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Title: Feeding preferences and chemical defenses of three Glossodoris nudibranchs and their diet sponges.

Approved: Valerie J Paul
Valerie J.Paul, Chairperson, Thesis Committee

Dorid nudibranchs are known to prey upon sponges, tunicates and bryozoans with high levels of secondary metabolites many of which have proved to be feeding deterrents. Nudibranchs frequently accumulate these deterrent compounds to use for their own defense. In this study three Glossodoris nudibranchs fed on Hyrtios sponges containing potent feeding deterrent compounds; yet, the nudibranchs showed no evidence of accumulating these deterrent compounds.

On a pinnacle in Apra Harbor, Guam, Glossodoris pallida was found feeding exclusively on a variety of Hyrtios erecta containing a high concentration of scalardial. Glossodoris hikeurensis and G. cincta preyed upon another variety of H. erecta from Cocos Lagoon, Guam which did not contain scalardial, but had high concentrations of heteronemin. Heteronemin was not found in H. erecta from Apra Harbor. When these two sponges were compared in laboratory preference tests G. pallida

strongly preferred H. erecta from Apra Harbor while G. hikeurensis and G. cincta preferred H. erecta from Cocos Lagoon.

The chemistry of G. pallida was similar to H. erecta from Apra Harbor, with some minor differences occurring, indicating that G. pallida receives its secondary metabolites through its diet. In laboratory preference tests Glossodoris pallida was not attracted to the crude extract or major metabolite of H. erecta from Apra Harbor. Thus, secondary metabolites did not appear to act as feeding cues for G. pallida. It is unknown how G. hikeurensis and G. cincta receive their secondary metabolites. Both of these nudibranchs contained four major metabolites in their organic extracts, none of which have been identified; but none were similar to heteronemin, the major metabolite of their diet sponge.

Organic extracts of the Hyrtios sponges were deterrent in at least two of the three feeding assays while organic extracts of nudibranchs were not deterrent under identical conditions. Minor metabolites may play a key role in determining feeding deterrence. Heteronemin was the only pure metabolite which proved to be a predator deterrent. Heteronemin was apparently consumed by both G. hikeurensis and G. cincta, but not stored. Deterrent compounds from diet Hyrtios sponges are probably excreted or altered by the Glossodoris nudibranchs.

FEEDING PREFERENCES AND CHEMICAL DEFENSES OF THREE GLOSSODORIS NUDIBRANCHS AND THEIR DIET SPONGES

BY

SHELLY D. ROGERS

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The members of the committee approve the thesis of Shelly D. Rogers presented March 31, 1989.

Valerie J. Paul, Chairman

Robert H. Richmond, Member

Ronald D. Smith, Member

ACCEPTED:

James A. McDonough
Dean, Graduate School and Research

Date

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INTRODUCTION

Dorid nudibranchs are interesting organisms for chemical ecological studies. Most specialize in feeding on sponges rarely eaten by other predators. The nudibranch's prey items typically contain a high level of secondary metabolites, particularly terpenoids, alkaloids, and acetogenins, that are biosynthesized by the prey organism but are apparently not needed for basic metabolism. Many of these secondary metabolites are biologically active, i.e. they possess antimicrobial activity, antiinflammatory activity, cytotoxicity or icthyotoxicity (reviewed by Minale 1978; Bakus 1981). Some are known feeding deterrents and may help to protect the sponge from being eaten by generalist predators. However, sponge chemical defenses do not work very well against specialist predators such as the nudibranch. Nudibranchs are typically not deterred by these noxious compounds and instead may accumulate and store them in specialized glands to be used for their own defense. The chemical relationship between nudibranchs and sponges has been reviewed by Thompson et al. (1982), Faulkner and Ghiselin (1983) and Karuso (1987).

Many dorid nudibranchs are currently assumed to possess chemical deterrents, especially if their known prey items contain a high concentration of secondary metabolites. This assumption may not be true for all

nudibranchs. The nudibranchs may be able to expel or alter the noxious compounds so that they are no longer deterrent. This could occur passively as the compounds decompose or be actively mediated by enzymes from the nudibranch. The advantage for the nudibranch would be not having to transport and store noxious compounds which could be harmful. It is also possible that the nudibranch may not accumulate enough of these defensive compounds to deter predators. At natural concentrations, the extracts of several species of Phyllidia nudibranchs were not found to be significant feeding deterrents against the sharpnose pufferfish Canthigaster solandri (Paul and Rogers, work in progress). Only a small portion of all dorid nudibranch species have been extracted and tested for feeding deterrence, although many nudibranchs are assumed to contain deterrent compounds (reviewed by Thompson et al. 1982; Faulkner and Ghiselin 1983; Karuso 1987). Furthermore, nudibranch extracts typically contain more than one compound. Generally, only the crude extract or most abundant metabolite have been tested for deterrence.

There are several possible mechanisms by which a nudibranch might accumulate feeding deterrent compounds. It may simply be taking advantage of an available food source which contains feeding deterrent compounds, and hence accumulates the secondary metabolites incidentally.

Possibly, the nudibranch actively selects for these metabolites when choosing its food, in which case, the nudibranch should be attracted to these secondary metabolites and may store these compounds in higher concentrations than other metabolites. A third alternative is that the nudibranch is capable of de novo synthesis of deterrent metabolites. The nudibranch Dendrodoris grandiflora (family Dendrodoridiidae) is able to produce its own feeding deterrent compounds from simple carbon molecules (Cimino et al. 1985). However, this is believed to be rare among nudibranchs. Most are believed to obtain their major secondary metabolites through their diet. A close relationship usually exists between the secondary metabolites of dorid nudibranchs and their dietary sponges (reviewed by Karuso 1987).

This study focuses on the chemical ecology of nudibranchs in the genus Glossodoris (family Chromodoridiidae), specifically Glossodoris pallida, G. hikeurensis and G. cincta. Glossodoris pallida is a small nudibranch, commonly known as a sea slug, varying between 0.1cm and 2.5cm in length and is nearly all white. Glossodoris hikeurensis and G. cincta are larger nudibranchs averaging roughly 8cm in length. Glossodoris hikeurensis and G. cincta look very similar to each other and are splotchy brown in color. Glossodoris nudibranchs typically feed on sponges in the order Dictyoceratida

(e.g. Schulte et al., 1980; Okuda and Scheuer, 1981; Thompson 1982; pers. obs.). Dictyoceratida is in a group of sponges called Desmospongiae. Desmospongiae are biologically unique in that they possess a fibrous rather than a mineral skeleton (Bergquist 1980) and contain a relatively high concentration of secondary metabolites, including a variety of terpenes, particularly sesterterpenes (Crews and Naylor 1985).

On Guam, Glossodoris pallida is found almost exclusively on the sponge Hyrtios erecta (order Dictyoceratida; family Thorectidae). Large, constant populations of the sponge and nudibranch exist at a depth of 20-30m on a pinnacle known as Sponge Mound in Apra Harbor, Guam (Fig. 1). Hyrtios erecta has been extensively collected and studied in Australia, the Red Sea, Palau and Polynesia (Crews et al. 1985; Crews and Bescansa 1986; Paul pers. comm.). Glossodoris pallida has not been reported to feed on H. erecta in any part of the world except Guam. The major metabolite of the sponge on Guam is scalardial (Fig. 2). Hyrtios erecta populations from Australia, the Red Sea, and Palau are reported to contain a different, but closely related secondary metabolite, heteronemin (Fig. 2), which is not found in H. erecta from Apra Harbor. Scalardial has been reported as a secondary metabolite of H. erecta from the Kingdom of Tonga, Polynesia, but in very low

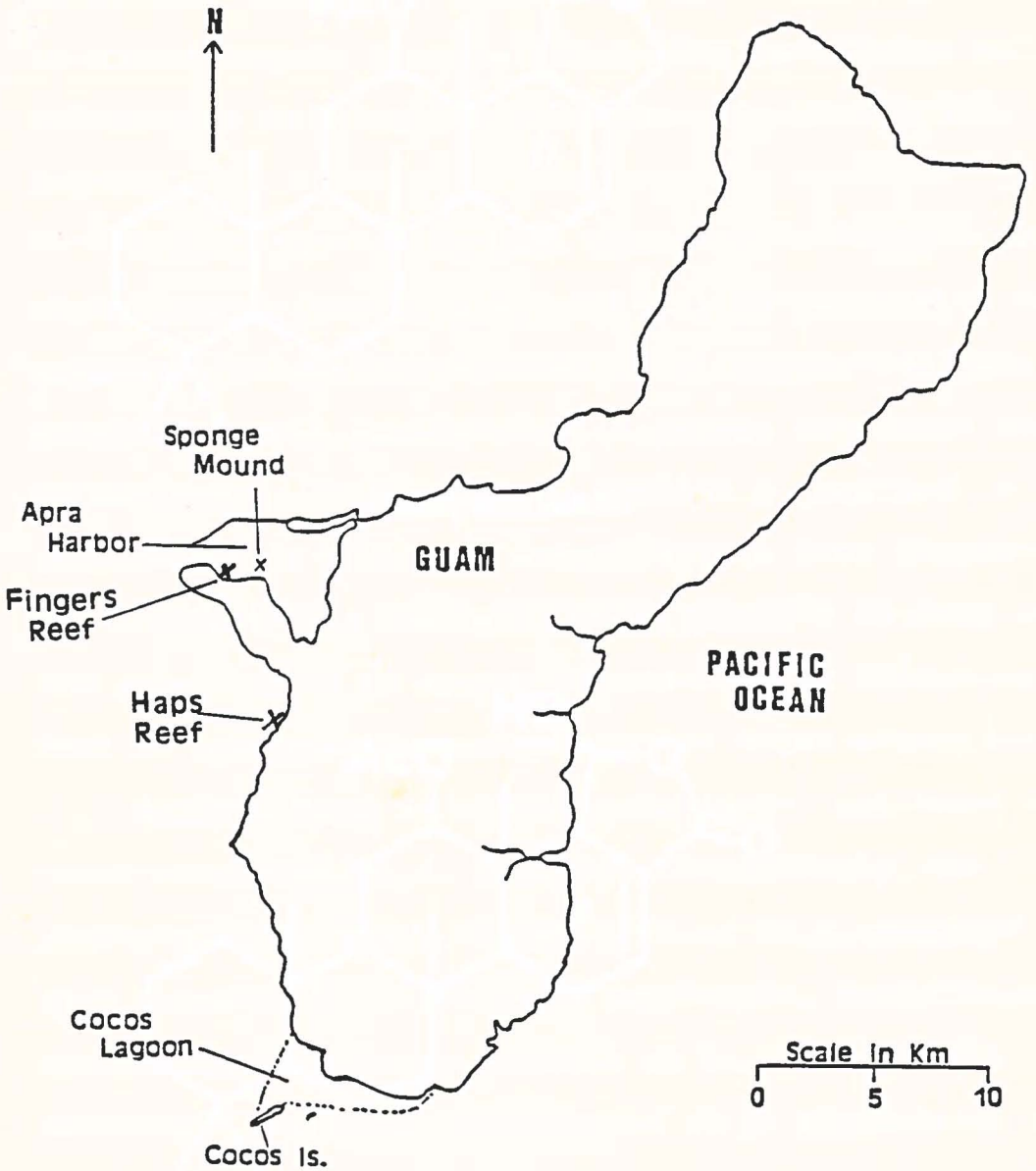
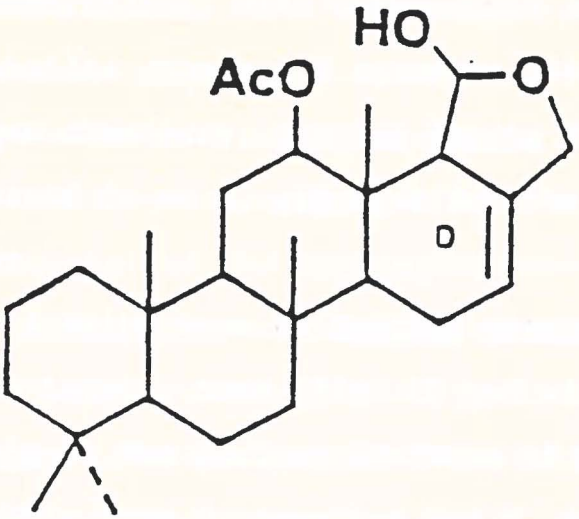
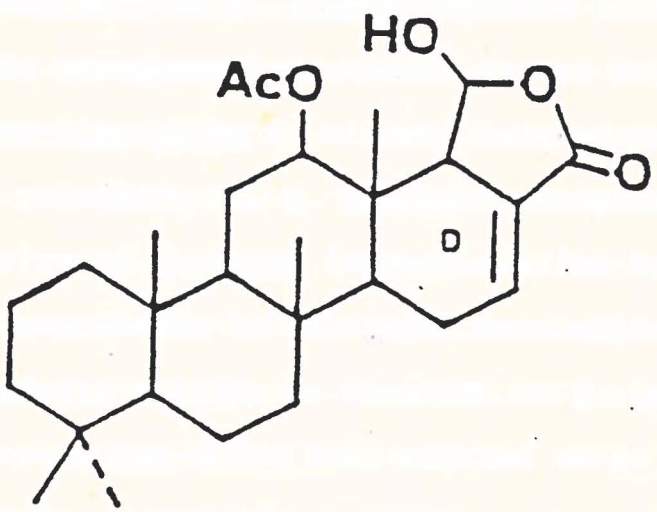


Fig. 1. Map of Guam showing study sites.



Deoxyscalarin



Scalarin

Fig. 3. Major metabolites of Hyrtios erecta-A, not previously pictured, deoxyscalarin and scalarin.

concentrations, with heteronemin still being the major metabolite (Crews and Bescansa 1986). Because of its unique chemistry, Hyrtios erecta from Apra Harbor will be referred to as H. erecta-A for the remainder of this paper.

A third type of Hyrtios erecta was discovered from Cocos Lagoon, Guam (Fig. 1) and will be referred to as H. erecta-C. The surface skeleton of H. erecta-C differs slightly from H. erecta-A and H. erecta from other parts of the world. It is uncertain whether H. erecta-C is a new species or simply an unusual growth form (Bergquist pers. comm.). The chemistry of H. erecta-C is similar to the chemistry of H. erecta reported in the literature in that it possesses heteronemin as its major metabolite.

Hyrtios altum is closely related to, and found in close proximity to, H. erecta-A. Surprisingly, the chemistry of H. altum is most similar to H. erecta-C in that heteronemin is the major metabolite. Even though Glossodoris pallida is abundant on H. erecta-A, the nudibranch has never been sighted on H. altum or on H. erecta-C.

In contrast, Glossodoris hikeurensis and G. cincta, on Guam, are found feeding on Hyrtios erecta-C in or near Cocos Lagoon, and have never been sighted feeding on H. erecta-A. This study focused on G. pallida because it

could be collected consistently and in larger numbers than other Glossodoris species.

The purpose of this study was to examine the biochemical relationship between the Glossodoris nudibranchs and the Hyrtios sponges. The hypothesis is that Glossodoris nudibranchs are attracted to and selectively accumulate feeding deterrent compounds found in their food sponges and that these metabolites are feeding deterrents.

Three questions are addressed:

(1) Can Glossodoris pallida detect and be attracted by secondary metabolites found in H. erecta-A?

(2) Do Glossodoris nudibranchs selectively accumulate and concentrate secondary metabolites, particularly scalardial and heteronemin, from their food sponges?

(3) Are the crude extracts or major secondary metabolites of any of the Hyrtios sponges or Glossodoris nudibranchs deterrent toward nudibranch potential predators?

MATERIALS AND METHODS

NUDIBRANCH FIELD STUDIES

Three techniques were used to determine nudibranch feeding preferences: field observations, cage experiments in the field, and laboratory preference experiments. Field observations involved weekly dives on Sponge Mound where Glossodoris pallida and Hyrtios erecta-A are abundant. Seventy-five Hyrtios erecta-A were tagged with flagging tape. The number of G. pallida on each tagged sponge was recorded approximately twice each month for 10 months, November 1987 through August 1988. At the same time, H. altum in the vicinity were examined for nudibranchs.

A caged field experiment with Glossodoris pallida was conducted to help answer two questions: (1) will G. pallida eat a sponge closely related to Hyrtios erecta-A if no other food source is available? (2) if G. pallida will feed on a related sponge, will its chemistry change? Three cages were set out at Sponge Mound with one type of Hyrtios sponge in each cage. Since H. erecta-C is the only sponge that occurs naturally at Cocos Lagoon, and it does not occur at Sponge Mound, a fourth cage was set up with H. erecta-C at Cocos Lagoon to help control for effects of moving the sponge to a new habitat. Six G. pallida were placed in each of the four cages.

The cages were brushed weekly to unclog the screen mesh and allow for increased water circulation. At the same time, nudibranch location with respect to substrate, sponge or screen, was recorded. Nudibranchs consistently found on their caged sponge were assumed to be feeding on that sponge. After 55 days, the cages were collected; and the nudibranchs and sponges were extracted. Extracts were compared by thin layer chromatography (TLC). Compounds are separated by TLC according to polarity, with each compound giving a characteristic staining pattern after visualizing it with 50% sulfuric acid. Thus, the presence or absence of secondary metabolites can be readily assessed (Paul and Fenical 1986). Similarities and differences in secondary metabolites between the caged nudibranch groups were noted.

Approximately 15 dives were made in Cocos Lagoon where G. hikeurensis and G. cincta were observed. The substrate they were crawling on was recorded. Before this study began, no G. hikeurensis had ever been sighted on Sponge Mound. To determine if G. hikeurensis could feed on Hyrtios erecta-A or H. altum, found in the area, three G. hikeurensis were introduced to the Sponge Mound reef. Periodic dives were made to determine what the nudibranchs were feeding on in the new area.

LABORATORY PREFERENCE STUDIES

In the laboratory, Glossodoris pallida was tested for attraction toward Hyrtios sponges and sponge metabolites. Twelve nudibranchs were placed individually into twelve, 300ml flow-through tanks. Pieces of sponge were weighted with lead sleeves; and one piece was placed in each tank with a nudibranch. One rock was also placed in each tank as a control. The rock was approximately the same size as the sponge that it was placed with. Once the experiment was set up, nudibranchs were not moved or disturbed for at least 15 test intervals. From previous observations, sixty minutes was determined as the minimum test interval which allowed sufficient time for the nudibranchs to move.

At the end of each interval, each nudibranch was checked to see if it was in contact with the sponge, the rock, or something else in the tank. To avoid biases, the nudibranch was recorded as being in contact with whatever object its mouth was touching. If its mouth was touching the sponge, it received a "+" score. If its mouth was touching the rock, it received a "-" score. If it was anywhere else in the tank, it did not receive a score for that interval. The test consisted of a minimum of 12 intervals with 12 active individuals. Nudibranchs which did not receive a single score during the entire test were excluded. At the end of the test, the "+"'s and

"-"'s were added separately for each individual. The Wilcoxon Signed-Ranks Test for paired comparisons was used to analyze the results (Sokal and Rohlf 1981).

Using similar techniques, several other preference experiments were conducted. Glossodoris pallida was tested to see if it preferred Hyrtios erecta-A over either H. erecta-C or H. altum. Afterwards G. pallida was tested to determine if there was a preference between H. erecta-C and H. altum. Preferences between the light inner matrix and the dark surface layer of H. erecta-A were also tested. These experiments were completed by matching portions of the matrix with similar size portions of the surface of the same size piece of H. erecta-A, then placing each matched pair in a test tank and observing the nudibranchs' reaction as before. Finally, several pieces of H. erecta-A that had been grazed by G. pallida in the field were collected. The portion of the sponge that was grazed by the nudibranch was cut out and offered to nudibranchs in replicate tanks along with a portion of the same sponge that had not been grazed.

To test for the nudibranch's attraction toward extracts and isolated metabolites, Hyrtios erecta-A was soaked repeatedly in a 1:1 mixture of ethylacetate:methanol until all visible organic metabolites were removed. Solvents were evaporated from

the sponge by leaving the sponge under a fume hood overnight. Pieces of the extracted sponge were cut and matched with equivalent-size pieces of fresh sponge. The pairs were placed into the test tanks to determine if Glossodoris pallida preferred the extracted or the fresh sponge.

In a later test, the organic metabolites from the extracted Hyrtios erecta-A sponge were replaced. This was done by first removing the water layer from the organic extract, then evaporating the solvents by rotary evaporator. The oily residue was diluted in diethyl ether and coated on the extracted sponge at the extract's natural concentration. Glossodoris pallida was then offered the choice between extracted sponge coated in sponge extract or extracted sponge coated in pure ether. The same procedure was also used to test scalardial, the major metabolite of the sponge Hyrtios erecta-A, at the sponge's natural concentration. Results were analyzed as before with the Wilcoxon Signed-Ranks Test for paired comparisons.

Glossodoris hikeurensis and G. cincta were tested for their preference between Hyrtios erecta-A and H. erecta-C. Hyrtios altum was not tested because it was not as abundant as the other two sponges. Five G. hikeurensis and three G. cincta were placed in a large flow-through aquarium with equivalent amounts of H. erecta-A and H.

erecta-C. Each morning for approximately 3 weeks, the aquarium was checked to determine which sponge, if any, each nudibranch was attached to. Due to variations in color patterns within each species, it was possible to tell the individual nudibranchs apart. Thus, a separate feeding record for each nudibranch was obtained. Results were analyzed using the Wilcoxon Signed-Ranks Test for paired comparisons.

CHEMICAL ANALYSIS

To prepare an extract, the nudibranch or sponge was first freeze-dried. Then, sponges were blended in a 1:1 mixture of acetone:methanol and filtered out immediately. This was repeated until all visible metabolites had been extracted from the sponge. One exception was made to this procedure. When comparing the surface and matrix layers of H. erecta-A, the sponge portions were soaked instead of blended, because of the small quantities used for the extractions. Nudibranchs were soaked in two consecutive baths of acetone, which were later combined. Then, the nudibranchs were filtered out. Water-soluble metabolites were extracted from H. erecta-A and H. altum. This was done by homogenizing the sponge in tap water in a blender, followed by filtering and freeze-drying. The percent yield of extract from each organism was calculated by dividing the weight of the extract by the

dry weight of the organism and multiplying by one-hundred.

All extracts were compared by thin layer chromatography (TLC). Compounds were usually identified by comparing the results to known standards. Major metabolites were isolated by flash chromatography followed by high performance liquid chromatography (HPLC). Metabolites were identified by proton nuclear magnetic resonance spectroscopy (NMR), comparing observed values to those reported in the literature. Concentrations of the metabolites within the extracts were determined based on the amounts isolated by HPLC.

To determine if a chemical change in the sponge occurred when Glossodoris pallida grazed on Hyrtios erecta-A, eight sponges with five or more nudibranchs on each and twelve sponges with no nudibranchs were collected. Comparisons of TLC plates and extract concentrations were used to determine if there were any qualitative or quantitative differences between the grazed and ungrazed sponges. Chemical differences between the matrix and the surface of H. erecta-A were also tested.

PREDATOR DETERRENCE

To determine if Glossodoris pallida has feeding deterrent compounds, ten nudibranchs were placed, one at a time, in an aquarium with hungry predatory fish. Although this technique was crude, it has proven to be useful in determining possibilities of feeding deterrent compounds (Herdman and Clubb 1892, Crossland 1911 and Thompson 1960). Pieces of fish meat, the same color and size as the nudibranch, were offered to the aquarium fish in between each offered nudibranch. The fishes' reactions were recorded. Fish used were Rhinecanthus aculeatus (Picasso trigger fish), Halichoeres trimaculatus (3-spotted wrass), Lutjanus monostigmus (snapper) and Epibulus insidiator (sling-jaw wrass).

Feeding deterrence of crude extracts and pure metabolites was tested against the pufferfish Canthigaster solandri under controlled laboratory conditions, and against general predatory fish under natural conditions, in the field. Canthigaster solandri feeds on benthic algae and small benthic invertebrates (Amesbury and Myers 1982) and could be assumed to be a potential predator of Glossodoris nudibranchs. Several Canthigaster solandri were trained to feed on agar-based food tablets in the laboratory. The food was made by heating agar, brine shrimp eggs, freeze-dried krill and

water to boiling, then allowing the solution to cool until it jelled.

In order to test a compound, it was dissolved in a small quantity of acetone or a mix of acetone and hexane or methanol, depending upon the polarity. The dissolved compound was added to the food mixture after heating, while it was still warm. The mix was stirred for thirty seconds while the solvent evaporated to ensure even distribution of the compound. Finally, the mix was poured into a tray and allowed to cool until firm. Control tablets were made the same way using pure solvents. All compounds were tested at natural concentrations or below. Concentrations of the treated tablets were calculated based on the percent of the metabolite by dry weight of the food tablet.

Four treated and four control tablets were offered to sets of two to three Canthigaster solandri in separate chambers within a large outdoor aquarium. A minimum of seven sets of fish was used. The amount eaten was determined by measuring the change of weight in the treated and control tablets for each set of fish. A t-test for paired comparisons was used to analyze the results (Sokal and Rohlf 1981). One treated-and-control pair was placed in a chamber without predators to insure that weight changes in treated and control tablets were equivalent. Tests resulting in a p value between .03 and

.07 were repeated. Repeated tests were analyzed together using a Three-Way Factorial ANOVA (Sokal and Rohlf 1981).

Feeding deterrence against generalist predators under natural conditions was tested at two sites, Fingers Reef, inside Apra Harbor, and Haps Reef, facing the open ocean (Fig. 1). To prepare a field assay, sets of four pieces of dried squid filets, approximately 1.5cm^2, were individually attached by a paper clip to 3-strand, polypropylene ropes 0.5m in length. Extracts or isolated metabolites were diluted in a volatile solvent, diethyl ether, acetone or 1:1 acetone:methanol, and coated evenly on the dry squid pieces. Control ropes were made by coating squid pieces in pure solvent.

SCUBA was used to conduct the assays. Matched pairs of ropes, consisting of one treated and one control, were attached to the reef at 3-6m depth and spaced 0.25m apart. Twelve to 15 pairs were used in each assay with each pair placed several meters apart. When at least four pieces of squid were consumed from a pair, the numbers of control and treated pieces eaten from that pair were recorded. The numbers of treated and control pieces eaten were compared with the Wilcoxon Signed-Ranks test for paired comparisons (Sokal and Rohlf 1981). Assays lasted approximately 15-20 minutes. Results were compared to test results from the laboratory experiments with Canthigaster solandri.

RESULTS

NUDIBRANCH FIELD STUDIES

A total of 75 sponges were labeled and examined during 19 dives over a 10-month period to determine the mean number of nudibranchs, Glossodoris pallida, per sponge, Hyrtios erecta-A, which was 1.92 ± 0.67 (Table 1). During the periodic dives, Glossodoris pallida was never sighted on H. altum, a closely related sponge found in the area. Over the 10 month period there was a gradual increase in the number of G. pallida per H. erecta-A, ranging from 1.0 on December 12, 1987 to 3.4 on July 13, 1987. Several of the tagged H. erecta-A died, probably for reasons related to the tagging and handling of these sponges. This may have caused the higher numbers in the latter part of the study, as there were fewer sponges available for nudibranchs to graze. When compared against a Poisson distribution of expected random frequencies, the data showed a significantly clumped distribution of nudibranchs (X^2=177, $p<0.001$; see Table 2). The number of nudibranchs on any particular sponge varied occasionally from week to week by one individual, indicating that nudibranchs occasionally moved from one sponge to another, but generally stayed on the same sponge.

Results of the caged preference experiments with G. pallida are summarized in Table 3. All six nudibranchs

Table 1. Number of Glossodoris pallida on Hyrtios erecta-A. The mean was 1.9 ± 0.7. N represents the number of sponges examined.

Month:	Oct.	Nov.	Nov.	Dec.	Dec.	Jan.	Feb.	Mar.	Mar.	Mar.
Date:	27	10	18	2	21	7	11	3	18	30
N:	36	43	37	43	40	34	31	27	22	27
Ratio:	2.0	1.3	1.6	1.4	1.0	1.3	1.4	1.0	1.4	1.7

Month:	Apr.	Apr.	Apr.	May	May	June	June	July	Aug.
Date:	7	13	27	11	26	2	14	15	29
N:	20	25	24	21	17	21	14	16	25
Ratio:	2.6	2.4	2.4	2.8	2.1	1.8	2.2	3.4	2.7

Table 2. Frequency of Glossodoris pallida on Hyrtios erecta-A. The number of nudibranchs were counted on 525 sponges over a ten month period. When compared with a Poisson distribution of expected frequency, the nudibranchs showed a significantly clumped distribution with the following values obtained: $X^2 = 177$; $p<0.001$; $Y=1.77$: $s^2=0.67$.

Nudibranchs/Sponge:	0	1	2	3	4	5	6+
Frequency:	175	113	81	66	49	21	20

Table 3. Caged *Glossodoris pallida* on *Hyrtios* sponges. Groups of 6 nudibranchs were caged with various sponges. Number of nudibranchs seen crawling on each sponge were recorded weekly. * denotes no nudibranchs remaining in that cage. *H.e.* denotes *Hyrtios erecta*.

Sponge	Location	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Final Week
H.e.-A	Apra	6	6	5	6	6	6	6
H. altum	Apra	0	0	0	0	0	*	*
H.e.-C	Apra	0	2	1	2	1	1	0
H.e.-C	Cocos	1	2	1	*	*	*	*

caged with Hyrtios erecta-A were in contact with, and apparently feeding on this sponge for every observation with the exception of one individual in the third week which was seen crawling on the screen. In contrast, no individuals caged with H. altum were ever seen on this sponge. In the fifth week, nudibranchs caged with H. altum began to disappear; and none were left by the end of the experiment. In the cage with H. erecta-C in Apra Harbor, no more than two individuals were observed crawling on their sponge during any given observation. Only one individual remained at the termination of the experiment. TLC analysis revealed that the chemistry of this nudibranch contained the same major metabolites, in roughly the same concentrations, as nudibranchs caged with H. erecta-A. All nudibranchs caged with H. erecta-C in Cocos Lagoon disappeared after three weeks. The waters in this area were very silty and there was a strong current. These factors may have caused the pores of the cage to clog and decrease water circulation, which resulted in the nudibranchs dying.

Glossodoris hikeurensis was found frequently feeding on Hyrtios erecta-C in the southern part of Guam, in or near Cocos Lagoon. This was the only sponge G. hikeurensis was ever seen feeding on, except for individuals transplanted to Sponge Mound. The three G. hikeurensis introduced to Sponge Mound were released on

top of H. erecta-A. All three nudibranchs appeared to begin feeding immediately. One month later two medium size G. hikeurensis were discovered and collected while they were feeding on H. altum at Sponge Mound. The following week one large G. hikeurensis was discovered at the same site, but it was not feeding at the time. It is believed that these were the same three nudibranchs that were introduced earlier. Glossodoris cincta was discovered feeding naturally on two different sponges, H. erecta-C in Cocos Lagoon and H. altum at Sponge Mound.

LABORATORY PREFERENCE STUDIES

In laboratory experiments, Glossodoris pallida was attracted to Hyrtios erecta-A, but not to H. erecta-C or H. altum (Table 4). Strong preference was shown for H. erecta-A over the two other sponges. No difference between H. erecta-C and H. altum was apparent.

Glossodoris pallida avoided extracted Hyrtios erecta-A preferring to crawl on control sponges ($N=12$, $p<.01$). Twenty-four nudibranchs were tested for preference between extracted, control H. erecta-A and extracted sponges treated with 6% of the sponge's natural metabolites; and none reacted. No nudibranch was seen on either the treated or the control sponge. Only four out of fourteen G. pallida reacted in the experiment testing scalardial, the major metabolite of H. erecta-A. All four

Table 4. *Glossodoris pallida* preference tests. Several *G. pallida* were offered choices between two objects in a tank. Two-tailed Wilcoxon Signed-Ranks test was used. Symbols represent: n.s. = not significant, $p>0.05$; * = $0.01<p<0.05$; ** = $p<0.01$; *H.e.* = *Hyrtios erecta*-A; *H.e.*-C = *Hyrtios erecta*-C. N represents the number of active nudibranchs reacting without ties.

Choice-1	Choice-2	Preference	N	T	Sig.
H.e.	Rock	*H.e.*	18	3	**
H.e.-C	Rock	none	17	76	n.s.
H. altum	Rock	none	13	18.5	n.s.
H.e.	*H.e.*-C	*H.e.*	16	17	**
H.e.	*H. altum*	*H.e.*	17	3	**
H.e.-C	*H. altum*	none	9	6.5	n.s.
H.e.-extracted	*H.e.*-fresh	fresh	12	3.5	**
H.e.-extracted-coated in 6% extract	*H.e.*-extracted coated in ether	none	0	0	n.s.
H.e.-extracted-coated in 2% Scalardial	*H.e.*-extracted coated in ether	none	4	3	n.s.
H.e.-surface	*H.e.*-matrix	surface	7	0	*
H.e.-grazed	*H.e.*-ungrazed	none	15	44	n.s.

active nudibranchs crawled on the control sponge, indicating that G. pallida is not attracted to scalardial. The non-active nudibranchs were probably not attracted to either the control or treated extracted sponge, preferring to crawl along the walls of the tank (Table 4). Glossodoris pallida showed no preference between grazed and ungrazed H. erecta-A (N=15, p>.05), but did prefer the surface tissue of H. erecta-A over the matrix (N=7, p=.02).

The laboratory preference experiments conducted with Glossodoris hikeurensis and G. cincta indicated that collectively these nudibranchs prefer Hyrtios erecta-C over H. erecta-A (N=8, p=.03). Results of tests with G. hikeurensis and G. cincta were combined because the two species responded very similarly and not enough individuals were available of either species to be tested separately.

CHEMICAL ANALYSIS

Table 5 shows yield of organic extract as a percentage of dry weight of the organism for Hyrtios sponges and Glossodoris nudibranchs. The yield of extract was approximately equal in Glossodoris pallida and its diet sponge Hyrtios erecta-A. The metabolites in the surface and the matrix of the sponge appeared to be qualitatively identical. However, the concentration of

Table 5. Percent yield of crude extracts by dry weight of organism.

Species	Crude Extract % Yield	s^2	N
Glossodoris pallida	14.8	7.8	19
G. hikeurensis	7.8	3.4	3
G. cincta	10.1		1
Hyrtios erecta-A	14.1	4.6	20
H. erecta-A-surface	8.9	2.6	9
H. erecta-A-matrix	4.7	1.3	9
H. erecta-A-grazed	14.4	4.8	8
H. erecta-A-ungrazed	14.0	5.0	12
H. erecta-C	5.7	2.3	3
H. altum	7.0		2

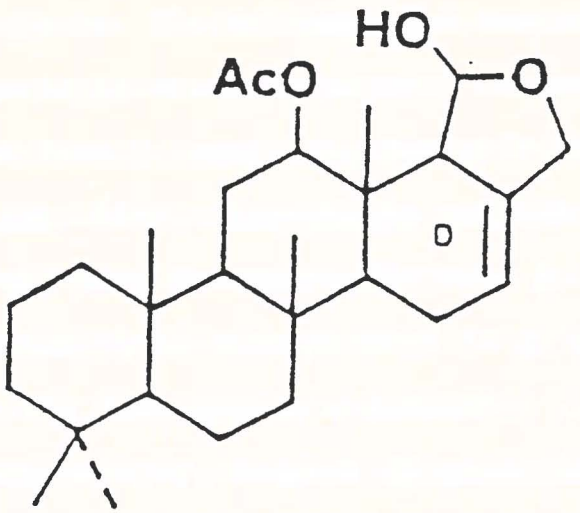
the crude extract of the surface layer was significantly higher, roughly double that of the matrix, 8.9 ± 2.6g extract per sponge g dry weight for the surface vs. 4.7 ± 1.3g extract per sponge g dry weight for the matrix (Table 5). Extracts from the surface layer were also much darker due to pigments. No chemical difference in quality or quantity of secondary metabolites could be detected between grazed and ungrazed pieces of Hyrtios erecta-A, suggesting that there were no induced chemical defenses over the time scales investigated.

Table 6 lists the major metabolites of each organism and the yield of the metabolites, when known, as a percentage of the dry weight of the organism. The major metabolites of Hyrtios erecta-A, in order of increasing polarity, were one unidentified compound, scalardial, deoxyscalarin and scalarin (Fig. 3). Several unidentified minor metabolites were also present. Scalardial was present in the highest concentration at approximately 2.4% dry weight of the sponge. TLC analysis of 19 Hyrtios erecta-A individuals indicated that the relative concentrations of metabolites may vary among individual sponges. These variations were not correlated with nudibranch grazing patterns.

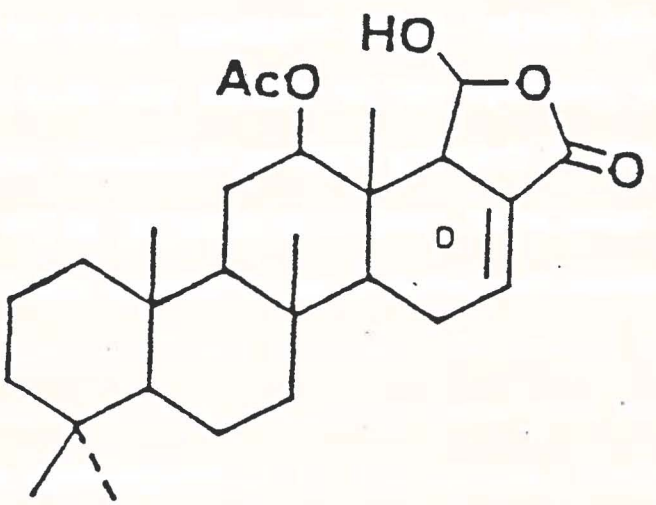
The organic extract of Glossodoris pallida was similar to that of H. erecta-A and contained three major metabolites: one unidentified compound, scalardial and

Table 6. Major metabolites of study organisms. * denotes the most abundant metabolite.

Species	Major Metabolites	% Yield
Glossodoris pallida	Unknown	
	Scalardial	
	*Deoxyscalarin	
G. hikeurensis	Unknown-1	
	Unknown-2	
	Unknown-3	
	Unknown-4	
Hyrtios erecta-A	Unknown	
	*Scalardial	2.4
	Deoxyscalarin	
	Scalarin	
H. erecta-C	*Heteronemin	1.0
H. altum	*Heteronemin	1.8



Deoxyscalarin



Scalarin

Fig. 3. Major metabolites of Hyrtios erecta-A, not previously pictured, deoxyscalarin and scalarin.

deoxyscalarin. The unidentified compound showed the same characteristic staining pattern as the unidentified compound from H. erecta-A. Scalarin was not detected in G. pallida. Also, Glossodoris pallida contained relatively more deoxyscalarin than H. erecta-A. Fewer minor metabolites were present in G. pallida's extract than in its diet sponge's extract.

Surprisingly, neither Glossodoris hikeurensis or G. cincta contained detectable concentrations of heteronemin, even though their natural food sponges, H. erecta-C and H. altum contained high concentrations of it (Table 6). In fact, heteronemin was the only major metabolite in H. erecta-C and H. altum at approximately 1.0% and 1.8% dry weight of the sponges, respectively. Four major metabolites were present in the organic extract of G. hikeurensis. None have been identified; but they did not appear to be related to heteronemin.

PREDATOR DETERRENCE

All hungry aquarium fish engulfed Glossodoris pallida, then rapidly spat them out. None of the nudibranchs were ever swallowed. One nudibranch was engulfed and spat out by five different fish before it reached the bottom of the tank. The fish would rapidly

swallow pieces of fish meat in between each nudibranch that was offered.

Results of the feeding assays varied and depended upon which species of fish were tested. Results of laboratory feeding assays with the pufferfish Canthigaster solandri are shown in Fig. 4. Organic extracts of Glossodoris pallida and G. hikeurensis were not deterrent toward Canthigaster solandri. Glossodoris pallida was tested at 6% while G. hikeurensis was tested at 5% dry weight of the food tablets. Thus, nudibranch extracts were tested at roughly one-half their natural concentrations. Not enough extracts were available to test at a higher concentration. The G. hikeurensis extract tended to be avoided by some fish (N=8, p=0.07) so that it is quite possible that this extract would be deterrent toward C. solandri at a higher concentration.

Both the organic and the water soluble extracts of Hyrtios erecta-A were strongly deterrent at 7% dry weight of the food tablets, slightly more than one-half of the natural concentrations in the sponge. Organic extracts of H. erecta-C and H. altum were both not deterrent, even when tested at above natural concentrations. The extract from H. erecta-A was more deterrent than that of H. altum when these two sponges were compared; and the metabolites in the matrix layer of H. erecta-A appeared to be more deterrent than those in the surface when compared at the

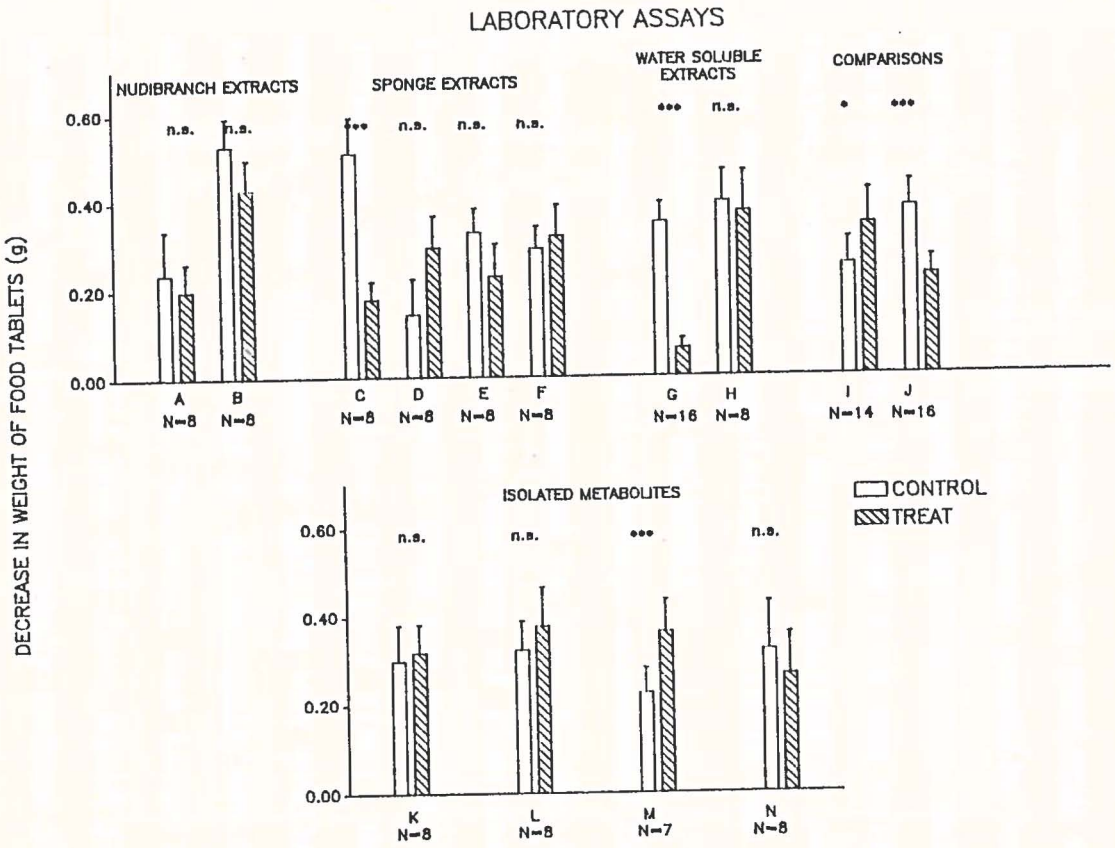


Fig. 4. Laboratory feeding assays with Canthigaster solandri. Values are reported at X ± SE. Symbols represent: n.s. = not significant, $p>.05$; * = $.01<p<.05$; *** = $p<.005$. Letters denote: A = Glossodoris pallida (6.0%); B = G. hikeurensis (5.0%); C = Hyrtios erecta-A (7.0%); D = H. erecta-C (7.0%); E = H. erecta-C (12.0%); F = H. altum (12.0%); G = Water extract of H. erecta-A (10.0%); H = Water extract of H. altum (7.0%); I = Hyrtios erecta-A (5.0%) vs. H. altum (5.0%) : J = Surface vs. Matrix of H. erecta-A (4.0%); K = Scalardial (1.6%); L = Scalarin (0.6%); M = Scalarin (1.0%); N = Heteronemin (2.0%). All concentrations are in percent dry weight of food tablets.

same concentrations. In the last two tests, two trials were completed for each comparison with the same set of fish used for each trial. Results were analyzed using Three-Way Factorial ANOVA. In the surface vs. matrix test there were significant fish*trial and fish*treatment interaction effects.

Scalardial, heteronemin and scalarin were the only metabolites available in enough quantity to test for feeding deterrence against Canthigaster solandri. They were tested at 1.6%, 2.0% and 1.0% dry weight of the food tablet, respectively. Neither scalardial nor heteronemin were deterrent. Surprisingly, scalarin was a feeding attractant toward C. solandri.

Results of feeding assays conducted in the field at Fingers Reef are presented in Fig. 5. The experiment at Fingers Reef had a wide variety of fish feeding which included several members from the families Labridae (wrasses) and Balistidae (trigger fish). Not enough extract from Glossodoris pallida was available to be tested. The extract of G. hikeurensis was not a significant deterrent. However, one large fish, Cantherhines dumerili ate all four squid pieces of at least four ropes indiscriminately before it was chased off. Until this fish appeared the test results seemed to show that the G. hikeurensis extract was deterrent. Not

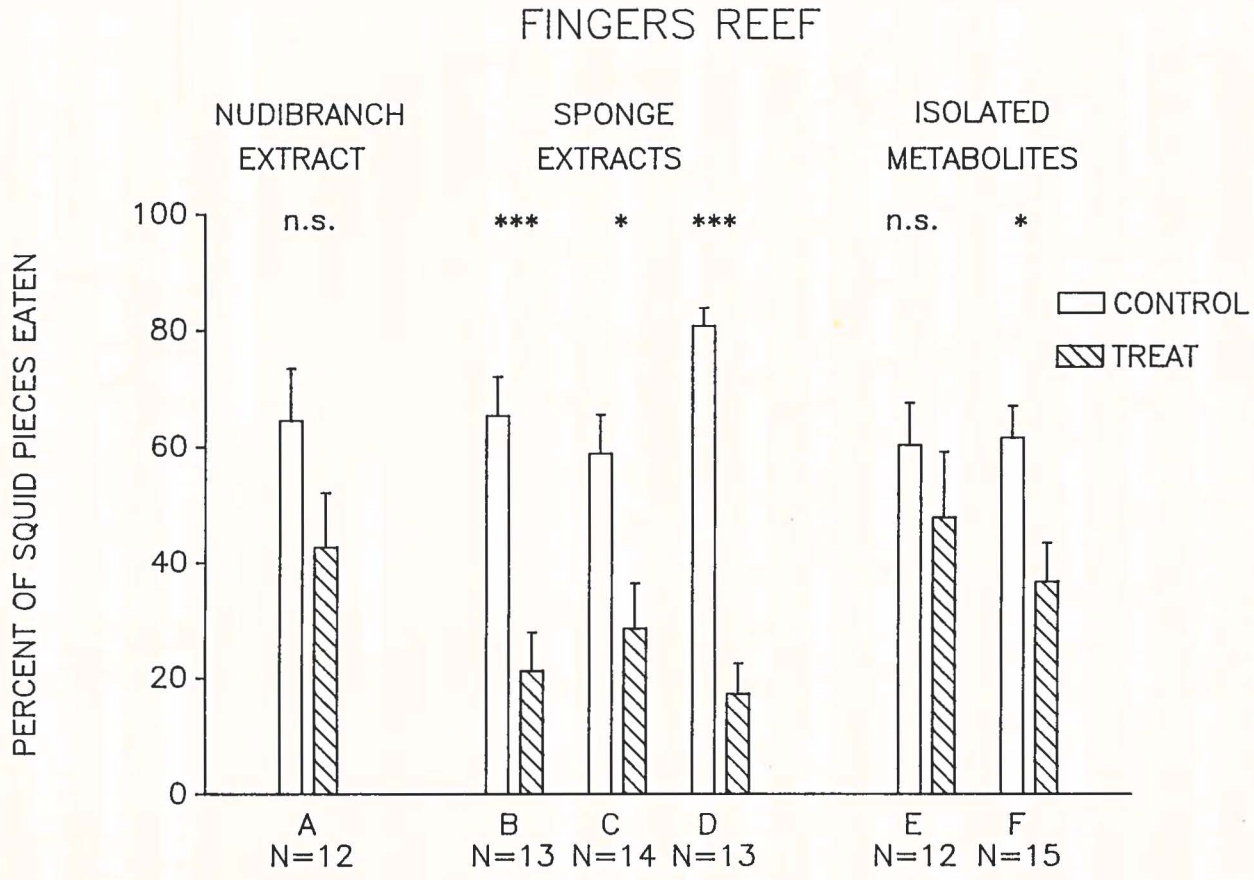


Fig. 5. Feeding assays conducted at Fingers Reef. Values are reported at X ± SE. Symbols represent: n.s. = not significant, $p>.05$; * = $.01<p<.05$; *** = $p<.005$. Letters denote: A = Glossodoris hikeurensis (7.0%); B = Hyrtios erecta-A (7.0%); C = H. erecta-C (7.0%); D = H. altum (7.0%); E = Scalardial (1.5%); F = Heteronemin (1.0%). All concentrations are in percent dry weight of squid pieces.

enough extract from G. hikeurensis was available to repeat the test.

All three sponges at 7%, and heteronemin at 1% of squid dry weight were deterrent toward generalist predators at Fingers Reef (Fig. 5). These percentages are at natural concentrations or below. Scalardial was not deterrent at 1.5% of squid dry weight.

At Haps Reef, one species of trigger fish, Melichthys vidua, was responsible for most of the feeding during the assays. Only the extracts from Hyrtios erecta-A, H. erecta-C and scalardial were tested at Haps Reef. Results are shown in Fig. 6. Surprisingly, the extract of H. erecta-A was not deterrent at 7% of squid dry weight, although it was significantly deterrent at Fingers Reef and in laboratory tests. H. erecta-C was strongly deterrent at 7%; and scalardial was not deterrent at 1.5% of squid dry weight at Haps Reef.

DISCUSSION

NUDIBRANCH PREFERENCES

Three species of nudibranchs, Glossodoris pallida, G. hikeurensis and G. cincta, feed on chemically rich Hyrtios sponges. On Guam, Glossodoris pallida is always found in close association with Hyrtios erecta-A while G. hikeurensis and G. cincta are most frequently sighted

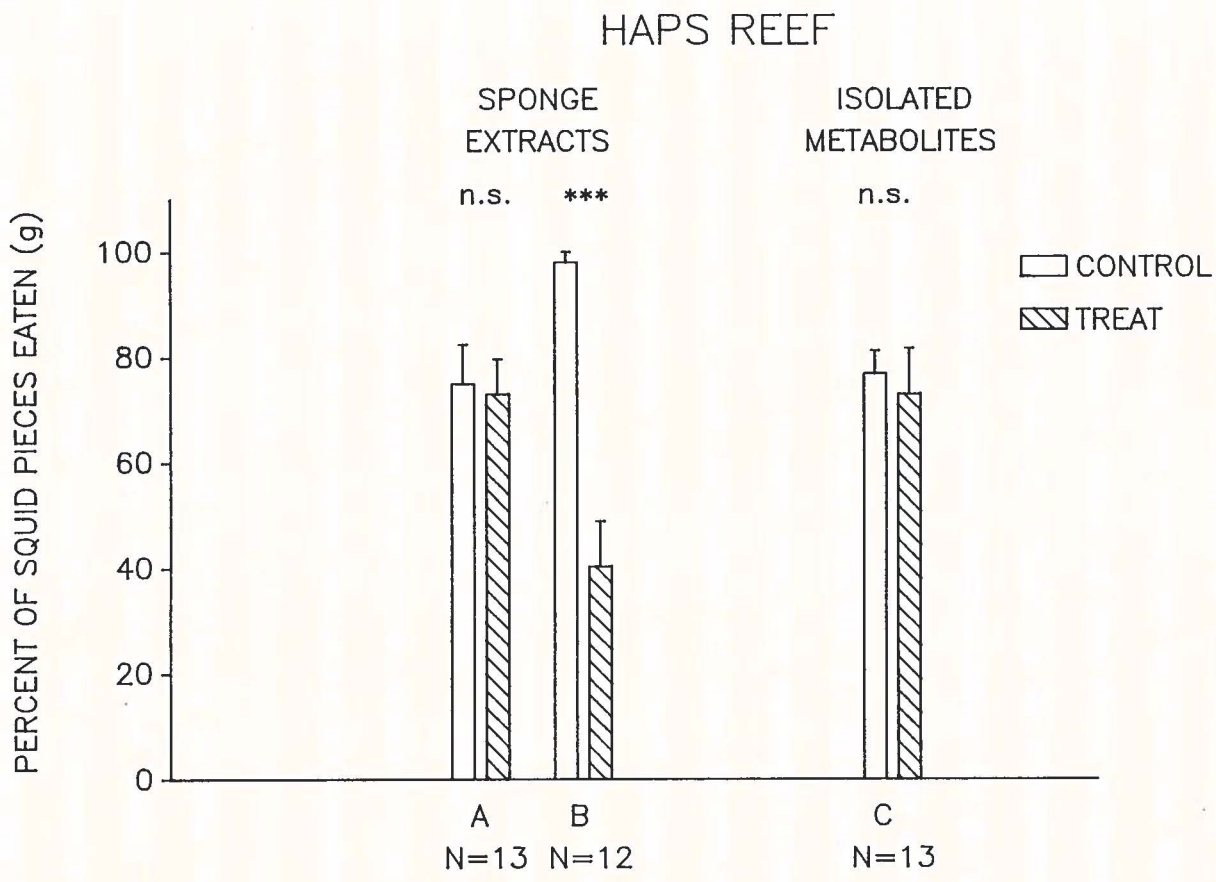


Fig. 6. Feeding assays conducted at Haps Reef. Values are reported at X ± SE. Symbols represent: n.s. = not significant, $p>.05$; * = $.01<p<.05$; ** = $.005<p<.01$; *** = $p<.005$. Letters denote: A = Hyrtios erecta-A (7.0%); B = H. erecta-C (7.0%); C = Scalardial (1.5%). All concentrations are in percent dry weight of squid pieces.

feeding on H. erecta-C. In the laboratory preference tests Glossodoris pallida was attracted to, and strongly preferred, Hyrtios erecta-A over H. erecta-C or H. altum. The reason for G. pallida's preference is still unknown. The texture of all three sponges varied slightly, but probably not enough to be a major factor in the nudibranch's selection. None of the data supports the original hypothesis that G. pallida is attracted to major secondary metabolites found exclusively in H. erecta-A. Glossodoris pallida is not attracted to the crude organic extract or the major metabolite of H. erecta-A which is scalardial. Possibly, there are key metabolites in H. erecta-A which were not included in the crude extract. Highly polar compounds could not extract into organic solvents; and highly volatile compounds would evaporate and not be included in the crude extract. Glossodoris pallida may cue in on a highly polar or highly volatile metabolite when selecting its food. This study tested only the compounds which were soluble in organic solvents and not highly volatile.

TLC analysis revealed variations in the relative concentrations of secondary metabolites among individuals of Hyrtios erecta-A. For example, the concentration of deoxyscalarin was much higher in some sponges than in others. If Glossodoris pallida did select for particular metabolites such as deoxyscalarin, then it should be

attracted toward individual sponges that had a higher concentration of that desired metabolite. However, nudibranch grazing patterns in the field showed no correlation with any particular chemical variations of the sponge.

Glossodoris pallida might be attracted by localized chemical concentration differences within a particular area on a sponge, instead of larger differences among sponges. Grazed portions of a sponge could have a higher concentration of metabolites which attract the nudibranch. However, G. pallida, in the laboratory, were not attracted towards small pieces which had been previously grazed in the field, suggesting that the grazed pieces did not contain more of the desirable metabolites. It is possible that G. pallida could induce localized chemical defenses in H. erecta-A. Since TLC analysis did not detect differences between grazed and ungrazed sponges; and G. pallida showed no preference or avoidance for grazed sponge pieces, it is unlikely that grazing by G. pallida induces chemical defenses in H. erecta-A.

METABOLITE SELECTION

The organic extract of Glossodoris pallida was very similar to that of its diet sponge Hyrtios erecta-A, indicating that G. pallida derives its major secondary

metabolites from its food. Some differences between nudibranch and sponge chemistry did exist. Glossodoris pallida contained relatively more deoxyscalarin and did not contain any scalarin, a major metabolite of H. erecta-A. Chemical differences could be due to several selection processes. As already discussed, nudibranch grazing patterns are probably not one of these selection processes. Since deoxyscalarin concentrations were higher in the nudibranch than in the sponge, it would have been interesting to determine if G. pallida was attracted to deoxyscalarin in a laboratory preference test. Unfortunately, not enough deoxyscalarin was available to conduct this test.

Chemical properties such as solubility and stability could play a major role in the selection process. For example, deoxyscalarin, may be more easily absorbed into the nudibranch's body. Scalarin may decompose more readily in the nudibranch's digestive tract or may be converted into related compounds, possibly deoxyscalarin, by the nudibranch's enzymes. It is conceivable that deoxyscalarin is synthesized by the nudibranch from simple carbon precursors. However, this seems unlikely considering that deoxyscalarin is very closely related to scalarin, a major metabolite found in the nudibranch's diet sponge.

Scalardial appears to be a relatively stable compound inside the nudibranch. The Glossodoris pallida that remained, after being caged with H. erecta-C for 55 days, still contained scalardial when it was extracted. Also, the seven G. pallida which appeared to be feeding on H. erecta-C in the laboratory for ten days showed no change in chemistry. It might be expected that G. pallida feeding on H. erecta-C would accumulate heteronemin, the only major metabolite of this sponge. However, no heteronemin was found in these nudibranchs.

Glossodoris hikeurensis and G. cincta can be found feeding on both Hyrtios erecta-C and H. altum in the field. Both of these sponges contain heteronemin as the major metabolite. These nudibranchs showed preference for H. erecta-C over H. erecta-A in laboratory preference experiments. Thus, G. hikeurensis and G. cincta prefer to feed on sponges with high concentrations of heteronemin. Yet, no heteronemin was ever detected in either of these two nudibranch species. Not enough G. hikeurensis or G. cincta were available to test if they were attracted to crude extracts or pure metabolites from their diet sponges. Possibly, G. hikeurensis and G. cincta manage to avoid areas on their food sponges with high concentrations of heteronemin; but this seems unlikely considering that they prefer sponges with high concentrations of heteronemin.

Heteronemin may be excreted by Glossodoris nudibranchs or may breakdown in the nudibranch's digestive tract. None of the major metabolites of G. hikeurensis or G. cincta have been identified; but they do not appear to be closely related to heteronemin. It is assumed that heteronemin is probably not converted to any of these compounds. Possibly, the nudibranch's major metabolites are derived from an undiscovered food source. However, this seems unlikely as these two nudibranchs were found consistently on Hyrtios erecta-C and H. altum and were never seen feeding on any other species of sponge in the field. The question remains, "What happens to the heteronemin in Hyrtios sponges when it is preyed upon by Glossodoris nudibranchs?". It may be an unstable compound, or it may be attacked rapidly by nudibranch enzymes.

PREDATOR DETERRENCE

Hungry aquarium fish refuse to accept healthy Glossodoris pallida as food, indicating that the nudibranch contains feeding deterrent compounds. However, the crude extract of the nudibranch is not deterrent when tested in a laboratory feeding assay against Canthigaster solandri. Probably, the deterrent compounds are not included in the nudibranch extract because they are either highly polar or highly volatile.

Results of the feeding assays were quite variable, depending largely upon which fish species were tested. The crude extract of Hyrtios erecta-A was strongly deterrent at half of its natural concentration against Canthigaster solandri in the laboratory and against general predators at Fingers Reef. Glossodoris pallida, the sponge's predator, contains many of the same major metabolites as the sponge, but was not deterrent when tested under identical conditions. Because of the limited availability of the G. pallida extract, it was only possible to test this extract in one assay. It is possible that the nudibranch extract would have been deterrent at higher concentrations or against other potential predators. The reason why the sponge extract was deterrent and the nudibranch extract was not is still unknown. Scalarin, a major metabolite in the sponge which was not found in the nudibranch, was hypothesized to be responsible for the deterrence in the sponge extract. This hypothesis was proven false when, under identical conditions, scalarin was shown to be a feeding attractant. With the same experimental design, scalardial, a major metabolite of both sponge and nudibranch, was also not deterrent. Scalardial and scalarin were the only metabolites from H. erecta-A available for testing. It is probable that minor secondary metabolites found in the sponge extract and not

in the nudibranch extract play a key role in the sponge extract's feeding deterrence. Several minor metabolites were noted in the sponge extract that were not present in the nudibranch extract.

The chemistry of Hyrtios erecta-C and H. altum were similar; and the results of the feeding assays of these two sponges were also similar. Neither the sponge extracts nor their major metabolite, heteronemin, were deterrent when tested against Canthigaster solandri in the laboratory. However, both sponges and the metabolite were significantly deterrent when tested against generalist predators at Fingers Reef. Heteronemin may be essential to the sponges in deterring many potential predators.

At Haps Reef, the extract of Hyrtios erecta-C was deterrent while its major metabolite was not. Minor metabolites may be causing the deterrence against the predators in this location. In all Hyrtios sponges minor metabolites may play a key role in predator deterrence. Having more than one deterrent metabolite would be a definite evolutionary advantage because a greater number of potential predators would be deterred.

Glossodoris pallida, G. hikeurensis and G. cincta are feeding upon sponges containing feeding deterrent compounds. Glossodoris pallida accumulates many compounds from its diet sponge, but does not appear to accumulate

the organically soluble feeding deterrent compounds. Glossodoris hikeurensis and G. cincta do not accumulate the major metabolite of their diet sponges which is known to be deterrent. By excreting or altering noxious compounds such as heteronemin, the nudibranchs might be able to take advantage of an available food source without accumulating compounds which could be harmful.

Generally, Glossodoris nudibranchs are found attached underneath or in a notch of the sponge where they are at least partially hidden from predators. Thus, Glossodoris nudibranchs are hidden from predators while they feed. The nudibranch's diet sponges have so few predators that the nudibranchs are not at risk of being eaten incidentally. Contrary to what is typically proposed for nudibranchs, Glossodoris nudibranchs appear to break down or excrete some of the noxious compounds consumed in their diet, rather than accumulate them for their own protection. At the same time they are at least partially protected from predation by hiding in a noxious sponge. Thus, by feeding on Hyrtios sponges, Glossodoris nudibranchs appear to be taking advantage of and receiving protection from an available food source without accumulating potentially harmful compounds.

In summary, this study was concerned primarily with organically soluble, nonvolatile compounds. Contrary to the original hypothesis, Glossodoris pallida was not

attracted to major secondary metabolites found in the crude organic extract of its diet sponge Hyrtios erecta-A. However, minor metabolites may play a key role in the nudibranchs' prey selection. Glossodoris pallida accumulates secondary metabolites from H. erecta-A; and some selection occurs as evidenced by the higher concentration of deoxyscalarin and disappearance of scalarin in the nudibranch. Glossodoris pallida does not accumulate heteronemin even when forced to feed upon sponges containing high concentrations of this metabolite. Glossodoris hikeurensis and G. cincta prefer to feed on Hyrtios sponges with a high concentration of heteronemin, but surprisingly do not accumulate heteronemin.

All the crude extracts of Hyrtios sponges were deterrent to at least some general predators while none of the nudibranch extracts were deterrent in any of the feeding assays. Heteronemin was the only pure metabolite tested that proved to be a significant feeding deterrent. It may be advantageous for Glossodoris nudibranchs to break down or excrete this compound, because it is detrimental. Thus, Glossodoris nudibranchs are able to take advantage of an available food source and not accumulate certain potentially harmful compounds found in that food source. The nudibranch may receive protection from their diet sponges simply by hiding in them.

LITERATURE CITED

Amesbury, S.S. and R.F. Myers. 1982. Guide to the Coastal Resources of Guam: Vol. 1. The Fishes. University of Guam Marine Laboratory Contribution No. 173. Mangilao, Guam. 141p.

Bakus, G.J. 1981. Chemical defense mechanisms on the Great Barrier Reef, Australia, Science 211:497-499.

Bergquist, P.R. 1980. A revision of the supraspecific classification of the orders Dictyoceratida, Dendroceratida, and Verongida (class Desmospongiae). New Zeal. J. of Zool. 7:443-503.

Cimino, G., S. De Rosa, S. De Stefano, R. Morrone and G. Sodano. 1985. Tetrahedron 41:1093.

Crews, P., P. Bescansa and G.J. Bakus. 1985. A non-peroxide norsesterterpene from a marine sponge Hyrtios erecta. Experientia 41:690-691.

Crews, P. and P. Bescansa. 1986. Sesterterpenes from a common marine sponge Hyrtios erecta. J. Nat. Prod. 49(6):1041-1052.

Crossland, C. 1911. Warning coloration in a nudibranch mollusc and in a chameleon. Proc. Zool. Soc. Lond. 79:1062.

Faulkner, D.J. and M.T. Ghiselin. 1983. Chemical defense and evolutionary ecology of dorid nudibranchs and some other opisthobranch gastropods. Mar. Ecol. Prog. Ser. 13:295-301.

Gosliner, T.G. 1987. Nudibranchs of Southern Africa. Sea Challengers. Monterey, California. 136p.

Gunthrope, L. and A.M. Cameron. 1987. Bioactive properties of extracts from Australian dorid nudibranchs. Mar. Bio. 94:34-93.

Herdman, W.A. and J.A. Clubb. 1892. Third report on the nudibranchiata of the L.M.B.C. district. Proc. Lpool Biol. Soc. 4:131-169.

Karuso, P. 1987. Chemical ecology of the nudibranchs. In Scheuer, P.J.(ed.) Bioorganic Marine Chemistry. Springer-Verlag, New York. pp31-60.

Minale, L. 1978. Terpenoids from marine sponges. In Scheuer, P.J.(ed.) Marine Natural Products: Chemical and Biological Perspectives, Vol.I. Academic Press, New York, pp175-240.

Okuda, R.K. and P.J. Scheuer. 1985. Latrunculin-A, ichthyotoxic constituent of the nudibranch Chromodoris elisabethina. Experientia 41:1355-1356

Paul, V.J. In press. Feeding deterrent effects of algal natural products. Bull. Mar. Sci. 41.

Paul, V.J. and W. Fenical. 1986. Chemical defense in tropical marine algal, order Caulerpales. Mar. Ecol. Prog. Ser. 34:157-169.

Rudman, W.B. 1982. The Chromodorididae (Opisthobranchia: Mollusca) of the Indo-West Pacific: Chromodoris quadricolor, C. lineolata and Hypselodoris nigrolineata color groups. J. Linn. Soc.(Zool.) 76:183-241.

Ravi, B.N., H.P. Perzanowski, R.A. Ross, T.R. Erdman and P.J.Scheuer. 1979. Recent research in marine natural products: the puupehenones. Pure Appl. Chem. 51:1893.

Schulte, G., P.J. Scheuer and O.J. McConnell. 1980. Two furanosesquiterpene marine metabolites with antifeedent properties. Helv. Chim. Acta 63:2159-2167.

Sokal, R.R. and F.J. Rohlf. 1981. Biometry. 2nd Ed. W.H. Freeman and Co. New York. 859p.

Thompson, T.E. 1960. Defensive adaptation in opisthobranchs. J. Mar. Biol. Ass. U.K. 39:123-134.

Thompson, J.E., R.P. Walker, S.J. Wratten and D.J. Faulkner. 1982. A chemical defense mechanism for the nudibranch Cadlina luteomarginata. Tetrahedron 38:1865-1873.