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REVIEW

O:N atomic ratio as a tool to describe zooplankton metabolism

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ABSTRACT: Use of the atomic O:N ratio as a metabolic index requires clarification of the biochemical complexities of zooplankton intermediary metabolism. We review the processes responsible for natural and experimentally-induced changes in the ratio and discuss their relative importance in relation to a variable natural environment. During starvation the O:N ratio is clearly linked to the availability of energy reserves and the use of body protein. Using theoretical computations, it can be shown that pure protein catabolism will yield O:N ratios in the range 3 to 16, while equal amounts of lipid and protein catabolism will correspond to values between 50 and 60. Under natural feeding conditions, the value of the ratio depends on the use by the animal of each biochemical fraction assimilated. Others factors, such as seasonal events in the life cycle, biochemical composition of the body and food quality are of importance for correct interpretation of the variations. The concepts behind the rates are discussed, suggesting that such measurements reflect the adaptability of the animals to their total environment.

INTRODUCTION

The growing use of metabolic rates, such as O:N or O:P (oxygen consumed versus nitrogen or phosphorus excreted), is largely based on simple assumptions and on theoretical computations originating from mammalian catabolic data (Harris 1959, Snow & Williams 1971, Mayzaud 1973, Ikeda 1974). More recent work on the relationship between glutamate dehydrogenase (GDH) and ammonia excretion by Bidigare & King (1981) and Bidigare et al. (1982) have utilized information on the pathways of zooplankton nitrogen catabolism in such calculations. The computation of O:N ratios from ETS (Electron Transport System) and GDH activity data (Bidigare et al. 1982, King 1984) further reinforces the need for clarification of processes responsible for the natural and/or experimental variations in the ratio and their probable interpretation.

The present review attempts to provide evidence to support the use of the O:N ratio as an index of metabolism, by discussing the assumptions and theoretical computations used by most authors and, in so far as possible, by relating the complexity of the environment to that of the metabolic processes.

INFLUENCE OF EXPERIMENTAL CONDITIONS

There are a limited number of studies on the influence of collection methods and experimental conditions on respiratory and excretory rates of zooplankton simultaneously. The effects of laboratory conditions on respiratory rate measurements have been extensively reviewed by Marshall (1973) and Ikeda (1976, 1977a, b, c), but, for the O:N ratio, there are only the few studies by Le Borgne (1979, 1982a, b) and by Ikeda & Skjoldal (1980). None has been able to show a consistent effect related to concentration of experimental populations or to the duration of incubation. Most showed that starvation is a major factor causing variation. Capture stress has been evoked to explain the general decrease in metabolic rate during the first few hours of incubation (Skjoldal & Båmstedt 1977) but the interpretation of the data varies (Ikeda & Skjoldal 1980), with both respiration and nitrogen excretion being affected similarly. No data comparing net and pump sampling are available, though harsh collection methods have been proposed to explain abnormally high ammonia excretion rates in gelatinous zooplankton (Biggs 1977, Cetta et al. 1986).

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O:N RATIO AND CATABOLIC ACTIVITY

Metabolism is the balance between anabolism (synthesis) and catabolism (degradation). Though anabolism is difficult to measure directly, it is often possible to differentiate between catabolism and total metabolism by starving experimental animals. It has been shown numerous times that, under starvation, metabolic rates decrease with time (Corner & Newell 1967, Mayzaud 1973, Nival et al. 1974) but the pattern of metabolic response can be variable. Although the rate of decrease in respiration appears more or less similar for different species, changes in ammonia excretion vary considerably from species to species (Mayzaud 1976, Ikeda 1977c) apparently related to the biochemical composition of the zooplankters. If we consider the first of the 3 species studied by Mayzaud (1976), stage V *Calanus finmarchicus* displayed a slowly increasing O:N ratio for the first 6 d of starvation, a more rapid increase between 6 and 13 d and then oscillations between high and low values for the next 21 d (Fig. 1). Low values corresponded to a period when protein was heavily used, while higher values were related to depletion of the lipid reserves. A similar oscillatory pattern was recorded by Ikeda (1977c) with *Calanus plumchrus* starved over 21 d, while steadily increasing ratios were observed with carnivorous species such as *Parathemisto pacifica*, *Pleurobrachia pileus* (Quetin et al. 1980) or deep water mysid such as *Gnathophausia ingens* (Hiller-Adams & Childress 1983a).

The O:N ratio for *Sagitta elegans* showed an initial increase during the first 4 d of starvation followed by a decrease during the next 5 d and a slight increase on the last day of the experiment (Mayzaud 1976). This pattern of variation agreed fairly well with that shown by the protein fraction which was heavily used during the last 7 d of the starvation period. *Acartia hudsonica* starved for 4 d did not show any significant changes ($F_{5,12} = 2.17$) in O:N ratio, although both respiration and ammonia excretion rates decreased during the period (Mayzaud 1976). Decreasing ratios with time of starvation seem common to all species with a predominantly protein-based metabolism and have been observed for several planktonic crustaceans (*Temora stylifera*, Nival et al. 1974; *Euphausia pacifica*, Ikeda 1977c; *Acartia australis*, Ikeda & Skjoldal 1980).

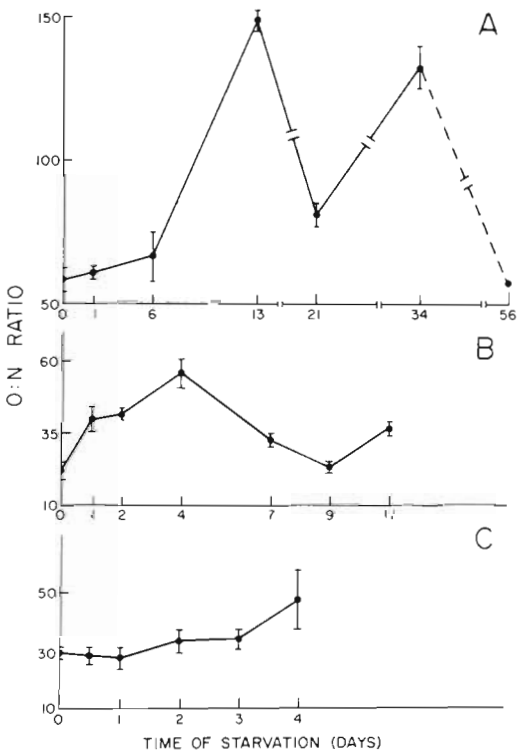

Fig. 1. Effect of starvation duration on O:N ratios of *Calanus finmarchicus* Stage V (A); *Sagitta elegans* adults (B); *Acartia hudsonica* mixed stages (C)

Table 1 *Calanus finmarchicus*. Correlation analysis between logarithm of O:N ratio, duration of starvation (T), logarithm of dry weight (W), and of nitrogen (N), carbon (C), protein (Prot), lipid (Lip) and total carbohydrate (Carb) content

Simple correlation analysis

Independent variables	Dependent variable	Correlation coefficient (r)	df
T	log O:N	0.749••	17
log W	–	−0.685••	–
log N	–	−0.475•	–
log C	–	−0.651••	–
log Prot	–	−0.577••	–
log Lip	–	−0.770••	–
log Carb	–	−0.426	–

Partial correlation matrix

	log O:N, W	log O:N, N	log O:N, C	log O:N, T
log W	–	0.562••	−0.292	0.062
log N	0.031	–	−0.144	0.276
log C	−0.072	−0.521•	–	−0.003
T	0.455•	0.691••	0.489•	–

(Multiple correlation coefficient R = 0.783••)

	log O:N, Prot	log O:N, Lip	log O:N, Carb	log O:N, T
log Prot.	–	0.044	−0.439	−0.002
log Lip.	−0.626••	–	−0.722••	−0.354
log Carb.	−0.097	0.184	–	0.572
T	0.585••	0.236	0.800••	–

(Multiple correlation coefficient R = 0.850••)

•• Correlation coefficient significant at $p < 0.01$
• Correlation coefficient significant at $p < 0.05$

The actual relation between O:N ratio and biochemical composition of the organisms remains poorly understood. Using data from Mayzaud (1976), it is possible to assess the influence of the biochemical variables on the changes in the O:N ratio. First, these were compared with time of starvation (in days), dry weight (in µg animal^{-1}) and chemical and biochemical composition (in µg animal^{-1}) by simple correlation analysis. In *Calanus finmarchicus*, the O:N ratio was positively correlated ($p < 0.01$) with duration of starvation (T) and negatively with dry weight (W), carbon (C), nitrogen (N), protein (Prot) and lipid (Lip) content (Table 1). No significant correlation could be established with the concentration of body carbohydrate. Because the 'independent' variables are intercorrelated, the influence of each on the others was removed by computing the partial correlation coefficients. Removing the effect of the other variables successively had (1) little effect on the strong positive correlation between log O:N and duration of starvation (T) but (2) a strong influence on the correlations between log O:N and log W and log C, significance being observed only when the influence of log N was removed (Table 1). This is not surprising as the carbon content is mainly responsible for dry weight. Total carbon is, therefore, the chemical fraction most closely linked to the O:N ratio changes. Removing the influence of the biochemical variables successively showed that, after time, lipid content was the most influential fraction affecting O:N; this is consistent with the conclusions above concerning the importance of the total carbon pool.

Using the same statistical procedure, O:N ratios for *Sagitta elegans* were compared with the same independent variables. Log O:N was negatively correlated with time and positively correlated with dry weight, carbon, protein, lipid and carbohydrate content (Table 2). No correlations were shown with the body nitrogen and non-protein nitrogen (NPN). As might be anticipated, removing the effect of these 2 variables did not change the correlations, in contrast with the effect of removing any of the other variables. Total carbon again appeared to be mainly responsible for dry

Table 2. *Sagitta elegans*. Correlation analysis between logarithm of O:N ratio, duration of starvation (T), logarithms of dry weight (W), and of nitrogen (N), carbon (C), protein (Prot), lipid (Lip), total carbohydrate (carb) and non-protein nitrogen (NPN)

Simple correlation analysis

Independent variables	Dependent variable	Correlation coefficient (r)	df
T	log O:N	−0.488*	21
log W	–	0.481*	–
log N	–	0.251	–
log C	–	0.535*	–
log Prot	–	0.661*	–
log Lip	–	0.586*	–
log Carb	–	0.578*	–
log NPN	–	0.415	–

Partial correlation matrix

	log O:N, W	log O:N, N	log O:N, C	log O:N, T
log W	–	0.431*	0.243	0.219
log N	0.087	–	−0.273	−0.237
log C	0.356	0.543**	–	0.253
T	−0.232	−0.482*	−0.027	–
	(Multiple correlation coefficient R = 0.609**)			

	log O:N, Prot	log O:N, Lip	log O:N, Carb	log O:N, NPN	log O:N, T
log Prot.	–	0.456*	0.479*	0.613**	0.511*
log Lip.	0.227	–	0.345	0.485*	0.288
log Carb.	0.302	0.328	–	0.517*	0.292
log NPN	0.281	0.175	0.290	–	0.213
T	−0.328	−0.224	−0.250	−0.306	–
	(Multiple correlation coefficient R = 0.748**)				

** Correlation coefficient significant at $p < 0.01$
* Correlation coefficient significant at $p < 0.05$

weight, but – contrary to the observations for *Calanus* – carbon was primarily associated with protein rather than with the lipid pool ($p < 0.05$, Table 2). Total nitrogen was not correlated with the O:N ratio because the large pool of non-protein nitrogen in *S. elegans* (Mayzaud & Martin 1975) did not undergo any significant change with time of starvation (Mayzaud 1976).

O:N RATIO AS INDEX OF CATABOLISM: DEFINITION OF THEORETICAL LIMITS

To establish the catabolic characteristics of a given species or population, the use of a metabolic index is certainly more convenient than measuring the variation in the main body constituents under starvation. To ascertain proper interpretation of the changes of the O:N ratio, the basic concepts underlying it must be explained and the theoretical range of values defined in order to allow discrimination between protein- and lipid-dominated metabolism.

When the animals are starving, carbohydrate catabolism can be ruled out, because sugars represent a small fraction of the organic content of zooplankton (Raymont & Krishnaswamy 1960, Raymont & Conover 1961, Mayzaud & Martin 1975). Various theoretical minimum values of the O:N ratio have been proposed by different authors for strictly proteinic catabolism, depending on the basis of calculation. Snow & Williams (1971) and Mayzaud (1973) reported a value of 7; Conover & Corner (1968) indicated a minimum of 8; and Bayne (1973), a minimum of 9.3. All these values are based on the experimental determination of a proteinic R. Q. made by Loewy (see West & Todd 1961) in which 1 g of urinary nitrogen was excreted on the catabolism of 6.25 g of protein after the absorption of 5.92 l of oxygen and the production of 4.76 l of CO_2 (R. Q. = 0.8), but differ in their method of computing the elemental composition of the protein. Using the same assumptions that 1 g of protein requires 0.94 l of oxygen in oxidative metabolism and that 1 g of lipid requires 2.04 l, it can be computed that an organism catabolizing equal amounts of proteins and lipids should have an O:N ratio of 24. Thus any value between 24 and infinity corresponds to a lipid-dominated catabolism.

As indicated by Mayzaud (1973), the minimum theoretical ratio varied somewhat with the metabolic pathway postulated in making the computation. If planktonic protein catabolism is assumed to proceed through transdeamination of amino acids to yield carboxylic acids (Braunstein 1957, Campbell 1973) then:

$$\underset{\text{NH}_2}{\text{R–CH–COOH}} + \text{H}_2\text{O} \xrightarrow[\text{FAD dehydrogenase}]{\text{aminotransferase}} \text{R–CO–COOH} + \text{NH}_3 + 2\text{H} \quad (1)$$

The volume of oxygen required for each atom of nitrogen excreted will be a function of the nature of the keto acid oxidized:

$$\text{R–CO–COOH} + n\text{O}_2 \rightarrow y\,\text{CO}_2 + z\,\text{H}_2\text{O}\ (\text{O:N} = 2n/1) \quad (2)$$

Assuming that the amino acid catabolism via the citrate cycle is quantitatively significant, the catabolic pathways show that the different amino acids can enter the Krebs cycle at 5 different sites (Fig. 2): α-ketoglutarate, succinyl CoA, fumarate, oxaloacetate and acetate. Oxidation of these 5 compounds in equal amounts would yield an average O:N ratio of 6.0. In fact, the biological oxidation proceeds by means of dehydrogenation involving one more intermediate hydrogen carriers (NADH, NADPH or FADH) rather than by means of direct oxidation as described by Equation 2. The hydrogens produced are then transferred along the electron transport system (Fig. 2) and used to reduce the terminal acceptor, e.g. oxygen. Thus, proper computation of the theoretical minimum O:N ratio should consider the production of hydrogen carriers through the catabolic pathway specific for each amino-acid (Lehninger 1975) (Table 3). As an example, let us consider the case of alanine:

2 alanine + 2 α-ketoglutarate → 2 pyruvate + 2 glutamate (transamination step)

$2\ \text{glutamate} + 2\ \text{NAD}_{ox} + 2\ \text{H}_2\text{O} \rightarrow 2\ \alpha\text{-ketoglutarate} + 2\ \text{NH}_3 + 2\ \text{NADH}$ (deamination step)

$2\ \text{pyruvate} + 30\ \text{Pi} + 30\ \text{ADP} + 6\ \text{O}_2 \rightarrow 6\ \text{CO}_2 + 36\ \text{H}_2\text{O} + 30\ \text{ATP}$ (oxidation through Krebs cycle)

$2\ \text{NADH} + 6\ \text{Pi} + 6\ \text{ADP} + \text{O}_2 \rightarrow 2\ \text{NAD}_{ox} + 4\ \text{H}_2\text{O} + 6\ \text{ATP}$ (electron transport system)

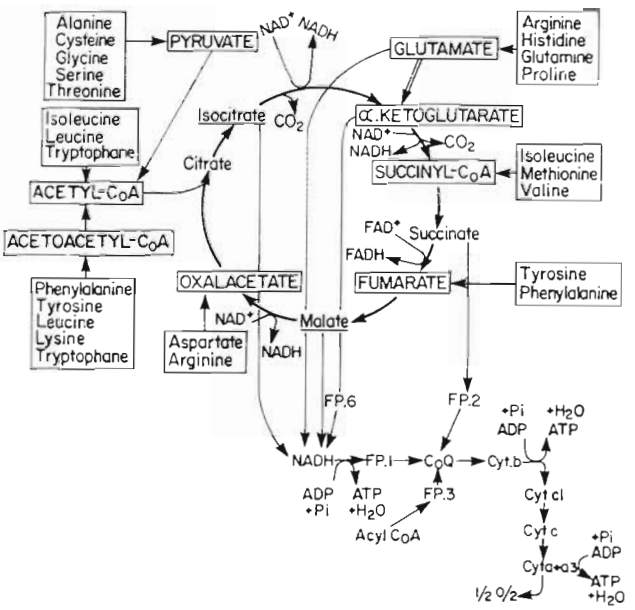

Fig. 2. Connecting metabolic pathways between amino-acid catabolism and electron transport system (i.e. respiration)

Table 3. Budget of metabolic oxidation of various amino-acids and resulting atomic O:N ratios. The metabolic pathways followed for computation are taken from Lehninger (1975). Pi: inorganic phosphorus

Amino-acids	Summarized oxidation equation	O_2 consumed	NH_3 produced	O:N
Alanine	2 Ala + 36 Pi + 36 ADP + 7 O_2 → 6 CO_2 + 42 H_2O + 2 NH_3 + 36 ATP	14	2	7.0
Glycine (major pathway)	2 Gly + 2 FH_4 + 6 Pi + 6 APD + O_2 → 2 H_5N_{10} methylene − FH_4 + 2 CO_2 + 8 H_2O + 2 NH_3 + 6 ATP	2	2	1.0
Threonine glycine (minor pathway)	Threo ⟶ Acetaldehyde + Gly Gly ⟶ Serine ⟶ Pyruvate + H_2O + NH_3	6	1	6.0
Serine	Pyruvate + 15 Pi + 15 ADP + 3 O_2 → 3 CO_2 + 13 H_2O + 15 ATP			
Cysteine	Cys + H_2O → Pyruvate + H_2S + NH_3	6	1	6.0
	or 2 Cys + 42 Pi + 42 ADP + 10 O_2 → 6 CO_2 + 52 H_2O + 2 SO_3^{--} + 2 NH_3 + 42 ATP	20	2	10.0
	or 2 Cys + 2 HCN + 36 Pi + 36 ADP + 7 O_2 → 6 CO_2 + 42 H_2O + 2 HSCH + 2 NH_3 + 36 ATP	14	2	7.0
Phenylalanine	2 Phe + 52 Pi + 52 ADP + 16 O_2 → 10 CO_2 + 61 H_2O + 2 NH_3 + 52 ATP	32	2	16.0
Tyrosine	2 Tyr + 52 Pi + 52 ADP + 14 O_2 → 10 CO_2 + 59 H_2O + 2 NH_3 + 52 ATP	28	2	14.0
Leucine	2 Leu + 2 CoA + 88 Pi + 88 ADP + 16 O_2 → 12 CO_2 + 42 H_2O + 2 NH_3 + 88 ATP + 2 CoASH	32	2	16.0
Lysine	2 Lys + 2 CoA + 74 Pi + 74 ADP + 15 O_2 → 12 CO_2 + 88 H_2O + 4 NH_3 + 74 ATP + 2 CoASH	30	4	7.5
Tryptophan	2 Trp + 2 CoA + 118 Pi + 118 ADP + 27 O_2 → 12 CO_2 + 2 Formate + 135 H_2O + 4 NH_3 + 118 ATP + 2 CoASH	54	4	13.5
Glutamine	2 Glu NH_2 + 22 Pi + 22 ADP + 3 O_2 → 2 CO_2 + 22 H_2O + 4 NH_3 + 22 ATP	6	4	1.5
Glutamic acid	2 Glu + 22 Pi + 22 ADP + 3 O_2 → 2 CO_2 + 24 H_2O + 2 NH_3 + 22 ATP	6	2	3.0
Arginine	2 Arg + 36 Pi + 36 ADP + 6 O_2 → 4 CO_2 + 38 H_2O + 2 NH_3 + 2 Urea + 36 ATP	12	2	6.0
Histidine	2 Hist + 22 Pi + 22 ADP + 3 O_2 → 2 CO_2 + 24 H_2O + 2 NH_3 + 22 ATP	6	2	3.0
Proline	2 Pro + 28 Pi + 28 ADP + 4 O_2 → 2 CO_2 + 28 H_2O + 2 NH_3 + 28 ATP	8	2	6.0
Methionine	2 Met + 2 Ser + 2 HCN + 2 CoA + 40 Pi + 40 ADP + 9 O_2 → 2 Adenine + 2 HSCN + 4 NH_3 + 50 H_2O + 40 ATP + 2 CoASH + 6 CO_2	18	4	4.5
Valine	2 Val + 2 CoA + 28 Pi + 28 ADP + 7 O_2 → 2 CO_2 + 36 H_2O + 2 NH_3 + 28 ATP + 2 CoASH	12	2	6.0
Isoleucine	2 Ile + 4 CoA + 46 Pi + 46 ADP + 8 O_2 → 4 CO_2 + 54 H_2O + 2 NH_3 + 46 ATP + 4 CoASH	18	2	9.0
Aspartic acid	2 Asp + 30 Pi + 30 ADP + 5 O_2 → 4 CO_2 + 34 H_2O + 2 NH_3 + 30 ATP	10	2	5.0
Asparagine	2 Asp NH_2 + 30 Pi + 30 ADP + 5 O_2 → 4 CO_2 + 34 H_2O + 3 NH_3 + 30 ATP	10	3	3.3

Table 4. Metabolic oxidation of fatty acids and acetyl CoA. Pi: inorganic phosphorus; PPi: inorganic polyphosphate

Summary equations
β-oxidation $CH_3–(CH_2–CH_2)_n–COOH + ATP + (n+1)\ CoASH + nNAD^+ + nE.FAD + nH_2O \longrightarrow (n+1)CH_3–CO–S–CoA + (ADP+Pi; AMP+PPi) + n\ NADH + nH^+ + nE.FADH_2$
NAD_{red} oxidation $2\ NAD_{red} + 6\ Pi + 6\ ADP + O_2 \longrightarrow 2\ NAD_{ox} + 8\ H_2O + 6\ ATP$
$FADH_2$ oxidation $2\ FADH_2 + 4\ ADP + 4\ Pi + O_2 \longrightarrow 2\ FAD + 5\ H_2O + 4\ ATP$
Cyclical oxidation of acetyl-CoA (Krebs cycle) $Acetyl\text{-}CoA + 3\ NAD^+ + FAD + GDP + Pi + 2\ H_2O \longrightarrow 2\ CO_2 + CoASH + 3\ NADH + 3H^+ + FADH_2 + GTP$ $3\ NADH + 3\ H^+ + FADH_2 + 2\ O_2 \longrightarrow NAD^+ + FAD + 4\ H_2O$

By adding the equations and eliminating the terms common on both sides, we can define the general oxidation equation of alanine:

$$2\ alanine + 36\ Pi + 36\ ADP + 7\ O_2 \rightarrow 6\ CO_2 + 38\ H_2O + 2\ NH_3 + 36\ ATP$$

and the corresponding atomic O:N ratio : $7 \times 2/2 = 7$. Similar computations, following the specific degradation pathway of each amino acid described by Lehninger (1975), are summarized in Table 4 and yield an atomic O:N ratio of 6.8, if all amino acids are degraded in proportion to their presence in the proteins (data from Cowey & Corner 1963, Table 2).

To be realistic, such computation should consider in some detail the ammonia-forming mechanisms and their relations with intermediary metabolism. As shown in Fig. 3, body protein level is regulated by the balance between the breaking down of tissues (catabolism) and the building up of tissues (anabolism). The free amino-acid pool represents the available supply of these substances throughout the body fluids and tissues which is replenished by catabolic processes, absorption from the gut and by synthesis (for non-essential amino-acids). In starvation only catabolic processes are maintained and the pool acts as a buffer between protein degradation and requirements of intermediary metabolism (energy, production of enzymes and other special molecules). Such a role is related to the non-random degradation of body protein during nutritional imbalance or starvation. In fish, it has been shown that albumins are metabolized first, followed by α and β globulins but not by γ-globulins (Sorvachev 1957, Dave et al. 1975). In the muscle, contractile fibers (especially myosin) are broken down much more rapidly than collagen or elas-

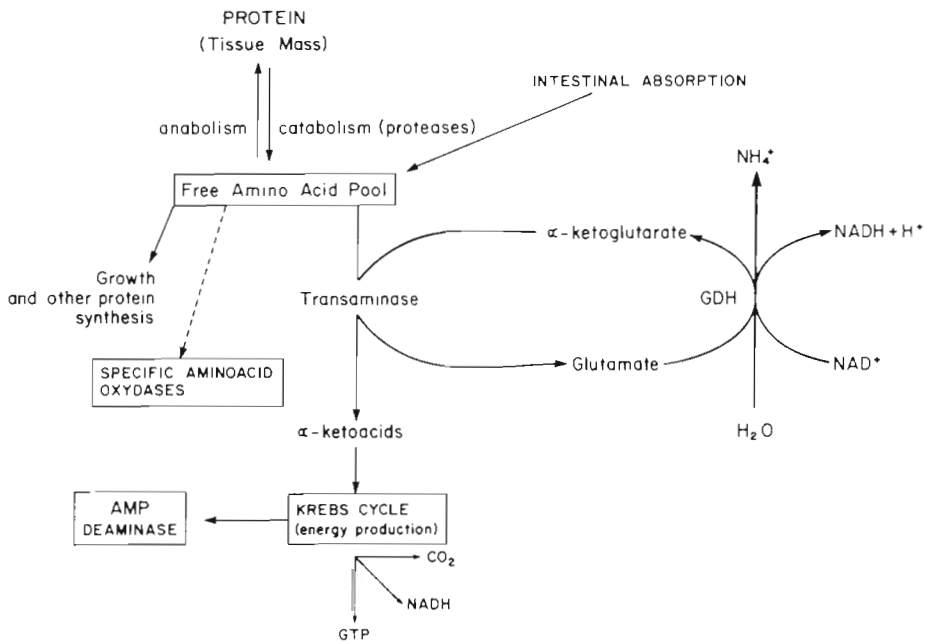

Fig. 3. Transdeamination pathway and nitrogen metabolism (modified from Bidigare 1983)

tin (Creach & Serfaty 1974), which makes sense from an energy conservation point of view and preserves vital organs and molecules.

Within the free amino-acid pool, catabolism is also a non-random process. Mammals (Felig et al. 1969), reptiles (Coulson & Hernandez 1983), fishes (Van Waarde 1983), insects (Stanilawski et al. 1979) and crustaceans (Wieser & Schweiser 1972, Herz-Heubner et al. 1973, Fair & Sick 1982) appear to dispose of non-essential amino-acids rapidly and essential ones slowly. This phenomenon suggests a general mechanism for conserving those amino-acids which cannot be manufactured by the organism. From data obtained by Zandee (1966), Cowey & Forster (1971), Shewbart et al. (1972), Van Marrewijk & Zandee (1975), Lasser & Allen (1976), Claybrook (1976) and Armitage et al. (1981) benthic or planktonic crustaceans should catabolize first arginine, glutamine, proline, glutamate, asparagine, aspartate, alanine, cysteine, glycine and serine, i.e. those acids which enter the Krebs cycle through α-ketoglutarate, oxaloacetate or pyruvate.

Besides the composition of the free amino-acid pool, we should also consider the different catabolic pathways involved in amino-acid deamination and ammonia production. As indicated in previous reviews (Campbell 1973, Mayzaud 1986), amino-acid catabolism proceeds through 4 possible pathways: transamination, transdeamination, specific oxidases and adenylate deamination. As indicated by Campbell (1973) most L-amino-acids can undergo transamination reactions either via glutamate-pyruvate transaminase (GPT) or via glutamate-oxaloacetate transaminase (GOT), i.e.

L-alanine + α-ketoglutarate ⟷ L-glutamate + pyruvate (GPT)

or

L-aspartate + α-ketoglutarate ⟷ L-glutamate + oxaloacetate (GOT).

Neither of these reactions yields ammonia but they insure the transfer of an amino-group to an α-ketoacid and the formation of L-glutamate. The removal of the α-aminogroup from glutamate as ammonia will involve an oxidative deaminative pathway (Fig. 3) catalyzed by glutamate dehydrogenase (GDH). The results obtained by Bidigare & King (1981), Bidigare et al. (1982), King (1984), Park (1986) and Park et al. (1986) for various species of zooplankton and King et al. (1985) or Batrel & Regnault (1985) for other crustaceans have shown: (1) NAD-dependent GDH operates in the oxidative deamination mode; (2) GDH activity and ammonia excretion rate are significantly correlated under various nutritional conditions (feeding and the first few days of starvation); (3) GDH activity could account for the ammonia produced; (4) GDH seems to be controlled by allosterism and fluctuations in the internal pool of glutamate. Most authors have concluded that GDH is one of the key enzymes in the catabolic pathway of ammoniogenesis. Such a conclusion is supported by the fact that the direct oxidation of amino-acids is mainly encountered in molluscs and is a poor substitute for transdeamination because it is inactive with several common amino-acids, e.g. glutamate, alanine, aspartate, proline, glycine, serine, etc. (Campbell 1973). Similarly, adenylate (AMP) deaminase has been found to be inversely related to the glutamate dehydrogenase activity in fish and polychaete annelids.

Under starvation it seems likely that excess glutamate is channelled into the Krebs cycle in the same way as those acids related to α-ketoglutarate, namely, arginine, histidine, glutamine and proline. Assuming that these different amino-acids are mobilized first, the resulting minimum O:N ratio should then lie between 3 and 3.5. Obviously different values will be observed for animals catabolizing amino-acids through other pathways. The recent finding by Batrel & Regnault (1985) and Park (1986) that GDH activity and ammonia excretion remains correlated during the early period of starvation, in shrimp and copepods, reinforces the claim that GDH is a key enzyme in the ammonia-producing pathways. The opposite changes observed by the same authors during prolonged starvation suggested a shift to AMP deamination to prevent a depletion of the energy charge (Chapman & Atkinson 1973, Raffin 1983) and confirmed that a change in the dominant pathway suppressed the relationship between excretion rate and GDH activity.

These theoretical computations illustrate that the minimum value of O:N is directly related to the nature of the amino-acids actually entering the Krebs cycle. Without detailed data on catabolism, values between 3 and 16 would be possible for protein-dominated catabolism.

Similarly, to compute the theoretical range of values for the O:N ratio with approximatively equal utilization of lipid and protein, we must consider the catabolic pathway of fatty acids. Such reactions are summarized in Table 4 and show that fatty acids produce acetyl CoA as end-product of β-oxydation. Assuming that the average fatty acid of neutral lipids has 16 to 18 carbons (Lee et al. 1971), the oxidation of the $NADH_2$ and $FADH_2$ produced would require 14 to 16 atoms of oxygen and yield 8 to 9 moles of acetyl CoA. Under starvation, we can assume that the acetyl CoA would also be channelled into biological oxidation and thus require 46 to 52 atoms of oxygen. Catabolism of equal amounts (molar basis) of glutamate and C16 fatty acids would then correspond to:

$$\text{Palmitoyl CoA} + 23\ O_2 + 131\ Pi + 131\ ADP \rightarrow CoA + 16\ CO_2 + 146\ H_2O + 131\ ATP$$

$$\text{Glutamic acid} + 3/2\ O_2 + 11\ Pi + 11\ ADP \rightarrow CO_2 + 12\ H_2O + NH_3 + 11\ ATP$$

giving an O:N ratio of 49. Similar calculation with leucine would produce a ratio of 54. With C18 (stearyl CoA) fatty acid, the ratio would vary between 55 and 60. Depending on the amino-acid and the fatty acid involved, a range of values between 50 and 60 should then be considered as typical for equal catabolism of fat and protein.

O:N RATIO AND METABOLIC ACTIVITY

Under natural conditions, a healthy, growing individual must display a positive nitrogen balance. Considering the metabolic pathways in Fig. 3, the dietary nitrogen intake must exceed that needed for growth and excretion (fecal and fluid). Because zooplankton use proteins as energy sources, the definition of the nitrogen requirement must consider the supply of amino-acids for growth and the input of carbon intermediates either for direct energy production or for lipid synthesis. Thus, the natural changes in O:N ratio will not only reflect the type of metabolic substrate oxidized but also the metabolic relationships between the animals and their food supply (Conover & Corner 1968, Snow & Williams 1971, Ikeda 1974, 1977c, Le Borgne & Dufour 1979, Le Borgne & Roger 1983, Ikeda & Dixon 1984).

Theoretical considerations

Starting from the elemental composition of the marine particulate matter, a theoretical O:N ratio of 17 has been computed, assuming that complete oxidation of particulate matter proceeds in zooplankton according to the Redfield et al. (1963) ratios, i.e. 276 atoms of oxygen are required to fully oxidize 106 atoms of carbon, 16 atoms of nitrogen and 1 atom of phosphorus (Harris 1959, Conover & Corner 1968, Corner & Cowey 1968, Le Borgne 1973, Ikeda 1977c). However, the oxidative reactions used by Redfield et al. (1963) included the oxidation of ammonia to nitrate, a reaction which never takes place in animals. A different type of computation, also based on elemental composition of the organic matter oxidized, was given by Ikeda (1977c). The theoretical values of O:N for the catabolism of carbohydrates, lipid and protein were respectively infinity, 415 and 6.8, but assumed similar nitrogen metabolic pathways for fed and starved individuals and depended on (1) oxidation of glycerophosphate-nitrogen moieties of phospholipids which does not take place under normal conditions, and (2) usage of constitutive oxygen as an oxidizing agent which is impossible. An alternative method considers that the average C:N ratio of the particulate matter is 106/16 = 6.6 from which, assuming that all amino acids are deaminated and all carbon oxidized, the average O:N ratio should be 13. The data obtained by Conover & Corner (1968) or Conover & Mayzaud (1976) illustrate how illusory these limits may be, as the O:N ratios measured varied from less than 20 to more than 50 for copepods feeding on particles with a C:N ratio around 6.

It seems more than probable that the actual value of the O:N ratio depends not so much on the chemical composition of the food as on the use made by the animals of each biochemical fraction assimilated. Thus the O:N ratio may be directly or indirectly related to the food supply, depending whether the organic substrates are used directly in the intermediary metabolism or used through anabolic pathways. In terms of processes, amino-acids do not accumulate in the gut lumen but are rapidly assimilated and, in part, transaminated into a limited number of nitrogen carriers. In fishes and reptiles these are glycine, alanine, aspartate and glutamate (Coulson & Hernandez 1983, Chew & Ip 1987), while in crustaceans the main products are glycine, alanine, aspartate, glutamate, serine, tyrosine, proline and hydroxyproline (Zandee et al. 1958, Huggins 1966, Zandee 1966, Van Marrewijk & Zandee 1975).

The fate of assimilated nitrogen depends essentially on the nutritional situation faced by the animal: (1) If the intake of protein is smaller than required per unit time, essential, as well as non-essential, amino-acids are continually removed from the pool for growth as well as the production of vital molecules (enzymes, hormones, etc.). Dietary carbohydrates and lipids become increasingly important as sources of energy and spare amino-acids from general oxidative purposes (Huggins & Munday 1968). (2) If the intake of protein is unbalanced, i.e. lacking in one or several essential amino-acids, the organism finds itself with a supply of amino-acids which, in a large part, cannot be utilized for synthesis. Consequently, they will be deaminated and the nitrogen excreted without corresponding use of α-keto-acids in the energy-producing pathways (West & Todd 1961). If the intake of protein exceeds the daily requirements then the keto-acids are used for the synthesis of neutral lipids (Van Waarde 1983, Sargent & Henderson 1986). So, only when the intake of protein is complete in terms of essential acids and in equilibrium with the metabolic demand will the O:N ratio reflect the catabolism of the food assimilated.

O:N ratio and seasonal changes

The relative scarcity of data on nitrogen metabolism in natural zooplankton makes it difficult to ascertain the real applicability of the processes described above. Nevertheless, a review of existing literature suggests that such generalizations show a fair level of validity. Conover & Corner (1968) indicated that large, primarily

herbivorous, calanoid copepods – storing considerable quantities of fat – displayed seasonal changes of the O:N ratio related mainly to their life cycle and to the variations in their biochemical composition. Thus *Calanus hyperboreus, C. finmarchicus* or *C. glacialis* showed changes in O:N ratio associated with periods of either lipid synthesis (high deamination activity, with a high nitrogen excretion level) or lipid consumption (fall and winter diapause) but not usually with food supply in the short run (Conover & Corner 1968, Båmstedt & Tande 1985). On the contrary, more omnivorous species, such as *Metridia longa,* with lower lipid stores showed little seasonal change in O:N ratio; both respiration and nitrogen excretion were more closely related to temperature and to the immediate food supply.

In a similar study on small neritic, omnivorous, copepod populations, Conover & Mayzaud (1976) confirmed the lack of marked seasonal changes, but described a complex pattern of variation. Seasonality of the O:N ratio is shown in Fig. 4 along with some measures of potential food supply such as chlorophyll (phytoplankton) and particulate organic carbon and nitrogen (total particulate organic matter). The average value of the ratio ranged from 30 to 50 most of the time but displayed several significant deviations from the long-term average. The value dropped below 30 in September and in November–December, and below 25 in February, again for deep-water animals in March and for the upper level animals in May. In each of these cases the nitrogen released as ammonia was dispropor-

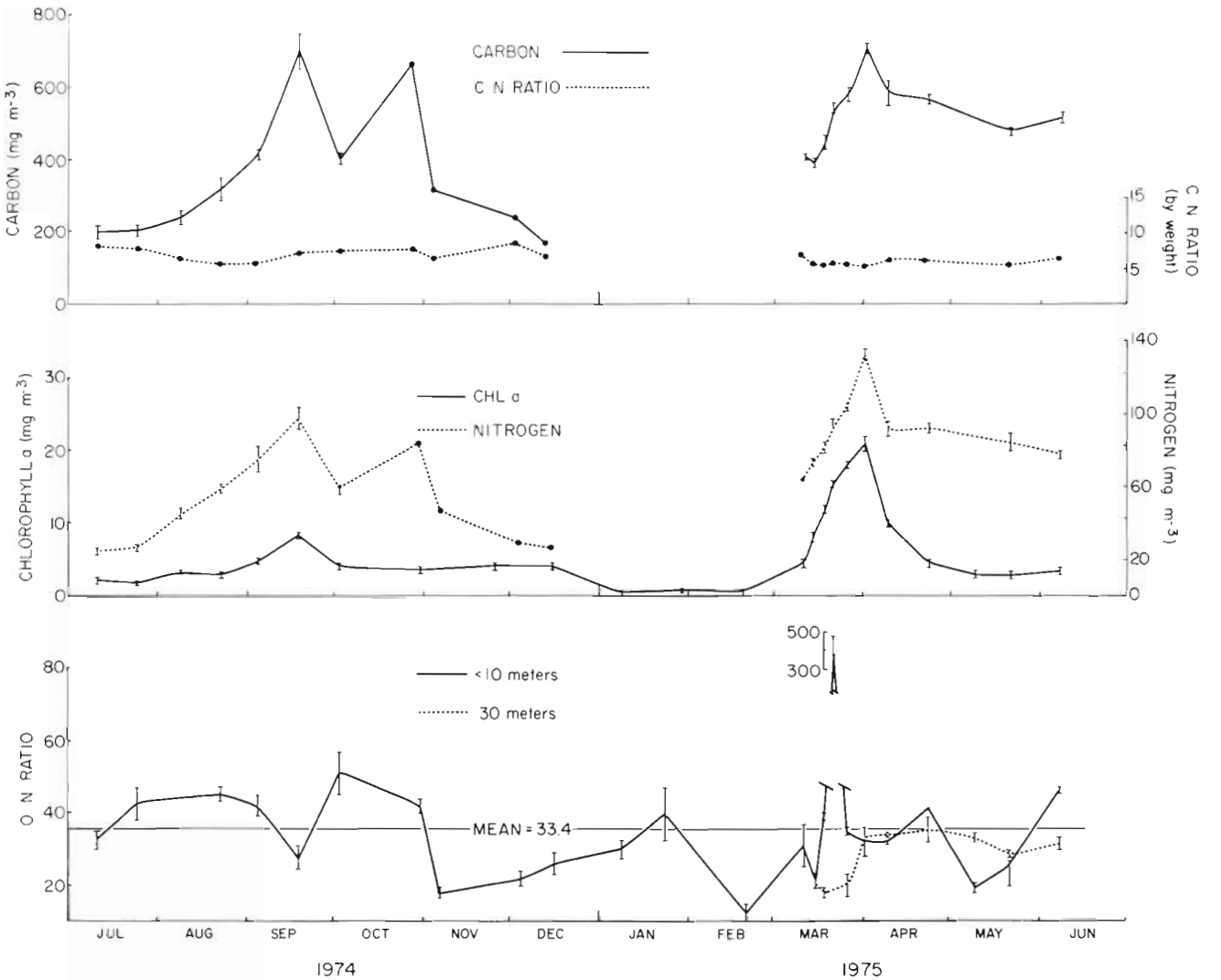

Fig. 4. Seasonal variation in Bedford Basin of POC, PON, chlorophyll, and C:N ratio of potential food supply (particulate organic matter) of the total copepod population and their corresponding O:N ratios. (Data from Conover & Mayzaud 1976 and Taguchi & Platt 1978)

tionately higher than would have been expected at that season if temperature or food levels were the only factors controlling the catabolic processes.

Potential food supply showed a marked seasonality with peaks in chlorophyll corresponding to spring and fall blooms of phytoplankton. Chlorophyll and nitrogen concentrations were greater in spring although the amount of carbon was roughly the same in both seasons. Fall increases in carbon and nitrogen, or prolonged peaks following the spring bloom, each occurring during periods of relatively low or decreasing chlorophyll, emphasized the importance of changes in quality. Each of these periods was accompanied by dramatic changes in particle size spectra, as measured with a Coulter Counter. Grouping of similar size spectra by principal component analysis (Mayzaud et al. 1984) showed that, in terms of size-heterogeneity, the seasonal changes of the particulate matter were more complicated than would have been expected from their elemental and chlorophyll composition (Fig. 5). Four size groups of particles accounted for the seasonal variability, and their relative importance in the spectrum at each time defines the seasonal cycle (Fig. 5, lower). Transition between these different periods corresponded to observations made on July 11, September 18, May 9 and 21. Coincidence between these dates and those for which low values of the O:N ratio were observed strongly suggests that, besides food quantity, the ratio was also influenced by changes in food quality. A positive correlation between O:N and Amylase:Trypsin ratios, as found by Gaudy & Boucher (1983), implies that the ease with which the particulate food is digested could be a factor in the value of the metabolic index.

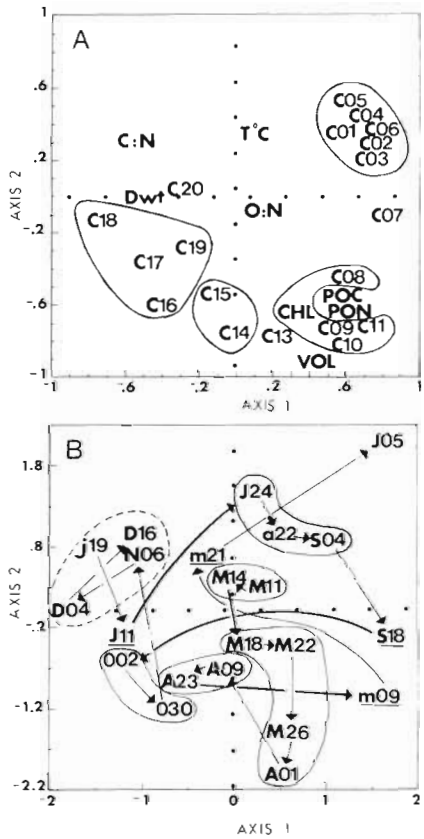

Fig. 5. Principal component analysis of correlation matrices between Channels C02 to C19 (2.52 to 128 µm equivalent diameter), with projection of Channels C01 and C20, chemical parameters of the particles (POC, PON, VOL, CHL), O:N ratio and dry weight (Dwt) of zooplankton and temperature (T°C) as 'supplementary variables'. (A) Factor loading on selected axes (but grouping of channels made considering the first 3 axes) C12 is hidden behind C09. (B) Factor scores; circled periods of time are typically associated with dominant size of particles. VOL: total volume of particles; CHL: chlorophyll *a*; POC: particulate organic carbon; PON: particulate organic nitrogen; C:N: carbon-to-nitrogen ratio of the particles; Dwt: dry weight per animal of the population. J, June; j, July; M, March; m, May; A, April; a, August; S, September; O, October; N, November; D, December

Seasonal changes in O:N ratio may show no clear relationship with food supply or body biochemical composition. Ikeda & Bruce (1986) found that, for animals of similar size, early summer krill *(Euphausia superba)* had a higher O:N ratio and lower body C:N ratio than mid-summer krill, despite higher concentrations of phytoplankton. The authors suggest that because *E. superba* has lived through a long winter of food shortage, at the start of feeding in early summer most of the amino-nitrogen assimilated is used for replenishing body protein rather than for meeting oxidative requirements.

O:N ratio and nutritional status

The effect of feeding on the O:N ratio has been demonstrated experimentally only for a few zooplankton species. Ikeda (1977c) studied the influence of constant food conditions for several herbivorous and carnivorous species over periods of several days to a month. The pattern of changes were different between fed and starved animals and depended on the type of substrate catabolized. Ikeda observed anomalous O:P and N:P ratios suggesting utilization of carbohydrates in starved *Calanus plumchrus* where, in fact, carbohydrate reserves were negligible. These observations serve as an example of the type of error which can be introduced by the use of only elemental composition data of the organic matter in the computations. In a more recent study, Ikeda & Dixon (1984) clearly established the influence of the nature of the food ingested and, as expected, found that phytoplankton-fed, or starved, krill displayed higher O:N ratios than individuals fed *Artemia* nauplii. The progressive decrease

in O:N with greater ingestion rate of the diatom *Fragilariopsis antarctica*, which they also observed, was probably related to increased deamination associated with excess protein intake, rather than greater utilization of the protein fraction of the algae as a metabolic substrate.

Effects of alternating periods of feeding and starvation on metabolic indices were studied by Hiller-Adams & Childress (1983b) in the carnivorous, bathypelagic mysid *Gnathophausia ingens*. The use of large lipid reserves, characteristic of this species, explained an increasing O:N ratio with increasing duration of starvation. In contrast, feeding shrimp meat induced a sharp decrease in the O:N value which varied somewhat with the previous 'trophic experience' of the animals. Under such experimental conditions there is a clear link between nitrogen excretion and protein content of the food assimilated.

O:N ratio and temperature acclimation

Conover & Corner (1968) suggested that *Calanus hyperboreus* stage V, containing much stored lipid, might have an increasing O:N ratio with increasing temperature while those which were lean might have a decreasing one. On the other hand, Conover & Mayzaud (1976) reported no correlation between O:N ratio and *in situ* temperature for populations of small neritic copepods, which might be evidence for short-term temperature acclimation. Two planktonic crustaceans with relatively low levels of lipid reserve, *Acartia clausi* (Copepoda) and *Meganyctiphanes norvegica* (Euphausiacea), showed opposite responses with increasing temperature (Fig. 6). As noted by Conover & Corner (1968) and Galkovskaya & Eismont-Karabin (1981) planktonic organisms do not behave in a consistent manner with temperature variation. Other species, *Phronima sedentaria* (Amphipoda) and *Sagitta setosa* (Chaetognatha), did not show significant variations with temperature (Mayzaud unpubl.) further confirming that O:N ratio does not reflect mechanisms of temperature adaptation in an easily interpretable way.

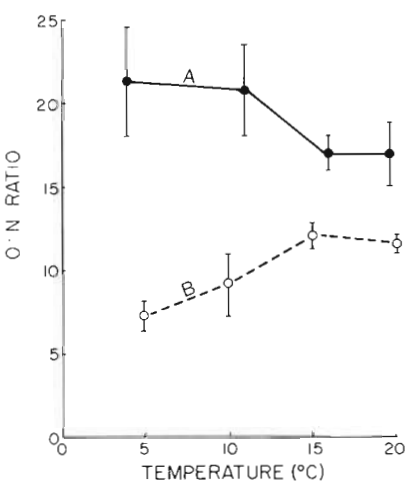

Fig. 6. Changes in O:N ratio with temperature. (A) *Meganyctiphanes norvegica*; (B) *Acartia clausi*

Most experiments have considered the acclimation to short-term temperature changes rather than long-term adaptation. Ikeda (1974) showed that the O:N ratios from tropical, subtropical and temperate species were generally lower than those from boreal species. He concluded that the ratio was affected most by the temperature of the habitat from which the animals were collected. Such a conclusion is consistent with observations on fishes that adaptation to low temperature increases the relative capacitiy of the organism for lipid oxidation (Van Waarde 1983). Unfortunately, because the measurements were made with starving animals, and boreal species accumulate more lipids than tropical or sub-tropical ones, it is not clear whether the results express 'true' adaptation of natural populations or simply an effect of starvation on animals with small lipid reserves.

The complexity of the processes which determine the ratio makes it difficult to understand the influence of physical parameters such as temperature. As suggested by Quetin et al. (1980), short-term variability can be influenced by a differential response to the laboratory or stress-causing experimental conditions. Muravskaya & Belokopytin (1975, cited in Quetin et al. 1980) showed that decreasing experimental temperature increased the O:N ratio for standard metabolism and lowered it for active metabolism in the black sea bass *Spicaria smaris*. Similar behaviour could explain why changes in O:N ratio with temperature for different zooplankton are not consistent. Moreover, the difficulty of recognizing what may be abnormal activity for small copepods could lead to extremely low O:N values (1 to 4), such as those reported by Harris (1959), Nishizawa et al. (1969), Taguchi & Ishii (1972) or Mayzaud (1973), perhaps caused by high metabolic activity in nutritionally unbalanced, natural populations under stress, yielding an artificially high excretion rate.

CONCLUSIONS

The use of metabolic indices such as the O:N ratio requires a general conceptual framework for proper interpretation. Our knowledge of the processes involved in the intermediary and nitrogen metabolism of zooplankton is still very limited, but the increasing number of studies on their enzymatic equipment allowed us to make some inference from the larger

body of literature on fishes and crustaceans with a certain degree of confidence. Further work is needed to evaluate the proposed hypotheses and to verify the generality of the processes.

Observations from starved and fed organisms reveal that different pathways of nitrogen metabolism feed the oxidative system. The existence of 2 pathways emphasizes that the O:N ratio is regulated by the fate of each biochemical fraction being catabolized and not by the elemental composition of the food or the animal itself.

A certain degree of generalization can be made if we consider that the O:N ratio reflects the overall balance of the animal metabolism. Such balance will be related, on one hand, to the internal energy requirements of the organism and, on the other hand, to various external variables. Under experimental starvation the external factors are usually kept constant, but not the internal requirements. Most zooplankters will initially rely on their energy reserves to maintain a certain degree of independence in their respiratory rate (e.g. Mayzaud 1976, Hiller-Adams & Childress 1983a, b) at the expense of their body constituents (lipid or protein). Species with large lipid storage are able to maintain some independence for prolonged periods of time while species without reserves are unable to maintain metabolic homeostasis for more than a few days (Nival et al. 1974, Mayzaud 1976, Ikeda & Skjoldal 1980).

Under natural conditions both external and internal factors are variable and, over seasons, any factors which induce a modification in the covariation of the 2 metabolic processes will cause a change in the ratio. Key factors will be the level of energy requirements and the nutritional status of the individuals or the population. Trophic history, and quantity and quality of food supply – because they exert a strong influence on the nitrogen metabolism – provide essential information for proper interpretation of observed changes.

The results to date strongly suggest that animals storing large quantities of energy reserves, and displaying a degree of independence from sudden change in food supply, will show variation in O:N ratio mainly associated with the seasonal cycle of synthesis and degradation of their reserves, which will be reflected by changes in their biochemical composition. In contrast, populations with a protein-orientated metabolism will strongly react to the changes of their nutritional environment. The lack of clear variation in neritic zooplankton populations probably reflects a more or less steady food supply, supplemented by non-living particles. Temporal or spatial instability in the quality of the food, depending on its duration, may stress certain components of the zooplankton population beyond their capacity to adapt, reducing assimilation (Mayzaud & Mayzaud 1985) and yielding lower O:N ratios.

The study of the processes involved in nitrogen- and oxidative-metabolism, as shown by the activity of key enzyme systems such as GDH and ETS, already yields measurements of O:N ratio, free from artifacts induced by stress of capture or incubation (Bidigare et al. 1982). Such a complementary approach might clarify our view of how planktonic organisms acclimate to environmental change. Used in conjunction with information on the life history, biochemical composition and food supply, an O:N ratio readily describes the nutritional status of natural populations and provides one of the few tools to assess zooplankton metabolism. Whether O:N ratios should be based on ammonia or total excretion, especially when large amounts of organic nitrogen are released (Webb & Johannes 1967, Eppley et al. 1973), cannot be decided until the sources and mechanisms of such excretion are known. Because of this uncertainty, it is probably preferable to use only values of excreted ammonia to compute O:N ratios at this time.

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