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Anti-predatory chemical defenses of ascidians: secondary metabolites or inorganic acids?

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Abstract

Both secondary metabolites and inorganic acids have been hypothesized to protect adult ascidians from predation, raising the possibility of alternative defensive strategies in these sessile, soft-bodied, benthic invertebrates. The objective of this investigation was to determine if ascidian species from the Western Atlantic have these chemical defenses against fish predators, and if so, to determine their location within the body of the ascidian. The palatability of crude organic extracts of whole ascidians, as well as the dissected tunics, viscera, and gonads (when possible) were determined at natural volumetric concentrations using laboratory feeding assays with the bluehead wrasse, *Thalassoma bifasciatum*. Acidified food pellets were also assayed to determine the effect of lowered pH on predation. Sixteen of the 17 species tested had deterrent organic extracts from some region of the body (*Aplidium constellatum*, *Aplidium stellatum*, *Ascidia interrupta*, *Ascidia nigra*, *Botrylloides* sp., *Clavellina picta*, *Didemnum candidum*, *Didemnum vanderhosti*, *Diplosoma listerianum*, *Ecteinasci-dia turbinata*, *Eudistoma capsulatum*, *Eudistoma hepaticum*, *Rhopalaea abdominalis*, *Styela plicata*, *Symplegma rubra*, and *Trididemnum solidum*). The location of the deterrent secondary metabolites was isolated in the gonad in all three solitary species, raising the possibility that these defenses are passed on to eggs or larvae. Nine ascidian species sequestered acid in their tunics (*A. interrupta*, *A. nigra*, *A. stellatum*, *D. candidum*, *D. vanderhosti*, *E. capsulatum*, *E. hepaticum*, *R. abdominalis*, and *T. solidum*) at levels that were effective in deterring fish predation ($pH \leq 3.0$). Only one species (*Botrylloides nigrum*) had neither chemical defense. Results of this study indicate that there is not a clear trade-off between the presence of secondary metabolites and inorganic acid defenses in ascidians, suggesting that

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these defenses are redundant, or that alternative chemical defenses may have evolved for different predators or for different stages in the life history of the ascidians producing them.

Keywords: Ascidian; Acid; Chemical defense; Predation; Secondary metabolites; Alternative defenses

1. Introduction

Ascidians are conspicuous members of marine fouling and benthic communities. Their soft-bodied morphology provides ascidians with little obvious structural defense from predation. Spicules, originally thought to provide some anti-predatory defenses for the marine invertebrates that secrete them in their tissues, do not deter fish feeding in assays performed with silicious spicules from sponges (Chanas and Pawlik, 1995, 1996) or calcareous spicules from ascidians (Lindquist et al., 1992). Therefore, chemistry has been implicated in defending ascidians from predators.

Ascidians have been a rich source of marine natural products (reviewed in Faulkner, 2000 and previous reviews cited therein), and the ecological roles of these metabolites have been investigated in a few studies (Paul et al., 1990; McClintock et al., 1991; Vervoort et al., 1998). Some ascidian larvae also possess anti-predatory chemical defenses (Young and Bingham, 1987; Lindquist and Fenical, 1991; Lindquist et al., 1992; Lindquist and Hay, 1995, 1996). Similarities have been noted in the chemistry of adults and larvae (Lindquist et al., 1992), particularly in tropical compound ascidians. But organic compounds are not the only potential defenses that ascidians may use against predation.

Inorganic acids are sequestered by a variety of marine organisms, such as algae, molluscs, and ascidians (Thompson, 1988). Ascidians that sequester acids within their tunic have a lower incidence of predation by gastropods (Young, 1986). Fish feeding assays conducted with pieces of ascidian tunic have demonstrated that species that contain acids are less palatable than those that lack them (Stoecker, 1980a). These assays, however, did not decouple the possible defenses of acids from secondary metabolites that may decrease palatability. Heavy metals, such as vanadium, have also been proposed to provide ascidians with anti-predatory and anti-fouling defenses (Stoecker, 1978, 1980a,b), but the validity of this hypothesis was subsequently disputed (Parry, 1984; Davis and Wright, 1989; reviewed in Pawlik, 1993; however, see Discussion).

While a diversity of unusual secondary metabolites have been isolated from ascidians, the location of the potential chemical defenses within the bodies of the ascidians that produce them is not clear. Localization of chemical defenses to specific regions of an organism is exhibited in terrestrial plants and marine algae (Zangerl and Rutledge, 1996; Dworjanyn et al., 1999; Van Alstyne et al., 1999), and has been observed in some invertebrates, such as sponges (Schupp et al., 1999), gorgonians (Harvell and Fenical., 1989), and molluscs (Pawlik et al., 1988). The allocation of defensive compounds to areas that are most vital for survival and fitness is a component of the optimal defense theory (Rhoades, 1979). With this theory in mind, it would be expected that any given species of ascidian would elaborate either an organic or an inorganic chemical defense, and that this

defense would be localized in body regions that maximize fitness. If fitness would best be served by protecting the adult ascidian, chemical defense of the tunic would be expected. If, however, defense of eggs or larvae would result in greater fitness, it would be expected that acids or metabolites would be associated with the gonads.

Considering the foregoing, we assessed the chemical defenses of 17 Western Atlantic ascidians to answer the following questions: (1) Are these species chemically defended? (2) Is the chemical defense organic (secondary metabolites) or inorganic (acid)? (3) Where are the chemical defenses located within the adult ascidian?

2. Materials and methods

2.1. Specimen collection

Ascidians were collected from four locations representing the warm-temperate to tropical northwestern Atlantic: (1) jetties and floating docks at Wrightsville Beach, NC, USA (34°12.46′ N, 77°47.66′ W) collected from September 1999 to June 2000; (2) North Dry Rocks Reef, Key Largo, FL, USA (25°07.85′ N, 80°17.52′ W) collected May 2000; (3) Sweetings Cay (26°33.05′ N, 77°53.00′ W), Northern Bahamas Islands, collected July 2000; and (4) Little San Salvador Island (24°34.63′ N, 75°57.72′ W), Central Bahamas Islands collected August 2000. Freshly collected specimens were used for the determination of surface and tissue acidity and for extracting lipid soluble organic metabolites. The ascidian species examined are listed as follows, with superscript numbers indicating the collecting sites listed above: *Aplidium constellatum*[1], *Aplidium stellatum*[1], *Ascidia interrupta*[1], *Ascidia nigra*[2], *Botrylloides nigrum*[2], *Botrylloides* sp.[4], *Clavellina picta*[3], *Didemnum candidum*[1], *Didemnum vanderhosti*[3], *Diplosoma listerianum*[1], *Ecteinascidia turbinata*[3], *Eudistoma capsulatum*[2], *Eudistoma hepaticum*[1], *Rhopalaea abdominalis*[4], *Styela plicata*[1], *Symplegma rubra*[1], and *Trididemnum solidum*[4]. Species were identified according to Goodbody (2000) and Van Name (1945) and taxonomic authorities for these species are contained therein.

2.2. Organic extraction

The volume of whole ascidians was determined by liquid displacement; specimens were then extracted twice in a 1:1 mixture of dichloromethane and methanol and once in methanol for 12 h each. The extracts were filtered through celite, dried by rotary evaporation, transferred to vials, and dried to completion on a vacuum concentrator.

In addition to the extraction of whole animals, the tissues of seven species were further dissected, tissues measured by volumetric displacement, and extracted as before. Four species of compound ascidians were dissected to separate the tunic from the visceral mass (zooid + gonad). Three species of solitary ascidians were dissected to separate the tunic, zooid (branchial basket + gut), and gonad. The majority of species collected for this study (10 of 17) were compound or colonial, with very small visceral masses that could not be reliably separated from the tunic; only whole-animal extractions were performed for these species.

2.3. Feeding assays

Assays were performed as described in Pawlik et al. (1995). Whole-animal and tissue extracts were incorporated at natural volumetric concentrations into a food matrix composed of 5.0-g freeze-dried squid mantle, 3.0-g alginic acid, and 100-ml distilled water. The mixture was stirred, loaded into a 10-ml volumetric syringe, and then extruded into a 0.25 M calcium chloride solution to harden. The resulting spaghetti-like strand was cut into 4-mm-long pellets. Control pellets were made in the same manner, but without the addition of extracts. Control and treated pellets were presented to 10 groups of three bluehead wrasses, *Thalassoma bifasciatum*, with each group held in separate partitions of laboratory aquaria. This species is one of the most abundant fishes on the reefs of the Caribbean, as well as a common inhabitant of offshore reefs in North Carolina during summer months, and is a generalist predator of a wide assortment of benthic invertebrates (Randall, 1967). Groups of fish were haphazardly chosen during feeding assays and offered either a treated or control pellet, followed by the other choice. If the second pellet was a treated pellet and rejected by the fish, another control pellet was offered to determine whether the fish had ceased feeding; groups of fish that would not eat control pellets were not used in assays (15–20 groups of fish were available for assays at any given time). A pellet was considered rejected if not eaten after a minimum of three attempts by one or more fish to take it into their mouth cavity, or if the pellet was approached and ignored after one such attempt. The significance of differences in the consumption of treated vs. control pellets was evaluated with the Fisher exact test (Zar, 1998). For any single assay of 10 replicates, an extract was significantly deterrent if four or more of the pellets were rejected ($p \leq 0.043$, one-tailed test); therefore, extracts were considered deterrent if the mean number of pellets eaten was less than or equal to 6. Replicate assays (three or more) were performed for each species (or dissected parts thereof) using specimens collected from geographically distant locations (>1 km) to prevent assaying asexually produced clones.

2.4. Testing acidity

Presence of acids sequestered within the ascidian tunics was determined with analytical pH strips (EM-Reagents 9580, 9581). External and internal pH values were taken for each species. Test strips were accurate to 0.3 pH units, but uncertainty in slight variations of color usually resulted in data being recorded at 0.5 pH unit increments. Three sets of pH values were taken: external values of fresh tunics drained of excess surface water; external values of tunics abraded with a stainless steel dissecting probe using slight pressure (approximately handwriting pressure); and internal values of tunics sliced tangentially to a depth of 1–2 mm with a scalpel. The presence of acid in the visceral masses of ascidians was also determined by grinding them (>5 for compound, one for solitary species) and taking a pH value with analytical strips. At least three specimens from separate collections were used to calculate an average pH value of both tunics and visceral masses.

To assay for the effect of acidity on palatability, food pellets were made from a mixture of 1.5-g freeze-dried squid mantle, 0.75-g Type I carrageenan, and 30.0-ml distilled H_2O. Treatment pellets were acidified with a few drops of 1.0 N sulfuric acid that provided the

desired pH, as indicated with analytical pH test strips. Control pellets were made the same way, but did not contain acid. Thompson (1988) identified sulfuric acid as the acid sequestered in ascidian tunics. This mixture was stirred and microwaved for 45 s. The heated mixture was poured into molds and allowed to harden. Food pellets were cut from the hardened block. Feeding assays were performed as before, but pellet pH was determined with analytical pH strips both prior to assay and after pellets were rejected to assure consistency in pellet acid values. The pH of successive assays was increased within a range starting at pH 1.0 up to pH 7.0. Control pellets had a pH of 7.0.

3. Results

Organic extracts from whole animals or from dissected tissues deterred feeding of *T. bifasciatum* for 16 of 17 ascidian species (Figs. 1–3). Whole-animal extracts were deterrent for all the compound ascidians that could not be further dissected, except for the extract of *B. nigrum* (Fig. 1). For the seven species that were dissected, six yielded palatable extracts when the whole organism was extracted and the extract assayed as a function of the volume of the whole body, but when the viscera, zooid or gonad was assayed alone, these were deterrent (Figs. 2 and 3). For the three solitary ascidian species, organic extracts of tunic tissue were palatable, while extracts of gonads were not (Fig. 3).

Acidity was found only on or inside the tunic for 9 of 17 ascidian species (Table 1); the visceral mass or internal tissues were never acidic. Some species appeared to have acidity

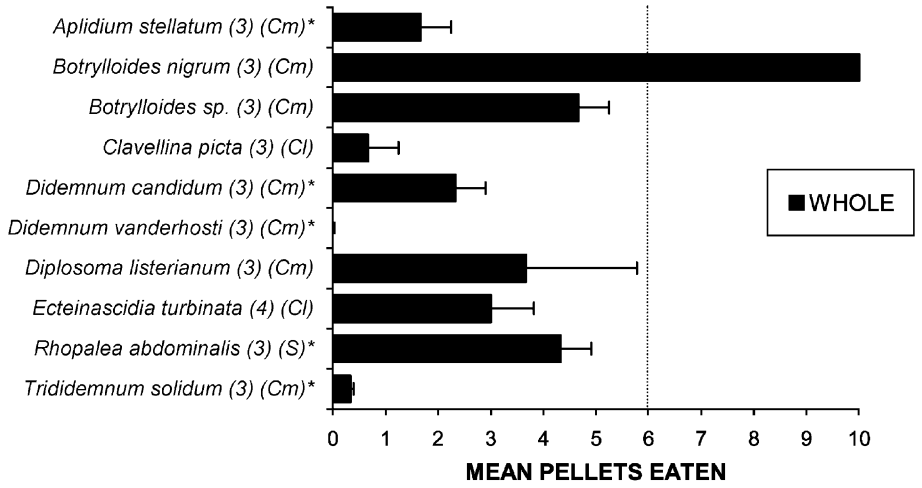

Fig. 1. Consumption by *Thalassoma bifasciatum* of food pellets (mean + SD) containing organic extracts of whole ascidians at natural volumetric concentrations. Fish consumed 10 control pellets in all cases. Following each species name is the number of replicate assays (each of 10 pellets) and an abbreviation indicating the level of coloniality (S = solitary, Cl = colonial, Cm = compound). * indicates tunic surface is strongly acidic (pH ≤ 2.0). For any individual assay, extracts were considered deterrent if the number of pellets eaten was less than or equal to 6 ($p \leq 0.043$, Fisher exact test, one-tailed), as indicated by the dotted line on the graphs.

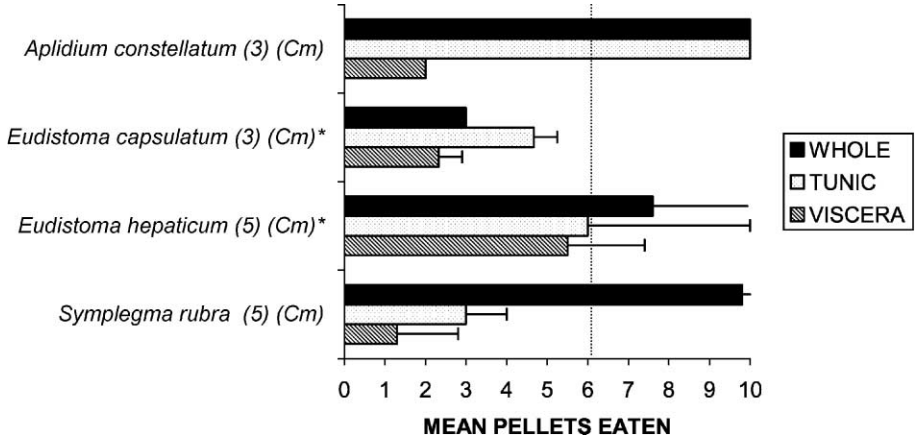

Fig. 2. Consumption by *Thalassoma bifasciatum* of food pellets (mean + SD) containing organic extracts of whole compound ascidians and dissected ascidian tissues at natural volumetric concentrations. Fish consumed 10 control pellets in all cases. Following each species name is the number of replicate assays and an abbreviation indicating the level of coloniality (S = solitary, Cl = colonial, Cm = compound). * indicates tunic surface is strongly acidic (pH ≤ 2.0). For any individual assay, extracts were considered deterrent if the number of pellets eaten was less than or equal to 6 ($p \leq 0.043$, Fisher exact test, one-tailed), as indicated by the dotted line on the graphs.

concentrated on the outside of the tunic (e.g., *A. interrupta*, *A. nigra*), while others had acidity concentrated within the tunic (e.g., *A. stellatum*, *D. vanderhosti*). While pH readings for most species were highly consistent between replicate samples, the drained

Fig. 3. Consumption by *Thalassoma bifasciatum* of food pellets (mean + SD) containing organic extracts of whole solitary ascidians and dissected ascidian tissues at natural volumetric concentrations. Fish consumed 10 control pellets in all cases. Following each species name is the number of replicate assays and an abbreviation indicating the level of coloniality (S = solitary, Cl = colonial, Cm = compound). * indicates tunic surface is strongly acidic (pH ≤ 2.0). For any individual assay, extracts were considered deterrent if the number of pellets eaten was less than or equal to 6 ($p \leq 0.043$, Fisher exact test, one-tailed), as indicated by the dotted line on the graphs.

Table 1
pH values of the tunics of ascidians

Species	Drained	Abraded	Cut
Aplidium constellatum	7.0	7.0	7.0
Aplidium stellatum	7.5	1.5	1.0
Ascidia interrupta	1.5	1.0	6.0
Ascidia nigra	1.0	1.0	7.0
Botrylloides nigrum	7.0	7.0	7.0
Botrylloides sp.	7.0	7.0	7.0
Clavellina picta	7.0	7.0	7.0
Didemnum candidum	1.0	1.0	1.0
Didemnum vanderhosti	5.0	2.0	1.0
Diplosoma listerianum	7.0	7.0	7.0
Ecteinascidia turbinata	7.0	7.0	7.0
Eudistoma capsulatum	ND	1.9	ND
Eudistoma hepaticum	(1.0–7.5)	1.5	1.5
Styela plicata	7.0	7.0	7.0
Symplegma rubra	7.0	7.0	7.0
Rhopalea abdominalis	1.2	2.7	7.0
Tridemnum solidum	1.0	1.0	1.0

Analytical pH strips were applied to the surface of the tunic drained of excess water, to the surface after slight abrasion with a dissecting probe, and to the cut surface after the tunic was sliced with a scalpel. Mean values are shown ($N=3$); there was little or no variance for these means, except for the drained surface of *Eudistoma hepaticum*, for which a range is indicated. ND = no data. Seawater pH = 7.5.

surface of *E. hepaticum* was highly variable, ranging from pH 1.0 to 7.5 (Table 1). This variability was present both between replicate samples, and in different locations on the same sample. The remaining eight tunicate species had tunic pH values of 7.0. Seawater pH using the same analytical test strips was 7.5.

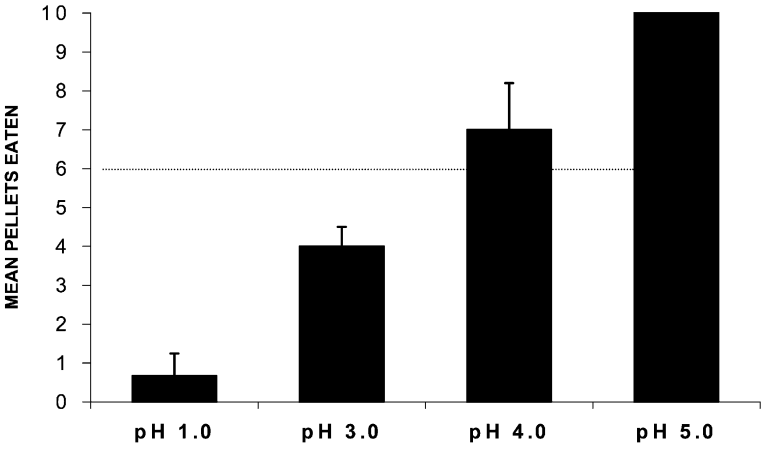

Fig. 4. Consumption by *Thalassoma bifasciatum* of food pellets (mean + SD) acidified with sulfuric acid. Fish consumed 10 control pellets in all cases. Treatments were considered deterrent if the number of pellets eaten was less than or equal to 6 ($p \leq 0.043$, Fisher exact test, one-tailed), as indicated by the dotted line on the graphs. $N=3$ for each pH value.

Food pellets with a pH of ≤ 3.0 deterred feeding by *T. bifasciatum* (Fig. 4). The pH of food pellets was the same immediately after being mouthed and rejected by *T. bifasciatum*.

All nine species that exhibited an acidic (pH $\sim$ 1.0) tunic also yielded deterrent organic extracts of either the whole animal, or of dissected tissues (Figs. 1–3). Seven of these species were colonial or compound, the remaining two species were solitary.

4. Discussion

Inorganic chemical defenses of ascidians were first explored by Stoecker (1978, 1980a,b) before the diversity of organic secondary metabolites in the tissues of these animals was fully realized. Subsequent studies have examined strategies used by ascidians to protect themselves from predation (e.g. Young, 1986; Young and Bingham, 1987; McClintock et al., 1991; Lindquist et al., 1992; Vervoort et al., 1998), but the present study represents the first survey of ascidians in which both the type and the location of chemical defenses were examined using feeding experiments. In our survey, the presence of either secondary metabolites or inorganic acids varied, with several species employing both defensive strategies (Figs. 1–3). Moreover, at least two species had redundant defenses in one tissue, with both inorganic acids and secondary metabolites located within the tunic (Fig. 2). Selective pressures for chemical defenses against predation are likely important in the evolution of ascidians because all but one of the species had one or both anti-predatory chemical defenses.

The redundancy and variability of chemical defenses in tissues of some of the ascidian species examined do not seem to support the theory of optimal defense, even though localization of deterrent chemistry was observed in some species. Assuming large, mobile predators (fish, crabs) are likely to prey on ascidians, chemical defenses would be predicted to exist in the tunic, with one or the other defense elaborated (acid or secondary metabolites), but not both. However, 3 of 17 species had a chemically undefended tunic, and 2 of 17 species had a tunic defended by both acid and organic metabolites. In fact, freshly chopped pieces of the tunic from *S. plicata* were readily consumed by *T. bifasciatum* in laboratory feeding assays (three replicate assays), but like the other solitary species, *S. plicata* concentrated deterrent compounds in the gonads (Fig. 3). Pieces of the tunic from *A. interrupta* were consistently rejected by *T. bifasciatum* (three replicate assays), because this species has an acidic tunic (Fig. 3).

The importance of feeding assays based on tissue volume, as opposed to dry mass or some other measure, is particularly clear in this study. For several species, extracts of whole animals were not deterrent at natural volumetric concentrations, but extracts of the viscera or gonad were strongly deterrent (Figs. 2 and 3). Gonad or viscera may make up only a small portion of the total volume of the whole animal, usually because the tunic is large and gelatinous. If the extract of the tunic is not defended by secondary metabolites, usually the extract of the whole animal is also not deterrent, because the volume of the tunic dilutes any deterrent chemistry that might be found in the much smaller viscera or gonads (e.g., *A. constellatum*, *A. interrupta*, *A. nigra*). Strangely, the whole extract of *S. rubra* was not deterrent, but extracts of both the tunic and viscera inhibited feeding (Fig. 2), a result that cannot be readily explained.

The allocation of defensive chemistry within reproductive tissues has previously been observed in some asteroids and molluscs (Lucas et al., 1979; Pawlik et al., 1988; McClintock and Vernon, 1990; Avila and Paul, 1997). These defenses may reduce predation on the gametes after they have been deposited or released into the water column. In many cases, the tunics of ascidians have very little nutritive value, whereas the bulk of usable protein and lipid is within the visceral mass, including the gonad (Steimle and Terranova, 1985; McClintock et al., 1991). It seems unlikely that defenses concentrated in the gonad would serve to protect an adult solitary ascidian, as opposed to its eggs or larvae, because (1) these localized defenses do not appear to be sufficient to protect the volume of the whole organism (Fig. 3), and (2) a predator would have to open the tunic of a solitary ascidian in order to encounter the localized defense, resulting in the death of the ascidian and providing no advantage to drive the evolution of this defense mechanism. It appears that for the majority of compound and colonial species, levels of defensive metabolites are sufficient, even if they are localized, to protect the volume of the whole organism (Fig. 1). Moreover, for these clonal ascidians, one could imagine that a predator targeting only the gonad could attack and kill a single clone within the colony, but be deterred from further feeding on a colony. This scenario would permit the evolution of chemical defenses localized in reproductive tissues, yet benefiting the adult organism. Moreover, if specialist predators of clonal ascidians target nutritionally rich tissues, multiple defenses may have arisen in some clonal species: acids or secondary metabolites in the tunic to protect the colony from "grazing" predators (fish, crabs), and secondary metabolites in the viscera or gonads to protect from specialists (e.g., polyclad flatworms). The data reported herein may support such a strategy for some species, but clearly not for others. Additionally, there may be alternate functions of acids or of secondary metabolites that have not been considered in the foregoing analysis.

As previously stated, the solitary ascidian *S. plicata* concentrates defensive compounds only in the gonad tissues. When the larvae of *S. plicata* were offered to *T. bifasciatum* they were not eaten (D. Pisut, personal observation), supporting the hypothesis that defensive chemistry in the gonads may be passed on to the larvae. These results, coupled with previous studies that have demonstrated that some ascidian larvae are chemically defended (Young and Bingham, 1987; Lindquist et al., 1992), suggest that there is a potential transfer of deterrent metabolites from the adult gonad to the egg and larva. Although deterrent compounds have not yet been isolated from both the gonads and larvae of any species of ascidian, we might expect that unpalatable adult gonads would yield unpalatable larvae, as most ascidian larvae are lecithitrophic, gleaning the majority of their metabolic requirements from the parent ascidian. Thus, assaying for the palatability of gonad chemistry may serve as a proxy for the palatability of larval chemistry. However, Lindquist and Hay (1996) reported that larvae of *A. constellatum* were not chemically defended, whereas our data indicate that the viscera of this species is defended by secondary metabolites, and neither acids nor unpalatable secondary metabolites were isolated from the tunic (Fig. 2). In this example, the defenses of the adult do not appear to be passed on to the larvae. Unless defense of the viscera could in some way deter more specialized predators, the allocation strategy for this species is unclear, as assays of the whole tissue extract were palatable at natural concentrations (Fig. 2).

If both low pH values and secondary metabolites are effective at deterring generalist fish predators, why produce both? Secondary metabolites may not equally deter all possible predators. Ascidians are preyed upon by an assortment of reef inhabitants, including fish, gastropods, and flatworms. Secondary metabolites that deter generalist fish predators of marine algae are often consumed by mesograzers, such as amphipods and ascoglossan gastropods (Hay, 1992). Also, contact with the ascidian surface may rupture acid vesicles that would deter some crawling predators.

It has been argued that acids would not serve to protect organisms against predation because they would be quickly neutralized by sea water. However, in both the artificial food assay and feeding assays with naturally acidic tunics, low pH values were maintained even after mouthing and rejection of pellets or tunic pieces by *T. bifasciatum*. In fact, strong base was required to effectively neutralize the tunic of *A. interrupta*. Micrographs of the tunic of *A. nigra* revealed that a large area of the upper tunic is composed of vacuolated cells containing sulfuric acid (Hirose, 1999).

In addition to acids, Stoecker (1980a,b) investigated the importance of heavy metals as anti-predatory chemical defenses of ascidians, focusing primarily on vanadium. Feeding assays conducted with fish and crabs yielded a minimum deterrent concentration of vanadyl sulfate or sodium vanadate of ~ 100 μg/g dry mass, which translated to a range of 1250–3333 μg/g wet tissue mass (Stoecker, 1980a; and references cited therein). Stoecker (1980b) found 2 of 35 ascidian species from Bermuda contained wet tissue concentrations of vanadium within this range. Interestingly, one of these species, *A. nigra*, was also surveyed in the present study and was found to contain deterrent secondary metabolites in its zooid and gonad (Fig. 3). Vanadium complexed in ascidian tissues, however, occurs at lower oxidative states than it does in the salts used for feeding assays (Stoecker, 1980a); therefore, the palatability of naturally occurring heavy metal complexes has yet to be established. In any case, the anti-predatory functions of heavy metals such as vanadium were not addressed in the present study and may represent an additional inorganic chemical defense for some ascidian species.

The use of either acids located within the tunic or secondary metabolites distributed in one or several tissues appear to provide most ascidians with defenses against generalist fish predators. Variability in the type and location of these chemical defenses in each of the 17 species examined suggests that either these defenses are redundant, or that alternative chemical defenses may have evolved for different predators or for different stages in the life history of the species producing them.

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References

Avila, C., Paul, V.J., 1997. Chemical ecology of the nudibranch *Glossodoris pallida*: is the location of diet-derived metabolites important for defense? Mar. Ecol.: Prog. Ser. 150, 171–180.

Chanas, B., Pawlik, J.R., 1995. Defenses of Caribbean sponges against predatory reef fish: II. Spicules, tissue toughness, and nutritional quality. Mar. Ecol.: Prog. Ser. 127, 195–211.

Chanas, B., Pawlik, J.R., 1996. Does the skeleton of a sponge provide a defense against predatory reef fish? Oecologia 107, 225–231.

Davis, A.R., Wright, A.E., 1989. Interspecific differences in fouling of two congeneric ascidians (*Eudistoma olivaceum* and *E. capsulatum*): is surface acidity an effective defense? Mar. Biol. 102, 491–497.

Dworjanyn, S.A., De Nys, R., Steinberg, P.D., 1999. Localisation and surface quantification of secondary metabolites in the red alga *Delisea pulchra*. Mar. Biol. 133, 727–736.

Faulkner, D.J., 2000. Marine natural products. Nat. Prod. Rep. 1, 7–55.

Goodbody, I., 2000. Diversity and distribution of ascidians (Tunicata) in the Pelican Cays, Belize. Atoll Res. Bull. 480, 302–326.

Harvell, C.D., Fenical, W., 1989. Chemical and structural defenses of Caribbean gorgonians (*Pseudopterogorgia* spp.): intracolony localization of defense. Limnol. Oceanogr. 34, 382–389.

Hay, M.E., 1992. The role of seaweed chemical defenses in the evolution of feeding specialization and in the mediation of complex interactions. In: Paul, V.J. (Ed.), Ecological Roles of Marine Natural Products. Comstock Press, Ithaca, pp. 93–118.

Hirose, E., 1999. Pigmentation and acid storage in the tunic: protective functions of the tunic cells in the tropical ascidian *Phallusia nigra*. Invert. Biol. 118, 414–422.

Lindquist, N., Fenical, W., 1991. New tambjamine class alkaloids from the marine ascidian *Atapozoa* sp. and its nudibranch predators: origin of the tambjamines in Atapozoa. Experientia 47, 504–506.

Lindquist, N., Hay, M.E., 1995. Can small prey be chemically defended? The case for marine larvae. Ecology 76, 1347–1358.

Lindquist, N., Hay, M.E., 1996. Palatability and chemical defense of marine invertebrate larvae. Ecol. Monogr. 66, 431–450.

Lindquist, N., Hay, M.E., Fenical, W., 1992. Defense of ascidians and their conspicuous larvae: adult vs. larval chemical defenses. Ecol. Monogr. 62, 547–568.

Lucas, J.S., Hart, R.J., Howden, M.E., Salathe, R., 1979. Saponins in eggs and larvae of *Acanthaster planci* (L.) (Asteroidea) as chemical defences against planktivorous fish. J. Exp. Mar. Biol. Ecol. 40, 155–166.

McClintock, J.B., Vernon, J.D., 1990. Chemical defense in the eggs and embryos of antarctic sea stars (Echinodermata). Mar. Biol. 105, 491–495.

McClintock, J.B., Heine, J., Slattery, M., Weston, J., 1991. Biochemical and energetic composition, population biology, and chemical defense of the antarctic ascidian *Cnemidocarpa verrucosa* Lesson. J. Exp. Mar. Biol. Ecol. 147, 163–175.

Parry, D.L., 1984. Chemical properties of the test of ascidians in relation to predation. Mar. Ecol.: Prog. Ser. 17, 279–282.

Paul, V.J., Lindquist, N., Fenical, W., 1990. Chemical defense of the tropical ascidian *Atapazoa* sp. and its nudibranch predators *Nembrotha* spp. Mar. Ecol.: Prog. Ser. 59, 109–118.

Pawlik, J.R., 1993. Marine invertebrate chemical defenses. Chem. Rev. 93, 1911–1922.

Pawlik, J.R., Kernan, M.R., Molinski, T.F., Harper, M.K., Faulkner, D.J., 1988. Defensive chemicals of the Spanish Dancer nudibranch, *Hexabranchus sanguineus*, and its egg ribbons: macrolides derived from a sponge diet. J. Exp. Mar. Biol. Ecol. 119, 99–109.

Pawlik, J.R., Chanas, B., Toonen, R.J., Fenical, W., 1995. Defenses of Caribbean sponges against predatory reef fish: I. Chemical deterrency. Mar. Ecol.: Prog. Ser. 127, 183–194.

Randall, J.E., 1967. Food habits of reef fishes of the West Indies. Stud. Trop. Oceanogr. 5, 665–847.
Rhoades, D.F., 1979. Evolution of plant chemical defenses against herbivores. In: Rosenthal, G.A. (Ed.), Herbivores: Their Interaction with Secondary Plant Metabolites. Academic Press, Orlando, pp. 4–55.
Schupp, P., Eder, C., Paul, V., Proksch, P., 1999. Distribution of secondary metabolites in the sponge *Oceanapia* sp. and its ecological implications. Mar. Biol. 135, 573–580.
Steimle, F.W., Terranova, R.J., 1985. Energy equivalents of marine organisms from the continental shelf of the temperate Northwest Atlantic. J. Northwest Atl. Fish. Sci. 6, 117–124.
Stoecker, D., 1978. Resistance of a tunicate to fouling. Biol. Bull. 155, 615–626.
Stoecker, D., 1980a. Chemical defenses of ascidians against predation. Ecology 61, 1327–1334.
Stoecker, D., 1980b. Relationships between chemical defense and ecology in benthic ascidians. Mar. Ecol.: Prog. Ser. 3, 257–265.
Thompson, T.E., 1988. Acidic allomones in marine organisms. J. Mar. Biol. Assoc. U. K. 68, 499–517.
Van Alstyne, K.L., McCarthy, J.J., Hustead, C.L., Kearns, L.J., 1999. Phlorotannin allocation among tissues of Northeastern Pacific kelps and rockweeds. J. Phycol. 35, 483–492.
Van Name, W.G., 1945. The North and South American ascidians. Bull. Am. Mus. Nat. Hist. 146, 1–476.
Vervoort, H.C., Pawlik, J.R., Fenical, W., 1998. Chemical defense of the Caribbean ascidian *Didemnum conchyliatum*. Mar. Ecol.: Prog. Ser. 164, 221–228.
Young, C.M., 1986. Defenses and refuges: alternative mechanisms of coexistence between a predatory gastropod and its ascidian prey. Mar. Biol. 91, 513–522.
Young, C.M., Bingham, B.L., 1987. Chemical defense and aposematic coloration in larvae of the ascidian *Ecteinascidia turbinata*. Mar. Biol. 96, 539–544.
Zangerl, A.R., Rutledge, C.E., 1996. The probability of attack and patterns of constitutive and induced defense: a test of optimal defense theory. Am. Nat. 147, 599–607.
Zar, J.H., 1998. Biostatistical Analysis, 4th ed. Prentice-Hall, Upper Saddle River, NJ.