

Feeding preferences of adult and juvenile rabbitfish *Siganus argenteus* in relation to chemical defenses of tropical seaweeds

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ABSTRACT: The role of algal secondary metabolites in determining feeding preferences of adult and juvenile rabbitfish *Siganus argenteus* was examined in outdoor tanks on Guam. Secondary metabolites could not explain the preferences of these herbivores, although the presence of secondary metabolites appeared to account for the low palatability of some seaweeds, such as *Tydemania expeditionis* and *Microcoleus lyngbyaceus*. Other species, including *Desmia hornemanni* and *Halimeda discoidea*, that contained secondary metabolites with feeding-deterrent properties were of medium preference. Further species that produced secondary metabolites, including *Asparagopsis taxiformis*, *Caulerpa* spp., *Chlorodesmis fastigiata*, *Dictyota* spp., and *Liagora farinosa* were rapidly eaten, and neither their extracts nor their isolated metabolites significantly deterred grazing. Responses to algal chemistry differed between adults and juveniles. Juvenile *S. argenteus* were not deterred by extracts of *T. expeditionis*, *Halymenia durvillaei*, *H. discoidea*, and *Enhalus acoroides*, or the isolated metabolites chlorodesmin and flexilin that deterred the adults. As regards morphological defenses, several calcified seaweeds were of medium preference for both adult and juvenile rabbitfish; however, none of the high-preference seaweeds were calcified, and calcification and toughness were associated with low feeding preference.

INTRODUCTION

Several recent reviews summarize evidence supporting the hypothesis that, in coral reef habitats, secondary metabolites produced by seaweeds can function as chemical defenses toward herbivores (Hay & Fenical 1988, Van Alstyne & Paul in press). The intense grazing activity of herbivores, especially fishes and sea urchins, on coral reefs has been extensively documented (Hay 1981a, b, 1984a, b, Littler et al. 1983, Hay & Taylor 1985, Lewis 1985, 1986, Lewis & Wainwright 1985, Carpenter 1986, Foster 1987, Morrison 1988, Hay & Fenical 1988). Thus, herbivory could be an important evolutionary pressure selecting for chemical and morphological defenses in tropical algae. Indeed, some of the hundreds of bioactive secondary metabolites isolated from tropical algae (Norris & Fenical 1982, Faulkner 1984, 1986) have been shown to function as effective chemical defenses against a variety of herbivores (Targett et al. 1986, Hay et al. 1987, 1988a, b, Paul 1987, Paul et al. 1987, in press, Wylie & Paul 1988).

One way to begin to unravel the complex associa-

tions between tropical herbivores and seaweeds is to compare the dietary preferences and responses to algal secondary metabolites of individual herbivore species (Targett et al. 1986, Hay et al. 1987, 1988c, Wylie & Paul 1988). In this study, we examined the feeding preferences of the rabbitfish *Siganus argenteus* (Quoy & Gaimard), a reef-dwelling herbivore common throughout the tropical western Pacific. Rabbitfish are important herbivores on Guam reefs particularly during episodic recruitment events that occur in April and May (Kami & Ikehara 1976). In some years, *S. argenteus* and *S. spinus* recruit in such large numbers that they consume virtually all of the palatable seaweeds and seagrasses on the reef flats around Guam (Tsuda & Bryan 1973, pers. obs). The role of algal secondary metabolites as defenses against grazing by these herbivores was the major focus of this investigation. We asked the following questions: (1) What are the seaweed preferences of juvenile and adult *S. argenteus*? (2) Do preferred seaweeds lack potential chemical and morphological defenses such as secondary metabolites, calcification, and/or toughness? (3) Do extracts and

isolated metabolites from seaweeds that produce secondary metabolites function as feeding deterrents toward *S. argenteus*? (4) Do juvenile and adult *S. argenteus* show similar responses to algal extracts and secondary metabolites?

METHODS

Seaweeds and fish used in this study were collected from various reef habitats around Guam, including reef slopes, reef flats, and seagrass beds. Information on the seaweeds of Guam, including taxonomic descriptions and authorities, is presented by Tsuda & Wray (1977). We selected species of seaweeds that were abundant enough to use in the preference trials and to provide sufficient material for chemical extractions. Seaweeds were maintained outdoors in large, flowing seawater tanks and were used in preference trials within 24 h of collection. Damaged or discolored plants were not used in the assays. Juvenile (6 to 8 cm TL) and adult (20 to 23 cm TL) *Siganus argenteus* were collected by dip net or cast net and maintained on food pellets and the seaweed *Enteromorpha* in outdoor flow-through tanks for periods ranging from several days to weeks prior to the study.

Feeding preference assays. Seaweed preferences of adult and juvenile *Siganus argenteus* were determined by providing an array of seaweeds to replicate fish or small groups of fish held in separate divisions within large, outdoor, flow-through tanks. Adults were tested individually in 12 large, 1 m × 1 m × 1.5 m, black, 1/4" (6 mm) plastic mesh compartments within two 5000 l tanks. Juveniles were tested in groups of 3 to 5 fish held in 16, 0.5 m × 0.5 m × 0.5 m, black, plastic mesh compartments in a 2000 l tank. The holding compartments physically and visually separated the fish and served as replicates for these assays. Juveniles were tested in groups rather than individually because individuals did not consume much alga per unit time.

Susceptibility of the various seaweeds to grazing was examined by inserting small, ca 6 cm long, equal-sized pieces, at 5 cm intervals, into a 0.5 m length of 3-strand polypropylene line. Lines were suspended 0.1 m above the bottom from PVC racks. Pieces of seaweed appearing similar in size were used so that all species should be equally apparent and available to the fish. Pieces of 5 to 14 seaweeds were inserted into the lines and offered to fish for ca 1 h. By this time, there was usually a clear distinction between the most and least susceptible plants. Similar methods have been used in seaweed susceptibility trials in field assays (Hay 1984a, Paul & Hay 1986). The thalli were weighed before and after the assays to determine the amount of each seaweed consumed. Seaweeds were

spun for 20 revolutions in a lettuce spinner and blotted dry on paper towels before weighing. The amount of each seaweed eaten was calculated for each replicate fish (adults) or group of fish (juveniles). Results were reported as the mean wet mass and percentage of each seaweed consumed. Because of the short duration of the assays, changes in weights of the seaweeds due to effects other than herbivory were assumed to be negligible.

Seaweeds used in the preference assays included the fleshy green seaweeds *Bryopsis pennata*, *Chlorodesmis fastigiata*, *Cladophoropsis membranacea*, *Enteromorpha clathrata*; the erect, uncalcified green seaweeds *Avrainvillea obscura*, *Caulerpa racemosa*, *Valonia fastigiata*; the calcified green seaweeds *Halimeda* spp., *Tydemania fastigiata*; the erect brown alga *Dictyota bartayresii*; the erect, lightly calcified brown alga *Padina tenuis*; the tough, leathery brown algae *Sargassum cristaefolium*, *Turbinaria ornata*; the erect, uncalcified red algae *Asparagopsis taxiformis*, *Desmia hornemanni*, *Halymenia durvillaei*; the erect, calcified red algae *Galaxaura marginata*, *Liagora farinosa*; the seagrasses *Enhalus acoroides*, *Halodule uninervis*; and the blue-greens *Calothrix crustacea* and *Microcoleus lyngbyaceus*. Most of these same seaweeds were extracted and used later in the chemical feeding deterrent assays.

Five different preference experiments were conducted for the adult rabbitfish, and 3 experiments were conducted for the juveniles. In most cases, the same fish were used for the different experiments. Each experiment used a different group of seaweeds, although some species were repeated in different experiments. Based on the results, the seaweeds were categorized as either low preference (0 to 20 % eaten), medium preference (21 to 60 % eaten), or high preference (> 60 % eaten). Relationships between the number of seaweeds in each of the 3 preference categories and the presence of both secondary chemistry and calcification were examined by Contingency Table Analysis with the G-test.

Chemical analyses. Seaweeds were blotted dry, cleaned of epiphytes, and extracted by homogenizing them in a blender with a 1:1 mixture of dichloromethane and methanol. The extracts were filtered, and the solvents were evaporated under reduced pressure with a rotary evaporator to yield a viscous oil.

Thin layer chromatographic analysis (TLC) (Norris & Fenical 1985) and proton nuclear magnetic resonance (NMR) spectroscopy were used to detect the presence or absence of secondary metabolites in the algae (Paul & Hay 1986). TLC plates were developed in 2 solvent systems, 100 % dichloromethane and 100 % diethyl ether, to screen for nonpolar and more polar metabolites. The plates were viewed under an

ultraviolet lamp to observe activity indicative of unusual metabolites. The plates were then sprayed with 50 % sulfuric acid and heated so that unusual color reactions indicating the presence of secondary metabolites could be observed.

Because TLC tells little about the chemical structures of compounds in the extracts unless the compounds can be related to known standards, we also examined the extracts by proton nuclear magnetic resonance spectroscopy (NMR). Known compounds can be readily detected and identified by NMR spectroscopy.

Individual secondary metabolites were isolated by silica gel column chromatography followed by high performance liquid chromatography (HPLC) (Paul & Fenical 1985, 1986).

Chemical feeding deterrent assays. Similar methods have been used in evaluating the feeding deterrence of crude extracts and isolated metabolites toward other herbivorous fishes (Wylie & Paul 1988, Paul et al. in press). Small pieces of a preferred alga *Enteromorpha clathrata* were pressed and blotted dry on paper towels and then coated with a solution of either the extract or the isolated metabolite dissolved in diethyl ether. The final concentrations on treated *E. clathrata* were 4 to 5 % of the estimated dry mass in trials with extracts and ca 1 % of the estimated dry mass in trials with pure metabolites. Dry mass of the wet *E. clathrata* was estimated by a previously calculated wet mass:dry mass ratio of 4.5:1. The concentrations approximated natural concentrations of most seaweed extracts which were determined during extraction. Organic extracts from uncalcified algae ranged from 5 to 8 % of the dry mass of the plants, and extracts from calcified algae were 3 to 6 % of the dry mass of the plants. Control pieces of *E. clathrata* were coated with diethyl ether only. *Cladophoropsis membranacea*, another preferred alga based on our studies, was used for approximately half of the assays with juvenile rabbitfish because *Enteromorpha* became unavailable during June and July 1988 when these assays were conducted.

For these assays, the fish were placed into separate compartments in flow-through tanks as described for the feeding preference assays. Adults were tested as individuals ($n = 8$ to 11 replicates), and juveniles were tested in separate groups of 3 to 5 individuals ($n = 9$ to 15 replicate groups). In assays with seaweed extracts, pieces of preferred algae weighed ca 5 g ($SD = 0.05$ g) for both control and treated pieces, while smaller, ca 2 g pieces were used both for testing isolated secondary metabolites toward the adults and in all of the assays with juveniles. For each assay, one piece of treated and one piece of control *Enteromorpha clathrata* were inserted at opposite ends of a strand of polypropylene line and offered to the rabbitfish for about 15 min. Lines were suspended 0.1 m above the

bottom from PVC racks as before. We observed the fish during the assays and removed the lines before all of either the control or treated algae had been consumed. The rabbitfish always attacked both treated and control algae initially and did not appear to detect any extract in the water around the algae. If the extract was deterrent, we often observed the fish biting and then rejecting the treated thalli, then the fish would rapidly consume the control algae before eating the treated algae. If the lines were left in the tanks too long, both of the *E. clathrata* pieces, treated and control, were often eaten. These replicates were excluded from the analyses. *E. clathrata* pieces were weighed before and after the assays. The pieces were carefully pressed and blotted dry with paper towels prior to weighing to determine the amount of control and treated algae consumed.

Both the extracts and the pure metabolites are lipophilic and adhere to the surfaces of the algae after the ether evaporates. McConnell et al. (1982), Hay et al. (1987), and Paul (1987) used similar methods and found that the compounds remained on the coated algae for at least 4 to 8 h. The duration of our assays was short (15 to 30 min), thus we assumed that loss of the compounds to seawater was negligible. In addition, we ran a TLC of the extract remaining on the surface of the algae after the assays to be sure that the secondary metabolites were still present and had not decomposed.

We tested each algal extract twice toward the same adult fish, randomizing the order in which the extracts were tested. We designated the 2 tests with the same extract as Trial 1 and Trial 2. Extracts were tested toward juvenile *Siganus argenteus* only once. All purified metabolites were also tested only once toward both adults and juveniles. The same fish were used throughout the assays. One assay was conducted per day, and fish were fed an abundance of algae and pelleted food between assays.

Assay results for adult *Siganus argenteus* were analyzed by a 3-way analysis of variance (ANOVA). The 3 factors used in the analyses were Treatment (control or treated), Trial, and Individual fish. A mixed model design without replication was used with Treatment and Trial as fixed factors and Individual fish as a random factor. For the assays with juveniles and the assays with isolated metabolites, we used a matched-pairs *t*-test to analyze the data. All statistical tests used the amounts (g) of control and treated algae eaten for the analyses. The statistical analyses were conducted with BMDP statistical programs available from the Health Science Computing Facility of the University of California at Los Angeles. Program BMDP 3D was used for the matched pairs *t*-test; BMDP 8V was used for the mixed model 3-way ANOVA (Dixon 1985).

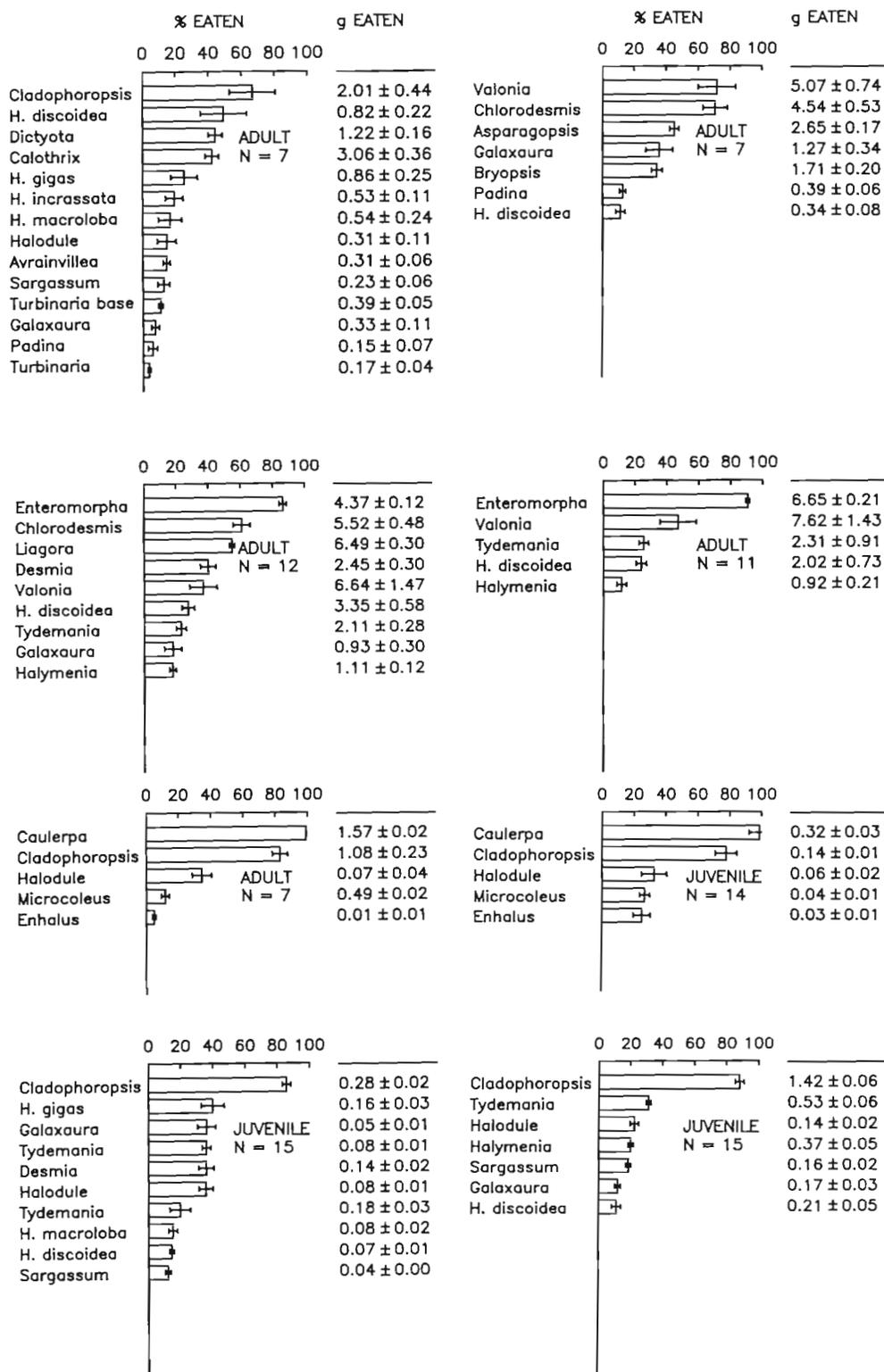


Fig. 1. *Siganus argenteus*. Feeding preferences of adults and juveniles. Bars represent % of each seaweed eaten ($\bar{X} \pm SE$). N: number of replicates with individual fish (adults) or groups of fish (juveniles). Numbers beside each bar are the amount (g) of seaweed eaten $\pm 1 SE$. Each histogram represents a separate experiment conducted on a different day

RESULTS

Feeding preferences

Fig. 1 and Table 1 present the seaweed preferences of the adult and juvenile *Siganus argenteus*. Fig. 1 presents the data as both the percentage and amount (g) of each seaweed consumed. Generally, the seaweed preferences were similar whether ranked by percentage or amount consumed, and the groupings into high, medium, or low preference did not vary. However, for a few species such as *Liagora farinosa*, *Calothrix crustacea*, and *Valonia fastigiata*, rankings based on the amount consumed differed from those based on the percent consumed. This discrepancy is an artifact of selecting pieces of seaweeds of comparable appearance rather than of comparable weight. *L. farinosa* and *C. crustacea* were ranked as medium preference based on percentage eaten (Table 1). Seaweed preferences

were similar for adult and juvenile *S. argenteus*, and there was no significant relationship between the presence of secondary chemistry and feeding preference for either adult or juvenile *S. argenteus* (Table 1). Morphological defenses (calcification and/or toughness) were more closely associated with feeding preference. Seaweeds with tough or calcified morphologies tended to be of low preference to adult *S. argenteus* and were significantly associated with low feeding preference by juveniles.

For the adults, 5 of 7 low-preference seaweeds, 8 of 10 medium-preference seaweeds, and 2 of 5 high-preference seaweeds produced secondary metabolites (3×2 Contingency Table Analysis, $G = 2.41$, $p > 0.25$; Table 1). Also, 4 of 4 species of low-preference seaweeds, 5 of 11 species of medium-preference seaweeds, and none of the 5 high-preference seaweeds were calcified or tough ($G = 5.50$, $0.1 > p > 0.05$; Table 1).

Table 1. *Siganus argenteus*. Feeding preferences of adults and juveniles. Only major secondary metabolites are listed under defenses. Structures of the major metabolites are shown in Fig. 2. Symbols are: C, calcified seaweed; T, seaweed with a tough or leathery morphology; –, no secondary metabolites detected; ?, presence of secondary metabolites uncertain, either results were not clear or seaweeds were not examined for presence of secondary metabolites. Low preference (L) = 0 to 20 % eaten; medium preference (M) = 21 to 60 % eaten; high preference (H) > 60 % eaten

Alga	Defenses	Adult preference	Juvenile preference
Cyanophyta			
<i>Calothrix crustacea</i>	?	M	
<i>Microcoleus lyngbyaceus</i>	Malyngamides	L	M
Chlorophyta			
<i>Avrainvillea obscura</i>	Avrainvilleol	L	
<i>Bryopsis pennata</i>	Polar lipids	M	
<i>Caulerpa racemosa</i>	Caulerpenyne	H	H
<i>Chlorodesmis fastigiata</i>	Chlorodesmin	H	
<i>Cladophoropsis membranacea</i>	–	H	H
<i>Enteromorpha clathrata</i>	–	H	
<i>Halimeda discoidea</i>	C, tetraacetate and halimediatrial	M	L
<i>Halimeda gigas</i>	C, pentaacetate	M	M
<i>Halimeda incrassata</i>	C, tetraacetate	L	
<i>Halimeda macroloba</i>	C, tetraacetate	L	L
<i>Tydemania expeditionis</i>	C, alcohol	M	M
<i>Valonia fastigiata</i>	–	H	
Phaeophyta			
<i>Dictyota bartayresii</i>	Dictyols	M	
<i>Padina tenuis</i>	C, –	L	
<i>Sargassum cristaefolium</i>	T, –		L
<i>Turbinaria ornata</i>	T, –	L	
Rhodophyta			
<i>Asparagopsis taxiformis</i>	Halogenated ketones and acetones	M	
<i>Desmia hornemanni</i>	Halogenated monoterpenes	M	M
<i>Galaxaura marginata</i>	C, –	M	M
<i>Halymenia durvillaei</i>	Polar lipids	L	M
<i>Liagora farinosa</i>	C, acetogenins	M	
Anthophyta (seagrasses)			
<i>Enhalus acoroides</i>	?	L	M
<i>Halodule uninervis</i>	–	M	M

For the results of experiments with juveniles, medium- and high-preference seaweeds were combined for analyses because only 2 species were of high preference. For these assays, 2 of 3 species of low-preference seaweeds, and 5 of 9 species of medium- to high-preference seaweeds, produced secondary metabolites (2×2 Contingency Table Analysis, $G = 0.11$, $p > 0.5$; Table 1). Of the low-preference seaweeds, all 3 species were calcified or tough, but only 3 of 10 species of the medium- to high-preference seaweeds were calcified or tough ($G = 5.73$, $p > 0.025$; Table 1).

Chemical feeding deterrents

Table 2 shows the results of algal extract assays with adult *Siganus argenteus*. Extracts of *Desmia hornemanni*, *Enhalus acoroides*, *Halymenia durvillaei*, *Halimeda discoidea*, *Microcoleus lyngbyaceus*, and *Tydemanina expeditionis* were all significantly deterrent ($p < 0.05$) toward adult *S. argenteus*. Extracts of *D. hornemanni* and *M. lyngbyaceus* that were significant deterrents overall were more strongly deterrent in one trial than in the other, and we observed significant effects attributed to the Trial factor as well as Treatment-Trial interactions in the ANOVA tests (Table 2). Many extracts showed significant Trial effects which indicated that, overall, different amounts of *Enteromorpha clathrata* were eaten in different trials. The trial effects are not important since the durations of the assays were approximate. Several extracts showed Treatment-Trial interactions which may have resulted from variation in algal chemistry among collections, or from differing physiological states, such as hunger, of the fish between trials. However, we always examined the extracts by TLC to ensure consistency among collections and did not use extracts that showed any evidence of chemical decomposition, so these potential sources of variation were eliminated.

The extracts of *Desmia hornemannii* and *Microcoleus lyngbyaceus* were deterrent toward juvenile *Siganus argenteus* as well as toward adults (Table 3). Thalli of *D. hornemannii* were of medium preference for both adult and juvenile *S. argenteus* (Fig. 1; Table 1), although the extracts and isolated metabolites were strong feeding deterrents.

Extracts of the preferred alga *Chlorodesmis fastigiata* did not deter either adults or juveniles (Tables 2 and 3). Extracts of *Halymenia durvillaei*, *Halimeda discoidea*, *Tydemanina expeditionis*, and the seagrass *Enhalus acoroides* were deterrent toward adult *S. argenteus* but not toward juveniles. We do not know what the chemical feeding deterrents are present in *H. durvillaei* or *E. acoroides*, and we are currently examining the extracts for secondary metabolites. *Halophila minor* and other

seagrasses from Guam have been reported to contain phenolic acids (Zapata & McMillan 1979).

Of the pure secondary metabolites isolated from the seaweeds used in this study, the only metabolite that was an effective deterrent toward both adults and juveniles was ochtodene, isolated from *Desmia hornemanni* (Table 4). A nonpolar mixture of ochtodene and acyclic halogenated monoterpenes was the only other isolate deterrent toward the juveniles, but the deterrence may have been due to ochtodene alone. Adults were also significantly deterred by chlorodesmin (although the crude algal extract was nondeterrent), by the structurally related alcohol from *T. expeditionis*, and by flexilin. None of the other metabolites were significantly deterrent.

DISCUSSION

Results indicate that some seaweed secondary metabolites are effective feeding deterrents toward the rabbitfish *Siganus argenteus* on Guam; however, there is no clear relationship between secondary metabolite production by seaweeds and low susceptibility to *S. argenteus*. Extracts from some of the low- or medium-preference algae producing secondary metabolites that functioned as feeding deterrents included: *Desmia hornemanni*, which produces halogenated monoterpenoids (Burreson et al. 1975b, c) including ochtodene (Paul et al. 1987); *Halimeda discoidea*, which produces diterpenoids including halimedatetraacetate and halimedatriol (Paul & Fenical 1986, Paul & Van Alstyne 1988); *Microcoleus lyngbyaceus*, which produces nitrogenous metabolites malyngamides A and B (Cardellina et al. 1978, 1979), and *Tydemanina expeditionis*, which produces acyclic diterpenoids related to chlorodesmin (Paul & Fenical 1985).

Interestingly, several algal species that produce high concentrations of secondary metabolites were eaten by the rabbitfish, and neither their extracts nor their isolated metabolites significantly deterred feeding. These included: *Asparagopsis taxiformis*, which produces halogenated ketones and acetones (Burreson et al. 1975a, McConnell & Fenical 1977, Woolard et al. 1979); *Caulerpa racemosa* which produces the metabolites caulerpin (Maiti et al. 1978) and caulerpenyne (Amico et al. 1978); *Dictyota bartayresii* which produces 'dictyol' diterpenoids including pachydictyol A (Faulkner 1984, Paul & Fenical unpubl.); *Liagora farinosa*, which produces acetylene-containing acetogenins (Paul & Fenical 1980), and *Chlorodesmis fastigiata*, which produces chlorodesmin and related acyclic diterpenoids (Wells & Barrow 1979, Paul & Fenical 1985). Chlorodesmin deterred adult but not juvenile rabbitfish. Several nondeterrent secondary metabolites were structurally

Table 2. *Siganus argenteus*. Results of chemical feeding deterrent trials with adult rabbitfish. All extracts were tested at 4 to 5% dry weight coated on *Enteromorpha clathrata*. Data were analyzed by a mixed-model 3-way ANOVA. Treatment and Trial are fixed factors, Fish is a random factor

Species	Trial	N	Mean % eaten \pm SE		p-values	
			Control	Treated	Treatment	Trial
Deterrents						
<i>Desmia hornemanni</i>	1	11	72.7 \pm 3.3	24.0 \pm 3.2	0.000	0.000
	2	11	79.5 \pm 3.0	69.0 \pm 3.7	Treatment-Trial interaction p = 0.001	
<i>Enhalus acoroides</i>	1	11	62.4 \pm 4.0	34.9 \pm 3.5	0.001	0.004
	2	11	45.0 \pm 5.3	19.3 \pm 3.9		
<i>Halimeda discoidea</i>	1	11	51.8 \pm 5.1	30.8 \pm 5.6	0.033	0.000
	2	11	77.5 \pm 4.3	72.1 \pm 2.1		
<i>Halymenia durvillaei</i>	1	11	53.2 \pm 7.2	42.1 \pm 2.6	0.044	0.000
	2	11	87.2 \pm 3.9	78.6 \pm 3.4		
<i>Microcoleus lyngbyaceus</i>	1	11	65.8 \pm 2.8	46.8 \pm 4.6	0.021	0.000
	2	11	77.3 \pm 1.9	77.6 \pm 2.0	Treatment-Trial interaction p = 0.007	
<i>Tydemania expeditionis</i>	1	11	29.4 \pm 5.1	23.3 \pm 4.3	0.038	0.580
	2	11	37.3 \pm 3.9	19.8 \pm 4.4		
Nondeterrents						
<i>Asparagopsis taxiformis</i>	1	8	59.6 \pm 3.9	48.0 \pm 9.2	0.051	0.000
	2	8	42.9 \pm 5.1	62.1 \pm 2.8	Treatment-Trial interaction p = 0.014	
<i>Avrainvillea obscura</i>	1	10	86.2 \pm 2.8	79.0 \pm 3.2	0.610	0.000
	2	10	42.2 \pm 5.2	54.2 \pm 5.6		
<i>Bryopsis pennata</i>	1	10	48.1 \pm 8.2	46.7 \pm 7.3	0.387	0.053
	2	10	39.3 \pm 4.5	30.4 \pm 3.0		
<i>Caulerpa racemosa</i>	1	11	79.4 \pm 3.6	65.6 \pm 4.5	0.978	0.000
	2	11	19.8 \pm 2.3	32.9 \pm 5.6	Treatment-Trial interaction p = 0.043	
<i>Chlorodesmis fastigiata</i>	1	11	54.8 \pm 5.1	48.5 \pm 6.0	0.388	0.077
	2	11	31.5 \pm 6.2	44.5 \pm 6.2		
<i>Dictyota bartayresii</i>	1	10	75.4 \pm 2.0	79.0 \pm 1.1	0.244	0.000
	2	10	39.0 \pm 10.3	18.1 \pm 5.7	Treatment-Trial interaction p = 0.038	
<i>Halimeda macroloba</i>	1	11	88.6 \pm 1.8	86.9 \pm 1.6	0.353	0.000
	2	11	66.1 \pm 2.2	61.3 \pm 3.8		
<i>Halophila minor</i>	1	11	63.4 \pm 3.9	57.3 \pm 4.9	0.260	0.000
	2	11	47.4 \pm 6.7	38.3 \pm 8.5		
<i>Liagora farinosa</i>	1	11	49.3 \pm 7.5	50.4 \pm 5.6	0.148	0.024
	2	11	71.9 \pm 5.6	52.6 \pm 4.5		
<i>Valonia fastigiata</i>	1	11	62.9 \pm 4.8	59.9 \pm 3.4	0.694	0.061
	2	11	67.2 \pm 4.3	65.6 \pm 3.7		

Table 3. *Siganus argenteus*. Results of chemical feeding deterrent trials with juvenile rabbitfish. All extracts were tested at 4 to 5% dry weight, coated on *Enteromorpha clathrata* (= E) or *Cladophoropsis membranacea* (= C). N: number of cages with different fish; p-values were determined by the paired t-test (2-tailed). * Significant feeding deterrent at $p < 0.05$

Algal extract	N	Alga used	Mean % eaten \pm SE		p-value
			Control	Treated	
<i>Asparagopsis taxiformis</i>	15	C	54.77 \pm 3.74	56.06 \pm 4.59	0.741
<i>Avrainvillea obscura</i>	14	C	54.80 \pm 3.55	49.71 \pm 3.53	0.196
<i>Bryopsis pennata</i>	14	C	71.60 \pm 2.58	66.29 \pm 3.44	0.174
<i>Caulerpa racemosa</i>	14	E	56.47 \pm 2.52	55.76 \pm 4.22	0.903
<i>Chlorodesmis fastigiata</i>	15	C	44.88 \pm 3.55	51.27 \pm 3.37	0.091
<i>Desmia hornemannii</i>	14	E	72.02 \pm 2.18	45.00 \pm 2.40	0.000*
<i>Dictyota bartayresii</i>	15	C	57.50 \pm 3.63	57.19 \pm 3.21	0.639
<i>D. cervicornis</i>	14	E	69.93 \pm 5.34	67.89 \pm 7.36	0.724
<i>Enhalus acoroides</i>	14	C	73.10 \pm 1.97	68.84 \pm 2.82	0.261
<i>Halimeda discoidea</i>	14	E	70.21 \pm 2.61	67.70 \pm 3.65	0.492
<i>Halimeda maculosa</i>	14	E	59.79 \pm 2.72	62.63 \pm 2.56	0.473
<i>Halophila minor</i>	9	C	66.96 \pm 5.10	71.04 \pm 3.69	0.280
<i>Halymenia durvillaei</i>	14	C	70.39 \pm 3.73	73.70 \pm 2.74	0.146
<i>Microcoleus lyngbyaceus</i>	15	C	52.77 \pm 2.64	37.16 \pm 2.56	0.001*
<i>Tydemanina expeditionis</i>	14	E	62.99 \pm 3.94	66.73 \pm 2.79	0.446
<i>Valonia fastigiata</i>	14	C	69.03 \pm 3.85	66.80 \pm 3.42	0.564

similar to deterrent metabolites (Fig. 2), therefore it is difficult to determine whether a compound will function as a feeding deterrent based on its chemical structure. For example, linear terpenoids from green algae including chlorodesmin, *Tydemanina* alcohol, and flexilin were deterrent while other structurally-related green algal terpenoids including caulerpenyne and udoteal were not (Fig. 2).

While fish only avoided the treated *Enteromorpha clathrata* after biting it, defended whole seaweeds were often avoided in feeding preference assays. It appears that fish initially perceive deterrents via their sense of taste but then learn to recognize chemically defended seaweeds visually.

Our results indicate that morphological defenses such as calcification and toughness were associated with low susceptibility to grazing by *Siganus argenteus*, especially for juveniles. Juveniles appeared to be more susceptible to seaweed morphology and less susceptible to secondary chemistry than the adults. Some seaweeds which produced both calcification and secondary metabolites, such as *Halimeda* spp. and *Tydemanina expeditionis*, were avoided by both adult and juvenile rabbitfish. The importance of combined calcification and chemical defenses in tropical seaweeds has been discussed previously (Hay 1984a, Paul & Hay 1986, Paul & Fenical 1986).

Our results are consistent with published studies on

Table 4. *Siganus argenteus*. Results of chemical feeding deterrent trials with rabbitfish. All isolated metabolites were tested at 1% dry weight coated on *Enteromorpha clathrata*. N: number of trials with different fish; p-values determined by the paired t-test (2-tailed). * Significant feeding deterrent. † Significant feeding attractant at $p < 0.05$. Blanks indicate compounds not tested

Metabolite	Juveniles				Adults			
	N	Mean % eaten \pm SE		p-value	N	Mean % eaten \pm SE		p-value
		Control	Treated			Control	Treated	
Avrainvilleol					12	69.97 \pm 4.24	68.97 \pm 5.16	0.757
Bryopsin					11	65.39 \pm 8.03	71.31 \pm 7.93	0.660
Caulerpenyne	14	75.46 \pm 1.67	70.44 \pm 2.07	0.113	12	57.00 \pm 6.62	69.54 \pm 3.93	0.215
Caulerpin					12	40.96 \pm 4.68	38.41 \pm 6.47	0.761
Chlorodesmin	14	86.79 \pm 1.11	86.20 \pm 1.06	0.553	11	53.63 \pm 4.89	42.28 \pm 3.96	0.028*
<i>Desmia</i> non-polar	14	86.68 \pm 1.54	70.09 \pm 1.59	<0.001*				
Flexilin	14	50.27 \pm 4.24	47.38 \pm 6.41	0.756	11	81.75 \pm 3.42	65.14 \pm 4.68	0.036*
Ochitodene	14	59.74 \pm 4.89	39.37 \pm 1.70	0.002*	8	92.38 \pm 1.52	86.00 \pm 2.11	0.026*
Pachydictyol A	14	78.48 \pm 2.23	76.32 \pm 3.43	0.608	11	62.96 \pm 5.18	73.14 \pm 4.04	0.071
Udoteal	14	77.65 \pm 1.97	77.54 \pm 2.48	0.924	11	81.93 \pm 2.28	80.50 \pm 2.74	0.842
<i>Tydemanina</i> alcohol					11	62.46 \pm 4.66	41.72 \pm 6.38	0.026*
Malyngamide B	14	48.27	2.75	63.20	4.58	0.020†		

isolated metabolites in field assays (Paul 1987), both the extracts of *Chlorodesmis fastigiata* and the isolated metabolite chlorodesmin were significant feeding deterrents to mixed-species populations of herbivorous fishes including rabbitfishes. The extract of *Halimeda macroloba* was not a significant deterrent in field assays, although the isolated metabolite halimedatetraacetate was deterrent at high concentrations (Paul 1987). Halimedatriol, which occurs in relatively high concentrations in *H. discoidea*, is a more potent feeding deterrent than halimedatetraacetate (Paul & Van Alstyne 1988). *H. discoidea* was the only *Halimeda* extract that showed significant deterrent activity toward *S. argenteus* (Table 2). Extracts of 3 *Caulerpa* species that contained caulerpin and caulerpenyne showed no feeding deterrent effects in field assays (Paul 1987).

Our results differ from those of Targett et al. (1986) for the parrotfish *Sparisoma radians* in the Caribbean and from studies of *Zebrasoma flavescens* on Guam (Wylie & Paul 1988). In the study by Targett and co-workers, extracts of *Halimeda incrassata* and the metabolites halimedatetraacetate and caulerpenyne were all significant feeding deterrents. Also, extracts of *Halimeda discoidea*, *Bryopsis pennata*, and *Dictyota* spp. and the isolated metabolite pachydictyol A from *Dictyota* spp. were all deterrents toward *Z. flavescens* (Wylie & Paul 1988) but not toward the rabbitfish. Apparently, different species of herbivorous fishes respond dissimilarly to algal secondary metabolites. Other examples of the differences among herbivorous fishes in their responses to algal secondary metabolites are presented by Paul et al. (in press). Thus, seaweed preferences of herbivorous fishes may differ markedly even between ecologically similar species.

We found that juvenile and adult *Siganus argenteus* also differed in their susceptibilities to seaweed chemical defenses. Several extracts and isolated metabolites that were deterrent toward the adults were not effective toward the juveniles. It is possible, however, that tank effects or interactions between juvenile fish that were tested in groups could confound the comparisons between adult and juvenile fish. Avoidance of secondary metabolites may involve a learning process, and the juveniles that we used for the assays had only limited prior exposure to seaweeds. Also since juveniles have a higher rate of metabolism per gram than adults, they may have a relatively higher motivation for feeding which makes them less easily deterred by secondary metabolites. Juveniles appeared to be more constrained by the morphology of the seaweeds.

This study provides one of a few direct tests of the hypothesis that secondary metabolites are major determinants of the food preferences of a herbivorous reef fish. Our results show that, although some algal sec-

dary metabolites provide effective chemical defenses against grazing by *Siganus argenteus*, many species that produce secondary metabolites are not avoided. The abilities of some reef fishes to consume species of seaweeds that produce high concentrations of secondary metabolites could allow them to exploit a food source that other herbivores avoid. Information on how herbivorous fishes could physiologically overcome plant defenses is lacking, and this may prove to be a promising area for future research.

Differential responses of herbivorous fishes to algal secondary metabolites may contribute to observed differences in seaweed communities among reef-slope habitats. For example, several species of *Dictyota* and *Caulerpa* are not abundant in reef-slope habitats on Guam where herbivorous fishes are common (pers. obs.), and the secondary metabolites produced by these algae do not appear to be effective defenses toward many herbivorous fishes on Guam (Paul 1987, Wylie & Paul 1988) including *Siganus argenteus*. In addition, while *Asparagopsis taxiformis*, *Caulerpa racemosa*, and *Liagora farinosa*, which produce secondary metabolites, are common in some reef habitats on Guam, we would predict low abundances of these algae in areas where rabbitfish are abundant, especially if more highly preferred seaweeds are not available.

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