehyde in filtered seawater at 0°C. The bryozoan tissue was then dehydrated through a graded ethanol series and infiltrated with amyl acetate by treatment with a graded ethanol/amyl acetate series and critical point drying. Samples were then sputter-coated with gold and observed with a Jeol JSM 840 scanning electron microscope.

Shell strengths of encrusted B. papyracea*, scraped* B. papyracea *and* B. cincta

The compressional forces required to the crack shells of encrusted *B. papyracea,* scraped *B. papyracea* and *B. cincta* were determined using an Instron 4301 materials testing system. Shells were evaluated with a 5 000-newton, internally calibrated load cell. Shells were placed on their apertures so that force was applied through a steel rod (3.4mm diameter) to the highest point of the first whorl of the shell. Force was applied continuously and without shock at a rate of 100mm per minute and the force required to cause shell failure recorded.

Results

J. lalandii *feeding assays*

The results of the *J. lalandii* feeding assays are shown in Figures 1–5 as cumulative totals of prey items consumed against day. The statistical analyses are summarised in Table 1.

In Assays 1A, 1B, 2B, 2C, 3A, 4A and 5 there was a significant feeding preference for control prey items (Yates' corrected χ^2, $p < 0.05$ in all cases), i.e. *J. lalandii* preferred scraped *B. papyracea* to encrusted (Assay 1A, B, Figure 1); *B. cincta* to encrusted *B. papyracea* (Assay 3A, Figure 3); *B. papyracea* treated with liquid nitrogen to untreated (Assay 4A, Figure 4); and control pellets to pellets containing either *A. nodosum* (Assays 2B and 2C, Figure 2) or an *A. nodosum* extract (Assay 5, Figure 5). In summary, *J. lalandii* avoided live *A. nodosum* and food pellets containing *A. nodosum* or its chemical extract.

The remaining assays (2A, 3B and 4B) showed no significant effects ($p > 0.05$). For Assay 2A (pellets containing freeze-dried *A. nodosum* vs control pellets) this was anomalous because the two repeats of this assay (Assays 2B and 2C) indicated a marked preference for control pellets (Figure 2). During the assays showing no effect using live prey (Assays 3B and 4B), five of the seven *J. lalandii* moulted and consumption of all prey items was extremely low. Little confidence is placed here in these results because they could reasonably be rejected *a priori*. Re-analysis excluding moulting *J. lalandii* showed a significant preference for *B. cincta* over encrusted *B. papyracea* in Assay 3B ($n = 5$, $\chi^2 = 5.200$, $p < 0.05$), but no difference between *B. papyracea* with live vs dead *A. nodosum* in Assay 4B ($n = 4$, $\chi^2 = 0.345$, $p > 0.05$).

Spectroscopic and toxicity screening

The NMR spectra obtained showed only those signals consistent with the presence of long-chain fatty acids, sterols and sugars. No deshielded resonances indicating the presence of unsaturated terpenes, acetogenins,

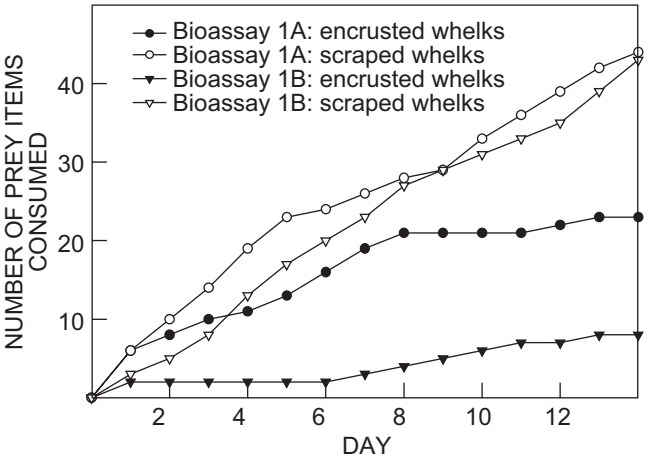

Figure 1: Cumulative totals of food items taken by *J. lalandii* in Assays 1A (n = 6) and 1B (n = 7): encrusted vs scraped *B. papyracea*

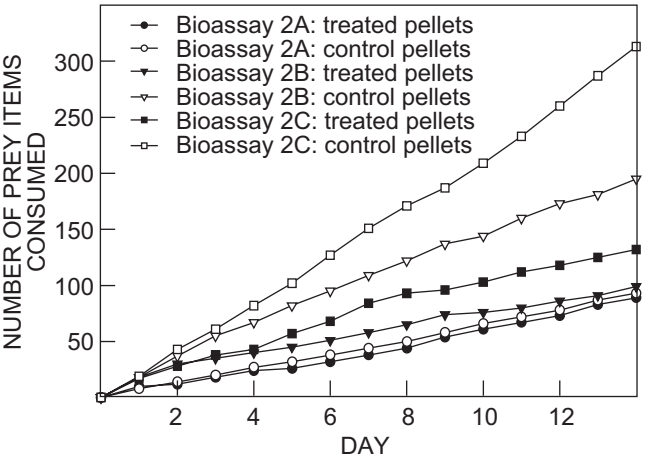

Figure 2: Cumulative totals of food items taken by *J. lalandii* in Assays 2A (n = 6), 2B (n = 7) and 2C (n = 12): pellets containing freeze-dried *A. nodosum* vs control pellets

Table 1: Summary of results of Yates' corrected χ^2 goodness-of-fit tests on *J. lalandii* feeding preference data performed on cumulative, pooled totals on termination of the assays

Bioassay	Replicate	χ^2	Significance
1. Encrusted vs scraped *B. papyracea*	A	6.597	$p < 0.05$
	B	24.039	$p << 0.001$
2. *Alcyonidium* treated pellets vs control pellets	A	0.093	ns
	B	31.350	$p << 0.001$
	C	73.622	$p << 0.001$
3. Encrusted *B. papyracea* vs *B. cincta*	A	59.899	$p << 0.001$
	B	0.944	ns*
4. Liquid N_2 treated vs untreated *B. papyracea*	A	12.266	$p < 0.001$
	B	0.156	ns
5. *Alcyonidium* extract treated pellets vs control pellets	A	67.205	$p << 0.001$

ns = Not significant ($p > 0.05$)
* $p < 0.05$ if moulting *J. lalandii* excluded

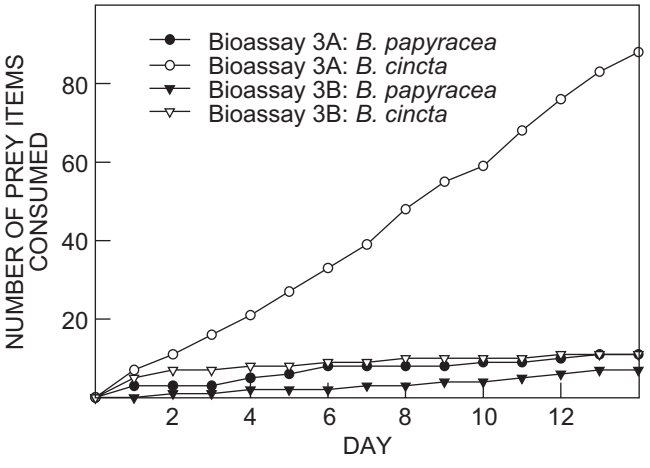

Figure 3: Cumulative totals of food items taken by *J. lalandii* in Assays 3A (n = 6) and 3B (n = 7): *B. papyracea* vs *B. cincta*

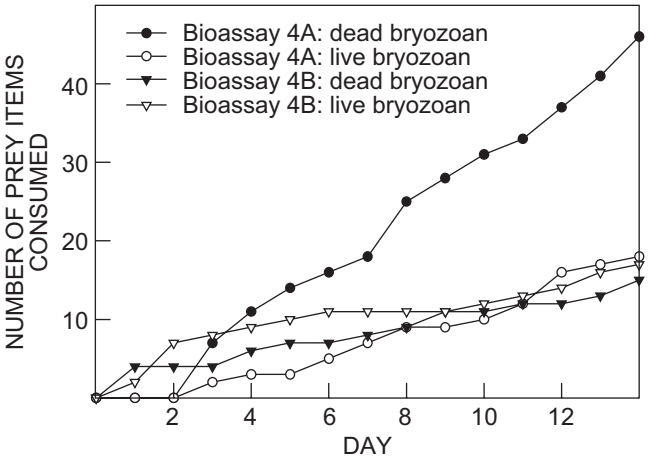

Figure 4: Cumulative totals of food items taken by *J. lalandii* in Assays 4A (n = 6) and 4B (n = 7): liquid nitrogen-treated vs untreated *B. papyracea*

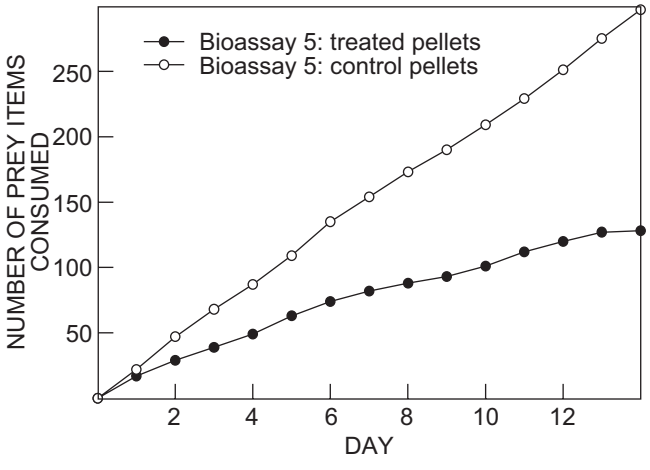

Figure 5: Cumulative totals of food items taken by *J. lalandii* in Assay 5 (n = 12): pellets containing *A. nodosum* extract vs control pellets

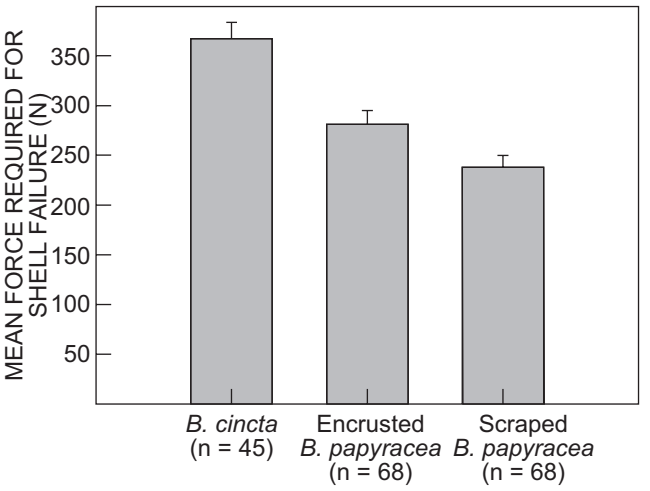

Figure 6: Comparison of shell strengths of *B. cincta*, encrusted *B. papyracea* and scraped *B. papyracea.* Error bars denote standard error

alkaloids, phenolics or peptides were apparent in the spectra. The *A. nodosum* extract did not display any toxic effects in the *Artemia* assay.

Analysis for volatile chemicals

In five out of the nine SPME bryozoan extractions, components were observed to elute from the column with retention times of between 15 and 20 minutes. These components did not elute in a regular pattern and elicited only very small responses from the FID detector (<1pA in all cases). The chromatograms of the remaining four bryozoan extractions did not differ from those obtained for the seawater control extractions. Because the presence and composition of volatiles appeared to be highly variable among colonies of *A. nodosum* and only extremely small amounts could be extracted when they were present, further characterisation of the volatile components by GC-MS was not attempted.

Inducible defences in A. nodosum

The surfaces of all bryozoan colonies appeared smooth and undulating with no obvious spines, spicules or other calcareous/chitinous structures. There was no difference in the surface appearance of bryozoans from whelks that had been exposed to *J. lalandii* compared to those from untreated whelks.

Shell strengths

Sample size, minimum and maximum shell lengths for treatments were: encrusted *B. papyracea* (n = 68, 17.4–62.9mm); scraped *B. papyracea* (n = 68, 16.3–62.9mm); and *B. cincta* (n = 45, 20.0–48.3mm). There was no significant difference in the mean size of whelks among groups (ANOVA, F-ratio = 1.430, $p > 0.05$).

The force required to crack shells (Figure 6) was independent of shell length (regression analysis, $r^2 < 0.05$ and $p > 0.05$ for all three groups), and the force required to fracture shells differed significantly for each whelk type (ANOVA, F-ratio = 19.987, $p << 0.001$ followed by Tukey's HSD). Mean failure load was ranked as *B. cincta* > encrusted *B. papyracea* > scraped *B. papyracea*.

Discussion

Over the past three decades, natural products chemists have described a plethora of novel secondary metabolites from an enormous range of benthic and pelagic marine organisms, including invertebrates, algae and microbes (Blunt *et al*. 2005 and previous reviews therein). Although the natural functions of most of these secondary metabolites remain unknown, many have been shown to have important roles in defence (against predators, competitors, fouling organisms or pathogens), reproduction and settlement or metamorphosis of larvae (e.g. Bakus *et al*. 1986, Pawlik 1992, Lambert and Todd 1994, McClintock and Baker 2001). The present results indicate that the defence conferred on *B. papyracea* by *A. nodosum* is likewise chemically rather than physically based.

Although the results of repeated, independent feeding assays were occasionally contradictory, experiments in which *J. lalandii* showed no preference between prey items were generally ones in which rock lobsters were moulting and simply not feeding, or else feeding at extremely low rates. Nevertheless, assays using rock lobsters gave results that are ecologically interpretable using statistical methods that analyse pooled data. The results confirm that *J. lalandii* shows a distinct preference for unencrusted *B. papyracea* (Assays 1A and 1B, Figure 1). The deterrent effect of encrusted whelks is not owing to physical factors, because *A. nodosum* does not produce additional external defensive structures on exposure to *J. lalandii* and, although the presence of *A. nodosum* on *B. papyracea* shells does increase shell strength, this does not appear to influence prey selection by rock lobsters. When given a choice between *B. papyracea* and *B. cincta*, *J. lalandii* preferentially fed upon the latter species, even though it has a stronger shell than encrusted *B. papyracea* (Assay 3A, Figure 3).

Other physical factors that may bias prey selection by *J. lalandii* could be alteration of the texture or appearance of the whelk by the bryozoan, so that rock lobsters do not recognise encrusted whelks as potential prey, or cannot manipulate them on account of the bryozoan shroud (cf. Bloom 1975). Whelks encrusted with *A. nodosum* are, however, conspicuous in the field because of the purple colour of the bryozoan, so that camouflage is presumably not an issue. If anything, the colouration may be aposomatic. In any event, rock lobsters tended to handle and thoroughly investigate all prey items in the assays before rejecting or accepting them. Similarly, the assay comparing predation between *B. papyracea* encrusted with live and dead *A. nodosum* showed that lobsters preferred whelks encrusted with dead *A. nodosum* even though the two sets of whelks were not physically or visibly different (Assay 4A, Figure 4).

The results of the food pellet assay showing no preference between pellet types (Assay 2A, Figure 2) prompted the analysis for volatile chemicals in the bryozoan that may have been lost during lyophilisation. Volatile components were extracted at very low levels but their occurrence in colonies was highly variable, thus hindering further characterisation by GC-MS. This analysis, however, was made redundant by the second and third replicate of the pellet assay (Assays 2B and 2C, Figure 2), which indicated a distinct preference for the control pellets.

Therefore, it appears that *A. nodosum* contains one or more chemical components that deter feeding by *J. lalandii*. Indeed, freeze-dried *A. nodosum* and a crude organic *A. nodosum* extract did affect prey selection in feeding assays (Assays 2B, 2C and 5, Figures 2, 5), even though spectroscopic and toxicity screening did not suggest the presence of biologically active metabolites. This could be a consequence of several effects. Decapod crustaceans are able to respond to nanomolar or even picomolar concentrations of certain common metabolites (Fuzessery and Childress 1975, Schmitt and Ache 1979). Therefore, the molecule (or molecules) deterring *J. lalandii* may be produced in quantities that are below the level of detection for standard chemical and spectroscopic techniques or the *Artemia salina* assay when in a crude extract. Alternatively, the signals relating to the active chemicals may be present in the ^{1}H NMR spectra, but hidden or swamped by the resonances corresponding to the large amounts of primary metabolites in the extract. Finally, the components responsible for the deterrent effect need not necessarily exhibit high broad-scale biological activity or unusual spectroscopic properties; they may simply be unremarkable, common metabolites that are unpalatable to *J. lalandii* (e.g. McClintock *et al.* 1994b).

The concentration of *A. nodosum* extract in test pellets was very similar to that in live *A. nodosum* and was sufficient to deter rock lobster feeding. *J. lalandii* does not have pincers, and attacks whelks by chipping away the lip of the shell using its mouthparts. Consequently, when attempting to feed on encrusted *B. papyracea*, *J. lalandii* would have to endure prolonged exposure to the bryozoan before being able to access or ingest any of the whelk flesh. These assays test for a deterrent effect owing to the presence of *A. nodosum* or its extract in the pellets rather than an effect following ingestion of the bryozoan. It is therefore concluded that *A. nodosum* does contain a non-volatile chemical constituent, or constituents, that deter feeding of *J. lalandii* and hence protect *B. papyracea* from this predator.

This study offers a good example of one species benefiting from the chemical defences of another, other than through sequestration. The selective advantages of chemical defence are often obvious, as are those of exploiting another species' defences. This is generally done through the behaviour of the non-defended species. For example, amphipods can create tubes from chemically defended macrophytes that are distasteful to predators (Hay *et al*. 1990); a good example is the abduction of chemically defended pteropods by the Antarctic amphipod *Hyperiella dilatata* (McClintock and Janssen 1990). In the present example, there is no apparent modification of behaviour, but

the selective pressures involved have presumably been intense because the association between whelk and bryozoan is an obligate one. The purple bryozoan shell covering is diagnostic of *B. papyracea* and *A. nodosum*, occurring exclusively on the shells of *B. papyracea* (O'Donoghue and de Watteville 1944, O'Donoghue 1956), and possibly two other species of *Burnupena* (Dempster and Branch 1999). The situation is similar to that described by Bloom (1975) in which a scallop and an encrusting sponge provided mutual protection from predation, but in that case there was no evidence of a chemical component. The present study provides a good example of how powerfully chemical defences can modify biotic interactions. The study was prompted by work at two adjacent South African islands, one dominated by filter-feeders and the other by *J. lalandii*. *B. papyracea* is one of only two whelks that coexist with *J. lalandii* on the second island (the other whelk is immune to predation because of its particularly heavy shell) and is involved in a dramatic predator/prey role reversal at the island dominated by filter-feeders (Barkai and McQuaid 1988). The effects of chemical defences can be subtle and complex (e.g. McGovern and Hellberg 2003, Tillmann 2003), but they can also profoundly influence the biology of marine species.

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References

BAKUS, G. J., TARGETT, N. M. and B. SCHULTE 1986 — Chemical ecology of marine organisms: an overview. *J. chem. Ecol.* **12**: 951–987.

BARKAI, A. and G. M. BRANCH 1988 — Energy requirements for a dense population of rock lobsters *Jasus lalandii*: novel importance of unorthodox food sources. *Mar. Ecol. Prog. Ser.* **50**: 83–96.

BARKAI, A., DAVIS, C. L. and S. TUGWELL 1996 — Prey selection by the South African Cape rock lobster *Jasus lalandii*: ecological and physiological approaches. *Bull. mar. Sci*. **58**: 1–8.

BARKAI, A. and C. [D.] McQUAID 1988 — Predator-prey role reversal in a marine benthic ecosystem. *Science, N.Y.* **242**: 62–64.

BEST, B. A. and J. E. WINSTON 1984 — Skeletal strength of encrusting cheilostome bryozoans. *Biol. Bull. mar. biol. Lab., Woods Hole* **167**: 390–409.

BLACKMAN, A. J., DAVIES, N. W. and C. E. RALPH 1992 — Volatile and odorous compounds from the bryozoan *Biflustra perfragilis*. *Biochem. Syst. Ecol*. **20**: 339–342.

BLOOM, S. A. 1975 — The motile escape response of a sessile prey: a sponge-scallop mutualism. *J. expl mar. Biol. Ecol.* **17**: 311–321.

BLUNT, J. W., COPP, B. R., MUNRO, M. H. G., NORTHCOTE, P. T. and M. R. PRINSEP 2005 — Marine natural products. *Nat. Prod. Rep*. **22**: 15–61.

CARLÉ, J. S. and C. CHRISTOPHERSEN 1980 — Dogger Bank Itch. The allergen is (2-hydroxyethyl) dimethylsulfonium ion. *J. Am. Oil Chem. Soc*. **102**: 5107–5108.

CARLÉ, J. S. and C. CHRISTOPHERSEN 1982 — Dogger Bank Itch. 4. An eczema-causing sulfoxonium ion from the marine animal *Alcyonidium gelatinosum* (Bryozoa). *Toxicon* **20**: 307–310.

CARROLL, S., McEVOY, E. G. and R. GIBSON 2003 — The production of tetrodotoxin-like substances by nemertean worms in conjunction with bacteria. *J. expl mar. Biol. Ecol.* **288**: 51–63.

CHANAS, B. and J. R. PAWLIK 1995 — Defences of Carribean sponges against predatory reef fish. II. Spicules, tissue toughness, and nutritional quality. *Mar. Ecol. Prog. Ser*. **127**: 195–211.

CHRISTOPHERSEN, C. and J. S. CARLÉ 1978 — Chemical signals from a marine bryozo. *Naturwissenschaften* **65**: 440.

DEMPSTER, Y. and G. M. BRANCH 1999 — A review of the genus *Burnupena* Iredale, 1918 (Gastropoda: Buccinidae) with descriptions of two new species. *Ann. Nat. Mus.* **40**: 173–204.

FUZESSERY, Z. M. and J. J. CHILDRESS 1975 — Comparative chemosensitivity to amino acids and their role in the feeding activity of bathypelagic and littoral crustaceans. *Biol. Bull. mar. biol. Lab., Woods Hole* **149**: 522–538.

GRIFFITHS, C. L. and J. L. SEIDERER 1980 — Rock-lobsters and mussels — limitations and preferences in a predator-prey interaction. *J. expl mar. Biol. Ecol.* **44**(1): 95–109.

HARVELL, C. D. 1984 — Predator-induced defence in a marine bryozoan. *Science* **224**: 1357–1359.

HARVELL, C. D. 1986 — The ecology and evolution of inducible defences in a marine bryozoan: cues, costs and consequences. *Am. Naturalist* **128**: 810–823.

HARVELL, C. D. 1990 — The ecology and evolution of inducible defences. *Q. Rev. Biol.* **65**: 323–340.

HARVELL, C. D. 1992 — Inducible defences and allocation shifts in a marine bryozoan. *Ecology* **73**: 1567–1576.

HAY, M. E., DUFFY, J. E. and W. FENICAL 1990 — Host-plat specialization decreases predation on a marine amphipod: an herbivore in plant's clothing. *Ecology* **71**: 733–743.

LAMBERT, W. J. and C. D. TODD 1994 — Evidence for a waterborne cue inducing metamorphosis in the dorid nudibranch mollusc *Adalaria proxima* (Gastropoda: Nudibranchia). *Mar. Biol.* **120**: 265–271.

MAYFIELD, S. and G. M. BRANCH 2000 — Interrelations among rock lobsters, sea urchins and juveile abalone: implications for community management. *Can. J. Fish. aquat. Sci.* **57**: 2175–2185.

McCLINTOCK, J. B. and B. J. BAKER (Eds) 2001 — *Marine Chemical Ecology*. Boca Raton; CRC Press: 610 pp.

McCLINTOCK J. B., BAKER B. J., HAMANN, M. T., YOSHIDA W., SLATTERY M., HEINE J. N., BRYAN P. J., JAYATILAKE G. S. and B. H. MOON 1994a — Homarine as a feeding deterrent in common shallow-water Antarctic lamellarian gastropod *Marseniopsis mollis*: a rare example of chemical defense in a marine prosobranch. *J. chem. Ecol.* **20**: 2539–2549.

McCLINTOCK, J. B., BAKER, B. J., SLATTERY, M., HEINE, J. N., BRYAN, P. J., YOSHIDA, W., DAVIES-COLEMAN, M. T. and D. J. FAULKNER 1994b — Chemical defense of common Antarctic shallow-water nudibranch *Tritoniella belli* Elliot (Mollusca: Tritonidae) and its prey, *Clavularia frankliniana* Rouel (Cnidaria: Octocorallia). *J. chem. Ecol*. **20**: 3361–3372.

McCLINTOCK, J. B. and J. JANSSEN 1990 — Pteropod abduction as a chemical defence in a pelagic Antarctic amphipod. *Nature, Lond.* **346**: 462–464.

McGOVERN, T. M. and M. E. HELLBERG 2003 — Cryptic species, cryptic endosymbionts, and geographical variation in chemical defences in the bryozoan *Bugula neritina*. *Mol. Ecol*. **12**: 1207–1215.

O'DONOGHUE, C. H. 1956 — Some South African bryozoa. *Trans. R. Soc. S. Afr.* **35**: 71–95.

O'DONOGHUE, C. H. and D. DE WATTEVILLE 1944 — Additional notes on bryozoa from South Africa. *Ann. Natal Mus*. **10**: 407–432.

PAWLIK, J. R. 1992 — Chemical ecology of the settlement of benthic marine invertebrates. *Oceanogr. mar. Biol. a. Rev*. **30**: 273–335.

PAWLIK, J. R., CHANAS, B., TOONEN, R. J. and W. FENICAL 1995 — Defences of Caribbean sponges against predatory reef fish. I. Chemical deterrency. *Mar. Ecol. Prog. Ser*. **127**: 183–194.

PECK, L. S., HAYWARD, P. J. and M. E. SPENCER-JONES 1995 — A pelagic bryozoan from Antarctica. *Mar. Biol*. **123**: 757–762.

SCHMITT, B. C. and A. W. ACHE 1979 — Olfaction: responses of a decapod crustacean are enhanced by flicking. *Science* **205**: 204–206.

SOLIS, P. N., WRIGHT, C. W., ANDERSON, M. M., GUPTA, M. P. and J. D. PHILLIPSON 1993 — A microwell cytotoxicity assay using *Artemia salina* (brine shrimp). *Plant. Med*. **59**: 250–252.

THORPE, J. P. and J. S. RYLAND 1979 — Cryptic speciation detected by biochemical genetics in three ecologically important intertidal bryozoans. *Estuar. Coast. mar. Sci*. **8**: 395–398.

TILLMANN, U. 2003 — Kill and eat your predator: a winning strategy of the planktonic flagellate *Prymnesium parvum*. *Aquat. Micro. Ecol*. **32**: 73–84.

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