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

LOTHAR D. J. KUIJPER*, THOMAS R. ANDERSON† AND SEBASTIAAN A. L. M. KOOIJMAN
VRIJE UNIVERSITEIT, FACULTY OF EARTH & LIFE SCIENCES, DEPARTMENT OF THEORETICAL BIOLOGY, DE BOELELAAN 1015, 1081 HV AMSTERDAM, THE NETHERLANDS AND †SOUTHAMPTON OCEANOGRAPHY CENTRE, WATERFRONT CAMPUS, UNIVERSITY OF SOUTHAMPTON, SOUTHAMPTON SO14 3ZH, UK
*CORRESPONDING AUTHOR: kuijper@bio.vu.nl

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 (Sterner 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 stoichiometric 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, \( \varphi_f \), can then be defined, below which C limits production, and above which N limits:

\[
\varphi_f^* = \frac{\varphi_Z K_N^*}{K_C^*} \tag{1}
\]

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 \( \varphi_Z \) 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; Kiorboe, 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, 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 \(K_Y\) 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 Kiorboe (Kiorboe, 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 \(j_{C:1}\) [mol C (mol C\(^{-1}\) h\(^{-1}\)]. This C is divided between nitrogenous substrates (proteins, nucleic acids: \(P\)) with a fixed C:N ratio, \(\varphi_P\), 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
where $\varphi_F$ 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

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Value</th>
<th>Units</th>
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</thead>
<tbody>
<tr>
<td>$H$</td>
<td>carbohydrate reserve biomass</td>
<td>variable</td>
<td>mol C (mol C)$^{-1}$</td>
</tr>
<tr>
<td>$j_{CI}$</td>
<td>carbon intake</td>
<td>variable</td>
<td>mol C (mol C)$^{-1}$ h$^{-1}$</td>
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<tr>
<td>$j_{CO_{2}M}$</td>
<td>CO$_2$ produced in maintenance</td>
<td>variable</td>
<td>mol C (mol C)$^{-1}$ h$^{-1}$</td>
</tr>
<tr>
<td>$j_{CO_{2}G}$</td>
<td>CO$_2$ produced in egg production</td>
<td>variable</td>
<td>mol C (mol C)$^{-1}$ h$^{-1}$</td>
</tr>
<tr>
<td>$j_{H,A}$</td>
<td>assimilated $H$</td>
<td>variable</td>
<td>mol C (mol C)$^{-1}$ h$^{-1}$</td>
</tr>
<tr>
<td>$j_{H,G}$</td>
<td>$H$ used for egg production</td>
<td>variable</td>
<td>mol C (mol C)$^{-1}$ h$^{-1}$</td>
</tr>
<tr>
<td>$j_{H,M}$</td>
<td>$H$ used for maintenance</td>
<td>variable</td>
<td>mol C (mol C)$^{-1}$ h$^{-1}$</td>
</tr>
<tr>
<td>$j_{H,R}$</td>
<td>$H$ rejected by production SUs</td>
<td>variable</td>
<td>mol C (mol C)$^{-1}$ h$^{-1}$</td>
</tr>
<tr>
<td>$j_{NH_{4}G}$</td>
<td>NH$_4^+$ produced in egg production</td>
<td>variable</td>
<td>mol N (mol C)$^{-1}$ h$^{-1}$</td>
</tr>
<tr>
<td>$j_{NH_{4}M}$</td>
<td>NH$_4^+$ produced in maintenance</td>
<td>variable</td>
<td>mol N (mol C)$^{-1}$ h$^{-1}$</td>
</tr>
<tr>
<td>$j_{P,A}$</td>
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<td>mol C (mol C)$^{-1}$ h$^{-1}$</td>
</tr>
<tr>
<td>$j_{P,G}$</td>
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<td>variable</td>
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</tr>
<tr>
<td>$j_{P,M}$</td>
<td>$P$ used for maintenance</td>
<td>variable</td>
<td>mol C (mol C)$^{-1}$ h$^{-1}$</td>
</tr>
<tr>
<td>$j_{P,R}$</td>
<td>$P$ rejected by production SUs</td>
<td>variable</td>
<td>mol C (mol C)$^{-1}$ h$^{-1}$</td>
</tr>
<tr>
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<td>variable</td>
<td>mol C (mol C)$^{-1}$ h$^{-1}$</td>
</tr>
<tr>
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<td>$X$ replaced in maintenance</td>
<td>variable</td>
<td>mol C (mol C)$^{-1}$ h$^{-1}$</td>
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<td>$h_{R}$</td>
<td>reserve mobilization rate</td>
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</tr>
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<td>$h_{M}$</td>
<td>maintenance turnover rate</td>
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<td>h$^{-1}$</td>
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<td>$K_{C}$</td>
<td>carbon gross growth efficiency</td>
<td>variable</td>
<td>mol C (mol C)$^{-1}$</td>
</tr>
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<td>$K_{N}$</td>
<td>nitrogen gross growth efficiency</td>
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<td>mol N (mol N)$^{-1}$</td>
</tr>
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<td>$m_{H}$</td>
<td>reserve carbohydrate density</td>
<td>variable</td>
<td>mol C (mol C)$^{-1}$</td>
</tr>
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<td>$n_{P}$</td>
<td>reserve protein density</td>
<td>variable</td>
<td>mol C (mol C)$^{-1}$</td>
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<td>$n_{CO_{2}}$</td>
<td>CO$_2$ formed per X produced in $P$-route</td>
<td>0.33</td>
<td>mol C (mol C)$^{-1}$</td>
</tr>
<tr>
<td>$n_{CO_{2}}$</td>
<td>CO$_2$ formed per X produced in $PH$-route</td>
<td>0.33</td>
<td>mol C (mol C)$^{-1}$</td>
</tr>
<tr>
<td>$n_{H,L}$</td>
<td>$H$ used per X formed in PH-route</td>
<td>0.71</td>
<td>mol C (mol C)$^{-1}$</td>
</tr>
<tr>
<td>$n_{NH_{4}}$</td>
<td>NH$_4^+$ formed per X produced in $P$-route</td>
<td>0.19</td>
<td>mol N (mol C)$^{-1}$</td>
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<td>$n_{P}$</td>
<td>$P$ used per X formed in PH-route</td>
<td>1.33</td>
<td>mol C (mol C)$^{-1}$</td>
</tr>
<tr>
<td>$n_{P}$</td>
<td>$P$ used per X formed in PH-route</td>
<td>0.63</td>
<td>mol C (mol C)$^{-1}$</td>
</tr>
<tr>
<td>$P$</td>
<td>protein reserve biomass</td>
<td>variable</td>
<td>mol C (mol C)$^{-1}$</td>
</tr>
<tr>
<td>$X$</td>
<td>biomass: structure, eggs</td>
<td>variable</td>
<td>mol C (mol C)$^{-1}$</td>
</tr>
<tr>
<td>$\beta_{N}$</td>
<td>average assimilation efficiency of N</td>
<td>variable</td>
<td>mol N (mol N)$^{-1}$</td>
</tr>
<tr>
<td>$\beta_{C}$</td>
<td>average assimilation efficiency of C</td>
<td>variable</td>
<td>mol C (mol C)$^{-1}$</td>
</tr>
<tr>
<td>$\varphi_{P}$</td>
<td>C:N of food</td>
<td>variable</td>
<td>mol C (mol N)$^{-1}$</td>
</tr>
<tr>
<td>$\varphi_{P}$</td>
<td>C:N of proteins</td>
<td>3.7</td>
<td>mol C (mol N)$^{-1}$</td>
</tr>
<tr>
<td>$\varphi_{X}$</td>
<td>C:N of structural biomass, eggs</td>
<td>5.9</td>
<td>mol C (mol N)$^{-1}$</td>
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<tr>
<td>$\kappa$</td>
<td>fraction H and P returned to reserves</td>
<td>0.5</td>
<td>–</td>
</tr>
<tr>
<td>$\theta_{F,L}$</td>
<td>lipid carbon fraction of diatoms</td>
<td>variable</td>
<td>mol C (mol C)$^{-1}$</td>
</tr>
<tr>
<td>$\theta_{F,H1}$</td>
<td>soluble carbohydrate fraction of diatoms</td>
<td>variable</td>
<td>mol C (mol C)$^{-1}$</td>
</tr>
<tr>
<td>$\theta_{F,H2}$</td>
<td>insoluble carbohydrate fraction of diatoms</td>
<td>variable</td>
<td>mol C (mol C)$^{-1}$</td>
</tr>
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<td>$\theta_{F,P}$</td>
<td>protein fraction of diatoms</td>
<td>variable</td>
<td>mol C (mol C)$^{-1}$</td>
</tr>
<tr>
<td>$\theta_{C}$</td>
<td>fraction of SUs in state xy</td>
<td>variable</td>
<td>–</td>
</tr>
<tr>
<td>$\theta_{P}$</td>
<td>steady-state fraction of SUs in state xy</td>
<td>variable</td>
<td>–</td>
</tr>
<tr>
<td>$\rho_{P}$</td>
<td>binding probability of $P$ to $\theta_{..}$</td>
<td>0.66</td>
<td>–</td>
</tr>
<tr>
<td>$\rho_{P}$</td>
<td>binding probability of $P$ to $\theta_{H..}$</td>
<td>0.95</td>
<td>–</td>
</tr>
<tr>
<td>$\rho_{P}$</td>
<td>binding probability of $H$ to $\theta_{..}$</td>
<td>0.66</td>
<td>–</td>
</tr>
<tr>
<td>$\psi_{P}$</td>
<td>C yield efficiency: reaction P</td>
<td>0.75</td>
<td>mol C (mol C)$^{-1}$</td>
</tr>
<tr>
<td>$\psi_{PH}$</td>
<td>C yield efficiency: reaction PH</td>
<td>0.75</td>
<td>mol C (mol C)$^{-1}$</td>
</tr>
<tr>
<td>$\Omega_{P}$</td>
<td>steady-state binding state for reaction P</td>
<td>variable</td>
<td>–</td>
</tr>
<tr>
<td>$\Omega_{PH}$</td>
<td>steady-state binding state for reaction PH</td>
<td>variable</td>
<td>–</td>
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</table>
elemental stoichiometry (i.e. both have C:N \( \varphi_x \)). The magnitudes of \( P \) and \( H \) are specified by normalizing to structural biomass of the females, i.e. with units \( \text{mol C/(mol C)} \). 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 (Kiørboe 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:

\[
n P \rightarrow X + n P C O_2 \quad (4a)
\]

\[
n PH \rightarrow X + n P C O_2 \quad (4b)
\]

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 \( \psi_{PH} \) in the \( PH \) reaction, and \( \psi_p \) for the \( P \) reaction, respectively), then the yield coefficients are:

\[
n P = \frac{1}{\psi_p} \quad (5a)
\]

\[
n CO_2 = \frac{1}{\psi_p} - 1 \quad (5b)
\]

\[
n NH_4^+ = \frac{1}{\psi_p \varphi_p} - \frac{1}{\varphi_x} \quad (5c)
\]

\[
n PH = \frac{1}{\psi_{PH}} - \frac{\varphi_p}{\varphi_x} \quad (5d)
\]

\[
n P = \frac{\varphi_p}{\varphi_x} \quad (5e)
\]

\[
n PH = \frac{1}{\psi_{PH}} - 1 \quad (5f)
\]

### 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 macrobiochemical 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. 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 \( H \)).

![Fig. 2. Conceptual diagram showing binding of \( P \) and \( H \) by SUs.](image-url)
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, $\rho$ ($0 \leq \rho \leq 1$). H is bound with affinity $\rho^m_H$. The protein-sparing PH reaction is favoured over the P reaction in the model by setting parameter $\rho^P_H$, the affinity for P if H has not yet been bound to the SU, to be lower than parameter $\rho^m_H$, 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: $\theta_e$ (neither site filled), $\theta_P$ (first site filled: P reaction proceeds), $\theta_H$ (second binding site filled), $\theta_{PH}$ (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 $\theta_P$ or $\theta_{PH}$ release their substrates and revert to $\theta_e$. Differential equations for the four binding states are:

$$\frac{d}{dt} \theta_e = k_M - \left( \frac{\rho^m_P}{n^m_P} k_E m_P + \frac{\rho^m_H}{n^m_H} k_E m_H \right) \theta_e \tag{6a}$$

$$\frac{d}{dt} \theta_H = \frac{\rho^m_H}{n^m_H} k_E m_H \theta_e - \frac{\rho^m_P}{n^m_P} k_E m_P \theta_H \tag{6b}$$

$$\frac{d}{dt} \theta_P = \frac{\rho^m_P}{n^m_P} k_E m_P \theta_e - k_M \frac{\theta_P}{\theta_P + \theta_{PH}} \tag{6c}$$

$$\frac{d}{dt} \theta_{PH} = \frac{\rho^m_P}{n^m_P} k_E m_P \theta_H - k_M \frac{\theta_{PH}}{\theta_P + \theta_{PH}} \tag{6d}$$

In this model the total dissociation rate (of $\theta_P$ and $\theta_{PH}$) 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 $\theta_P$ and $\theta_{PH}$ at steady state:

$$\theta_P^* = \frac{\rho^m_P}{n^m_P} m_P \tag{7a}$$

$$\theta_{PH}^* = \frac{\rho^m_P}{n^m_P} m_H \tag{7b}$$

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

$$j_{P,M} = k_M (n^P_P \Omega_{PH} + n^P_H \Omega_P) \tag{8a}$$

$$j_{H,M} = k_M n^P_P \Omega_{PH} \tag{8b}$$

where:

$$\Omega_P = \frac{\theta_P}{\theta_P + \theta_{PH}} \tag{9a}$$

$$\Omega_{PH} = \frac{\theta_{PH}}{\theta_P + \theta_{PH}} \tag{9b}$$

**Egg production**

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

$$j_{P,C} = k_E m_P - j_{P,M} \tag{11a}$$

$$j_{H,C} = k_E m_H - j_{H,M} \tag{11b}$$

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 $\theta_P$ and $\theta_{PH}$ 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 $\theta_e$ and $\theta_H$ binding combinations are:

$$\frac{d}{dt} \theta_e = \frac{\rho^m_P}{n^m_P} j_{P,C} \theta_H - \frac{\rho^m_H}{n^m_H} j_{H,C} \theta_e \tag{12a}$$

$$\frac{d}{dt} \theta_H = \frac{\rho^m_P}{n^m_P} j_{P,C} \theta_e - \frac{\rho^m_H}{n^m_H} j_{H,C} \theta_H \tag{12b}$$
are rejected and assumed to be respired as CO$_2$ or excreted as ammonium:

\[ \frac{\text{d}}{\text{d}t} \left( m_P + m_H + j_P + j_H \right) = j_P + j_H - j_{P,R} - j_{H,R} \]

The amount of X produced as eggs is:

\[ j_{X,G} = \frac{\rho}{n_p} \theta^* \left( \frac{\rho_p}{n_p} \theta^* + \frac{\rho_{PH}}{n_p} \theta^*_H \right) \]

Quantities of protein and carbohydrate used in egg production are:

\[ j_{P,G} = \frac{\rho}{n_p} \theta^* \left( \frac{\rho_p}{n_p} \theta^* + \frac{\rho_{PH}}{n_p} \theta^*_H \right) \]

\[ j_{H,G} = \frac{\rho_{PH}}{n_p} \theta^*_H \]

Fraction $\kappa$ of activated $P$ and $H$ left after maintenance and egg production is returned to the reserve pools. Remaining substrates, $j_{P,R}$ and $j_{H,R}$ for $P$ and $H$ respectively, are rejected and assumed to be respired as CO$_2$ or excreted as ammonium:

\[ j_{P,R} = (1 - \kappa) \left( k_E m_P - j_P - j_P \right) \]

\[ j_{H,R} = (1 - \kappa) \left( k_E m_H - j_H - j_H \right) \]

**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:

\[ \frac{\text{d}}{\text{d}t} m_P = j_{P,A} + \kappa (k_E m_P - j_P) - k_{EM} \]

\[ \frac{\text{d}}{\text{d}t} m_H = j_{H,A} + \kappa (k_E m_H - j_H) - k_{EM} \]

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

\[ \text{C:} \quad j_{P,A} + j_{H,A} = j_{X,M} + j_{CO_2,M} + j_{X,G} + j_{CO_2,G} + j_{P,R} + j_{H,R} \]

\[ \text{N:} \quad \frac{j_{P,A}}{\varphi_P} = \frac{j_{X,M} + j_{X,G}}{\varphi_X} + j_{NH_3,M} + j_{NH_3,G} + \frac{j_{P,R}}{\varphi_P} \]

**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 Kiorboe (Kiorboe, 1989).

A C:N ratio for nitrogenous substrates (proteins), $\varphi_P$, of 3.7 mol C (mol N)$^{-1}$ was used (Vollenweider, 1985). The ratio in eggs, $\varphi_X$, was set at 5.9 mol C (mol N)$^{-1}$, based on the C:N in eggs of *Acartia* (Kiorboe et al., 1985). The stoichiometric coefficients in the $PH$ and $P$ reactions (equations 5a–f) are then determined from parameters $\psi_{PH}$, $\psi_P$, 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 $\sim 0.75$ (Calow, 1977) and have accordingly used this value when setting $\psi_{PH}$, $\psi_P$. Equations 4a and b can accordingly be rewritten as:

\[ 0.63P + 0.71H \rightarrow X + 0.33CO_2 \]

\[ 1.33P \rightarrow X + 0.19NH_3 + 0.33CO_2 \]
A high affinity, 0.95, was set for the binding efficiency of $P$ to SU in state $\theta_H$ (parameter $\rho_{PH}^P$), thereby allowing the favoured $PH$ reaction to proceed readily. Setting values for the binding probabilities of $P$ and $H$ to SU in state $\theta_P$, respectively, is more problematic. One might expect the binding of $H$ to $\theta_P$ to have a lower affinity, suppressing the $P$ route, i.e. $\rho_P^P < \rho_{PH}^P$. However, if it is assumed that copepods can in principle fulfill maintenance requirements solely using protein reserves, a constraint in the model is that $\rho_P^P \geq \rho_{PH}^P$ (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 $\rho_P^P = \rho_{PH}^P = 0.66$.

The reserve mobilization rate, $k_M$, 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 $\varphi_F$ is 6.625 (the Redfield ratio), increasing to 10.5 for $\varphi_F$ of 35. The fractions of substrates rejected by the maintenance and production routes, $\rho_P^H$ and $\rho_{PH}^P$, respectively, is more problematic. One might expect the binding of $H$ to $\theta_P$ to have a lower affinity, suppressing the $P$ route, i.e. $\rho_P^P < \rho_{PH}^P$. However, if it is assumed that copepods can in principle fulfill maintenance requirements solely using protein reserves, a constraint in the model is that $\rho_P^P \geq \rho_{PH}^P$ (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 $\rho_P^P = \rho_{PH}^P = 0.66$.

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**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. The model and data is shown in Figure 3, achieved with $k_M = 0.0041$ h$^{-1}$ and $\rho_P^P = \rho_{PH}^P = 0.66$. 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, $\varphi_F$, 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...
that the fraction of N allocated to pellets is constant at 0.312. The fraction of C egested as pellets declines at high \( F \) 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; Kiorboe 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 Kiorboe et al. (Kiorboe 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

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**Fig. 4.** Predicted fate of C and N in food for algal C:N 5–35: (a) constant intake of C of 0.06 mol C (mol C\(^{-1}\)) h\(^{-1}\), (b) constant intake of N of 0.01 mol N (mol C\(^{-1}\)) 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.
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 CO₂ associated with maintenance is also a constant fraction of C intake because the \( \text{PH} \) and \( \text{P} \) reactions both yield the same amount of CO₂ (equations 19a and b). However, production of CO₂ associated with the energetic costs of egg production decline with increasing \( \varphi_F \) 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 \( \varphi_F \) 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 \( \varphi_F \) because the increased availability of \( H \) relative to \( P \) favours the \( \text{PH} \) 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 \( \varphi_F \) 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 \( \varphi_F \). 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 \( \varphi_F \) because there is a ready supply of \( H \) to favour the \( \text{PH} \) 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 \( \varphi_F \) is below 0.0162 then there is insufficient C to meet the requirements for maintenance and zero egg production (the minimum ration depends on \( \varphi_F \), increasing at high values). Results are consistent with experimental observations (Kiørboe 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 (Kiørboe, 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
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, $\varphi_F$, observed by Kiørboe (Kiørboe, 1989). It also captures the relatively low $K_N$, but predicts a decrease at high $\varphi_F$, 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, $\rho^P_F$, $\rho^P_H$, and $\rho^P_0$. With the exception of $\rho^P_0$ (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 $K_N$ 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 nl O$_2$ ind$^{-1}$ min$^{-1}$, converted assuming 4 µg 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 (Penaus 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 shown in Figure 6. 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_{16}H_{36}O_{2} and CH_{2}O 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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**APPENDIX I: CONSTRAINTS TO VALUES OF ρ PARAMETERS**

When there is insufficient supply of reserves to fulfill 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.

\[ j_{P,M} = k_M \left( \frac{n_P^P \rho_P^P}{\rho_P^H} j_{P,C} + \frac{n_P^{PH} \rho_{PH}^H}{\rho_P^H} j_{H,C} \right) \quad (A1a) \]

\[ j_{H,M} = k_M \left( \frac{n_P^P \rho_P^P}{\rho_P^H} j_{P,C} + \frac{n_P^{PH} \rho_{PH}^H}{\rho_P^H} j_{H,C} \right) \quad (A1b) \]

in which \( j_{H,C} = k_{EMH} \) and \( j_{P,C} = k_{EPM} \).

It is assumed that copepods can potentially fulfill 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:

\[ j_{H,C} = n_P^{PH} k_M - \frac{n_P^P \rho_P^P}{\rho_P^H} j_{P,C} \quad (A2a) \]

\[ j_{H,C} = \frac{n_P^P \rho_P^P}{\rho_P^H} \left( j_{P,C} - \frac{k_M j_{H,C}}{n_P^{PH}} \right) \quad (A2b) \]

Substitution of these two gives, alter 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:

\[ j_{P,C}^* = \frac{n_P^{PH} n_P^P k_M}{\rho_P^H + \rho_P^P \rho_{PH}^H \rho_P^P} \quad (A3) \]

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:

\[ \rho_P^P (n_P^{PH} - n_P^P) \geq \rho_P^P (n_P^{PH} - n_P^P) \quad (A4) \]

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 \( \rho_P^P \geq \rho_{PH}^H \). To obtain a preference for the \( PH \)-route, \( \rho_P^P \) has to be as large as possible and, therefore, we use \( \rho_P^P = \rho_{PH}^H \).