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Toxicity of harmful cyanobacterial blooms to bream and roach

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ABSTRACT

Aquatic ecosystems are facing increasing environmental pressures, leading to an increasing frequency of cyanobacterial Harmful Algal Blooms (cHABs) that have emerged as a worldwide concern due to their growing frequency and their potential toxicity to the fauna that threatens the functioning of ecosystems. Cyanobacterial blooms raise concerns due to the fact that several strains produce potent bioactive or toxic secondary metabolites, such as the microcystins (MCs), which are hepatotoxic to vertebrates. These strains of cyanobacteria may be potentially toxic to fish via gastrointestinal ingestion and also by direct absorption of the toxin MC from the water. The purpose of our study was to investigate toxic effects observed in fish taken from several lakes in the Ile-de-France region, where MCs-producing blooms occur. This study comprises histological studies and the measurement of MC concentrations in various organs. The histological findings are similar to those obtained following laboratory exposure of medaka fish to MCs: hepatic lesions predominate and include cell lysis and cell detachment. MC concentrations in the organs revealed that accumulation was particularly high in the digestive tract and the liver, which are known to be classical targets of MCs. In contrast concentrations were very low in the muscles. Differences in the accumulation of MC variants produced by blooms indicate that in order to more precisely evaluate the toxic potential of a specific bloom it is necessary not only to consider the concentration of toxins, but also the variants produced.

1. Introduction

Freshwater ecosystems provide diverse ecosystem goods and services (Sollie et al., 2008). They constitute a vital source of drinking water, sustain major economic activities and contribute to the well-being of the human populations living in their vicinity (Nienstedt et al., 2012). However, these ecosystems are also facing severe environmental pressures which are leading to an increasing frequency of cyanobacterial Harmful Algal Blooms (cHABs). HABs have emerged as a worldwide concern due to perceived increases in their occurrence and severity, and to their known acute and chronic toxic effects on plants and animals, including human beings. HABs are caused by massive and prolonged overgrowth of algae and other plant-like organisms, such as dinoflagellates, diatoms and cyanobacteria that threaten the functioning of these ecosystems and the ecosystem services they provide. The Ile-de-France region includes a large number of small and shallow lakes that undergo perennial or recurrent cHABs events (Catherine et al., 2008, 2012). Against a background of i) the high levels of cyanobacterial proliferation in recent years, ii) the diversity of potentially toxic cyanobacterial species identified, and iii) the high concentrations of

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cyanotoxins (microcystins and saxitoxins) measured during the summer season, cyanobacterial blooms raise concern due to the fact that several strains (*e.g.* of the genera *Microcystis*, *Planktothrix*, or *Anabaena*) produce potent bioactive or toxic secondary metabolites (Sivonen and Jones, 1999). One group of these toxins are the cyclic heptapeptides microcystins (MCs), which are potent inhibitors of serine threonine protein phosphatases and hepatotoxic to vertebrates (MacKintosh et al., 1990). These strains of cyanobacteria may also be toxic to fish both via gastrointestinal ingestion and by direct absorption of the toxin microcystin from the water (Cazenave et al., 2005). As in other vertebrates, after experimental inoculation of high doses of microcystins, the microcystins rapidly accumulate in the liver of the fish, inhibiting the protein phosphatases thus causing damage by cytoskeletal disorganisation and cellular disruption followed by intrahepatic haemorrhage that can prove fatal (Gupta et al., 2003). The purpose of the present study was to investigate toxic effects detected in fish taken from several lakes in the Ile-de-France region, where cHAB-producing microcystin blooms occur. Histological studies were performed and MC concentrations measured in various organs of the fish.

2. Materials and methods

2.1. Sampling

Fish were collected from 3 different lakes (the Enghien and La Grande Paroisse Lakes, and the Lake of the Base de Loisirs de Champs-sur-Marne) in the Ile-de-France region, during eight fishing campaigns that were carried out over a 2-year period at the times of peak cHAB productivity (September–October). The first 2 lakes experience seasonal or perennial blooms of *Plankthotrix,* and the third seasonal blooms of *Microcystis*. Both these organisms produce microcystins. A total of 93 fish were collected from these 3 sites, corresponding to 9 different species: roach (*Rutilus rutilus*), bream (*Abramis brama*), pumpkinseed (*Lepomis gibbosus*), perch (*Perca fluviatilis*), pikeperch (*Sander lucioperca*), pike (*Esox lucius*), ruffe (*Gymnocephalus cernua*), mirror carp (*Cyprinus carpio carpio*), and common carp (*C. carpio*). Since similar results were obtained for most of the fish, results are presented for only two of them: bream and roach, which gave very typical data. The cyanobacterial concentrations were monitored in the water of all 3 lakes, the variants of the microcystins produced were identified by mass spectrometry (Djediat et al., 2011), and the MC concentrations were estimated (in eq. MC-LR) using the PP2A test (Lecoz et al., 2008) or a commercial ELISA kit for MCs (Abraxis, Microcystins ADDA ELISA kit).

Parts of the livers, gills, intestines, female or male gonads of the fish were removed and immediately fixed for subsequent histological and ultrastructural examination. The other parts of the livers, gills, intestines and some muscles were collected and stored at −20 °C for further extraction of MCs. The livers and gonads of the remaining fish in the two groups were immediately frozen in liquid nitrogen, and stored at −80 °C for future proteomic studies.

2.2. Histochemical studies (photon and transmission electron microscopy)

Samples were prepared for both light and electron microscopy. Those used for photon microscopy were fixed with a mixture of paraformaldehyde (2%), glutaraldehyde, (0.5%), picric acid (0.5%), and sucrose (0.18 M) in 0.1 M, pH 7.4, Sørensen buffer. For the ultrastructural observations a similar fixing mixture was used, but with a higher level of glutaraldehyde (1%), and fixing was followed by postfixing with osmium tetroxide (1%). In both cases, the samples were washed three times with Sørensen phosphate buffer (0.1 M, pH 7.4) in three successive 10-min baths, and were then dehydrated in ethanol (50%, 70%, 90% and 100%), with three successive baths at each dehydration step, before being embedded in an epoxy mixture (Spurr's resin). Medium and ultrathin sections were sliced with diamond knives (Diatome) on a Reichert-Jung Ultracut microtome. The semi-thin sections (0.5–1 μm thick) were stained with methylene blue (1%) or with toluidine blue-basic fuchsin and examined under a Nikon photon microscope. They were examined and photographed under a photon microscope (Nikon and Imager Z1 Zeiss with Axovision software). The ultra-thin sections (50–70 nm) were stained with a saturated solution of uranyl acetate in 50% alcohol, and then observed under a transmission electron microscope (TEM), (Hitachi H-7100). The pictures were taken with a Hamamatsu CCD camera. All photos were post-processed using Adobe Illustrator 10 software.

Some samples were used for MC immunolocalization under a light microscope. After fixing they were treated with a monoclonal antibody against MCs (Adda specific, AD4G2, Alexis) that recognizes all MCs. A 1:250 dilution was used. Samples were immerged in the diluted antibody solution. The antibody was revealed with an immunoperoxidase reaction using a DakoCytomation EnVision + System, Peroxidase-HRP kit. Then samples were dehydrated in graded ethanol from 50% to 100%, before being embedded in Unicryl resin. Thin sections were obtained, stained and observed as described above.

2.3. Measurement of MCs concentration in various fish organs

The organs stored for the physical-chemical analyses were lyophilized for 72 h (Lyophilisator Beta 1.8 – Avantec, France). Free and total microcystins were extracted and LC/MS–MS analyses were conducted following the method described by Neffling et al. (2010) and Cadel-Six et al. (2013).

3. Results

3.1. Histological studies in roach (Rutilus rutilus)

Six fish were collected from the lake at Enghien where the concentration of toxins in Microcystin-LR equivalents was 2.88 μg L^{-1}. In the livers of the roach some hepatic lobules looked fairly normal (Fig. 1A) while other were definitely faded (Fig. 1B, C and D). In the latter zones, lytic areas were observed and were apparently responsible for a loss of the general architecture of the liver (Fig. 1B, D) and