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Cyanobacteria and microcystins in lake Furnas (S. Miguel island-Azores)

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ABSTRACT

Cyanobacteria and microcystins in lake Furnas (S. Miguel Island-Azores)

This study investigated the changes in the cyanobacterial population and quantified the occurrence of microcystins in Lake Furnas during the first decade of the 21st century.
The eutrophication of Lake Furnas has been recognized since the 1980s. The lake's phytoplankton population increased over the years in relation to this process of nutrient enrichment. Cyanobacteria began to dominate the phytoplankton and blooms of greater than $20 \cdot 10^3$ cells/ml occurred. After 2004, cyanobacterial blooms occurred regularly in the lake throughout the year.
From 2000 through 2009, 30 blooms were detected. Of these blooms, 13 were dominated by *Microcystis aeruginosa* and 11 by *Woronichinia naegeliana*. In the other 6 blooms, the dominant cyanobacteria were *Microcystis* spp., *Anabaena* spp., *Aphanocapsa* spp. and *Coelosphaerium kuetzingianum*. A number of blooms involved more than 2 species simultaneously. The highest cell density ($12.3 \cdot 10^6$ cells/ml) occurred during winter 2007. The predominant species in this bloom were *M. aeruginosa* ($11.9 \cdot 10^6$ cells/ml) and *W. naegeliana* ($83.8 \cdot 10^3$ cells/ml).
Because almost all of the cyanobacteria cited were considered toxin producers, a search for microcystins, the hepatotoxins most often found in freshwaters, was initiated in 2001. Samples were collected at four depths: surface, 2.5 m, 5.0 m and 0.5 m above the sediments.
From 2001 through 2009, soluble microcystins were detected six times during the summer, four times during the winter and autumn and three times during the spring (25 % of 129 samples). The average concentrations of soluble microcystins in the water column ranged from 0.1 μg/l to 0.5 μg/l. Intracellular microcystins were detected in 84 % of the samples. All samples collected after 2004 contained these cyanotoxins. The average concentrations of intracellular microcystins in the water column ranged from 0.1 μg/l to 11.2 μg/l. The highest value (154.5 μg/l) was found in a water sample collected from the lake surface during the winter of 2009, during a bloom dominated by *W. naegeliana*. The amounts of microcystins produced by cyanobacteria, expressed on a seston dry weight basis, varied between 24 mg/kg and 9737 mg/kg and showed an increase in 2008 and 2009. The concentrations of microcystins in samples from *M. aeruginosa* blooms ranged from 86 mg/kg to 1171 mg/kg and the highest values were recorded during the spring and summer of 2008.

Key words: Water quality, cyanobacteria, blooms, microcystins.

RESUMEN

Cianobacterias y microcistinas en la laguna Furnas (isla de S. Miguel-Azores)

Este estudio presenta la variación de la población de cianobacterias y la cuantificación de microcistinas en la Laguna Furnas en la primera década del siglo 21.
La eutrofización de la Laguna Furnas había sido señalada desde los años ochenta del siglo pasado. Debido a este proceso de enriquecimiento en nutrientes, la población de fitoplancton aumentó a lo largo de los años, las cianobacterias se volvieron dominantes y surgieron blooms (más de $20 \cdot 10^3$ células/ml). Después del 2004, los blooms de cianobacterias fueron permanentes en la laguna.

De 2000 a 2009, se detectaron 30 blooms, 13 dominados por Microcystis aeruginosa *y 11 por* Woronichinia naegeliana. *En los 6 restantes, las cianobacterias dominantes fueron* Microcystis *spp*., Anabaena *spp*., Aphanocapsa *spp*. *y* Coelosphaerium kuetzingianum. *A recesen algunas ocasiones, existieron más de dos especies en la fase de Bloom al mismo tiempo. La densidad celular más alta ($12.3 \cdot 10^6$ células/ml) tuvo lugar en el invierno de 2007, siendo* M. aeruginosa *($11.9 \cdot 10^6$ células/ml) y la* W. naegeliana *($83.8 \cdot 10^3$ células/ml) las especies predominantes.*

A partir de 2001 y una vez que casi todas las cianobacterias mencionadas fueron consideradas como productoras de toxinas, se inició la búsqueda de microcistinas, concretamente las hepatotoxinas ya que son las más frecuentes en aguas dulces. Las muestras se tomaron en cuatro profundidades: superficie, 2.5 m, 5.0 m y 0.5 m por cima del sedimento.

De 2001 a 2009, fueron detectadas microcistinas solubles seis veces en verano, cuatro veces en invierno y otoño y tres veces en primavera (25 % de 129 muestras). Sus concentraciones medias en la columna de agua variaron desde 0.1 µg/l hasta 0.5 µg/l. Fueron detectadas microcistinas intracelulares en el 84 % de las muestras y en todas las recogidas después del 2004. Sus concentraciones medias en la columna de agua variaron desde 0.1 µg/l hasta 11.2 µg/l. El valor más alto (154.5 µg/l) fue encontrado en una muestra recogida en la superficie durante el invierno de 2009, mientras ocurría un bloom dominado por W. naegeliana. *Las cantidades de microcistinas producidas por las cianobacterias, expresadas en base de peso seco del seston, variaron entre 24 mg/kg y 9737 mg/kg y mostraron un aumento en los años 2008 y 2009. Las concentraciones en muestras de blooms de* M. aeruginosa *variaron desde 86 mg/kg hasta 1171 mg/kg y los valores más altos fueron registrados en primavera y verano de 2008.*

Palabras clave: *Calidad del agua, cianobacterias, floraciones algales, microcistinas.*

INTRODUCTION

Lake Furnas is located in the eastern region of S. Miguel island, the largest island in the Archipelago of the Azores (37°46′N; 25°19′W). It is a warm monomictic lake and is located in the caldera of Furnas volcano. The surface of the lake is 280 m above sea level. The lake has an area of 1.9 km^2 and a maximum depth of 12 m. Over the past 30 years, a local meteorological station recorded average air temperatures in this region ranging from 12 °C in winter to 19 °C in summer (unpublished data supplied by Regional Environmental Secretary). The daily thermal amplitude was very narrow. The monthly average precipitation ranged from 96 l/m^2 in summer to 229 l/m^2 in winter, with a daily maximum of 802 l/m^2. The hydrological basin has an area of 13 km^2 and a perimeter of 14 km. The total runoff from the drainage basin to the lake is approximately $22.2 \cdot 10^6$ m^3/year and flows mainly from three small torrential streams.

The eutrophication of Lake Furnas, due to nutrient enrichment enhanced by human agricultural activities, began to be recognised during the 1980s. As a result of the first monitoring programme conducted in this lake in 1988/89, the lake was classified as eutrophic (average Carlson's TSI for chlorophyll a = 66). Later studies concluded that Lake Furnas was receiving nutrient loadings eleven and nine times higher than the maximum permissible loadings of nitrogen and phosphorus, respectively 17.0-67.0 g N/m^2 · year and 0.86-1.61 g P/m^2 · year. These nutrient inputs explained the trophic state of the lake.

In 1988/89, the phytoplankton densities in Lake Furnas ranged from $6.21 \cdot 10^3$ cells/ml to $9.34 \cdot 10^3$ cells/ml. The phytoplankton populations were dominated by Bacillariophyceae during the winter, Chlorophyceae during the spring and Cyanobacteria during the autumn and summer (primarily *Aphanizomenon flos-aquae*- $3.09 \cdot 10^3$ cells/ml to $5.96 \cdot 10^3$ cells/ml). A *Microcystis* sp. was also found, but its maximum count was $0.11 \cdot 10^3$ cells/ml (Rodrigues *et al.*, 1993). From 1992 to 1996, phytoplankton densities varied between $0.83 \cdot 10^3$ cells/ml and $13.0 \cdot 10^3$ cells/ml, and Bacillariophyceae still dominated during the winter, Chlorophyceae during the spring and cyanobacteria during the sum-

mer and autumn. The percentages of cyanobacteria during this period ranged from 0 to 93 %. The most abundant species found was again *Aphanizomenon flos-aquae,* with a maximum density of $12.1 \cdot 10^3$ cells/ml (INOVA, 1996-unpublished data). In 1997 and 1998, the maximum phytoplankton count increased to $27.9 \cdot 10^3$ cells/ml, but cyanobacteria represented only 35 % of the total phytoplankton. Among the cyanobacteria, *Aphanizomenon flos-aquae* almost disappeared, *Anabaena* sp. increased to a maximum of $0.78 \cdot 10^3$ cells/ml and *Microcystis aeruginosa* increased to $8.40 \cdot 10^3$ cells/ml during autumn 1997 and to $9.86 \cdot 10^3$ cells/ml during winter 1998. Chlorophyceae were dominant during the remainder of 1997/98. Their densities ranged from $0.97 \cdot 10^3$ cells/ml to $6.23 \cdot 10^3$ cells/ml (INOVA, 1998-unpublished data). These findings are normal under eutrophic conditions. In eutrophic waters, cyanobacteria often dominate the summer and early autumn phytoplankton population, whereas during the winter and spring they are replaced by diatoms and green algae (Tilman *et al*., 1986). Nevertheless, cyanobacteria can be present and even dominant throughout the year (Chorus & Bartram, 1999).

During the autumn of 1997 and the winter of 1998, severe rains occurred in the region. As a result, substantial amounts of nutrients were transported from the basin to the lake. Because the temperature and light in the S. Miguel island region are adequate for phytoplankton growth throughout the year and the nutritional conditions favour cyanobacteria development (Oliver & Ganf, 2000), blooms occurred in 2000. The term "bloom" is used here to mean a cell concentration greater than $20 \cdot 10^3$ cells/ml (Oliver & Ganf 2000).

Eutrophication has been recognised as a water quality problem of increasing concern since the 1950s. Subsequently, the proliferation of cyanobacteria and cyanobacterial toxins were considered to be human health problems deriving from eutrophication because cyanobacteria that flourish in aquatic environments can produce a diverse range of toxins that present risks of illness and even mortality to humans and animals (Chorus & Bartram, 1999). Cyanotoxins are secondary metabolites synthesised within the cells and include a wide variety of chemical compounds, primarily alkaloids and peptides (Sivonen & Jones 1999; Kardinaal & Visser, 2005; Falconer 2005), but also organophosphates and lipopolysaccharides (Chorus, 2001).

Freshwater cyanobacteria can produce microcystins and nodularins (both hepatotoxic cyclic peptides) and the cyclic guanidine alkaloid cylindrospermopsin as primary products. In addition, they can produce the neurotoxic alkaloids anatoxin-a, anatoxin-a(S) and saxitoxins, and lipopolysaccharides with pyrogenic properties (Chorus & Bartram, 1999; Chorus 2001). Microcystins are produced by the most abundant cyanobacteria found in freshwaters worldwide. Several planktonic genera, such as *Microcystis*, *Anabaena*, *Aphanizomenon* and *Planktothrix*x, are considered potentially toxigenic. The concentrations of microcystins monitored in several lakes revealed highly variable concentrations (μg/l and per unit seston dry weight-mg/kg_{dw}) (Chorus & Bartram, 1999; Chorus 2001; Codd *et al*. 2005; Falconer, 2005; Kardinaal & Visser 2005).

The aim of this study is to report the occurrence of cyanobacterial blooms and microcystins in Lake Furnas (S. Miguel Island-Azores) and their variability in space and time over the past ten years.

MATERIALS AND METHODS

Water sampling, physicochemical analysis and TSI

The monitoring programme was initiated in February 2000 and included 38 sampling trips. Three of these trips were made in 2000, two in 2001 and four (winter, spring, summer and autumn) every year from 2002 through 2009. The water samples were always collected from a boat between 11 a.m. and 12 a.m., at one sampling point located in the middle of the lake at its deepest zone. Samples were collected with a Van Dorn-type device at the surface, 2.5 m, 5 m and 0.5 m above the sediments and transported in refrigerated polyethylene bottles (or in glass for samples destinated for the analysis of phosphorus

and microcystins) to a laboratory. The samples were then analysed for physical and chemical parameters (nitrogen and phosphorus compounds and chlorophyl *a* (Standard Methods, 1998)). The water temperature, pH, dissolved oxygen, turbidity and conductivity were determined *in situ* with a multi-parameter meter (Horiba U 10, Horiba Ltd., Kyoto, Japan). Transparency was determined with a Secchi disc.

Carlson's trophic state index (TSI) based on chlorophyll *a* (chl *a*) concentrations was calculated using the following equation (Carlson, 1977): TSI = $10(6 - \log_2 7.7/\text{chl } a^{0.68})$.

Phytoplankton analyses

Samples for phytoplankton analyses were obtained by mixing equal volumes of water collected at the four depths. One aliquot was placed in a polyethylene flask and preserved with a 1 % Lugol solution. Phytoplankton identifications were performed with a Leica DML optical microscope. Utermöhl's method was used to determine the cell count (Lund *et al*. 1958).

Analyses of microcystins

The measurements of microcystins were made in the samples collected at the depths specified above from 2001 through 2009. The water samples were filtered through a glass fibre membrane (WhatmanGF/F, nominal pore size ≈ 0.7 μm). The filters with the biomass were kept frozen at −18 °C prior to toxin extraction with methanol. The filtered samples were passed through C18 cartridges (Sep-Pak Waters Corporation), preconditioned with methanol and washed with water at a flow rate not exceeding 10 ml/min. The cartridges were then eluted with 3 ml methanol acidified with 0.1 % TFA. The extracts were evaporated to dryness with a nitrogen flow. The residue was re-dissolved in 250 μl methanol and centrifuged for 10 min at 10 000 rpm. The filter discs were placed in glass beakers containing 20 ml of methanol and allowed to extract for 24 h at −4 °C. The samples were evaporated under pressure at 40 °C until they became dry. The residue was re-dissolved in 500 μl methanol and centrifuged for 10 min at 10 000 rpm. The centrifuged extracts were analysed using HPLC-UV with photodiode array detection using an Acclaim column (4.6×150 mm; 3 μm). The different microcystins were separated and their peaks were identified based on UV absorption spectra analysis. The chromatographic signals (peaks) were quantified using microcystin LR standard calibration curves. The concentrations of total (unidentified) microcystins in each sample were expressed in equivalent units of microcystin LR (Lawton *et al*. 1994).

Table 1. Nitrogen, phosphorus and chlorophyll *a* concentrations in the water of Lake Furnas during 2000-2009. *Concentraciones del nitrógeno, fósforo y clorofila* a *en el agua de la Laguna Furnas en el periodo de 2000-2009.*

		2000-2009			
		Aver.	Max.	Min.	n
Nitrogen (mg N/m^3)	Total	1474	2730	440	38
	Inorganic	316	940	80	38
Total Phosphorus (mg P/m^3)		49	93	17	38
Chlorophyll-*a* (mg/m^3)		28	91	8	38
TN:TP (mg N/mg P)		34	66	12	38

RESULTS

Physicochemical measurements and TSI

The water temperature at the surface of the lake at 11 a.m. ranged from 13 °C-14 °C during the late autumn and winter to 22 °C-23 °C during the summer. The transparency ranged from 0.2 m to 3.0 m. The average transparency was 1.0 m. The surface water pH ranged from 6.6 to 9.6.

The average nitrogen, phosphorus and chlorophyll *a* (Chl *a*) concentrations in the water column and the TN:TP mass ratio are shown in Table 1.The inorganic nitrogen concentrations (the sum of ammonium, nitrite and nitrate) were quite variable, ranging from 80 mg N/m^3 to 940 mg N/m^3, with an average (316 mgN/m^3) corresponding to a meso-eutrophic state. The total phosphorus concentrations (TP) ranged from 17 mg P/m^3 to 93 mg P/m^3. The average concentration of TP was 49 mg P/m^3, characteris-

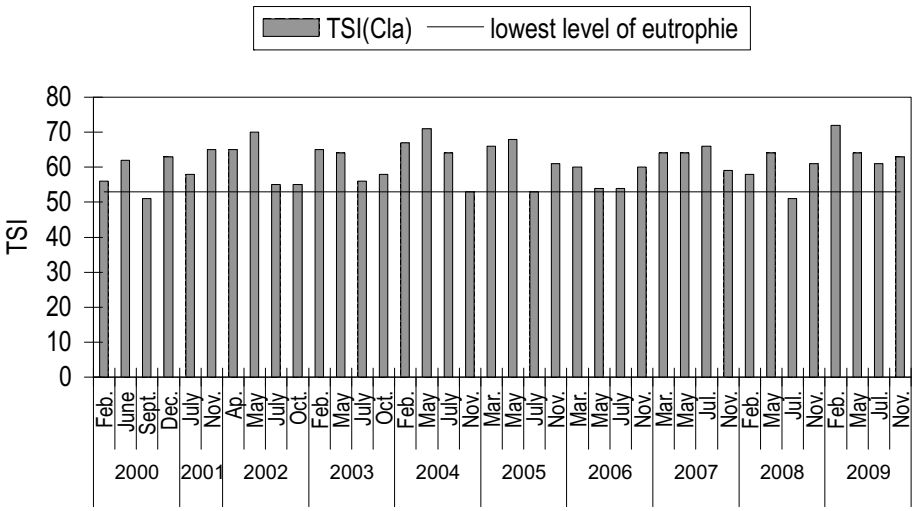

Figure 1. Chlorophyll (Carlson's Trophic State Index) in Lake Furnas (2000/2009). *Índice de Estado Trófico de Carlson (IET) para la clorofila en la Laguna Furnas (2000/2009).*

tic of eutrophic conditions (Vollenweider, 1968). According to OECD, 1982, the average concentration of TP and the concentration of Chl *a* (8 mg/m^3 to 91 mg/m^3, with an average of 28 mg/m^3) in Lake Furnas correspond to a eutrophic state. The maximum value of chlorophyll was already characteristic of a hypertrophic aquatic environment. The TN:TP mass ratio (mg N/mg P) ranged from 12 to 66, with an average of 34.

The Carlson's Trophic State Index (TSI) average, based on Chl *a* concentrations (Fig. 1), was 61, well above the eutrophic lower limit of 53 established by Kratzer & Brezonic (1981). A minimum TSI of 51 was found only twice in 38 sampling trips. TSIs above 70 were detected three times. These findings indicate that the lake is eutrophic with a tendency to hypertrophy.

Phytoplankton and cyanobacteria

Total phytoplankton increased during the years of the study. The percentages of cyanobacte-

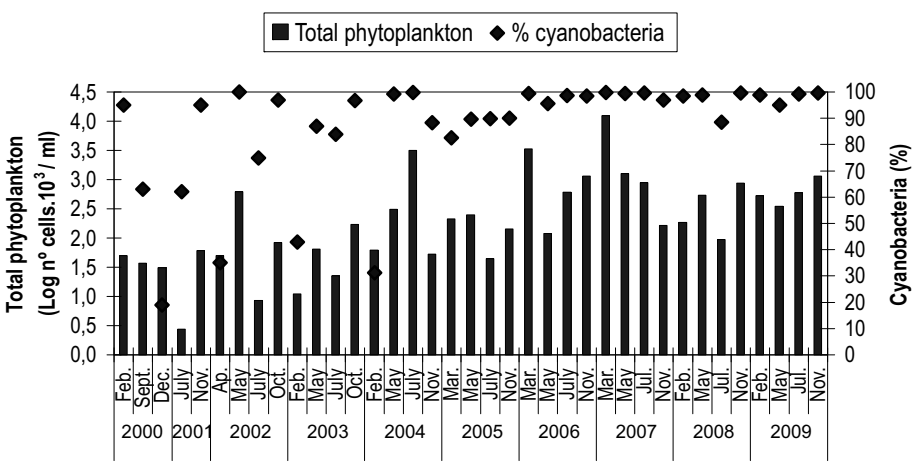

Figure 2. Total phytoplankton and relative percentage of cyanobacteria in Lake Furnas (2000/2009). *Porcentaje relativa en total de fitoplancton y cianobacterias en la Laguna Furnas (2000/2009).*

ria remained greater than 90 % after 2005. The worst situations occurred during 2006 and 2007. Cyanobacteria represented approximately 99% of the total phytoplankton (Fig. 2) in almost every instance throughout these years.

The first cyanobacterial bloom was observed in February 2000. The bloom was caused by a proliferation of *Microcystis aeruginosa* ($45.2 \cdot 10^3$ cells/ml). Subsequent to that event and until 2009, this same species participated in 17 additional blooms, alone on five occasions and dominant in 12 when other cyanobacteria were also blooming. During 2006, the blooms of this species remained in the lake throughout the year. The situation reached its peak ($11.9 \cdot 10^6$ cells/ml) during the winter of 2007. One unidentified *Microcystis* sp. bloomed ($150.2 \cdot 10^3$ cells/ml) during the autumn of 2003. Two other *Microcystis* species, identified as *M. flos-aquae* and *M. robusta*, were detected in three additional blooms. *M. robusta* bloomed alone during autumn 2007 ($153.6 \cdot 10^3$ cells/ml) and bloomed during winter 2008 ($56.5 \cdot 10^3$ cells/ml) with *Woronichinia naegeliana* ($121.2 \cdot 10^3$ cells/ml). *M. flos-aquae* bloomed ($270.3 \cdot 10^3$ cells/ml) with *M. aeruginosa* ($736.9 \cdot 10^3$ cells/ml) and *Coelosphaerium kuetzigianum* ($204.8 \cdot 10^3$ cells/ml) during autumn 2009 (Fig. 3).

The last mentioned cyanobacterium was first detected in November 2005 with a smaller cell density ($2.3 \cdot 10^3$ cells/ml). Its cell counts subsequently increased to $78.0 \cdot 10^3$ cells/ml during the summer and to $497.3 \cdot 10^3$ cells/ml during the autumn of the same year. In March 2007, during the largest *M. aeruginosa* bloom, *C. kuetzigianum* ($395.0 \cdot 10^3$ cells/ml) was the second cyanobacterium blooming in the lake. *W. naegeliana* was the third, with $83.8 \cdot 10^3$ cells/ml. Subsequently, *C. kuetzigianum* occurred in five additional blooms, with cell densities varying from $44.7 \cdot 10^3$ cells/ml to $610.6 \cdot 10^3$ cells/ml and always combined with two or more cyanobacteria species that were also blooming.

W. naegeliana blooms began in the spring of 2003 ($53.0 \cdot 10^3$ cells/ml) when it was the only species of cyanobacteria blooming in the lake. Subsequently, this cyanobacterium was present in 16 more blooms, with cell densities that ranged from $31.3 \cdot 10^3$ cells/ml to $3.18 \cdot 10^6$ cells/ml. The most significant bloom occurred in July 2004, when *M. aeruginosa* was also present. *W. naegeliana* was dominant in three more blooms ($121.0 \cdot 10^3$ cells/ml to $248.8 \cdot 10^3$ cells/ml) in association with *Microcystis* spp., *Anabaena* sp. or *C. kuetzigianum*. It occupied second or third place in seven other blooms. During autumn 2004 ($47.0 \cdot 10^3$ cells/ml), spring and summer 2005 ($224.6 \cdot 10^3$ cells/ml and $31.3 \cdot 10^3$ cells/ml) and winter and spring 2009 ($493.2 \cdot 10^3$ cells/ml and $322.1 \cdot 10^3$ cells/ml), *W. naegeliana* was the only species of cyanobacteria blooming in the lake.

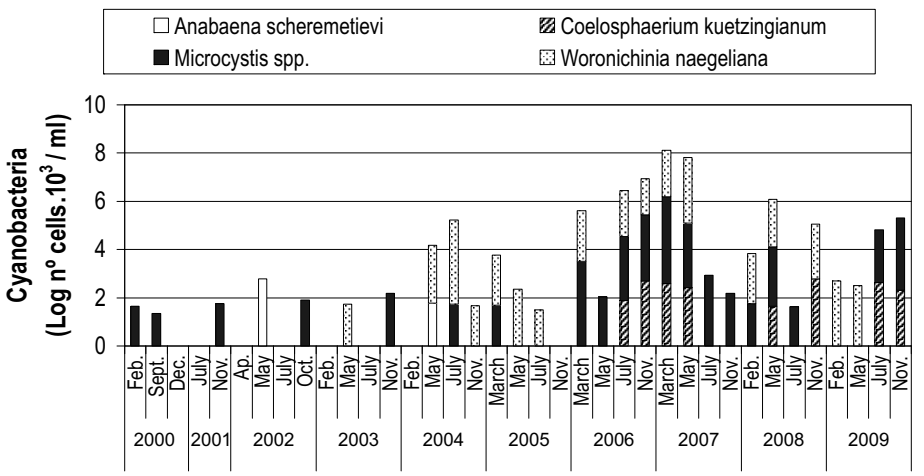

Figure 3. Relative abundance of the principal cyanobacterial species blooming in Lake Furnas (2000/2009). *Abundancia relativa de cianobacterias causadoras de blooms en la Laguna Furnas (2000/2009).*

Anabaena spp. blooms were detected only twice. *A. solitaria* bloomed alone ($606.2 \cdot 10^3$ cells/ml) during May 2002 and *A. scheremetievi* ($58.8 \cdot 10^3$ cells/ml) bloomed together with *W. naegeliana* ($248.8 \cdot 10^3$cells/ml) during May 2004.

No correlations were found between the cell densities of cyanobacteria and nitrogen or phosphorus concentrations (inorganic and total forms) or with the TN:TP mass ratio. For example, *Microcystis* sp. blooms occurred at TN:TP mass ratio values from 12 to 57 and *W. naegeliana* blooms were detected at TN:TP values from 18 to 66.

Microcystins

The detection of the blooms of 2000 was followed, beginning in 2001, by the analysis of microcystins in the water of Lake Furnas. A total of 129 samples were collected at several depths from 2001 through 2009. Microcystins in solution (extracellular) were detected in 25 samples (19 %). These microcystins were detected most often in samples from the lake's surface (11 samples) and less frequently in samples taken at a depth of 5 m (3 samples). Seven samples from the 2.5 m depth and five samples collected near the bottom were positive for dissolved microcystins.

The amount of dissolved microcystins (average concentrations in the water column) ranged from 0.05 μg $MC\text{-}LR_{equiv}$/l to 0.49 μg $MC\text{-}LR_{equiv}$/l (Fig. 4). The highest single concentration (1.95 μg $MC\text{-}LR_{equiv}$/l) was detected in a sample collected 0.5 m above the sediments in May 2007.

Intracellular microcystins (seston) were present in 108 samples (84 %) collected at several depths. Their concentration increased during 2008 and 2009. After 2005, all the samples analysed contained intracellular microcystins. The samples from the lake's surface normally contained higher concentrations of intracellular microcystins, but these compounds were also present in samples from other depths.

The most frequently observed concentrations were less than 20 μg $MC\text{-}LR_{equiv}$/l. However, during the winter of 2009, a value of 154.5 μg $MC\text{-}LR_{equiv}$/l was observed in a sample collected from the surface of the lake during a bloom of *W. naegeliana* ($493.2 \cdot 10^3$ cells/ml). At that time, *A. sheremetievi* ($9.5 \cdot 10^3$ cells/ml), *Aphanocapsa delicatissima* ($9.2 \cdot 10^3$ cells/ml), *Chroococcus dispersus* ($6.6 \cdot 10^3$ cells/ml) and *Lyngbya* sp. ($6.4 \cdot 10^3$ cells/ml) were also recorded, but none of these four species were blooming. On several occasions in this lake, the occurrence of *W. naegeliana* could be related to the detection of microcystins. During winter 2004, this bacterium was the only species of cyanobacteria found in the lake. Its cell density was lower

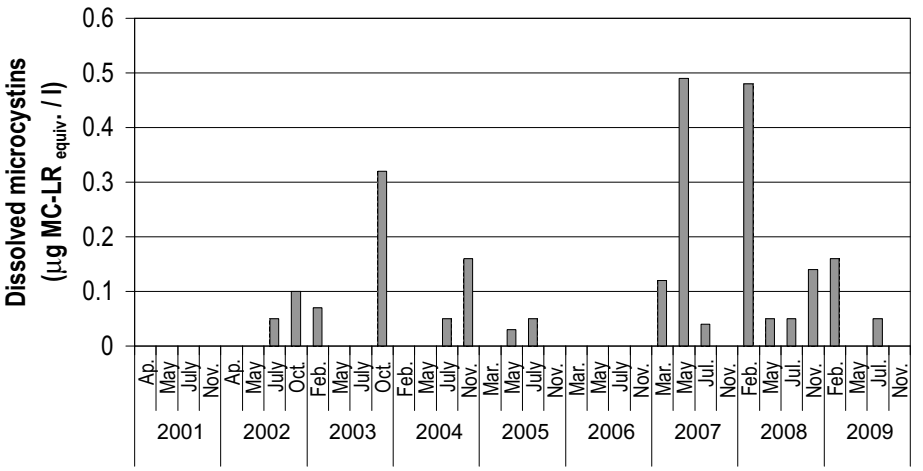

Figure 4. Concentrations of dissolved microcystins (μg MC-LR $_{equiv}$/l) detected in Lake Furnas samples collected from 2001 through 2009. *Concentraciones de microcistinas disueltas (μg $MC\text{-}LR_{equiv}$/l) detectadas en muestras de la Laguna Furnas cogidas entre 2001 y 2009.*

than that of a bloom ($19.4 \cdot 10^3$ cells/ml), and intracellular microcystins were detected at concentrations of 2.85 μg MC-LR_{equiv}/l to 4.33 μg MC-LR_{equiv}/l. Concentrations of 1.03 μg MC-LR_{equiv}/l to 1.71 μg MC-LR_{equiv}/l were also detected during the autumn of the same year when *W. naegeliana* ($47.0 \cdot 10^3$ cells/ml) was blooming alone in the lake. The other cyanobacterium present was *Phormidium mucicola*, occurring at a very low density ($0.02 \cdot 10^3$ cells/ml). *W. naegeliana* was also the only cyanobacterium blooming during spring 2005 ($224.6 \cdot 10^3$ cells/ml) and intracellular microcystins were detected in the water column at concentrations ranging from 0.56 μg MC-LR_{equiv}/l to 2.38 μg MC-LR_{equiv}/l. Other cyanobacteria were also identified at the same time, but their densities were very low (*A. scheremetievi* $-0.09 \cdot 10^3$ cells/ml, *Aphanocapsa elachista* and *Oscillatoria* sp. – both with $< 0.01 \cdot 10^3$ cells/ml). These results indicate that the *W. naegeliana* strain found in this Azorean lake is toxigenic.

No correlations were found between the average concentrations of total microcystins in the water column and the cell densities of cyanobacteria (Fig. 5). In fact, the highest amounts of microcystins did not correspond to the largest blooms of cyanobacteria. Moreover, no correlations were found between the densities of individual species blooming in the lake and the concentrations of microcystins.

The amounts of microcystins produced by cyanobacteria, expressed based on seston dry weight, were determined only after 2003. The average concentrations in the water column varied from 0 to 6060 mg MC-LR_{equiv}/kg_{dw} and showed an increase during 2008 and 2009 (Fig. 6). The highest concentration (9737 mg MC-LR_{equiv}/kg_{dw}) was recorded in a sample collected from the surface of the lake in May 2009 during a bloom of *W. naegeliana* ($322.0 \cdot 10^3$ cells/ml). *C. kuetzigianum* ($8.5 \cdot 10^3$ cells/ml) was also present, and other cyanobacteria were present at densities less than $1.0 \cdot 10^3$cells/ml. At that time, samples from 2.5 m and 5 m depths also contained high amounts of microcystins (6216 mg MC-LR_{equiv}/kg_{dw} and 8070 mg MC-LR_{equiv}/kg_{dw}, respectively). In samples from *M. aeruginosa* blooms, the concentrations of microcystins ranged from 86 mg MC-LR_{equiv}/kg_{dw} to 1171 mg MC-LR_{equiv}/kg_{dw}. The highest values were recorded during spring and summer 2008. The concentrations of microcystins per unit seston dry weight were not correlated with the cell densities of cyanobacteria.

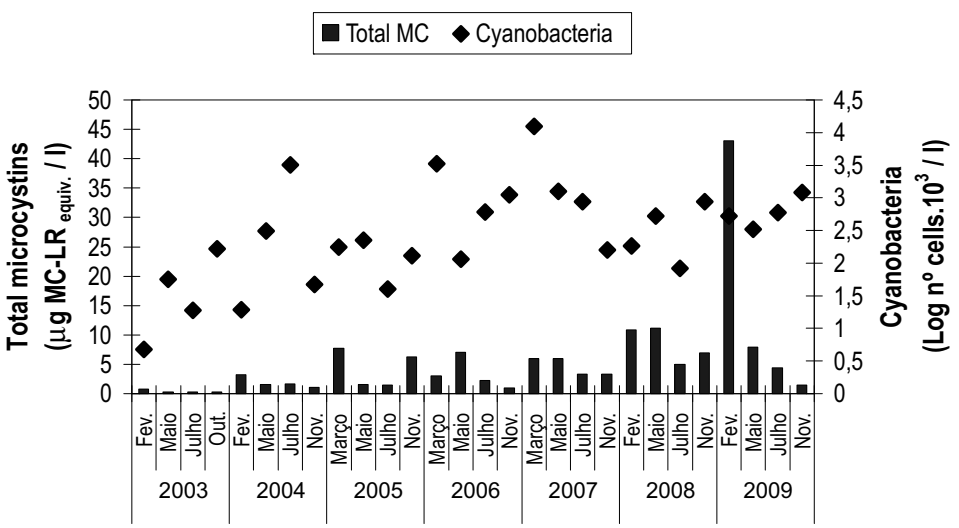

Figure 5. Average concentrations of total microcystins in the water column (MC-mg MC-LR_{equiv}/m^3) vs. cyanobacterial cell densities in Lake Furnas (2001/2009). *Concentraciones medias de microcistinas totales en la columna de agua (MC-mg MC-LR_{equiv}/m^3) vs. densidades celulares de cianobacterias en la Laguna Furnas (2001/2009).*

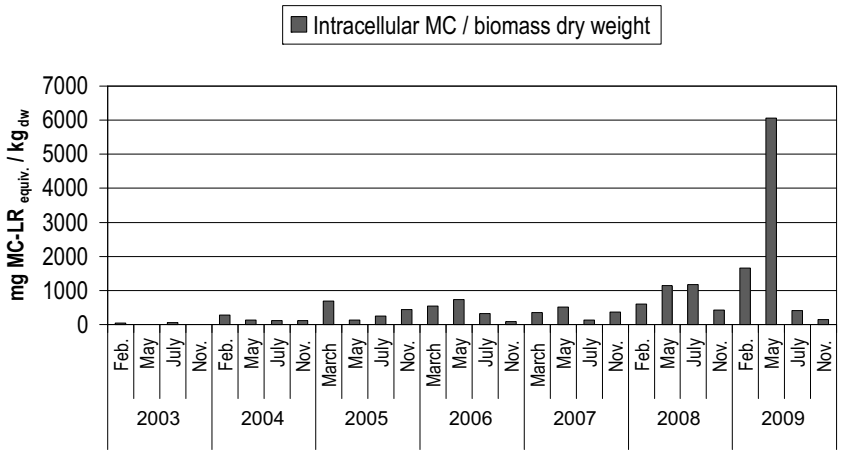

Figure 6. Average concentrations of intracellular microcystins in the water column per unit seston dry weight (mg MC-LR $_{equiv}$/kg$_{dw}$) in Lake Furnas (2003/2009). *Concentraciones medias de microcistinas intracelulares por unidad de peso seco de seston (mg MC-LR$_{equiv}$/kg$_{ps}$) en la Laguna Furnas (2003/2009).*

DISCUSSION

Although the process of eutrophication in Lake Furnas has been recognised since 1989, blooms of cyanobacteria only began to occur in the lake in 2000. These blooms followed the severe rains of 1997/1998 on the island of S. Miguel. Until 2004, blooms of cyanobacteria were primarily detected between spring and autumn. This pattern is similar to that known from many countries of the European continent, such as Continental Portugal (Vasconcelos, 1994) and Spain (Quesada *et al*., 2004; Moreno *et al*., 2005), where blooms can occur in rivers and reservoirs, especially during late summer and autumn. After 2004, cyanobacteria blooms occurred regularly in Lake Furnas, even during winter, due to high nutrient availability and also to environmental conditions. In fact, this study shows that the mild weather of the Azorean Archipelago favours the proliferation of cyanobacteria throughout the year. Previously, the occurrence of blooms of cyanobacteria was also reported from Lake Sete-Cidades, a mesotrophic lake located in the northwest zone of the island. These blooms lasted from winter to autumn (Santos *et al*., 2005).

The blooms occurring in Lake Furnas were dominated primarily by *Chroococcales* (*Microcystis* spp., especially *M. aeruginosa*, *C. kuetzingianum* and *W. naegeliana*). Blooms of *Anabaena* sp. (*Nostocales*) only occurred twice. The highest cell density of cyanobacteria occurred during winter 2007 ($12.3 \cdot 10^6$ cells/ml), in a bloom dominated by *M. aeruginosa* ($11.9 \cdot 10^6$ cells/ml). In this bloom, *C. kuetzingianum* ($395 \cdot 10^3$ cells/ml) was subdominant and *W. naegeliana* ($84 \cdot 10^3$ cells/ml) was also present. Cell densities as high as these were previously reported by several authors, e.g. Moreno *et al*., 2005 (Vitonogales and Valdelacalzada in Guadiana River, Spain) and Pawlik-Skowronska *et al*., 2004 (lakes in Poland).

The absence of a significant relationship between cyanobacterial cell densities and nutrient concentrations in Lake Furnas indicates that nitrogen and phosphorus were always plentiful in the lake during the period of investigation. In fact, inorganic nitrogen concentrations ranged from 80 to 940 mg N/m^3. These very high values also explained the dominance of cyanobacterial species that are not diazotrophic. Even the blooms of the nitrogen-fixing *Anabaena* spp. occurred at inorganic nitrogen concentrations of 250 mg/m^3 and 150 mg/m^3, higher than the threshold value of 50-100 mg/m^3 considered to be the limit below which nitrogenase activity is induced (Oliver & Ganf, 2000). These results also suggest that TN:TP ratios are not important for determining cyanobacterial dominance, as previously concluded by several authors cited

in Oliver & Ganf (2000). Smith (1983) concluded from a survey of data on 17 lakes that bloom-forming cyanobacteria will tend to dominate if the TN:TP mass ratio is less than 29. However, one half of the 30 cyanobacterial blooms in Lake Furnas reported here were associated with TN:TP mass ratios ranging from 29 to 66. The other half of these blooms was associated with TN:TP values varying from 12 to 29.

All the cyanobacteria blooming in Lake Furnas are found worldwide (Fristachi & Sinclair 2008) and are potentially toxigenic (Lawton *et al*., 1999; Whitton & Potts, 2000; Chorus, 2001; Falconer, 2004; Hudnell (ed.), 2008). The toxigenicity of the cyanobacteria found in Lake Furnas was confirmed by the findings that 19 % of 129 samples collected in the lake at several depths, from 2001 through 2009, contained soluble microcystins and 84 % of the samples revealed the presence of intracellular microcystins. These results are consistent with several reports from European countries. These reports showed that 61 % to 90 % of the samples collected from several water bodies during the 1980s and 1990s were toxic (Quesada *et al*, 2004).

The average concentrations of extracellular microcystins ranged from 0.05 μg MC-LR_{equiv}/l to 0.49 μg MC-LR_{equiv}/l, values similar to those found in the water of Lake Sete-Cidades (Santos *et al*., 2005). These results are in accordance with several surveys (Chorus, 2001) and enable us to conclude that extracellular microcystins are seldom found. Their concentrations rarely exceed 1 μg/l, and substantial concentrations are exceptional and transitory. The highest concentration of dissolved microcystins (1.95 μg MC-LR_{equiv}/l) was detected in a bottom sample collected during May 2007 and can be related to a substantial accumulation of dead cyanobacteria in the sediments, after the significant winter bloom caused by the three cyanobacterial species *M. aeruginosa*, *C. kuetzingianum* and *W. naegeliana*. The dead biomass of these cyanobacteria was still releasing toxins to the water four months after the bloom reached its highest density.

After 2005, intracellular microcystins were detected in all samples. Their concentrations increased during 2008 and 2009. The most frequent water column average values were less than 20 μg MC-LR_{equiv}/l, but a concentration of 154.5 μg MC-LR_{equiv}/l was recorded in the 2009 winter surface sample, during a bloom of *W. naegeliana* ($493.2 \cdot 10^3$ cells/ml). Even higher concentrations of total microcystins (greater than 500 μg/l) were previously found in 1997 in the Spanish part of the Tajo Basin (Gordo *et al*., 1999 cited by Quesada *et al*., 2004).

In the same 2009 winter sample, the amount of microcystins per unit seston dry weight was also very high (9737 mg MC-LR_{equiv}/kg). In comparison, maximal values reported from Germany, Czech Republic and Korea were from 1500 mg/kg to 5800 mg/kg and the highest value (7100 mg/kg) was recorded in Portugal (Chorus, 2001). More recently, Willame *et al*., 2005, reported that 2231 mg/kg was the highest concentration found in a study of lakes of Belgium and Luxembourg. This value was found in a sample of a bloom dominated by *W. naegeliana*. Grabowska & Pawlik-Skowronska (2008) also reported 7827 mg/kg as the maximum concentration found in a reservoir in the northeast region of Poland. However, this value was associated with an increase of *Oscillatoriales* in the water body and a simultaneous decrease of *Chroococcales*. In Lake Furnas, where similarly high amounts of microcystins were recorded, *Chroococcales* were almost always dominant.

The local authorities are concerned about the results presented here, especially in view of the progressive increase of the concentrations of microcystins over time. Nevertheless, several measures are already being taken in the basin of the lake to prevent the advance of eutrophication, and it is expected that the cyanobacteria blooms will diminish.

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