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Accumulation and detoxification dynamic of cyanotoxins in the freshwater shrimp *Palaemonetes argentinus*

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ABSTRACT

The uptake and accumulation of microcystin-LR (MC-LR) in the shrimp *Palaemonetes argentinus* was investigated using both laboratory and field assays. Shrimps were exposed in aquarium during 1, 2, 3 and 7 days to 1, 10 and 50 μg L^{-1} MCLR. Accumulation (0.7 ± 0.2 μg MC-LR g^{-1}) was observed after three days exposures to 50 μg L^{-1} toxin. Then, shrimps were relocated in fresh water (free of MCLR) to verify the detoxification dynamic, showing a drop to 0.18 ± 0.01 μg MCLR g^{-1} after three days. The activity of glutathione-S-transferase, measured in the microsomal fraction (mGST), was significantly increased during the exposure period, with further increment during the detoxification period. Furthermore, cytosolic GST (sGST) and glutathione reductase (GR) increased their activities during detoxification, while inhibition was observed for catalase (CAT) with no significant changes for glutathione peroxidase (GPx). Current results suggest that GSH conjugation could be an important MC detoxification mechanism in *P. argentinus* and that MCLR induce oxidative stress in this shrimp.

Field exposures were carried out in San Roque Reservoir (Córdoba, Argentina) after a cyanobacteria bloom. Nodularin (Nod) presence was measured for the first time in this waterbody (0.24 ± 0.04 μg L^{-1}), being the first report of Nod in South America freshwaters. Nod was also detected in *Palaemonetes argentinus* (0.16 ± 0.03 μg g^{-1}) after three weeks of exposure in this reservoir, with the concomitant activation of mGST, sGST and CAT.

Although internal doses of Nod were low throughout the exposure, they were enough to cause biochemical disturbances in *Palaemonetes argentinus*.

Further laboratory studies on Nod accumulation and antioxidant responses in *Palaemonetes argentinus* are necessary to fully understand these field results. *P. argentinus* should be considered a potential vector for transferring cyanotoxins to higher trophic levels in aquatic environments.

1. Introduction

Serious eutrophication accompanied with the presence of massive cyanobacterial blooms and cyanotoxins have been documented in many inland waters worldwide (Gurbuz et al., 2009). Among cyanotoxins, microcystins (MCs) and nodularins (Nod) are considered the most dangerous groups, mainly because both are potent hepatotoxins (for a review see: Zurawell et al., 2005; Wiegand and Pflugmacher, 2005) and tumor promoters (Nishiwaki-Matsushina et al., 1991, 1992). MCs are constituted by seven amino acid (one characteristic, 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid-ADDA, and 2 variable), while Nod is formed by five amino acid (preserving the characteristic amino acid ADDA; Sivonen et al., 1989).

In natural environments, MCs were found in a wide range of aquatic biota such as fish (Mohamed et al., 2003; Cazenave et al., 2005; Deblois et al., 2008; Chen et al., 2009; Amé et al., 2010), shrimps (Pflugmacher et al., 2005; Chen and Xie, 2005), gastropods (Chen et al., 2005; Zhang et al., 2012), bivalves (Gerard et al., 2009), and aquatic plants (Mitrovic et al., 2005). Cyanotoxins were present in diverse organs but also in the muscle and other edible parts of studied animals. On the other hand, the accumulation of Nod has been observed in flounders, mussels, clams (Sipiä et al., 2002) and eiders (Sipiä et al., 2008).

To our knowledge, there are not reports on the presence of cyanotoxins in *Palaemonetes argentinus*, which is omnivorous, feeding throughout the water column, preying small components of the plankton and benthos. *P. argentinus* is one of the most widely distributed decapods in the littoral region of Argentina, Paraguay,

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Uruguay and southern Brazil, inhabiting freshwater ponds and lakes (Morrone and Lopreto, 1995). Despite its important role in the food chain, little is known about the dynamics of accumulation–detoxification of cyanotoxins in this shrimp.

In according to Ito et al. (2002), the toxicity of MC depends on the balance between accumulation and metabolism. MC conjugation with glutathione (GSH) is catalyzed by glutathione-S-transferases (GST) in different aquatic organisms such as plant, mollusk, crustacean and fish. This conjugation is generally considered the primarily route for MC detoxification in aquatic organisms, by forming more polar compounds, facilitating the excretion (Pflugmacher et al., 1998; Beattie et al., 2003). However, upon uptake, absorption and distribution, it can inhibit in the liver the key regulatory enzymes: serine-threonine protein phosphatases (PPs) 1 and 2A (Yoshizawa et al., 1990; Honkanen et al., 1994; Annila et al., 1996). Furthermore, some evidences suggest a close connection between cellular hyperphosphorylation state and oxidative stress generation induced by the exposure to MCs (Amado and Monserrat, 2010). When oxidative stress takes place in the cell, the antioxidant system is activated. This system comprises enzymatic antioxidant defense such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPx) (Amado and Monserrat, 2010).

The main goal of this work was to examine the uptake, detoxification and accumulation of MCLR in shrimps, experimentally exposed to MCLR dissolved in water. Additionally, we aimed to verify laboratory results obtained by evaluating the accumulation of cyanotoxins in shrimps exposed in their natural habitat after a cyanobacteria bloom. The antioxidant enzymatic activities (CAT, GR, GPx and GST) were also measured to evidence the oxidative stress induced by cyanotoxin exposure.

2. Materials and methods

2.1. Standard cyanotoxins

MCs and Nod used in different assays and measurements were provided by Sigma–Aldrich (Sigma–Aldrich Argentina S.A.).

2.2. Animals

Freshwater shrimps (31.6 ± 6.2 mm, 0.15 ± 0.03 g), used for laboratory exposures, were obtained from free MCs natural water (San Antonio River, Córdoba, Argentina) and then acclimated to controlled aquarium conditions during 20 days (water reconstituted from deionized water with 100 mg L^{-1} sea salt, 200 mg L^{-1} $CaCl_2$, 103 mg L^{-1} $NaHCO_3$, light/dark cycle of 12 h:12 h, temperature 21 ± 1 °C). Shrimps were fed once a day with commercial fish pellet. Absence of MCs in both natural and reconstituted water was verified by HPLC–MS (see Sections 2.6 and 2.7).

2.3. Laboratory exposure

After acclimation, 144 shrimps were exposed to 1, 10 and 50 μg L^{-1} dissolved MCLR in order to measure the toxin accumulation. Forty-eight organisms were initially used at each concentration. After 1, 2, 3 and 7 days, 4 shrimps were collected and preserved as a pool (0.6 ± 0.1 g) at −20 °C until extraction. Experiments were performed in triplicate. The residual water was immediately processed to verify remaining MCLR. For this assay two controls were also performed. The first one verified the persistence of MCLR in the aquarium water (Control 1, without animals, where MC concentration was measured after 1 and 7 days). The second was a negative control, measuring MCLR in animal tissues after 7 days exposure to water without MCLR (Control 2).

A second experiment was carried out to observe the dynamic of accumulation/detoxification of MCLR and the antioxidant response in exposed organisms. Thus, 36 shrimps were exposed to 50 μg L^{-1} MCLR. After 3 days, 12 animals were collected and preserved as pools (4 individuals each) at −20 °C until analysis of MCLR. Other 18 shrimps were re-localized in free MCLR water during 3 additional days. Afterwards, this last group was collected and preserved as pools (4 individuals each) at −20 °C until analysis. Twelve additional shrimps were collected, six after 3 days exposure and six after 3 days detoxification. These last pools were preserved at −80 °C until the assessment of enzymatic activities. The concentration of MC and the enzymatic activity in shrimps exposed to free MC aquarium water was also evaluated.

2.4. Field exposure set up

2.4.1. Sampling site

San Roque Reservoir is an artificial lake, formed by a dam, which is in middle Argentina (31°21′ S: 64°30′ W), being classified as eutrophic to hypereutrophic with elevated concentrations of nutrients (Amé et al., 2003; Ruibal Conti et al., 2005). This waterbody has a surface area of 16 km^2 and a maximal depth of 35 m. Heavy surface blooms of cyanobacteria have occurred regularly during warm seasons in recent decades (Amé et al., 2003). Near the dam wall, there is a small bay where the wind frequently promotes the accumulation of cyanobacteria. This site is located on a government land without public access, which facilitated its selection as the site of exposure.

2.4.2. Assay set up

The field experiment was conducted in March, 2011, with an average temperature of 18.6 °C. During this month, the senescence of cyanobacterial blooms, with subsequent release of endotoxins into the surrounding water, is typically observed (Amé et al., 2003; Ruibal Conti et al., 2005). A total of 80 shrimps were collected upstream in the lake basin, at a quasi pristine location in the San Antonio River. After collection, shrimps were acclimated in the laboratory, using free-MC water, and then transported to San Roque Reservoir. The absence of cyanotoxins in a random selected pool of shrimps (10 shrimp) was confirmed previous to field exposition.

Palaemonetes argentinus were exposed at the selected site in two cages, with 40 individuals each, at a depth of 30–50 cm below the water surface. Cages consisted of polyvinylchloride boxes (48 cm × 20 cm × 20 cm) with 60 drilled holes of 0.4 cm diameter at each side to guarantee regular water flow. Water samples (1.5 L) and 16 exposed shrimps, taken randomly from both cages (0.10 ± 0.02 g), were collected at the beginning of exposure, and once a week during one month. Collected organisms were pooled (3 groups; 4 individuals each), shock-frozen using liquid nitrogen, transported to the laboratory and stored at −20 °C until MCs analysis. For enzyme analysis, 4 shrimps were individually stored at −80 °C until extraction. Water quality parameters were also determined at the beginning of exposure, and once a week during one month. Parameters measured include: cyanotoxins (μg L^{-1}), pH, temperature (°C), conductivity (μS cm^{-1}), dissolved oxygen (mg L^{-1}), chlorophyll (mg m^{-3}), ammonia (mg L^{-1}), nitrates (mg L^{-1}), nitrites (mg L^{-1}) and phosphorus (mg L^{-1}). Conductivity, pH, temperature and dissolved oxygen were field measured during sampling. All the parameters were measured in according to standard procedures, considering our previous experience (APHA, 2005; Pesce and Wunderlin, 2000; Amé et al., 2003).

2.5. Extraction of cyanotoxins from shrimps

Cyanotoxin extraction were carried out with two different extraction solvents in order to evaluate the best recovery of MC

from shrimps: MeOH 70%–TFA 0.01% (Lawton et al., 1994) and BuOH:MeOH:H_2O (1:4:15) (Barco et al., 2005). Recovery percentages were evaluated from spiked samples (0.3 ± 0.1 g WW for 4 pooled shrimp), added with pure MCLR (50 ng per sample).

Whole shrimps were homogenized with 25 mL of the extraction solvent and sonicated for 5 min in an ultrasonic bath. The extract was evaporated to dryness. Then, the residue was dissolved with 5% acetic acid and applied to a C-18 solid phase extraction cartridge (LiChrolut RP-18, 500 mg, Merck), previously washed with methanol and further conditioned with 5% acetic acid. Toxins were eluted using methanol (3 mL, HPLC grade). The eluate was evaporated to dryness at 40 °C under reduced pressure, suspended in 500 μL methanol (HPLC grade), and analyzed by HPLC–MS/MS (Amé et al., 2010). The evaluation of the recovery percentage was performed in triplicate. The matrix effect was assessed by adding MCLR standards to different shrimp pools before and after the extraction procedure (either to shrimp homogenate or to the MeOH elute after the procedure). Matrix effect was assessed comparing the peak areas corresponding to MCLR from both samples during analysis.

2.6. Extraction of cyanotoxins from water

For the evaluation of cyanotoxins in the residual water used during laboratory assays, and in natural water from field exposure, 50 and 1000 mL of sample were processed, respectively. To measure the total cyanotoxin content in natural water samples (particulated plus dissolved toxins), three freeze–thaw cycles followed by sonication for 5 min were made to 1 L raw. This procedure was repeated three times to ensure the break of cyanobacteria, releasing MCs to the water. Then, the suspended material was eliminated by filtration through a 0.45 μm filter. The filtrated sample was brought to 5% acetic acid and applied to a C-18 solid phase extraction cartridge. Further work up was similar to that used for the extraction of MC from shrimp (Section 2.5).

2.7. Quantification of cyanotoxins by HPLC–MS/MS

The quantification of cyanotoxins was made following the method described by Amé et al. (2010). Briefly, toxins were quantified by HPLC coupled a Varian 1200 triple quadrupole mass spectrometer, equipped with an ESI source operated in positive mode, using a column Varian Polaris 5 μm C18-A (50 mm × 2.0 mm). Solvent delivery was performed at 0.25 mL min^{-1} by two pumps Varian Prostar 210 Dynamax using water supplemented with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B) (total run time 25 min). Samples and standard solutions were introduced in HPLC using a Varian ProStar 410 autosampler equipped with a 20 μL loop, injecting 10 μL on column. The limit of detection (LOD) = 1.5 μg L^{-1} (15 pg on column) was established considering a signal to noise ratio of 10 (S/N ≥ 10); while the limit of quantification (LOQ) = 4.5 μg L^{-1} (45 pg on column) was decided on the basis of linear regression results (S/N ≥ 80), which are in good agreement with values reported in the literature (Barco et al., 2002), although slightly higher than reports using LC–ESI-MS/MS (MRM) (Mekebri et al., 2009). Linear standard plots were obtained between LOQ and 1000 μg L^{-1} ($R^2 > 0.9949$). Both samples and standard solutions were analyzed by triplicate. The LOD and LOQ corresponding to different analytical matrixes (water or shrimp), depend on the volume of water and the weight of shrimp used during the extraction procedure.

2.8. Enzyme analysis

Enzyme extracts were prepared from individual organisms (not pooled) in according to Monferrán et al. (2011). Thus, cytosolic and microsomal protein fractions were obtained from each analyzed shrimp.

Enzymatic activities were determined by spectrophotometry, using a UV/vis equipment (Lambda 25; Perkin-Elmer). The activity of soluble and membrane bound glutathione-S-transferase (sGST and mGST; EC 2.5.1.18) was determined using CDNB as substrate (Habig et al., 1974). Glutathione reductase activity (GR; EC 1.8.1.7) was assayed according to Tanaka et al. (1994). The activity of glutathione peroxidase (GPx; EC 1.11.1.9) was determined as reported by Drotar et al. (1985) using H_2O_2 as substrate. Catalase activity (CAT; EC 1.11.1.6) was also measured as previously reported, using H_2O_2 as substrate (Chang and Kao, 1998). The enzymatic activity was calculated in terms of the protein content of the sample, measured at 595 nm by the Bradford method (Bradford, 1976), and it is reported in nanokatals per milligram of protein (nkat mg $prot^{-1}$), where 1 kat is the conversion of 1 mol of substrate per second. Each enzymatic measurement was carried out in triplicate.

2.9. Statistical analysis

All data are reported as mean ± standard deviation. Normal distribution for data was analyzed by Shapiro–Willks test, while Levene test was used to check the homogeneity of variance. One-way ANOVA followed by Duncan's test were carried out for comparing different treatments when normal variables were analyzed. When data were not normally distributed, they were subjected to non-parametric statistical analysis on ranks (Kruskal–Wallis) followed by Dunn's post-test. Pearson correlation test was used to establish the association between different variables. Significance was accepted for $p < 0.05$. The InfoStat/P software (2001) was employed in all cases.

3. Results

3.1. Test of solvent efficiency for microcystin extraction from P. argentinus

Our current results reveal that the best solvent to extract MCLR from *Palaemonetes argentinus* was methanol 70%:TFA 0.1% (recovery = 112 ± 7% of MCLR spiked). On the other hand, extraction using BuOH:MeOH:H_2O (1:4:15) resulted in recoveries below 10–20%.

The extraction solvents used for the extraction of MCs from animal tissues vary greatly in the literature (Barco et al., 2005; Smith and Boyer, 2009). The solvent selected for *Palaemonetes argentinus* was successfully used also in the mussel (*Mytilus edulis*; Kankaanpää et al., 2007), in fish (*Corydoras paleatus*, *Jenynsia multidentata*, *Odontesthes bonariensis* and *Cyprinus carpio*; Cazenave et al., 2005; El Ghazali et al., 2010), in Lesser Flamingo (*Phoenicopterus minor*; Krienitz et al., 2003) and in blue crabs (*Callinectes sapidus*; Garcia et al., 2010) with similar percentages of recovery.

3.2. Laboratory assay

3.2.1. Accumulation

The concentration of MCLR diminished in control and residual water at every concentration tested. The toxin decay was similar in both control and exposure medium, recovering between 60 and 79% of the added MC (Table 1).

The uptake and accumulation of MCLR in shrimp under laboratory conditions was only detectable when animals were exposed during 3 days at 50 μg L^{-1} (0.73 ± 0.02 μg MCLR g^{-1} shrimp). It was not possible measuring the accumulation of MCLR at 50 μg L^{-1} after 7 days exposure due to the death of all exposed shrimps. No LD_{50} information has been previously established for this

Table 1
Percentage of recovered MCLR in water and MCLR in *P. argentinus* during 7 days exposure to dissolved MCLR (LOD in *P. argentinus* = 0.0025 $\mu g\,g^{-1}$).

Concentration	Day	% MCLR recovery water		MCLR $\mu g\,g^{-1}$ *P. argentinus*	
		Control 1	Residual	Control 2	Exposed
1 $\mu g\,L^{-1}$	1	102 ± 5	101 ± 6	ND	<LOD
	2	ND	ND	ND	<LOD
	3	ND	ND	ND	<LOD
	7	73 ± 7	76 ± 6	<LOD	<LOD
10 $\mu g\,L^{-1}$	1	98 ± 3	102 ± 5	ND	<LOD
	2	ND	ND	ND	<LOD
	3	ND	ND	ND	<LOD
	7	60 ± 2	70 ± 6	<LOD	<LOD
50 $\mu g\,L^{-1}$	1	105 ± 14	98 ± 4	ND	<LOD
	2	ND	ND	ND	<LOD
	3	ND	ND	ND	0.7 ± 0.2
	7	75 ± 5	79 ± 7	<LOD	ND

<LOD, below detection limit; ND, no data.

species. Previous laboratory studies, conducted with other aquatic crustacean, suggest that these species are tolerant at ecologically relevant concentrations of MCs, at least for short periods of time (Liras et al., 1998; Smith et al., 2008). However, exposure conditions used during the present work produced deleterious effect on *Palaemonetes argentinus*. Thus, a shorter exposure time was selected for the accumulation–detoxification assay.

Similar accumulation values (0.7 ± 0.1 μg MCLR g^{-1} *Palaemonetes argentinus*) were obtained when the dynamic accumulation–detoxification test was run at 50 $\mu g\,L^{-1}$ for 3 days. After the detoxification period (3 days in MCLR-free water) the amount of MCLR in shrimps decreased by about 74% (0.18 ± 0.01 μg MCLR g^{-1} *P. argentinus*; Table 2).

3.2.2. Enzymatic activities

Antioxidant and detoxification enzymes (GST, GR, GPX and CAT) were evaluated in *Palaemonetes argentinus* during laboratory assays (Fig. 1). So, when shrimps were exposed to 50 $\mu g\,L^{-1}$ MCLR, the activity of GST (cytosolic and microsomal) was increased after 3 days-exposure, being this rise only significant for the microsomal fraction. After the detoxification period, GST showed a significant increased activity in both fractions (Fig. 1). A similar response was observed for GR, with a threefold increment (with reference to the control) after 3-days detoxification. On the other hand, the GPx did not show significant changes throughout the studied period (exposure + detoxification), while CAT activity only showed a significant decay after 3 days exposure (Fig. 1).

3.3. Field assay

3.3.1. Cyanotoxins soluble

Water quality parameters in addition to concentrations of MC-LR, -RR, -LA, -YR and Nod were measured in both water and

Table 2
MCLR in water and MCLR in *P. argentinus* after accumulation–detoxification assay (LOD in water = 0.03 $\mu g\,L^{-1}$; LOD in *P. argentinus* = 0.0025 $\mu g\,g^{-1}$).

Day	MCLR water ($\mu g\,L^{-1}$)		MCLR $\mu g\,g^{-1}$ *P. argentinus*	
	Control 1	Residual	Control 2	Exposed
0	ND	50.0 ± 0.2	ND	ND
3 (accumulation)	<LOD	38 ± 1	<LOD	0.7 ± 0.1
6 (detoxification)	<LOD	<LOD	<LOD	0.18 ± 0.01

<LOD, below detection limit; ND, no data.

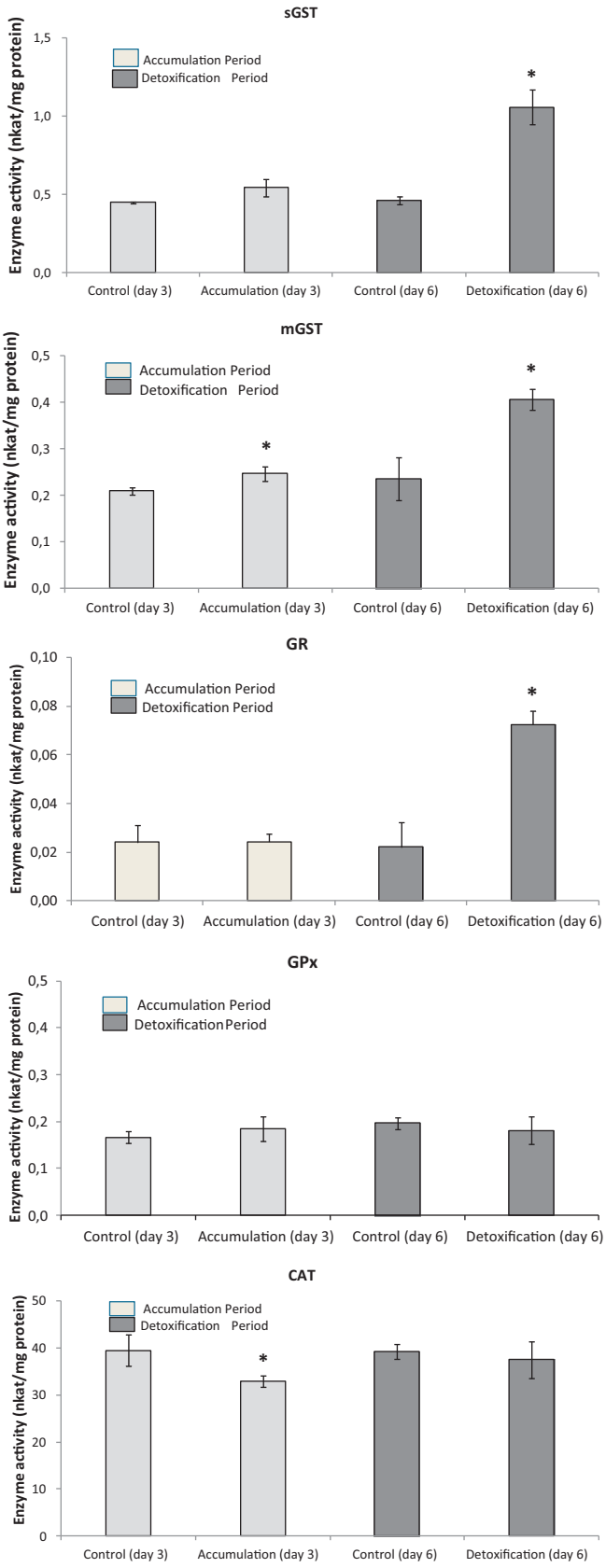

Fig. 1. Enzymatic activities of detoxification and antioxidants systems in *P. argentinus* during laboratory accumulation/detoxification assay. (A) Cytosolic fraction of glutathione-S-transferase (sGST); (B) microsomal fraction of glutathione-S-transferase (mGST); (C) glutathione reductase (GR); (D) glutathione peroxidase (GPx) and (E) catalase (CAT). *Statistical significance was inferred at $p < 0.05$ in reference to the control.

Table 3
Water quality parameters, MC-LR, -RR, -LA, -YR and nodularin concentrations measured in water and in *P. argentinus* evaluated during the exposure in San Roque Reservoir. Time 0 corresponding to the day when the assay begun (LOD in water = 0.03 μg L^{-1}; LOD in *P. argentinus* = 0.0025 μg g^{-1}).

Samples	Parameters	Time 0	Week 1	Week 2	Week 3	Week 4
Water	N-NO_3 (mg L^{-1})	0.9 ± 0.1	0.82 ± 0.01	0.44 ± 0.01	0.17 ± 0.04	0.28 ± 0.02
	N-NO_2 (mg L^{-1})	0.0103 ± 0.0004	0.0069 ± 0.0001	0.00686 ± 0.00002	0.0070 ± 0.0005	0.01247 ± 0.00009
	N-NH_3 (mg L^{-1})	0.69 ± 0.04	0.26 ± 0.02	0.36 ± 0.03	0.51 ± 0.02	0.033 ± 0.03
	P-PO_4 (mg L^{-1})	0.02 ± 0.01	0.78 ± 0.06	0.9 ± 0.2	2.7 ± 0.3	0.9 ± 0.3
	pH	9.5 ± 0.2	8.5 ± 0.1	7.95 ± 0.1	7.82 ± 0.1	8.51 ± 0.2
	T (°C)	25 ± 2	22 ± 1	21 ± 4	24 ± 2	22 ± 1
	Dissolved oxygen (mg L^{-1})	6.5 ± 0.7	6.4 ± 0.1	6.5 ± 0.1	7.5 ± 0.5	7.4 ± 0.4
	Conductivity (μS cm^{-1})	223 ± 10	214 ± 18	216 ± 15	207 ± 10	203 ± 12
	Nodularin (μg L^{-1})	0.20 ± 0.05	0.152 ± 0.003	0.145 ± 0.009	0.16 ± 0.03	0.10 ± 0.02
	MCLR (μg L^{-1})	<LOQ	<LOQ	<LOQ	<LOD	<LOD
	MCYR (μg L^{-1})	<LOQ	<LOQ	<LOQ	<LOD	<LOD
	MCRR (μg L^{-1})	<LOD	<LOD	<LOD	<LOD	<LOD
	MCLA (μg L^{-1})	<LOD	<LOD	<LOD	<LOD	<LOD
Shrimp	Nodularin (μg g^{-1})	<LOD	<LOD	<LOD	0.09 ± 0.02	0.08 ± 0.02
	MCLR (μg g^{-1})	<LOD	<LOD	<LOD	<LOD	<LOD
	MCYR (μg g^{-1})	<LOD	<LOD	<LOD	<LOD	<LOD
	MCRR (μg g^{-1})	<LOD	<LOD	<LOD	<LOD	<LOD
	MCLA (μg g^{-1})	<LOD	<LOD	<LOD	<LOD	<LOD

Palaemonetes argentinus during field-exposure in the San Roque Reservoir. Results are reported in Table 3.

Levels of nutrients and other physicochemical variables were similar to those previously reported for this reservoir (Amé et al., 2003; Cazenave et al., 2005). Therefore, San Roque Reservoir could still be classified as eutrophic to hypereutrophic, with an associated high biomass production. However, the concentrations of diverse MCs evaluated in the water were below LOD or LOQ throughout the studied period. The presence of MCRR and MCLA during the exposition was not detected. MCYR and MCLR were present when the exposure begun (during week 1 and 2), but below quantification limits afterward. Nod was the only cyanotoxin quantified in the water throughout the exposure time (0.24 ± 0.04 μg L^{-1}), showing significant differences among the first and the last week (Fig. 2 and Table 3).

3.3.2. Accumulation

The individuals of *Palaemonetes argentinus* used for the field exposure did not show accumulation of cyanotoxins before the field exposure. It was also not possible to detect MCs in shrimps after the exposure in San Roque Reservoir. This result is consistent with the absence or low level of these cyanotoxins in the water during the study period (Table 3). Conversely, bioaccumulation of Nod in *P. argentinus* was observed during the third week (0.09 ± 0.02 μg Nod g^{-1} shrimp), with a similar level during the fourth week (0.08 ± 0.02 μg Nod g^{-1} shrimp, Fig. 2 and Table 3).

3.3.3. Enzymatic activities

Fig. 3 shows the mean values for the activity of biotransformation and antioxidant enzymes, measured in *Palaemonetes argentinus* during the field exposure. GST activity showed a similar level since the beginning of the experiment (Time 0) throughout three weeks, with a significant increment during the fourth week in both cytosolic and microsomal fractions (Fig. 3). Conversely, the activity of GR was significantly increased in *P. argentinus* during the first, second and fourth week, compared with the starting time (Fig. 3). A similar response was observed for the activity of GPx, which was significantly increased during the second and fourth weeks (Fig. 3).

Finally, CAT showed significant augmented activities during the entire exposure period (Fig. 3).

Pearson correlation analysis was used to establish associations between levels of Nod in either reservoir water or exposed shrimps with the activity of antioxidant enzymes. However, no significant correlations were obtained between levels of Nod in reservoir water and the activity of antioxidant enzymes (data not shown). Conversely, significant correlations were found between activities of mGST and CAT and Nod levels in *Palaemonetes argentinus* ($r = 0.45$, $p < 0.05$ and $r = 0.51$, $p < 0.05$, respectively).

4. Discussion

4.1. Laboratory exposure

Exposure to cyanotoxins represents a health risk to aquatic organisms, wild life, domestic animals, and humans upon drinking or ingesting these compounds (Malbrouck and Kestemont, 2006). Although these toxins are rarely ingested by human in quantity high enough to reach a lethal acute dose, chronic toxic effects from exposure through food need to be considered, especially if there is long-term frequent exposure (Magalhães et al., 2001). *Palaemonetes argentinus* in all life cycle stages were found in ponds, lakes, rivers, and brackish-water systems (Spivak, 1997). Moreover, young and adults shrimps form part of the aquatic trophic web. They are preyed on by several fish and bird species (Montagna and Collins, 2007). This means that these shrimps could be considered a potential vector for transferring cyanotoxins to higher trophic levels through the food chain. By integrating the hazard identification, dose-response and exposure assessment information, health guideline values for microcystins in chain food can be derived, but it is important to first establish detoxification accumulation dynamics in different trophic levels (Mulvenna et al., 2012).

In the present study, the cyanotoxin MCLR was accumulated by *Palaemonetes argentinus* after 3 days of exposure in laboratory experiments. However, the bioaccumulation was evident only when the concentration of the dissolved MCLR reached 50 μg L^{-1}. The levels of MCLR measured in *P. argentinus* (0.7 μg g^{-1} FW or 2.8 μg g^{-1} DW) are similar to MCs measured in *Procambarus clarkii* during a laboratory exposure (2.9 μg g^{-1} DW; Vasconcelos et al., 2001), but higher if compared with other crustacean species like the *Palaemon modestus* (0.114 μg g^{-1} DW) and *Macrobrachium nipponensis* (0.051 μg g^{-1} DW) collected in an eutrophic lake of China (Chen and Xie, 2005). Even when the accumulation in other crustacean has also been reported, they will not be compared here, since most of them have been measured in different tissues instead of the whole body as in the present study (for a review see: Smith et al., 2008; Gutiérrez-Praena et al., 2013). According to Chen and Xie (2005) crustacean species in different genera of the same

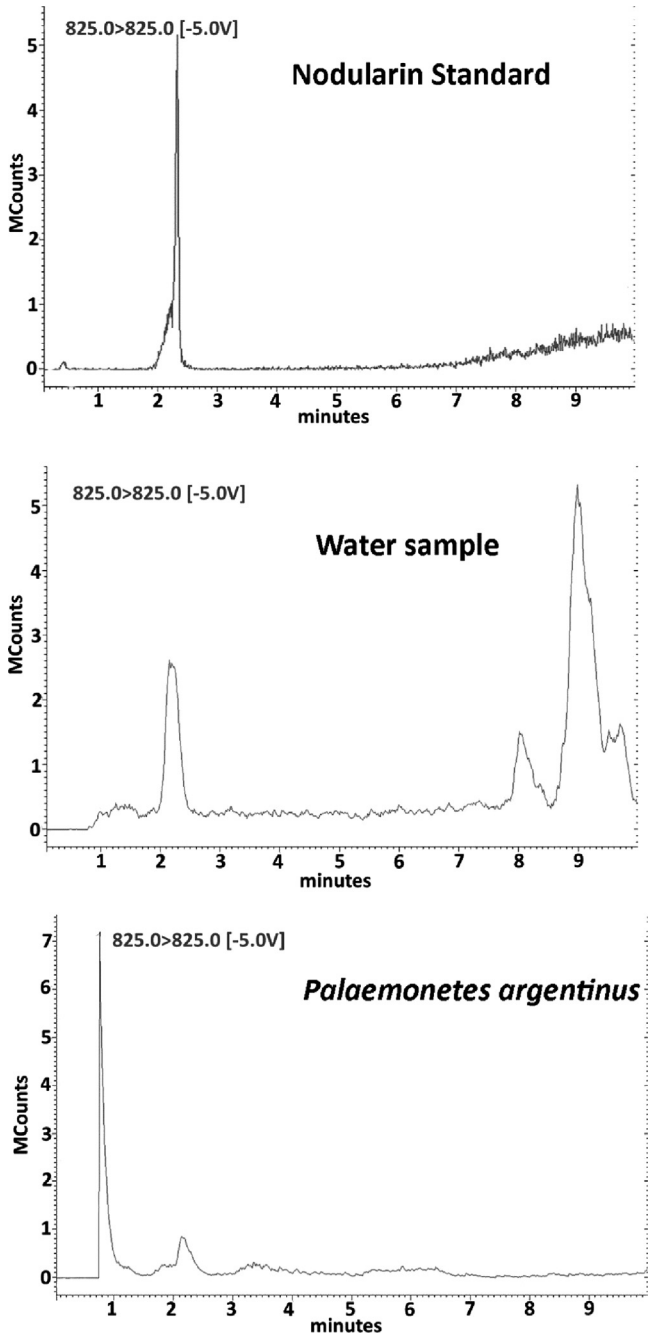

Fig. 2. HPLC–MS/MS detection of nodularin in water and *P. argentinus* samples, extracted ion chromatogram = *m*/*z* 825.0. (A) Nodularin standard solution; (B) San Roque Reservoir water sample; and (C) *P. argentinus* sample.

family may accumulate quite different levels of MCs and, therefore, the accumulation pattern must be known for each species to predict the bioaccumulation of MCs in various shrimps. Moreover, MCs accumulation in aquatic organisms might also be affected by various factors, such as the exposure route, exposure duration and exposure concentration, which also need to be considered. Thus, laboratory studies, using different approaches, can contribute to understand the dynamic of cyanotoxins accumulation.

Vasconcelos et al. (2001) showed that after two weeks of feeding with a toxic strain of *Microcystis aeruginosa*, *Procambarus clarkii* accumulated up to 2.9 μg MCs g^{-1} DW. During the subsequent two weeks of depuration, a fast decrease in the toxin level was recorded, in analogy with results reported in mussels

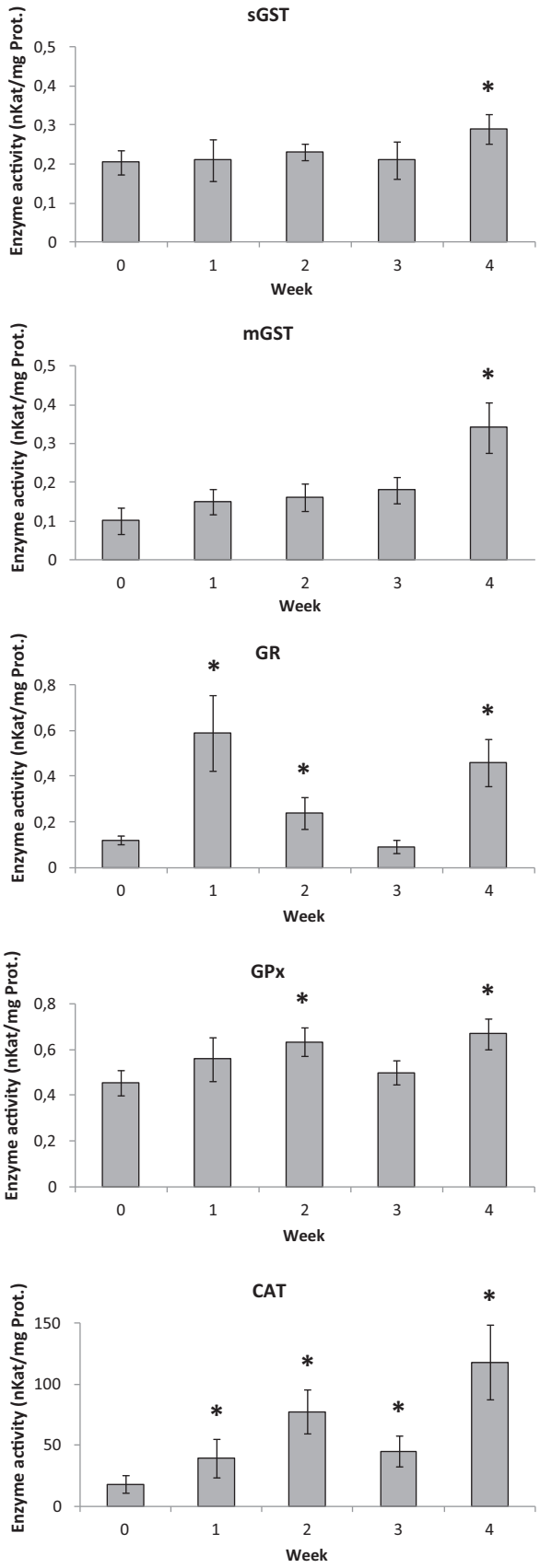

Fig. 3. Enzymatic activities of detoxification and antioxidants systems in *P. argentinus* during field exposure in San Roque Reservoir. (A) cytosolic fraction of glutathione-S-transferase (sGST); (B) microsomal fraction of glutathione-S-transferase (mGST); (C) glutathione reductase (GR); (D) glutathione peroxidase (GPx) and (E) catalase (CAT). *Statistical significance was inferred at $p < 0.05$ in reference to the control (Time 0).

(Shumway, 1990; Vasconcelos, 1995). Tricarico et al. (2008) also showed that the concentration of MCs significantly decreased with the time in the abdominal muscle of *P. clarkii*, but not in the intestine, independently of the sex and the feeding regime, after 21 days of depuration. In the present study, MCLR dropped from 0.7 to 0.18 $\mu g\ g^{-1}$ after 3 days (74%). According to Williams et al. (1997), mussels rapidly eliminated MCs when transferred to untreated salt water, showing that the total MCs content dropped from 337 μg to 11 $\mu g\ MC\ g^{-1}$ FW along 4 days, and was undetectable after this time. Lance et al. (2010) found that, at the end of the 3-week depuration period, the snail *Lymnaea stagnalis* tissues still contained up to 21 $\mu g\ g^{-1}$ DW of MCs. Moreover, Kankaanpää et al. (2005) reported a reduction of 80% of the original toxin concentration (130 $\mu g\ kg^{-1}$ DW) measured in the hepatopancreas of the prawn *Penaeus monodon* two hours after injection of MC-LR. Whether the detoxification of MCLR in *P. argentinus* is complete or not, would need the study of longer detoxification periods.

GSTs are the most important enzymes for MCLR detoxification. The formation of a MC-GSH conjugate via GST, which enhances the water solubility, was observed in different aquatic organisms (Pflugmacher et al., 1998). According to Gonçalves-Soares et al. (2012), the GSH conjugation can be an important MC detoxification mechanism in shrimps, similar to that observed in mammals and other invertebrates.

Increased activities of the GST system indicate a higher metabolism rate of the cyanobacterial toxin, whereas a decreased activity can be sign of saturation of this enzyme (Burmester et al., 2012).

When *Palaemonetes argentinus* was exposed to MCLR, the activity of mGST was significantly increased with relation to the control group (Fig. 1). This increment was larger when the crustaceans were relocated in free-MC medium. The mGST enhanced response during the detoxification period could be caused by an advantage taken by the detoxification system of the shrimp to remove the residual toxins in this new favorable situation.

The sGST activity of *Palaemonetes argentinus* showed a significant increase only after the depuration period. Several studies for other organisms reported different sGST and mGST responses after MCLR exposure (Burmester et al., 2012). Pflugmacher et al. (1999) observed an elevation of mGST activity in the aquatic macrophyte *Ceratophyllum demersum* exposed to MCLR up to 0.12 $\mu g\ L^{-1}$, followed by inhibition at higher concentrations, whereas sGST activity was increased upon exposure to 1.5 $\mu g\ L^{-1}$ MCLR. In *P. argentinus* the activity of mGST showed higher changes than the sGST after the accumulation–detoxification assay, with a significant increase after the accumulation period and more pronounced activity after the detoxification (Fig. 1).

On the other hand, the conjugation of MCLR and GSH mediated by GST could be not the only detoxification pathway. For instance, elevated expression and activity of P-glycoprotein (Mutixenobiotic Resistance system – MXR) were found in the bivalve *Dreissena polymorpha* as well as in the fish *Jenynsia multidentata* when exposed to MCLR (Contardo-Jara et al., 2008; Amé et al., 2009). This protein, also described in shrimps (Bard, 2000), could be favoring the excretion of MCLR, yielding a minor requirement of GST biotransformation activity.

More evidences have shown that oxidative stress, produced by reactive oxygen species (ROS) may play a significant role in the pathogenesis of MCs toxicity (Gonçalves-Soares et al., 2012). So, antioxidants are important cellular defenses against MC toxicity, by inactivating ROS and repairing oxidized biomolecules (Halliwell and Gutteridge, 2007). Thus, it could be expected that exposure to MC and ROS generation, would modulate the antioxidant enzymatic system at two levels: expression and activity.

In the preset study, the activities of antioxidant enzymes GPx, GR and CAT have been measured during the accumulation–detoxification assay. The methodology used to determine GST activity includes the antioxidant action carried out by GST α-class (catalyzing the reduction of organic hydroperoxides by GSH; Wang and Ballatori, 1998). Thus, changes in GST activity could mean both biotransformation and antioxidant function.

In addition to the induction of GST activity in shrimps, a significant drop in the activity of CAT was observed after the accumulation period, while GR activity was enhanced after the detoxification period (Fig. 1).

So far, after the first experimental phase (accumulation), no clear evidences of oxidative stress were observed.

On the other hand, the activation of GST and GR after the detoxification period could indicate that these enzymes were involved in the biotransformation of previously accumulated MCLR and probably also coping with ROS species generated.

A satisfactory explanation for the enzymatic behavior observed in shrimps during the accumulation–detoxification assay could be that MXR is favoring the elimination of intracellular MCLR during the accumulation period, with less intervention of GST. When the exposure ends, intracellular (accumulated) MCLR seems to be biotransformed by GST as described in other organisms. This biotransformation requires consumption of GSH, which could be regenerated by GR, with the concomitant increase in the activity of this enzyme. It is worthy mentioning here that GR catalyzes the reduction of oxidized glutathione (GSSG) to reduced GSH using electrons from NADPH (Reed, 1986). Moreover, the augment in GR activity despite no changes in GPx activity, support the idea that enhanced GSSG amounts emerged by non-enzymatic reactions with oxidant molecules. A similar pattern of antioxidant response has been reported by Contardo-Jara et al. (2009) and Sáenz et al. (2010) in mussels naturally exposed.

4.2. Field exposure

Cyanobacterial blooms have occurred in the San Roque Reservoir for about 40 years (Scarafia et al., 1995; Pizzolón et al., 1997). *Dolichospermum*, *Microcystis*, *Chroococcus*, *Oscillatoria*, *Pseudoanabaena*, *Phormidium*, *Lyngbya* and *Nodularia* are among the cyanobacteria genus more frequently described in this waterbody (Brandalise et al., 2007). MCs presence in the San Roque Reservoir has been informed since 1997, with maximum values in summer and autumn (Amé et al., 2003; Ruibal Conti et al., 2005). However, to our knowledge, there are not previous reports on the occurrence of other cyanotoxins (e.g. Nod) in the reservoir.

In the present study, MCYR and MCLR were detected during two weeks after starting the exposure but always below quantification limits. Conversely, Nod has been detected in all water samples analyzed during our study. The concentration of Nod showed a gradual decay along the exposure period (from 0.20 to 0.10 $\mu g\ L^{-1}$). No guidelines have been set for Nod, but since its toxicity resembles that of the MCs, the same guidelines can be used for both of these toxins (Kuiper-Goodman et al., 1999). Therefore, Nod concentrations did not surpassed the level suggested by WHO for drinking water nor exceeded the suggested recreational exposure guideline (WHO, 2003). Our current results are in good agreement with Nod levels reported by other authors in the Baltic Sea (0.004–565,000 $\mu g\ L^{-1}$; Mazur and Plinski, 2003; Henriksen et al., 2005). According to Dörr et al. (2010) the presence of Nod had not been reported in South America.

Nod content in *Palaemonetes argentinus* in San Roque Reservoir ranged from 0.06 to 0.11 $\mu g\ g^{-1}$ FW. However, the shrimp needed 3 weeks exposure to accumulate Nod at detectable levels, which is reasonable considering the low level found in the water. Bioaccumulation of Nod has been previously reported in crustaceans. Van

Buynder et al. (2001) reported 0.022 and 6.4 μg Nod g^{-1} FW in muscle and viscera of prawns, respectively. Kankaanpää et al. (2005) found 2000 μg Nod g^{-1} of DW in the black tiger prawn (*Penaeus monodon*), while Engström-Öst et al. (2002) measured 0.15–0.70 μg Nod g^{-1} DW in the mysid shrimp *Mysis relicta*, after feeding with copepods containing Nod. More recently, Wood et al. (2012) reported 0.01–0.22 μg Nod g^{-1} in the hepatopancreas of the freshwater crayfish *Paranephrops planifrons*. *P. argentinus* is not habitually used as human food. However, this shrimp is preyed by fish present in the reservoir, thus constituting an important link for translating this toxin to humans through the food chain.

Hence, in addition to previous reports on the presence of MCs (Amé et al., 2003; Cazenave et al., 2005; Ruibal Conti et al., 2005), spatial and temporal variations of Nod should be determined in the future, considering health risk for humans.

Although the accumulation of Nod in aquatic species appears well researched, less is known neither on its detoxification pathway nor on the health status of exposed organisms.

Previous studies showed that transplanting aquatic organisms from a reference site to a polluted area can be a feasible strategy for biomonitoring the effects of environmental changes in an aquatic system (Smolders et al., 2004). The response of biomarkers can be regarded as biological or biochemical effects after toxicant exposure, which makes them useful as indicators of both exposure and effects. According to van der Oost et al. (2003), most studied biomarkers at this level correspond to enzymes involved in the biotransformation and antioxidant systems.

During the field exposure conducted in the present study, differential response was observed for measured enzymes (Fig. 3), showing that *Palaemonetes argentinus* was able to reflect changes in the surrounding environment. The induction of the enzymatic antioxidant defenses (GST, GR, CAT and GPx) during the field exposure could be considered as an adaptive response; that is, a compensatory mechanism that enables the organism to overcome the threat produced by adverse environmental conditions (Di Giulio, 1991). Changes in enzymatic activities match changes in dissolved oxygen, nitrites and phosphates content in water (Table 3 and Fig. 3). However, the increase in antioxidant defenses as the dissolved oxygen rise should be carefully considered, since *Palaemonetes* is an oxyconformer (oxygen consumption varies according water oxygen availability; Anderson, 1985). According to Storey (1996), the ROS generation rate is closely related to the oxygen consumption and the amount of mitochondrion in the tissue. Thus, in a hyperoxic situation its oxygen consumption will be higher, thus augmenting ROS generation (Da Rosa et al., 2005).

An indication of the oxidative stress associated to the presence of Nod could be obtained correlating the cyanotoxin, either in the exposure water or bioaccumulated in *Palaemonetes argentinus*, with the activity of antioxidant enzymes measured in the shrimp. A significant correlation was obtained between mGST and CAT activities with Nod levels in *P. argentinus*.

Nodularin-GSH conjugates have been reported in the brine shrimp *Artemia salina* (Beattie et al., 2003) and mussels (Sipiä et al., 2002). Conversely, Pflugmacher et al. (2001) and more recently Lehtonen et al. (2003) reported no GSH conjugates of Nod in the reed plant *Phragmites australis* and clam *Macoma balthica*, respectively. Enhanced GST activity in addition to oxidative stress due to Nod exposure were described in laboratory exposures in the mussel *Perna viridis* (Davies et al., 2005), in the mussel *Mytilus edulis* (Kankaanpää et al., 2007), in the brown alga *Fucus vesiculosus* (Pflugmacher et al., 2007), in the red alga *Furcellaria lumbricalis* (Pflugmacher et al., 2010) and in the spinach *Spinachia oleracea* (Lehtimäki et al., 2011) among others.

Based on these previous studies and considering our current results, we could suggest that the conjugation of GSH to Nod could be an important Nod's detoxification mechanism in shrimp, similarly to that observed in other animals. The elevated CAT activity observed (Fig. 3) also suggests that oxidative stress could be occurring during the field exposure.

The bioaccumulation dynamics of Nod as well as the associated antioxidant response in *Palaemonetes argentinus* should be further study in laboratory assays to confirm current field results.

5. Conclusions

The present study confirms the bioaccumulation of MCLR in *Palaemonetes argentinus*. However, MCLR was partially eliminated from the shrimp after transferring to free-MC medium (74% after 3 days). Increased GST activity suggests that GSH conjugation can be an important MC detoxification mechanism also in *P. argentinus*. However, during laboratory exposure, it seems that GST in not mainly involved in the elimination of MCLR. Alternative mechanisms, like MXR, could be responsible for MCLR removal during the exposure period. After the exposure ends, GST and GR could be implicated in both conjugation with and restoration of GSH within the cell, leading to the detoxification of shrimps.

To the extent of our knowledge this is the first report of Nod in freshwaters of South America. Even when the measured concentrations during the studied period never surpassed the WHO guidelines, further monitoring should be conducted in San Roque Reservoir to evaluate both spatial and temporal variability to ensure that values remain within such limits of allowance.

The Nod was also detected in *Palaemonetes argentinus* after 3 weeks of exposure in this waterbody. This means that these shrimps could be considered a potential vector transferring cyanotoxins to higher trophic levels. Although the low internal levels of Nod in *P. argentinus*, such amounts were enough to cause biochemical disturbances in the shrimp.

Further controlled studies on Nod accumulation and molecular responses in *Palaemonetes argentinus* are necessary to fully understand this field results.

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References

Amado, L.L., Monserrat, J.M., 2010. Oxidative stress generation by microcystins in aquatic animals: why and how. Environment International 36, 226–235.

Amé, M.V., Díaz, M.P., Wunderlin, D.A., 2003. Occurrence of toxic cyanobacterial blooms in San Roque Reservoir (Córdoba – Argentina): a field and chemometric study. Environmental Toxicology 18, 192–201.

Amé, M.V., Baroni, M.V., Galanti, L.N., Bocco, J.L., Wunderlin, D.A., 2009. Effects of microcystin-LR on the expression of P-glycoprotein in *Jenynsia multidentata*. Chemosphere 74, 1179–1186.

Amé, M.V., Galanti, L.N., Menone, M.L., Gerpe, M.S., Moreno, V.J., Wunderlin, D.A., 2010. Microcystin-LR, -RR, -YR and -LA in water samples and fishes from a shallow lake in Argentina. Harmful Algae 9, 66–73.

Anderson, G., 1985. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (Gulf of Mexico) – grass shrimp. U.S. Fish Wildl. Serv. Biol. Rep. 82(11.35) U.S. Army Corps of Engineers, TR. EL-82-4. 19 pp.

Annila, A., Lehtimäki, J., Mattila, K., Eriksson, J.E., Sivonen, K., Rantala, T.T., Drakenberg, T., 1996. Solution structure of nodularin. An anhibito of serine/threonine-specific protein phosphatases. Journal of Biological Chemistry 271, 16695–16702.

American Public Health Association [APHA], American Water Works Association [AWWA], Water Environment Federation [WEF], 2005. In: Eaton, A.D., Clesceri, L.S., Rice, E.W., Greenberg, A.H. (Eds.), Standard Methods for the Examination of Water and Wastewater, Baltimore, Maryland21st ed..

Barco, M., Rivera, J., Caixach, J., 2002. Analysis of cyanobacterial hepatotoxins in water samples by microbore reversed-phase liquid chromatography–electrospray ionisation mass spectrometry. Journal of Chromatography A 959, 103–111.

Barco, M., Lawton, L., Rivera, J., Caixach, J., 2005. Optimization of intracellular microcystin extraction for their subsequent analysis by high-performance liquid chromatography. Journal of Chromatography A 1074, 23–30.
Bard, S.M., 2000. Multixenobiotic resistance as a cellular defense mechanism in aquatic organisms. Aquatic Toxicology 48, 357–389.
Beattie, K.A., Ressler, J., Wiegand, C., Krause, E., Codd, G.A., Steinbergd, C.E.W., Pflugmacher, S., 2003. Comparative effects and metabolim of two microcystins and nodularin in the brine shrimp *Artemia salina*. Aquatic Toxicology 62, 219–226.
Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72, 248–254.
Brandalise, M.V., Nadal, F., Rodriguez, M.I., Larrosa, N., Ruiz, M., Halac, S., Olivera, P., Licera, C., 2007. Índice de Calidad de Agua para uso Recreativo en Ambientes con Cianobacterias., http://www.ina.gov.ar/pdf/ifrrhh/02_007_Brandalise.pdf.
Burmester, V., Nimptsch, J., Wiegand, C., 2012. Adaptation of freshwater mussels to cyanobacterial toxins: response of the biotransformation and antioxidant enzymes. Ecotoxicology and Environmental Safety 78, 296–309.
Cazenave, J., Wunderlin, D.A., Bistoni, M.A., Amé, M.V., Krause, E., Pflugmacher, S., Wiegand, C., 2005. Uptake, tissue distribution and accumulation of microcystin-RR in *Corydoras Paleatus*, *Jenynsia multidentata* and *Odontesthes bonariensis*, a field and laboratory study. Aquatic Toxicology 75, 178–190.
Chang, C., Kao, C., 1998. H_2O_2 metabolism during senescence of rice leaves: change in enzyme activities in light and darkness. Plant Growth Regulation 25, 11–15.
Chen, J., Xie, P., 2005. Tissue distributions and seasonal dynamics of the hepatotoxic microocystins-LR and -RR in two freshwater shrimps, *Palaemon modestus* and *Macrobrachium nipponensis*, from a large shallow, eutrophic lake of the subtropical China. Toxicon 45, 615–625.
Chen, J., Xie, P., Guo, L.G., Zheng, L., Ni, L.Y., 2005. Tissue distributions and seasonal dynamics of the hepatotoxic microcystins-LR and -RR in a freshwater snail (*Bellamya aeruginosa*) from a large shallow, eutrophic lake of the subtropical China. Environmental Pollution 134, 423–430.
Chen, J., Zhang, D., Xie, P., Wang, Q., Ma, Z., 2009. Simultaneous determination of microcystin contaminations in various vertebrates (fish, turtle, duck and water bird) from a large eutrophic Chinese lake, Lake Taihu, with toxic *Microcystis* Blooms. Science of the Total Environment 407, 3317–3322.
Contardo-Jara, V., Pflugmacher, S., Wiegand, C., 2008. Multi-xenobiotic-resistance a possible explanation for the insensitivity of bivalves towards cyanobacterial toxins. Toxicon 52, 936–943.
Contardo-Jara, V., Galanti, L., Amé, V., Monferran, M., Wunderlin, D., Wiegand, C., 2009. Biotransformation and antioxidant enzymes of *Limnoperna fortunei* detect site impact in watercourses of Córdoba, Argentina. Ecotoxicology and Environmental Safety 72, 1871–1880.
Da Rosa, C.E., de Souza, M.S., Yunes, J.S., Proença, L.A.O., Nery, L.E.M., Moserrat, J.M., 2005. Cyanobacterial Blooms in estuarine ecosystems: characteristics and effects on *Laeonereis acuta* (Polychaeta, Nereididae). Marine Pollution Bulletin 50, 956–964.
Davies, W.R., Siu, W.H.L., Jack, R.W., Wu, R.S.S., Lam, P.K.S., Nugegoda, D., 2005. Comparative effects of the blue green algae *Nodularia spumigena* and a lysed extract on detoxification and antioxidant enzymes in the green lipped mussel (*Perna viridis*). Marine Pollution Bulletin 51, 1026–1033.
Deblois, C.P., Aranda-Rodriguez, R., Giani, A., Bird, D.F., 2008. Microcystin accumulation in liver and muscle of tilapia in two large Brazilian hydroelectric reservoirs. Toxicon 51, 435–448.
Di Giulio, R.T., 1991. Indices of oxidative stress as biomarkers for environmental contamination. In: Mayes, M.A., Barron, M.G. (Eds.), Aquatic Toxicology and Risk Assessment. American Society for Testing and Materials, Philadelphia, pp. 15–31.
Dörr, F.A., Pinto, E., Soares, R.M., Feliciano de Olivera e Azevedo, S.M., 2010. Microcystins in South American aquatic ecosystems: occurrence, toxicity and toxicological assay. Toxicon 56, 1247–1256.
Drotar, A., Phelps, P., Fall, R., 1985. Evidence for glutathione peroxidase activities in cultured plant cells. Plant Science 42, 35–40.
El Ghazali, I., Saqrane, S., Carvalho, A.P., Ouahid, Y., Del Campo, F.F., Oudra, B., Vasconcelos, V., 2010. Effect of different microcystin profiles on toxin bioaccumulation in common carp (*Cyprinus carpio*) larvae via *Artemia nauplii*. Ecotoxicology and Environmental Safety 73, 762–770.
Engström-Öst, J., Lehtiniemia, M., Greenb, S., Kozlowsky-Suzukid, B., Viitasaloa, M., 2002. Does cyanobacterial toxin accumulate in mysid shrimps and fish via copepods? Journal of Experimental Marine Biology and Ecology 276, 95–107.
Garcia, A.C., Bargu, S., Dash, P., Rabalais, N.N., Sutor, M., Morrison, W., Walker, N.D., 2010. Evaluating the potential risk of microcystins to blue crab (*Callinectes sapidus*) fisheries and human health in a eutrophic estuary. Harmful Algae 2009, 134–143.
Gerard, C., Poullain, V., Lance, E., Acou, A., Brient, L., Carpentier, A., 2009. Influence of toxic cyanobacteria on community structure and microcystin accumulation of freshwater molluscs. Environmental Pollution 157, 609–617.
Gonçalves-Soares, D., Zanette, J., Yunes, J., Yepiz-Plascencia, G.M., Bainy, A.C.D., 2012. Expression and activity of glutathione S-transferases and catalase in the shrimp *Litopenaeus vannamei* inoculated with a toxic *Microcystis aeruginosa* strain. Marine Environmental Research 17, 54–61.
Gurbuz, F., Metcalf, J.S., Gul Karahan, A., Codd, G.A., 2009. Analysis of dissolved microcystins in surface water samples from Kovada Lake, Turkey. Science of the Total Environment 407, 4038–4046.
Gutiérrez-Praena, D., Jos, Á., Pichardo, S., Moreno, I.M., Cameán, A.M., 2013. Presence and bioaccumulation of microcystins and cylindrospermopsin in food and the effectiveness of some cooking techniques at decreasing their concentrations: A review. Food and Chemical Toxicology 53, 139–152.
Habig, W., Pabst, M., Jakoby, W., 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry 249, 7130–7139.
Halliwell, B., Gutteridge, J.M.C., 2007. Free Radicals in Biology and Medicine, 4th ed. Oxford University Press, New York.
Henriksen, P., 2005. Estimating nodularin, content of cyanobacterial blooms from abundance of *Nodularia spumigena* and its characteristic pigments – a case study from the Baltic entrance area. Harmful Algae 4, 167–178.
Honkanen, R.E., Codispoti, B.A., Tse, K., Boynton, A.L., 1994. Characterization of natural toxins with inhibitory activity against serine/threonine protein phosphatases. Toxicon 32, 339–350.
InfoStat, 2011. Grupo InfoStat. Facultad de Ciencias Agropecuarias. Universidad Nacional de Córdoba, Córdoba, Argentina.
Ito, E., Takai, A., Kondo, F., Masui, H., Imanishi, S., Harada, K., 2002. Comparison of protein phospatase inhibitory activity and apparent toxicity of microcystins and related compounds. Toxicon 40, 1017–1025.
Kankaanpää, H.T., Holliday, J., Shröder, H., Goddard, T.J., Von Fister, R., Carmichael, W.W., 2005. Cyanobacteria and prawn farming in northern New South Wales, Autralia – a case study on cyanobacteria diversity and hepatotoxin bioaccumulation. Toxicology and Applied Pharmacology 203, 243–256.
Kankaanpää, H., Leiniö, S., Olin, M., Sjövall, O., Meriluoto, J., Lehtone, K., 2007. Accumulation and depuration of cyanobacterial toxin nodularin and biomarker responses in the mussel *Mytilus edulis*. Chemosphere 68, 1210–1217.
Krienitz, L., Ballor, A., Kotut, K., Wiegand, C., Pütz, S., Metcalf, J.S., Codd, G.A., Pflugmacher, S., 2003. Contribution of hot spring cyanobacteria to the mysterious deaths of Lesser Flamingos at Lake Bogoria, Kenya. FEMS Microbiology Ecology 43, 141–148.
Kuiper-Goodman, T., Falconer, I., Fitzgerald, J., 1999. Human health aspects. In: Chorus, I., Bartram, J. (Eds.), Toxic Cyanobacteria in the Water. E. & F.N. Spon, London, pp. 113–153.
Lance, E., Josso, C., Dietrich, D., Ernst, B., Paty, C., Senger, F., Bormans, M., Gérard, C., 2010. Histopathology and microcystin distribution in *Lymnaea stagnalis* (Gastropoda) following toxic cyanobacterial or dissolved microcystin-LR exposure. Aquatic Toxicology 98, 211–220.
Lawton, L., Edwards, C., Codd, G., 1994. Extraction and high-performance liquid chromatography method for the determination of microcystin in raw and treated water. Analyst 119, 1525–1530.
Lehtimäki, N., Shunmugam, S., Jokela, J., Wahlsten, M., Carmel, D., Keränen, M., Sivonen, K., Aro, E.M., Allahverdiyeva, Y., Mulo, P., 2011. Nodularin uptake and induction of oxidative stress in spinach (*Spinachia oleracea*). Journal of Plant Physiology 168, 594–600.
Lehtonen, K.K., Kankaanpää, H., Leiniö, S., Sipiä, V.O., Pflugmacher, S., Sandberg-Kilpi, E., 2003. Accumulation of nodularin like compounds from the cyanobacterium *Nodularia spumigena* and changes in acetylcholinesterase activity in the clam *Macoma balthica* during short-term laboratory exposure. Aquatic Toxicology 64, 461–476.
Liras, V., Lindberg, M., Nystrom, P., Annadotter, H., Lawton, L.A., Graf, B., 1998. Can ingested cyanobacteria be harmful to the signal crayfish (*Pacifastacus leniusculus*)? Freshwater Biology 39, 233–242.
Magalhães, V.F., Soares, R.M., Azevedo, S.M.F.O., 2001. Microcystin contamination in fish from the Jacarepaguá Lagoon (Rio de Janeiro, Brazil): ecological implication and human health risk. Toxicon 39, 1077–1085.
Malbrouck, C., Kestemont, P., 2006. Effects of microcystins on fish. Environmental Toxicology and Chemistry 25, 72–86.
Mazur, H., Plinski, M., 2003. Nodularia spumigena blooms and the occurrence of hepatotoxin in the Gulf of Gdansk. Oceanologia 45 (1) 305–316.
Mekebri, A., Blondina, G.J., Crane, D.B., 2009. Method validation of microcystins in water and tissue by enhanced liquid chromatography tandem mass spectrometry. Journal of Chromatography A 1216, 3147–3155.
Mitrovic, S.M., Allis, O., Furey, A., James, K.J., 2005. Bioaccumulation and harmful effects of microcystin-LR in the aquatic plants *Lemna minor* and *Wolffia arrhiza* and the filamentous alga *Chladophora fracta*. Ecotoxicology and Environmental Safety 61, 345–352.
Mohamed, Z.A., Carmichael, W.W., Hussein, A.A., 2003. Estimation of microcystins in the freshwater fish *Oreochromis niloticus* in an Egyptian fish farm containing a *Microcysts* blooms. Environmental Toxicology 18, 137–141.
Monferrán, M.V., Galanti, L.N., Bonansea, R.I., Amé, M.V., Wunderlin, D.A., 2011. Integrated survey of water pollution in the Suquía River basin (Córdoba, Argentina). Journal of Environmental Monitoring 13, 398–409.
Montagna, M.C., Collins, P.A., 2007. Survival and growth of *Palaemonetes argentinus* (Decapoda; Caridea) exposed to insecticides with chlorpyrifos and endosulfan as active element. Archives of Environmental Contamination and Toxicology 53, 371–378.
Morrone, J.J., Lopreto, E.C., 1995. Parsimony analysis of endemicity of freshwater Decapoda (Crustacea: Malacostraca) from Southern South America. Neotropica 41, 3–8.
Mulvenna, V., Dale, K., Priestly, B., Mueller, U., Humpage, A., Shaw, G., Allinson, G., Falconer, I., 2012. Health risk assessment for cyanobacterial toxins in seafood. International Jounal of Environmental Research and Public Health 9, 807–820.
Nishiwaki-Matsushina, R., Nishiwaki, S., Ohta, T., Yoszawa, S., Suganuma, M., Harada, K., Watanabe, M.F., Fujiki, H., 1991. Structure function relationships of microcystins, liver-tumor promoters, in interaction with protein phosphatase. Japanese Journal of Cancer Research 82, 993–996.

Nishiwaki-Matsushina, R., Oht, T., Nishiwaki, S., Suganuma, M., Yoszawa, S., Kohyama, K., Ishikaawa, Y., Carmichael, W.W., Fujiki, H., 1992. Liver tumor promotion by the cyanobacterial cyclic peptide toxin microcystin-LR. Journal of Cancer Research and Clinical Oncology 118, 420–424.

Pesce, S., Wunderlin, D., 2000. Use of water quality index to verify the impact of Córdoba city (Argentina) on Suquía river. Water Research 3, 2915–2926.

Pflugmacher, S., Wiegand, C., Oberemm, A., Beattie, K.A., Krause, E., Codd, G.A., Steinberg, C.E.W., 1998. Identification of an enzimatically formed glutathione conjugate of the cyanobaterial hepatoxin microcystin-LR: the first step of detoxification. Biochimica et Biophysica Acta 1425, 527–533.

Pflugmacher, S., Wiegand, C., Codd, G.A., Steinberg, C.E.W., 1999. Effects of the cyanobacterial toxin microcystin-LR on detoxification enzymes in aquatic plants. Environmental Toxicology 14 (1) 111–115.

Pflugmacher, S., Weigand, C., Beattie, K.A., Krause, E., Steinberg, C.E.W., Codd, G.A., 2001. Uptake, effects, and metabolism of cyanobacterial toxins in the emergent reed plant *Phragmites australis* (CAV.) Trin. Ex Steud. Environmental Toxicology and Chemistry 20, 846–852.

Pflugmacher, S., Wiegand, C., Werner, S., Shröder, H., Kankaanpää, H., 2005. Activity and substrate specificity of cytosolic and microsomal glutathione S-transferase in Australian black tiger prawns (*Penaeus monodon*) after exposure to cyanobacterial toxins. Environmental Toxicology 20, 301–307.

Pflugmacher, S., Olin, M., Kankaanpää, H., 2007. Nodularin induces oxidative stress in the Baltic Sea brown alga *Fucus vesiculosus* (Phaeophyceae). Marine Environmental Research 64, 149–159.

Pflugmacher, S., Olin, M., Kankaanpää, H., 2010. Oxidative stress response in the red alga *Furcellaria lumbricalis* (Huds) Lamour. due to exposure and uptake of the cyanobacterial toxin nodularin from *Nodularia spumigena*. Harmful Algae 10, 49–55.

Pizzolón, L., Tracanna, B., Silva, H., Prósperi, C., Fabricius, A.L.M., Emiliani, M.O.G., Otaegui, A.V., Amalfi, M., Labollita, H., Santinelli, N., Sastre, V., 1997. Inventario de ambientes dulceacuícolas de la Argentina con riesgo de envenenamiento por cianobacterias (toxic cyanobacterial blooms in Argentinean freshwaters). Ingeniería Sanitaria Ambiental 33, 26–34.

Reed, D.J., 1986. Regulation of reductive processes by glutathione. Biochemical Pharmacology 35 (1) 7–13.

Ruibal Conti, A.L., Reguerira, M., Guerrero, J.M., 2005. Levels of microcystins in two reservoirs used for water supply and recreation: differences in the implementation of safe levels. Environmental Toxicology 20, 263–269.

Sáenz, L.A., Seibert, E.L., Zanette, J., Fiedler, H.D., Curtius, A.J., Ferreira, J.F., Alves de Almeida, E., Freire Marques, M.R., Dias Bainy, A.C., 2010. Biochemical biomarkers and metals in Perna perna mussels from mariculture zones of Santa Catarina, Brazil. Ecotoxicology and Environmental Safety 73, 796–804.

Scarafia, M.E., Agnese, A.M., Cabrera, J.L., 1995. Microcystis aeruginosa: behaviour and toxic features in San Roque Dam (Argentina). Natural Toxins 3, 75–77.

Shumway, S.E., 1990. A review of the effects of algal blooms on shellfish and aquaculture. Journal of the World Aquaculture Society 21, 65–104.

Sipiä, V.O., Kankaanpää, H., Pflugmacher, S., Flinkman, J., Furey, A., Jame, K.J., 2002. Bioaccumulation and detoxification of Nodularin in Tissues of Flounders (*Platichthys flesus*), Mussels (*Mytilus edulis*), and Clams (*Macoma balthica*) from the northern Baltic Sea. Ecotoxicology and Environmental Safety 53, 305–311.

Sipiä, V.O., Nefling, M., Metcalf, J.S., Nybom, S.M.K., Meriluoto, J.A.O., Codd, G.A., 2008. Nodularin in feathers and liver of eiers (somateria mollisima) caught from the western Gulf of Finland in June–September 2005. Harmful Algae 7, 99–105.

Sivonen, K., Kononen, K., Carmichael, W.W., Dahlem, A.M., Rinehart, K.L., Kiviranta, J., Niemela, S.I., 1989. Occurrence of the hepatotoxic cyanobacterium *Nodularia spumigena* in the Baltic sea and structure of the toxin. Applied and Environmental Microbiology 55 (8) 1990–1995.

Smith, J.L., Boyer, G.L., Zimba, P.V., 2008. A review of cyanobacterial odorus and bioactive metabolites: impacts and management alternatives in aquaculture. Aquaculture 280, 5–20.

Smith, J., Boyer, G.L., 2009. Standardization of microcystin extraction from fish tissues: a novel internal standard as a surrogate for polar and non-polar variants. Toxicon 53, 238–245.

Smolders, R., Bervoets, L., Blust, R., 2004. In situ and laboratory bioassays to evaluate the impact of effluent discharges on receiving aquatic ecosystems. Environmental Pollution 132, 231–243.

Spivak, E.D., 1997. Life history of a brackish-water population of *Palaemonetes argentinus* (Decapoda: Caridea) in Argentina. Annales de Limnologie 33 (3) 179–190.

Storey, K.B., 1996. Oxidative stress: animal adaptations in nature. Brazilian Journal Of Medical and Biological Research 29, 1715–1733.

Tanaka, K., Sano, Y., Ishizuka, K., Kitta, K., Kawamura, Y., 1994. Comparison of properties of leaf and root glutathione reductases from spinach. Plant Physiology 91, 353–358.

Tricarico, E., Bertocchi, S., Brusconi, S., Casalone, E., Gherardi, F., Giorgi, G., Matromei, G., Parisi, G., 2008. Depuration of microcystin-LR from the red swamp crayfish *Procambarus clarkii* with assessment of its food quality. Aquaculture 285, 90–95.

van der Oost, R., Beyer, J., Vermeulen, N.P., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environmental Toxicology and Pharmacology 13, 57–149.

Van Buynder, P.G., Oughtred, T., Kirkby, B., Phillips, S., Eaglesham, G., Thomas, K., Burch, M., 2001. Nodularin uptake by seafood during a cyanobacterial bloom. Environmental Toxicology 16 (6) 468–471.

Vasconcelos, V.M., 1995. Uptake and depuration of the heptapeptide toxin microcystin-LR in *Mytilus galloprovincialis*. Aquatic Toxicology 32, 227–237.

Vasconcelos, V., Aliveira, S., Oliva Teles, F., 2001. Impact of a toxic and non-toxic strain of *Microcystis aeruginosa* on the crayfish *Procambarus clarkii*. Toxicon 39, 1462–1470.

Wang, W., Ballatori, N., 1998. Endogenous glutathione conjugates: occurrence and biological functions. American Society for Pharmacology and Experimental Therapeutics 50 (3) 335–355.

WHO (World Health Organization), 2003. Algae and cyanobacteria in fresh water. In: Guidelines for Safe Recreational Water Environments. Coastal and Fresh Waters, vol. 1. World Health Organization, Geneva, Switzerland, , pp. 136–158.

Wiegand, C., Pflugmacher, S., 2005. Ecotoxicological effects of selected cyanobacterial secondary metabolites a short review. Toxicology and Applied Pharmacology 203, 201–218.

Williams, D.E., Dawe, S.C., Kent, M.L., Andersen, R.J., Graig, M., Holmes, C.F.B., 1997. Bioaccumulation and clearance of microcystins from salt water mussels, *Mytilus edulis*, and *in vivo* evidence for colavently bound microcystins in mussel tissues. Toxicon 35 (11) 1617–1625.

Wood, S.A., Phillips, N.R., Winton, M., Gibbs, M., 2012. Consumption of benthic cyanobacterial mats and nodularin-R accumulation in freshwater crayfish (*Paranephrops planifrons*) in Lake Tikitapu (Rotorua, New Zealand). Harmful Algae 20, 175–179.

Yoshizawa, S., Matsushima, R., Watanabe, M., Harada, K., Ichihara, A., Carmichael, W.W., Fujiki, H.J., 1990. Inhibition of protein phosphatases by microcystin and nodularin associated with hepatotoxicity. Journal of Cancer Research and Clinical Oncology 116, 609–614.

Zhang, J., Wang, Z., Song, Z., Xie, Z., Li, L., Song, L., 2012. Bioaccumulation of microcystins in two freshwater gastropods from a cyanobacteria-bloom plateau lake, Lake Dianchi. Environmental Pollution 164, 227–234.

Zurawell, R.W., Chen, H., Burke, J.M., Prepas, E.E., 2005. Hepatotoxic cyanobacterial: a review of the biological importance of microcystins in freshwater environments. Journal of Toxicology and Environmental Health 69, 303–3018.