ATP CONTENT OF CALANUS FINMARCHICUS

Abstract

Measurements of adenosine triphosphate (ATP), carbon, and nitrogen were made on Calanus finmarchicus held in a food-free environment in the laboratory. Over a 23-day starvation period there were significant reductions of absolute amounts of carbon, nitrogen, and ATP, but no significant changes in the relationship between ATP and body carbon. Relative ATP levels in organisms remain sufficiently constant over a range of physiological conditions so as to make them good indicators of biomass. ATP:carbon ratios in lipid-storing copepods is also considered.

Measurements of adenosine triphosphate (ATP) have been used for estimating the biomass of planktonic organisms (e.g. Holm-Hansen 1969, 1970; Hamilton and Holm-Hansen 1967; Holm-Hansen and Booth 1966). Organisms ranging in size from bacteria to zooplankton have shown a consistent relationship between organic carbon and ATP, though published values for zooplankton are few (Holm-Hansen 1970). This paper reports some values obtained from the marine copepod Calanus finmarchicus.

In the course of feeding and starvation studies with C. finmarchicus (Balch 1971), measurements of particulate organic carbon, nitrogen, and ATP were obtained both from potential organic food particles in the sea and from zooplankton. The results reported here concern the amounts of ATP, expressed as a percent of body carbon, in stages IV and V C. finmarchicus over a starvation period of 23 days. The experimental animals were kept in darkness in Millipore-filtered (0.8 μ) seawater at 3°C. About 10 individuals were removed each week for measurement of carbon and nitrogen and 10 for measurements of ATP (Holm-Hansen 1969). Individual live animals were dropped into boiling Tris buffer where they were then broken up by forceps. Carbon and nitrogen were measured with a carbon-hydrogen-nitrogen analyzer (Hewlett-Packard model 185).

Results and Discussion

Since it is not possible to measure both ATP and carbon in the same animal, comparative values were obtained by pairing ATP and carbon data from individuals of similar size whenever possible, cephalothorax measurements having been made for all animals analyzed. Using only paired data, Table 1 shows values for ATP expressed as percent of total body carbon. The mean for all such data was 0.89%.

A major problem in comparing the ATP content of microorganisms with that of zooplankton is that some zooplankton species, including C. finmarchicus, store significant amounts of carbon as lipids which may be assumed to be free of ATP. Since the animals assayed in this study had vastly varying amounts of storage lipids, as evidenced by variability of oil sac size and of
C:N ratios, an expression of ATP as percent of total body carbon might be expected to display a large range of values, depending on the amount of storage lipid present. I therefore tried to partition total body carbon into its lipid and nonlipid fractions to see whether the relationship between ATP and nonlipid carbon might be significantly different from that between ATP and total carbon.

In an earlier series of experiments, assays of *C. finmarchicus* (stages IV, V, and adults) with no visible oil sacs, gave a mean C:N ratio of 3.52 (SD = 0.27, n = 26). This value agrees well with a mean of 3.8 (n = 45) and a minimum of 3.3 for *C. helgolandicus* (Mullin and Brooks 1970). On the assumption that the value of 3.52 was a reliable estimate of the C:N ratio of copepods with little or no storage lipids, I used it to partition total body carbon into its lipid and nonlipid fractions. Nonlipid is defined as that carbon which could be associated with measured nitrogen, assuming a C:N ratio of 3.52. Lipid carbon is defined as total body carbon minus the nonlipid fraction.

Values for ATP as a percent of nonlipid carbon are also shown in Table 1. As expected, they are considerably higher than the values for ATP as a percent of total carbon and probably represent a more stable and more useful index of copepod biomass.

Analyses of variance to determine whether any significant change in the ATP to carbon relationship occurred over the 23-day starvation period showed probabilities > 0.75, indicating that ATP as a percent of both total body carbon and of nonlipid carbon did not change significantly. This was in contrast to absolute amounts of carbon, nitrogen, and ATP, all of which did show significant changes over time, as indicated by probabilities < 0.001.

It might be expected that under starvation conditions lipid reserve carbon would be catabolized more rapidly than other forms of body carbon and that this would be reflected in changes in the ratio of lipid to nonlipid carbon. There was a drop of this ratio from 1.7 to 1.3 over the first 8 days, but no further drop, so that overall, any changes in the form of carbon catabolized did not influence ATP as percent of either total carbon or nonlipid carbon.

Since the relation of ATP to carbon did not change significantly over time, values from all animals measured over the 23-day period were grouped to allow a more accurate pairing of carbon and ATP values according to body size. The values for ATP as percent of body carbon obtained in this manner were 0.78% (SD = 0.02) for total carbon and 1.89% (SD = 0.06) for nonlipid body carbon. Differences between these values and the mean values in Table 1 result from the fact that with the larger number of copepods available for pairing of ATP and carbon data according to size, fewer animals had to be discarded for lack of suitable pairs. Since Helm-Hansen's (1970) value of 0.50% for *C. helgolandicus* was not accompanied with an estimate of

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**Table 1. Body carbon, nitrogen, ATP, and ATP expressed as percent of body carbon in Calanus under starvation conditions (SD = standard deviation)**

<table>
<thead>
<tr>
<th>Starvation period (days)</th>
<th>Nitrogen (µg/Calanus) mean (n) SD</th>
<th>Carbon (µg/Calanus) mean (n) SD</th>
<th>ATP (µg/Calanus) mean (n) SD</th>
<th>ATP as % of total carbon mean SD</th>
<th>ATP as % of nonlipid carbon mean SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>20.1 (9) 5.6</td>
<td>187.4 (9) 71.6</td>
<td>1642.2 (10) 507.6</td>
<td>0.87 0.33</td>
<td>2.23 0.70</td>
</tr>
<tr>
<td>8</td>
<td>18.7 (10) 4.2</td>
<td>153.0 (10) 44.3</td>
<td>1271.1 (10) 326.1</td>
<td>0.92 0.37</td>
<td>2.07 0.70</td>
</tr>
<tr>
<td>14</td>
<td>14.8 (10) 4.4</td>
<td>118.1 (10) 53.8</td>
<td>975.6 (10) 451.6</td>
<td>0.86 0.39</td>
<td>1.90 0.87</td>
</tr>
<tr>
<td>23</td>
<td>18.3 (12) 2.7</td>
<td>153.3 (12) 26.5</td>
<td>1268.6 (10) 544.0</td>
<td>0.91 0.33</td>
<td>2.04 0.78</td>
</tr>
</tbody>
</table>
variability, no statistical comparison can be made between it and the values reported here. However, the magnitude of the difference, plus the rather low standard deviations of my data, suggest that the two estimates are significantly different. This may have resulted from a more efficient ATP extraction in my work, where individual animals were broken up with forceps in the boiling Tris buffer; this was not done by Holm-Hansen (personal communication). In order that ATP extraction be complete, it must be carried out extremely rapidly. Although it is possible that because of the large size of the organisms I used extraction was not complete, the fact that relative amounts of ATP reported here are higher than in other published reports mitigates against this possibility.

This work suggests that Calanus is able to maintain a constant proportion of ATP in the face of starvation conditions, while absolute amounts of carbon, nitrogen, and ATP can be significantly reduced. This suggests that the rationale behind ATP measurements is sound; i.e. that relative ATP levels in organisms remain more or less constant over a range of physiological conditions and are therefore good indicators of biomass. However, the presence of storage lipids has to be taken into account when interpreting ATP measurements from lipid-storing copepods. Also, further work is needed to find whether ATP extraction from such large organisms is complete and whether the ATP to carbon relationship for various species of zooplankton is comparable to that of other organisms, as Holm-Hansen has stated. My work suggests that zooplankton, with between 0.78 and 1.89% of body carbon in the form of ATP, may have up to five times as much ATP per unit of body carbon as do unicellular algae, using Holm-Hansen's (1970) value of 0.35%. If this is so, then the inclusion of copepods in ATP measurements from natural seawater samples will bias the results. Since small copepods and other microzooplankton can at times be numerous and will often pass through the prestraining mesh designed to exclude zooplankton, they could significantly influence biomass estimates obtained from ATP measurements, if such estimates were based on ATP to carbon conversion ratios obtained only from unicellular organisms.

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Norval Balch

Institute of Oceanography,
Dalhousie University,
Halifax, Nova Scotia.

REFERENCES


1 Present address. Department of Biology, University of Victoria, Victoria, British Columbia.