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Microcystin–LR, –RR, –YR and –LA in water samples and fishes from a shallow lake in Argentina

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ABSTRACT

We evaluated the presence of four common microcystins (MC–LR, –RR, –YR and –LA) in water samples and tissues (liver and muscle) of *Odontesthes bonariensis* collected in Los Padres Lake (Argentina). MCs were quantified by HPLC–ESI-MS/MS. The total content of MCs in water samples (2.8 ± 5.6 μg L^{-1}) and in *O. bonariensis* muscle (3.9 ± 2.2 μg kg^{-1}) never surpassed guidelines values recommended by WHO for either recreational use of water or fish consumption. The total content of MCs in *O. bonariensis* liver was 33.6 ± 37.2 μg kg^{-1}. However, we observed both spatial and temporal changes in MC profile, suggesting the need of an intensive monitoring program at such lake to ensure the health of people living in its surrounding.

We observed a significant correlation between the content of total and dissolved MC in water and the concentration of these toxins in liver, showing the probable uptake of dissolved MC by *O. bonariensis*.

MC–RR was the dominant variant in water samples, followed by MC–LA and MC–LR. MC–YR was always present in water samples, but in the lowest concentrations of the congeners measured.

MC content in liver in *O. bonariensis* was ten-fold higher than the corresponding content in fish muscle. However, MCs were present in muscle of *O. bonariensis* during both wet and dry season, while we could not detect MCs in liver during the dry season. These results demonstrate that MC accumulation in liver and muscle of *O. bonariensis* occurred with different profiles.

Moreover, different uptake, tissue distribution and excretion pathways may occur between diverse MC variants. For instance, during the dry season, an increase in the relative percentage of MC–LR in water samples was accompanied with an augmentation in the relative percentage of this toxin in muscle of *O. bonariensis*. Conversely, a rise in the relative percentage of MC–LA in water samples during the dry season did not impact on the distribution of this toxin in fish muscle. These results suggest the need of further studies on dynamics of different variants of MC to pursuit a complete evaluation of human health risk associated to MC occurrence.

1. Introduction

Toxic cyanobacterial blooms occur worldwide in eutrophic lakes, rivers and reservoirs (Havens et al., 2003). However, there is little information of cyanotoxins presence in South America (Hirooka et al., 1999; Campos et al., 1999; Chellappa et al., 2000; Matthiensen et al., 2000; Neumann et al., 2000; Codd et al., 2005; dos S. Vieira et al., 2005) and only few reports in Argentina, even when toxic cyanobacteria have been identified in 13 out of 22 provinces along the country (Amé et al., 2003; Ruibal Conti et al., 2005; Cazenave et al., 2005; Andrinolo et al., 2007). Among cyanotoxins, microcystins (MCs) are considered to be one of the most dangerous groups, which are known to be potent hepatotoxin (Dawson, 1998) and tumor promoter (Nishiwaki-Matsushima et al., 1992). It is believed that MC is released from cyanobacteria after cell lysis, whereas only negligible amounts of toxins are apparently released from healthy cells. However, a high concentration of dissolved MC could be reached in water immediately after the collapse of a highly toxic bloom (Sivonen and Jones, 1999). Consequently, the concentrations of dissolved MC in the environment may vary from traces up to 1800 μg L^{-1} or higher (Chorus and Bartram, 1999).

Biota may take up and accumulate MC via two main routes: dissolved toxins via a transdermal route or ingestion via the intake of food (Ibelings and Chorus, 2007). There have been extensive studies on MC bioaccumulation in fishes (Råbergh et al., 1991;

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Sahin et al., 1996; Williams et al., 1997; Pflugmacher et al., 1998; Wiegand et al., 1999; Lawrence and Menard, 2001; Magalhães et al., 2001, 2003; Malbrouck et al., 2003; Soares et al., 2004; Cazenave et al., 2005; Li et al., 2004; Xie et al., 2004, 2005). However, most of these studies were performed in laboratories.

Over 70 structural analogues of these heptapeptides have been identified up to date, but only a few occur frequently in high concentrations. Minor structural changes may have major effects on uptake, organ distribution and excretion of these toxins (Dietrich and Hoeger, 2005). Nevertheless, as MC–LR is the most widely commercially available variant, most of accumulation studies carried out in laboratories are focused on this congener.

The main goal of this study was to evaluate the presence of four common microcystins (MC–LR, –RR, –YR and –LA) in water samples and fishes (liver and muscle) captured in Los Padres Lake (Argentina), looking to associate the levels of MC found in water with those observed in fish tissues.

2. Materials and methods

2.1. Site description

Los Padres Lake is a shallow, polimictic waterbody, 2.16 km^2 surface, located in the southeastern area of the Province of Buenos Aires, Argentina (37°56′17.09″ South; 54°44′11.17″ West). Los Padres Lake has one tributary, Los Padres Creek, and one effluent, La Tapera Creek. The flow of La Tapera Creek is regulated in rainy season to avoid the flood of adjacent fields. This stream discharges in the Atlantic Ocean. Giant bulrush (*Schoenoplectus californicus*) covers seventy percent of the surface of the lake; however, fishing, swimming, and sailing are some of the activities practiced in and around its waters. *Planktothrix agardhii*, *Anabaena* sp. and *Microcystis* sp. presence and bloom events have been previously reported (Esquiús et al., 2005, 2008; INIDEP, unpublished data).

2.2. Sampling

Two monitoring sites were selected for this study. Station 1 located in a small bay nearby the effluent of the lake and station 2 close to the mouth of the only tributary. Water samples from both sites and fish from station 1 were collected during 2007. Samplings were performed in July and August (dry season) and in November and December (wet season) of 2007. For MCs analysis, up to 1 L of lake water taken from the surface was filtered through membrane filters (0.45 μm pore size; Millipore, Billerica, USA) using a vacuum pump. The filters were air-dried, packed in aluminium foil and stored at −20 °C. Filtered water samples were maintained in glass bottles at −20 °C until analysis (Ballot et al., 2005; Cazenave et al., 2005). Every water sample was taken by triplicate. *Odontesthes bonariensis* (average weight: 58.00 ± 7.35 g; $n = 20$) were captured by rod fishing. This fish is ubiquitous in eutrophic lakes of Argentina (and widely distributed in South America), where is captured by both sport and professional fishermen. After capture, fish were sacrificed, ice-cooled, and transported to the laboratory, where they were immediately dissected, subsequently liver and muscle (ca. 1.5 g of liver and 5 g of muscle for each fish) were separated. Tissues were stored at −20 °C in aluminium foil until analysis.

Simultaneously, some water quality parameters we determined to establish the environmental conditions of sampling area. Analytical methods were standard; APHA (1998) method numbers are cited in parentheses. Measured parameters include: pH (4500-H+ B, field measured), dissolved oxygen (DO, 4500-O C), nitrites (4500-NO2) and orthophosphate phosphorus (4500-P).

2.3. Microcystins extraction

Determination of cellular MC in water was carried out according to Amé et al. (2003). Briefly, 0.45 μm air-dried filters were placed in glass tubes, covered with 1.5 mL of 5% acetic acid and sonicated for 5 min in an ultrasonic bath. The suspension was centrifuged at 9300 × *g* for 3 min, supernatant was retained and the filters re-extracted as before. Combined supernatants were centrifuged at 9300 × *g* for 10 min. Centrifuged supernatants were applied to a C-18 solid phase extraction cartridge (LiChrolut RP-18, 500 mg, Merck), which was previously conditioned with methanol (10 mL) and 5% acetic acid (10 mL). The cartridge was washed with 10 mL of 10, 20 and 30% aqueous methanol and toxins were eluted with 3 mL of pure methanol. The eluate was evaporated to dryness under reduced pressure (40 °C, 0.3 Torr) and resuspended in 500 μL of methanol prior to high performance liquid chromatography tandem MS/MS (HPLC–MS/MS) analysis (Amé et al., 2003).

For the determination of free dissolved MC, water samples (1 L) were conditioned with 5% acetic acid and applied to 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., 2003).

Total MC content in water was obtained by the addition of cellular and dissolved MC amount in the water sample.

For MC extraction from tissues, the method described by Cazenave et al. (2005) was used. Briefly: tissues (fresh weight) were homogenized with 70% methanol containing 1% (v/v) trifluoracetic acid, using an Ultra-Turrax homogenizer. Homogenates were introduced for 5 min in an ultrasonic bath (Ultrasonic 300), followed by centrifugation at 12,096 × *g* for 10 min. Supernatants were separated and evaporated to dryness at 40 °C under reduced pressure. Dried methanolic extraction residues of liver and muscle from *O. bonariensis* were added to 5% acetic acid. Acid extracts were 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. MCs were eluted with methanol (3 mL, HPLC grade). The eluate was evaporated to dryness at 40 °C under reduced pressure and suspended in 500 μL methanol (HPLC grade) (Amé et al., 2003). After this extraction and clean up procedures, clean extracts of fish tissues were analyzed by HPLC–MS/MS. The concentration of MC in tissues is expressed per kilogram of fresh weight.

2.4. Microcystins analysis by HPLC–MS/MS

MS/MS equipment: Varian 1200 triple quadrupole, equipped with an ESI ion source operated in positive mode with nitrogen as main gas (75.5 psi), drying gas (21 psi, 300 °C) and nebulizing gas (50 psi); needle 5000 V and shield at 600 V. MCs were recorded using MRM mode by selecting characteristic MC ions at the first quadrupole (Q1): 519.8 (MC–RR, $[MC+2H]^{2+}$), 910.6 (MC–LA, $[M+H]^{+}$), 995.6 (MC–LR, $[M+H]^{+}$), 1045.5 *m*/*z* (MC–YR, $[M+H]^{+}$). These ions were fragmented in Q2 using Ar and variable collision energy for each MC analogue to produce the ion *m*/*z* 135, characteristic of ADA moiety. Both characteristic MC ions and the fragment *m*/*z* 135 were analyzed at Q3; thus, using MRM mode. MCs were quantified by HPLC coupled to ESI-MS/MS using a column Varian Polaris 5μ 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), and a program starting with 20% B, changing to 80% B within 12 min, held by 5 min, returning to 20% B in one minute and keeping this condition

for 7 additional minutes to achieve column stabilization before next run (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. Quantification was performed using external standard method, with pure MC dissolved in methanol (1–1000 $\mu g\ L^{-1}$). The limit of detection (LOD) = 1.5 $\mu g\ L^{-1}$ (15 pg on column) was established considering a signal to noise ratio of 10 ($S/N \geq 10$); while the limit of quantification (LOQ) = 4.5 $\mu g\ L^{-1}$ (45 pg on column) was decided on the basis of linear regression results ($S/N \geq 80$), which are in good agreement with values reported in literature (Barco et al., 2002), although slightly higher than reports using LC–ESI-MS/MS (MRM) (Mekebri et al., 2009). Linearity on standard curves looks good between LOQ and 1000 $\mu g\ L^{-1}$ for all the MC variants ($R^2 > 0.9949$). Both sample and standard solutions were analyzed by triplicate. The LOD and LOQ corresponding to each sample (water or the analyzed organ), depend on the volume of water and weight of tissue used during the extraction procedure. 1000 mL of water were used to water analysis, thus giving a calculated LOD of 0.75 $ng\ L^{-1}$ and a calculated LOQ of 2.25 $ng\ L^{-1}$. During the analysis of *O. bonariensis* we used approximately 1.5 g of liver and 5.0 g of muscle, thus giving a calculated LOD of 0.5 and 0.15 $\mu g\ kg^{-1}$ and a calculated LOQ of 1.5 and 0.45 $\mu g\ kg^{-1}$ for liver and muscle respectively. Recovery percentages were evaluated from spiked samples. Thus, water samples were spiked with 1 and 10 $\mu g\ L^{-1}$ pure MC, followed by SPE extraction and further HPLC–MS/MS. Recovery percentage in water samples was always over 85%. On the other hand, recovery from organs was evaluated using 5.0 g muscle of *O. bonariensis* (free of MC), homogenized as previously described, and spiked with pure MC equivalent to 0.5 and 5 $\mu g\ kg^{-1}$, affording recoveries over 80% for all the MC variants tested. All recovery analyses were run by duplicate.

2.5. Statistics

All values are expressed as mean ± standard deviation. Normal distribution for data was analyzed by Shapiro–Willks test. One-way ANOVA followed by Duncan's test were carried out for comparing different treatments. Person correlation test was used to establish association between different variables. The InfoStat/P software (2001) was employed in all cases. Significance was accepted for $p < 0.05$.

3. Results

3.1. Water

Water quality, total MC, MC–LR, –RR, –LA and –YR concentrations in wet and dry season are reported in Table 1. All the water quality parameters revealed significant seasonal variation. Water was always fully saturated with oxygen, mainly in station 2 probably due to increased photosynthesis by the large quantities of algae. Similar nutrients and pH values have been observed in other mesotrophic or eutrophic waterbodies (Ryding and Rast, 1992; Amé et al., 2003; Cazenave et al., 2005).

The HPLC–MS/MS analysis showed the presence of MC in all the studied water samples. Total MC concentration varied from 0.16 to 15.77 $\mu g\ L^{-1}$. No significant differences were observed for total MC content in station 1 between seasons. However, station 2 showed significantly higher total MC concentration in the dry season as compared to the wet season.

Four variants: MC–LR, –RR, –LA and –YR were identified in water samples of Los Padres Lake. Both cellular and dissolved MC were measured. MC–RR was the main toxin found in both fractions (1.30 ± 2.92 and 1.50 ± 2.15 $\mu g\ L^{-1}$ cellular and dissolved respectively). MC–LA and MC–YR showed significant differences between dissolved and cellular content ($p < 0.05$), showing higher levels of cellular toxin for MC–LA (0.29 ± 0.73 and 0.13 ± 0.16 $\mu g\ L^{-1}$ cellular and dissolved respectively) and enhanced amounts of dissolved toxin for MC–YR (0.01 ± 0.01 and 0.04 ± 0.07 $\mu g\ L^{-1}$ cellular and dissolved respectively). MC–LR did not vary significantly between both fractions (0.05 ± 0.09 and 0.04 ± 0.05 $\mu g\ L^{-1}$ cellular and dissolved respectively).

The relative percentages of MC variants in water, sorted by station and season, are plotted in Fig. 1. The main differences observed between seasons were the absence of MC–RR during the dry season in station 1 and the elevation in the relative percentage of MC–LA during the dry season in station 2.

3.2. Fish

Examination of gastrointestinal tract of *O. bonariensis* showed presence of phytoplankton material, evidencing the ingestion of bloom material by this fish species.

Four variants: MC–LR, –RR, –LA and –YR were also detected by HPLC MS/MS in fish tissues collected in Los Padres Lake. The levels of these MC were measured in liver and muscle of *O. bonariensis* collected in station 1 during both wet and dry season (Table 2). The highest content of total MC was found in liver during wet season (67.3 ± 18.3 $\mu g\ kg^{-1}$). Unexpectedly, we did not detect MC in any liver sample corresponding to the dry season. On the contrary, four variants of MC were present in muscle of *O. bonariensis* during wet and dry season (Table 2).

The relative percentages of MC variants, sorted by tissue and season, are shown in Fig. 2. It is clear that the relative percentages of the four MC measured in liver and muscle were very similar during the wet season. However, relative percentages of MC–LR are significantly increased in muscle of *O. bonariensis* during the dry season.

3.3. Correlation analysis

Pearson correlation analysis was used to establish associations between MC in water and fish. The first correlation analysis was

Table 1
Water quality parameters, total MC, MC–LR, –RR, –LA and –YR concentrations in water from Los Padres Lake, evaluated during both dry and wet season of 2007.

Parameter	Station 1			Station 2		
	Wet	Dry	Average	Wet	Dry	Average
DO ($mg\ L^{-1}$)	8.94 ± 0.23	8.48 ± 0.15	8.81 ± 0.30	15.40 ± 0.00	14.26 ± 1.24	14.69 ± 1.12
Nitrites ($mg\ L^{-1}$)	0.070 ± 0.004	0.056 ± 0.002	0.067 ± 0.007	0.082 ± 0.004	0.049 ± 0.003	0.062 ± 0.017
pH	9.03 ± 0.13	8.80 ± 0.00	8.97 ± 0.15	8.27 ± 0.10	9.24 ± 0.05	8.88 ± 0.49
Phosphates ($mg\ L^{-1}$)	0.029 ± 0.005	0.031 ± 0.001	0.029 ± 0.004	0.037 ± 0.000	0.039 ± 0.000	0.038 ± 0.001
Total MC ($\mu g\ L^{-1}$)	0.43 ± 0.32	0.22 ± 0.05	0.37 ± 0.29	0.23 ± 0.01	14.96 ± 0.72	7.60 ± 8.08
MC–LR ($\mu g\ L^{-1}$)	0.01 ± 0.01	0.08 ± 0.03	0.03 ± 0.03	0.00 ± 0.01	0.32 ± 0.09	0.16 ± 0.18
MC–RR ($\mu g\ L^{-1}$)	0.39 ± 0.32	0.00 ± 0.00	0.29 ± 0.33	0.21 ± 0.02	12.37 ± 1.16	6.29 ± 6.70
MC–LA ($\mu g\ L^{-1}$)	0.02 ± 0.01	0.11 ± 0.01	0.04 ± 0.04	0.02 ± 0.01	2.14 ± 0.85	1.08 ± 1.28
MC–YR ($\mu g\ L^{-1}$)	0.01 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.00 ± 0.01	0.13 ± 0.00	0.07 ± 0.07

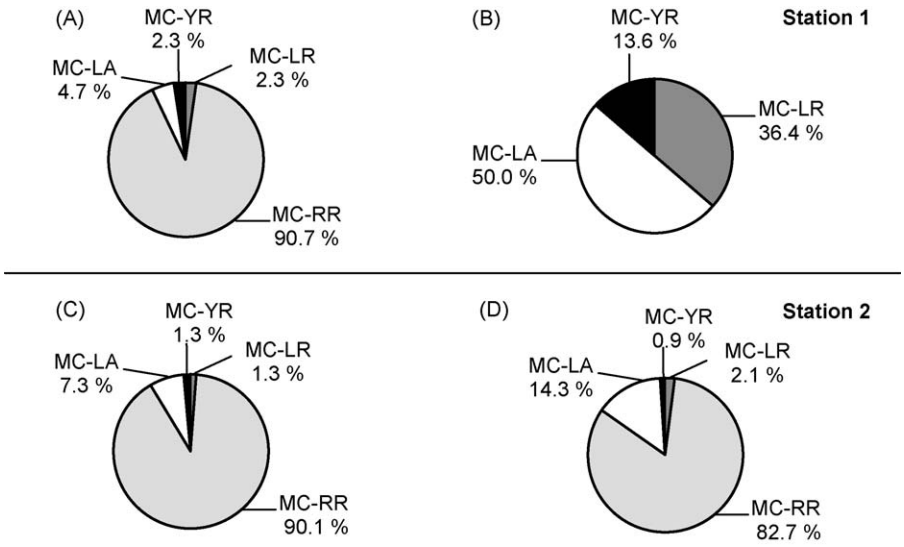

Fig. 1. Relative distribution of MC–LR, –RR, –LA and –YR in water samples from Los Padres Lake sorted by station and season: (A) wet season, station 1; (B) dry season, station 1; (C) wet season, station 2; (D) dry season, station 2.

performed considering the complete studied period, without discrimination between seasons. Total MC content in water showed a significant correlation with total MC content in *O. bonariensis* liver ($r = 0.60$, $p < 0.05$) but not in muscle ($r = -0.44$, $p > 0.05$). However, when the correlation was calculated considering either cellular or dissolved toxin in water and total MC content in liver and muscle, only dissolved MC showed significant correlation with MC content in fish liver ($r = 0.63$, $p < 0.05$). Not significant correlations between the amount of total MC in liver and total MC in muscle of fish were observed.

In view of the differences observed in fish tissues between dry and wet season (Table 2 and Fig. 2), another correlation analysis was performed between MC content (total and MC variants) in liver and muscle but now, considering only wet season. Significant correlation between MC contents in both liver and muscle were found ($r = 0.99, 0.93, 0.99, 0.73, 0.84$, for total MC, MC–LR, –LA, –RR and –YR respectively, $p < 0.05$).

4. Discussion

4.1. Total microcystins levels and human health risk in Los Padres Lake

To reduce risks caused by MC, the WHO has published protocols concerning their detection and recommendations for maximum permitted concentrations (1 $\mu g\,L^{-1}$) in water destined for human consumption (WHO, 1998). Recreational exposure to cyanotoxins can also pose substantial hazards to public health. However, complicating factors such as the age of users, many potential exposure pathways and varying durations of exposure (Chorus et al., 2000; WHO, 2003) mean that guidelines for acceptable recreational exposure range from the more conservative 20 $\mu g\,L^{-1}$ MC suggested by WHO (2003) to as high as 100 $\mu g\,L^{-1}$ MC (Fromme et al., 2000).

Table 2
MC concentration in liver and muscle of *O. bonariesis* collected in station 1, Los Padres Lake, during both dry and wet season of 2007.

	Concentration of microcystin ($\mu g\,kg^{-1}$ fresh weight)					
	Liver			Muscle		
	Wet	Dry	Average	Wet	Dry	Average
MC–LR	2.4 ± 0.9	<LOD	1.2 ± 1.4	<LOQ	1.0 ± 1.0	0.7 ± 0.9
MC–RR	49.2 ± 15.8	<LOD	24.6 ± 27.8	1.6 ± 1.	3.0 ± 2.6	2.5 ± 2.2
MC–LA	13.7 ± 1.6	<LOD	6.9 ± 7.3	0.5 ± 0.3	0.8 ± 0.8	0.7 ± 0.6
MC–YR	1.9 ± 0.7	<LOD	0.9 ± 1.1	<LOQ	<LOQ	<LOQ
Total MC	67.3 ± 18.3	<LOD	33.6 ± 37.2	2.2 ± 1.3	4.9 ± 2.0	3.9 ± 2.2

<LOD, below detection limit; <LOQ, below quantification limit.

MC has been detected in every sample analyzed during our study. However, MC concentrations rarely surpassed the level suggested by WHO for drinking water nor exceeded the suggested recreational exposure guideline. Our results are in good agreement with MC levels observed in South America. MC was present in 9 out of 50 (18%) water supplies from five regions of Paraná, Brazil, reaching 6.6 $\mu g\,L^{-1}$. Moreover, dos S. Vieira et al. (2005) reported MC at concentrations reaching 1.25 $\mu g\,L^{-1}$ in surface water collected from the Utinga Reservoir (Brazil). In Argentina, Ruibal Conti et al. (2005) reported 0.16 $\mu g\,L^{-1}$ MC in Paso de las Piedras reservoir. Also Ruibal Conti et al. (2005) reported 6.0 $\mu g\,L^{-1}$ MC in the center of San Roque Reservoir (Córdoba, Argentina).

Total MC content in muscle *of O. bonariensis* collected in Los Padres Lake ranged from 1.0 to 8.0 $\mu g\,kg^{-1}$ fresh weight. These results are in good agreement with previous reports (Magalhães et al., 2001, 2003; Chen et al., 2005, 2007; Shen et al., 2005; Sipia et al., 2006).

In view of the concentration of MC found in some fish, mussels and shellfish, the importance of food as exposure route must be also considered (Ibelings and Chorus, 2007). A tolerable daily intake (TDI) of 0.04 μg total MC per kilogram body weight per day has been proposed as a provisional guideline (Chorus and Bartram, 1999). Daily intake is typically based upon consumption of 100–300 g of food and 60–70 kg person (Ibelings and Chorus, 2007). Thus, in the worst situation found in Los Padres Lake during the studied period (MC content = 8.0 $\mu g\,kg^{-1}$ of muscle, consumption in a day of 300 g of fish muscle and a 60 kg person), the TDI propose by the WHO could be reached but not surpassed (calculated TDI = 0.04 μg total MC per kilogram body weight per day).

This is the first report on the presence of MC in Los Padres Lake and, fortunately, none of analyzed samples had gone above the guidelines values. However, in view of the extensive use of this waterbody and the variability in MC contents that has been extensively reported, monitoring programs need to be established to prevent intoxication with these toxins.

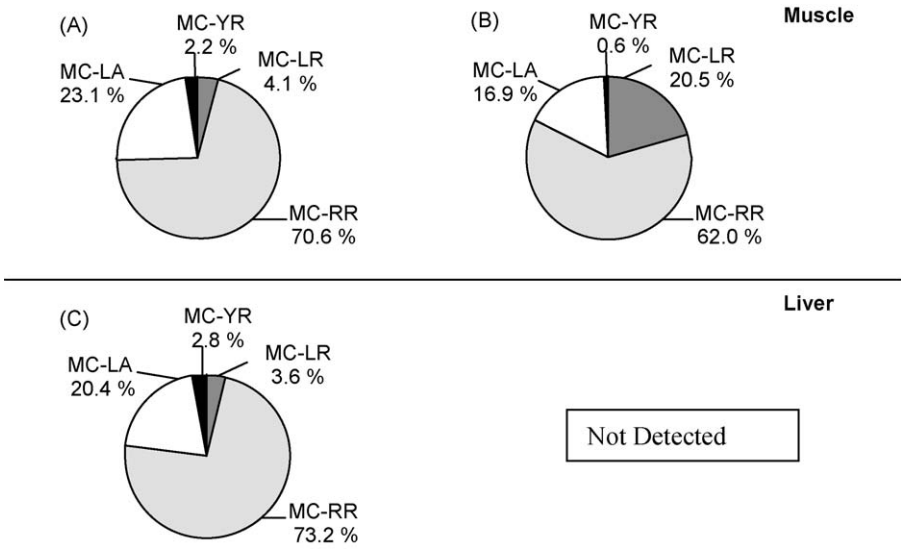

Fig. 2. Relative distribution of MC–LR, –RR, –LA and –YR in liver and muscle of *O. bonariensis* from Los Padres Lake separated by season: (A) muscle, wet season; (B) muscle, dry season; (C) liver, wet season.

4.2. Uptake and tissue distribution of microcystins in O. bonariensis

In the aquatic environment, cyanotoxins are mainly released to the surrounding water during cyanobacterial cell senescence, death and lysis. Thus, fish are naturally exposed to MC through ingestion of toxic cyanobacteria or contaminated food and, to a less extent, through dissolved toxin. In theory, MC could be absorbed by both gastrointestinal tract and gills (Cazenave et al., 2005).

O. bonariensis has opportunistic feeding habits. The presence of phytoplankton material has been observed in the fishes collected in our study. This result is coincident with the analysis of gastrointestinal tract of *O. bonariensis* collected in San Roque Dam (Argentina) where the presence of cyanobacterial cell was confirmed; evidencing that the ingestion of bloom material by this fish species is possible (Cazenave et al., 2005).

A rapid transference of MC from seston to fish was observed in a field study (Magalhães et al., 2001). In that study, the positive Pearson correlation between the levels of MC in seston and muscle of *Tilapia rendalli* is thought as the direct transference of MC to the fish by oral ingestion, probably from picoplankton cyanobacteria. Similar results were reported in Sepetiba Bay (Brazil), where a significant correlation was observed between the concentration of MC in seston and those found in fish muscle ($r = 0.96$, $p < 0.05$) (Magalhães et al., 2003).

In our current study, we did not observe correlation between the content of MC in water and its level in fish muscle. In contrast, we observed that the content of MC (total and dissolved) was positively related to the content of MC in fish liver. However, we did not find significant correlation between cell-bound MC and its amount in fish liver. Thus, our current results, which are in good agreement with previous studies (Cazenave et al., 2005), suggest that the uptake of dissolved MC would not be discarded as a pathway leading to bioconcentration of MC in *O. bonariensis*.

Once absorbed by either gills or intestinal epithelia, a rapid transport of the toxin throughout the fish body may take place. Thus, MC can be transported via the bloodstream and distributed to various organs or tissues (Cazenave et al., 2005; Amé et al., 2009). Previous field studies have shown that MC concentrations in fish tend to be highest in both gut and liver, rather lower in kidneys and gonads, and much lower in muscle tissue (Ibelings and Chorus, 2007 and other authors referenced therein). Our present results are in good agreement with those reports since MC content in liver was ten-fold higher than the corresponding in muscle. Chen et al. (2007) found that MC content in liver of bighead carp (*Aristichthys nobilis*) had a strong correlation with that in fish muscle ($r = 0.94$, $p < 0.01$). Similarly, in an Egyptian fish farm, there was a significant correlation in MC concentration between liver and muscle of a tilapia, *Oreochromis niloticus* ($r = 0.96$) (Mohamed et al., 2003). In our current study, we observed similar results but only during wet season.

Conversely, during the dry season we observed presence of MC in fish muscle but we were not able to detect MC in liver. Soares et al. (2004) demonstrated that MC accumulation in liver and muscle of *T. rendalli* occurred with different profiles. In a laboratory test, these authors found the highest concentration in liver on the sixth day of exposure to MC, while the maximum toxin concentration in muscle was reached during the depuration period, when fish were no longer exposed to toxic cyanobacteria. However, the concentrations of MC found in liver were always higher than in muscle. On the other hand, we did not find MC in liver during the dry season but in fish muscle (Fig. 2), even when different MC variants were present in water at the same time (Fig. 1). There are some suggestions that the water temperature could influence MC uptake (Zhang et al., 2009) but concurrence of different MC variants could also affect the absorption. Thus, our current results demonstrate the need of further laboratory studies on the dynamic of MC uptake and distribution, considering different tissues, temperatures, concurrent presence of different MC variants, dissolved or cellular MCs, etc.

4.3. Microcystins variants in water and tissues of O. bonariensis

In our study, MC–RR was the dominant variant of MC in water samples (Fig. 1). These results are in accordance with other reports (Amé et al., 2003; Dai et al., 2008; Messineo et al., 2009; Prakash et al., 2009). In contrast, MC–LA was rarely detected in natural blooms occurring on other countries (Messineo et al., 2009), while we always found MC–LA in water samples from Los Padres Lake, being MC–LA the second in quantity, even over MC–LR (Fig. 1). Also MC–YR was always present in the lake but with the lowest concentration (Fig. 1). The spatial and temporal variability observed for MC variants in water and seston has been extensively

reported (Amé et al., 2003; Ballot et al., 2005; Song et al., 2007; Dai et al., 2008; Messineo et al., 2009; Zhang et al., 2009). This variation could be a consequence of different environmental conditions as well as changes in the dominance of cyanobacterial species and strain composition along sampling periods.

However, the distribution of different MC variants in fish tissues, particularly in field studies, has been poorly investigated (Xie et al., 2005; Chen et al., 2007; Deblois et al., 2008).

MC–LR has been found in liver, gallbladder, spleen, kidney, gill and muscle of bighead carp (*A. nobilis*, Chen et al., 2007). However, other studies evidenced that MC–LR was present in liver, even when it was not found in muscle (Xie et al., 2004). We found MC–LR in both liver and muscle of O. *bonariensis* (Table 2). However, we observed significant difference in the relative distribution of MC–LR in muscle between wet and dry seasons (Fig. 2). This difference could be attributed to the distribution of different variants of MC in water, since the relative distribution of MC–LR during the dry season was significantly increased in station 1 (Fig. 1).

During a field study in San Roque Dam (Argentina), Cazenave et al. (2005) detected MC–RR in both external (gills) and internal tissues (liver, muscle) of *O. bonariensis*. Our current results are in good agreement with that report, although the profile of MC found in our case was significantly different. In our current report, the content of MC–RR in liver of *O. bonariensis* was $24.6 \pm 27.8\ \mu g\ kg^{-1}$, while the amount reported in San Roque Dam was $160 \pm 320\ \mu g\ kg^{-1}$. Similar differences can be observed in muscle of *O. bonariensis* from Los Padres Lake, where we found $2.5 \pm 2.2\ \mu g\ kg^{-1}$, while in San Roque Dam MC–RR reached $50 \pm 11\ \mu g\ kg^{-1}$. These differences could be due to the elevated concentrations of nutrients and high incidence of toxic cyanobacterial blooms reported in San Roque reservoir (Amé et al., 2003; Cazenave et al., 2005; Ruibal Conti et al., 2005). It is also remarkable that MC–RR was found in muscle of *O. bonariensis* during the dry season, even when this toxin was not present in water samples during the same period. Similar results were reported by Deblois et al. (2008) in two large hydroelectric reservoirs in southeastern Brazil. The presence of several MC variants in fish, which were absent from the seston on the sampling day, provide an indication of temporal variability in the toxicity of the bloom. It can be hypothesized that metabolic depuration is more effective with certain variants than others, leading to higher accumulation of those variants that cannot be easily eliminated from fish tissues (Deblois et al., 2008).

We also detected MC–LA in liver ($6.9 \pm 7.3\ \mu g\ kg^{-1}$) and muscle ($0.7 \pm 0.6\ \mu g\ kg^{-1}$) of *O. bonariensis* from Los Padres Lake. The relative percentages of MC–LA in muscle during dry and wet season were similar (Fig. 2). These comparable distributions are in contrast with the augment observed for MC–LR, since both MC–LA and MC–LR increased their relative percentage in water samples during dry season (Figs. 1A and B, 2A and B). These results could indicate that the uptake of MC–LA and MC–LR may be different, adding complexity to the association between MC present in water and seston with those found in biota.

MC–LA has been also found in liver and muscle of tilapia in two Brazilian hydroelectric reservoirs. The levels of MC–LA reported in liver of tilapia were higher than those observed in our present study ($0.8 \pm 0.5\ \mu g\ g^{-1}$; Deblois et al., 2008). The presence of MC–YR in fish seems to be more frequent than the corresponding to MC–LA. Deblois et al. (2008) found $3.3 \pm 1.6\ \mu g\ g^{-1}$ MC–YR in liver of tilapia, while Chen et al. (2007) reported $0.46\ \mu g\ g^{-1}$ MC–YR (average content in various tissues) in samples of bighead carp. Levels of MC–YR measured in tissues of *O. bonariensis* from Los Padres Lake were below these previous reports ($0.9 \pm 1.1\ \mu g\ kg^{-1}$ in the liver). Levels of MC–YR in muscle of *O. bonariesis* from Los Padres Lake were below LOQ, which does not enable further discussion on the uptake and distribution of MC–YR in the studied fish.

Minor structural changes, characteristic of different MC congeners, may have major effects on uptake, distribution and excretion of these toxins (Dietrich and Hoeger, 2005; Meriluoto et al., 1990). The affinity of organic ion transporters (OATP), which are responsible for the transport of MC across cell membrane, may differ among diverse variants of MC (Ibelings and Chorus, 2007). This phenomenon has been observed by several authors (Fischer et al., 2005; Lu et al., 2008; Feurstein et al., 2009). Dissimilar possibility to enter a cell would explain different levels and diverse toxic effects observed in tissues for MC congeners. According to Feurstein et al. (2009) MC–LF and MC–LW have a much higher potential for inducing neurotoxicity in mice than MC–LR. Atencio et al. (2008) has observed that although the lesions caused by MC–LR and MC–RR on tilapia fish were qualitatively identical, there were quantitative differences, with MC–RR showing mainly nephrotoxicity in contrast to the major hepatotoxicity induced by MC–LR. On the other hand, environmental factors could also affect different accumulation of MC variants in fish tissues, not only considering the variability of MC variants in water, but also affecting the uptake. Zhang et al. (2009) reported that temperature had close correlations both with intracellular toxin content in water, and with MCs content in snail hepatopancreas, suggesting the importance of water temperature on the MCs accumulation in snail hepatopancreas. Further studies on this direction would be necessary to understand differences observed in the uptake and tissue distribution of different variants of MCs in fish at different times, considering seasonal variation, uptake and distribution mechanisms.

5. Conclusions

MC–LR, –RR, –LA and –YR have been measured in water samples and tissues of *O. bonariesis* from Los Padres Lake at different seasons during 2007. None of the analyzed samples surpassed the WHO guidelines values neither for recreational use of water nor for fish consumption. However, considering both spatial and temporal variability, responsible authorities should consider the implementation of control measures to ensure that values remain within such limits of allowance. Furthermore, the persistence of different MC variants in fish muscle, even when these variant were absent in water samples, shows that further studies on the uptake and tissue distribution as well as evaluation on the transference of different MC congeners through the food web are necessary to fully understand and manage the presence of MC in freshwaters.

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