NUTRITIONAL STATUS RESPONSE OF *Daphnia laevis* AND *Moina micrura* FROM A TROPICAL RESERVOIR TO DIFFERENT ALGAL DIETS: *Scenedesmus quadricauda* AND *Ankistrodesmus gracilis*

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ABSTRACT

The accumulation of lipid reserves was investigated in two common cladoceran species typical of neotropical freshwaters. Experiments were performed in order to measure differential accumulation of lipid reserves in response to two algal diets, *S. quadricauda* and *A. gracilis*. The larger *D. laevis* fed with the *Ankistrodesmus* diet accumulated a higher amount of total lipids. The average lipid content for both diets was 11.1% and 22.1% dry weight for *Scenedesmus* and *Ankistrodemsus*, respectively. This difference was found to be highly significant. The superior nutritional quality of *A. gracilis* was confirmed by the experiments using the smaller *Moina micrura*. This cladoceran accumulated 11.4% and 19.9% of the average dry weight of lipids from *Scenedesmus* and *Ankistrodesmus*, respectively. The study also found that most lipid reserves are concentrated as triacylglycerols in both cladocerans. The relative contribution of this class of lipids also varied according to the diet. For *Daphnia*, for instance, the average triacylglycerol levels varied from 6.2 to 11.0 with the *Scenedesmus* and *Ankistrodesmus* diets, respectively.

Key words: variation nutritional, cladocerans, chlorophyceae, experiments.

RESUMO

Respostas ao estado nutricional de *Daphnia laevis* e *Moina micrura* em um reservatório tropical para diferentes dietas algais: *Scenedesmus quadricauda* e *Ankistrodesmus gracilis*

O presente estudo investigou o acúmulo de reservas de lipídeo em duas espécies comuns de cladóceros neotropicais, típicas de água doce. Foram desenvolvidos experimentos para medir o acúmulo diferenciado de reservas lipídicas em resposta a duas dietas algais, *S. quadricauda* e *A. gracilis*. O cladócero maior, *D. laevis*, alimentado com a dieta *Ankistrodesmus* acumulou uma quantidade maior de lipídeo total. As médias de lipídeo total para ambas as dietas foram 11,1% e 22,1% de peso seco para *Scenedesmus* e *Ankistrodesmus*, respectivamente. Essa diferença encontrada foi altamente significativa. A qualidade nutricional mais elevada de *A. gracilis* foi confirmada pelos experimentos em que foi utilizada uma espécie menor de cladócero, *Moina micrura*. Esse cladócero acumulou valores médios de lipídeo total de 11,4% e 19,9% de peso seco para *Scenedesmus* e *Ankistrodesmus*, respecitvamente. Este estudo também revelou que a maior parte dos lipídeos estocados em ambos os cladóceros referese a triglicérides. A contribuição relativa dessa classe de lipídeo também variou de acordo com a dieta. Para *Daphnia*, por exemplo, os teores médios de triglicérides variaram de 6,2% a 11,0% de peso seco total com as dietas de *Scenedesmus* e *Ankistrodesmus*, respectivamente.

Palavras-chave: variação nutricional, cladóceros, clorofíceas, experimentos.

INTRODUCTION

Lipids are the most important energy reserves for marine as well as freshwater zooplankton. Contrasting with the large literature about lipids on marine zooplankton, there is a scarcity of published works about lipids in freshwater zooplankton (Farkas, 1970; Goulden & Horning, 1980; Ahlgren *et al*., 1990; Vanderploeg *et al*., 1992; Ahlgren *et al.*, 1997). In planktonic crustaceans, the lipids may have important functional and structural properties. Since these compounds are of low density, high percentiles of lipids in the biomass may be helpful for animal flotation. Lipids are also colorless, an important characteristic that protects against visual predators in a highly transparent environment. The lipids also have the capacity to accumulate nearly twice as much energy as proteins and carbohydrates in their chemical bonds. Lipid levels can vary with several environmental factors. They also can be used as indicators of the degree of disturbance of an ecosystem (Arts, 1999). The crustacean zooplankton frequently contains a large amount of lipids (Sterner *et al*., 1992).

Most copepods and cladocerans accumulate large amounts of lipids as energy reserves. In marine copepods living in the deep sea or in the Arctic Ocean, up to 60% of their biomass can consist of lipids reserves, mainly as fatty acids. Copepods living in superficial water of temperate oceans and freshwater copepods and cladocerans have triacylglycerol as the main form of energy reserve. These compounds can reach up to 40% of body biomass. These reserves can be used by the adults to satisfy their metabolic needs during periods of food limitation (Goulden & Henry, 1988).

Abyssal marine copepods accumulate triacylglycerols and fatty acids. The triacylglycerols can be promptly used to sustain basal metabolism during brief periods of hunger. The fatty acids, in turn, are used when the animals are exposed to several months of low food levels. Species of marine copepods living at the surface accumulate only triacylglycerol that can be quickly metabolized to sustain vital activities when food is temporarily scarce, or when they are looking for a better feeding place. The energy reserves of freshwater species, such as *Daphnia*, are also mainly triacylglycerols (Goulden & Horning, 1980).

According to Goulden & Henry (1988), the energy reserves of cladocerans seem to be used in two different ways: a) the adults use them for their own metabolic needs; or b) the reserves are transferred to the offspring through eggs and are used by the young animals in their initial development. The plankton microcrustaceans represent one of the most important links between the producers and the fishes in the aquatic food chain. Thus, the bioenergetics of zooplankton affects the biochemical composition and energetic dynamics of fishes as well (Farkas, 1970). According to the same author, several methodological problems have contributed to the scarcity of published information concerning the dynamics of fats in planktonic crustaceans. These include sample treatment and appropriate methods since most of the existing procedures are not suitable for measuring lipids in such organisms (Gardner *et al*., 1985).

The availability of new methodologies of sample treatment (lyophilization, deep-freezing) as well as the adaptation or improvement of older analytical techniques have opened the possibility of performing simple, precise, and reliable quantitative measurements of different lipid classes such as triacylglycerols, phospholipids and fatty acids, in addition to total lipids (De Mott & Müller-Navarra, 1997; Lürling *et al*., 1997; Weers *et al*., 1997; Sekino *et al*., 1997; Weers & Gulati, 1997).

In the neotropics, zooplankton is typically dominated by small organisms, especially copepods of the genus *Thermocyclops* spp., cladocerans such as *Bosmina* and *Ceriodaphnia,* and several species of rotatoria (Lopes *et al*., 1997; Attayde & Bozelli, 1998). However, under certain circumstances, i.e., in the presence of eutrophication, the proportion of larger cladocerans in the zooplankton increases and they may often become dominant or sub-dominant. Some of these species are: *Daphnia laevis*, *Diaphanosoma birgei,* and *Moina micrura* (Pinto-Coelho, 1998).

The objective of the present investigation was to answer the following questions: a) how much lipid can a large tropical cladoceran accumulate under optimal food conditions? b) Is triacylglicerol the major lipid class accumulated by such organisms? c) Is there a significant difference in lipid accumulation between two often dominant cladocerans in neotropical freshwaters? d) To what extent can variations in food quality affect lipid reserves?

MATERIAL AND METHODS

Two zooplankton organisms, *Daphnia laevis* and *Moina micrura*, were collected in a nearby eutrophic reservoir, the Pampulha Reservoir, located in Belo Horizonte, Brazil, and cultivated in the laboratory. The organisms received a diet consisting of 3.0 to 4.0×10^4 cells.ml^{-1} of a mixed culture of *Scenedesmus quadricauda* and *Ankistrodesmus gracilis* algae. The water of these cultures was first changed every three days using reservoir water. It was then replaced with reconstituted water as proposed by Cetesb (1994).

The inocula of the chlorophyceae *Ankistrodesmus gracilis* and *Scenedesmus quadricauda* were obtained from the Laboratory of Phycology of the Department of Botany, Universidade Federal de Minas Gerais, Belo Horizonte, MG. The cultures and all experimental units were maintained under a photoperiod of 11 hours of light, with light intensity from 800 to 1,400 Lux and temperature of 21 ± 3°C, with food supplied daily. The experimental units consisted of large pyrex (2 liter) vessels. The experiment was started by transferring 20-40 females from the stock culture to every unit in triplicate for each treatment. All individuals were derived from a single female in order to avoid genetic variation between treatments. The experiments lasted five days. Stock cultures were also maintained in 2-liter Pyrex vessels.

At the end of every experiment, the organisms of each unit were concentrated separately using a 50 µm mesh plexiglas net. The animals were immediately transferred to Eppendorff tubes and frozen in liquid nitrogen and then freeze-dried (Edwards L5KR) for 24 hs. The dried organisms were maintained in a common freezer with silica gel in complete darkness to avoid lipid photooxidation (Berberovic & Pinto-Coelho, 1989). The dry weight of the samples was determined using a high precision Mettler 64 balance. Total lipids were measured using the traditional sulpho-phospho-vanilline method (Zollner & Kirsch, 1962) modified for aquatic invertebrates according to the procedure proposed by Meyer & Walther (1980). This method consists of oxidizing cellular lipids to small fragments after chemical digestion with hot concentrated sulfuric acid. After the addition of a solution of vanillin and phosphoric acid, a red complex is formed which is measured with a Shimadzu UV 1201 spectrophotometer (546 nm). The lipid values are expressed as dry weight percent.

The triacylglycerol analyses were carried out colorimetrically after lipid extraction using a modified Folch method (Folch *et al*., 1957). The lipid extraction procedure began with a conventional extraction using a chrophorm: methanol solution (2:1), followed by different washings and centrifugation with methanol and chlorophorm with the addition of NaCl and Foch solution (Chlorophormic: Methanol: water, 8:4:3). The samples were dried under pure gaseous N_2 and total lipid was determined gravimetrically. Triacylglicerol was determined using an enzymatic kit (Analisa) after the addition of isopropanol to the dried tubes. Triacylglyverol content was determined with a spectrophotometer at 505 nm after 15 minutes incubation at 37°C.

The data obtained in each experiment were analyzed statistically by the t test at the 5% level of probability. All calculations were made using the Statistica Program, version 4.3.

RESULTS

The total lipid level of the cladocerans studied varied as a function of the diets offered, ranging from 11.1% to 22.1% for *Daphnia laevis* and from 11.4% to 19.9% for *Moina micrura*. *D. laevis* exhibited a higher amount of lipids when fed the *Ankistrodesmus* diet, with an average of 22.1% of total dry weight (Fig. 1). These values were practically reduced by 50% with the *Scenedesmus* diet (11.1%). There was a significant difference between the two diets ($t = 5.37$; $df = 10$; $p < 0.001$). The second cladoceran studied behaved in a similar way since the mean percentage of total lipid for *Moina* was higher with the *Ankistrodesmus* diet, reaching a mean value of 19.9% of total dry weight (Fig. 2). However, there was no significant difference between the two diets ($t = 2.29$; $df = 6$; $p > 0.001$).

The allocation of energy resources in terms of total lipids was more affected by food quality if compared to the differences between the two cladocerans when fed the same diet.

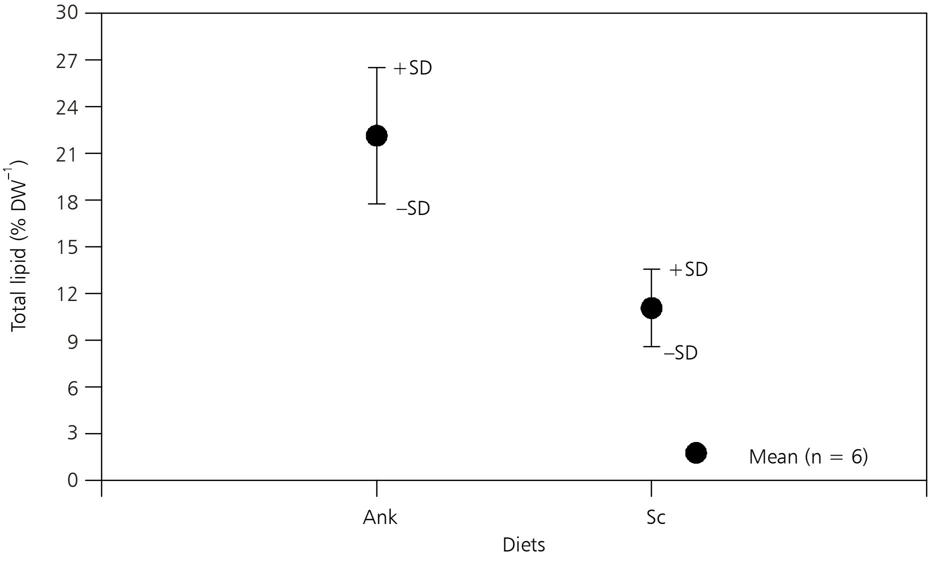

Fig. 1 — Values of total lipids (% DW) for *Daphnia laevis* fed the *Ankistrodesmus gracilis* (Ank) and *Scenedesmus quadricauda* (Sc) diets. N = total sample number.

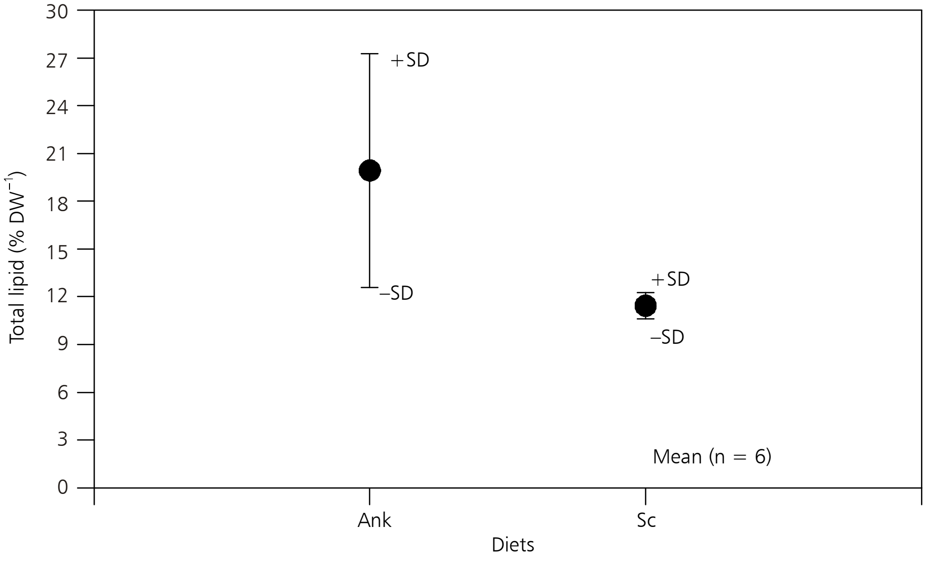

Fig. 2 — Values of total lipids (% DW) for *Moina micrura* fed the *Ankistrodesmus gracilis* (Ank) and *Scenedesmus quadricauda* (Sc) diets. N = total sample number.

Thus, there was no significant difference from the values observed for the two species when fed the same diet. The difference between *D. laevis* and *M. micrura* when fed *A. gracilis*, for example, produced the following statistical values: t = 0.28; df = 8; p > 0.001. This study also revealed that most of the lipid reserves in both cladocerans consisted of triacylglycerols, corresponding to 49.8% to 68.4% of total lipids. Triacylglycerol levels of *Daphnia* were higher with the *Ankistrodesmus* diet than with the *Scenedesmus* diet, reaching 11.0% and 6.2% of total dry weight, respectively (Fig. 3).

A significant difference in triacylglycerol levels was observed between the two diets ($t = 12.43$; $df = 5$; $p < 0.001$). With respect to *Moina*, triacylglycerol production was also higher with the *Ankistrodesmus* diet (Fig. 4), with average values of 7.8% and 11.2% for *S. quadricauda* and *A. gracilis*, respectively. However, no significant difference was found between the two diets ($t = 1.90$; $df = 5$; $p > 0.001$).

Although lower triacylglycerol levels were observed in both cladocerans when fed *Scenedesmus*, the relative contribution of triacylglycerol to the total lipids was higher with this algae, i.e., 55.8% for *D. laevis* and 68.4% for *M. micrura*. Finally, there was no significant difference between the two species ($t = 2.95$; $df = 6$; $p > 0.001$), in terms of triacylglycerol levels.

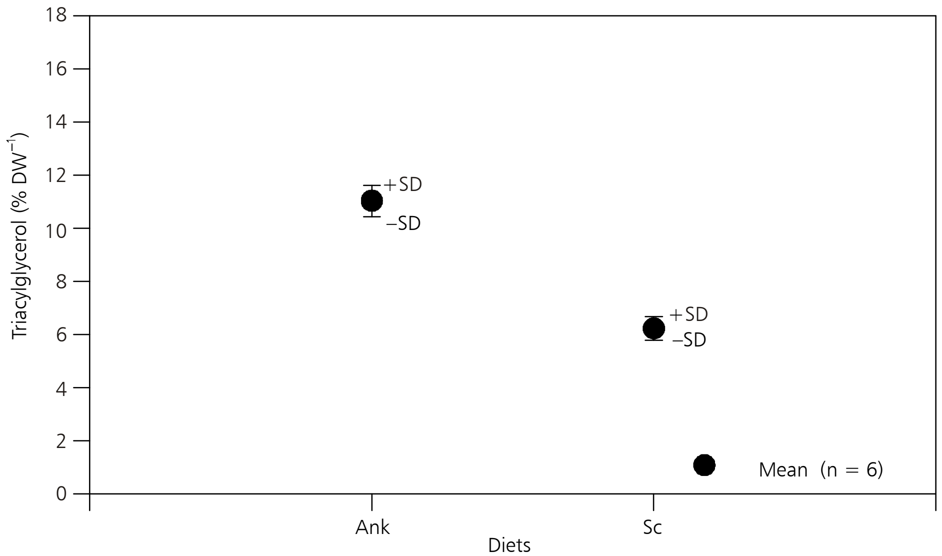

Fig. 3 — Values of triacylglycerol (% DW) for *Daphnia laevis* fed the *Ankistrodesmus gracilis* (Ank) and *Scenedesmus quadricauda* (Sc) diets. N = Total sample number.

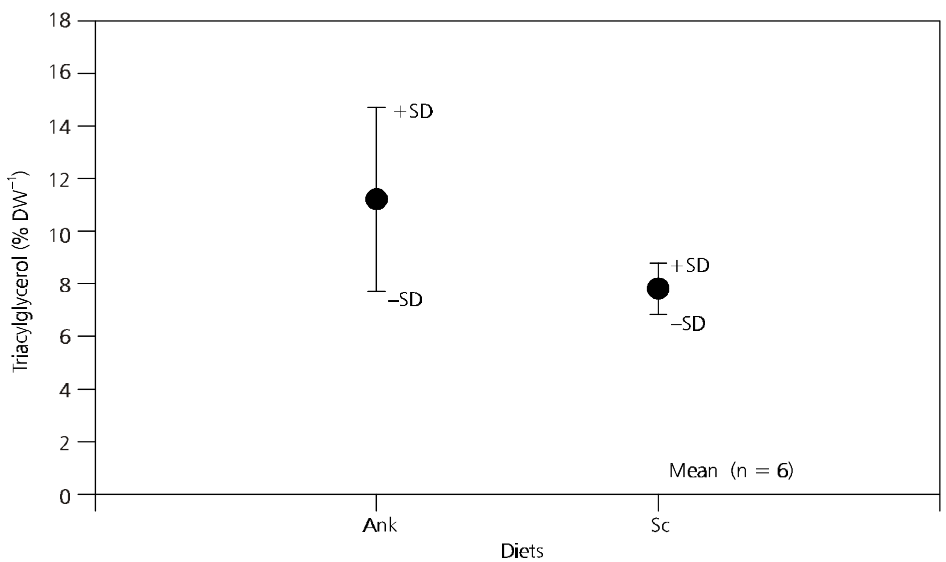

Fig. 4 — Values of triacylglycerol (% DW) for *Moina micrura* fed the *Ankistrodesmus gracilis* (Ank) and *Scenedesmus quadricauda* (Sc) diets. N = total sample number.

DISCUSSION

Lipid levels of freshwater zooplankton can suffer strong spatio-temporal fluctuations. Several patterns of temporal variability of lipids have been reported in the recent literature: long term or seasonal (Vanderploeg *et al*., 1992), and short term or diurnal (Pinto-Coelho *et al*., 1997a and b). These patterns are the result of several simultaneously acting environmental factors such as temperature, food availability, and plankton composition. Other ecophysiological factors such as feeding rhythms, reproductive stage etc., may also play an important role. *Daphnia magna*, for example, has lipid reserves ranging from 8% to 44% of total dry weight (Blazka, 1966). Nevertheless, a trend can be seen: Zooplankton organisms from tropical areas seem to have lower lipid levels (Table 1).

Most authors agree that triacylglycerols are the main lipid reserves used by the majority of freshwater zooplankton (Cavaletto *et al*., 1989). Farkas (1970) observed that 59.1% of the lipids of *D. cucullata* from Balaton Lake in Hungary were triacylglycerols.

However, not only fatty acids (triacylglycerol) but also phospholipids are present in daphnids (Weers & Gulati, 1997). According to Vanderploeg *et al*. (1992), the lipid levels found for late summer populations of calanoid copepods in Lake Michigan (39%-44%) are much higher than the reserves found for most freshwater cladocerans. In these copepods, mostly *Diaptomus sicilis*, the annual contribution of phospholipids to the total lipid content ranged from 61% to 90% (Vanderploeg *et al.*, 1992).

The authors observed that these copepods are able to survive for long periods at low temperatures without any food intake, probably because they maintain low metabolic rates fueled by high phospholipid content. These compounds are used not only for survival during periods of low food abundance but also as energy reserves to be allocated to the eggs.

Higher lipid levels were frequently observed in this animals reared in the laboratory compared to animals from natural waters (Table 1). This probably results from the more stable laboratory conditions which spare animals situations such as seasonal succession of phyto- and zooplankton or the typical instability of most physico-chemical conditions of natural waters. Additionally, this fact also suggests that the animals frequently face periods of food shortage in their natural habitats.

TABLE 1

Comparison of mean total lipid (TL) and triacylglycerol (TG) levels with literature values.

Site	Specie	TL	TG	Author
Lake Michigan	*D. sicilis*	3.90-44.00	13.20-17.60	Vanderploeg *et al*., 1992
Lake Michigan	*D. sicilis*	36.30	10.80	Cavaletto *et al*., 1989
Furnas reservoir	Zooplankton	7.70 -10.8	–	Pinto-Coelho *et al*., 1997a
Pampulha reservoir	Zooplankton	5.80-8.20	–	Pinto-Coelho *et al*., 1997a
Lake Ibirité	Zooplankton	4.20-7.52	–	Santeiro & Pinto-Coelho, 2000
Laboratory	–	–	6.70-11.80	Tessier *et al*., 1983
–	*D. magna*	12.80	3.50-6.60	Elendt, 1989
–	*D. magna*	16.6-22	11.62-15.4	Goulden & Place, 1990
–	*D. pulex*	19.8-30.6	13.86-21.42	Goulden & Place, 1990
–	Zooplankton	5.34-10.19	–	Santeiro & Pinto-Coelho, 2000
–	*Diaphanosoma*	12.20-25.10	–	Pinto-Coelho *et al*., 1997a
–	*D. laevis* (I)	13.27	11.03	This study
–	*D. laevis* (II)	6.63	6.22	This study
–	*M. micrura* (I)	12.00	11.21	This study
–	*M. micrura* (II)	6.86	7.81	This study

I = *Ankistrodesmus*.
II = *Scenedesmus*.

According to Goulden & Horning (1980), in laboratory cultures of *Daphnia*, animals use energy reserves immediately to maintain metabolic activities, as is also the case for marine copepods, but *Daphnia* also continues to reproduce and to transfer part of its energy reserves to offspring.

In the present study, *Scenedesmus quadricauda* was found to be a poor food for both cladocerans. The nutritional quality of a given algae can be associated with morphological as well as biochemical properties. The morphology of the *Scenedesmus* colony, with the presence of horns in the apical cells of the cenobia, may be associated with the lower assimilation of this chlorophyceae by cladocerans. Brett & Müller-Navarra (1997) verified that the variation in the food quality of phytoplankton was related to assimilation efficiency. They proposed a hierarchy in the alimentary quality of phytoplankton, where the green algae are considered of high quality in some cases and of low quality in others. They compared the cryptophyceae *Chlamydomonas*, that is easily digested and considered to be a food of higher quality, with *S. quadricauda*, that is difficult to digest and considered of low quality.

In the present study, the effect of food quality was the major source of significant shifts in the nutritional status of two cladoceran species. There was a significant difference between the two diets and both *Daphnia* and *Moina* presented lower values of total lipid and triacylglycerol when fed *S. quadricauda*. In contrast, both *D. laevis* and *M. micrura* accumulated moderate to high lipid levels when the algae *A. gracilis* was offered. The study also found that lipids are good predictors of nutritional status in cladocerans. Additionally, it was demonstrated that the bulk of lipid reserves of these cladocerans are triacylglycerols. Food caused the strongest and most significant differences in lipid accumulation. *Moina* individuals are smaller than *Daphnia* and have a faster growth rate (Macedo & Pinto-Coelho, 2000). However, there was no significant difference in lipid level between these cladocerans when they were fed the same algal food.

Both tested diets produced higher lipid reserves than most of those found in the zooplankton community of tropical reservoirs. This fact indicates that tropical zooplankton faces severe food limitation, even in eutrophic waters such as the Pampulha Reservoir (Pinto-Coelho *et al.*, 1997a).

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