J. Phycol. **46,** 674–678 (2010)

DOI: 10.1111/j.1529-8817.2010.00832.x

NOTE

PRESENCE AND DIVERSITY OF ALGAL TOXINS IN SUBTROPICAL PEATLAND PERIPHYTON: THE FLORIDA EVERGLADES, USA[1]

Brent J. Bellinger[2]

Soil and Water Science Department, University of Florida, Gainesville, Florida 32611, USA

and Scot E. Hagerthey

Everglades Division, South Florida Water Management District, West Palm Beach, Florida 33406, USA

Production of toxic secondary metabolites by cyanobacteria, collectively referred to as cyanotoxins, has been well described for eutrophied water bodies around the world. However, cohesive cyanobacterial mats also comprise a significant amount of biomass in subtropical oligotrophic wetlands. As these habitats generally do not support much secondary production, cyanotoxins, coupled with other physiological attributes of cyanobacteria, may be contributing to the minimized consumer biomass. Periphyton from the Florida Everglades has a diverse and abundant cyanobacterial assemblage whose species produce toxic metabolites; therefore, by screening periphyton representative of the greater Everglades ecosystem, six different cyanotoxins and one toxin (domoic acid) produced by diatoms were identified, ranging in content from 3×10^{-9} to 1.3×10^{-6} ($g \cdot g^{-1}$), with saxitoxin, microcystin, and anatoxin-a being the most common. While content of toxins were generally low, when coupled with the tremendous periphyton biomass (3–3,000 $g \cdot m^{-2}$), a significant amount of cyanotoxins may be present. While the direct effects of the toxins identified here on the local grazing community need to be determined, the screening process utilized proved effective in showing the broad potential of periphyton to produce a variety of toxins.

Key index words: **cyanotoxins; Everglades; grazing; periphyton; secondary production**

Abbreviations: **ANTX-A, anatoxin-a; CYN, cylindrospermopsin; DA, domoic acid; DAT, debromoaplysiatoxin; HPLC/MS, HPLC/mass spectrometry; LA, lyngbyatoxin-a; MC, microcystin; STX, saxitoxin; WCA, water conservation area**

Production of a variety of toxic secondary metabolites has been observed in association with blooms of cyanobacteria. These compounds, collectively referred to as cyanotoxins, are grouped according to their target organs, hepatotoxins, neurotoxins, and dermatotoxins (Hitzfeld et al. 2000), and also exhibit allelopathic activities (Smith and Doan 1999, Gantar et al. 2008). It has been hypothesized that cyanobacteria produce toxins in response to grazing (Thacker et al. 2005, Camacho and Thacker 2006, Berry et al. 2008 and references therein) and as a competitive inhibitor of bacteria, fungi, and algae (Valdor and Aboal 2007, Berry et al. 2008, Gantar et al. 2008), though they may also stimulate growth of organisms (Gantar et al. 2008). Effects on larger vertebrates include morphological and motor sensor abnormalities (Osswald et al. 2007) and impacts on protein production (Salierno et al. 2006, Mezhoud et al. 2008).

Production of toxins is not an obligate process and may be limited to a few select strains within a variety of cyanobacteria including *Anabaena*, *Aphanizomenom*, *Cylindrospermopsis*, *Microcystis*, *Nodularia*, *Nostoc, Phormidium*, *Lyngbya*, and *Oscillatoria* (Hitzfeld et al. 2000, Gugger et al. 2005, Teneva et al. 2005, Seifert et al. 2007). In addition, marine and estuarine *Pseudo-nitzschia* and *Nitzshcia* species can produce domoic acid, a toxin associated with amnesic shellfish poisoning (ASP) (Villac et al. 1993, Kotaki et al. 2004). Planktonic blooms and associated toxin concentrations in eutrophic waters have been the subject of most studies (Hitzfeld et al. 2000, Baker et al. 2001). More recently, toxins have been detected in cohesive cyanobacterial mats from rivers (Mohamed et al. 2006), reservoirs (Izaguirre et al. 2007), wetlands (Berry et al. 2004, Gantar et al. 2008), and inland seas (Surakka et al. 2005). Outbreaks of ASP have been reported along the coastlines of North America from the Arctic to the Gulf of Mexico (Villac et al. 1993). Studies vary in their aims, some sampling waterways for specific compounds associated with blooms, and others using culture incubations to determine the direct effects of an isolated compound on the biological activity and function of other organisms. From these studies,

[1]Received 2 July 2009. Accepted 28 January 2010.
[2]Author for correspondence: e-mail bjbellin@mtu.edu.

it is evident that nuisance blooms are not the only sources of cyanotoxins, but any aquatic habitat with significant cyanobacterial biomass can also contain toxins.

In the Florida Everglades, periphyton is prominent and widespread. Cyanobacteria are the primary algal component (Browder et al. 1994, McCormick et al. 1996), including known toxin-producing genera. Given production in other systems, the possibility exists for a variety of toxins to be present in the Everglades. Here, we present results of an initial screening of Everglades periphyton and show the presence of toxins from oligotrophic as well as eutrophic waters. We discuss the potential impacts of these toxins on the grazing communities present in the Everglades.

Periphyton was collected from six sites throughout the Everglades covering a range of water chemistry and periphyton types (Swift and Nicholas 1987, Browder et al. 1994, and see Gaiser et al. 2006 for a greater Everglades map) and coincided with times of significant biomass and areal coverage. From the ombrotrophic interior of WCA-1, amorphous periphyton communities composed of cyanobacteria, filamentous green algae, desmids, and diatoms were collected on 21 December 2007. At these sites, cyanobacterial abundances are lower but can still be a significant component (>50%) of the assemblage. Gelatinous clouds of cyanobacteria and green algae were collected from the eutrophic regions of WCA-2A on 31 January 2008 where cyanobacteria are often >90% of the algal assemblage. Cohesive cyanobacterial mats from the oligotrophic, mineral-rich interior of WCA-2A, WCA-3B, and Everglades National Park (ENP) were sampled on 6 September 2007 for the former, and 6 February 2008 for the latter two regions. At these sites, cyanobacterial abundances are typically >90% (see fig. 1 in Hagerthey et al. [in press] for further descriptions and images of the periphyton types). Multiple grab samples were collected within a slough and combined into a single composite sample. The periphyton was brought back to the laboratory on ice, frozen at −80°C, and lyophilized (Labconco freeze-drier, Kansas City, MO, USA) prior to shipment of 5–20 g dry weight (dwt) of periphyton to Greenwater Laboratories (Palatka, FL, USA) for toxin analysis.

Samples were screened for six cyanotoxins (cylindrospermopsin [CYN], microcystin [MC], saxitoxin [STX], anatoxin-a [ANTX-A], debromoaplysiatoxin [DAT], and lyngbyatoxin-a [LA]) and one toxin produced by diatoms (domoic acid [DA]). Approximately 0.5 g dwt of periphyton was used to quantify cyanotoxin content, which is reported as a unitless ratio ($g \cdot g^{-1}$; Tolhurst et al. 2005). Data were received as ng toxin $\cdot g^{-1}$ periphyton; however, Tolhurst et al. (2005) detailed the rationale for expressing content without this unit.

CYN, MC, and ANTX-A were extracted with 75% acidified MeOH, sonicated for 25 min, and centrifuged (IEC Centra CL12, Thermo Electron Corp, Madison, WI, USA) at 2,000 rcf for 10 min. The supernatant was collected, and extraction of the pellet repeated. The pooled supernatant was blown to dryness using a turbo-vap system (TurboVap LV, Caliper Life Sciences, Hopkinton, MA, USA), then reconstituted in 1 mL of double distilled (dd) H_2O. A 100 μL subsample was diluted to 1 mL and analyzed for CYN. The remaining 900 μL was used to extract MC and ANTX-A using reverse phase (Strata) solid phase extraction (SPE) cartridges (Phenomenex, Torrance, CA, USA). ANTX-A and MC were eluted with MeOH, blown to dryness, and reconstituted with ddH_2O (1:1). A 100 μL aliquot was diluted to 1 mL for MC analysis, and the remaining material filtered (0.45 μm syringe filter) for ANTX-A analysis. CYN and MC were determined by ELISA (Abraxis Bioscience, Los Angeles, CA, USA), and ANTX-A by HPLC/mass spectrometry (HPLC/MS) (Aversano et al. 2004). Not all samples required SPE, thus reducing potential losses of MC and ANTX-A.

STX was extracted with 20 mL 0.1 M HCl, boiled (105°C) with stirring for 5 min, cooled, and centrifuged at 1,560 rcf for 10 min. The supernatant was collected, filtered through a 0.45 μm polyvinylidene fluoride (PVDF) membrane (0.45 μm PVDF syringe filters, Whatman Inc., Florham Park, MA, USA), and quantified using ELISA (Abraxis Bioscience). DA was extracted with 10 mL 50% MeOH. Samples were sonicated in a water bath for 25 min and centrifuged at 2,000 rcf for 10 min. The supernatant was collected, and extraction of the pellet repeated. The pooled supernatant was filtered through GF/C (GF/C filter, Whatman Inc.), blown to dryness, and reconstituted in 1 mL of ddH_2O. Samples were analyzed via ELISA (Biosense Laboratories, Bergen, Norway) in duplicate after a 10-fold dilution. DAT and LA were extracted with 15 mL HPLC-grade acetone and sonicating bath for 25 min, with centrifugation at 2,000 rcf for 10 min. The extraction was repeated twice, and the supernatant was pooled, filtered (GF/C), blown to dryness, and reconstituted in 1 mL MeOH:H_2O (50:50). Prior to analysis by HPLC–MS/MS, samples were clarified using centrifugation (1,560 rcf for 10 min) and filtering through a 0.45 μm PVDF (Osborne et al. 2008).

All seven toxins were present in periphyton, the most common being STX, MC, and ANTX-A (Table 1). MC was detected in periphyton from ombrotrophic-minerotrophic as well as eutrophic-oligotrophic waters, while STX and ANTX-A were only found in cohesive cyanobacterial mats from minerotrophic waters with moderate or low nutrient content (Table 1). CYN was only detected in eutrophic habitats. Contents ($g \cdot g^{-1}$) of all toxins ranged from 9×10^{-9} DA to nearly 1.3×10^{-6} LA. Though present in very small amounts, the presence of DA is noteworthy. DA is typically associated with the

TABLE 1. Cyanotoxin content for the periphyton samples collected along a longitudinal gradient from the northern to the southern Everglades.

Location	Site/nutrient status	Periphyton type	Biomass (g AFDW · m^{-2})	MC/nodularin	CYN	STX	DA	ANTX-A	DAT	LA
WCA-1	Interior, oligotrophic	Metaphyton	40–225[a]	4.5×10^{-8}	–	–	–	–	–	1.3×10^{-6}
WCA-2A	Margin, eutrophic	Metaphyton	3–53[a]	5.2×10^{-8}	1.4×10^{-8}	–	3.0×10^{-9}	–	–	–
	Interior, mesotrophic	Metaphyton		5.6×10^{-7}	–	5.1×10^{-8}	9.0×10^{-9}	–	–	–
	Interior, oligotrophic	Metaphyton	1–157[a]	–	–	3.1×10^{-8}	–	1.0×10^{-8}	–	–
	Interior, oligotrophic	Epiphyton	1–120[a]	1.2×10^{-8}	–	4.0×10^{-7}	–	4.0×10^{-8}	1.0×10^{-6}	–
	Interior, oligotrophic	Epipelon	20–800[a]	–	–	3.1×10^{-8}	–	–	–	–
WCA-3B	Interior, oligotrophic	Metaphyton/*Utricularia*	1–24[b]	7.5×10^{-8}	–	3.6×10^{-8}	–	3.0×10^{-8}	–	–
ENP	Interior, oligotrophic	Metaphyton/*Utricularia*	286–3,665[c]	–	–	1.0×10^{-7}	–	2.0×10^{-8}	–	–

Toxin abbreviations: MC, microcystins; CYN, cylindrospermopsin; STX, saxitoxin; DA, domoic acid; ANTX-A, anatoxin-A; DAT, debromoaplysiatoxin; LA, lyngbyatoxin-A. AFDW, ash-free dry weight.

Cyanotoxin content is expressed as a unitless mass · mass ratio derived from g toxin · g^{-1} periphyton (Tolhurst et al. 2005); (–) indicates toxin was not detected. Biomass concentrations were obtained from the literature.

[a]McCormick et al. (1998)

[b]Turner et al. (1999).

[c]Gottlieb et al. (2006).

marine diatom *Pseudo-nitzschia* spp. (Villac et al. 1993) but was also recently observed in *Nitzschia navis-varingica* from brackish waters in Vietnam (Kotaki et al. 2004). Where DA was observed in this study, the waters are high in minerals (specific conductivity >1,000 $\mu S \cdot cm^{-1}$) and nutrients (total phosphorus >35 $\mu g \cdot L^{-1}$) (S. E. Hagerthey, A. Gottlieb, S. Newman, and P. V. McCormick, unpublished data). Several *Nitzschia* species have been identified at these sites; thus, further study is needed to confirm the presence of this toxin and to determine the species of origin.

The amorphous desmid/diatom assemblages from WCA-1 had MC and LA present, the latter having the greatest toxin content observed for any periphyton type in this study (Table 1). Known toxin-producing genera have been identified from WCA-1 including *Anabaena* sp. and *Planktothrix* sp. Gelatinous cyanobacterial and green algal periphyton from the mesotrophic and eutrophic waters in WCA-2A had the greatest content of MC and also contained CYN and DA (Table 1). From these mats, *Microcystis* sp., *Anabeana* sp., *Phormidium* sp., and *Planktothrix* sp., along with the aforementioned unidentified *Nitzschia* species, have all been observed.

Cohesive cyanobacterial mats from the oligotrophic interior of WCA-2A had the greatest total content and diversity of cyanotoxins of all periphyton screened. Concurrent with this, a large variety of toxin-producing genera were identified for these samples, including *Planktothrix agardhii* (B. J. Bellinger and S. E. Hagerthey, unpublished data). Toxins in the epiphyton included MC, STX, ANTX-A, and DAT, the last having the greatest content. Metaphyton had STX and ANTX-A, while epipelic mats only contained small amounts of STX (Table 1). Periphyton from WCA-3B contained MC, STX, and ANTX-A, the most abundant toxin being MC. In the ENP, cyanobacterial mats contained STX and ANTX-A, the former having the greater content. In both of these areas, the variety of toxin-producing genera was second only to those from WCA-2A.

A variety of Everglades cyanobacterial strains have been isolated and tested for biologically active metabolites (Berry et al. 2004, 2008, Gantar et al. 2008). This study expands upon these findings by screening for the potential abundance and diversity of cyanotoxins from intact periphyton common to the Everglades ecosystem. Here, seven different toxins were identified, the most widespread being STX, MC, and ANTX-A. Absolute detected amounts were comparatively low ($0.009–1.3 \times 10^{-6}$ $g \cdot g^{-1}$) to what has been observed for rivers, lakes, and reservoirs (e.g., $0.002–4.5 \times 10^{-3}$ STX, Velzeboer et al. 2000; $0.001–8.4 \times 10^{-3}$ MC, Kemp and John 2006) or extracted from cyanobacterial species' isolates (e.g., $0.01–4.3 \times 10^{-4}$ MC, Izaguirre et al. 2007; $2–4 \times 10^{-3}$ MC, Mohamed et al. 2006). However, in the Everglades, a tremendous amount of standing algal

biomass may be present (McCormick et al. 1996, Gottlieb et al. 2006, Iwaniec et al. 2006); thus, while absolute contents were low, overall concentrations may be significant. For example, using published biomass estimates typical of each region (Table 1), the concentration of cyanotoxins could exceed 300 $\mu g \cdot m^{-2}$ in WCA-1, 175 $\mu g \cdot m^{-2}$ in the interior of WCA-2A, and 440 $\mu g \cdot m^{-2}$ in ENP. Conversely, toxins may represent ''qualitative'' defenses, negating the need for large concentrations (Berry et al. 2008).

Wetlands can be areas of extreme conditions for higher trophic levels. Waters in the Everglades are generally very warm (in summer 20°C–35°C) and shallow (depths generally <60 cm) and, when algal biomass is low, may contain low amounts of dissolved oxygen (2–8 $mg \cdot L^{-1}$ at oligotrophic sites; <2 $mg \cdot L^{-1}$ at eutrophic sites) (Hagerthey et al. 2010). The added stress of cyanotoxin exposure could prove deleterious to fish survival or embryonic development. For example, Lefebvre et al. (2004) exposed larval zebrafish (*Danio rerio*) to STX at concentrations of 220–500 $\mu g \cdot L^{-1}$, resulting in severe swelling of eyes, pericardial cavity, and yolk sacs. In some cases, swim bladders did not inflate, and paralysis set in. However, removal of STX-spiked water eventually led to larval fish recovery.

Utilizing pahayokolide A, a toxin extracted from a *Lyngbya* strain isolated from Everglades floating cyanobacterial mats, Berry et al. (2004) observed 100% mortality of zebrafish at concentrations of 3–5 $\mu g \cdot mL^{-1}$. Zebrafish are being recognized as a model fish for toxicology studies (Lefebvre et al. 2004, Berry et al. 2007), and being a small, tropical freshwater fish makes it a good corollary to the native fishes of the Everglades. Thus far, >20 strains of cyanobacteria isolated from southern and central Florida, including the Everglades, have been shown to affect zebrafish development (Berry et al. 2007).

Additional physiological effects of toxins on fish species similar to, or occurring in, the Everglades have been tested. Concentrations of 1 μg microcystin-LR $\cdot mL^{-1}$ caused alterations of phosphoprotein and total protein content in medaka fishes (*Oryzias latipes*) (Mezhoud et al. 2008). Viability in carp (*Cyprinus carpio*) hepatocytes was reduced when exposed to microcystin levels of 10 $\mu g \cdot L^{-1}$ (Li et al. 2007). Accordingly, it is possible that fish in the Everglades could be similarly affected.

While toxic effects on higher trophic levels seem evident, their utility as a deterrent to grazing by invertebrates seems less certain (see Berry et al. 2008). Within Everglades periphyton, planorbid and hydrobiid snails are present, along with the grazing amphipod, *Hyalella* sp. (Liston and Trexler 2005). In a study of herbivore-induced toxin production by *Lyngbya wollei*, production of STX was induced by the snail *Pleurocera annuliferum*, but not by *Hyalella azteca* (Thacker et al. 2005). STX content in the snail treatments ranged from $1.0–3.0 \times 10^{-7}$, similar to the values observed in our study. Rather than toxins, the extracellular sheath of *L. wollei* has been shown to interfere with *Hyalella*'s ability to consume this cyanobacterium (Camacho and Thacker 2006). Invertebrate densities within metaphyton are greater than that of benthic mats (Liston and Trexler 2005), which may explain the greater STX content. Given that feeding strategies differ greatly among invertebrate herbivores (Hagerthey et al. 2002, Camacho and Thacker 2006) and instances of no deleterious effects due to ingestion have been reported (Berry et al. 2008), toxin effects are likely to be species specific.

Cyanobacteria are a major component of Everglades periphyton (Browder et al. 1994) that produce significant amounts of extracellular polymeric substances (EPS) (Bellinger et al. 2010) and are capable of precipitating calcium carbonate (Browder et al. 1994). Cyanobacteria also lack essential fatty acids (Demott and Müller-Navarra 1997). Our results indicate that algal toxins are also common to the many types of Everglades periphyton. All of these factors suggest that the Everglades periphyton may be a poor-quality food resource that contributes to low secondary production and the large standing stock of periphyton biomass (Turner et al. 1999, Geddes and Trexler 2003).

This work was supported by the South Florida Water Management District. Sampling efforts were part of the Cattail Habitat Improvement Project and in conjunction with long-term monitoring of stations throughout the greater Everglades Ecosystem. The authors are grateful to the anonymous reviewers for their comments and suggested revisions of this manuscript.

Aversano, C. D., Eaglesham, G. K. & Quilliam, M. A. 2004. Analysis of cyanobacterial toxins by hydrophilic interaction liquid chromatography-mass spectrometry. *J. Chromatogr.* 1028:155–64.

Baker, P. D., Steffensen, D. A., Humpage, A. R., Nicholson, B. C., Falconer, I. R., Lanthois, B., Fergusson, K. M. & Saint, C. P. 2001. Preliminary evidence of toxicity associated with the benthic cyanobacterium *Phormidium* in South Australia. *Environ. Toxicol.* 16:506–11.

Bellinger, B. J., Gretz, M. R., Domozych, D. S., Kiemle, S. N. & Hagerthey, S. E. 2010. Composition of extracellular polymeric substances from periphyton assemblages in the Florida Everglades. *J. Phycol.* 46 (in press).

Berry, J. P., Gantar, M., Gawley, R. E., Wang, M. & Rein, K. S. 2004. Pharmacology and toxicology of pahayokolide A, a bioactive metabolite from a freshwater species of *Lyngbya* isolated from the Florida Everglades. *Comp. Biochem. Phys. C* 139:231–8.

Berry, J. P., Gantar, M., Gibbs, P. D. L. & Schmale, M. C. 2007. The zebrafish (*Danio rerio*) embryo as a model system for identification and characterization of developmental toxins from marine and freshwater microalgae. *Comp. Biochem. Phys. C* 145:61–72.

Berry, J. P., Gantar, M., Perez, M. H., Berry, G. & Noriega, F. G. 2008. Cyanobacterial toxins as allelochemicals with potential applications as algaecides, herbicides and insecticides. *Mar. Drugs* 6:117–46.

Browder, J. A., Gleason, P. J. & Swift, D. R. 1994. Periphyton in the Everglades: spatial variation, environmental correlates, and ecological implications. *In* Davis, S. M. & Ogden, J. C. [Eds.]

Everglades: The Ecosystem and Its Restoration. St. Lucie Press, Boca Raton, Florida, pp. 379–418.

Camacho, F. A. & Thacker, R. W. 2006. Amphipod herbivory on the freshwater cyanobacterium *Lyngbya wollei*: chemical stimulants and morphological defenses. *Limnol. Oceanogr.* 51:1870–5.

Demott, W. R. & Müller-Navarra, D. C. 1997. The importance of highly unsaturated fatty acids in zooplankton nutrition: evidence from experiments with *Daphnia*, a cyanobacterium and lipid emulsions. *Freshw. Biol.* 38:649–64.

Gaiser, E. E., Childers, D. L., Jones, R. D., Richards, J., Scinto, L. J. & Trexler, J. C. 2006. Periphyton responses to eutrophication in the Florida Everglades: cross-system patterns of structural and compositional change. *Limnol. Oceanogr.* 51:617–30.

Gantar, M., Berry, J. P., Thomas, S., Wang, M., Perez, R. & Rein, K. S. 2008. Allelopathic activity among *Cyanobacteria* and microalgae isolated from Florida freshwater habitats. *FEMS Microbiol. Ecol.* 64:55–64.

Geddes, P. & Trexler, J. C. 2003. Uncoupling of omnivore-mediated positive and negative effects on periphyton mats. *Oecologia* 136:585–95.

Gottlieb, A. D., Richards, J. H. & Gaiser, E. E. 2006. Comparative study of periphyton community structure in long and short-hydroperiod Everglades marshes. *Hydrobiologia* 569:195–207.

Gugger, M., Lenoir, S., Berger, C., Ledreux, A., Druart, J. C., Humbert, J. F., Guette, C. & Bernard, C. 2005. First report in a river in France of the benthic cyanobacterium *Phormidium favosum* producing anatoxin-a associated with dog neurotoxicosis. *Toxicon* 45:919–28.

Hagerthey, S. E., Bellinger, B., Wheeler, K., Gantar, M. & Gaiser, E. In press. Everglades periphyton: a biogeochemical perspective. *Crit. Rev. Environ. Sci. Tech.*

Hagerthey, S. E., Cole, J. J. & Kilbane, D. 2010. Aquatic metabolism in the Everglades: the dominance of water column heterotrophy. *Limnol. Oceanogr.* 55:653–66.

Hagerthey, S. E., Defew, E. C. & Paterson, D. M. 2002. Influence of *Corophium volutator* and *Hydrobia ulvae* on intertidal benthic diatom assemblages under different nutrient and temperature regimes. *Mar. Ecol. Prog. Ser.* 245:47–59.

Hitzfeld, B. C., Hoger, S. J. & Dietrich, D. R. 2000. Cyanobacterial toxins: removal during drinking water treatment, and human risk assessment. *Environ. Health Perspect.* 108:113–22.

Iwaniec, D. M., Childer, D. L., Rondeau, D., Madden, C. J. & Saunders, C. 2006. Effects of hydrologic and water quality drivers on periphyton dynamics in the southern Everglades. *Hydrobiologia* 569:223–35.

Izaguirre, G., Jungblut, A. D. & Neilan, B. A. 2007. Benthic cyanobacteria (Oscillatoriaceae) that produce microcystin-LR, isolated from four reservoirs in southern California. *Water Res.* 41:492–8.

Kemp, A. & John, J. 2006. Microcystins associated with *Microcystis* dominated blooms in the southwest wetlands, Western Australia. *Environ. Toxicol.* 21:125–30.

Kotaki, Y., Lundholm, N., Onodera, H., Kobayashi, K., A Bajarias, F. F., Furio, E. F., Iwataki, M., Fukuyo, Y. & Kodama, M. 2004. Wide distribution of *Nitzschia navis-varingica*, a new domoic-acid producing benthic diatom found in Vietnam. *Fish. Sci.* 70:28–32.

Lefebvre, K. A., Trainer, V. L. & Scholz, N. L. 2004. Morphological abnormalities and sensorimotor deficits in larval fish exposed to dissolved saxitoxin. *Aquat. Toxicol.* 66:159–70.

Liston, S. E. & Trexler, J. C. 2005. Spatiotemporal patterns in community structure of macroinvertebrates inhabiting calcareous periphyton mats. *J. N. Am. Benthol. Soc.* 24:832–44.

McCormick, P. V., Rawlik, P. S., Lurding, K., Smith, E. P. & Sklar, F. H. 1996. Periphyton-water quality relationship along a nutrient gradient in the northern Florida Everglades. *J. N. Am. Benthol. Soc.* 15:443–9.

McCormick, P. V., Shuford, R. B. E., Backus, J. G. & Kennedy, W. C. 1998. Spatial and seasonal patterns of periphyton biomass and productivity in the northern Everglades, Florida, U.S.A. *Hydrobiologia* 362:185–208.

Mezhoud, K., Praseuth, D., Puiseux-Dao, S., Francois, J. C., Bernard, C. & Edery, M. 2008. Global quantitative analysis of protein expression and phosphorylation status in the liver of the medaka fish (*Oryzias latipes*) exposed to microcystin-LR I. Balneation study. *Aquat. Toxicol.* 86:166–75.

Mohamed, Z. A., El Sharouny, H. M. & Ali, W. S. M. 2006. Microcystin production in benthic mats of cyanobacteria in the Nile River and irrigation canals, Egypt. *Toxicon* 47:584–90.

Osborne, N., Seawright, A. & Shaw, G. 2008. Dermal toxicology of *Lyngbya majuscule*, from Moreton Bay, Queensland, Australia. *Harmful Algae* 7:584–9.

Osswald, J., Rellan, S., Carvalho, A. P., Gago, A. & Vasconcelos, V. 2007. Acute effects of an anatoxin-a producing cyanobacterium on juvenile fish – *Cyprinus carpio* L. *Toxicon* 49:693–8.

Salierno, J. D., Snyder, N. S., Murphy, A. Z., Poli, M., Hall, S., Baden, D. & Kane, A. S. 2006. Harmful algal bloom toxins alter c-Fos protein expression in the brain of killifish, *Fundulus heteroclitus*. *Aquat. Toxicol.* 78:350–7.

Seifert, M., McGregor, G., Eaglesham, G., Wickramasinghe, W. & Shaw, G. 2007. First evidence for the production of cylindrospermopsin and deoxy-cylindrospermopsin by the freshwater benthic cyanobacterium, *Lyngbya wollei* (Farlow ex Gornont) Speziale and Dyck. *Harmful Algae* 6:73–80.

Smith, G. D. & Doan, N. T. 1999. Cyanobacterial metabolites with bioactivity against photosynthesis in cyanobacteria, algae and higher plants. *J. Appl. Phycol.* 11:337–44.

Surakka, A., Sihvonen, L. M., Lehtimaki, J. M., Wahlsten, M., Vuorela, P. & Sivonen, K. 2005. Benthic cyanobacteria from the Baltic Sea contain cytotoxic *Anabaena*, *Nodularia*, and *Nostoc* strains and an apoptosis-inducing *Phormidium* strain. *Environ. Toxicol.* 20:285–92.

Swift, D. R. & Nicholas, R. B. 1987. *Periphyton and Water Quality Relationships in the Everglades Water Conservation Areas 1978–1982*. South Florida Water Management District, West Palm Beach, Florida, 44 pp.

Teneva, I., Dzhambazov, B., Koleva, L., Mladenov, R. & Schirmer, K. 2005. Toxic potential of five freshwater *Phormidium* species (Cyanoprokaryota). *Toxicon* 45:711–25.

Thacker, R. W., McLeod, A. M. & McLeod, S. W. 2005. Herbivore-induced saxitoxin production in the freshwater cyanobacterium *Lyngbya wollei*. *Arch. Hydrobiol.* 159:415–25.

Tolhurst, T. J., Underwood, A. J., Perkins, R. G. & Chapman, M. G. 2005. Content versus concentration: effects of units on measuring the biogeochemical properties of soft sediments. *Estuar. Coast. Shelf Sci.* 63:665–73.

Turner, A. M., Trexler, J. C., Jordan, C. F., Slack, S. J., Geddes, P., Chick, J. H. & Loftus, W. F. 1999. Targeting ecosystem features for conservation: standing crops in the Florida Everglades. *Conserv. Biol.* 13:898–911.

Valdor, R. & Aboal, M. 2007. Effects of living cyanobacteria, cyanobacterial extracts and pure microcystins on growth and ultrastructure of microalgae and bacteria. *Toxicon* 49:769–79.

Velzeboer, R. M. A., Baker, P. D., Rositano, J., Heresztyn, T., Codd, G. A. & Raggett, S. L. 2000. Geographical patterns of occurrence and composition of saxitoxins in the cyanobacterial genus *Anabaena* (Nostocales, Cyanophyta) in Australia. *Phycologia* 39:395–407.

Villac, M. C., Roelke, D. L., Villareal, T. A. & Fryxell, G. A. 1993. Comparison of two domoic acid-producing diatoms: a review. *Hydrobiologia* 269/270:213–24.