

Chemical properties of the test of ascidians in relation to predation

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ABSTRACT: The undamaged test of a range of aplousobranch ascidian species is shown to be chemically neutral. Acid is generated by surface test cells following cell lysis and is rapidly neutralised in the presence of calcareous test inclusions and/or sea water. Neither the capacity to produce acid nor the presence of vanadium prevents predation on ascidian species in the field.

INTRODUCTION

Stoecker (1980a, b, c) has proposed that low intracellular pH in the tunic fluids and high vanadium content of some aplousobranch and phlebobranch ascidians might prevent or inhibit predation. Intercellular acidity of ascidian tests has been observed in connection with investigations on *Prochloron* symbionts in the family Didemnidae which are either embedded in the test, or contained in the common cloacal cavity or loosely associated on the surface (Thin and Griffiths, 1977; Thorne et al., 1977). All symbionts, but especially those embedded in the test, would be subjected to a low pH if reports of intercellular acidity in the ascidian test are accurate. Attempts to culture *Prochloron* at low pH (Patterson and Withers, 1982) did not survive more than 3 generations (G. M. L. Patterson, pers. comm.). More compelling evidence that challenges the view that low intercellular pH is a normal property of the test of living aplousobranch ascidians is the fact that many of the living species in which it has been reported contain calcareous spicules in the test.

Further, the hypothesis that vanadium inhibits predation is not applicable to all members of the class. High concentrations of vanadium are present in Phlebobranch species but it is not commonly found in appreciable amounts in Aplousobranchia and is not found at all in the Stolidobranchia (Hawkins et al., 1983a).

The following report sets out investigations on the pH of ascidian tests and assessments of vanadium content and these are correlated with field observations on ascidian predation.

METHODS AND RESULTS

All species were collected from cryptic habitats just below low water mark at Heron Island, Great Barrier Reef.

Species tested: Family Polycitoridae: *Eudistoma amplus*, *Eudistoma* sp., *Cystodytes dellechiaiei*; Family Polyclinidae: *Aplidium* sp., *Polyclinum macrophyllum*; Family Didemnidae: *Didemnum molle*, *D. sphaericum*, *D. digestum*, *D. psammatoles*, *D. membranaceum*, *D. aff. chartaceum*, *D. proliferum*, *D. viride*, *Trididemnum* sp., *T. clinides*, *T. miniatum*, *T. cyclops*, *T. paracyclops*, *T. cerebriforme*, *Lissoclinum voeltzkowi*, *L. bistratum*, *L. patella*, *L. punctatum*, *L. ostrearium*, *L. fragile*, *Echinoclinum triangulum*, *Diplosoma virens*, *D. similis*, *D. listerianum*, *Leptoclinides* sp., *L. lissus*, *L. reticulatus*.

A range of pH indicator stains (neutral red, methyl red, bromocresol green and bromophenol blue) added to living colonies in sea water show that no part of the whole undamaged colonies are acid and pH was 6.5 to 7.0 in all cases. However, lysis of the external bladder cells with a probe produces acid at pH \leq 3.5 in the majority of species tested (Table 1). These measurements were made on at least 6 individuals of each species. In species in which the spicules are so crowded that the bladder cell layer is occluded, there was no acid reaction: *Lissoclinum bistratum*, *Didemnum sphaericum*, *D. proliferum*, *D. psammatoles*, *D. membranaceum*. The reaction seems not to be homologous with the reaction that generates a low pH on lysis of the blood cells of phlebobranch species (Hawkins et al., 1983b). The presence of calcareous

Table 1. Predators, vanadium content and pH of ascidians

Ascidian species	Predator	pH ^{1,a}	Vanadium ^{1,a} (ppm dry weight)	Latitudinal ^b Zone	Reference
<i>Leptoclinides</i> sp.	<i>Chromodoris</i> sp.				
	<i>Lamellaria</i> sp.	2.0	10000	TR	1
<i>Leptoclinides reticulatus</i>	<i>Turbo crassus</i>	< 3.5	10000	TR	1
<i>Eudistoma</i> sp.	<i>Trochus niloticus</i>	< 3.5	1200	TR	1
<i>E. amplus</i>	<i>T. niloticus</i>	< 3.5	880	TR	1
<i>Polyclinum macrophyllum</i>	<i>T. niloticus</i>	7.0	< 20	TR	1
<i>Lissoclinum ostrearium</i>	<i>T. niloticus</i>	< 3.5	< 20	TR	1
<i>Didemnum membranaceum</i>	<i>Cypraea</i> sp.				
	<i>Lamellaria</i> sp.	7.0	< 20	TR	1
<i>Cystodytes dellechiaiei</i>	<i>Cypraea</i> sp.	2.0	< 20	TR	1
<i>Atriolum</i> sp.	Cymatid mollusc	< 3.5	< 20	TE	2
Starfish					
<i>Amaroucium</i> spp.	<i>Mediaster aequalis</i>				
	<i>Pteraster tesselatus</i>				
	<i>Dermasterias imbricata</i>	(< 3.5)	–	TE	3
<i>Didemnum</i> spp.	<i>Pteraster tesselatus</i>				
	<i>Dermasterias imbricata</i>	(< 3.5)	(< 20)	TE	3
<i>Eudistoma psammion</i>	<i>Dermasterias imbricata</i>	≅ 2.0 ¹²	> 100 ¹²	TE	3
<i>Corella</i> spp.	<i>Solaster endeca</i>				
	<i>Mediaster aequalis</i>	–	< 20 ¹⁰	TE	3
<i>Pyura haustor</i>	<i>Solaster stimpsoni</i>				
	<i>Evasterias troschelii</i>	(7.0)	< 20 ¹⁰	TE	3
<i>Chelyosoma productum</i>	<i>Evasterias troschelii</i>	(< 3.5)	800 ¹¹	TE	3
Molluscs					
<i>Didemnum moseleyi</i>	<i>Hexabranhus sanguineus</i>	< 3.5 ¹	< 20 ¹	TR	4
<i>Diplosoma</i> sp.	<i>Trivia</i> sp.	(< 3.0)	(< 20)	TE	5,6
<i>Trididemnum</i> sp.	<i>Trivia monacha</i>				
	<i>T. arctica</i>	(< 3.5)	(< 20)	TE	6
<i>Polyclinum</i> sp.	<i>T. monacha</i>				
	<i>T. arctica</i>	7.0	< 20	TE	6
<i>Didemnum</i> sp.	<i>Lamellaria perspicua</i>	(< 3.5)	(< 20)	TE	6
<i>Phallusia</i> sp.	<i>Velutina velutina</i>	< 3.0 ¹	> 1000 ¹⁰	TE	6
<i>Ascidia mentula</i>	<i>Pleurobranchus membranaceus</i>	(< 3.0)	> 1000 ¹³	TE	7
<i>Diplosoma listerianum</i>	<i>Goniodoris nodosus</i>				
	<i>G. castanea</i>				
	<i>Ancula cristata</i>	< 3.0 ¹	< 20 ¹	TE	8
<i>Botryllus schlosseri</i>	<i>Goniodoris nodosus</i>				
	<i>G. castanea</i>				
	<i>Ancula cristata</i>				
	<i>Erato voluta</i>	7.0 ¹	< 20 ¹	–	5,8
Fish					
<i>Distaplia viridis</i>	<i>Penicipelta vittiger</i>	(< 3.5)	> 1000	TE	9
<i>Botrylloides</i> sp.	<i>P. vittiger</i>	(7.0)	(< 20)	TE	9

^a Damaged colonies, values in parentheses extrapolated from this work
^b TR, tropical; TE, temperate
¹ This work
² Kott, pers. comm.
³ Mauzey et al. (1968)
⁴ Thompson (1970)
⁵ Purchon (1977)
⁶ Fretter and Graham (1962)
⁷ Thompson and Slinn (1959)
⁸ Millar (1971)
⁹ Russ (1980)
¹⁰ Hawkins et al. (1983a)
¹¹ Danskin (1978)
¹² Swinehart et al. (1974)
¹³ Ciereszko et al. (1963)

spicules causes extremely rapid neutralisation of the acid generated. However, even without spicules, as in *Diplosoma* spp., sea water is sufficient buffer to neutralise the acid generated on cell lysis. Six colonies of

Diplosoma virens crushed in only 15 ml of sea water produced a pH 7.0.

The concentrations of vanadium were < 20 ppm dry weight in all species tested, except *Leptoclinides* spp.

in which the concentration is 10,000 ppm dry weight and *Eudistoma* spp. with 880 ppm dry weight. At least 4 individuals of each species were analysed for vanadium. The vanadium was determined by flame atomic absorption using either a Varian AA6 or Varian 875 spectrometer. In the determination of vanadium there is a large matrix effect which is corrected by the method of standard additions.

Results of field observations are presented in Table 1, together with other reports of predation from the literature.

DISCUSSION

Diverse communities of encrusting ascidians on the under surface of rubble behind the reef crest at Heron Island (Great Barrier Reef) are grazed by a number of species of molluscs, in particular, *Trochus niloticus* (Fig. 1). Some boulders may have up to 20 species of ascidians, many with acid producing cells, some with high vanadium and some with both; yet some of these boulders are completely denuded by *T. niloticus*, *Cypraea* sp. and many other species of molluscs.

There are many predators on ascidians ranging from molluscs to fishes (Table 1). Many molluscs feed on a wide range of ascidians: from stolidobranch ascidians, which have no vanadium and produce no acid, to Phlebobranchs and Aplousobranchs which have high vanadium content and produce acid. Molluscs are more frequently recorded predators – due to being less

active and spending more time in attacking their prey – and therefore are more readily observed. Only few ascidians have parts, calcareous spicules and in some the test, which sufficiently withstand digestion to facilitate identification. It is therefore not surprising that there are few records of ascidians forming part of the diet of fish and this may be in part the reason they are commonly regarded as distasteful (Millar, 1971). There are reports of fish predation; but they do not in all cases indicate the species of ascidians eaten (Randall and Hartman, 1968; Millar, 1971).

Nevertheless, *Trochus niloticus* was observed to feed on a wide range of ascidian species in the field; aquarium feeding experiments with *T. niloticus* over a period of 3 wk were unsuccessful (see also Mauzey, 1968).

The absence of epibionts on the surface of colonial aplousobranch ascidians may be a result of the ability of these surface cells to generate acid when damaged, as would occur when an epiphyte attempted to attach itself to the surface. However, the colonial stolidobranchs – *Botrylloides nigrum*, *B. magniceocus*, *Botryllus schlosseri* and *B. primigenus* – have no acid producing cells and no vanadium, yet they are also free of epibionts.

CONCLUSION

The internal environment of ascidians is never acid and *Prochloron* and other commensal organisms in the



Fig. 1 *Trochus niloticus* feeding on *Eudistoma* sp., Heron Island (Great Barrier Reef). Photograph: Professor J. H. Swinehart

test and in the common cloaca of these colonial organisms occupy a neutral environment. The rapid neutralisation of acid generated on cell lysis appears to preclude its suggested role as a defence against predation. Further, it appears to be very doubtful that the capacity to concentrate vanadium to a high level is a mechanism of chemical defense.

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