Vol. 396: 1–12, 2009
doi: 10.3354/meps08359

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published December 9

OPEN ACCESS

FEATURE ARTICLE

Feeding preferences and host associations of specialist marine herbivores align with quantitative variation in seaweed secondary metabolites

Finn A. Baumgartner[1, 2, 4,*], Cherie A. Motti[3], Rocky de Nys[1], Nicholas A. Paul[1]

[1]School of Marine and Tropical Biology, James Cook University, Townsville, Queensland 4811, Australia
[2]AIMS@JCU, James Cook University, Townsville, Queensland 4811, Australia
[3]Australian Institute of Marine Science, PMB 3, Townsville MC, Queensland 4810, Australia

[4]*Present address:* Department of Marine Ecology, Tjärnö Marine Biological Laboratory, Gothenburg University, 45296 Strömstad, Sweden

ABSTRACT: Consequences of congeneric quantitative variation in secondary metabolites of seaweeds on diet specificity and host association in specialist marine herbivores have received little attention. We investigated quantitative variation in caulerpenyne and oxytoxin 1 in 7 species of green seaweeds from the genus *Caulerpa*, along with the feeding preferences and host associations of 4 co-occurring sacoglossan molluscs. *C. taxifolia* and *C. sertularioides* contained high concentrations of metabolites and were preferred least by all herbivores. Algae with intermediate metabolite concentrations (*C. racemosa*, *C. serrulata*, and *C. cupressoides*) were preferred by *Elysia tomentosa* and *Lobiger viridis*. *Oxynoe viridis* and *Stiliger smaragdinus* had strong preferences for different low concentration *Caulerpa* species (*C. racemosa* var. *laetevirens* and *C. lentillifera*), suggesting not all feeding preferences are based exclusively on the major metabolites. *In situ* host associations of *L. viridis* and *S. smaragdinus* mirrored their feeding preferences, but this was not the case for *E. tomentosa*. Furthermore, those algal species with the highest and lowest metabolite concentrations had the lowest overall densities of sacoglossans. The results imply that the direct influence of quantitative variation in *Caulerpa* chemistry may only be limited to host associations in some sacoglossans. However, feeding pressure from multiple herbivore species with unique preferences could still contribute to variation in chemical defence amongst congeneric algae.

KEY WORDS: *Caulerpa* · Chemical cue · Coevolution · Dietary niche · Macroalgae · Opisthobranch · Plant–herbivore interactions · Congeneric variation

Mimicry by the sacoglossan sea slug *Stiliger smaragdinus* (center) of its exclusive host seaweed *Caulerpa lentillifera* (left, right and behind).

Photo: Nicholas Paul

INTRODUCTION

The selective force of specialist herbivores is considered an important driver in the evolution of quantitative and qualitative variation in plant chemical defences (Feeny 1992, Cornell & Hawkins 2003). For this reason, linking variation in seaweed chemical defences to the diet specificity of marine herbivores (e.g. Poore et al. 2008) is an important precursor to addressing theory derived from specialist associations in terrestrial systems (Futuyma & Moreno 1988, Feeny 1992, Cornell & Hawkins 2003), in particular coevolution (Hughes & Gliddon

*Email: finn.baumgartner@marecol.gu.se

1991, Sotka 2005). Because most marine herbivores (fishes, sea urchins, and gastropods) are generalists (Hay & Steinberg 1992), examples of chemically mediated, marine specialist interactions come from a broader phylogenetic pool of species than the traditional plant–herbivore model (e.g. sponge–predator, Becerro et al. 1998; indirect associations, Hay 1992, Stachowicz & Hay 2000). However, 2 groups of herbivores (amphipods and sacoglossan opisthobranch molluscs) do contain consumers with consistently narrow host ranges (Hay 1992, Poore et al. 2008). Of these, the sacoglossans are almost exclusively associated with chemically rich seaweeds (siphonous green algae, Jensen 1997, Williams & Walker 1999), which provides an opportunity to contrast the efficacy of chemical defences in closely related seaweeds against multiple specialised herbivores.

Sacoglossans have notable functional specialisations that suggest a close evolutionary relationship with their siphonous seaweed prey. These specialisations include intricate, modified teeth that pierce and suck the cytoplasm from seaweeds (Clark 1992), the sequestration of functional chloroplasts as a supplementary energy source through photosynthesis (Williams & Walker 1999, Evertsen et al. 2007), the sequestration of natural products, presumably for their own defence (Paul & Van Alstyne 1988, Gavagnin et al. 1994, Becerro et al. 2001, Marín & Ros 2004), and intriguing crypsis in both morphology (Marín & Ros 2004) and colouration (Clark & Busacca 1978). However, information on feeding specificity in sacoglossans is predominantly restricted to host associations and sacoglossan traits, such as feeding morphology and sequestration of functional chloroplasts (Clark & Busacca 1978, Jensen 1983, Thibaut et al. 2001, Gianguzza et al. 2002). Post-settlement behavioural responses of sacoglossans (such as diet preferences) have been less frequently described (Jensen 1989, Trowbridge & Todd 2001, Gianguzza et al. 2002, Trowbridge et al. 2009), with no demonstrable links between host chemical defences and sacoglossan associations or densities. These links should be complementary considerations in the evolution of specialisation (Futuyma & Moreno 1988).

One mechanism employed by seaweeds that directly influences diet preference and, presumably, host associations is chemical defence. Variation in marine secondary metabolites exists at a number of scales (Paul & Puglisi 2004) and the ecological consequences of such variation have been well described for community level comparisons (Hay et al. 1989, Becerro et al. 2003) and at much finer scales (e.g. intra-individual variation or inducible defences, Cronin & Hay 1996, Becerro et al. 1998). A notable gap is the consequence of congeneric variation in chemical defences (but see Trowbridge & Todd 2001), even though some seaweed genera, for example the conspicuous *Caulerpa*, are speciose with demonstrable quantitative variation in secondary metabolites (Meyer & Paul 1992, Amade & Lemée 1998, Jung et al. 2002). If specialist marine herbivores are important drivers of interspecific variation in seaweed chemical defences, then patterns of feeding preferences amongst congeners could reveal important modes of selection for both seaweed traits and herbivore diet specialisation.

On the Great Barrier Reef in tropical northeastern Australia there are a range of co-occurring species of *Caulerpa* (Kraft 2007), as well as many sacoglossan herbivores known to associate with siphonous green algae (e.g. *Elysia* sp. and *Cyerce nigricans* on *Chlorodesmis fastigiata*, Hay et al. 1989), but no broader information on feeding preferences exist. The sympatric diversity of both congeneric seaweed hosts and sacoglossans that include *Caulerpa* species in their diets provide an opportunity to correlate the concentrations of the major secondary metabolites among *Caulerpa* species with 2 aspects of sacoglossan ecology: feeding preferences and host association. Here we assess the chemical defence of *Caulerpa* species in the context of seaweed-specialist interactions by asking: (1) What are the congeneric quantitative differences in secondary metabolites between 7 species of *Caulerpa*? (2) What preferences do 4 species (different genera) of associated sacoglossan molluscs have for these *Caulerpa* species? (3) Is there any evidence that these preferences correlate with the concentration of secondary metabolites in the algae; and, based on hypotheses from the former questions? (4) Do host associations reflect the quantitative variation in seaweed chemical defences and/or feeding preferences?

MATERIALS AND METHODS

Algal collection. Seven native species of *Caulerpa* common to tropical northeastern Australia (Kraft 2007) were sampled from the shallow subtidal (<1 m depth) on 2 occasions, 5 wk apart in July and August 2007. *C. taxifolia*, *C. lentillifera*, and *C. racemosa* var. *laetevirens* were collected from Kissing Point, Townsville (19° 10′ S, 146° 45′ E), a predominantly muddy substratum with rock protrusions. *C. serrulata*, *C. sertularioides*, and *C. racemosa* var. *racemosa* were collected from the reef flat attached to coral rubble in Nelly Bay on Magnetic Island (19° 10′ S, 146° 50′ E), and *C. cupressoides* was collected from the sandy flat at Good Fortune Bay, 30 km north of Bowen (20° 0′ S, 148° 10′ E). Each of these species (and infraspecies) is unique in its gross morphology and was identified using Kraft (2007). We did not assign an infraspecies

for *C. cupressoides* (however, the likely variety is var. *mamillosa*, Kraft 2007). We shorten *C. racemosa* var. *racemosa* (Kraft 2007) to *C. racemosa*. Voucher specimens are lodged at the Royal Botanical Gardens, Sydney, Australia.

For chemical analyses, 10 whole (undamaged) individuals of each species were collected on each occasion, placed into separate press seal bags with seawater, and transported to the laboratory for extraction. For feeding assays, algae were collected and maintained at the Marine and Aquaculture Research Facilities Unit (MARFU), James Cook University (JCU), Townsville, Australia, in outdoor tanks with recirculating seawater prior to use (typically 1 to 2 d, but no longer than 1 wk). For sacoglossan abundance in the field, individual seaweeds (up to n = 23 per species per sample date) were carefully detached by dislodging the rhizoids and placed into separate press seal bags for analysis in the laboratory. This was done in 2008, over the same timeframe (July–August, n = 3 sample times) as the chemical analyses in 2007.

Herbivore collection. Sacoglossans were difficult to locate directly in the field; however, after earlier (pre-July) collections of all 7 *Caulerpa* species had been held in 350 l tanks (separated by sample site) for a few weeks, animals were identified when they became less cryptic after growing in size. Sacoglossans collected from *Caulerpa* species were identified (as per Burn 1998) as 2 shelled species, *Oxynoe viridis* and *Lobiger viridis*, and 2 unshelled species, *Elysia tomentosa* and *Stiliger smaragdinus*. The shelled *Lobiger* and *Oxynoe* species are considered *Caulerpa* specialists (Jensen 1983, 1997), whereas shell-less sacoglossans such as *Elysia* are also associated with other seaweed genera such as *Codium*, *Bryopsis*, *Udotea*, and *Halimeda* (Paul & Van Alstyne 1988, Jensen 1997, Becerro et al. 2001, Marín & Ros 2004, Trowbridge 2004). The collection of 4 different genera of sacoglossans (hereafter *Oxynoe*, *Lobiger*, *Elysia*, and *Stiliger*) provided a means to assess the preferences of a diverse group of sacoglossans against a broad assemblage of *Caulerpa* species.

At least 2 d prior to feeding assays, sacoglossans were transferred to a 68 l indoor tank supplied with flow-through seawater (35 to 36‰) with constant aeration. A 12 h light:12 h dark cycle was maintained with the temperature remaining constant at 27 to 29°C. Animals were fed a mixed assemblage of all 7 *Caulerpa* species prior to their use in feeding assays.

Isolation of *Caulerpa* secondary metabolites. Initial analysis of crude methanol (MeOH) extracts for each of the 7 species of *Caulerpa* by reverse phase HPLC electrospray ionisation mass spectrometry (RP-HPLC-ESI-MS) showed that 2 major metabolites were present in all species. These metabolites were isolated from the extract of cultured *C. taxifolia* (540 g) and separated using RP-C18 step gradient flash vacuum liquid column chromatography with Phenomonex Sepra C18 material and 10, 70, 80 and 100% MeOH in H_2O mixtures as eluent. The 80 and 100% fractions contained the 2 metabolites of interest as confirmed by RP-HPLC-ESI-MS. Both of these fractions were subjected to further purification using a Shimadzu HPLC system consisting of a SCL-10Avp system controller equipped with a LC-10AT pump, SPD-M10Avp photodiode array (PDA) detector, FRC-10A fraction collector and SIL-10A auto sampler (all Shimadzu) connected to a semi-preparative Phenomenex Luna C18, 5 µm, 250 × 21.2 mm HPLC column. All HPLC data were collected using Shimadzu Class-VP software. Chromatography was conducted at 8 ml min^{-1} with a gradient elution from 32% acetonitrile (ACN):H_2O to 95% ACN:H_2O over 60 min, held at 95% ACN:H_2O for 30 min before being ramped down to 32% ACN:H_2O over 5 min. Caulerpenyne (28.2 mg, Fig. 1a) and oxytoxin 1 (3.6 mg, Fig. 1b) were isolated from fractions collected from these 4 HPLC runs, as characterised by comparison of their proton (^1H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra (Bruker Avance 300 and 600 MHz NMR spectrometers) as well as mass spectra and UV spectra to literature values (Amico et al. 1978, Gavagnin et al. 1994). Standards were stable in MeOH stored at –20°C (as per Amade & Lemée 1998).

Quantification of *Caulerpa* secondary metabolites. Quantitative analysis of caulerpenyne and oxytoxin 1 in the 7 *Caulerpa* species was performed by RP-HPLC-ESI-MS using a method modified from Jung et al. (2002). Initial comparisons of extraction techniques (including preparation method and solvent type) demonstrated that extraction of fresh algae with MeOH (Amade & Lemée 1998) yielded the most consistent and reproducible quantitative analyses for *Caulerpa* species. MeOH extraction of *C. taxifolia* snap frozen with liquid nitrogen (Jung et al. 2002) gave variable yields of caulerpenyne and oxytoxin 1. Therefore, to quantify caulerpenyne and oxytoxin 1, fresh algae were collected, blotted dry of excess water, and 1.5 to 3 g portions (comprised of fronds, stolons, and rhizoids) were weighed for extraction. Algae were macerated in 2 ml of MeOH with 0.064 mg ml^{-1} coumarin as an internal standard and stored at –20°C in the dark to extract for 15 h.

MeOH extracts were subsequently filtered under reduced pressure vacuum (10 µm Teflon®) into 7 ml scintillation vials and returned to the freezer for 2 d prior to analyses. A 200 µl aliquot was taken and centrifuged for 2 min at 13500 × *g* to remove any algal debris. For each aliquot, 30 µl was then directly quantified by RP-HPLC-ESI-MS using an Agilent 1100 HPLC system comprising a degasser, binary pump,

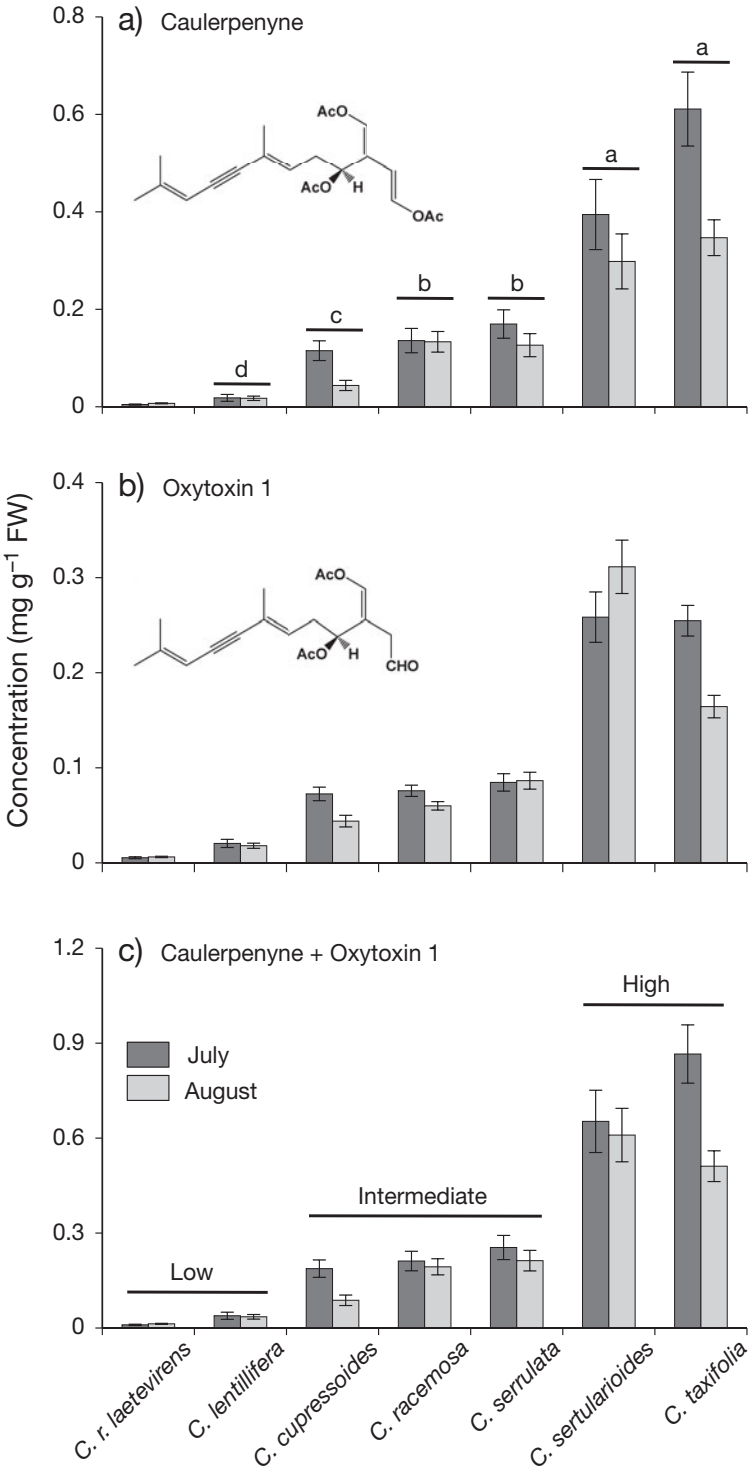

Fig. 1. *Caulerpa* spp. Concentration (mg g^{-1} fresh weight [FW]; mean ± SE) of secondary metabolites in 7 *Caulerpa* spp. at 2 sampling times (July and August; n = 10 per sp. at each sample time). Mass spectral data were analysed at 256 and 269 nm for caulerpenyne and oxytoxin 1, respectively. *Caulerpa* spp. sharing the same letter do not significantly differ at α = 0.05 (Tukey's honestly significant difference, HSD, multiple comparison). Groups of *Caulerpa* spp. with respect to total secondary metabolite concentrations are indicated as high, intermediate, or low

PDA, and Gilson 215 Liquid Handler autosampler/collector connected to a Phenomenex Gemini C18, 3 µm, 50 × 4.6 mm column coupled to a Bruker Daltonics Esquire 3000 plus mass spectrometer with an electrospray ionisation trap. Chromatography was conducted at a flow rate of 1 ml min^{-1} using gradient elution from 9.5% ACN:H_2O to 95% ACN:H_2O over 20 min, held at 95% ACN:H_2O for 5 min before being ramped down to 9.5% ACN:H_2O over 1 min and equilibrated at 9.5% ACN:H_2O for a further 5 min in readiness for the next injection. All RP-HPLC-ESI-MS data were collected using Bruker Daltonics Esquire Control v5.3 and Hystar v3.1.

Linear calibration curves for caulerpenyne, oxytoxin 1, and the internal standard (coumarin) were used for quantification. Chromatographic peaks were integrated for caulerpenyne at 256 nm and for oxytoxin 1 at 269 nm. The response was linear for caulerpenyne (5 repeat injections of 30 µl at 5 different concentrations), from 0.0055 to 0.35 mg ml^{-1} (r^2 = 0.998, $p < 0.001$), and for oxytoxin 1 (5 repeat injections of 30 µl at 4 different concentrations), from 0.0047 to 0.30 mg ml^{-1} (r^2 = 0.998, $p < 0.001$); the concentration of coumarin was 0.072 mg ml^{-1}.

Quantification was accomplished by measuring peak areas for each compound and the internal standard (peaks identified based on UV, mass, and retention time as determined above). The ratio of peak areas (compound:internal standard) was calculated for each metabolite (caulerpenyne and oxytoxin 1) and converted to mass (mg) by reference to the internal standard concentration and the response factor for each compound. Finally, the mass of the compounds were then standardised to the fresh weight (FW) of the algae extracted, to yield mg of metabolite per g FW.

FW concentrations were used for formal comparisons between species in the present study, as they provide a more meaningful measure of the amount of secondary metabolites a suctorial feeder encounters during feeding. However, FW data are also standardised to mg of metabolite per g dry weight (DW). These values were calculated using the mean wet:dry ratio of samples (n = 10 per species), separate to those used for chemical analyses and collected prior to feeding assays in August.

The quantification method was used to determine the concentrations of caulerpenyne and oxytoxin 1 in individuals (n = 10) of each *Caulerpa* species (n = 7) at 2 different sampling times that corresponded to the time over which feeding assays were conducted. Concentrations of the 2

secondary metabolites were analysed using a 2-factor ANOVA (SPSS 14.0) with *Caulerpa* species and sampling time as fixed factors. Caulerpenyne and oxytoxin 1 data were log-transformed prior to analysis to satisfy assumptions of homogeneity of variance. *C. racemosa* var. *laetevirens* data were not formally analysed because of small variance associated with very low concentrations of secondary metabolites. Post hoc comparisons of main effects using Tukey's honestly significant difference (HSD) multiple comparisons were made as required.

Combined (total) mass data of caulerpenyne and oxytoxin 1 were further combined to provide a means to assess total metabolite concentrations. No formal comparisons were made between species with the estimated concentrations of metabolites per g DW.

Feeding assays. A multiple choice feeding assay using an experimental design modified from Sotka & Hay (2002) and Prince et al. (2004) was conducted to measure the feeding preferences of each species of sacoglossan on the 7 *Caulerpa* species. Each replicate feeding assay consisted of a round 850 ml container (treatment, with herbivore) within a 1000 ml rectangular container (control, without herbivore). Small openings around the base of each treatment container allowed water flow to the control container.

Sacoglossans were held without food for 24 h and then simultaneously offered a choice of pre-weighed fronds of fresh tissue from each of the 7 species of *Caulerpa*. Two fronds (treatment and control) were removed from the same individual of each species, placed in seawater for a few minutes to allow wound plug formation, patted dry, and weighed (±1 mg). Treatment fronds from the 7 species were randomly placed around the edges of the treatment container with control fronds situated in the control container. Filtered seawater flowed constantly through the treatment container, into the control container, and back to the main system.

Sacoglossans (1 per replicate) were placed in the middle of the treatment container and feeding was observed every hour for 6 h, as most consumption occurred over this time, and every 3 h thereafter. A replicate was terminated if an animal had consumed at least one half of a species of algae or until 12 h had elapsed. Treatment and control fronds were subsequently patted dry, reweighed, and the change in wet mass of each piece determined. Herbivores were only used once (*Elysia*, n = 18; *Lobiger*, n = 20; *Stiliger*, n = 20; *Oxynoe*, n = 9) and their mass (FW, ±1 mg) was recorded post-assay.

Controls were used to account for changes in plant mass unrelated to sacoglossan feeding (autogenic change). The change in wet mass of each piece was scaled for autogenic change using the formula $[T_i \times (C_f/C_i)] - T_f$, where T_i and T_f represent the tissue subject to grazing and C_i and C_f represent the control tissue, before (i) and after (f) the experimental run (Sotka & Hay 2002) giving consumption (mg ind.$^{-1}$) on each *Caulerpa* species.

Non-parametric Friedman's tests (SPSS 14.0) were used to analyse feeding preference by each sacoglossan species, based on mean consumption (mg ind.$^{-1}$) of each *Caulerpa* species (Conover 1999). Prior to analysis, consumption data were rank-transformed within each replicate feeding assay. Friedman's post hoc comparisons were used to determine pairwise differences between mean consumption on each *Caulerpa* species by each sacoglossan species.

Host associations. Having established quantitative variation in metabolites and congener feeding preferences (see 'Results'), we subsequently tested whether host associations of sacoglossans reflected the chemical groupings of *Caulerpa* species. This was done over the same months that chemical analyses and feeding preferences were conducted (July and August, n = 3 sample times) in the following year. To do this we limited our field study to 2 of the 3 previous sites (Kissing Point and Nelly Bay), which contained a consistently high diversity of *Caulerpa* species.

Host associations of sacoglossans on *Caulerpa* were determined by haphazardly sampling each *Caulerpa* species from the shallow subtidal (n = 4 to 23 ind. per species, dependant on availability). This was done on 3 occasions approximately 4 wk apart from early July to late August 2008. *C. taxifolia*, *C. lentillifera*, and *C. racemosa* var. *laetevirens* were collected from Kissing Point. *C. sertularioides*, *C. serrulata*, *C. racemosa*, and *C. racemosa* var. *laetevirens* were collected from Nelly Bay. *C. cupressoides* was not present at either site at any time.

Each seaweed individual was carefully sorted and the numbers of sacoglossans recorded and weighed (FW, ±1 mg) for each *Caulerpa* species at each time, as well as seaweed biomass (FW, ±0.1 g). Density data were standardised between *Caulerpa* species by the number of animals per kg seaweed. Due to the high frequency of zero counts per individual (see 'Results'), no formal analyses of host distributional data were conducted. Furthermore, because our intent was to compare host association amongst *a priori* chemical groupings, *Caulerpa* species from high, intermediate, and low groupings were pooled across sites (e.g. *C. racemosa* var. *laetevirens* from Nelly Bay and Kissing Point were considered as 2 separate low concentration replicates). Data presented are means for each chemical grouping across sample times (n = 3 sample times), calculated in turn from the mean of low (n = 3, Species × Site), intermediate (n = 2) and high (n = 2) *Caulerpa* species for each sample time.

RESULTS

Quantification of *Caulerpa* secondary metabolites

Three groupings of *Caulerpa* species were apparent across both 2007 sampling times (July and August) for caulerpenyne (Fig. 1a), oxytoxin 1 (Fig. 1b), and total secondary metabolite (Fig. 1c) concentrations. *C. taxifolia* and *C. sertularioides* formed a high concentration group with mean concentrations of caulerpenyne and oxytoxin 1 ranging from 0.30 to 0.61 and 0.16 to 0.31 mg g^{-1} FW, respectively, over the 2 sampling periods. Three *Caulerpa* species comprised a group with intermediate concentrations of secondary metabolites (Fig. 1c). *C. racemosa*, *C. serrulata*, and *C. cupressoides* contained on average (calculated from the difference in group means) 3.4 times less caulerpenyne (0.044 to 0.17 mg g^{-1} FW; Fig. 1a) and 3.5 times less oxytoxin 1 (0.044 to 0.086 mg g^{-1} FW; Fig. 1b) than species within the high group. Species with the lowest concentration of secondary metabolites were *C. lentillifera* and *C. racemosa* var. *laetevirens*, for which levels of caulerpenyne and oxytoxin 1 were on average 10.2 (0.0046 to 0.018 mg g^{-1} FW; Fig. 1a) and 5.6 (0.0054 to 0.020 mg g^{-1} FW; Fig. 1b) times lower than *Caulerpa* species with intermediate levels, respectively.

There was significant variation in the concentration of caulerpenyne and oxytoxin 1 between species as well as variation in secondary metabolite concentrations over time (Table 1, Fig. 1a,b). Caulerpenyne was higher across all species in July compared to August ($p = 0.007$; Table 1), and species that grouped for total metabolite concentrations (Fig. 1c) still had significant differences in caulerpenyne content (e.g. *Caulerpa cupressoides* < *C. racemosa* = *C. serrulata*; *C. lentillifera* > *C. racemosa* var. *laetevirens*; Fig. 1a). A significant interaction between sampling month and species for oxytoxin 1 ($p < 0.001$; Table 1) was driven by decreases (up to 50%) in metabolite concentrations for 2 species (*C. cupressoides* and *C. taxifolia*) from July to August (Fig. 1b). A similar but non-significant ($p = 0.078$; Table 1) trend was observed for caulerpenyne (Fig. 1a). The variation between July and August in total (combined) secondary metabolite concentration did not alter the groupings of seaweeds into low, intermediate, and high concentrations (Fig. 1c).

Table 1. 2-factor ANOVA testing the effect of the fixed factors *Caulerpa* spp. and sample time on the concentration of caulerpenyne and oxytoxin 1. *Caulerpa racemosa* var. *laetevirens* data not included in analyses

Source	df	MS	*F*	p
Caulerpenyne				
Species	5	26.36	67.89	<0.001
Sample time	1	2.96	7.62	0.007
Species * Sample time	5	0.79	2.04	0.078
Error	108	0.39		
Oxytoxin 1				
Species	5	16.83	149.73	<0.001
Sample time	1	0.74	6.59	0.012
Species * Sample time	5	0.36	3.22	0.009
Error	108	0.11		

Caulerpa species had different wet:dry ratios ($F_{6,63} = 185.13$, $p < 0.001$), ranging from 21:1 for *C. lentillifera* to 9:1 for *C. cupressoides* (Table 2). No obvious trend existed between wet:dry ratios of each species and its categorisation as either high, intermediate, or low concentration (Table 2, Fig. 1c). The mean wet:dry conversions for each species were used to estimate the concentration of metabolites per unit dry weight (Table 2). The conversion from metabolite concentration per g FW to DW reduced the magnitude of differences in caulerpenyne and oxytoxin 1 concentrations amongst species for each month (Fig. 1c versus Table 2), but did not substantially alter the rank order of the concentrations of *Caulerpa* species.

Table 2. Wet:dry ratios (mean ± SE; n = 10) used for conversion of caulerpenyne and oxytoxin 1 concentrations from mg g^{-1} fresh weight (FW) (Fig. 1a,b) to mg g^{-1} dry weight (DW) for each replicate of all *Caulerpa* spp. from July and August. *Caulerpa* spp. maintained the broad categories of high, intermediate, and low concentrations, although they were not as distinct as the FW comparison (Fig. 1c). Significant differences in wet:dry ratios for species are indicated by different superscripted letters (1-factor ANOVA, Tukey's HSD, $p < 0.05$). Concentrations of mg g^{-1} DW were calculated from FW values and were not formally analysed

Species	Wet:dry	Caulerpenyne (mg g^{-1} DW)		Oxytoxin 1 (mg g^{-1} DW)		Total metabolite (mg g^{-1} DW)	
		Jul	Aug	Jul	Aug	Jul	Aug
C. r. laetevirens	19.24 ± 0.46[a]	0.09 ± 0.02	0.13 ± 0.02	0.10 ± 0.02	0.12 ± 0.01	0.19 ± 0.04	0.25 ± 0.03
C. lentillifera	21.41 ± 0.33[b]	0.39 ± 0.15	0.37 ± 0.10	0.44 ± 0.09	0.39 ± 0.06	0.83 ± 0.22	0.76 ± 0.13
C. cupressoides	8.99 ± 0.23[c]	1.03 ± 0.18	0.39 ± 0.09	0.65 ± 0.06	0.39 ± 0.06	1.69 ± 0.19	0.79± 0.10
C. racemosa	19.03 ± 0.37[a]	2.59 ± 0.48	2.54 ± 0.40	1.44 ± 0.11	1.14 ± 0.08	4.03 ± 0.48	3.68 ± 0.43
C. serrulata	11.73 ± 0.21[d]	1.99 ± 0.34	1.48 ± 0.28	0.99 ± 0.11	1.01 ± 0.10	2.98 ± 0.40	2.50 ± 0.34
C. sertularioides	12.13 ± 0.30[d]	4.79 ± 0.87	3.62 ± 0.68	3.14 ± 0.32	3.78 ± 0.34	7.92 ± 1.18	7.40 ± 0.92
C. taxifolia	9.50 ± 0.60[c]	5.80 ± 0.72	3.30 ± 0.35	2.42 ± 0.15	1.56 ± 0.11	8.22 ± 0.84	4.86 ± 0.39

Feeding assays

All sacoglossans had significant preferences for specific species of *Caulerpa*. These varied between herbivores, but only *Caulerpa* species containing intermediate or low concentrations of secondary metabolites were consumed (Fig. 2a–d). *C. taxifolia* and *C. sertularioides*, which contain high concentrations of secondary metabolites, were consistently the least preferred algal species across all herbivores assayed (Fig. 2a–d). Essentially, no herbivore consumed these species.

Caulerpa cupressoides, *C. racemosa*, and *C. serrulata*, which contained intermediate levels of caulerpenyne and oxytoxin 1, were preferred over all other species by both *Elysia* (Friedman's test, $\chi^2 = 29.52$, n = 18, df = 6, $p < 0.001$; Fig. 2a) and *Lobiger* ($\chi^2 = 60.13$, n = 20, df = 6, $p < 0.001$; Fig. 2b). In these assays, the highest mean consumption by both species of sacoglossan was on *C. racemosa* and *C. serrulata* (Fig. 2a,b).

Stiliger and *Oxynoe* had significant preferences for *Caulerpa* species with low secondary metabolite concentrations (Fig. 2c,d). *Oxynoe* showed significant preference for *C. racemosa* var. *laetevirens* (Friedman's test, $\chi^2 = 18.91$, n = 9, df = 6, $p < 0.01$; Fig. 2d); however, this preference was not exclusive, as 2 species with intermediate metabolite concentration, *C. serrulata* and *C. racemosa*, were also consumed at appreciable levels (Fig. 2d). Conversely, *Stiliger* preferred only *C. lentillifera* ($\chi^2 = 33.36$, n = 20, df = 6, $p < 0.001$; Fig. 2c), with consumption being almost exclusively on the species (Fig. 2c).

Host associations

There were some clear trends in the association of the 3 dominant sacoglossans (*Elysia*, *Lobiger*, and *Stiliger*) pertaining to the chemical groupings of *Caulerpa* species. The sacoglossans were partitioned in association between the 3 groupings, with the mean abundance of *Elysia* on high concentration *Caulerpa* species (Fig. 3a) similar to *Lobiger* on intermediate species (Fig. 3b) and *Stiliger* on low concentration species (Fig. 3c). Some *Caulerpa* species had much higher sacoglossan densities than others and *Caulerpa* associations differed markedly between sacoglossan species.

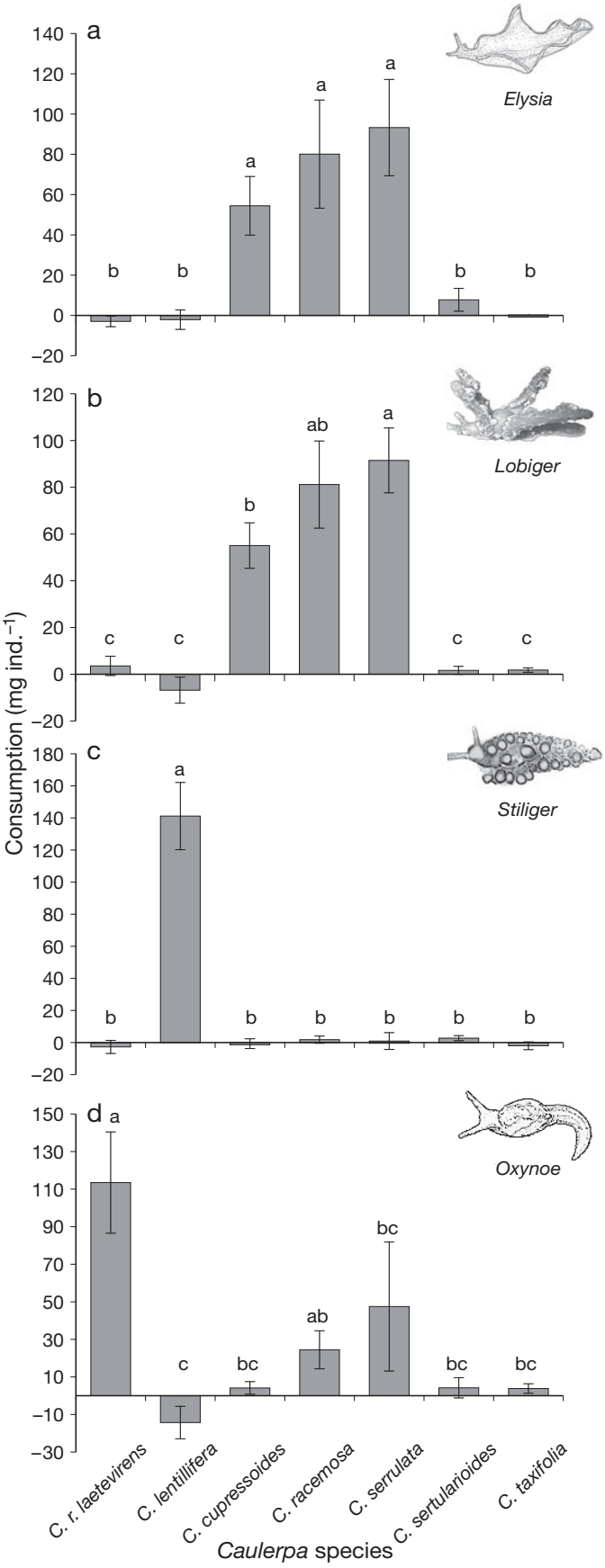

Fig. 2. *Caulerpa* spp. Consumption (mg ind.$^{-1}$; mean ± SE) by 4 sacoglossan species in multiple choice feeding assays. *Caulerpa* spp. sharing the same letter do not significantly differ at $\alpha = 0.05$ (Friedman's multiple comparison). Masses (mean ± SE) of sacoglossans were: *Elysia* = 0.911 ± 0.189 g (n = 18), *Lobiger* = 0.512 ± 0.113 g (n = 20), *Stiliger* = 1.966 ± 0.207 g (n = 20), and *Oxynoe* = 0.577 ± 0.115 g (n = 9)

In contrast to feeding preferences, *Elysia* were most commonly associated with *Caulerpa sertularioides* (16 ± 11 animals kg^{-1} seaweed; mean ± SE), a species that contains high levels of secondary metabolites (Fig. 2a). On average, *Elysia* were more abundant on high concentration *Caulerpa* species than those with intermediate or low levels of secondary metabolites (Fig. 3a). However, *Elysia* were not found on *C. taxifolia* (the highest chemical grouping species), although they were present at low densities on both intermediate and low concentration *Caulerpa* species (<1 animal kg^{-1} seaweed; Fig. 3a). Rarely were multiple *Elysia* found on an individual seaweed (e.g. 2 animals on 1 individual of *C. sertularioides* and 1 of *C. racemosa*).

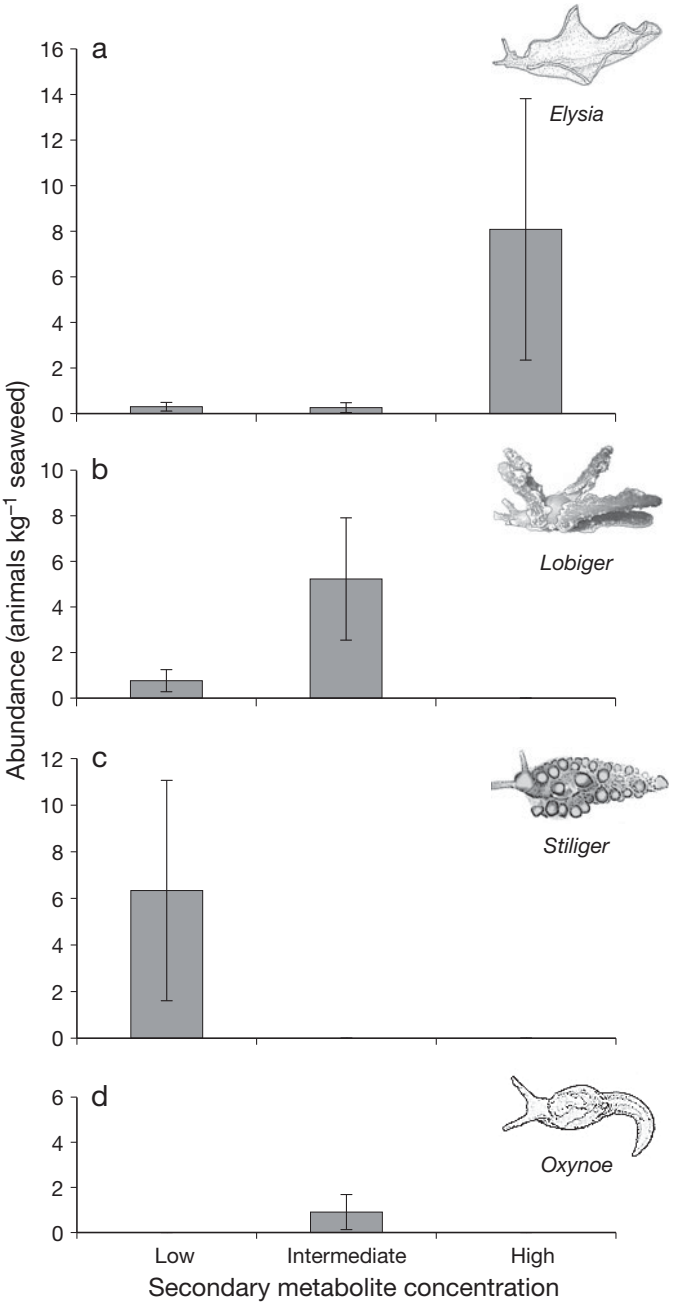

Fig. 3. *Caulerpa* spp. Abundance (animals kg^{-1} seaweed; mean of 3 sample times ± SE) of 4 sacoglossan species on chemical groupings of *Caulerpa* spp. (n = 6). Mean abundance on species for each chemical grouping (independent of site) were used to calculate overall means (low, n = 3; intermediate, n = 2; high, n = 2)

Lobiger abundance was greatest on *Caulerpa* species with intermediate levels of secondary metabolites (Fig. 3b), mirroring its feeding preferences (Fig. 2b). *Lobiger* mean density on intermediate level *Caulerpa* species, most commonly *C. serrulata* (10 ± 5 animals kg^{-1} seaweed; mean ± SE), were 7 times higher than on *Caulerpa* species with low secondary metabolite concentrations (Fig. 3b). They were not found on either of the high concentration species, *C. sertularioides* or *C. taxifolia*. *Lobiger* were consistently associated with *C. serrulata* and *C. racemosa*, but were also found at Kissing Point where neither species was present. In this case they associated with *C. racemosa* var. *laetevirens* (a low concentration species), and no animals were found on the other seaweeds, *C. taxifolia* or *C. lentillifera*. Sometimes multiple *Lobiger* were found on single seaweeds (e.g. 2 animals on 6 individuals of *C. serrulata* and 3 animals on a single *C. racemosa* individual).

The 2 sacoglossans with the narrowest diet preference, *Stiliger* and *Oxynoe* (Fig. 2c,d), were most limited in their distribution on *Caulerpa* species. Only 2 *Oxynoe* were found over the 3 sampling periods, and care should be taken with interpreting this data with respect to host association with intermediate chemical groupings (Fig. 3d). *Stiliger* was exclusively found on *C. lentillifera* (19 ± 14 animals kg^{-1} seaweed; mean ± SE).

Sacoglossans were present at densities in the range of 1 animal per 20 *Caulerpa* individuals for *Elysia* and *Lobiger*, 1 per 50 for *Stiliger* and 1 per 150 for *Oxynoe*. The size of sacoglossans (mean mass ± SE) were: *Elysia* 0.681 ± 0.162 g; *Lobiger* 0.304 ± 0.072 g; *Stiliger* 0.175 ± 0.094 g; and *Oxynoe* 0.077 ± 0.076 g. The total biomass of seaweeds and number of animals pooled across the sample times were: *C. taxifolia* 0.5 kg, no animals; *C. sertularioides* 0.7 kg, *Elysia* = 9; *C. serrulata* 1.8 kg, *Elysia* = 1, *Lobiger* = 17, *Oxynoe* = 1; *C. racemosa* 3.1 kg, *Elysia* = 3, *Lobiger* = 4, *Oxynoe* = 1; *C. lentillifera* 1.1 kg, *Stiliger* = 6; *C. racemosa* var. *laetevirens* from Nelly Bay 1.9 kg, *Elysia* = 2, *Lobiger* = 2; and *C. racemosa* var. *laetevirens* from Kissing Point 1.6 kg, *Lobiger* = 1.

Furthermore, there was some variation in sacoglossan abundance over the 3 sampling times that did not appear to depend on the presence of *Caulerpa* (e.g. more than 10 individuals of each *Caulerpa* species were collected at each site and sample time, except for

C. lentillifera on one occasion). This means that the absence of any animals on *C. taxifolia* was unlikely to be an artefact of sampling (i.e. the same number of individuals and a similar biomass to *C. sertularioides* were sampled). The only sacoglossans commonly associated with *Caulerpa* species at these sites were the 4 sacoglossan species assessed.

DISCUSSION

Sacoglossans are arguably the best described example of a group of marine specialist herbivores because of their narrow host ranges (Hay 1992, Poore et al. 2008) and unique morphological specialisations (Clark 1992, Marín & Ros 2004). In the present study, the distinct groupings of *Caulerpa* species into high, intermediate, and low concentrations of caulerpenyne and oxytoxin 1 provided an opportunity to examine potential consequences of congeneric quantitative secondary metabolite variation on feeding preferences and host associations of 4 specialist sacoglossans. The lack of substantial consumption by any sacoglossan of either high concentration species, *C. taxifolia* and *C. sertularioides*, suggests that quantitative variation in chemical defences can influence the diet selection of marine specialists, at least relative to less defended *Caulerpa* species. However, chemical data and feeding preferences did not entirely conform to host distributions. Only 2 sacoglossans (*Lobiger* and *Stiliger*) mirrored their preferences in field associations. *Elysia*, on the other hand, did not, and was associated with one high concentration seaweed. Therefore, any direct effects of quantitative variation in *Caulerpa* host chemistry on feeding preference and host association may be limited to a subset of sacoglossans.

Three concepts linking host seaweed chemistry and feeding preferences to sacoglossan field associations are supported by our data. The first relates to feeding preferences for *Caulerpa* species with intermediate levels of secondary metabolites, where both high and low threshold values may deter feeding and ultimately influence host associations of *Lobiger*. From the sacoglossan perspective this may represent a 'Goldilocks' scenario, with preferences influenced by secondary metabolite concentrations that are 'just right' in balancing the potentially toxic effect of the chemical defences with a requirement for food, as well as possible ecological roles for sequestered metabolites (e.g. Paul & Van Alstyne 1988). Secondly, *Elysia* and *Lobiger* had similar feeding preferences but very different patterns of host association. *Elysia* were most abundant on one of the high concentration seaweeds (*C. sertularioides*), although, in a similar manner to *Lobiger*, it was not found on the other high seaweed (*C. taxifolia*) and was rarely found on low concentration species. These contrasting patterns of host use cannot be attributed to the direct effects of quantitative variation in chemical defences. An alternative explanation is that resource partitioning by competing sacoglossans occurs between seaweed hosts (as can be inferred from Caribbean sacoglossan distributions, Clark & DeFreese 1987). The final concept is that species-specific feeding and/or host cues exist for some sacoglossans (e.g. *Stiliger* and *C. lentillifera*), but these cues are unlikely to be the major seaweed secondary metabolites (caulerpenyne and oxytoxin 1).

A clear empirical role for caulerpenyne in feeding deterrence has rarely been demonstrated (Targett et al. 1986, Pennings & Paul 1992, Erickson et al. 2006), and never for sacoglossans, yet many studies assume or suggest that caulerpenyne acts as a feeding deterrent or that it has broad spectrum effects against microbes (reviewed in Paul et al. 2007). The paradigm that chemical defences based on caulerpenyne are an important ecological strategy for *Caulerpa* species remains ambiguous because of the instability of the major compounds in their purified forms (Amade & Lemée 1998, Jung et al. 2002). Our correlative assessment of feeding preferences across seaweed species, specifically focused on specialists, supports the paradigm that caulerpenyne and related metabolites can be feeding deterrents at high concentrations. However, caulerpenyne is not effective at intermediate concentrations and, at these concentrations, may even be preferred by *Elysia* and *Lobiger*, both of which are known to sequester *Caulerpa* metabolites (Cimino et al. 1990, Gavagnin et al. 1994, 2000). Similar trends of preferred diets with intermediate to low levels of secondary metabolites (rather than very high) exist for other seemingly specialised opisthobranchs (Nagle et al. 1998, Ginsburg & Paul 2001, Vergés et al. 2008). Avoiding very high concentrations of secondary metabolites could relate to detoxification limits for seaweed chemical defences (e.g. Sotka & Whalen 2008), but for the present study it is unlikely, given that the majority of *Elysia* were found on a high concentration seaweed (*C. sertularioides*).

The different feeding preferences and varied host associations among sacoglossans have implications for specialist-driven selection of specific seaweed chemical defences. The possibility exists for diffuse coevolution involving multiple herbivores in this system (e.g. Fox 1981). Feeding preferences could have selected for high levels (an upper threshold) in *Caulerpa* chemical defence, in an attempt to 'radiate and escape' as suggested for terrestrial plants in response to specialisation by herbivores (Cornell & Hawkins 2003). Similarly, any preference for seaweeds with intermediate concentrations could manifest as an indirect defence for

low concentration *Caulerpa* species (e.g. *C. racemosa* var. *laetevirens*), where such species are common but are avoided by sacoglossans with broader diet niches (e.g. *Elysia* and *Lobiger*).

Both *Stiliger* and *Oxynoe* had strong but different preferences for low concentration seaweeds (*Caulerpa lentillifera* and *C. racemosa* var. *laetevirens*, respectively). The exclusive preference and association of *Stiliger* may be explained by qualitative variation in minor natural products and/or the release of metabolites from the seaweeds (i.e. chemical cues). Extremely narrow host ranges are common in limapontioids (= stiligerioids) (Jensen 1999); for example, *Stiliger aureomarginatus* only feeds on *Codium spongiosum* (Raven et al. 2001). A specific cue would provide an opportune trait for selection of host specificity between *Stiliger* and *C. lentillifera*, and may function in sacoglossan larval metamorphosis (e.g. Krug & Manzi 1999, Krug & Zimmer 2000). Unlike *Stiliger*, the preference of *Oxynoe* was not exclusive and field samples revealed individuals on *C. racemosa* and *C. serrulata*, despite that its preferred food was available at both sites. This apparent lack of specificity has also been documented for *Oxynoe viridis* in other parts of Australia feeding on multiple *Caulerpa* species (Raven et al. 2001).

Correlative studies have limitations, in particular an inability to definitively control aspects of seaweed and sacoglossan morphologies, as well as nutrition. For example, partitioning the effect of secondary metabolites from morphology requires manipulative experiments using artificial diets (e.g. Nagle et al. 1998). However, assays of this kind have not been employed for sacoglossans because their suctorial feeding mode precludes the use of traditional (e.g. agar-based) artificial diets. By using a diverse number of seaweeds and herbivores, some aspects were partially controlled in the assays and for field comparisons. Both high concentration species (*Caulerpa taxifolia* and *C. sertularioides*) possess a feather-like form and were avoided by all sacoglossans, yet it is known that sacoglossans can physically feed on them (Thibaut et al. 2001, authors' pers. obs.). Two superficially similar algae, *C. serrulata* and *C. cupressoides*, were also consumed in the present study. Furthermore, feeding preferences did not correlate with a gross measure of *Caulerpa* biology (in wet:dry ratios, Table 2) nor did they correlate with nutrient content (similar to Jensen 1983). For example, both *C. taxifolia* and *C. serrulata* have high nutrient values (% N fresh weight of >0.1%), whereas *C. lentillifera* and both *C. racemosa* varieties have contents almost 4 times lower (<0.03% N fresh weight, Paul & de Nys 2008). Given the difficulties associated with artificial diets, future studies could take advantage of environmental and/or between individual quantitative variation in secondary metabolites within *Caulerpa* species (e.g. July versus August, and within-species variance).

Here we have provided correlative links between chemical defence, feeding preferences, and host use by 2 of 4 sacoglossan herbivores, as well as evidence of contrasting strategies in chemical defence of sympatric *Caulerpa* species. The strikingly different degrees of investment in chemical defences are difficult to reconcile given the apparent ineffectiveness against specialist associations in the field, despite distinct preferences in feeding assays. Acknowledging that many factors influence diet preference and host association, including seaweed and herbivore morphology (Steneck & Watling 1982, Pennings & Paul 1992), as well as the predictability of host algae (e.g. annuals versus perennials) and competition between herbivores, some of the links between diet preference, host association, and the variation in secondary metabolite concentrations of congeners are compelling, specifically the intermediate preferences of *Lobiger*. Given the recent insights generated from the identification of signalling molecules and chemical cues in both plankton ecology (Pohnert et al. 2007) and opisthobranch alarm cues (Kicklighter et al. 2007), similar scrutiny of the chemical cues facilitating diet and host preference of sacoglossans—including the relationship between quantitative variation in chemical defences and post-settlement feeding cues—can reinvigorate discussion on chemically mediated specialisation and coevolution in seaweed–herbivore interactions.

Acknowledgements. The authors thank A. D. Wright for his input and comments on the manuscript, and S. Seymour for his assistance in the field. Four anonymous reviewers provided constructive feedback that improved the manuscript. Financial support was provided by the JCU Finfish and Emerging Species Research Advancement Program and an AIMS@JCU scholarship. Access to the Biomolecular Analysis Facility for chemical analyses was provided by AIMS, Cape Cleveland, Australia.

LITERATURE CITED

Amade P, Lemée R (1998) Chemical defence of the Mediterranean alga *Caulerpa taxifolia*: variations in caulerpenyne production. Aquat Toxicol 43:287–300

Amico V, Oriente G, Piattelli M, Tringali C, Fattorusso E, Magno S, Mayol L (1978) Caulerpenyne, an unusual sesquiterpenoid from the green alga *Caulerpa prolifera*. Tetrahedron Lett 19:3593–3596

Becerro MA, Paul VJ, Starmer J (1998) Intracolonial variation in chemical defenses of the sponge *Cacospongia* sp. and its consequences on generalist fish predators and the specialist nudibranch *Glossodoris pallida*. Mar Ecol Prog Ser 168:187–196

Becerro MA, Goetz G, Paul VJ, Scheuer PJ (2001) Chemical defenses of the sacoglossan mollusc *Elysia rufescens*

and its host alga *Bryopsis* sp. J Chem Ecol 27: 2287–2299

Becerro MA, Thacker RW, Turon X, Uriz MJ, Paul VJ (2003) Biogeography of sponge chemical ecology: comparisons of tropical and temperate defenses. Oecologia 135:91–101

Burn R (1998) Order Sacoglossa. In: Beesley PL, Ross GJB, Wells A (eds) Mollusca: the southern synthesis, fauna of Australia, Vol. 5, part B. CSIRO Publishing, Melbourne, p 961–974

Cimino G, Crispino A, Di Marzo V, Gavagnin M, Ros JD (1990) Oxytoxins, bioactive molecules produced by the marine opisthobranch mollusc *Oxynoe olivacea* from a diet-derived precursor. Experientia 46:767–770

Clark KB (1992) Plant-like animals and animal-like plants: symbiotic coevolution of ascoglossan (=sacoglossan) molluscs, their algal prey, and algal plastids. In: Reisser W (ed) Algae and symbioses: plants, animals, fungi, viruses, interactions explored. Biopress, Bristol, p 515–530

Clark K, Busacca M (1978) Feeding specificity and chloroplast retention in four tropical Ascoglossa, with a discussion of the extent of chloroplast symbiosis and the evolution of the order. J Molluscan Stud 44:582–586

Clark KB, DeFreese D (1987) Population ecology of Caribbean Ascoglossa (Mollusca: Opisthobranchia): a study of specialized algal herbivores. Am Malacol Bull 5:259–280

Conover WJ (1999) Practical nonparametric statistics, 3rd edn. John Wiley & Sons, New York

Cornell HV, Hawkins BA (2003) Herbivore responses to plant secondary compounds: a test of phytochemical coevolution theory. Am Nat 161:507–522

Cronin G, Hay ME (1996) Within-plant variation in seaweed palatability and chemical defenses: optimal defense theory versus the growth-differentiation balance hypothesis. Oecologia 105:361–368

Erickson AA, Paul VJ, Van Alstyne KL, Kwiatkowski LM (2006) Palatability of macroalgae that use different types of chemical defenses. J Chem Ecol 32:1883–1895

Evertsen J, Burghardt I, Johnsen G, Wägele H (2007) Retention of functional chloroplasts in some sacoglossans from the Indo-Pacific and Mediterranean. Mar Biol 151: 2159–2166

Feeny P (1992) The evolution of chemical ecology: contributions from the study of herbivorous insects. In: Rosenthal GA, Berenbaum MR (eds) Herbivores: their interactions with secondary plant metabolites, Vol. 2. Evolutionary and ecological processes. Academic Press, San Diego, CA, p 1–44

Fox LR (1981) Defense and dynamics in plant–herbivore systems. Am Zool 21:853–864

Futuyma DJ, Moreno G (1988) The evolution of ecological specialization. Annu Rev Ecol Syst 19:207–233

Gavagnin M, Marín A, Castelluccio F, Villani G, Cimino G (1994) Defensive relationships between *Caulerpa prolifera* and its shelled sacoglossan predators. J Exp Mar Biol Ecol 175:197–210

Gavagnin M, Mollo E, Montanaro D, Ortea J, Cimino G (2000) Chemical studies of Caribbean sacoglossans: dietary relationships with green algae and ecological implications. J Chem Ecol 26:1563–1578

Gianguzza P, Airoldi L, Chemello R, Todd CD, Riggio S (2002) Feeding preferences of *Oxynoe olivacea* (Opisthobranchia: Sacoglossa) among three *Caulerpa* species. J Molluscan Stud 68:289–290

Ginsburg DW, Paul VJ (2001) Chemical defenses in the sea hare *Aplysia parvula*: importance of diet and sequestration of algal secondary metabolites. Mar Ecol Prog Ser 215:261–274

Hay ME (1992) Seaweed chemical defenses: their roles in the evolution of feeding specialization and in mediating complex interactions. In: Paul VJ (ed) Ecological roles for marine secondary metabolites. Comstock Press, New York, p 93–118

Hay ME, Steinberg PD (1992) The chemical ecology of plant–herbivore interactions in marine versus terrestrial communities. In: Rosenthal GA, Berenbaum MR (eds) Herbivores: their interactions with secondary plant metabolites: ecological and evolutionary processes, Vol. 2. Academic Press, San Diego, CA, p 371–413

Hay ME, Pawlik JR, Duffy JE, Fenical W (1989) Seaweed–herbivore–predator interactions: host-plant specialisation reduces predation on small herbivores. Oecologia 81: 418–427

Hughes R, Gliddon C (1991) Marine plants and their herbivores: coevolutionary myth and precarious mutualisms. Proc Biol Sci 333:231–239

Jensen KR (1983) Factors affecting feeding selectivity in herbivorous ascoglossa (Mollusca: Opisthobranchia). J Exp Mar Biol Ecol 66:135–148

Jensen KR (1989) Learning as a factor in diet selection in *Elysia viridis* (Montagu) (Mollusca, Opisthobranchia). J Molluscan Stud 55:79–88

Jensen KR (1997) Evolution of the Sacoglossa (Mollusca, Opistobranchia) and the ecological associations with their food plants. Evol Ecol 11:301–335

Jensen KR (1999) Aggregative behaviour in the sacoglossan opisthobranch *Stiliger aureomarginatus* Jensen, 1993 in Rottnest Island, Western Australia. In: Walker DI, Wells FE (eds) The seagrass flora and fauna of Rottnest Island, Western Australia. Western Australian Museum, Perth, p 275–280

Jung V, Thibaut T, Meinesz A, Pohnert G (2002) Comparison of the wound-activated transformation of caulerpenyne by invasive and non-invasive *Caulerpa* species of the Mediterranean. J Chem Ecol 28:2091–2105

Kicklighter CE, Germann M, Kamio M, Derby CD (2007) Molecular identification of alarm cues in the defensive secretions of the sea hare *Aplysia californica*. Anim Behav 74: 1481–1492

Kraft GT (2007) Algae of Australia: the marine benthic algae of Lord Howe Island and the southern Great Barrier Reef. 1. Green algae. Australian Biological Resources Study, Canberra

Krug PJ, Manzi AE (1999) Waterborne and surface-associated carbohydrates as settlement cues for larvae of the specialist marine herbivore *Alderia modesta*. Biol Bull 197: 94–103

Krug PJ, Zimmer RK (2000) Development dimorphism and expression of chemosensory-mediated behavior: habitat selection by a specialist marine herbivore. J Exp Biol 203: 1741–1754

Marín A, Ros J (2004) Chemical defenses in sacoglossan opisthobranchs: taxonomic trends and evolutive implications. Sci Mar 68:227–241

Meyer KD, Paul VJ (1992) Intraplant variation in secondary metabolite concentration in three species of *Caulerpa* (Chlorophyta: Caulerpales) and its effects on herbivorous fishes. Mar Ecol Prog Ser 82:249–257

Nagle DG, Camacho FT, Paul VJ (1998) Dietary preferences of the opisthobranch mollusc *Stylocheilus longicauda* for secondary metabolites produced by the tropical cyanobacterium *Lyngbya majuscula*. Mar Biol 132:267–273

Paul NA, de Nys R (2008) Promise and pitfalls of locally abundant seaweeds as biofilters for integrated aquaculture. Aquaculture 281:49–55

Paul VJ, Puglisi MP (2004) Chemical mediation of interactions among marine organisms. Nat Prod Rep 21:189–209

Paul VJ, Van Alstyne KL (1988) Use of ingested diterpenoids by *Elysia halimedae* Macnae (Opisthobranchia: Ascoglossa) as antipredator defenses. J Exp Mar Biol Ecol 119:15–29

Paul VJ, Arthur KE, Ritson-Williams R, Ross C, Sharp K (2007) Chemical defenses: from compounds to communities. Biol Bull 213:226–251

Pennings SC, Paul VJ (1992) Effect of plant toughness, calcification, and chemistry on herbivory by *Dolabella auricularia*. Ecology 73:1606–1619

Pohnert G, Steinke M, Tollrian R (2007) Chemical cues, defence metabolites and the shaping of pelagic interspecific interactions. Trends Ecol Evol 22:198–204

Poore AGB, Hill NA, Sotka EE (2008) Phylogenetic and geographic variation in host breadth and composition by herbivorous amphipods in the family Ampithoidae. Evolution 62:21–38

Prince JS, Le Blanc WG, Macia S (2004) Design and analysis of multiple choice feeding preference data. Oecologia 138: 1–4

Raven JA, Walker DI, Jensen KR, Handley LL, Scrimgeour CM, McInroy SG (2001) What fraction of the organic carbon in sacoglossans is obtained from photosynthesis by kleptoplastids? An investigation using the natural abundance of stable carbon isotopes. Mar Biol 138:537–545

Sotka EE (2005) Local adaptation in host use among marine invertebrates. Ecol Lett 8:448–459

Sotka EE, Hay ME (2002) Geographic variation among herbivore populations in tolerance for a chemically rich seaweed. Ecology 83:2721–2735

Sotka EE, Whalen KE (2008) Herbivore offense in the sea: the detoxification and transport of algal secondary metabolites. In: Amsler CD (ed) Algal chemical ecology. Springer-Verlag, Berlin, p 203–228

Stachowicz JJ, Hay ME (2000) Geographic variation in camouflage specialization by a decorator crab. Am Nat 156: 59–71

Steneck RS, Watling L (1982) Feeding capabilities and limitation of herbivorous molluscs: a functional group approach. Mar Biol 68:299–319

Targett NM, Targett TE, Vrolijk NH, Ogden JC (1986) Effect of macrophyte secondary metabolites on feeding preferences of the herbivorous parrotfish *Sparisoma radians*. Mar Biol 92:141–148

Thibaut T, Meinesz A, Amade P, Charrier S and others (2001) *Elysia subornata* (Mollusca) a potential control agent of the alga *Caulerpa taxifolia* (Chlorophyta) in the Mediterranean Sea. J Mar Biol Assoc UK 81:497–504

Trowbridge CD (2004) Emerging associations on marine rocky shores: specialist herbivores on introduced macroalgae. J Anim Ecol 73:294–308

Trowbridge CD, Todd CD (2001) Host-plant change in marine specialist herbivores: ascoglossan sea slugs on introduced macroalgae. Ecol Monogr 71:219–243

Trowbridge CD, Hirano YM, Hirano YJ (2009) Interaction webs of marine specialist herbivores on Japanese shores. J Mar Biol Assoc UK 89:277–286

Vergés A, Paul NA, Steinberg PD (2008) Effects of sex and life history stage on herbivore responses to a chemically defended red alga. Ecology 89:1334–1343

Williams SI, Walker DI (1999) Mesoherbivore–macroalgal interactions: feeding ecology of sacoglossan sea slugs (Mollusca, Opisthobranchia) and their effects on their food algae. Oceanogr Mar Biol Annu Rev 37:87–138

Editorial responsibility: Joseph Pawlik, Wilmington, North Carolina, USA

Submitted: October 17, 2008; Accepted: October 7, 2009
Proofs received from author(s): November 2, 2009