Abstract: A stoichiometric approach is applied to model nutrient element content and population growth kinetics in phagotrophic flagellates. Available evidence is limited, but suggests that the nutrient composition of flagellates is not strictly homeostatic, but instead varies with the nutrient element composition of their food resources. A mathematical model is constructed that couples the C, N, and P contents of flagellates to their population growth rate and the nutrient fluxes assimilated from food resources. Variants of the model are explored to examine the effects of saturating ingestion, maintenance respiration, and selective feeding from food mixtures. In agreement with observations, the models predict non-homeostatic variation in the nutrient content of flagellates. Population growth rate is predicted to vary with both food quantity and quality (in terms of nutrient element content). It is proposed that lack of homeostasis and selective feeding on prey with high nutrient content enhance fitness of phagotrophic flagellates under some conditions.

Key words: ecological stoichiometry, homeostasis, heterotrophic nanoflagellates, phagotrophy, cell quota, Droop model.

Introduction

The rates at which organisms consume resources and use them for growth and reproduction are fundamental to many of the questions that have fascinated WINFRIED LAMPERT (e.g. LAMPERT 1977 a, b, ROTHHAUPT & LAMPERT 1992, KESSLER & LAMPERT 2004). For individuals, the energy and nutrient budgets summarized by these rates predict fitness in some environments and constrain it in others. For populations, rates of resource processing can determine com-
petitive ability and many other aspects of a species’ niche. Finally, when resources are partitioned among multiple species, the processing of resources becomes intimately tied to ecosystem dynamics and species diversity. Thus many levels of the biological hierarchy are linked through basic ecophysiological rates.

For many heterotrophs, theoretical representations of resource processing can often be simplified through assumptions of homeostasis. The approach of “ecological stoichiometry” has emphasized a strong version of homeostasis, in which the element composition of a consumer is strictly regulated (Sterner & Elser 2002). The rate at which consumers produce new biomass through growth and reproduction is then simply related to their rate of resource consumption, and a comparison of the element composition of consumers versus resources permits budgeting of assimilated and recycled nutrient fluxes. Strong homeostasis applies, as a good first approximation, to some zooplankton (Sterner 1990, Hessen 1990, Andersen & Hessen 1991) and underpins an extensive body of theory representing zooplankton-phytoplankton-nutrient interactions (Andersen 1997, Hessen & Bjorking 1997, Elser & Urabe 1999, Grover 2002, Hall 2004, Loladze et al. 2004).

On the other hand, it is well appreciated that the assumption of strong homeostasis applies poorly to many autotrophs, which can typically accumulate high levels of nutrient elements (Sterner & Elser 2002). Variations in stored nutrients, in terms of both identity and quantity, and their subsequent use in growth are important determinants of phytoplankton competitive fitness (Turpin 1988, Grover 1991), and of their quality as food for zooplankton (Mitchell et al. 1992, Sterner 1993, Sterner et al. 1993, Urabe et al. 1997, Demott et al. 1998).

The smallest zooplankton—phagotrophic flagellates—consume particulate matter like the larger zooplankton, though their foods are small compared to that consumed by metazoans. The ecophysiology of resource consumption and nutrient processing in phagotrophic flagellates has received less attention than have the same processes in other zooplankton. As yet, it is unclear whether the assumption of homeostasis is reasonable for phagotrophic flagellates. We suspect it is not since most clades of phagotrophic flagellates are phylogenetically related to certain of the algae, and some “algae” that retain plastids are predominantly phagotrophic.

This paper briefly summarizes observations suggesting that phagotrophic flagellates are not strictly homeostatic and proposes theoretical models to describe variable nutrient composition. Predictions of these models are presented for relationships between growth rates and food quantity and quality, and for the nutrient element composition of flagellates. The theory developed here for heterotrophic flagellates might also apply to mixotrophs under some conditions. The theory suggests hypotheses for further research on these organisms,
while contributing to an understanding of consumer-resource dynamics when strict homeostasis does not apply.

**The nutrient composition of phagotrophic flagellates**

There is little experimental data on the nutrient composition of phagotrophic flagellates. This is, perhaps, not surprising given the technical difficulties of separating flagellates from their bacterial prey and ensuring that undigested and unassimilated prey items within food vacuoles do not contribute significantly to the element composition attributed to the flagellate itself. Some phagotrophic flagellates are mixotrophs that can be grown autotrophically, and during such growth they can display variations in cellular nutrient content similar to those documented in strictly autotrophic algae. The P content of *Dinobryon cylindricum* varies up to tenfold (Sandgren 1988), while that of *Prymnesium patelliferum* varies a more modest twofold (Legrand et al. 2001). These observations suggest that mixotrophs share the relative lack of homeostasis characteristic of autotrophic algae, and point to a possible lack of strict homeostasis for phagotrophic flagellates in general.

**Stern & Elser (2002)** suggested examining homeostasis of nutrient competition with log-log plots of consumer versus resource nutrient ratios. A simple linear relationship having a slope approaching unity suggests a lack of homeostasis; the element composition of the predator varies linearly with the element composition of the prey. A slope of zero suggests strict homeostasis; predator element stoichiometry remains constant in the face of shifting element composition of prey items. A slope between one and zero implies weak physiological regulation in the direction of homeostasis; the element composition of the predator varies less than a strict one-to-one proportion with the element composition of the prey.

The most extensive data addressing the element composition of phagotrophic flagellates are those of Goldman et al. (1987) and Nakano (1994). Goldman et al. (1987) fed the flagellate *Paraphysomonas imperforata* two species of algae (*Phaeodactylum tricornutum* and *Dunaliella tertiolecta*) of varying C:N:P stoichiometry. Nakano (1984) fed an unidentified heterotrophic flagellate (thought to be *Paraphysomonas* or *Spumella*) four types of bacteria with varying C:N:P stoichiometry.

We have used these data to develop stoichiometric homeostasis plots for various nutrient ratios for phagotrophic flagellates and their prey (Fig. 1), which suggest that phagotrophic flagellates may not be strictly homeostatic. C:N ratios in phagotrophic flagellates, like that of bacteria (Chrzanowski & Kyle 1996), seem to be less variable than N:P or C:P ratios. The phagotrophic flagellate C:N ratio (panel A) also appears to be more highly correlated with prey element stoichiometry than does either phagotrophic flagellate N:P (panel B) or C:P (panel C) suggesting greater flexibility in regulation of P accumulation. Flexibility in P accumulation is consistent with models suggesting that P content (or Q, see below) must increase as a function of growth rate (Elser et al. 1996). Interestingly, the slopes of these relationships are similar in magnitude to those found for a wood-decaying fungus (Stern & Elser 2002), suggesting a similar degree of weak homeostasis in another group of heterotrophic eukaryotic microbes.
Fig. 1. Stoichiometric homeostasis plots for phagotrophic flagellates fed prey items of varying element stoichiometry. Dotted line is the 1:1 line. Circles – data from GOLDMAN et al. (1987); triangles – data from NAKANO (1994). All regression lines are statistically significant, P <0.01.
Theoretical models of flagellate growth and nutrient composition

Grover (2003, 2004) represented the population growth rate ($\mu$) of phagotrophic flagellates in relation to nutrient element composition with the equation

$$
\mu = \mu_{\text{max}} \left( 1 - \max_j \left\{ \frac{Q_j^{\text{min}}}{Q_j} \right\} \right) \quad \text{for } j = C, N, P
$$

(1).

Here, $Q_j$ are the cell quotas of nutrient elements (C, N, or P), i.e. the nutrient mass per cell (see Table I for notation). The parameter $Q_j^{\text{min}}$ is the minimal quota for nutrient $j$, at which the growth rate goes to zero. The parameter $\mu_{\text{max}}$ is the apparent maximal growth rate that would occur if quotas of all nutrients were infinite. Realized maximal growth rates are lower, due to physiological limitations described below. According to equation (1), the growth rate is limited by the nutrient whose quota is lowest in relation to its minimal value. If quotas of any two elements are sufficiently high, then the growth rate is an increasing and saturating function of the third element’s quota. Since its introduction by Droop (1974), equation (1) has been widely used to model algal growth, and it has also been applied to bacterial growth (Thingstad 1987).

Equation (1) for growth rate presumes complementary equations governing quota dynamics. Phagotrophic flagellates acquire nutrients through ingestion of bacterial prey. Let the ingestion rate (prey cells ingested per unit time) be denoted $\nu(X)$, where $X$ is prey density. Then the nutrient flux made available through ingestion is the product of the ingestion rate and the quota of nutrient $j$ in prey cells, $q_j$. Typically, only a fraction of this ingested nutrient flux is assimilated, while the remainder is released at a rate $R_j$. An additional nutrient flux is related to population growth: the partitioning of quota into new cells produced at a rate $\mu$ decreases quota at the instantaneous rate $\mu Q_j$. These assumptions lead to the differential equations

$$
\frac{dQ_j}{dt} = \nu(X)q_j - \mu Q_j - R_j
$$

(2).

Grover (2003, 2004) used a simple linear function of prey density for the ingestion rate:

$$
\nu(X) = \alpha X
$$

(3),

where $\alpha$ is the attack rate, and also the clearance rate, representing the volume of water cleared of food by ingestion per unit time.

In previous work, the nutrient release rate followed the expression

$$
R_j = \nu(X)q_j \left[ 1 - e_j \left( \frac{Q_j^{\text{max}} - Q_j}{Q_j^{\text{max}} - Q_j^{\text{min}}} \right) \right]
$$

(4),

where $Q_j^{\text{max}}$ is the physiological upper limit to cell quota and $e_j$ is the dimensionless, maximal assimilation efficiency of nutrient $j$. Equation (4) for nutrient release is equivalent to assuming that the net assimilation rate ($A_j$) is
\[ A_j = \mu(X)q_j \frac{Q_j^{\text{max}} - Q_j}{Q_j^{\text{max}} - Q_j^{\text{min}}} \] 

which shows that assimilation efficiency is maximal when quota approaches its minimal value, and is reduced to zero as quota approaches its maximal value. Equation (4)

### Table 1. Notation, dimensions and parameter values.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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<tbody>
<tr>
<td>( \mu )</td>
<td>Population growth rate</td>
</tr>
<tr>
<td>( Q_C )</td>
<td>Cell quota for carbon</td>
</tr>
<tr>
<td>( Q_N )</td>
<td>Cell quota for nitrogen</td>
</tr>
<tr>
<td>( Q_P )</td>
<td>Cell quota for phosphorus</td>
</tr>
<tr>
<td>( \mu_{\text{max}} )</td>
<td>Maximal growth rate occurring if cell quotas are infinite</td>
</tr>
<tr>
<td>( Q_{C}^{\text{min}} )</td>
<td>Minimal cell quota for carbon</td>
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<tr>
<td>( Q_{N}^{\text{min}} )</td>
<td>Minimal cell quota for nitrogen</td>
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<td>( Q_{P}^{\text{min}} )</td>
<td>Minimal cell quota for phosphorus</td>
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<td>( Q_{C}^{\text{max}} )</td>
<td>Maximal cell quota for carbon</td>
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<td>( Q_{N}^{\text{max}} )</td>
<td>Maximal cell quota for nitrogen</td>
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<td>( Q_{P}^{\text{max}} )</td>
<td>Maximal cell quota for phosphorus</td>
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<tr>
<td>( \varepsilon_C )</td>
<td>Maximal assimilation efficiency for carbon</td>
</tr>
<tr>
<td>( \varepsilon_N )</td>
<td>Maximal assimilation efficiency for nitrogen</td>
</tr>
<tr>
<td>( \varepsilon_P )</td>
<td>Maximal assimilation efficiency for phosphorus</td>
</tr>
<tr>
<td>( \rho_C )</td>
<td>Fixed release rate for carbon, representing maintenance respiration</td>
</tr>
<tr>
<td>( \rho_N )</td>
<td>Fixed release rate for nitrogen</td>
</tr>
<tr>
<td>( \rho_P )</td>
<td>Fixed release rate for phosphorus</td>
</tr>
<tr>
<td>( \alpha )</td>
<td>Attack rate</td>
</tr>
<tr>
<td>( \tau )</td>
<td>Handling time</td>
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<tr>
<td>( X_0 )</td>
<td>Threshold prey density at which flexible preferences occur</td>
</tr>
<tr>
<td>( X )</td>
<td>Prey density</td>
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<tr>
<td>( q_C )</td>
<td>Prey cell quota for carbon</td>
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<tr>
<td>( q_N )</td>
<td>Prey cell quota for nitrogen</td>
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<tr>
<td>( q_P )</td>
<td>Prey cell quota for phosphorus</td>
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### Other symbols
- \( j \) Index for nutrients C, N or P
- \( k \) Index for a non-limiting nutrient
- \( l \) Index for a limiting nutrient
- \( n \) Index for non-preferred prey type in a mixture
- \( p \) Index for a preferred prey type in a mixture
- \( R_j \) Release rate of nutrient \( j \)
- \( A_j \) Assimilation rate of nutrient \( j \)
I predicts that nutrient release rates go to zero when prey density, and hence ingestion rate, approach zero. For the inorganic nutrients N and P this is perhaps reasonable, but for C the release rate specified by equation (4) includes respiration. Thus maintenance respiration that occurs during starvation is neglected.

In this paper, the previous model (Grover 2003, 2004) is extended by including more realistic assumptions of saturating ingestion and maintenance respiration. Saturating ingestion is described by the function

$$I(X) = \frac{\alpha X}{1 + \alpha \tau X}$$

where $\tau$ is the handling time per prey cell and $\alpha$ is the attack rate. This latter rate also represents the maximal clearance rate exhibited under low prey density. Equation (6) corresponds to an ingestion function with a maximal ingestion rate of $I_{max} = \tau^{-1}$, reached asymptotically at high prey density, and half-saturation prey density of $K_i = \frac{I_{max}}{\alpha}$. Incorporating maintenance respiration, or other losses under starvation conditions, is accomplished by including a constant release rate $\rho_j$ into equation (4):

$$R_j = I(X)q_j \left[ 1 - e_j \left( \frac{Q_j^{\text{max}} - Q_j^{\text{min}}}{Q_j^{\text{max}} - Q_j^{\text{min}}} \right) \right] + \rho_j$$

Additional extensions of previous work permit a preliminary exploration of prey mixtures, which could represent nutritionally different bacterial species or strains. These are applied only with the most realistic assumptions detailed above, entailing saturating ingestion rates and maintenance respiration. Only mixtures of two prey types are considered here, with ingestion rates following an extension of equation (6):

$$I(X_j) = \frac{\alpha_i X_i}{1 + \alpha_1 \tau_1 X_1 + \alpha_2 \tau_2 X_2}$$

where subscript $i$ indicates prey type 1 or 2. Equation (1) is unchanged for prey mixtures, but the first term equation (2) must be replaced by the flux of nutrient $j$ obtained from ingesting both prey types: $I(X_1)q_{1j} + I(X_2)q_{2j}$. Preferences for ingesting different prey types could follow many scenarios, but two are examined here: fixed preferences quantified as differences in attack rate constants $\alpha_i$, and flexible preferences exhibited only when total prey density is high (Boenigk et al. 2002). For the latter, the attack rate on the less-preferred prey type was a decreasing function of total prey density above a threshold, but equal to the attack rate on the preferred prey type below the threshold. A simple function with these properties is

$$\alpha_s = \min \left\{ \alpha_r, \alpha_p \frac{X_0}{X_1 + X_2} \right\}$$

where subscripts $n$ and $p$ indicate less preferred and preferred prey types, respectively, and $X_0$ is the threshold prey density at which ingestion becomes selective.
Numerical analyses

These theories are explored to develop numerical predictions of flagellate growth rates and nutrient composition in relation to prey density and food quality as nutrient composition. The relationship between growth rate and prey density is commonly studied in laboratory cultures (e.g. FENCHEL 1982). Although nutrient composition of flagellates is technically more difficult to study, predictions are presented for comparison to the few data available (Fig. 1).

The model with linear ingestion and no maintenance respiration was previously parameterized (GROVER 2003) based on a number of laboratory studies of the flagellate genus Paraphysomonas (CARON et al. 1985, 1986, GOLDMAN & CARON 1985, GOLDMAN et al. 1985, 1987, ANDERSEN et al. 1986, NAKANO 1994, ECCLESTON-PARRY & LEADBETTER 1995). The same parameters are adopted here. For Paraphysomonas preying on bacteria, GOLDMAN & DENNETT (1992) found a maximal ingestion rate of about 800 prey cells d⁻¹, implying a handling time of 0.00125 d. Respiration rates of starving flagellates are typically about 2-4 % of respiration rates during exponential growth (FENCHEL 1982). CARON et al. (1986) observed respiration rates of about 2000 fmol C cell⁻¹ d⁻¹ for exponentially-growing Paraphysomonas, so a value ρ_C = 80 fmol C cell⁻¹ d⁻¹ is adopted here. Release of N and P under starvation is assumed not to occur, so ρ_N = ρ_P = 0. The ranges of prey quotas (q_j) considered here represent bacteria that vary two-fold in C and N content (THINGSTAD 1987), but six-fold in P-content (VADSTEIN 2000).

Three versions of models parameterized with values from Table 1 were examined for a single type of prey: (I) the original model with linear ingestion and no maintenance respiration (GROVER 2003); (II) a model with saturating ingestion but no maintenance respiration; and (III) a model with both saturating ingestion and maintenance respiration. For each version, steady state flagellate quotas for C, N and P are calculated across a range of prey densities X and nutrient contents q_j, from setting equations (2) to zero. Only one nutrient (denoted l) limits growth rate and its steady state quota is

\[ Q^*_l = \frac{\mu(C)q_l + \mu_{max}(Q_{lmax} - Q_{lmin})}{\mu(C)q_l + \mu_{max}(Q_{lmax} - Q_{lmin})} \]

This equation must be evaluated for all three nutrients, and the one producing the lowest growth rate according to equation (1) is identified as the limiting nutrient. The steady state quotas of the other, non-limiting nutrients (denoted k) then depend on the growth rate calculated from the limiting nutrient, according to

\[ Q^*_k = \frac{\mu(C)q_k + \mu(C)(Q_{kmax} - Q_{kmin})}{\mu(C)q_k + \mu(C)(Q_{kmax} - Q_{kmin})} \]

Note that in equations (10) and (11), the terms ρ_l and ρ_k are zero, except for the case of carbon (ρ_C) in model III with maintenance respiration.

For each of the three models, steady state growth rates in relation to density X of a single prey type were calculated from zero to \( 1 \times 10^8 \) cells ml⁻¹, for all combinations of
the highest and lowest nutrient quotas considered for bacterial prey in Table 1. Only selected examples are presented below to illustrate the effects of food quality (as nutrient composition) on growth rate. To explore food quality further, stoichiometric plots following Fig. 1 were constructed with varying nutrient composition of prey. Bacterial C quota $q_C$ was set to 1.6 fmol C cell$^{-1}$, and N quota was increased over the range indicated in Table 1, while P quota was decreased. Steady state nutrient composition of flagellates was then calculated according to equations (10) and (11) at a low prey density of $3 \times 10^6$ cells ml$^{-1}$, and a high prey density of $1 \times 10^8$ cells ml$^{-1}$. The higher prey density saturates growth under all conditions examined, while the lower prey density supports positive growth under all conditions examined. Model III with maintenance respiration predicts negative growth rates at very low prey densities, under which conditions equation (11) can predict negative cell quotas, and such computations are avoided here.

For prey mixtures, only model III with both saturating ingestion and maintenance respiration was considered. For simplicity, handling times for both prey types in equation (8) were set equal to the value used for single prey scenarios. There is only scant justification for assuming equal handling times of prey types. SHANNON (2006) found that digestion kinetics were invariant for a strain of Ochromonas fed prey of differing nutrient element composition, suggesting that some of the processes related to handling time might also be invariant for different prey types (but see BOENIGK et al. 2001a, b). For the fixed preference scenario, the attack rate on the less preferred prey type was set to $4.0 \times 10^5$ ml flagellate$^{-1}$ d$^{-1}$, about half the attack rate on the preferred prey type ($8.1 \times 10^5$ ml flagellate$^{-1}$ d$^{-1}$). For the flexible preference scenario, the attack rate on the preferred prey type was again set to this value, and the preference threshold $X_0$ was set to $1 \times 10^7$ cells ml$^{-1}$, roughly the prey density above which BOENIGK et al. (2002) observed selective ingestion in three flagellate species. Several combinations of preferences and nutrient contents for various mixtures of prey types were examined numerically, and only some selected results are presented to illustrate potential effects of prey mixtures on flagellate growth rates and nutrient composition.

Results

Growth on a single prey type

Although the simplest model examined here assumes that ingestion rate is a linear function of prey density, growth rate is a saturating function for this model (Fig. 2 A). The saturated growth rate is always less than the parameter $\mu_{max}$ and the half-saturating prey density depends on both prey nutrient content and the parameters describing flagellate physiology. The identity of the nutrient that limits growth depends primarily on prey nutrient content, rather than prey density. In one of the cases illustrated, with prey nutrient quotas at the lowest values in Table 1, N limits flagellate growth at all prey densities, while C limits in the other cases shown.
Fig. 2. Growth rate in relation prey density, for parameterized models of flagellates growing on bacteria of differing nutrient content: solid line – bacterial quotas of all nutrients set to low values of ranges in Table 1; dashed line – bacterial quotas of all nutrients set to high values of ranges in Table 1; dotted line – bacterial quota for C set to low value and quotas for N and P set to high values of these ranges. A. Model I with linear ingestion and no maintenance respiration. B. Model II with saturating ingestion and no maintenance respiration; C. Model III with saturating ingestion and maintenance respiration.
Fig. 3. Stoichiometric homeostasis plots for phagotrophic flagellates fed prey of varying element stoichiometry predicted from parameterized models. Dotted line is the 1:1 line. Solid line – low food density; dashed line – high food density. A–C. Model I with linear ingestion and no maintenance respiration. D–F. Model II with saturating ingestion and no maintenance respiration. G–I. Model III with saturating ingestion and maintenance respiration.
When saturating ingestion is incorporated in model II, growth rate is again a saturating function of prey density (Fig. 2B). The saturated growth rate is reduced compared to model I, because saturating ingestion constrains the nutrient fluxes made available for growth. The saturated growth rate also strongly depends on prey nutrient content. The half-saturating prey density for ingestion is $9.9 \times 10^6$ cells ml$^{-1}$, but the half-saturating prey density for growth ranges about 30–50% of this value, depending on prey nutrient content. The identity of the nutrient that limits growth depends primarily on prey nutrient content, rather than prey density. In one of the cases illustrated, with prey C quotas at the lowest value in Table 1 and prey N and P quotas at the highest values, C limits flagellate growth at all prey densities, while N limits in the other cases shown.

When both saturating ingestion and maintenance respiration are incorporated in model III, growth rate is a saturating function of prey density with a threshold required for positive growth (Fig. 2C). These thresholds range from about $8 \times 10^5$ cells ml$^{-1}$ for prey with high C content to $2 \times 10^6$ cells ml$^{-1}$ for prey with low C content. Saturated growth rates are not changed much by adding maintenance respiration to the model, and such respiration has a much stronger effect on growth at low prey density. The half-saturating prey density for growth ranges about 30–70% of the density that is half-saturating for ingestion, depending on prey nutrient content. Growth is always limited by C at low prey density, due to the C requirement for maintenance respiration. For prey with high C content, the nutrient that limits growth switches from C to N as prey density increases, usually at a prey density supporting growth at 50–70% of the saturated rate.

The models examined here usually predict that flagellate nutrient content displays weak homeostasis (Fig. 3), qualitatively similar to observations (Fig. 1). That is, the slopes of stoichiometric plots are similar for predictions (Fig. 3) and observations (Fig. 1). For model I, homeostasis is more closely approached at high prey density than at low prey density (Figs 3A–C). When saturating ingestion is incorporated in model II, the approach to homeostasis at high prey density disappears, and flagellate nutrient composition responds strongly to prey composition at all prey densities (Figs 3D–F). When both saturating ingestion and maintenance respiration are incorporated in model III, weak homeostasis at all prey densities is preserved (Figs 3G–I). However, low prey density strongly reduces the relative amount of C in flagellates, due to the C requirements of maintenance respiration.

**Growth on prey mixtures**

When flagellates feed on a mixture of two prey types with fixed preferences, growth rate is predicted to be a saturating function of total prey density with a
Fig. 4. Growth rate in relation prey density, for parameterized models of flagellates growing on mixtures of two prey types, one with high N and P and low C content, and the other with low N and P and high C content. Upper dotted line – growth on high NP prey alone; lower dotted line – growth on low NP prey alone; dashed line – growth on a mixture of two prey types with non-preferential feeding; solid line – growth on a mixture of two prey types with preferential feeding on the high NP prey. A. Fixed feeding preferences, mixtures with 30% prey of the high NP type. B. Flexible feeding preferences, mixtures with 20% prey of the high NP type.

threshold for growth (Fig. 4 A). The exact relationship depends on prey nutrient contents, flagellate preferences, and the proportion of each prey type in the mixture. For purposes of illustration, one prey type is assigned the lowest C
quota from the range in Table 1, and the highest quotas for N and P, while the other type is assigned the higher C quota and the lowest quotas for N and P. Thus one prey type is rich in the inorganic nutrients N and P relative to organic C, while the other has opposite characteristics.

The upper dotted line in Fig. 4A shows growth in relation to prey density when the flagellate feeds on only the high NP prey, and the lower dotted line shows growth when feeding only on the low NP prey. The solid line shows growth when feeding on a mixture with 30% prey of the high NP type, assumed to be the preferred type (with a higher attack rate parameter in equation 8). For comparison, the dashed line in Fig. 4A shows growth for the same mixture, but with no feeding preferences and both prey types assigned the same, high value for the attack rate. Thus, preference is modeled as a reduced rate of ingestion for the high C, low NP prey type. This preference is costly at low prey densities for which C limits flagellate growth, but is beneficial at high prey density, permitting more rapid growth than would non-preferential feeding. For mixtures with other proportions of prey types (not shown), preferential feeding on the low C, high NP prey type is always beneficial at sufficiently high prey densities, but costly at lower prey densities. Preferential feeding on the high C, low NP prey type is never predicted to be beneficial compared to non-preferential feeding.

The costs of preferential feeding on prey with low C content, but high NP content at low prey densities are ameliorated to some extent when feeding preferences are flexible, rather than fixed (Fig. 4B). At the lowest prey densities, feeding is non-preferential, and the predicted growth rate with feeding preferences (solid line in Fig. 4B) is identical to that for non-preferential feeding (dashed line in Fig. 4B). For intermediate prey densities, preferential feeding on the high NP prey type is costly, reducing growth below that achieved from non-preferential feeding. For sufficiently high prey densities, preferential feeding on the high NP prey type is beneficial, elevating growth above that achieved from non-preferential feeding. These results are illustrated in Fig. 4B for a mixture with 20% prey of the high NP type, but are qualitatively similar for various other mixtures fed upon with flexible preferences. For all mixtures examined, flexible, preferential feeding on the high C, low NP prey type is never beneficial compared to non-preferential feeding.

Preferential feeding on mixtures of prey types with different nutrient composition could have complex effects on flagellate nutrient composition (Fig. 5). With fixed preferences (Figs 5A–C), stoichiometric plots for C:N and C:P ratios display weak homeostasis, in agreement with results for a single prey type. However, something close to homeostasis is predicted for the flagellate N:P ratio under the conditions examined: mixtures with 0–100% prey of the low C, high NP type, at two total prey densities (3 × 10⁶ and 1 × 10⁸ cells ml⁻¹). Flexible preferences produce similar results at the low total
Fig. 5. Stoichiometric homeostasis plots for phagotrophic flagellates feeding on mixtures of two prey types, one with high N and P and low C content, and the other with low N and P and high C content. Average prey stoichiometry is shown on the horizontal axes, based on the proportion of two types in the mixture, varying from 0–100%. Dotted line is the 1:1 line. Solid line – low food density; dashed line – high food density. A–C. Fixed preference for the prey type with high NP content is assumed. D–F. A flexible preference for the prey type with high NP content is assumed.

prey density (Fig. 5 D–F), but at high total prey density, flagellates are predicted to be close to homeostatic for C:N and C:P.
Discussion

Based on admittedly limited data (Fig. 1), we have proposed that the nutrient composition of phagotrophic flagellates is weakly, but not strictly homeostatic. The theory presented here predicts weak homeostasis and permits exploration of its implications. Even if departures from strict homeostasis are relatively weak, compared to autotrophs for example, there are two reasons to explore their implications. Departures from homeostasis influence the competitive fitness of consumers, and they influence the biogeochemical impacts of consumer-resource interactions.

The fitness implications of weak homeostasis are related to the decoupling that causes the growth rate to saturate at a lower prey density than the ingestion rate. DROOP's equation (1) implies that as a consumer's growth rate becomes nutrient limited and is reduced from maximal, the quota for the limiting nutrient falls from the high level required for maximal growth. This in turn reduces the nutrient flux required for growth, while maintaining growth rate as high as possible, ultimately reducing the ingestion rate and prey density required to meet demands for growth. This permits the consumer to achieve a lower "R*" (sensu Tilman 1982, pp. 43-45) in terms of prey density. Other things being equal, the species with weaker homeostasis would thus have better competitive fitness in a constant environment.

It seems likely that weak homeostasis could also contribute to competitive fitness in non-constant environments. When DROOP's equation is applied to the theory of competition among algae for dissolved nutrients, increasing $Q_{\text{max}}$ and thus weakening homeostasis permits nutrient storage that is competitively advantageous when nutrient supply varies and long periods of starvation occur (Grover 1991). Therefore, in addition to proposing that phagotrophic flagellates display weak homeostasis in nutrient composition, we also propose that they are under selective pressure against strict homeostasis.

Weak homeostasis also affects the biogeochemical implications of consumer-resource interactions, which can be partly understood from plots such as those in Fig. 1. The intersection of the regression line with the 1:1 line estimates a resource stoichiometry that is "optimal" in that it matches consumer stoichiometry. Under such conditions the need for consumers to dispose of excess nutrients by excreting or otherwise releasing them is minimized. Owing to less than perfect assimilation of resources, consumers will likely still release nutrients in a ratio matching that of the resources. If the resource population relies on these released nutrients, as the bacterial and algal prey of flagellates often do, then stoichiometric matching of consumer and resource trophic levels implies an ecosystem "optimality", wherein trophic efficiency and nutrient retention are maximized. If resource stoichiometry does not match consumer stoichiometry, then the strength of homeostasis – related to the slopes
of the regression lines in Fig. 1 – determines how much excess nutrient is released by consumers. As the slope of such a line reduces to zero, strict homeostasis is approached and the release of excess nutrients is maximized. Released nutrients are often dissolved and at risk of transport from the ecosystem. Rotating the regression lines in Fig. 1 counter-clockwise weakens homeostasis, and implies that consumers retain a greater proportion of excess nutrients, potentially holding them within the ecosystem.

Despite qualitative agreement, the elevations of the observed stoichiometric plots (Fig. 1) differ consistently from the predictions developed here (Figs 3 and 5). The mathematical models portray flagellates with higher N contents than the observations, despite assigning parameters for the genus Paraphysomonas using data from some of the same studies. In part, this discrepancy might arise from simulating bacterial prey in the models, while the experimental observations involved a broader range of prey including small algae. The discrepancy could also arise from other simplifications made in constructing the models. With the assigned parameters, these models predict that flagellate growth is usually C- or N-limited under the conditions explored, and rarely P-limited. If flagellates were parameterized with relatively lower N contents and higher P contents, then P-limitation would be more often predicted. As yet, too little is known about the nutrient requirements for growth of heterotrophic flagellates to determine their relative susceptibility to limitation by different nutrient elements. The necessary information on the nutrient element composition of flagellates is difficult to obtain, as it requires careful separation from nutrient composition of their prey.

The most realistic parameterized model analyzed here included a saturating ingestion rate and maintenance respiration under starvation conditions. This latter property predicts that a threshold prey density is required for positive population growth. Although this is a realistic prediction (Rothhaupt 1996), we caution against applying our model to very low or negative growth rates. Modifications are needed to describe nutrient composition at negative growth rates, for which equation (11) can present negative solutions. Moreover, cannibalism or transformation to resting cysts or other special cell types occurs when some species are forced to low growth rates (Fenchel 1982, 1986). Nor has this body of theory been explored much under non-steady state conditions (but see Grover 2003, 2004). Coupling between ingestion and growth rates could be especially weak under such conditions, leading to large variations in cell composition.

Much more work is also needed to address feeding on mixtures of prey species and selective ingestion. There are long-standing observations of size-selective feeding flagellates (Chrzanski & Šimek 1990, González et al. 1990), and more recent observations of selectivity based on nutrient composition (John & Davidson 2001). Here, only a few of the conceivable patterns of
selectivity were examined. The results suggest that flagellates might sometimes exhibit a closer approach to homeostatic composition by selectively feeding on prey of different nutrient composition. This is but one issue to explore in future theoretical and experimental work.

If it is true that phagotrophic flagellates often display weak homeostasis as proposed here, it is possible that they could be useful model organisms for studying the implications of weak homeostasis in consumer-resource interactions, much as the cladoceran *Daphnia* has become a model for studying the implications of strict homeostasis. Winfried Lampert contributed much to the study of *Daphnia* biology, for example by developing continuous flow systems permitting steady state growth studies (Lampert 1975, 1976). Many of the references cited in this paper are written by people affiliated in some way with Winfried Lampert. We do not think this is a biased selection of the relevant literature, but rather a genuine outcome of his contributions to aquatic sciences.

References


Stoichiometry of phagotrophic flagellates


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