

Relationships Between Chemical Defense and Ecology in Benthic Ascidi

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ABSTRACT: Thirty-five species of nearshore, benthic ascidians were collected in Bermuda and notes were made on their habitat, microhabitat, susceptibility to epizoid recruitment, morphology, and acidity. Twenty-four ascidian species were later analyzed for vanadium and iron contents. Ten species had vanadium concentrations of over 100 ppm d. w. (dry weight); three species had vanadium concentrations of over 1000 ppm d. w. Iron contents were independent of vanadium contents. Thirteen species had acidic (pH \leq 2) tunic fluids. Tunic fluid pH was not associated with vanadium contents. Vanadium content was not significantly associated with habitat, microhabitat, susceptibility to epizoid recruitment, or coloniality. Tunic acidity was significantly associated with habitat and lack of epibionts. No acidic species had macroscopic epibionts. Coloniality was significantly associated with lack of epibionts. These findings are discussed in reference to previous experimental work, which has shown that high vanadium contents and acidity are involved in chemical defense in ascidians, and in reference to the role of chemical defenses in sessile, epibenthic, marine invertebrates.

INTRODUCTION

There has been little empirical work on the relationship of chemical defense to the natural history of epibenthic invertebrates. The benthic ascidians are a convenient taxon in which to examine this relationship because ascidians occur in a variety of habitats and microhabitats and include both solitary and colonial species. Many benthic ascidians concentrate transition elements from seawater and produce free sulfuric acid (Webb, 1939, 1956; Carlisle, 1968; Swinehart et al., 1974). Associations between the abilities to concentrate different transition elements and to produce acid have been investigated (Carlisle, 1968; Swinehart et al., 1974) but are still poorly understood.

Until recently little thought had been given to the role of toxic elements and acid as allelochemicals in ascidians. Vanadium, except in very low amounts, is a metabolic poison (NRC, 1974). Sulfuric acid, at a pH of two or less, is destructive to most tissues. Organic allelochemicals are known to be involved in chemical defense of vascular plants and insects against predators, parasites, and competitors (Whittaker and Feeny, 1971); inorganic allelochemicals can have similar

functions in marine invertebrates (Thompson, 1960; Stoecker 1978, 1979, and in press).

Vanadium concentrations of 100 ppm w. w. (wet weight) or greater can reduce the palatability of food to marine fish and crustaceans (Stoecker, 1979, and in press). High concentrations of vanadium in the surface deposit on the tunic of *Phallusia nigra* (= *Ascidia nigra* of several authors) may help prevent fouling (Stoecker, 1978). Acidity is important in repelling marine predators (Thompson, 1960, 1965; Stoecker, 1979, and in press) and is probably also important in preventing fouling of *P. nigra* (Stoecker, 1978, 1979).

The objective of this study was to determine if chemical defenses, in particular high vanadium contents and acidity, are related to habitat, microhabitat, tunic morphology, presence of epibionts, or coloniality in Bermuda ascidians. These relationships are discussed in reference to the evolution and ecological importance of chemical defenses in sessile, epibenthic, marine invertebrates.

METHODS AND MATERIALS

Collection of Specimens and Field Observations

Ascidians were collected during the spring and summer of 1978 in a variety of habitats in Bermuda includ-

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ing sheltered waters (Walsingham Pond and Mangrove Lake), near shore waters (Ferry Reach, Whalebone Bay, Flatts Inlet, Hungry Bay and the Causeway), and reefs (Three-Hills Shoals and North Rock). Specimens were collected at low tide from intertidal and shallow sub-tidal areas in Hungry Bay, Ferry Reach, Whalebone Bay, and along the Causeway. Individuals were collected from depths within a few meters of the surface at Walsingham Pond, Ferry Reach, Three-Hills Shoals and North Rock. Field observations were made on the habitat, microhabitat, and presence and type of macroscopic epibionts on ascidians.

Specimens for identification and for morphological observations were relaxed by adding enough crystals of chloral hydrate or menthol to sea water so that some crystals remained undissolved after two hours (menthol was more effective than chloral hydrate) and preserved in 5 % formaldehyde in seawater. Specimens for chemical analyses were dried at 60 °C and stored in a freezer. Classification and identifications are based on Van Name (1945), C. Monniot (1972a and b) and F. Monniot (1972).

pH Determinations

The pH of the tunic and body tissues of live specimens was determined by inserting a miniature pH probe (Microelectrodes Inc., Londonderry, N. H., USA) into the surfaces or by pressing pH paper (Hydriion comparator paper, 0.5 unit increments) against tunic or body tissues of solitary species and against the surface of colonial species. Determinations of pH were made on at least three specimens of each species (20 to 30 specimens of the more abundant species). The accuracy of the pH paper was checked against standard buffers and against standard pH meters. The pH paper was accurate in distinguishing between pH values less than 2 and greater than 2 (the region of significance in this study). At higher pH, 6–8, the paper was biased toward the acid.

Chemical Analysis

After storage, dried samples were redried at 60 °C to constant weight and then digested in hot concentrated nitric acid in a reflux system. Dissolved samples were analyzed for transition metals using flame atomic absorption spectrometry (Perkin Elmer Model 403). At least three specimens of each species were analyzed for vanadium and iron.

Statistical Methods

Independence or association of properties were statistically tested with RXC tests of independence using the G-statistic (Sokal and Rohlf, 1969). A significant G-statistic requires rejection of the null hypothesis that the two properties are independent (i. e. the two properties are statistically associated). With a non-significant (n. s.) G-statistic the null hypothesis cannot be rejected.

RESULTS

Thirty-five species of ascidians from seven families and three orders were collected in Bermuda (Table 1). *Clavelina picta*, *Ecteinascidia turbinata*, *Phallusia nigra*, and *Polycarpa spongiabilis* (= *Polycarpa objecta* of several authors) were most common in near-shore fouling communities on rock walls, pilings, and rubble. *C. picta* and *P. nigra* were also found at Three-Hills Shoals. *C. picta*, *E. turbinata*, and *P. nigra* were very conspicuous because of their size and coloration (*C. picta* is lavender; *E. turbinata* is orange; and *P. nigra* is black). Although *P. spongiabilis* grows in the same nearshore habitats, it was difficult to locate because of its dull color. This species is camouflaged by epibionts when it grows in non-cryptic microhabitats. *Clavelina oblonga* grows exposed in large masses on mangrove roots and rock walls in Walsingham Pond whereas in other habitats it is usually cryptic and found as smaller colonies. Other species found in great abundance in Walsingham Pond were *Lissoclinum fragile* and *Perophora* spp. In Walsingham Pond *L. fragile* colonies were large and found growing exposed on mangrove roots but in other habitats *L. fragile* colonies were small and found under rubble. Species found primarily on reefs were *Echinoclinium verrilli*, *Trididemnum savignii*, and *Cystodytes dellechiaiei*. Most other species were found most commonly under rocks near shore.

Epibionts

Twenty-one ascidian species were free of macroscopic epibionts; the other thirteen species had epibionts (Table 1). When found in non-cryptic microhabitats, *Polycarpa spongiabilis*, *Microcosmus exasperatus*, and *Pyura vittata* were usually completely covered, excepting the siphons, by a dense growth of epibionts, mostly algae. Species susceptible to epizoic recruitment but with short zooid lifespans, such as *Ecteinascidia turbinata* (Morgan, 1977), were lightly to moderately covered by epibionts. Species with

Table 1. Selected characteristics of Bermuda ascidians

Species	Solitary or colonial	Typical habitat	Typical microhabitat	Presence of epibionts	Tunic characteristics
Order Aplousobranchiata					
Family Polyclinidae					
<i>Aplidium exile</i>	C	Nearshore	Under rocks	0	Moderately firm; sometimes contains sand
<i>Aplidium bermudae</i>	C	Nearshore	Under rocks	0	Tough, hard, cartilaginous; sometimes contains sand
<i>Polyclinum constellatum</i>	C	Very sheltered waters	Rock wall	0	Soft
<i>Pseudodistoma saxicavum</i>	C	Nearshore and shallow reefs	Under rocks	0	Very soft, gelatinous
Family Didemnidae					
<i>Trididemnum savignii</i>	C	Shallow reefs	Under rocks, on coon oysters	0	Tough, firm; contains spicules
<i>Trididemnum</i> sp. (= <i>Didemnopis</i> sp. of several authors)	C	Shallow reefs	On rocks	0	Cartilaginous; no spicules
<i>Diplosoma macdonaldi</i>	C	Nearshore and reefs	Under rocks	0	Very soft, gelatinous
<i>Echinoclinum verrilli</i>	C	Shallow reefs	Crevice, among algae	0	Moderately soft; contains spicules
<i>Polysyncraton amethysteum</i>	C	Nearshore	Under rocks	0	Firm; contains spicules
<i>Lissoclinum fragile</i>	C	Nearshore very sheltered waters	On mangrove roots, under rocks	0	Firm; contains spicules
<i>Didemnum</i> spp.	C	Nearshore and reefs	Under rocks; on coon oysters; on algae	0	Firm; contains spicules
Family Polycitoridae					
<i>Cystodytes dellechiaiei</i>	C	Shallow reefs	Under rocks	0	Firm; contains spicules
<i>Eudistoma clarum</i>	C	Nearshore	Under rocks	0	Very soft; gelatinous
<i>Eudistoma obscuratum</i>	C	Nearshore and shallow reefs	Under and on rocks, on coon oysters	0	Soft
<i>Eudistoma olivaceum</i>	C	Very sheltered waters and nearshore	On or under rocks	+	Firm; sometimes contains sand at base
<i>Eudistoma</i> sp.	C	Nearshore	Under rocks	0	Soft
<i>Distaplia bermudensis</i>	C	Nearshore	Under rocks	0	Soft
<i>Clavelina picta</i>	C	Nearshore shallow reefs	On rocks, pilings, walls	+	Firm; not tough
<i>Clavelina oblonga</i>	C	Very sheltered waters; nearshore	On walls and rocks	+	Firm; not tough
Order Phlebobranchiata					
Family Perophoridae					
<i>Ecteinaiscidia turbinata</i>	C	Nearshore	On walls, pilings, and rocks	+	Thin; delicate
<i>Ecteinaiscidia conklini</i>	C	Nearshore	On and under rocks	+	Thin; delicate
<i>Perophora</i> sp.	C	Very sheltered waters	On rocks, walls, algae, and bryozoans	+	Thin; delicate
Family Ascidiidae					
<i>Ascidia interrupta</i>	S	Nearshore	Under rocks	+	Moderately thick, firm; not tough; coated with mud
<i>Ascidia curvata</i>	S	Nearshore	Under rocks	+	Firm; not tough; sometimes encrusted with sand
<i>Phallusia nigra</i> (= <i>Ascidia nigra</i> of several authors)	S	Nearshore shallow reefs	On pilings, walls, and on and under rocks	0	Thick, firm; not tough
Order Stolidobranchiata					
Family Styelidae					
<i>Botrylloides nigrum</i>	C	Nearshore shallow reefs	Under rocks	0	Firm

Table 1. Continued

Species	Solitary or colonial	Typical habitat	Typical microhabitat	Presence of epibionts	Tunic characteristics
<i>Botryllus planus</i>	C	Nearshore shallow reefs	Under rocks	0	Soft
<i>Symplegma viride</i>	C	Nearshore shallow reefs	Under rocks	0	Very thin
<i>Symplegma rubra</i>	C	Nearshore	Under rocks	0	Very thin
<i>Polyandrocarpa tincta</i>	C	Nearshore	Under rocks	+	Thick; very tough
<i>Polycarpa</i>	S	Nearshore	On pilings, walls, on and under rocks	+	Tough, sometimes encrusted with sand and shell fragments
<i>Spongiabilis obtecta</i> (= <i>Polycarpa obtecta</i> of several authors)					
<i>Styela partita</i>	S	Nearshore	On mangrove roots	+	Tough
Family Pyuridae					
<i>Microcosmus exasperatus</i>	S	Nearshore	Under rocks	+	Thick, very tough; sometimes encrusted with sand
<i>Pyura vittata</i>	S	Nearshore	Under rocks	+	Very tough; sometimes encrusted with sand
<i>Pyura minuta</i>	S	Nearshore	Under rocks	+	Tough; encrusted with sand

C = colonial; S = solitary; + = epibionts present; 0 = epibionts absent

epibionts found in cryptic habitats were primarily colonized by sponges and other ascidians.

Tunic Morphology

Ascidians differ greatly in their tunic characteristics. *Diplosoma macdonaldi*, *Eudistoma clarum* and *Pseudodistoma saxicavum* have large bladder cells and are extremely soft in consistency. All didemnids, except *Diplosoma* and an unidentified *Trididemnum* sp. (= *Didemnopsis* sp. of several authors: Hartmeyer, 1909; Lahille, 1890; Lafargue, 1968), had a gritty consistency due to the presence of spicules in their tunics. The only species with extremely tough, protective tunics were *Aplidium bermudae*, *Polyandrocarpa tincta*, *Microcosmus exasperatus*, and *Pyura vittata*.

Acidity and Metal Content

Determinations of tunic pH were made on all species (Table 2). Ascidians had either extremely acidic (pH ≤ 2) tunic fluids or non-acidic tunic fluids. The body pH values were all greater than 5.0. Acidity was limited to the families Polyclinidae, Didemnidae, Polycitoridae, and Ascidiidae.

Sufficient biomass (approximately 0.05 g dry weight for each determination) of 24 types of ascidians was collected for metal analyses. Vanadium contents greater than 50 ppm d. w. (dry weight) occur in the families Polycitoridae, Perophoridae, and Ascidiidae (Table 2). Vanadium concentrations of over 1000 ppm d. w. occur

in *Distaplia bermudensis*, *Ecteinascidia turbinata*, and *Phallusia nigra*. High vanadium and iron contents were not significantly associated (Table 3). Low pH of tunic fluids was not significantly associated with vanadium contents (Table 4); many species in the Family Didemnidae are extremely acidic but do not have high metal concentrations.

Associations Between Vanadium Content, pH, and Habitat

Habitat type (pond, nearshore, or reef) was independent of vanadium content (Table 5). There were relatively more acidic species in nearshore and reef habitats than in Walsingham Pond (Table 5). In the analysis of the relationship of microhabitat to vanadium content and pH (Table 6), data for Walsingham Pond were excluded because species usually found in cryptic habitats in most nearshore locations (*Clavelina picta*, *Lissoclinum fragile*, *Pseudodistoma saxicavum*, and *P. tincta*) were growing on exposed surfaces at Walsingham Pond. Vanadium content was not significantly associated with microhabitat (Table 6). However, the three species typically found on exposed surfaces, *C. picta*, *Ecteinascidia turbinata*, and *Phallusia nigra*, are relatively rich in vanadium (Table 2). Acidity was not significantly associated with microhabitat.

Lack of epibionts was independent of vanadium contents but was positively associated with acidity (Table 7). No acidic species had macroscopic epibionts. Coloniality was not significantly associated with vanadium content or acidity (Table 8).

Table 2. Acidity and vanadium and iron contents of Bermuda ascidians

Species	pH*	ppm Dry weight \pm S.D.	
		Vanadium	Iron
Order Aplousobranchiata			
Family Polyclinidae			
<i>Aplidium exile</i>	6-8	≤ 10	590 \pm 31
<i>Aplidium bermudae</i>	6-8		
<i>Polyclinium constellatum</i>	6-8	≤ 10	410 \pm 40
<i>Pseudodistoma saxicavum</i>	0-2		
Family Didenmidae			
<i>Trididemnum savignii</i>	1-2		
<i>Trididemnum</i> sp.	0-2	≤ 5	115 \pm 57
<i>Diplosoma macdonaldi</i>	1-2		
<i>Echinoclinum verrilli</i>	1-2		
<i>Polysyncraton amethysteum</i>	1-2	≤ 10	665 \pm 204
<i>Lissoclinum fragile</i>	5-6	≤ 2	1055 \pm 57
<i>Didemnum</i> spp.	1-2	≤ 2	311 \pm 177
Family Polycitoridae			
<i>Cystodytes dellechiaiei</i>	1-2	≤ 10	329 \pm 151
<i>Eudistoma clarum</i>	1-2		
<i>Eudistoma obscuratum</i>	0-2	88 \pm 48	874 \pm 704
<i>Eudistoma olivaceum</i>	5-6	91 \pm 19	627 \pm 102
<i>Eudostoma</i> sp.	1-2	67 \pm 36	1400 \pm 381
<i>Distaplia bermudensis</i>	1-2	3211 \pm 1278	499 \pm 118
<i>Clavelina picta</i>	5-6	194 \pm 55	745 \pm 455
<i>Clavelina oblonga</i>	4-6	114 \pm 19	2496 \pm 421
Order Phlebobranchiata			
Family Perophoridae			
<i>Ecteinascidia turbinata</i>	5-6	1127 \pm 312	555 \pm 155
<i>Ecteinascidia conklini</i>	5-6	923 \pm 541	1788 \pm 1044
<i>Perophora</i> sp.	5-6		
Family Ascidiidae			
<i>Ascidia interrupta</i>	5-6	337 \pm 27	2626 \pm 1408
<i>Ascidia curvata</i>	5-6		
<i>Phallusia nigra</i>	1-2	1979 \pm 628	1213 \pm 906
Order Stolidobranchiata			
Family Styelidae			
<i>Botrylloides nigrum</i>	5-7	≤ 10	689 \pm 69
<i>Botryllus planus</i>	6-7	≤ 10	476 \pm 216
<i>Symplegma viride</i>	6-7	≤ 12	1226 \pm 125
<i>Symplegma rubra</i>	6-7		
<i>Polyandrocarpa tincta</i>	6-7	≤ 3	707 \pm 160
<i>Polycarpa spongiabilis</i>	6-8	≤ 2	8003 \pm 3496
<i>Styela partita</i>	6-7		
Family Pyuridae			
<i>Microcosmus exasperatus</i>	5-6	20 \pm 3	1378 \pm 1077
<i>Pyura vittata</i>	6-8	≤ 10	583 \pm 357
<i>Pyura minuta</i>	6-8		

* pH measurements are of bruised tunics
S.D. = standard deviation

DISCUSSION

Acidity and Vanadium Content of Bermuda Ascidians

The occurrences of acidity in the families Didenmidae, Polycitoridae, Polyclinidae, and Ascidiidae, of high vanadium contents in the families Polycitoridae, Perophoridae, and Ascidiidae, and of high iron contents in several families are in general agreement with

previously reported results (Prenant, 1925; Webb, 1939; Levine, 1961; Ciereszko et al., 1963; and Swinehart et al., 1974). Previously it had been thought that very high vanadium concentrations (over 1000 ppm d.w.) were limited to the families Perophoridae and Ascidiidae (Goodbody, 1974). However, *Distaplia bermudensis* in the Polycitoridae exceeds this threshold (Table 2).

Acidity has usually been thought to be associated

Table 3. Association between iron (Fe) and vanadium (V) contents in Bermuda ascidians

	Number of species		
	V < 50 ppm	V = 50-1000 ppm	V > 1000 ppm
Fe < 500 ppm	5	0	1
Fe = 500-1000 ppm	5	3	1
Fe > 1000 ppm	4	4	1

RXC test of independence using the G-statistic (Sokal and Rohlf, 1969): G = 9.488, n.s. (not significant)

Table 4. Association between pH of tunic fluids and vanadium (V) content in Bermuda ascidians

	Number of species	
	V < 50 ppm	V ≥ 50 ppm
pH ≤ 2	4	4
pH > 2	10	6

RXC test of independence using the G-statistic (Sokal and Rohlf, 1969): G = 3.360, n.s.

Table 5. Association between vanadium content, pH and habitat in Bermuda ascidians

	Number of species			
	V < 50 ppm	V ≥ 50 ppm	pH ≤ 2	pH > 2
Walsingham Pond	6	3	1	10
Nearshore habitats	13	10	10	21
Reef habitats	7	3	9	5

RXC tests of independence using the G-statistic (Sokal and Rohlf, 1969): V content vs habitat, G = 0.650, n.s.; pH vs habitat, G = 9.058, p < 0.025
V = vanadium

Table 6. Association between vanadium content, pH and microhabitats in Bermuda ascidians

	Number of species			
	V < 50 ppm	V ≥ 50 ppm	pH ≤ 2	pH > 2
Cryptic microhabitats	13	7	12	19
Non-cryptic microhabitats	0	3	1	2

RXC tests of independence using the G-statistic (Sokal and Rohlf, 1969): V content vs. microhabitat, G = 5.592, n.s.; pH vs. microhabitat, G = 0.032, n.s.
V = vanadium

Table 7. Association between vanadium content, pH and presence of epibionts in Bermuda ascidians

	Number of species			
	V < 50 ppm	V ≥ 50 ppm	pH ≤ 2	pH > 2
Epibionts	4	6	0	12
No epibionts	10	4	13	10

RXC tests of independence using the G-statistic (Sokal and Rohlf, 1969): V content vs presence or absence of epibionts, G = 2.388, n.s.; tunic pH vs. presence or absence of epibionts, G = 14.688, p < 0.005
V = vanadium

Table 8. Association between vanadium content, pH and coloniality in Bermuda ascidians

	Number of species			
	V < 50 ppm	V ≥ 50 ppm	pH ≤ 2	pH > 2
Solitary	4	2	1	7
Colonial	10	8	12	15

RXC tests of independence using the G-statistic (Sokal and Rohlf, 1969): V content vs. coloniality, G = 0.230, n.s.; tunic pH vs. coloniality, G = 3.040, n.s.
V = vanadium

Table 9. Association between presence of epibionts and coloniality in Bermuda ascidians

	Number of species	
	Epibionts	No epibionts
Solitary	8	1
Colonial	7	19

RXC tests of independence using the G-statistic (Sokal and Rohlf, 1969): G = 11.232, p < 0.005

with ability to concentrate vanadium because both the vanadium chromogen and sulfuric acid are found within the vacuoles of vanadocytes in the families Ascidiidae, and probably also Perophoridae (Webb, 1939; Carlisle, 1968). Swinehart et al. (1974) suggested that one function of vanadium in ascidians is free acid production, but acidity occurs in the Polycitoridae, which have relatively high vanadium contents but lack typical vanadocytes (Swinehart et al., 1974), and in the Didemnidae, which lack both vanadocytes and vanadium.

Analyses for vanadium of replicate subsamples of homogenized, pooled ascidian tissue results in a standard deviation of less than 10 % around the mean. However, standard deviations of analyses of different

individuals are much greater than 10 % of the mean concentration for many ascidian species (Table 2). Several factors may account for the additional variation. At least for *Phallusia nigra*, there is a significant negative correlation between dry weight and vanadium content (Stoecker, 1979). Because most of the vanadium in ascidians is in zooid rather than tunic materials, variations in tunic to body tissue ratios may partially account for differences in vanadium content among different individuals or colonies of the same species (Swinehart et al., 1974). Presence of foreign materials such as micro-epibionts on the ascidians may also have influenced metal determinations.

Some ascidian species may be polymorphic for the ability to concentrate vanadium (Carlisle, 1968). *Distaplia bermudensis* is extremely variable in color (orange, black, red, pink and white, grey and white, and green and white colonies were collected) and in vanadium content (Table 2). Similar variations in color and vanadium content were observed by Swinehart et al. (1974) in *D. occidentalis*. These differences may represent true polymorphisms within species or may be due to differences among unrecognized species.

Defenses Against Epizoic Recruitment

It has been shown experimentally in *Phallusia nigra* that both acidity and high surface concentrations of vanadium may be important in preventing epizoic recruitment (Stoecker, 1978, 1979). Thirteen acidic species are found in Bermuda; none had macroscopic epibionts (Table 7). Acidity appears to be a widespread and important defense against epizoic recruitment in ascidians. *P. nigra* is unusual among high vanadium species in that it has a high surface concentration of vanadium (Stoecker, 1978). High vanadium content by itself does not appear to be important in preventing epizoic recruitment (Table 7). Some species which lack either acidity or a high surface concentration of vanadium are nevertheless free of epibionts. This apparent lack of epizoic recruitment may be due to fast growth rates in some colonial species such as *Symplegma*, to organic allelochemicals, or to mechanical properties of the tunic surface (Goodbody, 1974).

Defenses Against Predation

Both acidity and high vanadium contents (e. g. over about 1000 ppm d. w.) seem to be important defenses against predation in ascidians (Stoecker, 1979, and in press). Three species, *Distaplia bermudensis*, *Ecteinascidia turbinata*, and *Phallusia nigra* (Table 2) have

high enough vanadium contents to reduce their palatability. Acidic species (tunic pH ≤ 2) were rejected by potential predators in laboratory experiments (Stoecker, in press). Some species, such as *Clavelina picta*, may have organic allelochemicals. Other species which have highly palatable bodies, such as *Polycarpa spongiabilis*, *Pyura vittata*, and *Microcosmus exasperatus*, may be partially protected from predation by their tough tunics or camouflage (Stoecker, 1979, and in press).

Relationships Between Vanadium Content, pH, and Habitat

It is interesting that in Walsingham Pond non-acidic species are prevalent and that many ascidians grow on exposed surfaces. Walsingham is a land-locked salt-water pond, surrounded by mangroves, which has underground water exchange with the sea. Physical factors and reduced exposure to browsing and rasping fishes and other marine predators may explain the prevalence of non-acidic species on exposed surfaces in Walsingham Pond. Bakus (1969) has suggested that sponges and ascidians are not consumed by fishes in habitats such as mangrove swamps where the density of rasping fishes is low. Parrot fishes, surgeon fishes, and crabs seem to be uncommon in Walsingham Pond. Physical and chemical factors, such as reduced water turbulence and shading, may also account for the atypical ascidian fauna in Walsingham Pond.

Extremely acidic species and species high in vanadium occurred in cryptic habitats where they would not be normally exposed to fish predation. Chemical defenses may be important to cryptic species because of predation by invertebrates, competition, or epizoic recruitment. We know very little about the influences of invertebrate predators in tropical waters (Bakus, 1970). In Bermuda, as well as in other coral reef environments (Bakus, 1969; Patton, 1976), xanthid crabs and amphinonid polychaetes are common in crevices and under rocks; these invertebrates may be important predators in cryptic microhabitats. Jackson and Buss (1975) originally suggested that allelopathy may be important in cryptic coral reef communities where competition for space may be intense.

Relationships Between Vanadium Content, pH, and Coloniality

Jackson (1977) has predicted that, in general, colonial species should be more resistant to epizoic recruitment, and less palatable to predators than solitary species. High vanadium contents and coloniality are

not closely linked in tunicates (Table 8). The Ascidiidae, which are known for their high vanadium content, are all solitary. Although the association between acidity and coloniality is not statistically significant (Table 8), there does seem to be a tendency for colonial species to be more acidic than solitary species. In Bermuda, in general, colonial species were less palatable to predators than solitary species of ascidians (Stoecker, 1979, and in press). However, Russ (1980) found that the colonial species, *Distaplia viridis* and *Botrylloides nigrum*, were grazed by fish in Australia. In Bermuda, colonial species are in general less susceptible to epizoic recruitment than are solitary species (Table 9).

Evolution and Role of Chemical Defenses

Jackson and Buss (1975) have suggested that many cryptic invertebrates have evolved species-specific allelochemical effects against competitors and that these effects are important in the formation of competitive networks (Buss and Jackson, 1979). However, acidity is analogous to quantitative (Feeny, 1976) or generalized (Levins, 1976) chemical defenses in land plants. In ascidians of both non-cryptic and cryptic microhabitats, generalized defenses may be more important than species-specific defenses. However, some predators and competitors may have evolved defenses against these generalized toxins.

Allelochemicals are thought to be important in the ecology of many algae and benthic invertebrates (Bakus, 1964, 1969, 1970; Sieburth, 1968; Bakus and Green, 1974; Kittredge et al., 1974; Jackson and Buss, 1975; Jackson, 1977). Studies of sponges and holothurians have suggested that fish predation and grazing play an important role in selecting for toxicity in coral reef invertebrates (Bakus, 1964, 1969, 1970; Bakus and Green, 1974; Green, 1977). Bakus (1969) suggests that crypticity may have evolved as a protection against fish predation and that in warm waters most nontoxic shell-less invertebrates live relatively unexposed to fish predation whereas toxic species may live both unexposed and exposed to fish predation and grazing. However, in Bermuda, ascidians with chemical defenses effective against predators (Stoecker 1979, and in press) are found in both cryptic and non-cryptic microhabitats. It is impossible to ascribe the evolution of chemical defenses in ascidians to any one selective factor; fish and invertebrate predation, epizoic recruitment, and competition may all have been important.

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LITERATURE CITED

- Bakus, G. J. (1964). The effects of fish grazing on invertebrate evolution in shallow tropical waters. Occ. Pap. Allan Hancock Fdn 27: 1–29
- Bakus, G. J. (1969). Energetics and feeding in shallow marine waters. Int. Rev. gen. exp. Zool. 4: 275–369
- Bakus, G. J. (1970). An ecological hypothesis for the evolution of toxicity in marine organisms. In: De Vries, A., Kochva, E. (eds) Toxins of animal and plant origin. Gordon and Breach Science Publishers, New York, pp. 57–62
- Bakus, G. J., Green, G. (1974). Toxicity in sponges and holothurians: A geographic pattern. Science, N. Y. 185: 951–953
- Buss, L. W., Jackson, J. B. C. (1979). Competitive networks: Nontransitive competitive relationships in cryptic coral reef environments. Am. Nat. 113: 223–234
- Carlisle, D. B. (1968). Vanadium and other metals in ascidians. Proc. R. Soc. (Series B) 171: 31–42
- Ciereszko, L. S., Ciereszko, E. M., Harris, E. R., Lane, C. A. (1963). Vanadium content of some tunicates. Comp. Biochem. Physiol. 8: 137–140
- Feeny, P. (1976). Plant apparency and chemical defense. Recent Adv. Phytochem. 10: 1–40
- Goodbody, I. (1974). The physiology of ascidians. Adv. mar. Biol. 12: 2–232
- Green, G. (1977). Ecology of toxicity in marine sponges. Mar. Biol. 40: 207–216
- Hartmeyer, R. (1909). Tunicata (Manteltiere). Bronn's Kl. Ordn. Tierreichs 3 (Suppl.): 1281–1773
- Jackson, J. B. C. (1977). Competition on marine hard substrata: the adaptive significance of solitary and colonial strategies. Am. Nat. 111: 743–767
- Jackson, J. B. C., Buss, L. (1975). Allelopathy and spatial competition among coral reef invertebrates. Proc. natl. Acad. Sci. USA 72: 5160–5163
- Kittredge, J. S., Takahashi, F. T., Lindsey, J., Lasker, R. (1974). Chemical signals in the sea: Marine allelochemicals and evolution. Fish. Bull. U.S. 72: 1–11
- Lafargue, F. (1968). Les peuplements sessiles de l'archipel de Glénan. II. Les Didemnidae. Systématique. Ecologie. Vie Milieu 19: 353–446
- Lahille, F. (1890). Recherches sur les Tuniciers des côtes de France, Toulouse
- Levine, E. (1961). Occurrence of titanium, vanadium, chromium, and sulfuric acid in the ascidian *Eudistoma ritteri*. Science, N. Y. 133: 1352–1353
- Levins, D. A. (1976). The chemical defenses of plants to pathogens and herbivores. A. Rev. ecol. Syst. 7: 121–159
- Monniot, C. (1972a). Ascidiés stolidobranches des Bermudes. Bull. Mus. natn. His. nat., Paris 43: 617–743
- Monniot, C. (1972b). Ascidiés phlebobranches des Bermudes. Bull. Mus. natn. His. nat., Paris 61: 939–948

- Monniot, F. (1972). Ascidies aplousobranches des Bermudes. Polyclinidae et Polycitoridae. Bull. Mus. natn. His. nat., Paris 61: 949-962
- Morgan, T. O. (1977). Reproduction, growth, and longevity of three species of West Indian colonial ascidians. Proceedings of the Association of Island Marine Laboratories of the Caribbean 12: 38
- National Research Council, Committee on Biologic Effects of Atmospheric Pollutants. (1974). Vanadium. National Academy of Sciences, Washington, D. C.
- Patton, W. K. (1976). Animal associates of living reef corals. In: Jones, O. A., Endean, R. (eds) Biology and geology of coral reefs, Vol. III, Biology 2. Academic Press, New York, pp. 1-36
- Prenant, M. (1925). Contributions a l'etude cytologique du calcaire II. Sur les conditions de formation des spicules chez les didemnides. Bull. Biol. Fr. Belg. 59: 403-435
- Russ, R. G. (1980). Effects of predation by fishes, competition, and structural complexity of the substratum on the establishment of a marine epifaunal community. J. exp. mar. Biol. Ecol. 42: 55-69
- Sieburth, J. McN. (1968). The influence of algal antibiosis on the ecology of marine microorganisms. In: Droop, M. R., Woods, E. J. F. (eds) Advances in microbiology of the sea, Vol. 1. Academic Press, New York, pp. 63-94
- Sokal, R. R., Rohlf, F. J. (1969). Biometry, W. H. Freeman and Co., San Francisco
- Stoecker, D. (1978). Resistance of a tunicate to fouling. Biol. Bull. mar. biol. Lab., Woods Hole 155: 615-626
- Stoecker, D. (1979). The ecological roles of acid and vanadium in ascidians. Ph. D. thesis, State University of New York, Stony Brook
- Stoecker, D. (in press). Chemical defenses of ascidians against predators. Ecology
- Swinehart, J. H., Biggs, W. R., Halko, D. J., Schroeder, N. C. (1974). The vanadium and selected metal contents of some ascidians. Biol. Bull. mar. biol. Lab., Woods Hole 146: 302-312
- Thompson, T. E. (1960). Defensive adaptations in opisthobranchs. J. mar. biol. Ass., U. K. 39: 125-134
- Thomson, T. E. (1965). Epidermal acid secretion in some marine polyclad turbellaria. Nature, Lond. 206: 954-955
- Van Name, W. G. (1945). The North and South American ascidians. Bull. Am. Mus. nat. Hist. 84: 1-476
- Webb, D. A. (1939). Observations on the blood of certain ascidians, with special reference to the biochemistry of vanadium. J. exp. Biol. 16: 499-523
- Webb, D. A. (1956). The blood of tunicates and the biochemistry of vanadium. Naples Stazioni Zoologica, 28: 273-288
- Whittaker, R. H., Feeny, P. P. (1971). Allelochemicals: Chemical interactions between species. Science, N. Y. 171: 757-770

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