

## Amphipod herbivory on the freshwater cyanobacterium *Lyngbya wollei*: Chemical stimulants and morphological defenses

Frank A. Camacho and Robert W. Thacker<sup>1</sup>

Department of Biology, University of Alabama at Birmingham, Birmingham, Alabama 35294-1170

### Abstract

The freshwater cyanobacterium *Lyngbya wollei* forms dense mats in lentic systems throughout the southeastern United States and produces paralytic shellfish poisons (PSPs), such as saxitoxins, that could provide a chemical defense against herbivory. In addition, *Lyngbya* filaments are surrounded by a prominent extracellular polysaccharide sheath that might function as a structural defense against herbivory. We investigated the roles of PSPs and sheath structure in deterring consumption of *Lyngbya* by an omnivorous amphipod, *Hyaella azteca*. A series of two-choice feeding assays paired *Lyngbya* with a highly palatable green alga, *Rhizoclonium hieroglyphicum*. These assays included comparisons of whole *Lyngbya* and *Rhizoclonium*, freeze-dried and ground *Lyngbya* and *Rhizoclonium*, artificial food treated with *Lyngbya* crude extract, artificial food treated with pure saxitoxin, and artificial food containing *Lyngbya* sheath material, with and without *Lyngbya* crude extract. *Hyaella* preferred whole, live *Rhizoclonium* over fresh *Lyngbya*. Similarly, *Hyaella* preferred freeze-dried and ground *Rhizoclonium* over *Lyngbya*. However, *Hyaella* preferred treated foods containing *Lyngbya* crude extract or pure saxitoxin over control foods. The sheath constituted over 55% of the total dry mass of *Lyngbya*. In assays combining sheath material and crude extract in artificial foods, *Hyaella* avoided foods containing sheath material. Therefore, morphological defenses play an important role in deterring consumption of *Lyngbya* by *Hyaella*, whereas PSPs stimulate feeding. Our study indicates that structural defenses can partially explain the high abundance of filamentous cyanobacteria observed in aquatic communities under grazing pressure.

Freshwater cyanobacteria are common components of culturally affected aquatic ecosystems and major constituents of harmful algal blooms (Carmichael 1994). In recent years, considerable attention has been drawn to bloom-forming cyanobacteria because their toxic secondary metabolites have been implicated in numerous livestock, canine, and fish deaths worldwide (Chorus and Bartram 1999). Most freshwater cyanobacterial secondary metabolites can be grouped into two classes of toxins on the basis of their mode of biological activity: hepatotoxins (e.g., microcystins) and neurotoxins, such as paralytic shellfish poisons (PSPs; e.g., saxitoxin (STX) and anatoxin; Carmichael 1994; Kaebnick and Neilan 2001). Previous studies have demonstrated that PSPs can alter the feeding behavior and survivorship of freshwater zooplankton (Haney et al. 1995; Gilbert 1996). However, the effects of PSPs on larger freshwater invertebrates, particularly mesograzers capable of consuming filamentous cyanobacteria, remain largely unexplored. In marine systems, such organisms are important consumers of primary production and exert considerable grazing pressure (Paul et al. 2001). Chemical defenses against freshwater mesograzers could maintain the

persistence of noxious cyanobacteria after the initial onset of bloom formation.

Because of their morphological simplicity, few studies have considered whether structural features of cyanobacteria can serve as effective defenses against herbivory. Among aquatic macrophytes and marine algae, structural traits are often effective barriers to grazers (Hay et al. 1994; Cronin et al. 2002). The most prominent morphological feature of many cyanobacterial taxa is a glycocalyx, or sheath, that surrounds the cells and consists primarily of polysaccharides (Robbins et al. 1998; Stal 2000). Several hypotheses have been proposed for the function of these extracellular polysaccharides, including evidence for their roles as a barrier to oxygen in nonheterocystous nitrogen-fixing species (Paerl et al. 1991) and as a sink for excess fixed carbon (Otero and Vincenzini 2004). However, the robustness of the sheath in *Lyngbya* and the observation that many species are unpalatable to a broad suite of herbivores suggest that cyanobacterial extracellular polysaccharides could also function as a morphological defense.

The filamentous cyanobacterium *Lyngbya wollei* forms dense mats in lentic systems throughout the southeastern United States (Speziale and Dyck 1992) and produces several types of PSPs (Carmichael et al. 1997; Onodera et al. 1997). Sympatric with these *L. wollei* mats are other aquatic macrophytes, including a filamentous green alga, *Rhizoclonium hieroglyphicum*. A diverse assemblage of invertebrate mesograzers lives on and within the mats, particularly the amphipod *Hyaella azteca*. Although this amphipod is omnivorous, it readily consumes plant and algal tissues. Littoral densities of *Hyaella* in aquatic systems have been reported to be as high as 17,000 individuals per square meter (Alcocer et al. 1998), suggest-

<sup>1</sup> Corresponding author (thacker@uab.edu).

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ing that this species might be an important consumer in nearshore communities.

The purpose of this study was to analyze the roles of the chemical and structural elements of *L. wollei* in deterring herbivory by an omnivorous crustacean. In particular, we tested the hypotheses that (1) live *Lynngbya* is less palatable to *Hyaella* than a common filamentous green alga, *Rhizoclonium*, (2) crude extracts of *Lynngbya* and pure STX deter *Hyaella* from feeding, and (3) the extracellular polysaccharide sheath of *Lynngbya* is an effective structural defense against herbivory by *Hyaella*.

## Materials and methods

**Study organisms**—Mats of the cyanobacterium *L. wollei* and the filamentous green alga *R. hieroglyphicum* were collected from Lake Guntersville in northern Alabama, a reservoir with historically high densities of aquatic macrophytes and cyanobacteria (Doyle and Smart 1998). The freshwater amphipod *H. azteca* was collected by hand from mats of *Rhizoclonium* and *Lynngbya*. Before use in assays, amphipods were maintained in 40-liter laboratory aquaria provided with constant aeration and containing detritus and fresh *Lynngbya* and *Rhizoclonium*. Water from Lake Guntersville was passed through 0.2- $\mu\text{m}$  filters before use in all assays. All assays were conducted with constant aeration at 25°C under a 12:12 light:dark (LD) photoperiod, with fluorescent lighting providing an average of 10  $\mu\text{mol}$  quanta  $\text{m}^{-2} \text{s}^{-1}$ .

**Whole *Lynngbya* and *Rhizoclonium* assay**—For an initial test of palatability, approximately 0.135 g (wet mass, after blotting) each of live *Lynngbya* and *Rhizoclonium* were added to 20 petri dishes, each containing 40 mL of filtered lake water. All dishes were maintained with constant aeration at 25°C under a 12:12 LD photoperiod, with fluorescent lighting providing an average of 10  $\mu\text{mol}$  quanta  $\text{m}^{-2} \text{s}^{-1}$ . Ten dishes received six individual *Hyaella*; each of these dishes was paired to a control dish containing only *Lynngbya* and *Rhizoclonium*. This paired control was used to estimate the amount of autogenic change in *Lynngbya* and *Rhizoclonium* during the assay (Peterson and Renaud 1989). Amphipods grazed for 26 d; subsequently, the *Lynngbya* and *Rhizoclonium* samples from each control-treatment pair were removed and reweighed.

For each control-treatment pair, the relative change in mass of each *Lynngbya* or *Rhizoclonium* sample was calculated with the formula  $(X_f - X_i)/X_i$ , where  $X_i$  is the initial wet mass of *Lynngbya* or *Rhizoclonium* and  $X_f$  is the final wet mass of *Lynngbya* or *Rhizoclonium*. The relative change in mass of a grazed alga or cyanobacterium was then subtracted from the relative change in mass of its paired autogenic control (Peterson and Renaud 1989). Values were arcsine-transformed to meet the assumptions of a paired *t*-test comparing net relative change in *Lynngbya* to *Rhizoclonium* (Sokal and Rohlf 1995).

**Freeze-dried, ground *Lynngbya* and *Rhizoclonium* assay**—Feeding assays were conducted using lyophilized, ground *Lynngbya* and *Rhizoclonium* added to an agar matrix to

assess cyanobacterial palatability with the structural continuity of the extracellular polysaccharide sheath removed. We modified previously described methods (Hay et al. 1994, 1998; Thacker et al. 1998) and combined 0.31 g of agar with 10 mL of distilled water, then heated this mixture in a microwave for 15 s. After the mixture cooled to <60°C, a 0.4-g sample of either ground *Lynngbya* or *Rhizoclonium* was mixed into the agar, and the mixture was poured onto nylon-coated fiberglass screening before being pressed between wax paper and two panes of glass with 9 kg of force. The mixture was allowed to cool, and 10 screens each of *Lynngbya* and *Rhizoclonium* were cut with a razor blade. Each individual screen consisted of 100 squares of food in addition to one empty square that was cut to distinguish between control and treated food.

Individual replicates ( $n = 15$ ) consisted of a petri dish containing six amphipods and 40 mL of filtered water from Lake Guntersville. Each replicate received one *Lynngbya* and one *Rhizoclonium* screen. Amphipods were allowed to graze on both screens until at least half of the squares on one of the screens were cleared of food. Both screens in the dish were then removed and the number of squares of *Lynngbya* and *Rhizoclonium* completely cleared of food was counted. Replicates in which neither of the strips was adequately consumed at the end of 1 week were not scored. Differences in the consumption of *Lynngbya* and *Rhizoclonium* were compared by a paired *t*-test (Sokal and Rohlf 1995).

**Purification of *Lynngbya* sheath**—To determine the relative mass of the extracellular polysaccharide sheath surrounding *Lynngbya* filaments, samples of whole, freeze-dried *L. wollei* were weighed and digested for 24 h in 60 mL of water and 10% commercial bleach. Filaments were then rinsed under distilled water for at least 30 min to remove residual bleach as well as any visible pigment. Several filaments were removed from each sample and viewed under a compound microscope to ensure that all intracellular material was removed. Samples were then lyophilized to remove any remaining water before they were reweighed.

**Crude extract versus sheath assays**—We compared the palatability of *Lynngbya* crude extracts in the presence and absence of ground *Lynngbya* sheath material by again using artificial foods coated onto window screening. Control food strips contained freeze-dried, ground *Rhizoclonium* and agar, as described above. Treated foods added crude extracts, sheath, or both to the control food. Crude extracts were prepared according to the methods of Buckley et al. (1976). Weighed, freeze-dried *Lynngbya* were extracted overnight in 25% MeOH and 75% acetic acid (50 mmol  $\text{L}^{-1}$ ). Extracts were passed through 11- $\mu\text{m}$  filters (Whatman), then through 300-mg C-18 cartridges (Alltech). Samples were frozen at -80°C and lyophilized to remove the solvent. The relative concentration of crude extract per dry mass of *Lynngbya* was determined by weighing the remaining extract, whereas the amount of STX in the crude extract was estimated with the Ridascreen STX immunoassay (R-Biopharm, Inc.). To prepare powdered sheaths for

addition to artificial foods, freeze-dried *Lyngbya* was bleached and dried as described above before being ground with a mortar and pestle. Ground sheath was then sieved through 500- $\mu\text{m}$  mesh and stored at room temperature.

Three artificial food assays were performed: (1) control food versus control food plus crude extract, (2) control food versus control food plus pure STX, and (3) control food with and without crude extract in the presence or absence of *Lyngbya* sheath. For the crude extract assay, both control and treated foods consisted of a mixture of 10 mL of water and 0.15 g of agar that was microwaved for 15 s. After the mixture cooled to  $<60^\circ\text{C}$ , 0.4 g of ground, lyophilized *Rhizoclonium* ( $<500\ \mu\text{m}$  particle size) was added to the mixture. Treated food received a 1-mL aliquot of water containing 61.79 mg of crude extract, resulting in a food mixture that approximated the natural dry mass yield (10.1%) of crude extract. Control food received only 1 mL of water. Each food was stirred thoroughly before being spread onto fiberglass screening and pressed between wax paper and glass plates, as previously described. Screens were removed from the press after 5 min of cooling and cut into  $7 \times 7$  strips with a razor blade. One control and one treated food strip were then added to individual glass beakers ( $n = 20$ ), each containing 10 amphipods and 300 mL of filtered water from Lake Guntersville. Amphipods in each beaker were allowed to feed on the food strips until approximately half of one of the strips was eaten; replicates in which neither of the strips was adequately consumed at the end of 1 week were not scored. Differences in the consumption of control and treated foods were compared by paired *t*-test (Sokal and Rohlf 1995).

For the pure STX assay, artificial foods were prepared as described above. Treated food received 1 mL of water containing 497 ng of STX (R-BioPharm, Inc.), whereas control foods received 1 mL of water only. This amount of STX approximated the natural dry mass concentration (906 ng  $\text{g}^{-1}$ ) of PSPs in our *Lyngbya* samples. Food was molded and cut in the same manner as the crude extract assay. Thirteen replicate beakers, each containing 10 amphipods and 300 mL of filtered lake water, received one control and one treated strip. After allowing the amphipods to feed, strips were removed and scored as described above. Differences in the consumption of control and treated foods were compared by paired *t*-test (Sokal and Rohlf 1995). To assess the potential for STX to leach out of foods during the assay, we held eight control and eight treated food strips in identical conditions without herbivores. We extracted STX from these foods and quantified STX concentrations according to the procedures detailed above.

The potentially interactive effects of *Lyngbya* crude extract and sheath material on feeding by *Hyaella* were analyzed in a factorial design. Artificial food with and without *Lyngbya* crude extract received either purified *Lyngbya* sheath or no sheath. For each food type, 3 mL of water and 0.06 g of agar were microwaved for 20 s and cooled to  $<60^\circ\text{C}$ , after which 0.1 g of lyophilized, ground *Rhizoclonium* were added to each mixture. Because the extracellular sheath of *Lyngbya* constituted  $55.7\% \pm 3.6\%$

( $n = 8$ ) of the dry mass, sheath treatments incorporated 0.2 g of purified ground *Lyngbya* sheath ( $<500\ \mu\text{m}$  particle size) to simulate structurally defended food. Foods lacking sheath material received 0.2 mL of water. Crude extract was incorporated at natural dry mass concentrations (on the basis of the mass of agar, *Rhizoclonium*, and sheath in the artificial foods) by suspending 40 mg of extract in 0.2 mL of water and adding it to the food containing sheath material. We chose to keep the absolute amount of crude extract constant; therefore, food containing no sheath material also received 40 mg of crude extract; the dry mass concentration of crude extract in this food was 20%, almost double the concentration used in the previously described crude extract assay. The four food mixtures were each spread onto fiberglass mesh and pressed thinly, as described above, before  $5 \times 5$  strips of each food type were cut with a razor. One additional square was left open and nicked with a razor to distinguish between control and treated foods. A strip of each of the four food types was paired to a strip of control food; each pair was added to a beaker containing three amphipods and 300 mL of filtered lake water. The relative amount of food eaten for each replicate ( $n = 10$  per treatment) was calculated as  $(C - T) / (C + T)$ , where C and T were the number of squares of control and treated food consumed, respectively. Because the raw data did not meet the assumptions for analysis of variance, values were rank-transformed before analysis with sheath content and extract content as main effects (Sokal and Rohlf 1995).

## Results

For the assay with whole, live *Lyngbya* and *Rhizoclonium*, autogenic changes in dishes without amphipods were minimal (means  $\pm$  SE,  $n = 10$ ; *Rhizoclonium*,  $4 \pm 26$  mg; *Lyngbya*,  $11 \pm 28$  mg). Mats of both species showed no evidence of pigment loss during the assay. After correcting for autogenic changes, *Hyaella* consumed significantly more *Rhizoclonium* than *Lyngbya* ( $t = 4.071$ ,  $n = 10$ ,  $\text{df} = 9$ ,  $p = 0.003$ ; Fig. 1), resulting in a net loss of mass of the alga over the course of the assay. This change in *Rhizoclonium* mass was equivalent to a consumption rate of  $1.24\ \text{mg}\ \text{amphipod}^{-1}\ \text{d}^{-1}$ . Although *Lyngbya* growth was not significantly different from zero in the autogenic controls, amphipod grazing of *Rhizoclonium* was associated with significant *Lyngbya* growth, averaging a 15% increase in wet mass (Fig. 1). This growth was likely stimulated by the excretion of nutrients by amphipods and the release of nutrients stored in *Rhizoclonium* cells. Visual analysis of amphipod frass confirmed that both *Rhizoclonium* and *Lyngbya* cells were consumed by *Hyaella*; however, growth by *Lyngbya* in these mixed cultures prevented an estimate of the consumption rate of *Lyngbya* by *Hyaella*.

Removing the structural continuity of *Lyngbya* filaments by freeze-drying, grinding, and adding them to artificial food did not alter the preference of *Hyaella* for food containing *Rhizoclonium* ( $t = 4.08$ ,  $n = 8$ ,  $\text{df} = 7$ ,  $p < 0.005$ ; Fig. 2). Amphipods given a choice of food with and without crude extract at natural concentrations (10.1% dry mass) consumed more of the extract-containing food after

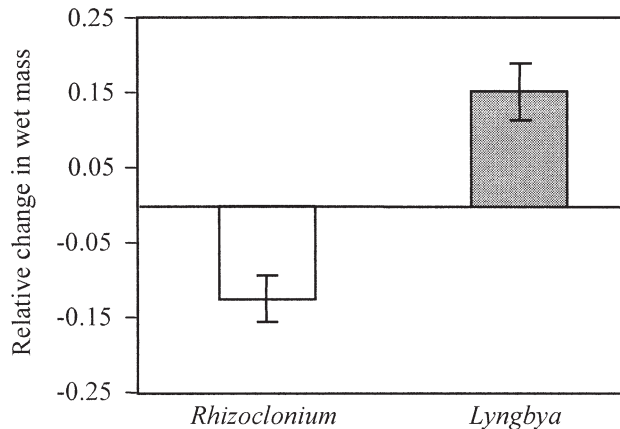


Fig. 1. *Hyalella* consumption of whole *Rhizoclonium* and *Lyngbya*. Bars represent means  $\pm$  1 SE. Treated dishes ( $n = 10$ ) contained six amphipods and were paired with control dishes to account for autogenic changes during the assay.

6 d of feeding ( $t = -3.97$ ,  $n = 18$ ,  $df = 17$ ,  $p < 0.001$ ; Fig. 3). When *Hyalella* was presented with control food paired with food containing STX at natural concentrations ( $906 \text{ ng g}^{-1}$ ), a similar pattern emerged after 3 d of feeding ( $t = -3.92$ ,  $n = 13$ ,  $df = 12$ ,  $p < 0.005$ ; Fig. 3). We examined the potential for STX to leach out of the treated food during this assay by measuring STX concentrations in control and treated food strips that were held without amphipods. We detected  $28.9 \pm 2.7 \text{ pg}$  of STX per treated food strip ( $n = 8$ ); control food strips were below the detection limit. The amount of STX remaining in treated food strips was approximately 20% of the total amount incorporated; thus, substantial amounts of STX leached out of the treated foods.

The extracellular polysaccharide sheath of *Lyngbya* constituted over  $55.7 \pm 3.6\%$  of the dry mass of the bleached samples, with sheath dry mass and total dry mass displaying a significant positive linear correlation ( $n = 8$ ;  $r^2 = 0.976$ ;  $p < 0.005$ ). For the feeding assays manipulating

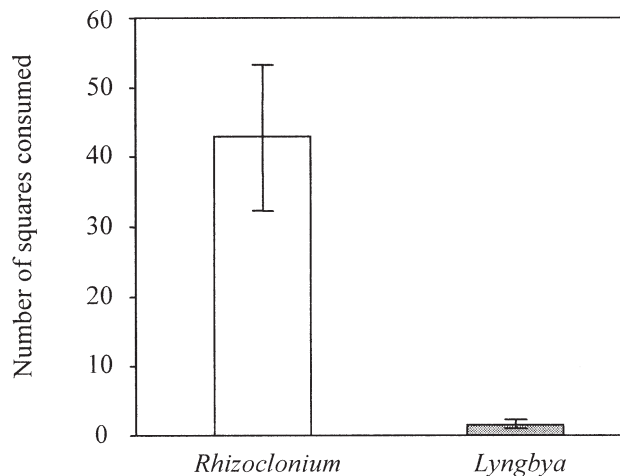


Fig. 2. *Hyalella* consumption of ground *Rhizoclonium* and *Lyngbya* in artificial food. The paired foods were placed in dishes ( $n = 8$ ) containing six amphipods. Bars represent means  $\pm$  1 SE.

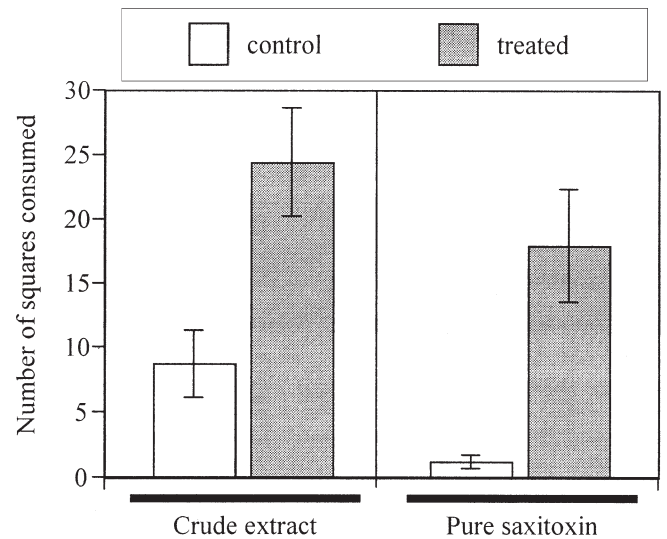


Fig. 3. *Hyalella* consumption of artificial foods containing *Lyngbya* crude extract ( $n = 18$ ) and pure STX ( $n = 13$ ). For each assay, paired control and treated food strips were placed in beakers containing 10 amphipods. Bars represent means  $\pm$  1 SE.

the presence of *Lyngbya* crude extract and sheath material, a significant interaction between the extract and sheath treatments was detected ( $p < 0.001$ ; Table 1). In the absence of sheath material, extract-containing food was preferentially consumed over control food; however, the presence of *Lyngbya* sheath material deterred *Hyalella* from consuming extract-containing food (Fig. 4).

## Discussion

Our experiments demonstrate that amphipods are stimulated to feed by the crude extract of *L. wollei* and pure STX but are deterred by the extracellular polysaccharide sheath that surrounds *Lyngbya* filaments. We initially hypothesized that *Lyngbya* would be resistant to herbivory by amphipods in light of the negative effect of cyanobacterial neurotoxins on zooplankton (Haney et al. 1995). As predicted, amphipods consumed more live *Rhizoclonium* than *Lyngbya*, decreasing *Rhizoclonium* growth while stimulating *Lyngbya* growth. Although we did not monitor damage to *Lyngbya* filaments, the presence of *Lyngbya* cells in amphipod frass indicated that amphipods were able to consume this cyanobacterium. Similarly, *Hyalella* preferentially consumed ground *Rhizoclonium* over *Lyngbya* when embedded within agar food strips in a paired feeding assay. Because the filaments were dried, ground, and sieved through  $500\text{-}\mu\text{m}$  mesh before addition to the foods, we assumed that any structural effects of the sheath were removed (Hay et al. 1998). However, the possibility that the sheath component of ground *Lyngbya* reduced digestibility or rendered the food unpalatable was not excluded (Hay et al. 1994).

The PSP concentration from our *Lyngbya* collection ( $906 \text{ ng g}^{-1}$  dry mass) was within the range of values reported for *Lyngbya* (Carmichael et al. 1997). Although STX has not been identified from *Lyngbya* in Lake

Table 1. Results of a two-way fixed-factor analysis of variance of the relative consumption of artificial foods. Sheath and extract content were independent variables ( $n = 10$  per treatment).

Factor	Sum of squares	df	Mean squares	F	p
Sheath	4,000.0	1	4,000.0	356.2	<0.001
Extract	193.6	1	193.6	17.2	<0.001
Sheath $\times$ extract	313.6	1	313.6	27.9	<0.001
Error	404.3	36	11.2		

Guntersville, other analogs, such as decarbamoyl STX and decarbamoyl gonyautoxin, have been isolated (Onodera et al. 1997). The concentration of pure STX that we added to artificial foods effectively deters cladocerans from ingesting toxic cells (Haney et al. 1995). However, we found that the addition of either crude extract or STX to artificial foods enhanced feeding by amphipods, even at double the natural concentration of PSPs. Although PSPs might have partially leached out of the artificial foods during the assays, we were able to detect STX in the treated foods, but not the control foods, at the end of the assays. The similarity in feeding response between pure STX and the crude extract suggests that both treatments have similar physiological effects on *Hyalella* and that PSPs are not effective feeding deterrents against *Hyalella*.

Because the *Lyngbya* crude extract and pure STX were feeding stimulants for *Hyalella*, we explored the role of the extracellular polysaccharide sheath as a feeding deterrent.

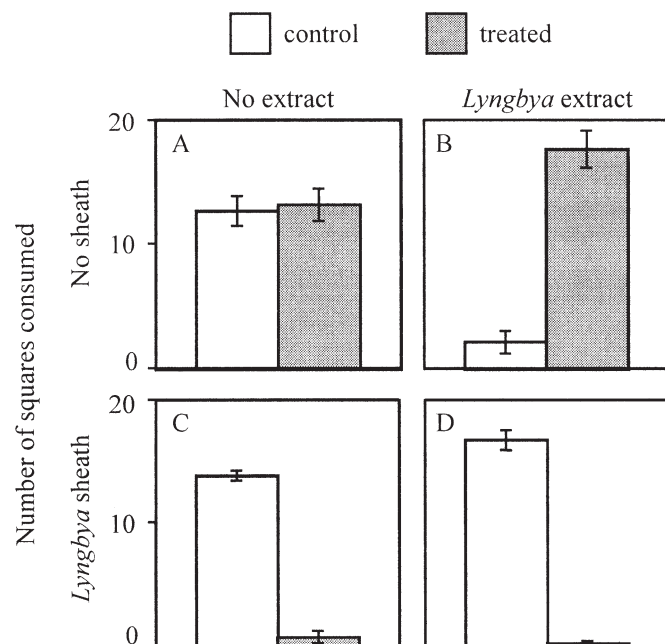


Fig. 4. *Hyalella* consumption of artificial foods that manipulated the presence or absence of *Lyngbya* crude extract and sheath material. In panel A, control and treated foods were identical in composition, containing *Rhizoclonium* and agar. In panel B, *Lyngbya* crude extract was added to the treated food. In panel C, *Lyngbya* sheath was added to the treated food. In panel D, both crude extract and sheath were added to the treated food. For each control-treatment pair ( $n = 10$ ), food strips were placed in beakers containing 3 amphipods. Bars represent means  $\pm$  1 SE.

The sheath was a considerable component of the *Lyngbya* filaments (55% of dry mass, or 15% of wet mass). Not surprisingly, the sheath of *L. wollei* is among the thickest of its genus (Speziale and Dyck 1992). In comparison, Robbins et al. (1998) determined that 0.6% of the wet mass of *L. aestuarii* was composed of sheath material. Amphipods were stimulated to feed on artificial foods containing crude extracts but were strongly deterred when sheath material was added to control or extract-treated foods. This reversal of amphipod food preferences is sufficient to explain our observations of feeding deterrence when amphipods were given a choice between live *Lyngbya* and *Rhizoclonium*. Similar interactions between chemical defenses and structural defenses have been reported for marine algae (Hay et al. 1994).

The pattern of enhanced feeding on foods treated with crude extract or STX was not expected and suggests that PSP-producing cyanobacteria with thin or absent extracellular polysaccharide sheaths could be more vulnerable to herbivory by *Hyalella*. *Hyalella* might also be able to tolerate or detoxify PSPs because amphipods can tolerate other types of chemical defenses. For example, *Hyalella* prefers senesced roots of the chemically defended plant *Berula erecta* over periphyton with no observed loss of fitness (Rowell and Blinn 2003). *Ampithoe longimana*, a marine amphipod, displays a genetically based tolerance of the secondary metabolites produced by *Dictyota*, a genus of brown algae (Sotka and Hay 2002). Even though *Lyngbya* crude extracts and pure STX stimulated feeding, it is still possible that PSPs could be deterrent at concentrations higher than those incorporated into our artificial foods. For example, Nagle et al. (1998) found that the opisthobranch sea hare *Stylocheilus longicauda* was stimulated to feed when given food with low to moderate concentrations of majusculamide from the marine cyanobacterium *Lyngbya majuscula* but was deterred from feeding at high concentrations of the toxin. Nevertheless, our observations are based on artificial foods that incorporated both crude extract and sheath material at concentrations similar to those that *Hyalella* would experience in Lake Guntersville.

Our feeding assays demonstrate that the relatively simple structural traits of cyanobacteria could be potent defenses against crustacean mesograzers. Similar morphologic traits have been identified in cyanobacteria as defenses against zooplankton. For example, DeMott et al. (2001) observed that structural interference by cyanobacterial filaments can inhibit *Daphnia* feeding rates. Similarly, Trabeau et al. (2004) found that seasonal increases in capsule thickness of *Microcystis* were correlated with declines in *Daphnia magna*

abundance. Fialkowska and Pajdak-Stós (1997) reported striking evidence for a defensive function of sheaths in *Phormidium*, observing that filaments tended to aggregate and form mucilage in the presence of ciliates; when filaments came into direct contact with a ciliate grazer, cells retracted into the sheath in an apparent antipredatory response.

Our finding that the sheath of *Lyngbya* deters feeding by crustacean mesograzers raises several intriguing questions. For example, variability in sheath thickness among cyanobacterial species might reflect a trade-off between defense and some other physiological function. It is not known whether *Lyngbya* sheaths can serve as sinks for excess carbon (Otero and Vincenzini 2004) or whether carbon invested in the sheath can be considered a cost of defense. In addition, the sheath might not be an effective defense against grazers that have more robust feeding structures, such as snails and fish. Finally, *Lyngbya* mats might provide a refuge from predation for *Hyalella*, yielding the possibility of tritrophic interactions among fish, *Hyalella*, *Lyngbya*, and surrounding algae. Future investigations of these interactions will not only advance our knowledge of freshwater chemical ecology, but also our understanding of harmful algal blooms in freshwater ecosystems.

## References

- ALCOCER, J., E. ESCOBAR, A. LUGO, AND L. PERALTA. 1998. Littoral benthos of the saline crater lakes of the basin of Oriental, Mexico. *Int. J. Salt Lake Res.* **7**: 87–108.
- BUCKLEY, L. J., M. IKAWA, AND J. J. SASNER. 1976. Isolation of *Gonyaulax tamarensis* toxins from soft shell clams (*Mya arenaria*) and a thin-layer chromatographic-fluorometric method for their detection. *J. Agric. Food Chem.* **24**: 107–111.
- CARMICHAEL, W. W. 1994. The toxins of cyanobacteria. *Sci. Am.* **270**: 78–86.
- , W. R. EVANS, Q. Q. YIN, P. BELL, AND E. MOCZYDŁOWSKI. 1997. Evidence for paralytic shellfish poisons in the freshwater cyanobacterium *Lyngbya wollei* (Farlow ex Gomont) comb. nov. *Appl. Environ. Microbiol.* **63**: 3104–3110.
- CHORUS, I., AND J. BARTRAM [EDS.]. 1999. Toxic cyanobacteria in water: A guide to their public health consequences, monitoring, and management. E & FN Spon.
- CRONIN, G., AND OTHERS. 2002. Crayfish feeding preferences for freshwater macrophytes: The influence of plant structure and chemistry. *J. Crust. Biol.* **22**: 708–718.
- DEMOTT, W. R., R. D. GULATI, AND E. VAN DONK. 2001. *Daphnia* food limitation in three hypereutrophic Dutch lakes: Evidence for exclusion of large-bodied species by interfering filaments of cyanobacteria. *Limnol. Oceanogr.* **46**: 2054–2060.
- DOYLE, R. D., AND R. M. SMART. 1998. Competitive reduction of noxious *Lyngbya wollei* mats by rooted aquatic plants. *Aquat. Bot.* **61**: 17–32.
- FIALKOWSKA, E., AND A. PAJDAK-STÓS. 1997. Inducible defense against a ciliate grazer, *Pseudomicrothorax dubius*, in two strains of *Phormidium* (cyanobacteria). *Proc. R. Soc. Lond., B* **264**: 937–941.
- GILBERT, J. J. 1996. Effect of temperature on the response of planktonic rotifers to a toxic cyanobacterium. *Ecology* **77**: 1174–1180.
- HANEY, J. F., J. J. SASNER, AND M. IKAWA. 1995. Effects of products released by *Aphanizomenon flos-aquae* and purified saxitoxin on the movements of *Daphnia carinata* feeding appendages. *Limnol. Oceanogr.* **40**: 263–272.
- HAY, M. E., Q. E. KAPPEL, AND W. FENICAL. 1994. Synergisms in plant defenses against herbivores: Interactions of chemistry, calcification, and plant quality. *Ecology* **75**: 1714–1726.
- , J. J. STACHOWICZ, E. CRUZ-RIVERA, S. BULLARD, M. S. DEAL, AND N. LINDQUIST. 1998. Bioassays with marine and freshwater macroorganisms, p. In K. F. Haynes and J. G. Millar [eds.], *Methods in chemical ecology*, v. 2, bioassay methods. Chapman and Hall.
- KAEBERNICK, M., AND B. A. NEILAN. 2001. Ecological and molecular investigations of cyanotoxin production. *FEMS Microbiol. Ecol.* **35**: 1–9.
- NAGLE, D. G., F. T. CAMACHO, AND V. J. PAUL. 1998. Dietary preferences of the opisthobranch mollusc *Stylocheilus longicauda* for secondary metabolites produced by the tropical cyanobacterium *Lyngbya majuscula*. *Mar. Biol.* **132**: 267–273.
- ONODERA, H., M. SATAKE, Y. OSHIMA, T. YASUMOTO, AND W. W. CARMICHAEL. 1997. New saxitoxin analogues from the filamentous cyanobacterium *Lyngbya wollei*. *Nat. Toxins* **5**: 146–151.
- OTERO, A., AND M. VINCENZINI. 2004. *Nostoc* (Cyanophyceae) goes nude: Extracellular polysaccharides serve as a sink for reducing power under unbalanced C/N metabolism. *J. Phycol.* **40**: 74–81.
- PAERL, H. W., L. E. PRUFERT, AND W. W. AMBROSE. 1991. Contemporaneous N<sub>2</sub> fixation and oxygenic photosynthesis in the nonheterocystous mat-forming cyanobacterium *Lyngbya aestuarii*. *Appl. Environ. Microbiol.* **57**: 3086–3092.
- PAUL, V. J., E. CRUZ-RIVERA, AND R. W. THACKER. 2001. Chemical mediation of seaweed-herbivore interactions: Ecological and evolutionary perspectives, p. 227–265. In J. McClintock and B. Baker [eds.], *Marine chemical ecology*. CRC.
- PETERSON, C. H., AND P. E. RENAUD. 1989. Analysis of feeding preference experiments. *Oecologia* **80**: 82–86.
- ROBBINS, R. A., J. BAULD, AND D. J. CHAPMAN. 1998. Chemistry of the sheath of the cyanobacterium *Lyngbya aestuarii* Lied. *Cryptogam. Algol.* **19**: 169–178.
- ROWELL, K., AND D. W. BLINN. 2003. Herbivory on a chemically defended plant as a predation deterrent in *Hyalella azteca*. *Freshw. Biol.* **48**: 247–254.
- SOKAL, R. R., AND F. J. ROHLF. 1995. *Biometry*, 3rd ed. W. H. Freeman.
- SOTKA, E., AND M. E. HAY. 2002. Geographic variation among herbivore populations in tolerance for a chemically rich seaweed. *Ecology* **83**: 2721–2735.
- SPEZIALE, B. J., AND L. A. DYCK. 1992. *Lyngbya* infestations: Comparative taxonomy of *Lyngbya wollei* comb. nov. (Cyanobacteria). *J. Phycol.* **28**: 693–706.
- STAL, L. J. 2000. Cyanobacterial mats and stromatolites, p. 61–120. In B. A. Whitton and M. Potts [eds.], *The ecology of cyanobacteria: Their diversity in space and time*. Kluwer.
- THACKER, R. W., M. A. BECERRO, W. A. LUMBANG, AND V. J. PAUL. 1998. Allelopathic interactions between sponges on a tropical reef. *Ecology* **79**: 1740–1750.
- TRABEAU, M., R. BRUHN-KEUP, C. McDERMOTT, M. KEOMANY, A. MILLSAPS, A. EMERY, AND B. DE STASIO, JR. 2004. Midsummer decline of a *Daphnia* population attributed in part to cyanobacterial capsule production. *J. Plankton Res.* **26**: 949–961.

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