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Impacts of microcystins on the feeding behaviour and energy balance of zebra mussels, *Dreissena polymorpha*: A bioenergetics approach

G. Juhel [a,*], J. Davenport [a], J. O'Halloran [a], S.C. Culloty [a], R.M. O'Riordan [a], K.F. James [b], A. Furey [b], O. Allis [b]

[a] *Department of Zoology, Ecology & Plant Science, and Environmental Research Institute, University College Cork, Distillery Fields, North Mall, Cork, Ireland*

[b] *PROTEOBIO, Mass Spectrometry Centre for Proteomics and Biotoxin Research, Department of Chemistry, Cork Institute of Technology, Cork, Ireland*

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Abstract

Microcystins are produced by bloom-forming cyanobacteria and pose significant health and ecological problems. To investigate the impacts of these biotoxins on the physiology of the zebra mussels, *Dreissena polymorpha*, a series of short-term feeding experiments were conducted in the laboratory. We used five microalgal diets consisting of single-cell suspensions of the green algae, *Chlorella vulgaris*, the diatom, *Asterionella formosa*, the cryptophyte, *Cryptomonas* sp. and two strains of the toxic cyanobacterium, *Microcystis aeruginosa* (strains CCAP 1450/06 and CCAP 1450/10). A sixth diet was a mixture of the diatom and the CCAP 1450/10 cyanobacterial strain. The low-toxicity strain CCAP 1450/06 contained 7.4 $\mu g\,l^{-1}$ of the MC-LR variant while the very toxic strain CCAP 1450/10 contained 23.8 $\mu g\,l^{-1}$ of MC-LR and 82.9 $\mu g\,l^{-1}$ of MC-LF. A flow-through system was designed to measure the following feeding parameters: clearance, filtration, ingestion and absorption rates. Ultimately the scope for growth (SFG) was determined as a net energy balance. We observed that mussels cleared the cyanobacterial species containing MC-LF (mean ± 95% confidence interval) at a significant lower rate (498 ± 82 ml h^{-1} g^{-1} for the single cell suspension and 663 ± 100 ml h^{-1} g^{-1} for the mixture diet) than all of the non-toxic species and the cyanobacterium containing MC-LR (all above 1 l h^{-1} g^{-1}). The same pattern was observed with all the feeding parameters, particularly absorption rates. Furthermore, MC-LF caused an acute irritant response manifested by the production of 'pseudodiarrhoea', unusually fluid pseudofaeces, rich in mucus and MC-LF-producing Microcystis cells, ejected through the pedal gape of the mussels. This overall response therefore demonstrates selective rejection of MC-LF-producing cyanobacteria by zebra mussels, enhancing the presence of the very toxic MC-LF-producing *M. aeruginosa* in mixed cyanobacterial blooms and in the benthos.

Finally, we observed that the SFG (mean ± 95% confidence interval) of mussels feeding on *M. aeruginosa* containing MC-LF was significantly lower (34.0 ± 18.8 J h^{-1} g^{-1} for the single cell suspension and 83.1 ± 53.0 J h^{-1} g^{-1} for the mixture diet) than for mussels ingesting non-toxic diets, except for *C. vulgaris* (all above 200 J h^{-1} g^{-1}). This reveals a sublethal, stressful effect of microcystins (particularly MC-LF) on the feeding behaviour and energy balance of the zebra mussel.

Keywords: Microcystins; Cyanobacteria; *Microcystis aeruginosa*; Mussel feeding behaviour; Scope for growth; Toxicity

1. Introduction

The zebra mussel, *Dreissena polymorpha* (Pallas), is a well-known introduced invasive freshwater bivalve that has colonized a large part of Northern Europe (including Ireland) and North America after previously being restricted to the Black and Caspian Seas in the 18th century (Hebert et al., 1989; Pollux et al., 2003; Astanei et al., 2005). Zebra mussel populations can reach very high densities in lakes and rivers, and therefore cause serious economic problems by fouling a wide range of immersed structures and by clogging water intake pipes (Nalepa and Schloesser, 1993). Zebra mussels' ability to filter water at high rates has important ecological impacts, particularly because they have a very high potential for removal of microalgae from the water column (Bastviken et al., 1998), leading to significant increases in water clarity of freshwater systems (Budd et al., 2001; Raikow et al., 2004). Moreover, the presence of zebra mussels in a water body has been associated with significant

* Corresponding author. Tel.: +353 214904650; fax: +353 214904664.
E-mail address: g.juhel@mars.ucc.ie (G. Juhel).

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changes in algal species dominance in phytoplankton communities (Heath et al., 1995; Raikow et al., 2004). Some studies have found that the arrival of the zebra mussel is associated with an increase in the dominance of diatoms or green algae (Smith et al., 1998; Dionisio Pires and Van Donk, 2002) but others have shown that the zebra mussel may promote blooms of toxic cyanobacteria, such as *Microcystis aeruginosa*, by selectively rejecting them in their pseudofaeces (Vanderploeg et al., 1996, 2001).

The interaction between zebra mussels and phytoplankton communities is complex, particularly because they can affect algal community composition both directly and indirectly (Bastviken et al., 1998). Indirect interactions are due to the mussels' impact on nutrient or light regimes, and to differential growth of algal species (Bastviken et al., 1998). Direct interactions are linked to the bivalves' feeding activity because they are capable of selective feeding (Baker et al., 1998; Baker and Levinton, 2003; Dionisio Pires et al., 2004a,b). This particle selection is particularly influenced by the quality of the food they feed on, which depends on the algal species they filter (Schneider et al., 1998). The presence of toxins in a phytoplankton species effectively makes that phytoplankton a poor quality diet that can affect zebra mussels' feeding behaviour and physiology (Engstrom et al., 2001). Because of this, the relationship between zebra mussels and the toxic cyanobacterium, *M. aeruginosa* (Kützing), has recently received much attention, particularly because the toxins they produce, microcystins (MC), are associated with harmful algal blooms (HABs) in freshwater bodies subject to eutrophication (Codd, 1992; Nicholls et al., 2002), therefore causing serious ecological and health problems to wildlife and humans (Carmichael, 1994; Bell and Codd, 1996).

Selective feeding in response to MCs has already been investigated in zebra mussels by laboratory studies of their feeding rates on toxic and non-toxic cyanobacteria (Vanderploeg et al., 2001; Dionisio Pires and Van Donk, 2002). However, all of these studies focused only on selective removal (preferential clearance) mechanisms in response to toxins. No research has so far considered the physiological response of zebra mussels to microcystins, that encompass all feeding processes: clearance, filtration, ingestion, absorption, pseudofaecal production, faecal production rates and ultimately energy balance, also known as scope for growth (SFG). SFG is a particularly useful measure, as it has proved to be an effective indicator of stress in marine bivalves (Widdows and Donkin, 1992).

A recent study by Juhel et al. (2006) showed that the highly toxic microcystin variant microcystin-LF (MC-LF) could have particularly detrimental effects on some of the feeding processes of the zebra mussel, including pseudofaeces production, by inducing an acute irritant response. That study suggested that MC-LF might be a useful tool for the study of the effects of MCs on zebra mussels' energy balance. Therefore, the aim of this study was to assess the impact of MCs (particularly MC-LF) on the physiology of zebra mussels by measuring their feeding response, from clearance rate to SFG (energy balance), when feeding on a range of microalgal diets including toxic (MC-producing cyanobacteria) and non-toxic (diatoms, cryptophytes and green algae) species.

2. Methods

2.1. Production of algal species

The algal species used for these experiments were chosen because of their abundance in Irish freshwaters (Bowman, 2000). All algal species were cultured under controlled conditions ($20 \pm 1\,^{\circ}C$; 12 h light/dark cycle). Five strains were cultured: *Asterionella formosa* (Hassal) (bacillarophycea) (SAG 8.95) obtained from Sammlung von Algenkulturen Göttingen (SAG, Göttingen, Germany), *Chlorella vulgaris* (Beijerinck) (chlorophycea) (CCAP 211/11B), *Cryptomonas* sp. (Ehrenberg) (cryptophycea) (CCAP 979/62) and two strains of the toxic cyanobacterium *M. aeruginosa* (CCAP 1450/10 and CCAP 1450/06) obtained from the Culture Collection of Algae and Protozoa (CCAP, Argyll, UK). *A. formosa* was cultured in Bacillariophycean Medium with Vitamins (Diat. + Vit. Mix, Schlösser, 1994) and the other species were cultured in Jaworsky medium (JM, Thompson et al., 1988). Each strain was used in its exponential stable phase of growth, corresponding to different cell concentrations for each culture: approximately 6.3×10^5 cells ml^{-1} for *A. formosa*, 1.2×10^6 cells ml^{-1} for *C. vulgaris*, 4.0×10^4 cells ml^{-1} for *Cryptomonas* sp., 8.1×10^6 cells ml^{-1} for *M. aeruginosa* CCAP 1450/06 and 1.3×10^7 cells ml^{-1} for *M. aeruginosa* CCAP 1450/10.

2.2. Toxin profiles of the two strains of M. aeruginosa

For each of the two strains of *M. aeruginosa*, culture samples (50 ml) were collected a day prior to the experiments and their toxic profiles was determined according to the general procedure of Ortea et al. (2004) also described in Juhel et al. (2006). Microcystins were extracted with optimised solid-phase extraction (SPE) columns (Bakerbond C_{18} Polarplus cartridge; Mallinckrodt Baker, Pillipsburg, NJ) based on the method developed by Lawton et al. (1994). Analyses of microcystins were carried out with liquid chromatography–tandem mass spectrometry (LC–MS/MS) using a HP100 series LC system with ultra violet-photodiode array (UV-PDA) detector (Agilent, Ipswitch, UK) linked with an LCQ ion-trap mass spectrometer (ThermoFinnegan, San Jose, CA). Four toxic variants were investigated MC-LR, MC-RR, MC-YR and MC-LF. These variants were chosen because they are the most abundant in nature (Dawson, 1998). Ortea et al. (2004) indicated a detection limit better than 0.1 ng for their method.

2.3. Mussel collection and handling

One hundred zebra mussels were collected with a garden hoe from the submerged area of a quay in Ballina Marina, Killaloe, County Tipperary, in March 2004, Ireland. Although cyanobacteria have been found in Lough Derg (Reynolds and Peterson, 2000), to date no reports of MC-forming cyanobacterial blooms exist in the literature in that lake. Cyanobacterial blooms usually occur in late spring–early summer (Reynolds and Peterson, 2000), meaning that mussels were not likely to have been recently exposed to MCs. Only adult mussels between 20