REVIEW ARTICLE

Oxidative stress and detoxification biomarker responses in aquatic freshwater vertebrates exposed to microcystins and cyanobacterial biomass

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Abstract Cyanobacterial blooms represent a serious threat to the aquatic environment. Among other effects, biochemical markers have been studied in aquatic vertebrates after exposures to toxic cyanobacteria. Some parameters such as protein phosphatases may serve as selective markers of exposure to microcystins, but under natural conditions, fish are exposed to complex mixtures, which affect the overall biomarker response. This review aims to provide a critical summary of biomarker responses in aquatic vertebrates (mostly fish) to toxic cyanobacteria with a special focus on detoxification and oxidative stress. Detoxification biomarkers such as glutathione (GSH) and glutathione-S-transferase (GST) showed very high variability with poor general trends. Often, stimulations and/or inhibitions and/or no effects at GSH or GST have been reported, even within a single study, depending on many variables, including time, dose, tissue, species, etc. Most of the oxidative stress biomarkers (e.g., superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase) provided more consistent responses, but only lipid peroxidation (LPO) seemed to fulfill the criteria needed for biomarkers, i.e., a sufficiently long half-life and systematic response. Indeed, reviewed papers demonstrated that toxic cyanobacteria systematically elevate levels of LPO, which indicates the important role of oxidative damage in cyanobacterial toxicity. In summary, the measurement of biochemical changes under laboratory conditions may provide information on the mode of toxic action. However, comparison of different studies is very difficult, and the practical use of detoxification or oxidative stress biomarkers as diagnostic tools or early warnings of cyanobacterial toxicity is questionable.

Keywords Biomarkers · Cyanobacteria · Detoxification · Fish · Oxidative stress · Microcystin

Toxic cyanobacteria—an introduction

Massive cyanobacterial water blooms represent a worldwide environmental problem in freshwater, brackish, and marine ecosystems (Smith 2003; Codd et al. 2005). Besides other adverse effects such as the disruption of hydrochemistry or sunlight in the water column, cyanobacteria are also known to produce various toxins, cyanotoxins (Blaha et al. 2009). The most widely studied cyanotoxins are microcystins, a family of cyclic heptapeptides with over 90 structural variants (Welker and Von Dohren 2006), and microcystin-LR, microcystin-RR, or microcystin-YR are the most dominant variants found in the environment (Blaha et al. 2009). Although microcystins have been associated with mass mortalities of fish, other factors including oxygen depletion are also of importance (Chellappa et al. 2008; Xu et al. 2010). Combined stressors (cyanobacteria vs. anthropogenic pollution, weather, or predators) have also been documented to decrease the resistance of fish to bacterial or viral infections (Zimba et al. 2001). Therefore, assessment of the impacts of cyanobacterial blooms on other aquatic organisms should always consider interactive effects of known toxins acting within a complex of other abiotic and biotic environmental factors (Yu et al. 2011).

With reference to human health, the main model organisms for toxicity studies involving cyanobacteria have been

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laboratory mammals (Dawson 1998; Gupta et al. 2003; Gehringer et al. 2004; Moreno et al. 2005). Several field observations have also linked toxic cyanobacteria to the deaths of freshwater aquatic birds (Onodera et al. 1997; Matsunaga et al. 1999; Krienitz et al. 2003; Lugomela et al. 2006), but recent laboratory studies with model bird species have documented rather moderate effects of cyanobacteria (Skocovska et al. 2007; Paskova et al. 2008; Pašková et al. 2011). Fish or amphibians are among the freshwater vertebrates most likely affected by cyanotoxins, and they are discussed in the present review in most detail.

Extreme toxic effects of chemical compounds (including cyanotoxins) such as mortality may be preceded by early changes in biochemical parameters, i.e., biomarkers, and their application as early warnings of potential toxicity to individuals or whole populations has been discussed (van der Oost et al. 2003; Jemec et al. 2010). With respect to isolated microcystins, the inhibition of protein phosphatases PP1 and PP2A is a well-characterized and selective biomarker in both mammals and aquatic biota (Mackintosh et al. 1990; Tencalla and Dietrich 1997; Fischer and Dietrich 2000a; Fischer and Dietrich 2000b; Malbrouck and Kestemont 2006). However, the composition of natural cyanobacterial blooms is very complex, inducing thus general toxicity responses such as modulations of blood chemistry, hematology, immunology, and behavior (for example, see Kopp and Hetesa 2000; Palikova et al. 1998, 2004; Fischer et al. 2000; Baganz et al. 2004; Li et al. 2007b). Of all the biomarkers studied in aquatic biota exposed to toxic cyanobacteria, biotransformation and oxidative stress parameters have been most extensively investigated. Their modulations have often been discussed as early warning signals of cyanobacterial toxicity, but, to our knowledge, no critical overview of responses has been reported.

Therefore, the major objective of the present review was to summarize and evaluate existing data from studies involving freshwater fish (and also amphibians) to assess the potential application of oxidative stress and detoxification biomarkers as diagnostic tools. Besides fish, invertebrates are also negatively affected by cyanobacteria, but much less information is available, and biomarkers have only rarely been studied (Beattie et al. 2003). Another specific aim was to explore the role of individual toxins—microcystins—in the overall context of cyanobacterial toxicity and biomarker responses. In many studies, complex samples (e.g., crude biomass extracts) were investigated, but the results were often interpreted and discussed as effects of individual toxins, which represented only a single component of the whole mixture. Therefore, we tried to discriminate between the outcomes of different studies, clearly separating investigations into isolated microcystins and studies with complex samples containing cyanobacterial cell (biomass) extracts. We also tried to simplify the complexity and variability by

defining two major exposure durations with an arbitrary limit of 7 days, i.e., acute exposure (≤7 days) and chronic exposure (>7 days). This approach allowed us to present and discuss the outcomes of individual studies in a tabular way (Tables 1 and 2) searching for possible general trends (summarized in Table 3).

Modulations of biotransformation parameters by toxic cyanobacteria

All xenobiotics—including cyanotoxins—undergo biotransformation in the organism, and the changes in biotransformation parameters may thus serve as biomarkers of exposure or effect (Pflugmacher et al. 1998; van der Oost et al. 2003). During the biotransformation, the parent compound is converted into a more water-soluble form, which may be excreted from the body. Phase I of biotransformation involves oxidation, reduction, or hydrolysis, while in phase II, xenobiotics are conjugated with endogenous compounds, such as glycine, glutathione, and glucuronate.

Detoxification phase I enzymes

The effects of cyanotoxins on the activities of phase I enzymes have only rarely been studied, as cyanotoxins are readily soluble compounds directly entering phase II. Nevertheless, studies with a model bird, the Japanese quail (*Coturnix japonica*), reported the stimulation of ethoxyresorufin *O*-deethylase (EROD) activities in the heart and brain after 10 days' exposure to cyanobacterial extract in drinking water (Skocovska et al. 2007; Paskova et al. 2008). An increase in EROD in the brain remained apparent even after 30 days. These findings need further confirmation, but they suggest the production of compounds modulating the arylhydrocarbon receptor (AhR) in cyanobacteria. These could be, for example, derivatives of aminoacid tryptophane such as formylindolo(3,2-b)carbazole, which has been described as a putative physiological ligand of AhR (Wincent et al. 2009).

Reduced glutathione

The modulation of biotransformation phase II parameters by toxic cyanobacteria have been studied more often. These mostly included changes in glutathione (GSH) levels and activities of glutathione S-transferase (GST), as detoxification of microcystin via GST conjugation with cysteine or GSH has been described (Kondo et al. 1992; Pflugmacher et al. 1998). As shown in Table 1, isolated microcystins suppressed GSH levels in vitro in common carp hepatocytes (Li et al. 2003), but in vivo effects of microcystins on GSH were rather weak, as documented for example in studies

Table 1 Effects of isolated microcystins on widely studied biomarkers of detoxification and oxidative stress in freshwater fish

Species	Exposure duration and concentrations	Organ	Studied parameter	Observed change of parameters	Investigated cyanotoxin	Reference
Zebra fish (*Danio rerio*) embryos	Acute—5 days; concentrations, 0.1, 0.5, 1, 2, 5 mg/L	Whole embryos	GST, GPx	▲	MC-LR	Wiegand et al. (1998)
Zebra fish (*Danio rerio*) embryos	Acute—24 h; concentration, 0.5 µg/L	Whole embryos	GST	▼	MC-LR and LPS (from *Microcystis* sp. and *Gleotricha* sp.)	Best et al. (2002)
			GST	■	MC-LR (from *Microcystis* sp. and *Gleotricha* sp.)	
Zebra fish (*Danio rerio*) embryos	Acute —24 h; concentrations, 25 µg/L (MC-RR), 25 µg/L (MC-LF)	Whole embryos	GST, CAT	▲	MC-LF, MC-RR	Cazenave et al. (2006a)
			GST	▼	MC-LF, MC-RR with NOM	
			GPx, GR	■		
Zebra fish (*Danio rerio*) eggs	Acute—concentration, 0.25 µg/L in medium for 24 h	Fish eggs	GST	▼	MC-LR, MC-RR	Pietsch et al. (2001)
Zebra fish (*Danio rerio*) adults 6 months	Acute i.p. injection of MCLR (50 µg/kg b.w.), studied after 3–48 h at three different temperatures (12, 22, and 32°C)	Liver	Transcription GST isoenzymes (rho1, zeta1, a1, mu3, theta1, p1 and p2)	▲	MC-LR	Zhang et al. (2011)
Tilapia fish (*Oreochromis* sp.)	Acute—single dose 500 µg/kg by i.p, measured after 7 days	Liver	GR, CAT, SOD, LPO	▲	MC-LR	Prieto et al. (2006)
			GPx	■		
		Kidney	GPx, CAT, SOD, LPO	▲		
			GR	■		
		Gills	CAT, SOD, LPO	▲		
			GPx, GR	■		
		Liver	CAT, LPO	▲	MC-RR	
			GPx, GR, SOD	■		
		Kidney	CAT, SOD, LPO	▲		
			GPx, GR	■		
		Gills	GPx, GR, SOD, LPO	▲		
			CAT	▲		
Tilapia fish (*Oreochromis* sp.)	Acute—orally exposed to MC-LR in feed; concentration,120 µg MC-LR/fish, measured after 24 h	Liver	LPO, GPx, GR, GST	▲	MC-LR (from water bloom *Microcystis* spp.)	Atencio et al. (2009)
			CAT, SOD, GSH/GSSG	▼		
		Kidney	LPO, SOD	▲		
			CAT, GR	▼		
			GPx, GST	■		

Table 1 (continued)

Species	Exposure duration and concentrations	Organ	Studied parameter	Observed change of parameters	Investigated cyanotoxin	Reference
Silver carp (Hypophthalmichthys molitrix)	Acute—single dose 2,000 µg/kg by i.p., measured after 1, 3, 12, 24, and 48 h	Liver	SOD CAT, GPx	▲ after 24 and 48 hours ■	MCs (MC-RR+MC-LR+ MC-YR)	Li and Xie (2009)
Silver carp (Hypophthalmichthys molitrix)	Acute—single dose 2,000 µg/kg by i.p., measured after 1, 3, 12, 24, and 48 h	Liver	GST GSH,GR	▲ after 24 and 48 h ■	MCs (MC-RR + MC-LR + MC-YR)	Li et al (2007b)
Silver carp (Hypophthalmichthys molitrix)—newly hatched (1 day) embryo	3- and 7-day exposure, 10 and 100 µg/L of MC-LR	Whole body homogenates	SOD, GSH, LPO	■	MC-LR	Sun et al. (2011)
Silver carp (Hypophthalmichthys molitrix)—21-day-old larvae	24-, 48-, and 96-h exposure, 10 and 100 µg/L of MC-LR	Whole body homogenates	GSH SOD LPO	▲ after 24 and 48 h ▲ after 48 and 96 h ▲ 48 h only	MC-LR	Sun et al. (2011)
Common carp (Cyprinus carpio) hepatocytes	Acute—in medium with hepatocytes, 10 µg/L, measured after 6 h	Hepatocytes	GSH GPx, CAT, SOD	▼ ▲	MC-LR	Li et al. (2003)
Goldfish (Carassius auratus) juveniles	Acute—concentration, 125 µg/kg body wt by i.p., measured after 24, 48, 96 h, and 21 days	Liver	GST	▼+recovery after 21 days	MC-LR	Malbrouck et al. (2003)
Goldfish (Carassius auratus) juveniles	Acute—single dose 125 µg MC-LR/kg body wt by i.p., measured after 6, 24, 48, and 96 h	Liver	GSH, GST	■	MC-LR	Malbrouck et al. (2004)
Corydoras paleatus	Acute—concentrations, 0.5, 2, 5, 10 mg/L in water for 24 h	Liver	GST, GPx, GR, CAT	▼ ◀	MC-RR	Cazenave et al. (2006b)
		Intestine	GST GR, CAT, LPO GPx	▶ ■ ◀		
		Brain	GST GR GPx, CAT LPO	▶ ◀ ■ ◀		
		Gills	GPx, GR, GST, LPO CAT	▶ ■		
Jenynsia multidentata	Acute—orally exposed to MC-LR in feed; concentration, 1 µg/g food, measured after 24 h	Liver, brain	GST	◀	MC-RR	Cazenave et al. (2008)

Table 1 (continued)

Species	Exposure duration and concentrations	Organ	Studied parameter	Observed change of parameters	Investigated cyanotoxin	Reference
	Acute—orally exposed to MC-LR in the food; concentration, 0.1 µg/g food, measured after 24 h	Liver, brain	GST	▼		
	Acute—orally exposed to MC-LR in the food; concentration, 0.01 µg/g food, measured after 24 h	Liver, brain	GST	■		Li et al. (2010)
Bighead carp (*Aristichthys nobilis*)	Acute—single dose 400 µg/kg by i.p., measured after 1, 3, 12, 24, and 48 h	Liver	CAT, SOD, GST GSH GR	▲ after 3 h ■ ▲ after 1 and 3 h	MC-LR, MC-RR	
	Acute—single dose 1,000 µg/kg by i.p., measured after 1, 3, 12, 24, and 48 h	Liver	CAT, SOD, GPx, GST GSH GR	▲ after 3 h ■ ▲ after 1, 3, and 24 h		
Common carp (*Cyprinus carpio*)	Acute—0.5, 1, 2, 4, 6 days; chronic—14 days; concentrations, 10 µg MC-LR/L	Liver	CAT GST LPO GSH	▼ after 4, 6, 14 days ▲ after 0.5, 1, 2 days+ recovery in time ▲ after 2, 4, and 6 days ▼ after 4 days	MC-LR	Jiang et al (2011)

Symbols and abbreviations: ▲ stimulation in comparison with controls, ▼ inhibition, ■ no effects, *CAT* catalase, *GPx* glutathione peroxidase, *GR* glutathione reductase, *GSH* glutathione, *GST* glutathione S-transferase, *LPO* lipid peroxidation, *NOM* natural organic matter, *SOD* superoxide dismutase

Table 2 Effects of cyanobacterial cells samples (extracts or whole biomass) on widely studied biomarkers of detoxification and oxidative stress in freshwater fish

Species	Exposure duration and concentrations	Organ	Parameters	Observed change of the parameter	Cyanobacterial biomass with dominant species	Reference
Zebra fish (*Danio rerio*) eggs	Acute—cyanobacterial biomass in water with concentration: 24.8 µg MC/L for 24 h	Fish eggs	GST	▲	*Microcystis*	Pietsch et al. (2001)
Tilapia fish (*Oreochromis* sp.)	Acute—orally exposed to cyanobacterial biomass mixed with the food; concentrations, 1,350 µg/g MC-LR, measured after 24 and 72 h	Liver	LPO	▲ after 72hours	*Microcystis*	Prieto et al. (2007)
			GR	▲ after 72hours		
			GPx, CAT, SOD	▼		
		Kidney	LPO	▲ after 72hours		
			GPx, GR, CAT, SOD	▼		
		Gills	LPO	▲ after 72hours		
			GR, CAT, SOD	■		
			GPx	▼		
Common carp (*Cyprinus carpio*)	Acute—exposed with cyanobacterial extract in two gavages with a 24-h interval; concentration, 25 and 50 µg/kg body wt	Liver	GST, GSH	▼	*Microcystis*	Amando et al. (2011)
			GR	■		
		Gills, muscle, brain	GST, GR	■		
Tilapia fish (*Oreochromis* sp.)	Chronic—orally exposed to cyanobacterial biomass mixed with feed; concentration, 3,230 µg MC-LR/g biomass dry wt, for 14 days	Liver, kidney, gills	GPx, GR, CAT, SOD, LPO	■	*Microcystis*	Jos et al. (2005)
	Chronic—orally exposed to cyanobacterial biomass mixed with feed concentration of 3,340 µg MC-LR/g biomass dry wt, for 21 days	Liver	GPx, GR, CAT, SOD, LPO	▲		
		Kidney	GPx, GR, CAT, LPO	▲		
			SOD	■		
		Gills	SOD, LPO	▲		
			GR, CAT	■		
			GPx	▼		
Tilapia fish (*Oreochromis niloticus*)	Acute—single oral dose of cyanobacterial cells in food; concentration, 120 µg MC-LR/fish, measured after 24 h	Liver	LPO, CAT, SOD, GPx, GR	▲	*Microcystis*	Prieto et al. (2008)
		Kidney	LPO	▲		
			CAT, SOD, GPx, GR	▼		
		Gills	LPO, CAT	▲		
			SOD, GPx	▼		
Tilapia fish (*Oreochromis niloticus*)	Chronic 21 day exposure, biomass with and without MCs added into food pellets. Effects at MC-containing biomass (60 µg MCs/fish/day) presented here. (weak effects at biomass without MC)	Liver	LPO, GST, GPx activity, GST-expression, GPx expression	▲	*Microcystis*	Puerto et al. (2011)
		Kidney	LPO	▲		
			GST activity, GPx expression	■		
			GPx activity	▲		
			GST expression	▼		

Table 2 (continued)

Species	Exposure duration and concentrations	Organ	Parameters	Observed change of the parameter	Cyanobacterial biomass with dominant species	Reference
Silver carp (*Hypophthalmichthys molitrix*)	Chronic—cyanobacterial biomass with concentration of 182–539 μg MCs/g biomass dry wt, in water for 5 weeks	Liver	GSH, GST; GPx, GR	◄; ■		Blaha et al. (2004)
Silver carp (*Hypophthalmichthys molitrix* Val.)	Chronic—cyanobacterial biomass with concentration 182–539 μg MCs/g biomass dry wt, in water for 9 weeks	Liver	GSH; GST, GPx, GR	▼; ■	*Microcystis*	Adamovský et al. (2007)
Silver carp (*Hypophthalmichthys molitrix*)	Chronic—cyanobacterial biomass with concentration 513 μg MCs/g biomass dry wt, in water for 25 days	Liver	GSH; LPO	◄; ■	*Microcystis*	Li et al. (2007a)
Silver carp (*Hypophthalmichthys molitrix*)	Chronic—cyanobacterial biomass with concentration of 182–539 μg MCs/g biomass dry wt, in water for 4 weeks	Liver	GPx, GR; GSH, GST	◄; ■	*Microcystis*	Li et al. (2007a)
Silver carp (*Hypophthalmichthys molitrix*)	Chronic—cyanobacterial biomass with concentration of 182–539 μg MCs/g biomass dry wt, in water for 9 weeks	Liver, kidney	GST, GPx, GR; GSH	■; ▼	*Microcystis*	Li et al. (2007a)
Hypophthalmichthys molitrix, *Aristichthys nobilis*, *Carassius auratus*, *Culter ilishaeformis*	Chronic—exposure through natural food uptake in lakes before and during cyanobacterial blooms	Liver	GST, GPx, CAT, SOD; GSH	◄; ▼	*Microcystis*	Qiu et al. (2007)
Silver carp (*Hypophthalmichthys molitrix*)	Chronic—exposure through natural food uptake in lakes before and during cyanobacterial blooms	Liver	GSH; GST, GPx, CAT, SOD	■; ◄	*Microcystis*	Qiu et al. (2007)
Common carp (*Cyprinus carpio*) embryos	Acute—fractions from cyanobacterial biomass with concentration 120, 40, 10, and 3 mg dry wt/L, measured after 120 h	Whole embryos	GST; GST	■; ▼	*Microcystis* *Aphanizomenon flos-aquae*, *Planktothrix agardhii*	Palikova et al. (2007a)
Common carp (*Cyprinus carpio*) embryos	Acute—cyanobacterial biomass and aqueous extract with concentration of 2,560 μg MCs/L of dry wt and 70 μg MCs/L of dry wt; concentration, 120, 80, and 40 mg/L, measured after 120 h	Whole embryos	GST	◄	*Microcystis*	Palikova et al. (2007b)
Common carp (*Cyprinus carpio*)	Chronic—cyanobacterial biomass with concentration of 182–539 μg MCs/g biomass dry wt, in water for 4 weeks	Liver	GSH, GST, GPx; GR	■; ◄	*Microcystis*	Adamovský et al. (2007)
Common carp (*Cyprinus carpio*)	Chronic—cyanobacterial biomass with concentration of 182–539 μg MCs/g biomass dry wt, in water for 9 weeks	Liver	GSH, GR; GST, GPx	◄; ■	*Microcystis*	Adamovský et al. (2007)
Tench (*Tinca tinca*)	Acute—orally exposed to cyanobacterial biomass mixed with feed; concentration, 5, 11, 25, 55 μg MC-LR/fish, measured after 96 h	Liver	CAT, SOD; LPO; GSH	▼; ◄; ■	*Microcystis*	Atencio et al. (2008)

Table 2 (continued)

Species	Exposure duration and concentrations	Organ	Parameters	Observed change of the parameter	Cyanobacterial biomass with dominant species	Reference
Loach (*Misgurnus mizolepis*)	Chronic—orally exposed to cyanobacterial biomass mixed with feed; concentration, 10 µg MC-RR/kg body wt, for 28 days	Liver	GPx, CAT, SOD LPO	▲ ■	*Microcystis*	Li et al. (2005)
African clawed frog (*Xenopus laevis*) embryos	Acute—FETAX, fractions from cyanobacterial biomass, measured after 96 h	Whole embryos	GSH, GPx, GR, GST	■	Five cyanobacterial biomasses: *Microcystis*, *Aphanizomenon*, *Anabaena*, *Planktothrix*	Buryskova et al. (2006)

For symbols and abbreviations, see Table 1

with silver carp *Hypophthalmichthys molitrix* (Li et al. 2007b), bighead carp *Aristichthys nobilis* (Li et al. 2010), and juvenile goldfish *Carassius auratus* (Malbrouck et al. 2004). Similarly, cyanobacterial cell samples (shown in Table 2) only rarely suppressed GSH (Amando et al. 2011), and highly variable responses (both stimulations and suppressions) were reported even in studies with a single species such as silver carp (Blaha et al 2004; Adamovský et al. 2007; Li et al. 2007a). Often, cyanobacterial cell or their extracts had no effect on GSH levels in fish or *Xenopus laevis* (e.g., Atencio et al. 2008; Qiu et al. 2007; Buryskova et al. 2006), which seems to indicate that GSH is a rather insensitive and also highly variable biomarker demanding complicated interpretation.

Glutathione S-transferase

Cyanobacteria were also shown to modulate multifunctional substrate-inducible phase II enzymes—glutathione S-transferases (GSTs; van der Oost et al. 2003). Microcystins are expected to generally induce GST activities (Kondo et al. 1992; Pflugmacher et al. 1998), but, interestingly, in vivo studies with freshwater fish showed only rare stimulations of GST (e.g., Atencio et al. 2009; Li et al. 2010). Most short-term studies with isolated toxins (Table 1) showed either no significant modulations (e.g., Malbrouck et al. 2004; Cazenave et al. 2008) or even GST inhibitions, as documented for example in juvenile goldfish (Malbrouck et al. 2003) and zebra fish at different developmental stages (Pietsch et al. 2001; Best et al. 2002; Cazenave et al. 2006a). In contrast to isolated toxins, cyanobacterial cell samples dominated by *Microcystis* sp. (Table 2) often induced GST activities in various fish species such as silver carp (Adamovský et al. 2007; Qiu et al. 2007; Li et al. 2007a), bighead carp and goldfish (Qiu et al. 2007), as well as zebra fish eggs (Pietsch et al. 2001) and common carp embryos (Palikova et al. 2007b). However, GST inhibitions in vivo were also observed in common carp exposed to other species of cyanobacteria, such as *Aphanizomenon* and *Planktothrix* (Palikova et al. 2007a). It should also be noted that several poorly characterized natural factors present in complex cyanobacterial bloom may interfere with detoxification. Such interference was shown, for example, with lipopolysaccharides, natural components of cyanobacterial cell walls (Best et al. 2002), and dissolved natural organic material and humic acids (Cazenave et al. 2006a), which significantly modulated GST activity in fish.

Summary—GSH and GST

The variability in responses is further summarized in Table 3. More systematic inductions apparently occur at GST only after prolonged exposures (>7 days) to complex

Table 3 Overview of available literature on modulations of selected biomarkers in the liver of freshwater fish or whole embryo homogenates after exposures to isolated microcystins or cyanobacterial cells. Table shows numbers of studies that demonstrated stimulatory, inhibitory or no effects following acute (≤7 days) or chronic exposures (>7 days). For abbreviations, see Table 1

Parameter	Stimulatory effect ▲	No effect ■	Inhibitory effect ▼
Isolated microcystins—acute exposures			
GSH	1	3	3
GST	7	3	6
LPO	5	0	1
SOD	5	1	1
CAT	6	1	2
GPx	5	4	0
GR	4	3	0
Complex samples—acute exposures			
GSH	1	3	0
GST	3	2	1
LPO	3	0	0
SOD	1	0	2
CAT	1	0	2
GPx	1	1	1
GR	2	2	0
Complex samples—chronic exposures			
GSH	3	2	2
GST	3	3	0
LPO	1	3	0
SOD	4	1	0
CAT	4	1	0
GPx	4	5	0
GR	3	3	0

cyanobacterial cell samples. The effects of (1) isolated microcystins and (2) acute exposures resulted in rather nonsystematic up- or downregulations of both GSH and GST. Elevated activities of phase II enzyme GST observed after prolonged exposures may indicate adaptive biotransformation responses among aquatic biota (Pflugmacher et al. 1998).

Besides fish, a recent study with birds (Japanese quails) showed significant stimulations of GSH levels and also GR activities discussed below (Pašková et al. 2011). The effects were apparent in both the liver and heart after chronic 30 day gavage applications of *Microcystis* biomass, and they were even more pronounced in birds coexposed to low levels of other stressors including toxic metal (lead) and/or virus vaccination. These model observations thus highlight the general importance of the mixture toxicity, and more studies are needed to fully elucidate the mechanisms of detoxification processes and the toxicity of complex cyanobacterial water blooms.

Chronic stimulations of detoxification processes by cyanobacteria are unwanted processes in fish and other biota, as these increase energy demands impairing thus animal health and activating other toxicity mechanisms such as oxidative stress discussed in the following paragraphs (De Coen and Janssen 2003).

Modulations of oxidative stress parameters by toxic cyanobacteria

Oxidative stress results from the overproduction of reactive oxygen species (ROS) such as superoxide anion radical O_2^-, hydroxyl radical $OH^.$, and hydrogen peroxide H_2O_2. ROS are produced by a number of biological processes, and organisms have developed efficient systems to cope with them. However, a pathological increase in ROS production beyond the antioxidant capacity leads to the oxidative damage of proteins, DNA, or membrane lipids (van der Oost et al. 2003; Cazenave et al. 2006b). Exposures to chemicals, including cyanobacterial toxins, have been linked to this kind of oxidative tissue damage. Although the exact mechanism by which microcystins or other cyanotoxins induce ROS is not known, some studies highlight impacts on mitochondrial membranes in the liver, leading to strong oxidative processes (Ding et al. 1998). Microcystins as well as whole cyanobacteria are also known to stimulate ROS production in fish (Cazenave et al. 2006a, Jiang et al. 2011), and modulations of several biomarkers related to oxidative stress have been investigated in aquatic organisms, including lipid peroxidation (LPO), levels of GSH (discussed in the previous section), and activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR).

Lipid peroxidation

The most widely used biomarker, which reflects direct damage to the cell, is the assessment of phospholipids peroxidation, and a number of studies have shown elevated LPO levels after exposures to isolated microcystins (e.g., Prieto et al. 2006; Atencio et al. 2009; Table 1). On the other hand, it must be noted that microcystins have also been discussed as having an antioxidant protective role in cyanobacterial cells (Zilliges et al. 2011). Furthermore, in the fish *Corydoras paleatus*, acute exposure to microcystin was shown to decrease LPO in gills (Cazenave et al. 2006b). Besides pure toxins, studies with cyanobacterial cell samples (Tables 2 and 3) showed either significant LPO stimulations (especially during acute exposures), as observed for example in tench (Atencio et al. 2008) or tilapia (Jos et al. 2005; Prieto

et al. 2007; Prieto et al. 2008), or there were no effects (especially after prolonged exposures), as documented, for example, in silver carp and loach (Blaha et al. 2004; Li et al. 2005). To our knowledge, none of the studies with cyanobacterial cells or extract samples reported a decrease in LPO. Besides LPO, the oxidation of proteins is another—hence less explored—biomarker of direct oxidative damage, and studies with tilapia showed significant inductions after exposures to both isolated microcystin-LR (Atencio et al. 2009) and cyano-bacterial cells (Prieto et al. 2007). Weaker effects on protein oxidation were also reported in a study with tench (Atencio et al. 2008). Taken together, the available data indicate that cyanobacteria (especially complex cell samples or extracts) induce direct oxidative damage in fish, and elevations of LPO seem to be a reasonable marker, especially after short acute exposure.

Catalase and superoxide dismutase

Other important parameters studied in relationship to cya-nobacterial exposure are SOD and CAT. Both enzymes catalyze the direct removal of reactive radicals—superoxide is removed by SOD and hydrogen peroxide by CAT, and their elevated levels have been discussed as important indi-rect markers of actual ROS overproduction (Stegeman et al. 1992). In most studies with freshwater fish, both SOD and CAT were stimulated by isolated microcystins (Table 1), as shown for example in tilapia or zebra fish embryos (Prieto et al. 2006; Atencio et al. 2009; Cazenave et al. 2006a). How-ever, high tissue variability can be expected, as demonstrated for example in a study with tilapia, where the same exposures resulted in elevations of SOD and CAT in the liver but inhibitions in the kidney (Atencio et al. 2009). In addition, more complex cyanobacterial cell samples (Table 2) seemed to stimulate SOD and CAT activities in different fish, especially after long-term exposures (Jos et al. 2005; Prieto et al. 2008; Li et al. 2005; Qiu et al. 2007). Some acute studies with biomass, however, reported inhibitions of both enzymes, e.g., in tench (Atencio et al. 2008) and tilapia (Prieto et al. 2007). Taken together, elevations in both SOD and CAT may serve as suitable biomarkers reflecting oxidative stress. However, actual measured en-zymatic activities must be carefully compared to the po-tential "control" situation, as both stimulations, inhibitions or no effects, have been recorded in different studies (Tables 1, 2, and 3).

Glutathione-related enzymes (GPx and GR)

Other antioxidant enzymes that have often been studied as oxidative stress biomarkers link detoxification of ROS with the metabolism of GSH (Stegeman et al. 1992; van der Oost et al. 2003). These include especially GPx [an enzyme removing hydrogen peroxide by the simultaneous oxidation of reduced GSH to its oxidized form glutathione disulfide (GSSG)] and GR (an enzyme catalyzing the conversion of GSSG back to its reduced bioactive form maintaining thus GSH/GSSG equilibrium). Morever, guaiacol peroxidase has similar functions to GPx, but its modulations have been explored in only a limited number of studies (Pietsch et al. 2001; Cazenave et al. 2006a,b). Isolated microcystins (Table 1) as well as cyanobacterial biomass (Table 2) often stimulated both GPx and GR in fish, but some experimental investiga-tions showed no effects, e.g., in zebra fish embryos (Cazenave et al. 2006a), silver carp (Li and Xie 2009), and *X. laevis* embryos (Buryskova et al. 2006), and rare inhibitions of both enzymes were also observed. For example, acute exposures to isolated microcystins inhibited GPx in the kidney of *C. paleatus*, although stimulations were observed in the liver (Cazenave et al. 2006b), and chronic studies with *Micro-cystis*-dominated bloom inhibited GPx activities in the gills of tilapia (Jos et al. 2005). Taken together, it seems that activities of both GSH-coupled enzymes GPx and GR are relatively sensitive biomarkers of cyanobacteria-induced oxidative stress. This is valid especially for long-term exposures and studies with liver tissues, where, to our knowledge, no inhibitions have been recorded, indicating thus the good discriminating potential of GPx and GR.

Discussion on variability in biomarker responses

Biomarkers potentially suitable for use as diagnostic tools or early warnings should possess various characteristics such as a systematic trend in responses (e.g., elevated levels in response to a certain factor) or sufficiently long half-life. These requirements are, however, not met by many of the biomarkers related to oxidative stress and detoxification. In this section, we try to discuss the major sources of variability and their influence on biomarker interpretation and the even-tual use of biomarkers in practice.

Isolated cyanotoxins vs. cyanobacterial cells: mixture effects

The composition of studied cyanobacterial samples is a major factor, which—unfortunately—has not been fully characterized in a number of studies. Many reports investi-gated complex extracts of cyanobacteria containing micro-cystins. However, toxins were usually present only as a component of the complex sample, often forming a minor fraction. The toxicities observed in studies with cyanobac-terial extracts have, however, often been interpreted as if they were caused by individual microcystins (several studies listed in Table 2; see also Falconer 2007). Some studies

directly demonstrated modulatory effects of other cyanobacterial metabolites such as lipopolysaccharides (Best et al. 2002) or dissolved organic material (Cazenave et al. 2006a). A wide range of peptide metabolites is also produced in various cyanobacterial strains (Welker and Von Dohren 2006), but their possible mixture effects are elusive. Although studies with isolated toxins should minimize the overall variability, even these standard assays provided highly variable results, as shown Table 1. Two structurally related microcystins such as MC-LR and MC-RR tested within the same study induced different responses in different tissues. For example, Prieto et al. (2006) showed that a single i.p. dose of MC-LR in tilapia led to stimulations of SOD and GR in the liver or gills, but the administration of MC-RR had no effect on these parameters.

Taken together, a shift in current perceptions of cyanobacterial toxicity is needed. Microcystins are certainly not the only or most toxic components in cyanobacterial cells (Falconer 2007), and quantifying the mixture toxicity is a major scientific challenge. This should be achieved by the careful design and interpretation of individual studies.

Experimental design, concentrations, and exposure duration

As also mentioned, the systematic response of the biomarker is an important parameter with respect to its potential suitability as an early warning signal. However, this requirement is not met with respect to parameters of oxidative stress or detoxification in studies with toxic cyanobacteria. An interesting example of variability is a non-systematic dose-dependent response reported, for example, in *Jenynsia multidentata* (Cazenave et al. 2008; Table 1). The authors used a simple study design—24 h oral exposure to MC-RR in three doses. Interestingly, the lowest tested dose (0.01 μg/g of food) had no effects on GST, the medium dose (0.1 μg/g) inhibited GST, and the highest dose (1 μg/g) stimulated GST in comparison to control (Cazenave et al. 2008). Moreover, in another two studies with zebra fish embryos, big differences were observed in spite of comparable design. Wiegand et al. (1998) observed stimulation of GST after 24 h exposure to 0.5 μg/L of MC-LR, but Pietsch et al. (2001) reported inhibitions of GST at a similar 0.25 μg/L concentration. Although these are biologically interesting findings, they seem to question the assessment of GST (or other biochemical changes) as suitable early warning biomarkers.

Interspecies variability

As fish are the most diverse vertebrates, high interspecies variability in any biological response is likely. The studies reviewed in the present paper covered mostly fish species from the *Cyprinidae* or *Cichlidae* families, which are among the most often applied biological models and also commercially important fish. Biomarker responses in other fish such as *Salmonidae* have been less studied as these fish species are less likely to be affected by cyanobacterial blooms in their natural habitats. Indeed, as is apparent from Tables 1 and 2, high interspecies variability was observed, but comparison is complicated by the different experimental setups used. Only a few reports investigated more than one fish species within a single study and design (Baganz et al. 2004; Adamovský et al. 2007), but even these demonstrated significant differences in bioaccumulation and biomarker responses. For example, closely related fish such as common carp and silver carp exposed to natural cyanobacterial bloom showed either GR activation (common carp) or no difference from control (silver carp), and the fish also differed in their temporal profiles of GSH (Adamovský et al. 2007).

Intraspecies variability and tissue-specific responses

Considering single species, pronounced differences in biomarker responses have been observed even within simple standard acute studies with the zebra fish embryo model using characterized and isolated toxins (Wiegand et al. 1998; Best et al. 2002; Cazenave et al. 2006a; Pietsch et al. 2001). As shown in Table 1, GST is a good example of a highly variable parameter, which tends to be up- or down-regulated or provide no response to toxic cyanobacteria even within a single species and similar developmental stage. Different profiles depend on specific toxins (MC-LR, MC-LF, and MC-RR), concentrations (cited studies used the range 0.25 μg/L–5 mg/L), or exposure durations (24 h–days). The most pronounced difference was the example already mentioned, i.e., up- vs. downregulation of GST observed in the studies of Wiegand et al. (1998) and Pietsch et al. (2001), which were very similar in their design and with respect to the concentrations used (Table 1).

Another important source of variability is the tissue-specific responses within the same organism. For example, in a chronic 21-day study with tilapia (Puerto et al. 2011), various parameters including levels of GSH and the activities and transcription levels of GPx and GST were systematically stimulated in the liver. By contrast, highly variable profiles (stimulations, inhibitions, and no effects) were recorded in the kidney in the same study (Puerto et al. 2011). Tissue-dependent profiles of biomarkers such as GPx were also recorded in tilapia (Prieto et al. 2006; Atencio et al. 2009) and *C. paleatus* (Cazenave et al. 2006b). Pronounced differences between tissues generally complicate the generalization of biomarker responses. With this respect, biomarkers should carefully be interpreted especially in the whole-body homogenates (used in the embryo studies), where the outcome of stimulation vs. inhibition in two different tissues could be "no effect."

Summary and conclusions

A number of various biochemical changes reflecting exposures to cyanobacteria have been studied in freshwater aquatic vertebrates. While some parameters such as protein phosphatases PP1 and PP2A selectively respond to individual microcystins (Dawson 1998), modulations of other biomarkers indicating general toxicity, detoxification, or oxidative stress are very complex (Falconer 2007), especially under environmentally realistic exposures to cyanobacterial cell extracts or blooms. From the studies reviewed, only elevated LPO, a biomarker of direct tissue damage by ROS, seems to consistently indicate stress, and it might be recommended as an early warning signal of cyanobacterial toxicity. By contrast, the interpretation of other biomarkers of detoxification or oxidative stress (GSH, GST, SOD, CAT, GPx, or GR) is very complicated with respect to nonsystematic responses (inductions and/or inhibitions). Effects depend on exposure type, material, duration, dose, age, organ, species, as well as many external environmental factors, which, however, have only rarely been characterized. During standardized laboratory experiments, the majority of confounding parameters may be controlled, and the assessment of biochemical changes often supports the outcomes of mechanistic studies. However, the application of these traditional biomarkers as diagnostic tools or potential early warnings of toxicity is questionable, especially under field conditions. To better understand adverse impacts of toxic cyanobacteria on biota in aquatic ecosystems, a shift from the current paradigm is needed (Falconer 2007). While most research has focused on individual toxic components (microcystins, cylindrospermopsin, LPS, etc.), their importance in the ecotoxicity of cyanobacteria needs to be critically reassessed, addressing properly the problems of mixture toxicity, interactions with anthropogenic contaminants, and other environmental factors.

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