Chapter 32: Cyanobacterial toxins: a qualitative meta–analysis of concentrations, dosage and effects in freshwater, estuarine and marine biota

Bas W Ibelings[1], Karl E Havens[2]

[1]Netherlands Institute of Ecology (NIOO–KNAW) – Centre for Limnology, Rijksstraatweg 6, 3631 AC, Nieuwersluis, The Netherlands; e–mail b.ibelings@nioo.knaw.nl; [2]University of Florida, Department of Fisheries and Aquatic Sciences, Gainesville, FL 33458, USA

Abstract

This paper reviews the rapidly expanding literature on the ecological effects of cyanobacterial toxins. The study employs a qualitative meta–analysis from the literature examining results from a large number of independent studies and extracts general patterns from the literature or signals contradictions. The meta–analysis is set up by putting together two large tables – embodying a large and representative part of the literature (see Appendix A). The first table (Table A.1) reviews the presence (concentrations) of different cyanobacterial toxins in the tissues of various groups of aquatic biota after exposure via different routes, experimentally in the lab or via natural routes in the environment. The second table (Table A.2) reviews the dose dependent effect of toxins on biota. The great majority of studies deal with the presence and effects of microcystin, especially of the MC–LR congener. Although this may partly be justified – MC–LR is an abundant and highly toxic protein – our review also emphasizes what is known about (i) other MC congeners (a number of studies showed a preferred accumulation of the less toxic variant MC–RR in animal tissues), (ii) nodularin (data on a range of biota from studies on the Baltic Sea), (iii) neurotoxins like anatoxin–a(s), which are conspicuously often present at times when mass mortalities of birds occur, (iv) a few studies on the presence and effects of cylindrospermposin, as well as (v) the first examples of

ecological effects of newly identified bioactive compounds, like microviridin–J. Data were reorganized to assess to what extent bioconcentration (uptake and concentration of toxins from the water) or biomagnification (uptake and concentration via the food) of cyanobacterial toxins occurs in ecosystems. There is little support for the occurrence of biomagnification, and this reduces the risk for biota at higher trophic levels. Rather than biomagnification biodilution seems to occur in the foodweb with toxins being subject to degradation and excretion at every level. Nevertheless toxins were present at all tropic levels, indicating that some vectorial transport must take place, and in sufficient quantities for effects to possibly occur. Feeding seemed to be the most important route for exposure of aquatic biota to cyanobacterial toxins. A fair number of studies focus on dissolved toxins, but in those studies purified toxin typically is used, and biota do not appear very sensitive to this form of exposure. More effects are found when crude cyanobacterial cell lysates are used, indicating that there may be synergistic effects between different bioactive compounds. Aquatic biota are by no means defenseless against toxic cyanobacteria. Several studies indicate that those species that are most frequently exposed to toxins in their natural environment are also the most tolerant. Protection includes behavioral mechanisms, detoxication of MC and NODLN by conjugation with glutathione, and fairly rapid depuration and excretion. A common theme in much of the ecological studies is that of modulating factors. Effects are seldom straightforward, but are dependent on factors like the (feeding) condition of the animals, environmental conditions and the history of exposure (acclimation and adaptation to toxic cyanobacteria). This makes it harder to generalize on what is known about ecological effects of cyanobacterial toxins. The paper concludes by summarizing the risks for birds, fish, macroinvertebrates and zooplankton. Although acute (lethal) effects are mentioned in the literature, mass mortalities of – especially – fish are more likely to be the result of multiple stress factors that co–occur during cyanobacterial blooms. Bivalves appear remarkably resistant, whilst the harmful effects of cyanobacteria on zooplankton vary widely and the specific contribution of toxins is hard to evaluate.

List of abbreviations.

Abbreviation	Definition
AchE	Acetyl Choline Esterase
ANA(a)(as)	Anatoxin–a; anatoxin–a(s)
BCF	Bioconcentration Facor (concentration of toxic compound in an organism as % of that in water)
BMF	Biomagnification Factor (concentration of toxic compound in an organism as % of that in its diet)
CAT	Catalase (one of the antioxidative enzymes)
CYN	Cylindrospermopsin
GI	Gastrointestinal tract
GPx	Glutathione Peroxidase (one of the antioxidative enzymes)
GR	Glutathione Reductase (one of the antioxidative enzymes)
GSH	Glutathione
GST	Glutathione–S–Ttransferase (catalyst of the formation of MC–GSH conjugates in detoxication)
H_2O_2	Hydrogen peroxide (one of the ROS formed during oxidative stress)
HP	Hepatopancreas
IP	Intraperitoneal injection
LC_{50}	Concentration at which 50 % of the test animals die from exposure to the toxin
LOEC	Lowest Observable Effect Concentration
LPO	Lipid Peroxidation (outcome of oxidative stress)
LPS	Lipopolysacharides
MC	Microcystin
NODLN	Nodularin
PP	Protein phosphatases (inhibition of PP by MC results in hyperphosphorilation of proteins)
PST	Paralytic Shellfish Toxin
ROS	Reactive Oxygen Species (formed during oxidative stress)
SAX	Saxitoxin
SOD	Super Oxide Dismutase (one of the antioxidative enzymes)
TDI	Tolerable Daily Intake (of a toxin like MC)
TEH	Total Extractable Hepatotoxins (sum of toxins and their biotransformation products)
TOSC	Total Oxygen Scavenging Capacity

Introduction

Until just a few years ago statements like "traditionally research has focused on the acute toxicity of microcystin–LR to laboratory mammals" and "there is a general lack of research involving aquatic organisms which may be exposed to toxin producing cyanobacterial blooms in their natural environment" (Zurawell et al. 1999) were fully justified. In contrast, there now exists a large literature from observational and experimental studies dealing with cyanobacterial toxins in aquatic systems. Yet major information gaps remain, and our ability to understand effects is limited by certain attributes of those studies. The aim of this paper is to review the extant literature, identify general patterns in results, and identify key areas where additional research is warranted.

This assessment is complex because in addition to microcystin–LR there are many other toxins produced by cyanobacteria. Some of these toxins, such as nodularin, are closely related to microcystin, while others are quite different (e.g., the neurotoxins anatoxin–a and a(s) and saxitoxin and the protease inhibitor cylindrospermopsin). There also exists an ever increasing list of bioactive compounds produced by cyanobacteria, some of which have been shown to be toxic to selected aquatic biota (like microviridin–J for *Daphnia* (Rohrlack et al. 2004), but many of which have not been studied in any detail. Furthermore, chronic and sub–chronic effects (see Havens et al elsewhere in this volume for definitions) may be more relevant to study than acute lethal effects. Exposure of biota in lakes supporting cyanobacterial blooms is likely to be repetitive and over much of an organism's lifespan. Although cyanobacterial toxins have been claimed to play a role in acute events like mass mortalities of fish and birds, there is usually insufficient evidence to link fish and bird kills directly to these toxins. This does not mean that there are no important sub–lethal effects resulting from chronic exposure in the aquatic ecosystem.

This study employs qualitative meta–analysis of data from the literature, examining results from a large number of independent studies and synthesizing summaries and conclusions addressing the issue of toxic effects. Meta–analysis aims to utilize the increased power of pooled data to clarify the state of knowledge on that issue, and may include quantitative statistical analyses when the data are consistent with that approach – this is not the case here. We set up the meta–analysis by putting together two large tables (see Appendix A). The first table (Table A.1) reviews the presence (concentrations) of cyanobacterial toxins in the tissues of aquatic biota after exposure via different routes, be it experimental or via natural routes in the field. The second table (Table A.2) reviews the dose dependent effects

of toxins on biota. While Tables A.1 and A.2 do not include 100% of the published papers on these subjects, they do embody a large and representative part of the literature.

Methodology

The literature was queried using the ISI–Web of Science. Results of the literature search are given in Havens et al (this volume). To assemble Table A.1 – concentrations in biota – the following data were extracted from the literature: (i) biota involved, four groups are distinguished: birds, fish, macroinvertebrates and zooplankton); (ii) type of cyanobacterial toxin studied; (iii) exposure route; (iv) toxin concentrations in biota; (v) concentration in the source (i.e. this could be dissolved purified toxin in an experimental setting, cells from cultures of toxic cyanobacteria or natural seston containing toxic cyanobacteria); and (vi) analytical analysis method that was used to quantify the toxin. In the discussion, data from Table A.1 will be organized in such a way that biomagnification (accumulation of the toxins in biota via consumption of food that contains the toxins) can be quantified. Bioaccumulation of cyanobacterial toxins is often speculated to increase the risk of exposure for aquatic biota (especially at higher trophic levels), but bioaccumulation has seldom been analyzed correctly (see Havens et al, this volume for definitions). Especially for MC it is well known that the analytical method may have a marked effect on the concentration that is measured. This is even more so when MC is measured in biota, rather than in the toxin producing cyanobacteria. Standard MeOH extraction does not include covalently bound MC and analysis using ELISA suffers from cross reactivity between MC or NODLN and their GSH conjugates formed in detoxication. The consequences of this for interpretation of the concentrations given in Table A.1 are discussed.

For Table A.2 the following data were compiled: (i) biota involved; (ii) exposure route; and (iii) dose and effect. In many studies it is not possible to establish true dose–effect relationships because organisms are exposed to only one or two different dosages, and Table A.2 will indicate in which cases sufficient data have been gathered to establish a relationship. Ideally the unit for dose would be units of toxin administered per unit of body weight and per unit of time (for comparison TDI for humans equals a dose of 0.04 μg kg bw^{-1}). It is more common however to find toxin contents of the source expressed in μg L^{-1}. Differences in units hamper interpretation across different studies. What will also emerge from Table A.2 is that the exposure route has a strong influence on biological effects. In fish IP injec-

tion of MC is often fatal however oral dosage hardly ever results in mortality. As will be discussed, fish–kills in lakes seem to be a consequence of multi–stress factors during blooms of toxic cyanobacteria rather than of direct intoxication. Tables A.1 and A.2 are included as an appendix of this paper.

Results and Discussion

The last 5 years have included a steady increase in the number of papers investigating cyanobacterial toxins in aquatic biota. Whether this increasingly large body of literature is sufficiently broad (in terms of the toxins / bioactive compounds and aquatic biota covered by the studies) and deep (in terms of yielding a detailed understanding of the effects these toxins have on aquatic biota) is another matter. Below we formulate an answer to this question – which is central to this paper – by analyzing the assembled literature presented in the tables.

Toxins in biota

Toxin producing species and their toxins

This review takes a somewhat unusual perspective in that the focus is not on toxin producing cyanobacteria but rather on the biota in the aquatic ecosystem that may be affected by the toxins. Cyanobacteria that (frequently) appear in the tables are the MC producing genera *Microcystis* (species *M. aeruginosa*, *M. flos aquae* and *M. viridis*) and *Planktothrix* (*P. agardhii* and *P. rubescens*), the main NODLN producing species *Nodularia spumigena* and the anatoxin–a and a(s) producing genus *Anabaena.* Although species within a genus may differ greatly in their ecology (*P. agardhii* for instance is commonly found in hypertrophic, turbid shallow lakes, whereas *P. rubescens* is typically found in clear, deep alpine lakes), we do not stress differences between toxin producers at the species level since often we know little about differences in toxin production within a genus like for instance *Microcystis*.

If there is one thing immediately striking about the tables it is the prominence of MC, especially of MC–LR (not all studies specify which MC congeners were present, although most studies express total MC as MC–LR equivalents since often MC–LR is the only standard used in HPLC analysis). This focus on the presence and effects of MC is probably (partly) justified because surveys of cyanobacterial toxins in several coun-

tries have indeed shown that microcystins are prominently present (e.g. in Denmark – Henriksen et al. 1997). The spotlight on MC–LR (one variety in a family of around 70 different MC congeners) may be more biased, and seems influenced by the laboratory work on mammals. MC–LR is relatively toxic, its LD_{50} in mice is 50 μg kg^{-1}, considerably lower than for instance the LD_{50} of MC–RR (600 μg kg^{-1}) (Spoof 2005). Yet as the data in Table A.1 indicate there are a number of studies demonstrating that especially the less toxic MC–RR is taken up into tissues of aquatic biota, for instance in silver carp (Jang et al. 2004); or in freshwater snails (only MC–RR present in foot of *B.aeruginosa* (Chen and Xie 2005) and hepatopancreas of *S.histrica* (Ozawa et al. 2003). On the basis of their observations on the ratio of MC–LR:MC–RR in different tissues and organs, Xie et al. (2004) suggested that MC–LR may be actively degraded during digestion, whereas MC–RR is transported across the intestines and embedded into body tissues. At the same time Xie suggested that MC–RR is not acutely toxic to the carp, since no mortality was observed despite the uptake of MC–RR into various organs. In a study using the *Thamnocephalus* bioassay LC_{50} of MC–RR (and of MC–YR) was actually very close to the LC_{50} of MC–LR. The real difference in this invertebrate was a much gentler slope of MC–RR compared to MC–LR (and YR) when LC_{10} and LC_{90} values were included (Blom et al. 2001).

A further relevant distinction within the microcystins is that between the methyldehydroalanine–containing microcystins which covalently bind to PP in the cell and MC that contains dehydrobutyirine and – like NODLN – that do not bind covalently to PP. For NODLN it has been suggested that because NODLN does not bind covalently its transfer in the food web is facilitated (Kankaanpaa et al. 2001). The same would be true for the dehydrobutyirine containing microcystins, but since concentrations of these have not been analyzed in biota there is no evidence for this. Overall it is clear that studies on MC–LR cover just a small part of the total complexity of interactions between microcystin producing cyanobacteria and aquatic biota, so that the bias indicated by Tables A.1 and A.2 is unjustified. More research is needed on the ecological effects of the whole spectrum of bioactive – potentially harmful – compounds produced by cyanobacteria, including MC congeners other than LR.

Some toxins other than MC have been analyzed in biota. NODLN features fairly prominently in the tables, due to considerable work that has been done on the Baltic sea. This is one of the few regions in the world where there are sufficient data to actually follow concentrations of NODLN and its effects throughout much of the food web (more in discussion on 'bioaccumulation'). Neurotoxins are not a notable group in the table. Interestingly anatoxins (anatoxin–a and/or anatoxin–a(s)) often seem

to play a role when toxic cyanobacteria have been implicated in the death of waterfowl (Henriksen et al. 1997; Krienitz et al 2003). Neurotoxicosis can be seen as convulsed extremities and arched back necks (Codd et al. 2005). The death of the lesser flamingos in Africa's Rift valley lakes is an interesting example where multiple cyanobactertial toxins (neurotoxins and hepatoxins) may play a simultaneous role and have synergistic effects (a more accurate description of the interaction between different toxins or toxins and other stress factors may be additive rather than synergistic effects). This is another area where very little is known today.

Biota involved and organs affected

Tables A.1 and A.2 distinguish four groups of aquatic organisms: waterfowl, fish, macroinvertebrates (i.a. bivalves, crabs, prawns, snails) and zooplankton. There are only a handful of studies involving birds (see above). There are many more studies on fish, the other group of aquatic vertebrates where mass mortalities have been attributed to blooms of toxic cyanobacteria. Toxins in fish have been analyzed after exposure via different routes, but the majority of work actually involves studies of fish caught in lake or sea, i.e. fish that has been exposed to toxins via natural routes (exposure through the food web or to dissolved toxins after lysis of blooms). In the ecosystem the feeding guild of a fish species appears to be a primary determinant of exposure to toxins. Phytoplanktivorous fish like silver carp (e.g. Jang et al. 2004) directly consume cyanobacteria. Zooplanktivorous fish like sticklebacks and smelt (Ibelings et al. 2005) feed on zooplankton that directly consume cyanobacteria. Likewise a species like flounder predates on filter feeding blue–mussels. Piscivorous fish that prey on zooplanktivorous fish are one step further removed from the toxin producing cyanobacteria. It may be expected that in the absence of biomagnification (but see discussion below) MC concentrations decrease in the order phytoplanktivorous > zooplanktivorous > piscivorous fish . Omnvirous fish may fit in anywhere. Indeed fish caught in the IJsselmeer, The Netherlands showed an increase in MC in fish–liver moving from larger perch (predatory) to ruffe (benthic) and zooplanktivorous smelt (Ibelings et al. 2005). In contrast Xie et al. (2005) found that MC in various tissues and organs varied as carnivorous > omnvivorous > phytoplanktivorous fish. Fischer and Dietrich (2000) explain that there are several differences in GI tract between a carnivorous fish like rainbow trout and planktivorous and herbivorous cyprinids like carp. Cyprinids (as well as cichlids) possess a much longer ileum with larger surface area and higher resorption capacities, so that carnivorous fish would accumulate less MC, i.e. the opposite of what was found in Chinese lakes. In contrast

Carbis et al. (1997) explain that the neutral or slightly basic conditions in the GI of carp limit absorption of MC, since cells of cyanobacteria would only be digested in an acid environment.

Overall no relationship between feeding guild and toxin concentrations in fish can be pulled out from the data in Table A.1. Data from different studies are hard to compare because toxin concentrations, exposure routes and a host of other biotic and abiotic factors differ between sites and studies. What is clear, and this is found across studies on fish, is that concentrations of MC are mainly present in the gut and liver, to a somewhat lesser extend in kidneys and gonads, and much less in muscle tissue (e.g. Kankaanpaa et al. 2005a; Li et al. 2004; Soares et al. 2004; Malbrouck et al. 2003; Xie et al. 2005). Microcystins are also found in fish faeces in substantial amounts (Jang et al. 2004; Xie et al. 2005) and in pseudofaeces of *Dreissena* (Babcock–Jackson et al. 2002; Pires et al. 2004). This could expose the benthic community to cyanobacterial toxins produced in the pelagic zone.

There is a surprisingly large number of studies on the presence of toxins in macroinvertebrates, especially in bivalves (mussels and clams). In contrast there are very few studies that describe effects of cyanobacterial toxins on these animals, perhaps because generally they seem insensitive (e.g. Saker et al. 2004). A common theme in the studies on macroinvertebrates is the analysis of time courses for accumulation and depuration of toxins. Accumulation is often found to be time dependent and proceeds in an orderly manner. Time lagged acccumulation occurred at deeper sites in the Baltic sea. Mussels at deep sites are primarily exposed to toxic *Nodularia* towards the end of the bloom period, when filaments sink to the sediment, where they over winter. Although mussels at deeper sites contained much less toxin, they did accumulate some NODLN (Sipia et al. 2002). Depuration from mussels is almost always found to be biphasic (Ozawa et al. 2003; Sipia et al. 2001b; Vasconcelos et al. 1995), sometimes concentrations of toxin even increase in the first phase of depuration (Amorim and Vasconcelos 1999). It has been suggested that this is a consequence of dynamics in production and degradation of PP to which the MC are bound (Vasconcelos et al. 2001), but more research is needed (Ozawa et al. 2003). Although depuration is commonly judged to be rapid (e.g. Sipia et al. 2002; Kankaanpaa et al. 2005b; Pereira et al. 2004) it is equally clear that depuration is incomplete even after a considerable period of time. Depuration is temperature dependent and slows down in winter, so that toxins may even be carried on to the next spring (Ozawa et al. 2003). In for instance Lake IJsselmeer this has consequences for thousands of diving ducks that arrive in autumn. Although summer *Microcystis* blooms have dispersed, the mussels (food for the ducks) still contain traces of toxins.

Thus the mussels may be considered a vector that prolongs the time when toxins are able to exert negative effects in that lake ecosystem. In macroinvertebrates hepatotoxins, but also CYN, were mainly found in the haemolymph and hepatopancreas, to a lesser extend also in gonads and muscle tissue (foot).

With respect to zooplankton we see the opposite of the macroinvertebrates papers: there are a large number of studies on effects of cyanobacterial toxins on zooplankton but much less on concentrations of toxins in the animals. The available results suggest that concentrations are relatively high in indiscriminately filter feeding taxa such as *Daphnia* (Ibelings et al. 2005; Kotak et al. 1996a; Thostrup and Christoffersen 1999), and perhaps lower in copepods, but again there is a general lack of data. Toxins seem to be taken up into the body of zooplankton, concentrations of toxin cannot be explained solely by the presence of toxic cyanobacteria in the gut.

Analytical analysis methods

The standard method for analysis of hepatotoxins (MC and NODLN) is HPLC, coupled to diode array UV detection (see Table A.1). ELISA is frequently used because of its high sensitivity (see Spoof 2005) for pros and cons of different methods. A drawback of ELISA is cross reactivity with detoxication metabolites, like the conjugates of MC and GSH. These conjugates have been shown to have a much lower toxicity (Metcalf et al. 2000). Conjugates can be detected using LC–MS, but this is still rarely undertaken (however see Karlsson et al. 2003 and Sipia et al. 2002). Because ELISA suffers from cross reativity some studies on biota in the Baltic Sea have introduced the term TEH – total extractable hepatotoxins, which includes the biotransformation products. TEH almost invariably exceeds the concentrations of untransformed hepatotoxins in biota – see for instance the comparison of NODLN (analyzed on LC–MS) and TEH (ELISA) in Kankaanpaa et al. (2005a), which differ by an order of magnitude, or Lehtonen et al. (2003) where NODLN was < 5 % of TEH in Baltic clams. Another important analytical issue is that of extraction of the toxins. A large number of MC congeners – those that contain methyldehydroalanine – covalently bind to PP in plant and animal cells; these covalently bound MC are not extracted using standard MeOH extraction. The handful of studies that have used Lemieux oxidation – a method that does extract covalently bound MC – demonstrated that a large part of the MC in biota is covalently bound (Williams et al. 1997; Pires et al. 2004) (see Table 2 in Havens et al, this issue). This means that almost all of the concentrations given in Table A.1 seriously underestimate the total amount of MC present in biota. What is unknown – and this is important – is whether all these

studies also underestimate the bioavailability and toxicity of MC. Is covalently bound MC in *Daphnia* still (equally) toxic to the fish that swallows cladocerans?

Bioaccumulation

Many studies have suggested that cyanobacterial toxins bioaccumulate in aquatic biota and that this may enhance the risk of exposure of biota higher up in the food web (e.g. Li et al. 2004; Sipia et al. 2001a; Negri and Jones 1995). Xie et al. (2005) present data that demonstrate that MC has a general tendency to accumulate up the food chain, with concentrations being highest in carnivorous and lowest in herbivorous fish species. PST concentrations in *Daphnia magna* grazing on *Aphanizomenon* exceeded those in the cyanobacterium (bioaccumulation factor > 1). Bioaccumulation in most papers however use a loose definition and usually it just means that toxins are present in biota. When a more formal – and informative – definition of bioaccumulation, and the related processes of bioconcentration and biomagnification are used (see Havens et al, this issue for definitions) there is very little evidence to support the notion of bioaccumulation of MC and NODLN in aquatic food webs. Rather the opposite, i.e. biodilution of hepatotoxins in the food web, is supported by the data (Karjalainen et al. 2005). Data in Table A.1 do show however that bioconcentration of NODLN may take place. In an experimental setting two copepods and a ciliate took up dissolved NODLN and accumulated this to concentrations far higher than in the water (BCF ranged from 12–22). Predators of these zooplankters and protozoa would be exposed to substantial concentrations of toxin in their food and may suffer consequences like decreased ingestion rates, as was shown for pike larvae and mysid shrimps feeding on the zooplankton (Karjalainen et al. 2005).

Biomagnification factors express the concentration of a toxin in biota as a percentage of that in their diet. BMF for the Baltic Sea and IJsselmeer are shown in Table 1.1. BMF in the Baltic biota and in *Daphnia* and *Dreissena* from the IJsselmeer are well below 100 %, indicating that the concentration was much below the concentration in the seston. Biomagnification obviously is absent in these cases. BMF in the Baltic of Copepods and clams are exceptionally low; only a very small part of NODLN which is present in the cyanobacteria is taken up by these grazers. The calculation in Table 1 of BMF for grazers of the phytoplankton is very sensitive to the toxin content of the seston used in the calculation, and this content may be highly variable. Values of BMF in the table should be taken as indicative rather than absolute. The BMF of ruffe and especially smelt in the IJsselmeer are > 100 and seem to indicate that biomagnification of MC has

taken place. In this case however concentrations of MC in a whole organism (like *Daphnia*) are compared to values for a selected organ where the toxin specifically accumulates (the liver), and this gives a skewed representation of biomagnification (Gray 2002). The difference in BMF between freshwater mussels in the IJsselmeer and their marine counterparts in the Baltic is striking. The very low concentration of MC in *Dreissena* led Ibelings et al. (2005) to the conclusion that the food web linked to filter feeding mussels is hardly exposed to toxins. In contrast Kankaanpaa et al. (2005a) concluded that in the Baltic food webs involving mussels are especially exposed to hepatotoxins. The tenfold difference in BMF supports these apparently opposing conclusions.

Table 1. Biomagnification factors Baltic Sea and IJsselmeer (The Netherlands). BMF were calculated as NODLN (Baltic) or MC (IJsselmeer) content in biota as a percentage of toxin in their diet (e.g. in eiders as a % of that in mussels). For comparison BMF is also calculated as percentage of the concentration in the seston (although this would only qualify as biomagnification for organisms that actually feed on seston, like Daphnia and the mussels). Data compiled from (Engstrom–Ost et al. 2002; Kankaanpaa et al. 2005a; Karjalainen et al in press; Lehtonen et al. 2003; Sipia et al. 2001b; Sipia et al. 2002; Sipia et al. 2004) for the Baltic sea. BMF for the IJsselmeer have been modified from Ibelings et al. (2005). Data on which calculation of BMF are based are taken from Table A.1.

Baltic biota	BMF seston	BMF diet	IJsselmeer biota	BMF seston	BMF diet
Copepods	0.3	0.3	Daphnia galeata	20	20
Blue mussel (Mytilus edulis)	8.9	8.9	Zebra mussel (Dreissena polymorpha)	0.9	0.9
Baltic clam (Macoma baltica)	0.6	0.6	Perch (Percia fluviatilis)	5.9	11
Mysid shrimp (Mysis relicta)	0.3	100	Ruffe (Gymnocephalus cernuus)	13.2	120
Pike larvae (Esox lucieus)	0.2	59	Smelt (Osmerus eperlanus)	53.5	286
Sticklebacks (Gasterosteus aculeatus)	0.05	24			
Flounder (Platichthys flesus)	1.6	19			
Eider (Somateria mollissima)	0.8	8			

Exposure routes

Laboratory studies

Early studies on fish (Tencalla et al. 1994; Kotak et al. 1996b) primarily used the method which is preferred for exposure of mammals in laboratory studies – intra–peritoneal injection. Direct injection of toxins like MC proved to be highly toxic to fish. The effects are comparable to those seen in mammals, but differences are seen as well. Whereas mammals die from haemorrhagic shock following hepatocyte insult, fish die from direct liver failure, necrosis (e.g. Malbrouck et al. 2003, Li et al 2004). The LC_{50} for MC–LR in perch (1500 μg g DW^{-1}, Ibelings et al unpublished data) is well above the LC_{50} for mice, indicating that these fish species are less sensitive to the toxin than warm blooded animals. Nevertheless Sipia et al. (2001a) notes that salmon hepatocytes seem more sensitive to algal toxins than rat hepatocytes. When MC was administered orally (up to 1150 μg MC kg^{-1} bw given by gavage 8 times over 96 h (a total dose of 9200 μg MC kg^{-1} bw) to perch from the IJsselmeer no mortality was seen, although histopathology of the livers showed that MC were having severely detrimental effects. Similar differences between IP injection and gavage can be found in Table A.2 (e.g. Tencalla et al. 1994).

Directly from the water

In ecotoxicology there is a general assumption that uptake from the water is a common route for aquatic vertebrates to accumulate xenobiotic substances (Karjalainen et al. 2003). Indeed some of the studies have demonstrated direct uptake of cyanobacterial toxins from the water, even to the extent that the concentration in biota exceeds those in the water. However, most studies where biota are exposed to dissolved toxins have been in the laboratory (see Table A.2) using purified toxins. These studies have proven valuable in finding the mechanisms through which biota are affected by cyanobacterial toxins but are less informative about the importance of uptake of dissolved toxins in the ecosystem. A specific effect of dissolved MC is inhibition of ATP–ase activity of Na^+ K^+ pumps in the gills of fish and crabs, resulting in ion imbalance (Best et al. 2003; Vinagre et al. 2003; Zambrano and Canelo 1996). Concentrations of dissolved MC are much increased when surface blooms of floating cyanobacteria lyse. When cyanobacteria float to the surface they are exposed to extreme conditions, in particular an increase in irradiance, potentially to damaging levels. Photoprotective mechanisms that may protect the cells from photooxidation are hampered by the co–occurrence of light stress and other stress factors,

notably an increase in temperature, desiccation and depletion of inorganic carbon (Ibelings and Maberly 1998). Lehtonen et al. (2003) suggested that the major fate of cyanobacterial blooms in the Baltic is to disintegrate in the water column so that very little reaches the bottom. If this were the case exposure to dissolved toxins would be a major event. Lysis of surface blooms is not unlikely, but we maintain that exposure of biota to high concentrations of dissolved toxin are the exception rather than the rule because processes like mixing, adsorption to clay particles, photolysis and bacterial degradation rapidly reduce the availability of dissolved toxins (Ozawa et al. 2003).

Moreover it has been shown by several authors that some aquatic biota are not sensitive to dissolved cyanobacterial toxins – e.g., brown trout (Best et al. 2001), pike–larvae (Karjalainen et al. 2005) and *Daphnia magna* (Lurling and van der Grinten 2003). Microcystins tend to be quite water soluble and polar, and do not readily pass the lipid bilayer of membranes. It is important to note however that whenever effects of purified dissolved toxins are compared to whole cell extracts biological effects tend to be much enhanced for the latter (Palikova et al. 1998; Oberemm et al. 1999), a possible indication of synergistic effects between MC and other bioactive compounds in cyanobacterial cells. There are exceptions, however. For example, in *A. salina* purified CYN showed a lower LC_{50} than crude *Cylindrospermopsis* extracts, and this may indicate that unidentified compounds in the cyanobacterial cell extracts lowered the bioavailability of the toxin (Metcalf et al. 2002).

Via food (vectorial transport)

Feeding seems to be the most important route for exposure of aquatic biota to cyanobacterial toxins. This seems natural for organisms that directly feed on seston that includes cyanobacteria. Zooplankton, filter feeding bivalves and phytoplanktivorous fish would be among the organisms that are directly exposed to toxins in their food (unless they manage to avoid toxic cyanobacteria – see below 'protective mechanisms'). For those biota that do not feed directly on cyanobacteria, toxins must reach them via the food web. The risk of being exposed to toxins via the food web is much increased if biomagnification takes place. This is commonly found for lipophilic toxicants like PCB, but is less likely for hydrophilic compounds like MC–LR. This congener has a very low octanol to water partition coefficient, but as demonstrated by Ward and Codd (1999) other variants may have higher coefficients, and toxicity to *Tetrahymena* has been shown to vary accordingly. As discussed above biomagnification of MC and NODLN is unlikely and not substantiated by data from the field. Of the

amount of toxin ingested with the food very little is actually taken up into the body (e.g. 2.7 % in *Daphnia* in Rohrlack et al. 2005). And even the little toxin that is actually taken up into the blood of *Daphnia* and transported to its organs is subject to detoxication (see later in this paper) and excretion. These processes that dilute toxin concentration act at every step in a food chain. Rather than biomagnification MC and other toxins may be subject to biodilution in the foodweb. Nevertheless toxins are found at higher trophic levels, so there must be some vectorial transport, and as will be discussed below in sufficient quantities to have harmful effects. The presence of toxins in grazers like the zooplankton indicate that cyanobacteria are indeed ingested, despite their reputation of being hard to handle because of their large size. Indeed Work and Havens (2003) found cyanobacteria in the gut of all crustacean zooplankton in a large subtropical lake, including taxa known to produce toxins such as *Anabaena*.

A special case of exposure via food is coprophagy, described in a study on blue mussels (Svensen et al. 2005). A fair number of studies have analyzed toxins in the faeces of various species. Concentrations may be relatively high compared to concentrations in organs and tissues, and the faeces laden with toxins provide a medium for further transport of toxins in aquatic systems, especially towards the benthic community. Examples in Tables 1 and 2 include the faeces of silver carp and *C. gibelio* (Jang et al. 2004), *M. galloprovincialis* (Amorim and Vasconcelos 1999) as well as *M. edulis* (Svensen et al. 2005), and faecal pellets of calanoid copepods (Lehtiniemi et al. 2002).

Effects on biota

Acute vs. chronic effects

Acute effects are those that result from a single exposure to a toxin. This is conceivable under laboratory settings or after large scale lysis of a surface bloom. Biota in the field however will mainly be exposed repeatedly to toxins over a long period of time. This is sub–chronic and chronic exposure (definitions in Havens et al., this issue). An example of a study of acute exposure is that by Kankaanpaa et al. (2002) on sea trout. The fish were exposed to a single bolus of toxic *Nodularia* and time dependent accumulation / depuration of NODLN was coupled to the analysis of damage and recovery of the liver. An example of a sub–chronic exposure study (i.e. on a time scale intermediate between acute and chronic) is that by Pinho et al. (2003) where estuarine crabs were exposed daily for 4–7 d to cell extracts from toxic *Microcystis* or the exposure of carp to *Microcystis*

during 28d (Li et al. 2004). Experimental chronic exposure studies where biota are exposed to toxins for the greater part of their lifespan have – necessarily – been restricted to organisms with short generation times, especially zooplankton. There are a fair number of studies in which the effects of cyanobacterial toxins on the life–history of *Daphnia* have been studied. Examples are the studies by (Lurling 2003) and (Hietala et al. 1997). Some of the bivalve studies (accumulation / depuration) lasted for several weeks (e.g., Pires et al. 2004 and Bury et al. 1996) exposed brown trout to MC–LR for a period of 63d, but the great majority of data on chronic exposure to toxic cyanobacteria come from field studies where animals are exposed to toxic cyanobacteria via natural routes (many examples in Tables A.1 and A.2) during extended periods of time.

Overall Table A.2 indicates wide ranging effects of different cyanobacterial toxins on various aquatic organisms. Effects vary from mortality to subtle changes in behavior. Effects in fish include changes in liver enzymology, liver damage and ionic imbalance. Effects of cyanobacterial toxins on the embryonic development of fishes have been studied in two species: zebra fish and loach. Whereas immersion of zebra fish embryos in a solution of purified MC did not result in morphological changes except at the very highest concentration (Oberemm et al 1999), embryonic development of loach was affected by exposure to MC (Liu et al. 2002). In the study on zebra fish it was seen – like in studies on other biota – that crude cell extracts had much stronger effects, resulting in malformations of the fishes.

Effect studies are especially rich in the zooplankton literature. In Table A.2 it can be seen that effects on zooplankton vary from feeding inhibition to reduced reproduction, growth and mortality. Feeding inhibition may actually serve as a protective mechanism, and there is some evidence that especially species that are highly susceptible to MC may protect themselves by strong inhibition of the intake of cyanobacteria (Demott 1999). Studies also have shown that zooplankton is relatively insensitive to dissolved toxins (Demott et al. 1991; Lurling and van der Grinten 2003) so that feeding inhibition may indeed be very effective in preventing harmful exposure to the toxins. A complicating factor in zooplankton studies is that also ‘non–toxic’ cyanobacteria induce effects like reduced growth and reproduction. Cyanobacteria are generally believed to be food of low quality to zooplankton, especially *Daphnia*, so that direct toxic effects can not always be separated from the effect of insufficient food of good quality (LaurenMaatta et al. 1997). Experimental tests in which toxicity effects were separated from food effects – by adding a sufficient amount of high quality food like the green alga *Scenedesmus* – clearly demonstrate however that nutritional insufficiency of *Microcystis* cannot be solely respon-

sible for the effects on *Daphnia.* Negative effects on survival, growth and population development persisted even when green algae were added. Moreover this was true even when a *Microcystis* mutant was used that no longer produces MC (Lurling 2003). Hence the author concluded that harmful effects by *Microcystis* cannot be the result of MC only. Feeding inhibition (starvation) and unknown bioactive compounds must also play a part.

The studies by Rohrlack and co–workers (Rohrlack et al. 1999b; Rohrlack et al. 2004; Rohrlack et al. 2005) enable direct insight into the effects of MC on *Daphnia* because the wild type *Microcystis* and its mutant only differ in their capacity to produce MC. Several interesting observations were made. Although MC was not responsible for feeding inhibition – the mutant had an equally strong effect – clearly MC had direct toxic effects. Visible first symptoms of MC poisoning included an inhibition of movements of thoracic legs, mandibles, foregut, second antennae, as well as stimulation of gut muscles leading to a permanent contraction of the midgut. These effects became apparent as soon as MC was taken up into the blood. Contraction of the midgut interferes with digestion, nutrient assimilation and uptake of ions. Eventually MC resulted in a breakdown of *Daphnia* metabolism, exhaustion and eventually death. In nature intake of microcystins will be modulated by various factors that were not considered in the study by Rohrlack et al. (2005) like *Microcystis* colony size, presence of alternative food, temperature or condition of the animal (see section below on 'modulating factors').

Dose effect relationships

In studies on Baltic Sea flounder as well as on fish from the IJsselmeer no relationship could be detected between liver histopathology and toxin concentrations (Ibelings et al 2005.; Kankaanpaa et al. 2005a). The lesions that are seen in fish livers caught from systems supporting dense blooms of cyanobacteria may be attributed to hepatotoxin exposure, but other factors like liver parasites and anthropogenic pollutants will also play a part. Kankaanpaa et al. (2005a) concluded that liver histopathology can not be used as a reliable bioindicator of exposure to cyanobacterial toxins. A complicating factor is the dynamic nature of liver damage and recovery. The acute exposure study mentioned earlier where flounders were given a single dose of toxin (Kankaanpaa et al. 2005a) demonstrated that damage is transient, and recovery from liver damage is rapid (on the order of days). Many studies have failed to relate effects to concentrations of toxin. Egg production of *Daphnia* in the IJsselmeer had no relationship with toxin content of the cyanobacteria in the lake (Ibelings et al. 2005). According to

Rohrlack et al. (1999) it may not be the presence of toxins in the seston but the actual intake of toxins that matters. *Daphnia* species that were presumed to differ in susceptibility to MC may actually be equally susceptible – where they actually differ may be in their ingestion rate of toxic *Microcystis* cells. Rohrlack et al. (2004) established a clear relationship between MC ingestion rate and LT_{50} (survival time) of *Daphnia*. Despite all the complicating factors, significant dose–effect relationships have been found and are included in Table A.2. Another example is the dose (and time) dependent mortality in brine shrimp exposed to CYN and MC (Metcalf et al. 2002).

Protective mechanisms

Aquatic biota are by no means defenseless against toxic cyanobacteria. Blooms of toxic *Nodularia* have been around for at least 7000 years in the Baltic, giving other biota sufficient time to adapt to these nuisance cyanobacteria (Bianchi et al. 2000). Several studies indicate that species which are most frequently exposed to the toxins have the highest physiological tolerance. Baltic shrimp are less sensitive than fish larvae, but these larvae only feed on phytoplankton during the first stages of their life. Baltic copepods feed upon and ingest toxic *Nodularia* and they survive and reproduce without apparent harmful effects of the toxins (Engstrom et al. 2000). Where *Thamnocephalus* exhibited reduced survival after grazing upon *Planktothrix* filaments, other zooplankton – naturally co–existing with toxic cyanobacteria– were unaffected (Kurmayer and Juttner 1999).

Sessile organisms like mussels cannot move away from cyanobacteria, but zooplankton and fish may migrate to parts of the system where concentrations of cyanobacteria are low, as has been suggested for fish in the Baltic (Karjalainen et al. 2005). Moreover zooplankton, mussels and fish may temporarily stop feeding when toxic cyanobacteria are present and avoid ingestion in this way. If toxic cyanobacteria can not be avoided and cells are indeed ingested, very little of the toxin present may actually be taken up into the body. Mucoid cyanobacteria like *Microcystis* are resistant to digestion, and there are barriers for the uptake of MC across the gut epithelium into the blood (Fischer and Dietrich 2000). Rohrlack et al. (2005) however showed that presence of *Microcystis* in the midgut of *Daphnia* caused the epithelium to loose cohesion. Cells loose contact with each other and this may facilitate the uptake of MC into the blood. Microcystin was transported by the blood to various organs, where beat rates were slowed down, until finally *Daphnia* died. Although both the MC producing wild type and the mutant (that no longer is capable of MC production) affected cohesion of the epithelium, beat rates were only affected by the MC

producing strain, a clear demonstration that MC – if taken up into the blood – is indeed highly toxic to *Daphnia*.

Feeding inhibition in the presence of toxic cyanobacteria is an efficient means to prevent ingestion of the toxins. However the avoidance of toxins must be balanced with the risk of starvation. Demott (1999) found that in *Dahnia magna* exposure to toxic *Microcystis* in a mixture with *Scenedesmus* resulted in a rapid feeding inhibition, but feeding recovered when exposure was continued. DeMott concluded that this pattern of inhibition and recovery may balance the benefits of reduced ingestion of toxin with the disadvantage of a reduced food intake. In an environment with a patchy occurrence of toxic cyanobacteria feeding inhibition would be adaptive if the environment could be sensed correctly (chemical cues) and animals are able to recover quickly from inhibition in the absence of toxic strains.

Detoxication and oxidative stress

Another important process is detoxication of MC, which now has been documented in many aquatic biota, including several animals and macrophytes (Pflugmacher 2004). Metabolic breakdown of MC results in conjugate formation, amongst others with GSH. The formation of these conjugates is catalyzed by the enzyme GST, which has a microsomal and a cytosolic fraction. The activity of cGST has been demonstrated to increase after exposure to MC in zebra fish (Wiegand et al. 1999) and brine shrimp (Beattie et al. 2003), although exceptions have also been described, where activity of the enzyme remained unchanged, e.g. in goldfish (Malbrouck et al. 2004) and carp (Li et al. 2003). GST activity (cGST and mGST) also increased in *Daphnia* after exposure to CYN and an unidentified hepatotoxin (Nogueira et al. 2004). The MC–GSH conjugates have a much reduced toxicity and may be subject to enhanced excretion. Detoxication thus is a significant mechanism that protects biota to acute toxic effects of MC, as long as the capacity for detoxication is not exceeded. As a result of detoxication the cellular GSH pool is depleted and this exposes cells to oxidative stress through the formation of ROS like hydrogen peroxide. Organisms have a wide range of protective mechanisms to oxidative stress, including enzymes like SOD, CAT and GSH–reductase. The latter enzyme needs GSH as a co–substrate and its activity may be reduced when GSH is depleted through its conjugation with MC. Thus exposure to MC may have damaging effects in direct (inhibition of PP) and indirect ways (disbalance in ROS). Jos et al. (2005) showed that crushed cyanobacterial cells (MC released) resulted in enhanced oxidative stress resulting in lipid peroxidation, despite the fact that also levels of defensive enzymes were enhanced.

Studies by several authors in Table A.2 (i.e. Best et al. 2002) indicate that LPS (which are present on the cell–surface of cyanobacteria and on bacteria associated with cyanobacterial blooms) interfere with the detoxication process. In the study by Best and others GST activity in zebra fish was reduced when MC and LPS were offered in combination. Since LPS from different bacterial sources are always present in the aquatic environment (although not all LPS from different sources equally disturbed detoxication when tested) it would be rewarding to study the process of detoxication in the field. Another study (Best et al. 2003) demonstrated that LPS stimulate drinking in fish, the increased volume of water in the gut potentially increases the opportunity for uptake of toxins (including MC) from the water and promotes osmoregulatory imbalance.

Modulating factors

A common theme in much of what has been discussed in this paper is that of modulating factors. The effects that these cyanobacterial toxins have on aquatic biota are seldom straightforward but are modulated by factors in the environment or the status of biota themselves. Examples of modulating factors include condition of the animals, temperature and pre–acclimation /adaptation to cyanobacterial toxins. Hepatotoxicity of MC–LR has been shown to increase in fasted compared to fed animals (Malbrouck et al. 2004). Fasted goldfish showed a more severe and rapid inhibition of PP, and this may be related to differences in the glycogen content of the livers and the rate of MC removal from the body via the bilary excretion system. The tolerance of *Daphnia pulex* to toxic *Microcystis* was shown to be temperature dependent (Hietala et al. 1997) and decreased with higher temperatures. Adaptation to toxic cyanobacteria may play an important role too. *Daphnia* from locations where it is repeatedly exposed to toxic blooms would develop a higher tolerance to the toxins (Gustafson and Hansson 2004). Whether this is truly an adaptive evolutionary response remains to be tested since adaptation during 4–6 generations in the experiments by Gustafson and Hansson (2004) seem insufficient (although adaptation in *Daphnia* has indeed been shown to be a rapid process (e.g. Ebert et al. 2000). The essential message from their work is clear however: whenever the ecological effects of cyanobacterial toxins on biota are considered it is important to understand the history of the species involved. Modulating factors are an important reason why it is so hard to generalize the effects toxic cyanobacteria have on the biota in their environment.

Knowledge gaps

Throughout this paper remarks have been made about knowledge gaps that limit our understanding of the 'true' ecological effects of cyanobacterial toxins. At this point there is no need however to list those gaps extensively, since this is the subject of the paper by Havens et al, this issue. To summarize their main findings, Havens et al recommend further study on the following subjects:

- Studies at the whole community level in the presence vs. absence of cyanobacterial toxins;
- Studies that mitigate the bias towards microcystin, especially MC–LR, i.e. more knowledge is needed about ecological effects of toxins like CYN;
- Studies into synergistic effects of combinations of cyanobacterial toxins and of cyanobacterial toxins and other bioactive compounds from cyanobacterial cells;
- Studies in which biota are exposed to toxins under environmentally relevant conditions (synergistic effects with other stressors like temperature, low oxygen etc);
- More emphasis should be placed into (sub)chronic studies having sub–lethal effects, including those on behavior or genotoxicty; these may be more relevant than acute lethal effects, and more knowledge is needed here;
- What is the fate of toxins produced by cyanobacteria in the ecosystem?; what is for instance the role of detoxication and covalent binding of MC on transfer of toxins in the foodweb?
- Effects of toxins on benthic communities are not well understood;
- In which way and to what extend does toxicity of cyanobacteria interfere with lake restoration?

Conclusions

The qualitative meta–analysis identifies the following general patterns for major groups of aquatic biota (birds, fish, macroinvertebrates and zooplankton).

Birds. On basis of the limited number of studies on the role of toxic cyanobacteria on waterfowl we conclude that aquatic birds are at risk of cyanobacterial toxicosis. Anatoxins seem to play a relatively large role, they are often present when dead birds are found and the symptoms in diseased birds indicate a neurotoxin. Birds may be at high risk because they may directly feed on floating scum of cyanobacteria (personal observation) and are warm blooded animals, like the mammals which have been shown to be sensitive to cyanobacterial toxins in laboratory studies. A disease that must be mentioned here is avian vacuolar myelinopathy (AVM), which is a neurologic disorder primarily affecting bald eagles (*Haliaeetus leucocephalus*) and American coots (*Fulica americana*). The agent of this disease is an uncharacterized neurotoxin produced by a novel cyanobacterial epiphyte of the order *Stigonematales* (Wilde et al. 2005).

Fish. On basis of their study of common carp exposed to *Microcystis*, Li et al. (2005) conclude that fish kills during blooms of cyanobacteria can be assumed to result from extensive liver damage. Zambrano and Canelo (1996) on the other hand state that blockage of the gill activity could be the cause of mass mortalities during blooms of *Microcystis*. Both papers have in common that they put forward that cyanobacterial toxicosis can directly be responsible for the death of fish. We maintain that this is unlikely. Studying the collected data in Table 2 it seems doubtful whether naturally occurring concentrations of cyanobacterial toxins (either dissolved in the water or contained in the cell) are sufficiently high to be directly lethal. Again, generalizations are difficult because there appear to be important differences between fish species. Fischer and Dietrich (2000) related the capacity for uptake of toxins to the morphology of the GI. Perhaps combinations of stress factors that co–occur during blooms of toxic cyanobacteria (high temperature and pH, enhanced levels of ammonia, low oxygen in addition to cyanobacterial toxins) are more likely to cause fish mortality. Important sub–lethal effects of cyanobacterial toxins in fish are more than probable, however. Harmful effects have been seen on embryonic development, on growth of juvenile fish and on adult species. Several organs may be affected (e.g., kidney, heart, gonads), but the liver is the main target. Several studies have shown that around 50 % of fish caught from lakes or estuaries that support cyanobacterial blooms show hepatic lesions that could – partially – be the result of exposure to cyanobacterial toxins.

Macroinvertebrates. Most of the studies on bivalves agree that these animals are quite resistant to different cyanobacterial toxins. This has been shown for freshwater and marine mussels and clams and has been found for hepatotoxins, neurotoxins and CYN. More attention is given to the potential accumulation of toxins in mussels, and especially the risk of vecto-

rial transport to predators (including man). However depuration studies have shown that mussels clear toxins fairly rapidly, so that there is little retention. Nevertheless depuration is seldom complete, and low concentrations may even be carried through to the start of the next cyanobacterial growing season.

Zooplankton. Whenever the ecological significance of cyanobacterial toxins is discussed the primary suggestion is often that they deter grazing by zooplankton. Highly selective grazers like copepods would exert a stronger selection pressure than less selective grazers like *Daphnhia,* but the study by Kurmayer and Juttner (1999) shows that *Daphnia* may play a persisting role in the evolution of MC production (see also studies by Jang et al. 2004) who demonstrated that MC concentrations increased up to five–fold when *Microcystis* was exposed to filtered zooplankton growth medium (*Daphnia* and *Moina* spp). The literature concerning the effects of toxic cyanobacteria on zooplankton is extensive (Table A.2 only shows a selection) but there appear to be many contradictions. This is not surprising since there are numerous complicating factors. Furthermore it is now well established that not all toxic effects can be traced back to the well known cyanobacterial toxins like MC. Although work by Rohrlack et al. (2005) has proven decisively that microcystins are toxic, the same work has shown that also the mutant incapable of producing MC has negative effects like inhibition of feeding. Effects of cyanobacterial blooms also exert effects at the community level. Zooplankton community composition may change towards dominance of smaller cladocerans which have a lower grazing pressure. In this way cyanobacterial blooms may stabilize the turbid state on which some of the cyanobacteria like *Planktothrix agardhii* depend (Scheffer et al. 1997) and interfere with lake restoration. The specific contribution of toxins – as opposed to general negative effects of cyanobacteria – at this high level of integration is unclear however.

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References

Agrawal MK, Bagchi D, Bagchi SN (2005) Cysteine and serine protease–mediated proteolysis in body homogenate of a zooplankter, Moina macrocopa, is inhibited by the toxic cyanobacterium, Microcystis aeruginosa PCC7806. Comp Biochem Physiol B–Biochem Mol Biol 141(1): 33–41

Amorim A, Vasconcelos V (1999) Dynamics of microcystins in the mussel Mytilus galloprovincialis. Toxicon 37(7): 1041–1052

Babcock–Jackson L, Carmichael WW, Culver DA (2002) Dreissenid mussels increase exposure of benthic and pelagic organisms to toxic microcystins. VerhInternatVereinLimnol 28: 1082–1085

Baganz D, Staaks G, Steinberg C (1998) Impact of the cyanobacteria toxin, microcystin–LR on behaviour of zebrafish, Danio rerio. Water Res 32(3): 948–952

Ballot A, Krienitz L, Kotut K, Wiegand C, Metcalf JS et al. (2004) Cyanobacteria and cyanobacterial toxins in three alkaline rift valley lakes of Kenya – Lakes Bogoria, Nakuru and Elmenteita. J Plankton Res 26(8): 925–935

Beattie KA, Ressler J, Wiegand C, Krause E, Codd GA et al. (2003) Comparative effects and metabolism of two microcystins and nodularin in the brine shrimp Artemia salina. Aquat Toxicol 62(3): 219–226

Best JH, Pflugmacher S, Wiegand C, Eddy FB, Metcalf JS et al. (2002) Effects of enteric bacterial and cyanobacterial lipopolysaccharides, and of microcystin–LR, on glutathione S–transferase activities in zebra fish (Danio rerio). Aquat Toxicol 60(3–4): 223–231

Best JH, Eddy FB, Codd GA (2003) Effects of Microcystis cells, cell extracts and lipopolysaccharide on drinking and liver function in rainbow trout Oncorhynchus mykiss Walbaum. Aquat Toxicol 64(4): 419–426

Best JH, Eddy FB, Codd GA (2001) Effects of purified microcystin–LR and cell extracts of Microcystis strains PCC 7813 and CYA 43 on cardiac function in brown trout (Salmo trutta) alevins. Fish Physiol Biochem 24(3): 171–178

Bianchi TS, Engelhaupt E, Westman P, Andren T, Rolff C et al. (2000) Cyanobacterial blooms in the Baltic Sea: Natural or human–induced? Limnol Oceanogr 45(3): 716–726

Blom JF, Robinson JA, Juttner F (2001) High grazer toxicity of [D–Asp(3) (E)–Dhb(7)]microcystin–RR of Planktothrix rubescens as compared to different microcystins. Toxicon 39(12): 1923–1932

Bury NR, Eddy FB, Codd GA (1996) Stress responses of brown trout, Salmo trutta L, to the cyanobacterium, Microcystis aeruginosa. Environ Toxicol Water Quality 11(3): 187–193

Bury NR, Eddy FB, Codd GA (1995) The Effects of the Cyanobacterium Microcystis–Aeruginosa, the Cyanobacterial Hepatotoxin Microcystin–Lr, and Ammonia On Growth–Rate and Ionic Regulation of Brown Trout. J Fish Biol 46(6): 1042–1054

Carbis CR, Rawlin GT, Grant P, Mitchell GF, Anderson JW et al. (1997) A study of feral carp, Cyprinus carpio L, exposed to Microcystis aeruginosa at Lake

Mokoan, Australia, and possible implications for fish health. J Fish Dis 20(2): 81–91

Chen J, Xie P (2005) Tissue distributions and seasonal dynamics of the hepatotoxic microcystins–LR and –RR in two freshwater shrimps, Palaemon modestus and Macrobrachium nipponensis, from a large shallow, eutrophic lake of the subtropical China. Toxicon 45(5): 615–625

Codd GA, Lindsay J, Young FM, Morrison LF & Metcalf J (2005). Harmful cyanobacteria. From mass mortalities to magament measures. Huisman J, Matthijs HCP, Visser PM. Harmful cyanobacteria. Springer (Dordrecht)

de Magalhaes VF, Soares RM, Azevedo S (2001) Microcystin contamination in fish from the Jacarepagua Lagoon (Rio de Janeiro, Brazil): ecological implication and human health risk. Toxicon 39(7): 1077–1085

Demott WR (1999) Foraging strategies and growth inhibition in five daphnids feeding on mixtures of a toxic cyanobacterium and a green alga. Freshw Biol 42(2): 263–274

Demott WR, Zhang QX, Carmichael WW (1991) Effects of Toxic Cyanobacteria and Purified Toxins On the Survival and Feeding of a Copepod and 3 Species of Daphnia. Limnol Oceanogr 36(7): 1346–1357

Ebert D, Lipsitch M, Mangin KL (2000) The effect of parasites on host population density and extinction: Experimental epidemiology with Daphnia and six microparasites. Am Nat 156(5): 459–477

Engstrom–Ost J, Lehtiniemi M, Green S, Kozlowsky–Suzuki B, Viitasalo M (2002) Does cyanobacterial toxin accumulate in mysid shrimps and fish via copepods? J Exp Mar Biol Ecol 276(1–2): 95–107

Engstrom J, Koski M, Viitasalo M, Reinikainen M, Repka S et al. (2000) Feeding interactions of the copepods Eurytemora affinis and Acartia bifilosa with the cyanobacteria Nodularia sp. J Plankton Res 22(7): 1403–1409

Ferrao–Filho AD, Azevedo S (2003) Effects of unicellular and colonial forms of toxic Microcystis aeruginosa from laboratory cultures and natural populations on tropical cladocerans. Aquat Ecol 37(1): 23–35

Ferrao AS, Azevedo S, DeMott WR (2000) Effects of toxic and non–toxic cyanobacteria on the life history of tropical and temperate cladocerans. Freshw Biol 45(1): 1–19

Fischer WJ, Dietrich DR (2000) Pathological and biochemical characterization of microcystin– induced hepatopancreas and kidney damage in carp (Cyprinus carpio). Toxicol Appl Pharmacol 164(1): 73–81

Gray JS (2002) Biomagnification in marine systems: the perspective of an ecologist. Mar Pollut Bull 45(1–12): 46–52

Gustafson S, Hansson LA (2004) Development of tolerance against toxic cyanobacteria in *Daphnia*. Aquat Ecol 38: 37–44

Henriksen P, Carmichael WW, An JS, Moestrup O (1997) Detection of an anatoxin–a(s)–like anticholinesterase in natural blooms and cultures of Cyanobacteria/blue–green algae from Danish lakes and in the stomach contents of poisoned birds. Toxicon 35(6): 901–913

Hietala J, LaurenMaatta C, Walls M (1997) Life history responses of Daphnia clones to toxic Microcystis at different food levels. J Plankton Res 19(7): 917–926

Ibelings BW, Maberly SC (1998) Photoinhibition and the availability of inorganic carbon restrict photosynthesis by surface blooms of cyanobacteria. Limnol Oceanogr 43(3): 408–419

Ibelings BW, Bruning K, de Jonge J, Wolfstein K, Pires LMD et al. (2005) Distribution of microcystins in a lake foodweb: No evidence for biomagnification. Microb Ecol 49(4): 487–500

Jang MH, Ha K, Lucas MC, Joo GJ, Takamura N (2004) Changes in microcystin production by Microcystis aeruginosa exposed to phytoplanktivorous and omnivorous fish. Aquat Toxicol 68(1): 51–59

Jos A, Pichardo S, Prieto AI, Repetto G, Vazquez CM et al. (2005) Toxic cyanobacterial cells containing microcystins induce oxidative stress in exposed tilapia fish (Oreochromis sp.) under laboratory conditions. Aquat Toxicol 72(3): 261–271

Kankaanpaa H, Vuorinen PJ, Sipia V, Keinanen M (2002) Acute effects and bioaccumulation of nodularin in sea trout (Salmo trutta m. trutta L.) exposed orally to Nodularia spumigena under laboratory conditions. Aquat Toxicol 61(3–4): 155–168

Kankaanpaa H, Turunen AK, Karlsson K, Bylund G, Meriluoto J et al. (2005a) Heterogeneity of nodularin bioaccumulation in northern Baltic Sea flounders in 2002. Chemosphere 59(8): 1091–1097

Kankaanpaa HT, Sipia VO, Kuparinen JS, Ott JL, Carmichael WW (2001) Nodularin analyses and toxicity of a Nodularia spumigena (Nostocales, Cyanobacteria) water–bloom in the western Gulf of Finland, Baltic Sea, in August 1999. Phycologia 40(3): 268–274

Kankaanpaa HT, Holliday J, Schroder H, Goddard TJ, von Fister R et al. (2005b) Cyanobacteria and prawn fanning in northern New South Wales, Australia – a case study on cyanobacteria diversity and hepatotoxin bioaccumulation. Toxicol Appl Pharmacol 203(3): 243–256

Karjalainen M, Kozlowsky–Suzuki B, Lehtiniemi M, Engström–Ost J, Kankaanpää H, Viitasalo M (in press). Nodularin accumulation during cyanobacterial blooms asnd experimental depuration in zooplankton

Karjalainen M, Reinikainen M, Lindvall F, Spoof L, Meriluoto JAO (2003) Uptake and accumulation of dissolved, radiolabeled nodularin in Baltic Sea Zooplankton. Environ Toxicol 18(1): 52–60

Karjalainen M, Reinikainen M, Spoof L, Meriluoto JAO, Sivonen K et al. (2005) Trophic transfer of cyanobacterial toxins from zooplankton to planktivores: Consequences for pike larvae and mysid shrimps. Environ Toxicol 20(3): 354–362

Karlsson K, Sipia V, Kankaanpaa H, Meriluoto J (2003) Mass spectrometric detection of nodularin and desmethylnodularin in mussels and flounders. J Chromatogr B 784(2): 243–253

Koski M, Schmidt K, Engstrom–Ost J, Viitasalo M, Jonasdottir S et al. (2002) Calanoid copepods feed and produce eggs in the presence of toxic cyanobacteria Nodularia spumigena. Limnol Oceanogr 47(3): 878–885

Kotak BG, Zurawell RW, Prepas EE, Holmes CFB (1996a) Microcystin–LR concentration in aquatic food web compartments from lakes of varying trophic status. Can J Fish Aquat Sci 53(9): 1974–1985

Kotak BG, Semalulu S, Fritz DL, Prepas EE, Hrudey SE et al. (1996b) Hepatic and renal pathology of intraperitoneally administered microcystin–LR in rainbow trout (Oncorhynchus mykiss). Toxicon 34(5): 517–525

Krienitz L, Ballot A, Kotut K, Wiegand C, Putz S et al. (2003) Contribution of hot spring cyanobacteria to the mysterious deaths of Lesser Flamingos at Lake Bogoria, Kenya. FEMS Microbiol Ecol 43(2): 141–148

Kurmayer R, Juttner F (1999) Strategies for the co–existence of zooplankton with the toxic cyanobacterium Planktothrix rubescens in Lake Zurich. J Plankton Res 21(4): 659–683

LaurenMaatta C, Hietala J, Walls M (1997) Responses of Daphnia pulex populations to toxic cyanobacteria. Freshw Biol 37(3): 635–647

Lehtiniemi M, Engstrom–Ost J, Karjalainen M, Kozlowsky–Suzuki B, Viitasalo M (2002) Fate of cyanobacterial toxins in the pelagic food web: transfer to copepods or to faecal pellets? Mar Ecol–Prog Ser 241: 13–21

Lehtonen KK, Kankaanpaa H, Leinio S, Sipia VO, Pflugmacher S et al. (2003) Accumulation of nodularin–like compounds from the cyanobacterium Nodularia spumigena and changes in acetylcholinesterase activity in the clam Macoma balthica during short–term laboratory exposure. Aquat Toxicol 64(4): 461–476

Li XY, Liu YD, Song LR, Liu HT (2003) Responses of antioxidant systems in the hepatocytes of common carp (Cyprinus carpio L.) to the toxicity of microcystin–LR. Toxicon 42(1): 85–89

Li L, Xie P, Chen J (2005) In vivo studies on toxin accumulation in liver and ultrastructural changes of hepatocytes of the phytoplanktivorous bighead carp i.p.–injected with extracted microcystins. Toxicon 46(5): 533–545

Li XY, Chung IK, Kim JI, Lee JA (2004) Subchronic oral toxicity of microcystin in common carp (Cyprinus carpio L.) exposed to Microcystis under laboratory conditions. Toxicon 44(8): 821–827

Liras V, Lindberg M, Nystrom P, Annadotter H, Lawton LA et al. (1998) Can ingested cyanobacteria be harmful to the signal crayfish (Pacifastacus leniusculus)? Freshw Biol 39(2): 233–242

Liu YD, Song LR, Li XY, Liu TM (2002) The toxic effects of microcystin–LR on embryo–larval and juvenile development of loach, Misguruns mizolepis Gunthe. Toxicon 40(4): 395–399

Lurling M (2003) Effects of microcystin–free and Microcystin containing strains of the cyanobacterium Microcystis aeruginosa on growth of the grazer Daphnia magna. Environ Toxicol 18(3): 202–210

Lurling M, van der Grinten E (2003) Life–history characteristics of Daphnia exposed to dissolved microcystin–LR and to the cyanobacterium Microcystis

aeruginosa with and without microcystins. Environ Toxicol Chem 22(6): 1281–1287

Magalhaes VF, Marinho MM, Domingos P, Oliveira AC, Costa SM et al. (2003) Microcystins (cyanobacteria hepatotoxins) bioaccumulation in fish and crustaceans from Sepetiba Bay (Brasil, RJ). Toxicon 42(3): 289–295

Malbrouck C, Trausch G, Devos P, Kestemont P (2003) Hepatic accumulation and effects of microcystin–LR on juvenile goldfish Carassius auratus L. Comp Biochem Physiol C–Toxicol Pharmacol 135(1): 39–48

Malbrouck C, Trausch G, Devos P, Kestemont P (2004) Effect of microcystin–LR on protein phosphatase activity and glycogen content in isolated hepatocytes of fed and fasted juvenile goldfish Carassius auratus L. Toxicon 44(8): 927–932

Metcalf JS, Beattie KA, Pflugmacher S, Codd GA (2000) Immuno–crossreactivity and toxicity assessment of conjugation products of the cyanobacterial toxin, microcystin–LR. FEMS Microbiol Lett 189(2): 155–158

Metcalf JS, Lindsay J, Beattie KA, Birmingham S, Saker ML et al. (2002) Toxicity of cylindrospermopsin to the brine shrimp Artemia salina: comparisons with protein synthesis inhibitors and microcystins. Toxicon 40(8): 1115–1120

Mohamed ZA, Carmichael WW, Hussein AA (2003) Estimation of microcystins in the freshwater fish Oreochromis niloticus in an Egyptian fish farm containing a Microcystis bloom. Environ Toxicol 18(2): 137–141

Negri AP, Bunter O, Jones B, Llewellyn L (2004) Effects of the bloom–forming alga Trichodesmium erythraeum on the pearl oyster Pinctada maxima. Aquaculture 232(1–4): 91–102

Negri AP, Jones GJ (1995) Bioaccumulation of Paralytic Shellfish Poisoning (Psp) Toxins from the Cyanobacterium Anabaena–Circinalis by the Fresh–Water Mussel Alathyria–Condola. Toxicon 33(5): 667–678

Nogueira ICG, Saker ML, Pflugmacher S, Wiegand C, Vasconcelos VM (2004) Toxicity of the cyanobacterium Cylindrospermopsis radborskii to Daphnia magna. Environ Toxicol 19(5): 453–459

Nogueira ICG, Pereira P, Dias E, Pflugmacher S, Wiegand C et al. (2004) Accumulation of Paralytic Shellfish Toxins (PST) from the cyanobacterium Aphanizomenon issatschenkoi by the cladoceran Daphnia magna. Toxicon 44(7): 773–780

Oberemm A, Becker J, Codd GA, Steinberg C (1999) Effects of cyanobacterial toxins and aqueous crude extracts of cyanobacteria on the development of fish and amphibians. Environ Toxicol 14(1): 77–88

Ozawa K, Yokoyama A, Ishikawa K, Kumagai M, Watanabe MF et al. (2003) Accumulation and depuration of microcystin produced by the cyanobacterium Microcystis in a freshwater snail. Limnology 4(3): 131–138

Palikova M, Kovaru F, Navratil S, Kubala L, Pesak S et al. (1998) The effects of pure microcystin LR and biomass of blue–green algae on selected immunological indices of carp (Cyprinus carpio L.) and silver carp (Hypophthalmichthys molitrix Val.). Acta Vet BRNO 67(4): 265–272

Pereira P, Dias E, Franca S, Pereira E, Carolino M et al. (2004) Accumulation and depuration of cyanobacterial paralytic shellfish toxins by the freshwater mussel Anodonta cygnea. Aquat Toxicol 68(4): 339–350

Pflugmacher S (2004) Promotion of oxidative stress in the aquatic macrophyte Ceratophyllum demersum during biotransformation of the cyanobacterial toxin microcystin–LR. Aquat Toxicol 70(3): 169–178

Pinho GLL, da Rosa CM, Yunes JS, Luquet CM, Bianchini A et al. (2003) Toxic effects of microcystins in the hepatopancreas of the estuarine crab Chasmagnathus granulatus (Decapoda, Grapsidae). Comp Biochem Physiol C–Toxicol Pharmacol 135(4): 459–468

Pires LMD, Karlsson KM, Meriluoto JAO, Kardinaal E, Visser PM et al. (2004) Assimilation and depuration of microcystin–LR by the zebra mussel, Dreissena polymorpha. Aquat Toxicol 69(4): 385–396

Prepas EE, Kotak BG, Campbell LM, Evans JC, Hrudey SE et al. (1997) Accumulation and elimination of cyanobacterial hepatotoxins by the freshwater clam Anodonta grandis simpsoniana. Can J Fish Aquat Sci 54(1): 41–46

Reinikainen M, Hietala J, Walls M (1999) Reproductive allocation in Daphnia exposed to toxic cyanobacteria. J Plankton Res 21(8): 1553–1564

Reinikainen M, Lindvall F, Meriluoto JAO, Repka S, Sivonen K et al. (2002) Effects of dissolved cyanobacterial toxins on the survival and egg hatching of estuarine calanoid copepods. Mar Biol 140(3): 577–583

Rohrlack T, Christoffersen K, Dittmann E, Nogueira I, Vasconcelos V et al. (2005) Ingestion of microcystins by Daphnia: Intestinal uptake and toxic effects. Limnol Oceanogr 50(2): 440–448

Rohrlack T, Henning M, Kohl JG (1999a) Does the toxic effect of Microcystis aeruginosa on Daphnia galeata depend on microcystin ingestion rate? Arch Hydrobiol 146(4): 385–395

Rohrlack T, Dittmann E, Henning M, Borner T, Kohl JG (1999b) Role of microcystins in poisoning and food ingestion inhibition of Daphnia galeata caused by the cyanobacterium Microcystis aeruginosa. Appl Environ Microbiol 65(2): 737–739

Rohrlack T, Dittmann E, Borner T, Christoffersen K (2001) Effects of cell–bound microcystins on survival and feeding of Daphnia spp. Appl Environ Microbiol 67(8): 3523–3529

Rohrlack T, Christoffersen K, Kaebernick M, Neilan BA (2004) Cyanobacterial protease inhibitor microviridin J causes a lethal molting disruption in Daphnia pulicaria. Appl Environ Microbiol 70(8): 5047–5050

Saker ML, Metcalf JS, Codd GA, Vasconcelos VM (2004) Accumulation and depuration of the cyanobacterial toxin cylindrospermopsin in the freshwater mussel Anodonta cygnea. Toxicon 43(2): 185–194

Scheffer M, Rinaldi S, Gragnani A, Mur LR, vanNes EH (1997) On the dominance of filamentous cyanobacteria in shallow, turbid lakes. Ecology 78(1): 272–282

Sipia VO, Kankaanpaa HT, Pflugmacher S, Flinkman J, Furey A et al. (2002) Bioaccumulation and detoxication of nodularin in tissues of flounder (Platichthys flesus), mussels (Mytilus edulis, Dreissena polymorpha), and clams

(Macoma balthica) from the northern Baltic Sea. Ecotox Environ Safe 53(2): 305–311

Sipia V, Kankaanpaa H, Lahti K, Carmichael WW, Meriluoto J (2001a) Detection of Nodularin in flounders and cod from the Baltic Sea. Environ Toxicol 16(2): 121–126

Sipia VO, Kankaanpaa HT, Flinkman J, Lahti K, Meriluoto JAO (2001b) Time–dependent accumulation of cyanobacterial hepatotoxins in flounders (Platichthys flesus) and mussels (Mytilus edulis) from the northern Baltic Sea. Environ Toxicol 16(4): 330–336

Sipia VO, Karlsson KA, Meriluoto JAO, Kankaanpaa HT (2004) Eiders (Somateria mollissima) obtain nodularin, a cyanobacterial hepatotoxin, in Baltic Sea food web. Environ Toxicol Chem 23(5): 1256–1260

Soares RA, Magalhaes VF, Azevedo S (2004) Accumulation and depuration of microcystins (cyanobacteria hepatotoxins) in Tilapia rendalli (Cichlidae) under laboratory conditions. Aquat Toxicol 70(1): 1–10

Spoof L (2005). Miocrocystins and nodularins. Meriluoto J and Codd GA. Cyanobacterial monitoring and cyanotoxin analysis. Åbo Akademi University Press

Svensen C, Strogyloudi E, Riser CW, Dahmann J, Legrand C et al. (2005) Reduction of cyanobacterial toxins through coprophagy in Mytilus edulis. Harmful Algae 4(2): 329–336

Tencalla FG, Dietrich DR, Schlatter C (1994) Toxicity of Microcystis–Aeruginosa Peptide Toxin to Yearling Rainbow–Trout (Oncorhynchus–Mykiss). Aquat Toxicol 30(3): 215–224

Thostrup L, Christoffersen K (1999) Accumulation of microcystin in Daphnia magna feeding on toxic Microcystis. Arch Hydrobiol 145(4): 447–467

Vasconcelos VM, Sivonen K, Evans WR, Carmichael WW, Namikoshi M (1995) Isolation and Characterization of Microcystins (Heptapeptide Hepatotoxins) From Portuguese Strains of Microcystis–Aeruginosa Kutz Emend Elekin. Arch Hydrobiol 134(3): 295–305

Vasconcelos V, Oliveira S, Teles FO (2001) Impact of a toxic and a non–toxic strain of Microcystis aeruginosa on the crayfish Procambarus clarkii. Toxicon 39(10): 1461–1470

Vinagre TM, Alciati JC, Regoli F, Bocchetti R, Yunes JS et al. (2003) Effect of microcystin on ion regulation and antioxidant system in gills of the estuarine crab Chasmagnathus granulatus (Decapoda, Grapsidae). Comp Biochem Physiol C–Toxicol Pharmacol 135(1): 67–75

Ward CJ, Codd GA (1999). Comparitive toxicity of four microcystins of different hydrophobicities to the protozoan Tetrahymena pyriformis. J. Appl. Microbiol. 86: 874–882

Wiegand C, Pflugmacher S, Oberemm A, Meems N, Beattie KA et al. (1999) Uptake and effects of microcystin–LR on detoxication enzymes of early life stages of the zebra fish (Danio rerio). Environ Toxicol 14(1): 89–95

Wilde SB, Murphy TM, Hope CP, Habrun SK, Kempton J et al. (2005) Avian vacuolar myelinopathy linked to exotic aquatic plants and a novel cyanobacterial species. Environ Toxicol 20(3): 348–353

Williams DE, Craig M, Dawe SC, Kent ML, Holmes CFB et al. (1997) Evidence for a covalently bound form of microcystin–LR in salmon liver and dungeness crab larvae. Chem Res Toxicol 10(4): 463–469

Work KA, Havens KE (2003) Zooplankton grazing on bacteria and cyanobacteria in a eutrophic lake. J Plankton Res 25(10): 1301–1306

Xie LQ, Xie P, Ozawa K, Honma T, Yokoyama A et al. (2004) Dynamics of microcystins–LR and –RR in the phytoplanktivorous silver carp in a sub–chronic toxicity experiment. Environ Pollut 127(3): 431–439

Xie LQ, Xie P, Guo LG, Li L, Miyabara Y et al. (2005) Organ distribution and bioaccumulation of microcystins in freshwater fish at different trophic levels from the eutrophic Lake Chaohu, China. Environ Toxicol 20(3): 293–300

Yokoyama A, Park HD (2003) Depuration kinetics and persistence of the cyanobacterial toxin microcystin–LR in the freshwater bivalve Unio douglasiae. Environ Toxicol 18(1): 61–67

Zambrano F, Canelo E (1996) Effects of microcystin–LR on the partial reactions of the Na+– K+ pump or the gill of carp (Cyprinus carpio linneo). Toxicon 34(4): 451–458

Zurawell RW, Kotak BG, Prepas EE (1999) Influence of lake trophic status on the occurrence of microcystin–LR in the tissue of pulmonate snails. Freshw Biol 42(4): 707–718

Appendix A

Table A.1. Concentrations of various cyanobacterial toxins in a range of aquatic biota. Where possible concentrations are expressed as µg g DW-1. Conversion of wet weight to dry weight in animals using a factor of 0.1 and conversion of C to DW requires multiplication by 1/0.526 {Winberg, 1971). Ash content of DW is neglected. MC is commonly expressed as MC–LR eq, since the majority of studies only used MC–LR as a standard in HPLC. Concentrations are usually presented as a range (lowest – highest values found). Concentration in the 'source' applies to either purified toxin (experiments), cultured cells of cyanobacteria, dissolved toxins in natural waters or cyanobacteria in the seston. For abbreviatons see 'list of abbreviations'.

Organism	Toxin	Exposure route	Conc. in organism (µg g^{-1} DW)	Conc. in source (µg g^{-1} DW)	Analysis method	Remark	Reference
BIRDS							
(i) coots (Fulica atra) (ii) grebes (Podiceps nigricollis, P. cristatus)	ANA(a-s), MC	Natural routes: feeding in foodweb from lake with Anabaena bloom	(i) ANA - Coot: 0.021 Grebe: 0.90 (ii) MC - 0.0005-0.001	ANA: 4-3300 MC: 0.1-0.9	ELISA (MC) AChE assay (ANA)	(i) Anabaena and its toxin found in stomach birds: cyanobacterial toxicosis? (ii) ratio ANAeq : MCeq 40 to > 1000	Henriksen et al, 1997
Lesser flamingo (Phoeniconaias minor)	MC-LR, RR, LF and YR, ANA(a)	Feeding on mats of cyanobacteria, as well as on planktonic Arthrospira	(i) stomach: MC 1.96 (all 4 variants); ANA 43.4 (ii) intestines: MC 0.36 (MC-LR only); ANA 7.62 (iii) faeces: MC 0.48 (all but MC-LF); ANA 2.45	(i) mats - MC: 221-845 ANA: 10-18 (ii) plankton – MC: max 4600 ANA: max 223	HPLC, MALDITOF-MS	(i) intoxication birds likely but lack of relevant data on susceptibility (ii) multiple stress factors	Krienitz et al, 2003; Ballot et al, 2004

Eider (Somateria mollissima)	NODLN	Feeding on mussels in Baltic sea	(i) 0.003-0.18 (ii) 0.1-5.8 µg per liver		ELISA and LC-MS		Sipiä et al, 2004
FISH							
Round goby (Neogobius melanostomus)	MC	Natural routes in lake	Goby liver: 0.6-3.0	91-820			Babcock-Jackson et al, 2002
Sticklebacks (Gasterosteus aculeatus)	NODLN	Feeding on copepods pre-exposed to cyanobacteria	(i) 0.15 (ELISA) (ii) 0.8 (PPase)		ELISA and PPase assay		Ensgtröm-Öst et al, 2002
(i) perch (Percia fluviatilis) (ii) ruffe (Gymnocephalus cernuus) (iii) smelt (Osmerus eperlanus)	MC	Natural routes in lake foodweb	(i) perch liver: 17-51 (ii) ruffe liver: 9-194 (iii) smelt liver: 59-874	7-3912	HPLC	Apparent biomagnification based upon skewed representation	Ibelings et al, 2005
(i) silver carp (Hypophthalmichthys molitrix), (ii) Carassius gibelio	MC-LR; MC-RR	Feeding on Microcystis cells	(i) homogenized tissues: < 5 (H. molitrix) < 0.8 (C. gibelio) (ii) faeces: 11 - 46 (H. molitrix) 21 (C. gibelio)			MC-RR levels in tissues >> MC-LR	Jang et al, 2004
Sea trout (Salmo trutta)	NODLN	Gavage with single dose of Nodularia	(i) liver: 0.019-1.2; max 1.6 (ii) muscle: max. 0.125		ELISA and HPLC	Single oral dose: loss liver architecture 1-2d, partial recovery 4-8 d, complete after 8 d: damage reversible	Kankaapää et al, 2002
Flounder (Platichthys flesus)	NODLN, MC	Feeding on mussels in Baltic sea	(i) 0.02-0.1 TEH pre-bloom exposure (ii) 0.02-2.23 TEH (ELISA) (iii) nd-0.47 (LC-MS)		ELISA and LC-MS	(i) NODLN variable between individuals: peak conc. in sub-populations (ii) 50 % livers small scale necrosis	Kankaapää et al, 2005a

Flounder (Platichthys flesus)	NODLN	Natural routes in sea	Liver: 0.82-6.37		LC-MS	No biotransformation products (e.g. glutathione adduct) found	Karlsson et al, 2003
(i) northern pike (Esox lucieus (ii) white sucker (Catostomus comersonii)	MC	Feeding on MC containing prey (i.a. gastropods)	Liver: not detected	1.2–6.1 µg L^{-1}	HPLC		Kotak et al, 1996a
Carp (Cyprinus carpio)	MC	Feeding on Microcystis scum in tanks	(i) hepatopancreas: 2.6 (ii) muscle: 0.4				Li et al, 2004
Tilapia rendalli	MC	Natural routes in lake	(i) viscera: 0 – 67.8 (ii) liver: 0 – 31.1 (iii) muscle: 0.003 – 0.026	max. 980 µg L^{-1}	HPLC, ELISA	MC found in 75 % fish samples	Magalhães et al, 2001
Fish (unspecified)	MC	Natural routes in lake	Muscle: 0.01 – 0.4	0.12–0.78 µg L^{-1}	ELISA		Magalhães et al, 2003
Goldfish (Carassius auratus)	MC-LR	IP injection	Liver: 0.5 - 3		PPase	Time course accumulation (first 48 h), depuration (48-96 h); patterns not affected by fastening	Malbrouck et al, 2003, 2004
Tilpapia (Oreochromis niloticus)	MC	On fish-farm to Microcystis bloom	(i) gut: 1.8 – 8.3 (ii) liver: 4.1 – 5.3 (iii) kidney: 3.6 - 4 (iv) muscle: 0.4 – 1.0	1120	ELISA	At times MC in liver > gut	Mohamed et al, 2003
Flounder (Platichthys flesus)	NODLN and MC	Natural routes in Baltic sea	Max 0.4 (TEH)	150-8700 THE; < 2400 NODLN	ELISA and MALDI-TOF-MS		Sipiä et al, 2001, 2002
Tilapia rendalli	MC	(i) Feeding on Microcystis cells + fish food; one exp cells disrupted prior feeding	(i) liver: 2.8 (ii) muscle: 0.08 (iii) faeces: 0.07 (all max values)	14.6	ELISA	(i) 15 or 42 d exposure (ii) accumulation MC less in presence alternative food (iii) depuration phase: only small percentage MC removed	Soares et al, 2004

Silver carp (Hypophthalmichthys molitrix)	MC-LR and RR	Feeding on Microcystis bloom in tanks	(i) faeces: 44.5; MC-LR:RR = 0.75 (ii) intestines: 49 – 115; MC-LR:RR = 0.17 (iii) blood (MC-RR): 0.4 – 50 (iv) liver (MC-RR): 8 - 18 (v) muscle (MC-RR): 0.5 – 1.4	MC: 286–866; MC-LR:RR = 0.57	PPase	(i) in the various tissues and organs always MC-RR found, rarely MC-LR (ii) active degradation of MC-LR during digestion (?)	Xie et al, 2004
Various fish species: phytoplanktivorous (Hypophthalalmichthys molitrix), herbivorous (Parabramis pekinensis), omnivorous (Carassius auratus), carnivorous (Culter ilishaeformis)	MC-LR and RR	Natural routes in lake	(i) intestines: 22 (26 % LR) (ii) blood:14.5 (45 % LR) (iii) liver: 7.8 (iv) bile 6.3 (48 % LR) (v) kidney: 5.8 (30 % LR) (vi) muscle: 1.8 (18 % LR)	240	HPLC	(i) MC in carnivorous > omnivorous > phytoplanktivorous fish (ii) fish at top of foodweb most at risk; general tendency MC to accumulate up the foodchain	Xie et al, 2005
Salmon (Salmo salar)	MC-LR	IP injection	(i) 2.6 – 263 (MeOH) (ii) 138 – 1181 (Lemieux)		(i) PPase after MeoH extract. (ii) Lemieux (GC-MS)	Covalently bound MC made up ~ 74 % of total	Williams et al, 1997a
MACROINVERTEBRATES							
Mytilus galloprovincialis	MC	Grazing on Microcystis cells	(i) mussel: 10.7 during accumulation rising to max of 16 on day 2 depuration (ii) faeces: 140	3.4 µg / 10^7 cells	ELISA	(i) no mussel mortality (ii) during depuration initial increase, followed decrease MC	Amorim & Vasconcelos (1999)
Zebra mussels (Dreissena polymorpha)	MC	Natural routes in lake	0.2	91-820			Babcock-Jackson et al, 2002

(i) freshwater shrimps (Palemon modestus; Macrobrachium nippo-nensis) (ii) red swamp crayfish (Procambarus clarkii)	MC-LR and RR	Natural routes in lake	(i) P.modestus - stomach: 4.53 hepatopancreas: 4.29 gonads: 1.17 eggs: 2.34 muscle: 0.13 gills: 0.51 (ii) M. nipponensis - stomach: 2.92 hepatopancreas: 0.53 gonads: 0.48 eggs: 0.27 muscle: 0.04 gills: 0.05 (iii) P. clarkia - stomach: 9.97 hepatopancreas: 0.08 gonads: 0.93 muscle: 0.05 gills: 0.27	LC-MS	(i) Proportion of MC-LR of total MC varied with type of tissue and the species; ratio of MC-LR:MC: P. modestus decreased gonad (94 %) > stomach (61 %) > eggs (56 %) > HP (30 %) > muscle (5 %) > gills (0 %) M. nipponensis: stomach (71 %) > muscle (48 %) > HP (39 %) > eggs (39 %)> gonads (33 %) > gills (0 %) P. clarkia: muscle (100 %) > intestine (68 %) > stomach (58 %) > gonad (57 %) > gills(52 %) (ii) considerable part toxin burden in eggs (29 % in P. modestus), i.e.MC transferred to offspring	Chen & Xie, 2005
Freshwater snail (Bellamya aeruginosa)	MC-LR and RR	Natural routes in lake	(i) digestive track: 0.8- 240 4.54 –MC LR:RR=0.44 (ii) HP: 1.06-7.42 - LR:RR = 0.63 (iii)) gonad: 0-2.62 - LR:RR=0.96 (iv) foot: 0.01	HPLC	MC hepatopancreas > digestive track: selective bioaccumulation?	Chen & Xie, 2005

Zebra mussels (Dreissena polymorpha)	MC-LR	Feeding on toxic Microcystis cells	(i) 11 – feeding solely Microcystis (ii) 3.9 feeding on mixture Microcystis and green algae	3.1	LC-MS; MMPB	(i) maximum share covalently bound MC 38 % total (ii) only 0.5 % offered MC found in mussels (iii) rapid depuration, after 3 wk nearly complete	Dionisio Pires et al, 2004
Mysid shrimp (Mysis relicta)	NODLN	Feeding on copepods pre-exposed to cyanobacteria	(i) 0.74 (ELISA) (ii) 0.52 (PPase)		ELISA and PPase	(i) time dependent accumulation in shrimps, not in fish (results ELISA) (ii) copepods as vectors to higher trophic levels	Ensgtröm-Öst et al, 2002
Zebra mussels (Dreissena polymorpha)	MC	Natural routes in lake foodweb	1-30	7-3912	HPLC		Ibelings et al, 2005
Black tiger prawns (Penaeus monodon)	MC, NODLN	(i) natural routes in ponds (ii) oral uptake NODLN via food in experiments (iii) injection MC-LR	(i) ponds - HP (TEH) 0.006-0.08 (ii) experiment (NODLN) - brain and heart: 0.36 HP: 0.25 (0.83 peak level) gut: 0.1 gills: 0.014 muscle: 0.01 (iii) experiment (MC) - HP: 0.130 (peak)	(i) ponds: TEH: 1.2 (ii) exp: 10 µg kg bw^{-1}	ELISA; HPLC	Rapid depuration from prawns	Kankaanpää et al, 2005b
Baltic Sea zooplankton: Acartia tonsa, Eurytemora affinis, Strombium sulcatum	NODLN	Exposure to dissolved NODLN (^{3}H-dihydronodularin)	A. tonsa: 0.37 µg g^{-1} C E.affinis: 0.60 S. aulcatum: 1.55	5 µg L^{-1}		(i) minimum BCF 12 – 18 for copepods; (ii) max BCF ciliate 22 (iii) possible vectorial transport with significant sublethal effects	Karjalainen et al, 2003

(i) pike larvae (Esox lucius) (ii) mysid shrimps (Neomysis integer)	NODLN	Fed with zooplankton pre-exposed to (i) Nodularia extract (ii) purified NODLN (20 µg L^{-1})	(i) pike larvae (12h): 0.47 (ii) Neomysis: (12h): 0.31			Only 0.12 (pike) and 0.03 % (shrimps) of ingested toxin was detected in animals	Karjalainen et al, 2005
Gastropods (Lymnea stagnalis, Helisoma trivolis, Physa gyrina)	MC	Grazing on (settled) lake phytoplankton	11 – 121	1.2 – 6.1 µg L^{-1} ~1220 µg g^{-1} DW	HPLC	MC in seston not expressed on DW basis	Kotak et al, 1996a
Baltic clam (Macoma balthica)	NODLN	Exposure to dissolved toxin and Nodularia cells in tanks	0.16 - 16.6 (24 h) – 30.3 (96 h) (TEH); < 5 % of this NODLN	10-50 µg L^{-1} (cells) 4-20 µg L^{-1} (dissolved)	ELISA, HPLC, MALDI-TOF-MS	No NODLN-GSH conjugates detected	Lehtonen et al, 2003
Signal crayfish (Pacifastacus leniusculus)	MC	Exposure to toxic and non toxic Planktothrix agardhii	MC present but not quantified	3610		(i) crayfish ingested 430 µg MC – no effects (ii) possible vectorial transport to fish, birds, mink	Liras et al, 1998
Crab (unspecified)	MC	Natural routes in lake	Muscle: 0.02 – 1.0	0.12 – 0.78 µg L^{-1}	ELISA		Magalhães et al, 2003
Freswhater mussel (Alathyria condola)	PST	feeding on toxic Anabaena	5.7	1580			Negri & Jones, 1995
Pearl oyster (Pinctada maxima)	SAX	(i) natural routes in sea; (ii) exposure juvenile oyster to Trichodesmium	0.73 in viscera diseased oyster	Not detectable	HPLC; mouse bioassay; sodium channel and saxiphilin binding assay	no mortality juvenile oysters in experiment	Negri et al, 2004

(i) freshwater snail (Sinotaia histrica) (ii) freshwater clam (Corbicula sandai)	MC-LR and RR	(i) natural routes in lake (ii) feeding on toxic Microcystis cells in exp.	(i) S. histrica (lake) intestine 2.7 – 19.5 (MC-LR + RR) HP 0 – 3.2 (MC-RR only) (ii) C. sinai: nd (iii) S. histrica (exp.) HP max. 436	51.8 – 284 (lake) 20.1 µg L^{-1} (exp.)	HPLC	(i) lag phase in depuration from snail tissue; biological half life 8.4 d (ii) MC in lake snail still present next spring	Ozawa et al, 2003
Freshwater mussel (Anodonta cygnea)	PST	Feeding on toxic Aphanizomenon - accumulation 14d, depuration 14d	0.26	1.9-2.6	HPLC	(i) Anodonta exposed to $1.4e^9$ cells L^{-1} d^{-1}, removed 65 % these; clearance rate negatively related to PST content (ii) slow-fast-slow depuration; s-shaped kinetics (8.2 % d^{-1})	Pereira et al, 2004
Freshwater clam (Anodonta grandis)		(i) exposure dissolved MC (ii) natural routes in lake	Viscera 0.59 (i) gills 0.31 (ii) muscle 0.36	MC 51-55 µg L^{-1} dissolved up to 8.3 µg L^{-1} in lake		(i) toxin burden evenly distributed over three body parts (ii) rapid depuration first 6d (~70% gone), stable for 15d afterwards (iii) suggestion of biocon-centration	Prepas et al, 1997

Freshwater mussel (Anodonta cygnea)	CYN	Feeding on Cylindrospermopsis culture	(i) haemolymph 61.5 (= 408 µg L^{-1}) (ii) viscera: 5.9 (iii) mantle: 0.13 (iv) foot + gonads: 0.75 (v) whole body extract: 2.9 (all maximum conc. after 10-16 d accumulation)	14-90 µg L^{-1}	HPLC	(i) no adverse effects on mussels despite bioaccumulation CYN in haemolymph to conc. higher than in water (ii) bi-phasic depuration, increase in CYN content from day 22-28	Saker et al, 2004
Blue mussel (Mytilus edulis)	NODLN	Natural routes in the sea	Max 2.15	Max 2400			Sipiä et al, 2001
(i) balthic clam (Macoma balthica) (ii) blue mussel (Mytilus edulis)	NODLN and MC	Natural routes in the sea	(i) mussels 1.5 (max of TEH) (ii) clams 0.1-0.13	150-8700	ELISA and MALDI-TOF-MS	(i) NODLN-GSH conjugates confirmed with MS (ii) mussels MC 30 fold increase summer (iii) time lagged accumulation deep sites	Sipiä et al, 2002
Blue mussels (Mytilus edulis)	NODLN	(i) grazing on Nodularia (ii) coprophagy (exposure to feces of mussels grazing on Nodularia)	(i) Mussels pre-exposure to Nodularia: 0.05-0.1 (ii) Mussels grazing on Nodularia - digestive track 245 body 80 gills 2 (iiii) feces when feeding on Nodularia 95 (iv) Body after coprophagy 0.065 (v) Feces coprophagy 1 (vi) PF from (ii) 714	Nodularia culture 16 µg L^{-1}	LC-MS	(i) high NODLN in PF indicative of selective feeding; (ii) cells Nodularia in PF may survive but growth inhibited	Svensen et al, 2005

Mytilus galloprovincialis	MC-LR	Grazing on Microcystis cells	10.5 (of which 96 % in digestive gland + stomach)	28 µg 10^8 cells^{-1}		Depuration bi-phasic and fairly rapid (13d)	Vasconcelos 1995
Crayfish (Procamabarus clarkia)	MC	Feeding on toxic (and non toxic) Microcystis cells	2.9 (max after 11 d), of which 53 % in intestine, 38 % HP and 9 % rest body	2300	ELISA	Juvenile crayfish enhanced mortality on non-toxic Microcystis: compounds other than MC more relevant to invertebrates (?)	Vasconcelos et al, 2001
Dungeness crab larvae (Cancer magister)	MC-LR	Natural routes in foodweb	(i) 0.006 (after MeOH extraction) (ii) 84.4 (after Lemieux)		(i) PPase after MeoH extract. (ii) Lemieux (GC-MS)	10.000 fold greater MC concentration using MMPB after Lemieux extraction	Williams et al, 1997a
Blue mussel (Mytilus edulis)	MC-LR	(i) feeding on toxic Microcystis cells (ii) natural routes in foodweb	(i) 3369 decreasing to 113 after 4 d depuration (Lemieux) (ii) 2 - dropping to 0.14 over 53 d depuration (MeOH)		(i) PPase after MeoH extract. (ii) Lemieux (GC-MS)	Less than 0.1 % of MC extractable with MeOH	Williams et al, 1997b
Freshwater snail (Bellamya aeruginosa)	MC-LR, RR		(i) HP 1.06-7.42 (ii) digestive track 0.8-4.54 (iii) gonad 0-2.62 (iv) foot 0-0.06 (MC-LR only)		HPLC, LC-MS	(i) bioaccumulation in HP (ii) ratio LP:RR increased from digestive track to HP to gonad (iii) suggestion LR more resistant to degradation?	Xie et al, 2005
Freshwater mussel (Unio douglasiae)	MC-LR	Exposure to Microcystis cells	HP: (i) at 15 oC – 130 (ii) at 25 ° C - 250	27 - 50 µg L^{-1}		(i) accumulation temperature dependent (ii) depuration relatively fast; but slower at 15 than 25 °C; halted in winter (iii) no adverse effects on mussels	Yokoyama & Park (2003)

Pulmonate snails (Lymnea stagnalis, Helisoma trivolis, Physa gyrina)	MC	Natural exposure in lakes of varying trophic status	Up to 144	0 – 1526	HPLC	Possible uptake routes via food and directly from water	Zurawell et al, 1999
ZOOPLANKTON							
Echinogammarus ischnus	MC	Natural routes in lake	2	91-820		Exposure of this detrivore via pseudofaeces Dreissena?	Babcock-Jackson et al, 2002
Community of different spp, i.a. Daphnia galeata	MC	Natural routes in lake	0-1352		HPLC		Ibelings et al, 2005
(i) calenoid copepods (Eurytemora affinis, Acartia tonsa) (ii) ciliate (Strombidium sulcatum)	NODLN	Uptake of dissolved NODLN from water during 15 min - 6 d	(i) E.affinis 1.14 (ii) A. tonsa 0.66 (iii) S. sulcatum 4.8 (maxima)	5 µg L^{-1}		(i) BCF copepods 12-18 (ii) ciliate 22 (iii) vectorial transport to shrimps and planktiv. fish	Karjalainen et al, 2005
Community of different spp., a/o Daphnia pulex	MC	Grazing on lake phytoplankton	Up to 67	1.2 – 6.1 µg L^{-1}	HPLC		Kotak et al, 1996
(i) Thamnocephalus, platyurus (ii) Eudiaptomus gracili, (iii) Daphnia hyaline (iv) Cyclops abyssorum	MC	(i) grazing on mixtures of Cryptomonas and Planktothrix (ii) Planktothrix at 0.05 or 0.1 mg C L^{-1} - MC(+) or MC(-)		4.7 ng MC-LR per µg C^{-1}	HPLC		Kurmayer & Jüttner, 1999

Calanoid copepod (Eurytemora affinis)	NODLN	(i) feeding on Nodularia and non-toxic flagellates (ii) natural seston	(i) fed with Nodularia: 0.032 ng copepod^{-1} (background) rising to 0.007 (ELISA) (ii) 0.0095 to 0.101 (PPase) (iii) feacal pellets - 0.0067 ng pellet^{-1} (ELISA) (iv) idem 0.0050 (PPase)		ELISA and PPase	(i) lower conc. when fed with natural seston (dominated by non-tox Aphanizomenon) (ii) ELISA and PPase different but not significant (iii) no acute effects on copepods despite accumulation (iv) vectorial transport to foodweb (v) toxin content feacal pellets low: no vector to coprophagous animals	Lehtiniemi et al, 2002
Daphnia magna	PST	(i) grazing on Aphanizomenon (1.2e^6 cells mL^{-1}) (ii) lyophilized material (1 mg mL^{-1})	(i) exposure Apha: 0.065-0.378 pmol PST animal^{-1} (ii) exposure lyophilized material: 0.007	(i) cells: 643-1170 pmol mL^{-1} (ii) lyoph.: 2745 pmol mL^{-1}	HPLC-FLD	Bioaccumulation factor > 1 after 12h feeding	Nogueira et al, 2004
Daphnia magna	CYN	Grazing on Cylindrospermopsis strains (+/- CYN)	(i) 0.025 ng animal^{-1} (24 h) (ii) 0.020 ng animal^{-1} (48 h)	(i) intracellular toxin 219.6 – 236.4 ng mL^{-1} (ii) extracellular toxin 12.4 – 47.4 ng mL^{-1} (increase in time) (iii) total CYN 234.2 – 278.4 ng L^{-1}	HPLC-MS	Bioaccumulation factors smaller than unity: 0.71 (24 h) and 0.46 (48 h); levels not high enough to indicate bioaccumulation	Nogueira et al, 2004(b)

Daphnia magna	MC	(i) grazing on Microcystis alone or mixed with Scenedesmus (3.2 mg C L^{-1}) (ii) exposure to lake water enriched with Microcystis from enclosures (8.9 mg C L^{-1})	Daphnia 0.2-24.5 µg g^{-1} DW (highest when exposed solely to Microcystis)	(i) Microcystis: 2000 µg g DW^{-1} (ii) MC varied between 5 – 156 µg L^{-1} in enclosures	ELISA	(i) calculation shows MC really accumulated in body, not just gut content (ii) calculation shows transport to roach results only in sublethal effects	Thostrup & Christoffersen, 1999

Table A.2. Effects of various cyanobacterial toxins on aquatic biota. Where possible dose and effect are shown.

Exposed organism	Toxin	Exposure route	Concentration (dose) and effect	Remark	Reference
BIRDS					
FISH					
Zebra fish (Danio rerio)	MC–LR	Purified MC, dissolved in water	(i) 0.5, 5 µg L^{-1}: increased motility (ii) 15, 50 µg L-1: decreased motility (iii) 50 µg L^{-1}: reduced spawning	(i) effects on behaviour dose dependent (reversal at higher conc.) (ii) LOEC < 0.5 µg L^{-1}	Baganz et al, 1998
Brown trout (Salmo trutta)	MC	i) purified MC ii) aqueous extracts of Microcystis	5, 50, 500 µg L^{-1} MC: cardiovascular effects	Purified MC no effect on (some) cardiac responses, in contrast to cell extracts – always effects; cardiac output only affected by MC at higher doses	Best et al, 2001
Zebra fish (Danio rerio)	MC–LR; LPS	In vivo exposure fish embryos to dissolved toxins; LPS different bacterial origins (including cyanobacterial)	LPS (up to 29.8 10^6 EU mg^{-1} DW) + MC (up to 12.6 ng mg DW^{-1}): reduced mGST + sGST	LPS from axenic bacteria or lake blooms reduced GST activity, interfered detoxication	Best et al, 2002
Rainbow trout (Oncorhynchus mykiss)	MC	Aqueous suspension whole or broken Microcystis cells	100 µg L^{-1} MC–LR: osmoregulatory imbalance, increased liver mass	Interaction MC with LPS	Best et al, 2003

Exposed organism	Toxin	Exposure route	Concentration (dose) and effect	Remark	Reference
Brown trout (Salmo trutta)	MC–LR	(i) purified MC (ii) lysed Microcystis cells	i) lysed tox Microcystis, containing 41 – 68 µg L^{-1} MC: ionic imbalance, reduced growth ii) purified toxin: effects on growth (less than toxic lysate) iii) lysed non–tox Microcystis: effects on growth (less than toxic lysate)	Ammonia similar effects to MC	Bury et al, 1995
Carp (Cyprinus carpio)	MC	Fish exposed to toxins via natural routes in the lake, including Microcystis scums	Up to 4000 µg g^{-1} DW: hepatic lesions	Lesions in > 50 % fish examined	Carbis et al, 1997
Carp (Cyprinus carpio)	MC–LR	Gavage freeze dried Microcystis cells (single sublethal bolus)	400 µg kg^{-1} bw: pathological changes in hepatopancreas, kidney, gastrointestinal tract (i.a. apoptosis)	In carp – cyprinid – compared to salmonids liver pathology develops faster and at lower MC concentrations	Fischer & Dietrich, 2000
Rainbow trout (Oncorhynchus mykiss)	MC	Gavage freeze dried Microcystis cells	5700 µg kg^{-1} bw: liver necrosis, hepatocyte apoptosis	Unbound MC results in fast effects, including PP inhibition and liver necrosis; covalently binding to MC results slower effects, i.a. apoptosis	Fischer et al, 2000
(i) Hypophthalmichthys molitrix (ii) Carassius gibelio	MC–LR; MC–RR	Feeding on cultured Microcystis cells	unspecified dose: reduced growth		Jang et al, 2004

Exposed organism	Toxin	Exposure route	Concentration (dose) and effect	Remark	Reference
Tilapia (Oreochromis sp)	MC–LR	Feeding on lyophilized Microcystis bloom (crushed and non–crushed cells)	60 μg $fish^{-1}$ day^{-1} MC–LR: oxidative stress in liver, kidney and gills	(i) crushed cells more effect non–crushed on LPO and levels antioxidant enzymes (ii) time dependent development oxidative stress: not yet present after 14d; 21 dlipid peroxidation + enhanced antioxidant enzyme activities	Jos et al, 2005
Sea trout (Salmo trutta)	NODLN	Gavage single dose Nodularia	210–620 μg kg^{-1} bw: rapid (1–2 d) but reversible (4–8 d) liver damage	(i) rapid detoxication, cross reaction conjugates in ELISA (ii) no effects on swimming	Kankaanpää et al, 2002
Rainbow trout (Oncorhynchus mykiss)	MC–LR	IP injection of purified MC	(i) 1000 μg kg^{-1}: 100 % mortality (ii) 400 μg kg^{-1}: no mortality; increased ratio liver to body mass, liver necrosis; kidney lesions	Fish less sensitive than mice: LD_{50} = 400 – 1000 μg kg^{-1}	Kotak et al, 1996b
Carp (Cyprinus carpio)	MC–LR	In vitro exposure hepatocytes to 10 μg L^{-1} MC–LR	Increase ROS, depletion GSH, increase SOD, CAT and GS–Px activity, GST unchanged: oxidative shock by exposure MC	Antioxoidant enxymes did not prevent oxidative shock: apoptosis and necrosis hepatocytes	Li et al, 2003
Carp (Cyprinus carpio)	MC	Feeding on Microcystis scum in tanks	50 μg kg^{-1} bw for 28 days: reduced growth, increased liver enzyme activity, damaged hepatocytes,	Long term subchronic effects	Li et al, 2004

Exposed organism	Toxin	Exposure route	Concentration (dose) and effect	Remark	Reference
Loach (Misguruns mizolepis)	MC–LR	Dissolved MC–LR	MC–LR at 1, 3, 10, 100, 1000 µg L^{-1}: (i) mortalilty, delayed hatching, liver (necrosis) and cardiotoxicity (ii) (late stage) embryos and larvae more sensitive than juvenile fish: LC_{50} larvae = 164; juveniles = 593 µg L^{-1}	(i) embryonic development loach affected by MC in contrast to zebrafish (ii) results dose dependent, i.e. increasing effects with increasing conc. MC. No mortality at the lower doses.	Liu et al, 2002
Goldfish (Carassius auratus)	MC–LR	IP injection	125 µg kg^{-1} bw: no change ionic homeostasis, liver lesions, changes liver enzyme activity	Liver damage reversible	Malbrouck et al, 2003
Goldfish (Carassius auratus)	MC–LR	IP injection of fed and fasted juvenile goldfish	(i) 125 µg kg^{-1} bw: inhibition PPase, complete after 6 h in fasted, less inhibition in fed fish (ii) recovery after 96 h (iii) GSH levels and GST unaffected	Feeding status has effect on toxicity MC, possibly by acting on bile formation and secretion	Malbrouck et al, 2004a
(i) silver carp (Hypophthalmichthys molitrix) (ii) carp (Cyprinus carpio)	MC–LR	(i) ip injection purified MC (ii) cyanobacterial biomass per os / anus	(i) 400 µg kg^{-1} bw (purified MC): no – minor effects (ii) 3 – 1,200 µg kg^{-1} bw (biomass): changes blood indices and immunological changes	(i) effects crude biomass much stronger purified toxin (ii) oxidative stress	Palikova et al, 1998

Exposed organism	Toxin	Exposure route	Concentration (dose) and effect	Remark	Reference
Seven fish (cyprinids) and three amphibian species	MC–LR, RR and YR, SAX, ANA(a)	(i) in vivo emersion (ii) exposure to dissolved purified toxin (iii) exposure crude cyanobacterial cell extracts	(i) MC 0 – 50 μg L^{-1}: no acute effects on embryonic development (ii) MC–RR > 0.5 μg L^{-1}; YR > 5 μg L^{-1}; LR > 50 μg L^{-1}: timing hatching affected (ii) morphological effects only at highest conc. MC–LR 10 mg L^{-1} (iii) SAX > 10 μg L^{-1} delayed hatching malformations at 500 μg L^{-1} (iv) ANA(a) 400 μg L^{-1} hearth rate affected, no chronic effects (v) far more pronounced effects with crude extracts: malformations and mortality	Crude cell extracts of several cyanobacteria gave severe effects (more so than purified toxin): cannot be attributed MC alone	Oberemm et al, 1999

Exposed organism	Toxin	Exposure route	Concentration (dose) and effect	Remark	Reference
Rainbow trout (Oncorhynchus mykiss)	MC–LR	(i) IP injection or oral dosage purified MC (ii) IP injection or oral dosage freeze dried Microcystis cells	(i) 550 µg kg^{-1} bw MC (ip): severe liver damage, death (ii) 550 µg kg^{-1} bw Microcystis (ip): severe liver damage, death (iii) 1200 µg kg^{-1} bw MC (oral): no effects (iv)1700 µg kg^{-1} bw Microcystis (oral): no effects (v) 6600 µg kg^{-1} bw Microcystis (oral): severe liver damage, death (vi) 550 µg kg^{-1} bw Microcystis (8 times oral dosage): modest – severe liver damage	Treatments included repeated (oral) exposure to MC, i.e. close to natural exposure but during 'limited' period of time (8 times at 12 h intervals)	Tencalla et al, 1994
Zebra fish (Danio rerio) embryos	MC–LR	Purified dissolved MC	(i) > 0.1 µg L^{-1}: increased sGST and GPx activity (ii) > 2.0 µg L^{-1}: effects on growth and survival	Dose dependent relationship MC~GST	Wiegand et al, 1999
Carp (Cyprinus carpio)	MC–LR	Purified dissolved MC	inhibition ATP–ase activity of Na^+K^+ pump in gills, disruption ion homeostasis	Harmful effects of dissolved MC (without ingestion of cells)	Zambrano & Canelo, 1996
MACRO–INVERTEBRATES					
Brine shrimp (Artemia salina)	MC–LR, MCHtyR and NODLN	Purified dissolved toxin	0.5 µg L^{-1}: elevation GST activity		Beattie et al, 2003

Exposed organism	Toxin	Exposure route	Concentration (dose) and effect	Remark	Reference
(i) pike larvae (Esox lucius) (ii) mysid shrimps (Neomysis integer)	NODLN	(i) purified NODLN (20 µg L^{-1}) (ii) crude extract Nodularia	(i) crude extract: pike larvae: decreased ingestion and faeces production rates shrimps: no effects on molting, faeces production, C:N or growth (ii) purified NODLN: no effects	Purified NODLN no effects on pike larvae; crude cell extracts stronger effects	Karjalainen et al, 2005
Baltic clam (Macoma balthica)	NODLN	(i) exposure to dissolved toxin (ii) exposure to toxic and non toxic Nodularia cells in tanks	4–20 µg NODLN per day: (i) conc. dependent neurotoxic effects (increase / decrease AChE activity when exposed to low and high NODLN respectively) (ii) some treatments low siphon activity	Abundant unidentified compound with NODLN like spectral characteristics (found in both toxic and non–toxic Nodularia treatments)	Lehtonen et al, 2003
Brine shrimp (Artemia salina)	CYN and MC	(i) purified dissolved CYN and MC (ii) extracts Cylindrospermposis, Microcystis	(i) CYN LC_{50} decreased from 4.48 to 0.71 µg mL^{-1} between 24 and 72 h (ii) likewise MC LC_{50} from 4.58 to 0.85 µg mL^{-1}	(i) dose and time dependent mortality (ii) LC_{50} cell extracts typically > than purified CYN (reduced bioavailabilty?)	Metcalf et al, 2002
Estuarine crab (Chasmagnathus granulatus)	MC	Cell extracts Microcystis	Injected daily for 4–7 d with 17.6 ng MC: (i) increased enzyme activity (GST, CAT) in hepatopancreas (ii) no change LPO – no oxidative damage (?) (iii) yet histological damage		Pinho et al, 2003

Exposed organism	Toxin	Exposure route	Concentration (dose) and effect	Remark	Reference
Estuarine crab (Chasmagnathus granulatus)	MC	Crabs injected twice with cell extracts Microcystis	(i) decreased Na^+, K^+–ATPase activity anterior gills (ii) increased GST posterior gills (iii) increased TOSC	Increased TOSC in response MC as protective mechanism against LPO (level unchanged by exposure to MC)	Vinagre et al, 2003
ZOOPLANKTON					
Moina macrocopa	Unidentified metabolites (possibly cyanopeptolins A–D)	Freeze dried Microcystis	Inhibition of proteases		Agrawal et al, 2005
Thamnocephalus platyurus	[D–Asp^3, (E)–Dhb^7]MC–RR, MC–LR, MC–YR, MC–RR, NODLN	Purified toxins	LC_{50} NODLN < [D–Asp^3, (E)–Dhb^7]MC–RR < other MC	LC_{50} insufficient to study response of organisms to exposure; LC_{10} + LC_{90} required to get slope	Blom et al, 2001

Exposed organism	Toxin	Exposure route	Concentration (dose) and effect	Remark	Reference
Daphnia pulicaria, D. Pulex, D. hyaline, Diaptomus birgei	MC–LR, NODLN, ANA(a)	Exposure to: (i) purified toxins (ii) cell extracts (iii) toxic or non–toxic cyanobacterial strains	(i) species specific responses to toxin exposure; not all daphnids equally sensitive in terms feeding inhibition (ii) LC_{50} for MC after 24 h (varied from > 50 μg L^{-1} in D. pulicaria to < 1.0 in D.birgei); for NODLN > 20 and < 0.6 respectively (iii) LC_{50} decreased with longer exposure	(i) feeding inhibition protects against toxic effects: less sensitive Daphnia stronger inhibiton (ii) zooplankters insensitive dissolved MC, more so dissolved ANA(a)	DeMott et al, 1991
Daphnia spp	MC	Feeding on mixtures Microcystis and Scenedesmus; 0, 50 or 80 % Microcystis in total food conc. of 0.5 mg C L^{-1}	(i) rapid feeding inhibition (but recovery after continued exposure to same mixture) (ii) reduced growth and reproduction (iii) reduction in growth/ingestion: direct toxic effects and feeding inhibition	Clear differences between Daphnia spp	DeMott, 1999
Temperate and tropical cladocerans (Ceriodaphnia cornuta, Daphnia pulex; D. pulicaria, D similes, Moina micrura, Moinodaphnia macleayi)	MC	(i) grazing on toxic Microcystis strains mixed with Ankistrodesmus, total conc. 1.0 mg C L^{-1}; (ii) acute and chronic exposure	MC contents 2810–4080 μg g^{-1} DW: (i) decreased survival in presence tox Microcystis (ii) toxic Microcystis inhibited feeding rate, even when just 5 % in mixture with greens (ii) non–toxic cyanobacterium as sole food:poor growth	(i) species from low productivity sites – adapted to starvation – showed lowest sensitivity to toxic Microcystis (ii) small and large bodied fast growing spp prone to starvation and most sensitive to MC; small bodied slow growing spp most resistant and least sensitive	Ferrão–Filho et al, 2000

Exposed organism	Toxin	Exposure route	Concentration (dose) and effect	Remark	Reference
Tropical cladocerans (Moina micrura, Ceriodaphnia cornuta)	MC	Grazing on: (i) large Microcystis colonies (ii) single cells and (small) colonies Microcystis	MC 2.0 – 16.0 µg L^{-1}: (i) toxic Microcystis inhibited growth reduced reproduction cladocerans; partly through feeding inhibition (ii) effects unicellular lab cultures stronger than colonial cultures or natural seston, especially with large colonies (although very toxic 3.9 mg MC g DW^{-1})	Colony forming cyanobacteria: little effect despite high toxicity; possible explanation why field studies fail to demonstrate effects of toxic blooms	Ferrão–Filho & Azevedo 2003
Daphnia magna	MC	Grazing on mixtures of Microcystis and Scenedesmus; max of 140,000 Microcystis cells mL^{-1} (1.0 mg C L^{-1})	(i) reduced growth, reproduction and survival, increasing effects with increased proportion Microcystis cells (0; 50 or 100 %)0 (ii) pre–exposure (acclimation) reduced harmful effects: development of tolerance		Gustafsson & Hansson, 2004
Daphnia pulex	MC–LR	(i) acute exposure to Microcystis cells 0–2.43 mg C L^{-1} = 0–360,000 cells mL^{-1} (ii) chronic exposure 30,000 cells mL^{-1}	MC–LR 7.6 10^{-5} ng $cell^{-1}$: (i) variation in acute tolerance (EC_{50}) to toxic Microcystis (ii) increase temperature: decrease EC_{50} (iii) chronic exposure, reduced survival and reproduction, clonal differences reversed compared to acute exposure	Suggestion made that more resistant clones show stronger feeding inhibition	Hietala et al, 1997

Exposed organism	Toxin	Exposure route	Concentration (dose) and effect	Remark	Reference
Calanoid copepods (Acartia bifilosa, Eurytemora affinis)	NODLN	Cultures of Nodularia added to enclosures	NODLN ~ 11µg mg DW^{-1} (3–4 µg mL^{-1}): Baltic copepds feed, ingest, reproduce and survive in presence toxic Nodularia, no negative effects	Perhaps hatching success more sensitive to toxins (not measured)	Koski et al, 2002
Eudiaptomus gracilis, Thamnocephalus platyurus, Daphnia hyaline, Cyclops abyssorum	MC	Grazing on (artificially shortened) filaments Planktothrix	(i) reduced survival Thamnocephalus (ii) survival naturally co–existing zooplankton unaffected; (iii) Eudiaptomus high sensitivity but also strict food avoidance (iv) Daphnia and Cyclops greater physiological resistance to MC, less avoidance – ingested filaments	(i) Daphnia feeding rates increased (not so for copepods) when prior toexposure MC were extracted from filaments (MC acts as feeding deterrent) (ii) unidentified lipophilic toxin present (iii) high avoidance linked to high sensitivity	Kurmayer & Jüttner (1999)
Daphnia pulex	MC–LR	Grazing on toxic Microcystis cells (0 – 320,000 cells mL^{-1}) + low or high density Scenedesmus (20,000 or 80,000 cells mL^{-1})	MC–LR 8.9 10^{-5} ng $cell^{-1}$: (i) decreased population density (ii) delayed maturity (iii) increased number ephippia ind^{-1}	Effects toxic Microcystis comparable to food of low quality or lack of food	Laurén Määttä et al, 1997

Exposed organism	Toxin	Exposure route	Concentration (dose) and effect	Remark	Reference
Daphnia magna	MC	Grazing on MC(+) wt and MC(–) mutant + Scenedesmus; Microcystis 0 –100 % of 5 mg C L^{-1}	(i) severe disturbance Daphnia population development (increased mortality, decreased reproduction) (ii) MC(+) killed Daphnia faster MC(–) (iii) also MC(–) had negative effects on survival and growth, even in presence Scenedesmus	(i) nutritional insufficiency Microcystis not responsible effects on Daphnia (ii) feeding inhibition and/or compounds other MC (cyanopeptolines?) may play role (iii) MC alone cannot explain harmful effects on Daphnia	Lürling, 2003
Daphnia magna	MC–LR	(i) grazing on MC(+) and MC(–) strains of Microcystis in max conc. of 5 mg C L^{-1} in mixtures with Scenedesmus + in some treatments in addition: (ii) exposure to purified, dissolved MC, max 3.5 µg L^{-1}	(i) no effects dissolved MC (ii) exposure cellbound MC: reduced feeding and growth (iii) reductions also in treatment 50 % Microcystis of MC(–) strain + 50 % Scenedesmus	Inhibition of Daphnia feeding and growth in presence of Scenedesmus and MC(–) strain Microcystis: unknown toxic compounds	Lürling & van der Grinten, 2003
Daphnia magna	PST	Grazing on Aphanizomenon	$1.2e^6$ cells mL^{-1} containing 643–1170 pmol mL^{-1} PST: (i) reduced fitness, growth and survival (ii) reduced activity cGST		Nogueira et al, 2004

Exposed organism	Toxin	Exposure route	Concentration (dose) and effect	Remark	Reference
Daphnia magna	CYN, unidentified hepatotoxin	Grazing on 2 different strains Cylindrospermopsis (+/– CYN); 1.8 – 3.6 10^6 cells mL^{-1};	CYN 4.78 ng mg cells DW^{-1}: (i) reduced growth and increased mortality, also in comparison to starvation treatment, (ii) above also true for CYN(-) strain (iii) sGST + mGST activity increased	Unknown toxic compounds present in strain that does not produce CYN	Nogueira et al, 2004(b)
Daphnia pulex; D. longispina	MC, unidentified toxins	Grazing on mixtures Scenedesmus and Microcystis cells (10,000 or 40,000 cells mL^{-1} ~ 0.076 – 0.304 mg C L^{-1})	(i) increased allocation resources to reproduction (ii) lower dose resulted smaller clutch size D. Pulex; D. longispina no effect (iiii) higher dose virtual inhibition reproduction D. Pulex; D. longispina reduced size neonates	(i) severe and dose dependent effects Microcystis on reproduction in Daphnia (ii) toxicity and food quality play a role	Reinikainen et al, 1999
Estuarine calanoid copepods (Eurytemora affinis; Acartia bifilosa)	MC–LR, ANA(a), NODLN	Purified dissolved toxins, single and in combination	(i) 1 µg mL^{-1} MC or ANA no effect on egg hatching (ii) 0, 0.25, 0.5 and 1 µg mL^{-1}l: reduced survival for MC > 0.1 µg mL^{-1} (iii) ANAa and NODLN only weak effects		Reinikainen et al, 2002
Daphnia galeata	MC	Daphnia feeding on toxic (wt) and non toxic (mutant does not produce MC) Microcystis strains	(i) wt toxic to Daphnia: decreased swimming + death, mutant not toxic (ii) both wt and mutant inhibit ingestion rate	Dose response relationship not between MC content of the food and effects in Daphnia but between ingestion rate and effects	Rohrlack et al, 1999

Exposed organism	Toxin	Exposure route	Concentration (dose) and effect	Remark	Reference
Daphnia pulicaria	Microviridin J	(i) Grazing on Microcystis strains containing 1.05 – 1.57 µg mm^{-3} microviridin (ii) Purified, dissolved microviridin (0–12 mg L^{-1})	Lethal molting disruption	Microviridin is a protease inhibitor	Rohrlack et al, 2004
Daphnia spp.	MC	Daphnia feeding on toxic MC(+) wt and non–toxic (MC(-) mutant Microcystis strains	(i) inhibition of feeding rate, equal inhibition for tox and non–tox Microcystis (ii) reduced survival time (LT_{50}) Daphnia when feeding on wt (iii) Daphnia feeding on mutant signs starvation	(i) MC major source of acute Daphnia poisoning (ii) clear relationship – dose–response – between LT_{50} and MC ingestion rate	Rohrlack et al, 2001
Daphnia galeata	MC	Daphnia feeding on MC(+) and MC(–) strain	Toxic strain 0.87 mg L^{-1} MC: (i) both tox and non–tox Microcystis negatively affect the cohesion of midgut epithelium (within 9 h) (ii) tox strain: uptake MC in blood, increase 0.25 to 1 ng L^{-1} (6–9 h) (iii) tox strain: decreased beat rates (5–9 h), constant contraction midgut, finally complete loss beat rates, death (32–41 h)	(i) midgut disrupting factor is not MC, disruption stimulates uptake bioactive compounds from cyanobacteria in blood Daphnia (ii) results not easily translated to field	Rohrlack et al, 2005
Daphnia magna	MC	(i) Daphnia exposed to suspensions of toxic Microcystis cells (ii) Daphnia exposed to lake water (filtered or not)	MC=2000 µg g DW^{-1}: reduced survival, fecundity and growth	Negative correlation between toxin content and growth (strong) and between toxin concentration and fecundity (weak)	Thostrup & Christoffersen, 1999