Hydrobiologia (2006) 553:319–326
DOI 10.1007/s10750-005-1129-x

Primary Research Paper

Chemical deterrence of a marine cyanobacterium against sympatric and non-sympatric consumers

Angela Capper[1,2,*], Edwin Cruz-Rivera[3], Valerie J. Paul[2] & Ian R. Tibbetts[1]
[1]*Centre for Marine Studies, School of Life Sciences, The University of Queensland, QLD 4072, St. Lucia, Australia*
[2]*Smithsonian Marine Station at Fort Pierce, 701 Seaway Drive, Fort Pierce, FL, 34949, USA*
[3]*Marine Sciences Institute, University of Texas at Austin, 750 Channel View Drive, Port Aransas, TX, 78373-5015, USA*
*(*Author for correspondence: Tel.: +1-(772)-465-6630; Fax: +1-(772)-461-8154; E-mail: capper@sms.si.edu)*

Received 20 December 2004; in revised form 30 May 2005; accepted 24 June 2005

Key words: Lyngbya majuscula, feeding deterrence, lyngbyatoxin-a, debromoaplysiatoxin, rabbitfish (*Siganus fuscescens*), sea hare (*Stylocheilus striatus*)

Abstract

This study investigates the influence of mesograzer prior exposure to toxic metabolites on palatability of the marine cyanobacterium, *Lyngbya majuscula*. We examined the palatability of *L. majuscula* crude extract obtained from a bloom in Moreton Bay, South East Queensland, Australia, containing lyngbyatoxin-a (LTA) and debromoaplysiatoxin (DAT), to two groups: (1) mesograzers of *L. majuscula* from Guam where LTA and DAT production is rare; and (2) macro- and mesograzers found feeding on *L. majuscula* blooms in Moreton Bay where LTA and DAT are often prevalent secondary metabolites. Pair-wise feeding assays using artificial diets consisting of *Ulva clathrata* suspended in agar (control) or coated with Moreton Bay *L. majuscula* crude extracts (treatment) were used to determine palatability to a variety of consumers. In Guam, the amphipods, *Parhyale hawaiensis* and *Cymadusa imbroglio*; the majid crab *Menaethius monoceros*; and the urchin *Echinometra mathaei* were significantly deterred by the Moreton Bay crude extract. The sea hares, *Stylocheilus striatus*, from Guam were stimulated to feed by treatment food whereas *S. striatus* collected from Moreton Bay showed no discrimination between food types. In Moreton Bay, the cephalaspidean *Diniatys dentifer* and wild caught rabbitfish *Siganus* fuscescens were significantly deterred by the crude extract. However, captive-bred *S. fuscescens* with no known experience with *L. majuscula* did not clearly discriminate between food choices. *Lyngbya majuscula* crude extract deters feeding by most mesograzers regardless of prior contact or association with blooms.

Introduction

Lyngbya majuscula is a widely distributed benthic cyanobacterium known to form prominent mats and blooms in coral reefs and seagrass beds (Dennison et al., 1999; Thacker & Paul, 2001; Thacker et al., 2001). It is a prolific producer of biologically active and structurally unique secondary metabolites (Nagle & Paul, 1999; Burja et al., 2001; Gerwick et al., 2001; Osborne et al., 2001) some of which have shown feeding deterrent properties against generalist herbivores including fish (Paul & Pennings, 1991; Pennings et al., 1996; Thacker et al., 1997; Nagle & Paul, 1998, 1999), sea urchins (Pennings et al., 1996; Nagle & Paul, 1998; Cruz-Rivera & Paul, 2002) and crabs (Pennings et al., 1996). However, this cyanobacterium also serves as host to a diverse array of small invertebrate consumers (i.e., mesograzers) (Cruz-Rivera & Paul, 2002) whose feeding behaviour is undocumented.

Temporal and spatial variation in the production of *L. majuscula* compounds is not uncommon (Nagle & Paul, 1999; Gerwick et al., 2001). Chemical variation poses the question of whether local grazers are tolerant to *L. majuscula* compounds. All other variables being equal, if different *Lyngbya* strains contain compounds that vary in their degree of deterrence against grazers, only the most deterrent strains would persist in areas of high herbivory. In areas with reduced herbivory or less tolerant consumers, *Lyngbya* strains with different compounds might be able to persist. While some *Lyngbya* compounds are known to be deterrent or toxic (Pennings et al., 1996; Nagle & Paul, 1999), the relative deterrence and ecological function of most secondary metabolites isolated from *L. majuscula* in different populations around the world remain unknown.

Some grazers readily feed on this cyanobacterium, e.g., cephalaspideans ('bubble snails') (personal observations), and the specialist sea hare, *Stylocheilus striatus* (*S. longicauda* – Rudman, 1999) which is known to sequester *Lyngbya* secondary metabolites (Paul & Pennings, 1991; Avila, 1995; Pennings et al., 1996; Capper et al., 2005), some of which can act as feeding stimulants (Pennings & Paul, 1993a; Nagle et al., 1998; Nagle & Paul, 1999; Cruz-Rivera & Paul, 2002). At high natural concentrations these secondary metabolites deter feeding and reduce growth in *S. striatus* (Pennings & Paul, 1993a; Nagle et al., 1998) which may in turn reduce fecundity (Pennings & Carefoot, 1995). Other mesograzers such as small crabs and amphipods have been observed consuming *L. majuscula* with varying preference (personal observations). Rabbitfishes (Siganidae) have also been observed feeding upon *L. majuscula* blooms (Hashimoto et al., 1969), with *Lyngbya* spp. often forming a common component of the adult diet (von Westerhagen, 1973; Bryan, 1975; Lundberg & Lipkin, 1979). Juvenile siganids find *L. majuscula* unpalatable (Paul et al., 1990; Nagle et al., 1996; Thacker et al., 1997; Nagle & Paul, 1998), so grazing by adult siganids may either indicate a tolerance for some secondary metabolites or selective feeding on *L. majuscula* without deterrent secondary metabolites (personal observations). The persistence of chemically distinct *L. majuscula* populations in different areas might reflect the relative abundance of diverse consumers or their relative tolerance for particular compounds.

To examine the feeding behaviour of a variety of consumers, we tested Moreton Bay *L. majuscula* crude extracts (containing the secondary metabolites lyngbyatoxin-a (LTA) and debromoaplysiatoxin (DAT)) with consumers from Australia and Guam. Our aims are to determine if the persistence of *L. majuscula* blooms in Moreton Bay is related to chemical deterrents in the cyanobacterium and, if so, to test whether these compounds are broadly deterrent or only locally deterrent. To accomplish this, we tested crude extracts from the same *L. majuscula* population against: (1) wild sympatric fish and invertebrates from Australia, (2) captive-bred sympatric fish that were artificially raised, and (3) allopatric *L. majuscula* grazers from Guam. Because *L. majuscula* also forms large blooms in Guam (Nagle & Paul, 1998; Thacker & Paul, 2001; Thacker et al., 2001), the grazers we tested are familiar with *L. majuscula*, but not with the secondary metabolites occurring in the Australian *L. majuscula*.

Materials and methods

Study organisms – Australia

Lyngbya majuscula was collected from a bloom at Adams Beach on North Stradbroke Island in Moreton Bay, South East Queensland, Australia (27° 51′ S, 153° 41′ E) and freeze-dried for chemical extraction. Associated sea hares (*Stylocheilus striatus*, $n = 12$) and cephalaspideans (*Diniatys dentifer*, $n = 14$) were collected from these blooms and maintained in 50 l tanks, prior to our feeding assays, in a closed circuit aquarium system Moreton Bay Research Station (MBRS), North Stradbroke Island. Salinities were kept between 34–36 ppt and temperature consistent with ambient (24 °C), with 12 h light and dark cycles.

All vertebrate collection and experimentation met with guidelines provided by The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Wild *Siganus fuscescens* were captured from Moreton Bay, South East Queensland using both seine and trawl nets. Wild fish were maintained in a 1000 l recirculatory seawater tank system at MBRS. Captive-bred

S. fuscescens (bred from aquarium stock in indoor ponds with no known experience with native macroalgae or seagrass species resident in the Bay) were obtained from Bribie Island Aquaculture Research Centre (BIARC) and maintained in a 5000 l recirculatory seawater tank system. Separately housed groups of fish were fed a diet of Ridleys Aqua-feed Native Starter 2 mm food pellets prior to testing. Individual fish were transferred to 50 l aquaria, with a recirculatory flow through, 96 h prior to testing to allow acclimation. Aquaria were covered to eliminate between tank interactions.

Study organisms – Guam

Animals were collected from shallow reef flats at Pago Bay and Piti Bombholes in Guam, Mariana Islands (13° 30′ N, 144° 45′ E). Algae and cyanobacteria were brought to the lab where they were examined for associated fauna. The most abundant mesograzers from these collections were maintained on a mixed diet of local algae and *Lyngbya* in aerated unfiltered seawater from Pago Bay. Four invertebrate species of mesograzers and one macrograzer were used: the small sea hare *Stylocheilus striatus*; ($n=11$), the majid crab *Menaethius monoceros* ($n=15$), the gammaridean amphipods *Cymadusa imbroglio* ($n=14$) and *Parhyale hawaiensis* ($n=12$), and the sea urchin *Echinometra mathaei* ($n=14$). *Stylocheilus striatus* and *C. imbroglio* were obtained from Piti Bombholes whereas the other three consumers were obtained from Pago Bay.

Except for amphipods, replicates consisted of individual animals placed in round plastic dishes (160 mm diameter, 60 mm height) filled with seawater. Because amphipods were smaller, we placed three *C. imbroglio* and two to three *P. hawaiensis* in each dish. This allowed a measurable feeding response within 48 h. For all mesograzers, water was changed and faeces were removed daily for the test duration. Sea urchins were placed in flow-through 15 l tanks.

Pair-wise choice assays

We tested the palatability of Australian *L. majuscula* crude extract to a range of resident consumers that readily encounter *L. majuscula* in Guam. In Australia, palatability was examined in two resident mesograzers known to consume *L. majuscula* and the macrograzer, *S. fuscescens*.

Artificial diets containing or lacking *L. majuscula* crude extract were created using methods outlined in Hay et al. (1998). Treatment foods were made using *L. majuscula* crude extract (extracted in 1:1 ethyl acetate:methanol, see methods below) diluted in ethyl acetate and coated at 4.84% dry mass ('natural' concentration based on quantity of crude extract obtained from 0.53 kg freeze-dried *L. majuscula* from Moreton Bay, Australia) onto freeze-dried, powdered *Ulva clathrata* (formerly *Enteromorpha*, Hayden et al., 2003). This method has been successfully used to test compounds from other *L. majuscula* populations (Nagle et al., 1998; Cruz-Rivera & Paul, 2002). *Ulva clathrata* was chosen because it is a broadly palatable alga that contains no known secondary metabolites (Pennings & Paul, 1993b). A rotary evaporator was used to remove all organic solvent allowing the hydrophobic components of the crude extract to adhere to the surface of *U. clathrata*. Control foods were made by treating the freeze-dried *U. clathrata* with ethyl acetate and evaporating the solvent as described above. Control and treatment foods were then incorporated into agar-based artificial diets (Hay et al., 1998) and poured onto plastic screen mesh to make food strips (Hay et al., 1998). Consumption was quantified as the number of mesh squares cleared of food over time.

Control and treatment food strips were offered simultaneously to consumers in a pair-wise choice design. A fixed-consumption stopping rule was applied (Lockwood, 1998) where tests were terminated once $>50\%$ of one of the foods had been consumed. Replicates in which no food was consumed were not used in data analysis because they provided no information on consumer feeding preference. Data were analysed using paired-sample *t*-tests with Statistica® software. Cochran and Levene's homogeneity of variance were used to ascertain normality of data. Prior to analyses, data that deviated from normal distribution were transformed using either square root or logarithmic transformations as required.

Chemical extraction and detection of secondary metabolites

Freeze-dried *L. majuscula* (0.53 kg) from Moreton Bay was extracted at the University of Guam Marine Laboratory using a 1:1 ethyl acetate:methanol mixture. After extracting four times and filtering under vacuum, solvents from the extract were removed under vacuum. This crude extract was used for all feeding assays in Guam and Australia. Thin layer chromatography (TLC) was used in Guam to check for the presence of LTA and DAT in the crude extract against known standards. In Australia, the extract was subjected to HPLC-MS/MS to quantify concentrations of LTA and DAT in *L. majuscula*. This was conducted approximately three months after the Guam assays. Three replicate samples of crude extract were dissolved in ethyl acetate and dried by rotary evaporation. Concentrations of secondary metabolites ($\mu g\ g^{-1}$) were determined by HPLC-MS/MS using a PE/Sciex API 300 mass spectrometer equipped with a high flow electrospray interface (TurboIonspray) coupled to a Perkin Elmer series 200 HPLC system. Separation was achieved using a 150×4.6 mm Altima C_{18} column (Alltech) run at 35°C, with a mobile phase consisting of 80/20 acetonitrile/hipure water containing 0.1% formic acid and 2 mM ammonium formate at a flow rate of 0.8 ml min^{-1}. The flow was split post column such that the flow to the mass spectrometer interface was 250 $\mu l\ min^{-1}$. Under these conditions, the retention times were 11.72 min for LTA and 9.3 min for DAT. The mass spectrometer was operated in the positive ion, multiple ion monitoring mode. Ions monitored with dwell times of over 300 ms were 438.3 $(M+H)^+$, 410.3 for LTA and 543.3 for DAT. Quantification was achieved by comparison to standards of DAT (kindly provided by Dr. R.E. Moore, Department of Chemistry, University of Hawaii at Manoa) and LTA (Calbiochem) run under the same conditions. Using a 20 μl injection the detection limit for both toxins was typically 0.01 mg l^{-1} in the extracted solution.

Results

Crude extracts of *L. majuscula* from Moreton Bay, Australia, significantly deterred feeding by all consumers tested in Guam (Fig. 1) except for *S. striatus*, which was strongly stimulated to feed by the crude extract ($p=0.003$, paired-sample *t*-test, Fig. 1a). Whereas, Australian *S. striatus* showed no preference for foods containing or lacking *L. majuscula* crude extract ($p=0.568$, paired-sample *t*-test, Fig. 2a). Cephalaspideans from Australia (*D. dentifer*) ate significantly less of the crude extract ($p<0.001$, paired-sample *t*-test, Fig. 2b).

Results from wild-caught versus captive bred *S. fuscecens* showed considerable differences. Wild *S. fuscescens* ate significantly less of the extract ($p=0.020$, paired-sample *t*-test, Fig. 2c), but captive-bred *S. fuscescens* had no feeding preference between control and treatment foods ($p=0.569$, paired-sample *t*-test, Fig. 2d).

Differences were observed in the secondary metabolites in the crude extract over time. TLC confirmed the presence of LTA and DAT in crude extract utilized in the Guam trials. However, quantification of the same crude extract used in the Australian trials three months later using HPLC-MS/MS revealed the presence of LTA only (11.99 mg g^{-1} crude extract ± 1.36 SE), suggesting DAT had degraded.

Discussion

Lyngbya majuscula crude extract from Moreton Bay, Australia was deterrent to most consumers regardless of geographic origin. Extracts deterred feeding by sea urchins (*Echinometra mathaei*), majid crabs (*Menaethius monoceros*), and two amphipod species (*Cymadusa imbroglio*, *Parhyale hawaiensis*) from Guam, as well as cephalaspideans (*Diniatys dentifer*) and wild-caught siganids (*Siganus fuscescens*) from Australia. In contrast, *L. majuscula* crude extract stimulated feeding by sea hares (*Stylocheilus striatus*) in Guam, but did not affect feeding in Australian sea hares or captive-bred siganids.

All consumers used in this study commonly encounter *L. majuscula* albeit from different populations. In previous studies, both amphipod and crab species from Guam were observed consuming local *L. majuscula* and mixed algal diets with varying preferences (personal observations), whereas *S. striatus* consumed cyanobacteria exclusively, with a strong preference for *L. majuscula*

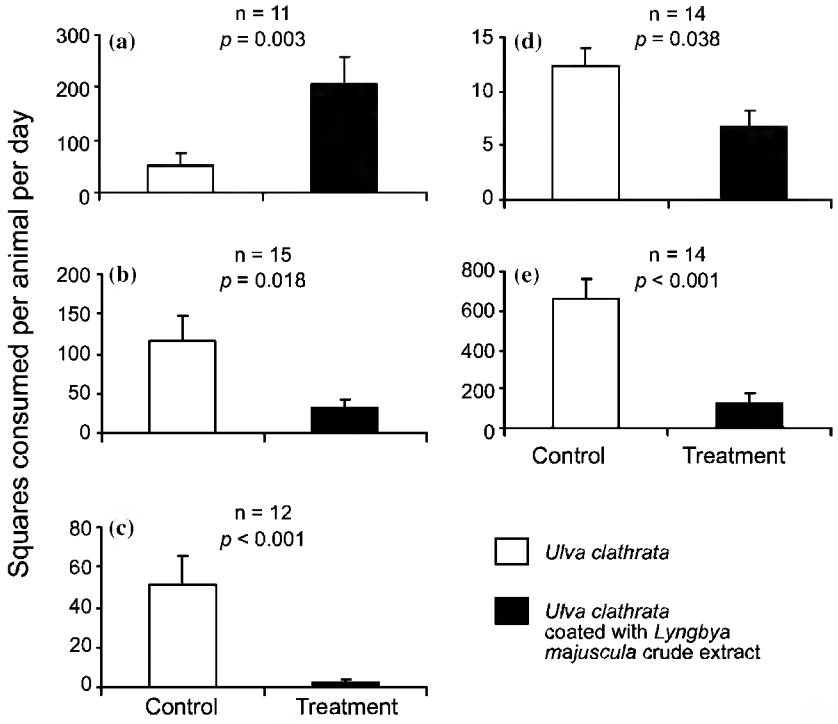

Figure 1. Guam pair-wise choice assays: (a) *Stylocheilus striatus*; (b) *Menaethius monoceros*; (c) *Parhyale hawaiensis*; (d) *Cymadusa imbroglio*; (e) *Echinometra mathaei*, using an artificial diet of *Ulva clathrata* only (control) or *U. clathrata* containing *Lyngbya majuscula* crude extract (treatment). Data are mean squares consumed per animal per day (±SE). A paired-sample *t*-test was used to analyse data. *n* = number of replicates. N.B. Different scales on individual graphs.

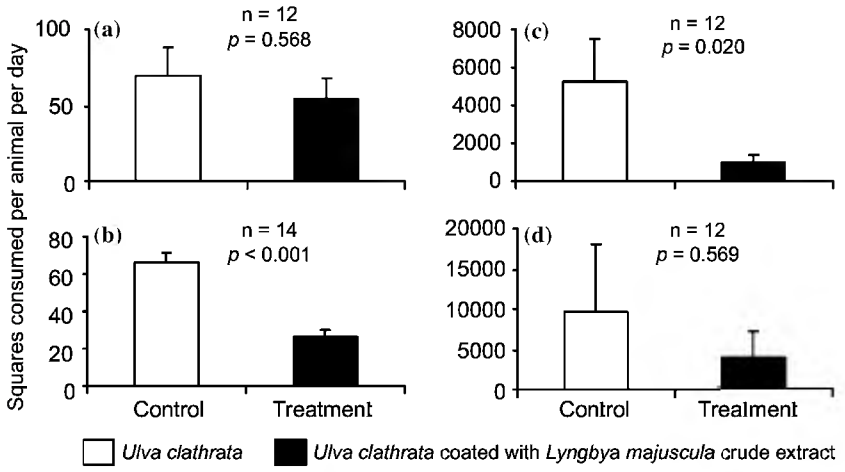

Figure 2. Australian pair-wise choice assays: (a) *Stylocheilus striatus*; (b) *Diniatys dentifer*; (c) wild *Siganus fuscescens*; (d) captive-bred *Siganus fuscescens*, using an artificial diet of *Ulva clathrata* only (control) or *U. clathrata* containing *Lyngbya majuscula* crude extract (treatment). Data are mean squares consumed per animal per day (±SE). A paired-sample *t*-test was used to analyse data. *n* = number of replicates. N.B. Different scales on individual graphs.

(Paul & Pennings, 1991). Therefore, the strong deterrence towards *L. majuscula* extracts shown by some of these mesograzers cannot be explained by simple aversion to *L. majuscula* or cyanobacteria in general.

We expected that Australian mesograzers directly associated with *L. majuscula* blooms would tolerate the *L. majuscula* secondary metabolites better than their Guamanian counterparts. This was not true since *S. striatus* and *D. dentifer* from the same bloom that yielded the crude extract tested were voracious consumers of *L. majuscula* in the laboratory prior to feeding assays (personal observations). Yet neither species was stimulated to feed by the presence of crude extract in artificial diets (as we had observed in *S. striatus* from Guam), and in fact *D. dentifer* was deterred by extracts. Although these results appear contradictory, changes in the chemistry of stored *L. majuscula* extracts might account for these patterns. Three months elapsed between trials in Guam and Australia, which led to measured changes in compound composition of the crude extract. Debromoaplysiatoxin (DAT) observed in the crude extract used in the Guam trials was not detected by HPLC-MS/MS in Australia in the same extract three months later. The degradation of DAT probably altered the way that consumers perceived the extract. In the case of *D. dentifer* the absence of DAT may have left the extract with only negative feeding cues. Alternatively, changes in the composition of other compounds not measured in those experiments may also have altered the palatability of crude extract to consumers.

While these limitations constrain the interpretation of our results from Australia, they do confirm a previous report (Nagle et al., 1998) that *S. striatus* is not deterred by LTA. Negative post-ingestive consequences of this metabolite are known to include: severe oral and gastro-inflammation in humans; intestinal haemorrhaging in mice (Sims & Zandee van Rilland, 1981; Ito et al., 2002); and suggestions of lethality in humans (Yasumoto, 1998). However, *S. striatus* as a specialist grazer of cyanobacteria, can sequester and detoxify LTA and DAT into less harmful acetates (Kato & Scheuer, 1975; Pennings & Paul, 1993b, Pennings et al., 1996; Gallimore et al., 2000).

The fate of these compounds in siganids has not yet been documented. Previous studies suggest that siganids consume *L. majuscula* in their diet (von Westerhagen, 1973; Bryan, 1975; Lundberg & Lipkin, 1979) however, the chemical composition of these blooms is unknown. Previous laboratory experiments show that wild *S. fuscescens* consume only small quantities of *L. majuscula* containing LTA, whilst they fed readily upon *L. majuscula* that had no detectable LTA or DAT (A. Capper, unpublished data). While wild-caught Australian *S. fuscescens* were deterred by *L. majuscula* extracts, captive-bred *S. fuscescens* were not. This difference may suggest that avoiding cyanobacterial metabolites like LTA might be a learned behavioral response. Some siganids may reject cyanobacterial compounds more strongly depending upon previous experience and hunger levels (Thacker et al., 1997). Initial encounters with unfamiliar compounds, such as LTA and DAT, may promote 'cautious' feeding behavior to reduce potential negative post-ingestive effects that may compromise the health of the organism (Freeland & Janzen, 1974; Pennings & Carefoot, 1995). This could account for the high variance in feeding of the captive-bred siganids and lack of preference between treatment and control foods. Repeated exposure to the same crude extract may increase feeding if no deleterious effects ensue (Lindquist & Hay, 1995; Thacker et al., 1997).

Our study shows that *L. majuscula* is deterrent to a diverse range of consumers from different locations. Only *S. striatus* from Guam and captive-bred siganids increased feeding on crude extracts. A thorough understanding of the chemistry of this cyanobacterium and how this affects consumer grazing patterns is required to help understand the potential of these nuisance blooms to persist.

Acknowledgements

This collaborative project was supported by NIH MBRS grant GM 44796 awarded to V.J.P. which provided travel subsistence for A.C. to visit the University of Guam Marine Laboratory. Research carried out at Moreton Bay Research Station (MBRS) was supported by a Centre for Marine Studies Scholarship (CMS, University of Queensland) awarded to AC. We thank M.J. Garson (Dept. of Chemistry, University of Queensland) for NMR assistance and G. Eaglesham (Queens-

land Health and Scientific Services) for HPLC-MS/MS assistance. We also thank D. Harrison for help with seine and trawl nets to catch wild siganids, and W. Knibb (BIARC) for supplying captive-bred siganids. We thank R. Ritson-Williams for feedback and valuable critique of the manuscript. This is contribution # 578 of the University of Guam Marine Laboratory and contribution # 618 of the Smithsonian Marine Station at Fort Pierce.

References

Avila, C., 1995. Natural products of opisthobranch molluscs: A biological review. Oceanography and Marine Biology: Annual Review 33: 487–559.

Bryan, P. G., 1975. Food habits, functional digestive morphology, and assimilation efficiency of the rabbitfish *Signaus spinus* (Pisces, Siganidae) on Guam. Pacific Science 29: 269–277.

Burja, A. M., B. Banaigs, E. Abou-Mansour, J. G. Burgess & P. C. Wright, 2001. Marine cyanobacteria- a prolific source of natural products. Tetrahedron 57: 9347–9377.

Capper, A., I. R. Tibbetts, J. M. O'Neil & G. R. Shaw, (2005). The fate of Lyngbya majuscula toxins in three potential consumers. Journal of Chemical Ecology 31(7): 1595–1606.

Cruz-Rivera E. & V. J. Paul, 2002. Coral reef benthic cyanobacteria as food and refuge: Diversity, chemistry and complex interactions. Proceedings of 9th International Coral Reef Symposium, Bali, Indonesia, pp. 515–520.

Dennison, W. C., J. M. O'Neil, E. J. Duffy, P. E. Oliver & G. R. Shaw, 1999. Blooms of the cyanobacterium *Lyngbya majuscula* in coastal waters of Queensland, Australia. Bulletin de l'Institut Oceanographique Monaco, Special 19: 501–506.

Freeland, W. J. & D. H. Janzen, 1974. Strategies in herbivory by mammals: the role of plant secondary compounds. American Naturalist 108: 269–289.

Gallimore, W. A., D. L. Galario, C. Lacy, Y. Zhn & P. J. Scheuer, 2000. Two complex proline esters from the sea hare *Stylocheilus longicauda*. Journal of Natural Products 63: 1022–1026.

Gerwick, W. H., T. Tan & N. Sitachitta, 2001. Nitrogen-containing metabolites from marine cyanobacteria. In Cordell, G. A. (ed.) The Alkaloids: Chemistry and Biology. 57 Academic Press, NY: 75–184.

Hashimoto, Y., S. Konosu, T. Yasnmoto & H. Kamiya, 1969. Ciguatera in the Ryukyu and Amami Islands. Bulletin of Japanese Society of Fisheries Science 35: 316–326.

Hay, M. E., J. J. Stachowicz, E. Cruz-Rivera, S. Bullard, M. S. Deal & N. Lindquist, 1998. Bioassays with marine and freshwater organisms. In Haynes, K. F. & J. R. Millar (eds), Methods in Chemical Ecology, Bioassays. Vol. 2 Chapman and Hall, New York: 39–141.

Hayden, H. S., J. A. Blomster, C. A. Maggs, P. C. Silva, M. J. Stanthorpe & J. R. Waland, 2003. Linnaeus was right all along: *Ulva* and *Enteromorpha* are not distinct genera. European Journal of Phycology 38: 277–294.

Ito, E., M. Satake & T. Yasumoto, 2002. Pathological effects of lyngbyatoxin A upon mice. Toxicon 40: 551–556.

Kato, Y. & P. J. Scheuer, 1975. The aplysiatoxins. Pure and Applied Chemistry 41: 1–14.

Lindquist, N. & M. E. Hay, 1995. Can small rare prey be chemically defended? The case for marine larvae. Ecology 76: 1347–1358.

Lockwood, J. R., 1998. On the statistical analysis of multiple-choice feeding preference experiments. Oecologia 116: 475–481.

Lundberg, B. & Y. Lipkin, 1979. Natural food of the herbivorous rabbitfish (*Siganus* spp) in Northern Red Sea. Botanica Marina 22: 173–181.

Nagle, D. G., Paul & V. J., 1998. Chemical defense of a marine cyanobacterial bloom. Journal of Experimental Marine Biology and Ecology 225: 29–38.

Nagle, D. G. & V. J. Paul, 1999. Production of secondary metabolites by filamentous tropical marine cyanobacteria: Ecological functions of the compounds. Journal of Phycology 35: 1412–1421.

Nagle, D. G., V. J. Paul & M. A. Roberts, 1996. Ypaoamide, a new broadly acting feeding deterrent from the marine cyanobacteria *Lyngbya majuscula*. Tetrahedron Letters 37: 6263–6266.

Nagle, D. G., F. T. Camacho & V. J. Paul, 1998. Dietary preferences of the opisthobranch mollnsc *Stylocheilus longicauda* for secondary metabolites produced by the tropical cyanobacterium *Lyngbya majuscula*. Marine Biology 132: 267–273.

Osborne, N. J. W., P. M. Webb & G. R. Shaw, 2001. The toxins of *Lyngbya majuscula* and their human and ecological health effects. Environment International 27: 381–392.

Paul, V. J. & S. C. Pennings, 1991. Diet-derived chemical defenses in the sea hare *Stylocheilus longicauda* (Quoy et Gaimard 1824). Journal of Experimental Marine Biology and Ecology 151: 227–243.

Paul, V. J., S. G. Nelson & H. R. Sanger, 1990. Feeding preference of adult and juvenile rabbitfish *Siganus argenteus* in relation to chemical defenses of tropical seaweeds. Marine Ecology Progress Series 60: 23–34.

Pennings, S. C. & V. J. Paul, 1993a. Secondary chemistry does not limit dietary range of the specialist sea hare *Stylocheilus longicauda* (Quoy et Gaimard 1824). Journal of Experimental Marine Biology and Ecology 174: 97–113.

Pennings, S. C. & V. J. Paul, 1993b. Sequestration of dietary secondary metabolites by three species of sea hares: Location, specificity and dynamics. Marine Biology 117: 535–546.

Pennings, S. C. & T. H. Carefoot, 1995. Post-ingestive consequences of consuming secondary metabolites in sea hares (Gastropoda: Opisthobranchia). Comparative Biochemistry and Physiology 111: 249–256.

Pennings, S. C., A. M. Weiss & V. J. Paul, 1996. Secondary metabolites of the cyanobacterium *Microcoleus lyngbyaceus* and the sea hare *Stylocheilus longicauda*: Palatability and toxicity. Marine Biology 126: 735–743.

Rudman, W. B., 1999. Stylocheilus longicauda and Stylocheilns citrina Nomenclature discussion. [In] Sea Slug Forum http://www.seaslugforum.net/factsheet.cfm?base=stylnome date accessed 23 May 2005.

Sims, J. K. & R. D. van Zandee Rilland, 1981. Escharotic stomatitis caused by the 'stinging seaweed' *Microcoleus lyngbyaceus* (formerly *Lyngbya majuscula*). Hawaii Medical Journal 40: 243–248.

Thacker, R. W. & V. J. Paul, 2001. Are benthic cyanobacteria indicators of nutrient enrichment? Relationships between cyanobacterial abundance and environmental factors on the reef flats of Guam. Bulletin of Marine Science 69: 497–508.

Thacker, R. W., D. G. Nagle & V. J. Paul, 1997. Effects of repeated exposures to marine cyanobacterial secondary metabolites on feeding by juvenile rabbitfish and parrotfish. Marine Ecology Progress Series 147: 21–29.

Thacker, R. W., D. W. Ginsburg & V. J. Paul, 2001. Effects of herbivore exclusion and nutrient enrichment on coral reef macroalgae and cyanobacteria. Coral Reefs 19: 318–3331.

von Westerhagen, H., 1973. The natural food of rabbitfish *Siganus oramin* and *S striolata*. Marine Biology 22: 367–370.

Yasumoto, T., 1998. Fish poisoning due to toxins of microalgal origins in the Pacific. Toxicon 36: 1515–1518.