Nodularin and cylindrospermopsin: a review of their effects on fish

Benoît Sotton, Isabelle Domaizon, Orlane Anneville, Franck Cattanéo, Jean Guillard

Rev Fish Biol Fisheries (2015) 25:1–19
DOI 10.1007/s11160-014-9366-6

REVIEWS

Nodularin and cylindrospermopsin: a review of their effects on fish

Benoît Sotton · Isabelle Domaizon · Orlane Anneville · Franck Cattanéo · Jean Guillard

Received: 27 January 2014 / Accepted: 29 July 2014 / Published online: 6 August 2014

Abstract Nodularin (NOD) and cylindrospermopsin (CYN) are hepatotoxic cyanotoxins that are present in numerous ecosystems where bloom episodes occur. In this review, the different effects of both of these cyanotoxins on the different ontogenic stages of various fish species were summarised to clarify the state-of-the-art scientific knowledge on this topic. It is clear that fish that are exposed to NOD and CYN were negatively impacted in every studied ontogenic stage. Indeed, these cyanotoxins can accumulate in various organs of fish, leading to deleterious effects on the physiology. This review highlights the fact that all of the previously published studies on the topic have focused only on the short-term effects of a given cyanotoxin on fish. However, during cyanobacterial blooms, fish can be exposed chronically to a variety of toxic compounds with which the fish interact, leading to stronger effects than those observed with a single toxin tested over a short timeframe. Thus, it is essential to conduct additional studies to better understand the actual toxic effects of cyanobacterial blooms on fish populations over medium- and long-term time scales.

Keywords Cyanobacteria · Cyanotoxins · Cylindrospermopsin · Effects · Fish · Nodularins

B. Sotton (✉) · I. Domaizon · O. Anneville · J. Guillard
INRA–UMR CARRTEL, 75 av. de Corzent,
74203 Thonon Les Bains, France
e-mail: bsotton@gmail.com

I. Domaizon
e-mail: isabelle.domaizon@thonon.inra.fr

O. Anneville
e-mail: orlane.anneville@thonon.inra.fr

J. Guillard
e-mail: jean.guillard@thonon.inra.fr

F. Cattanéo
HEPIA, Earth - Nature - Environment Institute, University of Applied Sciences Western Switzerland, Route de Presinge 150, 1254 Jussy, GE, Switzerland
e-mail: franck.cattaneo@hesge.ch

Introduction

The anthropogenic eutrophication of aquatic ecosystems during the twentieth century promoted toxic cyanobacterial blooms worldwide, leading to modifications in the stability and in the functioning of affected ecosystems and to water use issues (Codd et al. 2005; Ibelings and Havens 2008). Due to these negative impacts on ecosystems, the development of toxic cyanobacterial blooms has become a key environmental and sanitary concern in recent decades. In addition, ongoing climate changes are predicted to foster the occurrence and intensity of cyanobacterial blooms (Paerl and Paul 2011; O'Neil et al. 2012), suggesting that the resulting environmental issues will likely increase.

Many cyanobacterial genera produce a wide variety of cyanotoxins that can cause severe impairments in

aquatic and terrestrial organisms (Chorus and Bartram 1999; Wiegand and Pflugmacher 2005; Ibelings and Havens 2008). Cyanotoxins can be of diverse chemical natures and are usually classified into categories based on their biological targets: hepatotoxins, neurotoxins, cytotoxins, dermatotoxins, and others whose toxicological or ecotoxicological profiles are only partially known (Codd et al. 2005). When blooms occur, hepatotoxins are the most frequently detected toxic molecules and are most often implicated in intoxication cases. Microcystin (MC) and nodularin (NOD), which are both cyclic peptides, as well as cylindrospermopsin (CYN), which is an alkaloid also known as a cytotoxin, are considered hepatotoxins. The presence of these hepatotoxins in the water has already led to human and livestock acute intoxications, ultimately until the death of the exposed individuals (Azevedo et al. 2002; Codd et al. 2005).

In aquatic ecosystems, hepatotoxins may adversely affect all of the food web components. Phytoplankton, zooplankton, and fish are impacted by the presence of hepatotoxins (Leflaive and Ten-Hage 2007; Ibelings and Havens 2008). Information about the bioaccumulation and the negative effects of these toxins on fish is abundant in the literature because fish play a key role in the ecosystem functioning and as human food. The consumption by humans of contaminated fish could therefore lead to significant health hazards. Fish are exposed directly to hepatotoxins by feeding on phytoplankton or contaminated organisms and/or passively via their epithelium (gills and skin) when hepatotoxins are dissolved in the water (Malbrouck and Kestemont 2006). The disturbances that are induced by hepatotoxins on the behavioural, physiological, and histological parameters of fish have been widely investigated in recent years, but the results are variable and sometimes contradictory (Malbrouck and Kestemont 2006; Ibelings and Havens 2008; Ferrão-Filho and Kozlowsky-Suzuki 2011). The effects of MC on the physiological parameters of fish have been intensively investigated, and reviews on this topic are available in the literature (Zurawell et al. 2005; Malbrouck and Kestemont 2006; Martins and Vasconcelos 2009; Amado and Monserrat 2010; Paskerová et al. 2012). Conversely, information about the effects of NOD and CYN on fish is still scarce and, to our knowledge, has never before been reviewed.

The aim of this review is to synthesise data regarding the interactions between NOD, CYN and fish, including the accumulation processes, and their effects on the physiological parameters of the different ontogenetic stages of fish. To assemble this review, all of the references were identified using the ISI Database from the Web of Science and the keywords "nodularin" or "cylindrospermopsin" in combination with "fish". The number of references that have been published on the topic during the searched timeframe is given in Fig. 1. For each cyanotoxin, the in situ accumulation and biological effects of the toxin are presented separately. For the biological effects, the results are detailed according to the ontogenetic stages that were investigated.

Cylindrospermopsin

General presentation

Cylindrospermopsin is a stable tricyclic alkaloid of 415 Da that possesses a tricyclic guanidine moiety combined with hydroxymethyluracil (Fig. 2). CYN was first detected in *Cylindrospermopsis raciborskii* from tropical water samples and later in other cyanobacterial genera originating from various countries in different climates (Falconer and Humpage 2006). Currently, two structural variants have been identified: 7-epicylindrospermopsin (7-epi-CYN), which has a similar toxicity as that of the mother molecule, and 7-deoxycylindrospermopsin (7-deoxy-CYN), whose toxicity is still debated (Looper et al. 2005; Falconer and Humpage 2006).

Compared to other cyanotoxins that are generally intracellular components of cyanobacterial cells, CYN is mainly produced as an extracellular toxin (approximately 90 % of the total CYN) and is detected at variable concentrations (from 0 to more than 800 $\mu g\ l^{-1}$ total CYN) in samples from different ecosystems (Van Apeldoorn et al. 2007; Rücker et al. 2007; Gutiérrez-Praena et al. 2013a). From these observations, *Cylindrospermopsis* seems to be the main genera producing CYN, but other cyanobacterial genera such as *Aphanizomenon*, *Anabaena*, *Umezakia* and *Rhaphidiopsis* have also been identified as producers of this cyanotoxin (Falconer and Humpage 2006).

CYN has been characterised as a hepatotoxin because the liver of exposed mice is the most affected organ (Bernard et al. 2003). Nevertheless, this toxin has also been classified as a cytotoxin due to negative

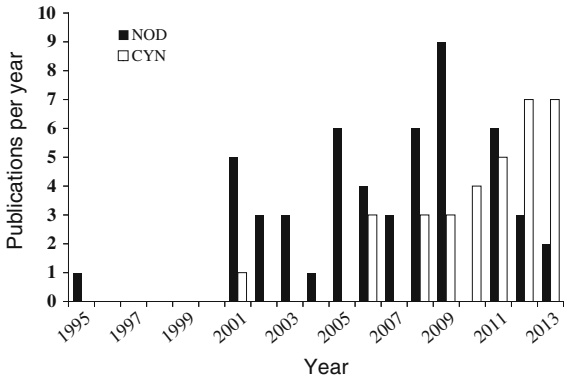
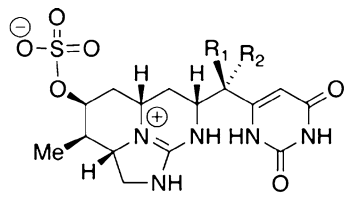


Fig. 1 Number of publications dealing with the effects of nodularin (*black*) and cylindrospermopsin (*white*) on fish



Compound name	R_1	R_2
Cylindrospermopsin	OH	H
7-Epi-cylindrospermopsin	H	OH
7-Deoxy-cylindrospermopsin	H	H

Fig. 2 Chemical structure of CYN and its variants

effects on other organs, such as the kidneys, lungs, thymus, spleen, adrenal glands, intestine, and heart (Falconer and Humpage 2006; Guzmán-Guillén et al. 2013b). The toxic mechanism of CYN has not been completely elucidated, but the results that were obtained from primary hepatocytes suggest a bi-modal action that seems to be both time- and concentration-dependent (Gutiérrez-Praena et al. 2011b, 2012). At low concentrations, the toxicity of CYN seems to be mediated via the rapid irreversible inhibition of protein synthesis, while at higher concentrations, a rapid toxic process implicating cytochrome P-450 (CYP450) might be the main mechanism (Froscio et al. 2003; Falconer and Humpage 2006). Other authors have reported that CYN causes a decrease in glutathione (GSH) synthesis (Runnegar et al. 1994), inducing oxidative stress (Guzmán-Guillén et al. 2013a, b). However, despite the wide occurrence of CYN in aquatic systems, few studies are available concerning both the accumulation and toxicity processes in fish.

Accumulation of CYN in fish

The accumulation processes of CYN in aquatic invertebrate and vertebrate organisms have been investigated in the past decade (Kinnear 2010; Gutiérrez-Praena et al. 2013a). To our knowledge, the first report of CYN accumulation in fish was published by Saker and Eaglesham (1999). In this paper, five rainbow fish (*Melanotaenia eachamensis*) were sampled from a crayfish pond during a *Cylindrospermopsis* bloom, and a concentration of 1.2 μg g^{-1} dry weight (DW) of CYN was detected in the viscera of fish (Table 1). More recently, Messineo et al. (2009) measured the CYN accumulation in the viscera (0.6–2.7 ng g^{-1} FW), muscle (0.1–0.8 ng g^{-1} FW), and ovaries (0.07 ng g^{-1} FW; Table 1) of two brown trout (*Salmo trutta*) specimens that were collected during an *Aphanizomenon ovalisporum* bloom in Lake Albano (Italy). Berry et al. (2012) collected various fish species in Lake Catemaco (Mexico) and found for the first time CYN in muscle ranging from 0.09 to 1.26 μg kg^{-1} FW (Table 1) and bioaccumulation factors ranging from 4 to 59 depending on the species. The highest levels of CYN accumulation were observed in herbivorous filter-feeding species (*Dorosoma mexicana* and *Bramocharax cabelleroi*) and herbivorous/omnivorous species (*Vieja fenestratus* and *Heterandria tuxtlaensis*), while the lowest levels were unexpectedly measured in the omnivorous introduced tilapia (*Oreochromis aureus*). These authors suggested that *Cylindrospermopsis* filaments could either be too small to be ingested or were specifically avoided by the omnivorous tilapia.

These results show that CYN and its variants can bioaccumulate in various fish species. However, the hydrophilic property of these molecules does not support the potential biomagnification process in the aquatic food web. Furthermore, the CYN concentrations in the different fish species were highly variable. Many factors, such as the concentration of cyanobacteria and of CYN present in the system, the absorption/elimination rates and pathways, and fish ages and analytical processes, could influence the bioaccumulation rates and measurements, thus explaining the differences that are observed between studies. Due to its bioaccumulation, CYN may impact fish at a physiological scale, leading to significant damages to the individual fish and more widely at the population scale.

Table 1 Cylindrospermopsin (CYN) accumulation in fish tissues as observed in field conditions

References	Sampling location	Cyanobacterial concentration	CYN concentration in water	CYN concentration in fish tissues	Measurement method
Saker and Eaglesham (1999)	Aquaculture pond	3.2×10^5 cell ml^{-1}	589 μg l^{-1}	*Salmo Trutta*: 1.2 μg g^{-1} DW in viscera	HPLC–MS/MS
Messineo et al. (2009)	Lake Albano	30×10^6 cell ml^{-1}	10–110 μg l^{-1}	*Melanotaenia eachamensis*: 2.7 ng g^{-1} FW in viscera 0.8 ng g^{-1} FW in muscle 0.07 ng g^{-1} FW in ovaries	ELISA
Berry et al. (2012)	Lake Catemaco	NS	ND	*Rhamdia* sp.: 0.24 μg kg^{-1} FW *Oreochromis aureus*: 0.09 μg kg^{-1} FW *Vieja* sp.: 0.42 μg kg^{-1} FW *Vieja fenestratus*: 0.81 μg kg^{-1} FW *Heterandria jonesii*: 1.26 μg kg^{-1} FW *Bramocharax cabelleroi*: 0.81 μg kg^{-1} FW *Cichlasoma urophthalmus*: 0.26 μg kg^{-1} FW *Cichlasoma helleri*: 0.15 μg kg^{-1} FW *Dorosoma Mexicana*: 0.80 μg kg^{-1} FW In the muscle of these species	ELISA

HPLC high-performance liquid chromatography, *MS* mass spectrometry, *ELISA* enzyme-linked immunosorbent assay, *DW* dry weight, *FW* fresh weight, *NS* not specified, *ND* not detected

Biological effects of CYN on fish

In vitro effects of CYN on hepatic fish cells

CYN toxicity on fish cells was investigated by in vitro studies to better understand the cellular interactions of CYN leading to disturbances of cell functioning. Interesting results have been observed in the hepatocytes of fish that were exposed to different CYN concentrations (Table 2). A decrease in cell viability reflected by a decrease (in a time- and concentration-dependent manner) in the protein content, neutral red uptake (NR) and tetrazolium salt reduction (MST) was observed (Gutiérrez-Praena et al. 2011b; Liebel et al. 2011). A decrease in the multixenobiotic resistance mechanism (MXR) was also observed for all of the CYN treatments (Liebel et al. 2011). These results show that the first-line defence mechanism, which is responsible for the efflux of xenobiotics, toxins, drugs and endobiotic metabolites, was negatively affected by pure CYN, even at low concentrations, suggesting a possible cellular accumulation of other substances at toxic levels. Similarly, a concentration-dependent increase in the reactive oxygen/nitrogen species

Table 2 In vitro effects of CYN on the hepatic cells of fish

References	Species	Mode, concentration and time exposure duration	Observable effects
Liebel et al. (2011)	*Prochilodus lineatus*	Immersion of hepatocytes in 0.1, 1 and 10 μg l^{-1} CYN during 72 h	↓ cell viability (0.1 and 1 μg l^{-1}), MRX (all treatments) ↑ RONS (all treatments), LPO (10 μg l^{-1}) ↔ GST, G6PDH, GSH/GSSG ratio, protein and DNA damages
Gutiérrez-Praena et al. (2011b)	*Poeciliopsis* lucida	Immersion of hepatocytes in 0.3, 0.6, 1.2, 2.5, 5, 10, 20 and 40 μg ml^{-1} CYN for 24 or 48 h	↓ protein content at 24 h for doses equal to or greater than 2.5 μg ml^{-1} and for all the treatments at 48 h ↓ NR from 5 μg ml^{-1} CYN for both doses ↓ MST from 10 μg ml^{-1} CYN at 24 h and from 2.5 μg ml^{-1} at 48 h ↑ ROS in a concentration-dependent manner at 24 h ↑ GSH and GCS at 2 μg ml^{-1} but ↓ at 8 μg ml^{-1} at 24 h

↑ = increase, ↓ = decrease, ↔ = no change, *MRX* multixenobiotic resistance mechanism, *RONS* reactive oxygen/nitrogen species, *LPO* lipid peroxidation, *GST* glutathione S-transferase, *G6PDH* glucose 6-phosphate dehydrogenase, *GSH* glutathione, *GSH/GSSG* reduced and oxidised glutathione ratio, *NR* neutral red uptake, *MST* tetrazolium salt reduction, *ROS* reactive oxygen species, *GSC* γ-glutamylcysteine synthetase

(RONS) and in lipid peroxidation (LPO) was observed (Table 2; Gutiérrez-Praena et al. 2011b; Liebel et al. 2011). A high concentration of RONS may lead to important damages to lipids (partially explaining the concomitant increase in LPO), proteins and even DNA. However, cells possess a robust protective mechanism to counteract the negative effect of CYN, thus maintaining cell viability. Additionally, glutathione S-transferase (GST) and glucose 6-phosphate dehydrogenase (G6PDH) do not seem to play a role in this protective mechanism because no differences in their activities were observed (Table 2; Liebel et al. 2011). The absence of protein and DNA damage was corroborated by the unaltered glutathione ratio (2GSH/Glutathione disulphide (GSSG)), suggesting that pure CYN did not affect the synthesis or cycling of glutathione (GSH), an important non-enzymatic antioxidant, and cofactors of glutathione-dependent enzymes that are involved in xenobiotic biotransformation and peroxide degradation (Liebel et al. 2011). However, the effects of CYN on GSH content and synthesis are not clear. Indeed, a decrease in the contents of both GSH and γ-glutamylcysteine synthetase (GSC), one of the enzymes that are involved in GSH production, was observed following exposure to pure CYN (Table 2; Gutiérrez-Praena et al. 2011b). Contrary to Liebel et al. (2011), these results suggest that an alteration in GSH contents and synthesis can be induced by the presence of CYN, which could lead to an increase in the contribution of oxidative stress to other damages, such as genotoxicity.

Biological effects of CYN on fish embryos

In every investigated ontogenic stage, reports of the biological effects of CYN on fish species are scarce. CYN is particularly present in the extracellular state; therefore, fish eggs and embryos may naturally come into contact with this toxin and thus be impacted by it. To our knowledge, only one publication has investigated the biological effects of CYN on young-stage fish (Berry et al. 2009). In this study, zebrafish (*Danio rerio*) embryos were exposed (1) by immersion to pure CYN (50 μg ml^{-1} for 5 days), (2) to a cyanobacterial extract containing CYN (14.3, 71.5 and 143 μg lyophilised CYN-containing cyanobacteria ml^{-1} for 1 day), or (3) by microinjection with various concentrations of pure toxin (50, 5, 1, 0.5, 0.2 and 0.1 μg ml^{-1} for 1 day). No developmental or mortality effects were observed in embryos that were exposed to pure CYN by immersion. Because CYN is highly hydrophilic, the authors suggest that CYN cannot readily permeate cellular membranes in

Table 3 Effects of CYN on the oxidative parameters of fish (*Oreochromis niloticus*)

References	Mode, concentration and exposure duration	Observable effects on *O. niloticus*
Gutiérrez-Praena et al. (2011a)	Gavage and injection with 200 μg kg^{-1} of CYN and observation at 24 h and 5 days	↑ NADPH activity at 24 h for both exposure mode (liver and kidneys) and at 5 days for fish that were orally exposed (liver)
		↑ LPO at 24 h for both exposure mode (liver and kidneys) and at 5 days for fish that were orally exposed (liver) and for both exposure mode (kidneys)
		↑ in protein oxidation at 5 days for both exposure mode (liver) and duration for fish that were injected (kidneys)
		↔ DNA oxidation in every exposure mode and duration
		↓ GSH and GSC contents at 24 h for both mode of exposure and ↔ at 5 days in every exposure mode
Puerto et al. (2011)	Gavage with 200 and 400 μg kg^{-1} of CYN and observation at 24 h	Dose-dependent ↑ of LPO (liver and kidneys)
		↔ GPx activity (liver) and ↓ for fish that were exposed to the highest dose (kidneys)
		↑ GPx gene expression for the lowest dose and ↓ for the highest (liver), ↑ GPx gene expression for both doses (kidneys)
		↑ GST for the lowest dose (liver) and for both doses (kidneys)
		Dose-dependent ↓ GST gene expression (liver) and ↑ GST gene expression for both doses (kidneys)
		↑ GST protein abundance for the lowest dose (liver) and for both doses (kidneys)
Puerto et al. (2012b)	Gavage with 200 and 400 μg kg^{-1} of CYN and observation at 24 h	Dose-dependent ↑ in protein oxidation (liver and kidneys)
		↑ NADPH activity for the lowest dose (liver) and for both dose (kidneys)
		↑ and ↓ GSH content for the lowest and highest dose respectively (liver) and ↓ GSH content for both doses (kidneys)
Gutiérrez-Praena et al. (2013b)	Gavage and injection with 200 μg kg^{-1} of CYN and observation at 24 h and 5 days	↔ GPx activity for every exposure duration and mode (liver and kidneys)
		↑ GPx gene expression at 24 h for fish that were orally exposed and for both exposure modes at 5 days (liver)
		↑ GPx gene expression at 24 h for fish that were orally exposed and ↓ for both exposure modes at 5 days (kidneys)
		↑ GST activity at 24 h both exposure modes, for injected fish at 5 days (liver) and for injected fish at both exposure durations (kidneys)
		↑ GST gene expression at 24 h for injected fish and for both exposure mode at 5 days (liver)
		↑ GST gene expression for both exposure modes at 24 h but only for injected fish at 5 days (kidneys)
		↑ GST protein abundance for both exposure modes at 24 h but only in orally exposed fish at 5 days (liver) and for fish that were injected at 5 days (kidneys)
Guzmán-Guillén et al. (2013a)	Immersion of fish in cyanobacterial cells containing 10 and 100 μg l^{-1} of CYN and observation at 7 and 14 days	↑ lipid peroxidation for both doses at 14 days (liver) and for the highest dose for every exposure duration (kidneys)
		↑ protein oxidation for the highest dose at 14 days (liver and kidneys)
		↑ DNA oxidation for the highest dose for every exposure duration (liver and kidneys) and for both doses for every exposure duration (kidneys)
		↑ GST activity for the lowest dose at 7 days and for both doses a 14 days (liver), ↓ GST activity for the highest dose at 14 days (kidneys)
		↔ GPx activity whatever the dose and exposure duration (liver), ↑ GPx activity for the highest dose at 7 days and for both doses at 14 days (kidneys)
		↑ SOD for the highest dose at 7 days and for both doses at 14 days (liver), ↑ SOD for the highest dose at 7 days but ↓ SOD for both doses at 14 days (kidneys)
		↓ and ↑ CAT for lowest and highest dose respectively at 7 days and ↓ CAT for the highest dose at 14 days (liver), ↓ CAT for every dose and exposure duration (kidneys)
		↑ GCS for the highest dose at 7 days (liver) and for both doses and exposure durations (kidneys)
		↓ GSH/GSSG ratio for the lowest dose at 7 days and dose-dependent ↓ at 14 days (liver), dose-dependent ↓ for every exposure duration (kidneys)

↑ = increase, ↓ = decrease, ↔ = no change, *LPO* lipid peroxidation, *GST* glutathione S-transferase, *GPx* glutathione peroxidase, *NADPH* nicotinamide adenine dinucleotide phosphate-oxidase, *GSH* glutathione, *GSC* γ-glutamylcysteine synthetase, *SOD* superoxide dismutase, *CAT* catalase, *GSH/GSSG* reduced and oxidised glutathione ratio

zebrafish embryos. However, developmental toxicity and strong mortality were observed in embryos that were treated with cyanobacterial extracts, suggesting that other metabolites in cyanobacteria could have more toxic effects on fish than CYN (Berry et al. 2009). To test the real toxicity of CYN on embryos and to circumvent possible difficulties in the penetration of CYN across cellular membranes, microinjections were also used. The obtained results showed a dose-dependent toxicity of CYN leading to rapid mortality and to deformations in less than 24 h, even at low concentrations. Nevertheless, CYN does not seem to be a negative factor for embryo development and survival, mainly because it does not easily permeate cellular membranes. However, in ecosystems, CYN-producing cyanobacteria can produce other toxic metabolites. Therefore, future studies need to consider the synergistic effects between these various toxic molecules to better comprehend the potential disturbances of both CYN and toxic metabolites to fish embryos.

Effects of CYN on adult fish

Oxidative parameters Oxidative stress is defined as an excess of pro-oxidants compared to antioxidants, leading to potential molecular damage (Amado and Monserrat 2010). To study the effects of toxic substances on oxidative stress parameters, the transcription and activity of enzymes that have been implicated in reactive oxygen species (ROS) scavenging or consequences of ROS effects are used as indicators. Superoxide dismutase (SOD) and catalase (CAT) provide the first defence against oxygen toxicity (Amado and Monserrat 2010). Guzmán-Guillén et al. (2013a) exposed tilapia sub-chronically (by immersion) to environmentally relevant concentrations of CYN for 7 or 14 days (Table 3). These authors observed an increase in SOD in the liver at the highest dose (100 μg l^{-1} CYN) for both exposure durations, to counteract ROS formation. In the kidneys after an initial increase in CYN, the SOD activity decreased after 14 days, correlating with an increased susceptibility of the kidneys to CYN (higher LPO, protein and DNA oxidation). The CAT activity increased in the liver after 7 days of exposure to control oxyradicals but decreased after 14 days when the oxidative damage was more pronounced. In the kidneys, the CAT activity decreased under both doses and exposure durations (Guzmán-Guillén et al. 2013a). Other ROS biomarkers have been investigated in fish that were exposed to cyanotoxins (Table 3). For example, an increase in NADPH oxidase activity, a biomarker of ROS formation, was observed in the liver and kidneys of fish that were exposed to CYN by gavage and injection. Many studies have investigated the GSH-dependent antioxidant system following of the exposure of fish to CYN. In this way, the activity and gene expression of glutathione peroxidase (GPx), an enzyme catalysing the reaction between GSH and hydrogen peroxide (H_2O_2), have been assessed by different authors (Puerto et al. 2011; Gutiérrez-Praena et al. 2013b; Guzmán-Guillén et al. 2013a). Changes in the GPx activity following the exposure of fish to CYN are not clear. In the liver, no change in the GPx activity was observed under any exposure mechanism, CYN dose or exposure duration. However, in the kidneys, the GPx activity decreased (Puerto et al. 2011) or increased in a dose-dependent manner (Guzmán-Guillén et al. 2013a) in fish that were exposed to CYN by gavage or immersion, respectively (Table 3). These results clearly demonstrate the influence of the exposure pathways in terms of enzyme responses, as well as the potential adaptive response of the kidneys rather than the liver to compensate for greater injuries. Concerning the gene expression of GPx, fish that were orally exposed and sacrificed after 24 h showed an increase in GPx expression in the liver and kidneys (Puerto et al. 2011; Gutiérrez-Praena et al. 2013b). However, at 5 days post-exposure, the GPx expression increased in the liver but decreased in the kidneys, suggesting a greater defensive capacity of the liver and thus a greater sensitivity of the kidneys to CYN.

Both the activity and gene expression of GST were assessed in the liver and kidneys of tilapia (Table 3; Puerto et al. 2011; Gutiérrez-Praena et al. 2013b; Guzmán-Guillén et al. 2013a). Concerning the GST activity, 24 h post-gavage, an increase was observed in both the liver and kidneys concomitantly with an increase in the GST protein abundance, but after 5 days, an activity similar to that of the control group was detected (Puerto et al. 2011; Gutiérrez-Praena et al. 2013b). These results suggest a potential recovery of the enzyme activity within a few days, traducing a rapid detoxification of negative cell components that is in accordance with the results of a previous study (Gutiérrez-Praena et al. 2011a). However, during blooms, fish are chronically exposed to cyanobacteria and to

their toxins and not only to a single one as is most often investigated. Guzmán-Guillén et al. (2013a) investigated the effects of CYN on fish that were exposed sub-chronically (7 and 14 days) to environmentally relevant concentrations of cyanobacterial extracts containing CYN. The GST activity increased in the liver of fish that were exposed to CYN for different exposure durations, suggesting a detoxification of peroxidised lipids that were detected in the liver. In the kidneys, the GST activity decreased when the fish were exposed to the highest concentration of CYN for 14 days. This difference between organs may be linked to a higher hepatic conjugation activity compared to that of the kidneys, leading to a greater sensitivity of the kidneys to CYN. Furthermore, GST gene expression was generally increased in both of the organs, depending on the time of measurement, and intraperitoneal injection produced greater effects with an increase in GST expression at every time of sacrifice and in every studied organ (Puerto et al. 2011; Gutiérrez-Praena et al. 2013b). In fish that were exposed both acutely and sub-chronically to CYN, the GSH content showed a decrease in both of the organs but with more pronounced effects in the kidneys, suggesting that CYN inhibits GSH synthesis and/or an increases the detoxification of ROS, implying the oxidation of GSH to GSSG (Gutiérrez-Praena et al. 2011a; Puerto et al. 2011; Guzmán-Guillén et al. 2013a). In this way, the GSC activity increased, mainly in the kidneys, at every dose and exposure duration, suggesting that GSH synthesis is not affected when the fish are exposed sub-chronically to environmental concentrations of CYN (Guzmán-Guillén et al. 2013a). At high exposure doses, GSH synthesis could be affected because a decrease in the GSC activity was observed in fish that were acutely exposed to CYN (Gutiérrez-Praena et al. 2011a).

The consequences of ROS formation in the organs of fish that were exposed to CYN have also been investigated (Table 3). An increase in LPO and protein oxidation following a time- and dose-dependent manner were observed in both of the organs of the exposed fish (Gutiérrez-Praena et al. 2011a; Puerto et al. 2011; Guzmán-Guillén et al. 2013a). In contrast to fish that were acutely exposed to CYN (Gutiérrez-Praena et al. 2011a), DNA damages were observed in the liver and more particularly in the kidneys of fish that were exposed sub-chronically to cyanobacterial extracts containing CYN (Guzmán-Guillén et al. 2013a). These results confirm that CYN can also affect organs other than the liver and that the kidneys appear to be the most sensitive organ to CYN. It has been suggested that the uracil group of CYN can interact with adenine group in RNA and DNA, leading to disturbances in DNA synthesis and thus inducing mutations by acting as a carcinogen (Zegura et al. 2011). This study is the first to demonstrate the possible involvement of oxidative stress in the genotoxic effects of CYN.

Histological parameters The studies investigating the histopathological damages that are induced by CYN contamination are presented in Table 4. Gutiérrez-Praena et al. (2012) showed that CYN induced histopathological damages in a wide variety of organs, such as the liver and kidneys, which are the main targets, but also in the heart, gills and intestines. The histological damages were more severe after intraperitoneal injection than after oral exposure (except for in the intestines). Furthermore, the exposure duration also affected the results of the histological parameters with more acute damages being observed after 5 days of exposure. Puerto et al. (2012a) observed structural alterations and degenerative processes in the liver, kidneys, heart, gills, and intestines but with more significant alterations at the highest exposure dose. In parallel with the histopathological alterations, authors have also observed changes in some oxidative biomarkers (increase in protein oxidation and in NADPH oxidase activity) in the liver and kidneys of exposed tilapia. The authors suggest that the increase in the observed NADPH oxidase activity could explain in part the vascular alterations that were observed in both the kidney and heart tissues.

To sum up, previous work unequivocally provided evidence for the deleterious effects of CYN at different biological scales and during various fish ontogenic stages. All of the studies report short-term effects. However, the main challenge for future work will be to assess the medium- to long-term effects of CYN at the fish population scale. While it is unlikely that CYN easily permeates the cellular membranes of fish embryos (which is supposed to be the most sensitive ontogenic stage of fish; e.g., Di Giulio and Hinton 2008), one should keep in mind that in ecosystems, fish are exposed simultaneously to multiple and potentially interacting toxic cyanobacterial metabolites, which could lead to a greater toxicity with species-dependent effects. However, it could be

Table 4 Effects of CYN on the histological parameters of fish (*Oreochromis niloticus*)

References	Mode, concentration and exposure duration	Observable effects on *O. niloticus*
Puerto et al. (2012b)	Gavage with 200 and 400 μg kg^{-1} of CYN and observation at 24 h	Histopathological changes more pronounced for the highest dose of exposure:
		Liver = disorganised parenchymal architecture, large hepatocytes with central nuclei and cytoplasmic vacuolisation and hyalinisation, cytoplasmic glycogen accumulation, dense nuclei surrounded by scarce cytoplasm with abundant granular glycogen and lipid accumulation
		Kidneys = glomerular atrophy, dilated Bowman's capsule, haemorrhage, dilated capillary lumen, congestion, irregular intramembranous electron-dense deposits, thickening of the basement membrane, elongation of podocytes
		Heart = myofibrolysis, absence of contractile material, scarce mitochondria
		Intestine = small size of enterocytes lining villi, basophil and irregular nuclei, necrotic intestinal cells, dilated lacteal vessels, oedema, pleomorphism of the villi and partial loss of microvilli
		Gills = loss of the lamellar structure, swelling, presence of inflammatory cells, thickening of the connective tissue, congestion, catarrhal inflammation, haemorrhages, destruction of branchial arches, tumefaction, cell infiltration
Gutiérrez-Praena et al. (2012)	Gavage and intraperitoneal injection with 200 μg kg^{-1} and sacrificed at 24 h and 5 days	Histological damages more pronounced for fish that were injected (excepted for intestinal tract) and for fish that were sampled at 5 days
		Liver = increase hepatocyte nuclear diameter, glycogen accumulation, steatosis, cytoplasmic vacuolisation, scarce cytoplasmic organelles
		Kidneys = atrophic and hyalinised glomeruli, hyperaemia, decreased width of the proximal and distal convoluted tubules, dilated Bowman's capsule, elongated podocyte primary foot processes, tubular cells with necrotic nuclei
		Heart = myofibrolysis, oedema, loss muscular fibres, pleomorphic fibres, scarce mitochondria
		Intestine = catarrhal enteritis process, oedema, hyperaemia, loss of microvilli, cytoplasmic vacuolisation, necrotic enterocytes, increased calceiform cells
		Gills = tumefaction processes, hyperaemia, inflammatory foci, desquamation of lamellae, haemorrhages

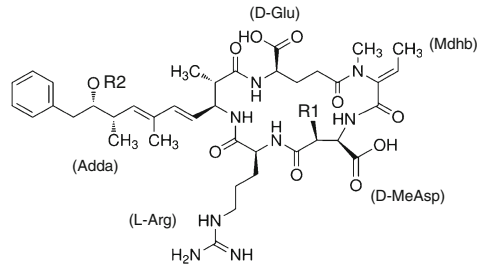
hypothesised that fish species can adapt (at the genetic scale) or accommodate cyanotoxins to counteract their toxic effects and thus to decrease their negative effects on the fish population.

Nodularin

General presentation

NOD was first isolated from *Nodularia spumigena*, a cyanobacterium that is typically present in estuaries and brackish waters (Rinehart et al. 1988). To date, seven naturally occurring variants of NOD have been identified (Pearson et al. 2010). NOD are 824 Da cyclic pentapeptides that are composed of (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (Adda), D-glutamic acid (D-Glu), L-arginine (L-Arg), D-erythro-β-methylaspartic acid (D-MeAsp) and 2-methylamino-2-dehydrobutyric acid (Mdhb; Fig. 3).

These cyanotoxins are only produced by the genera *Nodularia* and are mainly measured in the brackish water of the Baltic Sea, where the cyanobacteria form



Compound name	R1	R2	Other changes
nodularin	CH_3	CH_3	
[DMAdda^3]nodularin	CH_3	H	
[D-Asp^1]nodularin	H	CH_3	
[(6Z)-Adda^3]nodularin	CH_3	CH_3	Modifications of Adda moeity
[L-Val^2]nodularin	CH_3	CH_3	Change of L-Arginine residue by L-Valine residue
[L-Har^2]nodularin	CH_3	CH_3	Change of L-Arginine residue by L-Homoarginine residue

Fig. 3 Chemical structure of NOD and its variants

regular summer blooms (Buynder et al. 2001; Karjalainen 2005; Karjalainen et al. 2007). These toxins are mostly found in intracellular spaces, and experimentally, less than 10–20 % of the total toxin amount is released in the surrounding water (Chorus and Bartram 1999).

The toxicity of NOD comes from its capacity to inhibit the catalytic subunits of the serine/threonine-specific protein phosphatises (PPs) 1 and 2A (PP1 and PP2A), leading to the hyperphosphorylation of cells, causing cytoskeleton disintegration, the loss of cell junctions, and disturbances of cell metabolism and cell cycle control (Campos and Vasconcelos 2010). However, contrary to microcystines (MCs), which can covalently bind to PPs, NOD cannot interact covalently with PPs due to the lack of a methyldehydroalanine (Mdha) residue (MacKintosh et al. 1995; Martins and Vasconcelos 2009). NOD is also considered a tumour-promoter-possessing carcinogen (Karjalainen 2005; Van Apeldoorn et al. 2007).

At a macroscopic scale, NOD have been implicated in various disturbances of both primary and secondary consumers of aquatic ecosystems. In this way, the negative impacts of NOD have been observed in both the zooplankton and fish compartments (Van Apeldoorn et al. 2007). In fish, information about the physiological effects of NOD is still scarce, but several papers investigating the bioaccumulation of this toxin in various species of the Baltic Sea have already been published. Most studies have focused on the Baltic Sea because this area provides large amounts of fish for human consumption. As a consequence, the effects of NOD on fish were first investigated in terms of human food safety.

Accumulation of NOD in fish

The accumulation processes of NOD were investigated in different fish species from the Baltic Sea (Table 5). In flounder (*Platichthys flesus*), NOD concentrations were observed in different organs. The liver was the main organ of NOD accumulation in this fish species, with concentrations varying widely between specimens (Table 5) and ranging from non-detection (ND) to 1,100 ng g^{-1} DW (Sipiä et al. 2001a, b, 2002, 2006; Karlsson et al. 2003; Kankaanpää et al. 2005; Mazur-Marzec et al. 2007; Persson et al. 2009). Interestingly, Kankaanpää et al. (2005) investigated the NOD accumulation in the liver of flounder using two different measurement tools, LC/MS to evaluate the concentrations of pure NOD in fish samples and the ELISA method, which gives the total extractable hepatotoxin (TEH) concentration in the same samples. Concentrations ranging from ND to 390 ng g^{-1} DW of pure NOD and from 20 to 2,230 ng g^{-1} DW TEH were observed for the LC/MS and ELISA measurements, respectively. This result suggests the importance of the measurement method (1) in terms of the concentration relevance and the consequences that this relevance may have in

Table 5 Nodularin (NOD) accumulation in the fish tissues observed under field conditions

References	Sampling location	Cyanobacterial concentration	NOD concentration in ecosystem	NOD concentration in fish	Measurement method
Sipiä et al. (2001a)	Baltic Sea	NS	NS	*Platichthys flesus*: ND-140 ng g^{-1} DW in liver ND in the muscle *Gadus morhua*: 53–56 ng g^{-1} DW in the liver	ELISA PPI
Sipiä et al. (2001b)	Baltic Sea	NS	0.5–2.6 μg l^{-1} in water 0.5–2.1 μg g^{-1} DW in cyanobacteria	*Platichthys flesus*: ND-399 ng g^{-1} DW in the liver ND in the muscle	ELISA
Buynder et al. (2001)	Lake Victoria (Australia)	7,500–40,000 cells ml^{-1}	NS	Finfish: ND-152 ng g^{-1} FW in the viscera 0.7–2.5 ng g^{-1} FW in the muscle	HPLC–MS/MS
Sipiä et al. (2002)	Baltic Sea	NS	NS	*Platichthys flesus*: 250–420 ng g^{-1} DW in the liver ND in the muscle	ELISA LC–UV–MS/MS
Karlsson et al. (2003)	Baltic Sea	NS	NS	*Platichthys flesus*: 82–637 ng g^{-1} FW in the liver	MALDI-TOF–MS LC–MS
Kankaanpää et al. (2005)	Baltic Sea	NS	NS	*Platichtys flesus*: ND-390 ng g^{-1} DW in the liver (pure NOD) 20–2,230 ng g^{-1} DW in the liver (total extractable hepatotoxins)	ELISA LC–MS
Sipiä et al. (2006)	Baltic Sea	NS	NS	*Platichthys flesus*: ND-1,100 ng g^{-1} DW in the liver 5–100 ng g^{-1} DW in the muscle *Rutilus rutilus*: 3–900 ng g^{-1} DW in liver ND-200 ng g^{-1} DW in muscle	ELISA LC–MS
Mazur-marzec et al. (2007)	Baltic Sea	NS	6.8 μg l^{-1}	*Platichthys flesus*: 472 ± 14.6 ng g^{-1} DW in the liver 20.7 ± 14.6 ng g^{-1} DW in the guts 21.3 ± 2.4 ng g^{-1} DW in the gonads 1 ± 0.2 ng g^{-1} DW in the muscle	ELISA

Table 5 continued

References	Sampling location	Cyanobacterial concentration	NOD concentration in ecosystem	NOD concentration in fish	Measurement method
Sipiä et al. (2007)	Baltic Sea	NS	NS	*Gasterosteus aculeatus*: 2.8–16.5 ng g^{-1} DW in the viscera ND-700 ng g^{-1} DW in the viscera *Clupea harengus membras*: ND-5 ng g^{-1} DW in the liver ND-90 ng g^{-1} DW in the stomach *Salmo salar*: 10 ng g^{-1} DW in the liver	LC–MS
Karjalainen et al. (2008)	Baltic Sea	200–800,000 cells l^{-1}	NS	*Gasterosteus aculeatus*: 10–100 ng g^{-1} FW in the whole fish *Clupea harengus membras*: 10–30 ng g^{-1} FW in the whole fish *Sprattus sprattus*: <5–20 ng g^{-1} FW in the whole fish	ELISA
Persson et al. (2009)	Baltic Sea	NS	NS	*Platichthys flesus*: 22–257 ng g^{-1} DW in the liver ND in the muscle	HPLC–UV
Stewart et al. (2012)	Recreational lake in south-east Queensland	NS	NS	*Mugil cephalus:* 40.8–47.8 μg g^{-1} DW in the liver 32.3–56.8 ng g^{-1} DW in the muscle	HPLC–MS/MS

HPLC high-performance liquid chromatography, *LC* liquid chromatography, *UV* ultraviolet, *ELISA* enzyme-linked immunosorbent assay, *MALDI* matrix-assisted laser desorption/ionisation, *TOF* time-of-flight mass spectrometry, *NS* not specified, *ND* not detected

making comparisons between distinct studies and (2) in terms of toxicity evaluation. In addition, NOD were observed in various organs of flounder, indicating the presence of toxins in the muscle (from 1 to 100 ng g^{-1} DW), guts (20.7 ± 3.3 ng g^{-1} DW), and gonads (21.3 ± 2.4 ng g^{-1} DW; Sipiä et al. 2006; Mazur-Marzec et al. 2007). As a function of the toxicity of NOD and the presence of these toxins in the gonads of fish, it hypothesised that NOD could negatively interfere in reproduction processes and thus could lead to fish population disturbances. NOD were also found in the liver of cod (*Gadus morhua*), in the liver and muscle of roach (*Rutilus rutilus*), in the visceral tissues of the three-spined stickleback (*Gasterosteus aculeatus*), in the liver and stomach of herring (*Clupea harengus membras*), in the liver of salmon (*Salmo salar*) and in sprat (*Sprattus sprattus*), with a high degree of variation in the NOD concentrations according to the species (Sipiä et al. 2001a, b, 2006, 2007; Karjalainen et al. 2008). Australian ecosystems are also affected by blooms of *Nodularia spumigena* (Table 5). In Lake Victoria (New South Wales), Buynder et al. (2001) observed the presence of NOD in the viscera (152 ng g^{-1} FW) and muscle (2.5 ng g^{-1} FW) of finfish that were caught during a bloom of *Nodularia spumigena*. More recently, Stewart et al. (2012) measured the NOD concentration in sea mullet (*Mugil cephalus*) that were caught in a recreational lake in south-east Queensland and found concentrations reaching 47.8 µg g^{-1} DW in the liver and 56.8 ng g^{-1} DW in the muscle.

These results show that NOD can accumulate in various organs of fish during *Nodularia spumigena* blooms. However, most of these studies used ELISA measurements that quantify not only pure NOD but also other metabolites and detoxification products that are potentially toxic. In terms of safety food, this measurement method is most likely the most suited because it reports the worst case of contamination. In terms of toxicity evaluation, the concentrations of pure NOD are overestimated with this technique, thereby leading to the potential misinterpretation concerning the actual toxicity of NOD in tissues. Future studies are needed regarding the detoxification capacities and the effects of NOD on the reproduction processes of fish that are exposed to toxic *Nodularia* blooms both for short and long durations to better understand the potential risk of NOD contamination on fish populations. Furthermore, other ontogenic stages than adults must be investigated in terms of NOD accumulation in ecosystems.

Biological effects of NOD on fish

In vitro effects of NOD on fish cells

The first study assessing the effects of NOD on fish was conducted in vitro on the hepatic cells of juvenile Atlantic salmon (*Salmo salar*). Fladmark et al. (1998) exposed salmon hepatocytes to a medium containing pure NOD (824 µg l^{-1}). After incubation, many signs of an apoptotic response were observed with surface budding being particularly important, as well as cell shrinkage and chromatin condensation. Only recently have other authors investigated more precisely the in vitro effects of NOD on fish cells (Zhang et al. 2012). The lymphocytes of *Carassius auratus* were exposed to different concentrations (1, 5, 10 and 100 µg l^{-1}) of NOD to reveal the apoptotic reaction of lymphocytes in a dose-dependent manner, leading to intracellular changes, such as a condensed cytoplasm, nuclear chromatin agglutination and marginalisation. The DNA also presented fragmentation, and both the Ca^{2+} and ROS levels increased in a dose-dependent manner, indicating an important role of Ca^{2+} in the mechanism of NOD-induced programmed death. Furthermore, the mitochondrial membrane potential (MMP) decreased, suggesting an early apoptotic event. The levels of caspases (CAP) 3 and 8, which play an important role in the execution phase of apoptosis, increased in a dose-dependent manner, while such an increase was not observed for CAP 8. This result, together with other observations, suggests that NOD-induced apoptosis involves a mitochondrial pathway. At a biomolecular scale, the expression and protein detection of the apoptosis-related genes Bcl-2 and Bax changed, specifically in a dose-dependent manner for Bcl-2, while Bax increased at both the mRNA and protein levels. These findings suggest that the mitochondria-mediated pathway is involved in fish cell apoptosis after NOD exposure and thus that fish that are exposed to NOD-producing blooms could be rapidly and strongly affected by this cyanotoxin.

Biological effects of NOD on larvae and juvenile fish

To our knowledge, no study has investigated the effects of NOD on fish embryos, but a few studies have

investigated older stages. Karjalainen et al. (2005) studied the effects of NOD (and more widely of cyanobacterial extracts) on *Esox lucius* larvae that were fed daily (for 11 days) copepods containing NOD or cyanobacterial extracts. These authors observed that, when in contact with contaminated copepods, the ingestion rate, faeces production and growth rate decreased in pike larvae. The results showed significant differences with the control, but only for pike larvae that were exposed to cyanobacterial extracts, suggesting that the negative effects of these extracts were stronger than those of pure NOD, most likely due to the synergistic effects of NOD with other unidentified toxic metabolites that are produced by cyanobacteria. In the same way, based on a 15-day-long experiment, young-of-year (YOY) three-spined stickleback were exposed to *Nodularia*-exposed plankton either with (B-NOD) or without (N-NOD) *Nodularia* in water as well as to plankton that were unexposed to cyanobacteria (i.e., control; Pääkkönen et al. 2008). Interestingly, the specific growth rates were lower in B-NOD than in the other treatments despite the fact that the food consumption was higher in the B-NOD treatment. First, this result suggests that the turbidity that was caused by *Nodularia* did not affect the food consumption of the fish. Second, the highest consumption in this treatment was not correlated with the growth rate, and the decrease in protein synthesis was most likely due to the consumption of the energy intake for the detoxification of NOD and toxic metabolites. In this study, the most significant effects were observed in the B-NOD treatment, whereas for the N-NOD treatment, trends were observed but were generally not significant. This result is explained by the fact that in the B-NOD treatment, zooplankton and fish were chronically exposed to NOD and other toxic metabolites that are produced by *Nodularia*; the fish and zooplankton were thus more affected than in the N-NOD treatment where the NOD equivalent concentrations in both the zooplankton and fish decreased during the experiment (Pääkkönen et al. 2008). According to these results, the actual effects of NOD on young-stage fish seem too complex to highlight. However, the crude extract of *Nodularia* has an unequivocal effect on fish physiology. The exposure of fish to cyanobacterial extracts is relevant because in ecosystems, fish are exposed to *Nodularia* blooms that produce NOD and other toxic metabolites with potential interaction between these compounds, leading to stronger effects on fish than that of exposure to only one toxin. Future work should therefore consider all of the toxic substances that are produced by the cyanobacteria to precisely comprehend the potential effects of NOD-producing blooms on fish populations.

Biological effects of NOD on adult fish

Experimental accumulation and detoxification of NOD Few studies have investigated the biological effects of NOD on adult fish. Some investigations have focused on the accumulation process of NOD in adult fish using an experimental approach to better understand the detoxification process of fish to counteract the negative effects of NOD (Table 6). The first study was conducted by Kankaanpää et al. (2002) on Baltic Sea trout (*Salmo trutta* m. *trutta* L.) that were orally exposed to *Nodularia* (Table 6). In this study, no fish mortality was observed, and an ELISA analysis showed an increasing concentration of NOD and NOD-like compounds in the liver of sea trout at the end of experiment (approximately 1,200 ng g^{-1} DW after 8 days). However, the HPLC analysis did not detect NOD or NOD metabolites in the liver. The authors suggested that the discrepancy between the two measurement methods could be due to the transformation of NOD via conjugation with thiol-containing proteins, peptides and amino acids, such as GSH, methionine or cysteine, associated with the lack of NOD conjugate standards for the HPLC identification of the toxins and their metabolites. The lowest concentrations were found in the muscle, in which the concentration that was measured on the first day (125 ng g^{-1} DW) decreased and then stabilised at days 2 and 4 (35 and 43 ng g^{-1} DW, respectively). Furthermore, no modification in the swimming activity or behaviour of the fish were revealed under the effects of food containing NOD (Kankaanpää et al. 2002). A similar approach was used by Engström-Öst et al. (2002) using adult three-spined sticklebacks that were fed daily for 10 days NOD-contaminated copepods (Table 6). The results showed a peak but without a continuous increase in the NOD equivalent concentration in the liver of fish that were exposed. This result suggests the presence of a detoxification process capable of rapidly metabolising and excreting the toxins that were taken by the fish. More recently, Vuorinen et al. (2009) exposed adult flounder by force-

Table 6 Experimental effects of NOD on adult fish

Reference	Species	Mode, concentration and time of exposure	Observable effects
Kankaanpää et al. (2002)	*Salmo trutta* m. *trutta* L.	Sea trout exposed orally to a single dose of food containing 440 μg kg^{-1} BW of NOD sampled at 1, 2, 4 and 8 days	↑ NOD-like compounds concentrations in liver of fish during time with average concentrations of 19 and 1,200 μg kg^{-1} DW at day 1 and 8 respectively
			Lowest NOD-like compounds concentrations in muscle with 125 μg kg^{-1} DW, 35 μg kg^{-1} DW, 43 μg kg^{-1} DW at day 1, 2 and 4 respectively
			No mortalities
			No effects of the treatment on swimming activity and other behaviour
			Histology of liver severely affected after 1–2 days of exposition = Normal liver architecture lost, pyknosis, no visible nucleoli, disintegrated hepatocytes
			Healthier liver at day 8 but intestine inflamed and swollen
Engström-Öst et al. (2002)	*Gasterosteus aculeatus*	Fish fed with 40 copepods per day containing 1.251×10^{-5} ng NOD equivalent $copepod^{-1}$ during 10 days	Peak of toxin in total fish at day 5 (approximately 0.15 μg g^{-1} DW)
			No increase of concentrations with time
Vuorinen et al. (2009)	*Platichtys flesus*	Fish force-fed with (1) a single dose of NOD corresponding to 277 μg kg^{-1} BW and (2) 3 repeated doses of NOD corresponding to a total of 379 μg kg^{-1} BW and sampled 4 days after the last repeated dose or the single dose	NOD concentrations in liver reaching 220 ± 70 and 60 ± 5 ng g^{-1} DW in treatment (1) and (2) respectively. No GSH conjugates
			NOD-like compounds in bile with concentrations reaching 49 ± 5 and 37 ± 13 ng ml^{-1} in the treatment (1) and (2) respectively
			GST activity significantly highest in treatment (2)
			Architecture of liver incoherent in both treatment but not totally destroyed, irregular cell shape and round lipid vacuoles, degenerative hepatocytes, shrinkage of cytoplasm, loss of nucleolus and pyknosis
Persson et al. (2009)	*Platichtys flesus*	Fish exposed to NOD at concentration of 0, 2, 10 and 50 μg kg^{-1} BW by an intraperitoneal injection and sampled at 7 days or 14 days (7 days of recovery)	No difference in NOD accumulation between treatments and no detection of NOD in muscle
			↓ CAT and GST activities for fish exposed to 50 μg kg^{-1} BW but same activity levels as control after recovery
			No difference in MDA content between treatments

↑ = increase, ↓ = decrease, *GSH* glutathione, *GST* glutathione-S transferase, *CAT* catalase, *MDA* malondialdehyde

feeding with either a single dose or three repeated doses of a *Nodularia* slurry (Table 6). In the liver of flounder that were exposed to a single dose and repeated doses, NOD was found at mean concentrations of 220 ± 70 ng g^{-1} DW and 60 ± 5 ng g^{-1} DW, respectively. No GSH conjugates were observed in the liver of fish that were exposed, suggesting a rapid excretion/disintegration of NOD detoxification products in the fish. In parallel, an ELISA analysis was performed on the bile samples of fish and revealed the presence of NOD-like compounds at concentrations reaching 49 ± 5 and 37 ± 13 ng ml^{-1} for single and repeated exposures, respectively. A study of GST showed a significantly higher activity of the enzyme in the repeated treatment than in the single treatment and control (Table 6). In agreement with the decrease in the

NOD concentrations in the liver, this result suggests that fish that were repeatedly exposed to NOD, such as during bloom periods, can more rapidly and efficiently detoxify and excrete the toxin than can fish that were exposed to a single but higher concentration of the toxin.

Oxidative parameters Cyanotoxins in organs are responsible for the appearance of an oxidative stress state in exposed fish (Table 6). Persson et al. (2009) studied the effects of different concentrations of NOD on the oxidative stress state of flounder using intraperitoneal injections of NOD at different concentrations (Table 6). The fish were observed on the fourth day, and some of the fish that were injected with the highest dose (50 μg kg^{-1} BW) were sacrificed on the fourteenth day to evaluate the potential recovery of the oxidative stress enzymes. The lowest CAT and GST activities were observed in flounder that were exposed to the highest dose, but the activity levels of both of the enzymes returned to the control level after the recovery period. The authors suggested that the decrease in the GST activity could be explained by the reduction of GST protein synthesis at the molecular level or by the modification of the enzyme via the NOD-induced hyperphosphorylation state. The decrease in the CAT activity suggests a NOD-induced oxidative stress, leading to an increase in ROS that are directly scavenged by CAT. The authors also suggested that the CAT activity could decrease in response to direct damages to the enzyme that are caused by ROS. In parallel with these enzymatic measurements, the authors quantified the concentration of malondialdehyde (MDA), an oxidation product of polyunsaturated fatty acids in lipoproteins, in flounder livers, and observed no change, suggesting an efficient oxidative defence against ROS (Table 6). To our knowledge, there are no other publications investigating the effects of NOD on oxidative stress in fish. Again, most studies have investigated microcystins (MCs), which have similar cellular effects as NOD, and thus potentially the same effects at a larger biological scale. In this way, at a larger scale, histological analyses are widely used in terms of the evaluation of cyanotoxins effects on fish species.

Histological parameters We found two papers that investigated the effects of NOD on the histological integrity of the organs of exposed fish (Kankaanpää et al. 2002; Vuorinen et al. 2009). Following a single oral dose of *Nodularia* (Table 6), the livers of Baltic Sea trout were severely affected after 1 and 2 days (Kankaanpää et al. 2002). The authors observed a loss of the normal liver architecture with pyknosis, no visible nucleoli and some disintegrated hepatocytes with an unclear cell shape. However, on the 8th day of the experiment, the authors observed healthier livers but inflamed and swollen intestines (Kankaanpää et al. 2002). These results suggest that exposure to NOD through food can induce histological damages to the liver of fish but with a progressive recovery of the organ after a single NOD exposure. The exposure of flounder by the force-feeding of a single or repeated dose of NOD (Table 6) gave similar results (Vuorinen et al. 2009). Indeed, for both treatments, the liver architecture of the fish was incoherent but not totally destroyed. The cell shape was not strict, and the presence of round lipid vacuoles was highlighted. Furthermore, the focal area showed degenerative cells, cytoplasm shrinkage, and a loss of the nucleolus and pyknotic nuclei, leading to the same conclusions as in the first study (Table 6).

An overview of these results shows that NOD can accumulate in various organs of fish that are exposed to *Nodularia* blooms. However, the widespread use of the ELISA method to measure the NOD cannot discriminate between the different toxins that are produced by *Nodularia*, that can accumulate in fish and thus that may play a toxic role in fish physiology. NOD produce some cellular effects, such as oxidative stress, leading to apoptotic processes and, at a larger scale, causing physiological disturbances. However, fish can detoxify NOD when exposed to the toxins and thus to counteract the negative effects of NOD. Interestingly, few studies have investigated the biological effects of NOD on fish, most likely because most investigations have focused on the biological effects of MCs that have the same cellular effects as NOD. One way to contribute to the knowledge of the effects of NOD on fish would be to identify all of the toxic compounds that are produced by *Nodularia* blooms and which could accumulate in fish and affect their physiology.

Conclusions

Both CYN and NOD accumulate in the organs of fish that are exposed to cyanobacterial blooms. This

accumulation leads to negative effects in individual fish, with major effects being observed during the youngest ontogenic stages. To better comprehend the potential negative effects due to toxin accumulation, more accurate analytical methods need to be used to discriminate the pure toxins from the less toxic detoxification metabolites that accumulate in the organs of fish. Currently, it is difficult to determine the actual long-term potential impact of these toxic effects on fish populations that are exposed to cyanobacterial blooms. The different cyanotoxin effects that have been observed between fish species depend most likely on the recurrence of blooms in each ecosystem. In this way, in an ecosystem where blooms are recurring, fish adaptation (at the genetic scale) and/or accommodation (behavioural and physiological scales) phenomena could take place to avoid or counteract the deleterious effects of cyanotoxins. In fact, the available studies on the topic have only investigated the effects of a given toxin on a given fish species for a short duration. It is important to keep in mind that during cyanobacterial blooms, fish are exposed to a mixture of toxic compounds that can interact and increase the toxic effects on fish individuals. Furthermore, different fish species exhibit different detoxification capacities, which could explain the different toxic effects. Future studies need to integrate the synergistic and/or cumulative effects of toxic cyanobacterial metabolites on various fish species to better understand and anticipate their potential effects on fish populations after both short- and long-term exposure in ecosystems where blooms occur repeatedly.

References

Amado L, Monserrat J (2010) Oxidative stress generation by microcystins in aquatic animals: why and how. Environ Int 36:226–235

Azevedo SMF, Carmichael WW, Jochimsen EM, Rinehart KL, Lau S, Shaw GR, Eaglesham GK (2002) Human intoxication by microcystins during renal dialysis treatment in Caruaru-Brazil. Toxicology 181–182:441–446

Bernard C, Harvey M, Briand JF, Biré R, Krys S, Fontaine JJ (2003) Toxicological comparison of diverse *Cylindrospermopsis raciborskii* strains: evidence of liver damage caused by a French *C. raciborskii* strain. Environ Toxicol 18:176–186

Berry JP, Gibbs PDL, Schmale MC, Saker ML (2009) Toxicity of cylindrospermopsin, and other apparent metabolites from *Cylindrospermopsis raciborskii* and *Aphanizomenon ovalisporum*, to the zebrafish (*Danio rerio*) embryo. Toxicon 53:289–299

Berry JP, Jaja-Chimedza A, Davalos-Lind L, Lind O (2012) Apparent bioaccumulation of cylindrospermopsin and paralytic shellfish toxins by finfish in Lake Catemaco (Veracruz, Mexico). Food Addit Contam A 29:37–41

Buynder G, Oughtred T, Kirkby B, Phillips S, Eaglesham G, Thomas K, Burch M (2001) Nodularin uptake by seafood during a cyanobacterial Bloom. Environ Toxicol 16:468–471

Campos A, Vasconcelos V (2010) Molecular mechanisms of microcystin toxicity in animal cells. Int J Mol Sci 11:268–287

Chorus I, Bartram J (eds) (1999) Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management. E & FN Spon, London, p 416

Codd GA, Morrison LF, Metcalf JS (2005) Cyanobacterial toxins: risk management for health protection. Toxicol Appl Pharm 203:264–272

Di Giulio R, Hinton D (2008) The toxicology of fishes. Taylor & Francis, London, p 1096

Engström-Öst 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 276:95–107

Falconer IR, Humpage AR (2006) Cyanobacterial (Blue-Green Algal) toxins in water supplies: cylindrospermopsins. Environ Toxicol 21:299–304

Ferrão-Filho ADS, Kozlowsky-Suzuki B (2011) Cyanotoxins: bioaccumulation and effects on aquatic animals. Mar Drugs 9:2729–2772

Fladmark KE, Serres MH, Larsen NL, Yasumoto T, Aune T, Døskeland SO (1998) Sensitive detection of apoptogenic toxins in suspension cultures of rat and salmon hepatocytes. Toxicon 36:1101–1114

Froscio SM, Humpage AR, Burcham PC, Falconer IR (2003) Cylindrospermopsin-induced protein synthesis inhibition and its dissociation from acute toxicity in mouse hepatocytes. Environ Toxicol 18:243–251

Gutiérrez-Praena D, Jos A, Pichardo S, Cameán AM (2011a) Oxidative stress responses in tilapia (*Oreochromis niloticus*) exposed to a single dose of pure cylindrospermopsin under laboratory conditions: influence of exposure route and time of sacrifice. Aquat Toxicol 105:100–106

Gutiérrez-Praena D, Pichardo S, Jos A, Cameán AM (2011b) Toxicity and glutathione implication in the effects observed by exposure of the liver fish cell line PLHC-1 to pure cylindrospermopsin. Ecotox Environ Safe 74:1567–1572

Gutiérrez-Praena D, Jos A, Pichardo S, Moyano R, Blanco A, Monterde JG, Cameán AM (2012) Time-dependent histopathological changes induced in tilapia (*Oreochromis niloticus*) after acute exposure to pure cylindrospermopsin by oral and intraperitoneal route. Ecotox Environ Safe 76:102–113

Gutiérrez-Praena D, Jos A, Pichardo S, Moreno IM, Cameán AM (2013a) Presence and bioaccumulation of microcystins and cylindrospermopsin in food and the effectiveness of some cooking techniques at decreasing their concentrations: a review. Food Chem Toxicol 53:139–152

Gutiérrez-Praena D, Jos A, Pichardo S, Puerto M, Cameán AM (2013b) Influence of the exposure way and the time of

sacrifice on the effects induced by a single dose of pure Cylindrospermopsin on the activity and transcription of glutathione peroxidase and glutathione-S-transferase enzymes in tilapia (*Oreochromis niloticus*). Chemosphere 90:986–992

Guzmán-Guillén R, Prieto AI, Vasconcelos VM, Cameán AM (2013a) Cyanobacterium producing cylindrospermopsin cause oxidative stress at environmentally relevant concentrations in sub-chronically exposed tilapia (*Oreochromis niloticus*). Chemosphere 90:1184–1194

Guzmán-Guillén R, Prieto AI, Vázquez CM, Vasconcelos V, Cameán AM (2013b) The protective role of L-carnitine against cylindrospermopsin-induced oxidative stress in tilapia (*Oreochromis niloticus*). Aquat Toxicol 132–133: 141–150

Ibelings B, Havens K (2008) Cyanobacterial toxins: a qualitative meta-analysis of concentrations, dosage and effects in freshwater, estuarine and marine biota. Adv Exp Med Biol 619:675–732

Kankaanpää H, Vuorinen PJ, Sipiä V, Keinänen 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 61:155–168

Kankaanpää H, Turunen AK, Karlsson K, Bylund G, Meriluoto J, Sipiä V (2005) Heterogeneity of nodularin bioaccumulation in northern Baltic Sea flounders in 2002. Chemosphere 59:1091–1097

Karjalainen M (2005) Fate and effects of *Nodularia spumigena* and its toxin, nodularin, in Baltic Sea planktonic food webs. 34 pp

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

Karjalainen M, Engström-Ost J, Korpinen S, Peltonen H, Pääkkönen JP, Rönkkönen S, Suikkanen S, Viitasalo M (2007) Ecosystem consequences of cyanobacteria in the northern Baltic Sea. Ambio 36:195–202

Karjalainen M, Pääkkönen JP, Peltonen H, Sipiä V, Valtonen T, Viitasalo M (2008) Nodularin concentrations in Baltic Sea zooplankton and fish during a cyanobacterial bloom. Mar Biol 155:483–491

Karlsson K, Sipiä V, Krause E, Meriluoto J, Pflugmacher S (2003) Mass spectrometric detection and quantification of nodularin-R in flounder livers. Environ Toxicol 18:284–288

Kinnear S (2010) Cylindrospermopsin: a decade of progress on bioaccumulation. Mar Drugs 8:542–564

Leflaive J, Ten-Hage L (2007) Algal and cyanobacterial secondary metabolites in freshwaters: a comparison of allelopathic compounds and toxins. Freshw Biol 52:199–214

Liebel S, Oliveira Ribeiro CA, Silva RC, Ramsdorf WA, Cestari MM, Magalhães VF, Garcia JRE, Esquivel BM, Filipak Neto F (2011) Cellular responses of *Prochilodus lineatus* hepatocytes after cylindrospermopsin exposure. Toxicol In Vitro 25:1493–1500

Looper RE, Runnegar MTC, Williams RM (2005) Synthesis of the putative structure of 7-deoxycylindrospermopsin: C7 oxygenation is not required for the inhibition of protein synthesis. Angew Chem 44:3879–3881

MacKintosh RW, Dalby KN, Campbell DG, Cohen PTW, Cohen P, MacKintosh C (1995) The cyanobacterial toxin microcystin binds covalently to cysteine-273 on protein phosphatase 1. FEBS Lett 371:236–240

Malbrouck C, Kestemont P (2006) Effects of microcystins on fish. Environ Toxicol Chem 25:72–86

Martins J, Vasconcelos V (2009) Microcystin dynamics in aquatic organisms. J Toxicol Environ Health B 12:65–82

Mazur-marzec H, Tyminska A, Szafranek J, Plinski M (2007) Accumulation of nodularin in sediments, mussels, and fish from the gulf of Gdansk, southern Baltic Sea. Environ Toxicol 4:101–111

Messineo V, Melchiorre S, Corcia AD, Gallo P, Bruno M (2009) Seasonal succession of *Cylindrospermopsis raciborskii* and *Aphanizomenon ovalisporum* blooms with cylindrospermopsin occurrence in the volcanic Lake Albano, Central Italy. Environ Toxicol 25:18–27

O'Neil JM, Davis TW, Burford MA, Gobler CJ (2012) The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. Harmful Algae 14:313–334

Pääkkönen JP, Rönkkönen S, Karjalainen M, Viitasalo M (2008) Physiological effects in juvenile three-spined sticklebacks feeding on toxic cyanobacterium *Nodularia spumigena*-exposed zooplankton. J Fish Biol 72:485–499

Paerl HW, Paul VJ (2011) Climate change: links to global expansion of harmful cyanobacteria. Water Res 46:1349–1363

Paskerová H, Hilscherová K, Bláha L (2012) Oxidative stress and detoxification biomarker responses in aquatic freshwater vertebrates exposed to microcystins and cyanobacterial biomass. Environ Sci Pollut R 19:2024–2037

Pearson L, Mihali T, Moffit M, Kellmann R, Neilan B (2010) On the chemistry, toxicology and genetics of the cyanobacterial toxins, microcystin, nodularin, saxitoxin and cylindrospermopsin. Mar Drugs 8:1650–1680

Persson KJ, Legrand C, Olsson T (2009) Detection of nodularin in European flounder (*Platichthys flesus*) in the west coast of Sweden: evidence of nodularin mediated oxidative stress. Harmful Algae 8:832–838

Puerto M, Jos A, Pichardo S, Gutiérrez-Praena D, Cameán AM (2011) Acute effects of pure cylindrospermopsin on the activity and transcription of antioxidant enzymes in tilapia (*Oreochromis niloticus*) exposed by gavage. Ecotoxicology 20:1852–1860

Puerto M, Jos A, Pichardo S, Moyano R, Blanco A, Cameán AM (2012a) Acute exposure to pure cylindrospermopsin results in oxidative stress and pathological alterations in tilapia (*Oreochromis niloticus*). Environ Toxicol 29:371–385

Puerto M, Jos A, Pichardo S, Moyano R, Blanco A, Cameán AM (2012b) Acute exposure to pure cylindrospermopsin results in oxidative stress and pathological alterations in tilapia (*Oreochromis niloticus*). Environ Toxicol 29:371–385

Rinehart KL, Harada K, Namikoshi M, Chen C, Harvis CA, Munro MHG, Blunt JW, Mulligan PE, Beasley VR, Dahlem AM, Carmichael WW (1988) Nodularin, microcystin and the configuration of ADDA. J Am Chem Soc 110:8557–8558

Rücker J, Stüken A, Nixdorf B, Fastner J, Chorus I, Wiedner C (2007) Concentrations of particulate and dissolved cylindrospermopsin in 21 *Aphanizomenon*-dominated temperate lakes. Toxicon 50:800–809

Runnegar MT, Kong SM, Zhong YZ, Ge JL, Lu SC (1994) The role of glutathione in the toxicity of a novel cyanobacterial alkaloid cylindrospermopsin in cultured rat hepatocytes. Biochem Biophys Res Commun 201:235–241

Saker ML, Eaglesham GK (1999) The accumulation of cylindrospermopsin from the cyanobacterium *Cylindrospermopsis raciborskii* in tissues of the redclaw crayfish *Cherax quadricarinatus*. Toxicon 37:1065–1077

Sipiä V, Kankaanpää H, Lahti K, Carmichael WW, Meriluoto JA (2001a) Detection of nodularin in flounders and cod from the Baltic Sea. Environ Toxicol 16:121–126

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

Sipiä VO, Kankaanpää HT, Pflugmacher S, Flinkman J, Furey A, James KJ (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 53:305–311

Sipiä VO, Sjövall O, Valtonen T, Barnaby DL, Codd GA, Metcalf JS, Kilpi M, Mustonen O, Meriluoto JAO (2006) Analysis of nodularin-R in eider (*Somateria mollissima*), roach (*Rutilus rutilus* L.), and flounder (*Platichthys flesus* L.) liver and muscle samples from the western Gulf of Finland, northern Baltic Sea. Environ Toxicol Chem 25:2834–2839

Sipiä V, Kankaanpää H, Peltonen H, Vinni M, Meriluoto JA (2007) Transfer of nodularin to three-spined stickleback (*Gasterosteus aculeatus* L.), herring (*Clupea harengus* L.), and salmon (*Salmo salar* L.) in the northern Baltic Sea. Ecotox Environ Safe 66:421–425

Stewart I, Eaglesham GK, McGregor GB, Chong R, Seawright AA, Wickramasinghe WA, Sadler R, Hunt L, Graham G (2012) First report of a toxic *Nodularia spumigena* (Nostocales/Cyanobacteria) bloom in sub-tropical Australia. II. Bioaccumulation of nodularin in isolated populations of mullet (Mugilidae). Int J Environ Res Pub Health 9:2412–2443

Van Apeldoorn ME, Van Egmond HP, Speijers GJA, Bakker GJI (2007) Toxins of cyanobacteria. Mol Nutr Food Res 51:7–60

Vuorinen PJ, Sipiä VO, Karlsson K, Keinänen M, Furey A, Allis O, James K, Perttilä U, Rimaila-Pärnänen E, Meriluoto JAO (2009) Accumulation and effects of nodularin from a single and repeated oral doses of cyanobacterium *Nodularia spumigena* on flounder (*Platichthys flesus* L.). Arch Environ Contam Toxicol 57:164–173

Wiegand C, Pflugmacher S (2005) Ecotoxicological effects of selected cyanobacterial secondary metabolites a short review. Toxicol Appl Pharmacol 203:201–218

Zegura B, Straser A, Filipic M (2011) Genotoxicity and potential carcinogenicity of cyanobacterial toxins—a review. Mutat Res 727:16–41

Zhang H, Shao D, Wu Y, Cai C, Hu C, Shou X, Dai B, Ye B, Wang M, Jia X (2012) Apoptotic responses of *Carassius auratus* lymphocytes to nodularin exposure *in vitro*. Fish Shellfish Immunol 33:1229–1237

Zurawell RW, Chen H, Burke JM, Prepas EE (2005) Hepatotoxic cyanobacteria: a review of the biological importance of microcystins in freshwater environments. J Toxicol Environ Health B 8:1–37