

Morphological abnormalities in cladocerans related to eutrophication of a tropical reservoir

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ABSTRACT

In zooplankton communities, morphological changes in Cladocera (Crustacea: Branchiopoda) may be resulting from water pollution by anthropogenic activities and/or natural events. The removal of vegetation cover, urbanization, agriculture and sewage release accelerate the eutrophication process in the aquatic environment. The present study seeks to demonstrate the occurrence of morphological abnormalities in cladocerans and relate the changes in the morphology and species composition to the physical and chemical parameters of the water. Samplings were made monthly in five stations on the Sapucaí River compartment of Furnas Reservoir, located in the state of Minas Gerais, Brazil, from July 2013 to February 2014. The Furnas Reservoir has intense occupation of the surrounding areas by agriculture, urban and industrial activities and the installation of net cage fish cultures, which contribute to the water quality deterioration. Cladocera samples were collected using a suction pump and plankton net (68 µm mesh size) and concentrated from a volume of 400 L. The measures of physical and chemical parameters of the water were obtained by a Horiba U-50 multi-sensor on the surface of water column and the density and morphology of Cladocera were made by microscopy. Twenty-three species of Cladocera were recorded with high organism densities of Chydoridae family species. Morphological abnormalities were observed in *Daphnia gessneri*, *Ceriodaphnia silvestrii*, *Bosmina longirostris*, *Bosmina tubicen* and *Chydorus pubescens*. The highest densities of *C. pubescens* with abnormalities were observed at sampling stations which had littoral characteristics and influences of sewage release. For *C. pubescens*, abnormalities were observed and classified into two types. The type 1 abnormality was considered an increase of length of intestine and size of its intestinal loop, whereas for type 2 was considered the occurrence of an intestine prolapse. The morphological abnormalities in cladocerans were described and compared to the ones described in the literature. From the results, it may observe that the abnormalities were probably resulting from continuous eutrophication process which has been occurring in the reservoir due to anthropogenic activities around the reservoir and a decrease in the water volume of the reservoir, caused by an unusual dry weather period in this region in the last years.

Key words: Morphological alterations; anthropic action; Chydoridae, zooplankton.

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INTRODUCTION

Changes in the structure and composition of aquatic communities and morphology of organisms may result from contamination of water by anthropogenic activities (Jakhar, 2013). The removal of vegetation cover, urbanization, agriculture and sewage release accelerate the eutrophication process in aquatic environments (Brito *et al.*, 2011). Net cage fish cultures in reservoirs also contribute to increasing nutrient concentrations in water due to the waste of feed and fish's excreta (Santos *et al.*, 2009; Mallasen *et al.*, 2012).

The modifications in behavior and species composition of zooplankton community may occur with enrichment by nutrients and bloom of cyanobacteria, such as *Microcystis aeruginosa*, which produces toxic substances

to aquatic biota (Lampert, 1982, 1987; Nanazato and Yasuno, 1985; Forsyth *et al.*, 1990; Hanazato, 1991; Reichwaldt *et al.*, 2013). Although many toxicity tests with cyanobacteria and zooplankton have already been carried out (Hanazato and Yasuno, 1987; Fulton and Paerl, 1988; Billoir *et al.*, 2009; Cerbin *et al.*, 2010; Shao *et al.*, 2014), but the mechanism action of these substances on the zooplankton remains little known. In addition to the nutrient enrichment originated from the above causes, the continuous supply of pollutants such as hormones (Hense *et al.*, 2004), drugs (Flaherty and Dodson, 2005) and pesticides (Hanazato, 2001) may decrease the organism reproduction rate, causing the removal of larger species, interfering in the energy transference of food chain (Sibley and Hanson, 2011) and in the biogeochemical cycles (Henry *et al.*, 2004; Romo *et al.*, 2013).

Some authors have recorded Cladocera (Crustacea: Branchiopoda) morphological alterations generated by anthropogenic activities (Omair *et al.*, 1999; Elmoor-Loureiro, 2004; Sinev, 2000; Zanata *et al.*, 2008; Sousa *et al.*, 2011). Omair *et al.* (1999) reported abnormalities in cladocerans and copepods, in the Lake Michigan, and suggested that the abnormalities are a recent global phenomenon and probably due to the anthropogenic actions. Sinev (2000) reported abnormalities in sex-linked characters to males and ephippial females with a post-abdomen for *Alona affinis*, similar to that of the adult male, in a Russian lake. These changes may be due to a high metabolic rate and the rapid life cycle of cladocerans, which become them susceptible to chemical, physical or biological disturbances on environment (Tundisi and Matsumura-Tundisi, 2008).

The Chydoridae family (order Anomopoda) is the most diverse and usually occurs in the coastal region of water bodies associated with macrophytes, periphyton and sediment (Forró *et al.*, 2008). Species of Chydoridae have short life cycle, active and dormant forms, asexual (parthenogenesis) and sexual reproduction (gamogenesis) (Dodson and Frey, 1991) as ecological attributes, among others, and some morphological attributes. The species richness for *Chydorus* genus is attributed to its successful body shape, which is globular or spherical, and with the bilateral compression and its adaptation capacity. These characters allow that species of this genus get into small gaps and became them abundant in a great variability of habitats (Fryer, 1968), besides they can have a wide geographic distribution (Dole-Olivier *et al.*, 2000). The intense occupation of surrounding areas in the Furnas reservoir (Brazilian Southeast region) by agriculture, urban and industrial activities, and the installation of net cage fish cultures contribute to the water quality deterioration. Furthermore, there have been low rainfall levels in this region in recent years, leading to a decrease in the useful reservoir volume. In this context, this paper aims to relate the physical and chemical water parameters with the abnormalities in the morphology and species composition of cladocerans.

METHODS

Study area

The hydroelectric power plant of Furnas reservoir (20°42'49" S, 46°06'40" W) is located in the Grande River Basin, southern of Minas Gerais State, Brazil (Fig. 1). It was formed by the damming of Grande and Sapucaí Rivers. It offers maximum length of 220 km and a perimeter of 3500 km, occupies a flooded area of 1440 km² and has a total water volume of 22.95 billion m³ (ANA, 2014).

Five stations on the Sapucaí River compartment of Furnas Reservoir were selected for studies. The stations

were named 1A, 1B, 2, 3A and 3B. The stations 1A (21°23'50" S, 46°04'05" W) and 1B (21°23'50" S, 46°04'05" W) are located at the entrance of the São Tomé River. They are influenced by agriculture activities and it is used for potable water supply for Alfenas Town. The station 2 is located at the entrance of the Pântano Stream (21°23'52" S, 45°59'06" W). In this station, there is the contribution of residual water from wastewater treatment plant of Alfenas town. The stations 3A (21°26'36" W, 46°04'38" W) and 3B (21°27'04" S, 46°05'48" W) are located below the station 2 and there is installation of net cage fish cultures for production of Nile tilapia.

Sampling and data analysis

The samples were monthly collected from July 2013 to February 2014, in the five stations. The water temperature, pH, electrical conductivity and dissolved oxygen concentration were obtained by a Horiba U-50 multi-sensor on the surface of water column. Water transparency was estimated by Secchi disk. The total phosphorus and total dissolved phosphorus concentrations were determined by spectrophotometry analysis described in the Standard Methods for the Examination of Water and Wastewater (Rice *et al.*, 2012). The chlorophyll *a* concentrations were obtained by extraction with 90% cold acetone analysis (Golterman *et al.*, 1978). The Carlson method (1977), modified by Toledo Junior *et al.*, (1983), was used to calculate the trophic state index (TSI) considering the water transparency, chlorophyll *a*, total phosphorus and total phosphorus dissolved values.

Cladocerans were collected using a suction pump and plankton net (68 µm mesh size) and concentrated from a volume of 400 L. The samples were fixed with 4% formaldehyde solution. Qualitative samples were analyzed in full under stereoscopic microscope Zeiss Stemi 2000 with magnification up to 40x and the organism bodies were identified and photographed under light microscope Zeiss Scope A1, with image acquisition and magnification up to 400x. Quantitative samples were analyzed on subsamples. For cladocerans count, subsamples ranged from 5 mL to the entire sample were used and the organisms were counted in checkered acrylic sheets under stereoscopic microscope, with magnification up to 40x. The specialized literature was used for taxon identification. The cladoceran typical or atypical structure were detected based on a comparison with descriptions in specialized literature (Smirnov, 1996; Elmoor-Loureiro, 1997).

Canonical Correspondence Analysis (CCA) were performed by CANOCO 4.5 software (ter Braak and Šmilauer 2002). It was used to verify the influence of limnological variables on cladocerans densities (with and without morphological abnormalities) and correlated them with the stations and sampling dates.

RESULTS

During the study period, noticeable changes in the physical and chemical properties of the water in Furnas Reservoir were observed, reflecting well-defined the wet (December 2013 to February 2014) and dry (July-November, 2013) seasonal climatic periods.

The average pH was near neutrality. The water temperature ranged from 19.4°C to 31.4°C and the highest temperatures were registered during the wet period. Electric conductivity (36 to 84 $\mu\text{S cm}^{-1}$) was considered low when compared to other reservoirs, being the highest value recorded at station 3B, that is a littoral region and it is influenced by Pântano Stream, which receive residual water from wastewater treatment plant of Alfenas town

and by presence of net cage fish culture. In this station also occurred the highest total phosphorous concentration, 35.2 $\mu\text{g L}^{-1}$. Oxygen concentrations (average of 8.5 mg L^{-1}) were high evidencing that the reservoir is a well oxygenated. The suspension matter values ranged from 4.5 up to 11.5 mg L^{-1} , being the highest values recorded during the wet season due to the carrying by rainwater.

There was an increase of the trophic level of the aquatic environment due to the reduction of water reservoir volume and the continuous release of organic and inorganic materials from natural and anthropogenic activities. The stations 1A 1B, 3A and 3B were classified as eutrophic during the dry season and oligotrophic in the wet season, whereas the station 2 was classified as eutrophic throughout the study period. Also, there was the occurrence of bloom of cyanobacteria *Microcystis aeruginosa* during the dry sea-

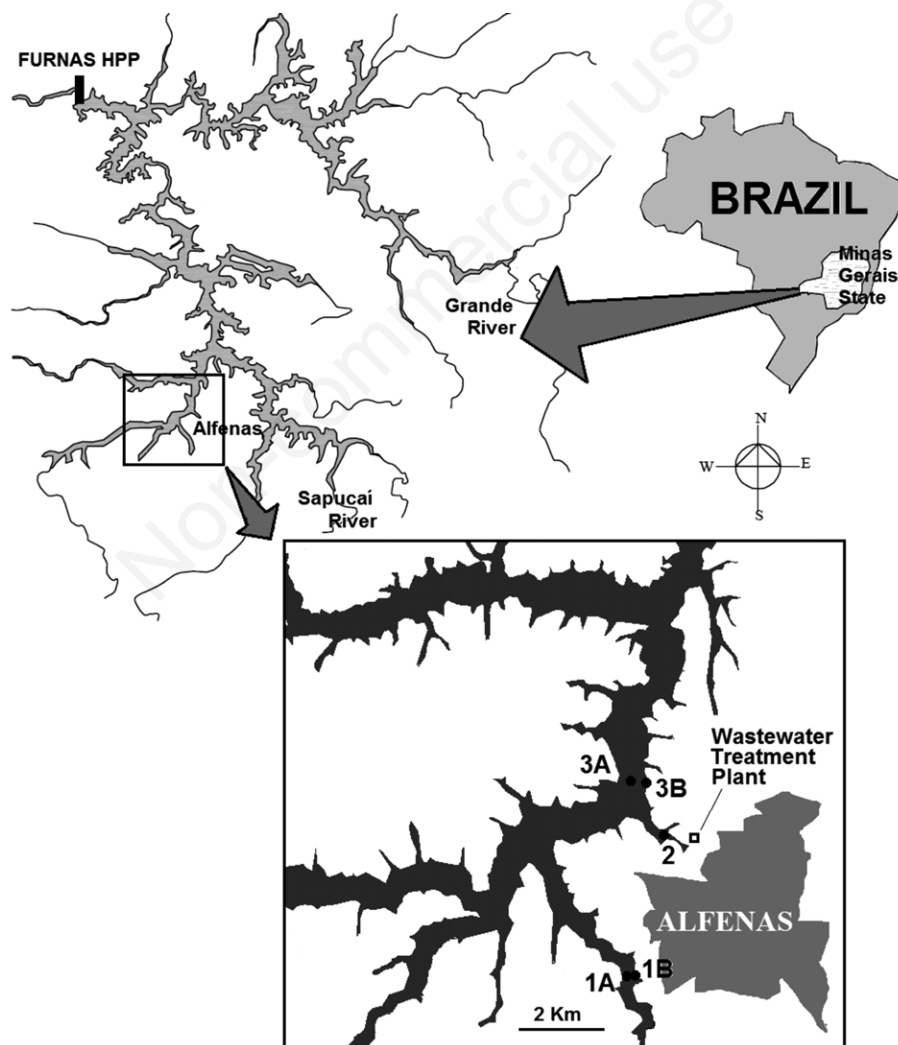


Fig. 1. Furnas Reservoir (20°42'49" S, 46°06'40" W) region. The five sampling stations (1A, 1B, 2, 3A and 3B) are shown, in Sapucaí River compartment.

son, for every sampling station. It was probably caused by the enrichment of the aquatic ecosystem, indicated by the highest concentrations of chlorophyll *a* in the dry season (from 24.9 to 35.2 $\mu\text{g L}^{-1}$) when compared to the wet season (1.0 to 10.4 $\mu\text{g L}^{-1}$) and the high densities of cyanobacteria observed in the analysis of zooplankton.

Twenty-three taxa of Cladocera were recorded: Macrothricidae (1), Moinidae (1), Sididae (3), Daphnidae (5), Chydoridae (8) and Bosminidae (4) family. High individuals density of Chydoridae occurred from July to November 2013, mainly in stations 2, 3A and 3B. It probably occurred due to the reduction in the reservoir water volume, becoming these stations shallow and with high presence of macrophytes. This family is typical of littoral region and lives associated to macrophytes and sediment. *Chydorus pubescens* Sars, 1991 was dominant among Chydoridae and occurred in high densities in stations 3A and 3B, in December 2013. Also, it was observed the occurrence of ephippial females and males of *Alona* sp.

Morphological abnormalities were observed in some species of cladocerans. *Daphnia gessneri* Herbst, 1967 shows a deformation of the rostrum and a folded tail spine

in only one individual, at the station 3A, in August 2013 (Fig. 2). Also, abnormalities were observed in the intestine of *Ceriodaphnia silvestrii* Daday, 1902, and *Bosmina longirostris* (O. F. Müller, 1785) in a single individual of each species. Abnormalities for *Bosmina tubicen* Brehm, 1953 were found at 6.3% from the population at the station 3B. However, for *C. pubescens* the abnormalities occurred at high population rate and were classified in type 1 and type 2 abnormalities. Type 1 had increased length of intestine and size of its intestinal loop, whereas the type 2 had an intestine prolapse (Fig. 3).

The abnormalities were observed for every station studied in the dry season for *C. pubescens*, mainly at 3A and 3B stations, in October and December 2013, where were observed high abnormality densities for type 1 (from 5.64 up to 22.33 ind m^{-3}) and high type 2 abnormality density (8.89 ind m^{-3}) at station 2 in October (Fig. 4). The total abnormalities (type 1 + type 2) occurred at ~75% and 42% of the total of individuals of this species in dry and wet seasons respectively, in average. The highest rates, associated to high densities of individuals, were registered at 1B (97%), 3A (98%) and 3B (93%) stations, in October.

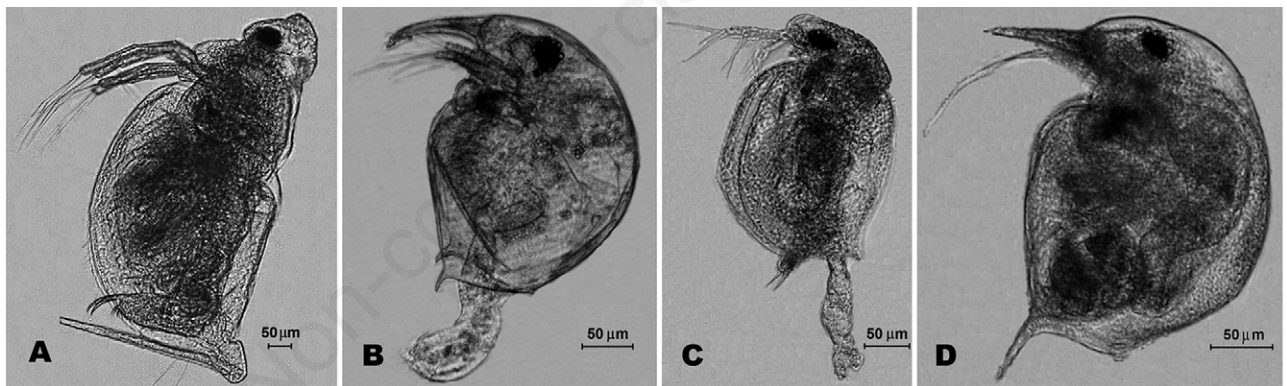


Fig. 2. Abnormalities in A, *Daphnia gessneri*; B, *Bosmina longirostris*; C, *Ceriodaphnia silvestrii*, and D, *Bosmina tubicen* species.

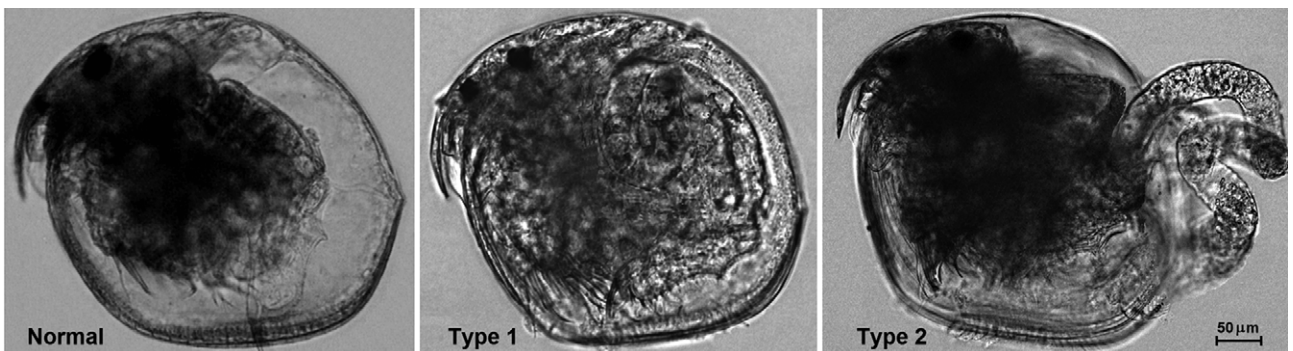


Fig. 3. *Ch. pubescens* female: normal (without abnormalities), type 1 and type 2 abnormalities.

These sampling stations are in the littoral zone or with low depth and they have been influenced by sewage release. During the wet season, mainly in January and February 2014, *C. pubescens* had a low density and/or no abnormalities, probably due to dilution caused by rainwater and subsequent decreasing of the trophic level.

The Canonical Correspondence Analysis (CCA) indicated that presence of normal individuals of *C. pubescens* only was correlated with lower depth, presence of suspended matter, lower pH and higher temperature of the

station samplings (Fig. 5). Presence of *C. pubescens* organisms with type 1 abnormality was more correlated with lower total phosphorus and higher inorganic phosphate concentrations, while type 2 abnormality was more correlated with higher chlorophyll *a* concentration. The most part of sampling months of stations 3A and 3B were correlated with higher total phosphorus, inorganic phosphate and chlorophyll *a* concentrations, probably due to the release of residual water from wastewater treatment plant of Alfenas Town.

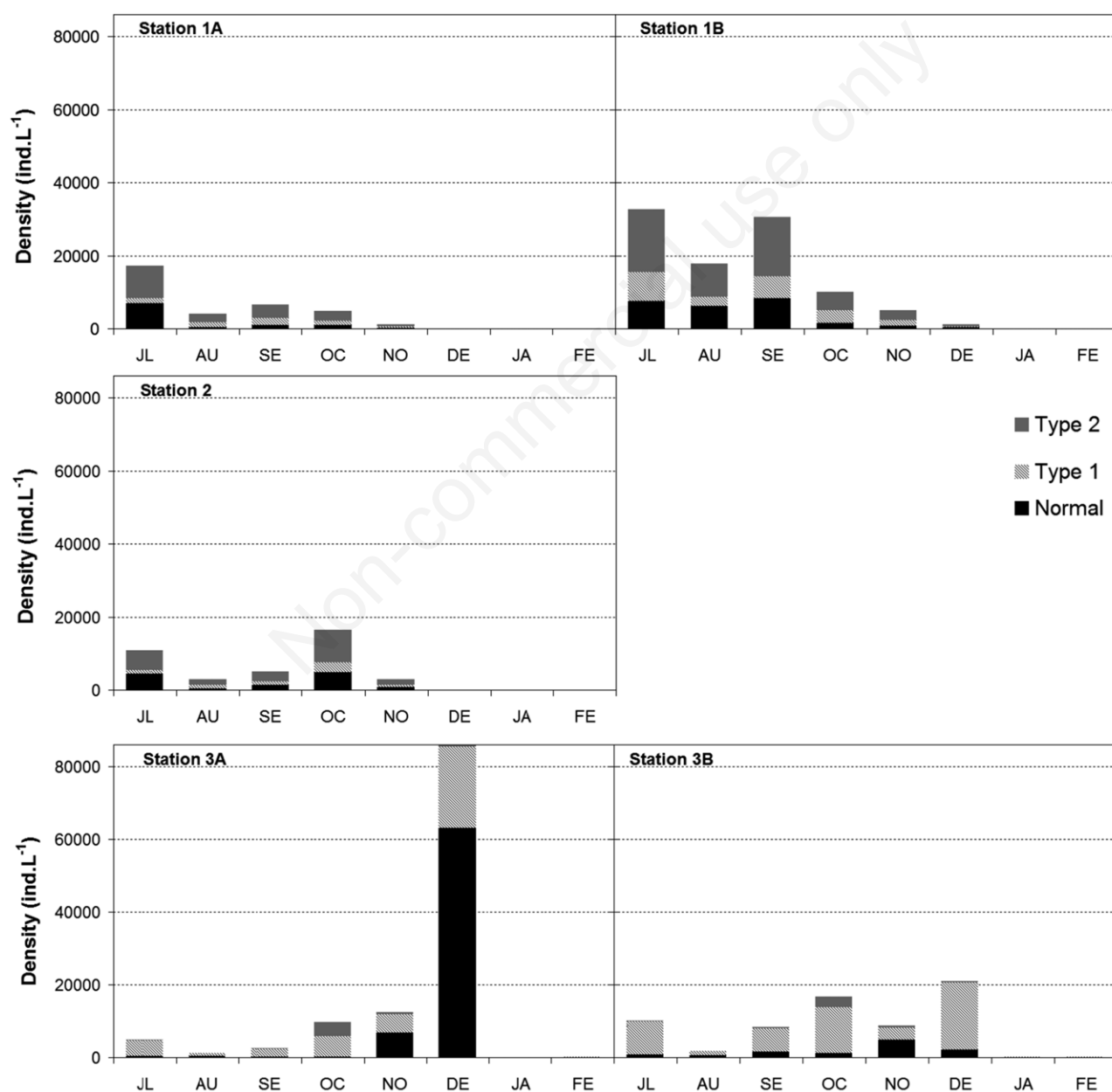


Fig. 4. Organism density with type 1 and type 2 abnormalities and Normal (no abnormalities).

DISCUSSION

The dry season (2013 and 2014) was an unusual period due to lack of rain when compared to previous dry seasons, in Brazil Southeastern region. Furthermore, the electric power production caused a decreasing of the useful reservoir volume from 57.4% in 2013 to 24.9% in 2014, from its maximum capacity (ONS, 2014). This reduction associated with continued releasing of particulate matter, which caused the increasing of nutrients concentration in the aquatic environment and intensified the eutrophication in the study area. Pinto-Coelho *et al.* (2005) noted that this reservoir is changing from oligotrophic to mesotrophic, and even it has been classified as eutrophic in some its portions. Santos *et al.* (2010) mentioned that the Sapucaí River compartment of Furnas Reservoir is still oligotrophic, but observed occasional the trophic level decrease and occurrence of cyanobacteria blooms, suggesting the nutrient enrichment. The increase of trophic level index was also observed in this study. The

station 2 was classified as eutrophic throughout the study period due to the release of residual water from wastewater treatment plant from Alfenas Town.

Species richness of cladocerans (23 species) was higher than that observed by Brito *et al.* (2011), which recorded 15 species. At eutrophic stations, there was the dominance of smaller species such as Chydoridae representatives that are less sensitive to cyanobacteria toxicity, low dissolved oxygen and food concentrations, found in these environments (Chislock *et al.*, 2013). The highest species richness of the Chydoridae (8 species) was expected, since the stations were shallow and there was occurrence of macrophytes, which support the development of typical species of the littoral zone (Sousa and Elmoor-Loureiro, 2008). Several male individuals of *Alona* sp. were found between July to October 2013, at stations 1A, 1B, 2 and 3B. It could mean that the environment conditions were unfavorable, because male individuals are generated to perform sexual reproduction and thus increase the species survival chances.

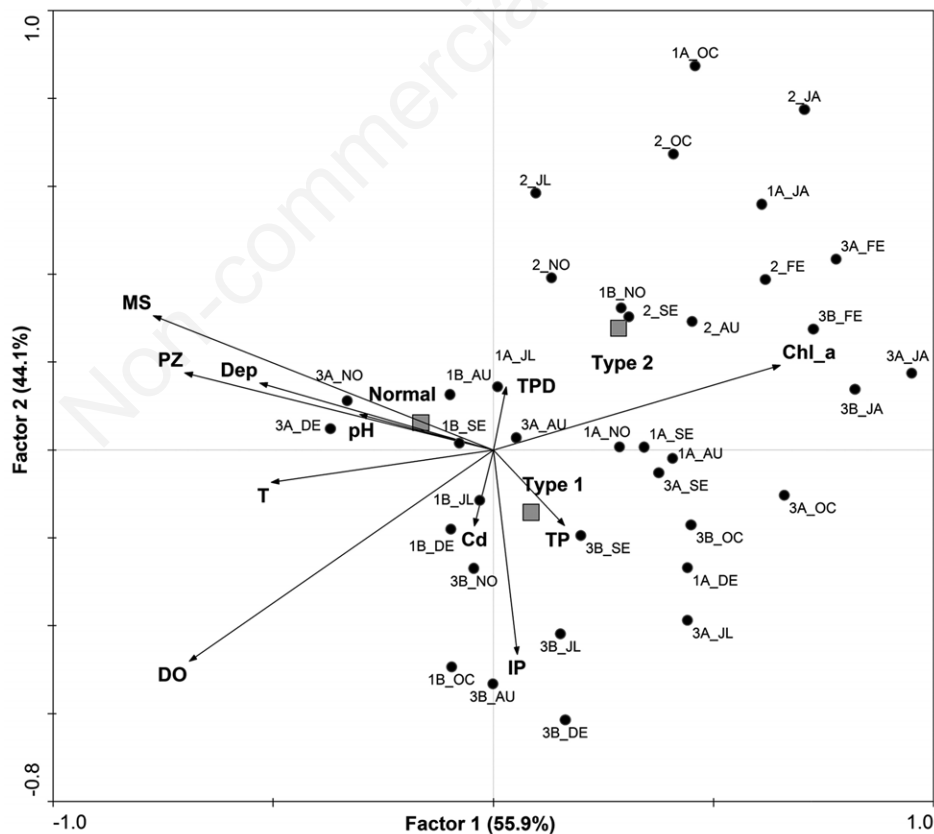


Fig. 5. CCA ordination diagram to *C. pubescens* organisms' density without abnormalities (Normal) and with Type 1 and Type 2 abnormalities. T, temperature; Cd, electrical conductivity; Dep, depth; PZ, photic zone; TP, total phosphorus; IP, inorganic phosphate; TPD, total dissolved phosphorus; Chl, chlorophyll *a*; MS, suspension matter (organic and inorganic); DO, dissolved oxygen concentrations, from Furnas Reservoir samplings (1A, 1B, 2, 3A and 3B). The last two letters attached to station names correspond to the sampling month.

According to Smirnov (1974), abnormalities of various parts of body are sometimes common in the Chydoridae and usually are detected at small minority of the population. They could be from embryonic origin or results of some physical injury during postembryonic development. In the Furnas Reservoir, the morphological abnormalities were observed in several species of Cladocera and in high rate for *C. pubescens*. It may have been resulting of anthropogenic influence that occur in the surroundings of the Furnas Reservoir and have caused deterioration in water quality and constant blooms of *Microcystis aeruginosa*. Several studies showed that Cyanobacteria are harmful to herbivores zooplankton (Lampert, 1982; Hanazato and Yasuno, 1987; Gliwicz and Lampert, 1990). They have low nutritional value, cause blockage of the digestive tract, blocking food intake, and produce toxins. A study carried out in a hypereutrophic pond in Rio de Janeiro found that blooms of *Microcystis aeruginosa* are potentially damaging for populations of cladocerans (Ferrão-Filho, 2009). Hanazato and Yasuno (1987) observed that the rate of growth and reproduction of *Moina micrura* Kurz, 1874 were affected by *Microcystis* sp when cultured in the laboratory. Likewise, Reinikainen *et al.* (1994) found in their studies that low *Microcystis* sp. concentrations were responsible for the death causes of *Daphnia pulex* Leydig, 1860. Christoffersen (1996) reported that cyanotoxins has effects on growth, survival, feeding, behavior in the development and establishment of susceptible species, bioaccumulation of toxins in the food chain, and also species diversity decreasing. The *C. pubescens* abnormalities cannot be associated directly to Cyanobacteria bloom because this species probably does not feed on *Microcystis*. Besides *C. pubescens* is morphologically similar to *Chydorus sphaericus* and, according to Fryer (1968), this species feed fine organic and inorganic particles and rarely diatoms and other no identified large particles.

The zooplankton community can be affected by release of pollutants. The effect of toxic substances in sewage on cladocerans were reported by several studies (Abdellatif, 1993; Monda *et al.*, 1995; Arauzo and Valladolid, 2003; Sotero-Santos *et al.*, 2006; Xiang *et al.*, 2012). In Furnas Reservoir were detected high pesticide concentrations in the water near the coffee cultures farms, which can cause changes in the zooplankton community (Santos-Neto and Siqueira, 2005). Stampfli *et al.* (2011) evaluated the impact of pesticides on zooplankton community in experiments with mesocosms and noted that the largest organisms were eliminated, causing changes in the trophic chain. The reproductive cycles for other aquatic species were also affected.

In this study, abnormalities were observed in the intestine morphology of various specimens of *C. pubescens*. For this, they were classified to type 1 and type 2 abnor-

malities. Smirnov (1974) and Røen (1968) also reported malformation of the post-abdominal claws and on the arrangement of the anal spinules. Dias (1999) observed prolapse (type 2) and intestinal partial disruption of chitin among segments in *Acartia lilljeborgi* (Crustacea Copepoda). Montú and Gloeden (1982) also observed these abnormalities in other species of Copepoda. Toxicity tests with cyanotoxin Microcystin - LR, which are present in *Microcystis aeruginosa*, show that at high concentrations of this cyanotoxin there was bioaccumulation and caused structural changes in the epithelial cells of the alimentary duct. There was a loss of structures which perform the link between cells and the cells with epidermis (Chen *et al.*, 2005; Rohrlack *et al.*, 2005). In this study, the abnormalities were observed in *Chydorus pubescens* and occurred in every station in the dry season for every sampling in high rates (75% in average) of population. On the other hand, there were low rates (42% in average) in wet season. The difference can be explained by useful water volume of Furnas Reservoir (lowest in dry season), which change of nutrients concentration, and the weather conditions.

According to CCA results, the occurrence of *C. pubescens* was more correlated to depth and the photic zone of water column. This species is typical of regions with coastal characteristics and presence of macrophytes, since it has scraper habit (Santos-Wisniewski *et al.*, 2006). The presence of the type 1 abnormality was more correlated to total phosphorus and dissolved phosphorus concentrations, at stations 3A and 3B, which can be associated with presence of organic matter from releasing of residual water (and nutrients) of the wastewater treatment plant and feeding and fish excreta from net cage fish cultures (Santos *et al.*, 2009). The type 2 abnormality was more associated with chlorophyll *a* concentration at stations 2, 3A and 3B, probably due to the bloom of cyanobacteria which occurred at these stations. However, the cited variables contributed to the CCA factors for both abnormality types.

Abnormalities in other species of cladocera were also observed for *Ceriodaphnia silvestrii*, *Daphnia gessneri*, *Bosmina longirostris* and *Bosmina tubicen*, in this study. Kotov *et al.* (2009) also observed morphological variability in *Bosmina tanakai*, a species which occur in Japan. Other authors observed abnormalities in cladocerans which may be associated with environmental factors, in Brazilian water bodies, as this study. Chemical contaminants or bacteria and viruses, may have caused changes in helm, rostrum and the ventral margin of the carapace of some species of daphnids, in reservoir cascade of medium and low Tietê River (Zanata *et al.*, 2008). Iron bioaccumulation by *Coronatella monacantha* Sars, 1901 may have caused abnormalities in the labrum of the keel in species of a stream in Ceará State, Brazilian Northeast (Sousa *et al.*, 2011). Organic pollution in Apipucos Reservoir may have caused the high percentage of abnormali-

ties in *Ilyocryptus spinifer* Herrick, 1882 post-abdomen (Elmoor-Loureiro, 2004).

CONCLUSIONS

The results of this study indicate a strong correlation between high concentrations of nutrients and bloom of cyanobacteria with the occurrence of abnormalities in some species the Cladocera, especially for *C. pubescens* species, probably intensified by an unusual period of dry weather in the study period, in Furnas Reservoir.

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