

# Grazer interactions with four species of *Lyngbya* in southeast Florida

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## Abstract

Blooms of the toxic cyanobacteria *Lyngbya* spp. have been increasing in frequency and severity in southeast Florida in recent years. *Lyngbya* produces many active secondary metabolites which often act as feeding deterrents to generalist herbivores, possibly increasing the longevity of these nuisance blooms. Whilst diverse arrays of small invertebrate consumers are often found in association with *Lyngbya*, little is known of their grazing selectivity among species of *Lyngbya*. This study examines the feeding preference of grazers for four local *Lyngbya* species (*Lyngbya majuscula*, *Lyngbya confervoides*, *Lyngbya polychroa* and *Lyngbya* spp.). *Stylocheilus striatus* and *Haminoea antillarum* showed no dietary selectivity between *L. polychroa*, *L. majuscula* and *Lyngbya* spp. in multiple choice feeding assays, whereas *Bulla striata* showed a distinct preference for *L. polychroa* ( $P < 0.001$ ). To determine whether preference might be related to species-specific secondary metabolites, *L. majuscula*, *L. confervoides* and *L. polychroa* non-polar and polar extracts were incorporated into artificial diets and offered to a range of mesograzers. No significant difference was noted in feeding stimulation or deterrence amongst extracts and the controls for any of the grazers. When fed a monospecific diet of *L. polychroa*, *S. striatus* consumed more ( $P < 0.001$ ) and attained a higher daily biomass ( $P = 0.004$ ) than *S. striatus* fed *L. confervoides*. As *L. polychroa* and *L. confervoides* often co-exist on local coral reefs and yield dense numbers of *S. striatus*, host switching to a more palatable species of *Lyngbya* may have important implications regarding top-down control of local blooms leading to proliferation of one species and decimation of another. *S. striatus* fed a diet of *L. polychroa* consumed more ( $P = 0.003$ ), had a greater increase in body mass ( $P = 0.020$ ) and higher conversion efficiency ( $P = 0.005$ ) than those fed *L. confervoides* regardless of host origin. Possible explanations for host switching between species of *Lyngbya* related to morphology, toxicity and nutrient requirements for growth are discussed.

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**Keywords:** Cyanobacteria; Secondary metabolites; Toxins; Nutrients; Top-down control; Feeding preference; Host switching

## 1. Introduction

### 1.1. Background and significance

Blooms of the toxic cyanobacteria *Lyngbya* spp. have been increasing in frequency and severity in southeast Florida in recent years (Landsberg et al., 2003; Paul et al., 2005). Three major *Lyngbya* species have been identified as ‘nuisance’ blooms. *Lyngbya polychroa* and *Lyngbya confervoides* are often found co-existing on coral reefs in Broward County and the northern Florida Keys and have persisted throughout the spring-fall for the last 4 years (Paul et al., 2005). *Lyngbya majuscula* on the other hand, is more ephemeral and blooms sporadically during the summer months in the Indian River Lagoon with distinct morphological variations, i.e. ‘hair-like’,

‘slimy’ and mixed assemblage ‘mat’ forms (Capper, pers. obs.). The temporal and spatial variation for different species of *Lyngbya* is poorly understood. Bloom dynamics are likely to be affected by a range of physico-chemical and biological parameters including temperature, light attenuation, nutrient availability and grazer preference (Paerl and Millie, 1996; Dennison et al., 1999; O’Neil, 1999; Thacker and Paul, 2001; Capper et al., 2004; Albert et al., 2005; Watkinson et al., 2005).

Whilst diverse arrays of small invertebrate consumers are often found in association with *Lyngbya* (Cruz-Rivera and Paul, 2002, 2006), little is known of their grazing interactions amongst *Lyngbya* species. *Lyngbya* is a prolific producer of many active secondary metabolites (see reviews by Nagle and Paul, 1999; Gerwick et al., 2001; Burja et al., 2001; Osborne et al., 2001) which are often deterrent to generalist herbivores including crabs (Pennings et al., 1996), sea urchins (Pennings et al., 1996; Nagle and Paul, 1998; Cruz-Rivera and Paul, 2002) and fish (Pennings et al., 1996; Thacker et al., 1997; Nagle and Paul, 1998, 1999), possibly increasing the longevity of these

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nuisance blooms. However, one group of consumers, the opisthobranch molluscs, are able to tolerate some compounds and may even be stimulated to feed by them (Nagle et al., 1998; Capper et al., 2005, 2006a), allowing them to capitalize on this transient food source. Bulk consumption by these opportunistic grazers leads to rapid growth rates often promoting a ‘boom-or-bust’ relationship with blooms (Capper et al., 2006b).

The ‘specialist’ grazer *Stylocheilus striatus* Quoy and Gaimard 1832, formerly *S. longicauda* (Rudman, 1999) is often found in association with *Lyngbya* blooms (Pennings et al., 1996; Nagle and Paul, 1999). Its larvae preferentially settle upon *L. majuscula* (Switzer-Dunlap and Hadfield, 1977) and adults preferentially feed and grow well upon a monospecific diet of *L. majuscula* (Paul and Pennings, 1991; Capper et al., 2006b). *S. striatus* sequesters *L. majuscula* secondary metabolites from its cyanobacterial host, often storing them in the digestive gland (Watson, 1973; Pennings and Paul, 1993a; Capper et al., 2006b) where it can convert them to less harmful acetates (Pennings et al., 1996). *Bursatella leachii* de Blainville, 1817 also utilizes chemical cues from *L. majuscula* to induce settlement leading to rapid development of larvae (Paige, 1988). Whilst *B. leachii* consumes and grows well on a diet of *Lyngbya* (Capper et al., 2006b), a broader dietary spectrum has been reported for this species (Paige, 1988; Ramos et al., 1995). A more expansive dietary repertoire could reflect a need for additional nutritional requirements. Alternatively, *B. leachii* may lack the digestive and physiological capabilities to detoxify or store some *Lyngbya* compounds (Capper et al., 2005).

Another group of grazers found in association with *Lyngbya* blooms are cephalaspideans. These are one of the most abundant groups of organisms observed in *L. majuscula* blooms in Gnam (Cruz-Rivera and Paul, 2006) and whilst grazing of *L. majuscula* has been observed, it has not yet been quantified (Cruz-Rivera, pers. comm.). *Bulla striata* Bruguiere 1792 and *Haminoea antillarum* d’Orbigny, 1841 are micro-herbivores and feed upon diatoms and detritus from algal mats (Marin et al., 1999). Settlement cues and sequestration mechanisms for these two species are poorly understood.

Fortuitous findings of these natural consumers of *Lyngbya* often occur during bloom events in southeast Florida. Yet little is known of host specificity and migration between co-existing species of *Lyngbya*. Whilst opisthobranchs often use chemical cues from *Lyngbya* for settlement from a planktonic larval phase, is it not known if they will choose to switch hosts to a more palatable *Lyngbya* species should one become available. *L. majuscula* blooms in the Indian River Lagoon often yield large quantities of *B. striata* and *H. antillarum*, yet these are rarely observed in *L. polychroa* and *L. confervoides* blooms in Broward County (pers. obs.). *S. striatus*, on the other hand, is often found in *L. polychroa* and *L. confervoides* in Broward County, but rarely collected from *L. majuscula* in the Indian River Lagoon (pers. obs.). *B. leachii* are seldom found in local *Lyngbya* blooms. If host switching does occur, is this related to *Lyngbya* morphology, grazer nutritional requirements or could it reflect a tolerance, attraction or deterrence towards some secondary metabolites? Host switching could affect bloom

dynamics resulting in the decimation of one species of *Lyngbya*, but the persistence of another. This is particularly important in sites in Broward County where *L. confervoides* and *L. polychroa* often co-exist on coral reefs.

## 1.2. Aim and objectives

Grazer interactions associated with *Lyngbya* blooms are poorly understood. The ecological significance of the role these consumers play is important to help understand potential top-down control of local blooms. This study examines the feeding preference of opisthobranchs among four local *Lyngbya* species (*L. majuscula*, *L. confervoides*, *L. polychroa* and *Lyngbya* spp.) and asks the following questions: (1) does feeding preference and palatability vary among *Lyngbya* spp.; (2) does one species of *Lyngbya* promote higher growth rates in grazers than another; (3) is diet choice related to host specificity; (4) are preferences and host switching a factor of *Lyngbya* morphology, nutritional and/or secondary metabolite composition?

## 2. Methods

### 2.1. Study organisms and sites

Opisthobranch molluscs are often found in association with cyanobacterial blooms. Four species of opisthobranch molluscs were collected from three different bloom locales in southeast Florida: (1) *S. striatus* from blooms of *L. polychroa* and *L. confervoides* in Broward County (Hillsboro Inlet 26°15'1344"N, 80°03'9077"W; Port Everglades 26°05'9902"N, 80°05'0184"W); (2) *B. striata* and *H. antillarum* from *L. majuscula* blooms at Little Jim Island (29°28'23"N, 080°18'41"W); (3) *B. leachii* from holding tanks at St. Lucie Nuclear Power Plant (27°20'201"N, 80°16'268"E) where no *Lyngbya* was observed. Opisthobranchs were maintained in small aquaria (1 l) at Smithsonian Marine Station at Fort Pierce (SMSFP). Salinities were kept between 34 and 36 ppt and temperature consistent with ambient (24 °C) with 12:12 h light and dark cycles. Water was changed every 24 h.

Persistent ‘nuisance’ blooms of *Lyngbya* have been reported at a number of sites in Broward County in southeast Florida. These sites are coral reef dominated and were impacted by *Lyngbya* blooms throughout spring-fall of 2002 through 2006. Two sites were also identified in the Indian River Lagoon (IRL) in St. Lucie County: Jensen Beach (27°13'674"N, 80°12'668"E, 27°14'563"N, 80°13'376"E) where a large bloom of *L. majuscula* was observed in August 2004; and Little Jim Island which was dominated by *L. majuscula* and *Lyngbya* spp. (unidentified mat forming, mixed assemblage of *Lyngbya* species, D. Littler pers. comm.) blooms during July 2005 and April–July 2006. These IRL sites are predominantly seagrass, macroalgae and bare sediment. *Lyngbya* collections were returned to SMSFP and maintained in 5 gallon buckets of aerated seawater at 35 ppt at 24 °C with 12:12 h light and dark cycles. Water was changed every 24 h. *Ulva intestinalis* (formerly *Enteromorpha*, Hayden et al., 2003) was collected in Jensen Beach for use in feeding assays and was maintained in the same manner as *Lyngbya*. *Gracilaria tikvahaei* was

obtained from Harbor Branch Oceanographic Institution and freeze-dried for use in palatability assays.

## 2.2. Multiple choice feeding assay

Dietary preferences of opisthobranchs were determined by conducting multiple choice feeding assays using *L. polychroa* from Broward County and *L. majuscula* and *Lyngbya* spp. from Little Jim Island. Due to the sporadic nature of blooms, *L. confervoides* could not be collected at the same time. *S. striatus* (collected from *L. polychroa*,  $n = 6$ ), *B. striata* (collected from *L. majuscula*,  $n = 10$ ) and *H. antillarum* (collected from *L. majuscula*,  $n = 10$ ), were acclimated for 48 h in 1 l aquaria and maintained on host diet. *Lyngbya* samples were rinsed with clean seawater (all visible epiphytes and particulate matter were removed by hand) and blotted dry. The number of blots was standardised for each *Lyngbya* species depending upon its water retention capacity. The algal material was randomly assigned to predetermined positions in individual replicate aquaria. To commence the experiment, individual opisthobranchs of similar weight (blotted wet weight,  $g_{wwt}$ ) were placed on the mid-floor of each aquarium. Control tanks (those without opisthobranchs) with algal material were interspersed between treatment tanks (those with opisthobranchs) to account for autogenic changes in the food mass (Peterson and Renaud, 1989). Consumption was determined using the formula  $[T_i \times (C_f/C_i)] - T_f$ , where  $T_i$  is the initial food mass,  $T_f$  is the final food mass,  $C_i$  is the initial control mass and  $C_f$  is the final control mass. The amount of food consumed was expressed as a proportion of total food consumed by each opisthobranch. A fixed-time design (Lockwood, 1998) of 96 h was allocated to the experiment, after which the test was terminated and food items were removed, re-blotted and re-weighed. Data were analysed using Friedman's ANOVA with Sigmasat<sup>®</sup> software. Tukey's *post hoc* tests were carried out on all significant results (i.e.  $P < 0.05$ ), to allow comparisons among multiple non-independent treatments based on ranks (Conover, 1980).

## 2.3. Chemical extraction of *Lyngbya*

To obtain crude extracts for use in palatability assays, all *Lyngbya* samples were frozen at  $-20^\circ\text{C}$  and then freeze-dried. Dried material was then extracted four times during a 48-h period in ethyl acetate:methanol (1:1) for the non-polar extract; then three times during a 36-h period in ethanol:distilled water (1:1) for the polar extract. Samples were filtered and dried by rotary evaporation. Presence of secondary metabolites in crude extracts was confirmed using proton nuclear magnetic resonance (NMR) spectroscopy.

## 2.4. Palatability assays using *Lyngbya* extracts

To determine whether preference might be related to species-specific secondary metabolites, both non-polar and polar extracts were incorporated into artificial diets and offered to a range of meso- and macro-grazers. Artificial diets

containing or lacking *Lyngbya* crude extracts were created using methods outlined in Hay et al. (1998) where treatment foods were made using non-polar and polar crude extracts. Extracts were resolubilised in ethyl acetate and methanol and then coated at natural concentration by percentage dry mass onto freeze-dried, powdered *Gracilaria tikvahiae*. This natural concentration is based on quantity of crude extract obtained from a known quantity of freeze-dried *Lyngbya*, i.e. *L. majuscula* non-polar at 6.72% and polar at 6.03%; *L. polychroa* non-polar at 20.39% and polar at 17.13%; *L. confervoides* non-polar at 10.97% and polar at 18.22%. This method has been successfully used to test extracts and compounds from *L. majuscula* populations (Nagle et al., 1998; Capper et al., 2006a; Cruz-Rivera and Paul, 2006). *G. tikvahiae* was chosen because it is a broadly palatable alga that is high in protein with a high energy value (McDermid and Stuercke, 2004). In a pre-trial feeding preference test, *G. tikvahiae* was significantly preferred by *S. striatus* to *Ulva* spp. (Friedman's ANOVA,  $P < 0.001$ ), and was thus used in all subsequent palatability assays. A rotary evaporator was used to remove all organic solvent allowing the hydrophobic and hydrophilic components of the crude extracts to adhere to the surface of *G. tikvahiae*. Control foods were made by coating freeze-dried *G. tikvahiae* with ethyl acetate and methanol and then evaporating the solvent as described above. Control and both treatment foods were then incorporated into agar-based artificial diets as described by Hay et al. (1998). The mixture was then poured onto plastic screen mesh to make food strips. Consumption was quantified as the number of mesh squares cleared of food over time.

In the first set of assays, control, non-polar and polar treatment food strips from *L. majuscula*, *L. confervoides* and *L. polychroa* were offered simultaneously to individual consumers in a choice experiment. A second set of choice assays was used to compare non-polar extracts of *L. polychroa* and *L. confervoides* with a control ( $n = 10$ ) to determine which component acted as a feeding stimulant. This was followed by both polar extracts and a control ( $n = 9$ ). A fixed-consumption stopping rule was applied (Lockwood, 1998) where tests were terminated once  $>50\%$  of total food 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. Replicates where  $<10\%$  or  $>90\%$  of total food had been consumed were also eliminated from the test. Data were analysed using Friedman's ANOVA with Sigmasat<sup>®</sup> software. Tukey's *post hoc* tests were carried out on all significant results (i.e.  $P < 0.05$ ).

## 2.5. No-choice assay and associated biomass increase

*S. striatus* were chosen for this assay as they are voracious consumers of *Lyngbya* and can double their body weight every 2 days on a monospecific diet of *L. majuscula* (Capper et al., 2006b). To determine increase in biomass of *S. striatus* fed a monospecific diet of *L. polychroa*, *L. confervoides* or *U. intestinalis*, five sea hares for each diet type were each housed individually in 1 l aquaria over a 10-day period (treatment), with seawater changed every other day. Replicate aquaria



containing the same plant material and no sea hares were randomly interspersed between treatment aquaria to ascertain autogenic changes in the food mass (control) (Peterson and Renaud, 1989). Both species of *Lyngbya* tested were obtained from Broward County. *Ulva* samples were collected from Jensen Beach. Samples were blotted and weighed (as per multiple choice feeding assay) and placed into the treatment and control aquaria. Plant material was removed, weighed and replaced with fresh plant material on Day 5 and Day 10 during a 10-day exposure period.

Conversion of cyanobacterial and algal mass (from a monospecific diet of *L. polychroa*, *L. confervoides* and *U. intestinalis*) to body mass ( $E_{\text{conv}}$ ) was determined from each treatment group using data obtained from the no-choice biomass assay above, as total change of sea hare mass ( $\Delta_{\text{shm}}$ ). This was subjected to a conversion efficiency calculation (Eq. (1)) that consisted of the total change of sea hare mass divided by the quantity of food consumed (control adjusted for autogenic changes in mass,  $L_{\text{cons}}$ ), the sum of which was divided by the experimental duration ( $t = x$  days) (Rogers et al., 1995).

$$E_{\text{conv}} = \frac{\Delta_{\text{shm}}/L_{\text{cons}}}{t} \quad (1)$$

The mean starting weight of all sea hares used was 0.39 g<sub>wwt</sub> ( $\pm 0.02$  S.E.). Sea hares were also weighed at the mid- and end-point of the test.

Biomass changes in animals following consumption of *L. polychroa* or *L. confervoides* in no-choice assay data were subjected to repeated measures ANOVA followed by Tukey's *post hoc* analysis (Statistica<sup>®</sup>). Homogeneity of variance was confirmed using Cochran and Levene's homogeneity of variance. Prior to analyses, consumption data that deviated from normal distribution was transformed using  $\log + 1$ . Consumption rates were then analysed in the same manner. Conversion efficiency data met normal data criterion and was analysed using one-way ANOVA and Tukey's *post hoc* tests (Statistica<sup>®</sup>).

## 2.6. Host switching assays

### 2.6.1. No-choice assay

*S. striatus* collected from Broward County *L. confervoides* ( $n = 12$  total) and *L. polychroa* blooms ( $n = 16$  total) were split into two groups for each host species of *Lyngbya*. One group from each host species of *Lyngbya* was given a monospecific diet of either *L. confervoides* or *L. polychroa* for a period of 15 days. Food was removed, weighed and replaced with fresh material every 3 days during the 15-day exposure period (see Section 2.2). Replicate aquaria containing the same plant material and no sea hares were randomly interspersed as previously described and followed the same weighing regime. Sea hares were removed and blotted wet weight recorded every 3 days to ascertain increase or decrease in biomass during the test period. Mean initial weight of sea hares was: (1) *L. confervoides* host fed *L. confervoides* diet 0.208 g<sub>wwt</sub> ( $\pm 0.006$  S.E.); (2) *L. confervoides* host fed *L. polychroa* diet 0.284 g<sub>wwt</sub>

( $\pm 0.009$  S.E.); (3) *L. polychroa* host fed *L. confervoides* diet 0.153 g<sub>wwt</sub> ( $\pm 0.021$  S.E.); (4) *L. polychroa* host fed *L. polychroa* diet 0.182 g<sub>wwt</sub> ( $\pm 0.031$  S.E.).

Initial weight of sea hare data deviated from normal distribution patterns and was log transformed to ensure homogeneity of variance and analysed using one-way ANOVA (Statistica<sup>®</sup>). Biomass changes in animals following consumption of *L. polychroa* or *L. confervoides* were subjected to repeated measures ANOVA (Statistica<sup>®</sup>) followed by Tukey's *post hoc* analysis after  $\log^3$  transformation. Homogeneity of variance was confirmed for all tests using Cochran and Levene's tests. Consumption rates were analysed in the same manner. Conversion efficiencies were analysed using one-way ANOVA (Statistica<sup>®</sup>) and Tukey's *post hoc* tests.

### 2.6.2. Pair-wise choice assay

*S. striatus* collected from Broward County *L. confervoides* ( $n = 8$ ) and *L. polychroa* blooms ( $n = 8$ ) were split into host-specific groups for each species of *Lyngbya*. One group from each host species of *Lyngbya* was offered a diet choice of *L. confervoides* and *L. polychroa* for a period of 24 h. Plant material was blotted and weighed as described above with concurrent control replicate aquaria with no sea hares. Two-way ANOVA (Statistica<sup>®</sup>) and Tukey's *post hoc* tests were used to analyse data.

## 2.7. Nutritional analysis

Carbon and nitrogen elemental analysis of *L. polychroa*, *L. confervoides* and *L. majuscula* was carried out at the University of Wyoming Stable Isotope Facility by M. Clementz. Samples were analysed via micro-Dumas combustion using Fison's 1108 Elemental Analyzer. Data reduction was performed by Eager 200 software. Four replicate samples were used for *L. polychroa* and *L. confervoides* and three replicate samples for *L. majuscula*. Three standards were used for each *Lyngbya* species (std (ACE) = acetanilide (71.09% C; 10.36% N)). C:N and %N data were analysed using one-way ANOVA (Statistica<sup>®</sup>) and Tukey's *post hoc* test.

## 3. Results

### 3.1. Multiple choice feeding assays

Three species of opisthobranch mollusc were used to determine feeding preferences amongst three species of *Lyngbya* (*L. polychroa*, *L. majuscula* and *Lyngbya* spp.). *S. striatus* and *H. antillarum* showed no preference amongst *Lyngbya* species (Fig. 1A, C), whereas *B. striata* significantly preferred *L. polychroa* even though it originated from *L. majuscula* (Friedman's ANOVA,  $P < 0.001$ , Fig. 1B).

### 3.2. Chemistry

Preliminary NMR data show all three species of *Lyngbya* contain secondary metabolites typical of lipopeptides produced by cyanobacteria. The predominant compounds in the non-

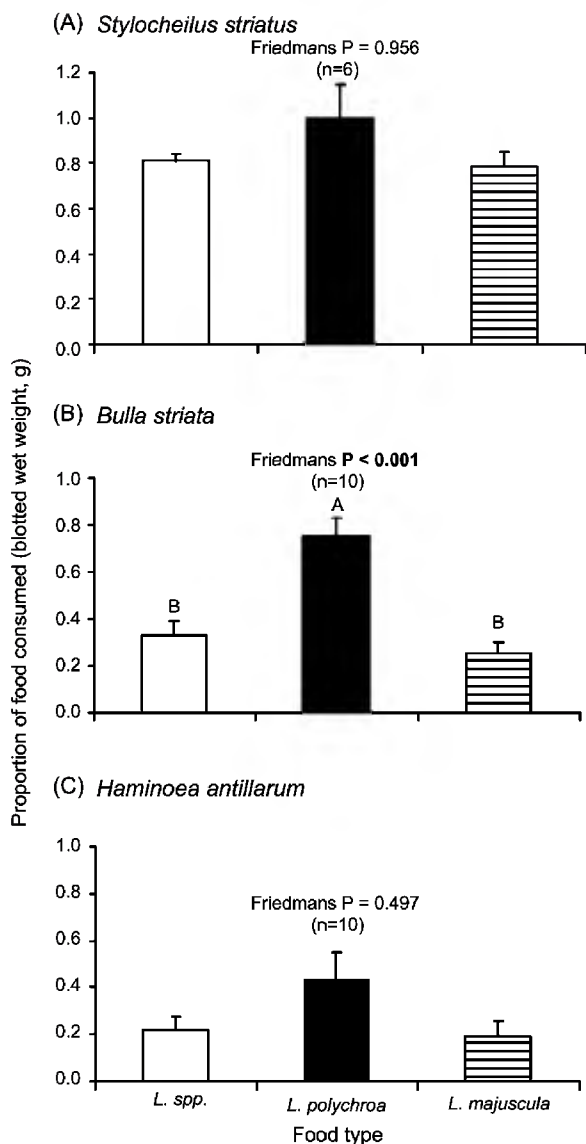


Fig. 1. Multiple choice feeding assays. Data are mean proportion of *Lyngbya* spp. (□), *L. polychroa* (■) and *L. majuscula* (▨) consumed (blotted  $g_{wwt}$ ) control adjusted  $\pm$ S.E. by: (A) *Stylocheilus striatus*; (B) *Bulla striata* and (C) *Haminoea antillarum*. Friedman's ANOVA was used to analyse data. Letters above bars denote a significant difference using Tukey's *post hoc* analysis ( $P < 0.05$ ). ( $n$  = number per replicate).

polar extract of *L. polychroa* are microcolins A and B (T. Meickle, pers. comm.), previously isolated from *L. majuscula* (Koehn et al., 1992). An assortment of compounds was isolated from *L. confervoides* including largamides and lyngbyastatin 4 in the non-polar extract (H. Luesch, pers. comm.; Plaza and Bewley, 2006; Matthew et al., 2007).

### 3.3. Palatability assays

Opisthobranchs showed no preference for foods containing or lacking *L. confervoides* or *L. majuscula* non-polar or polar crude extracts (Figs. 2 and 3). Whilst a non-polar crude extract of *L. polychroa* was preferred to the polar extract by *S. striatus* (Friedman's ANOVA,  $P = 0.028$ , Fig. 4A), this was not significantly different from the control in *post hoc* analysis.

*L. polychroa* polar extracts significantly stimulated feeding in *S. striatus* compared to *L. confervoides* polar extracts (Friedman's ANOVA,  $P = 0.0016$ , Fig. 5B). No significant differences were observed between non-polar extracts (Friedman's ANOVA,  $P = 0.199$ , Fig. 5A).

### 3.4. No-choice assay with associated increase in biomass

*S. striatus* fed a monospecific diet of either *L. confervoides* or *L. polychroa* both exhibited the same increase in daily rate of mass change ( $\delta_{mass}/t$ ) during laboratory trials. Both groups

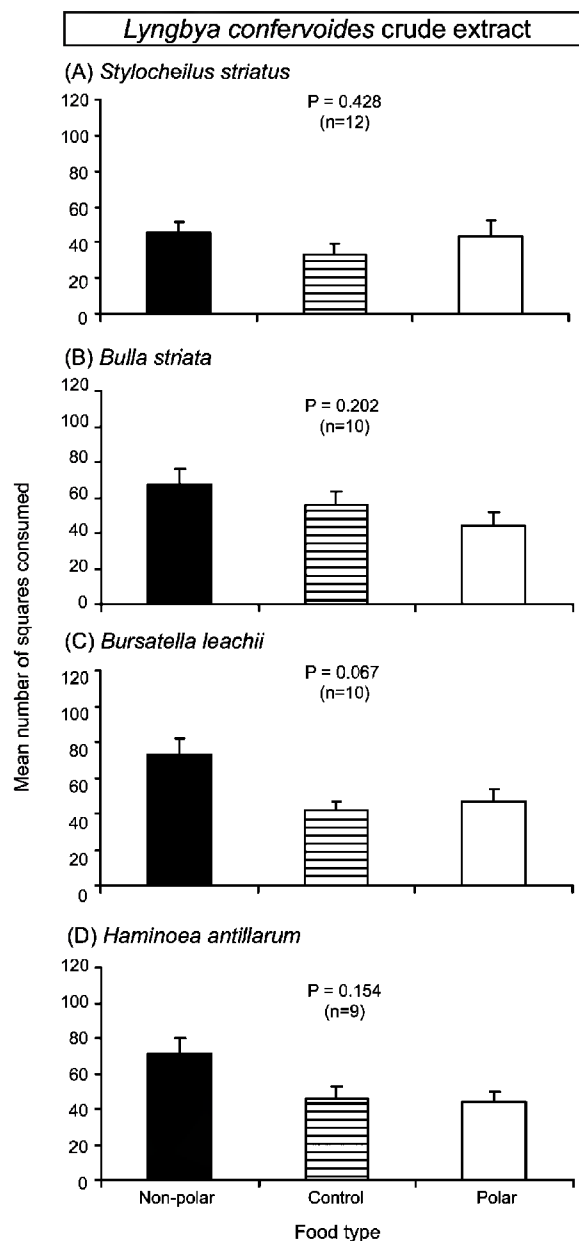


Fig. 2. Palatability assay for *Lyngbya confervoides* crude extracts: (A) *S. striatus*; (B) *B. striata*; (C) *Bursatella leachii* and (D) *H. antillarum*, using an artificial diet of solvent treated *Gracilaria tikvahiae* (▨), control; or *G. tikvahiae* with non-polar (■) or polar (□) crude extracts of *L. confervoides* treatments. Data are mean squares consumed per animal ( $\pm$ S.E.). Friedman's ANOVA was used to analyse data. ( $n$  = number of replicates).

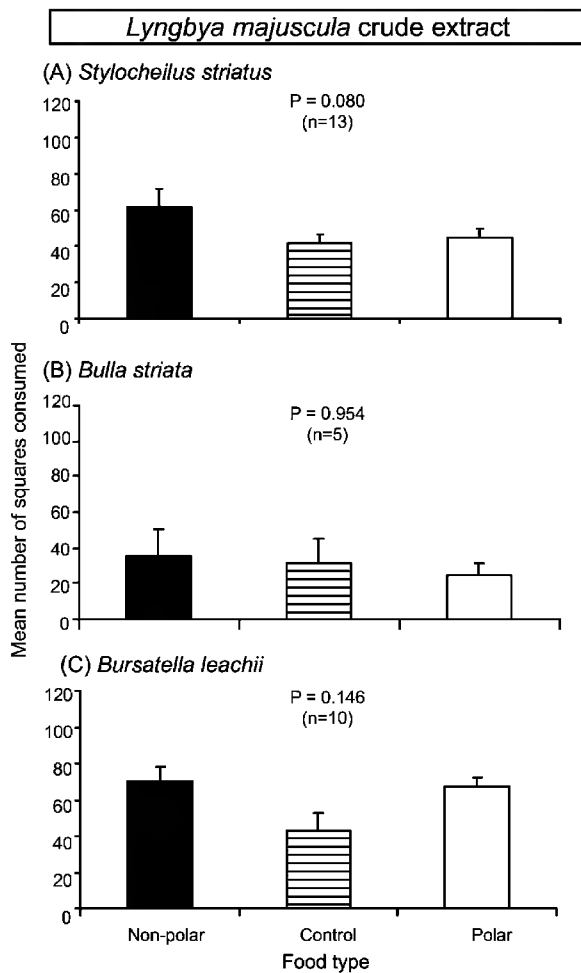


Fig. 3. Palatability assay for *Lyngbya majuscula* crude extracts: (A) *S. striatus*; (B) *B. striata* and (C) *B. leachii* using an artificial diet of solvent treated *G. tikvahiae* (▨), control; or *G. tikvahiae* with non-polar (■) or polar (□) crude extracts of *L. majuscula*, treatments. Data are mean squares consumed per animal ( $\pm$ S.E.). Friedman's ANOVA was used to analyse data ( $n$  = number of replicates).

differed significantly in mass from *S. striatus* fed a diet of *U. intestinalis* (repeated measures ANOVA,  $P = 0.004$ , Fig. 6A). The starting biomass of all *S. striatus* was not significantly different regardless of treatment type (one-way ANOVA,  $P = 0.465$ ). *S. striatus* fed *L. polychroa* actually consumed significantly larger amounts of food between Days 1–5 and Days 5–10 than those fed *L. confervoides* or *U. intestinalis* (repeated measures ANOVA,  $P < 0.001$ , Fig. 6B). However, the total amount of food consumed decreased in the latter half of the test. The efficiency of algal mass conversion to body weight ( $E_{\text{conv}}$ ) did not differ significantly between *S. striatus* fed either *Lyngbya* diet, whereas those fed *U. intestinalis* showed a negative conversion rate (ANOVA,  $P < 0.001$ , Fig. 6C).

### 3.5. Host switching assays

#### 3.5.1. No-choice assay

*S. striatus* fed a monospecific diet of *L. polychroa* had the greatest increase in biomass over a 15-day period (repeated measures ANOVA,  $P = 0.003$ , Fig. 7A). *S. striatus* originating

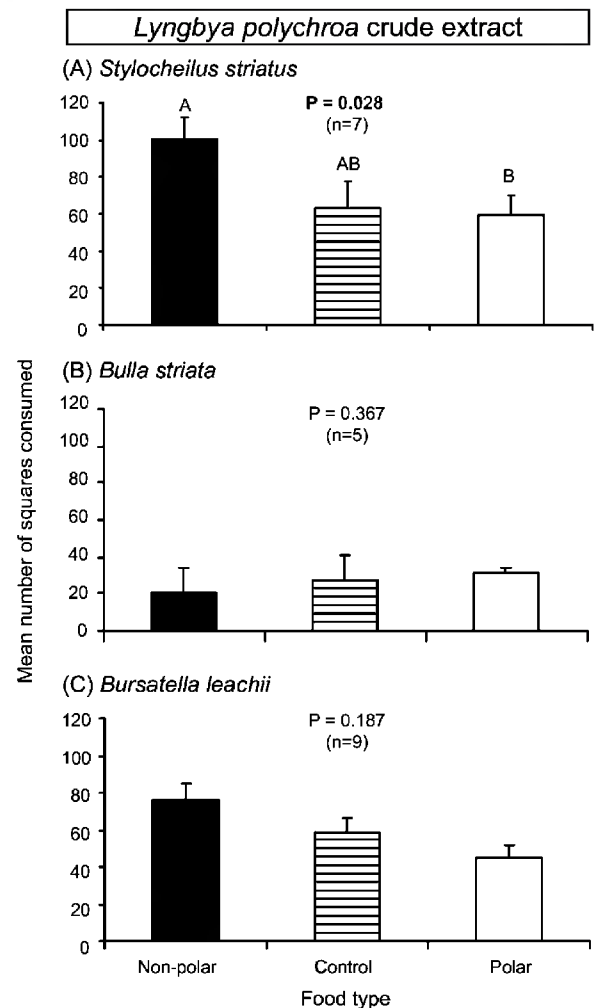


Fig. 4. Palatability assay for *Lyngbya polychroa* crude extracts: (A) *S. striatus*; (B) *B. striata* and (C) *B. leachii* using an artificial diet of solvent treated *G. tikvahiae* (▨) control; or *G. tikvahiae* with non-polar (■) or polar (□) crude extracts of *L. polychroa*, treatments. Data are mean squares consumed per animal ( $\pm$ S.E.). Friedman's ANOVA was used to analyse data. Letters above bars denote a significant difference using Tukey's *post hoc* analysis ( $P < 0.05$ ) ( $n$  = number of replicates).

from a *L. confervoides* host fed *L. polychroa* consumed significantly more food than those from a *L. polychroa* host fed *L. confervoides* (repeated measures ANOVA,  $P = 0.020$ , Fig. 7B). Consumption rates and associated increase in biomass were lowest in *S. striatus* originating from *L. polychroa* host fed a diet of *L. confervoides* (Fig. 6A and B). The efficiency of cyanobacterial mass conversion to body mass ( $E_{\text{conv}}$ ) differed significantly between treatment types (ANOVA,  $P = 0.005$ , Fig. 7C) with *L. polychroa* host *S. striatus* fed a *L. polychroa* diet showing the highest conversion efficiency whilst *L. polychroa* host fed *L. confervoides* and *L. confervoides* host fed *L. confervoides* exhibited the lowest. No significant difference was observed between the initial weights of all four groups of *S. striatus* (one-way ANOVA,  $P = 0.863$ ).

#### 3.5.2. Pair-wise choice assay

When offered a choice, *S. striatus* consumed significantly more *L. polychroa* than *L. confervoides*,

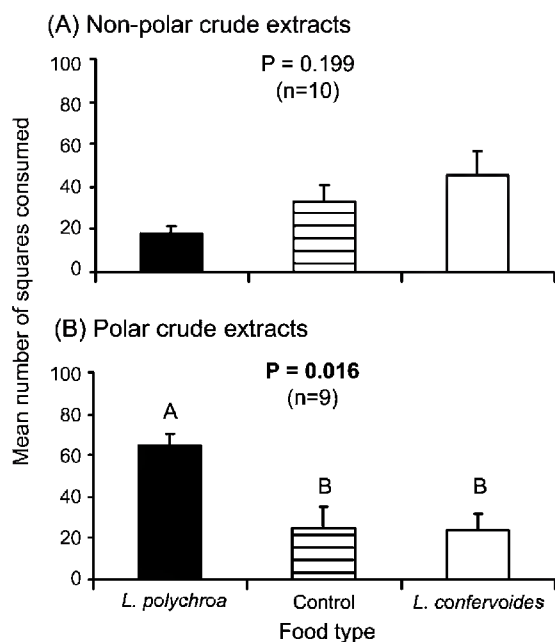


Fig. 5. Palatability assay for *S. striatus* using *L. polychroa* and *L. confervoides*: (A) non-polar and (B) polar crude extracts. An artificial diet of *G. tikvahiae* treated with *L. polychroa* (■) or *L. confervoides* (□) crude extracts or solvent-treated *G. tikvahiae* (▨) were used. Data are mean squares consumed per animal ( $\pm$ S.E.). Friedman's ANOVA was used to analyse data. Letters above bars denote a significant difference using Tukey's *post hoc* analysis ( $P < 0.05$ ) ( $n$  = number of replicates).

regardless of host origin (one-way ANOVA,  $P < 0.001$ , Fig. 8).

### 3.6. Nutritional analysis

*Lyngbya confervoides* exhibited the lowest C:N (one-way ANOVA,  $P < 0.001$ , Fig. 9A) and highest nitrogen levels (%N) (one-way ANOVA,  $P < 0.0001$ , Fig. 9B).

## 4. Discussion

This study focussed on dietary selectivity amongst four species of *Lyngbya* and four species of opisthobranchs commonly found in association with blooms in Florida. Opisthobranchs consumed all species of *Lyngbya* tested, suggesting they play an important ecological role in *Lyngbya* bloom dynamics. Many mesograzers associate themselves with a chemically defended host to not only provide a food source but also a refuge from predation (Hay and Fenical, 1988; Hay et al., 1998; Stachowicz and Hay, 1999; Ginsburg and Paul, 2001). The wide range of mesograzers found in association with *Lyngbya* blooms are likely to exhibit varying degrees of specificity related to both food and refuge (Cruz-Rivera and Paul, 2006). Opisthobranchs however, actively seek out *Lyngbya* as a food source (Cruz-Rivera and Paul, 2002). This study sought to identify differences in opisthobranch dietary preference amongst species of *Lyngbya* and ascertain how optimal foraging strategies might be related to nutritional requirements and/or secondary metabolite composition and ultimately consumer fitness.

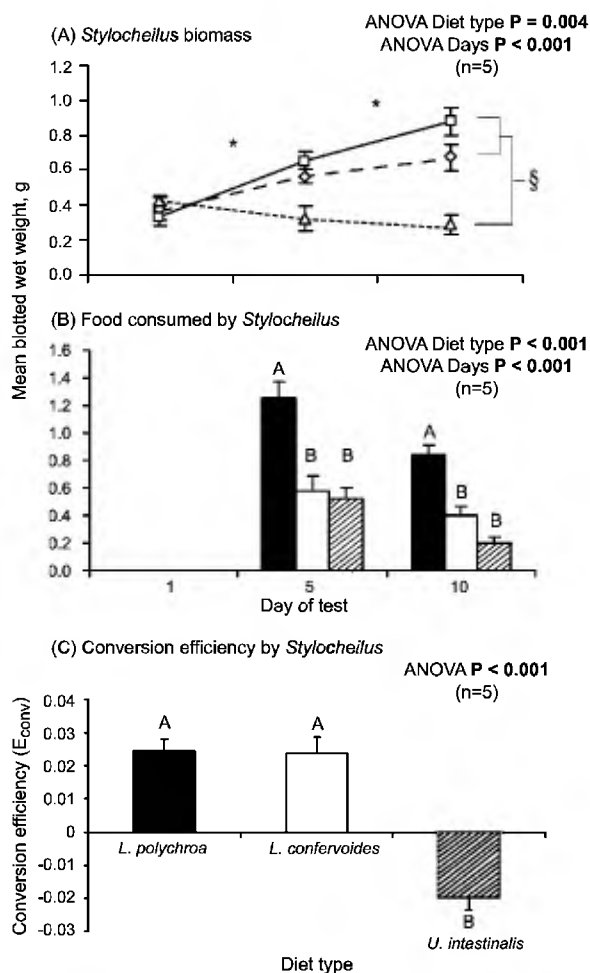


Fig. 6. *S. striatus* no-choice assay, associated biomass increase and conversion efficiency. (A) Mean blotted wet weight ( $g_{wwt}$ ) of *S. striatus* fed: *L. polychroa* (□); *L. confervoides* (◇); or *U. intestinalis* (△) over a period of 10 days. Data were analysed using repeated measure ANOVA. \* indicates significant difference ( $P < 0.05$ ) in *S. striatus* biomass increase between days for each treatment type using Tukey's *post hoc* analysis. § indicates a significant difference ( $P < 0.05$ ) between treatment/diet types using Tukey's *post hoc* analysis. (B) Mean proportion of *L. polychroa* (■), *L. confervoides* (□) and *U. intestinalis* (▨) consumed by *S. striatus* over the same time period (control adjusted  $\pm$ S.E.). Repeated measures ANOVA was used to analyse the data. Letters above bars indicate a significant difference ( $P < 0.05$ ) between treatments using Tukey's *post hoc* analysis. (C) Conversion of alga or cyanobacterial mass to body mass expressed mean proportional change in *S. striatus* blotted wet weight/food consumed ( $g_{wwt}$ /day  $\pm$ S.E. (Rogers et al., 1995)). One-way ANOVA was used to analyse the data. Letters above bars indicate a significant difference ( $P < 0.05$ ) between treatments using Tukey's *post hoc* analysis ( $n$  = number of replicates).

In multiple choice feeding assays, ambivalence was displayed by *S. striatus* and *H. antillarum* among three species of *Lyngbya*, whereas *B. striata* showed a preference for *L. polychroa* even though it originated from *L. majuscula*. In areas where *Lyngbya* species co-exist, an ability to consume all would be advantageous providing a broader menu of dietary options, allowing grazers to exploit an often ephemeral food source. In the Indian River Lagoon, both *L. majuscula* and *Lyngbya* spp. (mixed assemblage mats) co-exist. Both of these were consumed equally by both *B. striata* and *H. antillarum*. Yet *L. polychroa* was preferred by *B. striata* suggesting that



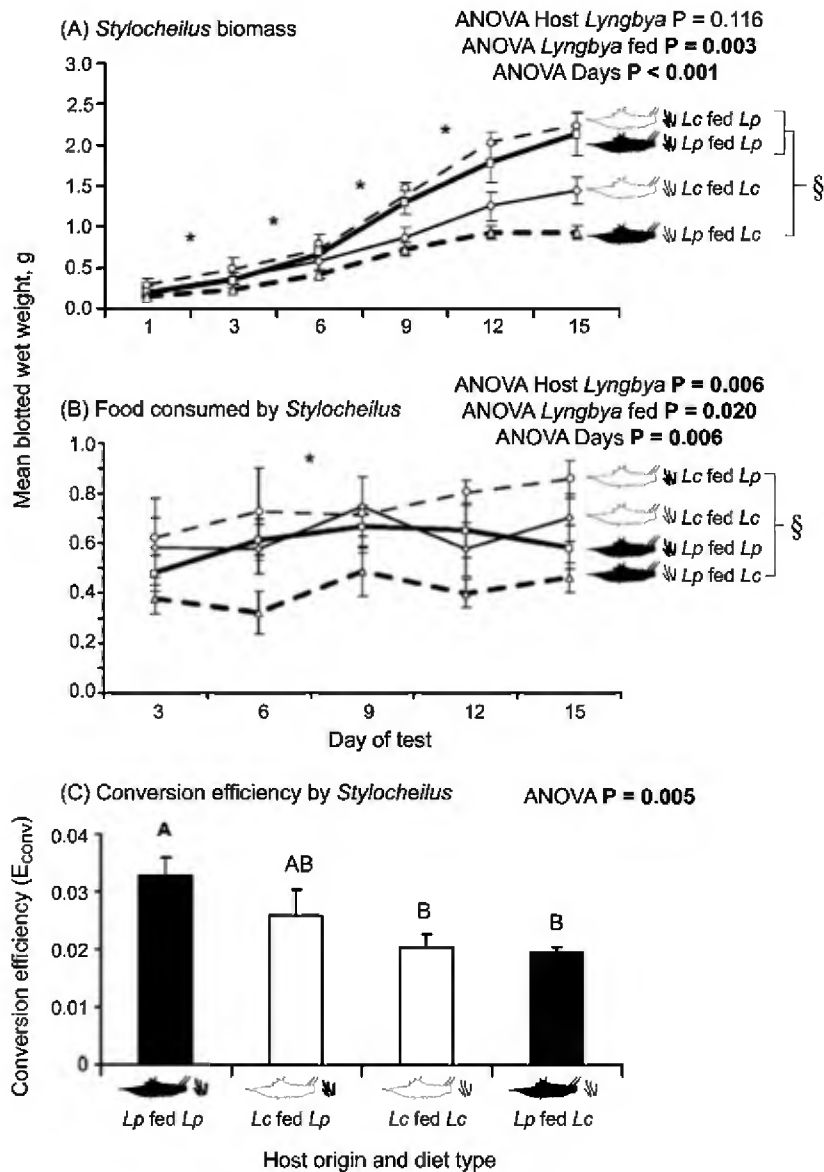


Fig. 7. *S. striatus* no-choice assay to ascertain influence of host switching on consumption patterns, associated biomass increase and conversion efficiency. (A) Mean blotted wet weight ( $g_{\text{wwt}}$ ) of *S. striatus* from: (1) *L. polychroa* fed *L. polychroa* ( $\square$ ),  $n = 8$ ; (2) *L. polychroa* fed *L. confervoides* ( $\triangle$ ),  $n = 8$ ; (3) *L. confervoides* fed *L. confervoides* ( $\diamond$ ),  $n = 6$  and; (4) *L. confervoides* fed *L. polychroa* ( $\circ$ ),  $n = 5$ , over a period of 15 days. Data were analysed using repeated measure ANOVA. \* indicates significant difference ( $P < 0.05$ ) in *S. striatus* biomass increase between days for each treatment type using Tukey's *post hoc* analysis. (B) Mean blotted wet weight ( $g_{\text{wwt}}$ ) of food consumed by *S. striatus*: (1) *L. polychroa* fed to *L. polychroa* host ( $\square$ ); (2) *L. polychroa* fed to *L. confervoides* host ( $\triangle$ ); (3) *L. confervoides* fed *L. confervoides* host ( $\diamond$ ) and; (4) *L. confervoides* fed *L. polychroa* host ( $\circ$ ) over a period of 15 days (control adjusted  $\pm$  S.E.). Repeated measures ANOVA were used to analyse the data. Letters above bars indicate a significant difference ( $P < 0.05$ ) between bars using Tukey's *post hoc* analysis. (C) Conversion of *Lyngbya* mass to body mass expressed as mean proportional change in *S. striatus* blotted wet weight/food consumed ( $g_{\text{wwt}}$ )/day  $\pm$  S.E. (Rogers et al., 1995). One-way ANOVA were used to analyse the data. Letters above bars indicate a significant difference ( $P < 0.05$ ) between treatments using Tukey's *post hoc* analysis ( $n =$  number of replicates).

some mesograzers may switch host to a more palatable species of *Lyngbya* should one become available. Whilst migration to a new food source may be somewhat limited by locomotor activity in these relatively sedentary grazers, more motile opisthobranchs such as *S. striatus* are less restricted and may be able to relocate by 'floating' in the water column using extended parapodia. This phenomenon has been described as 'sea hare rain' in Hawai'i ([www.turtles.org/limu/limu.htm](http://www.turtles.org/limu/limu.htm)) due to the vast numbers of animals observed during *Lyngbya* blooms. Once a palatable item is located sea hares drop out of the water column and resume feeding (pers. obs.). Location of

new food sources could be dependent upon chemical cues. Opisthobranchs use chemical cues from *Lyngbya* for settlement and metamorphosis of planktonic larvae (Switzer-Dunlap and Hadfield, 1977; Paige, 1988). These same chemical cues could be used by adults to locate new palatable food sources.

The palatability of *Lyngbya* to grazers is an important factor in determining which species of *Lyngbya* are more likely to be consumed than others. *Lyngbya* species are prolific producers of many secondary metabolites (Nagle and Paul, 1999; Gerwick et al., 2001; Burja et al., 2001; Osborne et al., 2001). Whilst many of these compounds are feeding deterrents against



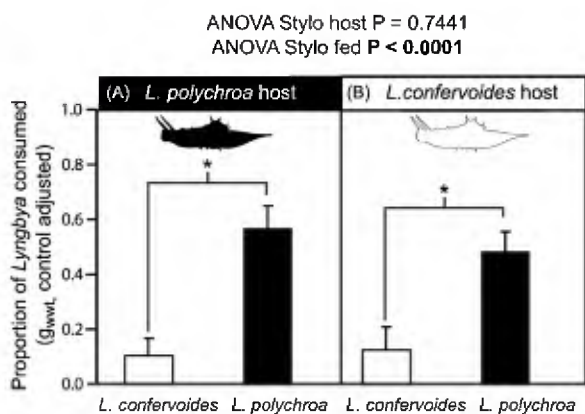


Fig. 8. *S. striatus* pair-wise choice assay. Mean blotted wet weight (g<sub>wwt</sub>) of: (A) *L. polychroa* (■) and *L. confervoides* (□) fed to *S. striatus* from a *L. polychroa* host; and (B) *L. polychroa* (■) and fed *L. confervoides* (□) fed *S. striatus* from a *L. confervoides* host over a 24 h period (control adjusted  $\pm$ S.E.). Two-way ANOVA was used to analyse data. \* indicates a significant difference ( $P < 0.05$ ) in consumption of foods using Tukey's *post hoc* analysis ( $n = 8$ ).

generalist herbivores including fish, sea urchins and crabs (Paul and Pennings, 1991; Pennings et al., 1996; Thacker et al., 1997; Nagle and Paul, 1998, 1999; Cruz-Rivera and Paul, 2002), some *Lyngbya* secondary metabolites can act as feeding stimulants to sea hares. Nagle et al. (1998) found that barbamides stimulated feeding at natural concentrations in *S. longicauda* whereas, malyngamides and majusculamides stimulated feeding at lower concentrations and inhibited feeding at higher concentrations. They also observed that *Stylocheilus* was more likely to be found on *Lyngbya* containing malyngamides A and B and majusculamides A and B. As the synergistic effects of cyanobacterial crude extracts often elicit a stronger response

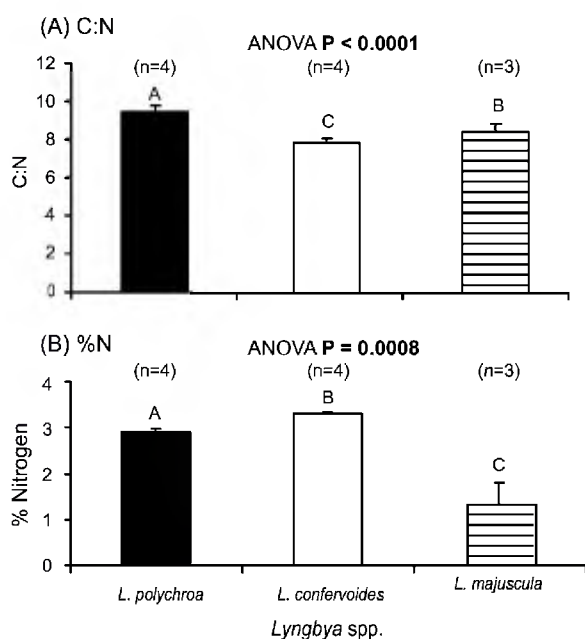


Fig. 9. C:N and %nitrogen content in three different species of *Lyngbya*. One-way ANOVA was used to analyse data C:N and %N. Letters above bars represent significant differences ( $P < 0.05$ ) between species using Tukey's *post hoc* analysis ( $n =$  number of samples per replicate).

than the pure compounds themselves (Pietsch et al., 2001), only non-polar and polar extracts were tested in our study. None of these promoted a strong feeding response in any grazer except *L. polychroa* polar extract in *S. striatus*. Pure compounds which have since been isolated from *L. polychroa* (microcolins A and B) and *L. confervoides* (largamides and lyngbyastatin 4) non-polar extracts have not yet been tested against these opisthobranchs.

Palatability may also be based upon the role a compound plays in opisthobranch physiology, such as compounds acquired for possible defence purposes. This question has initiated some debate (Paul and Pennings, 1991; Pennings et al., 1999, 2001; Rogers et al., 2000) as most compounds are compartmentalised in the digestive gland affording little protection as the sea hare must be eaten by the predator before it encounters the deterrent (Pennings et al., 2001). Accumulation of extremely high concentrations of dietary-derived metabolites in the digestive gland is more likely to assist detoxification of a chemically rich diet than deter predators (Pennings et al., 1996, 1999). Does this compound acquisition come at a cost to the consumer? *Stylocheilus* often bioaccumulates *Lyngbya* secondary metabolites and stores them in the digestive gland (Watson, 1973; Pennings and Paul, 1993a; Capper et al., 2006b). Conversion or possible detoxification to less harmful acetates (Pennings and Paul, 1993a,b; Pennings et al., 1996) may greatly reduce the overall energy gained from ingestion (Porter and Orcutt, 1980), resulting in poor growth rates. Whilst malyngamides are sequestered by *S. longicauda* in the digestive gland (Pennings and Paul, 1993a), addition of malyngamides to an artificial diet suppressed sea hare growth (Pennings and Paul, 1993b). Dietary administration of malyngamide B also resulted in reduced growth rates of *Aplysia* spp. (Pennings and Carefoot, 1995). Due to the temporal and spatial variation in the production of compounds in *Lyngbya* spp. (Nagle and Paul, 1999; Gerwick et al., 2001), an ability to detect changes in secondary metabolite concentrations could be advantageous to the consumer to help reduce potential negative post-ingestive consequences (Pennings and Carefoot, 1995; Rogers et al., 1995; Thacker et al., 1997).

In this study, consumption rates, animal growth (i.e. increase or decrease in blotted animal wet weight, g<sub>wwt</sub>) and conversion of food mass to body mass were used as performance indicators to ascertain consumer fitness based on *Lyngbya* species. Whilst *S. striatus* fed monospecific diets of *L. polychroa* or *L. confervoides* grew at equal rates, those fed *L. polychroa* consumed significantly more to achieve an equivalent biomass increase. *S. striatus* fed *U. intestinalis* decreased in biomass suggesting this is not a typical food source for this organism. All groups of *S. striatus* tested in this assay originated from a *L. polychroa* bloom in Broward County. This posed the question as to whether host origin influences consumption patterns, i.e. whether *S. striatus* originating from *L. polychroa* blooms are more likely to consume *L. polychroa* whilst those originating from *L. confervoides* blooms are more likely to consume *L. confervoides*. If this is the case, how does host switching influence the fitness of the sea hare?

*S. striatus* from each host species of *L. polychroa* and *L. confervoides* were split into two groups and maintained on a monospecific diet of either host or alternative species of *Lyngbya*. Growth rates were significantly different based on diet type regardless of host origin. *S. striatus* originating from *L. confervoides* had a significantly greater increase in biomass when fed a monospecific diet of *L. polychroa* compared to its counterpart originating from a *L. polychroa* host maintained on a diet of *L. confervoides*. This group also consumed significantly more food over the same time period. This suggests that continual consumption of *L. confervoides* by *S. striatus* may compromise the overall fitness of the animal if growth is used as a measurement parameter. One possible explanation for the differences in growth patterns observed could be related to one of the secondary metabolites present in *L. confervoides*. *L. confervoides* contains cyclic depsipeptides known as largamides, some of which inhibit chymotrypsin (Plaza and Bewley, 2006) and lyngbyastatin 4, which is also a chymotrypsin inhibitor (Matthew et al., 2007). Chymotrypsin is a digestive enzyme that performs proteolysis facilitating the cleavage of peptide bonds by hydrolysis. Inhibition of this process may result in proteinaceous material passing through the sea hare gut system without nutritional gain. Switching to a food item that does not contain such enzyme inhibitors may therefore be beneficial to the sea hare. Whilst this study only looked at growth as an indicator of performance, other parameters could also be measured such as fecundity and survivability on monospecific diets or artificial foods containing known compounds.

These findings suggest that a rapid growth increase can be attained by switching from a *L. confervoides* host to *L. polychroa*. The larger the sea hare grows, the more it will consume. As a potential top-down controller of blooms, host switching to a more palatable *Lyngbya* is likely to affect bloom dynamics resulting in the decimation of one *Lyngbya* spp. (such as *L. polychroa*) but persistence of another (such as *L. confervoides*). This is particularly important in sites in Broward County where *L. confervoides* and *L. polychroa* often co-exist on coral reefs. These findings were further confirmed in a simulated assay of host switching. When offered *L. polychroa* and *L. confervoides* simultaneously, *S. striatus* consumed significantly more *L. polychroa* regardless of host origin.

If one species of *Lyngbya* is more readily consumed and promotes better growth rates than another, one might expect this choice to be related to nutritional content. Nitrogen is often limiting in marine environments (Mattson, 1980), therefore some herbivorous grazers have adapted to utilizing cyanobacteria as a food source (O'Neil, 1999). Ready access to high N source that is not generally exploitable by other grazers may be an important trade-off for existence in marine depurate environments (O'Neil, 1999). Specialization upon a single species that supports maximal nutritional value is advantageous if it can also support increased herbivore growth (Barile et al., 2004). If *L. polychroa* is the food of choice for *S. striatus*, one might expect it to have a low C:N value and high

%N. However, this was not the case. *L. confervoides* had the lowest C:N value and highest %N compared to *L. polychroa* and *L. majuscula*. Nutritional values may not therefore be a good indicator for assessing dietary preference in *S. striatus*. This also appears to be the case for other sea hares with Rogers et al. (1995) suggesting that animals may supplement nutritional deficiencies through activities of gut symbionts. It therefore appears that host switching is more likely to be related to chemical cues and/or attractants than nutritional requirements.

## 5. Conclusion

In summary, all opisthobranchs tested consumed all species of *Lyngbya* in multiple choice feeding assays, and only *B. striata* showed a significant preference for *L. polychroa*. The palatability of non-polar vs. polar extracts of *L. polychroa*, *L. confervoides* and *L. majuscula* to opisthobranchs did not differ from the controls. However, when comparing polar extracts, *L. polychroa* significantly stimulated feeding over *L. confervoides* in *S. striatus*. Consumption of *L. polychroa* promoted higher growth rates in *S. striatus* with a more efficient conversion of food to body mass when fed a monospecific diet compared to *S. striatus* fed *L. confervoides*. Host specificity did not affect diet choice suggesting that *S. striatus* are likely to switch to a *L. polychroa* diet should one become available regardless of host origin. This could have important ecological implications for bloom dynamics and suggest opisthobranchs may indeed play a role in top-down control of blooms. It appears that preference for *L. polychroa* is more likely to be a factor of secondary metabolite composition than nutritive value, raising the question as to whether particular compounds are acquired for a physiological purpose such as defence or avoided due to digestive or storage incapability's which may ultimately compromise consumer fitness.

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