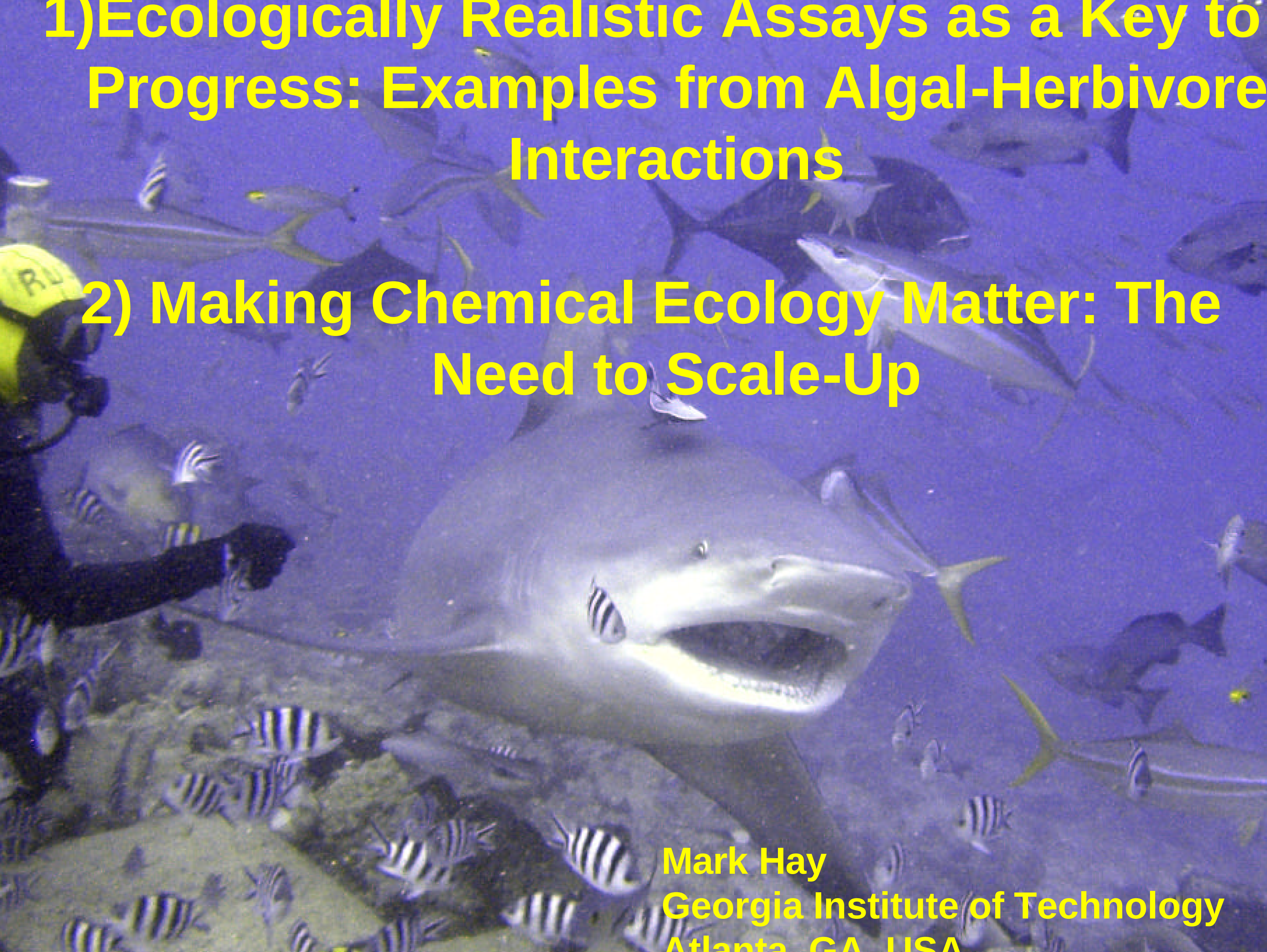
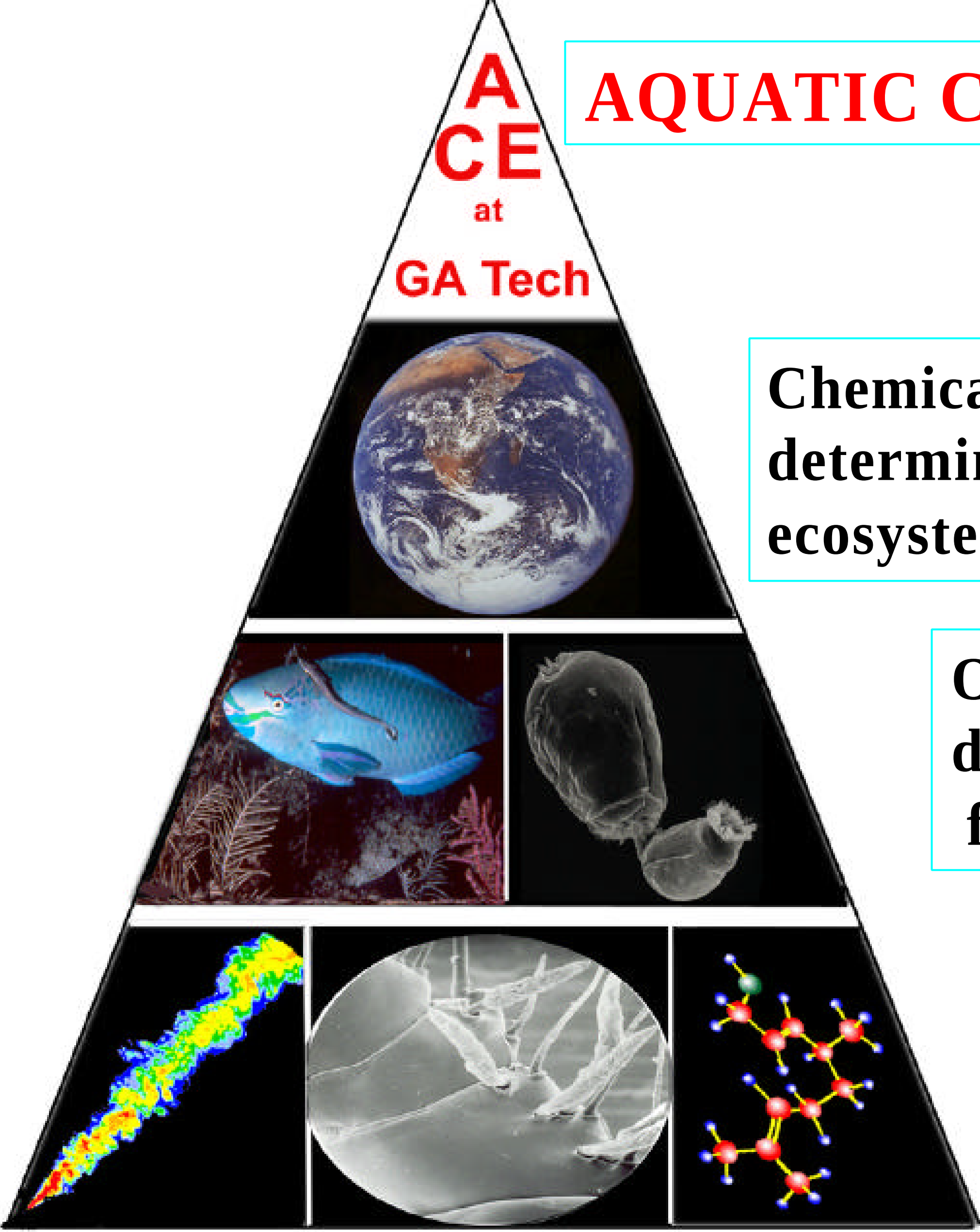


1)Ecologically Realistic Assays as a Key to Progress: Examples from Algal-Herbivore Interactions

2) Making Chemical Ecology Matter: The Need to Scale-Up

Mark Hay
Georgia Institute of Technology
Atlanta, GA, USA


THE DUKES OF HAZZARD
AUGUST 5
www.cgstock.com

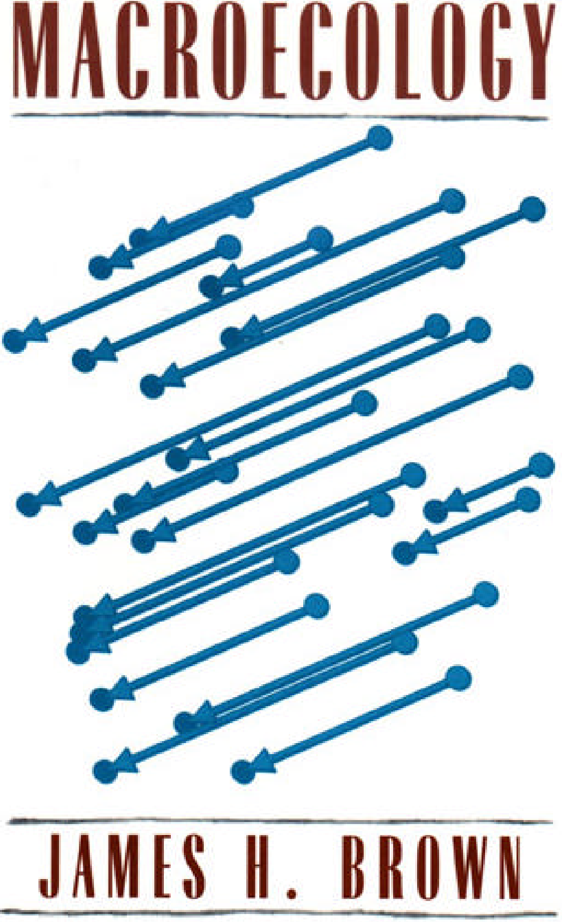


AQUATIC CHEMICAL ECOLOGY

Chemically-mediated interactions determine community structure and ecosystem function.

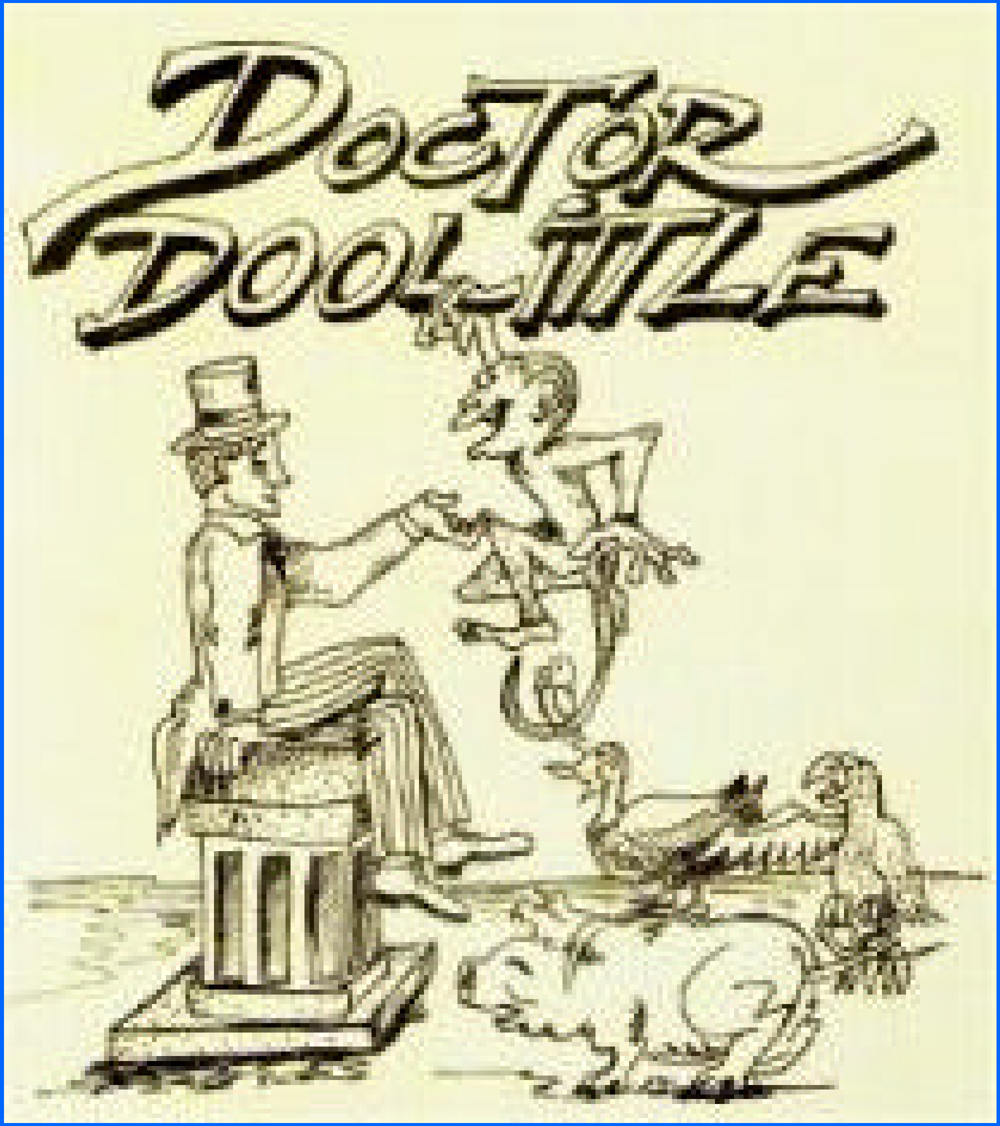
Organisms use chemicals to deter enemies, sense danger, find mates, and feed.

Chemistry, physics and sensory physiology are the base. Chemicals transporte in moving fluid interact with receptors.



Macroecology is self-consciously expansive and synthetic. In this r
spect it does differ philosophically from much of traditional ecolog
which I would characterize as becoming increasingly reductionist ar
specialized.[1] Rather than trying to use ever more powerful microscop
to study the fine details of ecological phenomena, macroecology tries
develop more powerful macroscopes that will reveal emergent patter
and processes. To make an analogy, the goal is not to understand a tape
try in terms of warp and woof and the chemistry of fibers and dyes, b
to see and interpret the entire scene. In order to visualize the big pictu
it is necessary to stand back and take a distant view. Accordingly, macro
cology attempts to increase the spatial and temporal scale of ecologi
inquiry, and also to expand the kinds of questions asked and the range
phenomena studied. It tries to achieve synthesis by exploring the relatio
ships between ecological phenomena and the patterns and process
studied by basic and applied scientists in other disciplines.

1. An extreme example is the subdiscipline of chemical ecology, which has taken an exceptionally reductionist, high-technology approach to studying chemically mediated processes, such as plant defense against herbivores. Many compounds important in ecological interactions have been isolated and their chemical formulae and structures characterized. In order to obtain this level of reductionist precision, however, much of the research is being done by chemists who have little knowledge of or interest in the effects of these compounds on free-living individuals, populations, communities, and ecosystems.



Chemical signaling IS the language of life. Understanding the language allows access to deeper ecological and evolutionary insights in the fields of Ecology/Evolution/Behavior.

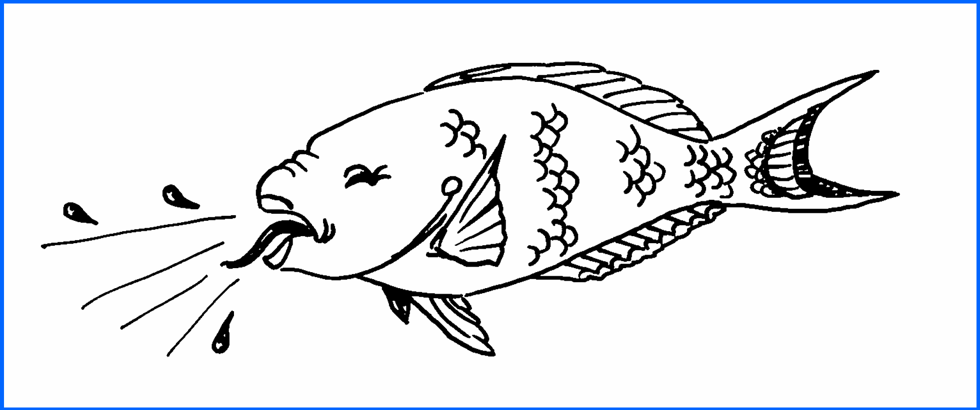
To Prove Jim Brown Wrong:

Studies of interactions need to be imbedded within a community context. Pairs of interactions alone will not be adequately informative.

Chemical defenses and the cascades of ecological and evolutionary interactions they produce need to be elaborated (complex interactions are the norm - not the exception)

Think Dials (variables) not Switches (dichotomies). Variance is the norm, appreciate it and work with it. Variance is the fuel of evolution, don't ignore it.

THE ECOLOGY OF YUCK



How do Consumers Affect Prey Traits?
How do Prey Traits Affect Consumers?
The Cascade of this through Multiple Trophic Levels
Effects at Various Spatial and Temporal Scales

Chemical Signals Structure Populations, Communities, and Ecosystems

In the Beginning

Soaking and injecting

=

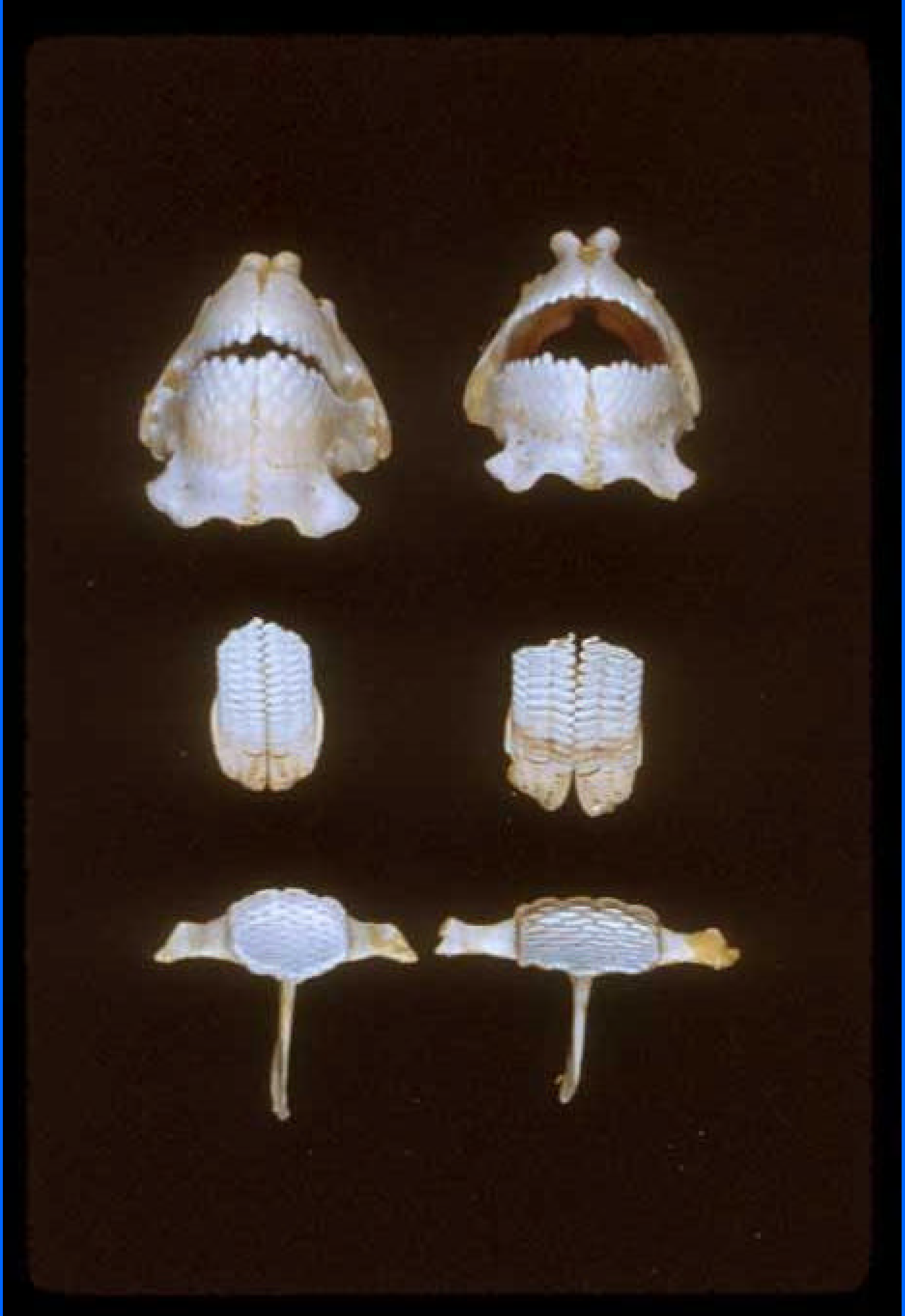
=

NOT <u>ecologically</u> valuable

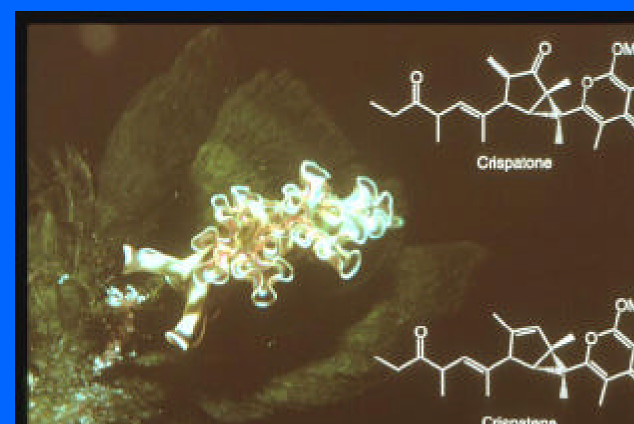
Defense against consumers must be tested by feeding assays – no matter how dangerous the animals

Smokin' Al's
Famous
BBQ JOINT

Some Natural History: Which herbivores matter, which systems to use, where and how to conduct assays?



Which Herbivores Select for Defenses? (Large mobile generalists vs. smaller, less mobile specialists - the buffalo vs. insect contrast)



(Lewis 1986 Ecological Monographs


10 weeks of growth - gone in 24-48h

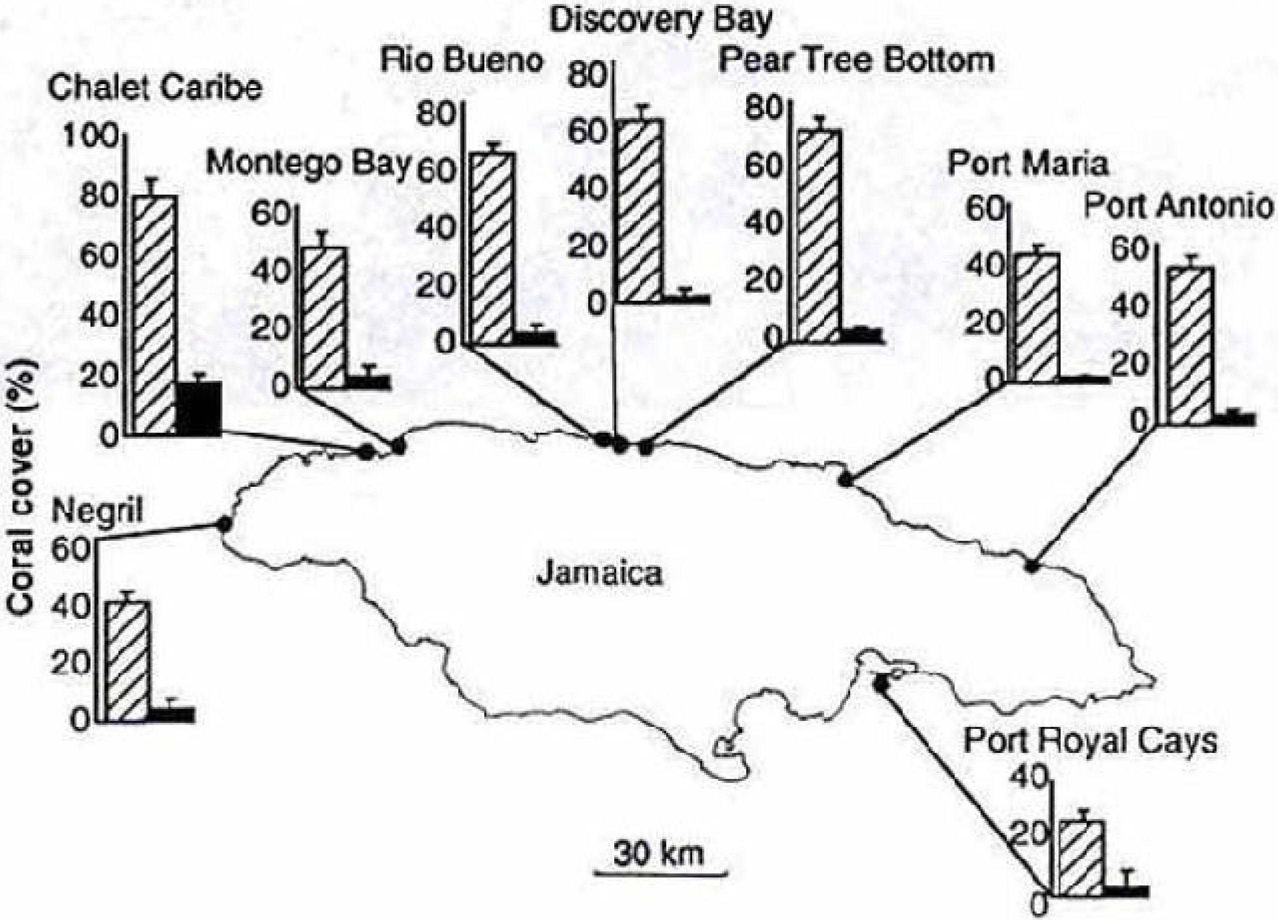


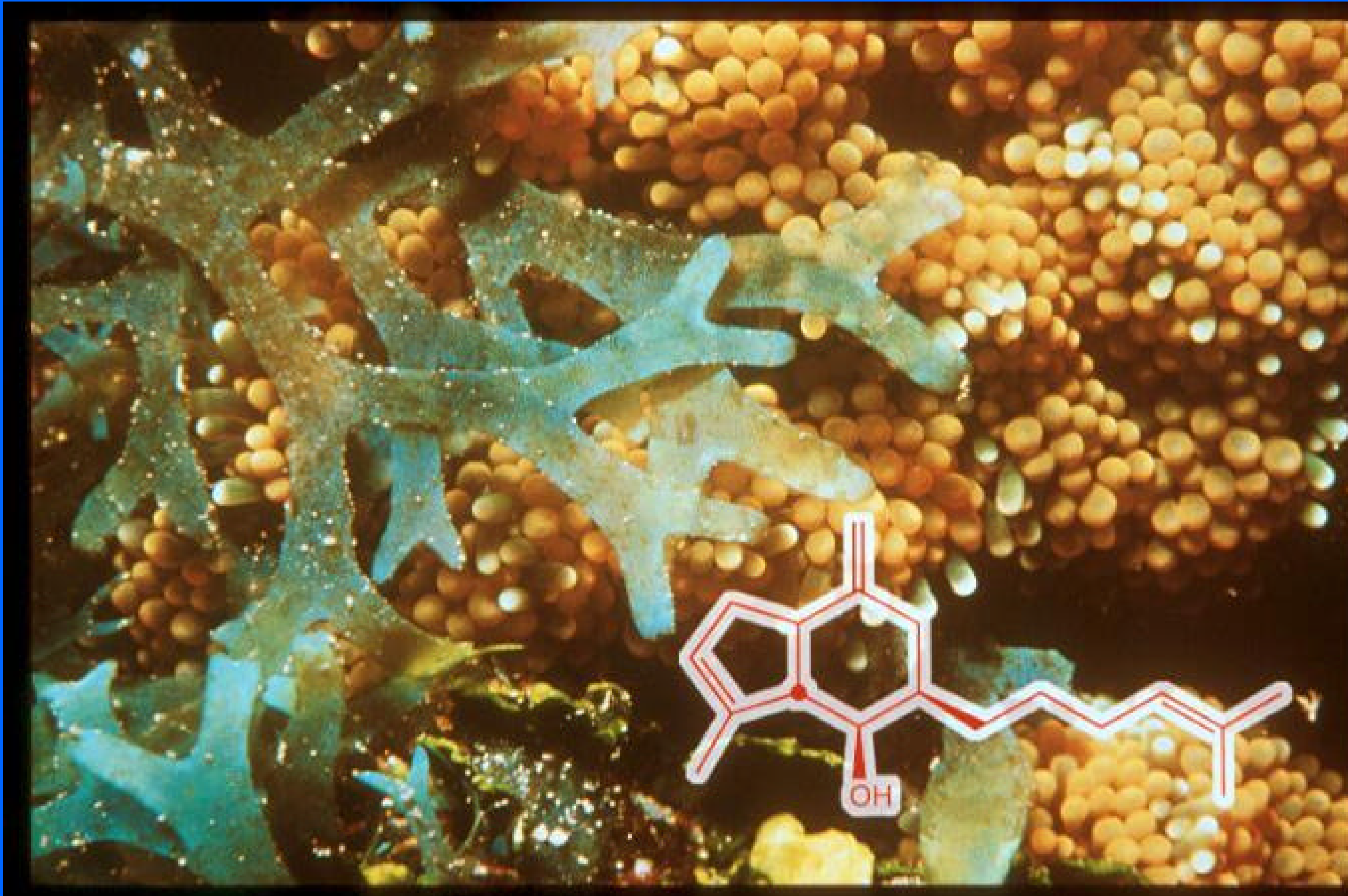
Fig. 5. Large-scale changes in community structure at fore-reef sites along >300 km of the Jamaican coastline, surveyed in the late 1970s (1977, hatched bars) and the early 1990s (1993, solid bars) (*34*).

(Hughes 1994 Science)

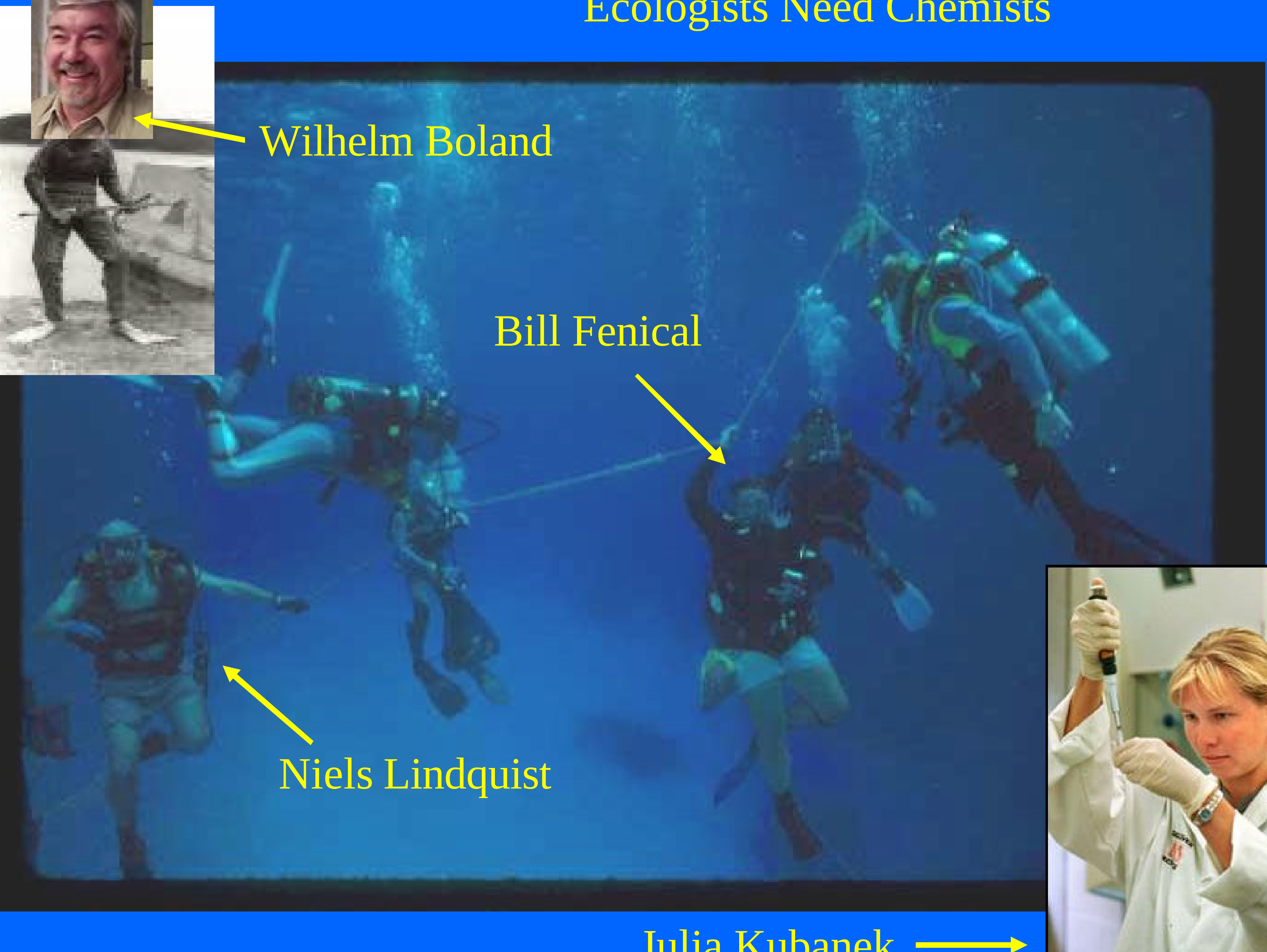
he question matters:

1) does compound “X” defend Dictyota by deterring feeding?

2) What traits allow Dictyota to co-exist with herbivores?

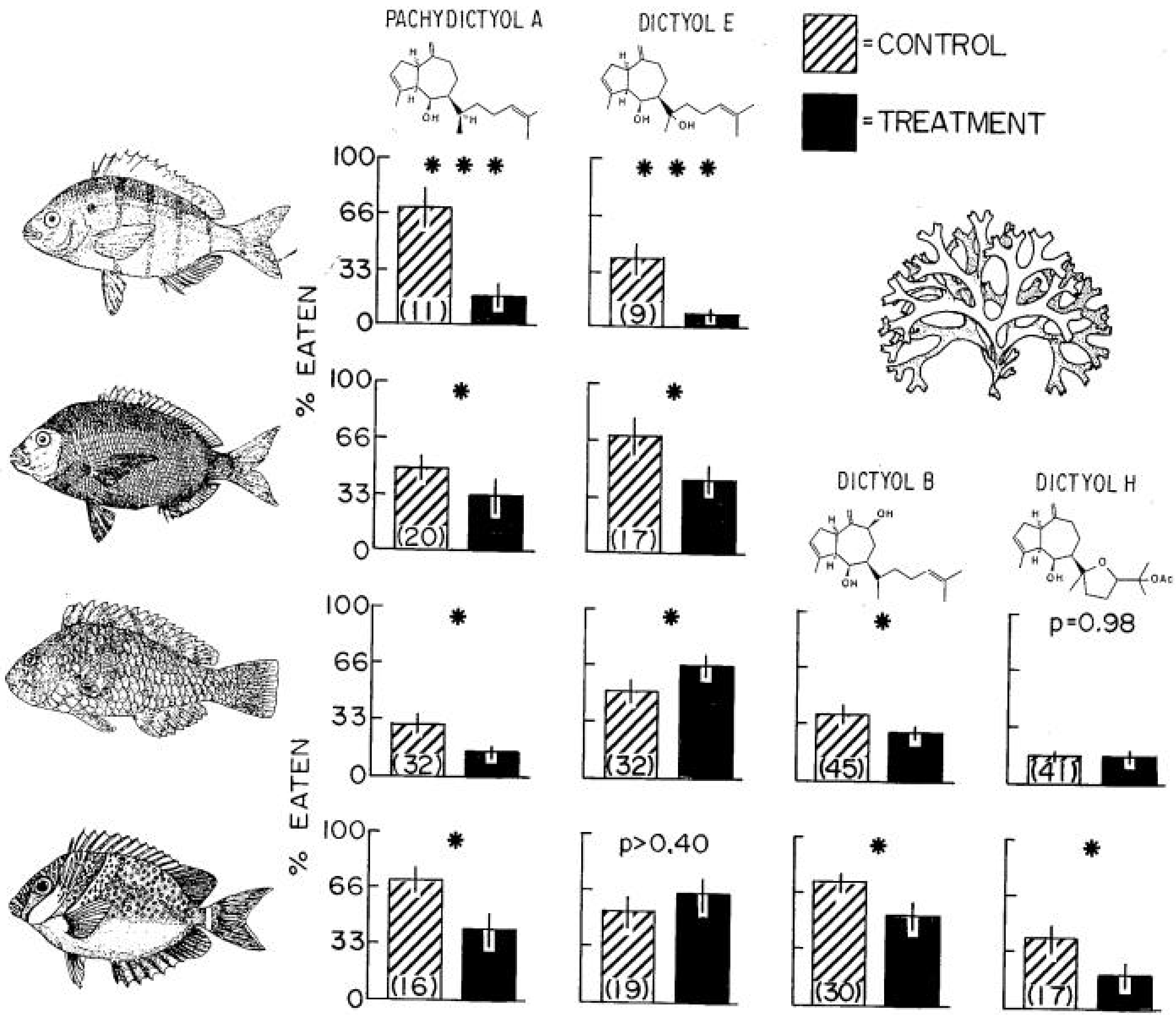


Ecologists Need Chemists



Patch Size – Sampling effects? Duration? Compound solubility and stability?

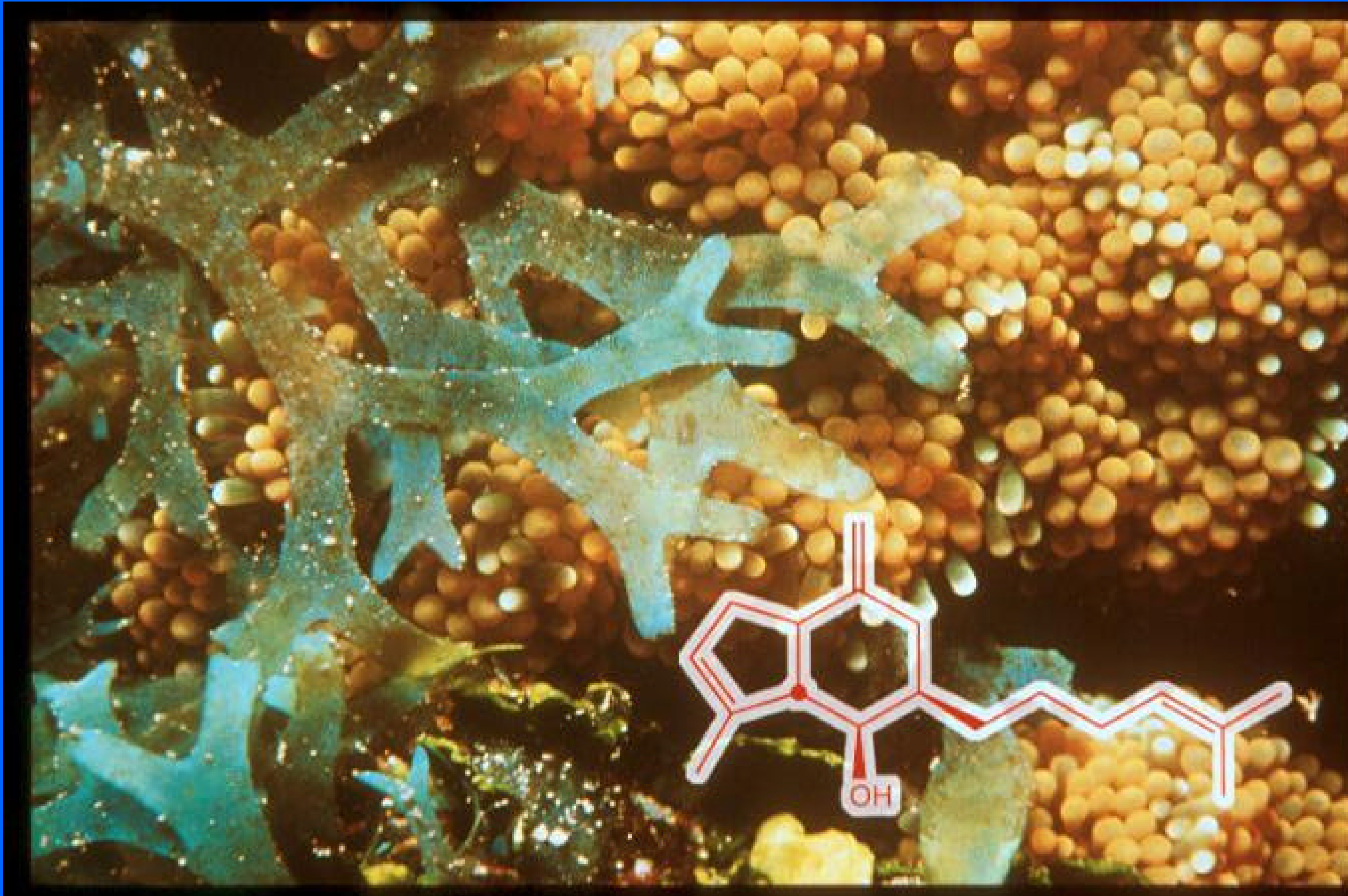
DICTYOLS TESTED AT 1% OF DRY MASS



he question matters:

1) does compound “X” defend Dictyota by deterring feeding?

2) What traits allow Dictyota to co-exist with herbivores?



Methods: Feeding Assays

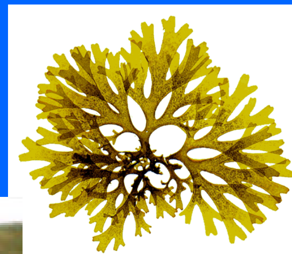
Whole Organism

Eaten

Avoidance Strategy?

Rejected

Freeze Dry & Grind



Feeding Assay

Nutritional?

Rejected

Eaten

Structural Defense?

Extract with Solvents

Incorporate Extract into Appropriate Food

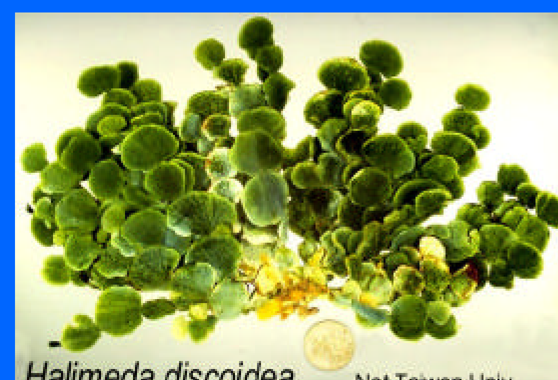
Feeding Assay

Rejected

Eaten

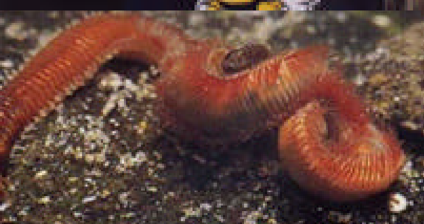
Chemical Defense?

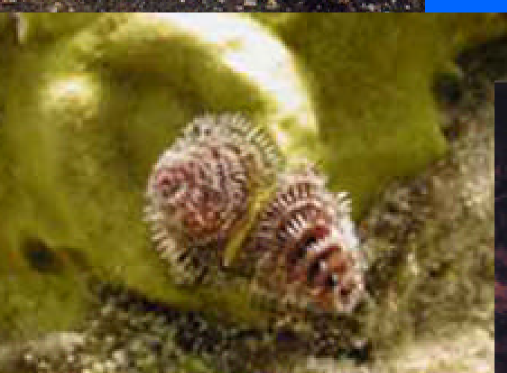
Decomposed chemistry, Inaccessable chemistry, Other

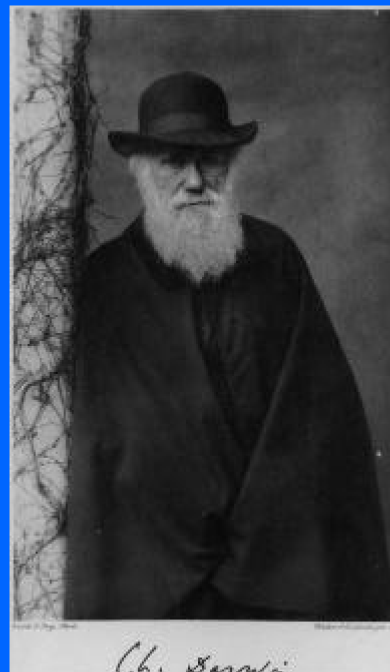


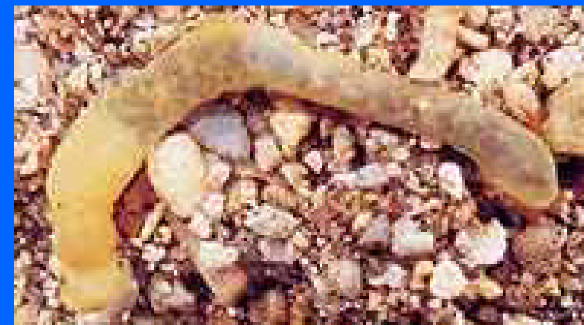
The Approach Works with ALMOST Any Taxonomic Group

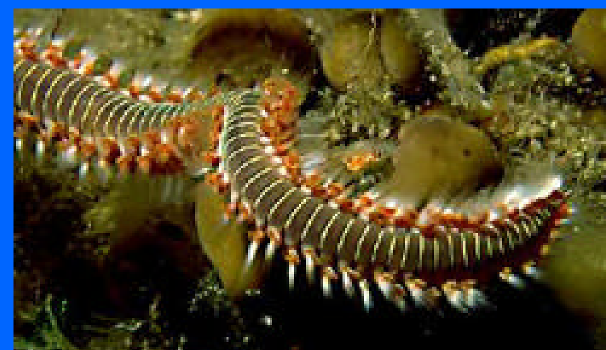
“WORMS have played a more important part in the history of the world than most persons would at first suppose”.—Darwin, 1883







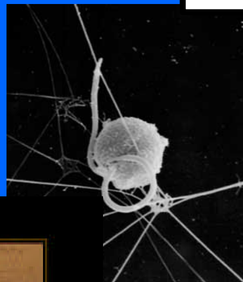




Phyto… aaarugh!!!

Whole Organism → Eaten → Avoidance Strategy?

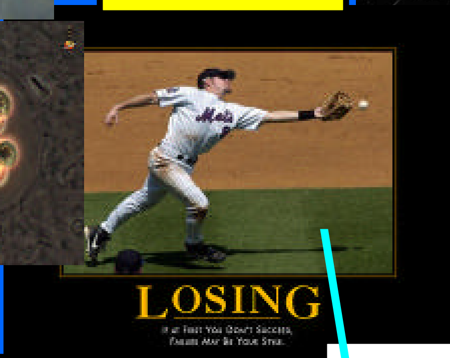
Whole Organism → Rejected → Freeze Dry & Grind → Feeding Assay



Feeding Assay → Eaten → Structural Defense?

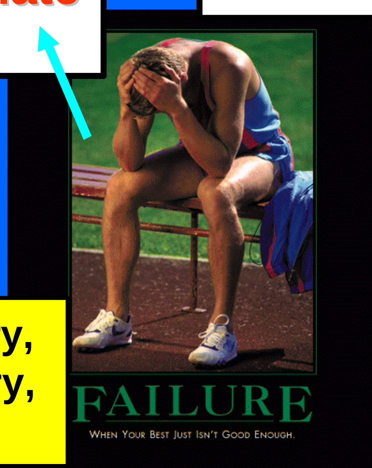
Feeding Assay → Rejected → Nutritional?

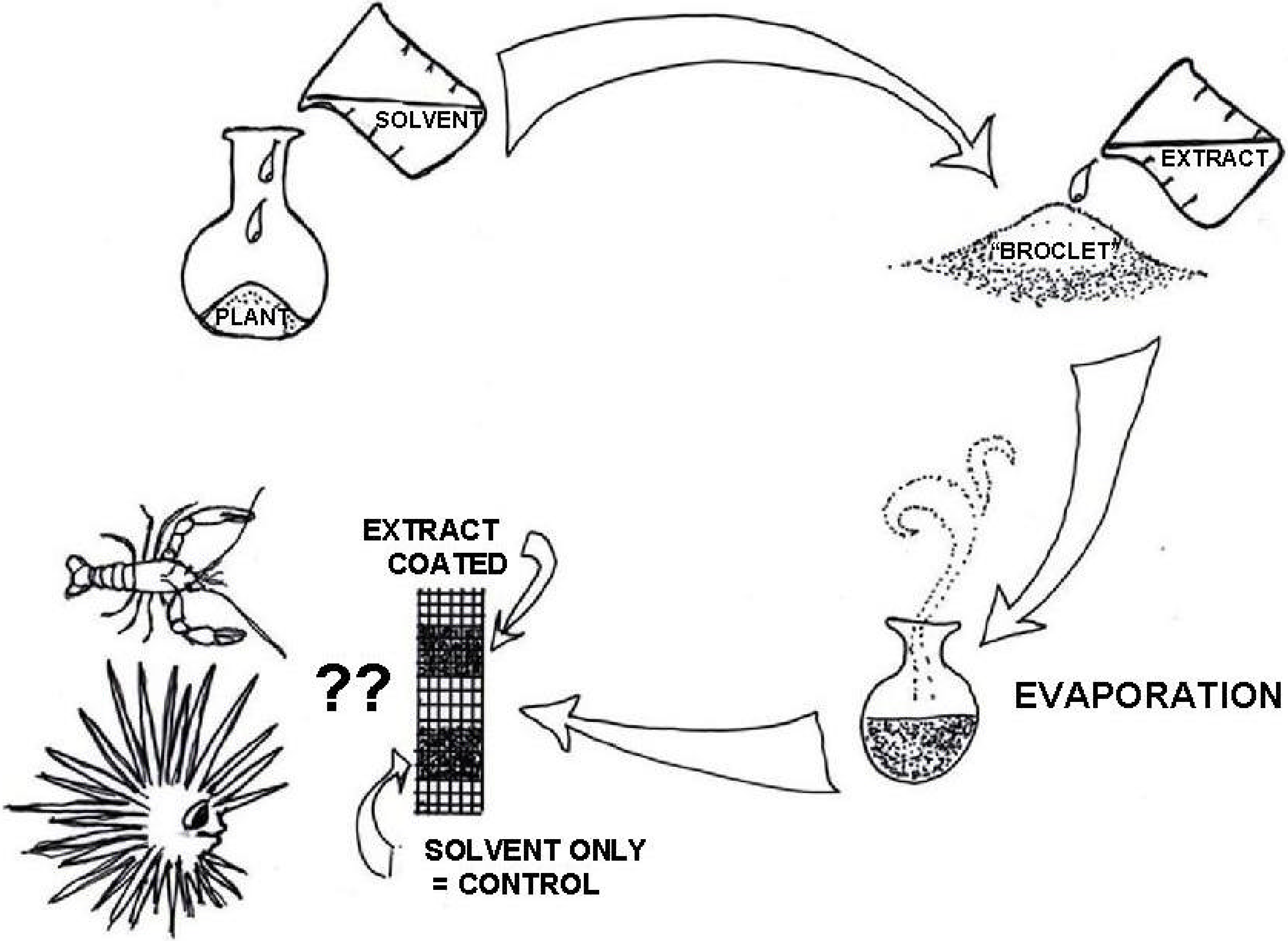
Rejected → Extract with Solvents → Incorporate Extract into **Appropriate** Food → Feeding Assay

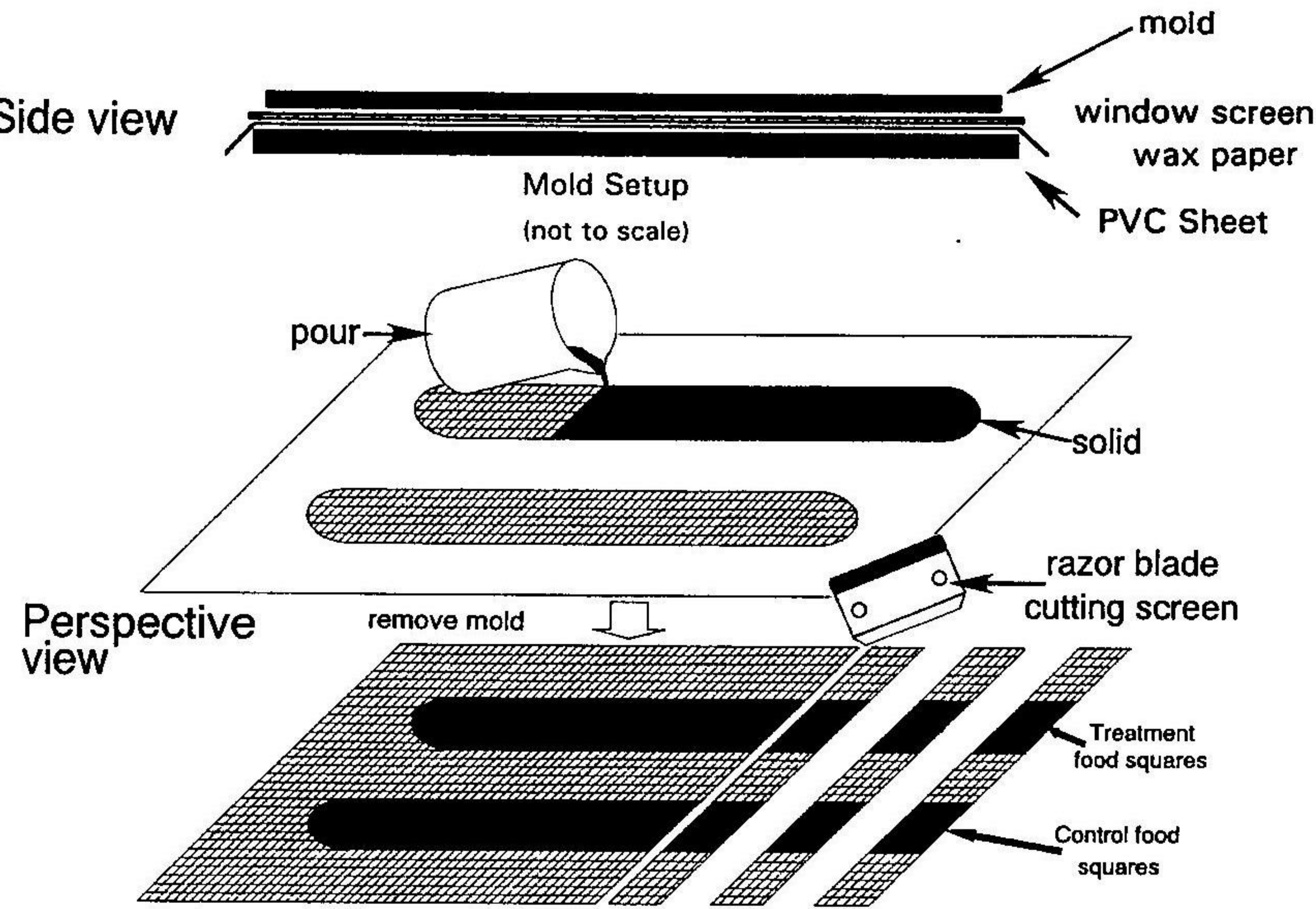


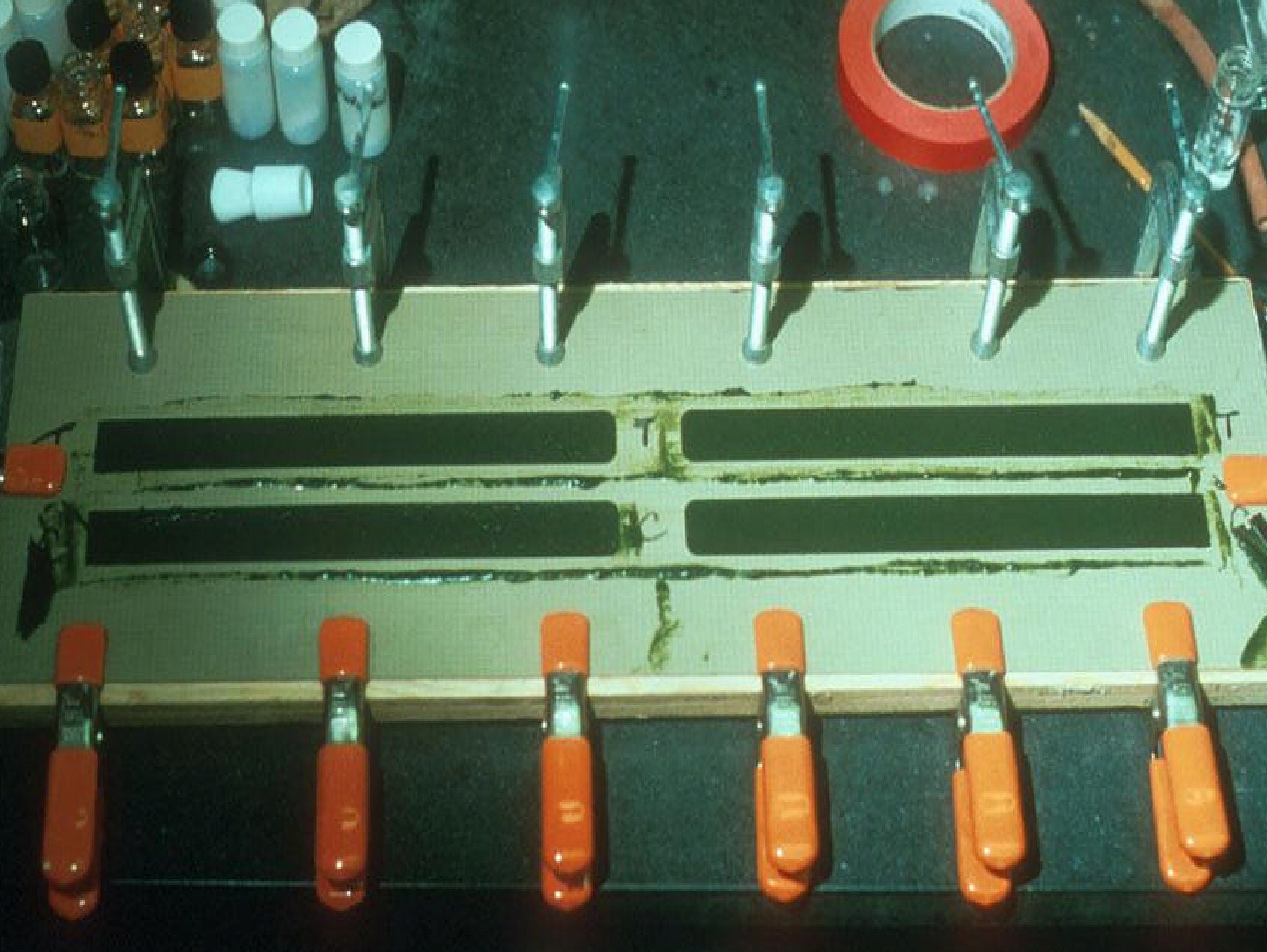
Feeding Assay → Rejected → Chemical Defense?

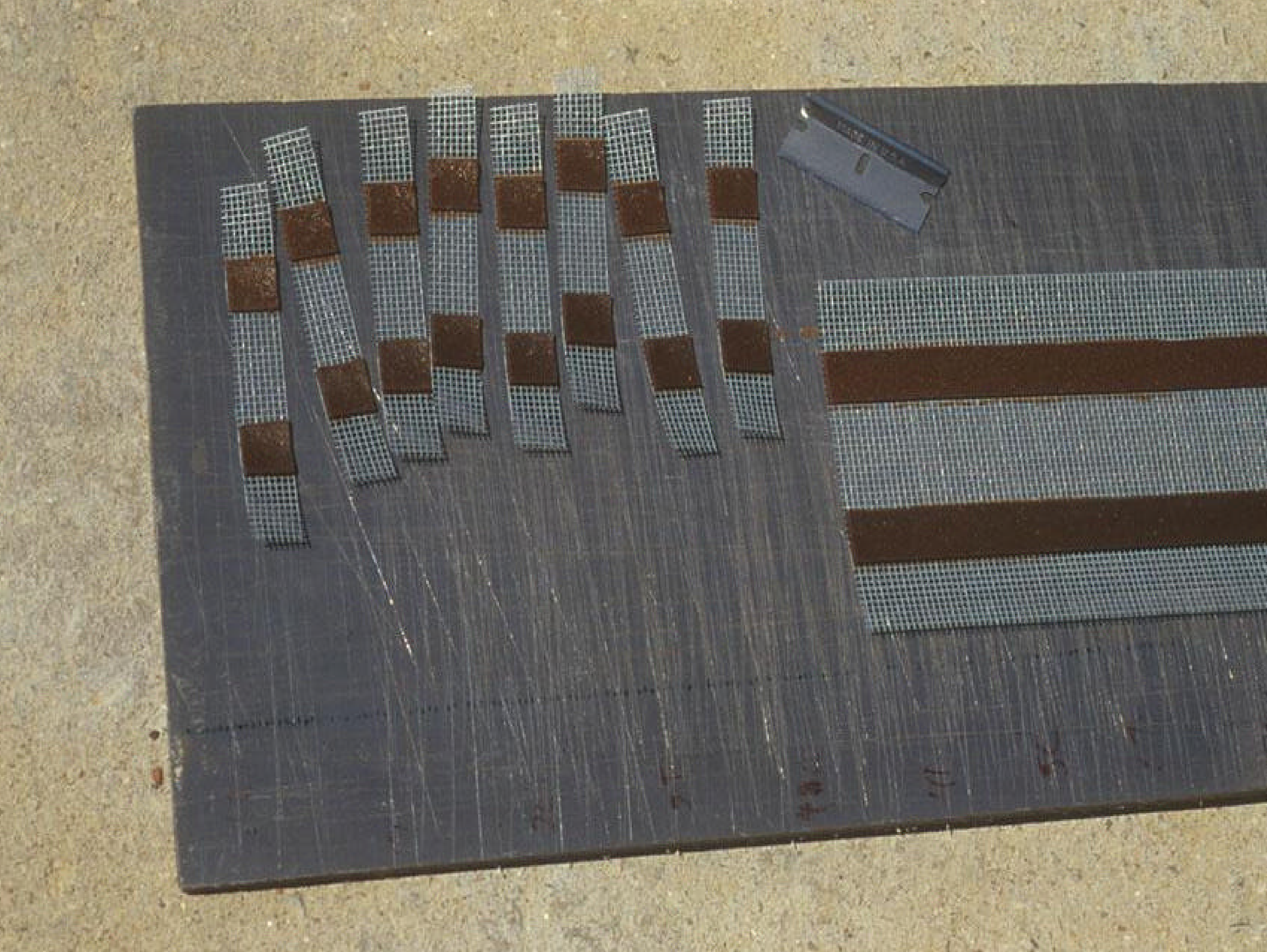
Feeding Assay → Eaten → Decomposed chemistry, Inaccessable chemistry, Other

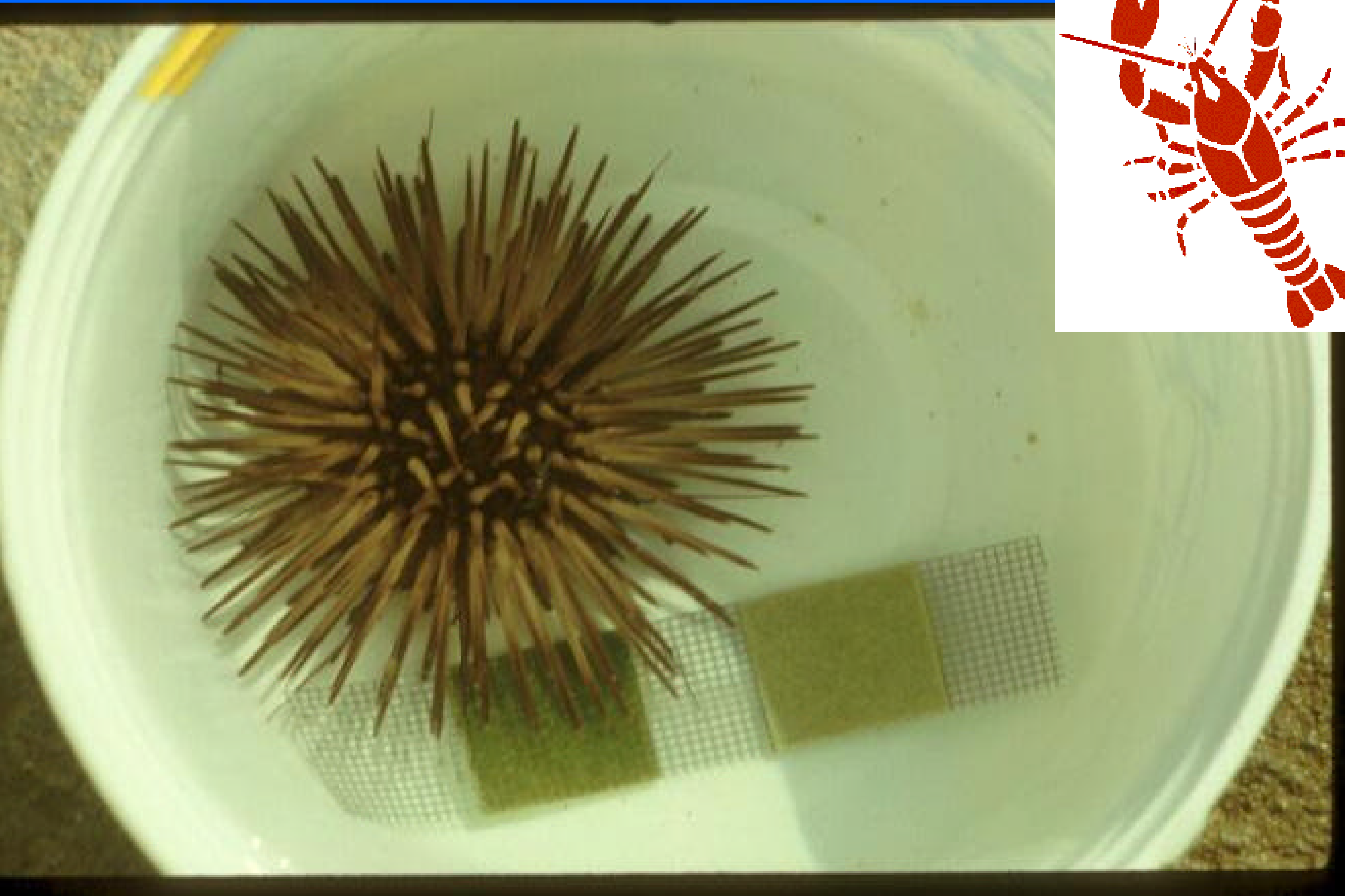



SOLVENT
PLANT
EXTRACT
"BROCLET"
EVAPORATION
EXTRACT
COATED
??
SOLVENT ONLY
= CONTROL

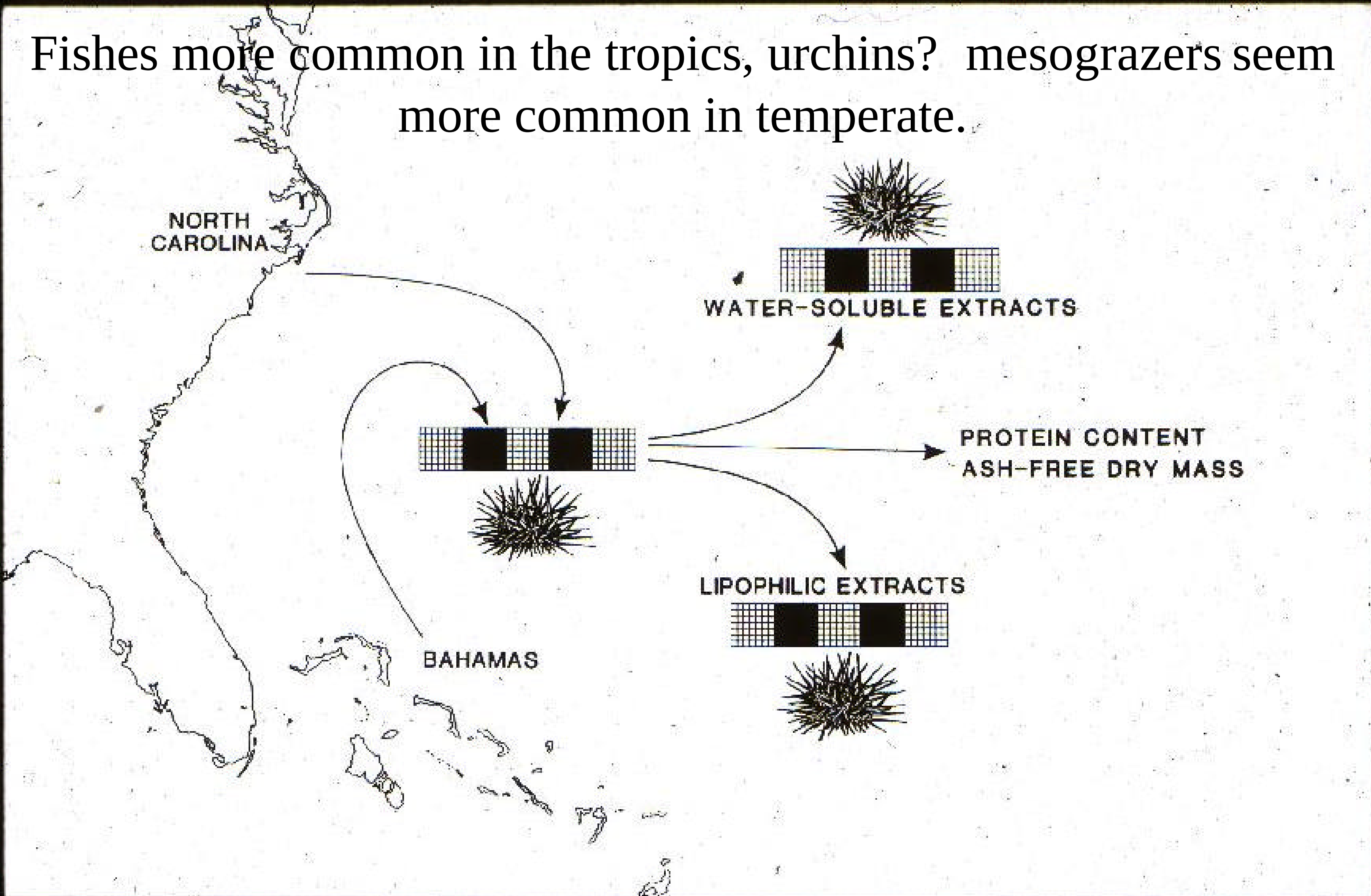

mold
Side view
window screen
wax paper
Mold Setup
(not to scale)
PVC Sheet
pour
solid
razor blade
cutting screen
Perspective view
remove mold
Treatment food squares
Control food squares



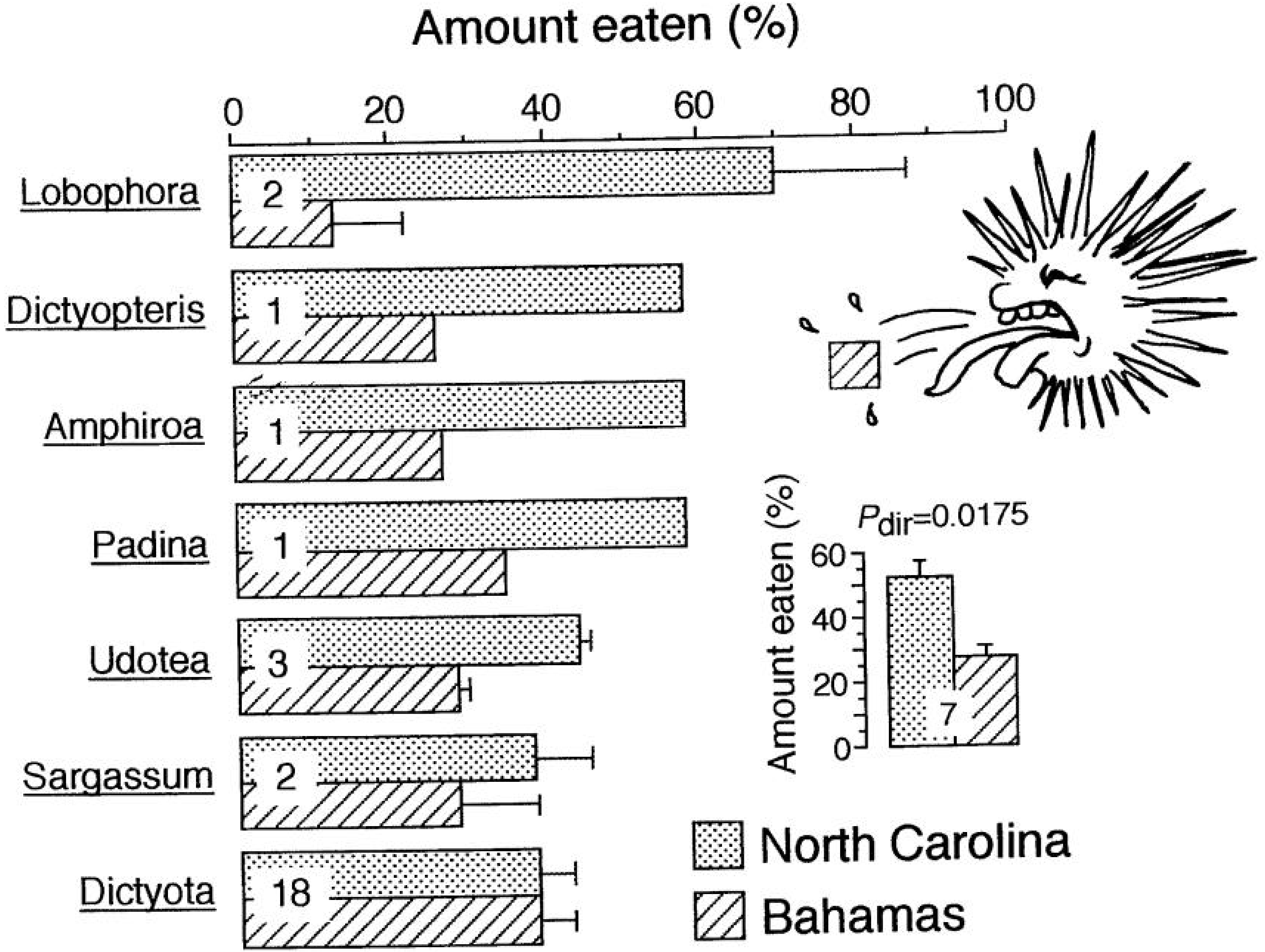




Fishes more common in the tropics, urchins? mesograzers seem more common in temperate.



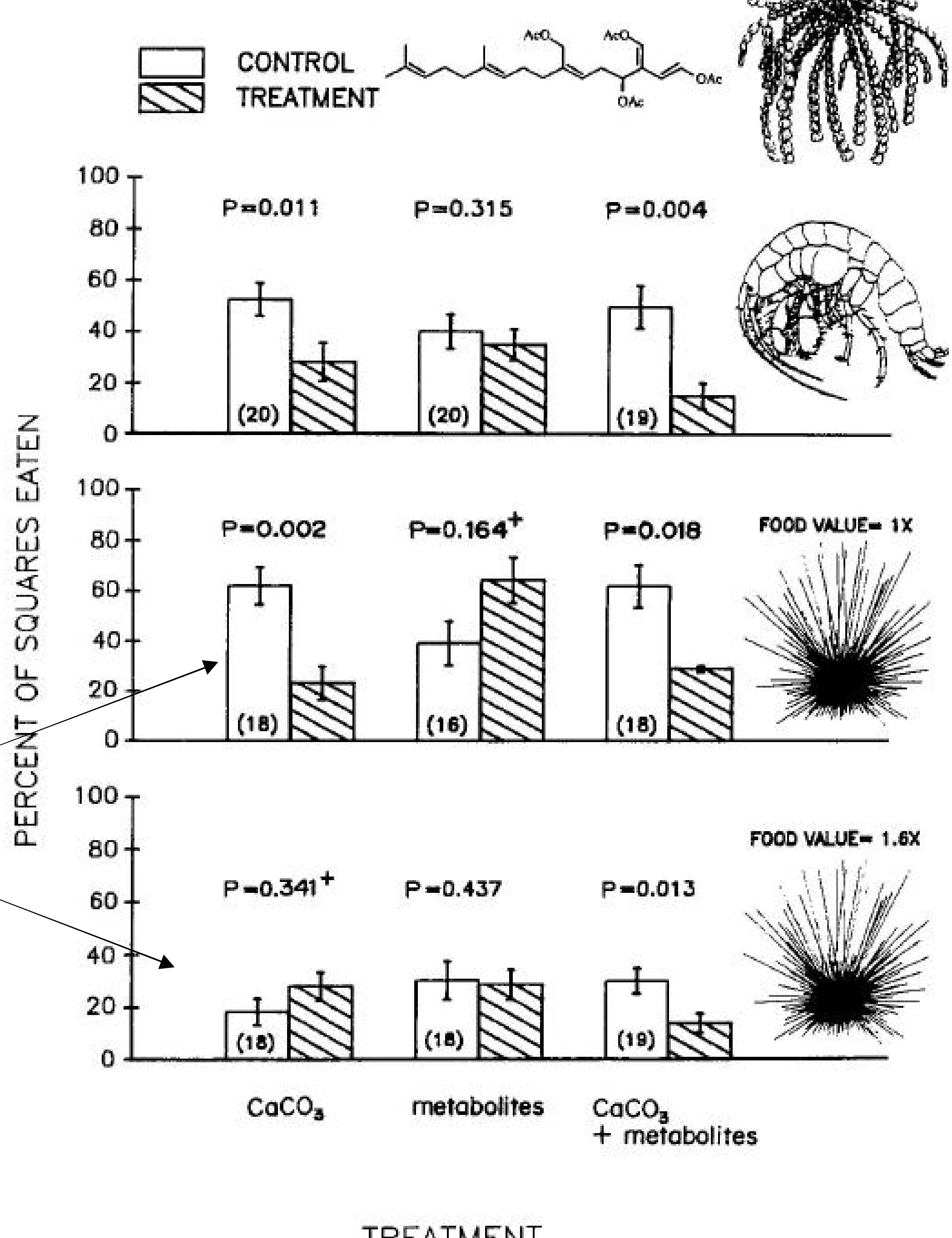
Bolser and Hay 1996 Ecology

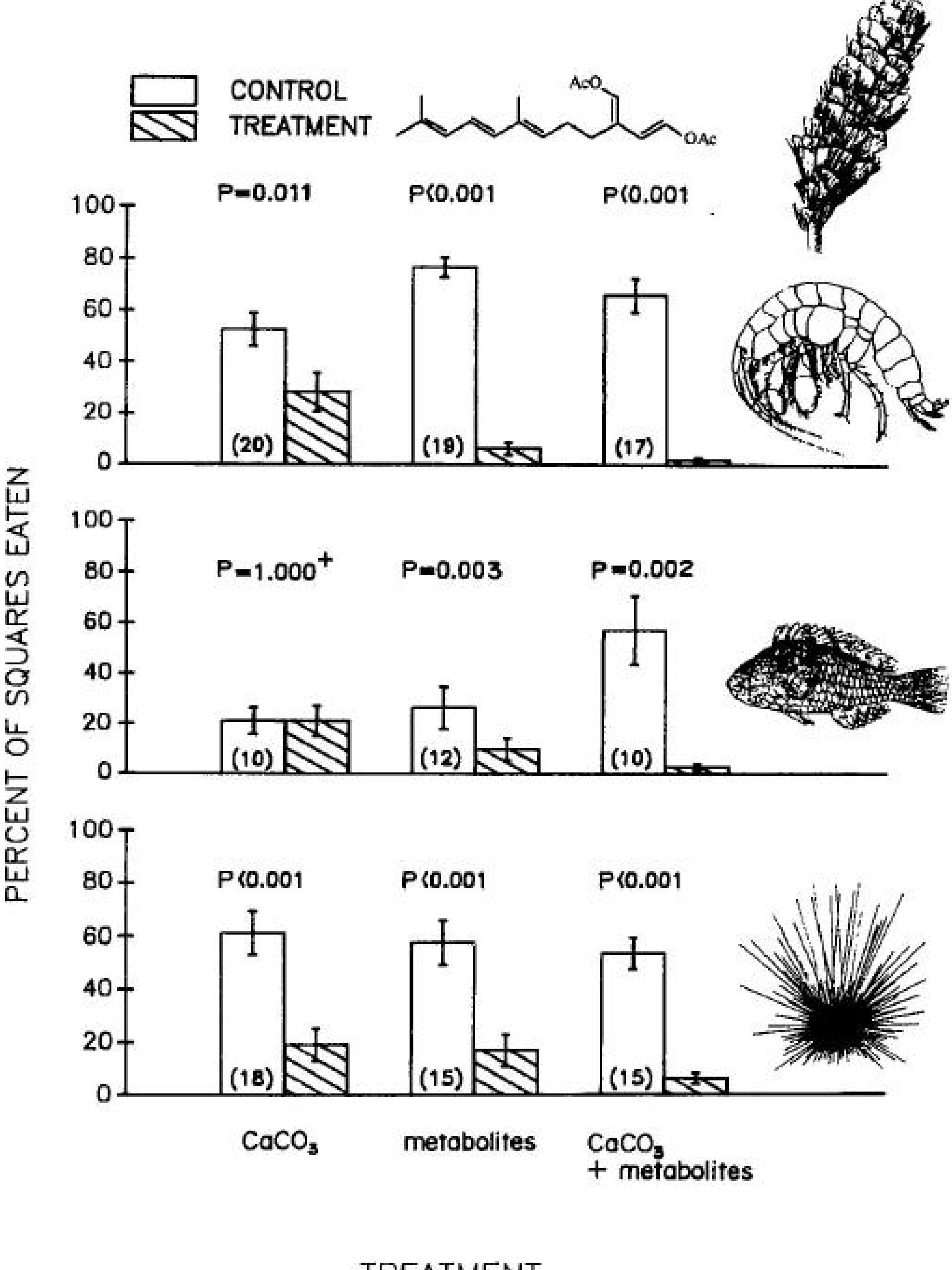

Amount eaten (%)
0
20
40
60
80
100
Lobophora
2
Dictyopteris
1
Amphiroa
1
Padina
1
Udotea
3
Sargassum
2
Dictyota
18
Amount eaten (%)
Pdir=0.0175
60
40
20
0
7
North Carolina
Bahamas

ntegrated Pest Management

r Synergisms of Defenses

Value can counter defense,
ll return to this later





Each deters

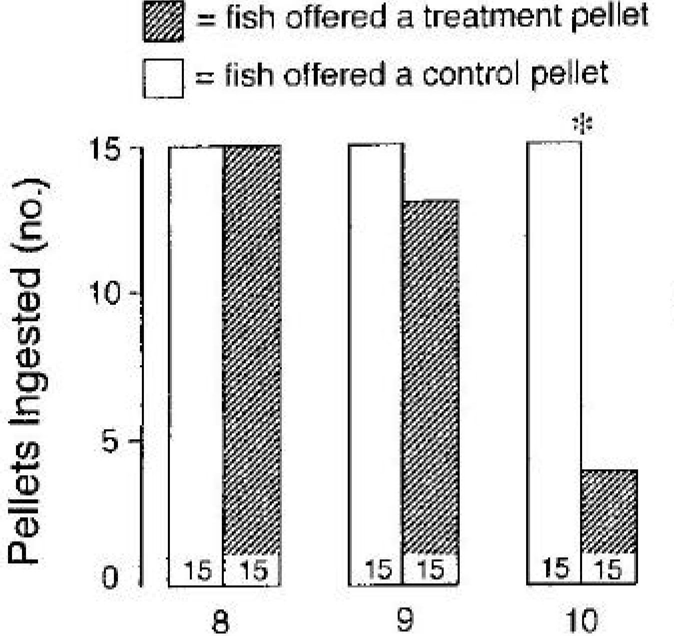
Chem. deters, but chem. and calc. are better than either alon

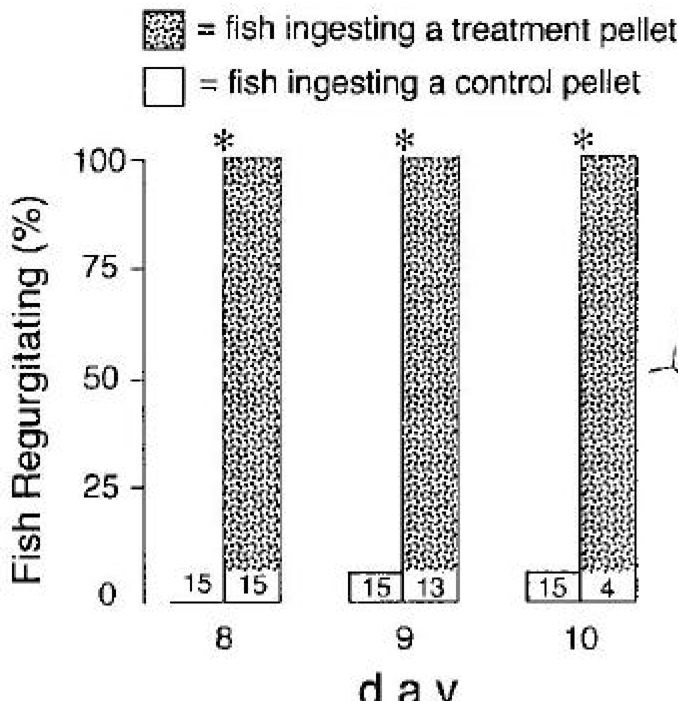
Either alone is as good as both

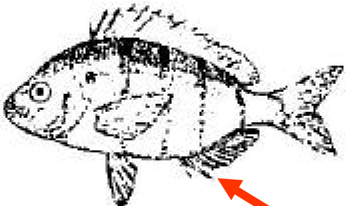
Can assess additive and synergistic effects

Why evolve to detect and avoid compounds?

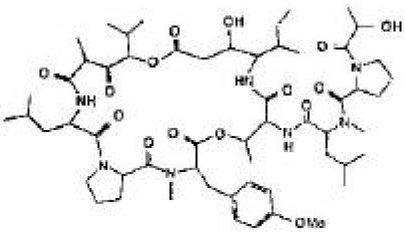
- Can a few feeding “mistakes” really be costly?
- All consumers have some ability to detoxify
- Do defended foods just taste bad or might they reduce consumer fitness – thus selecting for detection and avoidance?







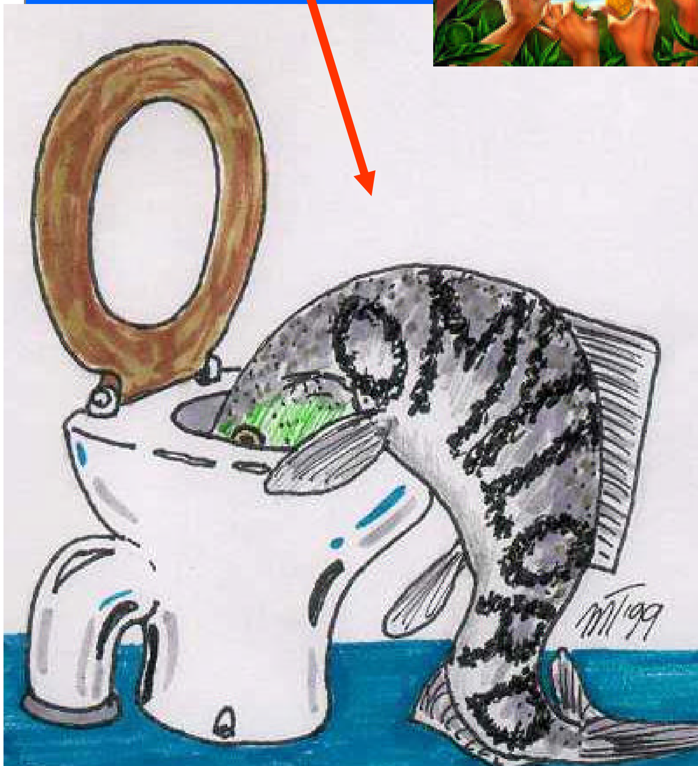
Lagodon rhomboides

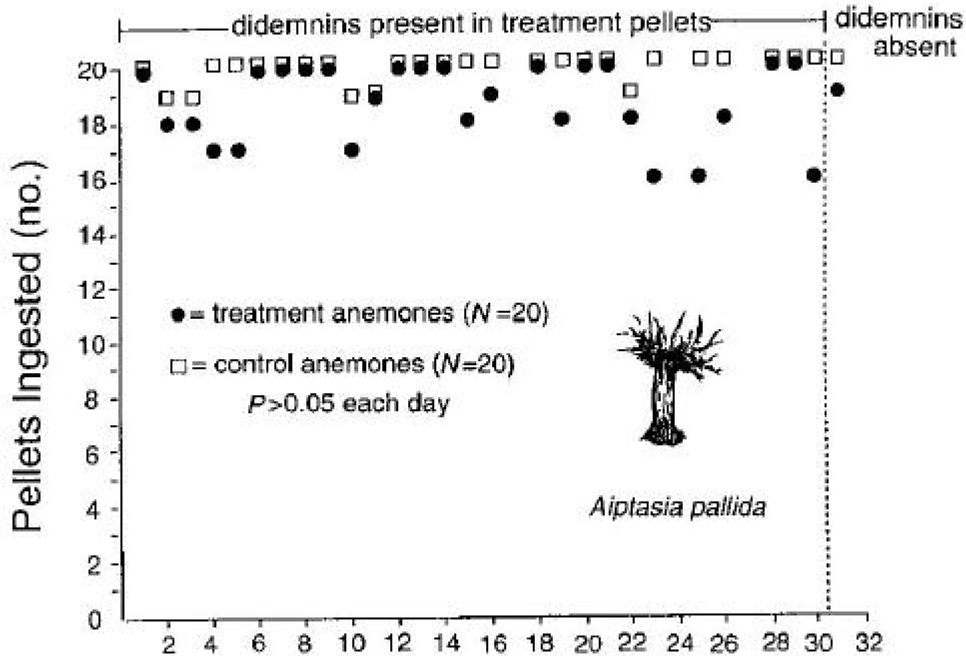


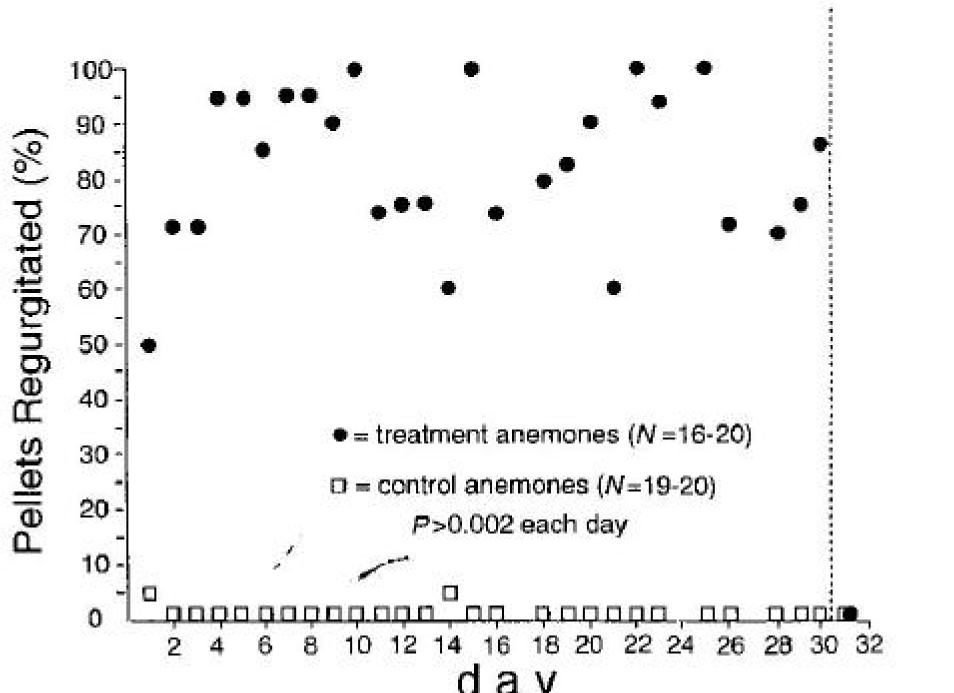
didemnin B

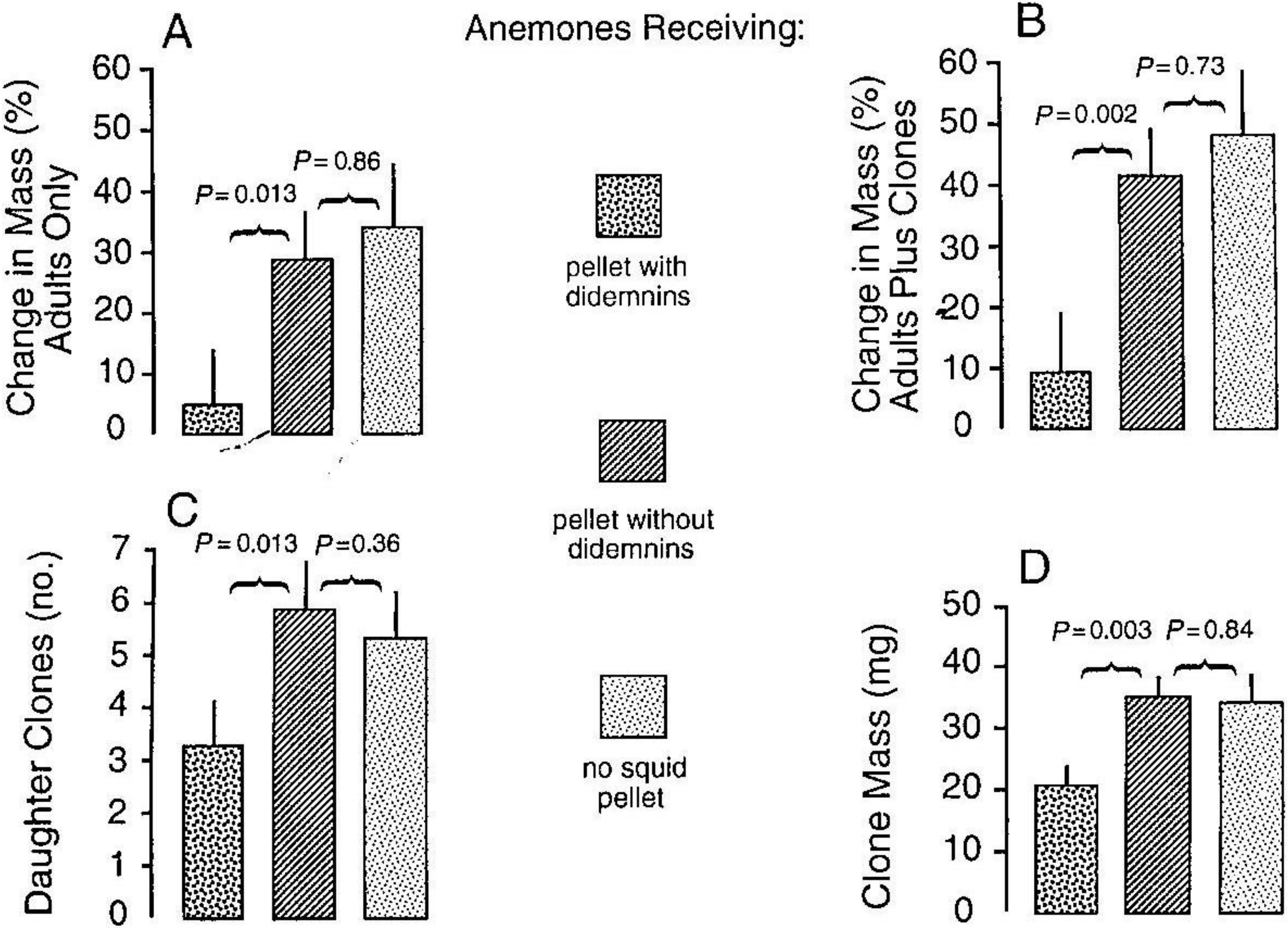
Too smart

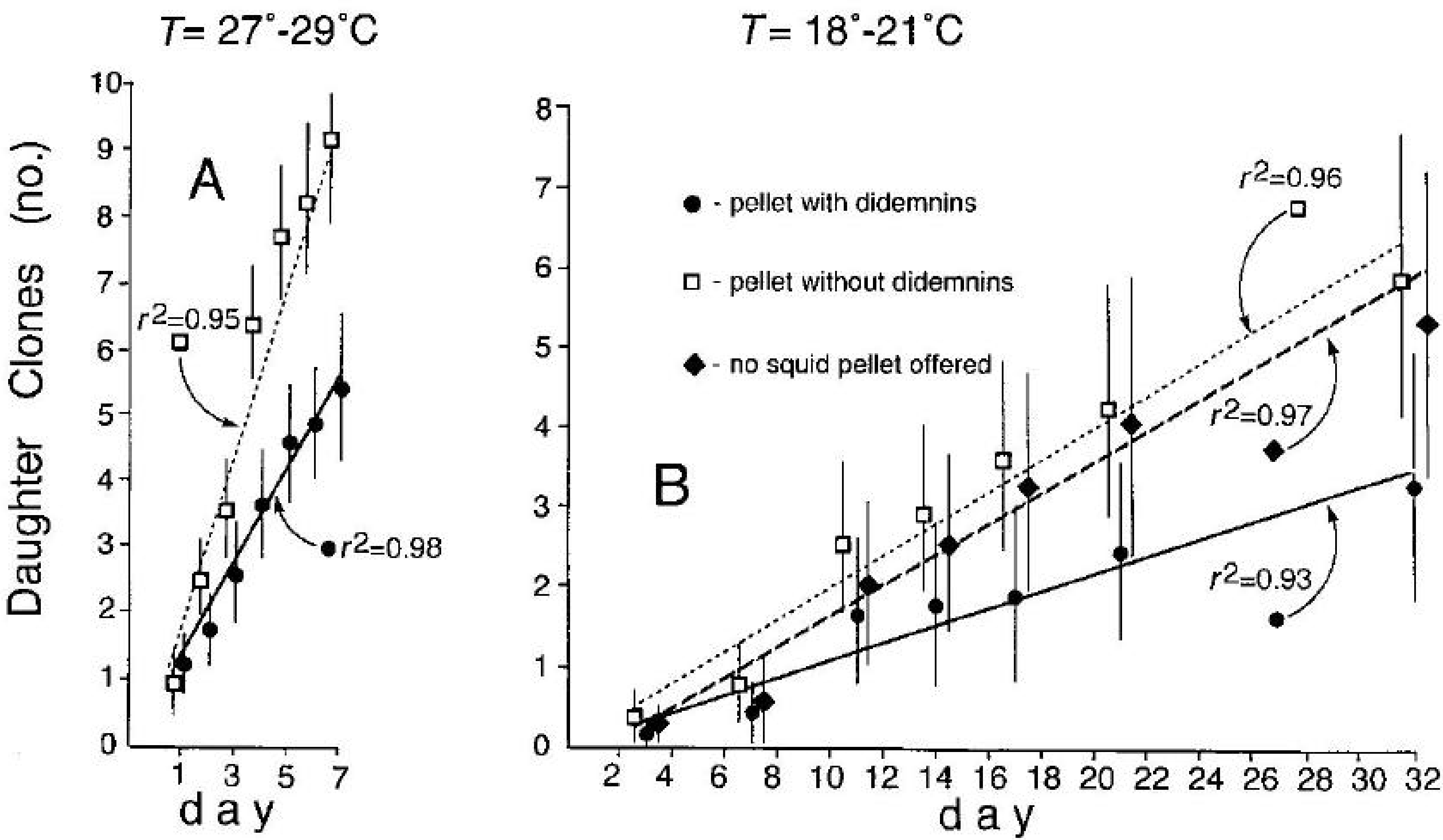
Learns Quickly:
The tequila effect




didemnins present in treatment pellets
didemnins absent
Pellets Ingested (no.)
0
2
4
6
8
10
12
14
16
18
20
● = treatment anemones (N =20)
□ = control anemones (N=20)
P>0.05 each day
Aiptasia pallida
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32


Pellets Regurgitated (%)
0
10
20
30
40
50
60
70
80
90
100
● = treatment anemones (N =16-20)
□ = control anemones (N=19-20)
P>0.002 each day
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
day


A
Anemones Receiving:
B
Change in Mass (%)
Adults Only
P = 0.013
P = 0.86
pellet with didemnins
Change in Mass (%)
Adults Plus Clones
P = 0.002
P = 0.73
C
Daughter Clones (no.)
P = 0.013
P = 0.36
pellet without didemnins
D
Clone Mass (mg)
P = 0.003
P = 0.84
no squid pellet



T= 27°-29°C
T= 18°-21°C
Daughter Clones (no.)
A
r2=0.95
r2=0.98
day
B
● - pellet with didemnins
□ - pellet without didemnins
◆ - no squid pellet offered
r2=0.96
r2=0.97
r2=0.93
day

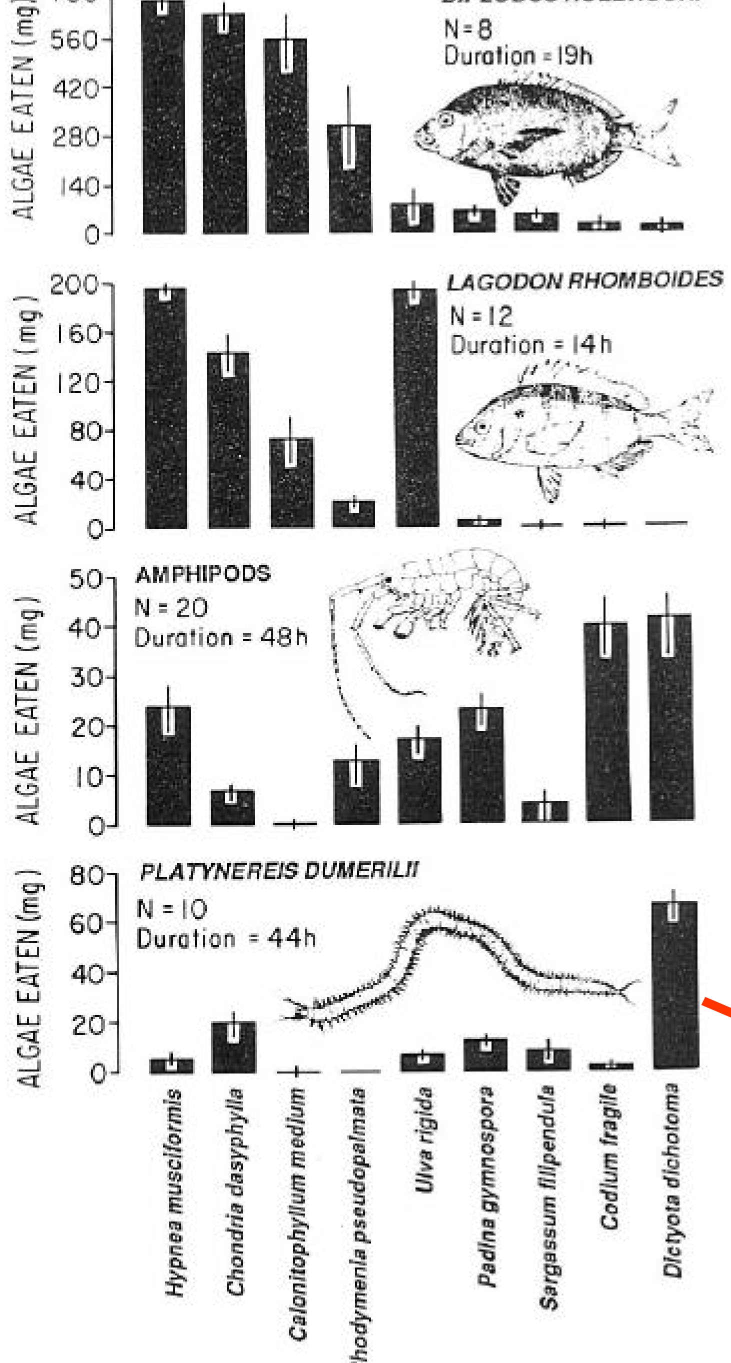
So Far **- Secondary Metabolites Commonly Deter Feeding by Large Generalists Such as Fishes and Urchins, with Traits sometimes working Synergistically and with Herbivores Consuming "Defended" Foods if they are more Valuable (i.e., context matters).**

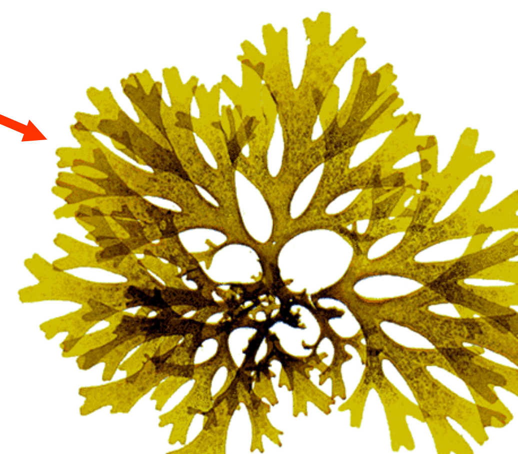
Next **- Once a Seaweed is Successfully Defended Against These Consumers, What Then?**

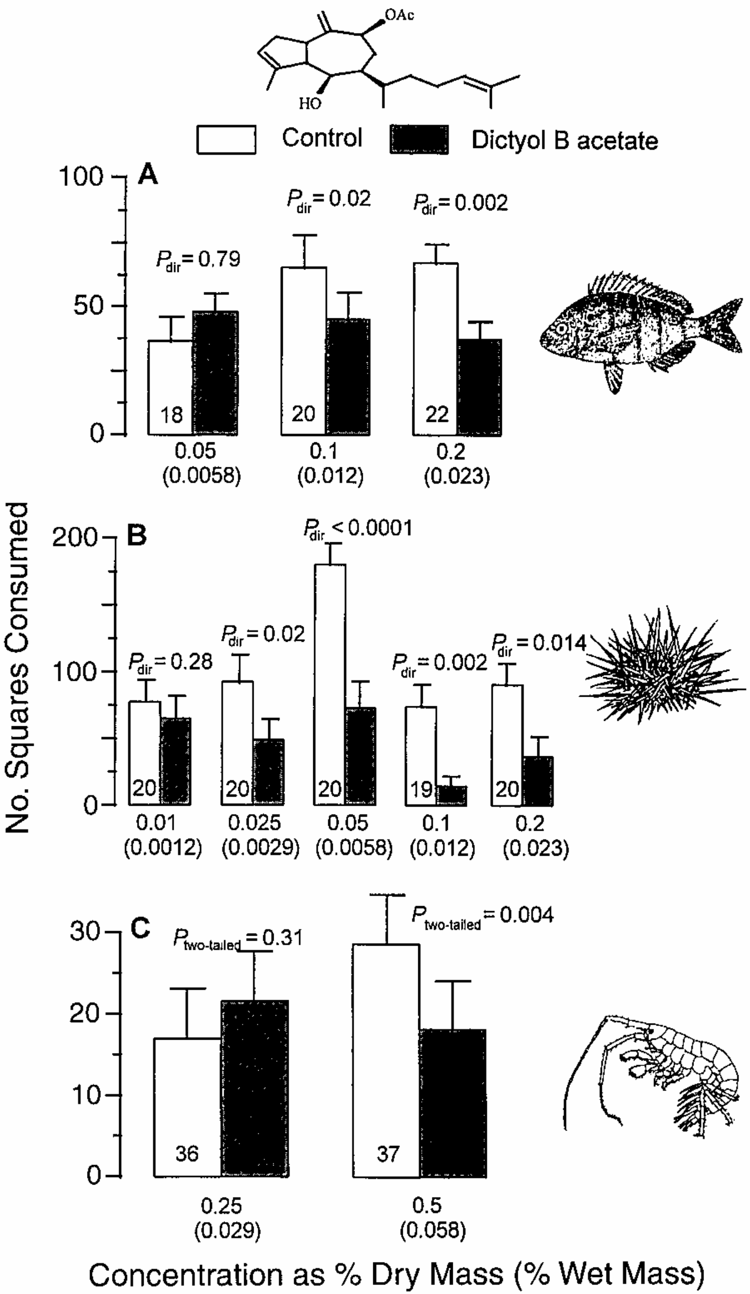
Who Gets Around These Defenses and Why?

Hypothesis: **Small Sedentary Herbivores that Live ON Seaweeds Can't Separate Food Choice from Habitat Choice. Seaweeds Eaten by Fishes will be Unsafe Habitats. Thus, Small Sedentary Herbivores Should Selectively Use Chemically Defended Hosts. "Feeding" Specialization may Really be Habitat Specialization.**

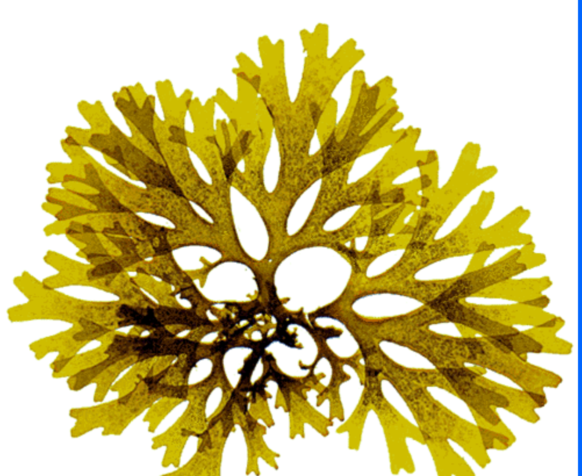
Getting Lunch Without Being Lunch


ALGAE EATEN (mg)
560
420
280
140
0
N=8
Duration = 19h
LAGODON RHOMBOIDES
N = 12
Duration = 14h
200
160
120
80
40
0
AMPHIPODS
N = 20
Duration = 48h
50
40
30
20
10
0
PLATYNEREIS DUMERILII
N = 10
Duration = 44h
80
60
40
20
0
Hypnea musciformis
Chondria dasyphylla
Calonitophyllum medium
hodymenia pseudopalmata
Ulva rigida
Padina gymnospora
Sargassum filipendula
Codium fragile
Dictyota dichotoma





To deter the amphipod, we need 5X to 20X more compound than to deter the fish and sea urchin, respectively



Amphipods that are Resistant to *Dictyota*'s Chemical Defenses Persist on *Dictyota* when Fishes are Common. Species that Cannot Live on and Feed on *Dictyota*, become Locally Extinct During this Period.

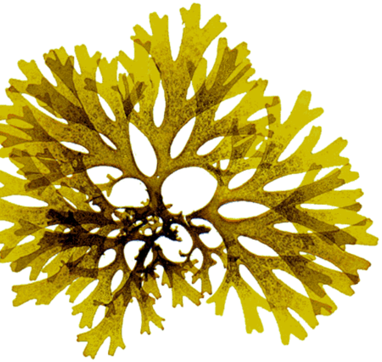
When Super-Glued to *Dictyota* vs. More Palatable Species and Placed in the Field, Amphipods on *Dictyota* Persist while Those on Palatable Seaweeds get Eaten by Fishes.

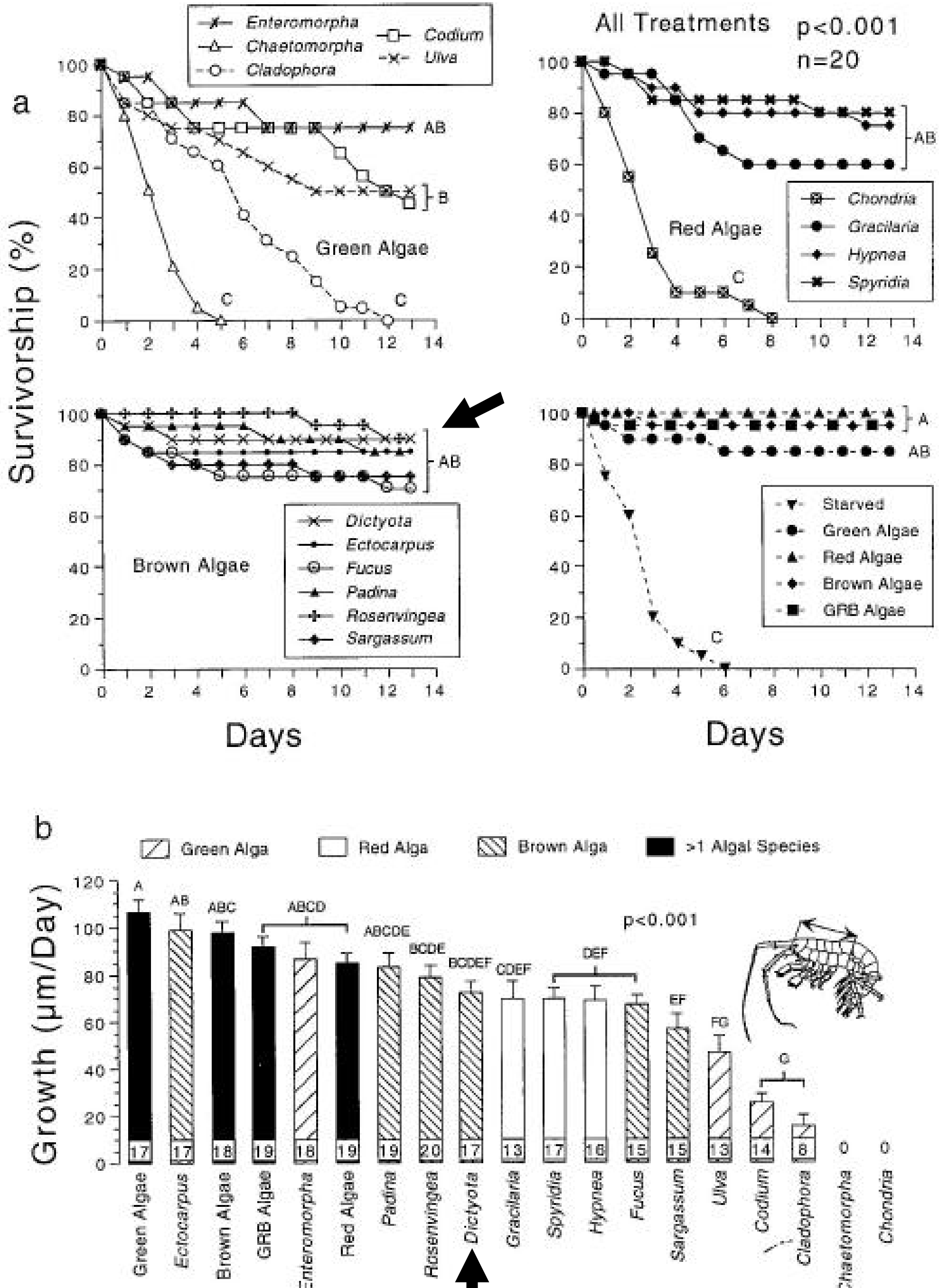
(Duffy and Hay 1994 Ecology)

So *Dictyota* Provides Refuge for *Ampithoe longimana* but

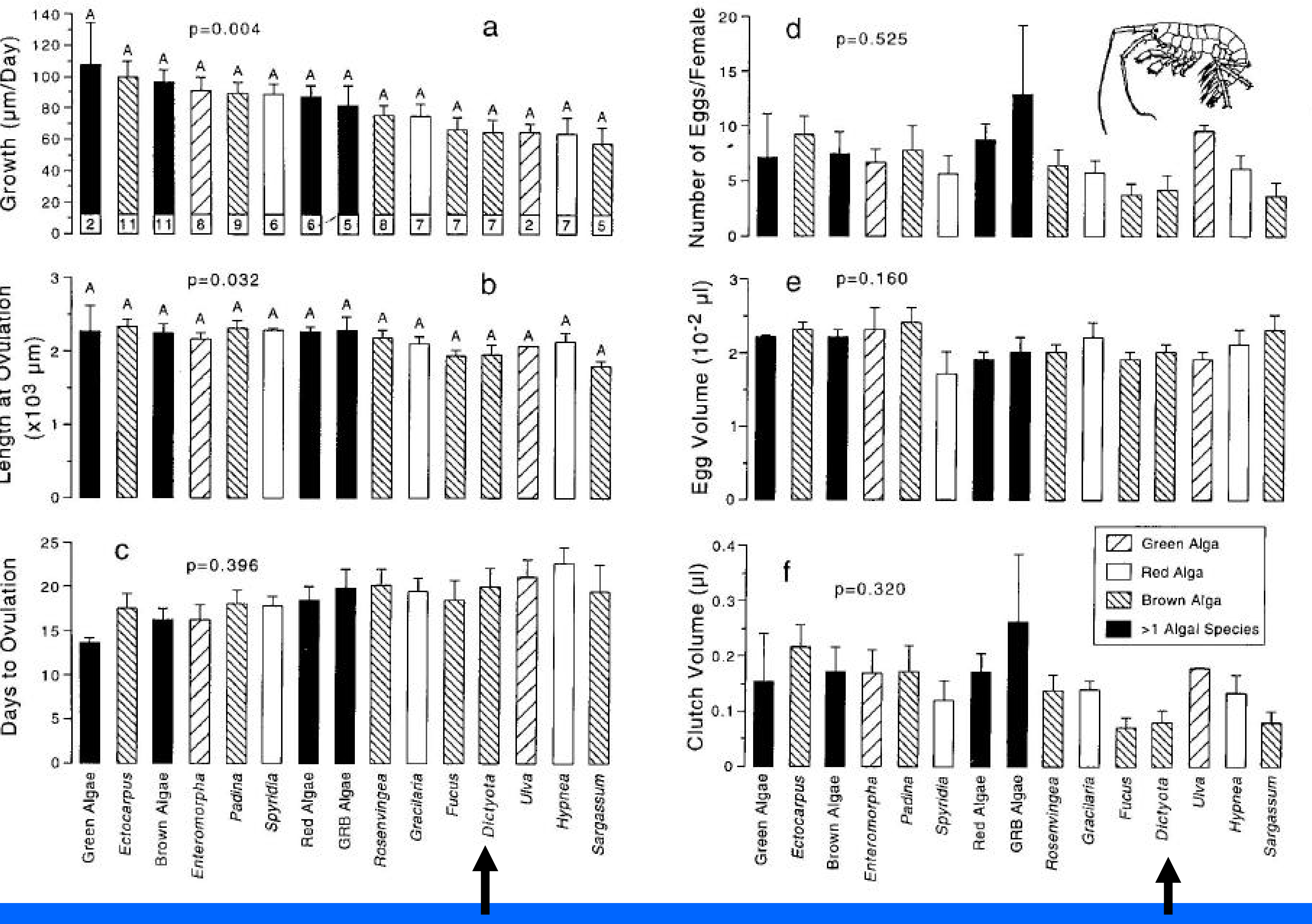
Is it a Good Food?

Is the Unique Value that Drives Preferential Utilization
Shelter or Food?





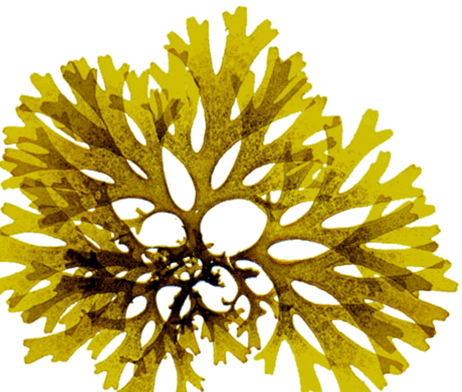
Cruz-Rivera &
Hay 2001 MEP

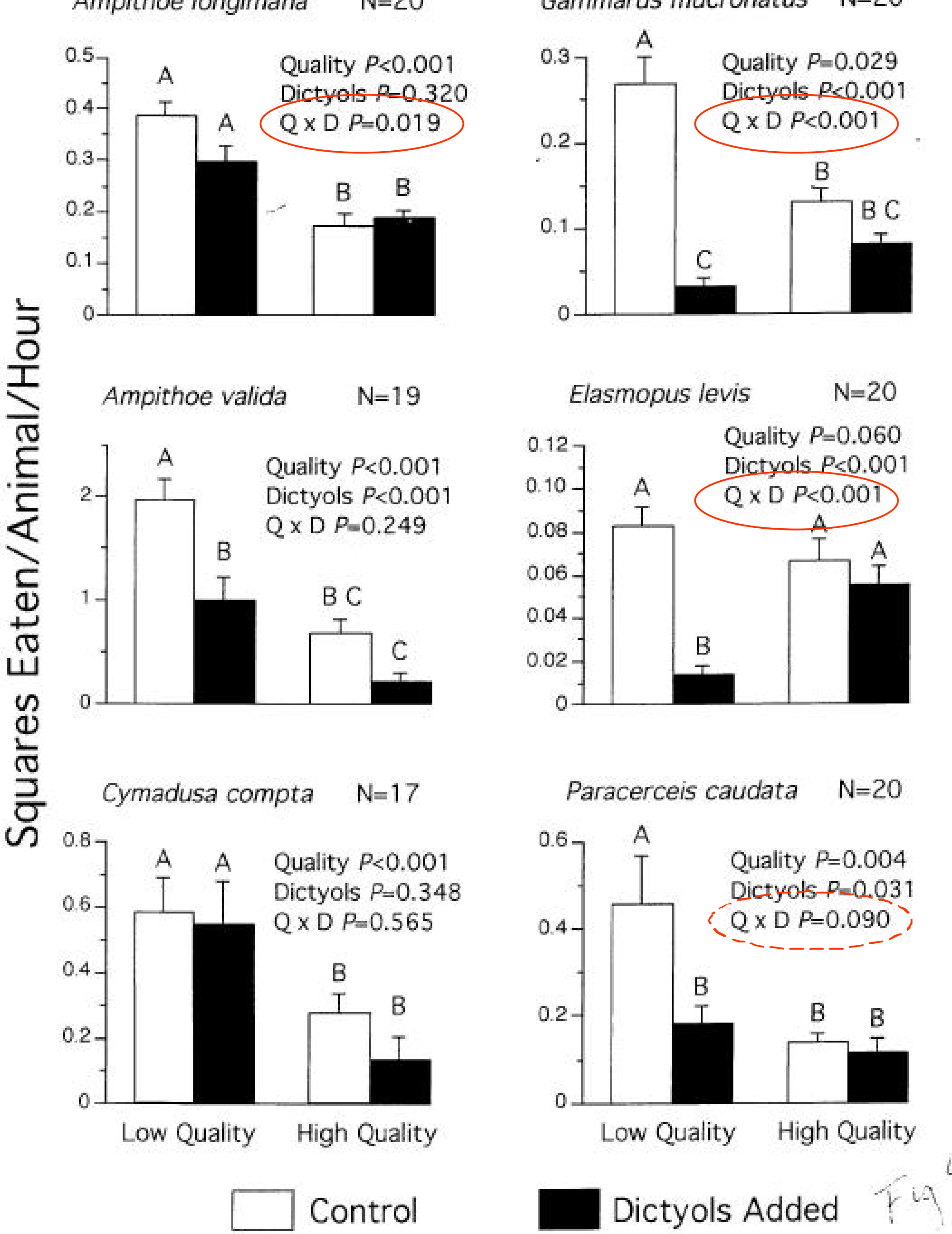


Cruz-Rivera & Hay 2001 MEP

ood Quality X
efense Interactions

) Quality matters
) Chem. sometimes matters
) INTERACTIONS





Cruz-Rivera & Hay 2003

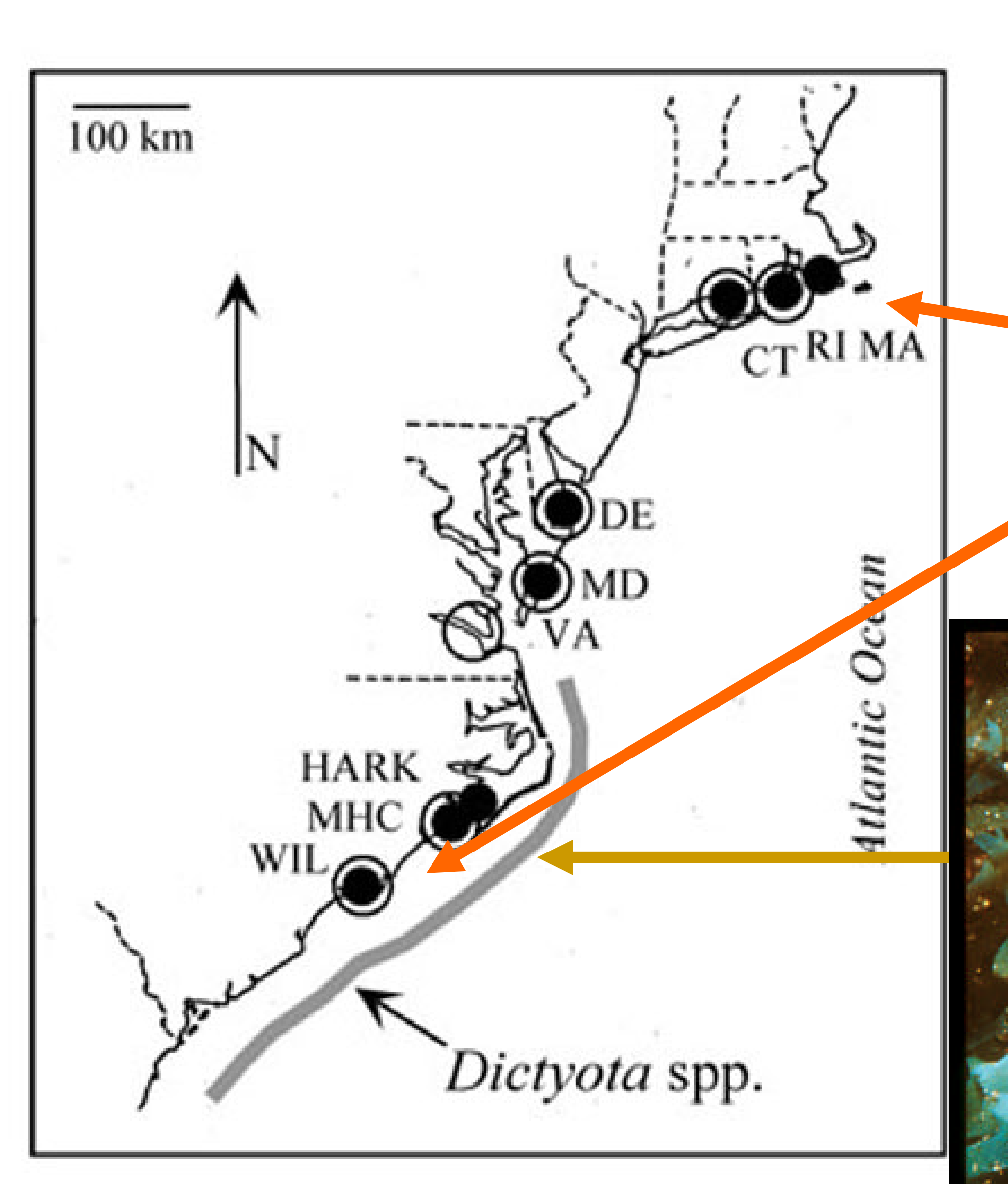
So *Dictyota* is Nothing Special as a Food and Seems to be Favored Due to its Value as a Habitat

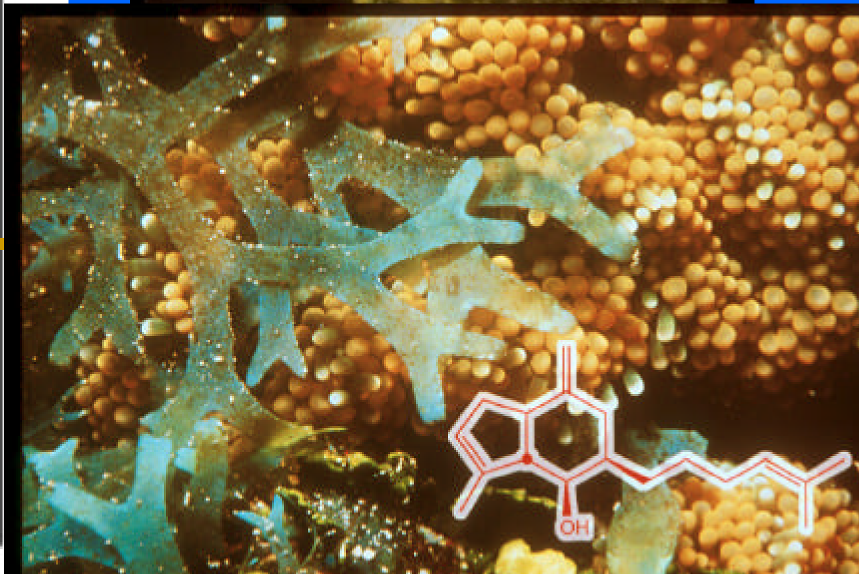
Its Value as a Habitat Lies in its Resistance to Fishes, and this Resistance is Based on its Production of Deterrent Chemicals

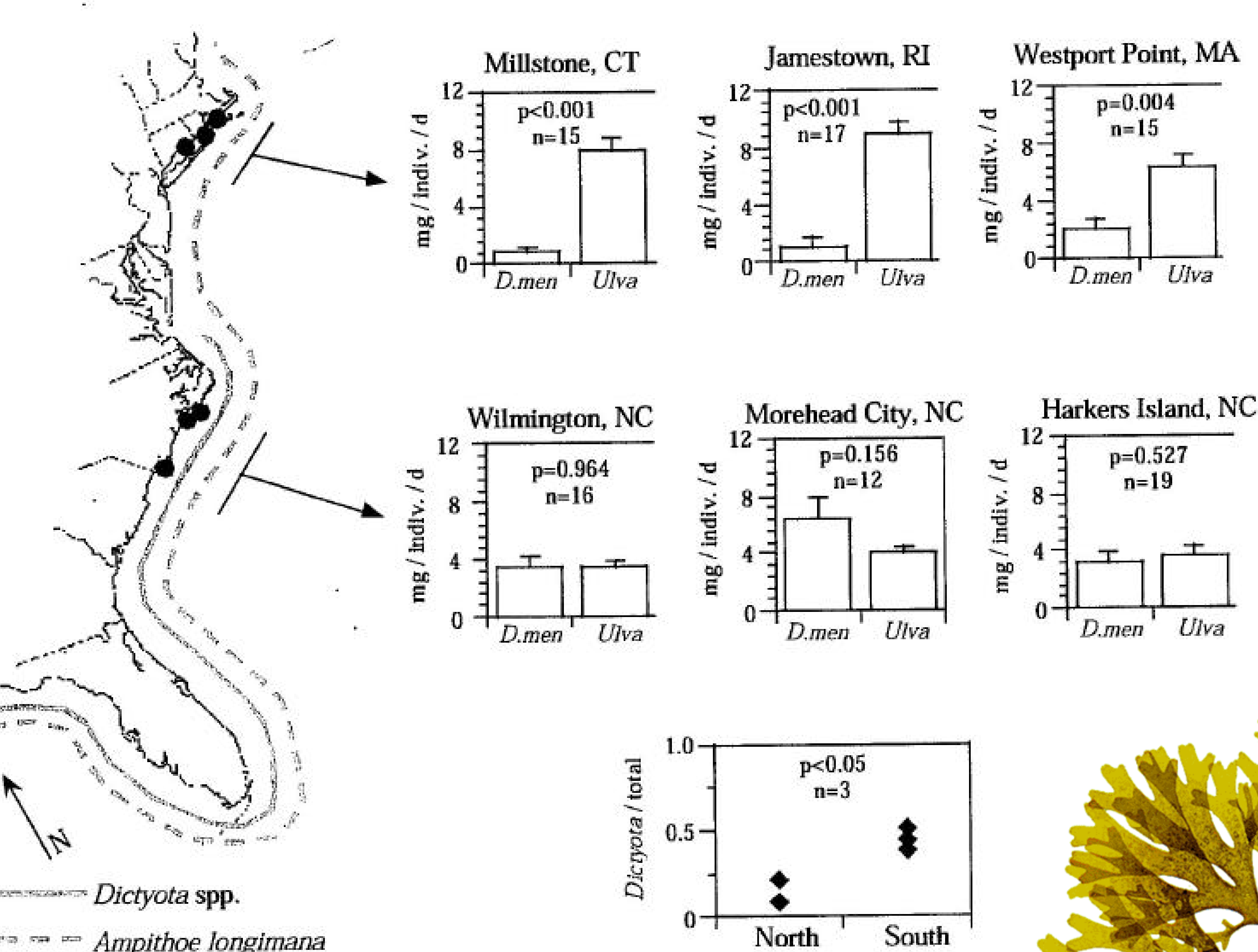
If this is true then,

Ho: where the amphipods co-occur with *Dictyota*, they should be selected to use it to avoid fishes, where they do not overlap with *Dicytota*, this selection should not occur

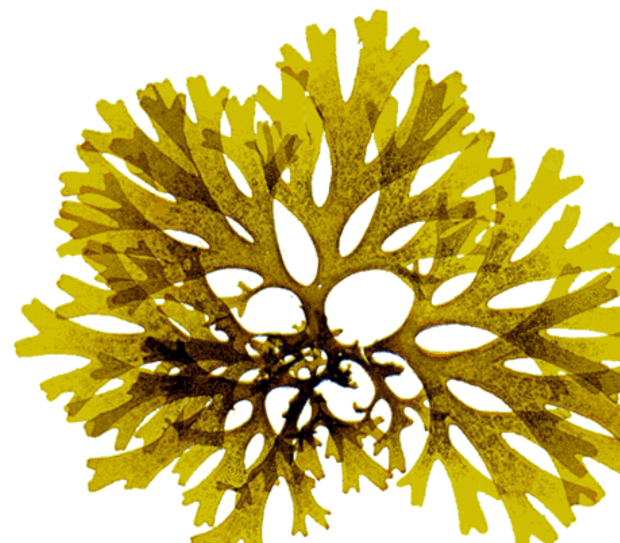
What about Intraspecific Patterns where *A. longimana* Does, or Does not, Co-occur with Dictyota?

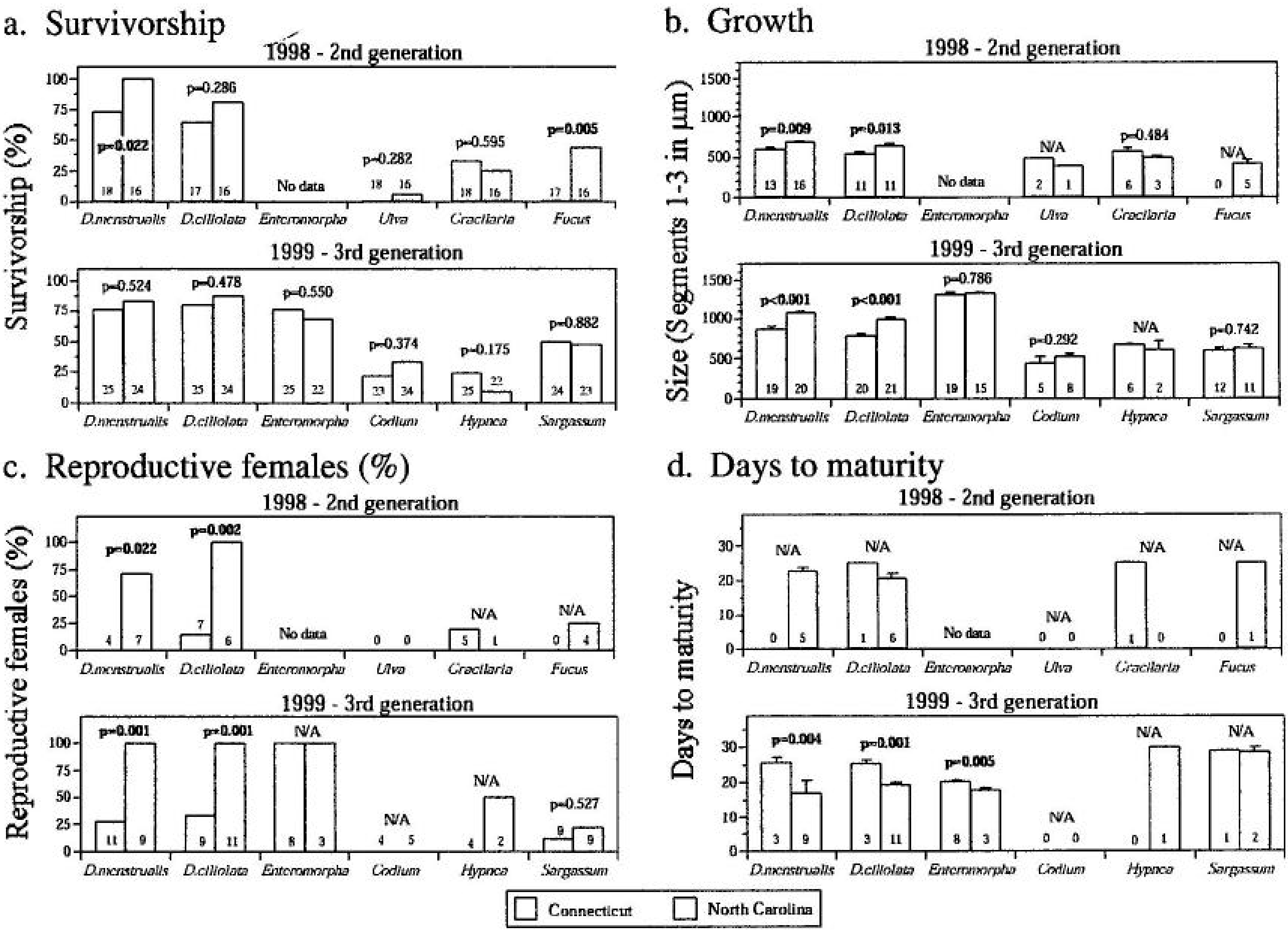




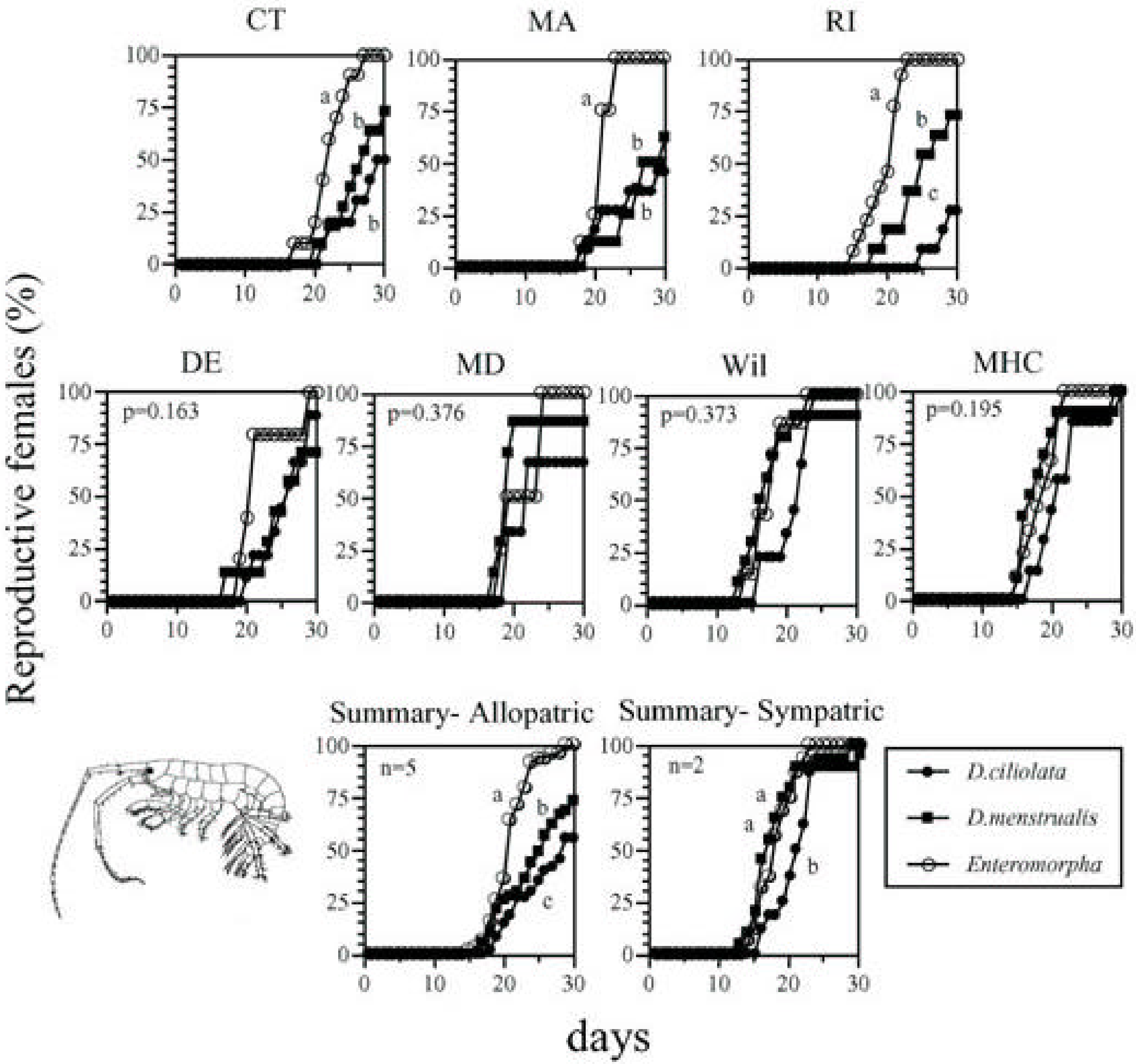

Millstone, CT
p<0.001
n=15
Jamestown, RI
p<0.001
n=17
Westport Point, MA
p=0.004
n=15
Wilmington, NC
p=0.964
n=16
Morehead City, NC
p=0.156
n=12
Harkers Island, NC
p=0.527
n=19
mg / indiv. / d
D.men
Ulva
12
8
4
0
Dictyota / total
p<0.05
n=3
North
South
1.0
0.5
0
N
Dictyota spp.
Ampithoe longimana

アナアオサ





Sotka and Hay 2002 Ecology



Sotka et al. 2003 Evol.)

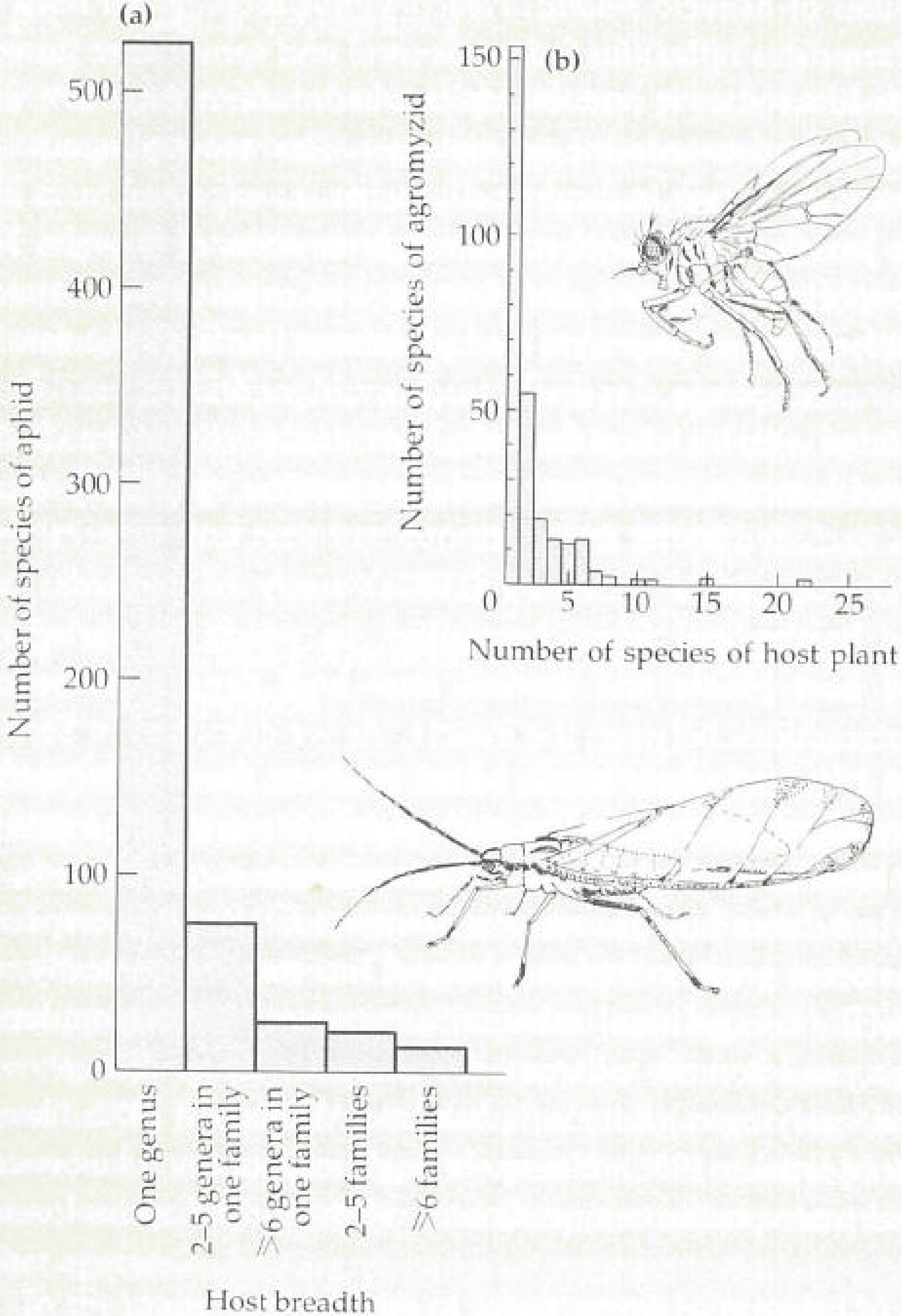
The dictyols cause this altered feeding

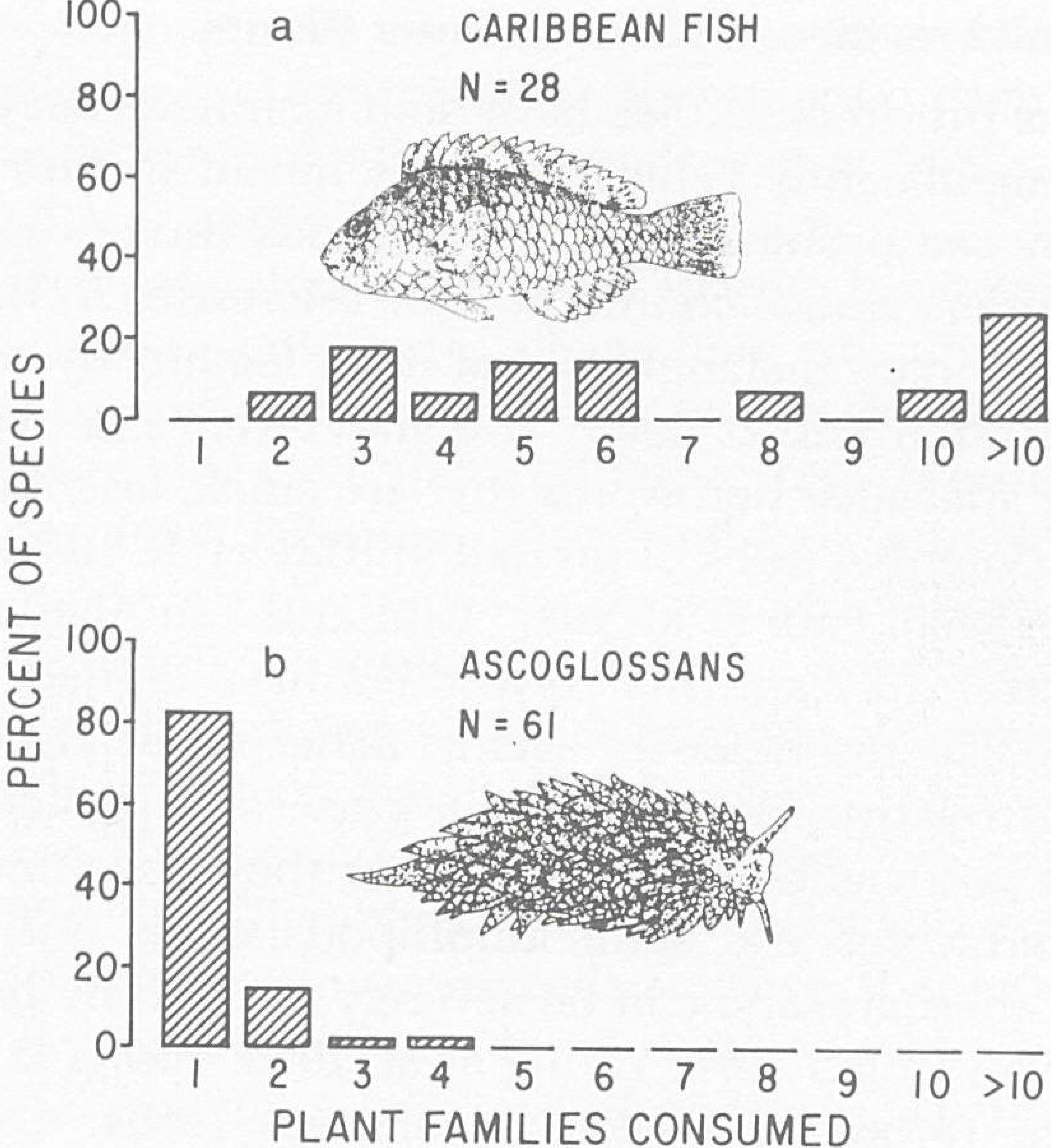
They also diminish fertility in allopatric amphipods, but not in sympartic ones

These patterns persist through 3 generations raised in common gardens

Seaweed Chemical Defenses Affect the Evolution of Herbivores

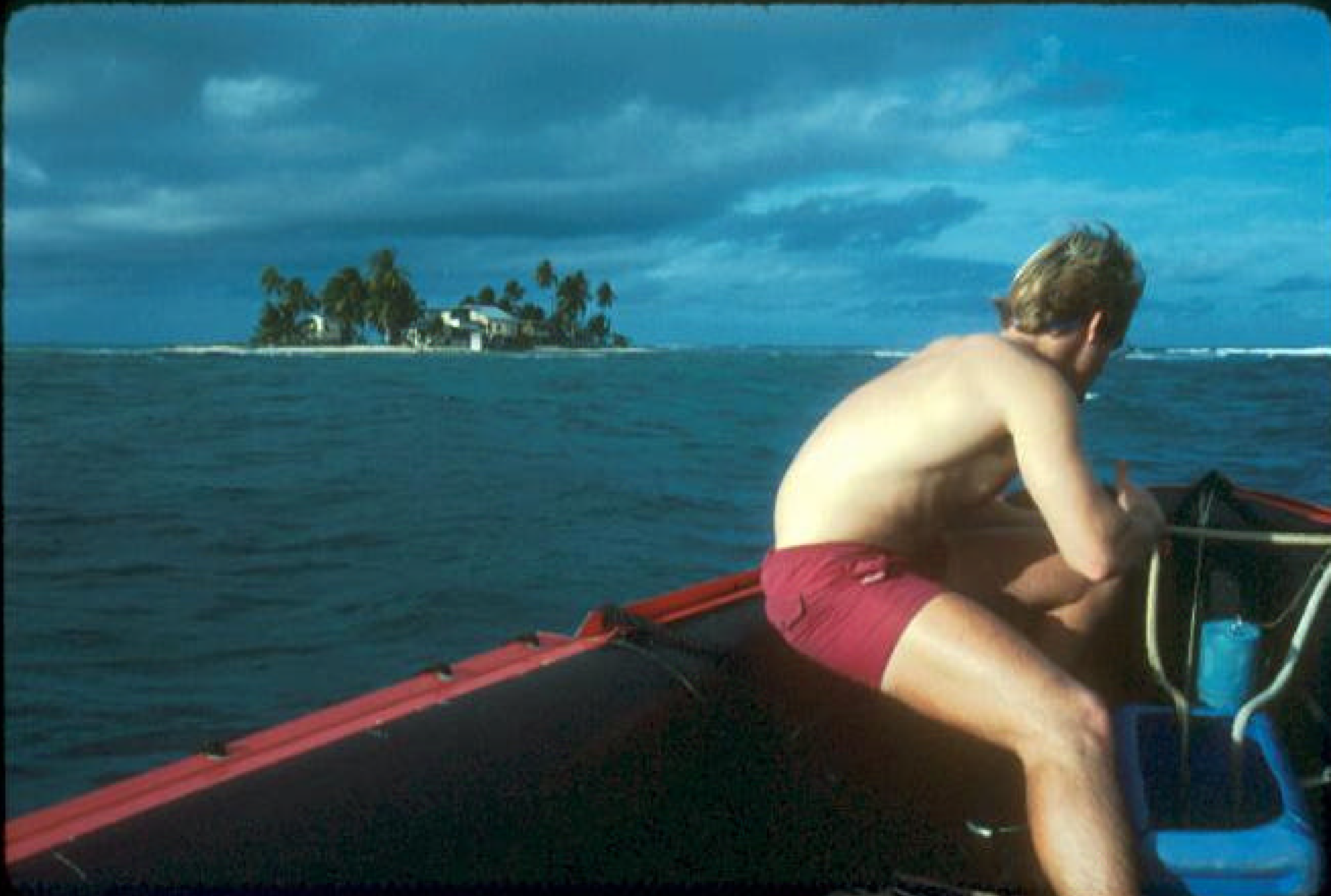
Could the Processes We Documented Affecting *A. longimana* go Further, and Select for Restrictive Feeding Specialization Rather than just Feeding Preference?

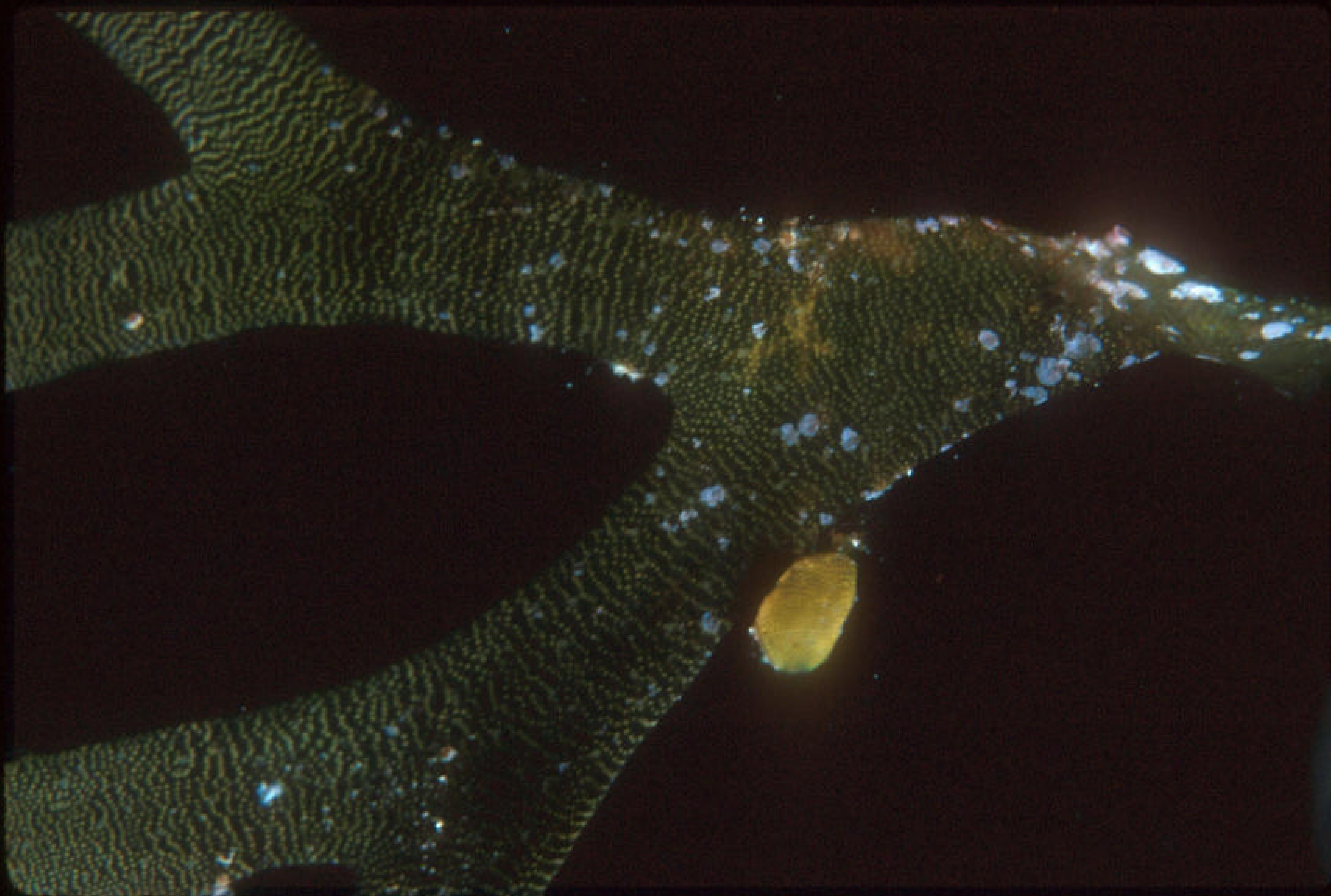

(a)
Number of species of aphid
500
400
300
200
100
0
One genus
2–5 genera in one family
≥6 genera in one family
2–5 families
≥6 families
Host breadth
(b)
Number of species of agromyzid
150
100
50
0
5
10
15
20
25
Number of species of host plant

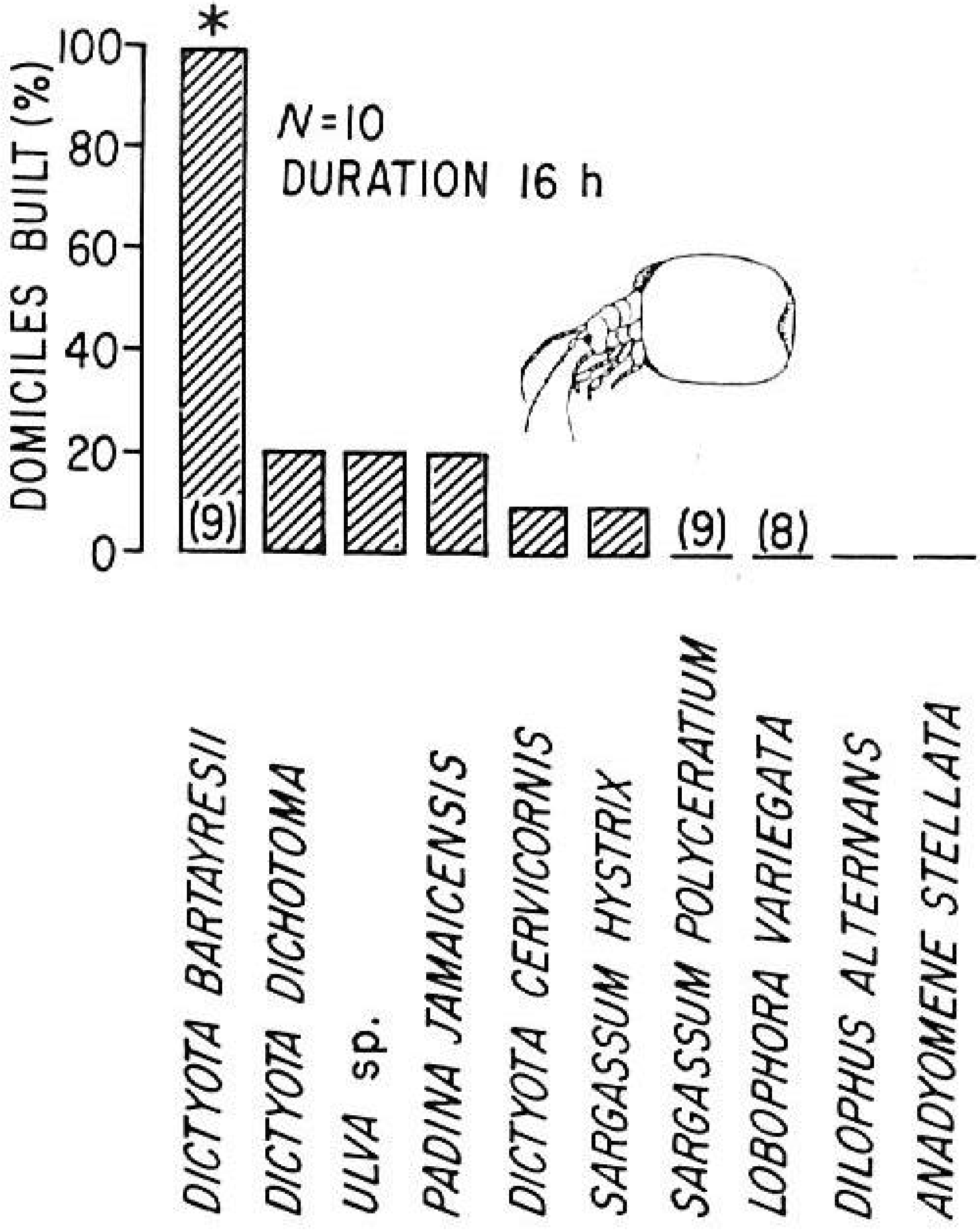

a
CARIBBEAN FISH
N = 28
b
ASCOGLOSSANS
N = 61
PERCENT OF SPECIES
0
20
40
60
80
100
1
2
3
4
5
6
7
8
9
10
>10
PLANT FAMILIES CONSUMED

Advantages of Marine Communities

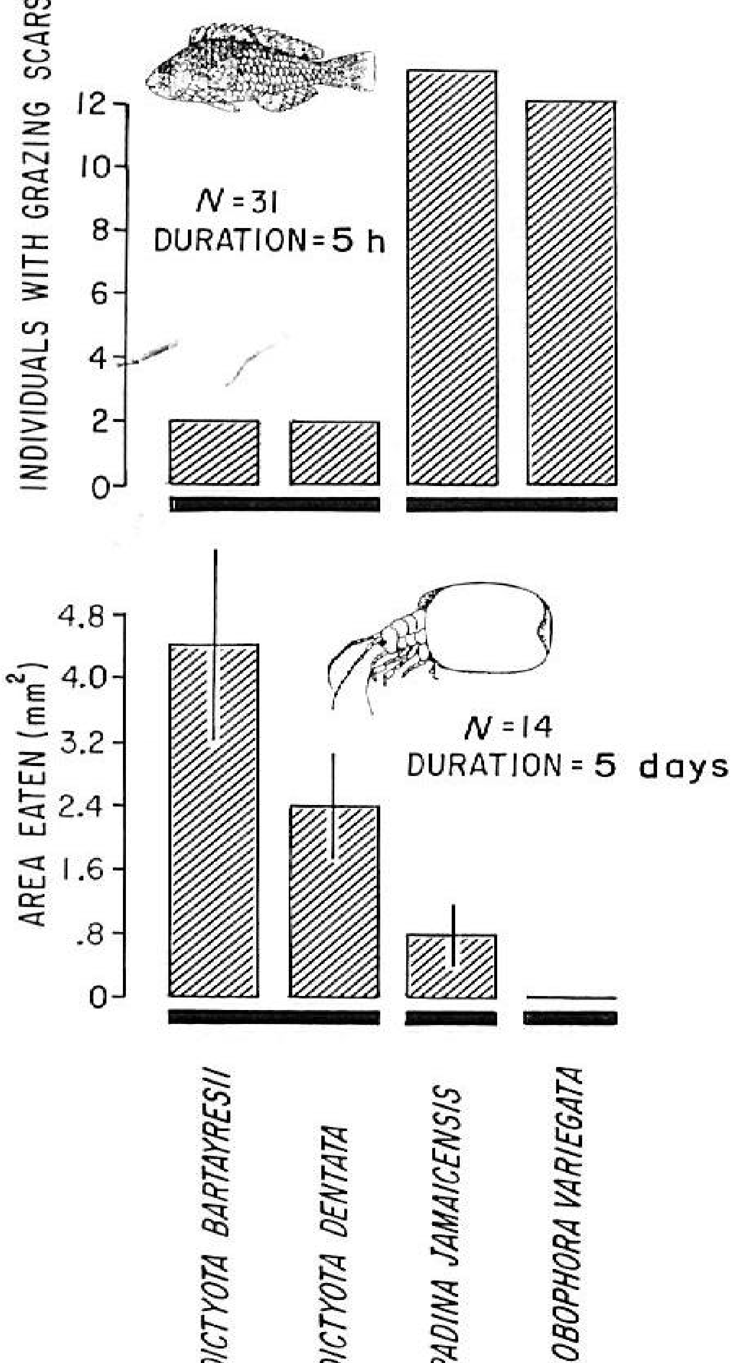
	Terrestrial	Marine
Paleodisturbance (1,000 - 1,000,000 yrs)	High	Low
Recent Disturbance (100s of years - agriculture)	High	Low
Number of Specialists Spp. (can’t see forest for trees)	High	Low
Selection Against Specialization	Low	High



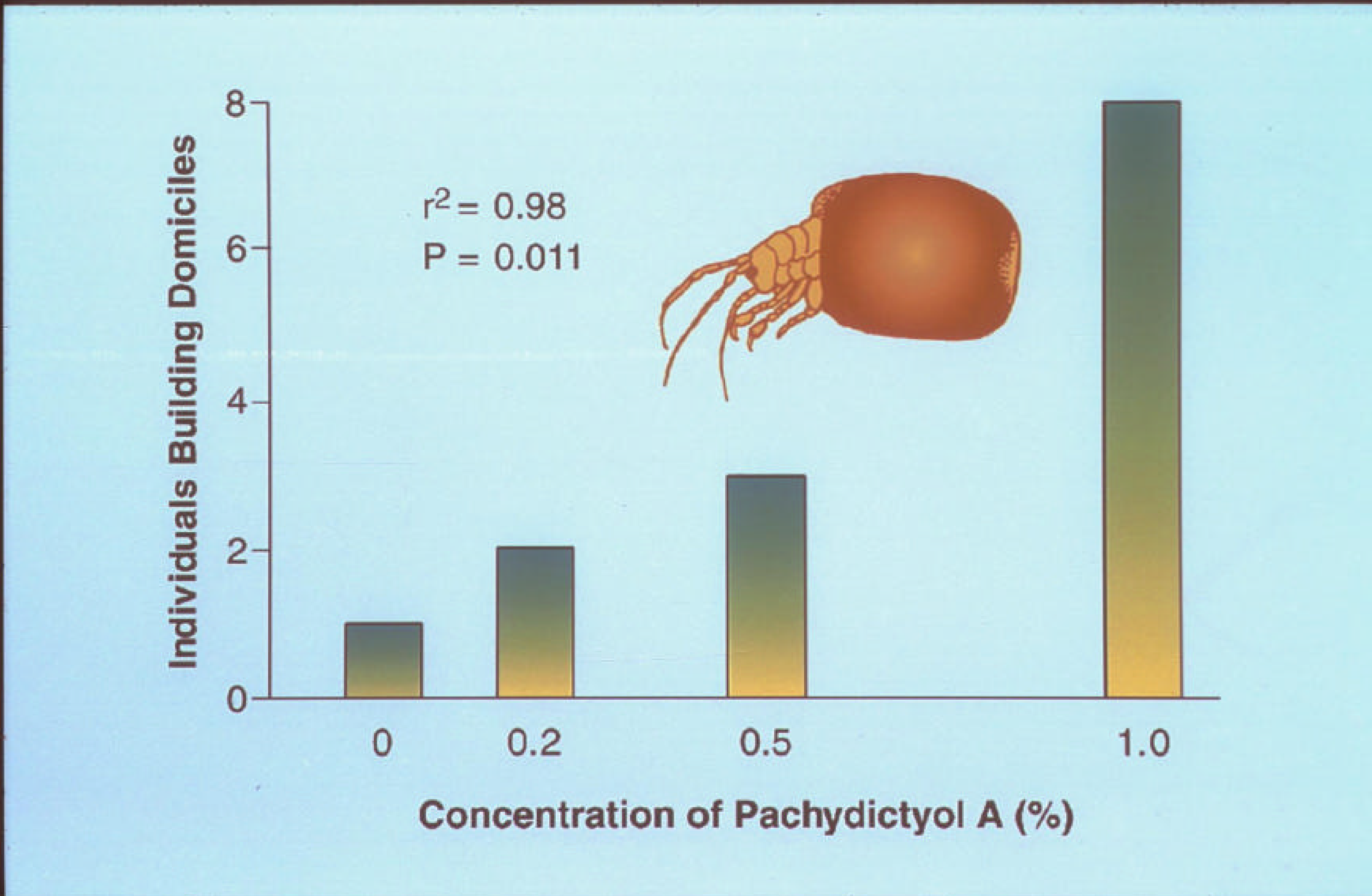




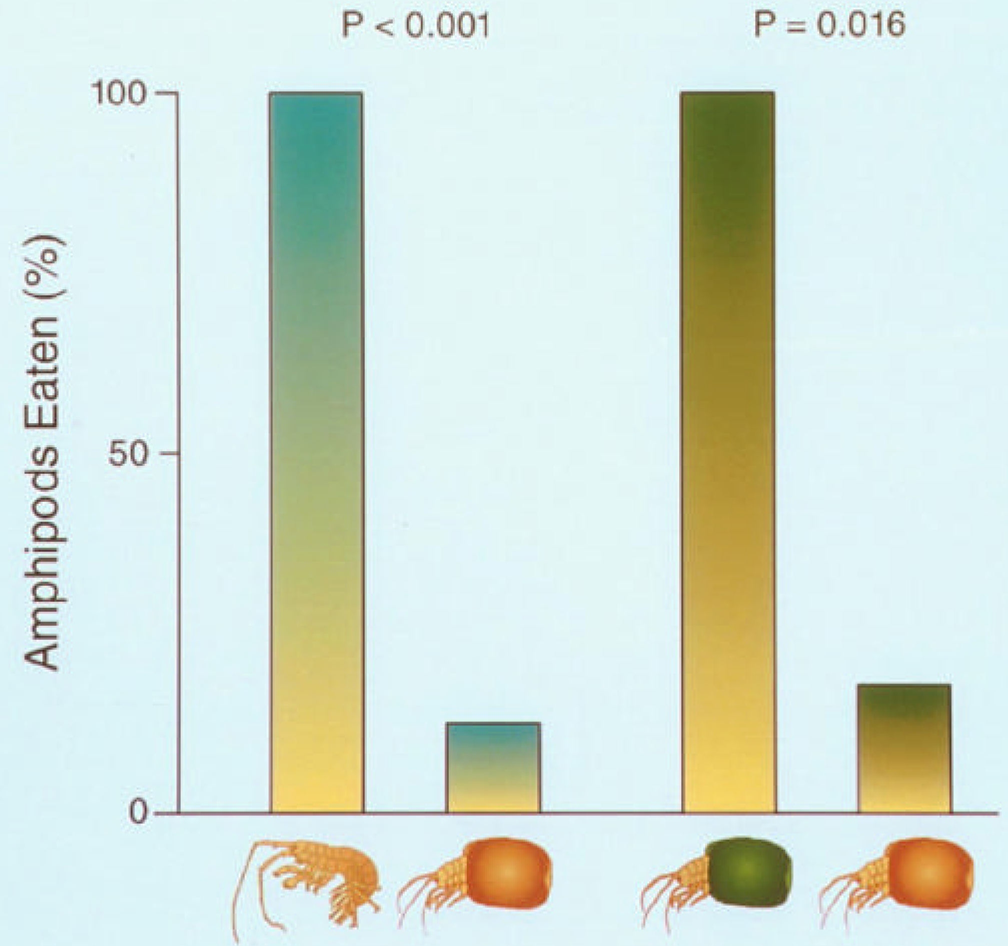
DOMICILES BUILT (%)
100
80
60
40
20
0
*
N=10
DURATION 16 h
(9)
(9)
(8)
DICTYOTA BARTAYRESII
DICTYOTA DICHOTOMA
ULVA sp.
PADINA JAMAICENSIS
DICTYOTA CERVICORNIS
SARGASSUM HYSTRIX
SARGASSUM POLYCERATIUM
LOBOPHORA VARIEGATA
DILOPHUS ALTERNANS
ANADYOMENE STELLATA



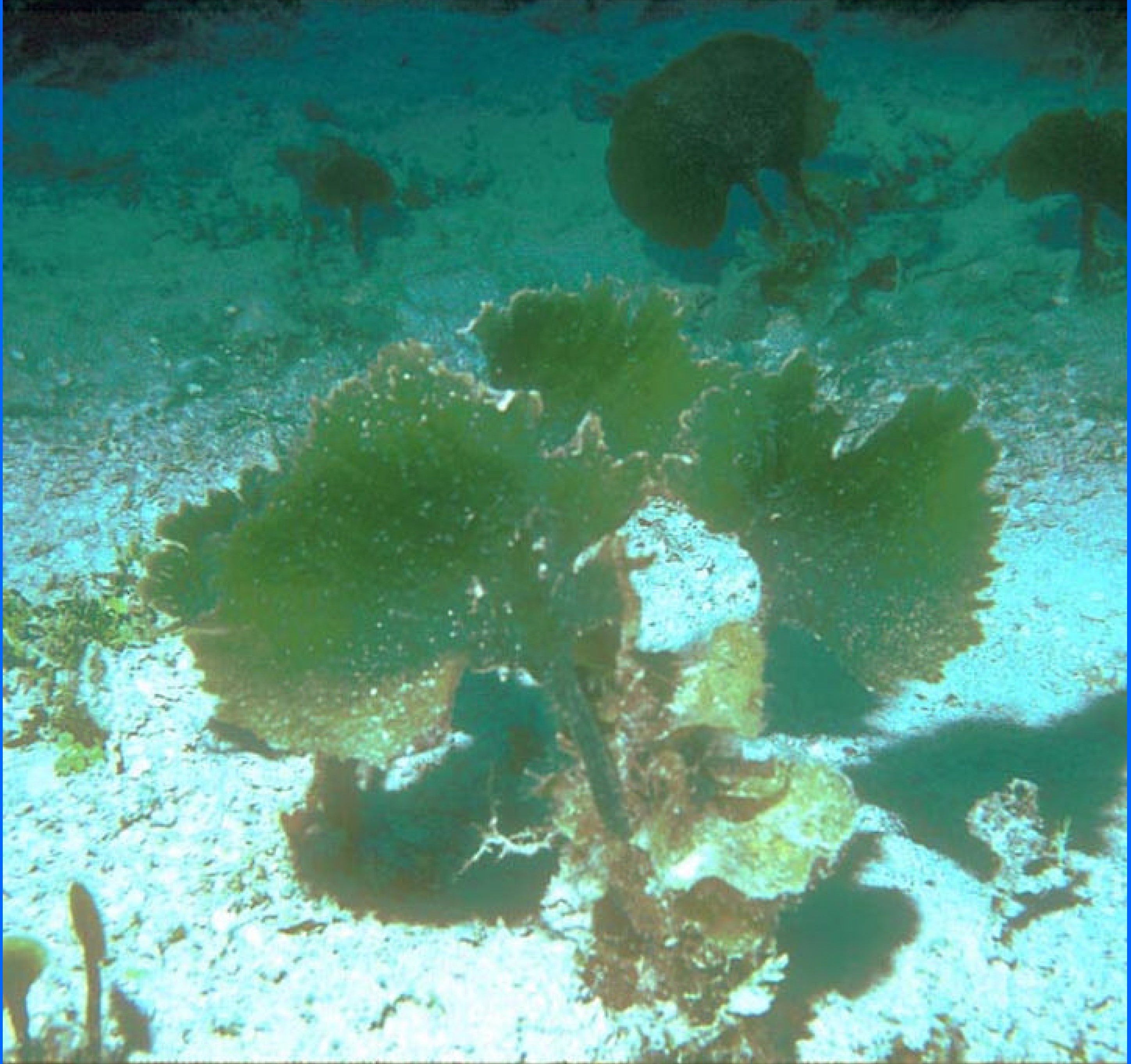
INDIVIDUALS WITH GRAZING SCARS
12
10
8
6
4
2
0
N = 31
DURATION = 5 h
AREA EATEN (mm²)
4.8
4.0
3.2
2.4
1.6
.8
0
N = 14
DURATION = 5 days
DICTYOTA BARTAYRESII
DICTYOTA DENTATA
PADINA JAMAICENSIS
OBOPHORA VARIEGATA



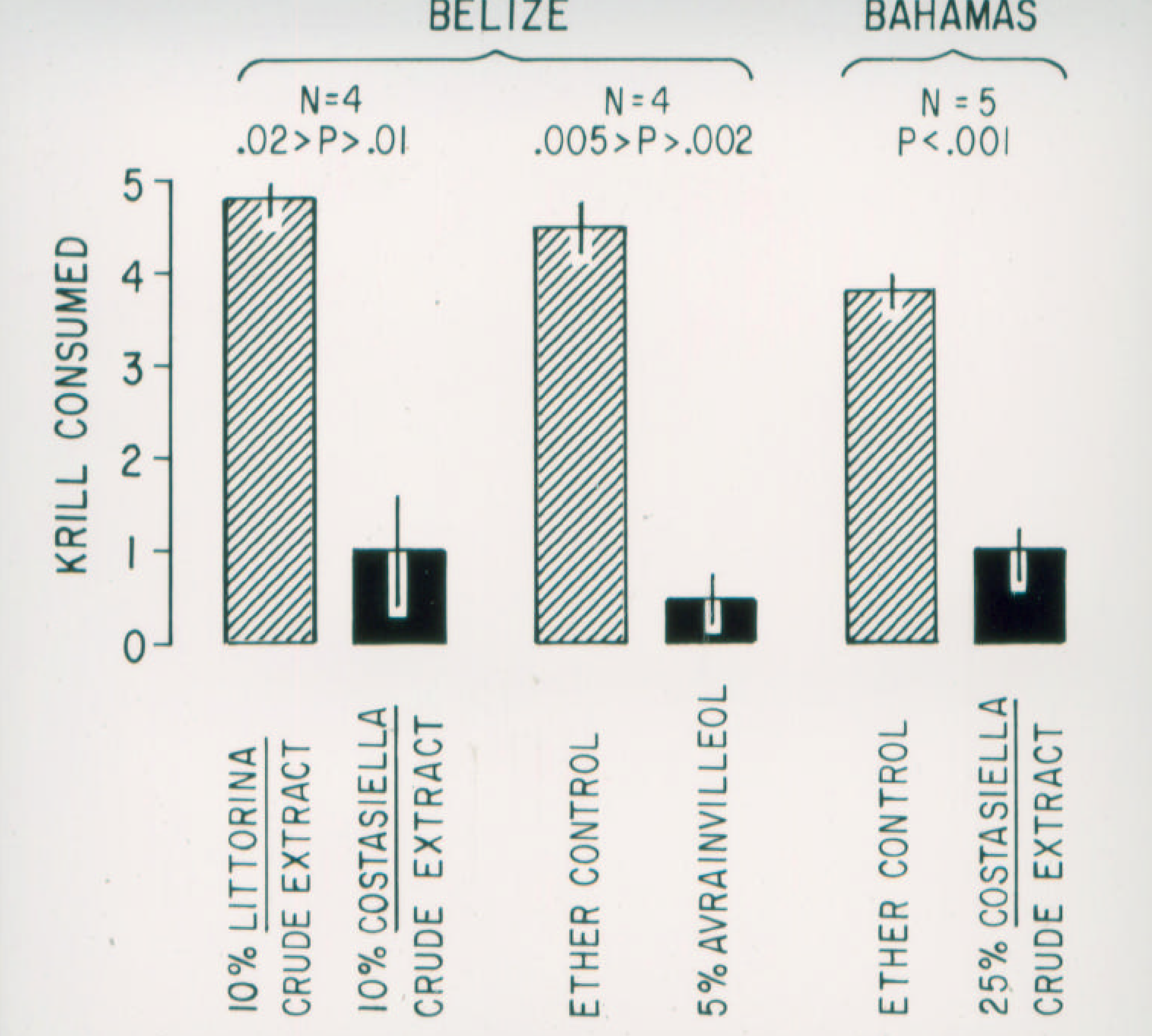
Individuals Building Domiciles
8
6
4
2
0
r^2 = 0.98
P = 0.011
0
0.2
0.5
1.0
Concentration of Pachydictyol A (%)

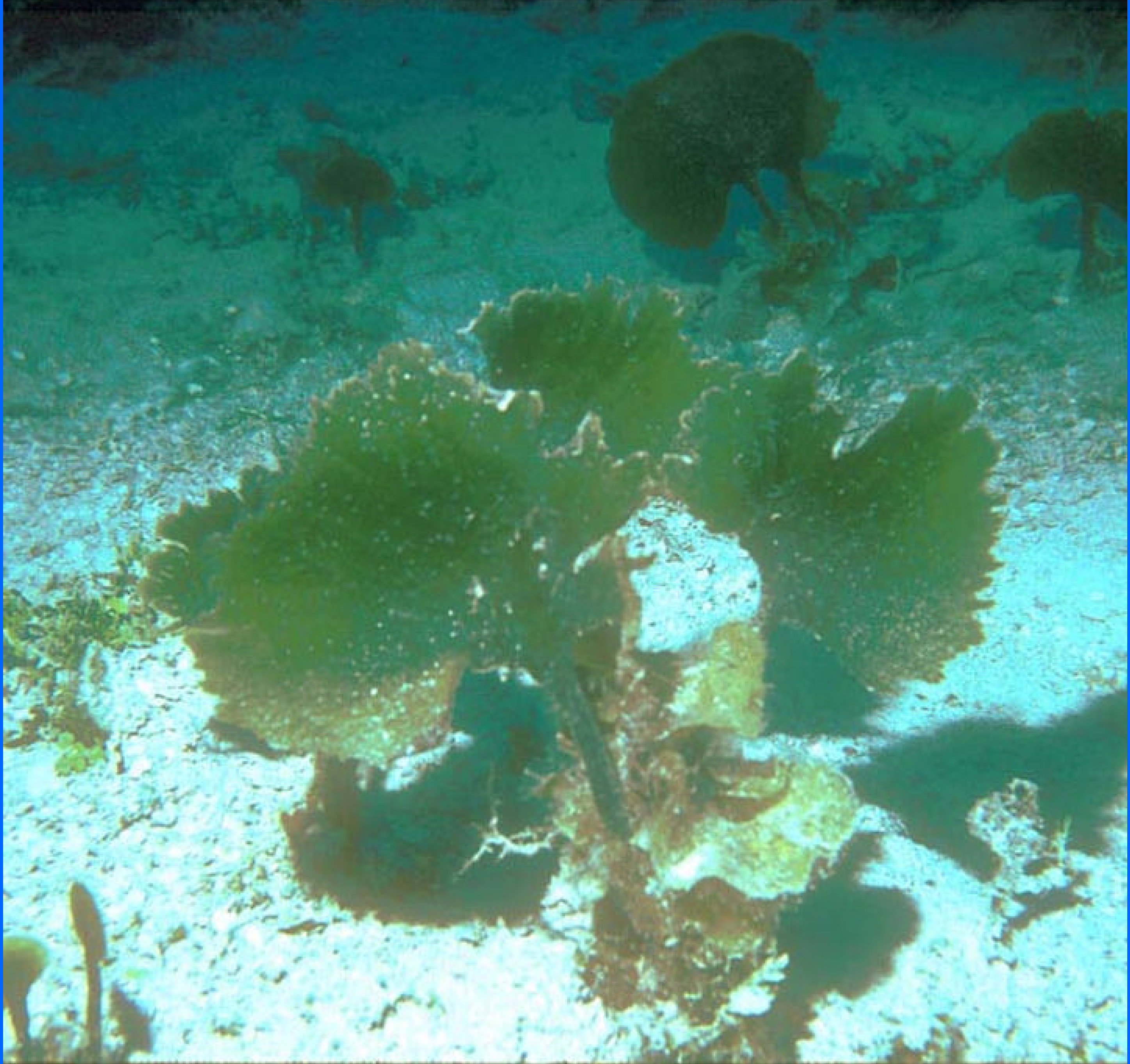


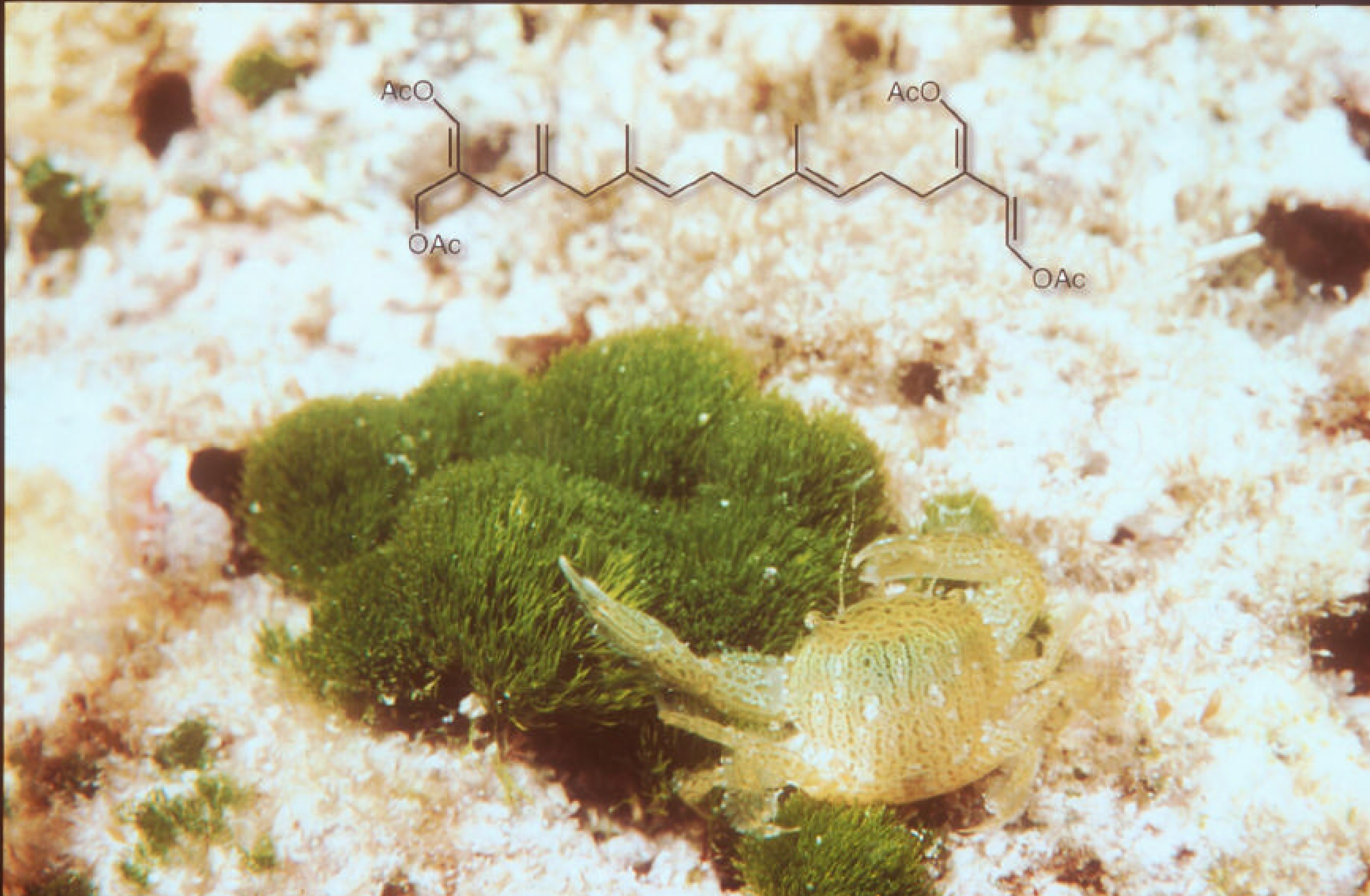
P < 0.001
P = 0.016
100
50
0
Amphipods Eaten (%)

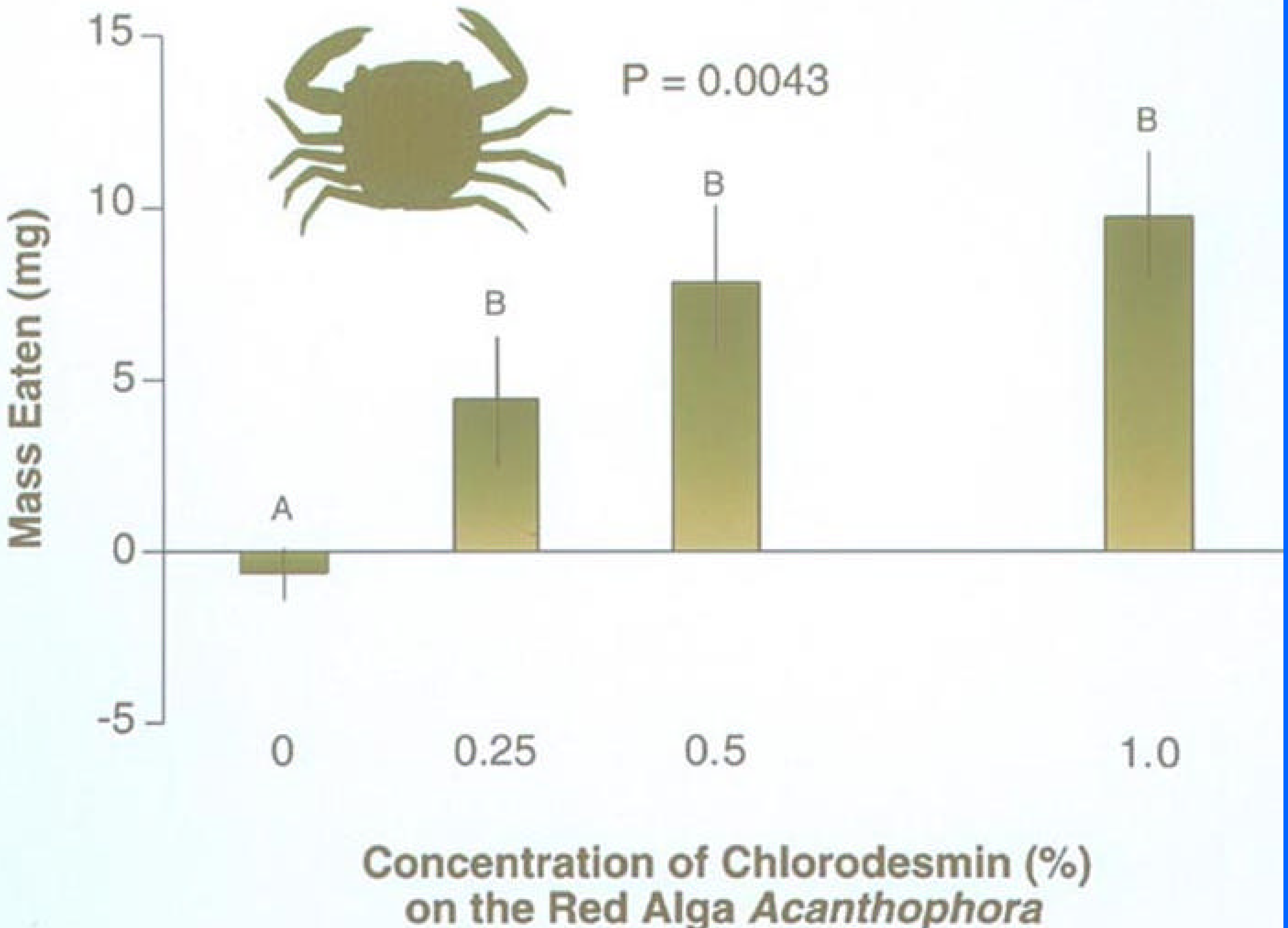



CH2OH
Br
Br
OH
OH
OH

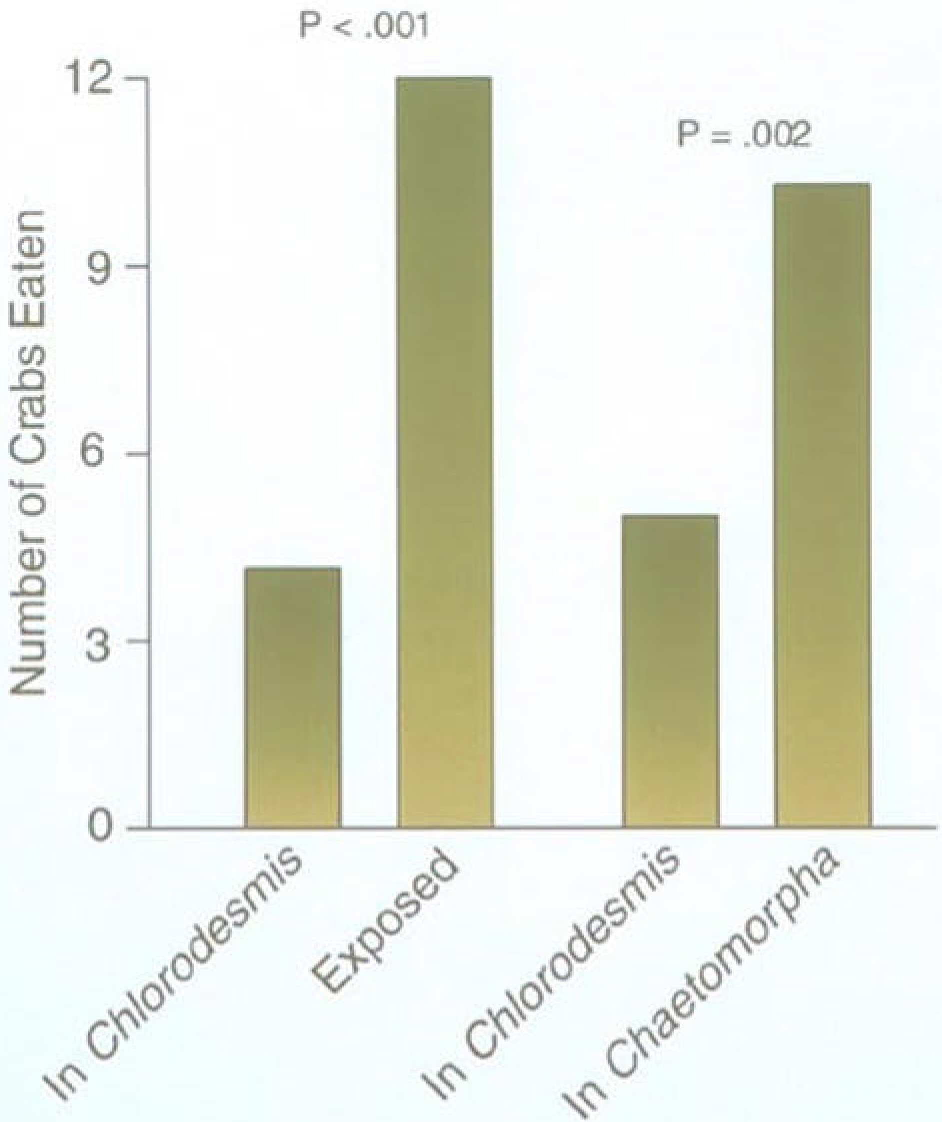

BELIZE
BAHAMAS
N=4
.02>P>.01
N=4
.005>P>.002
N = 5
P<.001
KRILL CONSUMED
5
4
3
2
1
0
10% LITTORINA CRUDE EXTRACT
10% COSTASIELLA CRUDE EXTRACT
ETHER CONTROL
5% AVRAINVILLEOL
ETHER CONTROL
25% COSTASIELLA CRUDE EXTRACT



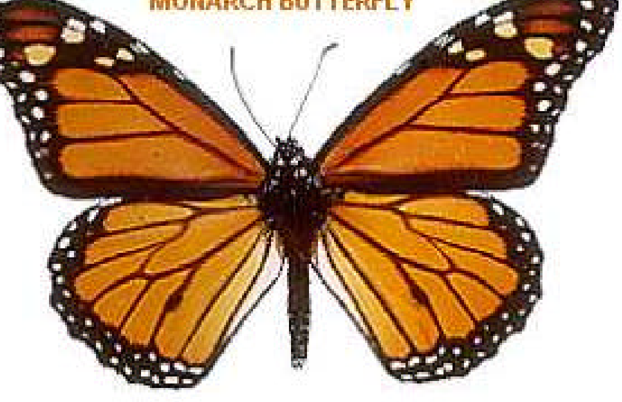

AcO
OAc
AcO
OAc

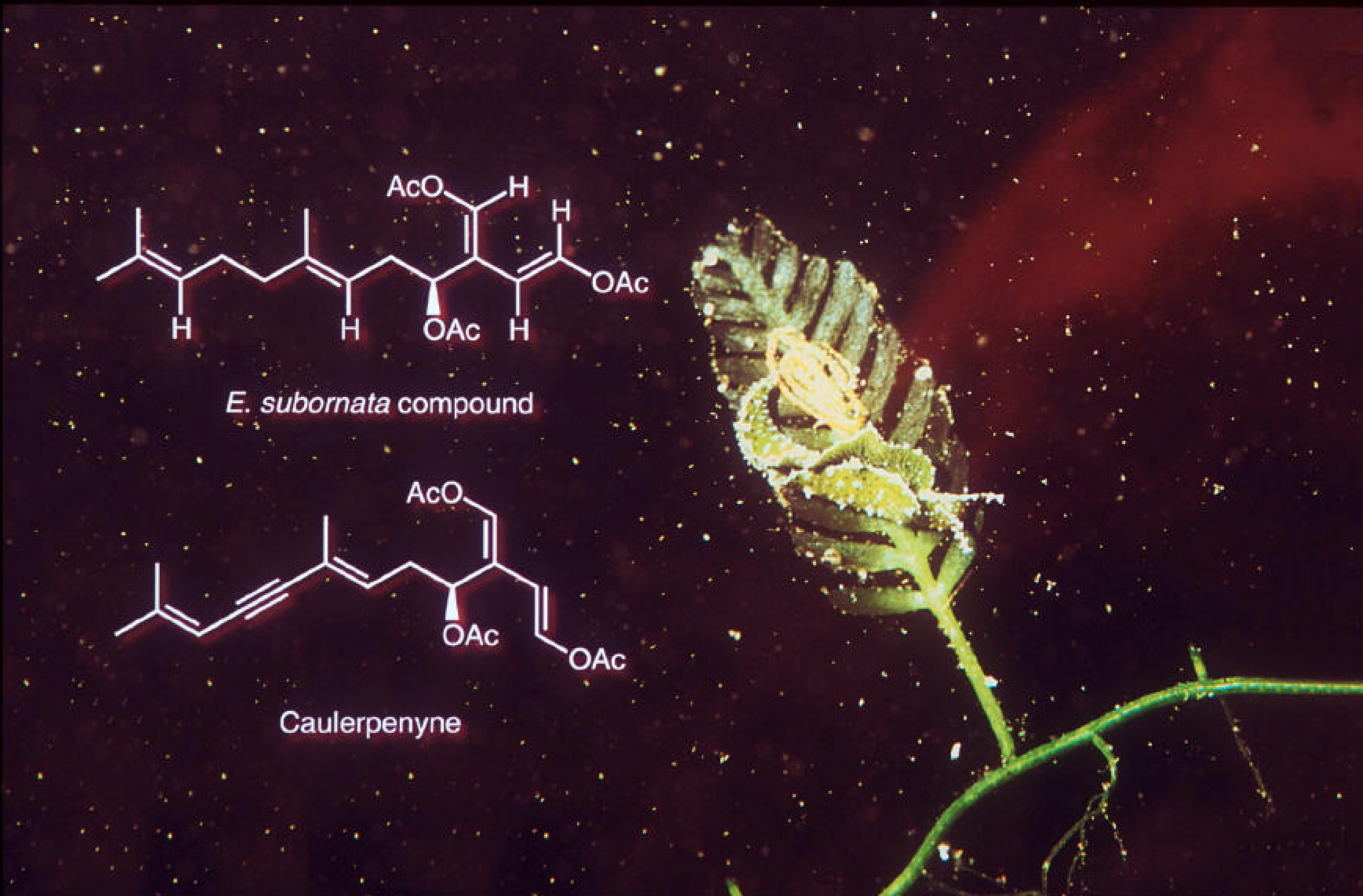


P = 0.0043
Mass Eaten (mg)
15
10
5
0
-5
A
B
B
B
0
0.25
0.5
1.0
Concentration of Chlorodesmin (%)
on the Red Alga *Acanthophora*



P < .001
P = .002
Number of Crabs Eaten
12
9
6
3
0
In Chlorodesmis
Exposed
In Chlorodesmis
In Chaetomorpha

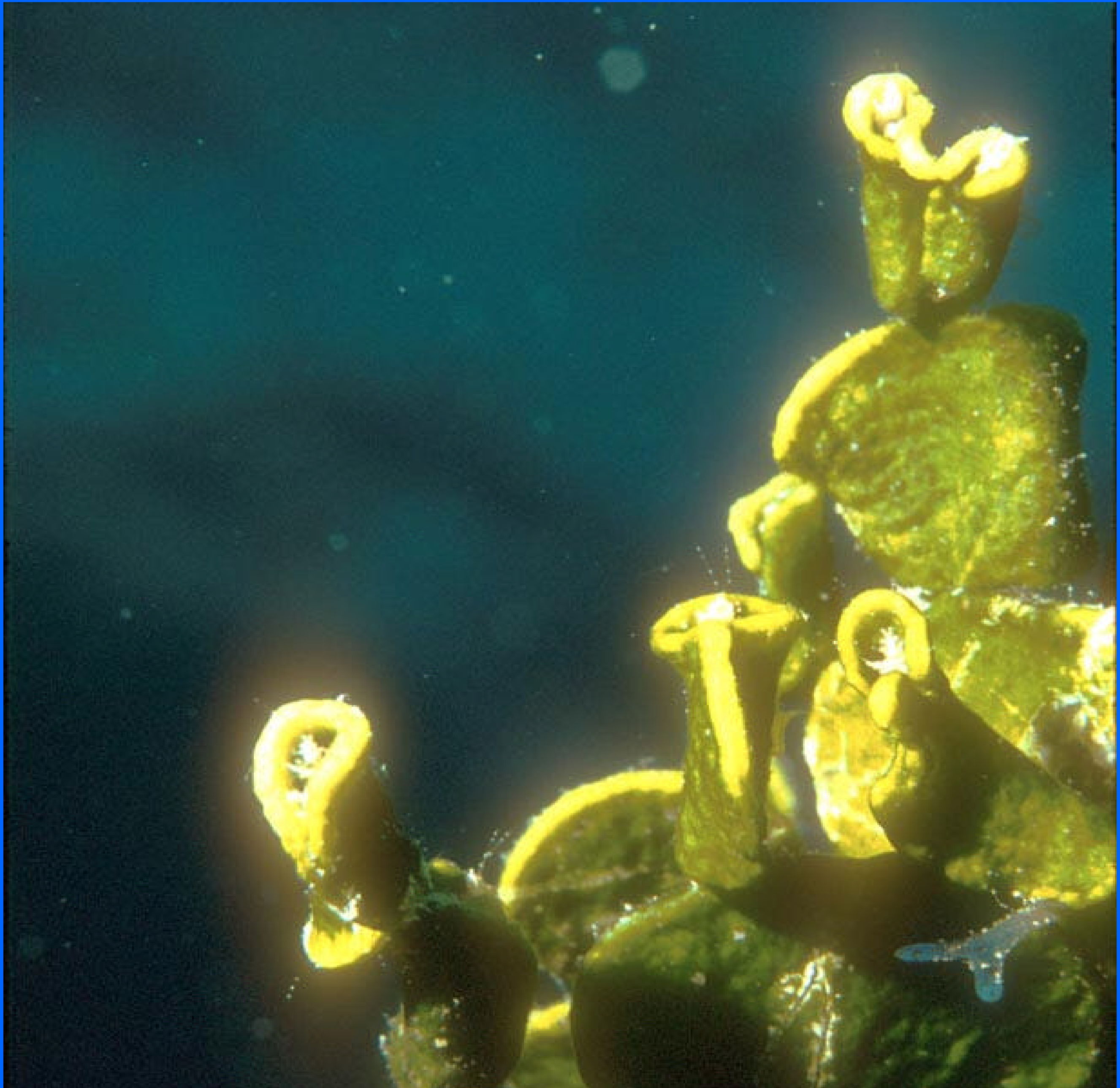

MONARCH BUTTERFLY

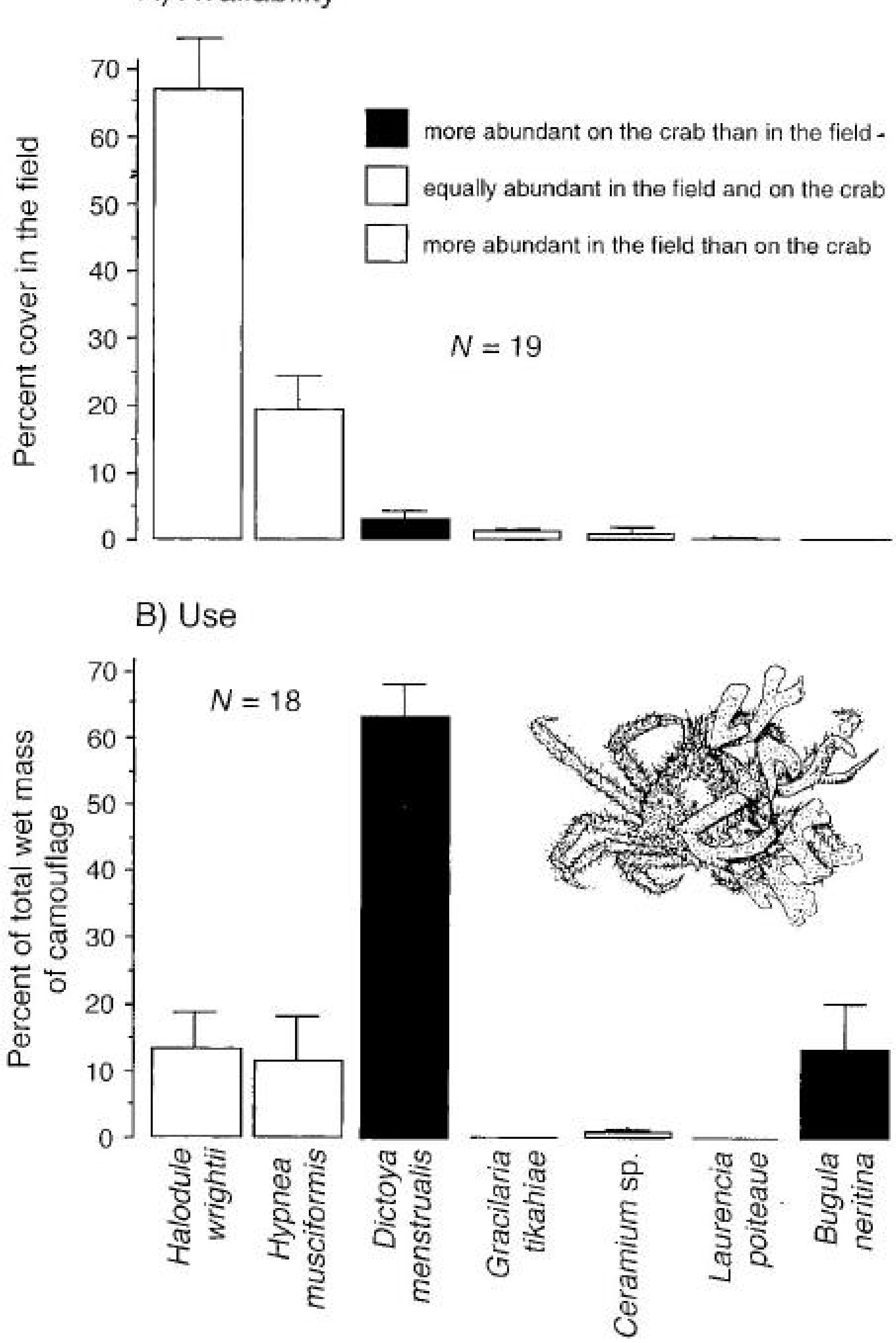

AcO
H
H
OAc
H
H
OAc
H
E. subornata compound
AcO
OAc
OAc
Caulerpenyne

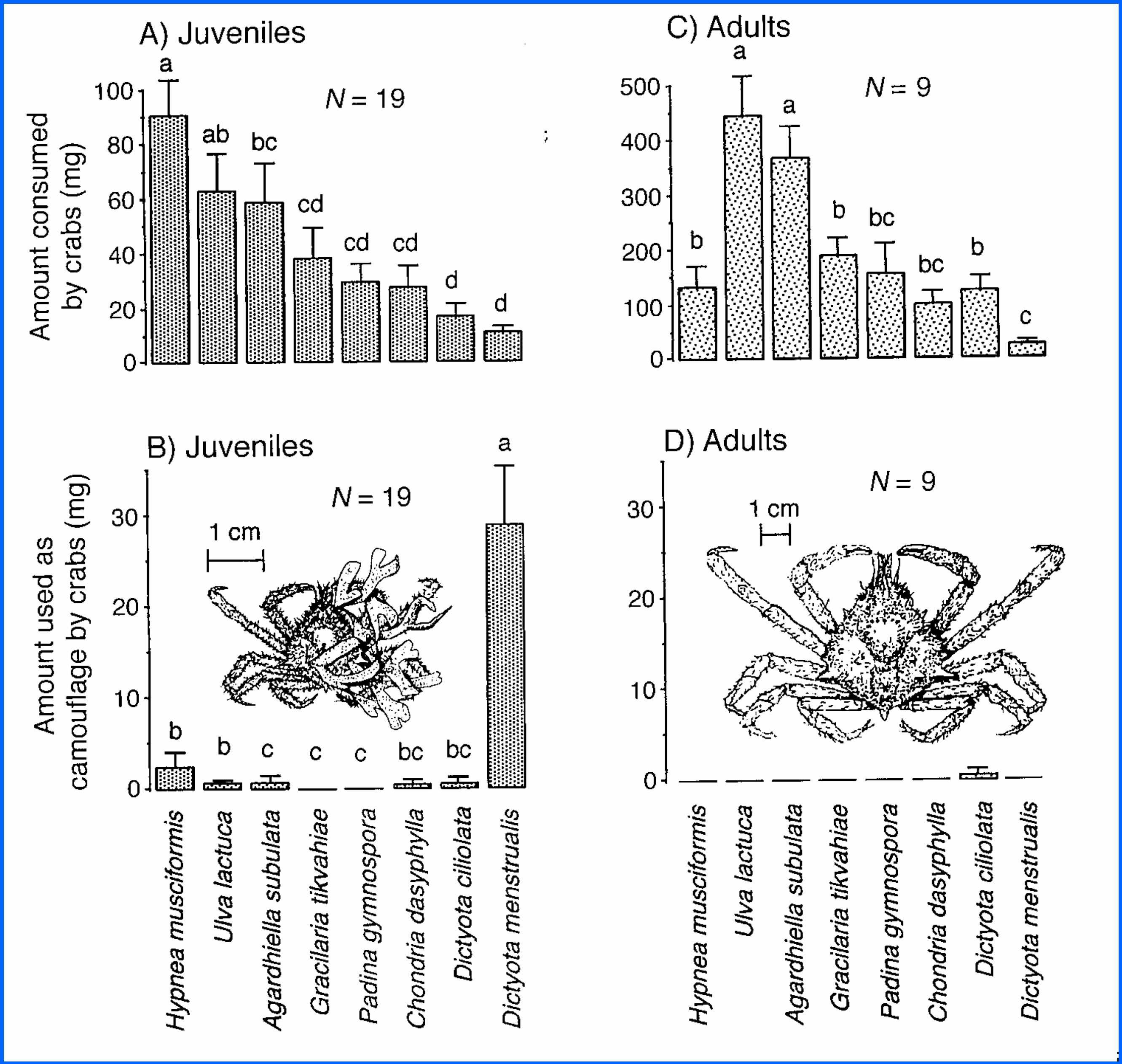
Specialized Feeding on Chemically-Rich Seaweeds is Predictably Associated with Escape from, or Deterrence of, Consumers (Atlantic, Caribbean, Indo-Pacific; Crabs, Amphipods, Gastropods…) . Thus, Feeding Patterns Among Small Sedentary Herbivores Appear to be Driven by the Need to Diminish Attack Rather than by Nutritional Aspects of the Food.

However, in the Cases I've Shown, <u>Food and Deterrence are Confounded.</u>

To get Around This, We Looked for <u>Seaweed Specialists that Didn't Eat Seaweeds</u> - Did They also Reduce Their Susceptibility to Consumers?



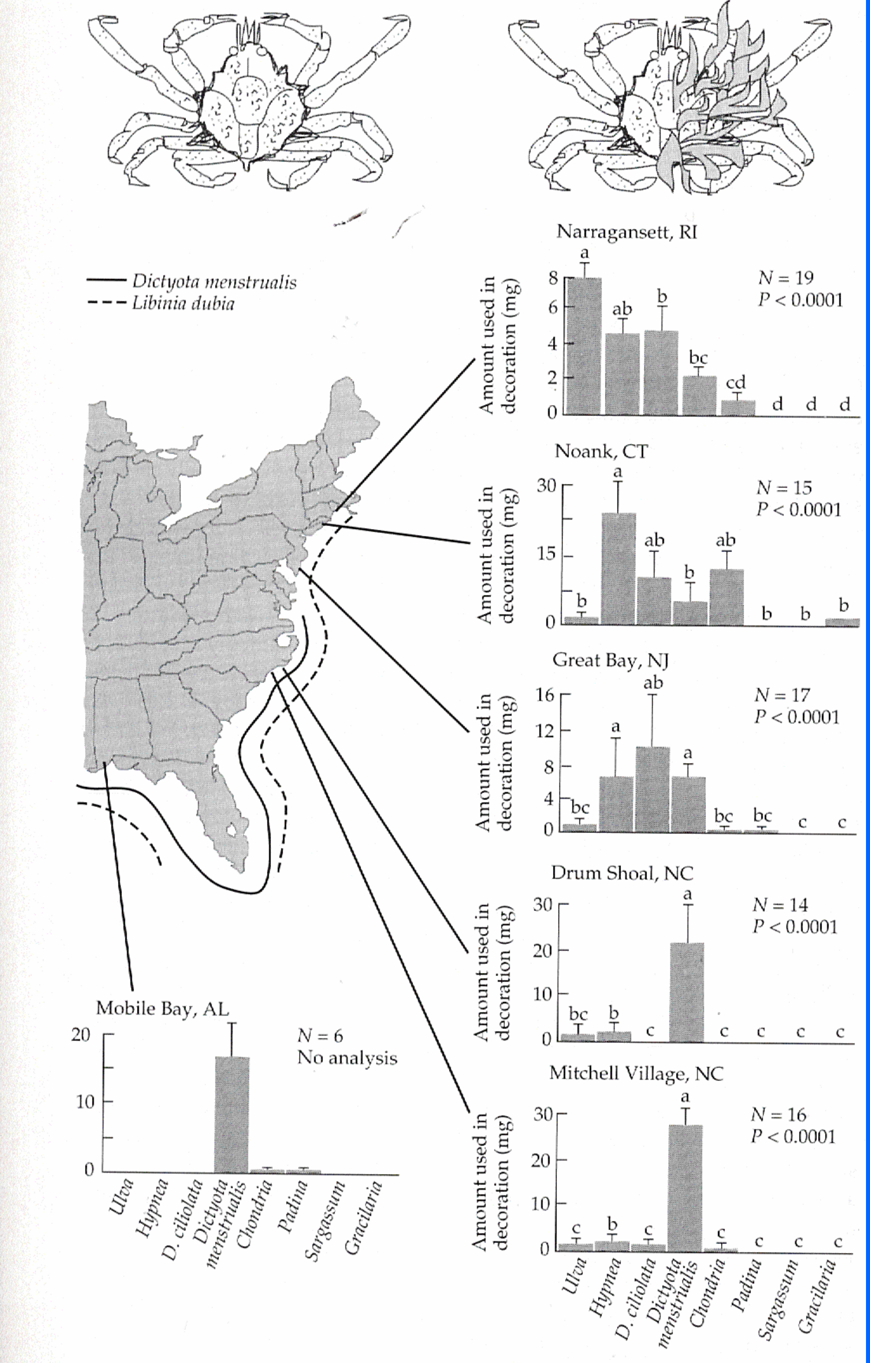

Percent cover in the field
70
60
50
40
30
20
10
0
more abundant on the crab than in the field
equally abundant in the field and on the crab
more abundant in the field than on the crab
N = 19
B) Use
Percent of total wet mass of camouflage
N = 18
Halodule wrightii
Hypnea musciformis
Dictoya menstrualis
Gracilaria tikahiae
Ceramium sp.
Laurencia poiteaue
Bugula neritina



(Stachowicz & Hay 1999 Ecology)

Of the Multiple Secondary Metabolites in *Dictyota menstrualis*, the Single Metabolite that Most Strongly Deterred Fishes was the Metabolite the the Crab used to Select Decorating Materials.

(Stachowicz & Hay 1999 Ecology)



(Stachowicz & Hay 2000 Am. Nat.)

Even Non-Herbivorous Species Can Specialize on Plants.

Like Herbivorous Species, These Non-Herbivores Escape Consumers Through Specialization

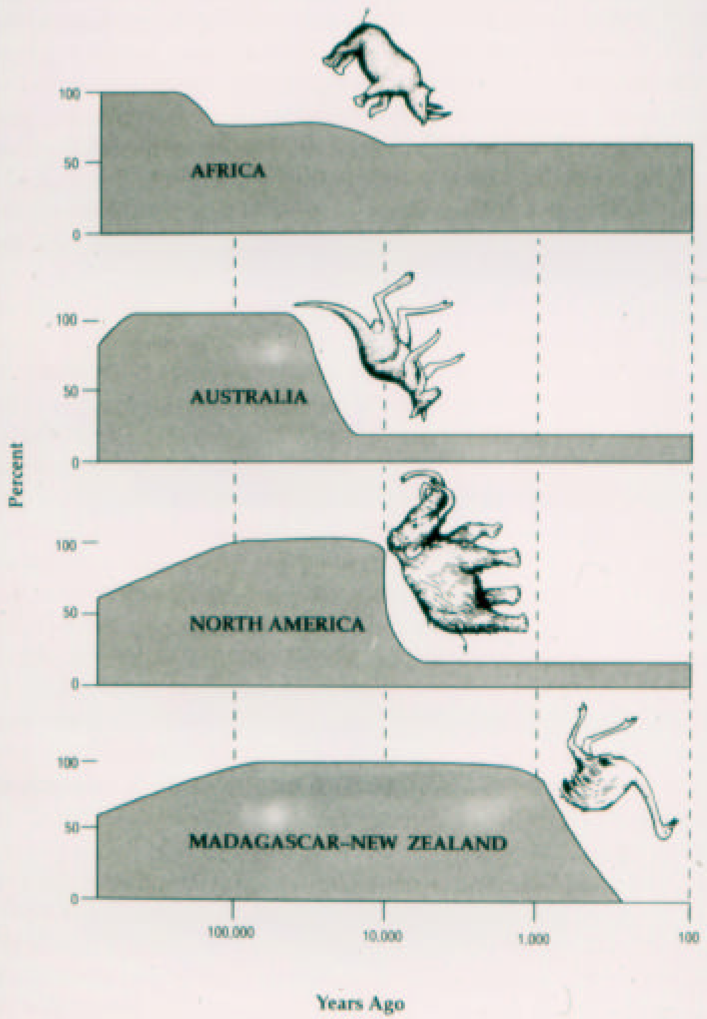
It Appears that Specialization Can Be, and Often Is, Driven by the Need to Avoid Consumers

Could this Explain Terrestrial Specialists as Well?

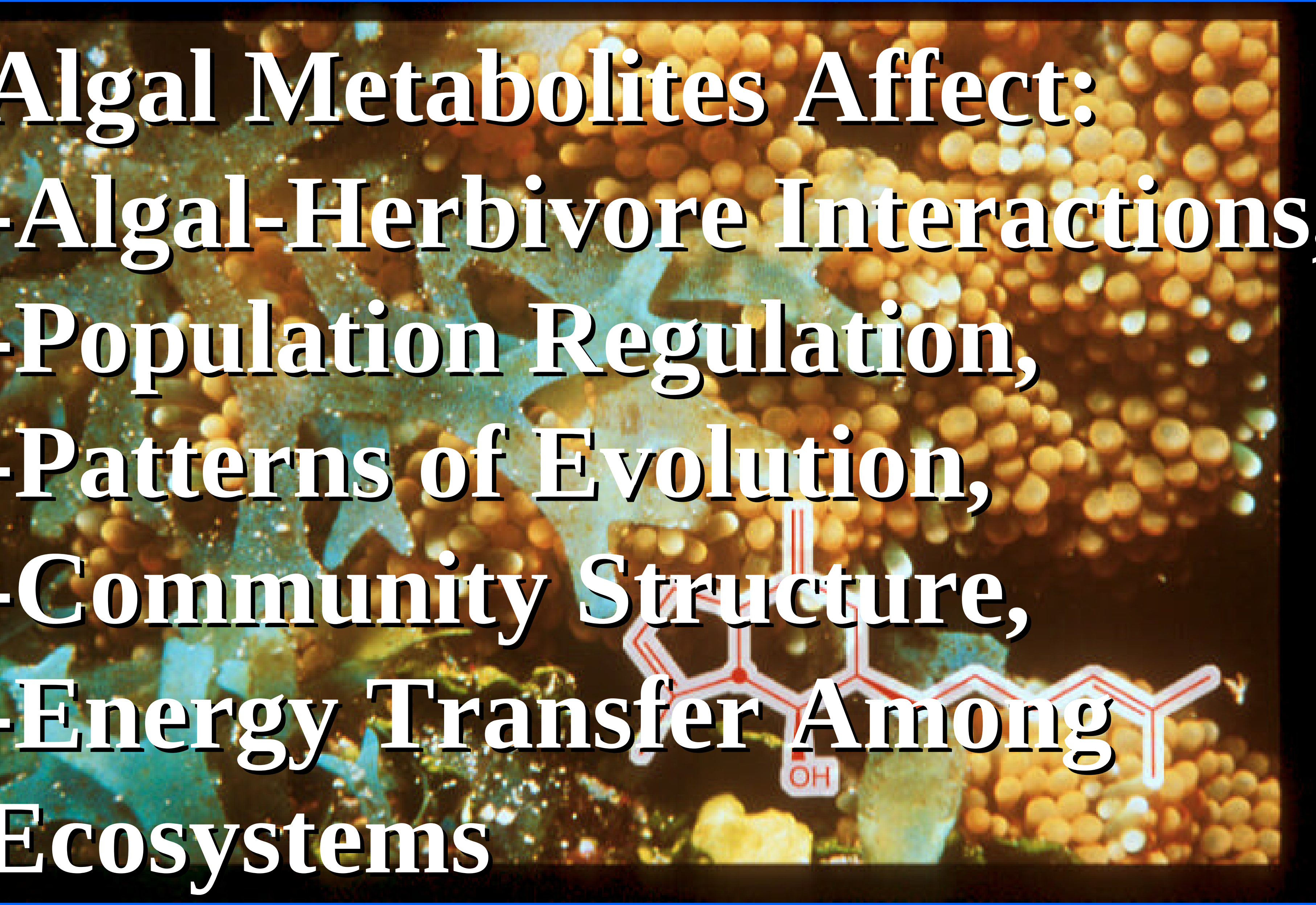
Can Marine Patterns Provide Insights into Terrestrial Processes?

“Insects drive plant evolution, vertebrates are less important.”
“We don’t have parrotfish equivalents that would indirectly consume insects.”
Etc…..

Evolution is a response to YESTERDAY, not today, and not tomorrow!

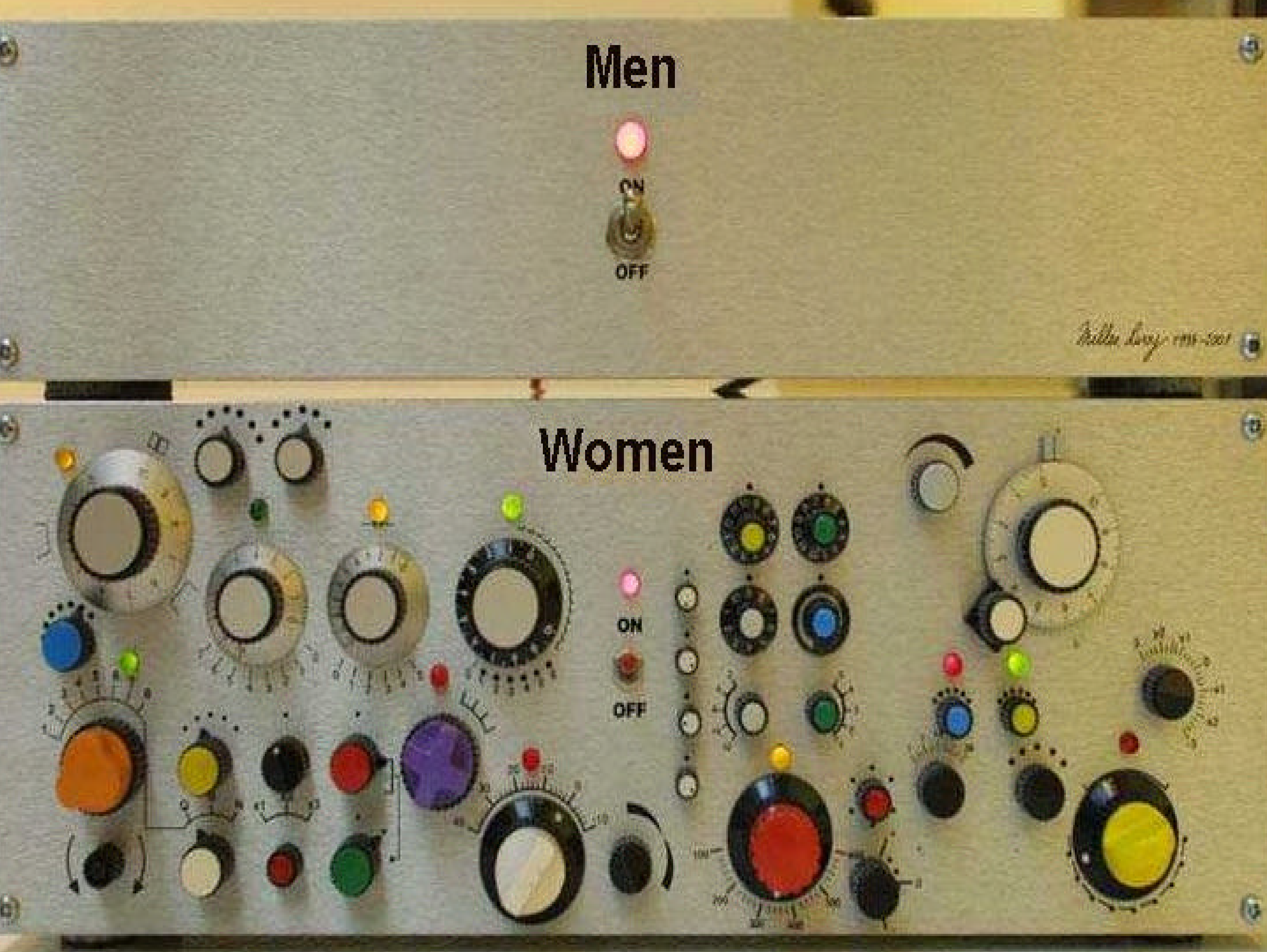


The extinction of large mammals and flightless birds coincided closely with the arrival of humans in North America, Madagascar, and New Zealand, and less decisively earlier in Australia. In Africa, where humans and animals evolved together for millions of years, the damage was less severe.


Algal Metabolites Affect:
-Algal-Herbivore Interactions,
-Population Regulation,
-Patterns of Evolution,
-Community Structure,
-Energy Transfer Among Ecosystems
OH

Two Final Thoughts on Chemical Ecology:

1) Context is Critical: Think dials not switches in most cases. Defensive against which consumer, in which situations, under which stresses, etc. (there are some people for which life is full of simple dichotomies, but for the thinking ones, it is much more situation specific – you know this from your own experiences


Men
ON
OFF
Women
ON
OFF

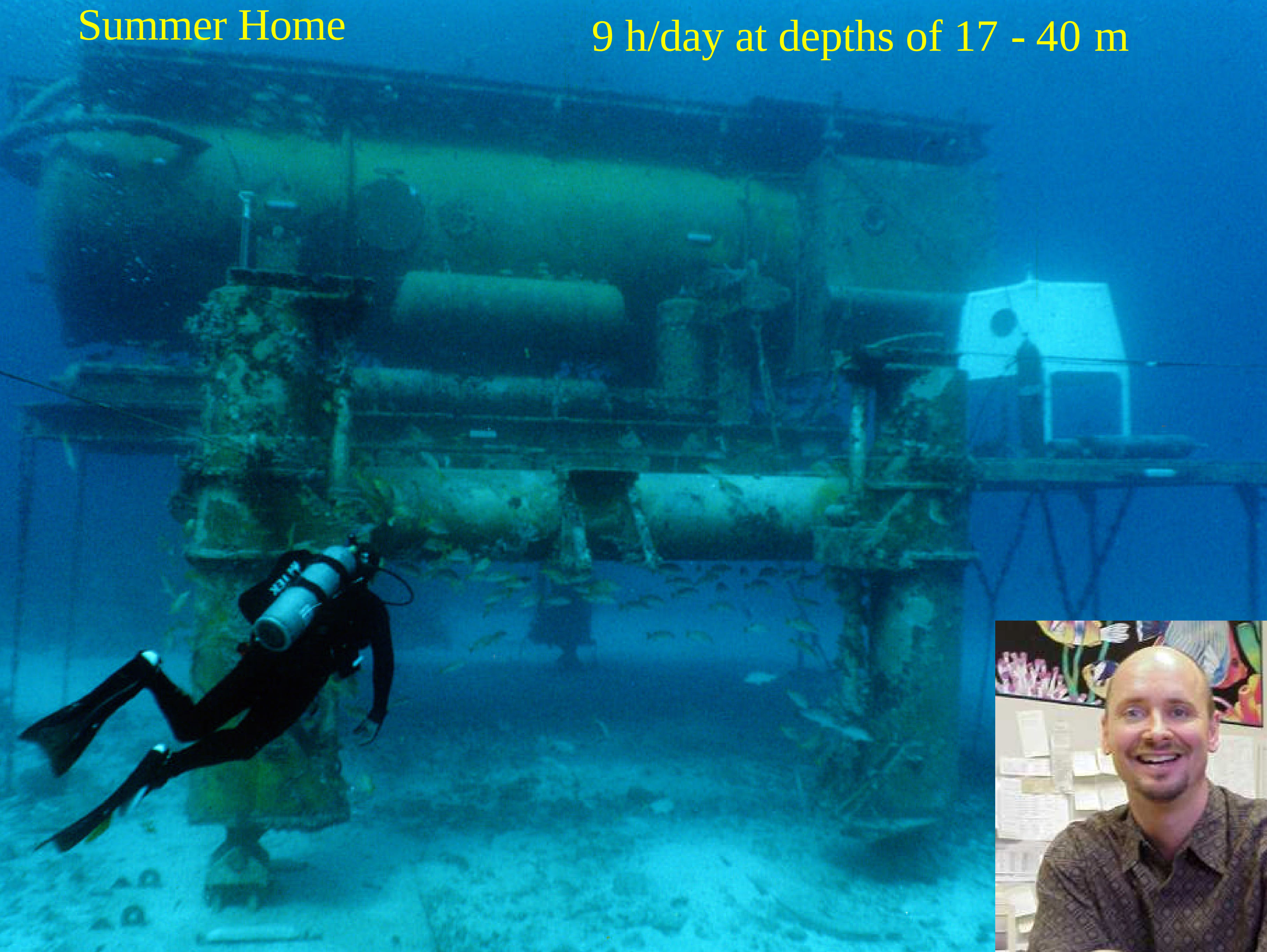
2) To do Science Well and Productively, It must Remain Fun, Challenging, and Meaningful


Gunilla can advise you




Summer Home

9 h/day at depths of 17 - 40 m




NOAA


SEAWEEDS OF THE SOUTHEASTERN UNITED STATES
UNCW


UNCW
NOAA


GOLDEN SHADOW
HAMILTON

The Hot-Tub ship….**NOT** our standard research ship

“Scientific” meeting of Biology graduate students at Ga Tech

To the organizers – many thanks!
But only Henrik has to wear the sh