Tissue-specific palatability and chemical defenses against macropredators and pathogens in the common articulate brachiopod *Liothyrella uva* from the Antarctic Peninsula

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Abstract

The punctate terebratulid brachiopod *Liothyrella uva* is the most common brachiopod species in Antarctica. Whole brachiopods, either live or freeze dried and ground into a powder and suspended in alginate, were unpalatable to the sympatric macropredators *Odontaster validus* (an abundant, omnivorous sea star) and *Notothenia coriiceps* (an abundant, omnivorous, epibenthic fish). The unpalatability of these ground tissues coupled with that of lipophilic extracts of whole *L. uva* presented in alginate pellets to *O. validus* suggests an involvement of chemical defenses. Several isolated brachiopod tissues were also unpalatable to *O. validus* after being freeze dried, ground and suspended in alginate, but only the pedicle was unpalatable in such preparations to both *O. validus* and *N. coriiceps*. This observation is consistent with the Optimal Defense Theory since the pedicle is the only tissue not protected inside the brachiopod shell. There was, however, no correlation between the energetic content and unpalatability of any of the individual tissues. Organic extracts of tissues involved in feeding (lophophore and intestine–stomach) had relatively strong antimicrobial activity when assayed against several strains of Antarctic bacteria. However, the lophophore was palatable to both macropredators, suggesting nonoverlapping chemical defenses are involved in protection against predators and pathogens.

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1. Introduction

Evidence from the fossil record indicates that articulate brachiopods dominated macrobenthic assemblages during the Paleozoic but then declined considerably during the Mesozoic (Thayer, 1986). This decline in dominance may have been the result of increased diversification of predators (Stanley, 1977, 1979), a hypothesis supported by direct evidence of increased predation damage in extinct clades of articulates during the Mesozoic (Alexander, 1986). Today, only three of the original six articulate clades remain (Terebratulida, Rhynchonellida, Thecideids). One possible explanation for patterns of extinction among articulate clades is that increased predation pressure may have selected for those species that were unpalatable as all extant clades have unpalatable representatives (Thayer and Allmon, 1990). While direct demonstration of chemical defenses are generally lacking in articulates (Thayer, 1985; Thayer and Allmon, 1990; but see McClintock et al., 1993), it is likely that the demonstrative unpalatability of brachiopod soft tissues is related to defensive chemistry.

Articulates are somewhat unique with respect to their defensive attributes in that a lack of tissue palatability is combined with a shelled armature (Thayer and Allmon, 1990). Chemical defenses commonly characterize groups of marine invertebrates such as sponges, soft corals and nudibranchs that lack significant physical defenses such as shells (Paul, 1992; Pawlik, 1993; McClintock and Baker, 2001). Nonetheless, there are some examples of shelled marine invertebrates that rely in part on defensive chemicals for their protection. Pawlik et al. (1986) describe a novel triterpene used to defend the intertidal limpet Colisella limatula, and Kvitok et al. (1991) found that diet-derived neurotoxins are used to defend the burrowing soft sediment bivalve Saxidomus giganteus. Clearly, theories examining patterns of evolution in articulate brachiopods based on their predator–prey dynamics will allow a more complete picture of the palatability, and more specifically, chemical defenses that may distinguish extant species.

The Antarctic benthos is characterized by abundant and diverse marine communities (Dayton et al., 1974, 1994; Starmans, 1977; Dayton, 1990; Clarke, 1992, 1996; Brey et al., 1994; Gutt and Starmans, 1998; Gray, 2001). Articulate brachiopods are no exception to this pattern, with densities in both sub-Antarctic and Antarctic environments that range to over 3000 individuals per meter square (Foster, 1974; Peck et al., 2001). Below the shallow depths impacted by anchor ice and ice scour (generally down to 25–30-m depth; Dayton et al., 1970), the rich Antarctic benthic communities are dominated by sessile suspension feeders including sponges, soft corals and articulate brachiopods. These deeper communities are considered “biologically accommodated” as predation and competition structure the benthos (Dayton et al., 1974; see also Dayton, 1990; Gutt and Starmans, 1998). The combination of a benthic fauna isolated for at least 20 million years (Dayton et al., 1994), and the constraints of predation and competition has selected for chemical defenses in Antarctic marine plants and a wide assortment of sessile and sluggish marine invertebrates (reviewed by McClintock and Baker, 1997a, 1998; Amsler et al., 2000, 2001a,b). While Antarctic predators such as fish and sea stars are capable of consuming articulate brachiopods, evidence suggests that they are rarely included in diets (Brand, 1974; Temnikov et al., 1976; Dearborn, 1977; Sloan, 1980; Eastman, 1994; McClintock, 1994).
The most well-studied Antarctic brachiopod is the punctate terebratulid *Liothyrella uva* which occurs in abundance down to 300-m depth (Bellisio et al., 1972; Dell, 1972; De Castillanos, 1973; Foster, 1974; Arnaud, 1977; Picken, 1985a,b; Peck et al., 1986, 1997; Voss, 1988; Peck and Robinson, 1994). Near Palmer Station on the Antarctic Peninsula, populations of *L. uva* may comprise up to 6% of the biomass at 20–30-m depth (Zamorano, 1983). McClintock et al. (1993) investigated aspects of the morphometry, biochemical and energetic composition of whole soft body tissues and shells, and chemically based behavioral tube foot retraction responses of sea star predators exposed to whole tissue filtrates of *L. uva*. They also performed laboratory feeding assays using agar pellets containing a krill feeding stimulant and finely ground lyophilized whole-body tissues of *L. uva* employing two allopatric fish predators. Six sea star species and one of the two fish species were deterred by brachiopod tissue filtrates or pellets containing finely ground whole brachiopod tissues, respectively.

The present study significantly extends the evaluation of chemical defenses in the common articulate *L. uva* by examining chemical deterrence in somatic [lophophore, intestine–stomach (including digestive diverticula), pedicle] and reproductive (male and female gonads) body components using feeding deterrence bioassays employing ecologically relevant sympatric sea star and fish predators. These data are evaluated within the context of the Optimal Defense Theory (ODT) (Rhoads, 1979; reviewed by Cronin, 2001), which assumes that resources are limited and predicts that chemical defenses should be allocated to those tissues most vulnerable to predation or most likely to maximize fitness. Moreover, in order to extend the evaluation of chemical deterrents to functions other than that of defending against macropredators, the present study examines the antimicrobial activity of somatic and reproductive tissues of *L. uva* using sympatric marine bacteria cultured from the surfaces of benthic Antarctic marine invertebrates. This provides an evaluation of the presence of bioactive compounds that may serve as antifoulants or inhibit bacterial infection.

2. Materials and methods

The brachiopod *L. uva* and the sea star *Odontaster validus* were collected subtidally from several sites within 3.5 km of Palmer Station on Anvers Island, Antarctica (64°46′ S, 64°04′ W; cf. Amsler et al., 1995). Antarctic rockfish, *Notothenia coriiceps*, were collected by hook and line from the shoreline at Palmer Station. Animals used in most assays were collected in March and April 2000 but brachiopods and fish used in one assay (ground whole brachiopod, see below) were collected in November 2001 (brachiopods) and December 2001 through January 2002 (fish). Sea stars and fish as well as those brachiopods that were utilized live were maintained at ambient temperatures (−1 to 1 °C) in flow through filtered seawater tables until use.

A portion of the collected brachiopods were sorted as male, female or juvenile, and used live in bioassays. Males and females examined were reproductively active at the time of collection as evidenced by the presence of spermatozoa and oocytes in the gonads, as well as brooded embryos, gastrulae and three-lobed larvae in the lophophores of some females greater than 30 mm in length. “Juveniles” were defined as ≤ 15 mm in length.
based on the report of Meidlinger et al. (1998). Additional adult animals were separated by sex and frozen whole at $-70^\circ$C before being freeze dried. Other adult brachiopods were dissected and the lophophore, intestine–stomach (including digestive diverticula), pedicle and gonads (ovaries or testes) were separated, weighed and then freeze dried. The tissues were used for feeding bioassays, for extraction and use in microbial bioassays, or for tissue energetic studies. These tissues were chosen because they comprise the major internal components on a biomass basis. The small adductor and diductor muscles were included in whole-body homogenates and extractions but not used as isolated tissues.

2.1. Feeding bioassays

Whole, live *L. uva* were used in some feeding bioassays. In others, either whole, freeze-dried *L. uva* excluding the shells or separated, freeze-dried tissues, including the lophophore, pedicle, intestine–stomach, male reproductive tissues and female reproductive tissues were individually macerated and suspended in a 2% alginate solution. Each homogenate suspension was formed into artificial food pellets or disks for feeding bioassays. This was done by dripping the homogenate suspension into a 1 M CaCl$_2$ solution where it solidified into pellets or by slowly pouring 1 M CaCl$_2$ over the homogenate in a petri dish where it was subsequently cut into disks. Both pellets and disks were prepared at a final homogenate concentration of 5% tissue in the 2% alginate. Ground, freeze-dried Antarctic krill (*Euphausia superba*) at a concentration of 5% tissue in 2% alginate served as feeding controls (McClintock and Baker, 1997b). This level of tissue homogenate in the artificial foods was chosen as it has proven effective in previous bioassays with Antarctic predators (e.g., McClintock et al., 1993; McClintock and Baker, 1997b) and falls well within the range used in artificial foods in studies of feeding preferences in a variety of other systems (e.g., Duffy and Hay, 1991; Hay et al., 1994; Pawlik et al., 1995; Bolser and Hay, 1998; Nagle et al., 1998; Kelman et al., 1999).

Hydrophobic extracts for feeding bioassays were prepared by extracting whole, freeze-dried adults in three changes (24 h each change) of 1:1 CH$_2$Cl$_2$/methanol. The extracts from the individual changes of each solvent mixture were combined, filtered through glass wool and the solvents removed by evaporation under reduced pressure. Extract yields were calculated on a wet weight basis and added to 5% krill at their natural wet weight equivalent concentration.

A bioassay utilizing the common, omnivorous sea star *O. validus* was based on methods previously described by McClintock and Baker (1997b). After collection, *O. validus* were placed in a 2-m diameter circular holding tank (3200 l) equipped with running ambient seawater. They were allowed to acclimate for at least 96 h before any feeding assays were started. Sea stars commonly climb the sides of the aquarium up to air–water interface and then extend one or two arms out directly under the water surface. In the bioassays, individuals at the surface were offered a live whole brachiopod or an experimental pellet. Experimental pellets consisted of either ground whole brachiopod (without shell) in alginate, a hydrophobic extract of whole brachiopod added to ground krill in alginate, or an individual tissue ground in alginate. The pellet was placed within the ambulacral groove of a single arm, equidistant between the arm tip and oral opening. Acceptance was recorded when the animal moved the brachiopod or pellet to the oral opening. Rejection was
recorded when the animal dropped the brachiopod or pellet, or moved it away from the mouth out of the ambulacral groove or towards the arm tip. Twenty minutes after the animal either accepted or rejected the experimental pellet or tissue, it was given a control pellet consisting of 5% krill in 2% alginate (McClintock and Baker, 1997b). Sample size was 10–13 individual sea stars for each treatment except whole brachiopods ground in alginate where the total sample was 37 individual sea stars. No animal was used more than once. Differences between tissue pellets and corresponding controls were determined using a two-tailed Fisher’s Exact Test of Independence (Sokal and Rohlf, 1995).

The Antarctic rockfish *N. coriiceps* was also used for feeding bioassays. Fish were held in 1 × 2 m seawater tables equipped with flowing seawater approximately 0.25 m in depth. Each table was divided into three compartments using fine mesh dividers with a single fish in each compartment. Two separate experimental series were run. In one series, all fish were fed one of each of the following tissue disk types in a random order per trial: lophophore, pedicle, intestine–stomach, male reproductive tissues, female reproductive tissues and a control disk composed of freeze-dried krill. Tissue preparations were presented in random order and no fish was given an experimental disk type more than once throughout the experimental series. Assays were performed every 12 h until the experiments were concluded. Five minutes after being presented with the experimental disk, the fish was given a disk consisting of 5% krill in 2% alginate. If the fish did not eat the postexperimental control disk in a given trial, those results were discarded. Based on the results of these bioassays, we decided to also test disks prepared from whole *L. uva* excluding the shells (collections in November 2001). These were assayed as above but with a different group of 12 *N. coriiceps*. Acceptance of a given disk was recorded when the fish ate a pellet and did not regurgitate it. Rejection was recorded when the fish took the disk into its mouth and subsequently spit it out. Significance of difference between tissue disks and corresponding controls was determined using a two-tailed Fisher’s Exact Test of Independence (Sokal and Rohlf, 1995).

### 2.2. Microbial bioassays

Antimicrobial assays utilized four strains of psychrotrophic marine bacteria that have been described previously (DeMarino et al., 1997). Strains McM13.3, McM18.1 and McM32.2 were isolated from the surfaces of Antarctic marine macroinvertebrates and strain McM11.5 was isolated from the near-shore Antarctic water column. All were capable of growth at both −1.0 and 20.0 °C. Voucher specimens of the bacteria are available in the Department of Biology, University of Alabama at Birmingham and in the Department of Chemistry, University of South Florida.

Isolated brachiopod tissues were weighed wet, freeze dried and then extracted in three changes (24 h each change) of 1:1 CH₂Cl₂/methanol, resulting in hydrophobic extracts. This was followed by three changes (24 h each change) of 1:1 methanol/water, resulting in hydrophilic extracts. The extracts from the individual changes of each solvent mixture were combined, filtered through glass wool and the solvents removed by evaporation under reduced pressure. Extract yields were calculated on a wet weight basis. The extracts were resuspended in either methanol (hydrophobic extracts) or 1:1 methanol/water (hydrophilic extracts) at 1 ml per g of wet tissue originally extracted. Paper antimicrobial assay disks
(BBL Microbiology Systems 31039) were prepared by placing 20 µl of these extract solutions or of solvent only onto each disk. This volume essentially saturates a disk. Consequently, this method results in an extract concentration that approximates tissue concentrations on wet weight and volumetric bases. For bioassays, the bacteria were spread onto Difco marine Agar 2216 (Difco Laboratories). Extract-containing disks were placed onto the culture plates and the cells allowed to grow for 2 days (strains McM13.3, McM18.1 and McM32.2) or 3 days (strain McM11.5) at 20 (± 1.5) °C. Antimicrobial activity was defined as visible inhibition of cell growth in a region surrounding the paper disk.

2.3. Tissue energetics

Freeze-dried brachiopod tissues were returned to the University of Alabama at Birmingham for calorimetric analysis. The lophophore, pedicle, female reproductive tissues, male reproductive tissues and intestine–stomach tissues were each pressed into small pellets and calorically analyzed with a Parr 1421 Semimicro Calorimeter. Three determinations were made for each tissue. Energy content (kJ/g dry wt.) was calculated against a benzoic acid standard and included a fuse correction. Trials were repeated three times for each tissue measured. Differences in the energy content of tissues was determined by one-way analysis of variance and by a Ryan–Einot–Gabriel–Welsch (REGWQ) post hoc test using SPSS software (SPSS, Chicago, IL).

3. Results

3.1. Feeding bioassay

The sea star *O. validus* rejected whole male, female and juvenile *L. uva* and a hydrophobic extract of *L. uva* added to the krill feeding stimulant (Fig. 1). However, lophophore tissue was accepted in all trials (Fig. 1).

![Percent acceptance by the sea star *O. validus* of whole *L. uva* individuals or *L. uva* lophophores (LOP) (dark bars) and hydrophobic extracts of *L. uva* (hatched bar) in comparison to corresponding controls (open bars). Asterisks indicate significant differences from controls (Fisher’s Exact Test, *p* < 0.05).](image-url)
Homogenized *L. uva* tissues suspended in alginate caused a differential response in the sea star and fish feeding bioassays. *Odonaster validus* rejected ground whole brachiopods, male reproductive tissues, the stomach–intestine and the pedicle (Fig. 2). In the first *N. coriiceps* bioassay, the only individual ground tissue rejected by the fish was the brachiopod pedicle (Fig. 3). In the second *N. coriiceps* bioassay with all soft tissues combined, the fish significantly rejected the ground brachiopod disks (Fisher’s Exact Test, *p* = 0.0006). Only 1 of 12 experimental disks was accepted (8.3%) compared to 10 of 12 controls (83%).

### 3.2. Microbial analysis

Antimicrobial activity was greatest in extracts of the lophophore and of the intestine with extracts of female reproductive tissues having somewhat less antimicrobial activity (Table 1). Hydrophobic extracts from the lophophore, intestine–digestive diverticula and female reproductive tissues and hydrophilic extracts from the lophophore and intestine–stomach had growth inhibition effects on two of the four strains of bacteria tested,
respectively (Table 1). Neither male reproductive tissue extracts nor pedicle extracts had any visible effect on bacterial growth (Table 1). Likewise, control disks prepared with either solvent alone did not inhibit growth in any bacterial strain.

### 3.3. Tissue energetics

There were significant differences between specific tissues from *L. uva*. The brachiopod pedicle was significantly lower in caloric content than the intestine–stomach, male reproductive tissue and the lophophore (Fig. 4). Female reproductive tissue was intermediate in caloric content and not significantly different from any of the other tissues examined (Fig. 4).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Extract</th>
<th>Lophophore</th>
<th>Intestine–stomach</th>
<th>Female rep.</th>
<th>Male rep.</th>
<th>Pedicle</th>
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<tr>
<td>McM11.5</td>
<td>HB</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>HB</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>McM13.3</td>
<td>HB</td>
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<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>HB</td>
<td>++</td>
<td>++</td>
<td>0</td>
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<tr>
<td>McM18.1</td>
<td>HB</td>
<td>0</td>
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<td>0</td>
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<td></td>
<td>HB</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>McM32.2</td>
<td>HB</td>
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<td>+</td>
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<tr>
<td></td>
<td>HB</td>
<td>++</td>
<td>0</td>
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<td>3.36</td>
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<tr>
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<td>9.92</td>
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<td>5.06</td>
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</tbody>
</table>

“0” = no effect. “+” = growth inhibition halo present but < 1 mm wide. “++” = growth inhibition halo 1 mm wide. “+++” = growth inhibition halo 2 mm wide. Extract mass indicates mg of dry extract added to antimicrobial paper assay disk (see text). HP: hydrophilic extract. HB: hydrophobic extract.

Fig. 4. Calorimetric data from the specific tissues of *L. uva*. Means ± 1 S.E.M. Analysis of variance indicated significant differences between tissues ($F_{4,14} = 5.844$, $p = 0.011$). Post hoc test (REGWQ, $p < 0.05$) results identifying differences between means are symbolized by letters above bars. Means with different letters are significantly different. See Fig. 2 for definitions of abbreviations.
4. Discussion

These data confirm our previous report (McClintock et al., 1993) that *L. uva* is unpalatable to sea stars and fish. Moreover, these data extend the previous report by directly demonstrating lack of palatability in feeding assays with sympatric predators. Although bioassays based on sea star tube foot retraction correlate well with known sea star feeding preferences on sponges in nature (reviewed by Amsler et al., 2000, 2001a,b; McClintock et al., 2000), such a correlation has not been made for other invertebrate prey. The present study, however, provides one data point that may be used to help make such correlations. The use of allopatric predators in feeding deterrence assays was not uncommon in the past and was necessary for logistical reasons with fish predators in our previous study, but lacks the direct ecological relevance afforded by assays with sympatric, potential predators.

Sea stars demonstrated strong rejection of whole live brachiopods regardless of their sex or age (feeding data in present study), while more qualitative observations revealed that fish (*N. coriiceps*) also repeatedly spit out whole adult brachiopods (C.D. Amsler, personal observations). Fish rejected ground, whole-body tissue embedded in alginate disks, but the lipophilic extract added to a feeding stimulant in alginate was not rejected. Feeding deterrence in fish could be the result of hydrophilic compounds but, due to logistical constraints, we were not able to test a hydrophilic extract with fish. The basis for these rejection responses to whole brachiopods by sea stars is definitively chemical in nature, at least in part, as lipophilic extracts of whole-body tissues embedded in alginate pellets were rejected. Because half or more of the internal tissues of *L. uva* may be composed of calcareous support spicules and other inorganic matter (McClintock et al., 1993; Peck, 1993), physical defenses and/or overall low nutritional value could also play a role in unpalatability as has been suggested by Peck (1993, 2001). Calcified cell walls are known to act synergistically with chemical defenses in reducing herbivory on some marine macroalgae (e.g., Hay et al., 1994; Schupp and Paul, 1994). In animals, calcareous sclerites produced by gorgonian corals can act synergistically with chemical defenses against predation but are often ineffective defenses by themselves and are rarely the sole defensive mechanism (van Alstyne et al., 1994; Koh et al., 2000; Puglisi et al., 2002). Siliceous spicules produced by sponges appear to have no defensive roles against a variety of predator types including sea stars (Chanas and Pawlik, 1995, 1996; Waddell and Pawlik, 2000a,b). The potential for physical defenses in *L. uva* that might act in addition to the observed chemical defenses against sea stars remains to be determined. It is of note that the structural integrity of *L. uva* spicules would have been destroyed by our homogenization procedure but that similar treatment usually did not effect the defensive properties of gorgonian sclerites (Puglisi et al., 2002).

Our direct measurements of the caloric contents of *L. uva* soft tissues (13–19 kJ/g dry weight) are somewhat higher than overall contents indirectly estimated from tissue biochemistry by McClintock et al. (1993) and Peck (1993) (9 and 12 kJ/g, respectively). This could potentially be due to differences in the individuals sampled but more likely is because of differences in the technique (cf. Paine, 1971). Regardless, although lower than most invertebrates (Brey et al., 1988), even the lower estimates of *L. uva* caloric contents are comparable to or greater than the contents of organisms in groups such as sponges,
echinoderms, ascidians and macroalgae (Paine, 1964; McClintock, 1986; Brey et al., 1988) that are commonly preyed upon by Antarctic sea stars including *O. validus* (McClintock, 1994).

The presence of chemical defenses in tissues of a shelled animal is probably less surprising in polar waters than in more temperate seas. Polar molluscs commonly have thin and poorly calcified shells (Nicol, 1967; Vermeij, 1978), perhaps due to the relatively high energetic cost of precipitating calcium carbonate in very cold water (Clarke, 1993). *L. uva* is similar to polar molluscs in having a very thin shell and must be handled carefully during collection to prevent breakage (A. Mahon, C. Amsler, personal observations).

The present study of *L. uva* provides an opportunity to evaluate the tenets of the ODT (Rhoads, 1979; reviewed by Cronin, 2001) which assumes that resources are limiting and predicts that chemical and/or physical defenses should be localized in tissues that are most exposed to predators and/or that maximize fitness. Discrete brachiopod body tissues “exposed” to sea star and fish predators in the natural environment include the lophophore, a branched filter feeding appendage that is repeatedly extended beyond the confines of the shell valves then rapidly withdrawn, and the pedicle, a stalk-like structure comprised of tough connective tissue that functions to affix the individual to the substrata.

In the present study, sea stars and fish readily accepted whole lophophores and freeze-dried ground lophophore embedded in artificial foods. In contrast, ground pedicle embedded in artificial foods was unpalatable to both sea stars and fish. These results conform to the predictions of the ODT in that the likelihood of sea stars or fish successfully attacking and consuming the rapidly retractable lophophore is low, while the constantly exposed pedicle is highly vulnerable. Therefore, in the context of ODT, investing defensive resources in the pedicle protects not only the most exposed and vulnerable body component, but is an allocation pattern most likely to maximize fitness, as consumption of the pedicle would result in the dislodgement of the individual from the substrata and subsequent probable mortality.

There was little variation in our measurements of the energetic content of tissues comprising discrete body components in *L. uva*. The pedicle contained the least energy per unit mass compared to the lophophore, reproductively active gametic tissues (gametogenesis is asynchronous in *L. uva*, Meidlinger et al., 1998) and the gut (intestine–stomach). The intact pedicle has a relatively small mass (unpublished data) when compared to other body components (Meidlinger et al., 1998) and would, thus, represent a smaller investment when considering the total allocation of energy to discrete body components. Even if as discussed above, the magnitude of our energetic content measurements is slightly high, the relative differences between components should be correct. In summary, there is no evidence for an increased investment of defenses to those tissues highest in energy content as has been suggested in some marine and terrestrial plants (Simms, 1992; Zangrel and Bazzaz, 1992; Cronin, 2001). Moreover, the allocation of defenses for the purpose of preventing predation upon internal body components seems unlikely as even a partially eaten individual is likely to die. Thus, the lack of palatability observed in sea stars rejecting food pellets containing homogenates of gametic tissues (weakly unpalatable) and intestine (strongly unpalatable) is more likely explained by other selective factors such as chemical defenses against pathogens.
Our analysis of the antimicrobial activity (growth inhibition) of organic extracts of the body components of *L. uva* exposed to four strains of sympatric Antarctic marine bacteria detected potent antibacterial activity in both hydrophilic and hydrophobic extracts of the lophophore and gut (intestine–stomach). Weaker antimicrobial activity was detected in the gonad (ovary) tissues. The presence of potent antimicrobial activity in the lophophore and gut provides compelling evidence that the internal organs least defended against predators, particularly the lophophore, possess bioactive secondary metabolites that provide defense against pathogens. Both the lophophore and gut are among those tissues most exposed to water column borne pathogens and, therefore, most likely to require antimicrobial defenses. Our data indicate that there is not a “cross-reaction” of bioactive compounds whereby the same defensive secondary metabolites serve multiple functions (e.g., see Paul, 1992; Schmitt et al., 1995 and references therein), but rather *L. uva* produces secondary metabolites that specifically provide for either defense against pathogens or macropredators, and that are allocated in such a functional manner to defend the most appropriate body components. We believe this is one of the few studies to date to demonstrate such secondary metabolite target specificity in a marine invertebrate.

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