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Diel Variations in Respiration, Excretion Rates, and Nutritional Status of Zooplankton From the Pampulha Reservoir, Belo Horizonte, MG

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ABSTRACT This investigation is focused on the experimental determination of diel cycles of metabolic activity of zooplankton in a tropical reservoir. Water and zooplankton used in laboratory experiments were collected from the Pampulha reservoir. The experimental units were incubated in the light (1500 Lux) and in the dark at 25.0 ± 1.0°C during different periods of the diel cycle. At the end of each experiment, the following variables were measured: temperature, dissolved oxygen, ammonia, and orthophosphate as well as the composition, abundance and dry weight of the zooplankton. The specific respiration and excretion rates were determined considering the differences in concentration between experimental and control units. The effect of diurnal cycle on respiration rates was clearly more intense than the effect of light. The average values of respiration rates obtained in the morning hours oscillated between 0.015 and 0.016 $mgO_2mgDW \cdot hr^{-1}$ (light and dark incubations). At night, these rates were higher and ranged from 0.020 to 0.035 $mgO_2mgDW \cdot hr^{-1}$. Increased biomass of zooplankton and longer incubation times produced lower respiration rates. The excretion rates of ammonia were higher at night, reaching a mean value of 4.2 $\mu gN\text{-}NH_4/mg\ DW \cdot hr^{-1}$ in illuminated units. The phosphate excretion rates were more elevated in the morning, reaching 0.58 $\mu gP\text{-}PO_4/mg/DW \cdot hr^{-1}$ illuminated vessels. The nanoplankton was able to actively absorb ammonia as well as phosphate. The highest ammonia absorption rates were measured at night, whereas the nanoplankton absorbed phosphorus only in the morning hours. The nutritional status of zooplankton also showed short-term variations. The mean phosphorus content of zooplankton biomass also varied between day and night as well as with incubation time. It ranged from 0.58–2.17%, whereas organic matter variation was more conservative, oscillating around 70–92% in all occasions. *J. Exp. Zool. 286:671–682, 2000.* © 2000 Wiley-Liss, Inc.

Among the several physiologic processes involving the metabolism of zooplankton, respiration has been studied intensely as a physiologic acclimatization of organisms to environmental changes (Mayzaud, '73; Layborn and Finley, '76; Banse '82; Fenchel and Finlay, '83; Andrew et al., '89; Pagano and Saint Jean, '94). In some communities dominated by small organisms and high metabolic rates, like zooplankton, respiration rates have also been used to estimate the magnitude of biological production (Pourriot, '82). A detailed knowledge of the alterations in the assimilation or respiration of zooplankton could also be applied to evaluate the feeding rhythms of zooplankton (Duval and Geen, '75, '76).

In aquatic ecosystems, a complex dynamics of nutrient flow exists between the biotic and abiotic pools as well as inside these compartments. The zooplankton community can modify the environmental conditions for the algae populations through the regeneration of nutrients (Urabe, '93) and the released nutrients are quickly available to the phytoplankton (Lehman, '84). Thus, the rates of excretion of zooplankton organisms can greatly affect the availability of elements of crucial importance for the producers and, thus, for the metabolism of the entire aquatic system.

Among the essential nutrients for the development of the aquatic community, nitrogen and phos-

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phorus are considered to be the most important (Wetzel, '75). The concentration of these nutrients can be influenced by such internal processes as the excretion of the zooplankton and the absorption and liberation of nutrients for the algae, bacteria, and macrophytes. Several studies (Hargrave and Geen, '68; Ganf and Blazka, '74; LaRow et al, '75; Weisse and Rudstam, '89; Pagano and Saint-Jean, '94; Pinto-Coelho et al., '97) have demonstrated the ecological importance of excretion of the zooplankton, which liberates nutrients essential to the growth of algae and bacteria in the aquatic environments.

Most of the studies focusing on the importance of the zooplankton as a nutrient-regenerating mechanism have been conducted on marine continental waters (Conover and Corner, '68; Hargrave and Geen, '68). Few investigations about the excretory activity of zooplankton have been conducted on tropical lakes, where the temperature frequently exceeds 25°C (Pagano and Saint-Jean, '94). According to Ganf and Blazka ('74), in tropical aquatic environments, diel changes are of greater import than seasonal ones. Therefore, it is important to investigate the daily physiologic activities of animals in these ecosystems.

The objective of the present study was to determine whether respiration and excretion rates can be used to identify the possible existence of diel variations in the metabolism of zooplankton in a eutrophic tropical reservoir. The effects of light intensity as well as the influence of time of day on the previously mentioned rates were investigated. In addition, short-term variations in zooplankton body phosphorus, organic matter, and ash were also studied.

MATERIALS AND METHODS

The experiments were conducted in the laboratory using animals from Pampulha Reservoir, located in Belo Horizonte city (19°51′18.1′S, 43° 58′46.3′W), Brazil. This is a shallow and eutrophic reservoir with about 2.4 km^2 of flooded area, a volume of about 12 million m^3, and with a mean depth of 5 m. The general characteristics of the reservoir have been described elsewhere (Pinto-Coelho, '92; Giani, '94).

Water samples and organisms were collected from a central point near the Iate Tênis Clube (Yacht Tennis Club) (depth = 5.0 m). Previous studies (Giani et al., '88) demonstrated that this point satisfactorily represents the limnological characteristics of the whole lake.

Experiments

Four experiments were performed (Table 1). Water and zooplankton were collected in the early morning (9:00 hr) or in the late afternoon (16:00 hr). The water was collected with a Kemmerer bottle at 1.0 m of depth and immediately transferred to clean and dry plastic carboys of 5 liters.

The mesozooplankton was collected with a 90 μm mesh net in a vertical drag covering the water column, between 0 and 4 meters. This mesh guaranteed the inclusion of most of the organisms of the mesozooplankton, i.e., those with a linear dimension of more than 200 μm (Sieburth, '78). In the Pampulha reservoir, this fraction is composed basically of Copepoda (excluding the nauplii), Cladocera, and Rotifera, especially *Brachionus calyciflorus*. The use of a 90-μm mesh net for mesozooplankton is explained by the fact that the width of many of these organisms is less than the 200-μm limit for the individuals' total length. The organisms collected with the net were carefully transferred to an opaque wide-mouthed thermos flask for transportation to the laboratory (30 min).

The following determinations were performed in each sampling: water transparency using a Secchi disk, temperature, and conductivity profiles (using a YSI 30M T-C device) and dissolved oxygen (YSI 55 dissolved oxygen digital meter).

In the laboratory, the lake water was sequentially filtered through a 50-μm inox gauze for exclusion of rotifers, protozoa, and nauplii, i.e., the microzooplankton (Sieburth, '78) and through a 20-μm filter for removal of excess algae, the net phytoplankton. The filtered water was placed in a 16-liter aquarium and a mixture of antibiotics was added (0.12 g/liter penicillin G-potassium and 0.24 g/liter streptomycin) to eliminate bacteria. The water was oxygenated for approximately 40

TABLE 1. Summary of the experiments conducted to determine respiration and excretion rates of zooplankton, with date, schedule, tested hypothesis and duration of each experiment

Experiment	Date	Hypothesis	Begin	End
1	May 17, 1996	Light effect (morning)	11:00 hr	15:17 hr
2	May 24, 1996	Light effect (night)	18:22 hr	23:50 hr
3	May 31, 1996	Incubation time effect	11:00 hr	17:50 hr
4	June 25, 1996	Dry weight effect	12:00 hr	17:00 hr

min. After filtration through a 160-μm gauze, the zooplankton contained in the thermal bottle were transferred to a beaker containing the water from the aquarium.

Transparent wide-mouthed 0.5-liter borosilicate vessels were used as experimental units. Each bottle was filled with filtered and oxygenated lake water. The zooplankton was transferred with a 10-ml nonselective Hensen pipette that permitted us to control the amount of dry weight transferred to each experimental unit. The control units were filled only with aquarium water. During the process of vessel filling with filtered water, care was taken to prevent air bubbling.

The experimental units were incubated in a germination cabinet for 2, 4, or 6 hr, depending on the experiment. The temperature was maintained at 25 ± 1.0°C. Some experimental units were fully covered with aluminum foil to prevent light penetration and incubated in complete darkness. At least four replicates per each treatment were considered.

At the end of each experiment, the following parameters were determined: water temperature, dissolved oxygen, ammonia, orthophosphate, and chlorophyll-a, as well as zooplankton composition, abundance, and dry weight. Oxygen and temperature were determined with a high-precision Jenway 3440 electrochemical-temperature analyzer previously calibrated by the iodometric method of Winkler. The ammonia and orthophosphate were determined using conventional spectrophotometric methods (Mackeneth et al., '78).

Water samples from the Pampulha Reservoir with and without antibiotics added were used for heterotrophic bacterial counts and to determine the presence of bacteria. The counts were performed in triplicate on Petri dishes containing NWRI agar medium in pour plate method (Greenberg et al., '92). Serial dilutions of the water without antibiotics were performed to permit the counts. The plates were incubated for 48 hr/24°C, conditions necessary for colony growth.

The dry weight of each experimental unit was gravimetrically determined after lyophilization. Zooplankton from each experimental unit was filtered through a 160-μm mesh inox gauze and transferred to an acrylic Petri dish measuring 5 cm in diameter. The dish was immediately transferred to a plastic box with silica gel and stored in a freezer at –20°C. After 24 hr, the organisms were freeze-dried (Edwards L5KR) for 24 hr. The zooplankton was weighed on a Mettler AE200 balance. This procedure was repeated 24 hr later to confirm dehydration.

The specific respiration rate (SRR) was determined using the "closed bottle" method (Lampert, '84). In this method, the consumption of oxygen is estimated by means of variations in the concentration of oxygen at the beginning and at the end of the incubation. Since the incubation time was the same for control and experimental units, the biomass specific respiration rate was estimated in the following way:

$$SRR\ [mgO_2 \cdot mgDW \cdot h^{-1}] = \frac{C_u - E_u}{DW * t_{inc}} \quad (1)$$

where Eu and Cu were experimental and control units, DW was the dry weight of zooplankton trapped in a given unity and t_{inc} was the incubation time (in decimal units).

The excretion rates of ammonia (AE) and phosphorus (PE) were determined considering the difference of the concentrations between experimental units with zooplankton (Eu) and control units, without zooplankton (Cu), divided by the dry weight of zooplankton present in each experimental unit multiplied by the duration of the time of incubation in hour decimals; see equations 2 and 3, respectively:

$$AE[\mu gN - NH_4 mgDW^{-1} \cdot h^{-1}] = \frac{NH_4E_u - NH_4C_u}{DW \cdot t_{inc}} \quad (2)$$

$$PE[\mu gP - POmgDW^{-1} \cdot h^{-1}] = \frac{P\text{–}PO_4E_u\text{–}P\text{–}PO_4C_u}{DW * t_{inc}} \quad (3)$$

The absorption rates of ammonia (AAR) and of orthophosphate (PAR) for the nanoplankton were determined considering the difference of the concentrations between control units at the beginning (b) of incubation and control units after (a) incubation, divided by the chlorophyll concentration (*chl–a*) present in each unit multiplied by the duration of the time of incubation in decimal hours, respectively; see equations 4 and 5:

$$\mathrm{AAR}[\mu gN - NH_4 \cdot \mu gchl - a^{-1} \cdot h^{-1}] = \frac{N - NH_4b - N\text{–}NH_4a}{chl \cdot a * t_{inc}} \quad (4)$$

$$PAR\,[\mu gP - PO_4 \cdot \mu g\ chla^{-1} \cdot h^{-1}] = \frac{P - PO_4b - P\text{–}PO_4a}{chl - a * t_{inc}} \quad (5)$$

Additional zooplankton samples were also taken from the reservoir, fixed with formalin, and

stained with Rose Bengal for further identification and counting.

Organic Matter and Ash

The content of mineral elements was obtained gravimetrically by the determination of ash and organic matter. During the incineration process, the whole organic matter was burned at 480°C, for 2 hr. To calculate the percentage of organic matter, a certain aliquot of dry weight was used. Small aluminum vessels were used (d = 6; h = 11 mm) and porcelain containers were precombusted in a furnace Zezimaq. About 4 mg dry weight of freeze-dried zooplankton was placed in these vessels and the mass was determined. The containers were then combusted for 2 additional hr. The total mass of organic matter and ash of the sample was calculated as follows (equations 6 and 7):

$$o.m.(\%) = \frac{[dw - cw] * 100}{dw} \quad (6)$$

$$ash(\%) = 100 - o.m.(\%) \quad (7)$$

where: dw = dry weight of sample, and cw = weight of the combusted aliquot (ash)

Determination of phosphorus content

A 3-mg aliquot of dried zooplankton was placed in 50-ml glass tubes; 0.1 ml of distilled water was added to each tube and the zooplankton sample was mechanically ground. The homogenized material was transferred to 250-ml Erlenmeyer flasks for analysis of phosphorus concentration and submitted to chemical digestion with potassium persulfate (10 ml). The phosphorus content in the extract was colorimetrically measured according to the technique of Murphy and Riley ('62). Body phosphorus was calculated according to the following formula (equation 8):

$$P - content(\%) = \frac{\mu gP - PO_4}{\mu gDW} * 100 \quad \cdot (8)$$

RESULTS

Limnological conditions of the lake

Four experiments were performed on the following dates: May 17, 24, and 31, 1996, and June 25, 1996 (Table 1). The temperature, dissolved oxygen, and electric conductivity were quite similar for the different sampling days (Fig. 1). Temperature ranged from 20–22°C in all experiments. Dissolved oxygen was very low, being always below 3.0 mg $O_2 \cdot liter^{-1}$ at all depths. Conductivity ranged from 270–281 $\mu S \cdot cm^{-1}$.

The composition of zooplankton was practically the same in all experiments (Fig. 2). *Daphnia laevis* (Birge, 1878) was by far the dominant organism when dry weight was considered (Fig. 2, top). The zooplankton was numerically dominated by rotifers, mainly *Brachionus calyciflorus* (Pallas, 1766), followed by cladocerans *Daphnia laevis* (Birge, 1878) (Fig. 2, bottom). Other organisms such as *Thermocyclops decipiens* (Kiefer, 1929) and *Diaphanosoma birgei* (Korineck, 1981) were also observed but in low numbers.

The phytoplankton community was dominated by green algae such as *Chlorella vulgaris* (Beijerinck, 1890) and by some flagellates such as *Trachelomonas volvocina* (Fritsch, 1918). The order Chlorococcales showed a larger number of organisms belonging to the three following families: Oocystaceae, Dictyosphaeriaceae and Radiococcaceae. The other groups were Cryptophyta and Euglenales, with one species each (data not represented).

Experiments

The zooplankton was incubated in opaque and transparent flasks in order to determine whether the illumination conditions affect its metabolic rates. Experiment 1 was done in the morning (09:00–11:00 hr). The dry weight of the zooplankton in the different experimental units ranged from 20–30 mg $DW \cdot hr^{-1}$. The *t*-test revealed that there was no significant difference in dry weight between treatments ($P < 0.008$). The specific respiration rates ranged from 0.015–0.043 mg O_2 mg $DW \cdot hr^{-1}$ (Fig. 3, bottom) in all treatments.

The average values of ammonia excretion ranged from 1.15–2.35 μg $N\text{-}NH_4$/mg $DW \cdot hr^{-1}$ in the dark and light incubations conducted during the morning. Lower values were found for the phosphorus excretion but PE rates were also largely affected by different illumination conditions. In incubations conducted using transparent vessels under constant illumination the P-excretion rate wasμ 0.58 g $P\text{-}PO_4$ mg $DW \cdot hr^{-1}$. In the dark, the average PE decreased to 0.25 μg $P\text{-}PO_4$. mg $DW \cdot hr^{-1}$ (Fig. 3, middle).

Experiment 2 was performed at the beginning of the night. The dry weight of the zooplankton in the different experimental units oscillated between 10 and 20 mg $DW \cdot hr^{-1}$, with no significant difference among them ($P < 0.009$). The

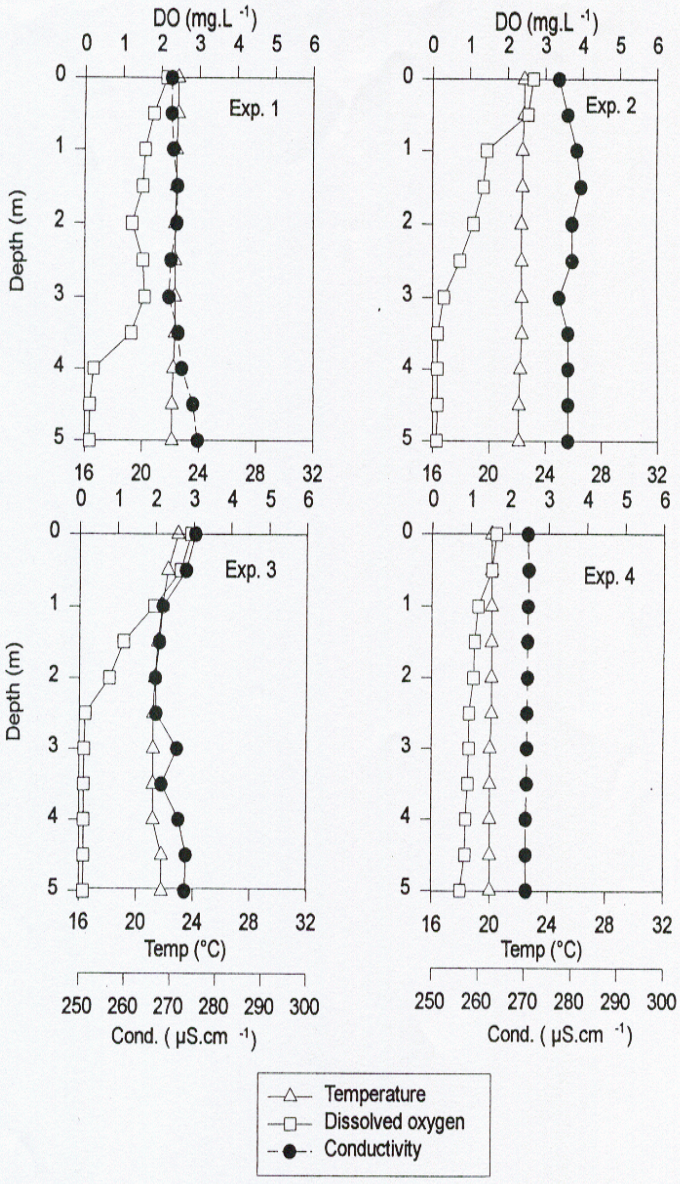

Fig. 1. Water temperature (°C), dissolved oxygen (mg O_2 · liter^{-1}) and electric conductivity (µS · cm^{-1}) in the Pampulha reservoir. Experiment 1: May 17, 1996; Experiment 2: May 24, 1996; Experiment 3: May 31, 1996, Experiment 4: June 25, 1996.

respiration rates were much higher, since average SRR values ranged from 0.027 to 0.043 mg O_2 mg DW · hr^{-1} in the illuminated and transparent vessels. In the dark incubations, the respiration rates decreased sharply oscillating between 0.014 and 0.028 mg O_2 mg DW · hr^{-1} (Fig. 3).

The mean value of AE was 4.20 µg N-NH_4/mg DW · hr^{-1} in incubations conducted at night, under constant illumination. This value dropped to values close to zero in the darkness (Fig. 3, top). The values of phosphate excretion were about 0.20 µg P-PO_4/mg DW · hr^{-1} in the transparent illuminated vessels. The PE mean value increased to 0.49 µg P-PO_4/mg DW · hr^{-1} in the dark incubations (Fig. 3, middle).

Significant differences in respiration rates were found between the incubations performed during the morning hours and those performed during

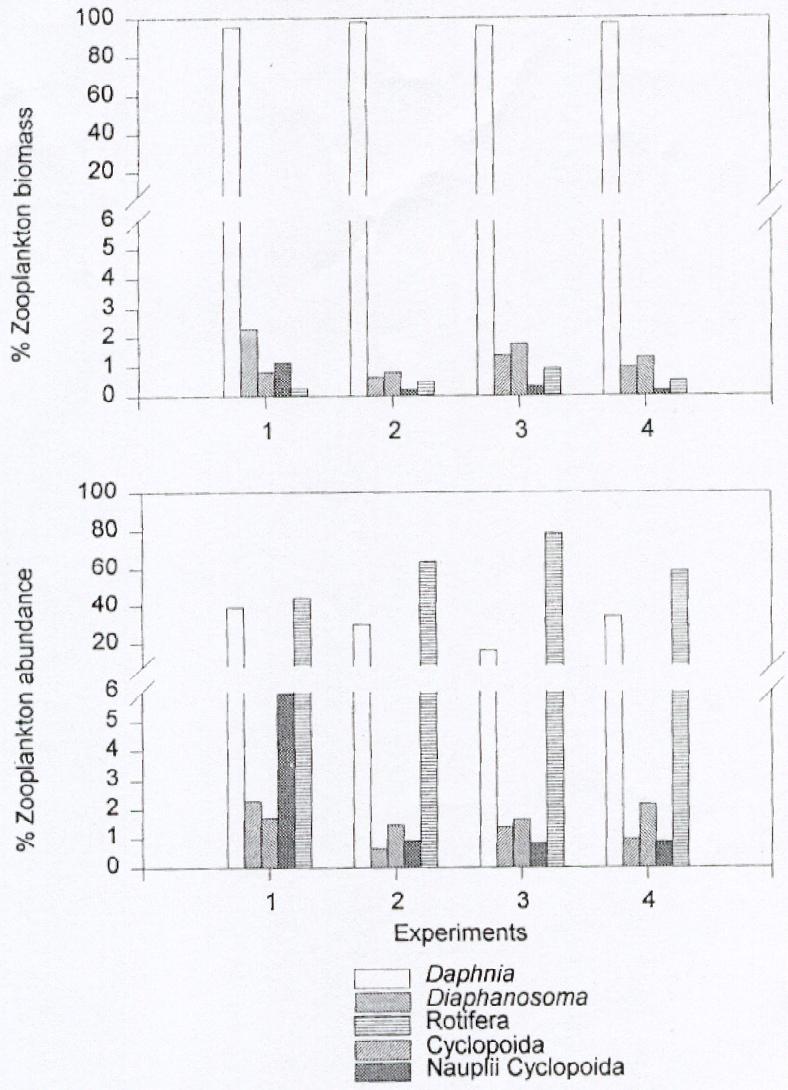

Fig. 2. Zooplankton dry weight and abundance in the Pampulha reservoir in four experiments. Experiment 1: May 17, 1996; Experiment 2: May 24, 1996; Experiment 3: May 31, 1996; Experiment 4: June 25, 1996.

the night hours for both treatments: illuminated vessels, $F = 62.376$, $P < 0.001$, and dark vessels, $F = 59.875$, $P < 0.002$.

Phosphate excretion rates were highest in the morning (light incubations). Ammonia excretion was highest in the light incubations performed at night. There was a significant difference in P-excretion between the different conditions both during the morning (ANOVA, $F = 62.874$, $P < 0.001$) and during the night (ANOVA, $F = 51.925$, $P < 0.002$).

Experiment 3 was done to determine the influence of time of incubation on specific respiration rates (SRR). Time of incubation presented a significant inverse linear correlation with SRR ($r = 0.544$, $P < 0.006$, $N = 11$), indicating that respiration tends to decrease with increasing time of incubation (Fig. 4).

Experiment 4 was performed to determine whether the respiration rates varied according to zooplankton biomass. Linear regression between

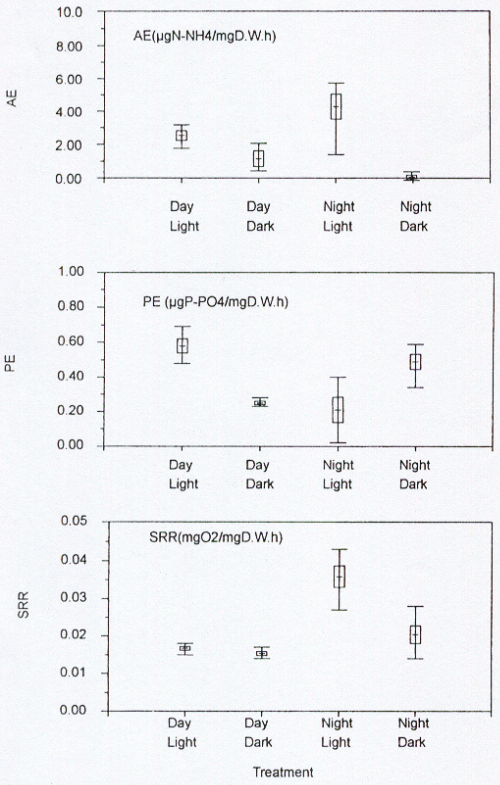

Fig. 3. Variation in respiration and excretion rates in different times of the day submitted to the light and dark treatments. The mean and standard error and range are given for each experiment (n = 5).

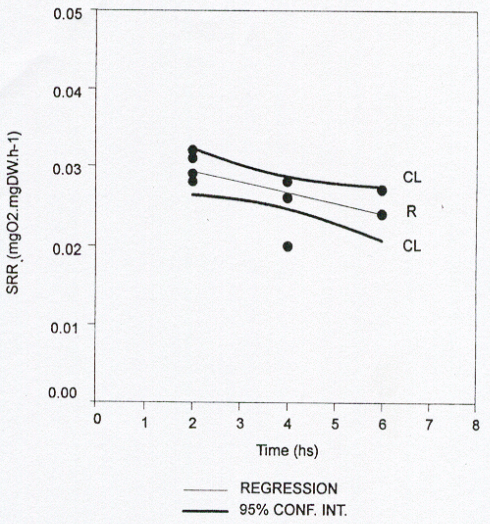

Fig. 4. Linear regression model relating specific respiration rates (SRR) of the zooplankton community of Pampulha Reservoir to incubation time. Upper and lower bold lines represent 95% confidence intervals.

respiration rates and dry weight showed a highly significant inverse correlation ($r = 0.828$, $P < 0.001$, $N = 12$) (Fig. 5). The relationship between dry weight and SRR can be described by the model SRR = 0.009 – 0.808 * dry weight ($r = 0.828$, $N = 12$). Therefore, it is evident that increases in the dry weight levels in the experimental units cause a decrease in SRR.

Since the experimental determination of N and P excretion rates could be potentially affected by the absorption of nutrients by the nanoplankton, short-term experiments were run in order to determine the magnitude of those rates. Phytoplankton absorbed ammonia in higher rates. The maximum value was 35.79 µg $N\text{-}NH_4$/µg chlorophyll-a · hr^{-1}, observed in the illuminated vessels

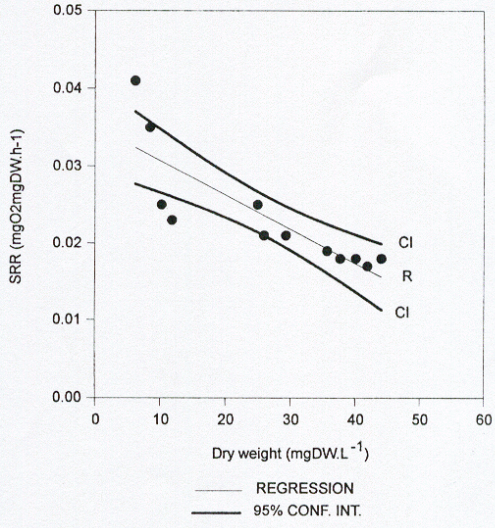

Fig. 5. Linear regression model relating specific respiration rates (SRR) of the zooplankton community of Pampulha Reservoir to dry weight. Upper and lower bold lines represent 95% confidence intervals.

incubated during the early night hours (Table 2). On the other hand, the nanoalgae absorbed phosphorus in higher rates during the morning. A maximum value of 0.39 μg $P\text{-}PO_4$/μg chlorophyll-$a \cdot hr^{-1}$ was determined in illuminated vessels. This means that experimental determination of ammonia excretion rates was more affected by uptake rates of nannoplankton in the night. Alternatively, the estimates of P-excretion of zooplankton were more affected by algal uptake rates during the day.

The bacterial counts demonstrated the efficiency of antibiotic addition in the experimental units (Table 3). This treatment caused a drastic reduction in the numbers of heterotrophic bacteria.

Organic matter and ash

The percentage of organic matter in zooplankton ranged from 72–92%, with an average of 80.1 ± 3.5% of dry weight. By comparing experiments 1 and 2 (Fig. 6, top and middle panels), it can be seen that the highest values of organic matter were found at night in the dark treatment (92%). For the morning, they were practically identical in the two treatments (81–84%). The content of organic matter measured at night (92%) was significantly different from values obtained in the morning incubations ($P < 0.03$).

Longer incubation times also caused a reduction in the content of organic matter and a increase in ash content (Fig. 6, bottom). Organic matter was the highest in the shortest incubations, with a mean value of 85.9% passing to 85.4% and 74.7%, in 4 hr and 6 hr, respectively. The difference in organic matter between the incubations of 2 hr and 6 hr as well as between incubations of 4 hr and 6 hr were significantly different ($P < 0.015$). Conversely, the amount of ash increased with incubation time, passing from 14.1% to 25.4%.

Determination of phosphorus content

The P-content of zooplankton was also affected on a daily basis. The amount of phosphorus in the biomass of zooplankton ranged between 1.03% and 2.17%, with mean values varying from 1.39% to 1.71% (Fig. 7, top). Values were always higher in vessels incubated under illuminated conditions. In the dark vessels, the P-content was always below 1.50 %. These differences, however, were not statistically significant. The P-contents showed,

TABLE 2. Ammonia uptake rates (μg $N\text{-}NH_4 \cdot$ μg chlorophyll-a $\cdot hr^{-1}$) and phosphate uptake rates (μg $P\text{-}PO_4 \cdot$ μg chlorophyll-a $\cdot h^{-1}$) by nanoplankton (<20 μm)

	Morning		*Night*	
	Light	Dark	Light	Dark
AAR	8.09	1.03	35.79	0
PAR	0.39	0.03	0	0

TABLE 3. Number of colonies of heterotrophic bacteria in vessles without antibiotics and in vessels with antibiotics

	Antibodies	Concentration (g/liter)	Dilution sample	No. of colonies
Antibiotics	Penicillin G-K	0.12	0	3
	Streptomycin	0.24		
Control	Without antibiotics	0.00	10	3000

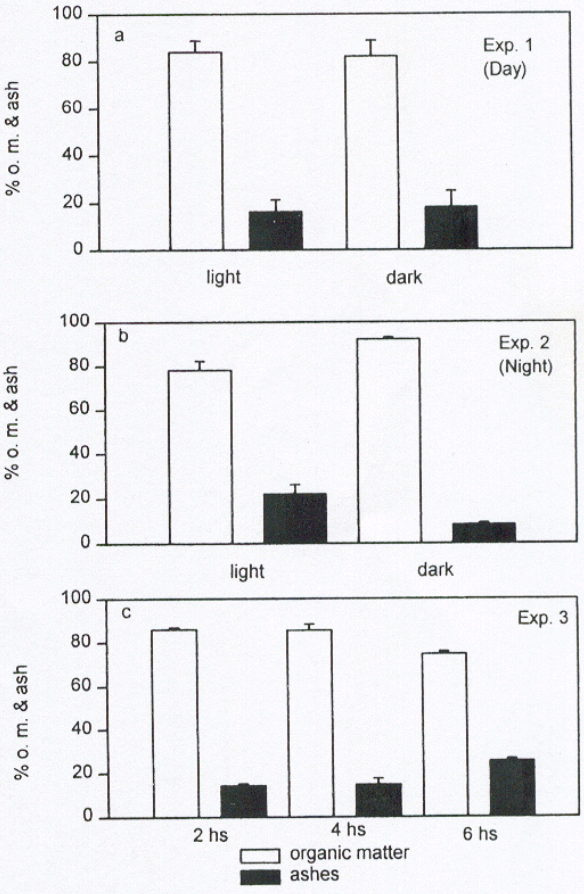

Fig. 6. Variations in the relative content of organic matter in the zooplankton in different situations. (**a**) Experiment 1 (in the morning, May 17, 1996); (**b**) Experiment 2 (at night, May 24, 1996), and (**c**) Experiment 3 (incubations, May 31, 1996).

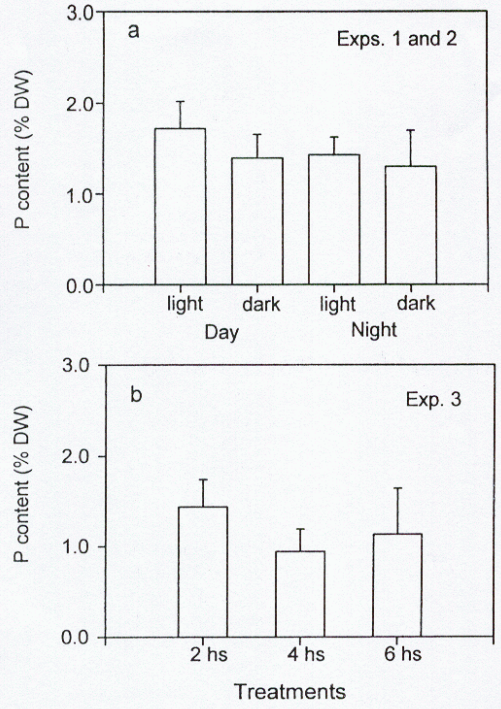

Fig. 7. Variations in the P-content of zooplankton in different situations. (**a**) Experiment 1 (in the morning, May 17, 1996), and experiment 2 (at night, May 24, 1996); (**b**) experiment 3 (incubations, May 31, 1996).

however, larger differences in response to different incubation times. They also decreased with longer incubation time, passing from 1.44% (2 hr) to 0.94 % (4 hr). This difference was significant ($P < 0.053$) possibly suggesting that nutritional conditions of zooplankton trapped in the experimental vessels rapidly decreased (Fig. 7, bottom).

DISCUSSION

Several factors influence the excretion rates and respiration of zooplankton, among them the nutritional status of the animal. Some studies have demonstrated relationships between feeding, excretion, and respiration (Ganf and Blaska, '74; Lampert, '84). The excretion rates are also correlated with environmental characteristics such as temperature (Hargrave and Geen, '68; LaRow et al., '75; Laybourn and Finlay, '76).

The metabolism of the animals is strongly affected by temperature. Temperature increases of about 10°C usually cause a doubling of the metabolic rate of the animal in general (Lampert, '84). This increase is described by the metabolic coefficient Q_{10} (Pough et al., '93). A Q_{10} value of 1.8, for example, means that an increase of 10°C causes an elevation of metabolic rate by a factor of 1.8. The incubation temperatures of this study were high (25–26°C) if compared to those used in studies on zooplankton from temperate regions (Mayzaud, '73; Weisse and Rudstam, '89). Therefore, the metabolic rates obtained in tropical areas clearly differ from those reported for temperate sites.

The elevated respiration rates typical of animals living in the tropics impose a higher demand in terms of energy resources to be allocated to the maintenance of basal metabolism. If we consider a respiration rate of just 0.02 mg O_2 mg DW · hr^{-1}, what does this mean in terms of energy demand? Respiration rates in this order of magnitude imply an energy allocation into the basic metabolism of about 38% of animal weight in a day in terms of carbon biomass (Macedo and Pinto-Coelho, '97). In other words, the animal has to consume an equivalent to its own weight (in C-content) every 2.6 days.

Pagano and Saint-Jean ('94), working with *Acartia clausi* in coastal environments in tropical Africa, found a still higher daily metabolic rate involving a carbon consumption corresponding to 36–87% of the animal's weight. This was probably due to the fact that the animals were submitted to temperatures between 26 and 32°C. Thus, the tropical zooplankton is conditioned to maintain higher metabolic rates, with a consequent greater energy consumption.

In this study, we observed higher respiration rates at night and in the illuminated vessels. Duval and Geen ('75, '76) also found higher respiration rates in the evening. Andrew et al. ('89) suggested that a great part of the increase in respiration activity at night can be due to the increase of the swimming activity of the animals, which seems to be more intense at the beginning of the night. Another reason that could explain this increase would be the enhancement of the filtration rates at night. The respiration rates can also vary due to exogenous factors, such as the composition of the zooplankton (Pourriot, '82). The possible effects of differences in the composition of the zooplankton were minimized in the present study since there was a clear predominance of *Daphnia laevis* in the total dry weight for all four experiments.

The excretion of nutrients also depends on several factors that affect the metabolism of the animal (Barlow and Bishop, '65). However, information on the variables that influence the rate of nutrient release in zooplankton is still limited (Urabe, '93). Animal size and nutritional status may be possible factors.

Peters and Lean ('73) suggested that the form in which phosphorus and nitrogen are liberated from zooplankton is as important as the liberation rates, because both determine the ecological role of the excretion. Data reported by Rigler ('61) demonstrated that all the phosphorus excreted by *Daphnia magna* was in the orthophosphate form. Most of the nitrogen liberated by zooplankton is immediately available to the phytoplankton, mainly as ammonia (Lehman, '85).

Ammonia excretion rates increased conspicuously during the night hours, mainly in the light incubations. The same kind of temporal pattern was found for respiration rates since SRR values were also higher at the beginning of the night. According to Mayzaud ('73), a relationship exists between respiration and nitrogen excretion and the O/N ratio gives estimates of the general metabolism, reflecting the level of activity of the oxidative metabolism and of proteins in the studied animal. In contrast, there was a reduction of excreted phosphorus at night in the light incubations. Thus, the highest excreted amounts of phosphorus were liberated in the morning in the light treatment and at night in the dark treatment.

Excretion rates are also affected by several factors associated with the experimental conditions. The composition as well as the physiological condition of zooplankton inside the experimental units belong to this category. In the present study, the strong dominance of *D. laevis* and the use of a nonselective pipette provided high homogeneity in the composition of zooplankton for the experimental units. In addition, the short incubation used in most experiments (2 hr) assured that most animals were active and in good physiological condition, both factors preventing substantial changes in nutritional status of the animals trapped inside experimental vessels.

In most of the pelagic ecosystems, the nitrogen and phosphorus supply is an important variable that limits the growth rate of the algae. The importance of zooplankton under these nutrient-limiting conditions is stressed considering that the excreted products are soluble and quickly available to primary producers (Urabe, '93). According to Ejsmont-Karabin ('90), the composition of phytoplankton species is determined by the rate of regeneration of nutrients by the zooplankton.

In the present study, there was a predominance of green algae, and the rate of nitrogen and phosphorus absorption by the nanoplankton could be determined considering the nutrient levels in the control units (without zooplankton) at the beginning and at the end of the incubation time, on all occasions. The phytoplankton were able to absorb higher levels of ammonia. The highest rates of ammonia absorption occurred at night when ammonia excretion by the zooplankton was also at a maximum. The highest rates of phosphorus absorption occurred in the morning in the light treatment, when the zooplankton also presented high rates of phosphorus excretion.

Algae and bacteria are potentially able to alter the oxygenation levels in the water and can actively absorb nitrogen and phosphate, a fact that may interfere with the determination of the respiration and excretion rates of zooplankton. Thus, both the control and the zooplankton units should have low and comparable levels of these organisms. Prefiltering (20 μm) guaranteed that only nanoplanktonic algae were maintained inside the experimental units. Furthermore, the addition of an antibiotic mixture was highly efficient in drastically reducing bacterial activity in all the experimental units, as shown by the bacterial colony counts.

According to Mayzaud ('73), the addition of antibiotics at levels of about 100 mg/liter^{-1} does not cause significant alterations in the basal metabolism of zooplankton. The decrease or even depression of microbial activity is very important in this type of experiment, because the zooplankton can alter the bacterial levels during incubation through the feces. These differences between control and experimental units represent a serious methodological interference.

The coincident patterns of diel variations in the nutrient uptake rates of phytoplankton and in the excretion rates of zooplankton may indicate the existence of fine mutual regulatory mechanisms linking the metabolism of producers and consumers inside the plankton community. Considering the simultaneous peaks in the excretion and uptake rates on a daily basis, the real excretion rates should be even higher that those measured in this study. However, completely eliminating the phytoplankton from experimental vessels might cause an even greater bias in the excretion rates, since this study also demonstrated that the nutritional status of zooplankton was rapidly affected by food shortage.

The ratio of ash/organic matter can be a good indicator of the nutritional status of the organisms. Variations in the amount of organic matter/ash are expected in response to modifications in their nutritional status. Thus, a high content of ash can be a result of a long period of not feeding or other stressful conditions. In the present study, the amount of ash increased with incubation time, a fact possibly caused by food shortage after a period of incubation of 6 hr.

The percentages of body phosphorus found in this study were similar to the values found by Andersen and Hessen ('91), which ranged from 1.3–1.7% DW. According to these investigators, there may be a relation between high P content in the dry weight of zooplankton and the dominance of *Daphnia* spp. The high P content of zooplankton found in the present study seems to confirm this relation since these cladocera predominated in the composition of zooplankton in dry weight terms. In addition, the P-content also showed short term variations confirming that zooplankton suffer from food shortage in longer incubations.

CONCLUSION

The present investigation demonstrated that the diel variation in the metabolic activity of zooplankton can be studied using the respiration and excretion rates. The experiments conducted at different times of day and under different illumination conditions demonstrated unequivocally the existence of diel variation with respect to the respiration and excretion rates of zooplankton. It was also demonstrated that these variations are linked to the "biological clock" of the animals rather than being simple responses to stimuli caused by variations in light intensity.

Furthermore, this study also provided evidence that the nutritional status of zooplankton is linked to the metabolism of excretion and respiration, as it can be assessed using the P-content as well as the ash/organic matter content.

This study also showed that some experimental conditions, such as incubation time and biomass levels, can alter significantly the magnitude of the excretion and respiration rates.

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