The collapse of a *Daphnia laevis* (Birge, 1878) population in Pampulha reservoir, Brazil*.*

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ABSTRACT: The collapse of a *Daphnia laevis* (Birge, 1878) population in Pampulha reservoir, Brazil. **The possible role of some regulatory factors determining short-term fluctuations of *D. laevis* in the Pampulha reservoir was investigated. In 1996, the population of this cladoceran showed a steady reduction during the dry season, reaching the lowest level in September. When this critical period arrived, a diel cycle was carried out during one of the first rain events closing the dry season. An abrupt decline of *Daphnia laevis* (ca. 50 ind.l^{-1} to zero), the dominant zooplankter, was found. The paper first shows that the mass death of *Daphnia* could not be attributed to methodological (bias in sampling or enumerating) or physical variables such as the washout factor. Next, evidence is given indicating that *D. laevis* was food limited prior to the mass death event. The possible role of contaminants such as trace metals or biocides was also investigated. Despite the presence of detectable amounts of trace metals in the zooplankton, no abnormal increase of such contaminants was found. Laboratory tests indicated that the local *Microcystis* strains might play an important role in mass mortality of D. *laevis*. The experiments showed, however, that this toxic effect is highly variable on a time scale. Field data also indicate that *Daphnia* can coexist for long periods of time with extensive *Microcystis blooms* but the total lipid reserves of zooplankton tend to be lower during *blooms* of these algae.**
Key-words: ***Daphnia*, diel cycle, Cyanobacteria, trace metals.**

RESUMO: O colapso de uma população de *Daphnia laevis* (Birge, 1878) no reservatório da Pampulha, Brasil. **O possível papel de alguns fatores reguladores nas flutuações de curto prazo em *D. laevis* no reservatório da Pampulha foi estudado. Em 1996, a população deste cladócero mostrou uma redução constante durante a estação seca, alcançando o nível mais baixo em setembro. Um declínio abrupto (50 ind.l^{-1} para zero) de *Daphnia laevis*, dominante no zooplâncton, foi encontrado em um ciclo diurno realizado durante uma das primeiras chuvas que encerram a época da seca. Este artigo mostra primeiramente que a morte maciça de *Daphnia* não poderia ser atribuída às variáveis físicas (temperatura, efeito diluidor de chuvas p. ex.) ou metodológicas, tais como artifícios de coleta ou contagem de amostras. Em seguida, evidências são dadas indicando que *D. laevis* estava limitada por alimento antes do evento da morte maciça na população. O possível papel dos contaminantes, tais como metais traço ou biocidas também foi investigado. Apesar da presença de quantidades detectáveis de metais traço no zooplâncton, nenhum aumento anormal de tais contaminantes foi encontrado naquela ocasião. Testes de laboratório indicaram que as cepas locais de *Microcystis* poderiam desempenhar um importante papel na mortalidade maciça de *D. laevis*. Os experimentos mostraram, entretanto, que este efeito tóxico é altamente variável dependendo da época do ano em que o experimento foi realizado. Os dados do campo indicam também que *Daphnia* pode coexistir por longos períodos de tempo com extensivas florações de *Microcystis*, mas as reservas de lipídeos totais do zooplâncton tendem a ser mais baixas durante a floração destas algas.**
Palavras-chave: ***Daphnia*, ciclo diário, Cianobactéria, metais traço.**

Introduction

One of the most important goals in ecology is to explain the different patterns of temporal and spatial distribution of organisms in a given ecosystem (Krebs, 1994). Most

organisms suffer large variations in abundance on different time scales as well as in space. This occurs in both terrestrial and freshwater ecosystems.

In eutrophic lakes, large cladocerans such as *Daphnia* are often the dominant organisms (Pinto-Coelho, 1991a). These organisms are able to maintain higher filtering and ingestion rates than most other planktonic invertebrate herbivores such as rotifers and small cladocerans (Dodson, 1974). Several species of *Daphnia*, like *D. galeata*, *D. hyalina* or *D. pulex* are key herbivores in many lakes (Balvay et al., 1987; Angeli et al., 1995; Geller, 1985, 1989). In these lakes, the filtering activity of *Daphnia* is associated with the clear-water phase, a phenomenon of abrupt increased transparency caused by massive removal of algal particles from phytoplankton (Lampert, 1978a). This capacity of *Daphnia* of clearing up large algal populations is of interest since it opens the way to using this organism in biomanipulation programs focused on the recovery of eutrophic lakes and reservoirs (Shapiro & Wright, 1984).

Daphnia populations, however, are very unstable, suffering intense fluctuations (Threlkeld, 1979; Wright & Shapiro, 1990). Many authors believe that these oscillations are caused by food limitation (Dodson et al., 1976; Lampert, 1978b, 1986; Muck & Lampert, 1984; Lampert & Muck, 1985).

Most of the knowledge about *Daphnia* ecology comes from temperate regions (Gillooly & Dodson, 2000). In tropical lakes, *Daphnia* populations are considered not so important as in the temperate region (Fernando et al., 1987). However, in some tropical eutrophic lakes, like the Pampulha Reservoir, in Brazil, they can be the dominant organism (Pinto-Coelho, 1998).

Pampulha Reservoir is a small and shallow water body situated in Belo Horizonte city (19°55'09"S, 43°56'47"W), Brazil. The reservoir has a surface of 2.4 km^2 , a volume of 12 x 10^6 m^3 and a mean depth of 6 m. The region under study has a tropical climate with a moderate hydric deficit, with a mean annual rainfall ranging from 1400 to 1600 mm (Pinto-Coelho, 1998). Since the seventies, it has been suffering from eutrophication. In the mid eighties, phytoplankton was dominated by the filamentous cyanobacteria *Cylindrospermopsis raciborskii* and the cyclopoid *Thermocyclops decipiens* was the leading zooplankter. In the nineties, extensive blooms of *Microcystis* appeared, forming dense hyperscums during the winter months and the zooplankton was dominated by two species of *Daphnia*, *D. gessneri* and *D. laevis* (Pinto-Coelho, 1998). The populations of these two species have experienced, however, extensive and abrupt fluctuations during the seasonal cycle.

One interesting feature of this ecosystem is the coexistence of large populations of *Daphnia* with extensive blooms of cyanobacteria (*Microcystis* spp.) for long periods (2-3 months) mostly during the dry season. The toxic effects of some *Microcystis* strains on the survival of zooplankton are well known in the literature (Watanabe et al., 1988; De Mott et al., 1991). The occurrence and the maintenance of hyperscums of cyanobacteria depends, however, on stable weather conditions such as low wind speed, strong incident light and higher temperatures (Zohary & Breen, 1989a; Zohary & Robarts, 1989b).

What are the major reasons that induce *Daphnia* populations to collapse from time to time in Pampulha Reservoir? Our monitoring program indicated that the population of *D. laevis* was on the edge of a collapse at the beginning of September 1996. Therefore, the main goal of this study was to investigate the short-term dynamics of a population of *D. laevis* coexisting with a bloom of cyanobacteria during a rainy day at the end of the dry season. The following ecological factors were examined in detail: (a) the washout factor, (b) food limitation, (c) the role of some contaminants coming into the reservoir through polluted tributaries and (d) the toxicity of cyanobacteria, especially *Microcystis* spp.

Material and methods

In 1996, plankton and water samples were taken every 15 days at a sampling point located in the central region of the reservoir. The water column at this point has a maximum depth of z_m = 8.5 m. Previous investigations have shown that this point is quite representative of the whole lake (Giani et al., 1988). At the beginning of September, an intensive sampling was performed during a single diel cycle. Samples were taken every

4 hours. Rainfall data were obtained at the meteorological station of Pampulha airport, which is located 0.5 km downstream of the dam (INFRAERO- Brazilian Airport Authority).

Temperature, conductivity and dissolved oxygen were measured *in situ* with Yellow Springs devices. Suspended matter was measured gravimetrically on 250 ml lake water samples filtered through pre-combusted Schleicher & Schüell GF-A glass fiber filters, 47 mm in diameter. Particulate and dissolved organic carbon was estimated using the volumetric method with previous potassium bicromate digestion (APHA, 1994). This is a simple and reliable method for detecting carbon in eutrophic waters (Torres et al., 1998). Chlorophyll-a was measured spectrophotometrically using 90% acetone extraction and Lorenzen (1967) formulas.

Phytoplankton was collected in the euphotic zone (0.0 , 0.5 and 1.0 m) using a 1.2 liter Kemmerer bottle. A 500 ml sample was immediately fixed using lugol acetic solution. All samples were kept at laboratory temperatures under dark conditions until they were processed. Counting was performed using an Utermöhl chamber using an inverted Zeiss microscope. A minimum of 100 individuals was always counted for the most common organism in each sample. Biovolume were calculated using approximate geometric formulas given by Rott (1981).

Duplicate zooplankton samples were drawn biweekly with a 5.1 liter Schindler trap equipped with a collector with 90 μm mesh size. The mesh size, 90 μm, collected all cladocerans and copepods and most rotifers present in the lake water the major components of mesozooplankton (Sieburth et al., 1978). Different depths covering the water column were considered: 0, 2, 4 and 6 m. For the diel cycle, samples of zooplankton were taken with a conical net 37 cm in diameter and with a mesh size of 90 μm equipped with a plexiglass collector at the bottom. The net tows covered the whole water column. Zooplankters were fixed using a buffered (pH=7.0) solution containing 37 % formalin, sucrose (250 $g.l^{-1}$) to achieve a final concentration of 4% of formalin. At the laboratory, each organism of each species in the sample was counted and measured using a Leica stereomicroscope. Sub-sampling was made using a 5 ml Hensen pipette, only if the total number of organisms exceeded 2000 in the sample. Biomass was calculated using allometric equations (McCauley, 1984). The biomass of rotifers (mostly *Brachionus calyciflorus*) was calculated from biovolume determinations according to Ruttner-Kollisko (1977).

The level of *Daphnia* gut content was determined as follows. A set of 50 animals was taken randomly from each sample and the gut content index was estimated for each individual. Following arbitrary indexes were considered: 0: gut completely empty; 1: gut with a few food particles; 2: food particles reaching less than 40% of total gut volume 3: food particles reaching 40-80% of total gut volume; 4: gut completely filled with food particles.

Zooplankton samples for biochemical analysis (lipid) and contaminant (trace metal and biocide) determinations were taken using a 90 μm mesh plankton net. The organisms trapped in a plexiglass vial at the bottom of the net were immediately transferred to clean plastic vials, avoiding any contact with metallic parts or devices. These vials were kept in a thermos-box with ice until they were transferred to a conventional freezer (-20 °C). On the next day, zooplankton samples were freeze-dried in an Edwards freeze-drier. To avoid the contamination of large colonial algae in the biochemical analysis of zooplankters, a manual separation of freeze dried animals was performed on stereo-microscope prior to further analytical procedures. Lipid analyses were carried out using 0.6-1.5 mg subsamples of freeze-dried zooplankton samples. Organisms suffered mechanical and chemical digestion prior to lipid extraction and lipids were determined as proposed by Meyer & Walther (1988).

For trace metal analysis, the samples were mechanically ground prior to sub-sampling. Samples were digested with nitric acid and analyzed by graphite tube atomic absorption spectrophotometry (AAS). The following elements were investigated: As, Cd, Pb and Zn. Zooplankton organisms were also investigated for traces of organic Cl- and P- biocides and carbamates. Samples for these analyses were processed using the same procedure as described for trace metals. Analysis for pesticides was performed using gas chromatography. All analyses of trace metals and pesticides were performed at the

Hidroquímica- Centro de Pesquisas Especiais - CEPE, a reference laboratory of the Labour Ministry of the Brazilian Government.

To investigate the toxicity of *Microcystis* blooms on *Daphnia* survivorship, fresh algal samples collected from the reservoir were offered to *D. laevis* individuals of different ages picked from cultures cultivated in the laboratory. These bioassays were performed on three different occasions between June and July 1997. *Microcystis* hyperscums, which are normally abundant in surface waters from Pampulha Reservoir, were sampled using a large opening PVC mug (250 ml). The material collected was immediately transferred to a 5 liter thermos flask. At the laboratory, an aliquot of 80 ml from this material was bi-filtered through 50 mm INOX gauze. All remaining zooplankton organisms were collected from the filtrate using a stainless steel microloop under a stereomicroscope (40 X). Microscopic observations also showed that this solution contained almost exclusively *Microcystis* spp. colonies. A 5.0 ml aliquot of this solution was filtered through a 25 mm GF-C glass fiber filter for chlorophyll-a determinations. This '*Microcystis* solution' had a concentration of 260 to 350 mg.l^{-1} chlorophyll- a.

For the bioassay, 10 individuals of *Daphnia laevis* (typically 5-6 young, 3-4 adult females and 1 egg carrying female) from a clonal culture kept in the laboratory were transferred to 12 experimental vessels consisting of 250 ml glass vials. These vials contained 70 ml of pre-filtered (20 mm) lake water and of a mixture *of Ankistrodesmus gracilis* and *Scenedesmus quadricauda* monospecific exponentially growing cultures (final concentration: 4.0-6.0 x 10^5 cells.ml^{-1}). To six randomly selected vials, 10 mg chlorophyll-a.l^{-1} equivalent amount of the '*Microcystis* solution' was added. The control units were the six remaining vessels, which were *Microcystis* free. After 2 hours and every 24 hours the number of survivors (lx), neonates (rx) and the number of dead organisms (dx) were counted in each experimental unity. Each bioassay lasted at least 72 hours.

Results

Seasonal development of food resources and zooplankton

In 1996, the rainfall in the reservoir's basin exhibited a clear seasonal pattern. The dry season extended from April to September. During this period, the monthly rainfall never exceeds 70 mm. The rainy season usually begins in October and ends in March. During this period, the monthly rainfall was always higher than 100 mm, sometimes reaching levels as high as the 500 mm measured in November 1996. The vertical arrow in Fig. 1 indicates the day of intensive sampling. During this diel cycle (6-7 September), the weather remained heavily cloudy all day long and the total daily rainfall amounted to 15 mm.

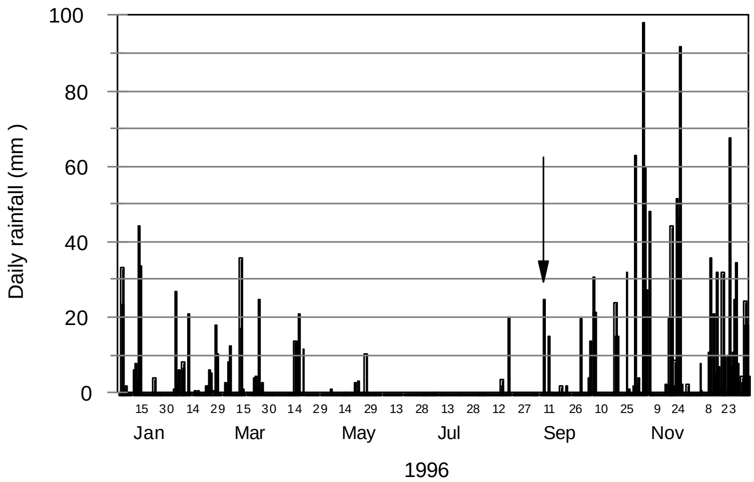

Figure 1: Daily rainfall during 1996, at the Pampulha Airport, Belo Horizonte, Brazil. The airport is located 0,5 km downstream of the dam. Data from the airport authority, the Infraero.

A distinct seasonal pattern can also be seen in some physical and chemical variables. In summer, the column remained well stratified (Fig. 2 a). Conversely, a homogeneous column was typical for the winter months. Surface temperatures oscillated between 27.4 °C in March, to 19.6 °C in June. Bottom temperatures ranged from 18.7 °C (August) to 24.1 °C (March). The reservoir typically has a low availability of dissolved oxygen near the bottom (Fig. 2b). Surface oxygen levels oscillated sharply with several peaks (values higher than 8.0 mg $O_2.l^{-1}$) being observed during the seasonal cycle (Fig. 2b). Conductivity varied between 199 and 370 $\mu S.cm^{-1}$, both values observed at the surface (Fig. 2c). This variable also has a seasonal cycle with higher values being observed at the end of the dry season. The vertical dashed line in Fig. 2 refers to the date when the intensive sampling was conducted. On that day, the water column remained stratified, the concentration of dissolved oxygen was about 2.2 mg $O_2.l^{-1}$ on the surface and nearly absent on the bottom. A small drop in conductivity was observed, possibly reflecting the diluting effect of the rainfall.

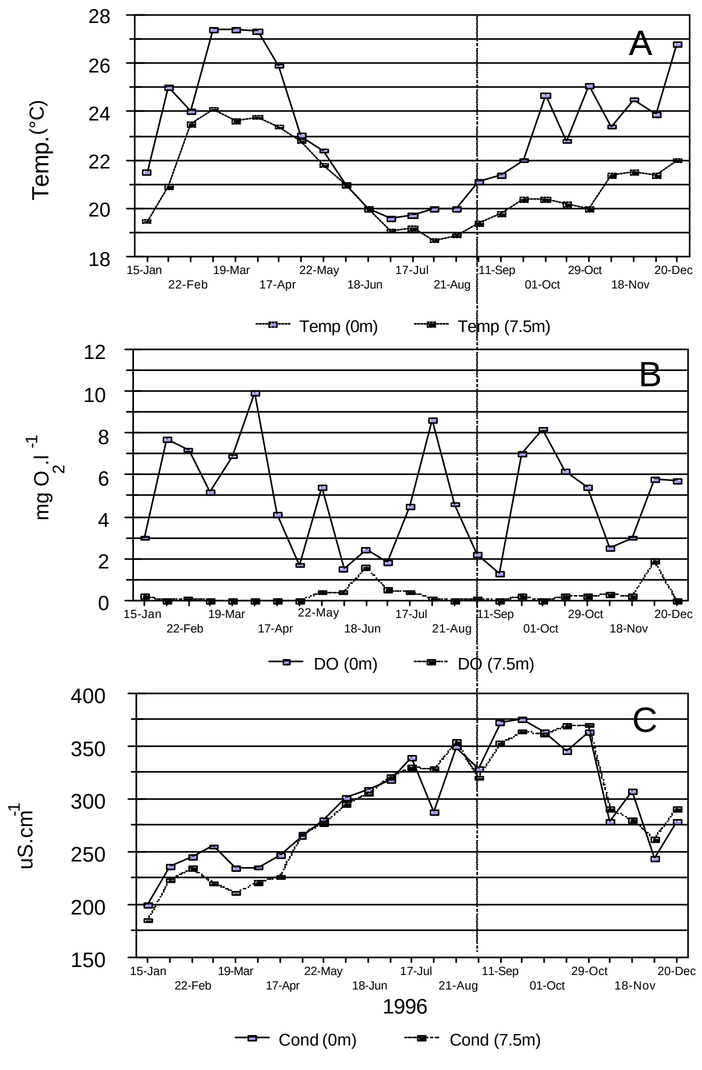

Figure 2: Annual course of water temperature (A), dissolved oxygen (B) and electric conductivity (C) in a central station of Pampulha Reservoir. The vertical line refers to date of the diel cycle, in September 1996.

The seasonal cycle of phytoplankton in Pampulha Reservoir is closely related to the annual pattern of rainfall (Fig. 3a). As soon as the summer rains diminished, in March/April, there was the first annual peak in algal biomass. Phytoplankton was dominated by a diverse array of green algae and phytoflagellates. The most important species (in terms of biovolume) of this first group were *Oocystis lacustris*, *Eutretamorus planctonicus* and *Chlorella vulgaris*. The green algae *O. lacustris*, for example, reached a maximum of 3.8 x 10^6 mm^3.ml^{-1}, in February 1996. Cryptophyceae, like *Cryptomonas brasiliensis*, *C. erosa* and *C. curvata*, were also especially abundant during this period. Later on, cyanobacteria bloomed showing two or more biomass peaks, most of them occurring at the end of the dry season (August-October). The most important species in this second group were *Microcystis viridis* and *M. flos-aquae,* which exhibited two maxima, one in early August and another in October, when a biovolume of 1.3 x 10^7 mm^3.ml^{-1} was recorded. Bacillariophyceae biomass was higher in November, at the beginning of the rainy season. Euglenophyta, represented mostly by *Trachelomonas* spp. and *Lepocinclis salina*, was another group relatively important in terms of biomass. This last group, however, did not show any conspicuous seasonal pattern.

The zooplankton of Pampulha Reservoir was dominated by a simple array of species, most of them typical for eutrophic tropical freshwater. During 1996, *Thermocyclops decipiens*, *Daphnia laevis* and *Brachionus calyciflorus* were the most abundant organisms. The population of *D. laevis* from Pampulha Reservoir exhibited two maxima in its biomass during the dry season of 1996, one in June/July and the other in November (Fig. 3b). These maxima occurred always after periods of high algal biomass. This species reached a biomass maximum

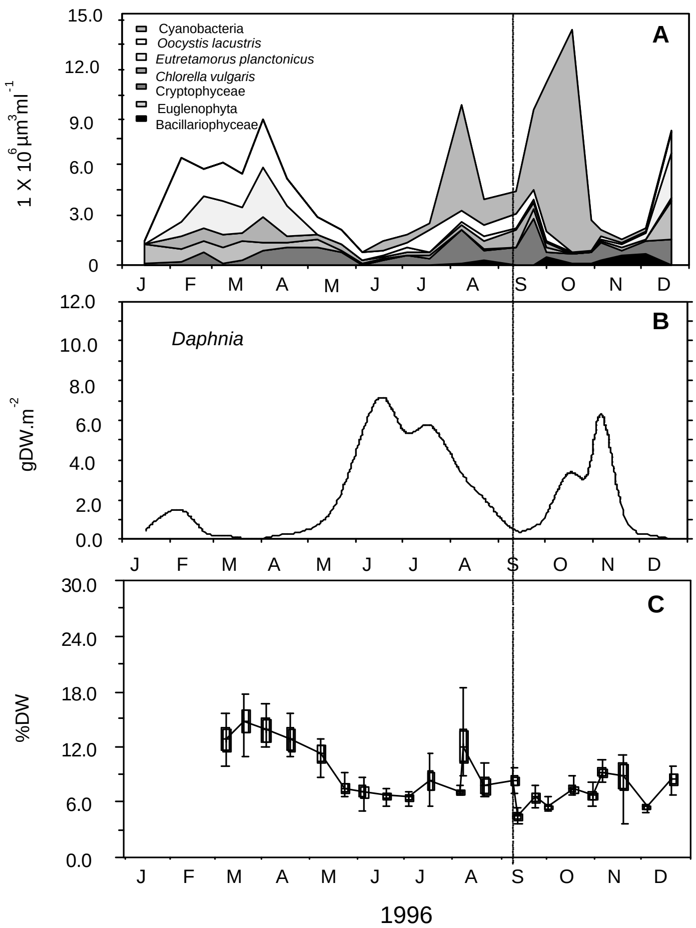

Figure 3: Annual course of phytoplankton biovolume (A), integrated biomass of *Daphnia laevis* (B) and total lipids of zooplankton in Pampulha Reservoir,1996 (C). Only the most important groups of algae (in terms of biovolume) are here represented.

higher than 6.0 g DW.m^{-2} on both occasions. Between these two maxima, *D. laevis* suffered a relatively long biomass decline after the first peak, coinciding with the months of July and August, collapsing at the beginning of September, when the diel cycle was performed. At that time, the zooplankton community was strongly dominated numerically by daphnids and rotifers, especially *Brachionus calyciflorus*.

Zooplankton showed total lipid levels ranging from 4.4 % (September) to 14.8 % dry biomass (March). A seasonal pattern was observed with higher values between March and May, when phytoplankton was dominated by chlorococcales. The lipid levels remained lower throughout the dry season, reaching an annual minimum at the beginning of September (Fig, 3c).

Chlorophyll-a concentrations exhibited a bimodal temporal pattern with two maxima: one at the end of the rainy season, in April, when green algae were dominant and the other at the end of dry season, in September-October, when cyanobacteria were blooming in the lake (Fig. 4a).

The seasonal variations of particulate organic carbon had a different pattern when compared to chlorophyll-a (Fig. 4b). Excluding some fluctuations during the rainy season, the POC concentrations remained in the range of 15-30 gC.m^{-2} throughout the year. There was no special depletion of POC values in September.

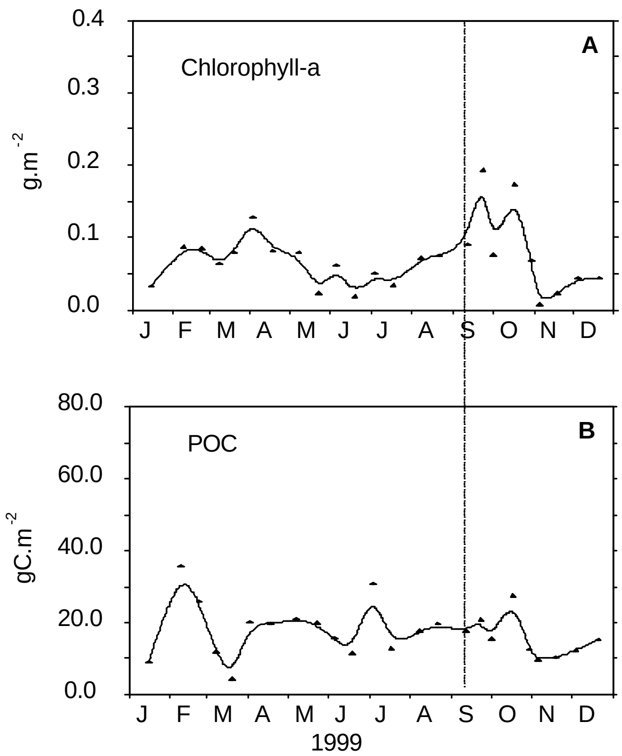

Figure 4: Seasonal course of chlorophyll-a (A), particulate organic carbon (B) at a central sampling point (z = 8m) in Pampulha Reservoir, Brazil during 1996.

Diel Cycle

In late July, an extensive bloom of cyanobacteria had become established in the lake. This trend was partially broken by two rain events that occurred in August and at the beginning of September, respectively (Fig. 1 and 2a). The diel cycle was conducted during this second rainy day, on 6-7 September 1996. Intensive sampling started at 10:00 hs and ended 24 hs later. The Secchi disk, measured only in the first and last excursion, was low, about 0.80 m, and did not vary. It rained in the first two sampling periods: at

10:00 hs and 14:00 hs and the weather remained heavily covered on all other occasions, i.e. 17:00, 22:00, 04:00 and 08:50 hs. The water temperature oscillated from 19 to 21 °C. Differences between surface and bottom remained always below 2°C, and a slight stratification was observed in the afternoon hours. Conductivity was high, varying from 320 to 332 μS.cm^{-1}. These values are typical for the end of the dry season in Pampulha Reservoir.

Suspended matter suffered a steady increase during the diel cycle. Particulate inorganic matter increased by a factor of six from 1.8 mg.l^{-1} to 12.9 mg.l^{-1} (Fig 5a). This variable suffered a steady increase in the diurnal cycle with highest values being observed

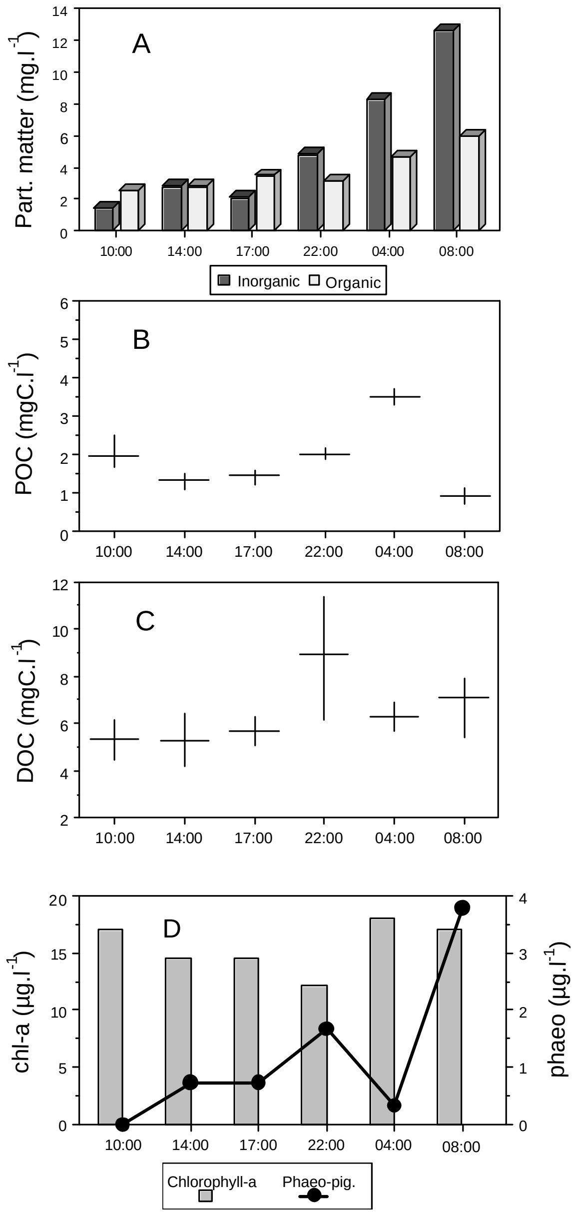

Figure 5: Diel variations in the concentrations of suspended matter (inorganic and organic) in Pampulha Reservoir in 6-7 September 1996 (A); particulate organic carbon - POC (B); dissolved organic carbon-DOC (C). For plots B and C, horizontal lines correspond to averages and vertical lines represent range of replicates; diel variations in the concentrations of chlorophyll-a (bars) and phaeo-pigments (lines) in Pampulha Reservoir (D). All data refer to samples taken at the depth of 0.5 m.

on the last excursion. Particulate organic matter also suffered an increase, varying from 2.6 mg.l^{-1} at the beginning of the diel cycle to 6.0 mg.l^{-1} at the end the study (Fig. 6a).

During the diel cycle, POC concentrations even increased from 1.67 mgC.l^{-1} (6 Sept, 10 hs.) to 3.51 mgC.l^{-1} (7, Sept. 4 hs.) (Fig. 5b). However, POC values in 1996 remained lower than those observed in previous years. The concentrations of dissolved carbon were higher. The average concentrations of this fraction (DOC) varied from 5.3 to 8.9 mgC.l^{-1}, with a peak concentration occurring at 22:00 hs. (Fig. 5c).

Chlorophyll-a concentrations ranged from 12.0 to 18.0 mg.l^{-1} (Fig. 5d). They suffered a steady decrease from 10:00 through 22:00 hs, increasing thereafter during the second half of the night remaining high in the morning hours. Phaeo-pigments showed an opposite trend, with increased values occurring from 10:00 am to 22:00 hs, when 1.68 mg.l^{-1} was measured. The concentration of this degradation form of pigment decreased at 4:00 hs and reached a maximum of 3.84 mg.l^{-1} in the last excursion on the following morning (Fig. 5d).

During the diel cycle, the density of total zooplankton dropped from 170-200 ind.L^{-1} at the beginning of the diurnal cycle to less than 50 ind.L^{-1} on the following morning (Fig 6a). The abundance of *Daphnia* also suffered an acute reduction along the diurnal cycle. This population always showed more than 50 ind.L^{-1} until 22:00 hs., a fairly high value. On the following morning, just ten hours later, daphnids disappeared from the lake (Fig. 6a). The disappearance of daphnids can be clearly observed by considering the time course of the relative proportions of *Daphnia* to total biomass of zooplankton (Fig. 6b). Figures of total biomass remained between 1.0 and 3.0 mgDW.l^{-1} until 22:00 hs. Thereafter, they decreased drastically, reflecting the dominance of *Daphnia* in terms of total zooplankton biomass. Particulate matter inversely followed the decrease of zooplankton density and biomass, increasing from 5.0 to nearly 18.0 mg.l^{-1} during the diel cycle (Fig. 6a,b).

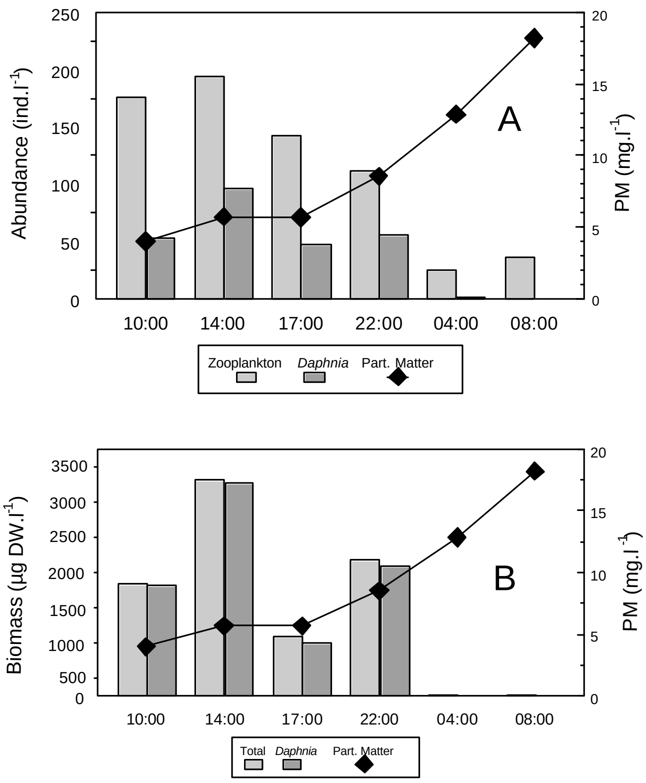

Figure 6: Temporal variations in the abundances of total zooplankton and *Daphnia laevis. S*uspended matter concentrations are also represented (A). Bottom: Total zooplankton and *D. laevis* biomass and suspended matter (B). All data refer to the diel cycle of 6-7 September 1996 in Pampulha Reservoir, Brazil.

The allometric structure of the *Daphnia* population showed that there was a dominance of large individuals (mostly adults) in the population. The size class distributions of individuals for all times shows that the mean size was higher than 1.15 mm on all occasions (Fig. 7a).

During the diel cycle, zooplankton exhibited lower mean of total lipid levels ranging from 6.3 to 9.1% of dry weight during the diel cycle (Fig. 7b). These numbers basically reflect the nutritional status of *D. laevis* since zooplankton was strongly dominated by this cladoceran. An increase of lipid levels was observed at the end of the day and during the first night hours.

The gut contents of daphnids were also examined. Animals were found to have very different food levels in their guts along the diel cycle. During most sampling times, the majority of individuals were observed with empty guts. At 22:00 and 04:00

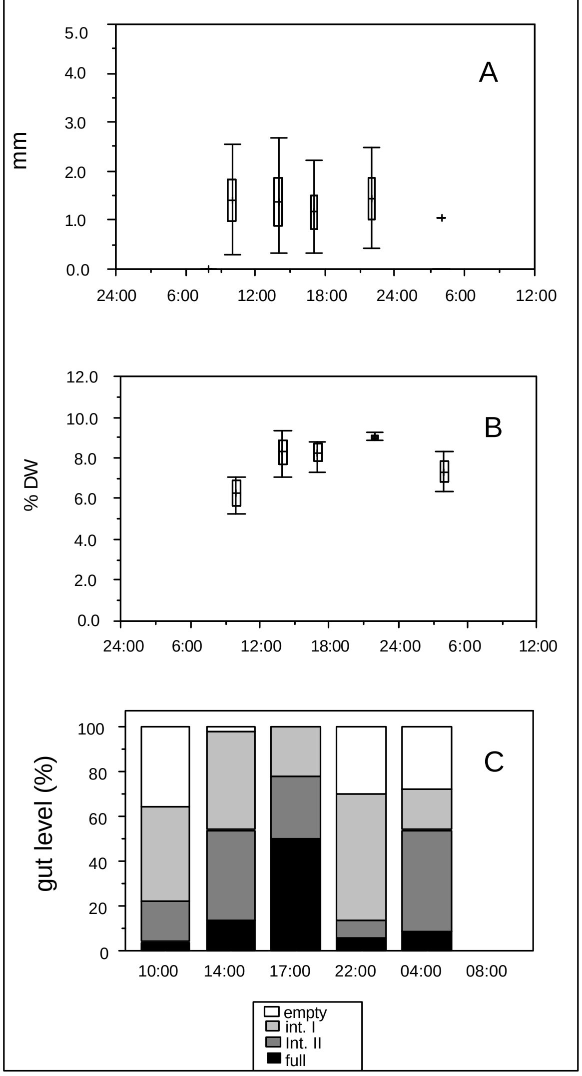

Figure 7: Allometric structure of the population of *Daphnia laevis* in Pampulha Reservoir from the diel cycle 6-7 September 1996 (A); total lipid content of zooplankton in Pampulha Reservoir (B). For plots A and B, mean, standard errors and ranges are given for each sample. Amount of food particles in the guts of *D. laevis* at different times of the day (C). At 4:00 hs am there was only a few individuals to perform the gut contents but the density as expressed by individuals per liter was negligible. All data refers to the diel cycle (6-7 September 1996) in Pampulha Reservoir.

hs, for instance, the guts were either empty or in intermediary stages 1 and 2 (Fig. 7c). Only at 17:00 hs., about 50% of individuals were found with full guts. On the following morning, there were not enough individuals to perform this analysis. The Spearman correlation matrix showed that gut levels found at 17:00 hs were inversely or not correlated with all other times.

Trace metals

Some tributaries of Pampulha reservoir are heavy polluted with domestic and industrial wastewater and therefore we have being investigating the bioavailability of trace metals in the biomass of zooplankton. It was possible that the mass mortality of daphnids could be associated with toxic effects related to the presence of such toxic elements. *Daphnia* is widely used in ecotoxicology bioassays since it has a high sensivity to a wide range of contaminants.

The bioavailability of four major trace metals in zooplankton was examined in six different diel cycles conducted between April 1994 and the present study (September 1996). Zinc was the metal with the highest concentrations in biomass, with a mean value of 177.15 ± 95.30 ppm ($mg.kg^{-1}$DW), followed by lead with a mean of 22.51±17.91 ppm (Fig. 8a). Arsenic and Cadmium were never detected with concentrations higher than 2.0 ppm. The mean values for the pooled data were 1.35±0.42 ppm and 0.97±0.42 ppm for As and Cd, respectively (Fig. 8b). Zinc and lead suffered the highest variation coefficients, which

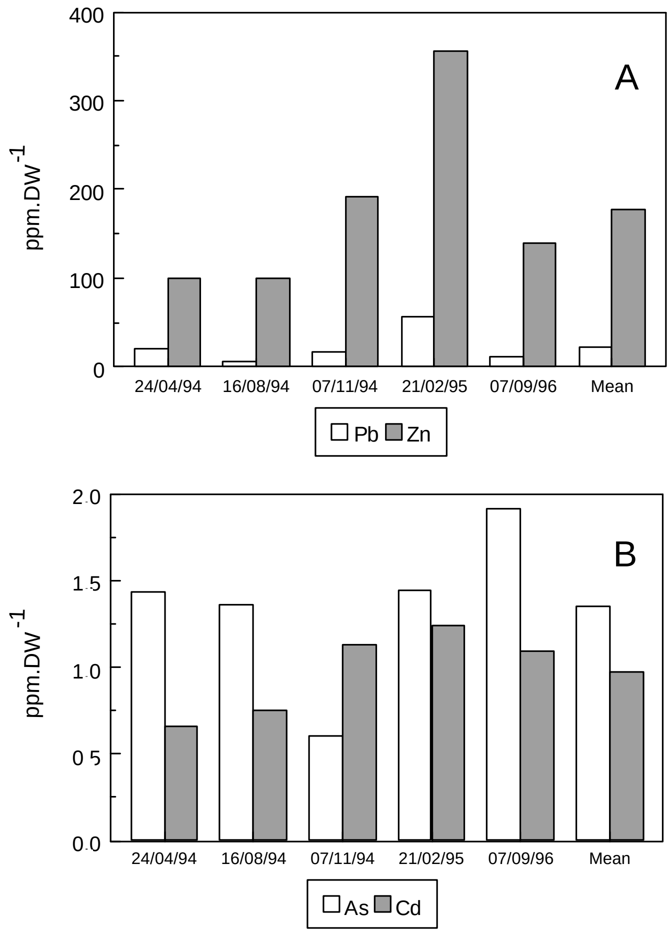

Figure 8: Relative concentrations (ppm = parts per million) of trace metals in the biomass of zooplankton of Pampulha Reservoir in five different occasions in the years 1994-1996. Top panel (A): concentrations of lead (Pb) and zinc (Zn); bottom panel (B): concentrations of arsenium (As) and cadmium (Cd).

were approximately 80% for both. Comparison of September 1996 with the other occasions revealed that the amounts of Cd, Pb and Zn were quite similar or even lower than the mean values obtained for the pooled data. There was, however, a slight increase in the concentrations of As in September 1996, when it reached a concentration 1.85 ppm. We also surveyed the existence of traces of standard biocides (Cl- and P- organic biocides) but no traces of these substances were detected.

Microcystis bioassays

The possible toxic effects of *Microcystis* on the survival of *D. laevis* were examined by means of short-term bioassays (96 hs.). Three different experiments were carried out. Only in the third bioassay, did we find strong evidence that *D. laevis* was affected by these algae (Fig. 9). In the first experiment, the number of survivors and neonates (lx+rx) was 4.4±2.7 and 1.8±2.9 (n=6), for control and *Microcystis* units, respectively. These differences were not significantly different (Tab. I). There was also no clear toxic effect of *Microcystis* on *D. laevis* in a second experiment. The number of daphnids remaining in control units was even lower than units containing *Microcystis,* 22.7±7.9 and 25.8±3.1, respectively. Again, this difference was not significant (Tab. I). A third experiment showed, however, a toxic effect of *Microcystis* since the mean number of living *D. laevis* individuals was reduced from 14.0±2.6 to 8.5±2.9 in the control and *Microcystis* vessels, respectively. These numbers were significantly different (t=2.733, P= 0.0041, F=11.71, P= 0.007).

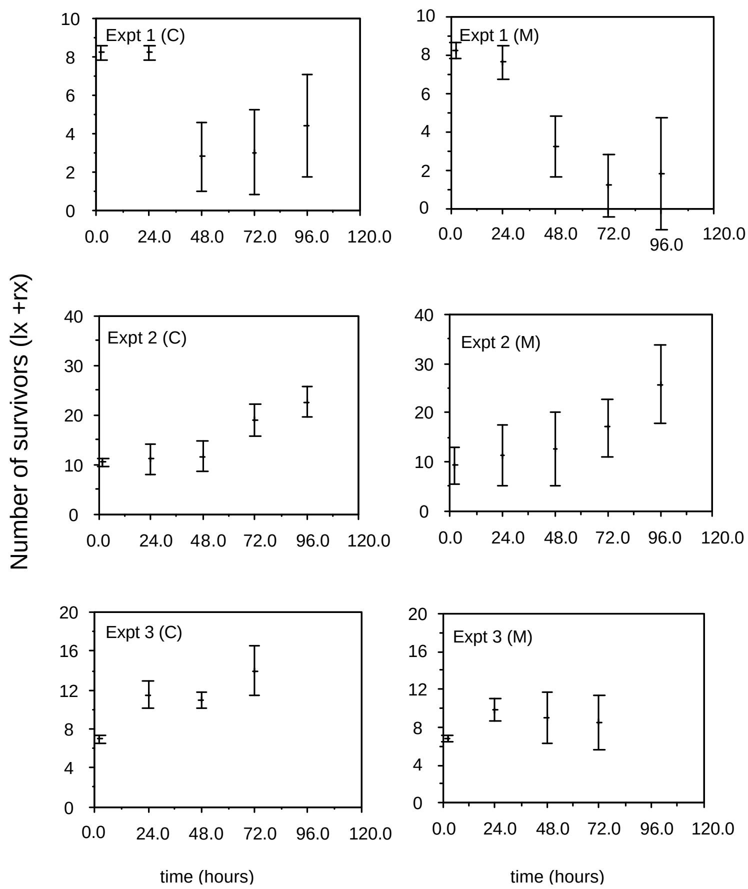

Figure 9: Number of survivors of *Daphnia laevis* in three bioassays. Experiment (1), top, Experiment (2): middle and Experiment (3): bottom. Mean and range for all replicates are represented. (C): control vessels, (M): *Microcystis* vessels.

Table I: Mean number of survivors (lx) and statistical analysis (ANOVA and T-TEST) of three different bioassays evaluating the toxic effect of the cyanobacterium *Microcystis spp.* on *Daphnia laevis.*

Bioassay	Treatment	x± sd (2h)	x±sd (24h)	x±sd (48h)	X±sd (72h)	x±sd (96h)	ANOVA	T-TEST
1	C	8.2±0.4	2.8±1.8	3.0±2.2	4.4±2.7	-----	F=2.113	T=-1.249
	M+	7.6±0.9	3.2±1.6	1.2±1.6	1.8±2.9	-----	P=0.184	P=0.267
2	C	10.5±0.8	11.2±3.1	11.8±3.0	19.2±3.3	22.7±3.1	F=0.638	T=1.452
	M+	9.3±3.7	11.3±6.2	12.7±7.4	17.0±5.8	25.8±7.9	P=0.443	P=0.206
3	C	7.0±0.4	11.5±1.4	11.0±0.9	14.0±2.6	-----	F=11.71	T=2.733
	M+	6.8±0.4	9.8±1.2	9.0±2.7	8.5±2.9	-----	P=0.007	P=0.041

Discussion

The seasonal dynamics of *D. laevis* in this reservoir revealed close associations with climatic conditions as well as with food availability. During the rainy period the population of this cladoceran was usually low. As soon as the phytoplankton community built large stocks of edible algae (chlorococcales and flagellates), a first peak of *Daphnia* occurred. There was a clear and inverse relationship between the *Daphnia* biomass and the biovolume of most algae. In the course of the dry season, especially from August on, a steady reduction of the *Daphnia* population was observed. This reduction was partially accompanied by a similar trend in total zooplankton lipids. This association suggests that food quality possibly plays a role in the seasonal dynamics of this cladoceran.

The population of *D. laevis* was drastically reduced during the diel cycle. Microscopic observations of the daphnids did not show any evidence of pathogens such as epibiontic protozoan or large numbers of bacteria around the carapaces. Our regular sampling program confirmed this disappearance of *Daphnia* from the lake, since its individuals continued to be absent in the samples collected three days later. This population recovered only one month later, in October. Therefore, four hypotheses can be suggested to explain this event: a) washout effect; b) food limitation c) allochthonous input of toxic substances and d) toxic effects of bloom forming cyanobacteria. Another possibility, would be related to the erratic spatial (horizontal) migration but this factor will not be discussed here.

The “washout” effect

Several investigations have suggested that zooplankton from reservoirs can be drastically affected by rainfall (i.e.: Pinto-Coelho 1987). In most cases, the rainfall is seen as a loss factor (Campbell et al., 1998). In other cases, the rainfall can even lead to increases in abundance of zooplankters (Sendacz, 1984). Nevertheless, it is quite probable that the washout effect played only a minor role as causing a mass removal of daphnids during this diel cycle. The total amount of rain between 6 and 7 September was only 15 mm. The annual course of the integrated *Daphnia* biomass reveals that a large population was present in the lake in November. During this month, there were six days when the total daily rainfall exceeded 40 mm.

The rain event of September 6-7 caused, however, some effect on water quality. The steady increase in inorganic matter observed during the diel cycle probably reflected the input of allochthonous material into the lake associated with the rain. This effect was clear during the first six hours of the diel cycle. Furthermore, the electric conductivity also showed a slight decrease on this day, a possible effect of some dilution caused by the rain.

Diel rhythm of food intake and food limitation of *D. laevis*

It is known that most *Daphnia* species exhibit marked diel fluctuations in nutrition in most temperate lakes (Angeli at al., 1995; Pinto-Coelho, 1991b), with an increase in filtering activities during the night hours. The present study confirms the existence of a diel rhythm in the food intake of *D. laevis* in Pampulha Reservoir. The variations in gut contents reached the highest index at 17:00 hs. Thereafter, most animals were observed with their guts predominantly empty or in intermediary stages. Total lipids also remained in low levels (< 10 %) on all occasions, although they increased slightly from 10:00 through 22:00 hs. At 04:00 hs, this trend was inverted and lipid values decreased. The temporal course observed in these variables indicates that zooplankton was able to convert food particles into lipids up to 22:00 hs but was not able to maintain this trend further.

Most *Daphnia* species are known to be efficient herbivores (Dodson, 1974). The decrease in chlorophyll-a concentrations observed at 22:00 hs occurred after the "peak" observed in the gut levels at 17:00 hs. With an opposite trend, phaeophytin concentrations increased following this maximum observed in gut levels. Thus, the magnitude of zooplankton food intake in Pampulha Reservoir probably affects the concentration of photosynthetic pigment and phaeo-pigment in lake water. Since *D. laevis* was the dominant zooplankter, the pigment concentrations and its degradation products, phaeo-pigments, are linked to the nutrition of daphnids as reflected by the gut index. Some authors have already observed associations between zooplankton grazing and increases of chlorophyll-a degradation products (Mackas & Bohrer, 1976; Baars & Osterhuis, 1984).

Several studies have demonstrated that food limitation is often associated with the disappearance of daphnids (Threlkeld, 1979; Wright & Shapiro, 1990). Most authors consider the threshold level of carbon availability for daphnids a value around 0.3 $mg.C.l^{-1}$ (Lampert & Muck, 1985). At the beginning of September, the concentration of POC was 1.8-2.0 $mgC.l^{-1}$ in the euphotic zone. In previous year (1994-1995), they usually remained above 2.0 $mgC.l^{-1}$ (Torres et al., 1998). Thus, it is quite probable that this factor is not the immediate cause for a mass death event such as that observed in this study. However, even considering the availability of high levels of POC and chlorophyll-a in lake water and the existence of a diel cycle of food intake, the study provides evidence that the population of *D. laevis* was suffering from food limitation. Three different lines of evidence support this hypothesis: (a) allometric structure of *Daphnia* population, (b) lipid reserves, and (c) food quality.

The allometric structure of the *D. laevis* population can be seen as evidence supporting the existence of limiting food conditions. The high prevalence of adults in the population also indicates that the reproductive rate was very low or near zero.

Despite the existing diel variation, the annual course of lipid reserves of zooplankton also suggests that some kind of food limitation might be occurring at the beginning of September. The lipid reserves showed a steady decrease along the seasonal cycle (with one exception in August), reaching an annual minimum in September. The annual course of lipids was only based on daily (9-11 hs) sampling, a period of lower lipid profiles on a diel basis. The prevalence of cyanobacteria may be the proximate cause for those lower values of lipid reserves of zooplankton. Several investigations have demonstrated the poor nutritional value of most blue greens for zooplankton (Lampert, 1981).

The lipid contents of zooplankton from Pampulha reservoir are typically lower than those of zooplankton from most temperate lakes (Pinto-Coelho et al., 1997). In Canadian lakes of different sizes and trophic status, Wainman et al. (1993) showed that the total lipids of zooplankton remained above 10% of dry weight during different phases of the growing season, despite conspicuous seasonal variation. In Pampulha reservoir, total lipid values during the diel cycle were also restricted to the 6.0-9.0% range. These lower lipid reserves for zooplankton in Pampulha Reservoir may be a result of the metabolic limitation that organisms must offset in warm waters. The *Daphnia laevis* population can consume up to 40 % of its C-weight per day at temperatures ranging from 22 to 25 °C, which are common in the reservoir (Macedo & Pinto-Coelho, 1997).

The last evidence of limiting food conditions comes from the composition of phytoplankton in Pampulha Reservoir during the diel cycle. At that time, it was dominated by *M. viridis*. Hanazato (1991), provided a long list of references indicating the association between decline in standing crop, productivity and feeding of zooplankton populations when living under blooms of blue green algae. In many cases, cyanobacteria blooms are associated with high mortality rates of vertebrate and invertebrate organisms due to the presence of cianotoxins (Watanabe et al., 1988).

Trace metals

Two main tributaries of the reservoir, the Ressaca and Sarandi streams bring wastewater from one of the largest industrial districts of Brazil, the industrial district of Contagem. In this region there are several large industries (chemical, steel, pharmaceutical, oil refinery, vehicle assembling plants, etc). A previous study demonstrated that zooplankton in this reservoir has detectable amounts of As, Pb, Cd and Zn in its biomass (Pinto-Coelho & Greco, 1998). The input of toxic substances such as heavy metals or pesticides can be another source of *Daphnia* mortality. These cladocerans are extremely sensitive to a diverse contaminant array such as trace metals, pesticides and oil refinery outputs (Das & Konar, 1988). In the polluted Weser estuary in Germany, for instance, the crustacean fauna has lower concentrations of Cd, Pb and Zn than zooplankton from Pampulha Reservoir. In the German estuary, for instance, the pelagic filter-feeding crustacean *Neomysis integer* had 0.14±0.08 ppm of Cd, 1.70±1.1 ppm of Pb and 77.0±35.0 ppm of Zn, in the years 1984-1986, all values referring to dry weight (Schirmer & Scheffel, 1991). In the present study, however, no abnormal increase in the bioaccumulation of most trace metals was found during the diel cycle.

Toxic effects of cyanobacteria

Like other eutrophic aquatic ecosystems in the tropics, several cyanobacteria species occur in the phytoplankton of Pampulha Reservoir. Some of them, like the colonial *Microcystis viridis* and *M. flos-aquae* often bloom for long periods of time during the dry season (May-September). *Microcystis* often forms hyperscums, which are surface accumulations of densely packed cyanobacteria, measuring several decimeters in thickness (Zohary & Robarts, 1990). According to Zohary and Breen (1989), the algal scums usually appear after prolonged periods of calm weather (reduced or no winds), with a clear sky with intense solar radiation and stable thermal stratification of the water column.

Short after the rainy season of 1996, in March-April, phytoplankton was practically cyanobacteria free, being dominated by small chlorococcales. During the course of the dry season, however, this situation changed drastically and colonial cyanobacteria established relatively large populations in the lake. *Microcystis viridis* dominated the phytoplankton community until the end of the dry season, in November, exhibiting two population maxima, one in August and another in October. At the beginning of September, the absolute densities of these algae were not especially high but they still were the dominant group of phytoplankton.

The bioassays suggest that the cyanobacteria of Pampulha Reservoir can have a toxic effect sometimes on *D. laevis*. The clonal culture isolated from the reservoir was not resistant to the toxins from *Microcystis* in one out of three bioassays. Recently, it was demonstrated that some populations of cladocerans (i.e: *Ceriodaphnia* sp.) can develop resistance to blooms of toxic cyanobacteria (Ferrão-Filho et al., 2000). Previous studies have demonstrated the prevalence of microzooplankton organisms coexisting with blooming cyanobacteria (Hanazato, 1991; Sellner et al., 1993). The finding that a large cladoceran, *D. laevis,* is able not only to survive but also to dominate the zooplankton community in the presence of blooming *Microcystis* is against the common belief that only small zooplankton can live under such circumstances. Furthermore, a recent work on Pampulha Reservoir showed that *Daphnia laevis* is even able to consume *Microcystis* (Eskinazi-Sant´Ana et al., 2002). Some authors believe that there may be little or no inhibitory effect of cyanobacteria and that zooplankton may only suffer from their poor food quality (Hanazato, 1991). The present study shows that the association between *D. laevis* and blooming cyanobacteria is essentially unstable, since inhibitory or even toxic effects can appear under certain circumstances such as an unexpected liberation of endotoxins caused by abrupt changes in climatic conditions. The 'price' for such strategy is the risk of mass mortality from time to time.

Conclusions

This study confirmed that the *Daphnia laevis* population is extremely unstable in Pampulha Reservoir. Intensive sampling showed that the vast majority of the individuals of this population died within a 6-8 hours. The *Daphnia* population continued to be practically absent from the lake until the beginning of October 1996.

The amounts of trace metals in zooplankton were not especially high prior to the mass mortality event, if they are compared with similar data obtained on other occasions. Therefore, the contamination with trace metals can possibly be excluded. No traces of P-Pesticides, Cl-Pesticides or carbamates were found in the biomass of zooplankton.

This study provides evidence that the *Daphnia* population was food limited prior to the mass mortality event. Zooplankton had a low profile of lipid reserves and there was a dominance of large individuals in the population of *Daphnia*. Daphnids showed a marked diel cycle of food intake, clearly reflected by the gut index and the lipid content and most individuals had empty guts just a few hours before they died. However, food limitation is not likely to be causing a synchronous mass mortality event. Low food quality, however, may have caused the long term reduction in the population observed for several weeks, in July and August 1996. Nevertheless, a food limited *D. laevis* population is probably more sensitive and less resistant to liberation of endotoxins present in the cells of *Microcystis*.

The bioassays showed that individuals of different size classes of *D. laevis* are potentially susceptible to the toxins of colonial cyanobacteria that frequently bloom in the reservoir. Therefore, this study suggests that the liberation of toxins of the blooming cyanobacteria, *Microcystis viridis*, into lake water may have been the proximate cause of the mass mortality of daphnids in Pampulha Reservoir. We suggest a further investigation aimed to follow the temporal dynamics of cyanobacteria toxins in the lake.

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