Oecologia (1995) 103: 170–179

ORIGINAL PAPER

Nancy M. Targett · Anne A. Boettcher
Timothy E. Targett · Nicholas H. Vrolijk

Tropical marine herbivore assimilation of phenolic-rich plants

Received: 5 December 1994 / Accepted: 20 February 1995

Abstract Phenolics in marine brown algae have been thought to follow a latitudinal gradient with high phenolic species in high latitudes and low phenolic species in low latitudes. However, tropical brown algae from the western Caribbean have been shown to be high in phlorotannin concentration, indicating that latitude alone is not a reasonable predictor of marine plant phenolic concentrations. This study shows that the range of high phenolic phaeophytes is not limited to the western Caribbean but encompasses the western tropical Atlantic, including Bermuda and the Caribbean, where algal phlorotannin concentrations can be as high as 25% dry weight (DW). Assimilation efficiencies (AEs) of phenolic-rich and phenolic-poor plants were examined in three tropical marine herbivores (the parrotfish, *Sparisoma radians*, and the brachyuran crab, *Mithrax sculptus*, from Belize and the parrotfish, *Sparisoma chrysopterum*, from Bermuda). AEs of phenolic-rich food by each of the three herbivore species were uniformly high, suggesting that high plant phenolic concentrations did not affect AEs in these species. This is in contrast to some temperate marine herbivores where phenolic concentrations of 10% DW have been shown to drastically reduce AE. The apparent contradiction is discussed in light of the effects of specific herbivore gut characteristics on successful herbivory of high phenolic brown algae.

Key words Phlorotannin · Polyphenolics · Herbivore · Assimilation efficiency · Brown algae

N. M. Targett (✉) · A. A. Boettcher · T. E. Targett
N. H. Vrolijk[1]
University of Delaware,
Graduate College of Marine Studies,
Lewes, DE 19958, USA

Present address:
[1]University of Maryland, Center of Marine Biotechnology,
Baltimore, MD 21202, USA

Introduction

Phlorotannins are common secondary metabolite constituents of marine brown algae, with a putative role in plant defense against marine herbivores and fouling organisms (Ragan and Glombitza 1986; Hay and Fenical 1988; Steinberg 1992). They are polymers of acetate-malonate derived 1,3,5 trihydroxybenzene (phloroglucinol) and are analogous to terrestrial condensed tannins (Ragan and Glombitza 1986; Waterman and Mole 1994). Phlorotannins range in size from the monomer, phloroglucinol, to 650 kDa. Spatial variability in brown algal phenolic concentrations seems to fall along broad geographical lines, and biogeographical comparisons of brown algal phenolic concentrations (see Steinberg 1992 for review: Targett et al. 1992) have shown that: (1) temperate browns are a mixture of high phenolic [> 2% dry weight (DW), Swain 1979] and low phenolic species; (2) phaeophyte phenolic levels in temperate Australasia are, on average, much higher than phenolic levels in brown algae from other temperate regions of the world; (3) brown algae in the tropical Pacific and Indo-Pacific are low in phenolic levels; (4) phaeophytes in the western Caribbean, like temperate browns, have a mixture of high and low phenolic species. In view of the latter finding, the hypothesized physical constraints (e.g. temperature, trace metal deficiency) on tropical phaeophytes regarding phenolic production (Steinberg and Paul 1990; van Alstyne and Paul 1990), as well as the assertion that polyphenolic concentrations are probably too low in tropical brown algae to have an important effect against herbivores (Steinberg and Paul 1990; Steinberg et al. 1991) are negated. More data are necessary to establish the generality of the occurrence of high phenolic browns throughout the western tropical Atlantic.

The responses of marine herbivores to brown algal phlorotannins, based upon both behavioral and physiological assays, are mixed. Studies of north temperate,

marine herbivores indicate that polyphenolics consistently deter feeding (Geiselman and McConnell 1981; Steinberg 1984, 1985, 1988; Steinberg and van Altena 1992) and can affect herbivore assimilation efficiencies (Irelan and Horn 1991; Steinberg and van Altena 1992; Boettcher and Targett 1993) at ecologically relevant concentrations. Temperate Australasian herbivores differ significantly from the north temperate pattern in that they are generally not deterred from feeding on algae rich in polyphenolics (see Steinberg 1992 for review; Steinberg and van Altena 1992). Studies with tropical herbivores from the Indo-Pacific (Steinberg and Paul 1990; van Alstyne and Paul 1990; Steinberg et al. 1991) produced seemingly conflicting results. Steinberg et al. (1991) found no correlation between grazing susceptibility and phenolic content of different temperate phenolic-rich and tropical phenolic-poor whole plant *Sargassum* species for tropical fishes in western Australia, while van Alstyne and Paul (1990), in their experiments in Guam with plant extracts coated onto a palatable algal species, found that tropical fish grazing was generally deterred by temperate high-phenolic plant extracts, although not by temperate or tropical low-phenolic ones.

Based upon data from terrestrial systems (for reviews see: Rosenthal and Janzen 1979; Bernays et al. 1989; Rosenthal and Berenbaum 1991a, b; Appel 1993) the effect of phenolics on herbivore feeding deterrence and assimilation efficiency is thought to be dependent upon characteristics of the phenolics themselves (Zucker 1983; Ragan and Glombitza 1986) and upon the environment in which they are acting, that is, the herbivore gut. These include plant phenolic characteristics such as molecular size, type, and concentration, and herbivore gut characteristics such as pH, redox potential, ion concentration, and surfactant activity (Feeny 1969; Berenbaum 1980; Bernays 1981; Martin and Martin 1984; Martin et al. 1985; Bernays et al. 1989; Appel and Martin 1990; Appel and Schultz 1992; Barbehenn and Martin 1992; Appel 1993).

For marine phlorotannins, the linkage between herbivore gut characteristics and phenolic activity is just beginning to be made (Irelan and Horn 1991; Tugwell and Branch 1992; Boettcher and Targett 1993). Four gut types have been described for marine herbivorous fishes (Horn 1989). These vary in pH, length of gut, triturating ability, and presence of hindgut caecum for microbial fermentation. Fishes categorized as type I have a thin walled stomach, a long intestine, and rely primarily upon acid lysis in the stomach to release cell contents. Type II fishes grind food in a gizzard-like stomach which is near neutral to neutral. They also have long intestines. Type III fishes grind food in a pharyngeal mill. They have no stomach and a moderately long intestine which has a neutral to basic pH. Fishes in the type IV category have a well-defined acidic stomach, a long intestine and a hindgut caecum for fermentation. Marine invertebrates are also known to have varying gut characteristics and exhibit differences in pH, surfactant characteristics, and microbial flora (e.g. Purchon 1977; Dall and Moriarty 1983; Tugwell and Branch 1992). Based upon data from terrestrial systems regarding the importance of the gut environment in determining the effects of polyphenolics in both herbivorous insects and vertebrates, it might be expected that differences in marine herbivore gut characteristics could result in herbivore-dependent responses to marine plant phlorotannins. Boettcher and Targett (1993) showed that in the stichaeid fish, *Xiphister mucosus* (type I gut), the effects of polyphenolics at naturally occurring concentrations (1% wet weight) on assimilation efficiency are related to phenolic molecular size. Phenolics >10 kDa reduced assimilation efficiency, phenolics between 1 to 10 kDa gave mixed results, while those <1 kDa (including the phloroglucinol monomer) showed no effect. Assimilation efficiency in another species with a type I gut, *Girella nigricans*, was also negatively affected by high molecular weight phenolics (>10 kDa, Boettcher 1992). A comparison of studies examining *G. nigricans* and the southern hemisphere temperate odacid, *Odax cyanolmelas*, which has a gut pH of 8–9 (type III gut) and feeds on brown algae with high concentrations of polyphenolics, suggests that the levels of polyphenolics in algae negatively affected the feeding preference of *G. nigricans*, but not *O. cyanolmelas* (Andrew and Jones 1990; P. D. Steinberg personal communication). It is possible that the apparent conflicts observed in marine herbivore susceptibility to phenolic plant defenses reflect differences in herbivore gut characteristics and/or differences in the plant phenolics themselves.

Clearly, more information is needed on the response of herbivores to high phenolic plant species. In this study, we examine the assimilation efficiencies of three tropical herbivores from two different locales (the parrotfish, *Sparisoma radians* and the brachyuran crab, *Mithrax sculptus* from Belize, and the parrotfish, *Sparisoma chrysopterum* from Bermuda) feeding on high phenolic tropical brown algae. In addition, we expand the range of Caribbean brown algae tested for phenolic concentration in an effort to determine if phenolic-rich phaeophyte species occur over a wider range of the tropical Atlantic.

Materials and methods

Plant collection for extraction of phlorotannins

In the warm temperate and tropical Atlantic, phaeophytes were collected from Belize (June 1990, July 1991, March 1993), Cozumel (June 1990), Bahamas (August 1993), Turks and Caicos (July 1994), Bermuda (June 1994), and North Carolina (July 1991). In the tropical Pacific, phaeophytes were collected from Hawaii in July 1991 (Table 1). Non-phaeophyte plants were collected for phenolic tests to establish baseline data for non-phlorotannin-containing species. These included the chlorophyte, *Ulva lactuca* (Delaware, November 1990 and February 1991), the rhodophyte, *Acanthophora spicifera*

(Carrie Bow Cay, Belize, December 1990), and the seagrass, *Thalassia testudinum* (Carrie Bow Cay, Belize, December 1990). A cold temperate phaeophyte, *Ascophyllum nodosum* (Maine September 1989 and May 1991) was also used for comparison of phenolic concentrations with literature values.

Determination of phlorotannin content

To quantify total phenolic concentration, plants were extracted in aqueous methanol according to the method described in Boettcher and Targett (1993). Phenolic concentrations of whole extracts were determined using the Folin-Denis assay (phloroglucinol standard, λ_{max} = 725 nm; Swain and Hillis 1959; Ragan and Jensen 1977; Steinberg 1988). This assay has fewer drawbacks than other colorimetric assays for polyphenolic compounds. However, because the Folin-Denis reagent can react with other plant constituents in addition to polyphenolics (e.g. amino acids and proteins: Andersen and Todd 1968; Yates and Peckol 1993) and because there are other interfering compounds (e.g. ascorbic acid, urea, detergents, and diethyl ether: Ragan and Glombitza 1986; Hagerman and Butler 1991) we also ran comparisons with samples of *Lobophora variegata* (decumbent, ruffled and encrusting forms; Coen and Tanner 1989) and *Ascophyllum nodosum* treated with insoluble polyvinylpolypyrrolidone (PVPP). PVPP specifically binds to phenolics in an acid environment, and the absorbance difference between PVPP and non-PVPP treatments can be expected to reflect only the concentration of phenolics (Yates and Peckol 1993). Algal extractions were as described in Boettcher and Targett 1993. The PVPP assay was optimized in a series of preliminary experiments using phloroglucinol and purified low and high molecular weight phlorotannins. For PVPP treatments, the algal extracts were acidified to pH 3.5 with acetic acid. PVPP was added to one half of the acidified extract (15 mg/ml). The extract was agitated for 10 min, centrifuged to remove precipitate, and the supernatant removed. The process was repeated 2 additional times so that each extract was partitioned a total of 3× with PVPP. The other half of the acidified extract was used for paired comparisons in the Folin-Denis assay. The Folin-Denis assay procedure was as described above for non-acidified, non-PVPP treated extracts. The standard curve was generated with phloroglucinol.

Phlorotannin content was based upon plant dry weight. Plant dry:wet weight ratios were determined from samples of each algal and seagrass species (n = 2–4, or as reported in Table 1) blotted and weighed fresh or fresh frozen and after drying at 60 °C for 24 h.

Total phlorotannin content (% dry weight, mean ± 1 SD where $n \geq 3$) for each plant was determined from the samples assayed. Statistical comparison of standard and PVPP extract treatments was done via a paired-sample t-test (α = 0.05).

Herbivore assimilation efficiency bioassays

Sparisoma radians (55–89 mm total length) were collected by trawling in seagrass beds located off the southeast side of Twin Cays, Belize (July 1990). Fish were transported to the Carrie Bow laboratory where they were held under natural light conditions in flowing seawater aquaria for 48 h, feeding ad libitum on their preferred food, the seagrass, *T. testudinum* (Lobel and Ogden 1981; Horn 1989). Prior to the feeding experiments, fish were held overnight without food. For the experiments, fish were selected from a pool of 60 fish. Each treatment had five replicates with five fish per replicate. Each replicate was run in an individual aquarium with flowing seawater. They were allowed to feed on a particular food item (their preferred food, *T. testudinum*, having 2.75–3.28% DW phenolics, or on ruffled, deep decumbent or encrusting forms of *L. variegata*, having 10.23% ± 0.67%, 13.39% ± 3.50%, and 8.33% ± 0.94% DW phenolics, respectively; Targett et al. 1992) for 1 h to insure that fish had cleared their guts of any other food material (Targett and Targett 1990). Fecal material was then collected and discarded. Fish were allowed to continue feeding for 2 h during which time fecal material was collected every 30 min. Fecal material was held on ice between collections and, at the conclusion of the 2 h experiment, was frozen in liquid nitrogen along with a food sample (n = 3) for transport to Lewes, Del, United States. The samples were held at −80 °C until analysis to determine assimilation efficiencies. Eleven fish were euthanized and dissected to determine anterior and posterior intestinal pH (ColorpHast pH 5–10 indicator sticks, EM reagents).

Mithrax sculptus (ca. 20–30 mm carapace width) were collected from *Porites porites* coral heads in a shallow (<1 m) back reef habitat at the northeast end of Carrie Bow Cay, Belize and returned to the Carrie Bow laboratory where they were held for at least 24 h in a flow-through seawater aquarium under natural light, and fed a preferred food (*Laurencia papillosa* in experiment 1 or *Acanthophora spicifera* in experiment 2; Coen 1988) ad libitum. For experiments, crabs were held individually in 10 cm × 10 cm plastic trays at ambient salinity, temperature and light. Water in the trays was changed daily. Crabs were allowed to feed on the treatment food [experiment 1 in July 1991: *Laurencia papillosa*, a rhodophyte, or ruffled, deep decumbent, or encrusting *Lobophora variegata*, containing 10.23% ± 0.67%, 13.39% ± 3.50%, and 8.33% ± 0.94% DW phenolics, respectively (Targett et al. 1992); experiment 2 in March 1993: *Acanthophora spicifera* (1.03%–1.14% DW), *Padina sanctae-crucis* (1.79% ± 0.36% DW), or ruffled *Lobophora variegata* (10.23% ± 0.67% DW); Targett et al. 1992] for 24 h to clear their guts of other food material. All fecal material produced during this time was collected. The crabs were then allowed to continue feeding on the treatment food for 2–3 days. Fecal material was collected daily and samples held in liquid nitrogen between collections. Three to five replicates per food type were run with fecal samples pooled from five crabs per replicate. Food samples (n = 3) of each food type were saved along with fecal samples for assimilation efficiency calculations. Fecal and food samples were frozen in liquid N_2 and transported to Lewes, Del, where they were held at −80 °C until analysis. Seven crabs were euthanized and dissected to determine digestive tract pH (ColorpHast pH 5–10 indicator sticks, EM reagents).

Sparisoma chrysopterum (230–410 mm total length) were collected by net in rubble adjacent to seagrass beds in Bailey's Bay, Bermuda (June 1994). Fish were transported to the Bermuda Biological Station where they were held in flowing seawater aquaria under natural light for 48 h, feeding ad libitum on *Padina gymnospora*. Prior to feeding experiments, individual fish (n = 4–5) were placed into individual, flow-through aquaria and allowed to feed ad libitum on either *Lobophora variegata* (shallow decumbent form) collected at Devil's Hole (6.31% ± 0.95% phlorotannins DW), and Shark Hole (7.51% ± 1.72% phlorotannins DW), Bermuda, or *P. gymnospora* (3.10%– 4.74% phlorotannins DW). Forty-eight hours after introducing the fish to the treatments, collection of fecal samples began and continued during the daytime at 1–2 h intervals for 5–10 days. Fecal (n = 4–5) and treatment food samples (n = 4) were frozen in liquid N_2 to transport to Lewes, Del, where they were held at −80 °C until analysis. Ten fish were euthanized and dissected to determine anterior and posterior intestinal pH (MicroElectrodes pH microprobe).

Calculation of assimilation efficiency

Lyophyllized food and fecal samples from each treatment were individually ground and homogenized using a Cresent Wig-L-Bug. Ash and organic contents were determined for each food and fecal sample, and total and organic assimilation efficiencies (AEs) were determined for each herbivore. In addition, protein AEs were determined for *S. chrysopterum* treatments. For all of the AE calculations, fecal ash, organic, and protein values derived from individual treatments were paired with corresponding food values for the same treatments. Total, organic, and protein AEs were determined via the ash

indicator method using the formulae outlined in Boettcher and Targett (1993). This method is a widely used indirect method in aquatic systems where quantitative collection of food input and fecal output is difficult (Montgomery and Gerking 1980; Edwards and Horn 1982; Horn and Neighbors 1984; Lassuy 1984; Horn et al. 1985, 1986; Horn 1989; Targett and Targett 1990; Irelan and Horn 1991; Boettcher and Targett 1993). Comparisons of direct versus indirect methods have resulted in similar AE values (Gerking 1984; Anderson 1988) with losses due to dissolution of, or bacterial activity on, the fecal pellet proving to be negligible over 24 h periods (Elliot 1976; Geesey et al. 1984; Brafield 1985; A. A. Boettcher and N. M. Targett, unpublished data; J. M. Montgomery and T. E. Targett, unpublished data). Ash and organic contents were determined for duplicate 10–20 mg fecal and food samples. The protein content was determined as described in Horn and Neighbors (1984) on duplicate 3–5 mg samples. Ashing was done in pre-ashed borosilicate test tubes in a muffle furnace at 450 °C for 24 h. The mean percentage of ash, organic, and protein contents were used in these calculations.

Total and organic AEs for each plant treatment upon which *Sparisoma radians* and *Mithrax sculptus* fed were examined among treatment groups, using a one-way model I ANOVA and Tukey's multiple comparisons test ($\alpha = 0.05$). Total, organic, and protein AEs for *Lobophora variegata* and *P. gymnospora* treatments upon which *S. chrysopterum* fed were compared using a Student's *t*-test ($\alpha = 0.05$).

Results

Plant phlorotannin content

Both high and low phenolic concentrations were found to occur in phaeophytes throughout the western tropical Atlantic (Table 1; Targett et al. 1992). *L. variegata* had high concentrations (> 2% DW; Swain 1979) at all locations assayed. Phenolic concentrations for *L. variegata* collected in North Carolina were lower than phenolic concentrations values for *L. variegata* collected in various western tropical Atlantic locales, but higher than the values reported for *L. variegata* in the Pacific and Indo-Pacific (Steinberg and Paul 1990; Targett et al. 1992). Phenolic concentrations for Pacific phaeophytes assayed were low (< 2%; Table 1) except for *Padina japonica*. Treatment of brown algal extracts with PVPP resulted in removal of phenolics that averaged 92.2% ± 3.3% (Table 2). A comparison of the phenolics between the standard treatment and as calculated from the PVPP difference method on a percentage DW basis, indicated that interference from non-phenolics was minimal (Table 2). Low Folin-Denis values for the rhodophyte, *Acanthophora spicifera* (1.03–1.14% DW), and the chlorophyte, *Ulva lactuca* (0.31–0.60% DW), non-phlorotannin-containing macroalgae, further supported the lack of general interference in the assay. Values for the seagrass, *Thalassia testudinium* (2.75–3.28% DW), were comparable to previously reported values (Zapata and McMillan 1979).

Herbivore assimilation efficiencies

The pH of both the anterior and posterior intestine of *Sparisoma radians* and *S. chrysopterum* were neutral to basic (pH range of 7–9; Table 3). The total and organic AEs of *S. radians* fed the high phenolic *L. variegata* forms were not significantly different from AEs of *S. radians* fed its preferred food, *T. testudinum* (Fig. 1) indicating that the herbivore's AE was not affected by the phlorotannin-rich alga *L. variegata*. *S. chrysopterum* showed high assimilation of both *L. variegata* and *Padina gymnospora* (Fig. 2) again indicating that AE was unaffected by food plants containing high levels of phlorotannins. Values for total, organic, and protein assimilation indicated that AEs were significantly higher for fish fed *L. variegata* than *P. gymnospora*. *P. gymnospora* had a higher ash content than *L. variegata* (41.9% versus 25.19%) and this accounted for some of the differences between treatments in total AE. However, both mean organic and protein AEs also showed significant differences between the two algal

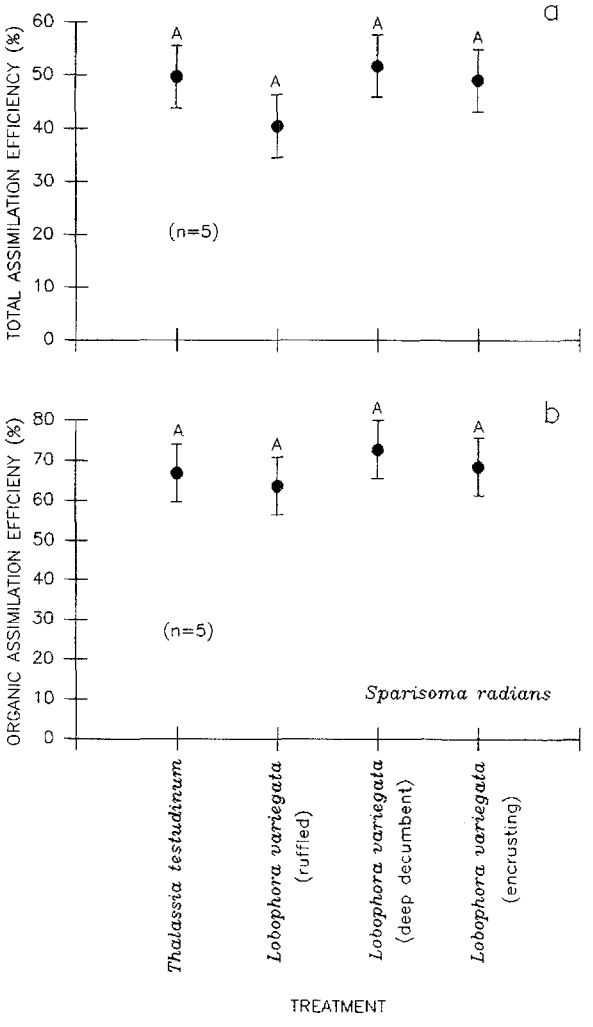

Fig. 1a, b Total **a** and organic **b** assimilation efficiency of *Sparisoma radians* fed *Thalassia testudinum* (2.75–3.28% dry weight, DW, phenolics) or *Lobophora variegata* (ruffled = 10.23% ± 0.67%, decumbent = 13.39% ± 3.50%, encrusting = 8.33% ± 0.94% DW phenolics). *Vertical bars* on means indicate 95% Tukey's multiple-comparison intervals. Treatment results with the same letter above error bar are not significantly different at $P < 0.05$

Table 1 Comparison of brown algal phenolic concentrations (percentage dry weight) from warm temperate, tropical western Atlantic, and tropical Pacific locations measured quantitatively via Folin-Denis tests (phloroglucinol standard, $\lambda_{max} = 725$ nm). Means reported are ±1 SD (where $n \geq 3$), n = no. of plants sampled

Algal species (collection site)	n	Phenolic concentration (% dry weight)
Belize		
Lobophora variegata (Carrie Bow) (shallow decumbent)	3	11.21 ± 3.84
Lobophora variegata (Blue Hole) (deep decumbent)	3	10.82 ± 1.03
Lobophora variegata (Man-o-War) (deep decumbent)	3	14.66 ± 0.90
Stypopodium zonale (Carrie Bow) (deep)	4	3.25 ± 1.46
Sargassum fluitans (Carrie Bow)	3	0.74 ± 0.13
Bahamas		
Lobophora variegata (Highborne Cay) (deep decumbent)	3	11.87 ± 2.32
Lobophora variegata (Aklins Island) (deep decumbent)	3	25.45 ± 4.42
Lobophora variegata (Alkins Island) (ruffled)	3	10.32 ± 0.77
Cozumel, Mexico *Lobophora variegata* (deep decumbent)	3	11.09 ± 3.96
Turks and Caicos, BWI		
Lobophora variegata (The Bight, Providenciales)	3	6.95 ± 1.90
Sargassum hystrix v. *buxifolium* (The Bight, Providenciales)	1	2.94
Sargassum platycarpum (The Bight, Providenciales)	1	0.98
Bermuda		
Colpomenia sinuosa (Devil's Hole)	3	1.74 ± 0.30
Sargassum fluitans (Ft. St. Catherine)	3	0.77 ± 0.13
Dictyota dichotoma (Shark Hole)	3	0.88 ± 0.69
Dictyota spp. (Bailey's Bay)	3	0.52 ± 0.37
Dictyopteris justii (Shelly Bay)	3	5.62 ± 0.81
Stypopodium zonale (Shelly Bay) (shallow)	3	15.85 ± 0.14
Stypopodium zonale (Hog Breaker) (deep)	3	1.81 ± 0.69
Lobophora variegata (Castle Harbor) (deep decumbent)	3	19.38 ± 3.31
Lobophora variegata (Hog Breaker and Twin) (deep decumbent)	3	8.68 ± 0.14
Lobophora variegata (Devil's Hole) (shallow decumbent)	3	6.31 ± 0.95
Lobophora variegata (Shark Hole) (shallow decumbent)	3	7.51 ± 1.72
Padina gymnospora (Devil's Hole)	2	3.10–4.74
North Carolina		
Lobophora variegata (deep decumbent)	3	4.27 ± 0.21
Hawaii		
Turbinata ornata	4	1.56 ± 0.36
Sargassum echinocarpum (non-reproductive)	4	2.03 ± 0.60
Sargassum echinocarpum (reproductive)	4	1.38 ± 0.25
Padina japonica	4	3.79 ± 0.52
Dictyota divaricata	4	1.07 ± 0.23

[a] Shallow sites are < 1 m, deep sites are >10 m

species, indicating some differences in organic digestibility. The differences were not attributable to plant phlorotannin concentration since *L. variegata*, with ca. twice the phenolic concentration (percentage DW), had higher organic and protein AEs.

The pH of the digestive tract of the crab, *Mithrax sculptus*, was 5.5. Despite the moderately acid conditions in its gut, *M. sculptus* readily assimilated the high phenolic *Lobophora variegata* (Figs. 3, 4). In experiment 1, total AEs for each of the three forms of

Table 2 Effectiveness of polyvinylpolypyrrolidone (PVPP) in removing phenolics from solutions of purified phlorotannins (including the monomer) and from plant extracts expressed as a percentage of phenolics removed. The difference between PVPP treated and untreated extracts is reported as a percentage dry weight (%DW, for *Lobophora variegata* (ruffled, encrusting, and deep decumbent forms) and *Ascophyllum nodosum* (*N/A* not applicable)

Treatment	% Phenolics removed by PVPP	Difference in % DW
Phloroglucinol	97.62	N/A
Purified phlorotannins from *Fucus vesiculosus*		
Low molecular wt	99.31	N/A
High molecular wt	97.22	N/A
High molecular wt	89.70	N/A
Aq/MeOH extract from *Lobophora variegata*		
Ruffled (Belize)	95.45	0.37
Encrusting (Belize)	94.33	0.67
Deep decumbent (Belize)	87.28	1.20
Deep decumbent (Bahamas)	93.57	1.74
Aq/MeOH extract from *Ascophllum nodosum*	90.37	0.52

Table 3 Gut pH values for herbivores tested in assimilation efficiency assays. Values are reported as means ±1 SD and were determined with ColorpHast pH 5–10 indicator sticks (*Sparisoma radians* and *Mithrax sculptus*) or with MicroElectrodes pH microprobe (*Sparisoma chrysopterum*)

Herbivore	Gut pH		
	Anterior intestine	Posterior intestine	Digestive tract
Sparisoma radians (n = 11)	7.8 ± 0.5	8.8 ± 0.2	–
Sparisoma chrysopterum (n = 10)	8.2 ± 0.5	8.3 ± 0.9	–
Mithrax sculptus (n = 7)	–	–	5.5 ± 0.0

L. variegata were significantly higher than for *Laurencia papillosa* (Fig. 3a), a preferred red algal food (Coen 1988), although all treatments were significantly different. The differences in total AE correlated with differences in algal ash content. *Laurencia papillosa* had the highest % ash (61%) and the lowest total AE (14.75%). The encrusting, ruffled, and decumbent forms of *Lobophora variegata* were 38.9%, 33.1% and 22.7% ash, respectively, with total AE values of 37.6% (encrusting), 48.1% (ruffled) and 63.5% (decumbent). Organic AE values (Fig. 3b) indicated that the organic fraction in the non-phlorotannin-containing rhodophyte, *Laurencia papillosa*, was less digestible than any of the *Lobophora variegata* forms, despite the fact that the *Lobophora variegata* plants had phenolic concentrations of 8–13% DW. In experiment 2, the total AEs of the high phenolic phaeophyte, *Lobophora variegata* and the low phenolic phaeophyte, *Padina sanctae-crucis*, were not significantly different, although both of these were significantly higher than a preferred food, the rhodophyte, *Acanthophora spicifera* (Fig. 4a). Again, the species with the lowest total AE had significantly higher ash (*A. spicifera* = 49% ash, *P. sanctae-crucis* = 38.6% ash, *L. variegata*, ruffled form = 32.5% ash). Organic AE values (Fig. 4b) indicated that the organic fraction of the

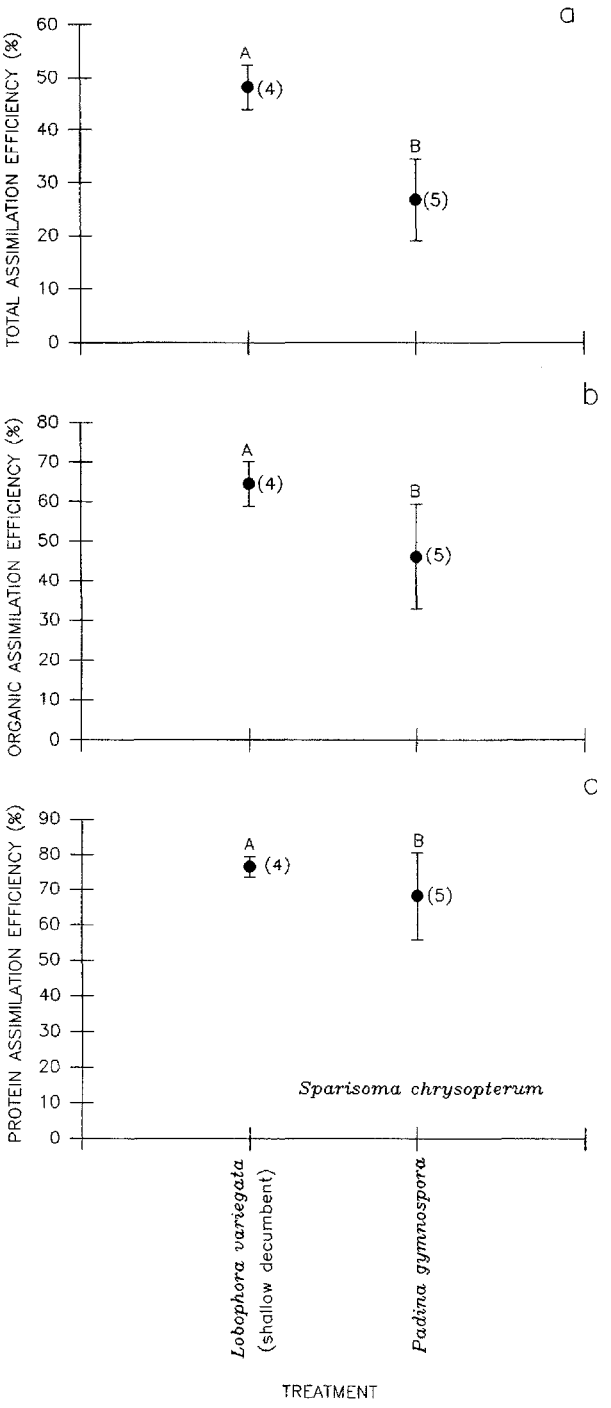

Fig. 2a–c Total **a**, organic **b** and protein **c** assimilation efficiency of *Sparisoma chrysopterum* fed *Lobophora variegata* (shallow decumbent, 6.31% ± 0.95% dry weight, DW, or 7.51% ±1.72% DW phenolics) or *Padina gymnospora* (3.10%–4.74% DW phenolics). *Vertical bars* on means indicate ± 1 SD. Treatment results with the same letter above error bar are not significantly different at $P < 0.05$

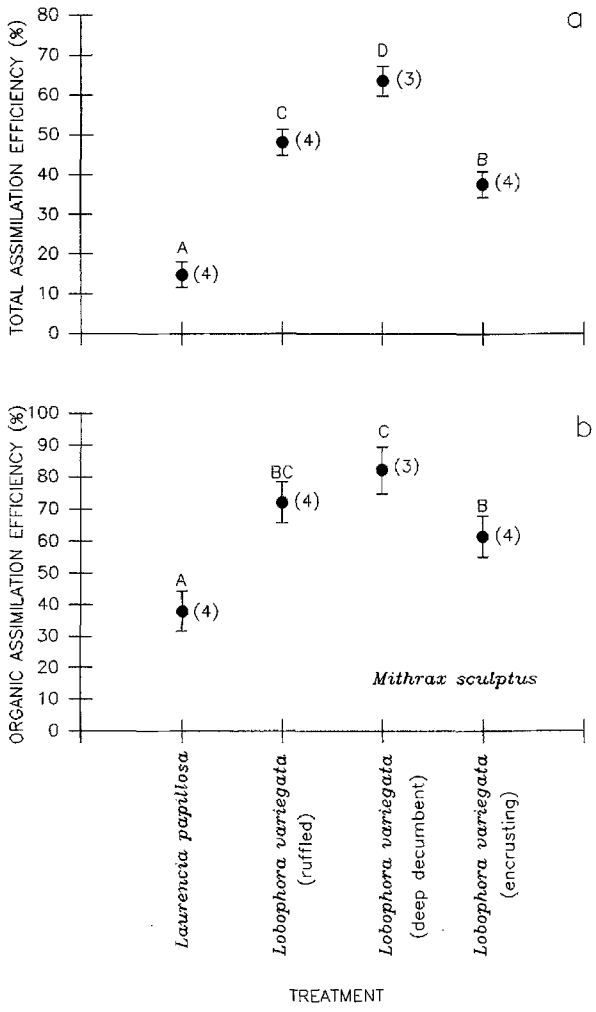

Fig. 3a, b Total **a** and organic **b** assimilation efficiency of *Mithrax sculptus* fed *Laurencia papillosa* (a rhodophyte with no appreciable phlorotannin concentration) or *Lobophora variegata* (ruffled = 10.23% ± 0.67%, decumbent = 13.39% ± 3.50%, encrusting = 8.33% ± 0.94% dry weight phenolics). *Vertical bars* on means indicate 95% Tukey's multiple-comparison intervals. Treatment results with the same letter above error bar are not significantly different at $P < 0.05$

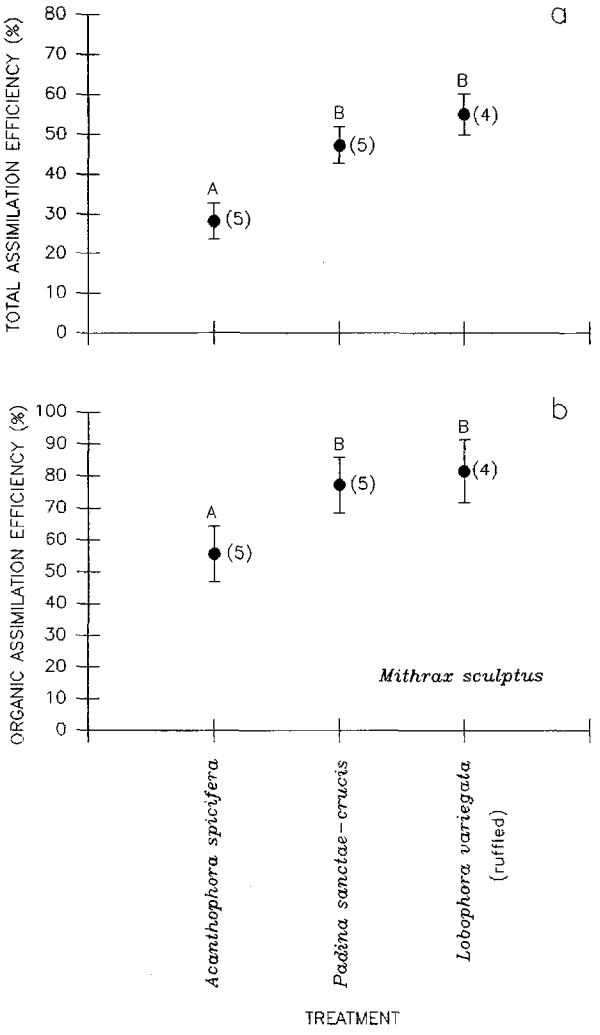

Fig. 4a, b Total **a** and organic **b** assimilation efficiency of *Mithrax sculptus* fed *Acanthophora spicifera* (a rhodophyte with no appreciable phlorotannin concentration), *Padina sanctae-crucis* (1.79% ± 0.36% dry weight, DW, phenolics), and *Lobophora variegata* (10.23% ± 0.67% DW phenolics). *Vertical bars* on means indicate 95% Tukey's multiple-comparison intervals. Treatment results with the same letter above error bar are not significantly different at $P < 0.05$

non-phlorotannin-containing rhodophyte, *A. spicifera*, was less digestible than either the low phlorotannin phaeophyte *P. sanctae-crucis* or the high phlorotannin phaeophyte *L. variegata* (ruffled form). Thus, there was no correlation in *M. sculptus* between a plant's phlorotannin concentration and its digestibility.

Discussion

Polyphenolic metabolites have been an enigma, particularly in the marine environment where the suggested latitudinal gradient of high phenolics in high latitudes and low phenolics in low latitudes countered the observed distributions of other secondary metabolites (van Alstyne and Paul 1990). This study expands upon the earlier work of Targett et al. (1992) which found high phenolic brown algal species occur in the western Caribbean and confirms that high phenolic concentrations are found in brown algae throughout the western tropical Atlantic. Where there was an overlap of species (forms for *L. variegata*) from the various geographic locales, there was close corroboration between phenolic content. Shallow and deep forms of *Stypopodium zonale* from both Belize and Bermuda consistently showed high (ca. 15% DW) and low (2%–3% DW) phenolic levels respectively. The deep decumbent form of *L. variegata* (> 10 m) which was examined from four of five tropical Atlantic locations had phlorotannin levels > 11% DW (this study; Targett et al. 1992). Phenolic values for *L. variegata* ranged up to 25% DW. The shallow decumbent form of *L. variegata* collected in Bermuda tended to have lower phlorotannin concentrations than the shallow

decumbent form collected in Belize and any of the deep decumbent form, although at 6–8% DW they were still high. *Dictyota* spp. and *Sargassum fluitans* from Bermuda and Belize (this study; Targett et al. 1992) were uniformly low in phlorotannin concentration. Therefore, the concept of a latitudinal gradient for phenolics (high levels in temperate regions, low levels in tropics) is not supported in the Atlantic.

Many of the high phenolic tropical Atlantic species are members of the Dictyotales. Folin-Denis derived phenolic concentrations from members of this order have been viewed as suspect since other reactive nonpolar metabolites are found in many Dictyotalean species (Steinberg 1992). However, the fact that there is no significant difference in the total phlorotannin content as calculated from standard Folin-Denis assays and from Folin-Denis difference assays in which PVPP in an acid environment has been used to specifically remove the polyphenolics, indicates that this is not an issue with these samples. Yates and Peckol (1993) found that brown algal samples treated with Folin-Denis without PVPP resulted in an overestimate of polyphenolic levels. Differences of this type did not materialize in our data. The close corroboration between our standard and PVPP difference Folin-Denis assays is likely attributable to two factors: (1) optimization of the PVPP difference assay and (2) an early hexane partition of the plant extract to remove lipophilic contaminants. Preliminary experiments with phloroglucinol and purified low and high molecular weight polyphenolics and PVPP indicated that multiple partitions were necessary to remove phenolics from solution. Tests which did not include multiple partitions did show large discrepancies between PVPP and non-PVPP treated extracts because the PVPP had saturated and was therefore unable to remove all of the phenolics. With multiple partitions ($3 \times$ was optimum in our assays) our preliminary experiments indicated that we averaged $> 90\%$ removal of purified polyphenolics. For algal extracts, calculation of phlorotannin concentrations for PVPP versus non-PVPP treated samples showed at most a 1.2% DW difference in phenolics; well within limits of error.

This study has shown that high phenolic species are assimilated as well or better than species with lower phenolic concentrations by the tropical herbivorous fishes *S. radians* and *S. chrysopterum* and the tropical herbivorous crab *M. sculptus*. The differences seen in the effects of polyphenolics on some temperate marine herbivores (Irelan and Horn 1991; Steinberg and van Altena 1992; Boettcher and Targett 1993) may be the result of differences in their gut characteristics and digestive mechanisms. For example, *Xiphister mucosus*, whose organic assimilation efficiency is reduced to ca. 10% at high molecular weight phlorotannin concentrations of 10% dry weight (Boettcher and Targett 1993), have acidic stomachs (pH 2–3) and digest algae via acid lysis (type I according to Horn 1989). A similar response is exhibited by *Girella nigricans* which also has an acidic stomach (Boettcher 1992). In this type of gut environment, hydrogen bonding between polyphenolics and macromolecules can occur, possibly preventing assimilation of nutrients. In contrast, *S. radians* and *S. chrysopterum* triturate their food and have a basic gut pH (8–9). Under the basic gut conditions hydrogen bonding would not occur. Ionic and covalent bonds are possible under basic conditions (Appel 1993) although it is unclear how important they are for phlorotannins, which are characterized by meta-substitution of hydroxyl groups. However, the results of this work indicate that at least for the two herbivorous fishes studied, the effect is minimal. The temperate odacid, *Odax cyanolmelas,* which, like *S. radians* and *S. chrysopterum*, has a gut pH of 8–9 readily feeds on high phenolic brown algal species and is apparently unaffected by high phenolic concentrations (Horn 1989).

Feeding studies by Steinberg et al. (1991) and by van Alstyne and Paul (1990) reported conflicting results in the response of Indo-Pacific fishes to high phenolic plant species or phenolic-extract-coated preferred plants placed in the field on polypropylene lines. In typical tropical settings, fishes representing several different gut types co-occur on the reef. Which type is in abundance may determine the outcome of experiments at specific sites. In both papers, the major herbivorous fishes are identified as acanthurids, siganids, pomacentrids and scarids. Acanthurids are characterized as having type I or type II guts depending upon whether they rely on acid lysis (type I) or mechanical grinding with a near neutral to neutral gut pH (type II). Pomacentrids also have guts that are categorized as type I. Siganids are thought to have guts with type I characteristics based upon their gut morphology (thin-walled stomach and long intestine), although gastric pH has not been measured (Horn 1989). Scarids have a type III gut which is characterized by no stomach, a neutral-basic intestine, and a pharyngeal mill to grind food. The discrepancy in the responses of marine fishes to high phenolic and extract-coated plants may be the result of differences in the specific suite of herbivores at each site coupled with the fact that the different herbivores have different gut characteristics.

This study showed that AEs in the crab *M. sculptus* were also not well correlated with the phenolic content of the algal species eaten. *M. sculptus* has a slightly acidic gut although at a pH of 5.5 it is much less acidic than type I fishes (Horn 1989). Tugwell and Branch (1992) established the presence of surfactants in vitro in the guts of seven marine invertebrates (3 isopods, 3 limpets, rock lobster) of which two (isopods) appeared to negatively affect polyphenolic activity. By analogy with terrestrial systems, other differences in marine herbivore gut environments may also be factors, although the identity of the specific factor for *M. sculptus* remains unknown.

Plant toughness can confound the interpretation of assimilation efficiency results if plants utilized in assays differ in tissue structure. A structurally tough plant fed to a herbivore that is not morphologically capable of triturating it would show low assimilation efficiency regardless of phenolic content. In this study, each of the three herbivores chosen for whole plant assays have robust mechanisms for triturating plant material. *S. radians* and *S. chrysopterum* both have pharyngeal mills and feed on seagrass as well as calcareous and fleshy algae (Lobel and Ogden 1981; Targett et al. 1986; Horn 1989). *M. sculptus* has a gastric mill and feeds on marine macrophytes (Coen 1988). Fecal material from these herbivores is finely ground. Therefore, for plants and herbivores utilized in this study, plant toughness is not a factor. Plant carbohydrate disgestibility is also hypothesized to affect herbivore assimilation efficiency (Montgomery and Gerking 1980). In this study, although there are differences between treatments in herbivore organic AE, it is important to note that the values are relatively high when compared with a range of AEs for other plant species (Edwards and Horn 1982; Table II in Horn 1989; Targett and Targett 1990; Irelan and Horn 1991; Boettcher and Targett 1993).

Based upon the uniformly low phlorotannin concentrations that have been encountered in phaeophytes in the Pacific and Indo-Pacific, it has been speculated that polyphenolics are probably unimportant as defenses in tropical brown algae (Steinberg and Paul 1990; Steinberg et al. 1991). In light of our information that high phenolic species are common in the tropical western Atlantic (this study; Targett et al. 1992), that conclusion may be premature for all tropical herbivores. Successful herbivory on high phenolic brown algae may be more a matter of specific herbivore gut characteristics. Variability in the effects of polyphenolics from one geographical region to another has already been documented in temperate areas where polyphenolics from temperate brown algae have been found to be very effective against herbivores in some temperate regions (e.g. temperate North America: Geiselman and McConnell 1981; Steinberg 1985, 1988; van Alstyne 1988) and much less effective in others (e.g. temperate Australia: Steinberg and van Altena 1992). A major difference in these two temperate regions is the occurrence in temperate Australia of odacids and kyphosids as major components of the herbivore community (Horn 1989). These fishes with their neutral to basic guts and hindgut fermentation chambers, type III and type IV guts, respectively (Horn 1989), might be expected to tolerate high phlorotannin concentrations in their food. More intriguing than temperate/tropical comparisons of phlorotannin concentrations in marine brown algae, is the question of why phenolic levels seem to be uniformly low in the tropical Indo-Pacific brown algae, an area of intense herbivory with a broad herbivore community, and, in a broader sense, what inter-ocean differences might result in the presence of high phenolic brown algae in one tropical ocean and not the other.

Acknowledgements We thank W. Mitchell, L. Furbert, and J. Ward of the Bermuda Aquarium, and Dr. Brian Luckhurst of the Bermuda Fisheries Division for logistical support and fish collection in Bermuda and Dr. Robbie Smith and Dr. Loren Coen for field assistance in Bermuda and Belize, respectively. We thank Dr. Mark Hay and Dr. Celia Smith for samples of brown algae from North Carolina and Hawaii, respectively, and Tom Arnold, Mark Hay, and an anonymous reviewer for critical review of the manuscript. Research was supported by NSF Grants OC E-9314351 to NMT and TET and SPI-8996152 to Loren Coen, Chris Tanner and NMT. This paper is contribution no. 442 from the Smithsonian Institution's CCRE Program and no. 1389 from the Bermuda Biological Station for Research.

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