Harmful Algae 9 (2010) 134–143

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Evaluating the potential risk of microcystins to blue crab (*Callinectes sapidus*) fisheries and human health in a eutrophic estuary☆

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ARTICLE INFO

Article history:
Received 23 February 2009
Received in revised form 24 August 2009
Accepted 25 August 2009

Keywords:
Blue crab
Callinectes sapidus
Cyanobacteria
Harmful algal blooms
Human health
Microcystins

ABSTRACT

Toxin producing cyanobacteria commonly inhabit fresh waters and brackish estuaries, and blooms of these species may be escalating worldwide due to eutrophication. The most common cyanobacterial toxins occurring in fresh and brackish waters are microcystins (MC), which are known to accumulate in aquatic organisms. Prey preference for filter-feeding organisms, such as clams and mussels, make the blue crab, *Callinectes sapidus*, a candidate for microcystin contamination, therefore making this commercially important edible crab species a potential vector of these toxins to humans. The present study was conducted in a hyper-eutrophic freshwater lake, Lac des Allemands, located in the Barataria estuary system of southeastern Louisiana, and was aimed at documenting the presence and abundance of toxic cyanobacteria and assessing microcystin concentrations in surface water and blue crabs taken from this region. *Microcystis* sp. were the dominant cyanobacteria, with alternating blooms of *Microcystis* and *Anabaena* spp. occurring during the 8-month study. Enzyme-linked immunosorbent assay (ELISA) was used to evaluate concentrations of microcystins from surface water and hepatopancreas, viscera, and muscle tissues of blue crabs. The highest concentration of microcystins found in surface water (1.42 $\mu g\ MC\ l^{-1}$) was above the tolerable daily intake (TDI) guideline for microcystins in drinking water (1.0 $\mu g\ MC\ l^{-1}$) set by the World Health Organization (WHO). Highest concentration of microcystins occurring in crab tissue were 820 $\mu g\ MC\ kg^{-1}$ in hepatopancreas, 65 $\mu g\ MC\ kg^{-1}$ in viscera, and 105 $\mu g\ MC\ kg^{-1}$ in muscle, which were close to or exceeding the WHO-TDI guidelines for human consumption (0.04 $\mu g\ MC\ kg^{-1}$ body weight day^{-1}) based on human body weight and amount of crab tissue consumed. This study documents the presence of microcystins in both surface water and blue crab tissue and therefore, demonstrates the potential for *Microcystis* and *Anabaena* blooms to produce toxins that may be accumulated in the tissues of blue crabs and transferred to higher level consumers, including humans.

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1. Introduction

Algal blooms are increasing worldwide due to eutrophication of aquatic environments (Anderson et al., 2002). Anthropogenic nutrient enrichment of rivers and estuaries can have a direct impact on algal species composition and the formation of noxious and toxic blooms and surface scums. The Barataria-Terrebonne Estuarine System (BTES) of Louisiana is an example of a eutrophied system, and toxic and noxious phytoplankton blooms may be increasing as a consequence of excess nutrients (Rabalais et al., 1995).

Although many algal classes including diatoms are capable of producing toxins, most toxic phytoplankton can be classified as either dinoflagellates or cyanobacteria (Glibert et al., 2005). Some cyanobacteria are of particular concern due to their production of a broad assortment of toxic metabolites. Species in the *Microcystis*, *Anabaena*, *Cylindrospermopsis*, and *Raphidiopsis* genera are capable of producing multiple toxins, with some cyanobacterial toxins having as many as 60 known structural variants (e.g., microcystins) (Codd et al., 1999).

Cyanotoxins in contaminated drinking and recreational water and food sources can pose a serious hazard to both wild and

☆ Disclaimer: Statements, findings, conclusions, and recommendations are those of the authors and do not necessarily reflect the views of the project funding sources.

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1568-9883/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.hal.2009.08.011

domestic animals, and to humans alike. Cyanobacterial toxins are classified, based on their modes of toxicity, as: hepatotoxins (chemicals causing liver injury), neurotoxins, cytotoxins, dermatoxins, and irritant toxins (Codd et al., 1999; Sotero-Santos et al., 2008). The most common cyanobacterial toxins found in blooms from fresh and brackish waters are cyclic peptides known as microcystins (Sivonen, 1996; Sivonen and Jones, 1999). These toxins are highly water-stable and resistant to boiling, and thus pose a threat to water and food quality if not properly monitored (Falconer and Humpage, 2005). Microcystins are produced by several genera including: *Microcystis*, *Planktothrix* (*Oscillatoria*), *Anabaena*, *Nostoc*, *Anabaenopsis*, and *Hapalosiphon* (Carmichael et al., 2001). The hepatotoxicity of microcystins depends on their degree of inhibition of protein phosphatases (MacKintosh et al., 1990), leading to symptoms of weakness, cold extremities, labored breathing, vomiting and diarrhea, possibly causing death due to liver hemorrhaging and respiratory arrest (Codd et al., 1999). Long-term exposure to sub-lethal concentrations of microcystins has been implicated in the promotion of tumors (Ito et al., 1997), making prolonged blooms of microcystin-producing cyanobacteria perilous.

The upper Barataria estuary receives multiple nutrient inputs from runoff of lake-shore development, intense agriculture, and unsewered areas, as well as diverted waters from the Mississippi River. These factors, in combination with other issues of residence time, temperature, food web dynamics, concentrations of micronutrients, and quantity and relative abundances of major nutrients supplied into the estuary, may have significant impacts on phytoplankton species composition, frequency and intensity of harmful algal blooms, and toxin production (Anderson et al., 2002, 2008 and references therein). Blooms of *Anabaena*, *Microcystis*, *Cylindrospermopsis*, *Raphidiopsis*, and *Aphanizomenon* species occur in the fresh and brackish waters of the upper Barataria estuary, and nutrient additions, primarily nitrogen, in bioassay microcosms with water from this area stimulated the growth of these cyanobacteria (Ren et al., 2008, 2009).

Few cases of cyanobacterial toxin poisonings have been documented in humans, but this may be due to a lack of information regarding vectors, inability to identify and link symptoms, and inadequate methods of toxin detection (Carmichael et al., 2001). Intoxication of aquatic organisms following exposure to microcystins is better documented, especially in freshwater fish and other pelagic organisms (see Magalhães et al., 2001; Mohamed et al., 2003; Simoni et al., 2004; Xie et al., 2005). However, exposure to microcystins has also been shown to adversely impact benthic and burrowing organisms, including bivalves, crayfish, and crabs (Amorim and Vasconcelos, 1999; Vasconcelos et al., 2001; Simoni et al., 2004; Dewes et al., 2006; Chen and Xie, 2007). Studies to describe benthic or demersal vectors of these toxins, however, are very limited.

The blue crab, *Callinectes sapidus*, is an opportunistic feeder known to be cannibalistic, omnivorous, and/or a carrion feeder. Blue crabs congregate on clam, mussel, and oyster beds (Hughes and Seed, 1981; Kennedy and Cronin, 2006), making them strong candidates for phycotoxin contamination via these filter feeders. Among the dominant prey items for blue crabs is the non-selective, filter-feeding freshwater clam, *Rangia cuneata* (Darnell, 1958). *R. cuneata* is abundant in the upper BTES (personal communication, K. Galván), and may represent an important link between cyanotoxins and blue crabs within this system. The area is also well known for its production of several species of demersal catfish, including *Ictalurus furcatus* (blue catfish) and *I. punctatus* (channel catfish). Deceased fish, often associated with intense algal blooms and low dissolved oxygen concentrations, could also serve as a potential food source for the carrion-feeding blue crab. To date there has been no study evaluating microcystin concentrations in the edible estuarine crab species, *C. sapidus*, a widely consumed demersal species.

Louisiana is the leader in production of blue crabs in the United States, with a majority of them being harvested from the 13 parishes within the BTES (McKenzie et al., 1995). The close proximity of the edible blue crab with their filter-feeding prey in this region, may establish a dietary link between cyanotoxin-producing algae and human consumers of blue crab.

The aims of this study were to analyze the efficiency of current microcystin extraction methods, document the presence and abundance of toxic cyanobacteria in the freshwater lake, Lac des Allemands in southeastern Louisiana, and assess microcystin concentrations in surface water and blue crabs taken from Lac des Allemands. The data collected in this study were further used to assess potential risk to higher trophic level organisms, including humans.

2. Materials and methods

2.1. Description of the study site

Lac des Allemands (29°55′58.1″N, 90°34′27.96″W) is a 49 km^2 freshwater lake (salinity <1 psu) in the uppermost part of the Barataria estuary in southeastern Louisiana (Fig. 1). The trophic status of the lake (Carlson, 1977) is hyper-eutrophic with chlorophyll *a* (Chl *a*) biomass levels of 40–185 $\mu g\ l^{-1}$ (Ren et al., 2008, 2009). Nutrient inputs into Lac des Allemands are primarily resulting from discharges of wastewater treatment plants, agricultural runoff, and storm water pumps (Rabalais et al., 1995). Fig. 1 shows Vacherie Canal, Bayou Lassene, Bayou Boeuf and Bayou Chevreuil in relation to Lac des Allemands. Vacherie Canal, which has a series of unsewered fishing camps at its terminus with Lac des Allemands, Bayou Lassene, and Bayou Boeuf all drain extensive sugar cane fields to the northwest and southwest. Following rain storms, Bayou Chevreuil becomes turbid with runoff from fertilizer plants along the Mississippi River to the west, contributing to the hyper-eutrophic status of this lake.

2.2. Sampling and water quality data

In situ water quality data for temperature and salinity were obtained at 30-min intervals using a YSI (Yellow Springs Instrument) Model 6600 sonde deployed (29°55′6.78″N, 90°33′42.00″W) to 0.3 m below the water surface in Lac des Allemands. The YSI sonde was checked for fouling during each sampling trip, and was switched out once for calibration. YSI data were checked for anomalous values upon the conclusion of each sampling trip. Surface whole water samples (2 l) were collected in clean Nalgene bottles monthly between December 2006 and February 2007, biweekly during March 2007, and weekly between April and June 2007 from four stations (Table 1) within Lac Des Allemands (Fig. 1). Coordinates and depths of all sampling sites are summarized in Table 1. Subsamples for the determination of Chl *a* and microcystin concentrations were transferred on ice to the laboratory. For the determination of Chl *a* concentrations, replicate 100 ml aliquots of surface water were filtered (<50 kPa) onto 4.7 cm diameter glass fiber filters (Whatman GF/F), immediately frozen, and stored at −80 °C. The filter papers were shipped overnight on dry ice to Dr. James L. Pinckney at the Department of Biological Sciences, University of South Carolina, Columbia, for high performance liquid chromatography (HPLC) analysis (Pinckney et al., 1996). Subsamples (100–400 ml aliquots of surface water) for determination of particulate microcystin concentrations were collected between April and July 2007, filtered (<40 kPa) onto 2.5 cm diameter glass fiber filters (Whatman GF/F), and kept