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A study of nitrogen excretion in the marine copepod Temora longicornis

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Abstract

Frequent collections were made of the marine planktonic copepod *Temora longicornis* and a series of measurements made to determine the level of nitrogen excretion in this copepod. An algal diet consisting of the marine diatoms *Skeletonema* and *Thalassiosira* labelled with ^{14}C was provided in the laboratory. Both excreted ammonia nitrogen and amino nitrogen were measured, as was the total nitrogen in the copepods. The copepods were kept for a maximum of one day and excretion measured one hour after feeding. Oxygen consumption was measured as an indicator of metabolic rate and as a basis for calculations of the O:N ratio and estimations of the efficiency of nitrogen utilisation.

Introduction

KETCHUM (1962), in his paper on the regeneration of nutrients by zooplankton, indicated that the excretion of phosphate and nitrogen by zooplankton played a substantial part in the cycle of nutrients in the sea. He used data from HARRIS (1959) who was one of the first workers to measure nitrogen excretion in zooplankton. HARRIS (1959) simultaneously measured respiration and nitrogen excretion which he used to calculate 0:N ratios. The 0:N ratio is considered to provide a fairly accurate indication of the metabolic state of an organism.

Zooplankton are generally considered to be ammonotelic; however, the work of JAWED (1969) and MAYZAUD (1973) indicates that zooplankton may excrete small but significant amounts of organic nitrogen.

In this study the respiration and nitrogen excretion of the marine copepod *Temora longicornis* were measured. Measurements were made as soon as possible after collection in order to minimise the effects of handling and laboratory conditions upon the copepods. 0:N ratios were calculated from these measurements.

Material and methods

Copepods were collected in a standard zooplankton tow net trailed behind a boat at a depth of approximately 1 metre. The zooplankton was brought back to the laboratory and maintained at 10°C. All subsequent measurements were carried out within 4 hours. *Temora longicornis* were carefully selected from the sample with a pipette by means of a low-power binocular microscope and transferred to 50 mls of seawater that had been filtered through a 0.22 μm millipore filter and autoclaved to render it bacteria free. Active undamaged copepods were selected by attracting them to a strong lamp.

An algal diet consisting of the marine diatoms *Skeletonema* and *Thalassiosira* was provided. Cultures of these diatoms were obtained from the Culture Centre of Algae

and Protozoa, Cambridge, U.K. and maintained in the laboratory using methods described by PAFFENHÖFER (1970). To monitor ingestion by the copepods the algal cultures were labelled by growing them in a medium containing $^{14}CO_2$. The algal diet ensured that the copepods would not starve during their 4 hour stay in the laboratory. The results of these labelled feeding experiments are to be reported later.

The copepods were allowed to feed on this mixed algal diet for 2 hours. After this period uningested algal cells were removed by filtration, the copepods were rinsed in sterilised seawater and placed in Gilson Differential Respirometer flasks containing 50 mls of seawater and maintained at a temperature of 10°C for 2 hours. During this period several readings of oxygen consumption were taken.

After this 2 hour period the copepods were removed from the experimental medium by filtering them through a fine mesh, then rinsed in distilled water, blotted dry and their wet weights measured. They were subsequently analysed for total body nitrogen. The following measurements were made on the remaining seawater medium:

1) Total nitrogen excretion.
2) Ammonia nitrogen excretion.
3) – amino nitrogen excretion.

Total excreted nitrogen was measured using a ninhydrin nitrogen positive reaction based upon a modification of the method of SNOW and WILLIAMS (1971). As this method does not discriminate between ammonia nitrogen and amino acid nitrogen, the ammonia nitrogen was removed from a portion of the sample by partial evaporation in a rotary evaporator at 100°C and pH 10. The ninhydrin positive nitrogen measured in this partially evaporated sample can be attributed to amino acid nitrogen products. The ammonia nitrogen excretion was deduced from the difference between the total nitrogen excretion and the amino nitrogen excretion measurements.

Total body nitrogen was measured by a micro Kjeldahl technique which involved digestion of the copepod sample in a selenium dioxide / sulphuric acid digest mixture and subsequent addition of the digest to a phenol / hypochlorite solution.

Results

A mean respiration rate of 1.05 $\mu l\ O_2$/mg wet weight/h was found (Table 1). From examination of the mean rate and standard error it appears that there is little correlation between the rate of respiration and the weight (and thus the numbers) of copepods used in each sample. This is important because the relatively small size of *Temora* made it necessary to use large numbers of copepods in each sample to obtain accurate measurements of respiration and nitrogen excretion. MAYZAUD (1973) found no appreciable effect of crowding upon either respiration or nitrogen excretion in the copepod *Acartia clausi*, but in the present study copepod densities were kept as low as possible in the respirometer flasks.

In respect of total nitrogen excretion there is a significant difference between mean measurements made in late spring and those made in early summer. The main nitrogen product was ammonia with 0.41 μgNH_3/mg wet weight/h excreted in the spring rising to 1.72 μgNH_3/mg wet weight/h during the summer measurements. This represents 70 percent of total nitrogen excretion in the spring and 84 percent during the summer.

There appear however to be significant amounts of organic nitrogen products in the form of amino and α–amino nitrogen excreted. A value of 0.17 μgNH_3/mg wet weight/h

Table 1
Respiration and nitrogen excretion of *Temora longicornis*

Date	Average wet weight/ sample (mg)	Respiration (μl O_2/mg wet weight/h)	Total N excretion (μgNH_3/mg wet weight/h)	NH_3 excretion (μgNH_3/mg wet weight/h)	-amino N excretion (μgNH_3/mg wet weight/h)
Spring	43.5	1.08 ± 0.34	0.58 ± 0.24	0.41 ± 0.14	0.17 ± 0.09
Summer	60.5	1.05 ± 0.06	2.03 ± 0.3	1.72 ± 0.28	0.30 ± 0.03

* ± Standard error

amino nitrogen was found in the spring measurements rising to 0.30 μgNH_3/mg wet weight/h during the summer.

Most of the body nitrogen found in copepods is in the form of protein amino acids but COWEY and CORNER (1963) found significant amounts of free amino acids in *Calanus*. The values for total body nitrogen in *Temora* are given in Table 2. These represent a combined value for protein amino acids, free amino acids and other nitrogenous compounds. The mean value of 13.8 μgN/mg wet weight was not significantly different between the spring and summer measurements. This mean value for total body nitrogen represents 1.3 percent of the wet weight of *Temora*. Also presented in Table 2 is nitrogen excretion expressed as percentage body nitrogen excreted per hour.

Table 2
Nitrogen excretion and total body nitrogen of *Temora longicornis*

Date	Total body nitrogen (μgNH_3/mg wet weight)	Total nitrogen excretion (%body N/h)	NH_3 excretion (%body N/h)	-amino excretion (%body N/h)
Spring	15.8 ± 2.8	3.65	2.58	1.07
Summer	15.7 ± 0.28	12.85	10.88	1.9

* ± Standard error

Table 3
The atomic 0:N rations for *Temora longicornis*

Date	Atomic ratio 0:N Total nitrogen	Atomic ratio 0:N Ammonia nitrogen
Spring	3.16	4.48
Summer	1.00	1.21

The 0:N rations for *Temora*, as presented in Table 3, represent the average of the 0:N ratios as calculated from the spring and summer measurements. 0:N rations were calculated on the basis of both total nitrogen excretion and ammonia nitrogen excretion. This total 0:N ratio was 3.16 in the spring and 1.0 during the summer measurements.

Discussion

The results presented for nitrogen excretion indicate that *Temora longicornis* is primarily ammonotelic. This agrees with CORNER and DAVIES (1971) who concluded in their literature review that all studied species of zooplankton are mainly ammonotelic. From Table 2 it can be seen that there is considerable variation in nitrogen excretion between spring and summer measurements. This seasonal variation in nitrogen excretion is to be expected considering the changes which occur in temperature and food level during the year. CONOVER and CORNER (1968) found a seasonal variation in nitrogen excretion in several species of marine zooplankton.

In the present study *Temora* was found to excrete a significant amount of organic nitrogen in the form of amino and α–amino acid nitrogen. This represents 30 percent of the total nitrogen excretion in the spring and 16 percent during the summer measurements. There has been some debate concerning the importance of organic nitrogen excretion in zooplankton. WEBB and JOHANNES (1967) found that mixed zooplankton excreted large amounts of free amino acids. However other workers have found that smaller quantities of organic nitrogen are excreted. JAWED (1969) measured excretion rates for organic nitrogen of between 18 – 24 percent of total nitrogen excretion in *Euphausia pacifica* and *Neomysis rayii*. MAYZAUD (1973) found that the copepod *Acartia clausi* excreted 16 percent of its total nitrogen excretion in the form of organic nitrogen.

The respiration rate measured during the course of this study was found to be relatively constant at 1.5 μl O_2/mg wet weight/h. This level of respiration is higher than that found in several species of larger copepod such as *Calanus*. However one would expect the respiration rate to be higher in the smaller copepod *Temora* due to the relative increase in metabolic rate found in smaller organisms.

Examination of the 0:N ratio can provide information about the nature of the substrate being oxidised by the organism. A high 0:N ratio implies that fat or carbohydrate are being oxidised to provide energy. A lowering in the value of the ratio suggests a shift from fat or carbohydrate breakdown to the oxidation of protein. REDFIELD et al. (1963) calculated an 0:N ratio of 17 for the oxidation of a substrate containing 'typical' amounts of carbohydrate, fat and protein. MAYZAUD (1973) proposed a minimum 0:N ratio of 4 for zooplankton oxidising a strictly protein substrate. In this study 0:N ratios below this value have been found. This could be caused by unsuitable experimental conditions which either lowered respiration or increased nitrogen excretion. As regards respiration the values found in this study are similar to those found by CONOVER (1959) when he measured the respiration of *Temora longicornis* from Southampton water. The effect of crowding has been previously discussed and assumed to have little influence upon either respiration or nitrogen excretion.

MAYZAUD (1973) found an 0:N ratio of 1.34 in the copepod *Acartia clausi*. He considered that this low value was due to the effect of starvation upon the copepod. However, the copepods used in the present study were measured within 4 hours of collection and as they were provided with an algal diet they were not suffering from starvation in the laboratory. From Table 2 it can be seen that the total nitrogen

excretion during the summer measurements was 12.85 percent of total body nitrogen per hour. This nitrogen loss could not be sustained for any length of time in a starving animal. Thus some other explanation has to be found to account for the low O:N ratio found during the summer measurements. This low ratio implies that nitrogen is being excreted more rapidly than carbon is being oxidised and thus it appears that the copepods are able to rapidly deaminate and excrete ingested nitrogen in the form of protein.

Several proposals can be put forward to explain these low O:N ratios. The zooplankton may have ingested more phytoplankton than they could utilise for energy or growth and they simply excreted excess carbohydrate, fat and protein. HARRIS (1959) proposed this explanation to account for an O:N ratio of 7.7 which he measured in mixed zooplankton. Alternatively there may be a protein 'leak' caused by the selective excretion of nitrogen whilst other portions of the diet are retained. This could be brought by a modification of the copepods metabolism enabling an increase in deamination to occur whilst the rate of respiration remains constant. Unusual values for the respiratory quotient have been found during lipid synthesis in fowl (KLEIBER 1961) and similar processes might account for the unusual ratios between oxygen and nitrogen found in copepods during the course of this study. However, as very little is known about the nitrogen metabolism in copepods further work needs to be done to investigate metabolic pathways in marine invertebrates.

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