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Trophic transfer of microcystins through the lake pelagic food web: Evidence for the role of zooplankton as a vector in fish contamination

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HIGHLIGHTS

- Zooplanktons ingest cyanobacterial filaments and concentrate MCs.
- Trophic transfer of MCs from herbivorous to carnivorous zooplankton is observed.
- Zooplankton DVM is an important process in the fish contamination in lakes.
- MCs are present in both liver and muscle of whitefish.

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ABSTRACT

An in situ study was performed to investigate the role of zooplankton as a vector of microcystins (MCs) from *Planktothrix rubescens* filaments to fish during a metalimnic bloom of *P. rubescens* in Lake Hallwil (Switzerland). The concentrations of MCs in *P. rubescens* and various zooplanktonic taxa (filter-feeders and predators) were assessed in different water strata (epi-, meta- and hypolimnion) using replicated sampling over a 24-hour survey. The presence of *P. rubescens* in the gut content of various zooplanktonic taxa (*Daphnia, Bosmina* and *Chaoborus*) was verified by targeting the cyanobacterial nucleic acids (DNA). These results highlighted that cyanobacterial cells constitute a part of food resource for herbivorous zooplanktonic taxa during metalimnic bloom periods. Furthermore, presence of MCs in *Chaoborus* larvae highlighted the trophic transfer of MCs between herbivorous zooplankton and their invertebrate predators. Our results suggest that zooplanktonic herbivores by diel vertical migration (DVM) act as vectors of MCs by encapsulating grazed cyanobacteria. As a consequence, they largely contribute to the contamination of zooplanktonic predators, and in fine of zooplanktivorous whitefish. Indeed, we estimated the relative contribution of three preys of the whitefish (i.e. *Daphnia*, *Bosmina* and *Chaoborus*) to diet contamination. We showed that *Chaoborus* and *Daphnia* were the highest contributor as MC vectors in the whitefish diet (74.6 and 20.5% of MC-LR equivalent concentrations, respectively).

The transfer of MCs across the different trophic compartments follows complex trophic pathways involving various trophic levels whose relative importance in fish contamination might vary at daily and seasonal scale.

1. Introduction

The massive eutrophication of freshwater ecosystems during the 20th century has caused the destabilisation of the structure and function of pelagic food webs (Smith and Schindler, 2009). One of the most common consequences of these enhanced nutrient inputs is a shift in the composition of the primary producers due to the appearance of noxious autotrophic bacteria, such as cyanobacteria (O'Neil et al., 2012). The presence of cyanobacteria in freshwater ecosystems is generally associated with the appearance of ecological disturbances. Indeed, cyanobacteria are known to produce a large variety of toxic metabolic compounds that interact with other trophic compartments (Martins and Vasconcelos, 2009; Van Apeldoorn et al., 2007; Wiegand and Pflugmacher, 2005). Microcystins (MCs) are likely the most widespread cyanotoxins found in freshwater lakes producing multiple adverse effects on phytoplankton, zooplankton and fish (Babica et al., 2006; Malbrouck and Kestemont, 2006; Wilson et al., 2006).

The effects of cyanobacteria on metazooplankton are well documented in the literature, especially as it is known that metazooplankton is a key food web component responsible for the transfer of matter and energy from primary producers up to the fish compartment in freshwater ecosystems (Lampert, 1997). Generally, cyanobacteria have negative

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effects on the total biomass and composition of the zooplankton community due to their shape/morphology and their toxin production (Hansson et al., 2007). However, zooplankton is known to contain MCs, indicating that it is able to graze on cyanobacteria and/or contaminated preys (Davis et al., 2011; Ferrão-Filho and Kozlowsky-Suzuki, 2011; Oberholster et al., 2006). Thus, zooplanktonic organisms could transport MCs and could therefore act as vectors of cyanotoxins for fish species in freshwater ecosystems where cyanobacterial blooms occur although such demonstrations are rare in literature (Teegarden et al., 2003).

Indeed, zooplanktonic organisms constitute a major part of the diet of many fish, either throughout their life or, at least, during early ontogenic stages (DeVries and Stein, 1992). In in situ conditions, several studies have previously highlighted the presence of MCs not only in various fish species (Ferrão-Filho and Kozlowsky-Suzuki, 2011; Martins and Vasconcelos, 2009; Sotton et al., 2011), but also in different zooplankton species, revealing the presence of MCs at multiple trophic levels (Ibelings et al., 2005; Kotak et al., 1996; Lehman et al., 2010). However, we still lack knowledge of the main vectors of MCs in fish contamination. This contamination pathway may vary according to the ecosystem, type of cyanobacteria (e.g., surface or metalimnic blooms), the ability/advantage of zooplankton to avoid cyanobacteria as a food resource, and the diet and spatial distribution of zooplanktivorous fish.

In Lake Hallwil (Switzerland), *Planktothrix rubescens*, a toxic cyanobacterium that produces primarily MC-RR and MC-LR, and their (de)methylated analogues (Briand et al., 2005; Ernst et al., 2009), produces annual metalimnic blooms. It also occasionally creates surface scums. In this meso-eutrophic lake (Ostermaier and Kurmayer, 2010), a massive mortality of zooplankton, especially *Daphnia*, has been observed during a *P. rubescens* bloom, but the underlying toxic mechanisms (e.g., consumption of filaments, balneation in MCs) were not identified (Baumann and Jüttner, 2008). The whitefish (*Coregonus suidteri*) dominates the pelagic fish community of Lake Hallwil (Enz et al., 2001), and is particularly important in the entire peri-alpine region due to its commercial value. Therefore, the risk of the MC accumulation in this species is of particular importance to human health. Whitefish occur below the thermocline due to their temperature preference (Mehner et al., 2010) in the water layer where metalimnic blooms of *P. rubescens* occur (Sotton et al., 2011). Their position in the water column increases the risk of the direct ingestion of filaments or the accumulation of dissolved MCs. However, for this zooplanktivorous species, a suggested contamination pathway is the trophic transfer of MCs by zooplanktonic or invertebrate preys (Sotton et al., 2012a, 2011). However, these preys might have various levels of contamination by MCs according to their diet, their migratory pattern and their contact/ingestion of cyanobacterial filaments. The circadian dynamics of vertical distribution of zooplankton is linked to migration movements between the deep water layers and the upper epilimnion (Hays, 2003) during which zooplankton could ingest *P. rubescens* filaments and then transfer MCs to fish. Consequently, fish that are not in direct contact with a *P. rubescens* bloom can be contaminated though the consumption of contaminated zooplankton.

In order to verify, in situ, the existence of a multi-trophic levels' transfer of MCs during a metalimnic bloom, and then to quantify the relative importance of various zooplanktonic taxa in whitefish contamination by MCs, we designed a multi-compartment in situ approach in order to (1) verify the ability of the different zooplanktonic preys of whitefish to ingest *P. rubescens* filaments (for this purpose, we applied DNA-based analyses specifically targeting *P. rubescens* in zooplankton gut contents), (2) assess the MC contamination in herbivorous/predator zooplankton, and the quantities of MCs transported by the different zooplanktonic organisms in water column (according to their biomass and their vertical distribution), and (3) estimate, from fish diet study, the relative contribution of various zooplanktonic preys as vectors of cyanotoxins for the zooplanktivorous whitefish.

2. Materials and methods

2.1. Study site and sampling design

Lake Hallwil is a meso-eutrophic lake (Ostermaier and Kurmayer, 2010) in northern Switzerland (47°18′N, 08°12′E). Its surface area is 10.3 km^2, and its maximum depth is 48 m (average depth of 28.6 m). Massive blooms of *P. rubescens* have been occurring since the early nineties (Stöckli, 2012).

Measurements and sampling were performed in August 2011 (26–27th) at one central station of the lake, corresponding to the maximum depth. Sampling was performed over a 24-h cycle. Samples were taken at dawn (05:30–07:30 h local time (LT)), during the day (13:30–15:30 h LT), at dusk (20:30–22:30 h LT) and at night (00:30–02:30 h LT) in the epilimnion (0–12 m), metalimnion (12–19 m) and hypolimnion (19–40 m). These water layers were defined according to the vertical thermal profiles obtained at each sampling time with a multiparameter probe (see below).

2.2. *P. rubescens* abundance from in-situ measurements and microscope counting

At each sampling time, a calibrated BBE Fluoroprobe (Moldaenke, Germany) was used to estimate the vertical distribution of *P. rubescens* abundances according to the fluorescence activity of chlorophyll *a* (Chl *a*) of the cyanobacterial filaments (Leboulanger et al., 2002). Simultaneously, a multiparameter probe (Seabird 19plus, Sea-Bird Electronics, Inc., USA) was used to determine the thermocline depth and the different water layers (epilimnion, metalimnion and hypolimnion). Water samples were collected in each of the 3 layers by pumping 20 L of water from each 'stratum'. Then, for each water layer, 3 subsamples (100 mL) were preserved in Lugol's iodine solution and used to obtain counts of *P. rubescens* with the Utermöhl technique (Uthermöhl, 1958) and an inverted microscope (Axiovert 135, Zeiss). Similarly, for each water layer, 3 other subsamples (250 mL) were filtered through a 1-μm filter (Nucleopore, Whatman) and stored at −20 °C prior to MC analysis (see Section 2.6).

2.3. Zooplankton sampling and counting

Zooplankton was collected from integrated samples ($n = 5$ per sampling time) in each water stratum (epilimnion, metalimnion, hypolimnion) with a 100-cm long, 22-cm wide and 175-μm mesh biconical closing net (Asptein's type) towed at a low speed through each water layer. According to the closing net radius and the height of each water layer, the total volumes filtrated per closing net sample were 0.542, 0.31, and 0.95 m^3 in epilimnion, metalimnion and hypolimnion, respectively.

For zooplankton counting, 1 closing net sample was fixed in 4% formaldehyde to constitute a 100 mL sample. Counts were made using 3 subsamples (2 mL each containing at least 300 individuals) for each water layer and at each sampling time. Subsamples were enumerated with a counting chamber and an Olympus BX 40 stereo microscope at 10× magnification.

Chaoborus larvae were observed using hydroacoustic methods, frequently used in many surveys (Malinen et al., 2005; Knudsen et al., 2006; Jurvelius et al., 2008). The same echo-sounder frequency (70 kHz) was used in this survey and in previously documented surveys (Gerdeaux et al., 1989).

To collect enough zooplanktonic material for microcystins and a DNA-based diet analysis of *Daphnia*, *Bosmina* and *Chaoborus*, 4 closing net samples were collected in each water layer at each sampling time, and then pooled to constitute one large sample per water layer and