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Experimental model of microcystin accumulation in the liver of *Oreochromis niloticus* exposed subchronically to a toxic bloom of *Microcystis* sp.

Charles P. Deblois[a], Alessandra Giani[b], David F. Bird[a,*]

[a] Dép. Sciences Biologiques, Université du Québec à Montréal (UQAM), C.P. 8888, Succ. Centre-Ville, Montréal, QC, Canada H3C 3P8
[b] Dept. Botanica – Instituto de Ciencias Biologicas, Universidade Federal de Minas Gerais, C.P. 486, 31270-901 Belo Horizonte, MG, Brazil

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ABSTRACT

Although accumulation of the liver toxin microcystin in phytoplanktivorous fish has been demonstrated in captive fish and in natural ecosystems, the relation between microcystin in ingested algae and the pattern of buildup of microcystin in fish is poorly known. In this month-long study performed at a Brazilian fish farm, 45 mature *Oreochromis niloticus* were fed daily with fresh seston periodically dominated by toxic *Microcystis* sp. Microcystin was measured daily in the food and every 5 days in liver and muscle samples. Control fish received a diet of seston that was low in toxic cyanobacteria. Initially, in treatment ponds, microcystin available for fish increased from 6.5 to 66.9 ng microcystin fish^{-1} day^{-1}, which was accompanied by an increase from 5.5 to 35.4 ng microcystin g liver^{-1}. Microcystin in muscle was below our detection limit of 4 ng g tissue^{-1} for the entire study. In the bloom phase, available microcystin reached its highest concentration (4450 ng MC fish^{-1} day^{-1}) then decreased to 910 ng microcystin fish^{-1} day^{-1} on day 31. During this period, microcystin reached its highest concentration of 81.6 ng MC g liver^{-1} and stayed high until the end of the experiment. A model based on rapid uptake, saturation, and exponential loss was built with these experimental results, and verified with data from the literature. Our model showed that accumulation was up to 50% of ingestion at low doses, but at intermediate doses, the onset of elimination led to a decline of liver burden. Although the accumulation rate confirms the high contamination potential of microcystin, it was balanced by a high depuration rate and this efficient systemic elimination may explain the tolerance of these fish to toxic blooms in the wild.

1. Introduction

The occurrence of toxic cyanobacterial blooms in eutrophic lakes and reservoirs is a world-wide problem (Codd, 2000). The toxins produced by cyanobacteria during these events are known to be harmful to aquatic wildlife, domestic animals and humans (Chorus and Bartram, 1999; Codd et al., 2005a). Among the toxins frequently encountered, microcystins (MC) are a group of monocyclic heptapeptides, mainly but not exclusively produced by *Microcystis* species (Kardinaal and Visser, 2005; Mankiewicz et al., 2003). These toxins are hepatotoxic and inhibit the activity of protein phosphatases 1 and 2A, which are key proteins in the regulation of many eukaryotic cell cycles including apoptosis (Mackintosh et al., 1990; Janssens and Goris, 2001). Perhaps for that reason, MC also acts as a tumour promoter in liver (Falconer and Humpage, 1996).

During cyanobacterial blooms, MC has been found to accumulate in a variety of aquatic organisms such as zooplankton, bivalves, crustaceans and fish, and also in aquatic vertebrates such as turtles, ducks and water birds (Cazenave et al., 2005; Chen et al., 2005, 2009; Deblois et al., 2008; Ferrao-Filho et al., 2002; Magalhães et al., 2003; Xie et al., 2005, 2007; Zhang et al., 2009). It is well known that consuming microcystin-contaminated water or seafood poses a risk to human health (Codd et al., 2005a,b; Ibeling and Chorus, 2007). To minimize this risk, the World Health Organization (WHO) has set a guideline of 1 μg l^{-1} of MC in drinking water, and established a tolerable daily intake (TDI) of 0.04 μg kg^{-1} day^{-1} (Chorus and Bartram, 1999). An important exposure route for humans is through the ingestion of contaminated fish. Microcystin-LR is weakly hydrophilic at basic pH but becomes hydrophobic in acid conditions (de Maagd et al., 1999), such as occur in the digestive tract; its uptake is further aided by the bile acid transport system. In fish, studies have demonstrated that MC accumulates mainly in liver, but can also be transported through the blood to other organs such as muscle, kidney, gill and brain (Cazenave et al., 2005; Martins and Vasconcelos, 2009). At an Egyptian fish farm the level of MC

Abbreviations: MC, microcystin or microcystins; MC-LR, microcystin-LR; AD, Assimilation–Depuration; ADX, Assimilation–Depuration–Excess; max$_{MC}$, asymptotic maximum liver burden of microcystin; DW, dry weight; FDW, freeze-dried weight; Chl a, chlorophyll a; v:v, volume to volume.

* Corresponding author. Tel.: +1 514 987 3000x7859; fax: +1 514 987 4647.
E-mail addresses: charlespdeblois@yahoo.com (C.P. Deblois), giani.alessandra@gmail.com (A. Giani), bird.david@uqam.ca (D.F. Bird).

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encountered in the muscle of Nile tilapia was found to exceed the TDI by up to 5-fold (Mohamed et al., 2003). In Brazil, Magalhães et al. (2001) reported that during a three-year monitoring of a brackish lake in Rio de Janeiro, 74% of the fish exceeded the TDI while a short-term study in two Brazilian reservoirs showed that 26% of tilapias sampled represented a risk for human consumption (Deblois et al., 2008).

In a study on microcystin accumulation and depuration in juvenile *Tilapia rendalli* fed with toxic *Microcystis*, MC was found to accumulate rapidly in the liver, whereas high MC concentrations were found in the muscle only during the depuration period (Soares et al., 2004). This result suggested that a complex biological pattern of accumulation and depuration occurs in the fish, and that fish muscle can become toxic even after the termination of a toxic bloom (Soares et al., 2004). It was also demonstrated that tilapia can detoxify and excrete MC as a way to avoid its toxicity (Mohamed and Hussein, 2006). The evidence is that these mechanisms are efficient in tilapia; Zhao et al. (2006) showed that this group of fish are more tolerant to high concentrations of dietary microcystin than are common carp (*Cyprinus carpio*) and gibel carp (*Carassius auratus gibelio*).

To date, most studies have looked at the link between MC in water and seston and MC measured in fish tissues through monitoring of both fishes and water contamination level (Cazenave et al., 2005; Chen et al., 2009; Deblois et al., 2008; Magalhães et al., 2001; Martins and Vasconcelos, 2009; Mohamed et al., 2003). Although it is essential to assess the contamination level of fish tissue for human safety, this approach requires a constant monitoring of the aquatic environment in order to properly alert the population when consumption of fish can pose a health risk (Ibeling and Chorus, 2007). Measuring MC concentration in water is relatively fast and easy, while measuring MC in fish tissue requires expensive facilities, can be tedious and is time consuming. One way that could help to diminish the amount of energy and time spent on monitoring of fishes without risking local health population could be provided by a model that can link MC concentration in water or seston to MC in fish tissues. This link was estimated by Zhao et al. (2006) in a laboratory study with *Tilapia* fingerlings and the dietary intake per fish was linked to MC in tissue through quadratic equations. This kind of predictive tool could be very useful for the prediction of contamination level of fish without direct sampling. It can also help to establish a threshold value below which it is not necessary to invest the time and energy to assess fish contamination. Such a model could also illuminate the dynamics of accumulation and depuration in fish.

In this paper, we present results on the accumulation of MC in fish during a 31-day experiment where reared *Oreochromis niloticus* were fed with seston collected from a toxic bloom of *Microcystis* sp. in a fish farm in Brazil. From these data we developed models that predict MC accumulation in liver from daily availability of MC per fish and from the average MC exposure of the fish to toxic seston. These models showed how accumulation and depuration dynamically change the net accumulation in tilapia.

2. Materials and methods

2.1. Fish

Tilapia (*O. niloticus*) were collected by net from a pond at a fish farm in Minas Gerais, Southern Brazil, at the beginning of the dry season (December 2002). The fish farm belongs to the Brazilian hydroelectric company, Furnas Centrais Eletricas S.A. The fish were selected to be roughly uniform in size, resulting in an average body mass of 250 g and average length of 22 cm. Fish were separated into two groups, control and treated fish, of 100 individuals each, and reared in 8000 l concrete tanks. They were allowed a three week acclimation period in the tank before the experiment began. During that period, no MC was detected in the five fish sampled from each treatment per week, confirming that fish were free of MC at the outset of the experiment.

2.2. Plankton production

Five 10,000 l concrete tanks were enriched once a week with manure for mass production of plankton. Algal succession in these tanks leads over a period of several weeks to a typical endpoint, which is of a toxic cyanobacterial community. The fish farm staff normally avoids this problem by emptying the tank and restarting with new water before cyanobacteria become abundant. Maintaining different tanks at different successional stages allowed us to maintain one tank with a bloom of *Microcystis aeruginosa*, while other tanks, monitored frequently by microscope observation, were relatively free of cyanobacteria. Plankton from these tanks was harvested each day with a 40 μm pore-size net to give two 80 l containers of seston concentrate containing either toxic cyanobacteria or non-toxic algae. These concentrates were sampled daily for dry weight and microcystin level analysis, and pigments and taxonomy were assessed every five days. Dry weight and pigments were measured (APHA, 1995), and taxonomy was done according to the Utermöhl technique (Utermöhl, 1958). Algal biovolume was determined from biometrical measurements of 20 individuals (when available) of each species identified, which were transformed with simple geometrical forms into $mm^3\,cell^{-1}$ (Sun and Liu, 2003).

Buoyant *Microcystis* cells from 15 ml of the concentrated seston were isolated by centrifugation at 2000 × *g* for 5 min. The buoyant *Microcystis* were concentrated in the top layer of the supernatant, and could be separated from the zooplankton and other algae which remained in the pellet. During the centrifugation, the zooplankton was concentrated in the bottom of the vial and a green algal layer was visible on the top of the seston mass. This made the algal layer relatively easy to separate with the help of a dissecting microscope, tweezers and scalpel, and we were able to weigh it separately from the zooplankton fraction. All three lyophilized fractions (zooplankton, non-floating algae and buoyant cyanobacteria) were weighed for freeze-dried weight estimates (FDW) and kept at −20 °C prior to extraction and determination of MC according to Sections 2.4 and 2.6 of this paper.

2.3. Experimental design

Fish were fed daily with the concentrates at the same time of day over an experimental period of 51 days. Since toxic cyanobacteria appeared only after the third week, we restricted our treatment of the data to that bloom interval, which represented a treatment period of 31 days. Every five days, five fish were sampled from each tank and anaesthetized with 2-phenoxyethanol. The fish tissues (liver and muscle) were dissected and preserved at −20 °C until microcystin was extracted and quantified.

2.4. Extraction of microcystin from seston

Microcystin was extracted from 20 to 100 mg of lyophilized cyanobacteria or seston fraction in 1.5 ml of 80% (v:v) MeOH:H_2O. The sample was then sonicated at low intensity for two periods of 30 s, on ice, and shaken for 1 h at room temperature. The sample was centrifuged at 14,000 × *g* and the supernatant collected and stored at −20 °C in a borosilicate tube. The pellet was reextracted twice and the supernatants were pooled together (final volume of 4.5 ml).