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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 hours) and high animal concentrations; others conduct 24-h long experiments at low plankton concentrations.

The purpose of this paper is to compare respiration and excretion rates and ratios obtained with the two methods using identical animal populations and to ascertain whether they are constant during a 24-h period. Three series of experiments were performed during *R.V. Capricorne* CAP 7706 cruise in July 1977 in the Atlantic

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 ($\pm$0.1 mg). The health condition of the animals was carefully controlled because injured or dead animals would release nutrients and lead to an over-estimation of excretion rates measured on short periods. Moreover, Mullin *et al.* (1975) pointed out that the excretion N/P ratio may be lower because release of phosphorus by dead animals is quicker than that of ammonium. For the three sets of experiments, only a few individuals were seen motionless, on the bottom of the flasks.

Excretion and respiration are the concentration differences between the zooplankton containing flasks and the mean value of the controls because no difference was observed between the controls of the six periods. Excretion and respiration rates are expressed per 1 mg dry wt and for 24 h. The following ratios were calculated: N_T/P_T (total nitrogen excreted : total phosphorus excreted), NH_4/PO_4 (ammonium excreted : inorganic phosphorus excreted), NH_4/N_T, PO_4/P_T, O/PO_4 (oxygen consumption : inorganic phosphorus excreted), O/P_T, O/NH_4, and O/N_T, all being atomic ratios.

RESULTS

The time evolution of excretion and respiration rates will be considered first and then the evolution of the ratios, the variations of which should be less because they are not influenced by dry weights. The consideration of respiration and excretion ratios should point to results which are not always obvious with the rates analysis.

STATISTICAL ANALYSIS

Figs 1, 2,and 3 show a correlation between ratios or rates and length of incubation but the relationship is far from being linear. Instead of dealing with the transformation of data, the Spearman rank correlation coefficient was used (Siegel, 1956).

In many cases, the figures show a steady rate or ratio with increasing length of incubation. To test whether data differ significantly for different periods, the non-parametric Kruskal-Wallis one-way analysis of variance was used. This method has an efficiency of 95.5% of the parametric *F* test (Siegel, 1956). This test was first applied to all six incubation periods. If the null hypothesis – *i.e.* data of different times coming from the same population – was rejected at the 5% level of significance, the test was then applied to 5, 4, ..., incubation periods until the null-hypothesis was accepted. Steady rates or ratios may be shown in this manner.

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.

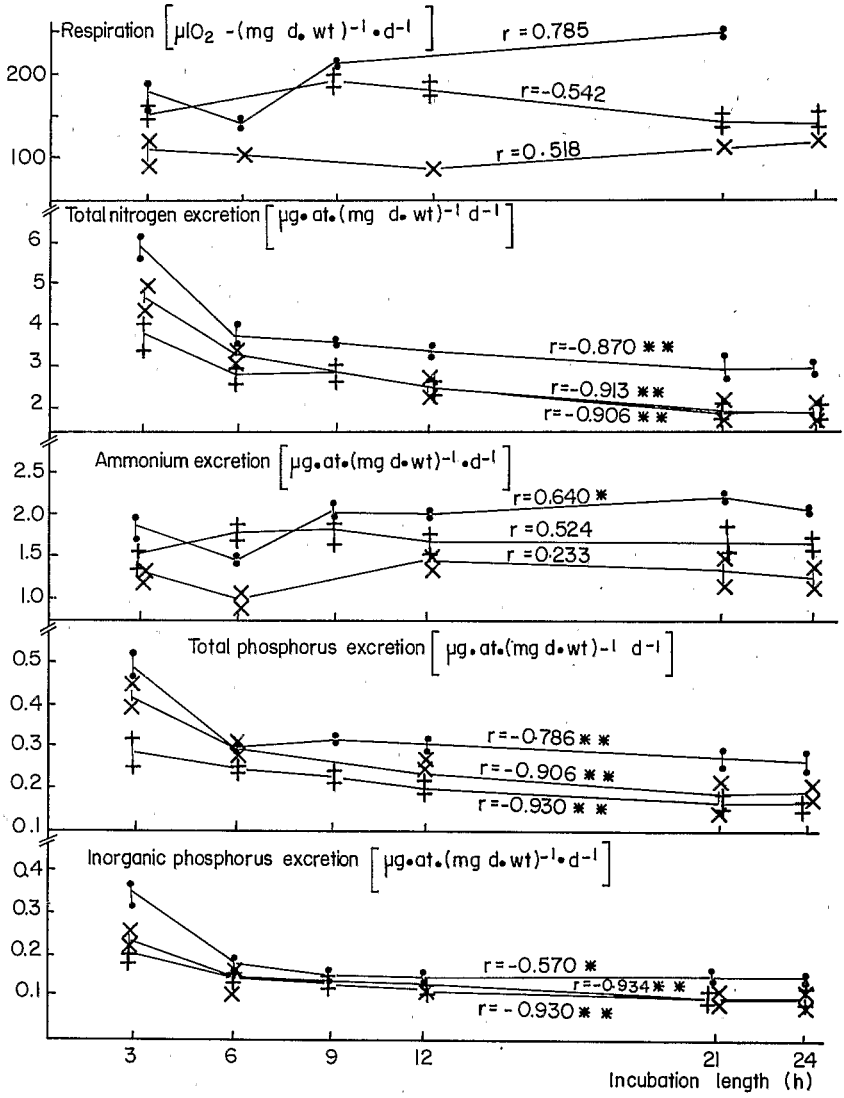

Fig. 1. Influence of duration of incubation on respiration and excretion rates: Spearman rank correlation coefficients of Series 1 (×), 2 (•), and 3 (+); *, 5% level of significance; **, 1% level of significance.

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 length of incubation (Fig. 2): Spearman correlation coefficient is not significant and Kruskal-Wallis test is not rejected when applied to the six periods. The same conclusion may be drawn for N_T/P_T and PO_4/P_T ratios (Fig. 3), although the null hypothesis is rejected when considering all periods (3 to 24 h). These ratios are, however, constant from 6 to 24 h. Ratios with respiration (O/P_T, O/PO_4, and O/N_T) at first increase with duration of incubation (Fig. 2), but become steady after 9 h (null hypothesis is not rejected for 9-, 12-, 21-, and 24-h series). The ratio of ammonium to total nitrogen (NH_4/N_T) increases, steady values being seen from 21 to 24 h. NH_4/PO_4 ratio increases up to 9 h, then remains steady (Fig. 3).

DISCUSSION

The decrease in rate of excretion with time of incubation is a commonly found result, and there are two possible explanations. First, the stress effect due to the sampling procedures and which increases the animals activity during the following hours. Secondly, the effect of starvation on metabolism. Skjoldal & Båmstedt (1977) measured the energy charge, *i.e.* (ATP + 1/2 ADP): (ATP + ADP + AMP), and found that it was much lower after capture. It takes about 24 h to recover a normal energy charge. This is the consequence of more active metabolism after capture and the subsequent ATP degradation. Many authors leave the animals for several hours before measuring respiration and excretion because of this stress. Nevertheless, after leaving their animals overnight, Nival *et al.* (1974) still found that ammonium excretion was reduced during the first hours of incubation. Satomi & Pomeroy (1965) were aware of the effect of starvation and thought the exact rate was the one observed for the first hours after capture. Actually, the distinction should be made between short-term starvation (for several hours) which animals generally meet in the natural medium and the consequence of which is a rather low decrease in metabolic activity, and long-term starvation (for several days) during which the physiology

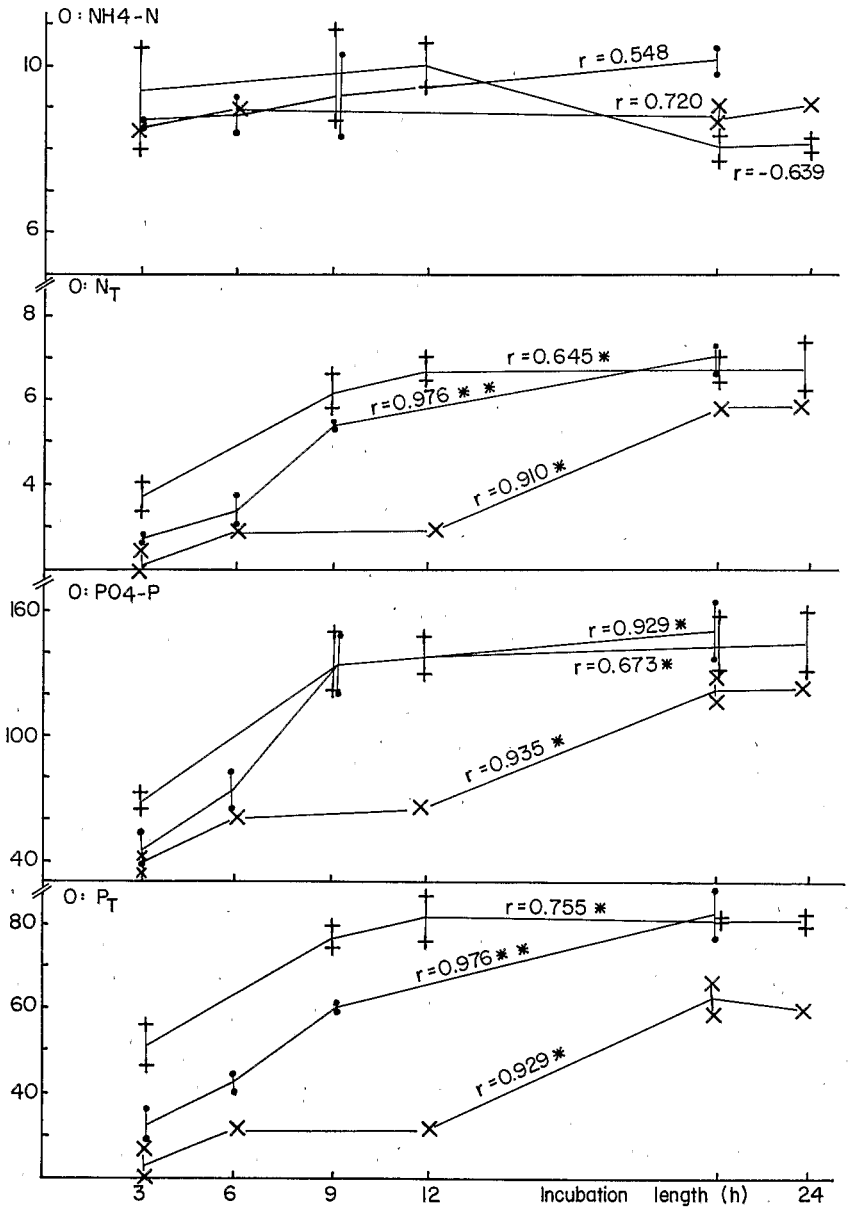

Fig. 2. Influence of duration of incubation on respiration/excretion ratios (in atoms): Spearman rank correlation coefficients of Series 1 (×), 2 (•), and 3 (+); *, 5% level of significance; **, 1% level of significance.

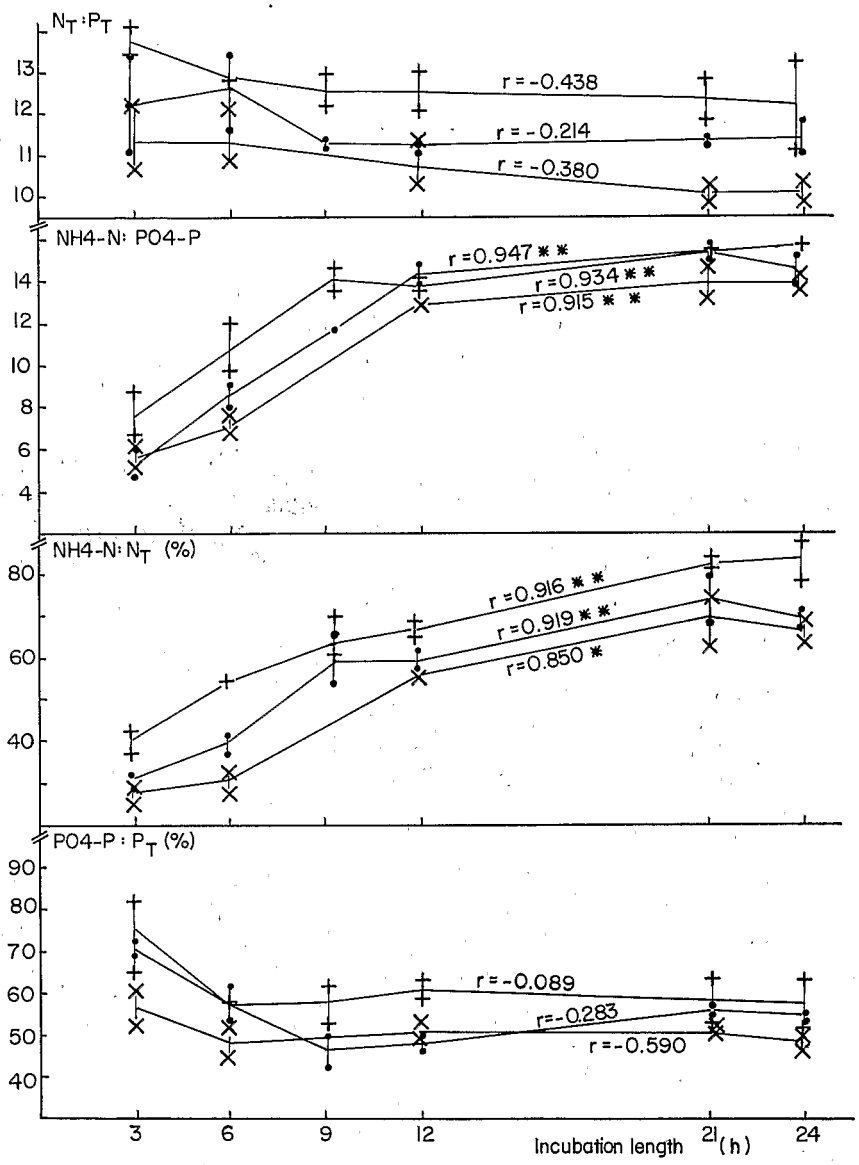

Fig. 3. Influence of duration of incubation on N/P ratios and percentages of inorganic excretion: Spearman rank correlation coefficients of Series 1 (×), 2 (•), and 3 (+); *, 5% level of significance; **, 1% level of significance.

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

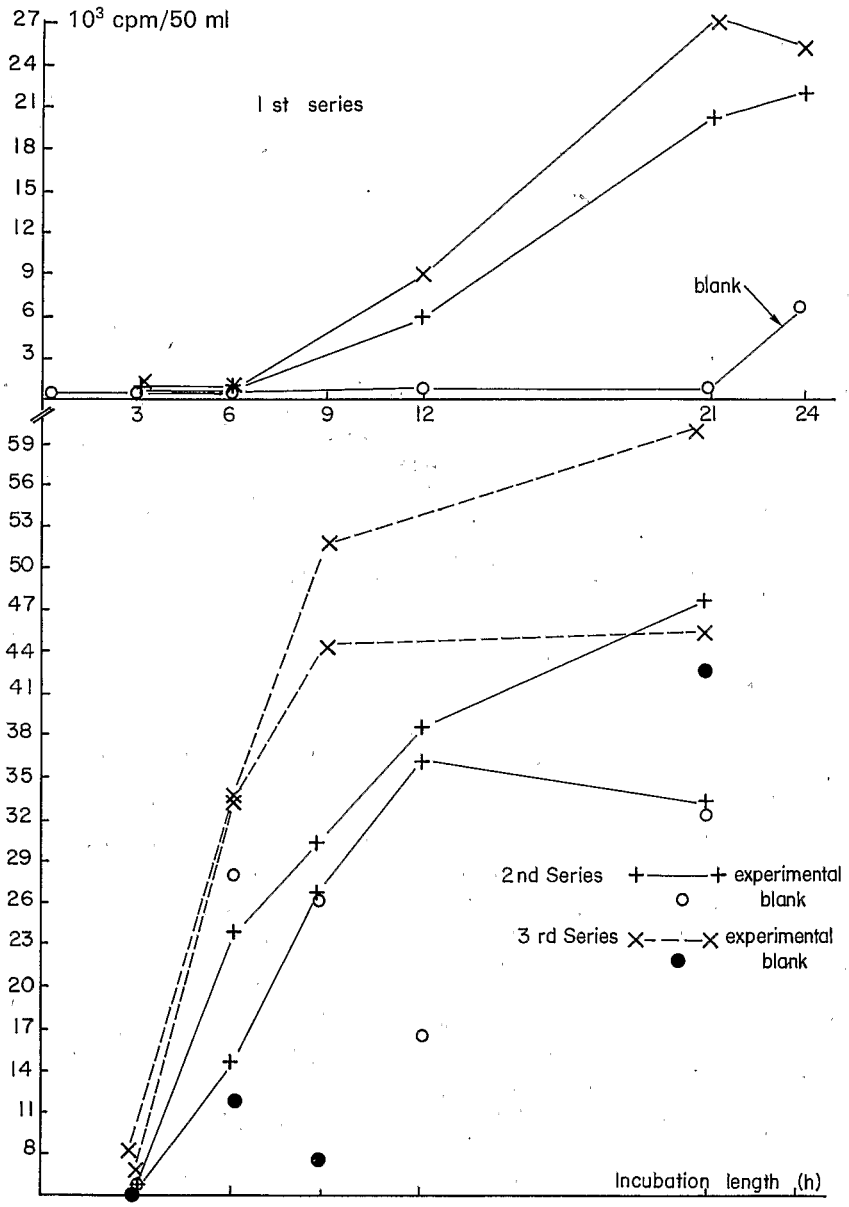

Fig. 4. Evolution of heterotrophic activity in experimental flasks and controls with duration of incubation.

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*.

Two ratios are constant whatever the period of incubation *viz*. O/NH_4 and N_T/P_T. The use of O/NH_4 ratio as an index of the type of nutrition (fat–carbohydrate metabolism when it is high, protein metabolism when it is low) is then justified. The steadiness of the N_T/P_T ratio makes the calculation of the net growth efficiency K_2 possible following Ketchum's (1962) method, which was improved by Butler *et al.* (1969) (see Le Borgne, 1978, for details). Such a calculation would be hazardous with the NH_4/PO_4 ratio because it increases during the first 9 h. Other ratios are constant from 9 to 24 h for temperatures ranging between 20 and 23 °C. It is worth mentioning that 24-h experiments may integrate diurnal variations which were reported by Eppley *et al.* (1973), Ganf & Blazka (1974), and Duval & Geen (1976). Our results do not show such variations, that may have been hidden by starvation.

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