

# ON THE NATURAL DIET OF *Daphnia laevis* IN THE EUTROPHIC PAMPULHA RESERVOIR (BELO HORIZONTE, MINAS GERAIS)

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## ABSTRACT

The aim of this study was to assess the major food items ingested by adult specimens of *Daphnia laevis* within the eutrophic Pampulha reservoir in Belo Horizonte, Minas Gerais, Brazil. The gut content was analyzed after addition of sodium hypochlorite and also through the examination of dissected guts under scanning electron microscopy. The results showed that Chlorophyceae was the main food item ingested, representing c. 80.5% of the total ingested food. Moreover, *Eutetramorus fottii*, *Coelastrum pseudomicroporum* and *Oocystis lacustris*, the dominant phytoplankton species within the reservoir, were the most frequent cells found in the gut contents. Euglenophyta also represented an important food item accounting for 15% of the ingested material, including mainly *Trachelomonas volvocina* and *Euglena oxyuris*, although less abundant in the reservoir (< 10% of total phytoplankton). Blue-green algae occurred at much lower percentages in the guts than in the phytoplankton. A small amount of undigested *Microcystis aeruginosa* colonies were also found in the gut content of *D. laevis*. Scanning electron microscopy results showed that, besides phytoplankton cells, a great amount of abiogenic material was also ingested. The amount of this inorganic material increased considerably in the tract (from 15% to 75% of the gut content), when a peak of *D. laevis* was observed in the reservoir. Our assumption is that the ingestion of this inorganic material can be a strategy used by *D. laevis* to obtain additional food supply.

*Key words:* *Daphnia laevis*, natural diet, reservoir, eutrophication.

## RESUMO

### Dieta natural de *Daphnia laevis* no reservatório eutrófico da Pampulha (Belo Horizonte, Minas Gerais)

Com o objetivo de verificar a composição dos itens alimentares utilizados por *Daphnia laevis* no reservatório eutrofizado da Pampulha (Belo Horizonte, MG), foi analisado o conteúdo do trato digestivo de indivíduos adultos, por intermédio das técnicas de clarificação e microscopia eletrônica de varredura. Os resultados obtidos mostraram que Chlorophyceae foi o principal item alimentar ingerido por *Daphnia laevis* (80,5%). As espécies mais frequentes no trato digestivo foram: *Eutetramorus fottii*, *Coelastrum pseudomicroporum* e *Oocystis lacustris*, que corresponderam às mais abundantes no reservatório. Euglenophyta também representou importante item alimentar (15%), especialmente *Trachelomonas volvocina* e *Euglena oxyuris*, embora tenham apresentado abundância reduzida no ambiente (< 10% do fitoplâncton total). Cyanophyceae esteve praticamente ausente no trato digestivo, tendo sido encontrada apenas pequena quantidade de colônias não digeridas de *Microcystis aeruginosa*. Os resultados obtidos na microscopia eletrônica de varredura mostraram, além do fitoplâncton, a presença de material abiogênico. A participação desse material inorgânico no tubo digestivo aumentou consideravelmente (de 15% para 75% de preenchimento do trato digestivo) quando um pico de *D.*

*laevis* foi observado no reservatório. Os resultados obtidos sugerem que a ingestão de material abiogênico parece ser uma estratégia na captação de recursos alimentares adicionais.

*Palavras-chave:* *Daphnia laevis*, dieta natural, reservatório, eutrofização.

## INTRODUCTION

In aquatic ecosystems, the structure and dynamics of zooplanktonic populations are strongly influenced by food availability, which is the result of the prevailing trophic conditions of the system. Several studies have correlated changes in species composition, size-structure and biomass in aquatic ecosystems with eutrophication (Bays & Crisman, 1983; Infante & Riehl, 1984; Mayer *et al.*, 1997). Since the herbivorous zooplankton plays a key role within aquatic food chains, the interactions between zooplankton and phytoplankton in eutrophic systems are of paramount importance to the understanding of the structure and functioning of these ecosystems (Nöges, 1997).

Despite the fact that the feeding behaviour of the zooplankton is directly involved with the nutrient cycling, energy transference, and productivity of the aquatic ecosystems, *in situ* studies showing direct observations on the ingested material are still scarce, especially in tropical systems. Furthermore, the majority of the studies concerning Cladocera feeding habits have been conducted under laboratory conditions, using unialgal cultures or limited food sources (Hanazato & Yasuno, 1987; Demott, 1988; Hartmann & Kunkel, 1991; Repka, 1997).

The objective of the present study was to assess the natural diet of *Daphnia laevis* within a eutrophic reservoir, through analysis of the gut content under both optical and scanning electron microscopy.

## STUDY AREA

Pampulha reservoir is a shallow urban water body, located in the southeast of Brazil (19°55'09''S; 43°56'47''W). The reservoir has an accumulated volume of 12 million m<sup>3</sup>, an area of 2.4 km<sup>2</sup>, and an average depth of 6 m (Fig. 1). The reservoir was built in 1938, re-built in 1952 and since then has been under a continuous

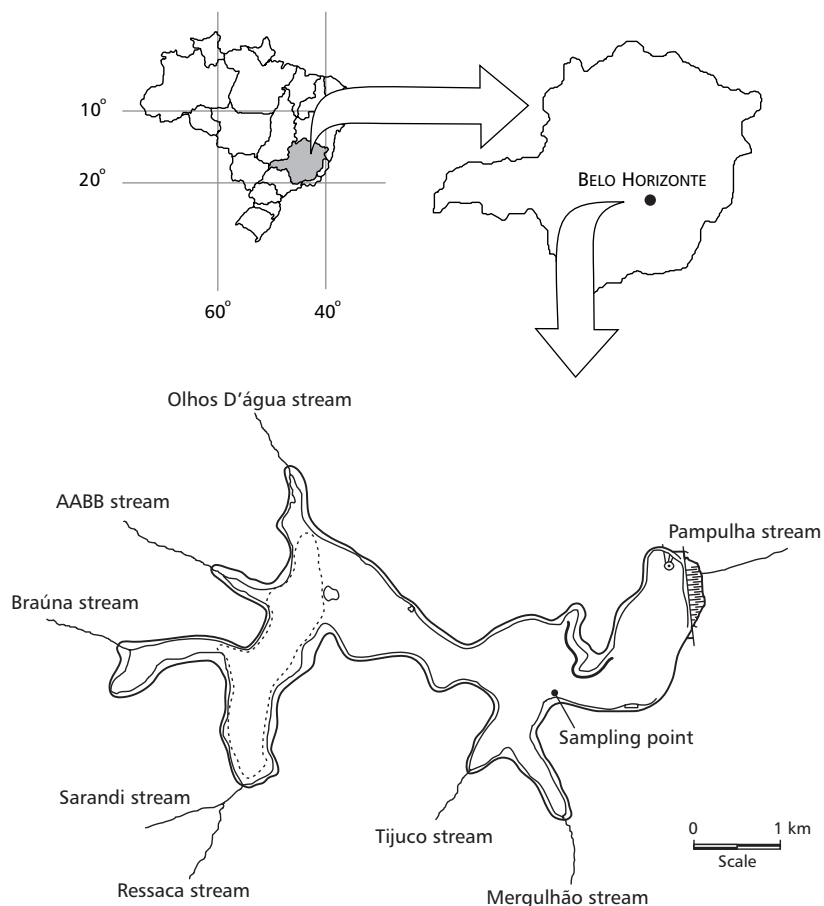
eutrophication process mainly due to domestic and industrial discharges, mostly untreated. Three of its major tributaries (Ressaca, Sarandi and Água Funda) are responsible for *c.* 98% of all the nitrogen entering the reservoir (6,000 and 3,000 µg/L, respectively) rendering charges of 406 kg/day (dry season) and 454 kg/day (raining season). For phosphorous, these streams are also the major contributors with concentrations higher than 600 and 200 µg/L respectively bringing to the reservoir charges of 45 kg/day (dry season) and 44 kg/day (raining season) of total phosphorus. Of these total inputs, *c.* 66-69% of nitrogen and *c.* 94.7-99.8% of total phosphorus are retained in the reservoir, mostly in organic forms (Barbosa *et al.*, 1998).

Several episodes of macrophyte's proliferation and some fish killings had been reported, as well as algae blooms, particularly of *Microcystis aeruginosa*, *M. flos-aquae*, *M. viridis* and *Woronochinia naegeliana* (Goodwin, 1997).

## MATERIALS AND METHODS

Samples for phytoplankton quantitative analysis were collected bi-weekly from July-December 1996 and January-March 1997 at a fixed sampling station in the reservoir (Fig. 1), using a Van Dorn bottle. Sub-samples were preserved with lugol solution. Qualitative samples were obtained through horizontal throls with a 20 µm net and sub-samples preserved with neutralized formaldehyde solution (4%). Quantitative analyses were performed under an inverted microscope as described in Sourmia (1984) and density expressed according to Villafañe & Reid (1995). Biovolume of cells and colonies were obtained through the use of a micrometer eye-piece and the formulae described by Edler (1979) and Wetzel & Likens (1991).

Zooplankton samples were taken with a 68 µm plankton net and short horizontal throls (*c.* 1 minute each), and the organisms were immobilized by addition of carbonated water and then preserved with 4% neutralized formaldehyde solution.



**Fig. 1** — Map of Pampulha reservoir showing location of the sampling station.

Adult specimens of *D. laevis* (> 1.8 mm) were isolated in Petri dishes, rinsed with distilled water, and mounted in glass slides. The analysis of the gut content was performed after clarification with sodium hypochlorite according to Infante (1978). The identification and counting of food items were performed using an inverted microscope. The gut content was determined through the counting of the present algae species and the proportion of the digested material. For the scanning microscopy analysis, 30 specimens of *D. laevis* were rinsed with distilled water, fixed with 1% osmium tetroxide for 2 hours. After dehydration, the digestive tracts were dissected and mounted in glass slides with Poly-L-Lisine. The gut content was air dried for 24 hours,

metalized with gold and examined under a ZEISS DSM 950 microscope.

## RESULTS

From the 22 microalgae species recorded in the reservoir, 12 were found within the gut content of *Daphnia laevis*. Chlorophyceae species represented the major food item ingested, from which *Eutetramorus fottii*, *Coelastrum pseudomicroporum*, *Oocystis lacustris*, *Chlorella vulgaris*, and *Chlorococcum* sp., were recorded in 90% of the examined tracts. Furthermore these species were the most abundant within the reservoir (Fig. 2).

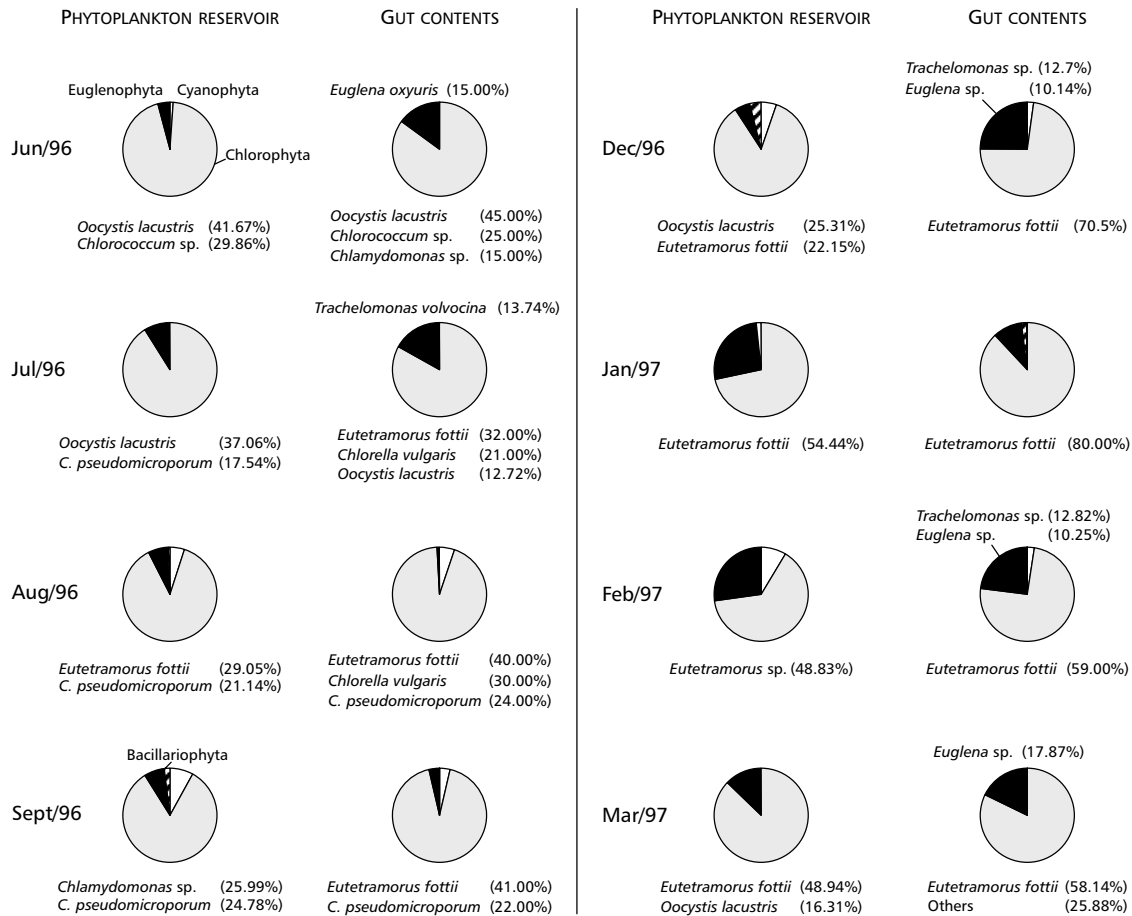


Fig. 2 — Distribution (%) of total phytoplankton in the reservoir and in the gut content of *Daphnia laevis*, during the study period.

Besides Chlorophyceae, Euglenophyceae also constitute an important food source, including *Euglena oxyuris* and *Trachelomonas volvocina* as major species. Cyanobacteria mainly represented by *Microcystis aeruginosa* and *Anabaena* sp. appeared in low abundances within the reservoir, representing less than 15% of total phytoplankton as well as within the digestive tract of *D. laevis* (< 10% of ingested food items). Furthermore, non-digested colonies of *M. aeruginosa* were also found among the gut content.

*Microcystis aeruginosa* was recorded within the digestive tract just following an observed peak of *D. laevis* in the reservoir (July 1996), when this species accounted for almost 60% of the total zooplankton (Fig. 3) decreasing markedly in August (< 10% of the total zooplankton) increasing again from December 1996 until March 1997.

The algae and *D. laevis* densities within the reservoir showed an inverse proportional pattern, thus an increase in phytoplankton density was recorded in July (the time of *D. laevis* peak) and September, followed by a decrease of algae species and an increase of *D. laevis* population (Fig. 6). The total phytoplankton density within the reservoir oscillated between 35,000 and 540,000 cels.L<sup>-1</sup>.

Scanning microscopy analysis of the digestive content revealed that besides the ingestion of phytoplankton species, *D. laevis* also ingested abiogenic material, among which algae fragments were recorded (Fig. 4). During the quantitative peak of *D. laevis*, the proportion of amorphous material increased considerably, reaching 75% of the total gut content, while during other periods, this material accounted for less than 15% of the total gut content.

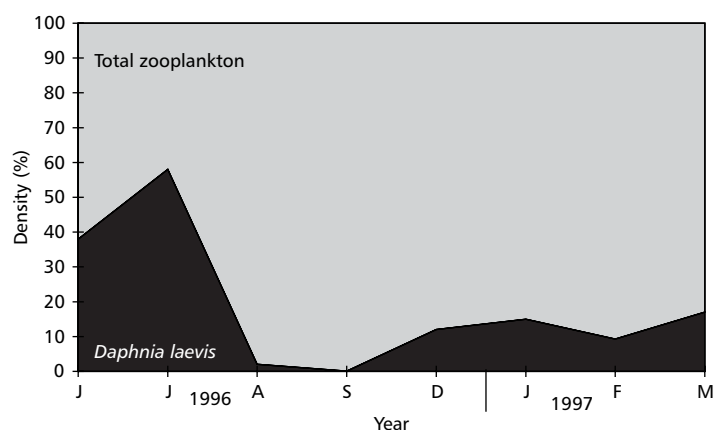


Fig. 3 — Density (%) of total zooplankton and *D. laevis*.

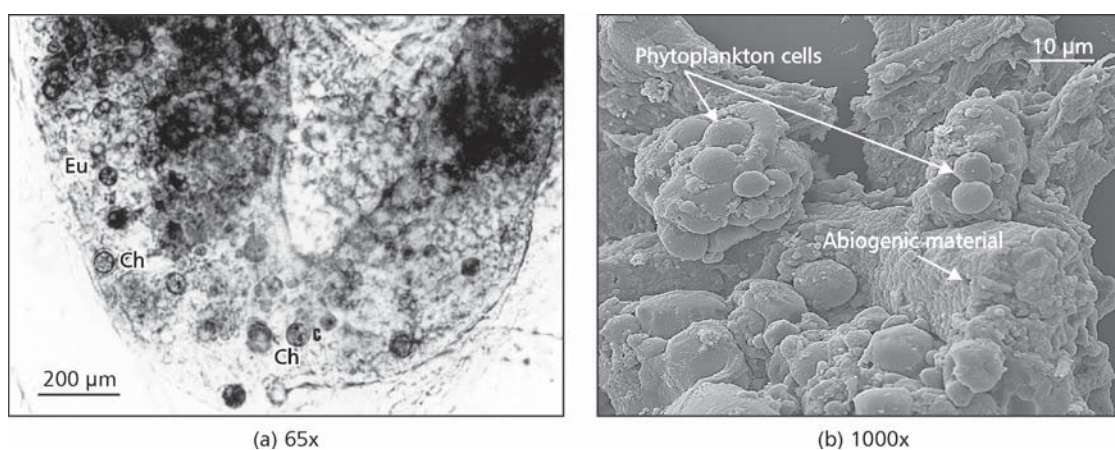


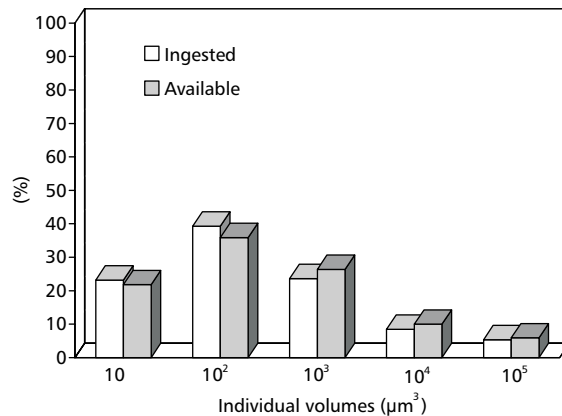
Fig. 4 — Optical and scanning electron microscopy of the gut content of *D. laevis*: (a): Ch = *Chlamydomonas* sp.; Eu = *Eutetramorus fottii*. (b): abiotic material and colonies of unidentified phytoplankton cells.

The distribution of algal biomass within the digestive tract of *D. laevis* was the following: 45% of the ingested phytoplankton was represented by algae in between 10 and 10  $\mu\text{m}^3$ , c. 25% of species of up to 10  $\mu\text{m}^3$  and 22% of algae with 1,000  $\mu\text{m}^3$  (Fig. 5). The phytoplankton cells possessing biomass higher than 10,000  $\mu\text{m}^3$  accounted for less than 15% of the ingested material. Within the reservoir, the most abundant biomass fraction was similar to the one formed by algae in between 10 and 1,000  $\mu\text{m}^3$ , a pattern that changed in August and September when cells with biomass higher than 1,000  $\mu\text{m}^3$  were dominant, mainly represented by colonies of *Eutetramorus fottii*, *Coelastrum*

*pseudomicroporum*, and *Microcystis aeruginosa* as well as *Euglena oxyuris* and *Phacus* sp.

## DISCUSSION

The most abundant phytoplankton species within the reservoir were the predominant ones found in the gut content of *D. laevis* (*Eutetramorus fottii*, *Coelastrum pseudomicroporum* and *Oocystis lacustris*) thus suggesting no food selection pattern, as similarly recorded by Tóth *et al.* (1987) for *Daphnia galeata*. Furthermore, several non-digested cells of *Euglena* sp. were observed within the digestive tract.



**Fig. 5** — Algal biomass distribution in the digestive tract of *D. laevis* and in Pampulha reservoir, according to cell biovolumes ( $\mu\text{m}^3$ ).

Similarly the presence of non digested colonies of *Microcystis aeruginosa* was observed, which according to Gliwicz & Siedlar (1980), can pass through the digestive tract without being digested due to their mucilage which provide protection against digestive enzymes. Furthermore, the ingestion of *M. aeruginosa* was restricted to the peak period of *D. laevis*, which seems to be associated with the food availability since both density and species composition of the algae changed during this period.

The viability of cyanobacteria as a food source for the herbivore zooplankton still remains controversial. Some authors suggest that growth and development of planktonic crustaceans can be strongly affected by the ingestion of small blue-green algae (Alcaraz *et al.*, 1980; Price *et al.*, 1983; Repka, 1998) despite the fact that they have been considered a food source of low nutritional quality (Ahlgren *et al.*, 1990). However, De Bernardi *et al.* (1981), Holm *et al.* (1983) and Rietzler & Espíndola (1998), suggest that the ingestion of blue-green algae can be a strategy to arrest additional food sources without affecting their intrinsic population rates. Furthermore, the possibility of an indirect ingestion of small blue-greens has been discussed, since their mucilage can also contain adhered detritus, bacteria, and protozoa (Amemiya, 1988).

Phytoflagellates were not recorded within the gut content of *Daphnia laevis*, in spite of the fact that these organisms can represent a reasonable

food source considering their small size and quick digestion due to the lack of shell or rigid cell walls (Giani, 1991). The rapid processing of these organisms within the digestive tract probably can explain the difficulty of identifying its fragments in the gut contents, even through the utilization of scanning electron microscopy.

The abiogenic material recorded within the gut content of *D. laevis* showed a considerable variation, coinciding with the quantitative fluctuation of this species in the reservoir. According to Infante & Edmondson (1985), food resources can interfere with the population dynamics of herbivorous zooplankton. Populations of *Daphnia* can double their densities in the presence of certain type of food resource. On the other hand, under food limitation the population may decline, thus suggesting some restriction in the nutritional quality of the available food source.

The zooplankton utilization of detritus has been described by several authors as a strategy in arresting additional food supplies. The presence of attached bacteria, organic compounds, mainly proteins and carbohydrates, and live phytoplankton cells turn these particles into a rich and diverse food source (Pechen-Finenko, 1987; Navarro *et al.*, 1993; Steinberg, 1995). Furthermore, other factors associated with the occurrence of seasonal patterns of zooplankton distribution, such as predation and changes of physical and chemical parameters of the water also can effectively influence population dynamics. Pinto-Coelho

(1998) recorded a marked seasonal variation of *Daphnia* sp. populations in Pampulha reservoir and pointed to the quality of available food source as the key factor in regulating their temporal variations.

The amount of detrital material ingested by *D. laevis* showed a temporal variation, representing the major food item inside the gut content during the time of the peak of this species. This variation in the predominantly ingested material seemed to have influenced the population dynamics of *D. laevis*, since its densities showed a reduction of c. 60% when the ingestion of detritus was enhanced.

The ingestion of this material seems to be correlated with the type of available food (size and nutritional quality) and also with intra-specific competition for food resources. During the peak of *D. laevis*, the chlorophytes *Eutetramorus fottii* and *Coelastrum pseudomicroporum*, represented the major available food item within the reservoir that was ingested by *D. laevis*. However, just after the boom of *D. laevis*, the biomasses of the phytoplankton species increased considerably from 100 to 10,000  $\mu\text{m}^3$ . Other micro-algae such as the euglenophytes *Euglena oxyuris* and *Phacus* sp., and colonies of *Microcystis aeruginosa*, also increased both percentages and biomasses.

An analysis of algae biovolumes ingested by *D. laevis* showed that 45% of the ingested phytoplankton was formed by cells in between 10 and 100  $\mu\text{m}^3$ . The change in the availability of cells with this volume range could be constraining the potentially assimilated algae by *D. laevis*, which could be found in the suspended material and additional food source.

The present results suggest that the population dynamics of *D. laevis* can be strongly affected by the type of available food resource that comprises the composition and size of the dominant phytoplankton. In this way, changes in the structure of phytoplankton community induced by processes such as eutrophication can exert a considerable influence on the temporal dynamics of *D. laevis* in Pampulha reservoir.

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