Chemical Ecology of Benthic Invertebrates

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Methods in Marine Chemical Ecology
Tjärno Marine Biological Laboratory
Strömstad, Sweden   9-14 Sep 2006


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Pacific Ocean
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HAWAII

DEFINITIONS:

Marine natural products = organic metabolites isolated from marine organisms

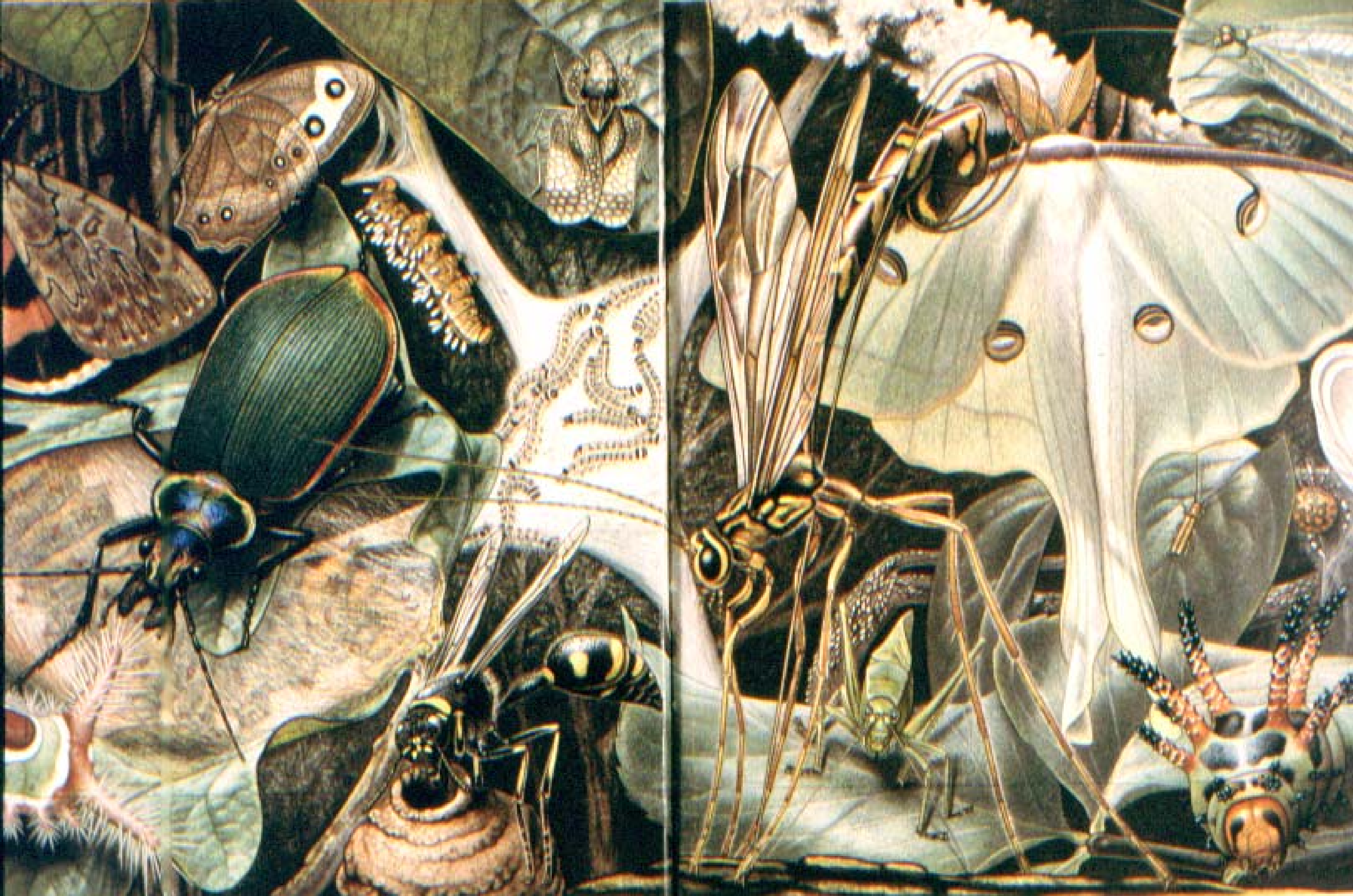
Secondary metabolites = metabolites not known to be involved in primary metabolism

Marine chemical ecology = study of the influence of secondary metabolites on the distribution and abundance of marine organisms





Terrestrial natural products chemistry




1960s – birth of marine natural products chemistry

1970s – peak isolation of non-polar metabolites

1980s – isolation work slows, diversification
birth of marine chemical ecology

1990s – isolation of polar metabolites
further multidisciplinary diversification

Categories of Marine Natural Products

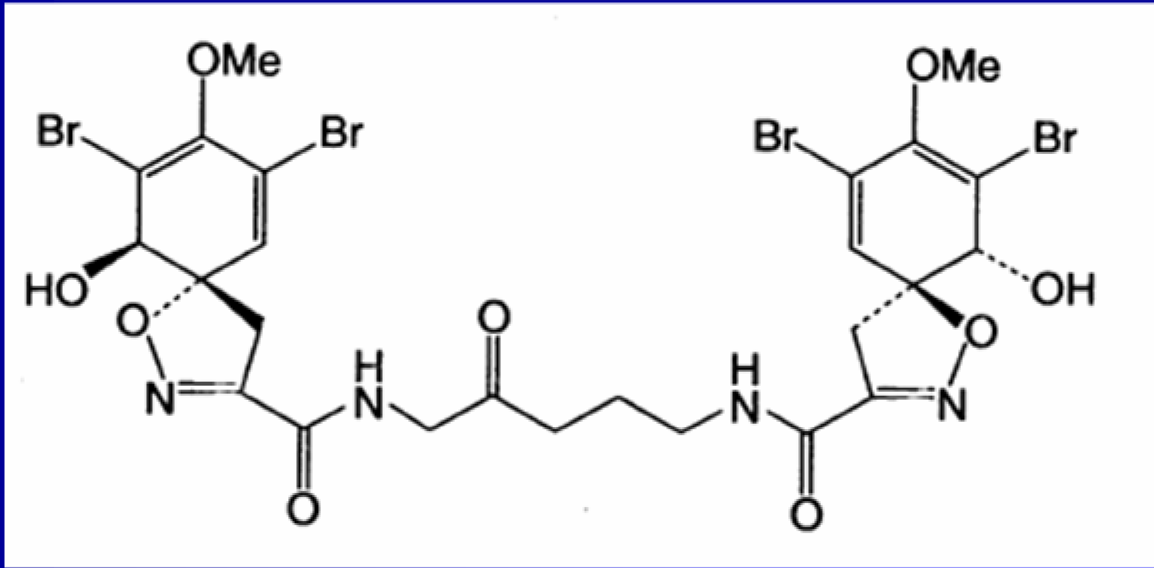
Biosynthetic Pathway	Structural Classes
Isoprenoid	Terpenes
	Steroids
	Carotenoids
	Prenylated quinones and hydroquinones
Acetogenin	Polyketides
	Polyphenols
	Fatty acids
	Prostaglandins
Amino Acid	Peptides (including cyclic)
	Alkaloids
Shikimate	Cinnamic acid derivatives
	Flavonoids
	Coumarins
Nucleic Acid	Nucleic acids
	Nucleo bases
Carbohydrate	Sugars
	Polysaccharides

Summary of Phylogenetic Distribution of Secondary Metabolites from Marine Organisms

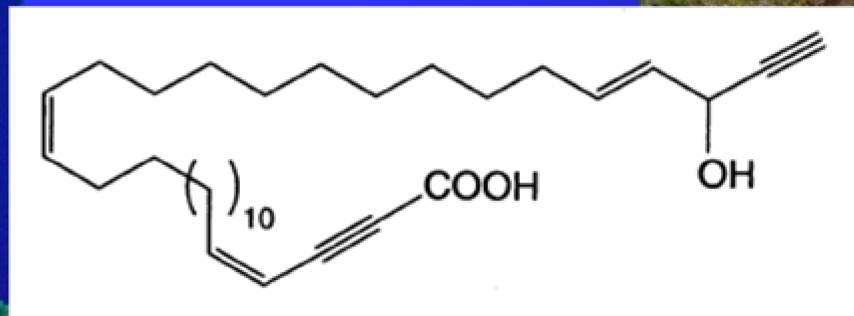
Phylum	Total	Isoprenoid	Acetogenin	Amino Acid	Shikimate	Nucleic Acid	Carbohydrate
Plants							
Seagrasses	12	1 (8)	1 (8)	0	8 (67)	0	2 (17)
Chlorophyta	177	109 (62)	30 (17)	24 (14)	8 (4)	0	6 (3)
Rhodophyta	938	429 (46)	356 (38)	89 (9)	45 (5)	1 (<1)	19 (2)
Phaeophyta	759	513 (68)	116 (15)	15 (2)	91 (12)	0	24 (3)
Subtotal	1886	1052 (56)	503 (27)	128 (7)	152 (8)	1 (<1)	51 (3)
Invertebrates							
Porifera	2609	1295 (50)	574 (22)	681 (26)	37 (1)	19 (<1)	3 (<1)
Cnidaria	1499	1236 (82)	142 (9)	112 (7)	4 (<1)	4 (<1)	1 (<1)
Ctenophora	0	0	0	0	0	0	0
Platyhelminthes	12	0	2 (17)	10 (83)	0	0	0
Nemertea	0	0	0	0	0	0	0
Nematoda	2	1 (50)	0	1 (50)	0	0	0
Sipuncula	0	0	0	0	0	0	0
Echiura	0	0	0	0	0	0	0
Mollusca	575	310 (54)	138 (24)	104 (18)	13 (2)	2 (<1)	8 (1)
Annelida	28	0	6 (21)	16 (57)	5 (18)	0	1 (4)
Arthropoda	28	2 (7)	1 (4)	25 (89)	0	0	0
Echinodermata	464	317 (68)	73 (16)	25 (5)	31 (7)	0	18 (4)
Brachiopoda	0	0	0	0	0	0	0
Ectoprocta	102	5 (5)	29 (28)	65 (64)	0	3 (3)	0
Hemichordata	22	8 (36)	2 (9)	12 (55)	0	0	0
Urochordata	452	73 (16)	71 (16)	299 (66)	6 (1)	3 (<1)	0
Subtotal	5793	3247 (56)	1038 (18)	1350 (23)	96 (2)	31 (1)	31 (1)

Note: Numbers in parentheses represent percentages.

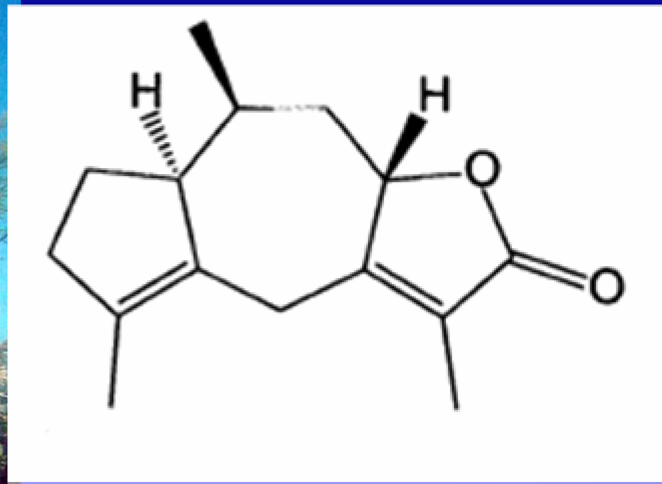
Not included: bacteria and fungi



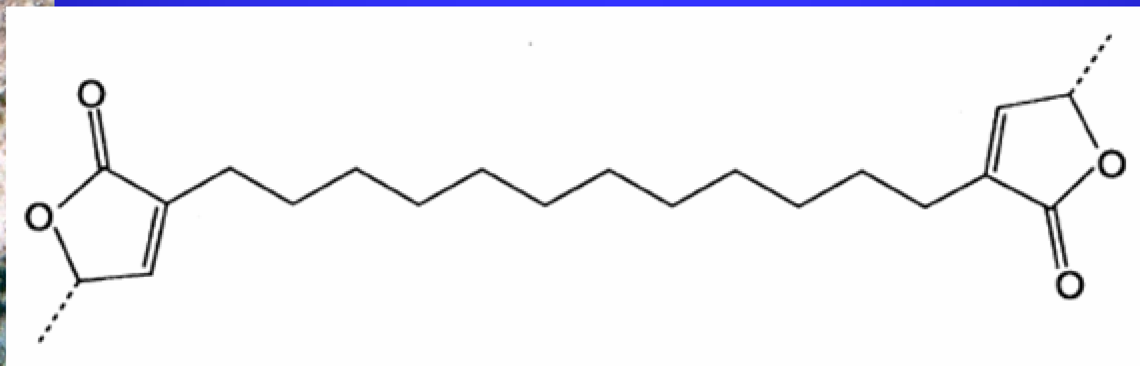
Oxohomoaerothionin – *Aplysina cavernicola*



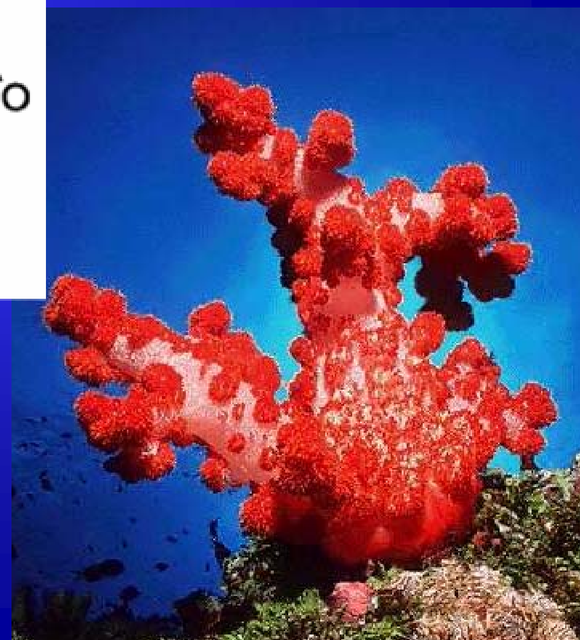
Pellynic acid – *Pellina triangulata*

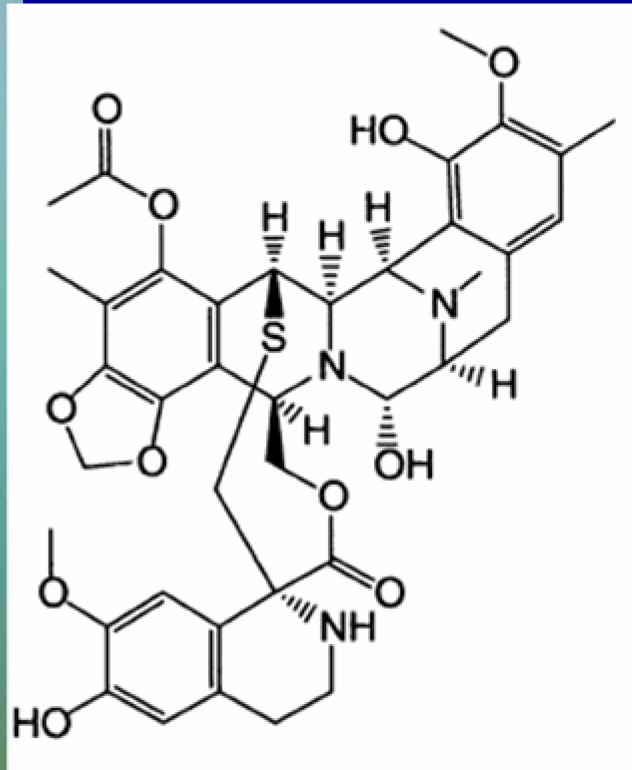


Americanolide F – *Pseudopterogorgia americana*

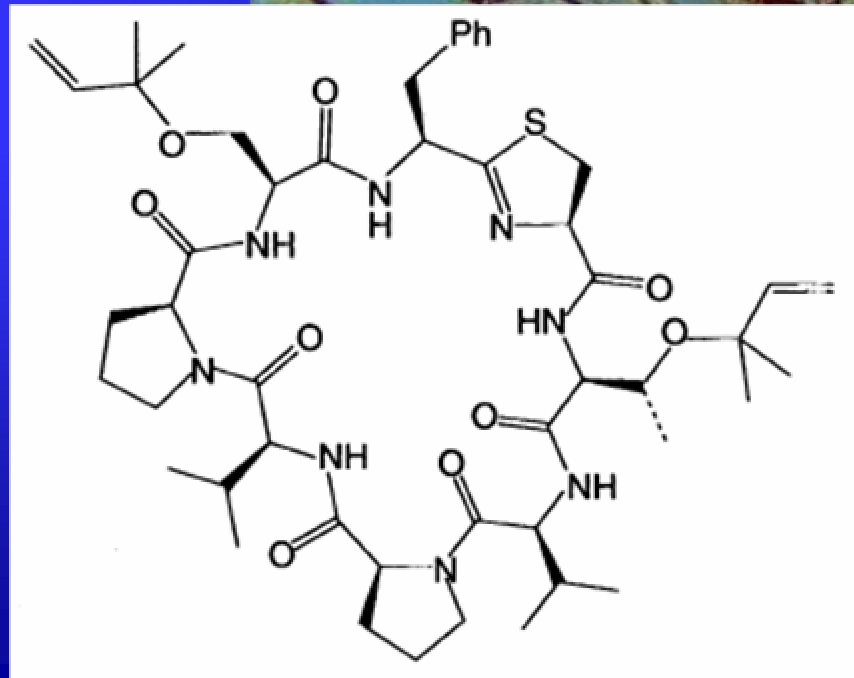


Ancepsenolide – *Pterogorgia anceps*





Ecteinascidin – *Ecteinascidia turbinata*



Meridine – *Amphicarpa meridiana*

INTERDISCIPLINARY SCIENCE:

- Synergistic
- Interests differ:

    chemist : molecular structure, synthesis

    pharmacologist : method of action

    ecologist : ecological function, evolution

Marine chemical ecology:

- Ecological theory from terrestrial ecology
- Follows "scientific method" of experimentation
  - -construct a null hypothesis and falsify
  - -multiple working hypotheses
- Tends to *assume* adaptive function of secondary metabolites based on "cost"

THEORY:

- Resources are partitioned: maintenance, acquisition, growth, reproduction, *defense*
- Resources are finite
- Natural selection results in *optimization*

TYPES OF CHEMICAL DEFENSES:

- Anti-predatory
- Anti-overgrowth (allelopathic)
- Antifouling
- Anti-pathogen
- UV radiation protection (sunscreens)

ANTI-PREDATOR DEFENSE:

- Behavioral (run away, hide)
- Associational (live among spines)
- Structural (physical - bear spines)
- Nutritional (make yourself poor food)
- Chemical (taste bad)

ANTI-PREDATORY CHEMICAL DEFENSES

Pre-1980s: Assessed by correlation or toxicity studies

Subsequent: Development of more ecologically relevant bioassays

- **Feeding assays**
- **Realistic consumers**
- **Natural concentrations of metabolites**

SCIENCE, VOL. 211, 30 JANUARY 1981

Chemical Defense Mechanisms on the Great Barrier Reef, Australia

GERALD J. BAKUS
Allan Hancock Foundation, University of Southern California, Los Angeles 90007

Abstract. *Seventy-three percent of all exposed common coral reef invertebrates, from four phyla (42 species) tested, are toxic to fish. This represents the first evidence of the high incidence of toxicity in the marine tropics among several phyletic groups comprising dominant species. Most of the remaining exposed species have structural defense mechanisms against predation by fish. Of cryptic invertebrates, 25 percent are toxic to fish. The relation between toxicity, fish feeding behavior, community structure, and evolutionary theory is discussed.*

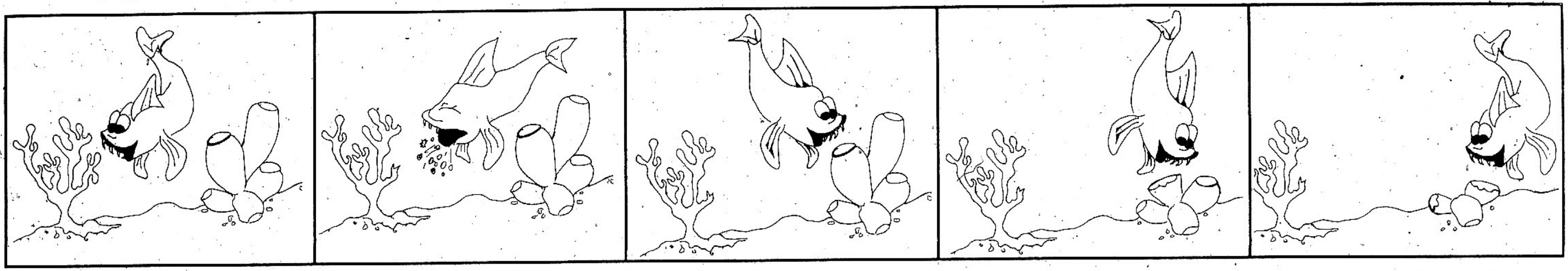
Goldfish toxicity assays to assess chemical defenses using tissue homogenates

Table 1. Bioassays of the toxicity to fish of common coral reef animals from Lizard Island, Great Barrier Reef, Australia. A total of 42 frozen species were tested in Sydney; 73 percent of all exposed organisms tested were toxic to goldfish. By exposed is meant that part of the body is exposed to fish or that the animal is exposed to fish during part of the day.

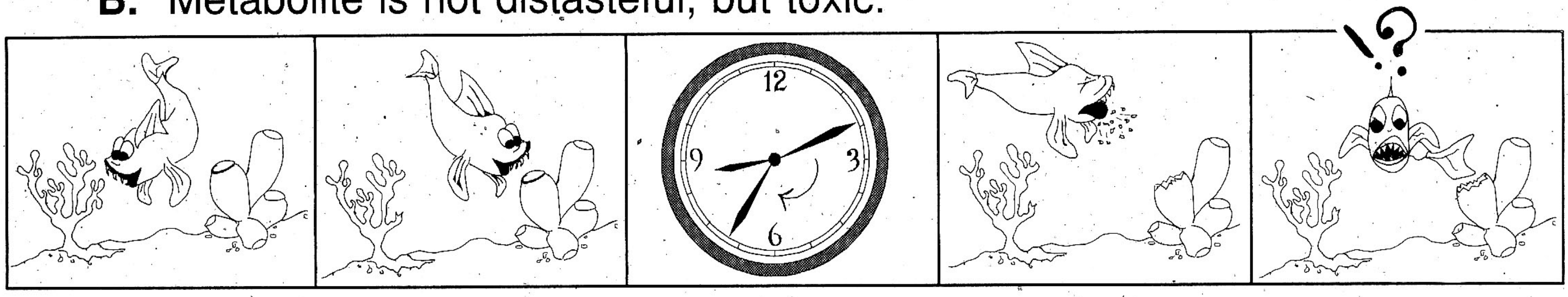
Taxonomic group	Number of species				
	Tested	Exposed		Cryptic	
		Toxic	Non-toxic	Toxic	Non-toxic
Sponges	16	6 (60)*	4	2	4
Soft corals	8	7 (88)	1		
Gorgonians	2	2 (100)			
Asteroids	1		1		
Crinoids	1	1 (100)			
Holothurians	12	6 (100)			6
Ascidians	2		2		

*Numbers in parentheses represent the percent of the total exposed.

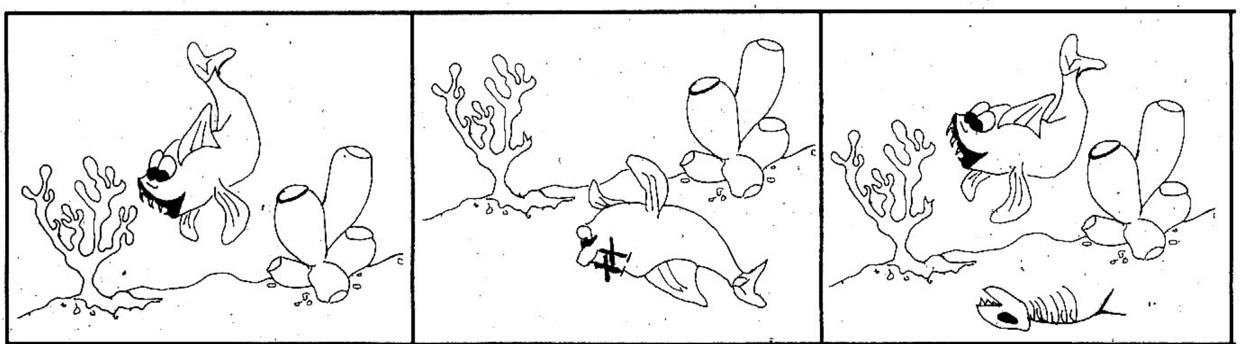
A. Metabolite is distasteful.



B. Metabolite is not distasteful, but toxic.

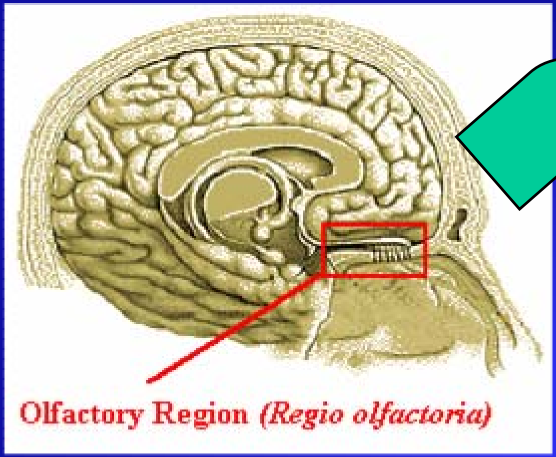


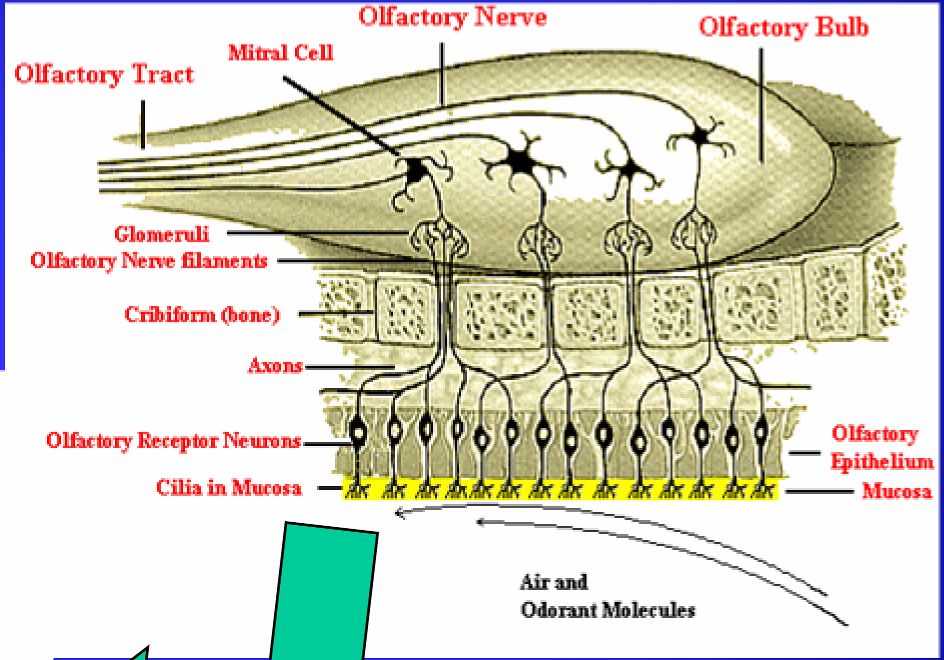
C. Metabolite is extremely toxic.

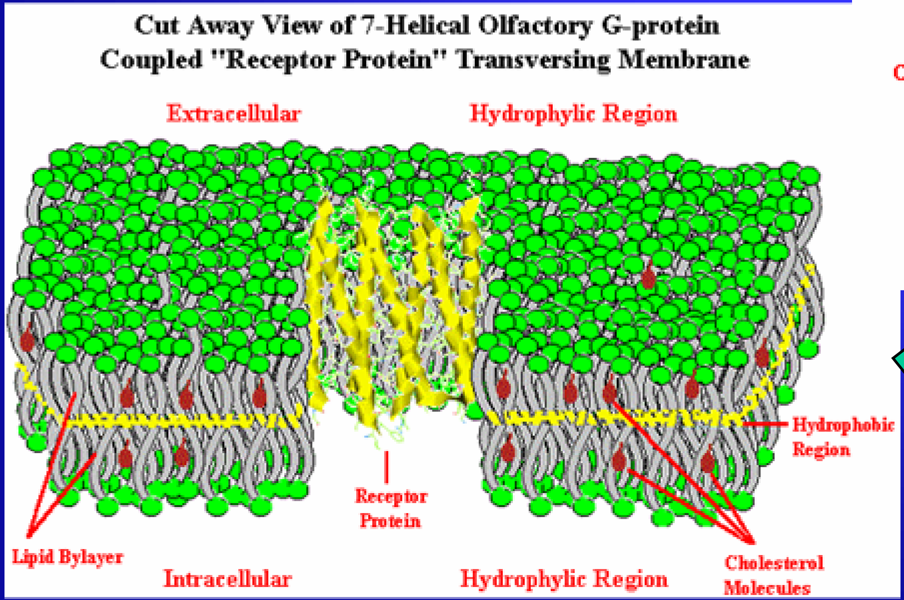


"Learned avoidance" by predator only occurs in (A): selection for distastefulness, not toxicity. Visual predator learns when very young.

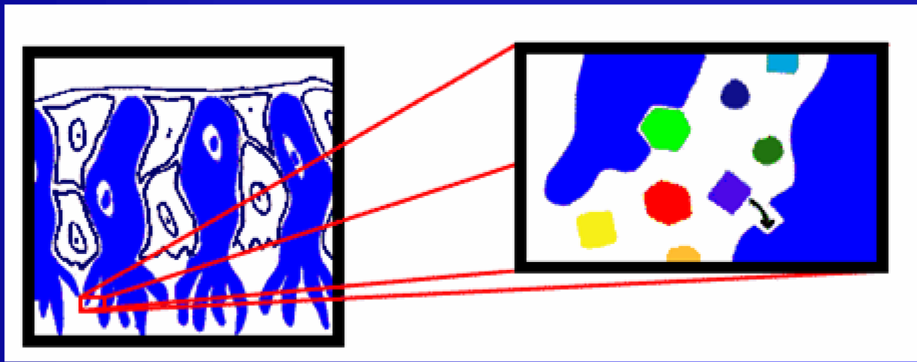
CHEMOSENSORY PERCEPTION



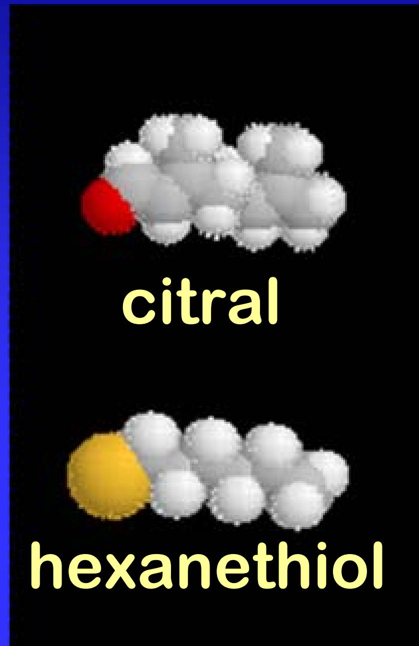




STRUCTURE-FUNCTION RELATIONSHIP



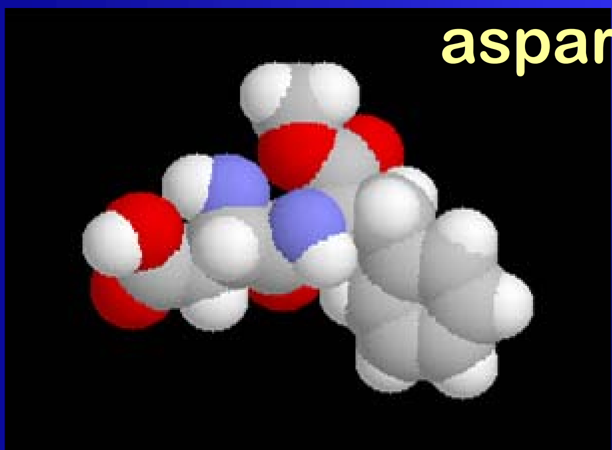
Olfactory responses

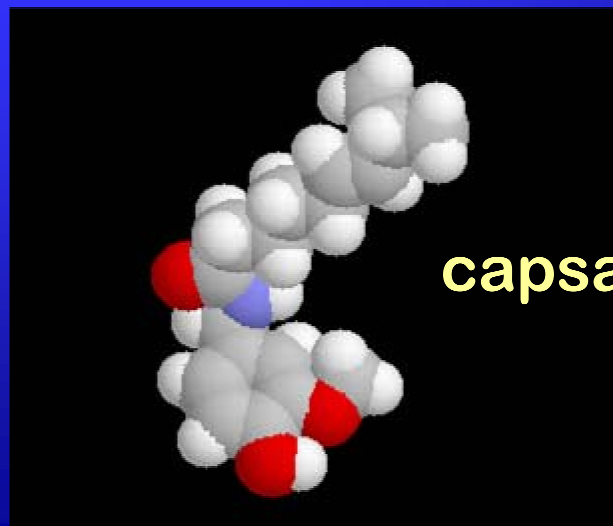


= LEMONY

= SKUNKY

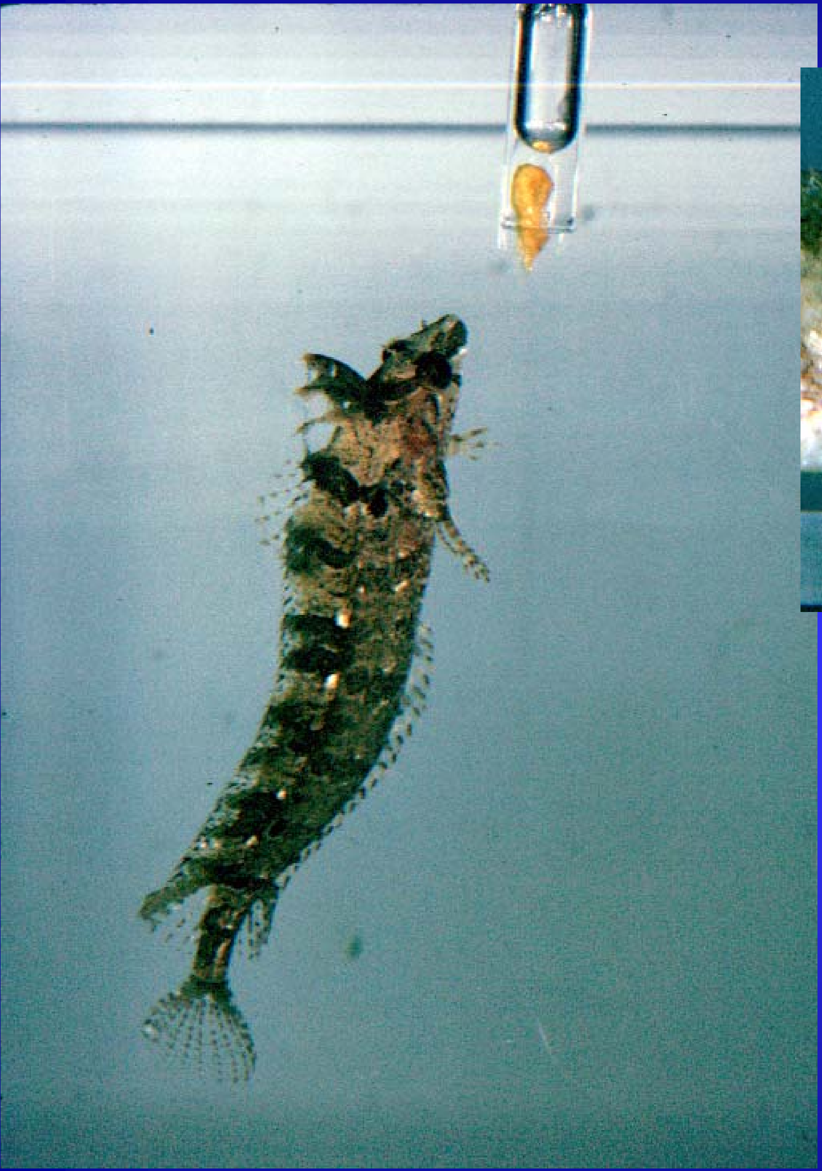
Gustatory responses

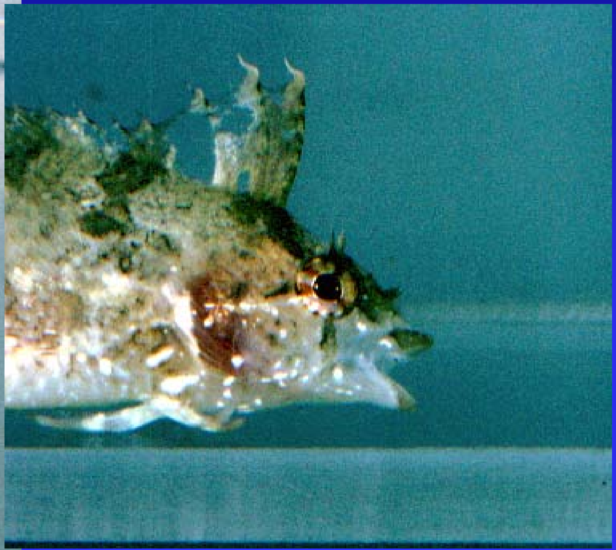




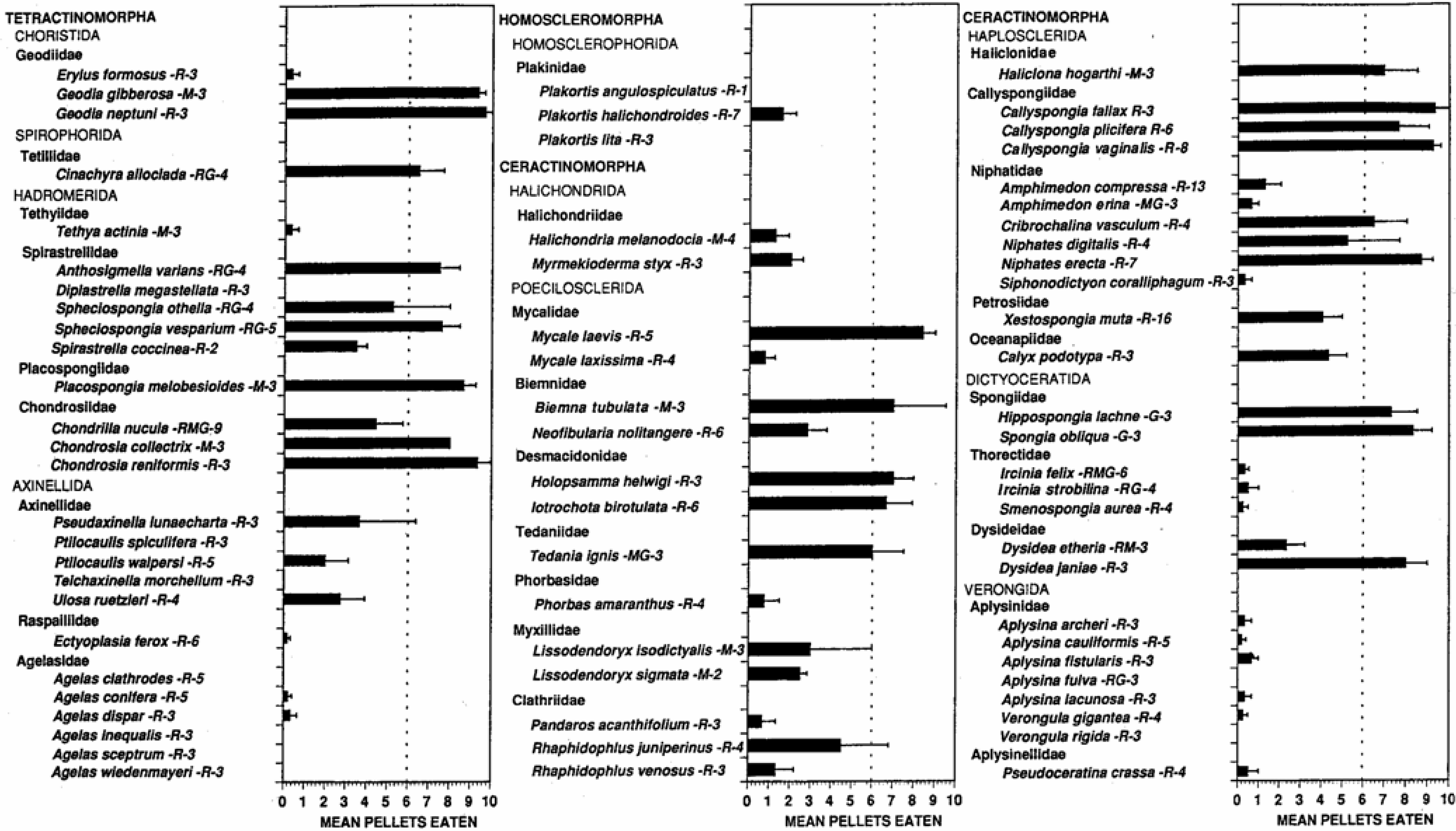
The ultimate bioassay

Mar. Ecol. Prog. Ser. 30: 251–260, 1986

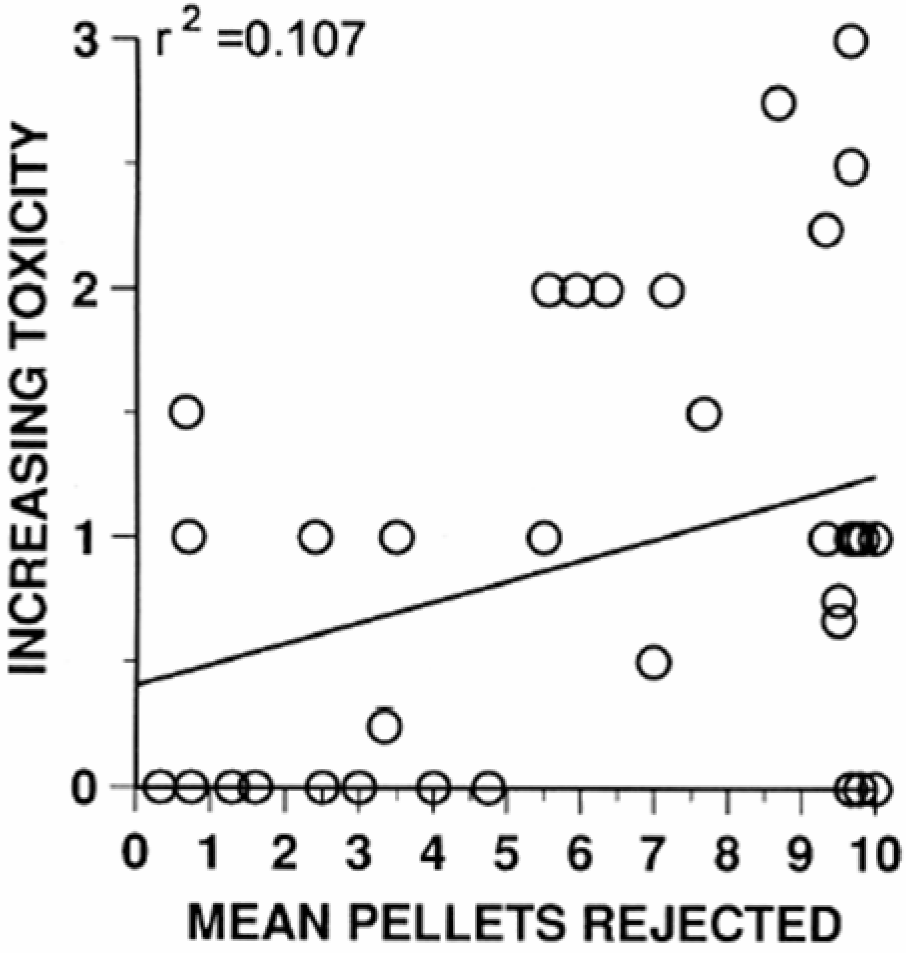




Aquarium assay



Pawlik, Chanas, Toonen & Fenical, (1995) MEPS 127:183-194



Pawlik et al.: Defenses of sponges. I. Chemical
Mar Ecol Prog Ser 127: 183–194, 1995

Fig. 3. Correlation of the deterrency of organic extracts with the mean toxicity of organic extracts of tissue from 36 species of Caribbean demosponges. Deterrency data are from this study (mean pellets eaten subtracted from 10), while toxicity data are from previous investigations (see 'Materials and methods')

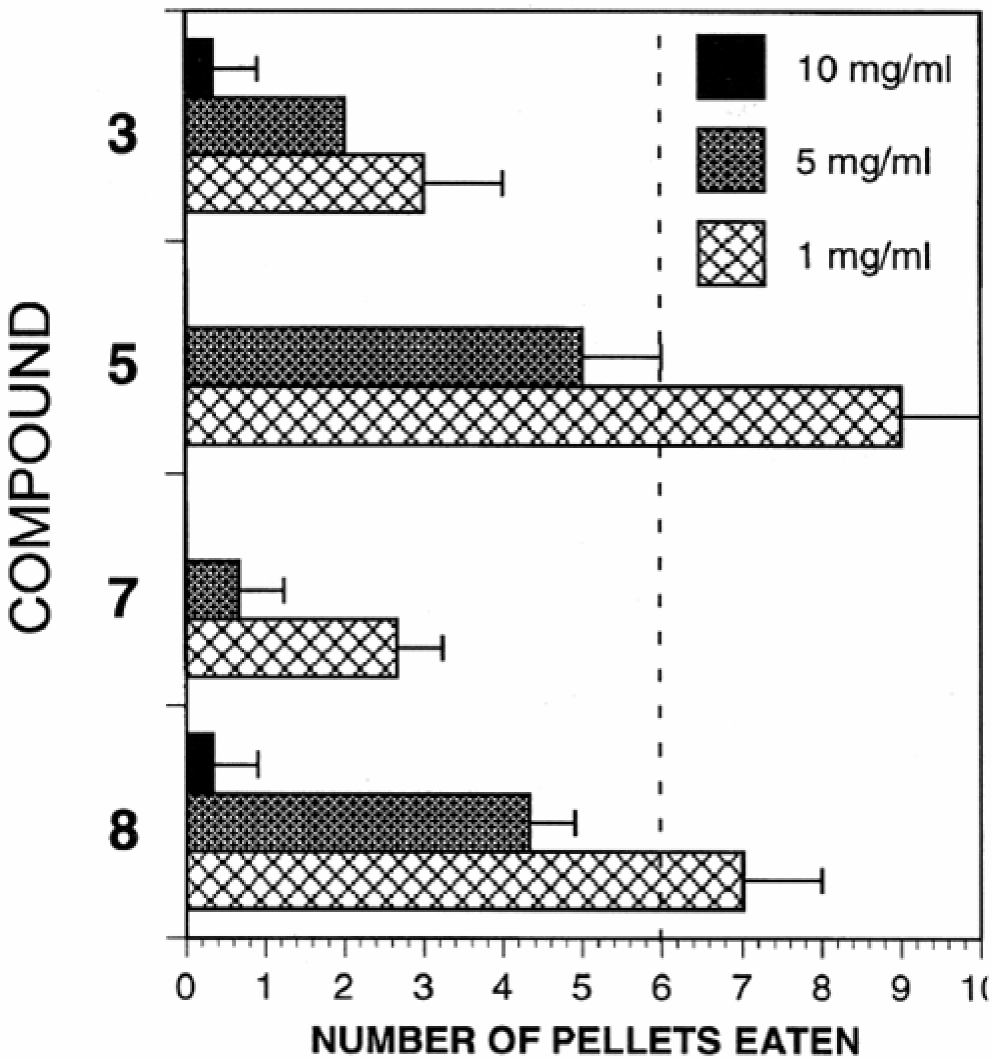
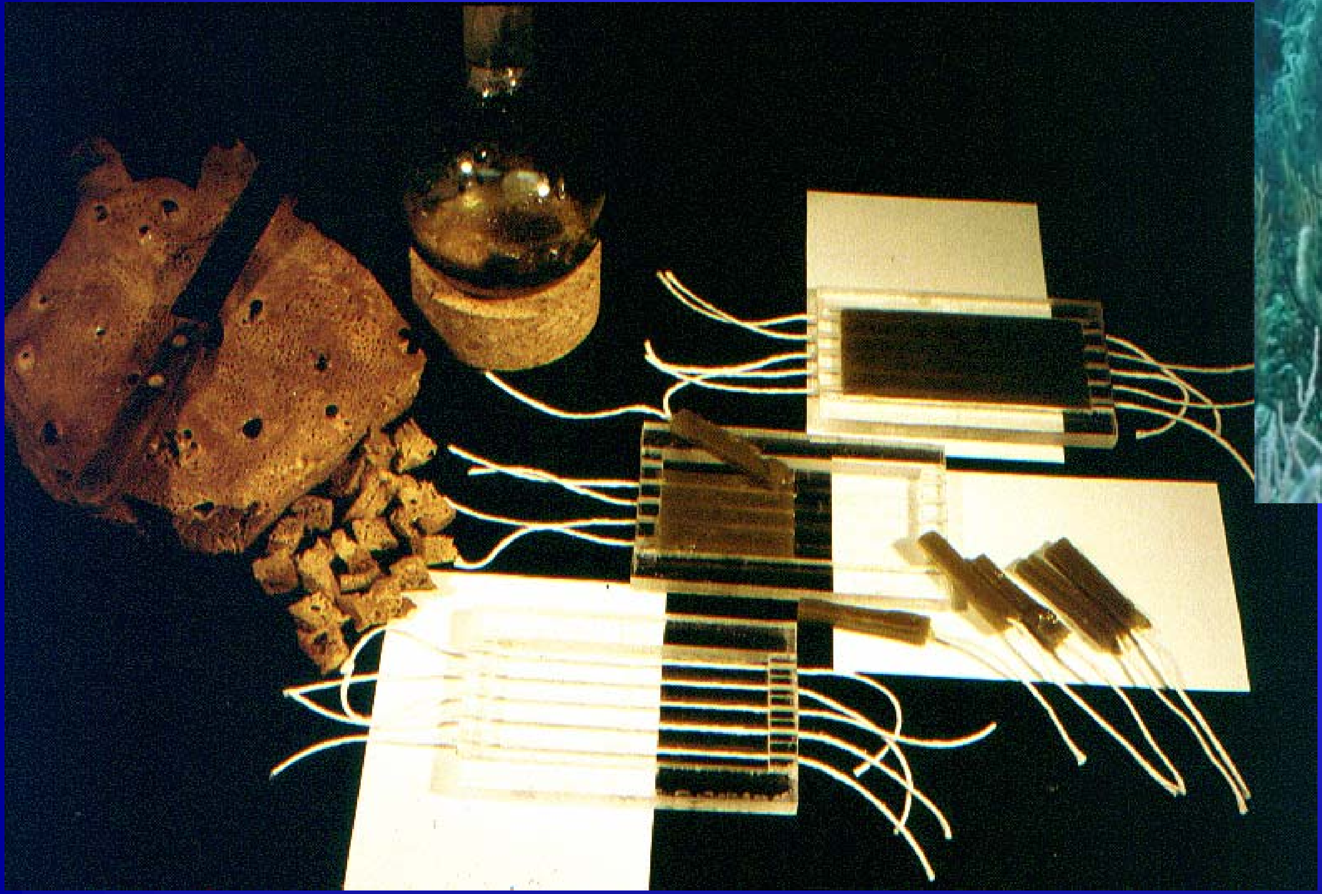


Fig. 3. Aquarium assay results of feeding by *Thalassoma bifasciatum* on pellets treated with sceptrin (**3**), dibromosceptrin (**5**), bromoageliferin (**7**), and dibromoageliferin (**8**) isolated from *Agelas conifera* at concentrations of 1, 5, and 10 mg ml^{-1}. All control pellets were eaten in all assays. Three replicate assays were performed at each concentration. One SD above the mean number of food pellets eaten is indicated. For any individual assay, a treatment was considered deterrent if the number of pellets eaten was less than or equal to 6 ($p < 0.043$, Fisher exact test, 1-tailed) as indicated by the dashed line

Assmann et al.: Chemical defense of *Agelas*
Mar Ecol Prog Ser 207: 255–262, 2000

Field assay



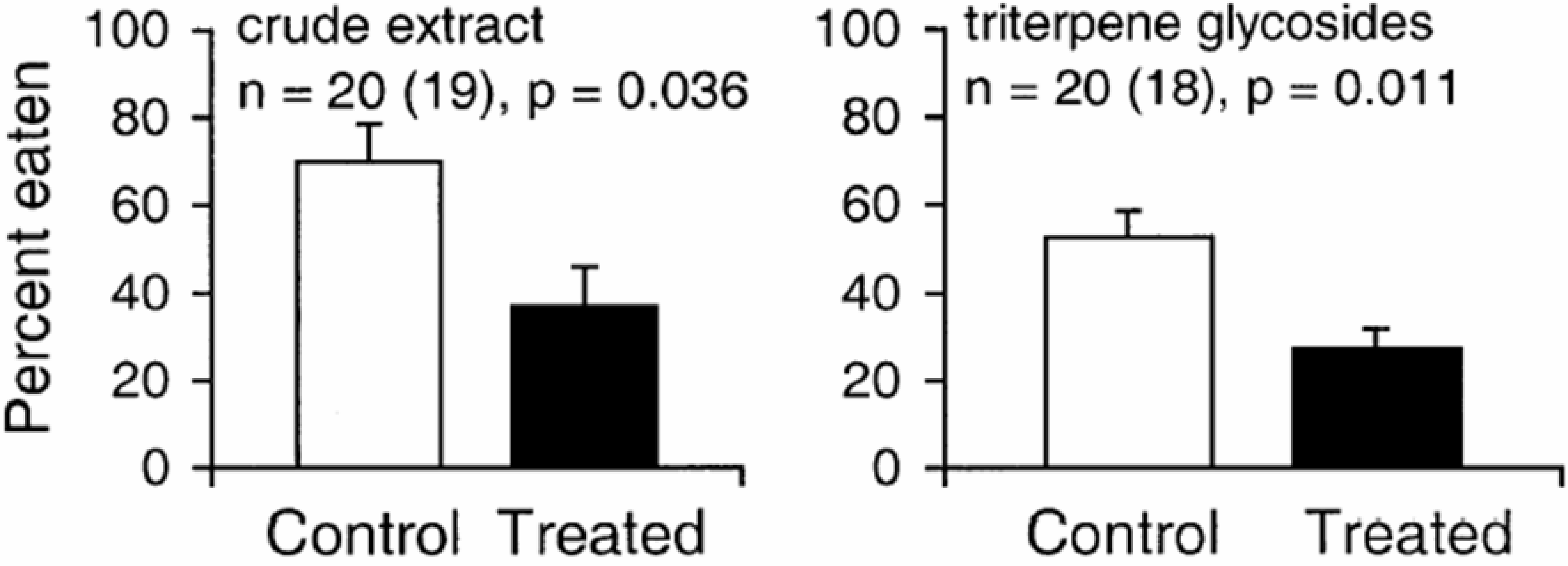
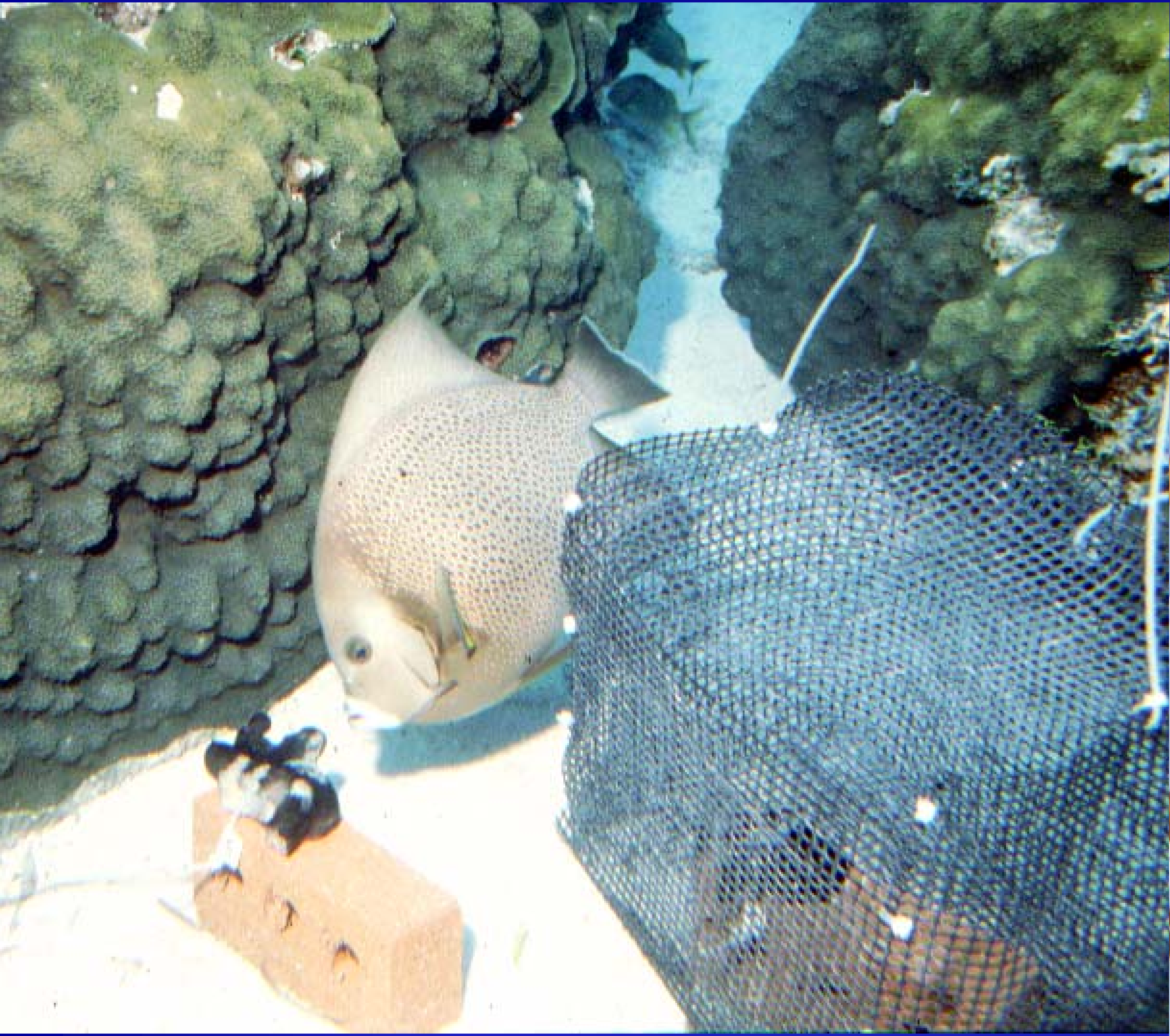
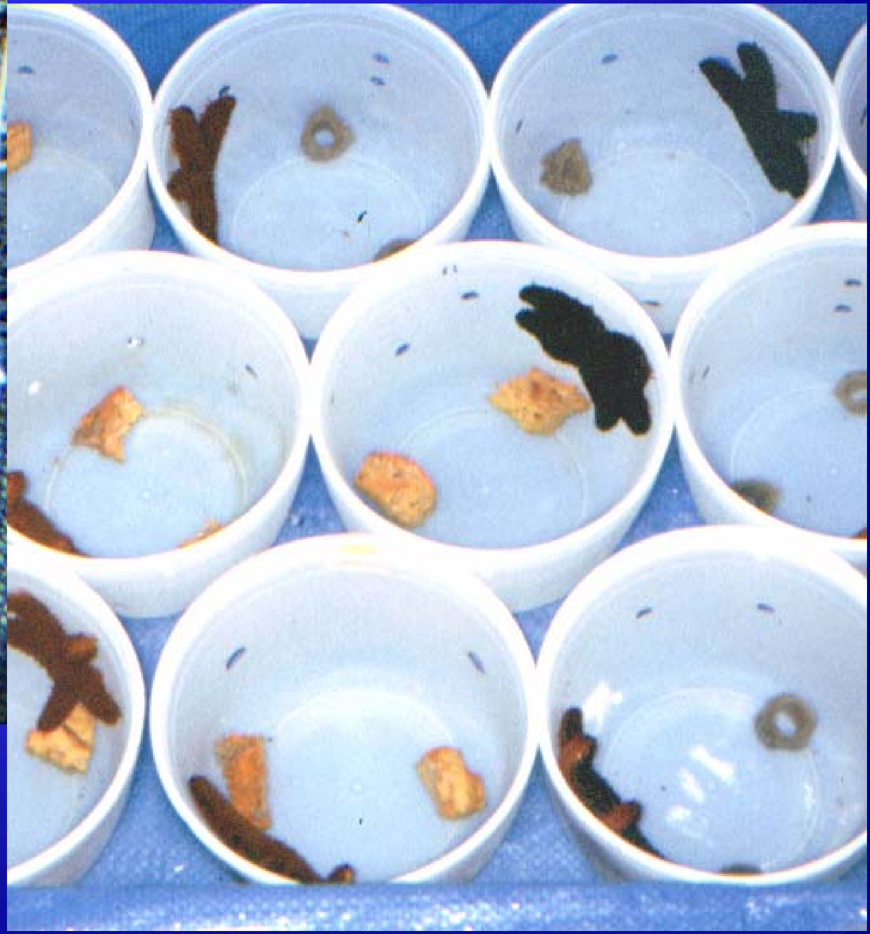


Fig. 3 Results of field assays assessing antipredatory effects of sponge compounds from *Ectyplasia ferox* against natural assemblages of reef fishes in the Bahamas. Error bars indicate±1 SE. *P*-values are for one-tailed paired *t*-test. [Assays using crude extracts and triterpene glycosides from *Erylus formosus* indicated similarly deterrent effects (Kubanek et al. 2000)]

Oecologia (2002) 131:125–136





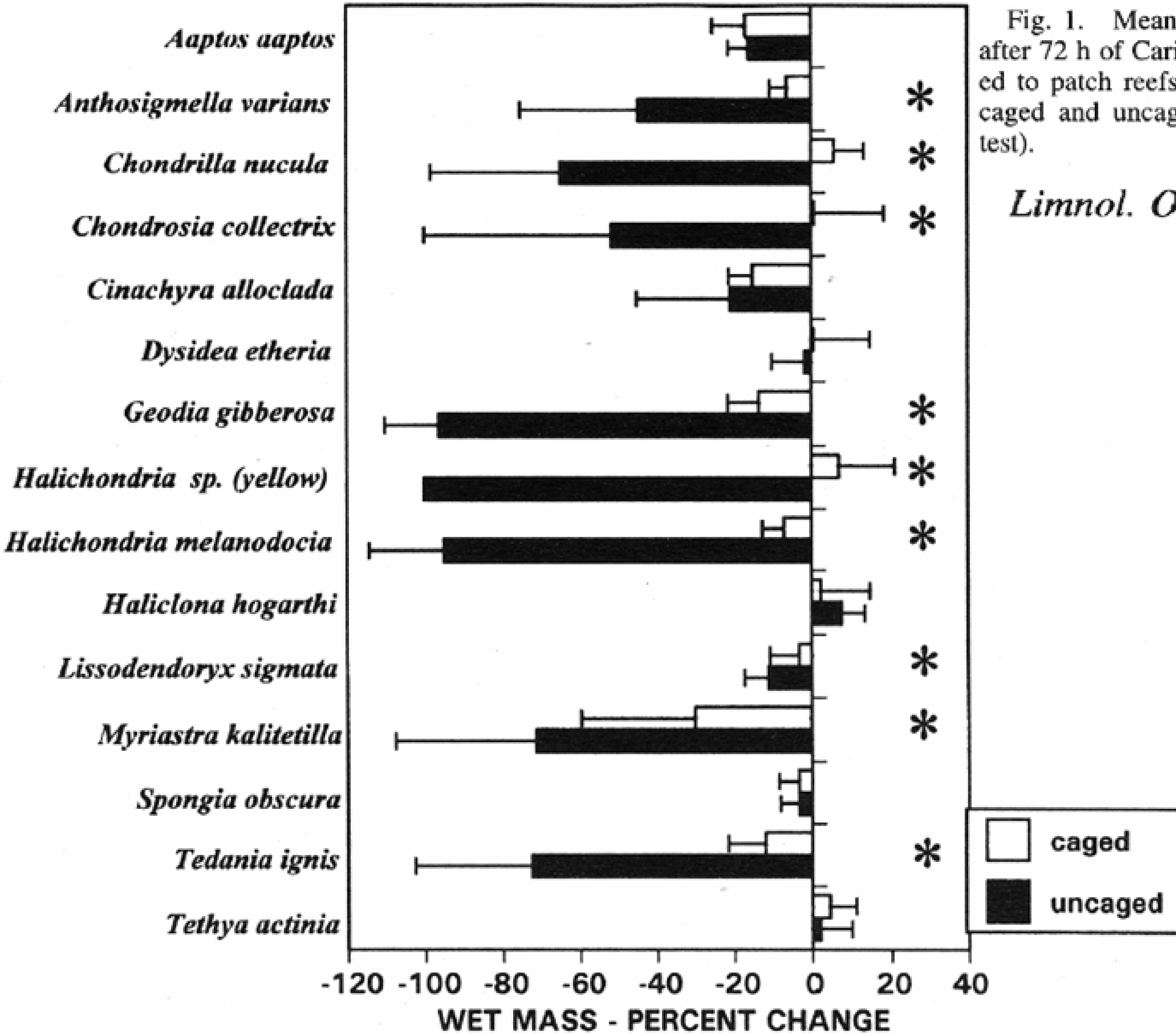
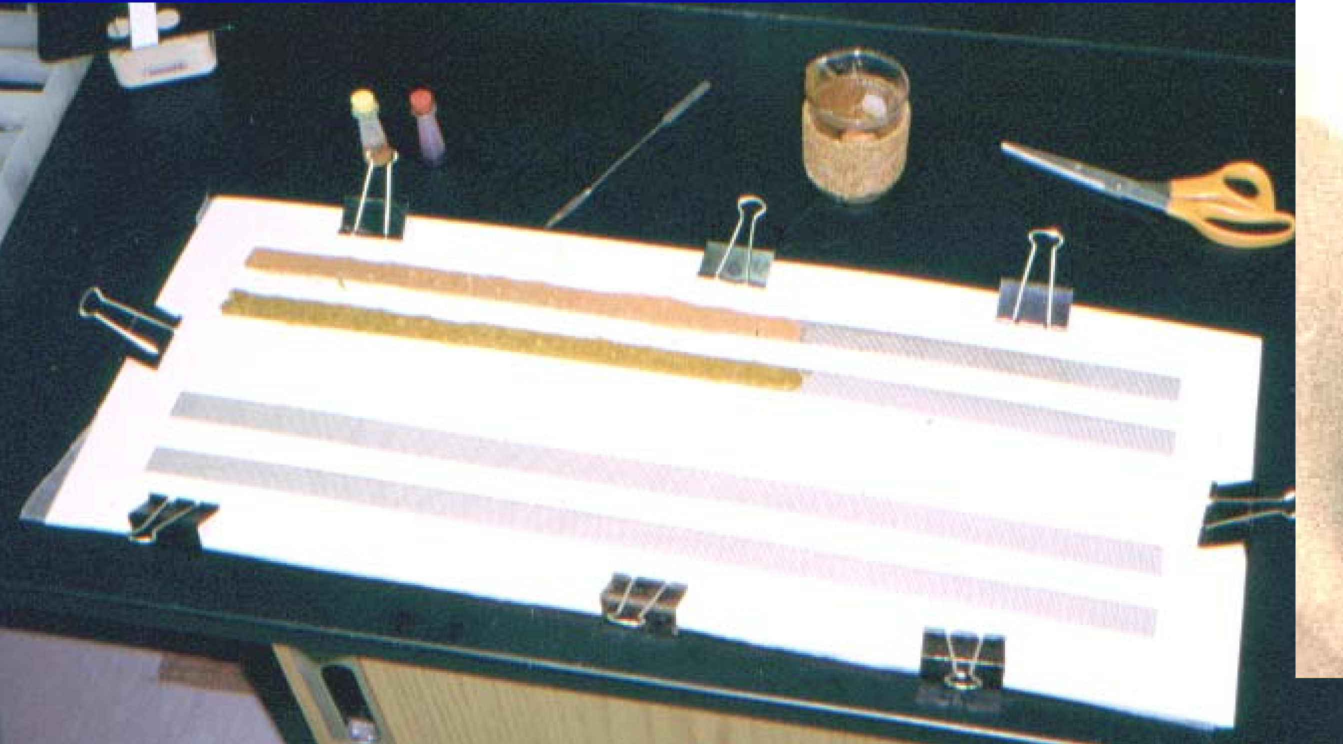
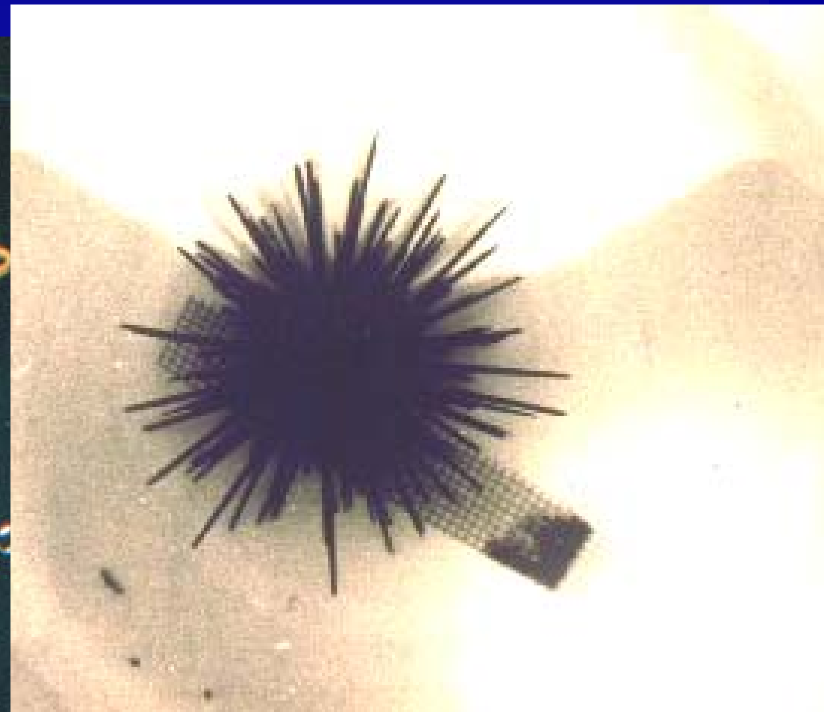
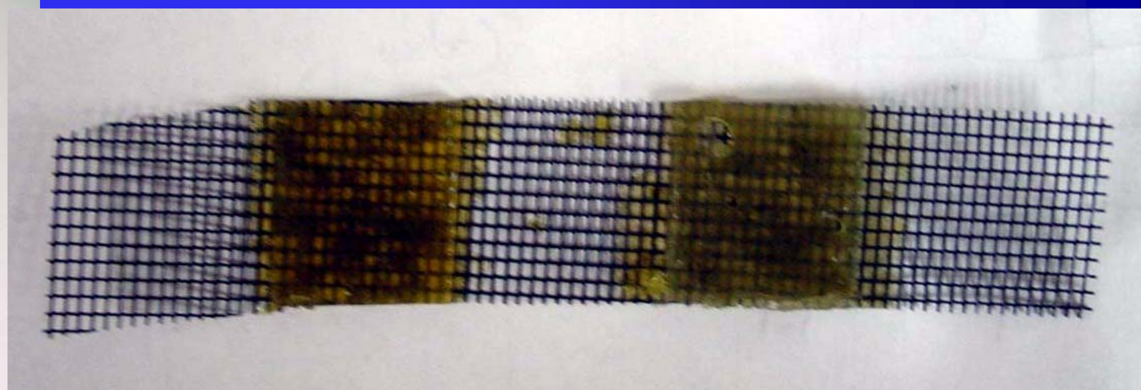


Fig. 1. Mean percentage change (±SD; $N = 15$) in wet mass after 72 h of Caribbean grassbed and mangrove sponges transplanted to patch reefs. * Significant difference in mass between paired caged and uncaged sponges ($P < 0.05$, Wilcoxon paired-sample test).

Limnol. Oceanogr., 43(6), 1998, 1396–1399







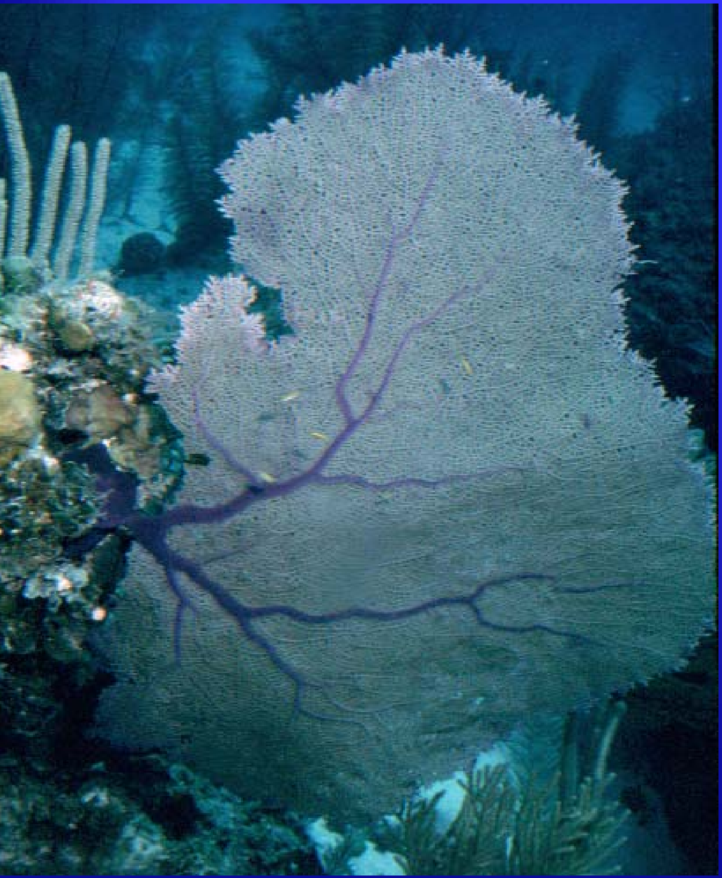
ANTI-PREDATORY CHEMICAL DEFENSES

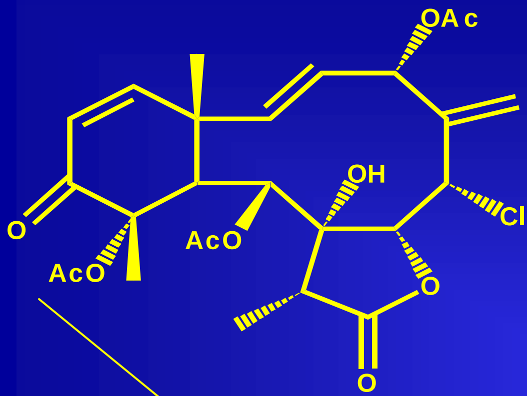
Subsequent: Development of more ecologically relevant bioassays

- **Feeding assays**
- **Realistic consumers**
- **Natural concentrations of metabolites**

Gravimetric vs. VOLUMETRIC!

Chemical vs. Physical Defenses in Caribbean Gorgonians

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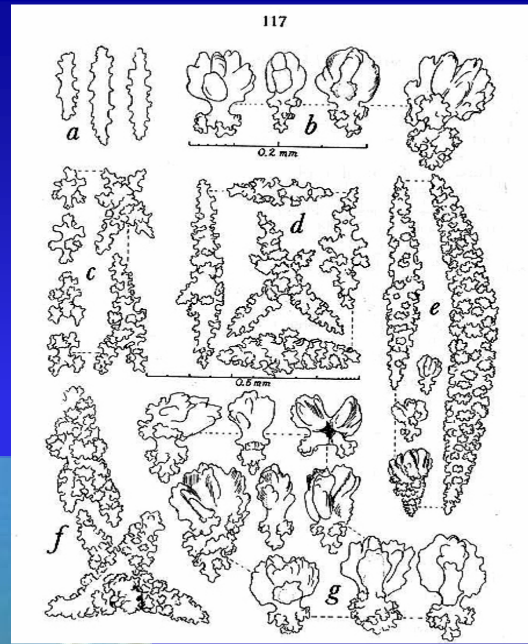
117
a
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f
g

Problems with Pawlik et al. (1987):

- Sample handling may have affected results
- Results based on gravimetric analyses

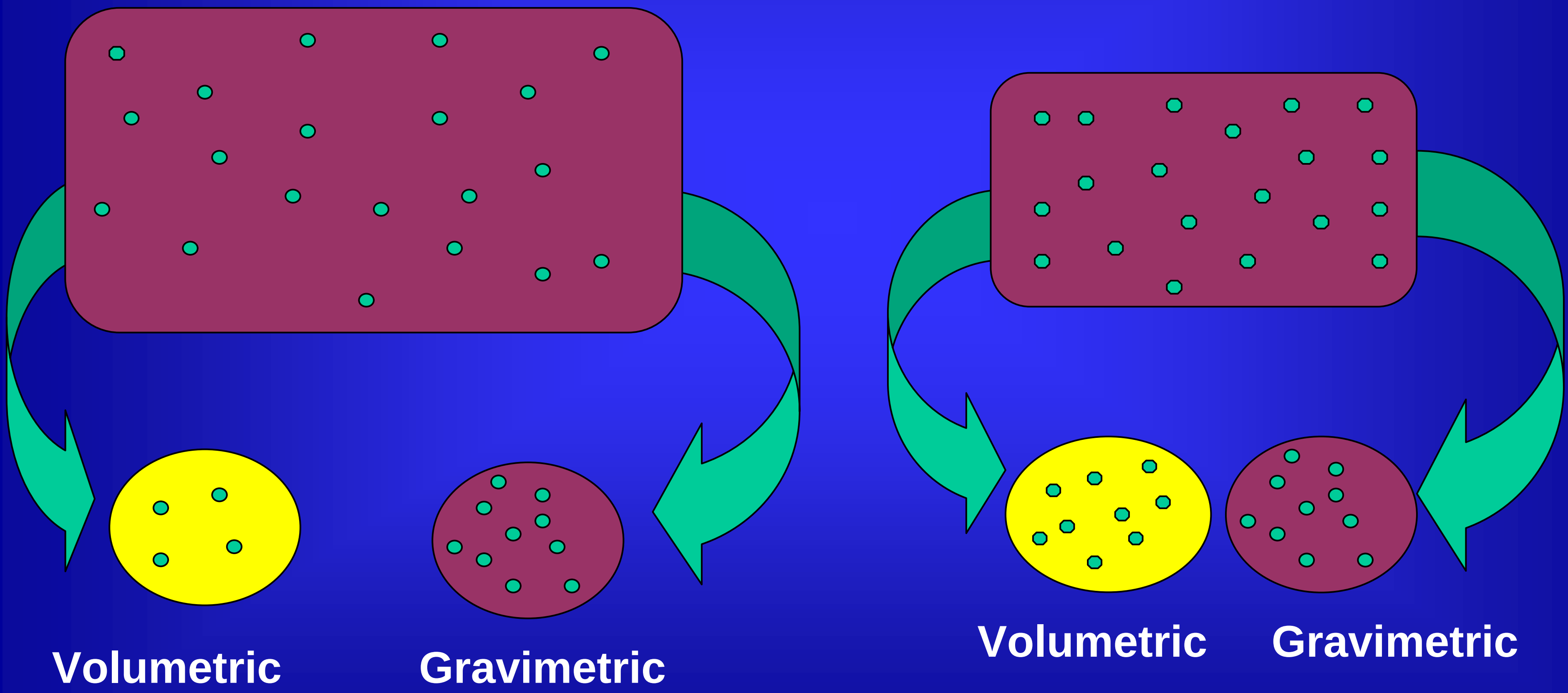
J. Exp. Mar. Biol. Ecol., 1987, Vol. 108, pp. 55–66

TABLE I

Preliminary survey of the palatability of gorgonian extracts to the reef fish *Thalassoma bifasciatum*. TLC, thin-layer chromatographic evidence of secondary metabolites: −, no evidence; +, positive evidence; ?, no reliable conclusions possible.

Voucher number	Family	Species identification[a]	Percentage (dry wt) of extract in: Gorgonian	Pellet	TLC
Group I. Highly unpalatable (zero or one of five pellets eaten)					
23	Anthothelidae	*Erythropodium caribaeorum*	2.6	6.3	+
7	Briareidae	*Briareum asbestinum* (erect)	4.3	17.0	+
24		*Briareum* sp. (encrusting)	5.2	20.0	+
12	Gorgoniidae	*Pseudopterogorgia acerosa*	6.3	8.4	+
38		*Pseudopterogorgia acerosa*[b]	8.0	10.2	+
39		*Pseudopterogorgia acerosa*[b]	10.0	12.2	+
22		*Pseudopterogorgia rigida*	15.0	34.0	+
40		*Pseudopterogorgia rigida*[b]	10.0	12.0	+
41		*Pseudopterogorgia rigida*[b]	12.5	8.0	+
1		*Pterogorgia anceps*	6.7	27.0	−
9		*Pterogorgia citrina*	2.1	6.9	−
15		*Pterogorgia guadalupensis*	11.0	42.0	−
5	Plexauridae	*Eunicea laciniata*	6.0	21.0	+
19		*Eunicea mammosa*	5.5	43.0	+

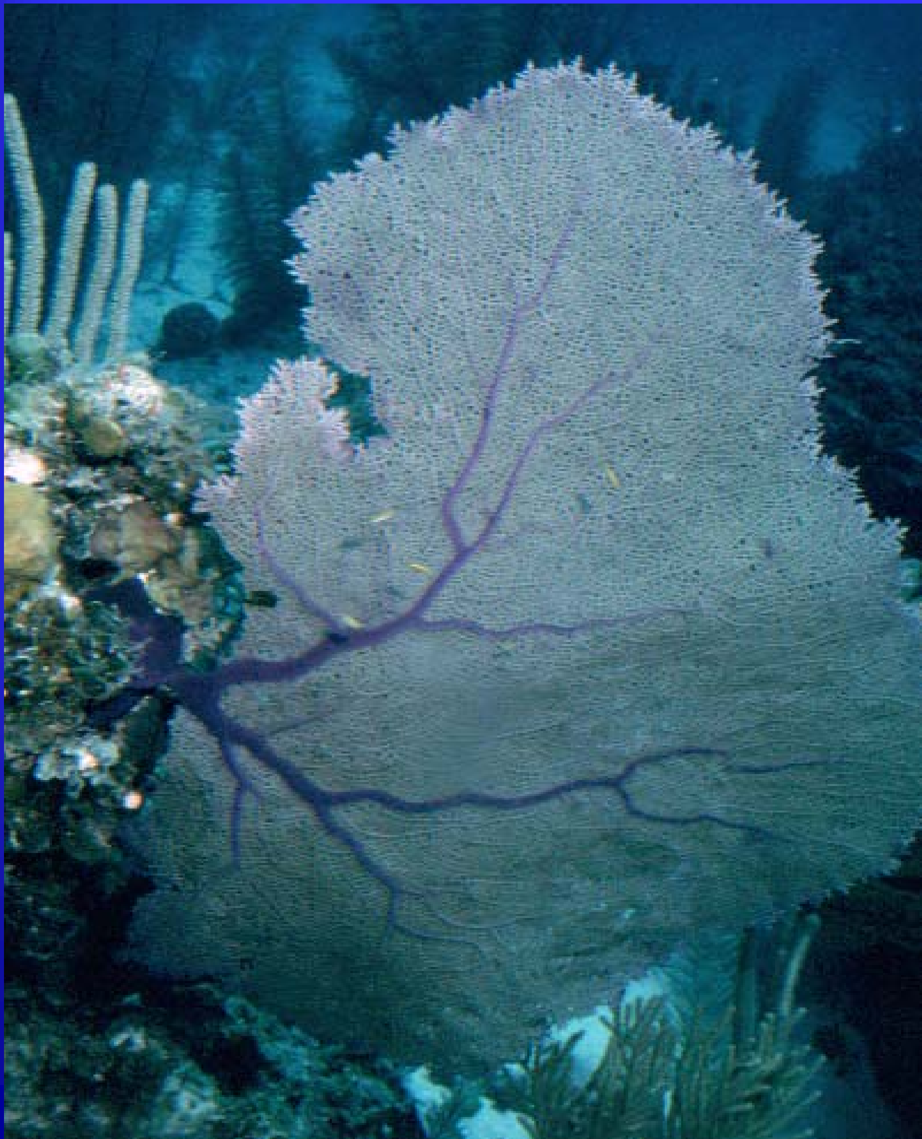
Importance of volumetric vs. gravimetric measurement:



Spicule Extractions

- 5 ml of gorgonian dissolved in sodium hypochlorite (bleach)
- Spicules washed in H_2O and acetone
- Spicules dried by suction filtration and assayed

Volumetric vs. Gravimetric



- Compare 2 samples of *Gorgonia ventalina*:
  - Sample A:
    - 19.72 g
    - 27 ml
  - Sample B:
    - 22.86g
    - 57 ml
- Mass of B was 16% greater than A
- Volume of B was 111% greater than A

TYPES OF CHEMICAL DEFENSES:

- Anti-predatory
- Anti-overgrowth (allelopathic)
- Antifouling
- Anti-pathogen
- UV radiation protection (sunscreens)

Proc. Nat. Acad. Sci. USA
Vol. 72, No. 12, pp. 5160–5163, December 1975
Zoology

Allelopathy and spatial competition among coral reef invertebrates

(species interactions/community structure)

J. B. C. JACKSON AND LEO BUSS

Department of Earth and Planetary Sciences, The Johns Hopkins University, Baltimore, Maryland 21218; and Discovery Bay Marine Laboratory, Box 35, Discovery Bay, Jamaica, West Indies

Communicated by Hans P. Eugster, August 5, 1975

ABSTRACT Species of ectoprocts and solitary encrusting animals were subjected in aquaria to homogenates of 11 sympatric species of sponges and colonial ascidians. Five of the nine sponge species and one of the two ascidian species exhibited species-specific allelochemical effects. Evidence suggests that allelochemical interactions provide a wide-spread, specific, and complex mechanism for interference competition for space among natural populations of coral reef organisms. The existence of such species-specific mechanisms may provide a basis for maintenance of diversity in space-limited systems in the absence of high levels of predation and physical disturbance.

Table 1. Results from addition of sponge and colonial ascidian homogenates to aquaria containing ectoprocts and solitary invertebrates

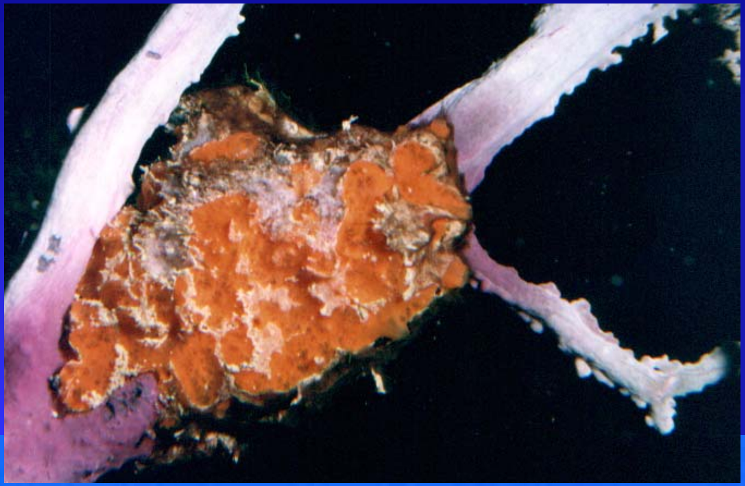
	Species exposed to homogenates							
	Ectoprocts				Solitary animals			
Homogenates	*Steganoporella magnilibris* (Busk)	*Stylopoma spongites* (Pallas) type 1	*Stylopoma spongites* (Pallas) type 2	*Reptadeonella violacea* (Johnston)	Serpulid 1	Serpulid 2	*Argyrotheca johnsoni* (Cooper)	*Basilomya goreaui* (Bayer)
Sponges								
Mycale laevis (Carter)	D^1	NTE	—	—	NTE	NTE	NTE	NTE
?*Tenaciella* sp.	NTE	NTE	—	—	NTE	NTE	NTE	NTE
?*Toxemna* sp.	NTE	D	—	—	NTE	NTE	NTE	NTE
?*Halisarca* sp.	NTE	NTE	—	—	NTE	NTE	NTE	—
Sponge 1	NTE	NTE	—	NTE	NTE	NTE	NTE	—
Plakortis ?sp.	D^1?	NTE	—	D^1	—	—	—	—
Sponge 2	NTE	NTE	—	—	—	—	—	—
Agelas ?*sceptrum* (Lamarck)	MF	NTE	—	—	—	—	—	—
Ectyoplasia ferox (Duchassaing and Michelotti)	NTE	D	D	D^1	—	—	—	—
Colonial ascidians								
Didemnum sp.	NTE	NTE	—	—	NTE	NTE	NTE	—
Ascidian 2	MF	D	—	—	NTE	NTE	NTE	—
No. species tested	11	11	1	3	7	7	7	3
No. species showing toxic effect	4	3	1	2	0	0	0	0

NTE, no apparent toxic effect; normal movement and feeding of ectoproct zooids; MF, no movement or feeding; ectoproct zooids intact; D, ectoproct colonies dead; zooids deteriorating; D^1, brown body formation in deteriorating zooids; —, no experiment run. Results were the same for all five replicates.

Fouling invertebrate toxicity assays to assess allelopathy using tissue homogenates

ALLELOPATHIC AGENTS are metabolites that alter the health, growth, or behavior of organisms of another species.

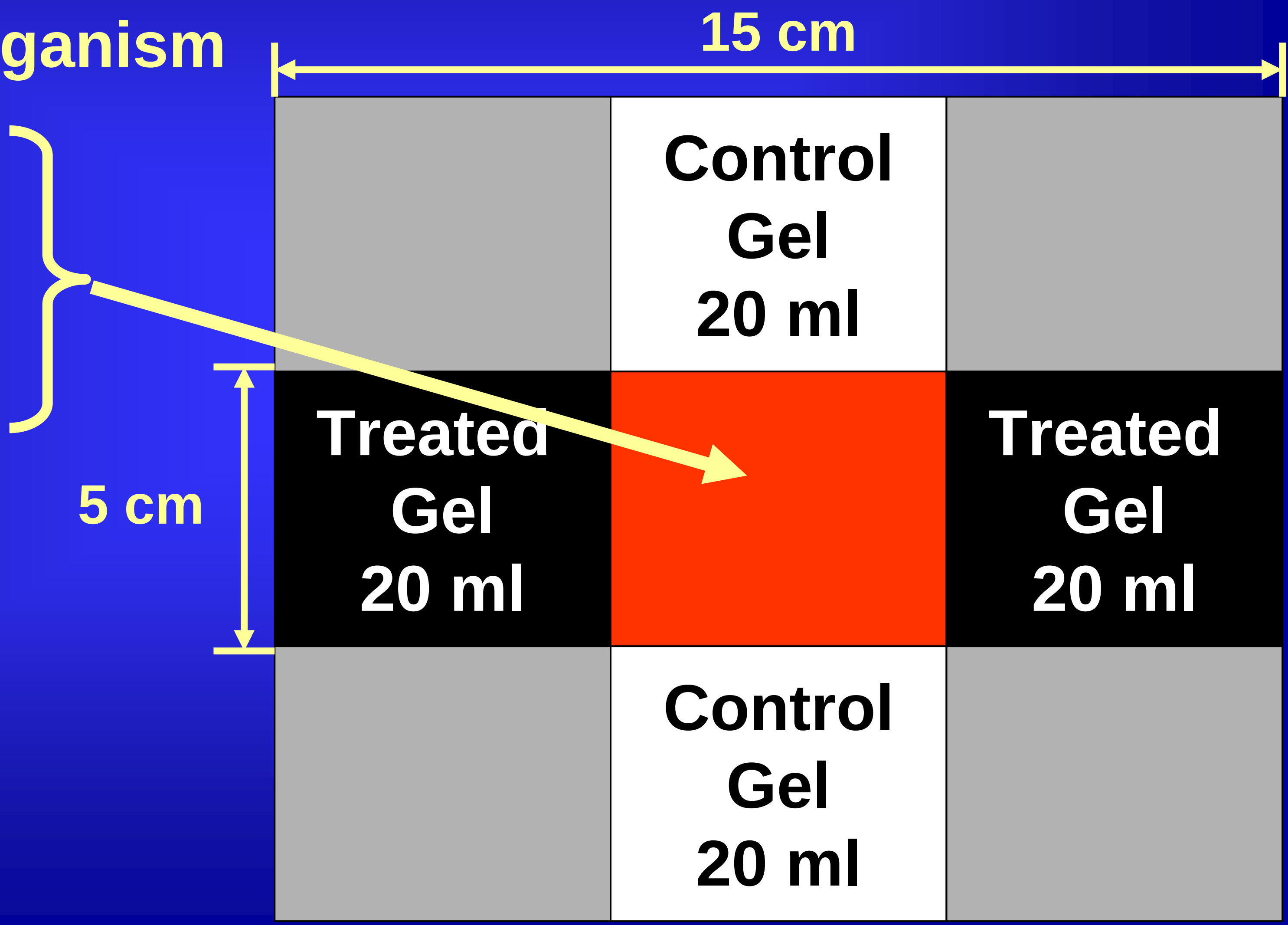
- Allomones: benefit the organism PRODUCING the metabolite.
- Kairomones: benefit the organism RECEIVING the metabolite.

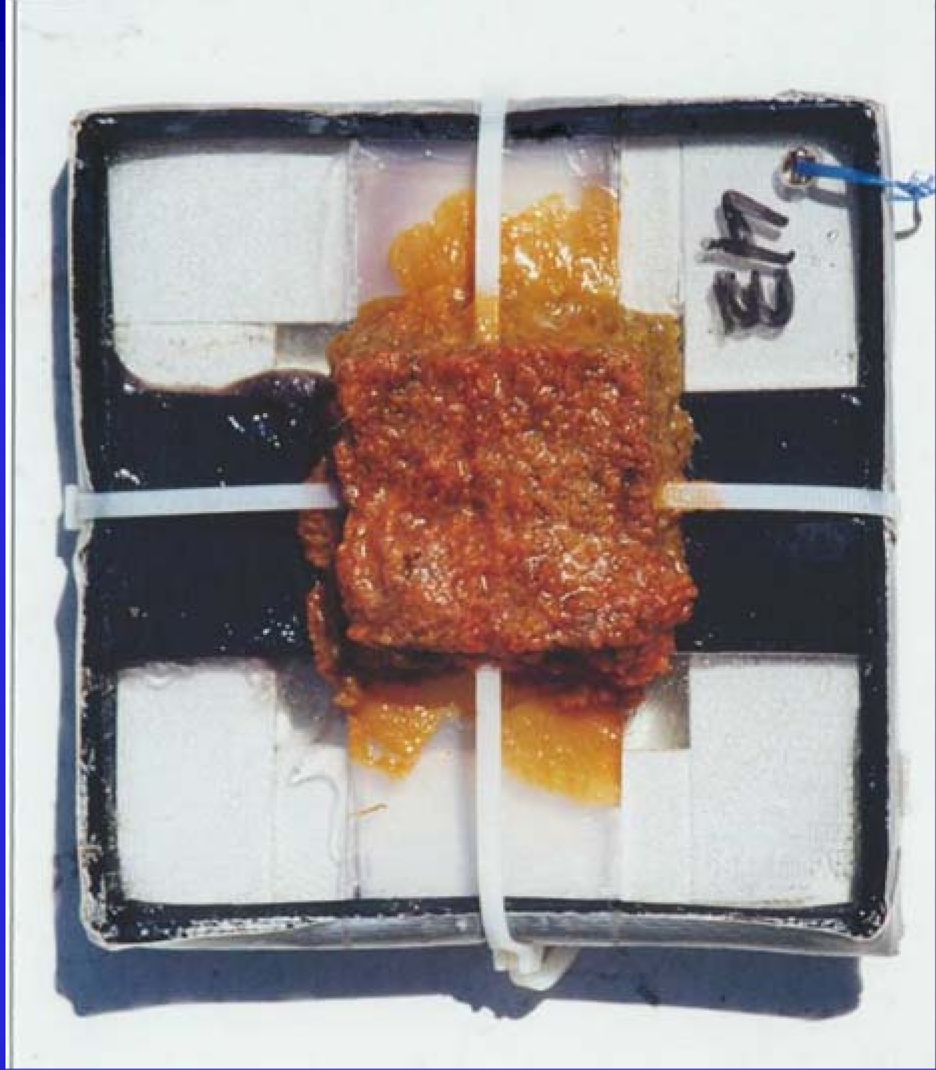




Overgrowth assay plate

Overgrowth organism

- Sponges
- Tunicates
- Bryozoans

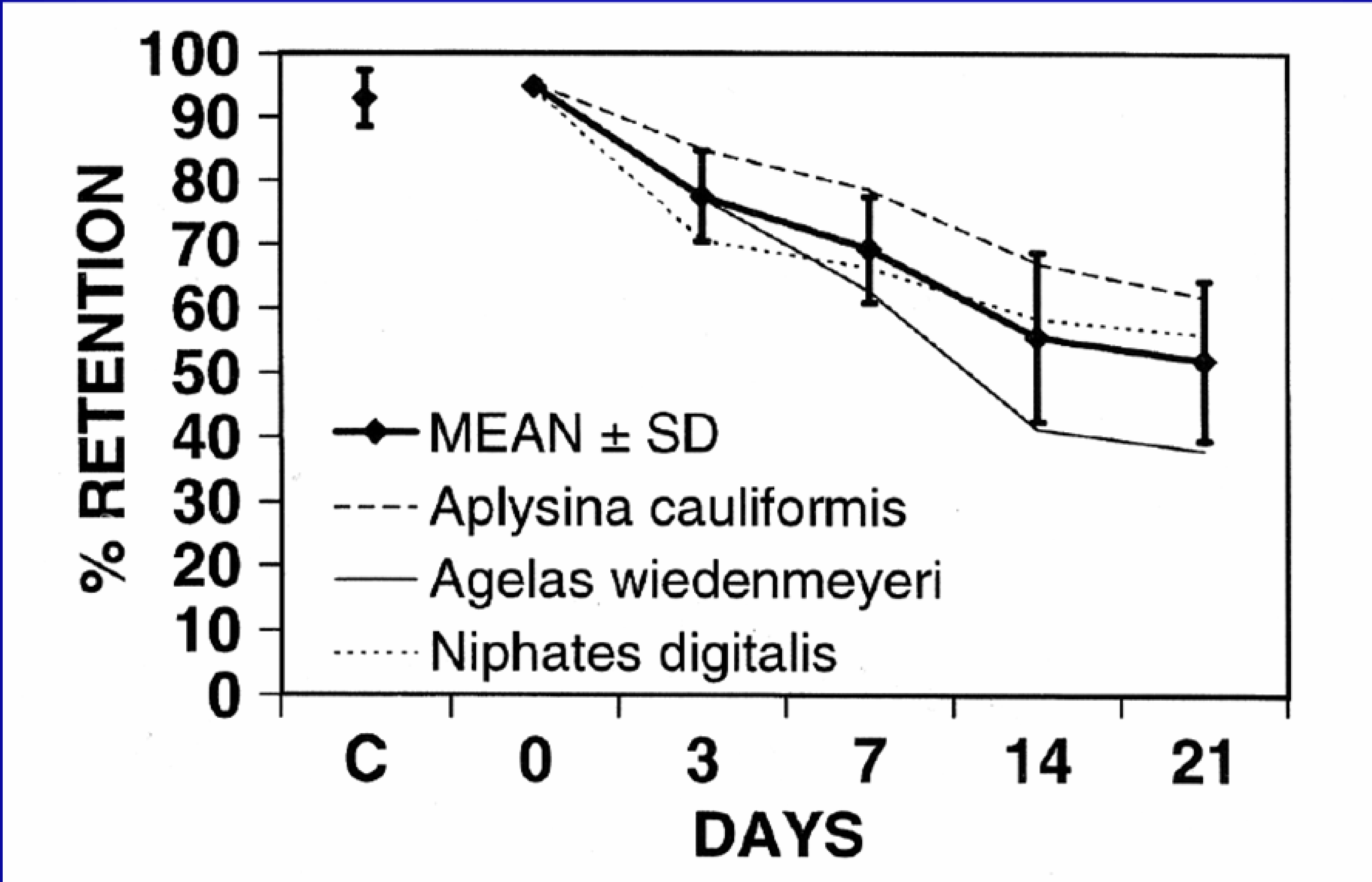




Extract: ***Aplysilla longispina***

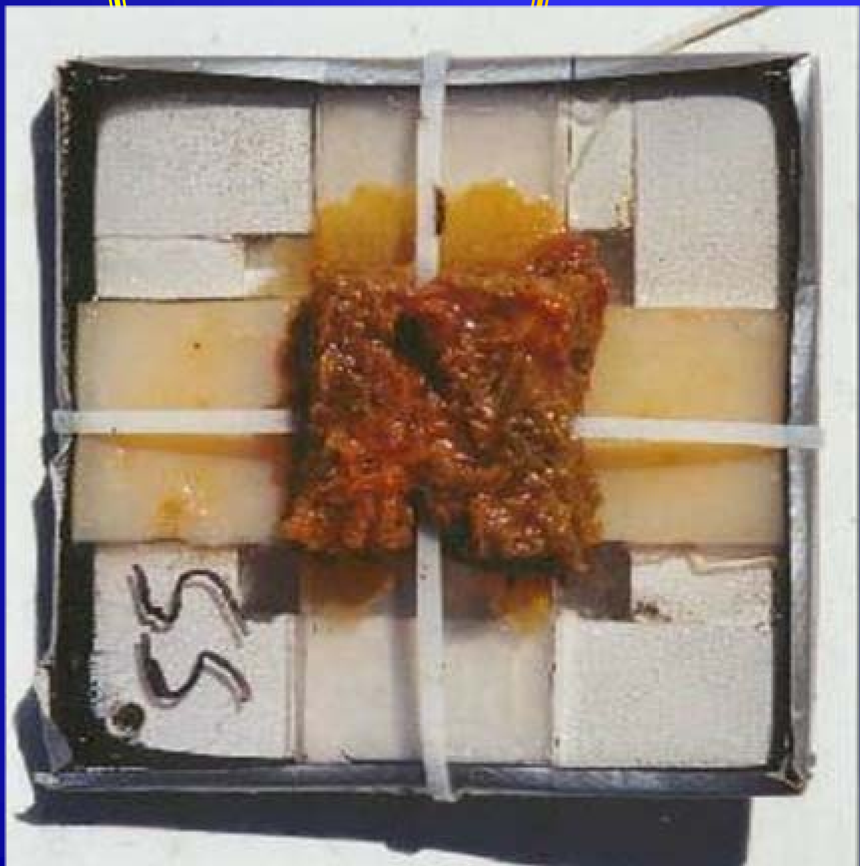
Overgrowth Sponge: ***Tedania ignis***

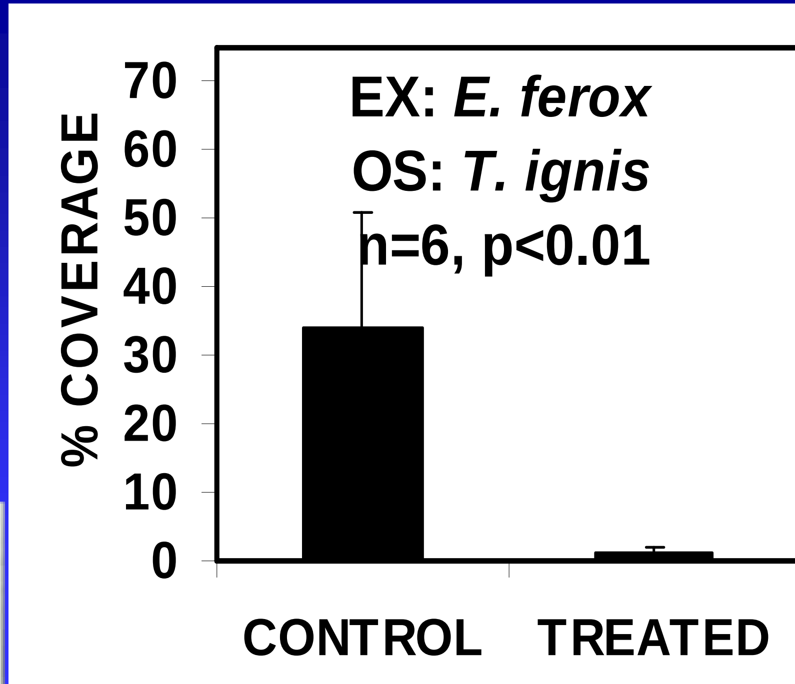
Extracts diffuse slowly from gels

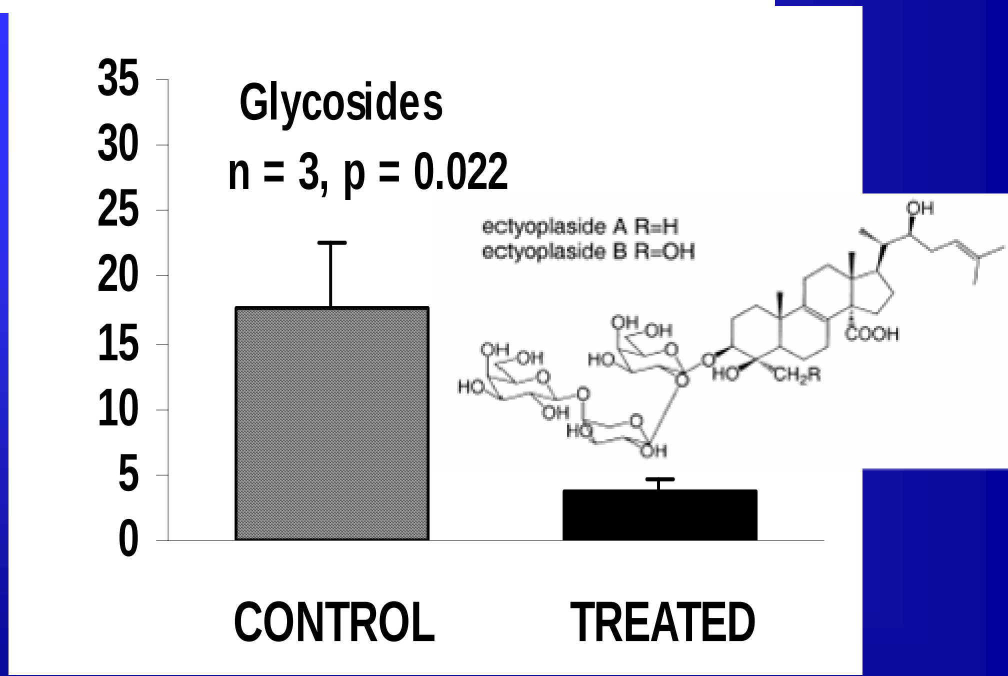


Ectyoplasia ferox

Tedania ignis







RESULTS:

Engel & Pawlik: Allelopathic activity of sponge extracts
Mar Ecol Prog Ser 207: 273–281, 2000

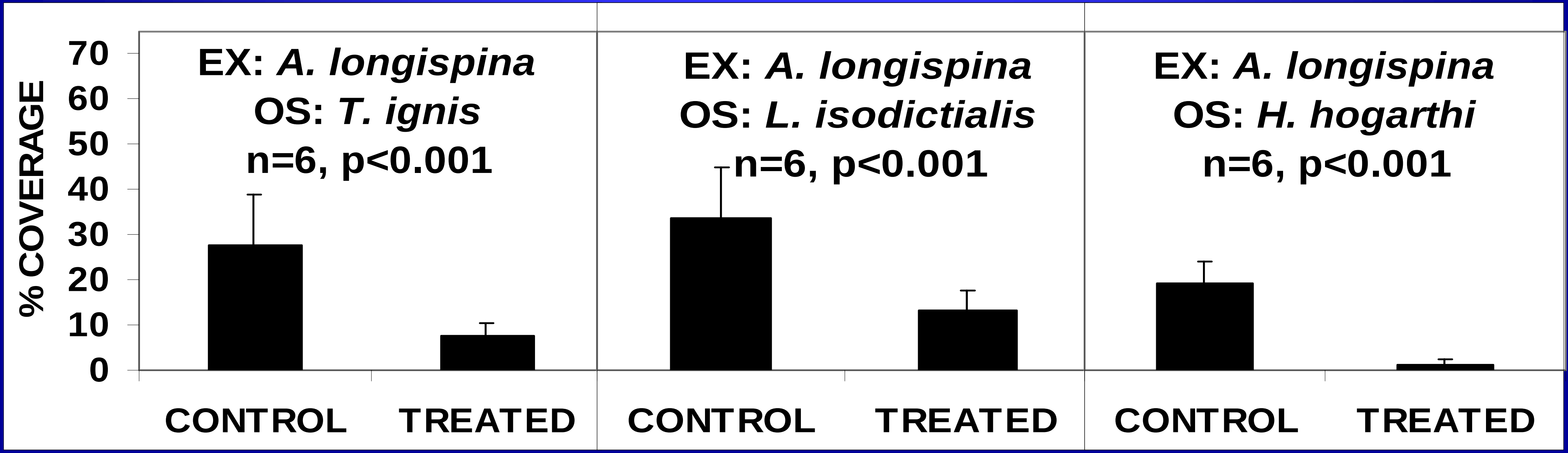
20 species of reef and mangrove sponges

6 (30%) Inhibited overgrowth

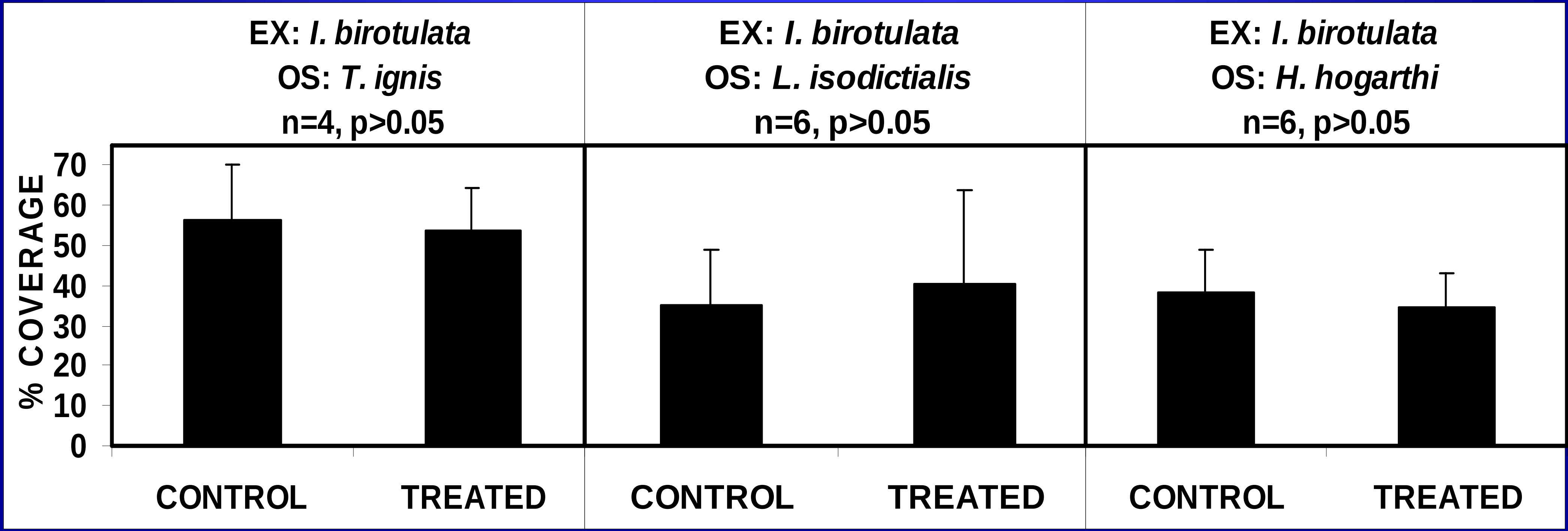
11 (55%) No effect on overgrowth

3 (15 %) Promoted overgrowth !!!

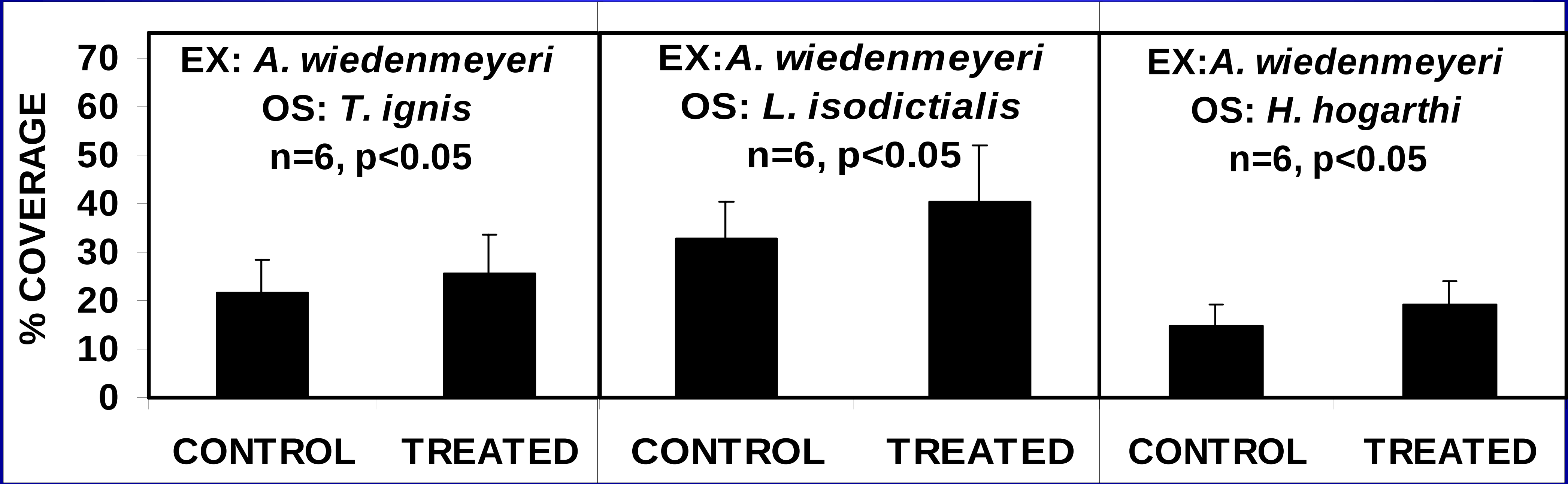
Extracts of *Aplysilla longispina* Inhibited Overgrowth



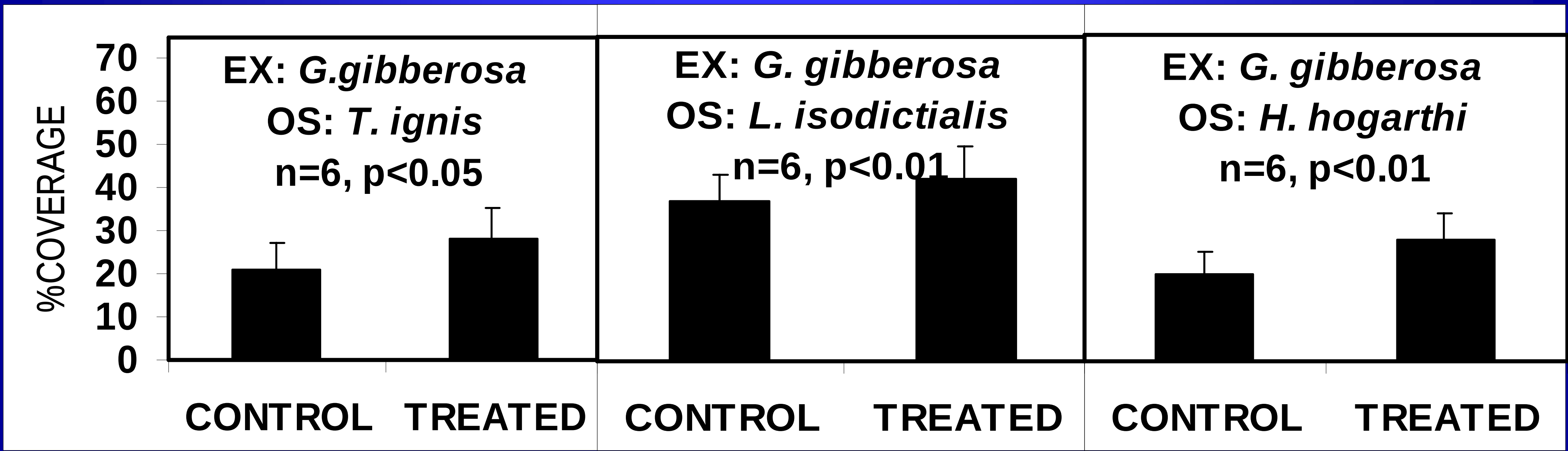
Extracts of *Irotrochorta birotulata* Do Not Affect Overgrowth



Extracts of *Agelas wiedenmeyeri* Promoted Overgrowth



Extracts of *Geodia gibberosa* Promoted Overgrowth



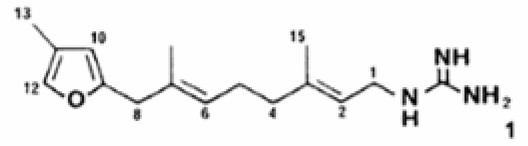
Conclusions

1. Gels provide a suitable substratum for overgrowth by sponges and others
2. Extracts diffuse gradually from gels
3. Observed 3 responses

   I) inhibited overgrowth

   II) no effect on overgrowth

   III) promoted overgrowth

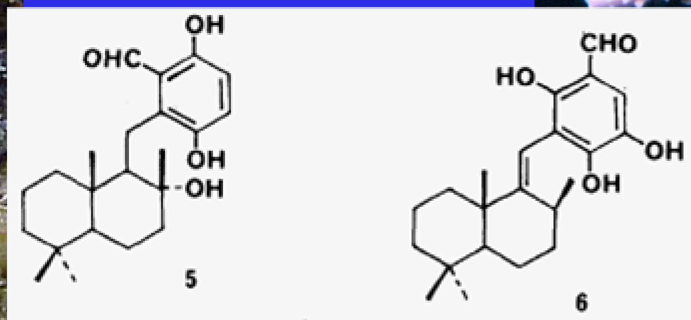


Science 221:1175 (1983)

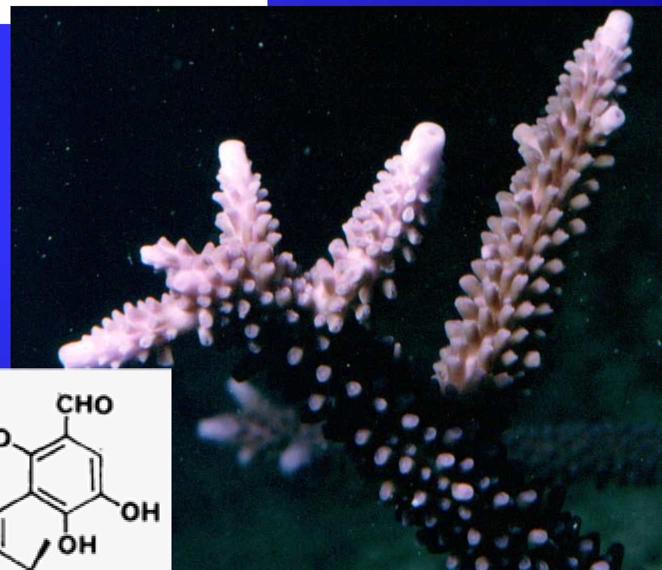
Siphonodictidine, a Metabolite of the Burrowing Sponge *Siphonodictyon* sp. That Inhibits Coral Growth

Abstract. *Siphonodictidine is the major secondary metabolite of an undescribed Indo-Pacific sponge* Siphonodictyon *sp. that burrows into living coral heads. The structure of siphonodictidine was determined from spectral data. Laboratory bioassays suggest that siphonodictidine and, by analogy, the siphonodictyals from* S. coralliphagum *are responsible for maintaining zones of dead coral polyps around the oscular chimneys of these sponges.*

BRIAN SULLIVAN
D. JOHN FAULKNER
Scripps Institution of Oceanography, La Jolla, California 92093
LEITH WEBB
James Cook University of North Queensland, Townsville, Queensland 4811, Australia

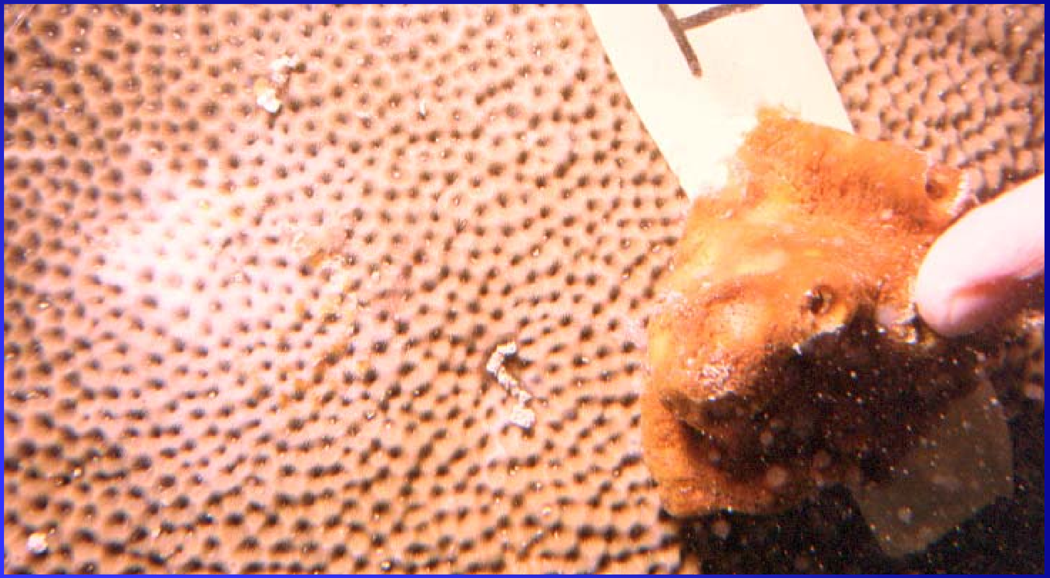


Siphonodictyals A & B



Siphonodictyon = Aka

First attempts (mid-1990s):



- Field assay
- Natural concentrations
- Comparison with live sponge

BUT:

HOW TO QUANTIFY???

PAM - pulse-amplitude modulated fluorometry:

- measures efficiency of photosystem II
- non-invasive
- quantitative
- correlates with O_2 evolution, C^{14} uptake
- very rapid

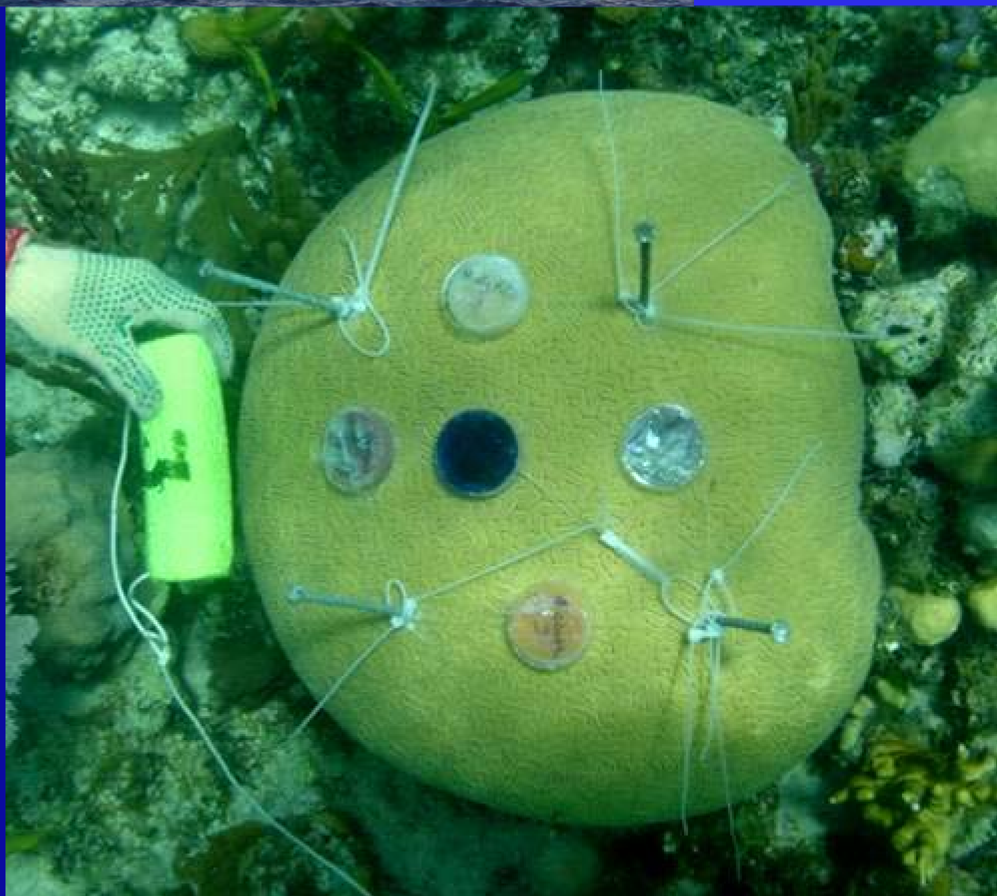
Use physiological condition of zooxanthellae as proxy for coral

Sweetings Cay, Bahamas

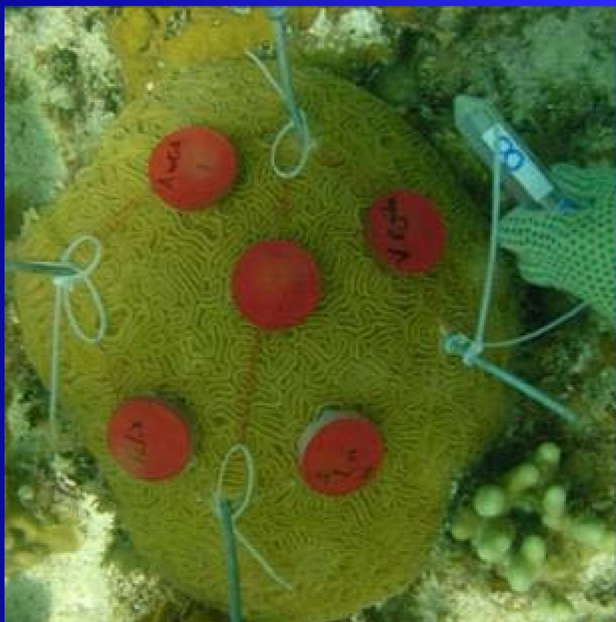
June 2003

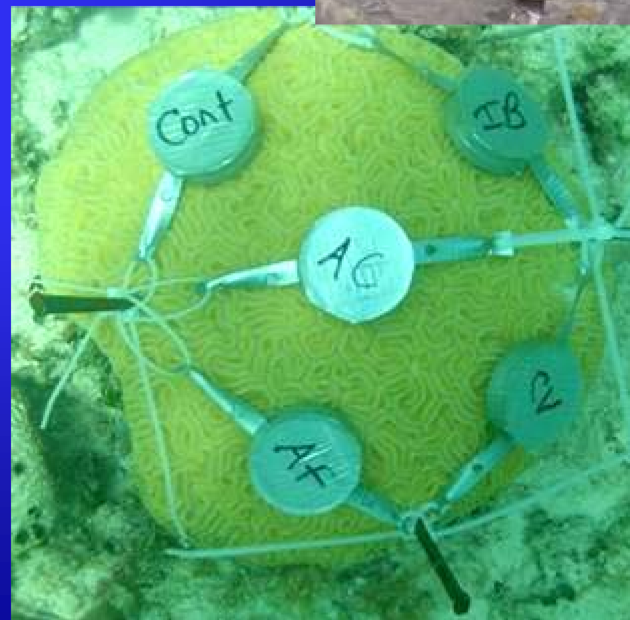
18 hr experiments

Coral: *Diploria labyrinthiformis*



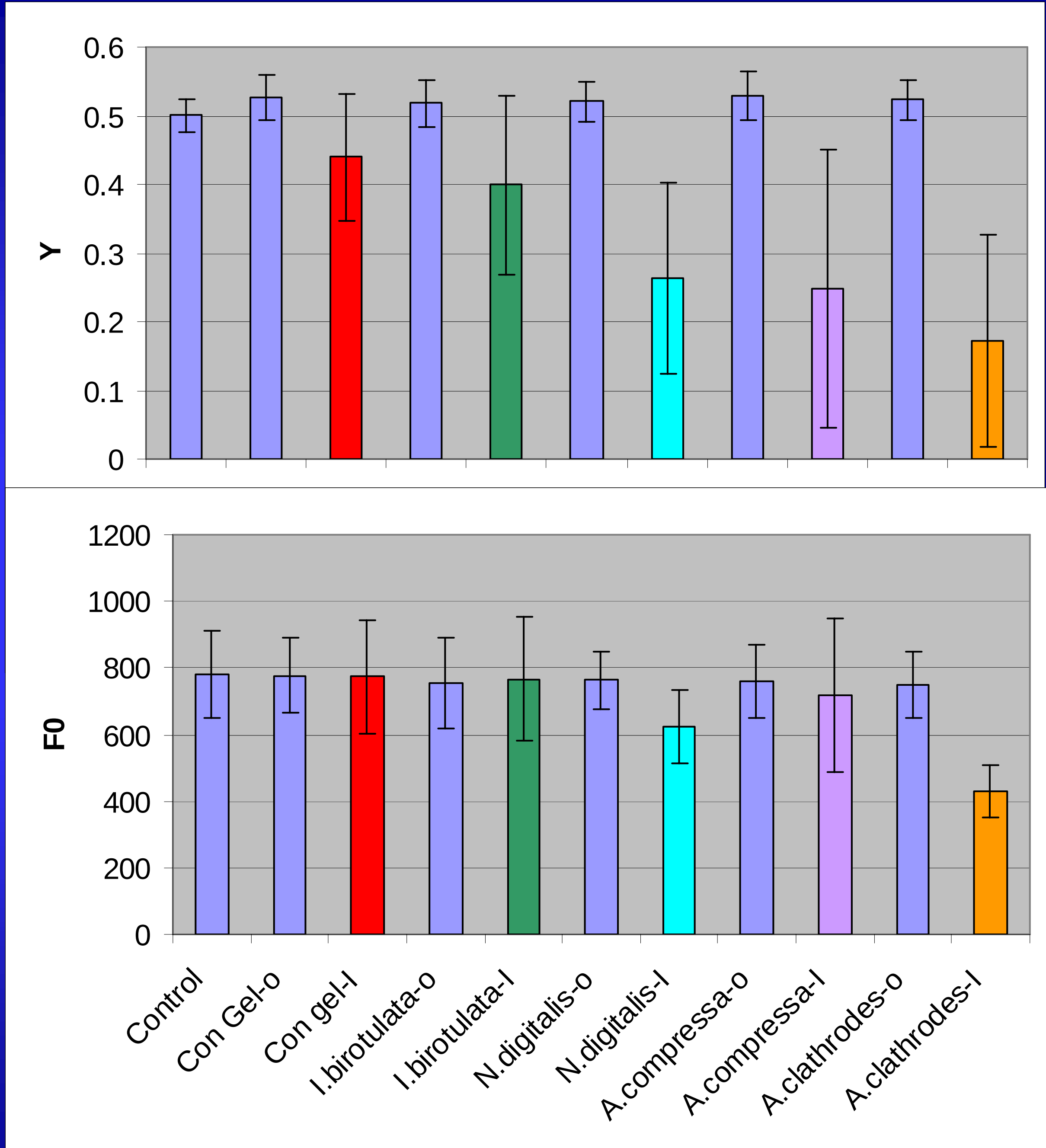
TECHNIQUE DEVELOPMENT



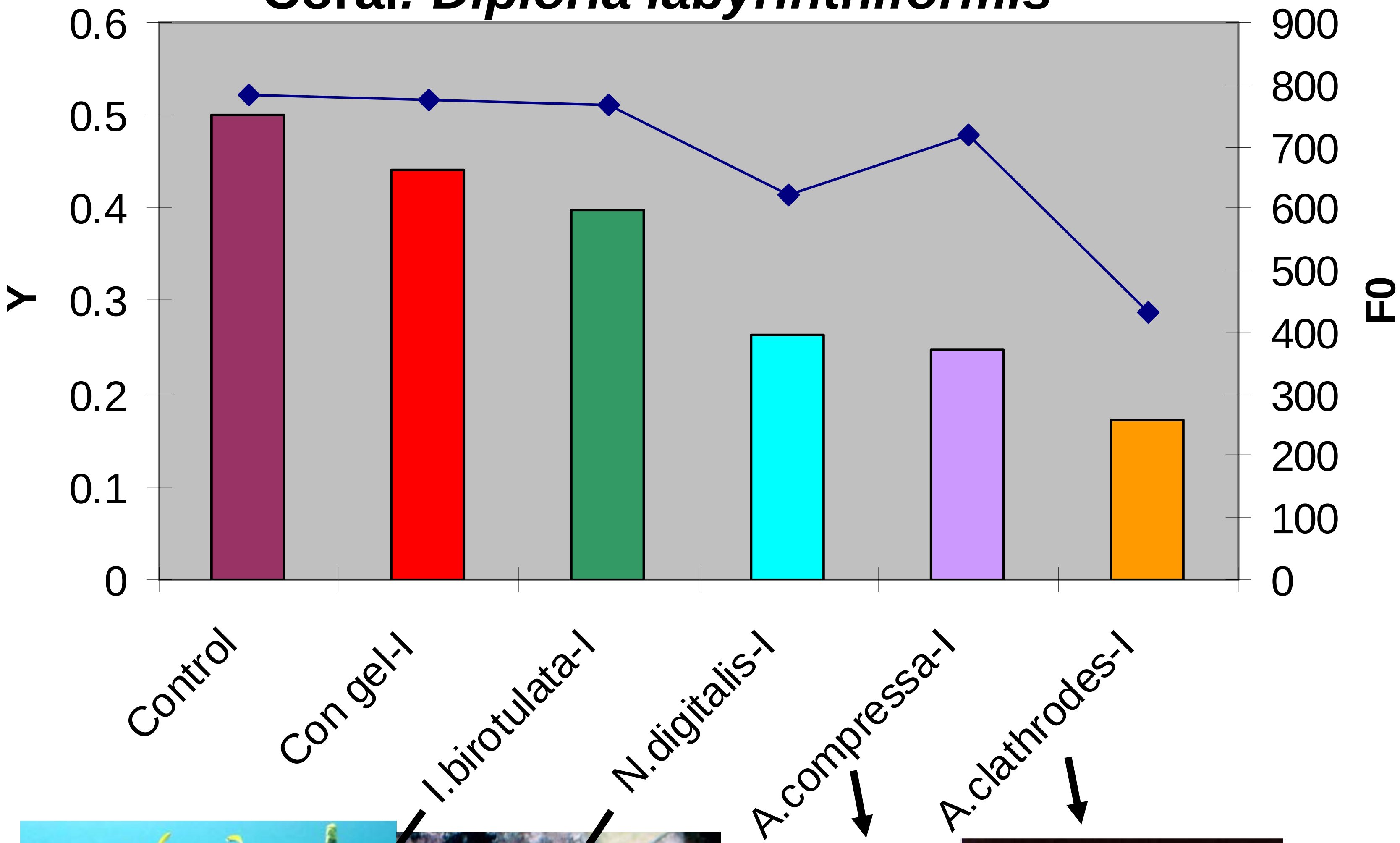


Y = effective quantum yield (health)

F = fluorescence of dark adapted coral (# of zooxanthellae)



Coral: *Diploria labyrinthiformis*



NEXT:

- More techniques development
- Survey of sponge-overgrowth spp.
- Bioassay-guided fractionation
- Isolation of active metabolites
- Test *Siphonodictyon* metabolites

TYPES OF CHEMICAL DEFENSES:

- Anti-predatory
- Anti-overgrowth (allelopathic)
- Antifouling
- Anti-pathogen
- UV radiation protection (sunscreens)

Assessing antifouling in the field

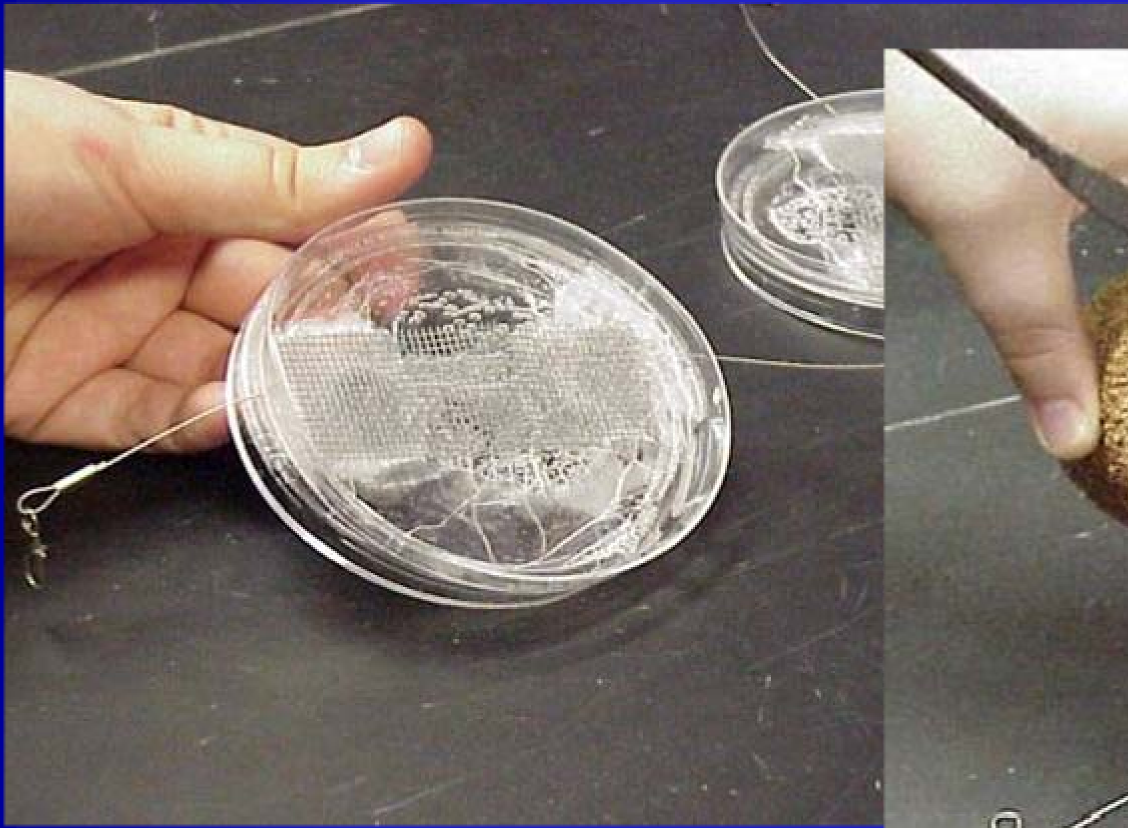
Advantages of field assay:

- natural flow regime and conditions
- natural population of propagules
- no “concentration artifacts”

Preparation of Gels:

made with Phytagel

natural volumetric concentrations



Assay Method:

•Crude organic extracts of 44 species of Caribbean sponge were incorporated into stable gels at natural and higher concentrations.
*Gels were deployed in the field for an average of 24 d.

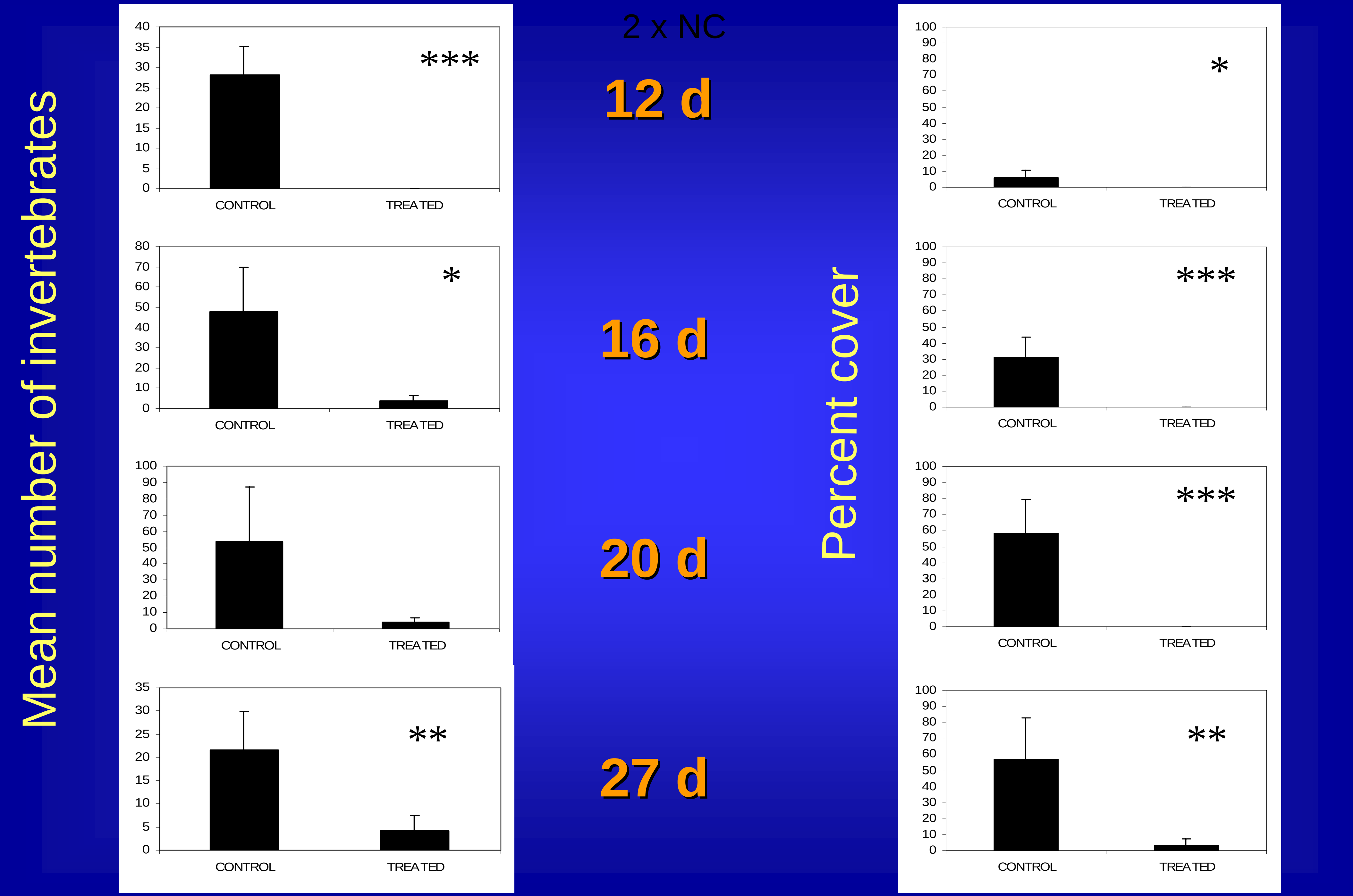
•Common settlers were:
- -barnacles
- -spirorbid worms
- -hydroids
- -bryozoans
- -tunicates
- -algae

Assay Method:

- Percentage cover was measured using a dot-grid estimate method

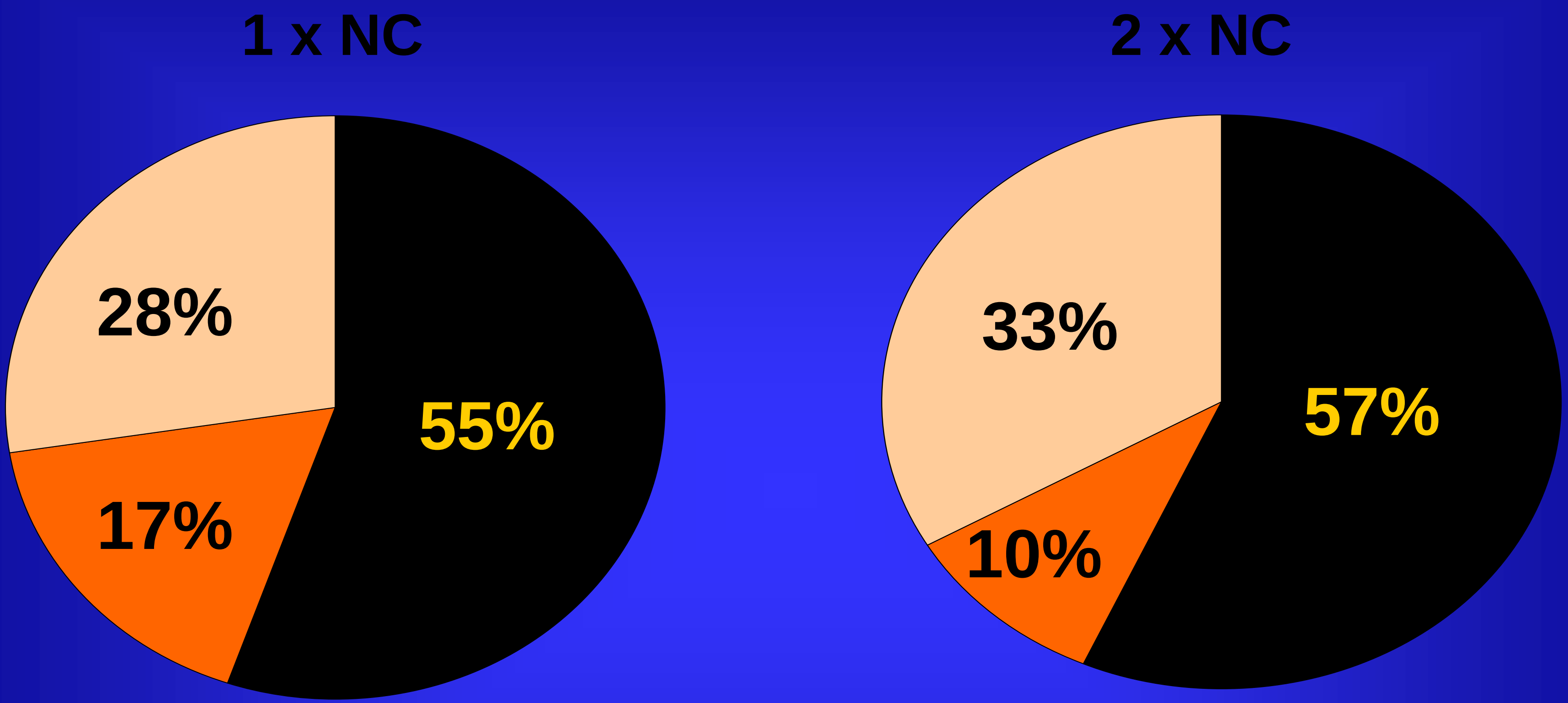
- Total number of individuals and colonies of sessile invertebrates were recorded

- Data was analyzed using a two tailed paired t-test ($p<0.05$)



2 x NC
Mean number of invertebrates
Percent cover
12 d
16 d
20 d
27 d
CONTROL
TREATED
***
*
**

Results: Total Percent Cover

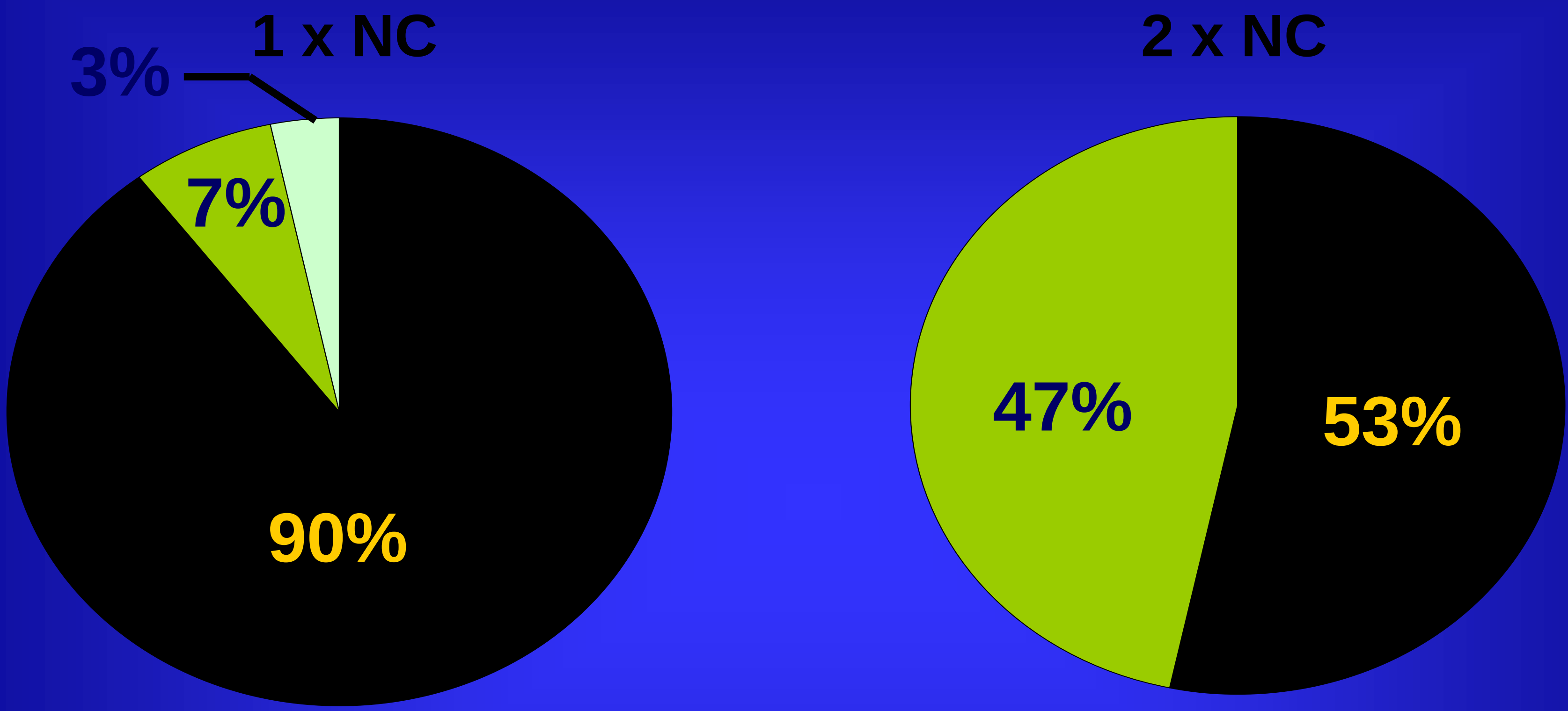


Percentage of extracts that were:

- Non significant
- Deterred algae and invertebrates
- Enhanced algae and invertebrates

April-August 1999

Results: Invertebrate counts

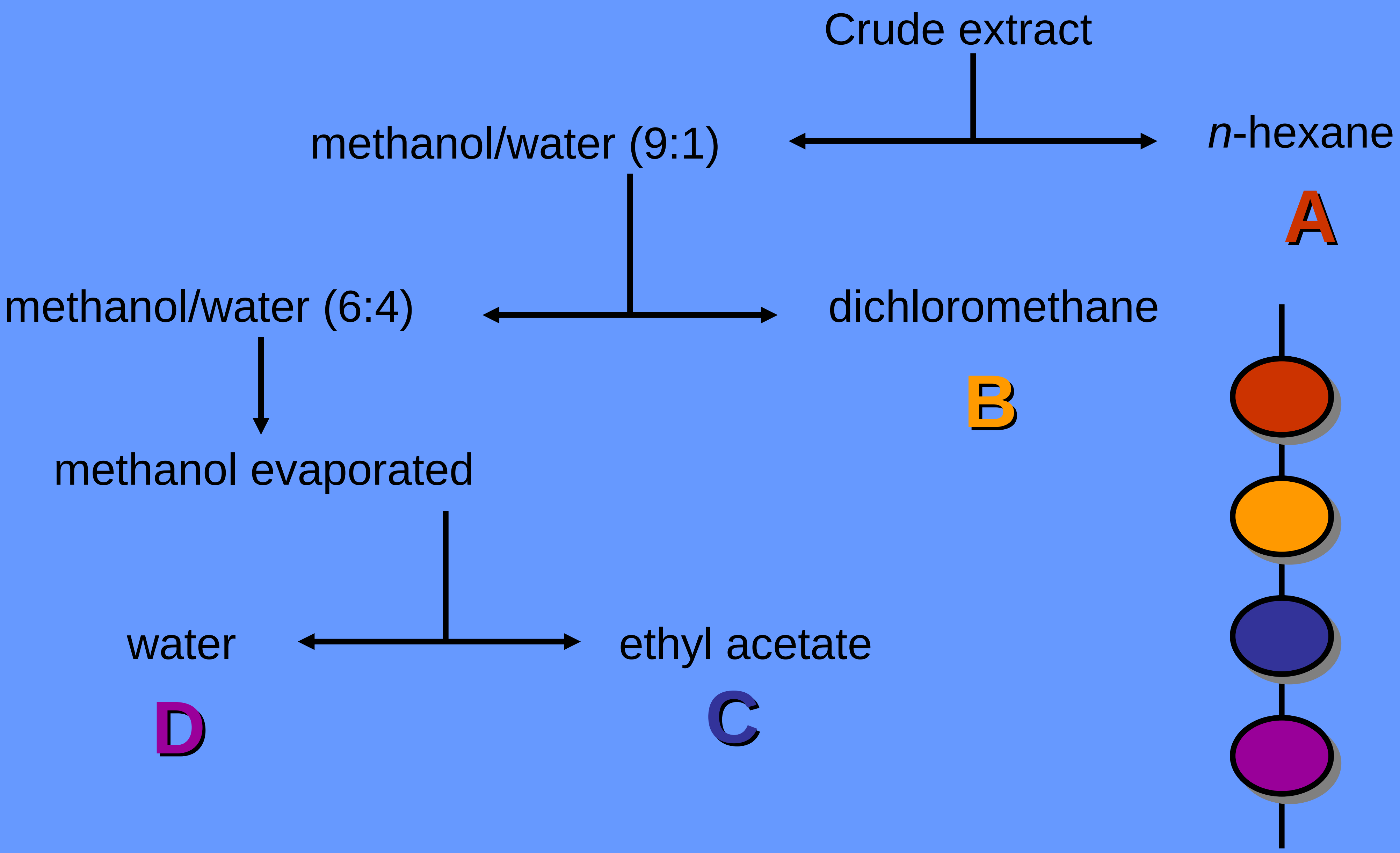


Percentage of extracts that:

- Non significant
- Deterred invertebrates only
- Enhanced invertebrates only

April-August 1999

Bioassay guided fractionation

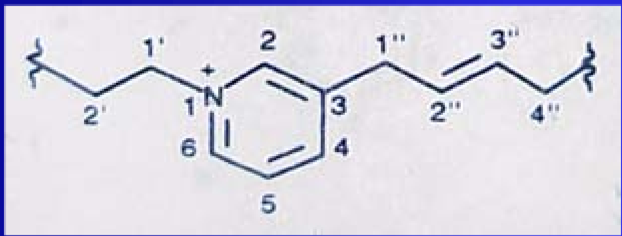


Results: Semi-purified extracts

	1 x NC		2 x NC					
	TPC	IMC	TPC	IMC	Hex	DCM	EtAc	Water
Callyspongia vaginalis	+		-	-	- (IMC)			
Ectyoplasia ferox	n/a	n/a		-				
Haliclona hogarthi	n/a	n/a		-			- (IMC)	
Lissodendoryx isodictyalis			+			+ (TPC)		
Siphonodictyon coralliphagum	-	-	n/a	n/a	- (TPC)			- (IMC)
Tedania ignis	n/a	n/a		-				
Xestospongia muta	+			-				+ (TPC)

semi-purified extracts assayed at 2x NC

Gels treated with organic extracts of *Amphimedon compressa*



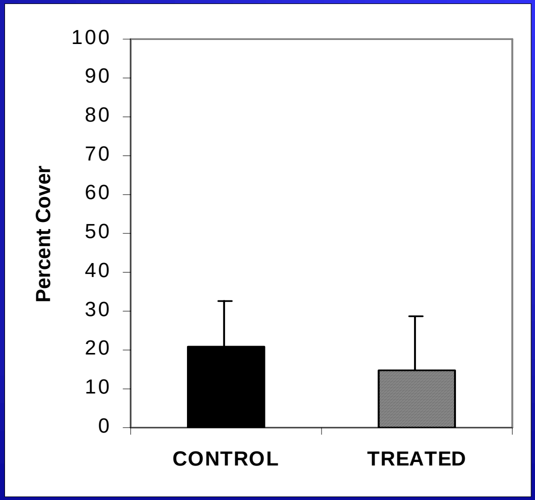
Treated Gel After 27 d in the field Control Gel

Gels treated with triterpene glycosides from *Ectyoplasia ferox*

Treated Gel

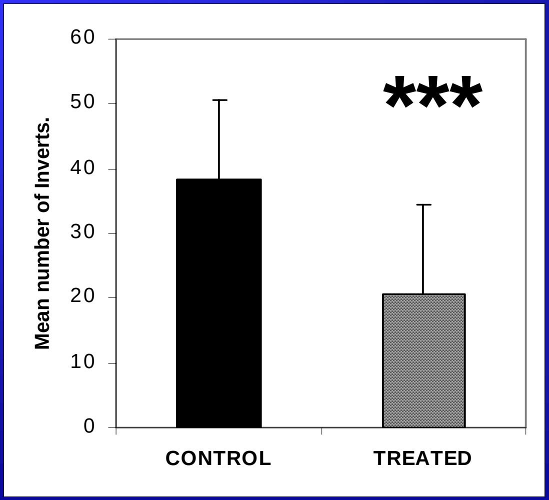
Control Gel

Total Percent Cover



Day 16

Invertebrate Mean Cover

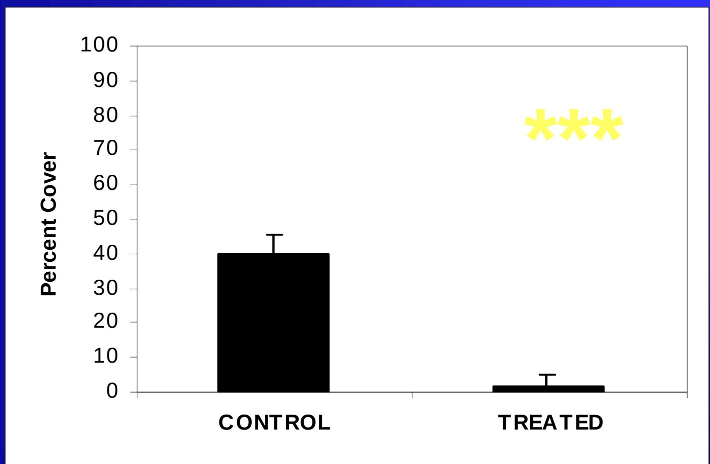


Gels treated with triterpene glycosides from *Erylus formosus*

Treated Gel **Control Gel**

(After 22 d in the field)

Total Percent Cover



Day 27

Invertebrate Mean Cover