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INFLUENCE OF DURATION OF INCUBATION ON ZOOPLANKTON RESPIRATION AND EXCRETION RESULTS

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Abstract: Respiration (O), ammonium (NH_4), phosphate (PO_4), total nitrogen (N_T) and phosphorus (P_T) excretions were measured on mixed zooplankton during 3-, 6-, 9-, 12-, 21-, and 24-h incubation periods at 20–23°C. The excretion rates of PO_4, N_T, and P_T decrease during a 21-h period, while rates of respiration and excretion of NH_4 are constant. The percentage of inorganic nitrogen excreted increases regularly from 3 h (30–40% of total nitrogen) to 21 h (70–80%) and it could be either due to a bacterial activity which was measured or to a decrease with time of organic nitrogen excreted because of starvation. O/N_T, O/PO_4, O/P_T, and NH_4/PO_4 ratios increase during the first 9 h of incubation; the percentage of inorganic phosphorus excreted is higher at the very beginning and then remains constant from 6 to 24 h. O/NH_4 and N_T/P_T ratios are constant during a 24-h term, which makes them useful metabolic indexes.

INTRODUCTION

Zooplankton respiration and excretion measurements are interesting for two reasons. First, the rôle of planktonic animals in nutrient regeneration may be quantified; secondly, simultaneous data for respiration and excretion rates – expressed as $\mu l\ O_2$, μg-at. N or P $\cdot$mg dry wt^{-1} $\cdot$day^{-1} – and C/N/P excretion and respiration ratios, allows the calculation of net production of zooplankton.

Until now, the sole technique used to measure excretion consisted of leaving animals in sea water and measuring increases in the nitrogen and phosphorus content after a known time. This technique was also generally used for measuring respiration, although there is now a means for measuring the instant rate of the respiratory electron transport system activity (E.T.S.) (Packard, 1969). In spite of incubation conditions maintained as close as possible to those in the sea (same water and temperature, use of large vessels), animals have to be concentrated, otherwise experiments must last a long time, if one wishes to observe significant differences in nutrient concentrations. In fact a compromise is usually made between animal concentration and duration of experiment. Some workers use short-term experiments (a few

Equatorial Area, at 0°11′N, 0°02′S, and 2°36′S for stations 1, 2, and 3 respectively, on the 4°20′W meridian.

MATERIAL AND METHODS

For each of the three series of experiments, the durations of incubation were 3, 6, 9, 12, 21, and 24 h. Animals of the same series were caught together and transferred at increasing concentrations to 2-l flasks while incubation period decreased (Table I).

TABLE I

Zooplankton concentrations (mg dry wt/l) in the experimental vessels and incubation time (h) (the same flask was used for two consecutive periods, a volume correction being made for the second).

Series 1		Series 2		Series 3	
Duration of incubation	Zooplankton concentration	Duration of incubation	Zooplankton concentration	Duration of incubation	Zooplankton concentration
3 and 6	14.1 20.1	3 and 6	15.1 13.7	3 and 6	21.9 16.2
12	7.8 9.6	9 and 12	10.5 9.2	9 and 12	12.6 11.1
21 and 24	9.7 8.0	21 and 24	6.5 7.0	21 and 24	8.7 9.6

Sea water taken by a 30-l Niskin bottle was filtered through a 200-μm bolting silk and poured into 2-l flasks. Zooplankton caught with a WP-2 net (UNESCO, 1968) towed vertically from 200 to 0 m was poured into beakers as soon as it was captured. It was then introduced into some of the 2-l flasks randomly by aspiration. The rest of the flasks had no animals: they were the controls. No antibiotics were added as they interfere with the U.V. method of total nitrogen analysis. No specific identification of the zooplankton was made. There were no apparent carnivores, such as medusae, siphonophores, ctenophores or chaetognaths because they were eliminated before introduction into the flasks. Le Borgne (1977) showed previously that in the same area and period, small individuals dominated in the WP-2 net catches: 60% of the 200–7000 μm mesozooplankton was made of the 200–500 μm fraction.

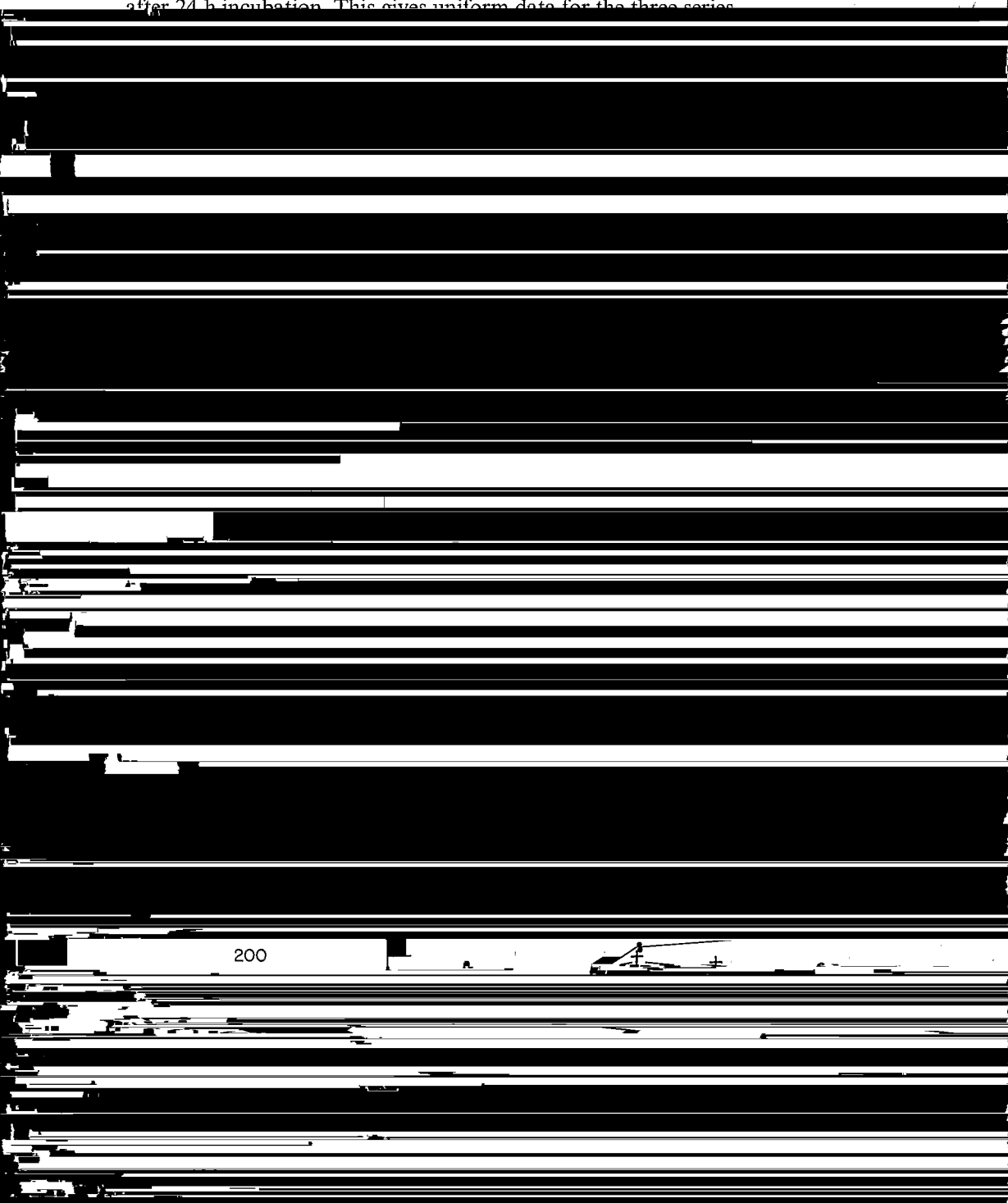
In each series of experiments there were two flasks with animals and one as control. All experiments were performed in total darkness in a constant temperature bath (20 °C for Series 1 and 2 and 23 °C for Series 3).

At the end of an experiment, the sea water was siphoned off through a 200-μm silk (to avoid escape of animals) into a 125-ml bottle for the oxygen determinations by the Winkler method, a 120-ml flask for total nitrogen and phosphorus analysis by U.V. irradiation (Armstrong & Tibbitts, 1968), and several ml for am-

monium and inorganic phosphorus analysis by the Technicon auto-analyzer (Slawyk & McIsaac, 1972, method for NH_4-N; Strickland & Parsons, 1968, for PO_4-P). A 50-ml sample was also incubated in darkness at constant temperature (20 °C) for 4 h with ^{14}C glucose. Heterotrophic activity was subsequently measured by the method of Herbland & Bois (1974).

At the end of an experiment, animals were collected on a 200-μm silk, and transferred to a preweighed Whatman GF/C fibre-glass filter, oven dried at 60 °C for 24 h and kept at −20 °C until being weighed (±0.1 mg). The health condition of the

Excretion rates and ratios depend on the type of zooplanktonic or particulate populations and these may be different for the three series. So, the Spearman correlation coefficient was calculated for each series; this was, however, not possible for the Kruskal-Wallis test because there were too few results. All three series were, therefore, mixed, using the differences between the observed value and the mean after 24 h incubation. This gives uniform data for the three series.



EXCRETION AND RESPIRATION RATES

The effect of the time of incubation is the same for all three series (Fig. 1). There is no significant influence on rates of respiration and ammonium excretion. The null-hypothesis of the Kruskal-Wallis test is not rejected when considering the six periods of incubation at the 5% confidence level, and the Spearman correlation coefficient is not significant. The excretion of total nitrogen and total and inorganic phosphorus decreases, however, from 3 to 21 h, the values not being significantly different between 21 and 24 h.

O/N, O/P, N/P RATIOS AND PERCENTAGE INORGANIC EXCRETION

The results are again similar for the three series (Figs 2, 3). From what has been previously noticed, it is not surprising that the O/NH$_4$ ratio is independent of the

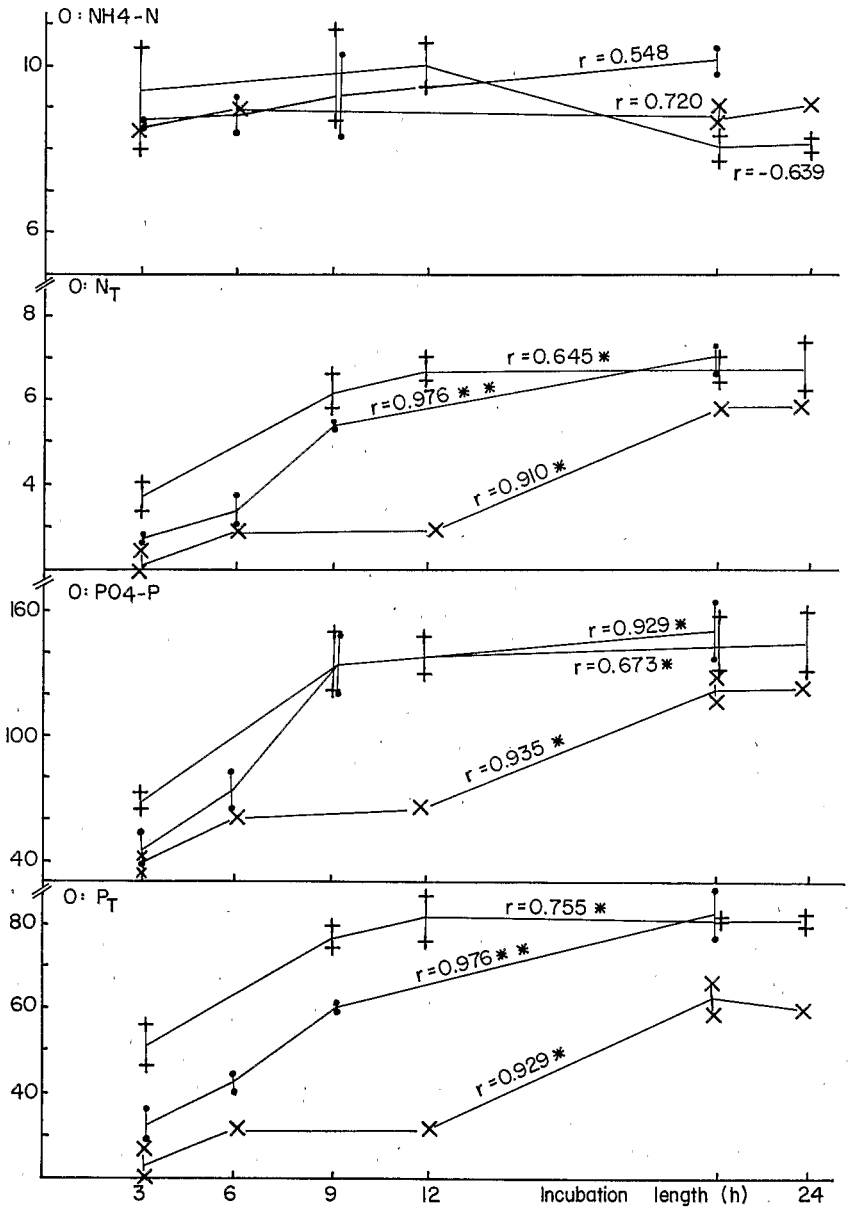


Fig. 2. Influence of duration of incubation on respiration/excretion ratios (in atoms): Spearman rank correlation coefficients of Series 1 (×), 2 (•), and 3 (+); *, 5% level of significance; **, 1% level of

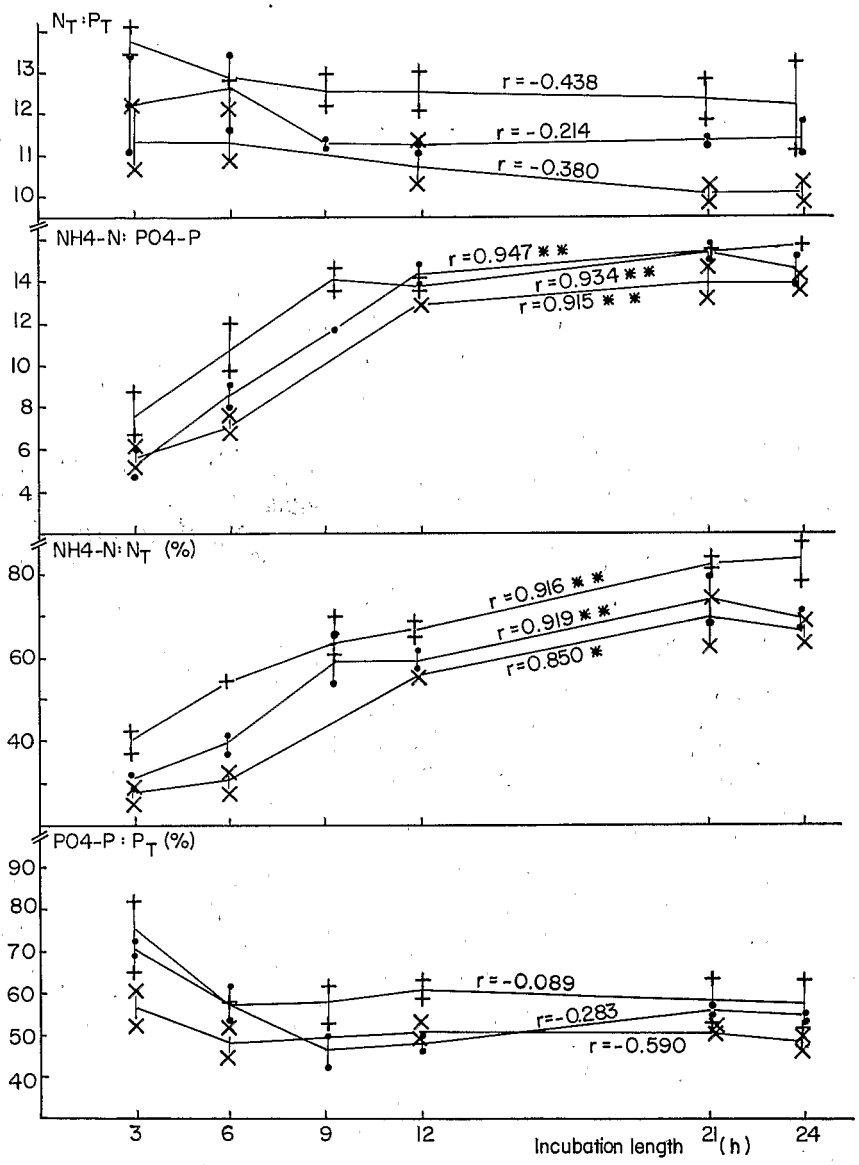


Fig. 3. Influence of duration of incubation on N/P ratios and percentages of inorganic excretion: Spearman rank correlation coefficients of Series 1 (×), 2 (•), and 3 (+); *, 5% level of significance; **, 1% level of significance.

of the animals is more or less altered as it was studied by Conover (1964), Ikeda (1971), and Mayzaud (1976). Although our results make it impossible to be in favour of the first (stress) or second (starvation) hypothesis, Roger's (1978) very recent work on ammonium and inorganic phosphorus excretion by *Meganyctiphanes norvegica* (euphausiid) is worth mentioning. The excretion rate he observed at the beginning of the experiments was the same as that of animals experimentally fed under natural prey concentrations.

The observed diminution does not, however, concern respiration or ammonium excretion rates. One could conclude, therefore, that there is no starvation effect during 24-h incubations. But, why is this not the case for rates of excretion of total phosphorus and nitrogen? Probably, the answer is the influence of bacterial activity, the consequence of which is an increase in respiration and the mineralization of excreted organic nitrogen into ammonium as shown by Mayzaud (1973). In that case, the reduction of respiration and ammonium excretion rate would be masked by bacterial respiration and ammonium formation. This mineralization of organic nitrogen could be shown by the increase of the percentage of inorganic nitrogen in total nitrogen excretion from 30–40% initially to 70–80% after 21–24 h (Fig. 3). Indeed, heterotrophic activity measurements made at each incubation period, show a spectacular increase after 3–6 h and higher values in flasks containing animals than in controls (for Series 1 and 3, Fig. 4). The explanation by bacterial activity is, however, not entirely satisfactory, for two reasons. First, the increase in the percentage of ammonium excreted in total nitrogen (NH_4/N_T ratio) is greater during the first 9 h than later on (Fig. 3), while one would have thought of an increase of this percentage, because of a cumulative effect. It is not impossible that part of the excreted organic nitrogen takes a longer time to be mineralized and that it is the same fraction which is transformed into ammonium, the result being the installment of a plateau. Secondly, bacterial activity only affects nitrogen excretion. As we noticed previously, the percentage of inorganic phosphorus excretion is steady from 6- to 24-h incubation. Still, one generally regards phosphorus as being more quickly mineralized than nitrogen. The explanation by bacterial activity is, therefore, not entirely satisfactory.

Two conclusions may be drawn from what has just been said. First, there probably has not been any noticeable ammonium uptake by autotrophic particles (experiments were made in the dark). Secondly, the controversy about the importance of the excreted organic nitrogen is met again, and according to our results, would depend on the duration of incubation. During short incubations Johannes & Webb (1965), Webb & Johannes (1967), and Eppley *et al.* (1973) found a rather high organic nitrogen excretion, whereas Corner *et al.* (1965) and Corner & Newell (1967) found it to be low during 24-h incubations. For Johannes & Webb there would be a bacterial activity during long experiments; Corner suggested, on the contrary, that there would be a crowding effect in short-term experiments. It is worth noticing that Corner *et al.* (1976) still found the same result but with 4-h incubations and, on the

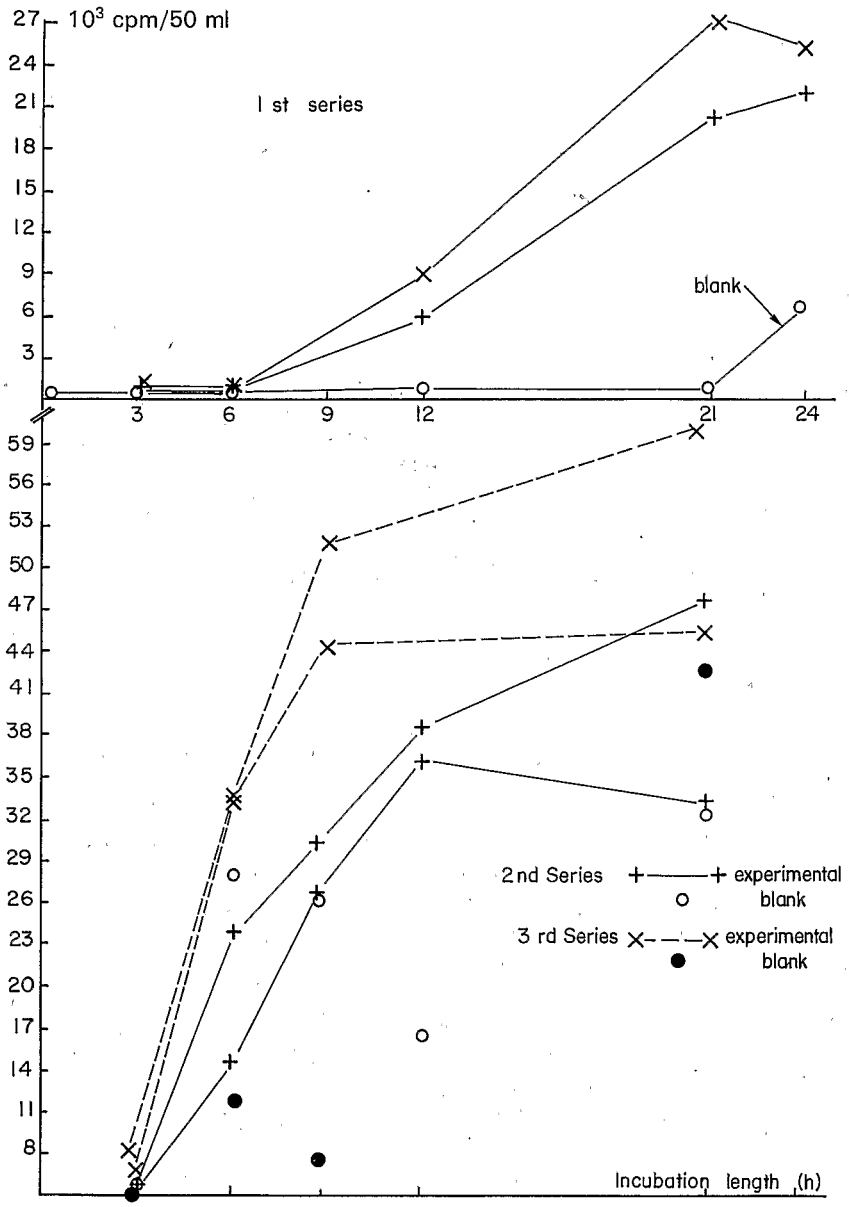


Fig. 4. Evolution of heterotrophic activity in experimental flasks and controls with duration of incubation.

contrary, Le Borgne (1973) found a high organic excretion (50%) in 24-h experiments in the Mauritanian upwelling. According to Corner *et al.* (1976), such differences could be due to different types of metabolism, and perhaps to temperature influence. An interesting explanation is put forward by McCarthy (in Eppley *et al.*, 1973):

organic nitrogen excretion, as urea, would only happen at the beginning when animals have recently fed. If this explanation were correct, ammonium excretion might actually be constant, total excretion decreasing because of the diminution of organic excretion. As far as inorganic phosphorus excretion is concerned, our results show a greater part in total excretion at the beginning, confirming the observations of Peters & Lean.(1973) on *Diaptomus* and *Daphnia.*

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