Assessment of microcystin distribution and biomagnification in tissues of aquatic food web compartments from a shallow lake and evaluation of potential risks to public health

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Abstract The objectives of this study were: (1) to examine the distribution and bioaccumulation of microcystins in the main components of the food web (phytoplankton, zooplankton, crayfish, shrimp, mussel, snail, fish, frog) of Lake Pamvotis (NW Greece), (2) to investigate the possibility of microcystin biomagnification and (3) to evaluate the potential threat of the contaminated aquatic organisms to human health. Significant microcystin concentrations were detected in all the aquatic organisms during two different periods, with the higher concentrations observed in phytoplankton and the lower in fish species and frogs. This is the first study reporting microcystin accumulation in the body of the freshwater shrimp *Atyaephyra desmsaresti*, in the brain of the fish species common carp (*Cyprinus carpio*) and in the skin of the frog *Rana epirotica*. Although there was no evidence for microcystin biomagnification, the fact that microcystins were found in lake water and in the tissues of aquatic organisms, suggests that serious risks to animal and public health are possible to occur. In addition, it is likely to be unsafe to consume aquatic species harvested in Lake Pamvotis due to the high concentrations of accumulated microcystins.

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Introduction

Algal blooms are increasing worldwide due to eutrophication of aquatic environments (Anderson et al. 2002). Anthropogenic nutrient enrichment of freshwaters has a direct impact on algal species composition and the formation of noxious and toxic blooms and surface scums. The most common phytoplankton organisms associated with eutrophication of freshwater systems are cyanobacteria (Codd 1995). Cyanobacteria have the ability to form several toxic metabolites (Ressom et al. 1994) known as cyanotoxins. Among them, microcystins are the most abundant group. Microcystins are produced by various genera of cyanobacteria, the predominant one being *Microcystis* sp. (Carmichael 1997; Codd 2000; Beresovsky et al. 2006). These toxins are highly water-stable and resistant to boiling, and thus pose a threat to water and food quality if not properly monitored (Falconer and Humpage 2005).

Exposure to microcystins represents a health risk to aquatic organisms, wild life, domestic animals, and humans upon drinking or ingesting cyanobacteria in the water (Duy et al. 2000). It is well recognized that among their toxigenic mechanisms they are potent inhibitors of protein phosphatase 1 and 2A that cause increased protein phosphorylation, which is directly related to their cytotoxic effects and tumor-promoting activity (Hooser et al. 1989; Carmichael 1994; Hooser 2000). Chronic toxic effects to humans from exposure through drinking water or contaminated food are particularly probable, especially if there is long-term frequent exposure (Magalhães et al. 2001;

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Zhang et al. 2009). High incidence of primary liver cancer in China is considered to be related to microcystins in drinking water (Yu 1995).

Bioaccumulation of microcystins in aquatic food chains may be another important factor, suggesting a potential risk for higher trophic level species. The ability of zooplankton and other invertebrates to accumulate microcystins may lead to a transfer of toxins through the food web to more sensitive organisms (Liras et al. 1998). Bivalves, as sessile filter-feeding animals, may be among the most threatened group of aquatic organisms in the presence of toxic cyanobacterial blooms. Gastropods are important links between primary producers and higher consumers (Habdija et al. 1995), thus toxin accumulation by these organisms, increases the risk for higher consumers. In the same way, toxin accumulation by amphibians increases the risk for terrestrial animals. The occurrence of toxic cyanobacteria in many water bodies where macroinvertebrates and large crustacea are abundant leads to the possibility of toxin accumulation and transfer through the food chain. Moreover, people consume a lot of freshwater fish, thus large quantities of cyanotoxins could also be transferred by this way.

However, only a few studies have tracked the presence of microcystins in single or multiple components of aquatic food webs (Kotak et al. 1996; Babcock-Jackson et al. 2002; Magalhães et al. 2001, 2003; Ibelings et al. 2005). Other studies have analyzed the uptake of microcystins by aquatic organisms in an experimental setting (among others, zooplankton (Demott 1999; Rohrlack et al. 2001; Thostrup and Christoffersen 1999), mussels (Amorim and Vasconcelos 1999; Prepas et al. 1997; Yokoyama and Park 2003), or fish (Malbrouck et al. 2003), often using an acutely toxic dosage and purified microcystin or cultured cyanobacteria (Xie et al. 2004). These results, therefore, are not readily transferable to a lake food web.

Many Greek lakes suffer from the presence of cyanobacterial blooms (Vardaka et al. 2005). Moreover, significant microcystin concentrations have been recorded in Greek freshwaters, including Lake Pamvotis (Kagalou et al. 2008; Papadimitriou et al. 2010). Our study was triggered by the high microcystin concentrations recorded in the tissues of an omnivorous fish species (*Carassius gibelio*), originated from Lake Pamvotis during the last years (Kagalou et al. 2008; Papadimitriou et al. 2010). This led us to consider about the dynamics of microcystin concentrations in significant aquatic food web components of Lake Pamvotis.

The major objectives of this study were: (1) to examine the distribution and bioaccumulation of microcystins in some aquatic food web compartments (phytoplankton, zooplankton, crayfish, shrimp, mussel, snail, fish, frog, (2) to investigate the possibility of microcystin biomagnification, (3) to evaluate the potential risk of the contaminated aquatic organisms to human health.

Materials and methods

Sample collection and preparation

Study area

The study was conducted in Lake Pamvotis, NW Greece (39°40′N, 20°53′E) which is a Mediterranean, warm shallow lake (mean depth, 5.5 m). In lake water, many activities take place, like recreational activities, fishing and water uptake for irrigation (Kagalou et al. 2003; Papadimitriou et al. 2010). During the last three decades the trophic status of the lake has changed as a result of anthropogenic activity (among others irrigation and domestic sewage discharge), resulting in serious problems (Kotti et al. 2000; Kagalou et al. 2008). Previews studies, have proven high cyanobacterial biovolumes in the lake (Vardaka et al. 2005). Dense scum of toxic cyanobacterial blooms occurs frequently in the water surface of the lake, mainly in the warm seasons every year (Papadimitriou et al. 2011).

Sampling of phytoplankton and collection of cyanobacterial blooms

Water samples (volume of 2 l) for phytoplankton and microcystins analysis were collected monthly from the surface of the water to a depth of 1 m using polyethylene bottles, during 2008. Subsamples of 50 mL were preserved with both Lugol's solution and formaldehyde. Phytoplankton identification was performed according Anagnostidis and Economou (1980), using an inverted microscope.

To estimate microcystin concentrations in the lake water (intracellular and extracellular toxins) 1 L of the water sample was filtered through a Whatman glass-fiber filter (Whatmann GF/C 0.45-μm filter). Seston onto the glass-fiber filter and the filtrate were used to detect intracellular and extracellular toxins, respectively.

Cyanobacterial blooms were collected from the surface water with a plankton net (mesh size, 63 μm) during August and November 2008, and immediately frozen at −20°C and then lyophilized for toxin analysis.

Sampling of zooplankton

Zooplankton from which the microcystin content was analyzed was collected from 0.5 m depth, by filtration of 500 L of lake water over a plankton net with a 250 μm mesh size. Collection was performed during August and November of 2008. A subsample was resuspended in 45 mL tap water and preserved with 5% formaldehyde for zooplankton identification. This was done under a microscope based on descriptions of Pennak (1978). The remaining volume of the sample was resuspended in 2 L tap water for microcystin

analysis. Before the analytical procedure could be started, samples had to be prepared to avoid contamination with phytoplankton, which would probably contain microcystin. According to Ibelings et al. (2005), the following steps were followed: (1) the water was left standing overnight in measuring cylinders at in situ lake temperature and under dimmed light, allowing detritus to sink to the bottom and buoyant cyanobacteria to float to the top; (2) scums of cyanobacteria were removed, and the remainder of the sample (but excluding the detritus) was rinsed carefully with tapwater and transferred to clean beakers; (3) the water containing the zooplankton was flushed with CO_2, by which treatment the zooplankton was sedated and sank to the bottom of the beakers—the overlying water was removed; (4) these steps were repeated until zooplankton was free of cyanobacteria (checked microscopically).

Sampling of macroinvertebrates

During August and November of 2008 (after November, the collection of aquatic organisms is difficult due to the ability of many aquatic organisms to reduce their metabolic activities), a suite of aquatic macroinvertebrates (10 specimens from every species) was collected and analyzed for microcystins. These included water snails (*Viviparus contectus*), freshwater shrimps (*Atyaephyra desmaresti*), crayfish (*Astacus astacus*) and mussels (*Anodonta cygnea*). These invertebrates were collected with a dip net from the shoreline or from the littoral zone from the side of the boat. In the cases of mussels and crayfish each individual was weighted sacrificed and the hepatopancreas, stomach, mantle, foot and gills (for mussels) and gills, hepatopancreas, brain, muscle, stomach and gonads (for crayfish) were collected, weighted and immediately frozen at −80°C. While for shrimps and water snails (without of shell) the whole body individually was weighted and frozen at −80°C.

Sampling of fish and frogs

Ten specimens of the fish species common carp (*Cyprinus caprio*) and the endemic Epirus frog (*Rana epirotica*) were captured in the same as the invertebrate's period. The collected fish and frogs were measured, weighed (mean weight: 849 g for fish and 29.3 g for frogs), and sacrificed immediately. Liver, intestines, kidney, gonads, brain and muscle (for fish) and intestine, liver, pancreas, gonads, skin and muscle (for frogs) were collected, weighed and immediately frozen at −80°C.

In addition, six specimens of common carp (*Cyprinus carpio*) originated from artificial propagation, which were kept for 16 months in aquariums with pure recycled water used as control sample.

Microcystin analysis

Microcystins in the seston (intracellular microcystins) of water column and zooplankton samples were analyzed quantitatively: microcystins in the seston and in zooplankton samples extracted with 100% methanol and stirring overnight at room temperature (20–22 C) followed by centrifugation at $1,300 \times g$ for 15 min. Extraction procedure was repeated three times and the supernatants of the extractions were pooled. The organic solvent was removed by placing the extract under nitrogen-stream. The concentrated sample extract remaining after removing of the organic solvent was subjected to ELISA assay (ABRAXIS, 520011, USA).

For analysis of microcystins dissolved in water, the filtered water was applied directly to ELISA.

For analysis of microcystins in bloom samples, lyophilized material was extracted with 100% methanol and sonicated for 10 min in ice. After centrifugation at $4,000 \times g$ rpm (20 min), the supernatant was retained and filtered through a 0.2 μm filter to exclude cell derbis. Microcystin detection and quantification in bloom extracts were done according to Lawton et al. (1994), using a Shimadzu HPLC system (Duisburg, Germany) equipped with a photodiode array detector (SPD-MZOA) and a CTO 10AS column oven. The column was a ThermoHypersil (ODS) C18 (250 mm × 4.6 mm, 5 μm). The mobile phase system was: (A): aqueous trifluoracetic (TFA) 0.05% (v/v) and (B): acetonitrile (TFA) 0.05% (v/v). A gradient elution program was employed as follows: 50% A isocratically for 10 min, to 40% for 5 min, to 10% A for 5 min and finally to 50% A for 10 min. The HPLC run program time was 30 min. The sample volume injected was 50 μL and the mobile phase run at 0.6 mL min^{-1}. Microcystins were identified on basis of a characteristic spectrum between 237 and 242 nm. The number of different microcystins was determined by the number of peaks that eluted with different retention times and showed a typical microcystin UV spectrum. Hence microcystins could be differentiated but not identified on basis of HPLC retention times. All different microcystins were transformed to microcystin-LR (MC-LR) equivalents for quantification of the total microcystin content. MC-LR (ALEXIS BIOCHEMICALS) was the only standard that was used.

Microcystins were extracted from the tissues of macroinvertebrates, fish and frogs, with 100% methanol (Magalhães et al. 2001), stirred overnight at room temperature and then centrifuged at $1,300 \times g$ for 15 min in a Universal 32 centrifuge. The supernatant was collected and stored overnight at 4°C. A 5 mL aliquot of the supernatant was concentrated to 350 μL under nitrogen stream. A 100-μL of the concentrated sample extract was diluted to 900 μL distilled water according to Sipia et al. (2002). The

final sample was clarified using membrane filters (pore size, 0.45 μm, and diameter 4 mm). Sample solutions were analyzed immediately with ELISA test.

For each ELISA test, the negative control and four calibrates were assayed at least in duplicate. A commercial ABRAXIS-Microcystin ELISA kit (520011, USA) was used following the instructions of the manufacturer. The ELISA is an indirect-competitive method used for the quantitative analysis of all the microcystin analogues and nodularins. This ELISA test, using the β-amino acid 6E-ADDA as the epitope for antibody recognition, has a limit of quantification of 0.02–0.07 ng/mL, lower than the WHO-proposed guideline (1 ng/mL) for drinking water, irrespective of the sample matrix.

The recovery of the method was determined by analyzing real samples before and after the addition of pure microcystin and then subtracting the concentration of microcystin present in the sample prior to spiking. The matrix effect (i.e., effect of animal tissue) was checked by spiking control tissues with MC-LR standard (2 μg/g) and the response was compared to 100% methanol spiked with the same amount of the standard. Results are expressed as nanograms of MC-LR equivalents per gram of tissue.

Statistics

The general linear model (GLM) was used in order to examine if there are significant differences between animals, tissues and sampling periods in relation to the microcystin concentrations. Concentrations of microcystin in the tissues of individual animals were compared using One-way analysis of variance (ANOVA). Data were explored using the statistical package SPSS 19.0 for Windows.

Biomagnification of microcystins was examined, through the calculation of the biomagnification factor (BMF). BMF is determined as the average microcystin content in biota (ng MC-LReq g^{-1} DW) as a percentage of the average value in the diet of the respective organisms (microcystin in organism/microcystin in diet × 100).

Results

Intracellular and extracellular toxins in the water column and concentration in surface blooms and in zooplankton

According to phytoplankton identification, the lake dominated of *Microcystis* sp. during the study period, with the exception of June and July, when *Anabaena* was the dominant genus. Zooplankton identification showed that

the dominant genus was *Alona* in November and *Polyarthra* in August.

Microcystin concentrations in seston (intracellular microcystins) varied considerably among months, ranging from 0.29 μg MC-LReq/L (in March) to 15.83 μg MC-LReq/L (in September) (Fig. 1). In August, microcystin concentrations reached the 14.2 μg MC-LReq/L (or 473.33 μg MC-LReq/g D.W.). In November, microcystin concentrations reached the 6.21 μg MC-LReq/L (or 390.56 μg MC-LReq/g D.W.). The lowest concentration of dissolved in the water column microcystins (extracellular microcystins), was found in December (0.32 μg MC-LReq/L). However, the highest concentration was found in October (4.12 μg MC-LReq/L) (Fig. 1). The microcystin concentration in surface cyanobacterial blooms ranged from 502 μg MC-LReq/g D.W. in November to 2,300 μg MC-LReq/g D.W. in August ($F = 149.05$, $P < 0.05$) (Fig. 2).

Microcystin concentrations were found in all the zooplankton samples. In November, the microcystin concentration reached the 80 μg MC-LReq/g D.W. In August, microcystin concentration was higher and reached the 152 μg MC-LReq/g D.W ($F = 132.19$, $P < 0.05$) (Fig. 2).

Distribution of microcystins in the tissues of aquatic animals

Microcystin concentrations were detected in all the animals examined. In contrast, the control samples did not contain any detectable concentrations of microcystins. The GLM analysis showed that there were significant differences between the animals ($F = 48.79$, $P < 0.05$), the tissues ($F = 121.82$, $P < 0.05$) and the sampling periods ($P < 0.05$,) concerning microcystin concentration. The recovery of microcystins that we obtained from the spiked samples was 74 ± 2.3% for liver, 71 ± 3.5% for kidneys, 72.5 ± 5.2% for brain, and 70.6 ± 1.46% for muscle.
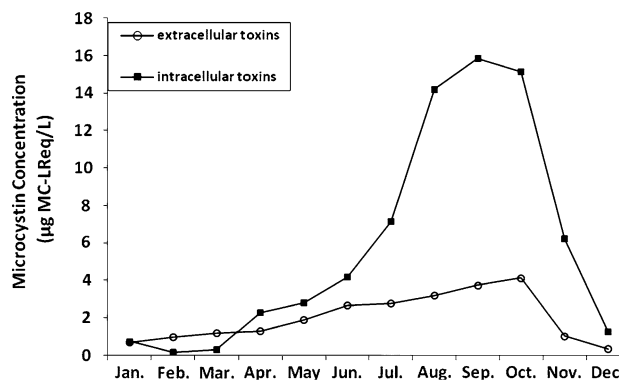
Fig. 1 Monthly variation of intracellular and extracellular microcystin concentrations (μg MC-LReq/L) in Lake Pamvotis, during 2008
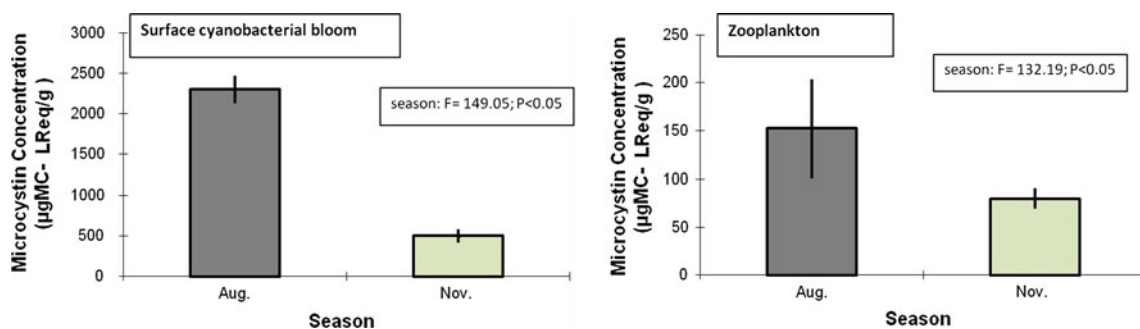
Fig. 2 Microcystin concentrations (μg MC-LReq/g D.W.) in surface cyanobacterial bloom and zooplankton of Lake Pamvotis, during August and November 2008

The matrix effect was negligible (from 0.08 to 5.2% of differences between matrix and methanol results with an average of $1.9 \pm 0.7\%$). Similar results about the matrix effect with the ELISA test were observed by Ernst et al. (2005).

Distribution of microcystins in macroinvertebrates

Microcystin concentrations were detected in all the macroinvertebrates (Fig. 3). The highest microcystin concentrations were found in the mussel *Anodonta cygnea*. Microcystin concentrations were significant different among the tissues of the mussel ($F = 132.81$, $P < 0.05$). Also, microcystin concentrations in the tissues of the mussel differed between the sampling periods ($F = 156.94$, $P < 0.05$) with higher concentrations observed in August. High microcystin concentrations were detected in

the hepatopancreas of the animal (1346.98 ± 164.78 ng MC-LReq/g and 884.97 ± 56.37 ng MC-LReq/g in August and November, correspondingly). Lower microcystin concentrations were detected in stomach (1188.53 ± 58.23 ng MC-LReq/g and 382.53 ± 86.01 ng MC-LReq/g in August and November, correspondingly), mantle (1150.49 ± 33.65 ng MC-LReq/g and 674.69 ± 137.38 ng MC-LReq/g in August and November, correspondingly), foot (1171.55 ± 108 ng MC-LReq/g and 799.07 ± 184.03 ng microcystin-LReq/g in August and November, correspondingly) and gills (1197.52 ± 52.17 ng MC-LReq/g and 841.69 ± 79.32 ng MC-LReq/g in August and November, correspondingly) (Fig. 3).

Lower microcystin concentrations were detected in the shrimp *Atyaephyra desmaresti* and in the water snail *Viviparus contectus* (Fig. 3). Microcystin concentration in the body of the shrimp was higher in August (2250.48 ± 123.58 ng
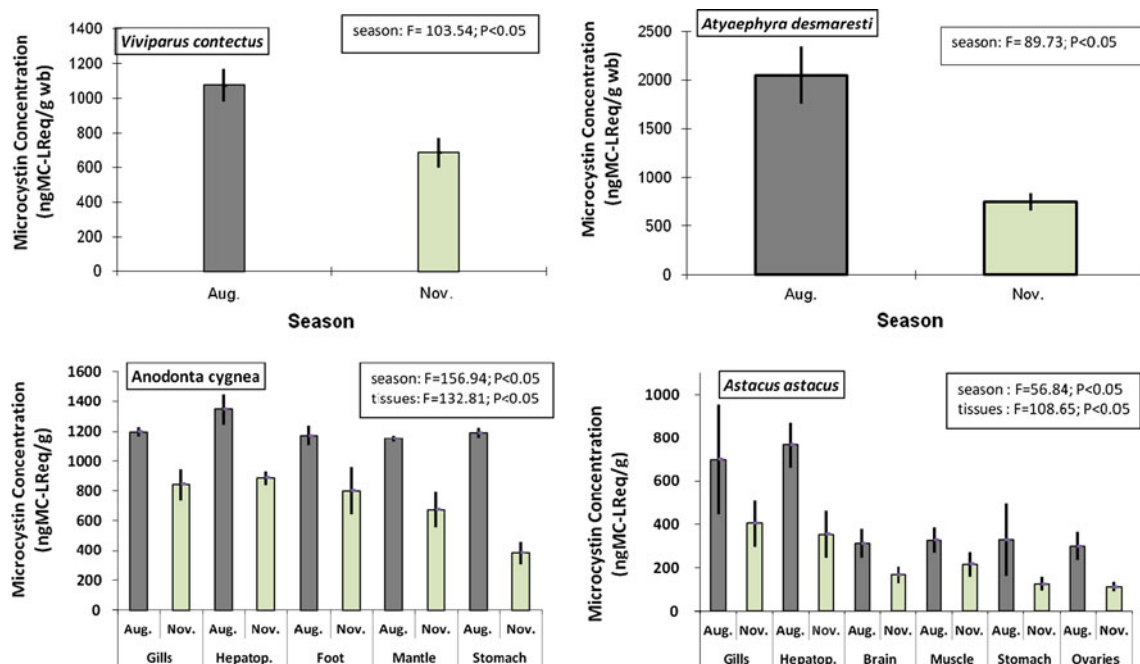
**Fig. 3** Microcystin concentrations (ng MC-LReq/g) in macroinvertebrates of Lake Pamvotis, during August and November 2008

MC-LReq/g) than in November (749.15 ± 83.61 ng MC-LReq/g) ($F = 89.73$, $P < 0.05$). The water snail characterized by lower microcystin concentrations (1074.07 ± 147.84 ng MC-LReq/g and 684.96 ± 40.11 ng MC-LReq/g in August and November, correspondingly) ($F = 103.54$, $P < 0.05$).

Finally, the lowest microcystin concentrations among the macroinvertebrates, were detected in crayfish *Astacus astacus* (Fig. 3). Microcystin concentrations were significant different among the tissues of the crayfish ($F = 108.65$, $P < 0.05$). Also, microcystin concentrations in the tissues of the crayfish differed between the sampling periods ($F = 56.84$, $P < 0.05$) with higher concentrations observed in August. High microcystin concentrations were detected in the hepatopancreas of the animal (766.99 ± 148 ng MC-LReq/g and 354.82 ± 98.34 ng MC-LReq/g in August and November, correspondingly). Lower microcystin

concentrations were detected in gills (701.09 ± 176.98 ng MC-LReq/g and 405.43 ± 91.46 ng MC-LReq/g in August and November, correspondingly), stomach (331.25 ± 70.41 ng MC-LReq/g and 126.94 ± 37.73 ng MC-LReq/g in August and November, correspondingly), muscle (328.61 ± 95.29 ng MC-LReq/g and 216.41 ± 65.71 ng MC-LReq/g in August and November, correspondingly), brain (312.93 ± 67.09 ng MC-LReq/g and 168.54 ± 42.64 ng MC-LReq/g in August and November, correspondingly) and gonads (301.76 ± 85.65 ng MC-LReq/g and 113.74 ± 26.45 ng MC-LReq/g in August and November, correspondingly) (Fig. 3).

The specimens of common carp (*Cyprinus carpio*) examined during the present study contained microcystins (Fig. 4). Significant statistical differences were found between the concentrations of microcystins in the tissues ($F = 165.28$; $P < 0.05$) and between the sampling periods ($F = 56.95$; $P < 0.05$). The highest level of microcystins

Fig. 4 Microcystin concentrations (ng MC-LReq/g) in tissues of fish (*Cyprinus carpio*) and frogs (*Rana epirotica*) originated from Lake Pamvotis, during August and November 2008
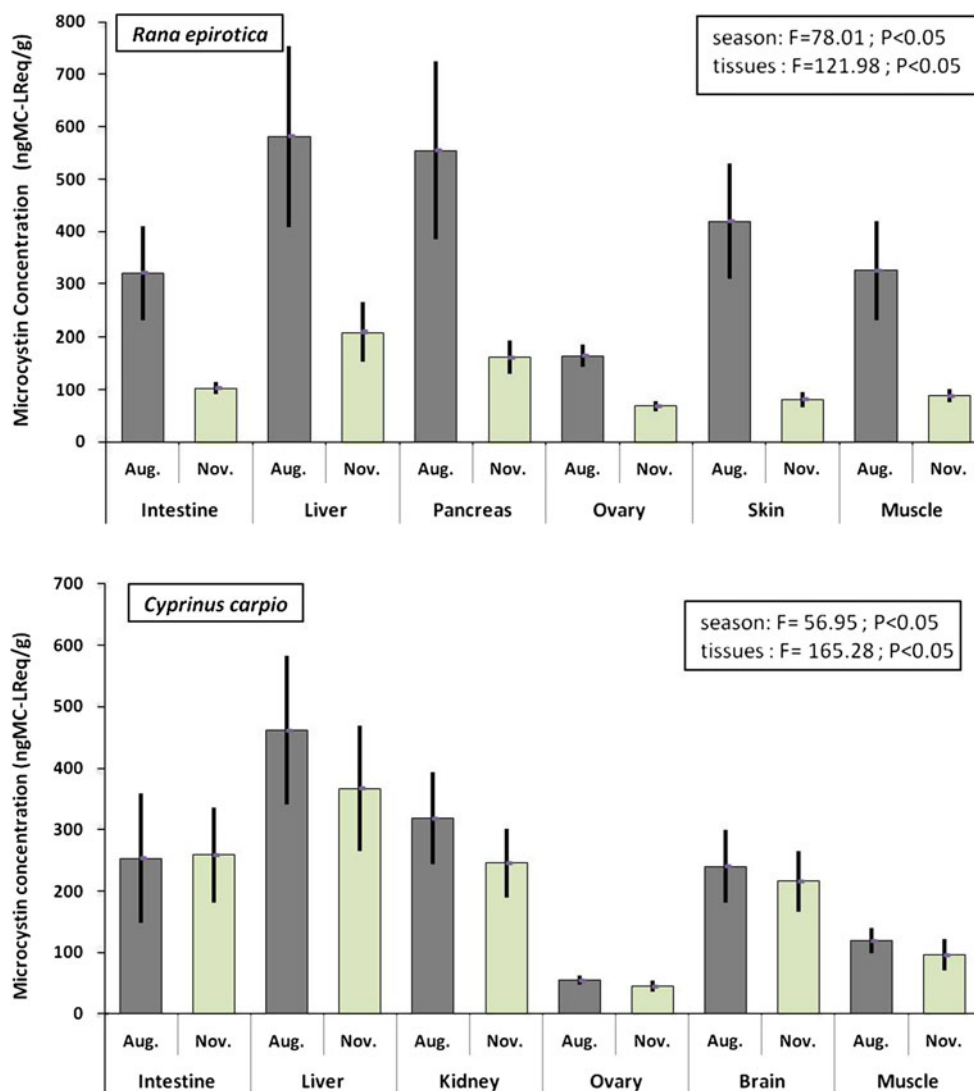
was found in the liver (462.09 ± 125.54 ng MC-LReq/g and 367.09 ± 89.58 ng MC-LReq/g in August and November, correspondingly), followed by the kidney (318.76 ± 105.85 ng MC-LReq/g and 245.47 ± 13.98 ng MC-LReq/g in August and November, correspondingly), intestines (253.43 ± 106.25 ng MC-LReq/g and 258.69 ± 105.40 ng MC-LReq/g in August and November, correspondingly), and brain (240.01 ± 95.41 ng MC-LReq/g and 215.89 ± 79.83 ng MC-LReq/g in August and November, correspondingly). The lowest concentrations were detected in the muscle (119.07 ± 32.99 ng MC-LReq/g and 95.78 ± 30.79 ng MC-LReq/g in August and November, correspondingly) and gonads (54.63 ± 11.66 ng MC-LReq/g and 45.35 ± 13.98 ng MC-LReq/g in August and November, correspondingly) (Fig. 4).

Microcystins in the tissues of fish and frogs

Samples of the endemic frog Rana epirotica also contained microcystins (Fig. 4). Significant statistical differences were found between the concentrations of microcystins in the tissues ($F = 121.98$; $P < 0.05$) and between the sampling periods ($F = 78.01$; $P < 0.05$). The highest level of microcystins was found in liver (581.46 ± 92.83 ng MC-LReq/g and 208.69 ± 89.58 ng MC-LReq/g in August and November, correspondingly), followed by the pancreas (554.06 ± 98.06 ng MC-LReq/g and 141.59 ± 51.32 ng MC-LReq/g in August and November, correspondingly), intestine (320.56 ± 63.98 ng MC-LReq/g and 102.59 ± 18.74 ng MC-LReq/g in August and November, correspondingly), and skin (419.52 ± 91.62 ng MC-LReq/g and 80.92 ± 23.56 ng MC-LReq/g in August and November, correspondingly). The lowest concentrations were detected in the muscle (325.49 ± 87.01 ng MC-LReq/g and 88.12 ± 20.85 ng MC-LReq/g in August and November, correspondingly) and gonads (164.62 ± 34.02 ng MC-LReq/g and 68.97 ± 15.74 ng MC-LReq/g in August and November, correspondingly) (Fig. 4).

The recovery of microcystins that we obtained from the spiked: (1) bloom samples was 92%, (2) zooplankton samples was 76%, (3) macroinvertebrate samples was 74%, (4) fish samples was 74%, (5) frog samples was 68%. The matrix effect was negligible (from 0.08 to 5.2% of differences between matrix and methanol results with an average of 1.9 ± 0.7%).

Biomagnification of microcystins

Biomagnification factors (BMF) ranged from 0.19 (in *Atyaephyra desmaresti*), to 32.1 (in zooplankton) (Table 1). Microcystin concentration in none of the food web compartments exceeded the concentration in seston. In general no evidence for biomagnification was found. Nevertheless,

Table 1 Biomagnification factors of microcystins in food web components of Lake Pamvotis

	August	November
Zooplankton*	32.1	20
*Atyaephyra desmaresti***	0.5	0.19
*Anodonta cygnea****	1.5	1
*Cyprinus carpio*****	3.7	5.9

* 'Diet' is seston for zooplankton

** According to studies of Anastasiadou Ch et al. (2004), the contribution of seston in the diet of the shrimp *Atyaephyra desmaresti* is estimated about 80%

*** According to studies of Mackie (1984), the diet of the mussel *Anodonta cygnea* contains 70% seston and 30% zooplankton

**** According to studies of Khan (2003), the contributions of zooplankton and *Atyaephyra desmaresti* in the diet of the fish species *Cyprinus carpio* are 25 and 36%, correspondingly

it is remarkable that all biomagnification factors were higher in August than November, with the exception of the biomagnification factor in fish species *Cyprinus carpio*. In August, BMF in *Cyprinus carpio* was 3.7, lower than in November (5.9).

Discussion

Cyanobacterial blooms have received the attention of many scientists worldwide, due to their destructive impact on the quality of many fresh and brackish waters. The production of cyanotoxins hinders the function and structure of waterbodies (Codd 2000). Moreover, cyanotoxins could be accumulated in animals through food chain, causing health problems, even to humans (Vasconcelos 2006). According to our results, significant concentrations of microcystins were found in the water along with the total of aquatic organisms of Lake Pamvotis. This is in agreement with study of Papadimitriou et al. (2011), who found significant microcystin concentrations (extacellular microcystins: 0.0121–7.88 μg/L, intracellular microcystins: 0.15–15.21 μg/L) in water of Lake Pamvotis, along with toxicity effects of natural cyanobacterial bloom on fish embryos. Phytoplankton appeared with the higher microrocystin concentrations, followed by zooplankton, macroinvertebrates, frogs and fish. Microcystin concentrations found in seston of Lake Pamvotis were higher in warm months when *Microcystis* sp. was the dominant phytoplankton species. The above concentrations were similar to microcystin concentrations found to other Mediterranean lakes located in Turkey, Spain, France and Algeria (Briand et al. 2002; Albay et al. 2003; Romo et al. 2011). Moreover, cyanobacterial bloom from Lake Pamvotis characterized by high concentrations of microcystins, similar to those found Ghazali et al. (2009) in Lake Lalla Tarkekoust (Morocco)

and Zhang et al. ([2009](#)) in Lake Taihu (China) while recently, cyanobacterial species as *Anabaena flos-aquae*, *Aphanizomenon flos-aquae* and *Microcystis* sp. were recorded as dominant species along with the presence of the microcystin producing gene mcy-A which also has been detected (Vareli et al. [2009](#)).

The increase of microcystin concentrations in seston of Lake Pamvotis was followed by an increase of microcystin concentrations in zooplankton. This is the first record of microcystin concentrations in zooplankton of a Mediterranean lake. According to Ferrão-Filho and Kozlowsky-Suzuki ([2011](#)), zooplankton is clearly the best bioaccumulator of microcystins, reaching values over 1,000 µg g^{-1} DW and an average value of about 383 µg g^{-1} DW. However, recent studies have shown that some zooplankton species not only are able to co-exist with toxic cyanobacteria and even growth in their presence (Leonard and Pearl [2005](#); Hansson et al. [2007](#)), but also that a sensitive species can indeed develop tolerance when pre-exposed to toxic cyanobacteria (Gustafsson and Hansson [2004](#); Gustafsson et al. [2005](#); Guo and Xie [2006](#)). For example negative physiological effects and negative fitness consequences on *Daphnia* spp. have been observed (Rohrlack et al. [2001](#), [2005](#); Gustafsson et al. [2005](#)) while some populations of *Daphnia galeata* and *Daphnia pulicaria* exposed to cyanobacteria can adapt to grow on and being more tolerant (Sarnelle and Wilson [2005](#)). Microcystin concentrations in zooplankton of Lake Pamvotis are similar to those detected in zooplankton of Lake Ijsselmeer in Netherlands (57–192 µg MC-LReq/g D.W.) (Ibelings et al. [2005](#)) but higher than those detected in zooplankton of Jacarepagua Lagoon in Brazil (0.3–16.4 µg MC-LReq/g D.W.) (Ferrão-Filho et al. [2002](#)). These differences in microcystin concentrations are probably associated with the different zooplankton community structure and functional complexity of each lake. Moreover, high intraspecific (clonal) variation in the sensitivity to toxic cyanobacteria has also observed, maybe due to different trophic state of the lakes (Sarnelle and Wilson [2005](#)). Some observations suggest that smaller species of herbivorous zooplankton such as rotifers, copepods, and small cladocerans are less susceptible to cyanobacteria than larger zooplankton (Ferrão-Filho et al. [2002](#)). According to Papadimitriou ([2010](#)), zooplankton community of Lake Pamvotis is dominated by small-sized species, thus, zooplankton of Lake Pamvotis could avoid feeding on cyanobacteria and consequently the accumulation of high microcystin concentrations due to its small size.

Microcystins can also be accumulated on other aquatic invertebrates such as bivalves, crustaceans and gastropods. According to our results, high concentrations of mycrocystins detected in tissues of *Anodonta cygnea*, *Atyaephyra desmaresti* and *Viviparus contectus*. According to previews

studies, gastropods may contain concentrations up to 436 µg g^{-1} DW (*Sinotaia histrica* in hepatopancreas, Ozawa et al. [2003](#)), with an average of 58 µg g^{-1} DW. Although bivalves are efficient filter-feeders, maximum concentration was 630 µg g^{-1} DW (*Unio douglasiae* hepatopancreas, Yokoyama and Park [2003](#)), and the average was only 57 µg MC g^{-1} DW. Larger crustaceans (i.e., Decapoda) seem to be the less efficient accumulators of microcystins, with an average of 4.2 µg g^{-1} DW and a maximum concentration of 12.4 µg g^{-1}DW (*Macrobrachium nipponensis* stomach, Chen and Xie [2005b](#)). According to our results, higher microcystin concentrations were accumulated in the hepatopancreas of *Anodonta cygnea* indicating that this is the target organ for microcystins. Nevertheless, *Anodonta cygnea* accumulated microcystins also in other tissues, possible due to the well documented process of microcystin circulation (Fischer et al. [2005](#)). However, bioaccumulation patterns depend on the mussel species (Chen and Xie [2005a](#); Martins and Vasconcelos [2009](#)). It is worth note that according to our results, zooplankton accumulated higher microcystin concentrations than the mussel *Anodonta cygnea*. An explanation would be that the mussels are more resistant than zooplankton to microcystins, appearing selective rejection of cyanobacteria (Ibelings et al. [2005](#)). Microcystin concentrations found in the body of *Viviparus contectus* from Lake Pamvotis were lower than those (19.5 µg MC-LReq/g) found in the gastropod *Sinotaia histrica* from Lake Biwa in Japan (Ozawa et al. [2003](#)). However, accumulation of microcystins in gastropods is of specific interest, as these organisms are important links between primary producers and higher consumers. *Viviparus contectus* from Lake Pamvotis probably accumulated the toxins through consumption of *Microcystis* sp. presented in lake water and/or on the leaves of aquatic plants. This is in accordance with studies of Lance et al. ([2006](#)) who suggested that gastropods accumulate microcystins mainly by grazing toxic phytoplankton, and to a lesser extent, via uptake of dissolved toxins.

In the present study, the presence of high microcystin concentrations in the freshwater shrimp *Atyaephyra desmaresti* is reported. As far as we know there is no previous evidence concerning the accumulation ability of this species. *Atyaephyra desmaresti* feeds on algae and decaying matter (Anastasiadou Ch et al. [2004](#)) and probably accumulates microcystins through its diet or/and during the continuous contact with the aquatic environment. *Atyaephyra desmaresti* is included in the diet of many fish species, thus it may be a key organism for the transfer of microcystins through the food chain. We also observed microcystins in tissues of crayfish *Astacus astacus*. It was confirmed that microcystins are accumulated primarily in their hepatopancreas, however microcystin concentrations

were found also in the other tissues. Unexpectedly, significant concentrations of microcystins were found in the brain, suggesting the ability of microcystins to be transported to the brain. The presence of microcystins in the gonads of *Astacus astacus*, is in accordance with the studies of Chen and Xie (2005b) about the transport of microcystins from adults crustaceans to embryos crustaceans (*Palaemon modestus* and *Macrobrachium nipponensis*). Lower microcystins accumulated in the muscles of *Palaemon modestus* and *Macrobrachium nipponensis*. This, in addition to the fact that *Astacus astacus* has a commercial value, increases the risk of human contamination through the consumption of this animal. Chen and Xie (2005b) also found microcystin concentrations in hepatopancreas (8.40 μg MC-LReq/g) and in muscle (0.53 μg MC-LReq/g) of the shrimp *Palaemon modestu*. The same authors also found microcystin concentrations in the muscle (0.05 μg MC-LReq/g), stomach (9.97 μg MC-LReq/g) and hepatopancreas (0.08 μg MC-LReq/g) of the crayfish *Procambarus clarkii*.

Microcystins are toxic to fish at concentrations as low as a few micrograms per Liter (mg/L) or possibly even fractional μg/L (reviewed by Wiegand and Pflugmacher 2005; Malbrouck and Kestemont 2006). Considering that microcystins have been measured in concentrations up to 25,000 μg/L in water ecosystems with cyanobacterial blooms (reviewed by W.H.O. 1998), it is not surprising that potential impacts on fish are receiving increased attention. Fish typically are exposed to microcystins either during ingesting cyanobacteria or prey that has fed on cyanobacteria (Malbrouck and Kestemont 2006). Previews studies, have focused on toxicity of microcystins in *Cyprinus carpio*, under laboratory conditions (Li et al. 2004; Palikova et al. 2007; Adamovsky et al. 2007) thus there is only limited information on microcystin accumulation in tissues of *Cyprinus carpio*, under field conditions at environmental relevant concentrations. According to our results, the microcystin content (extracellular and intracellular) of the lake water was sufficient to promote microcystin accumulation in the tissues of fish species *Cyprinus carpio*. Even though the target organ for microcystins is thought to be the liver, microcystins were also found in the rest of tissues, including muscle tissue. Unexpectedly, it was also found high levels of microcystins in the brain of the fish. Thus, our results give additional evidence on the ability of microcystins to cross through the blood–brain barrier and rose questions on the probable neurotoxicity of microcystins. Common carps are generally omnivorus but obligate herbivorus and also known as cyanobacterivorous (Fischer and Dietrich 2000a) while it is thought that *M.aeruginosa* comprises a significant portion of their diet when bloom formation occurs (Carbis et al. 1996). Zhang et al. (2009) also found significant microcystin concentrations in liver

(0.025 μg MC-LReq/g), intestinal wall (0.032 μg MC-LReq/g), gonads (0.027 μg MC-LReq/g) and muscles (0.067 μg MC-LReq/g) of *Cyprinus carpio* originated from the eutrophic Lake Taihu in China providing more evidence about the increased succesibility of carps on cyanotoxins in eutrophicated lakes (Tencalla et al. 1994; Xie et al. 2007). It seems that in eutrophicated lakes cyprinids can accumulate toxins surviving at sub-lethal concentrations.

We also found significant microcystin concentrations in various tissues of the endemic frog (*Rana epirotica*). Bioaccumulation of microcystins in amphibians may be critical if these represent linkages to terrestrial environments (White et al. 2007). For example, pre-metamorphic stages (tadpoles) may be consumed by terrestrial birds, thus allowing toxin to enter terrestrial food webs. However, until now, only one report refers to accumulation of microcystins in amphibians (Gkelis et al. 2006) and few reports exist on microcystin toxicity in amphibians (Gromov et al. 1997; Oberemm et al. 1999; Fischer and Dietrich 2000b; Dvorakova et al. 2002; White et al. 2007). The higher microcystin concentrations accumulated in liver and pancreas confirming the preferential accumulation in those organs. Lower concentrations were found in intestines, gonads and muscle probably as a result of microcystin transport via the bloodstream, or bypassing the hepatopancreas, or downstream of the hepatopancreas subsequent to an overload of presystemic hepatic elimination capacities (Fischer and Dietrich 2000a). Significant microcystin concentrations were detected in the skin of the frog, suggesting that transdermal uptake of extracellular microcystins is possible. This is in agreement with studies of Gromov et al. (1997) who found that larvae of the frog *Rana temporaria* died after exposure with lyophilised *Microcystis aeruginosa* biomass. These larvae were so young that they would not have been feeding during the experimental period, thus only transdermal uptake of extracellular toxin was possible. Gkelis et al. (2006) have also found similar microcystin concentrations in viscera (465 ng MC-LReq/g) and muscle (262 ng MC-LReq/g) of *Rana epirotica* from Lake Pamvotis. Our study along with other related studies, support that algal toxin exposure may be a contributing factor in the recent worldwide decline of amphibians, as reported in Kiesecker et al. (2001), especially since it is well known that cyanobacterial toxic blooms pose an increased risk globally (de Figueiredo et al. 2004).

The risk of being exposed to toxins through food consumption would be further increased if biomagnification were to take place, since some popular food, like fish species may stand at the top of the food web. Thus, the estimation of the BMF in a lake with presence of toxic cyanobacteria would be valuable for the protection of public health. The calculation of BMF in Lake Pamvotis, showed that there is no magnification of microcystins

through foodweb. BMF was higher when microcystin concentrations in seston were higher too. However, BMF concerning the fish species *Cyprinus carpio* was higher in November, probably due to the ability of fish to reduce their metabolic activities during the cold period. Rather than biomagnification, the opposite process, i.e., biodilution of hepatotoxins in the food web is supported by part of the literature (Engström-Öst et al. 2002; Sipiä et al. 2004; Ibelings et al. 2005; Karjalainen et al. 2005). However, the fact that microcystins are found in the tissues of organisms caught from Lake Pamvotis, clearly indicates that transfer of microcystins within the foodweb takes place, thus may be a potential health risk to human. The species *Astacus astacus* and *Cyprinus carpio* are considered as commercial species concerning inland fisheries while the latter is among the most important aquaculture products in many regions of the world included Greece. Additionally, the frog *Rana epirotica* is considered as a local food which attracts the liking of many tourists.

Estimated daily intake (EDI) calculations, included in our discussion, are aimed at assessing the potential exposure of humans to toxins thus contributing in the context of public health. Regarding at the risk to humans, EDI estimation is necessary before comparing our measured concentrations of microcystins in the edible tissue samples to the World Health Organization (WHO) tolerable daily intake (TDI) provisional guidelines. At the levels found in muscle tissue of *Astacus astacus* in our study the calculated EDI was two times (in November) and six times (in August) higher than the advisable TDI value (0.04 μg/kg of body weight). In muscle of *Cyprinus carpio* in our study the calculated EDI was 11 times (in November) and 14 times (in August) higher than the advisable TDI value. In muscle of *Rana epirotica* the calculated EDI was one time (in November) and six times (in August) higher than the advisable TDI value (Fig. 5). Therefore, *Astacus astacus*, *Cyprinus carpio* and *Rana epirotica* from Lake Pamvotis could possibly pose a health hazard and they were not safe for human consumption. Finally, it is necessary to point out that only uptake of microcystins from aquatic organisms was used to evaluate exposure risk of human to microcystins. However, water is often the major route for human microcystins' intoxication. In the present study, concentration of dissolved microcystins in the water samples of Lake Pamvotis ranged from 0.32 μg MC-LReq/L to 4.12 μg MC-LReq/L, with the values in warm period to be well above the safety limit of 1 μg/L MC-LR for drinking water. However, the amount of dissolved and intracellular microcystins found in Lake Pamvotis, poses moderate health risks through recreational activities (W.H.O. 1998). Although Lake Pamvotis is not used as source of drinking water, the combined risks from microcystin contaminated water and consumption of contaminated aquatic organisms,
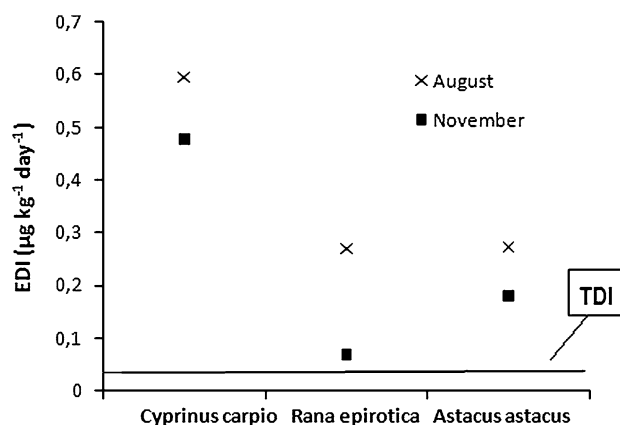
Fig. 5 Estimated daily intake (EDI) of microcystins based on a 60 kg person ingesting a 300 g serving of *Cyprinus carpio* or 50 g serving of *Astacus astacus*, or 50 g of *Rana epirotica* from Lake Pamvotis, during November and August of 2008. The *horizontal line* represents the tolerable daily total intake (TDI) established by WHO

to human health cannot be overlooked in the region around Lake Pamvotis.

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