ELSEVIER

Aquatic Toxicology 79 (2006) 140–148

AQUATIC TOXICOLOGY

www.elsevier.com/locate/aquatox

Interactions between cyanobacteria and Gastropods I. Ingestion of toxic *Planktothrix agardhii* by *Lymnaea stagnalis* and the kinetics of microcystin bioaccumulation and detoxification

Emilie Lance, Luc Brient, Myriam Bormans, Claudia Gérard *

Département d'Ecologie Fonctionnelle, UMR Ecobio 6553, Université de Rennes I, Campus de Beaulieu, Avenue du Général Leclerc, 35042 Rennes Cedex, France

Received 24 February 2006; received in revised form 1 June 2006; accepted 2 June 2006

Abstract

The last two decades have been marked by an increasing occurrence of toxic cyanobacterial blooms in aquatic ecosystems. These pose an expanding threat to the environment and to human health. Among the intracellular toxins produced by cyanobacteria, microcystins (hepatotoxins) are the most frequent and widely studied. As an ubiquitous herbivore living in eutrophic freshwaters, the freshwater snail *Lymnaea stagnalis* (Gastropoda: Pulmonata) is particularly exposed to cyanobacteria. The toxic filamentous *Planktothrix agardhii* is common in temperate lakes and is, therefore, a potential food resource for gastropods. We have studied the consumption of *P. agardhii* by *L. stagnalis* juveniles and adults in the presence or absence of non-toxic food (lettuce) over a 5-weeks period. Intoxication was followed by a 3-week detoxification period when snails were fed only on lettuce. The kinetics of microcystin accumulation and detoxification in the gastropods were established using the ELISA analytical method. The results showed an ingestion of toxic *P. agardhii* by *L. stagnalis*, even in the presence of lettuce, and the absence of food selection regardless of the age of the snails. Juveniles and adults consumed the same number of cells per millilitre and consumption was proportional to food availability. On average, 63% of cyanobacteria available were taken up during the first 24 h. After 5 weeks of intoxication, 61% of the toxins present in the ingested cyanobacterial cells had accumulated in snail tissues (95% in the digestive–genital gland complex) with a concentration up to 80.4 ± 4.9 μg g DW^{-1}. Toxin accumulation was greater in the gastropods fed on *P. agardhii* alone than those fed on the mixed diet, and was also greater in juveniles than in adults. After the removal of toxic cyanobacteria, detoxification was rapid: 64% of the toxins disappeared from snail tissues during the first week, but microcystins were still detected after 3 weeks (on average, 3.5 ± 0.9 μg g DW^{-1}). These results are discussed in terms of potential contamination to the food web.

Keywords: *Lymnaea stagnalis*; Cyanobacteria; Microcystins; Bioaccumulation; Detoxifcation

1. Introduction

Cyanobacteria can form massive blooms in freshwater bodies and produce a wide range of toxins such as hepatotoxins, neurotoxins and lipopolysaccharides. Hepatotoxins have a more widespread occurrence and are found in 40–75% of cyanobacterial blooms (Chorus and Bartram, 1999). The most studied hepatotoxins are the microcystins, cyclic heptapeptides of which 80 variants have been identified (Dietrich and Hoeger, 2005). Contamination of organisms can occur by exposure to soluble toxins, direct consumption of cyanobacterial cells and by consumption of contaminated prey. Microcystins have been recognized to accumulate and induce extensive damage in several organisms including zooplankton, bivalves and fish, after ingestion of cyanobacterial cells (for review: Zurawell et al., 2005).

Freshwater gastropods have rarely been considered in toxic cyanobacteria studies. However, these organisms represent an important part of freshwater macroinvertebrate biomass. They are important links between primary producers and higher consumers, and they often play key roles in structuring aquatic communities (Habdija et al., 1995). Recently, pathogenic effects of dissolved microcystin-LR on life-traits have been demonstrated in laboratory experiments on two gastropod species: the prosobranch *Potamopyrgus antipodarum* has shown a decrease in survival, growth and fecundity (Gérard

* Corresponding author. Tel.: +33 223235037; fax: +33 223235054.
E-mail address: claudia.gerard@univ-rennes1.fr (C. Gérard).

0166-445X/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquatox.2006.06.004

and Poullain, 2005) while the pulmonate *Lymnaea stagnalis* has shown a decrease in fecundity (Gérard et al., 2005).

Freshwater pulmonates like lymnaeids inhabit shallow littoral zones and are predominantly herbivores. They are considered as indiscriminate grazers searching for food in the entire water column and on various substrates of the littoral and adapt their diet to the relative abundance of available resources (Bovbjerg, 1968; Reavell, 1980; Brendelberger, 1997). As cyanobacteria can dominate phytoplankton community and colonize littoral waters in bloom periods, it is therefore relevant to ask whether these grazers would consume large quantities of toxic cyanobacteria and whether they would be affected by this consumption. The few field studies which have included gastropods (Kotak et al., 1996; Zurawell et al., 1999; Ozawa et al., 2003; Chen et al., 2005) have hypothesized a consumption of toxic cyanobacteria following a positive relationship found between toxin concentration in phytoplankton and microcystin accumulation in gastropod tissues.

L. stagnalis is characteristic of eutrophic aquatic systems (Clarke, 1979) which are more prone to cyanobacterial blooms. *Planktothrix agardhii*, a filamentous planktonic species is the most common cyanobacterium in eutrophic lakes of temperate areas (Scheffer et al., 1997; Brient et al., 2004) and has a greater cellular toxin production than other colonial or unicellular species (Christiansen et al., 2003). Although this species is planktonic, wind events have been shown to concentrate filaments in littoral zones. Indeed, Webster and Hutchinson (1994) predict that a blue-green population should be more strongly concentrated towards the downwind end of a lake. This prediction is in accord with measured distributions in lakes. Densities of toxic cyanobacteria can thus become very high in shallow waters and with the filaments trapped and accumulating in dense macrophytes, on rocks and littoral sediments, increasing the probability of grazing by snails.

This study is the first part of a research program on freshwater cyanobacteria–gastropod interactions and examines the potential of *L. stagnalis* to ingest *P. agardhii* and to accumulate microcystins. The second part of our work focuses on the negative impact of toxic cyanobacteria ingestion on the life traits of gastropods (survival, growth, fecundity), and the plasticity of the response according to their development stage (juveniles or adults). Results will be presented in a separate publication.

Consumption of cyanobacterial cells by gastropods in the presence or not of non-toxic food was monitored during 5 weeks to study the feeding behaviour of *L. stagnalis*. Toxin production by *P. agardhii* was evaluated to assess the quantity of microcystins ingested by snails. Additionally, we investigated the potential of *L. stagnalis* to accumulate microcystins by establishing the kinetics of accumulation and detoxification of microcystins in gastropod tissues during a 5-weeks intoxification period followed by a 3-weeks detoxification period, and by calculating the ratio of accumulated/ingested toxins.

We focused the discussion on the potential accumulation of cyanotoxins by lymnaeid snails in the field and contamination risk for the food web, since these gastropods are consumed daily by numerous invertebrates (crayfish, leeches, aquatic insects as adult coleopterans or larval tabanids) and vertebrates (fish, waterfowl) (for review: Michelson, 1957), which in turn are consumed by aquatic or terrestrial predators like fish, amphibians, musk rats and birds.

2. Material and methods

2.1. Biological material

Prior to the experiments, juvenile and adult *L. stagnalis* (14 ± 1 and 25 ± 1 mm shell length, respectively) were acclimatized at constant temperature ($20 \pm 1\,^{\circ}$C) and photoperiod (12-h light:12-h dark cycle) and fed on dried lettuce ad libitum during 7 days. The filamentous cyanobacterium *P. agardhii*, originating from the recreational watersport site at Viry (Essone, France), was maintained in a modified medium (20 mL of liquid BG11 per litre of dechlorinated water). Cyanobacteria were placed in an incubation room at constant temperature ($25 \pm 2\,^{\circ}$C) and photoperiod (12-h light:12-h dark cycle) at an irradiance of $40\,\mu\mathrm{E\,m^{-2}\,s^{-1}}$. The algal concentrations of 200,000 cells $\mathrm{mL^{-1}}$ were provided twice a week to the gastropods. These algal suspensions produced dmMC-LR and MC-RR (detected by HPLC–MS), with a total concentration of $5\,\mu\mathrm{g\,L^{-1}}$ expressed as microcystins-LR equivalents (MC-LReq) and measured by high-performance liquid chromatography (HPLC, Section 2.3.2). The density of cyanobacteria used in this study is similar to that commonly found, and often exceeded in natural systems worldwide (Chorus and Bartram, 1999).

2.2. Experimental setup

Following the period of acclimatization, snails were divided into four groups according to age and diet for a 5-weeks intoxication experiment: (1) cyanobacterial suspension as the only food source for juveniles (juv cyano) and (2) for adults (ad cyano), (3) a mixture of cyanobacterial suspension and lettuce ad libitum for juveniles (juv cyano + let) and (4) for adults (ad cyano + let). Each group consisted of 35 replicates, i.e. 35 isolated individuals, in glass containers of 15 mL for each juvenile and 40 mL for each adult. Preliminary observations showed that gastropods consumed the *P. agardhii* suspension of 200,000 cell $\mathrm{mL^{-1}}$ in 3 days, hence cyanobacteria suspension was renewed twice a week. Control glass containers of 15 and 40 mL were filled in the absence of gastropods, with cyanobacteria suspension with and without lettuce during 5-weeks. Preliminary experiments performed over 20 days on 30 replicates with and without lettuce showed that the *P. agardhii* growth was similar and constant. It was assumed that two replicates per treatment were sufficient to measure cyanobacterial growth, and the influence of the presence of lettuce on the development of cyanobacteria, as the conditions for growth (light, temperature, nutrient) were identical. After the intoxication period, all gastropods were fed solely on lettuce ad libitum and maintained in dechlorinated non-toxic water during a 3-weeks detoxification period.