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Short communication

Debromoaplysiatoxin in *Lyngbya*-dominated mats on manatees (*Trichechus manatus latirostris*) in the Florida King's Bay ecosystem

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ABSTRACT

Proliferation of the potentially toxic cyanobacterium, *Lyngbya*, in Florida lakes and rivers has raised concerns about ecosystem and human health. Debromoaplysiatoxin (DAT) was measured in concentrations up to 6.31 μg/g wet weight lyngbyatoxin A equivalents (WWLAE) in *Lyngbya*-dominated mats collected from natural substrates. DAT was also detected (up to 1.19 μg/g WWLAE) in *Lyngbya*-dominated mats collected from manatee dorsa. Ulcerative dermatitis found on manatees is associated with, but has not been proven to be caused by DAT.

Over the past century, widespread increases in eutrophication rates have led to a global magnification of harmful algal blooms in aquatic ecosystems (Hallegraeff, 1993; Phlips, 2002). One of the most prolific benthic species of algae in Florida is *Lyngbya* (Quinlan et al., 2008), which has become a management challenge because of its ability to form expansive mats, displacing more desirable plant populations, and its resistance to traditional chemical control methods (Phlips et al., 1992; Dubose et al., 1997). In addition to disruption of ecosystem structure, one of the greatest potential threats posed by *Lyngbya* is toxin production (Carmichael et al., 1997; Osborne et al., 2001; Berry et al., 2004). The marine form of *Lyngbya*, *Lyngbya majuscula*, has been shown to produce over 70 toxins, with effects ranging from skin, eye, and respiratory irritation to neuroparalytic and cytotoxic effects (Osborne et al., 2001; Shimizu, 2003; Capper et al., 2005).

Although the majority of research has focused on *L. majuscula*, two freshwater *Lyngbya* species are known to be toxigenic, *Lyngbya wollei* (Carmichael et al., 1997) and *Lyngbya aerugineo-coerulea* (Teneva et al., 2003). Both species have been shown to produce saxitoxin analogues and aplysiatoxins that have demonstrated toxicity to fish and mammals (Carmichael et al., 1997; Onodera et al., 1997; Teneva et al., 2003; Rodgers and Johnson, 2007).

Lyngbya is a dominant cyanobacterium in the benthic communities of many Florida ecosystems, such as the Homosassa River, the site of this study. The "No-name storm of 1993", or the March "Storm of the Century", in 1993 dramatically altered the Homosassa area, including King's Bay (Langtimm and Beck, 2003). A tidal surge of high salinity water killed salt-sensitive populations of aquatic plants, such as *Hydrilla*, the previous dominant flora of the ecosystem (Mataraza et al., 1999), which was replaced by *Lyngbya*. Since these events, *Lyngbya* remains one of the dominant flora in the ecosystem (Mataraza et al., 1999). The objective of our study was to identify toxins associated with algal mats in a manatee enclosure at the Homosassa River spring head (28 53′00.50″N 82

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35′21.09″W), as well as *Lyngbya*-dominated mats growing on manatee dorsum.

Lyngbya sp. samples were collected quarterly, during the winter, spring, summer, and fall of 2006, for species identification and toxicologic analysis. *Lyngbya* was opportunistically scraped from the dorsum of manatees during concurrent health assessments and placed in clean glass jars with PTFE-lined screw caps. Samples of free-living algae were also collected from rocks and tank walls and placed in standard plastic sealed bags. Species identification of live algae was performed using light microscopy with currently accepted keys (Komárek et al., 2003).

Algal samples were assayed for lyngbyatoxins A–C, aplysiatoxin, and debromoaplysiatoxin. These toxins were selected based on a reported toxicosis (Capper et al., 2005; Nagai et al., 1996) in which aplysiatoxin and lyngbyatoxin A were isolated from *Lyngbya* sp. cells coating the surfaces of *Gracilaria coronopifolia*, a nontoxic edible red alga frequently consumed by humans. Using methods described by Nagai et al. (1996) and Capper et al. (2005), all samples were extracted and analyzed via liquid chromatography (HP1100; Hewlett-Packard Company, Wilmington, DE) with mass spectrometric detection (Finnigan LCQ Ion Trap Mass Spectrometer; Finnigan MAT, San Jose, CA). Also as described in Nagai et al. (1996) and Capper et al. (2005), component identification was based on retention time and the fragmentation pattern of ions (m/z ratios) that formed during analysis and that were characteristic for each analyte. Because there was an available standard for LA, identification for this analyte was comparative against an authentic standard. For DAT and the remaining *Lyngbya* analogues, identification was based on relative retention times and reported ions, such as the molecular ion $(M+H)^+$ at m/z 593.3 and the $(M+Na)^+$ and $(M+K)^+$ ions at m/z 615 and m/z 631, respectively, for DAT. Debromoaplysiatoxin (DAT) and lyngbyatoxin A (LA) were first isolated from *L. majuscula* and evaluated structurally by NMR in 1977 and 1979, respectively (Mynderse and Moore, 1977; Cardellina et al., 1979). Due to the limited commercial availability of cyanotoxin standards, all quantifications were determined in equivalents of lyngbyatoxin A (EMD Chemicals, Calbiocem brand, San Diego, CA), against a standard curve containing at least five points and having a correlation coefficient $\geq$0.995.

The algal mat samples collected from manatee dorsum were dominated by *Lyngbya* spp. with noticeable amounts of other filamentous algae, diatoms and detritus. Other algae were present in very low quantities and varied in composition. *Lyngbya*-dominated mats were also collected from other regions of the spring run, as well as from the walls of the manatee holding tanks at Homosassa Springs. *Lyngbya* was also collected as epiphytes on the aquatic plant *Najas guadalupensis*. Manatee feces was sampled from the anal opening using rubber gloves and glass jars, when positioning allowed and feces was available.

Lyngbyatoxins A–C were below the detection limit (BDL) in all samples except for feces in which 0.085 and 0.095 μg/g WWLAE of lyngbyatoxins A and B, respectively, were identified. Aplysiatoxin was BDL in all samples except for a single sample from a manatee dorsum in which 0.70 μg/g WWLAE of aplysiatoxin was measured. Both of these samples were collected in October 2006. Debromoaplysiatoxin (DAT), a dermatitic polyacetate and weak tumor promoter (Fujiki et al., 1983, 1984) was identified in multiple samples (Table 1).

The presence of DAT in algal mats dominated by *Lyngbya* may have health implications for manatees and potentially for humans using environments containing high concentrations of the cyanobacterium. In the 1970s, human cutaneous inflammation was shown to be associated with pure extracts of DAT from the marine form, *L. majuscula*. Topical application of DAT produced an irritant pustular folliculitis in humans and a severe cutaneous inflammatory reaction in rabbits and hairless mice (Fujiki et al., 1983; Solomon and Stoughton, 1978). DAT produces dermatitis on the murine ear at 0.005 nmol (2.7 ng) per ear (Fujiki et al., 1983). The mechanism of tumor promotion and induction of the inflammatory response is likely through activation of calcium-activated, phospholipid-dependent protein kinase (protein kinase C) (Fujiki et al., 1984).

Over 70 chemically diverse metabolites with recognized biological activities have been isolated from *L. majuscula* (Shimizu, 2003). These include ichthyotoxins (antillatoxin, a sodium channel activator, and kalkitoxin, a sodium channel blocker), cytotoxins (lyngbyabellin A and B), a molluscicide (barbamide), along with immunosuppressive peptides (microlin A and B), a microtubulin inhibitor (curacin A), and the protein kinase C activators (aplysiatoxin, debromoaplysiatoxin, and lyngbyatoxins) which promote tumor formation and have also been the main causative agents for contact dermatitis and occasional incidents of human poisoning after accidental ingestion (Fujiki et al., 1985; Ito and Nagai, 1998; Yasumoto, 1998). Due to the numerous toxins that may be produced in varying concentrations, it is possible for *Lyngbya* to induce

Table 1
Debromoaplysiatoxin in *Lyngbya* sp. mats associated with manatees and other algae by LC–MS

Sample ID (n)	Debromoaplasiatoxin[a] [lyngbyatoxin A equiv (μg)/g wet wt: (mean/median) range]		
	18.0 min	21.5 min	Total
Apr-06			
Manatee dorsum (4)	(0.061/0.005) BDL–0.234	(0.015/0.005) BDL–0.050	BDL–0.284
Chlorophyta (1)	3.640	1.001	4.641
Lyngbya mat (1)	0.049	BDL	0.049
Aug-06			
Manatee dorsum (2)	BDL	BDL	BDL
Homosassa lyngbya (1)	BDL	BDL	BDL
Oct-06			
Manatee dorsum (6)	(0.099/0.092) BDL–0.257	(0.017/0.003) BDL–0.057	BDL–0.314
Nyad (1)	1.250	0.263	1.513
Lyngbya (1)	0.169	0.031	0.2
Tank wall algae (1)	4.513	1.796	6.309
Fecal sample (1)	1.861	0.080	1.941
Dec-06			
Manatee dorsum (2)	0.031, 1.048	BDL, 0.139	BDL, 1.187

BDL, below detection limit.
[a] Identical fragmentation patterns. Probable isomers.