

C and N gross growth efficiencies of copepod egg production studied using a Dynamic Energy Budget model

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*Simple stoichiometric models based on the principle that limiting elements are used with high efficiency have been unable to capture the apparently constant and low nitrogen gross growth efficiency that characterizes egg production in marine copepods. A new model of egg production is presented based on Dynamic Energy Budget theory. The model splits substrates between nitrogenous and non-nitrogenous compounds, distinguishes between structural and reserve components of animal biomass, and requires that assimilated substrates are utilized to meet maintenance costs prior to allocation for egg production. Carbon and nitrogen gross growth efficiencies of egg production by *Acartia* in response to food C:N are predicted realistically. Production tends to be maximized when consuming N-rich food, indicating a general N-limitation, the benefits being greatest at high intake rates. However, food carbon also plays a role in regulating egg production by supporting protein-sparing biochemical pathways. The model indicates that zooplankton are unable to utilize dietary N efficiently for egg production, even when it is scarce in the diet, because of the N demands for maintenance by the adult. The work emphasizes the need to consider the C and N requirements for maintenance in order to understand dietary constraints to mesozooplankton production.*

INTRODUCTION

The ability to quantify the efficiency with which grazers utilize ingested prey items for growth and reproduction is central to the development of mathematical models for studying the flows of carbon and nutrients in ecosystems. Quantifying growth efficiency requires an understanding of the nutritional aspects of food which limit production. Evidence to support nutrient element limitation of freshwater zooplankton is strong, particularly for *Daphnia* spp. where the potential for phosphorus limitation is well documented (Sternner and Hessen, 1994; DeMott *et al.*, 1998). The situation is less clear in the case of marine zooplankton. Strong correlations between egg production of copepods and food N have been shown in laboratory experiments (Checkley, 1980; Kiørboe, 1989). At first sight such results support the case for N-limitation in these organisms. However, correlation does not of itself conclusively demonstrate a causal limitation by the nutrient element (Brett, 1993).

The potential limiting effects of C and N in marine zooplankton can be examined using simple stoichio-

metric models (Anderson, 1992; Anderson and Hessen, 1995). In these models, the limiting element is identified as the one in least supply relative to demand. A threshold elemental C:N ratio in food items, φ_F^* , can then be defined, below which C limits production, and above which N limits:

$$\varphi_F^* = \frac{\varphi_Z K_N^*}{K_C^*} \quad (1)$$

where K_N^* and K_C^* are the maximum (upper bound) efficiencies with which N and C (respectively) can be used for production of eggs, and φ_Z is the C:N ratio of zooplankton biomass. The limiting element is assumed to be used with maximum efficiency, with excesses of other elements released as waste products. Deciding on appropriate values for K_N^* and K_C^* is, however, not easy. Previous studies have assumed that N and P can be used with a high maximum efficiency, e.g. 1.0 (Anderson, 1992; Urabe and Watanabe, 1992), whereas K_C^* is lower because catabolic processes necessarily result in the evolution of CO₂. The maximum C growth efficiency

based solely on the energetic costs of converting food substrates to biomass is ~ 0.75 (Calow, 1977). However, K_C^* also needs to take into consideration other C requirements of organisms, such as the energetic costs of locomotion and the maintenance costs of existent structures.

In the instance of zooplankton consuming food with a typical C:N for marine seston (e.g. the Redfield ratio, 6.625), C-limitation of zooplankton is indicated if values for K_C^* as low as 0.2–0.3 are inserted into equation (1). Stoichiometric theory would, however, require that N growth efficiency increases with increasing food C:N under C-limiting conditions. The experimental evidence shows *Acartia* using N for egg production with a low and constant efficiency of ~ 0.4 (Checkley, 1980; Kjørboe, 1989) and an even lower efficiency of 0.081 throughout the whole life cycle (Jones *et al.*, 2002). These low efficiencies contrast strongly with the high efficiencies which might, at least in theory, be expected for limiting elements. Protein sparing is a well known phenomenon in a range of organisms [e.g. (McGoogan and Gatlin, 1999; Roxburgh and Pinshow, 2000; Arnould *et al.*, 2001)] as a means of maximizing the utilization of limiting elements for growth. So if marine copepods are limited by elemental N, why do they utilize it with such low efficiency?

Justus von Liebig's 'Law of the Minimum', upon which stoichiometric theory is based, has been repeatedly invoked in plankton ecology (DeBaar, 1994). However, the simplicity and empirical nature of the stoichiometric approach, e.g. the difficulty in prescribing values for K_N^* and K_C^* , render it difficult to apply when examining C versus N limitation of marine copepods. New approaches to modelling resource use by consumers need to consider in greater detail the complex interplay between elements, biochemical arguments and physiology (Kooijman, 1998; Anderson *et al.*, 2004). One such approach is Dynamic Energy Budget (DEB) theory which provides a theoretical framework that is conceptually applicable to all organisms (Kooijman, 2001). Substrates assimilated from food items are directed to reserve pools which in turn are used for processes such as maintenance, growth, development and reproduction. An important concept incorporated into the DEB approach is the 'synthesizing unit' (SU). Enzymatic processes that handle a range of potentially limiting substrates and form one or more products are represented on the basis of probability theory of substrates being attached to binding sites on SUs. In this way smooth transitions between states between different dominant limiting factors can be represented (Kooijman, 1998).

Here we use a DEB model which includes SUs to investigate how the production of marine copepods is

influenced by C:N ratio in food (phytoplankton). Carbon is divided between nitrogenous substrates (proteins, nucleic acids: P) and non-nitrogenous substrates (carbohydrates, lipids: H) (Anderson, 1992). Substrates are firstly utilized for maintenance of the adult, with those remaining being available for egg production. Utilization follows two pathways: a P -only pathway in which energetic demands are met using P , and a PH pathway in which energetic demands are met using H , thereby sparing P . The relative dominance of the two pathways depends on the availabilities of P and H and the kinetics of the SUs. The limiting roles of C and N in egg production thus manifest themselves in this advanced treatment of P and H utilization, rather than on the basis of elemental ratios as in simple stoichiometric models. The model is calibrated to fit the experimental data of Kjørboe (Kjørboe, 1989), and results discussed in context of the ongoing debate regarding the roles of C and N in the nutrition of marine zooplankton.

MODEL DESCRIPTION

The model (Figure 1) is based on DEB theory (Kooijman, 1995). Assimilated food is passed to the reserve pools, from where it is available for use by SUs to meet the demands for maintenance and egg production by the organism. A list of model variables and parameters is provided in Table I. DEB notation is used throughout.

Ingestion and assimilation

Carbon is ingested at a constant rate $j_{C,I}$ [mol C (mol C) $^{-1}$ h $^{-1}$]. This C is divided between nitrogenous substrates (proteins, nucleic acids: P) with a fixed C:N ratio, φ_P , and non-nitrogenous substrates (carbohydrates, lipids: H). The biochemical composition of phytoplankton (the food) is variable in nature depending on growth conditions. Nitrogen-deficient cells usually exhibit low protein to carbohydrate ratios (high C:N ratios) reflecting accumulation of storage carbohydrates (Barlow, 1982). These compounds are assimilated by the predator much more easily than structural carbohydrates (Anderson, 1994). The application of a single assimilation efficiency for C in a model being used to examine the influence of food C:N on growth is therefore inappropriate. We employ the model of Anderson (Anderson, 1994) which, based on data for diatoms, divides C into four fractions, soluble (storage) and insoluble (structural) carbohydrates, lipids and proteins (H_1 , H_2 , L , P respectively), with assimilation efficiencies of 0.881, 0.487, 0.373 and 0.688 respectively. Non-nitrogenous substrates, H , incorporate the sum of H_1 , H_2 and L . Equations for these components as fractions of

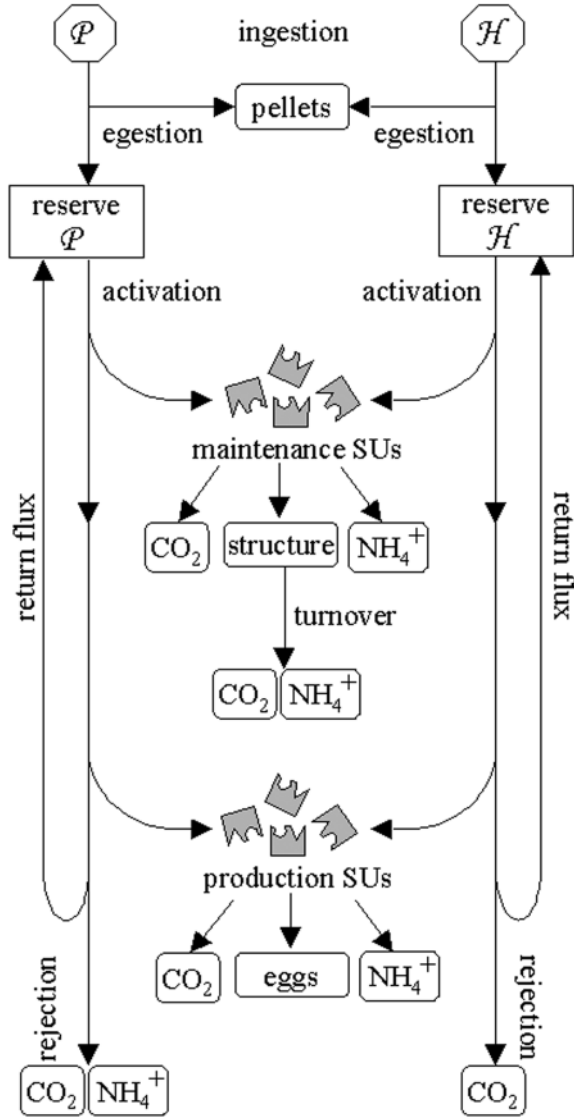


Fig. 1. Flow diagram of model illustrating how ingested proteins (P) and carbohydrates (H) are passed to reserve pools, and subsequently utilized by SUs for maintenance and egg production.

total cell C, $\theta_{F,P}$, $\theta_{F,H1}$, $\theta_{F,L}$, $\theta_{F,H2}$, are (Anderson, 1994):

$$\theta_{F,P} = \frac{\varphi_P}{\varphi_F} \quad (2a)$$

$$\theta_{F,H1} = 0.0232\varphi_F + 0.0118 \quad (2b)$$

$$\theta_{F,L} = 0.141(1 - \theta_{F,H1}) \quad (2c)$$

$$\theta_{F,H2} = (1 - \theta_{F,H1})(1 - 0.141) - \theta_{F,P} \quad (2d)$$

where φ_F is the total C:N of ingested food. Amounts of P and H assimilated [$j_{P,A}$, $j_{H,A}$, mol C (mol C) $^{-1}$ h $^{-1}$] are then:

$$j_{P,A} = 0.688\theta_{F,P}j_{C,I} \quad (3a)$$

$$j_{H,A} = (0.881\theta_{F,H1} + 0.487\theta_{F,H2} + 0.373\theta_{F,L})j_{C,I} \quad (3b)$$

These empirical relationships break down for $\varphi_F < 5$. Assimilated substrates enter reserve pools, m_P and m_H , quantified as densities normalized to structure. A novel feature of the model is that it includes separate reserve pools for P and H , unlike previous DEB models which have only a single reserve [e.g. (Muller *et al.*, 2001)]. The two reserve pools are subsequently mobilized to meet the requirements for maintenance and egg production at constant rates k_E (h $^{-1}$). Unassimilated substrates are egested as pellets.

Stoichiometry

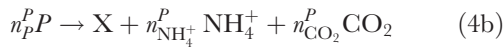
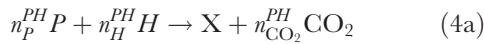
DEB theory divides the biomass of organisms between structure and reserves. Structure is permanent and has an associated maintenance cost, which is met using reserves. A similar concept is embraced by Droop cell quota models, in which the minimum subsistence quota represents structural material, the remainder operating as an active pool available for growth (Droop, 1983; Zonneveld, 1996). It is common to think of the primary reserves in animals as carbohydrates and lipids, such as triacylglycerol in high-latitude copepods [e.g. (Swadling *et al.*, 2000)]. However, reserves of protein also play major functional roles in organisms, particularly for reproduction [e.g. (MacCluskie and Sedinger, 2000; Telang *et al.*, 2002)]. Moreover, the very fact that organisms such as marine zooplankton typically have high N excretion rates reflects the extensive use of metabolically active nitrogenous compounds for respiration.

The model is set up here for egg production of adult *Acartia tonsa*. Zero growth of female structure is assumed, and so structural biomass is not explicitly included as a state variable in the model. Available substrates are allocated to reproduction after maintenance costs have been met. Changes in body weight of adult females can occur in reality (Hirst and McKinnon, 2001). Such changes are possible in the model due to changes in the size of reserve pools. Eggs, although composed largely of reserve material, typically show little variation in C:N [e.g. (Anderson and Pond, 2000)] and so egg production can be treated in the same way as production of structure in other DEB models. We therefore assume that maintenance and egg production (biomass products denoted X in our terminology) both conform to the same

Table I: List of variables and parameters

Symbol	Description	Value	Units
H	carbohydrate reserve biomass	variable	mol C (mol C) ⁻¹
$j_{C,I}$	carbon intake	variable	mol C (mol C) ⁻¹ h ⁻¹
$j_{CO_2,M}$	CO ₂ produced in maintenance	variable	mol C (mol C) ⁻¹ h ⁻¹
$j_{CO_2,G}$	CO ₂ produced in egg production	variable	mol C (mol C) ⁻¹ h ⁻¹
$j_{H,A}$	assimilated H	variable	mol C (mol C) ⁻¹ h ⁻¹
$j_{H,G}^g$	H assigned to egg production	variable	mol C (mol C) ⁻¹ h ⁻¹
$j_{H,G}$	H used for egg production	variable	mol C (mol C) ⁻¹ h ⁻¹
$j_{H,M}$	H used for maintenance	variable	mol C (mol C) ⁻¹ h ⁻¹
$j_{H,R}$	H rejected by production SUs	variable	mol C (mol C) ⁻¹ h ⁻¹
$j_{NH_4^+,G}$	NH ₄ ⁺ produced in egg production	variable	mol N (mol C) ⁻¹ h ⁻¹
$j_{NH_4^+,M}$	NH ₄ ⁺ produced in maintenance	variable	mol N (mol C) ⁻¹ h ⁻¹
$j_{P,A}$	assimilated P	variable	mol C (mol C) ⁻¹ h ⁻¹
$j_{P,G}^g$	P assigned to egg production	variable	mol C (mol C) ⁻¹ h ⁻¹
$j_{P,G}$	P used for egg production	variable	mol C (mol C) ⁻¹ h ⁻¹
$j_{P,M}$	P used for maintenance	variable	mol C (mol C) ⁻¹ h ⁻¹
$j_{P,R}$	P rejected by production SUs	variable	mol C (mol C) ⁻¹ h ⁻¹
$j_{X,G}$	X produced in egg production	variable	mol C (mol C) ⁻¹ h ⁻¹
$j_{X,M}$	X replaced in maintenance	variable	mol C (mol C) ⁻¹ h ⁻¹
k_E	reserve mobilization rate	0.10	h ⁻¹
k_M	maintenance turnover rate	0.0041	h ⁻¹
K_C	carbon gross growth efficiency	variable	mol C (mol C) ⁻¹
K_N	nitrogen gross growth efficiency	variable	mol N (mol N) ⁻¹
m_H	reserve carbohydrate density	variable	mol C (mol C) ⁻¹
m_P	reserve protein density	variable	mol C (mol C) ⁻¹
$n_{CO_2}^P$	CO ₂ formed per X produced in P -route	0.33	mol C (mol C) ⁻¹
$n_{CO_2}^{PH}$	CO ₂ formed per X produced in PH -route	0.33	mol C (mol C) ⁻¹
n_H^{PH}	H used per X formed in PH -route	0.71	mol C (mol C) ⁻¹
$n_{NH_4^+}^P$	NH ₄ ⁺ formed per X produced in P -route	0.19	mol N (mol C) ⁻¹
n_P^P	P used per X formed in P -route	1.33	mol C (mol C) ⁻¹
n_P^{PH}	P used per X formed in PH -route	0.63	mol C (mol C) ⁻¹
P	protein reserve biomass	variable	mol C (mol C) ⁻¹
X	biomass: structure, eggs	variable	mol C (mol C) ⁻¹
β_N	average assimilation efficiency of N	variable	mol N (mol N) ⁻¹
β_C	average assimilation efficiency of C	variable	mol C (mol C) ⁻¹
φ_F	C:N of food	variable	mol C (mol N) ⁻¹
φ_P	C:N of proteins	3.7	mol C (mol N) ⁻¹
φ_X	C:N of structural biomass, eggs	5.9	mol C (mol N) ⁻¹
κ	fraction H and P returned to reserves	0.5	–
$\theta_{F,L}$	lipid carbon fraction of diatoms	variable	mol C (mol C) ⁻¹
$\theta_{F,H1}$	soluble carbohydrate fraction of diatoms	variable	mol C (mol C) ⁻¹
$\theta_{F,H2}$	insoluble carbohydrate fraction of diatoms	variable	mol C (mol C) ⁻¹
$\theta_{F,P}$	protein fraction of diatoms	variable	mol C (mol C) ⁻¹
θ_{xy}	fraction of SUs in state xy	variable	–
θ_{xy}^*	steady-state fraction of SUs in state xy	variable	–
ρ_P^P	binding probability of P to $\theta_{..}$	0.66	–
ρ_P^{PH}	binding probability of P to $\theta_{.H}$	0.95	–
ρ_H^{PH}	binding probability of H to $\theta_{..}$	0.66	–
ψ_P	C yield efficiency: reaction P	0.75	mol C (mol C) ⁻¹
ψ_{PH}	C yield efficiency: reaction PH	0.75	mol C (mol C) ⁻¹
Ω_P	steady-state binding state for reaction P	variable	–
Ω_{PH}	steady-state binding state for reaction PH	variable	–

elemental stoichiometry (i.e. both have C:N φ_X). The magnitudes of P and H are specified by normalizing to structural biomass of the females, i.e. with units $\text{mol C} (\text{mol C})^{-1}$. The maintenance term in DEB represents not only the production of biomass to replace that lost through protein turnover, but also other costs to the organism such as the energetic expenditure associated with locomotion, filtration, defence mechanisms, membrane transports and the maintenance of osmotic potential. However, by giving maintenance the same stoichiometry as egg production, we have assumed that protein turnover and associated costs dominate maintenance. The costs of movement and other feeding-related costs in animals have indeed generally been shown to be low (Kjørboe *et al.*, 1985; Flood, 1991; Karasov, 1992). The energetic costs of egg production and maintenance can be met using either H or P as substrates; the former are expected to be the preferred option, thereby sparing P . The following two reactions are possible:



where the parameters n are the yield coefficients which must stoichiometrically balance. The first equation (4a) is the ‘ PH reaction’, i.e. both P and H are reactants, whereas the second (4b) is the ‘ P reaction’. If the efficiencies with which carbon is used to make structure are known for each of the above reactions (efficiencies ψ_{PH} in the PH reaction, and ψ_P for the P reaction, respectively), then the yield coefficients are:

$$n_P^P = \frac{1}{\psi_P} \quad (5a)$$

$$n_{\text{CO}_2}^P = \frac{1}{\psi_P} - 1 \quad (5b)$$

$$n_{\text{NH}_4^+}^P = \frac{1}{\psi_P \varphi_P} - \frac{1}{\varphi_X} \quad (5c)$$

$$n_H^{PH} = \frac{1}{\psi_{PH}} - \frac{\varphi_P}{\varphi_X} \quad (5d)$$

$$n_P^{PH} = \frac{\varphi_P}{\varphi_X} \quad (5e)$$

$$n_{\text{CO}_2}^{PH} = \frac{1}{\psi_{PH}} - 1 \quad (5f)$$

Synthesizing units

The PH and P reactions (equations 4a and b) are controlled by synthesizing units, which merge potentially limiting substrates, in this case proteins and carbohydrates, into metabolic products. SUs are enzymes for which the dissociation rate of enzyme–substrate complexes without transformation is taken to be negligibly small, simplifying their mathematical formulation. Moreover, the kinetics of SUs are based on fluxes rather than concentrations. Using the latter is cumbersome, as they tend to be ill-defined in spatially structured environments, such as living cells. SUs can be used to predict rates of macro-biochemical reactions, depending on the supply rates of substrates. They may also be used to assess contributions of simultaneous metabolic pathways when substrates can be used in different functions. For a detailed description of the SU concept, we refer to Kooijman (Kooijman, 1998).

The SUs for maintenance and production have the same properties, such that the underlying reactions are stoichiometrically identical. Each SU has two binding sites, the first binding P and the second binding H (Figure 2). A reaction proceeds as soon as P is bound to the P binding site. If at that instant the H binding site has

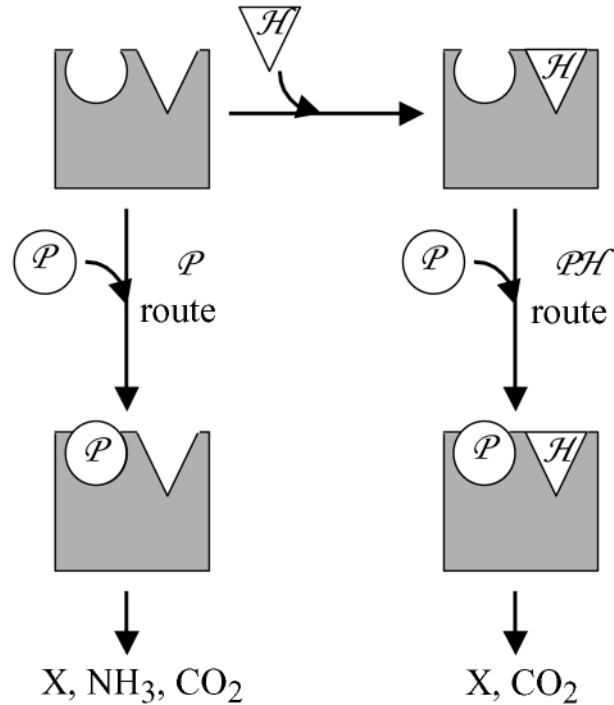


Fig. 2. Conceptual diagram showing binding of P and H by SUs. Products (biomass: X , NH_4^+ , CO_2) are formed as soon as P is bound. Thus if P is bound without H being present then the P pathway is followed (energetic demands met using P), otherwise the protein-sparing PH pathway proceeds (energetic demands met using H).

already been filled, then the PH reaction proceeds. Conversely, if the H binding site is empty, then the P reaction takes place. Binding of P and H to their respective binding sites is controlled by affinity parameters, ρ ($0 \leq \rho \leq 1$). H is bound with affinity ρ_H^{PH} . The protein-sparing PH reaction is favoured over the P reaction in the model by setting parameter ρ_P^P , the affinity for P if H has not yet been bound to the SU, to be lower than parameter ρ_P^{PH} , the affinity for P if H has already been bound. It is assumed that any one organism has a very large number of SUs, such that their binding states can be calculated on a probabilistic basis. Four binding states are possible: $\theta_{..}$ (neither site filled), θ_P (first site filled: P reaction proceeds), θ_H (second binding site filled), θ_{PH} (both sites filled: PH reaction proceeds).

Maintenance

There is a continuous requirement for substrates to meet the ongoing requirements of maintenance. These requirements are met first in the model, and any remaining substrates are then used for egg production. Let the cost of maintenance be k_M [mol C (mol C) $^{-1}$ h $^{-1}$]. Maintenance products are formed when maintenance SUs in binding state θ_P or θ_{PH} release their substrates and revert to $\theta_{..}$. Differential equations for the four binding states are:

$$\frac{d}{dt}\theta_{..} = k_M - \left(\frac{\rho_P^P}{n_P} k_E m_P + \frac{\rho_H^{PH}}{n_H^{PH}} k_E m_H \right) \theta_{..} \quad (6a)$$

$$\frac{d}{dt}\theta_H = \frac{\rho_H^{PH}}{n_H^{PH}} k_E m_H \theta_{..} - \frac{\rho_P^{PH}}{n_P^{PH}} k_E m_P \theta_H \quad (6b)$$

$$\frac{d}{dt}\theta_P = \frac{\rho_P^P}{n_P^P} k_E m_P \theta_{..} - k_M \frac{\theta_P}{\theta_P + \theta_{PH}} \quad (6c)$$

$$\frac{d}{dt}\theta_{PH} = \frac{\rho_P^{PH}}{n_P^{PH}} k_E m_P \theta_H - k_M \frac{\theta_{PH}}{\theta_P + \theta_{PH}} \quad (6d)$$

In this model the total dissociation rate (of θ_P and θ_{PH}) is fixed, because the maintenance requirements, k_M , are fixed. The relative contributions of the P and PH routes in meeting k_M are proportional to the relative magnitudes of θ_P and θ_{PH} at steady state:

$$\theta_P^* = \frac{\rho_P^P}{n_P^P} m_P \quad (7a)$$

$$\theta_{PH}^* = \frac{\rho_H^{PH}}{n_H^{PH}} m_H \quad (7b)$$

The quantities of P and H consumed in maintenance are then:

$$j_{P,M} = k_M (n_P^{PH} \Omega_{PH} + n_P^P \Omega_P) \quad (8a)$$

$$j_{H,M} = k_M n_H^{PH} \Omega_{PH} \quad (8b)$$

where:

$$\Omega_P = \frac{\theta_P^*}{\theta_P^* + \theta_{PH}^*} \quad (9a)$$

$$\Omega_{PH} = \frac{\theta_{PH}^*}{\theta_P^* + \theta_{PH}^*} \quad (9b)$$

Amounts of X produced (h $^{-1}$), ammonium excreted and CO_2 [mol (mol C) $^{-1}$ h $^{-1}$] respired as products of maintenance (normalized to structure) are:

$$j_{X,M} = k_M \quad (10a)$$

$$j_{\text{NH}_4^+,M} = k_M n_{\text{NH}_4^+}^P \Omega_P \quad (10b)$$

$$j_{\text{CO}_2,M} = k_M (n_{\text{CO}_2}^P \Omega_P + n_{\text{CO}_2}^{PH} \Omega_{PH}) \quad (10c)$$

Egg production

The fluxes of P and H assigned to the production SUs are the remainders of the catabolic flux after maintenance is paid:

$$j_{P,C}^g = k_E m_P - j_{P,M} \quad (11a)$$

$$j_{H,C}^g = k_E m_H - j_{H,M} \quad (11b)$$

The production SUs have the same properties as the maintenance SUs. Products are formed as soon as P is bound, whether or not H is present. The fractions of SUs in binding states θ_P and θ_{PH} therefore remain at zero. Contrary to the maintenance SUs, the production SUs dissociate at a rate proportional to the abundance of the substrate-SU complexes. Equations for the $\theta_{..}$ and θ_H binding combinations are:

$$\frac{d}{dt}\theta_{..} = \frac{\rho_P^{PH}}{n_P^{PH}} j_{P,C}^g \theta_H - \frac{\rho_H^{PH}}{n_H^{PH}} j_{H,C}^g \theta_{..} \quad (12a)$$

$$\frac{d}{dt}\theta_H = \frac{\rho_H^{PH}}{n_H^{PH}} j_{H,C}^g \theta_{..} - \frac{\rho_P^{PH}}{n_P^{PH}} j_{P,C}^g \theta_H \quad (12b)$$

giving rise to steady-state fractions:

$$\theta_{..}^* = \frac{\frac{\rho_P^{PH}}{n_P^{PH}} j_{P,C}^g}{\frac{\rho_P^{PH}}{n_P^{PH}} j_{P,C}^g + \frac{\rho_H^{PH}}{n_H^{PH}} j_{H,C}^g} \quad (13a)$$

$$\theta_{.H}^* = \frac{\frac{\rho_H^{PH}}{n_H^{PH}} j_{H,C}^g}{\frac{\rho_P^{PH}}{n_P^{PH}} j_{P,C}^g + \frac{\rho_H^{PH}}{n_H^{PH}} j_{H,C}^g} \quad (13b)$$

The amount of X produced as eggs is:

$$j_{X,G} = j_{P,C}^g \left(\frac{\rho_P^P}{n_P^P} \theta_{..}^* + \frac{\rho_P^{PH}}{n_P^{PH}} \theta_{.H}^* \right) \quad (14a)$$

Amounts of ammonium excreted and CO₂ respired as products of egg production are:

$$j_{NH_4^+,G} = j_{P,C}^g \frac{\rho_P^P n_{NH_4^+}^P}{n_P^P} \theta_{..}^* \quad (14b)$$

$$j_{CO_2,G} = j_{P,C}^g \left(\frac{\rho_P^P n_{CO_2}^P}{n_P^P} \theta_{..}^* + \frac{\rho_P^{PH} n_{CO_2}^{PH}}{n_P^{PH}} \theta_{.H}^* \right) \quad (14c)$$

Quantities of protein and carbohydrate used in egg production are:

$$j_{P,G} = j_{P,C}^g (\rho_P^P \theta_{..}^* + \rho_P^{PH} \theta_{.H}^*) \quad (15a)$$

$$j_{H,G} = \frac{\rho_H^{PH} n_H^{PH}}{n_P^{PH}} j_{P,C}^g \theta_{.H}^* \quad (15b)$$

Fraction κ of activated *P* and *H* left after maintenance and egg production is returned to the reserve pools. Remaining substrates, $j_{P,R}$, $j_{H,R}$ for *P* and *H* respectively, are rejected and assumed to be respired as CO₂ or excreted as ammonium:

$$j_{P,R} = (1 - \kappa)(k_E m_P - j_{P,M} - j_{P,G}) \quad (16a)$$

$$j_{H,R} = (1 - \kappa)(k_E m_H - j_{H,M} - j_{H,G}) \quad (16b)$$

Equations of state variables

The model is configured for a copepod with a fixed structural biomass (e.g. an adult female) ingesting C at a continuous rate $j_{C,I}$. The only state variables required

in order to study the fate of C and N in ingested food are therefore those of the reserve pools, m_P and m_H . Equations for these variables are:

$$\frac{d}{dt} m_P = j_{P,A} + \kappa(k_E m_P - j_{P,M} - j_{P,G}) - k_E m_P \quad (17a)$$

$$\frac{d}{dt} m_H = j_{H,A} + \kappa(k_E m_H - j_{H,M} - j_{H,G}) - k_E m_H \quad (17b)$$

At steady state the total assimilated C and N are balanced by the sum of the products of the SUs and rejected substrates:

$$\text{C: } j_{P,A} + j_{H,A} = j_{X,M} + j_{CO_2,M} + j_{X,G} + j_{CO_2,G} + j_{P,R} + j_{H,R} \quad (18a)$$

$$\text{N: } \frac{j_{P,A}}{\varphi_P} = \frac{j_{X,M} + j_{X,G}}{\varphi_X} + j_{NH_4^+,M} + j_{NH_4^+,G} + \frac{j_{P,R}}{\varphi_P} \quad (18b)$$

Parameter values

Where possible, model parameters are derived for the copepod *A. tonsa* consuming diatoms, permitting comparison of our results with the experimental results of Kiørboe (Kiørboe, 1989).

A C:N ratio for nitrogenous substrates (proteins), φ_P , of 3.7 mol C (mol N)⁻¹ was used (Vollenweider, 1985). The ratio in eggs, φ_X , was set at 5.9 mol C (mol N)⁻¹, based on the C:N in eggs of *Acartia* (Kiørboe *et al.*, 1985). The stoichiometric coefficients in the *PH* and *P* reactions (equations 5a–f) are then determined from parameters ψ_{PH} , ψ_P , i.e. the efficiencies with which C is used in these reactions. We expect the efficiency with which *P* and *H* are used to produce eggs to be close to the theoretical maximum of ~0.75 (Calow, 1977) and have accordingly used this value when setting ψ_{PH} , ψ_P . Equations 4a and b can accordingly be rewritten as:

$$0.63P + 0.71H \rightarrow X + 0.33CO_2 \quad (19a)$$

$$1.33P \rightarrow X + 0.19NH_4^+ + 0.33CO_2 \quad (19b)$$

Costs of maintenance are highly variable between organisms and environments, tending to be highest for small organisms experiencing high temperatures. We tuned the cost of maintenance (parameter k_M) to fit Kiørboe's (Kiørboe, 1989) data (see below), obtaining a value of 0.0041 h⁻¹ which is reasonable for *Acartia* at 17°C (the temperature of Kiørboe's experiments).

A high affinity, 0.95, was set for the binding efficiency of P to SUs in state θ_H (parameter ρ_P^{PH}), thereby allowing the favoured PH reaction to proceed readily. Setting values for the binding probabilities of P and H to SUs in state $\theta_.$, parameters ρ_P^P and ρ_H^{PH} respectively, is more problematic. One might expect the binding of H to $\theta_.$ to have a high affinity, setting up the PH route, and of P to $\theta_.$ to have a lower affinity, suppressing the P route, i.e. $\rho_P^P < \rho_P^{PH}$. However, if it is assumed that copepods can in principle fulfil maintenance requirements solely using protein reserves, a constraint in the model is that $\rho_P^P \geq \rho_P^{PH}$ (Appendix I). For simplicity we therefore assume that the two parameters have the same value, minimizing this dichotomy. The value was tuned by fitting to data (see below), giving $\rho_P^P = \rho_H^{PH} = 0.66$.

The reserve mobilization rate, k_E , was set to 0.1 h^{-1} . The fates of C and N at steady state in the model, the focus of the work here, are not affected by these parameters, although the sizes of the P and H reserve pools are. Predicted total (structure plus storage) C:N ratios of zooplankton are 6.2 when φ_F is 6.625 (the Redfield ratio), increasing to 10.5 for φ_F of 35. The fractions of substrates rejected by the maintenance and production SUs returned to the reserve pools, parameter κ , was set to 0.5. This setting gives realistic predicted total body compositions (structure plus reserves) for zooplankton.

RESULTS

The model is used to study the fate of algal C and N for copepods ingesting food items with a wide range of C:N ratios. Results are then compared with those of the egg production experiments of Kiørboe (Kiørboe, 1989). In these experiments adult female *A. tonsa* were fed the diatom *Thalassiosira weissflogii* with C:N ratios manipu-

lated through the concentration of nutrient in the algal growth medium. Carbon ingestion by *Acartia* was independent of algal C:N at $\sim 0.06 \text{ mol C (mol C)}^{-1} \text{ h}^{-1}$ and so we use this value for I_C in the model. This intake rate appears to be near the maximum for this species, which may be $\sim 0.08 \text{ mol C (mol C)}^{-1} \text{ h}^{-1}$, based on a maximum intake of $0.333 \mu\text{g C ind}^{-1} \text{ h}^{-1}$ and an individual weight of $4 \mu\text{g C}$ (Besiktepe and Dam, 2002). We show only steady-state solutions of the model, obtained numerically using a time step of 0.1 day.

Fit to data

Parameters for maintenance and SU binding affinities, k_M , ρ_P^P and ρ_H^{PH} , were tuned in order to fit the model to the data for C and N gross growth efficiency (K_C , K_N) of egg production versus algal C:N as found by Kiørboe (Kiørboe, 1989). Noting the constraint that $\rho_P^P \geq \rho_P^{PH}$ (Appendix I), these two parameters were assumed to be equal in value, effectively fitting them together. A least-squares fit to the K_C and K_N data was carried out, with a weighting (factor 10) toward matching K_C which shows a clear relationship with φ_F . The agreement between model and data is shown in Figure 3, achieved with $k_M = 0.0041 \text{ h}^{-1}$ and $\rho_P^P = \rho_H^{PH} = 0.66$. A close fit is achieved for K_C . The model predicts that K_N declines at high algal C:N because an increasing proportion of food N has to be used for maintenance purposes (see below). The data do not support this decline, although only a single point is in marked disagreement with the trend.

Fate of C and N: influence of algal C:N

The predicted fate of algal C and N in the fitted model, for algal C:N, φ_F , 5–35, is shown in Figure 4a. Nitrogen in food is assumed to be solely in the form of proteins which are assimilated with a fixed efficiency of 0.688, so

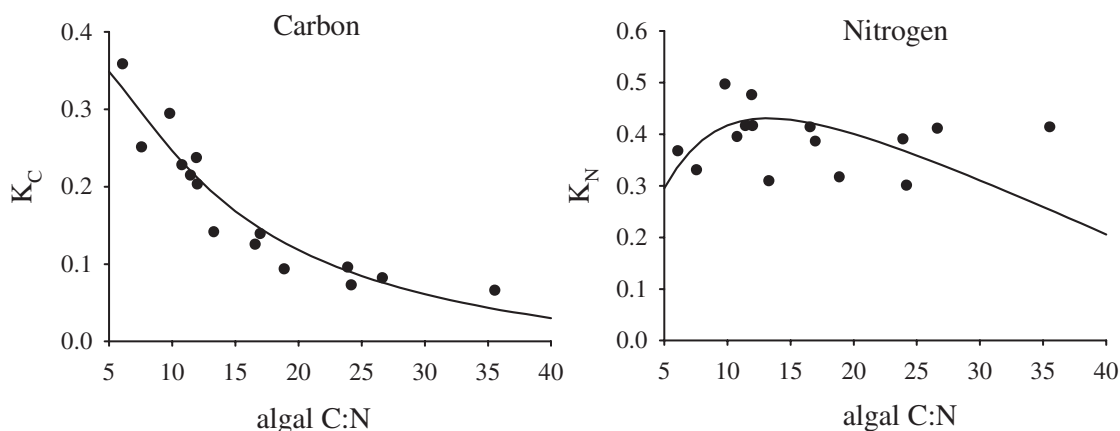


Fig. 3. Model fit (line) to the data of Kiørboe (Kiørboe, 1989): gross growth efficiencies for C and N, K_C and K_N , versus algal C:N ratio (molar). Fitted parameters: $k_M = 0.0041 \text{ h}^{-1}$ and $\rho_P^P = \rho_H^{PH} = 0.66$.

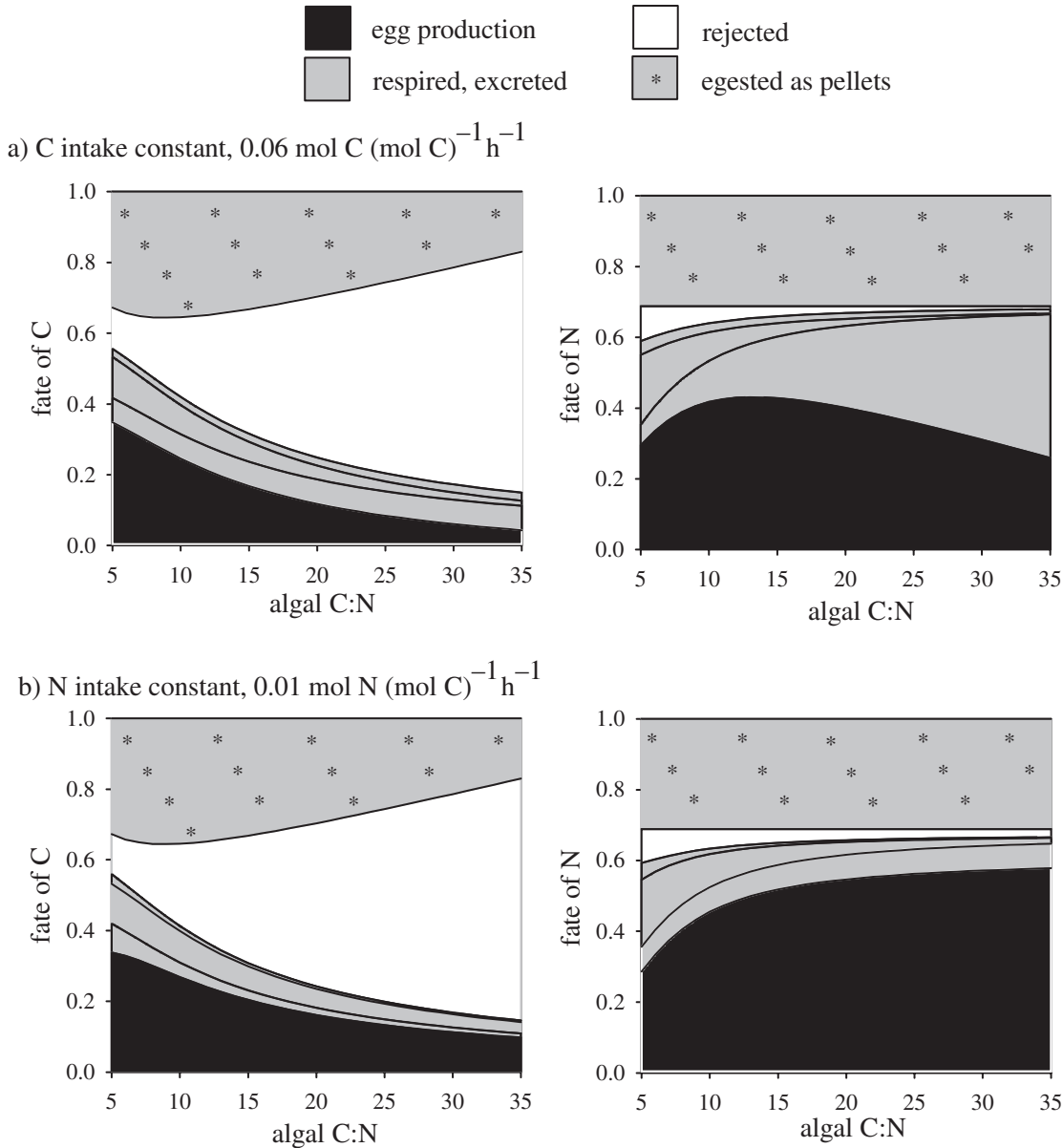


Fig. 4. Predicted fate of C and N in food for algal C:N 5–35: **(a)** constant intake of C of $0.06 \text{ mol C (mol C)}^{-1} \text{ h}^{-1}$, **(b)** constant intake of N of $0.01 \text{ mol N (mol C)}^{-1} \text{ h}^{-1}$. Respiration and excretion are subdivided into three components (from the bottom up): maintenance (production of biomass for maintenance is assumed to be balanced by an equivalent loss of CO_2 or NH_4^+ of material turned over), energetic costs of egg production, and energetic costs of maintenance.

that the fraction of N allocated to pellets is constant at 0.312. The fraction of C allocated to pellets declines at high φ_F because of the increasing proportion of storage carbohydrates in the algae which are easily assimilated (Anderson, 1994). Fractions of C and N allocated to egg production correspond to K_C and K_N as shown in Figure 3. These fractions are consistent with the observations of other studies involving *Acartia* (Checkley, 1980; Kiørboe *et al.*, 1985; Pagano and Saint-Jean, 1994). The fractions of intake respired (C) or excreted (N) compare

favourably with fractions of 0.15 respired and 0.25 excreted observed by Kiørboe *et al.* (Kiørboe *et al.*, 1985) for *A. tonsa* feeding on *Rhodomonas baltica* with a C:N of 5.75 (at high food concentration). Several processes contribute to respiration and excretion. Production of structure through maintenance to replace degrading tissues does not contribute to egg production and hence K_C and K_N . This production is assumed to be balanced by equivalent losses of CO_2 or ammonium from the organism, i.e. the degrading material. The

C and N requirements for maintenance in terms of production of X are proportional to the structural biomass of the zooplankter, which is held constant in the model. The fraction of ingested C required for maintenance therefore remains a constant proportion of C intake. Production of CO_2 associated with maintenance is also a constant fraction of C intake because the *PH* and *P* reactions both yield the same amount of CO_2 (equations 19a and b). However, production of CO_2 associated with the energetic costs of egg production decline with increasing φ_F in line with decreasing carbon gross growth efficiency.

A fixed maintenance requirement means that an increasing proportion of N in food must be allocated for this purpose as φ_F increases (for a fixed C intake, in which case N intake declines). This increasing allocation decreases the N available for egg production, resulting in lower K_N . The fraction of N needed to meet the energetic costs of egg production and maintenance decreases with increasing φ_F because the increased availability of *H* relative to *P* favours the *PH* route (equation 3a) rather than the *P* route (equation 3b) thereby sparing N. Perhaps the most striking asymmetry in the modelled fates of C and N is in the fluxes rejected by SUs. At high φ_F the SU binding sites for *H* rapidly become saturated. A fraction $(1 - \kappa)$ of the excess C arriving at these binding sites is rejected, the remainder being returned to reserves. In contrast, N is always in short supply relative to the demands for maintenance and egg production, and so is effectively bound by SUs, with little rejection.

The fates of C and N predicted by the model for an animal ingesting a constant N ration are shown in Figure 4b. The main difference with the results described

above is that K_N , the fraction of N allocated to production, now increases with increasing φ_F . There is no longer a requirement for an increasing proportion of N to be allocated to maintenance because the availability of N remains fixed. The N in food is used more efficiently for egg production at high φ_F because there is a ready supply of *H* to favour the *PH* route (which has a N efficiency of 100% for producing X: equation 19a) rather than the *P* route (which has an efficiency of 47%: equation 19b).

Fate of C and N: influence of food ration

The influence of food intake on the predicted fate of C and N, for an algal C/N of 15, is shown in Figure 5. Lower intake increases the cost of maintenance as a fraction of assimilated substrates, and so decreases growth efficiencies for C and N. Indeed if φ_F is below 0.0162 then there is insufficient C to meet the requirements for maintenance and zero egg production (the minimum ration depends on φ_F , increasing at high values). Results are consistent with experimental observations (Kjørboe *et al.*, 1985) showing increasing production, and decreasing respiration and excretion, as fractions of intake, as intake increases.

DISCUSSION

Laboratory experiments in which copepods were fed food with varying C:N showed egg production being proportional to intake of N (Kjørboe, 1989), apparently indicating limitation by that element. Previous models based on classical stoichiometric theory (Anderson and Hessen, 1995) have been unable to capture the decreasing carbon gross growth efficiency (K_C), and apparently

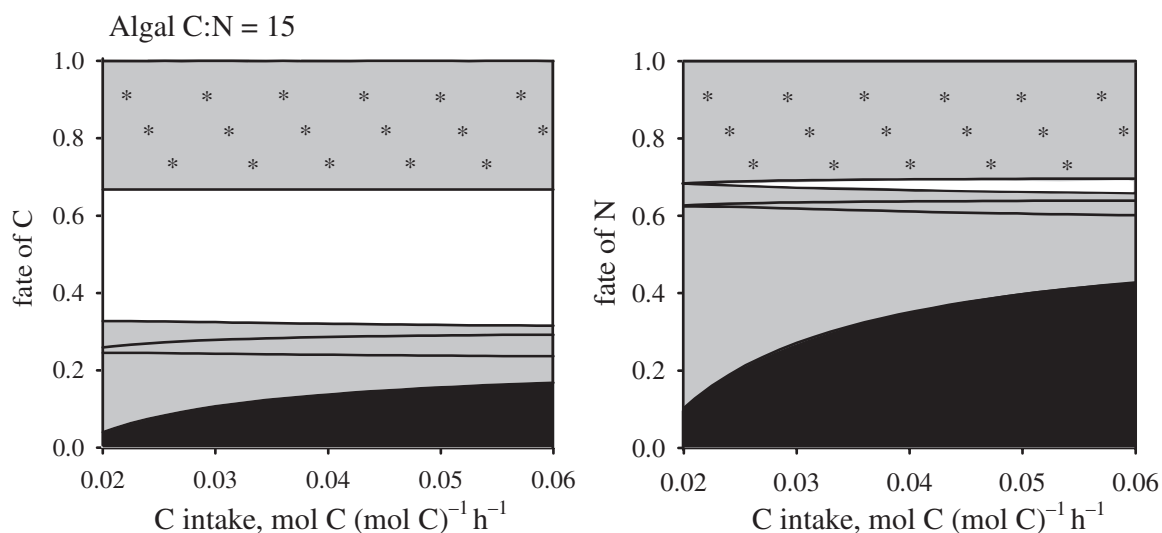


Fig. 5. Predicted fate of C and N in food for algal C:N of 15, C intake 0.02–0.06 mol C (mol C)^{−1} h^{−1}. Fractions as in Figure 4.

low (~ 0.4) and constant K_N , of egg production with increasing food C:N observed in these experiments. Here we used a new model based on DEB theory to examine the roles of food C and N in controlling zooplankton egg production. The model splits substrates between nitrogenous and non-nitrogenous compounds (Anderson, 1992), distinguishes between structural and reserve components of animal biomass, and requires that assimilated substrates are utilized to meet maintenance costs prior to allocation for egg production. Conversion of substrates to products is controlled by synthesizing units. The model is able to capture realistically the trend of decreasing K_C of egg production with increasing food C:N, φ_F , observed by Kiørboe (Kiørboe, 1989). It also captures the relatively low K_N , but predicts a decrease at high φ_F , although only a single data point is in obvious disagreement with the model (Figure 3).

Before discussing the implications of the results regarding the nutrition of marine zooplankton, it is worth commenting on the parameterization of the model. Many of the model parameters were set based directly on experimental data for *Acartia*, or on a sound theoretical basis. The main unknown parameters are the maintenance requirement, k_M , and the affinity parameters of the SUs, ρ_P^P , ρ_P^{PH} , ρ_H^{PH} . With the exception of ρ_P^{PH} (which was given a theoretical value), these parameters were assigned values by fitting to data (Kiørboe, 1989). The model is sensitive to k_M which is therefore well constrained by the data, but less so to the affinity parameters. Alternative values for these affinities, in combination with small adjustments to k_M , can result in other parameter combinations that also provide a good fit to the data. However, the basic patterns of C and N utilization exhibited by the model remain unchanged, and so we argue that the insight into zooplankton nutrition thus provided is real.

Classical stoichiometric theory dictates that limiting elements are used with high efficiency, with non-limiting elements being recycled to the environment [e.g. (Hessen, 1992)]. Indeed previous modelling studies have assumed that N can be used with a maximum efficiency of 100% (Anderson, 1992; Urabe and Watanabe, 1992). Models of this type are therefore unable to predict the low K_N of ~ 0.4 that is seen in egg production experiments. This inconsistency led Anderson and Hessen (Anderson and Hessen, 1995) to conclude that 'stoichiometric models do not support the view of a predominant N limitation in marine copepods'. An important difference between these previous models and the DEB model described here is that the latter has a significant N requirement for maintenance. The new model successfully generated the low K_N seen in the laboratory experiments, the maintenance demand for N playing a key role. Although it may appear self-evident

that maintenance should feature as a component in the N budget, the advantage of our model is that it provides a quantitative evaluation of this process in context of the N budget as a whole, based on physiological principles. Nitrogen gross growth efficiency in the model depends not only on food C:N, but also the ration. So if N becomes scarce in food then what little there is is allocated to maintenance, and K_N is low, not high (Figure 5). The maintenance parameter in the model, k_M , was estimated by fitting to data. The resulting value for k_M of 0.0041 h^{-1} is slightly higher than the respiration rate measured by Thor (Thor, 2003) for *Acartia* during starvation of 0.003 h^{-1} ($0.4 \text{ nl O}_2 \text{ ind}^{-1} \text{ min}^{-1}$, converted assuming $4 \mu\text{g C ind}^{-1}$, respiratory quotient of 1, 1 mole gas occupies 24 L at 18°C). Published estimates of N turnover in marine crustaceans are scarce. Fry and Arnold (Fry and Arnold, 1982) noted rapid carbon turnover during growth of brown shrimp (*Penaeus aztecus*). Frazer *et al.* (Frazer *et al.*, 1997) found that larval krill reared at 1.5°C replaced 13–22% of body N during 8–10 week experiments. The egg production experiments to which the model is fitted were however carried out at 17°C , in which instance a considerably higher protein turnover rate would be expected. In general, improved estimates of N turnover are needed for zooplankton, as well as other organisms (Frazer *et al.*, 1997).

Carbon gross growth efficiency of copepods is typically low, between 0.2 and 0.3, in common with other planktonic consumers (Straile, 1997). We have already discounted 'obligatory' respiration as a major C sink in copepods. The model indicates that less than 20% of food C is respired due to maintenance and the energetic costs of maintenance and egg production (Figure 4). The relative demands for C and N by the modelled copepods are reflected in the extent to which substrates are bound by the SUs. Very little N is rejected by SUs, whereas the low K_C in the model is due to high rejection of C by SUs. In other words, the predicted low carbon gross growth efficiency is due to stoichiometric regulation, effectively N-limitation (see below), and not energetic demands for C-rich compounds. One can speculate about the ultimate fate of this rejected material. One possibility is that it is simply respired or excreted, or it could be egested in pellets (Checkley and Entzeroth, 1985).

Low rejection of N by SUs is consistent with the idea that zooplankton are N-limited. Predicted egg production is inversely related to food C:N for animals ingesting a constant carbon ration (Figure 4a). A feature of the model is, however, that it is not restricted to the simple Liebig-type C or N limitation. The simple stoichiometric models which do operate on Liebig principles would show zero sensitivity to C in food under conditions of N-limitation. Yet the DEB model shows that an animal ingesting a food item with a fixed quantity of C and N

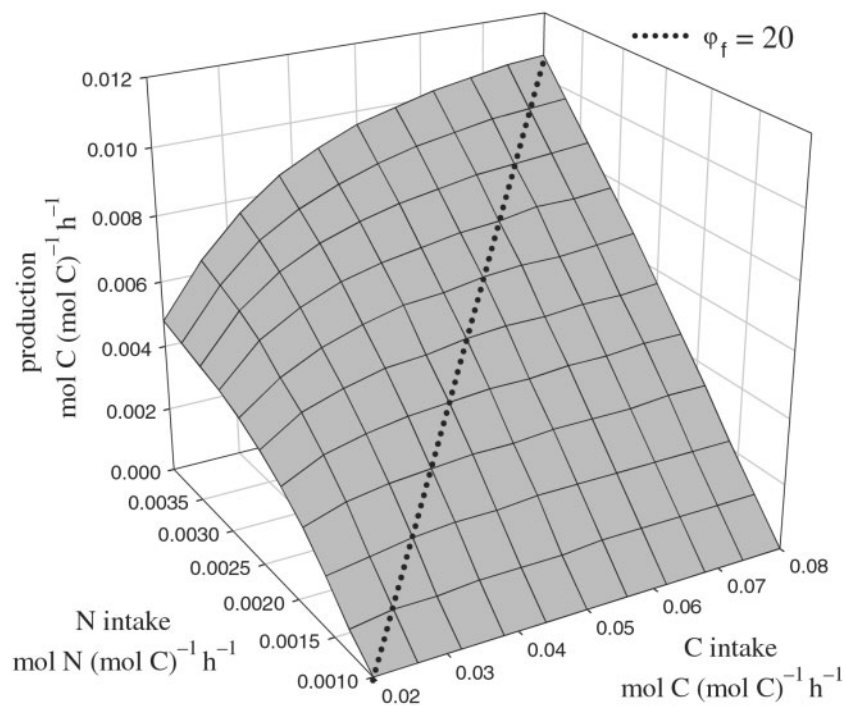


Fig. 6. Production as predicted by the model, as influenced by quantities of C and N in ingested food. Dotted line indicates food C:N = 20 (higher C:N to right, lower to left).

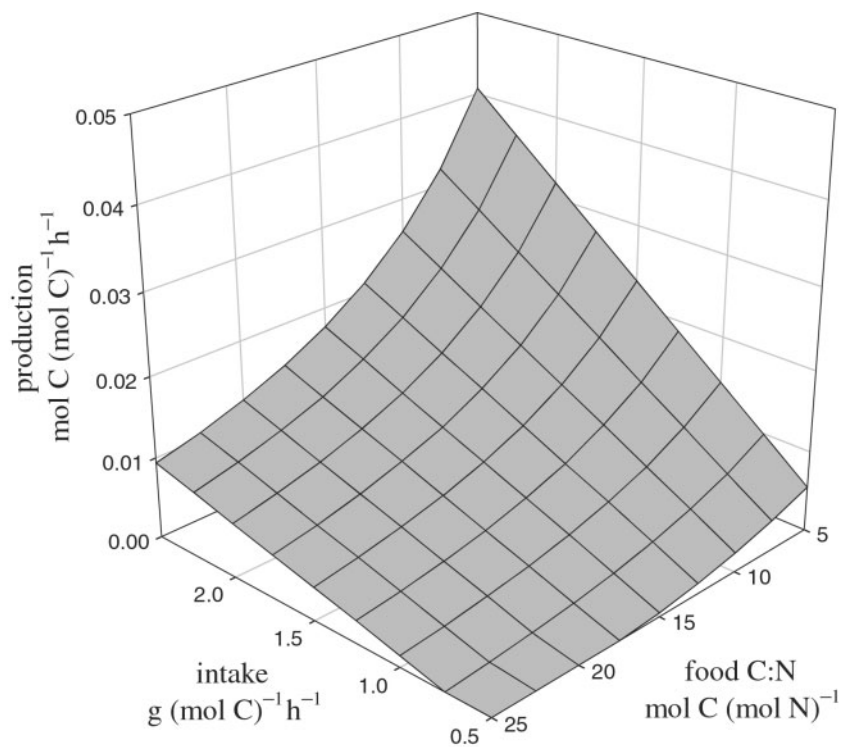


Fig. 7. Production as predicted by the model, as influenced by food intake (in units of dry weight) and food C:N.

would increase production if extra C is added to that food (Figure 4b). This extra C increases the prevalence of the *PH* reaction pathway relative to the *P* pathway, permitting protein sparing for egg production. Simple stoichiometric models predict the fate of C and N based only on the relative elemental ratios of consumer and prey, and assumed growth efficiencies. The fate of C and N in the model described here depends on both the quantity and quality of the food, demands for maintenance and egg production, and the dynamic action of the SUs. The separate influences of C and N in ingested food on production, as predicted by the new model, are shown in Figure 6. At high C:N ratios (>20) production appears to be strongly N-limited, with little or no response to further supplements of C in the diet. At low C:N ratios, and particularly when total intake of C is also low, production is stimulated by addition of either C or N to the diet.

As a further examination of the relationship between intake and food C:N, the former can be quantified in terms of dry weight, i.e. it is assumed that copepods are obtaining a certain ration on a per gram basis. It is possible to convert the biochemical fractions used in the model to a per unit weight scale by assuming fixed elemental compositions for these fractions: $C_{59}N_{16}H_{94}O_{19}S_{0.5}$ for protein (Vollenweider, 1985) and $C_{18}H_{36}O_2$ and CH_2O for lipid and carbohydrate respectively (Anderson, 1992). The relative dominance of these fractions is related to food C:N (equations 2a–d). On this basis, the production versus intake and algal C:N is shown in Figure 7. These results confirm that egg production is maximized by consuming as much as possible, and by selecting food items rich in nitrogen.

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REFERENCES

- Anderson, T. R. (1992) Modelling the influence of food C:N ratio, and respiration on growth and nitrogen excretion in marine zooplankton and bacteria. *J. Plankton Res.*, **14**, 1645–1671.
- Anderson, T. R. (1994) Relating C:N ratios in zooplankton food and faecal pellets using a biochemical model. *J. Exp. Mar. Biol. Ecol.*, **184**, 183–199.
- Anderson, T. R. and Hessen, D. O. (1995) Carbon or nitrogen limitation in marine copepods? *J. Plankton Res.*, **17**, 317–331.
- Anderson, T. R. and Pond, D. W. (2000) Stoichiometric theory extended to micronutrients: comparison of the roles of essential fatty acids, carbon, and nitrogen in the nutrition of marine copepods. *Limnol. Oceanogr.*, **45**, 1162–1167.
- Anderson, T. R., Boersma, M. and Raubenheimer, D. (2004) Stoichiometry: Linking elements to biochemicals. *Ecology*, in press.
- Arnould, J. P. Y., Green, J. A. and Rawlins, D. R. (2001) Fasting metabolism in Antarctic fur seal (*Arctocephalus gazella*) pups. *Comp. Biochem. Physiol. A*, **129**, 829–841.
- Barlow, R. G. (1982) Phytoplankton ecology in the Southern Benguela Current. I. Biochemical composition. *J. Exp. Mar. Biol. Ecol.*, **63**, 209–227.
- Besiktepe, S. and Dam, H. G. (2002) Coupling of ingestion and defecation as a function of diet in the calanoid copepod *Acartia tonsa*. *Mar. Ecol. Prog. Ser.*, **229**, 151–164.
- Brett, M. T. (1993) Comment on ‘Possibility of N or P limitation for planktonic cladocerans: an experimental test’ (Urabe and Watanabe) and ‘Nutrient element limitation of zooplankton production’ (Hessen). *Limnol. Oceanogr.*, **38**, 1333–1337.
- Calow, P. (1977) Conversion efficiencies in heterotrophic organisms. *Biol. Rev.*, **52**, 385–409.
- Checkley, D. M., Jr (1980) The egg production of a marine copepod in relation to its food supply: laboratory studies. *Limnol. Oceanogr.*, **25**, 430–446.
- Checkley, D. M., Jr and Entzeroth, L. C. (1985) Elemental and isotopic fractionation of carbon and nitrogen by marine, planktonic copepods and implications to the marine nitrogen cycle. *J. Plankton Res.*, **7**, 553–568.
- DeBaar, H. J. W. (1994) Von Liebig Law of the Minimum and plankton ecology (1899–1991). *Prog. Oceanogr.*, **33**, 347–386.
- DeMott, W. R., Gulati, R. D. and Siewertsen, K. (1998) Effects of phosphorus-deficient diets on the carbon and phosphorus balance of *Daphnia magna*. *Limnol. Oceanogr.*, **43**, 1147–1161.
- Droop, M. R. (1983) 25 years of algal growth kinetics. *Bot. Mar.*, **26**, 99–112.
- Flood, P. R. (1991) Architecture of, and water circulation and flow rate in, the house of the planktonic tunicate *Oikopleura labradoriensis*. *Mar. Biol.*, **111**, 95–111.
- Frazer, T. K., Ross, R. M., Quetin, L. B. and Montoya, J. P. (1997) Turnover of carbon and nitrogen during growth of larval krill, *Euphausia superba* Dana: a stable isotope approach. *J. Exp. Mar. Biol. Ecol.*, **212**, 259–275.
- Fry, B. and Arnold, C. (1982) Rapid $^{13}C/^{12}C$ turnover during growth of brown shrimp (*Penaeus aztecus*). *Oecologia*, **54**, 200–204.
- Hessen, D. O. (1992) Nutrient element limitation of zooplankton production. *Am. Nat.*, **140**, 799–814.
- Hirst, A. G. and McKinnon, A. D. (2001) Does egg production represent adult female copepod growth? A call to account for body weight changes. *Mar. Ecol. Prog. Ser.*, **223**, 179–199.
- Jones, R. H., Flynn, K. J. and Anderson, T. R. (2002) Effect of food quality on carbon and nitrogen growth efficiency in the copepod *Acartia tonsa*. *Mar. Ecol. Prog. Ser.*, **235**, 147–156.
- Karasov, W. H. (1992) Daily energy expenditure and the cost of activity in mammals. *Am. Zool.*, **32**, 238–248.
- Kjørboe, T. (1989) Phytoplankton growth rate and nitrogen content: implications for feeding and fecundity in a herbivorous copepod. *Mar. Ecol. Prog. Ser.*, **55**, 229–234.
- Kjørboe, T., Møhlenberg, F. and Hamburger, K. (1985) Bioenergetics of the planktonic copepod *Acartia tonsa*: relation between feeding, egg production and respiration, and composition of specific dynamic action. *Mar. Ecol. Prog. Ser.*, **26**, 85–97.
- Kooijman, S. A. L. M. (1995) The stoichiometry of animal energetics. *J. Theor. Biol.*, **177**, 139–149.

- Kooijman, S. A. L. M. (1998) The synthesizing unit as a model for stoichiometric fusion and branching of metabolic fluxes. *Biophys. Chem.*, **73**, 179–188.
- Kooijman, S. A. L. M. (2001) Quantitative aspects of metabolic organization; a discussion of concepts. *Philos. Trans. R. Soc. B*, **356**, 331–349.
- MacCluskie, M. C. and Sedinger, J. S. (2000) Nutrient reserves and clutch-size regulation of Northern Shovelers in Alaska. *Auk*, **117**, 971–979.
- McGoogan, B. B. and Gatlin, D. M. (1999) Dietary manipulations affecting growth and nitrogenous waste production of red drum, *Sciaenops ocellatus*. I. Effects of dietary protein and energy levels. *Aquaculture*, **178**, 333–348.
- Muller, E. B., Nisbet, R. M., Kooijman, S. A. L. M., Elser, J. J. and McCauley, E. (2001) Stoichiometric food quality and herbivore dynamics. *Ecol. Lett.*, **4**, 519–529.
- Pagano, M. and Saint-Jean, L. (1994) *In situ* metabolic budget for the calanoid copepod *Acartia clausi* in a tropical brackish lagoon (Ebrié Lagoon, Ivory Coast). *Hydrobiologia*, **272**, 147–161.
- Roxburgh, L. and Pinshow, B. (2000) Nitrogen requirements of an old world nectarivore, the orange-tufted sunbird *Nectarinia osea*. *Physiol. Biochem. Zool.*, **73**, 638–645.
- Sterner, R. W. and Hessen, D. O. (1994) Algal nutrient limitation and the nutrition of aquatic herbivores. *Annu. Rev. Ecol. Syst.*, **25**, 1–29.
- Straile, D. (1997) Gross growth efficiencies of protozoan and metazoan zooplankton and their dependence on food concentration, predator–prey weight ratio, and taxonomic group. *Limnol. Oceanogr.*, **42**, 1375–1385.
- Swadling, K. M., Nichols, P. D., Gibson, J. A. E. and Ritz, D. A. (2000) Role of lipid in the life cycles of ice-dependent and ice-independent populations of the copepod *Paralabidocera antarctica*. *Mar. Ecol. Prog. Ser.*, **208**, 171–182.
- Telang, A., Buck, N. A. and Wheeler, D. E. (2002) Response of storage protein levels to variation in dietary protein levels. *J. Insect Physiol.*, **48**, 1021–1029.
- Thor, P. (2003) Elevated respiration rates of the neritic copepod *Acartia tonsa* during recovery from starvation. *J. Exp. Mar. Biol. Ecol.*, **283**, 133–143.
- Urabe, J. and Watanabe, Y. (1992) Possibility of N or P limitation for planktonic cladocerans: an experimental test. *Limnol. Oceanogr.*, **37**, 244–251.
- Vollenweider, R. A. (1985) Elemental and biochemical composition of plankton biomass; some comments and explorations. *Arch. Hydrobiol.*, **105**, 11–29.
- Zonneveld, C. (1996) Modelling the kinetics of non-limiting nutrients in microalgae. *J. Mar. Syst.*, **9**, 121–136.

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APPENDIX I: CONSTRAINTS TO VALUES OF ρ PARAMETERS

When there is insufficient supply of reserves to fulfil maintenance demands, the copepods will die. Equations 8a and b quantify the amounts of reserves used for

maintenance. After some rearranging, they read as follows.

$$j_{P,M} = k_M \frac{\rho_P^P j_{P,C} + \frac{n_P^{PH}}{n_H^{PH}} \rho_H^{PH} j_{H,C}}{\frac{\rho_P^P}{n_P^P} j_{P,C} + \frac{\rho_H^{PH}}{n_H^{PH}} j_{H,C}} \quad (\text{A1a})$$

$$j_{H,M} = k_M \frac{\rho_H^{PH} j_{H,C}}{\frac{\rho_H^{PH}}{n_H^{PH}} j_{H,C} + \frac{\rho_P^P}{n_P^P} j_{P,C}} \quad (\text{A1b})$$

in which $j_{H,C} = k_{EMH}$ and $j_{P,C} = k_{EMP}$.

It is assumed that copepods can potentially fulfil maintenance requirements by respiration of protein reserves solely. The organisms are at the brink of starvation when all proteins and all carbohydrates are invested in maintenance. This is the case when $j_{P,C} = j_{P,M}$ and $j_{H,C} = j_{H,M}$. Using equations A1a and A1b, this gives:

$$j_{H,C} = n_H^{PH} k_M - \frac{\rho_P^P n_H^{PH}}{\rho_H^{PH} n_P^P} j_{P,C} \quad (\text{A2a})$$

$$j_{H,C} = \frac{\rho_P^P n_H^{PH} j_{P,C} \left(k_M - \frac{j_{P,C}}{n_P^P} \right)}{\rho_H^{PH} (j_{P,C} - k_M n_P^{PH})} \quad (\text{A2b})$$

Substitution of these two gives, after some algebra, the starvation boundary of $j_{P,C}$, which is the amount of proteins that is just sufficient for survival. The quantity of protein used when all available proteins and carbohydrates are invested in maintenance is:

$$j_{P,C}^* = \frac{\rho_H^{PH} n_P^{PH} k_M}{\rho_H^{PH} + \rho_P^P \frac{n_P^{PH}}{n_P^P} - \rho_P^P} \quad (\text{A3})$$

In the absence of carbohydrates, the quantity of required proteins can be derived from equation A1a with $j_{H,C} = 0$, giving $j_{P,M} = k_M n_P^P$. If $j_{P,C}^* < k_M n_P^P$, a certain amount of carbohydrates is required to produce enough $j_{H,M}$. This violates the assumption that copepods can potentially survive in the absence of carbohydrates. Therefore, we need $j_{P,C}^* \geq k_M n_P^P$, which translates into the constraint:

$$\rho_H^{PH} (n_P^{PH} - n_P^P) \geq \rho_P^P (n_P^{PH} - n_P^P) \quad (\text{A4})$$

As the *PH*-route is more efficient with respect to the usage of nitrogen than the *P*-route (see equations 4a and 4b), we have $n_P^P \geq n_P^{PH}$, so that $\rho_P^P \geq \rho_H^{PH}$. To obtain a preference for the *PH*-route, ρ_H^{PH} has to be as large as possible and, therefore, we use $\rho_P^P = \rho_H^{PH}$.