Building the “perfect beast”: modelling mixotrophic plankton

KEVIN J. FLYNN* AND ADITEE MITRA
INSTITUTE OF ENVIRONMENTAL SUSTAINABILITY, WALLACE BUILDING, SWANSEA UNIVERSITY, SWANSEA SA2 8PE, WALES, UK

*CORRESPONDING AUTHOR: k.j.flynn@swansea.ac.uk

Received April 6, 2009; accepted in principle May 18, 2009; accepted for publication May 22, 2009; published online 28 June, 2009

 Corresponding editor: John Dolan

A mechanistic model is described for carbon–nitrogen–phosphorous-based interactions within a protistan mixotroph. The model describes interactions between photosynthesis (with photoacclimation), inorganic nutrient acquisition and the consumption of prey, making use of a flexible structure to allow an exploration of alternative modes of interaction. Operation can be varied with respect to differential growth rates under pure phototrophy, phago-heterotrophy or mixotrophy, substitutional or additive interactions between modes of C acquisition, the suppression of digestion by C flow from photosynthesis (including, if applicable, that from kleptochloroplasts), competition for volume within the cell between chloroplasts and food vacuole, the need for some level of obligatory photosynthetic activity, activation of mixotrophy in response to general growth limitation, or to specific nutrient limitations. Simulations under dynamic conditions include considerations of predation on bacteria, and on microalgae. These show the potential for mixotrophs, but also indicate the importance of using an appropriate description of their physiology, with different mixotrophy configurations having significant effects on system dynamics. The potential value of kleptochloroplasts for support of mixotroph growth is highest when the food vacuole is large, when the ingested phototroph prey is of good nutritional status and when digestion of prey is repressed by photosynthesis. Analyses of the behaviour of the new model demonstrate that simulations which do not consider the stoichiometric implications of mixotrophy cannot reflect the reality of the trophic interaction both for the mixotroph and for the associated ecosystem.

We dedicate this work to the memory of Mike Fasham FRS, without whose enthusiasm and guidance none of this would have come to pass.

INTRODUCTION

Phytoplankton use primarily light and dissolved inorganic nutrients. Bacteria use dissolved inorganic and organic nutrients (with access to particulate nutrients via the action) of extra-cellular enzymic action. Zooplankton obtain their nutrition through capture and then digestion of particulate organic particles. Protistan mixotrophs combine all these modes of nutrition, making them what may be considered ideal organisms, the “perfect beasts” of the title of this work. Much has been written about these organisms (e.g. reviews by Jones, 1997; Raven, 1997; Stoerck, 1998; Jones, 2000), but until relatively recently a role for mixotrophy amongst protists typically labelled as phytoplankton has been largely ignored in mainstream plankton research. While some research groupings have long been considering the importance of mixotrophs, notably those interested in harmful algal blooms (Burkholder et al., 2008), recent evidence suggests that many of populations hitherto labelled as phytoplankton are actually capable of displaying some level of phagocytic mixotrophy (Jeong et al., 2005b; Unrein et al., 2007; Zubkov and Tarran, 2008). Throughout this work, unless otherwise indicated, reference to heterotrophy in mixotrophs is in respect to phagotrophy.


© The Author 2009. Published by Oxford University Press. All rights reserved. For permissions, please email: journals.permissions@oxfordjournals.org
Table I: Mixotroph classifications after Jones (Jones, 1997) and Stoecker (Stoecker, 1998)

<table>
<thead>
<tr>
<th>Primary mode</th>
<th>Mixotrophy stimulus</th>
<th>Jones, 1997</th>
<th>Mixotrophy sub-stimulus</th>
<th>Stoecker, 1998</th>
<th>Model configuration</th>
</tr>
</thead>
</table>
| "Ideal" balanced mixotroph | N/A | – | N/A | I | $\mu_{mix}^*\mu_{phot}$ | $P_{bal,crit}=0$
| Heterotroph | Prey limitation | A | C-limiting | IIa | $P_{bal,crit}=0$
| | | | Supplement C using kleptochloroplasts | IIb | $P_{bal,crit}=0$
| | | | | $S_{mix}=1$
| Phototroph | Light limitation (C-limitation) | B | | IIc | $P_{bal,crit}>0$
| | Nutrient limitation | C | Macro nutrient (e.g. DIN) | IIa | $P_{bal,crit}>0$
| | | | Trace nutrient | IIb | $P_{bal,crit}>0$
| | Prolonged darkness (stress survival) | D | – | IIc | $P_{bal,crit}>0$

The final column shows the critical configurations required by the model to conform to these classifications (see Table III).

The complexity of the food webs and the associated biogeochemical dynamics involving mixotrophs is readily apparent. For example, the mixotrophic ciliate *Myrionecta* (= *Mesodinium*) can consume bacteria (Myung *et al*., 2006), which can access nutrient unavailable directly to eukaryotes, as well as cryptophytes (Yih *et al*., 2004). The ciliate can subsequently engage in phototrophy using the kleptochloroplasts ingested from the cryptophyte together with inorganic nutrients which would not normally be accessible to a heterotrophic protist. *Myrionecta* may itself be predated upon by the mixotrophic dinoflagellate *Dinophysis* (Kim *et al*., 2008), which then can use the kleptochloroplasts originally of crypto-phyte origin, together with direct assimilation of the prey biochemical composition and additional dissolved nutrients to further its own growth.

The interactions between the contrasting modes of nutrition exhibited by mixotrophs have been considered previously on a conceptual basis. Table I shows the classifications according to Jones (Jones, 1997) and Stoecker (Stoecker, 1998). The “ideal” balanced mixotroph, with broadly equal growth potential in either nutrient mode (Stoecker type I) appears rare. Most show a clear disposition towards being primarily phototrophic or heterotrophic, at least with respect to their growth rates and/or their most frequently observed nutritional mode. Ultimately, the division between being primarily phototrophic (Table I; Jones types B, C, D; Stoecker type II) or heterotrophic (Table I; Jones type A; Stoecker type III) comes down to whether some level of photosynthesis is essential for growth, or not. Stoecker (Stoecker, 1998) lists type IIIa as those mixotrophs that have their own plastids, but rapidly depress their involvement in growth, while type IIIb are protists that engage in photosynthesis only through the use of kleptochloroplasts.

Another fundamental difference between phototrophy and heterotrophy is that in the former, components are taken up as required; in phago-heterotrophy, complete food packages are taken up and any excess voided. Mixotrophs engaging in heterotrophy will tend towards being C-limited, rather than non-C limited, unless their prey contains a high C content. Usually, prey of high C content (i.e. high C:(N:P)) have been growing under nutrient limitation. A stimulus for the mixotroph to switch to phototrophy due to prey limitation (Jones type A) under such a condition would not be obviously advantageous because this same nutrient limitation would adversely affect the growth of the mixotroph. In consequence, in reality, a stimulus for a primarily heterotrophic mixotroph to engage in phototrophy through a lack of prey is most likely to be associated with some level of C-limitation (Stoecker type III).

For a primarily phototrophic organism, a shortage of any potential element (C, N, P, Fe etc.) may be expected to stimulate the engagement of heterotrophy; the division between Stoecker types IIa and IIb likely reflects part of a continuum, related to the nutrient status of the environment in which a particular species evolved. However, the consequences of engaging in mixotrophy for a phototroph run far beyond satisfying just the nutrient shortage that initially stimulated feeding. Most likely the demands for other (non-limiting) nutrients will be down-regulated in consequence of assimilating prey biomass. Further, by adding in additional nutrients, growth of the mixotroph may be enhanced. The stoichiometric and growth-limiting interplay between factors that may be expected to (de)press facets of cellular physiology require recognition in models of mixotrophs.

Attempts to model this important plankton functional type have been restricted largely to simple combinations of descriptors for phytoplankton plus microzooplankton sharing a common biomass (Thingstad *et al*., 1996; Baretta-Bekker *et al*., 1998; Stickney *et al*., 2000; Hammer and Pitchford, 2005), or to more detailed descriptions of particular species (e.g. Zhang *et al*., 2003; Hood *et al*., 2006). Such models have pointed to the potential important role of mixotrophic organisms not only as vectors for nutrient transformations, but...
also as stabilizers of system dynamics (Jost et al., 2004; Hammer and Pitchford, 2005). A common perception is that these organisms are most important in nutrient impoverished systems (Baretta-Bekker et al., 1998; Dolan and Perez, 2000; Flöder et al., 2006). However, Stickney et al. (Stickney et al., 2000) disagree with Baretta-Bekker et al. (Baretta-Bekker et al., 1998) over the functional role of mixotrophs as mediators of the microbial loop. Further, the value of any upgrading of seston C:N:P (Ptacnik et al., 2004), improving its stoichiometric value, depends on the changing trophic status of mixotrophs (Weithoff and Wacker, 2007). To properly consider such ecological processes require a more considered attempt to construct and then analyse mixotrophic behaviour than is possible using simple, usually non-stoichiometric-based, models.

The need for a mechanistic mathematical description of planktonic mixotrophs can be justified for both autecological and ecological studies. However, despite a rich qualitative and semi-quantitative literature on mixotrophs, there are few data sets, representing the activity of only a few species, suitable for parameterization of their physiological processes. The situation is even worse than it may appear at first sight because of problems associated with taxonomy; for example, Gymnodinium galatheanum, Karlodinium micrum and Karlodinium veneficum are now considered to be the same organism (Bergholtz et al., 2005). As an example of those data that are available, data for the activity of the dinoflagellate Fragilidium subglobosum feeding on Ceratium sp. (Skovgaard, 1996, Hansen and Nielsen, 1997; Hansen et al., 2000; Skovgaard et al., 2000) were transformed and collated (Fig. 1). The general patterns shown are consistent with those seen elsewhere for mixotrophs that consume photosynthetic prey. Figure 1A and B shows the decreased Chl:C content and photosynthesis of feeding...

Fig. 1. Data for the activity of the dinoflagellate Fragilidium subglobosum feeding on Ceratium sp., collated from the works of Skovgaard et al. (Skovgaard, 1996; Skovgaard et al., 2000), panels (A and B), and from Hansen et al. (Hansen et al., 2000) and Hansen and Nielsen (Hansen and Nielsen, 1997), panels (C and D). Data from the original works have been transformed to give C-specific and mass ratio values using information from the source papers. Data are shown co-plotted, hence multiple lines and symbols for some plots. Photon flux density (PFD) has units of μmole photons m^-2 s^-1.
cells (noting that this inevitably may include some level of kleptochloroplastic activity and pigment), and that the ingestion rate may show some inter-relationship with light (and hence with concurrent photosystem operation). Growth rate under mixotrophic nutrition may exceed that under pure phototrophy by a significant margin, though the implication from Figure 1C is that assimilation efficiency decreases as total C uptake saturates. For this organism, which ingests its prey, cell size increases markedly during mixotrophy (Fig. 1D).

From such works, it is possible to construct a conceptual mechanistic model to drive the development of a mathematical description; this is portrayed in Fig. 2. There are several points of interaction. The (de)repression interactions between feeding, digestion and phototrophic nutrition (Int 1; Fig. 2) may be considered as primarily substitutional (as one increases the other is repressed), or additive (though constrained by the maximum possible growth rate). The volume occupied by ingested prey material (or complete prey in phagocytic species) may compete with that occupied by photosystems in mixotrophs (Int 2; Fig. 2). This potential competition for space will be accentuated under low-light conditions where photoacclimation would require an increased volume for chloroplasts. Other interactions may include a repression of prey digestion if the C flow from photosynthesis is high (a mechanism which may also depress digestion of the active prey photosystems in kleptochloroplasts). A role for kleptochloroplasts in ciliates is clear (Stoecker et al., 1987; Stoecker and Silker, 1990; Johnson et al., 2006, 2007); it is logical to consider similar activity in mixotrophs consuming phototrophic prey, even if digestion is relatively rapid (half lives for digestion may be significant fractions of a day—Li et al., 2001). There may also be an obligatory need for photosynthesis in some mixotrophs (Li et al., 1999; Adolf et al., 2007a, b; Cf. Skovgaard et al., 2003).

Another point of interaction, and one that separates mixotrophs from other microzooplankton, is their inherent ability to re-assimilate nutrients which would be otherwise lost (regenerated) during heterotrophic metabolism. This is possible because phototrophs contain the enzymes for inorganic nutrient assimilation, while the capacity for photosynthesis itself provides the C skeletons for (re)assimilation. This may not always be so, however. Gustafson et al. (Gustafson et al., 2000) note that while Myrionecta (“Mesodinium”) can use inorganic nutrients, other plastid-retaining ciliates cannot.

The aim of this work is to construct a mathematical description of protistan mixotrophic activity which contains sufficient flexibility to allow for an exploration of alternative biological strategies using a common modelling structure. To enable this, the model was constructed so that photo- and hetero-trophic components could interact with each other in different ways. Inevitably, given the paucity of data suitable for the support of modelling, the work described here is in part theoretical, considering different metabolic scenarios which one may consider as playing a part in the evolution of these organisms. Validation of the models’ output depends on qualitative behaviour (Rykiel, 1996) while an analysis of model behaviour helps to shape our understanding of what features may be of particular importance in future models of this plankton functional group.

**METHODS**

**Model description**

The basis of the model is a merging of the carbon–nitrogen–phosphorous (CNP) stoichiometric zooplankton model of Mitra (Mitra, 2006) and the normalized...
quota-based, photoacclimative CNP phytoplankton model of Flynn (Flynn, 2001). The means by which these components are interlinked defines the operation of the total model. The model contains eight state variables (Table II) describing C, N, P and Chl associated with the core mixotroph biomass (mC, NC, PC, ChlC) and also the same constituents associated with the contents of the food vacuole (namely, FC, FNC, FPC and FChlC). The term “food vacuole” refers throughout this work to material of prey-origin which is physically associated with the mixotroph, be it within the bounds of the mixotroph cell, or outside of it, held within a feeding veil. The amount of material associated with the food vacuole is relative to the core mixotroph C-biomass. Thus, the total C associated with the mixotroph is mC/(1+FC) (gC L⁻¹). Changes in these state variables are described as follows:

$$\frac{dmC}{dt} = C\text{ fixation} + C\text{ assimilation}$$

$$- C\text{ respiration} - C\text{ void} \tag{1}$$

$$\frac{dXC}{dt} = \text{inorganic X uptake} + X\text{ assimilation}$$

$$- X\text{ void} - X\text{ regeneration} - \mu \cdot XC \tag{2}$$

$$\frac{dChlC}{dt} = f\{C\text{ demand}\} - f\{C\text{ excess}\}$$

$$- \mu \cdot ChlC \tag{3}$$

$$\frac{dFW}{dt} = W\text{ ingestion} - W\text{ digestion} - \mu \cdot FW \tag{4}$$

In equation (2), X is either N or P; in equation (4), W is C, N, P or Chl in the food vacuole described as a ratio to core C-biomass (Table II); f is function of the term in \{\}. Note that because all state variables other than mC [equation (1)] are mass ratios, there is a dilution term (of the form \(\mu \cdot Y\)) which decreases the state variable value with mixotroph growth rate (\(\mu, \text{ C}^{-1} \cdot \text{day}^{-1}\)).

In brief, carbon flows into mC from photosynthetic activity (from both the mixotrophs’ own photosystems and from the activity of any functional photosystems held within the food vacuole) and also from C assimilated from prey digestion (Fig. 2). Carbon is removed from mC to support basal respiration, metabolic respiration (associated with biosynthesis) as well as for the reduction of nitrate to intracellular ammonium and subsequent ammonium assimilation. Hansen et al. (Hansen et al., 2000) report a 2-fold difference in respiration rates between cells engaging in phototrophy rather than heterotrophy. Flows of N and P into the mixotroph biomass come from the uptake of inorganic nutrients (ammonium, nitrate and phosphate), as well as from ingested biomass, with losses from regeneration and voiding. A description of these components follows, together with the inclusion of switches, to enable the consideration of alternative physiological interactions (e.g. Int1 and Int2 in Fig. 2). Descriptions of the parameters used in the following equations are given in Tables II–V. The model was constructed and run within Powersim Constructor v 2.51 (Isdalstø, Norway), using either Euler or fourth-order Runge–Kutta (variable step) integration methods (which gave near-identical results).

As in our previous models (e.g. Flynn, 2001, 2003; Mitra, 2006), we make frequent use of normalized sigmoidal functions to provide feedback response curves. These take the form shown in equation (5), where \(S\) is a quotient describing the stimulus for the process, \(K\) is a half saturation constant, \(H\) is the Hill number (which controls the shape of the sigmoidal curve, a value of \(H = 1\) returning a rectangular hyperbolic form) and RF is the response factor quotient.

$$RF = \frac{(1 + K^H) \cdot S^H}{S^H + K^H} \tag{5}$$

The use of these equations has several benefits. First, they can provide a robust and stable form of feedback, enabling the use of higher integration steps than when using rectangular hyperbolae or crude switches; sensitivities to changes in \(K\) and \(H\) are typically low. Secondly, the form of these responses is in keeping with the allosteric, multi-factorial, feedback processes that typically apply in biology. They also allow readily the deployment of different response factors by simply changing \(K\) and \(H\), rather than having to alter model code.

Table II: State variables for the mixotroph model and the equation defining its value

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Equation</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChlC</td>
<td>Core mixotroph Chl:C</td>
<td>11 gChl (gC⁻¹)</td>
<td></td>
</tr>
<tr>
<td>FC</td>
<td>Food vacuole C content relative to mC</td>
<td>25 gC (gC⁻¹)</td>
<td></td>
</tr>
<tr>
<td>FChlC</td>
<td>Food vacuole Chl content relative to mC</td>
<td>26 gChl (gC⁻¹)</td>
<td></td>
</tr>
<tr>
<td>FNC</td>
<td>Food vacuole N content relative to mC</td>
<td>Text near 26 gN (gC⁻¹)</td>
<td></td>
</tr>
<tr>
<td>FPC</td>
<td>Food vacuole P content relative to mC</td>
<td>Text near 26 gP (gC⁻¹)</td>
<td></td>
</tr>
<tr>
<td>mC</td>
<td>Core mixotroph biomass</td>
<td>48 gC L⁻¹</td>
<td></td>
</tr>
<tr>
<td>NC</td>
<td>Core mixotroph N:C</td>
<td>46 gN (gC⁻¹)</td>
<td></td>
</tr>
<tr>
<td>PC</td>
<td>Core mixotroph P:C</td>
<td>46 gP (gC⁻¹)</td>
<td></td>
</tr>
<tr>
<td>ChlC</td>
<td>Core mixotroph Chl:C</td>
<td>11 gChl (gC⁻¹)</td>
<td></td>
</tr>
</tbody>
</table>
Another feature common with our previous models is the deployment of normalized C-quotas of N and P to describe the nutrient status of the mixotroph (Flynn, 2002, 2008a, 2008b). Thus, quotient XCu (either NCu or PCu) relates to mixotroph N:C (NC) or P:C (PC), within minimum and maximum quota values (XCmin, XCmax), with a curve-defining constant KQX [equation (6)]. Flynn (Flynn, 2008a, 2008b) discusses values for KQN and KQP; KQN is typically high, giving a near-linear response curve, while KQP is small, giving a strongly curved relationship and hence a significant decrease in P:C from PCmax causes a change in the quotient.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Equation</th>
<th>Value and units</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAsyn</td>
<td>Cost for amino acid synthesis</td>
<td>8, 40</td>
<td>1.5 gC (gN)^{-1}</td>
</tr>
<tr>
<td>AEmax</td>
<td>Maximum AE</td>
<td>28, 29</td>
<td>0.75</td>
</tr>
<tr>
<td>AEmin</td>
<td>Minimum AE</td>
<td>28, 29</td>
<td>0.5</td>
</tr>
<tr>
<td>BR</td>
<td>Basal respiration rate</td>
<td>8, 23, 41</td>
<td>0.05 gC (gC^-1) day^{-1}</td>
</tr>
<tr>
<td>CChl</td>
<td>Carbon associated with photosystem</td>
<td>12</td>
<td>12 gC (gCChl)^{-1}</td>
</tr>
<tr>
<td>ChlCabs</td>
<td>Absolute maximum Chl:C</td>
<td>12</td>
<td>0.06 gChl (gC^-1)</td>
</tr>
<tr>
<td>CN</td>
<td>Slope of grazing rate</td>
<td>15</td>
<td>0.01 day^{-1} \times \mu gC L^{-1}</td>
</tr>
<tr>
<td>FChl</td>
<td>Maximum feeding vacuole size</td>
<td>14</td>
<td>0.5 gC (gC^-1)</td>
</tr>
<tr>
<td>FCmin</td>
<td>Minimum feeding vacuole size</td>
<td>14</td>
<td>0 gC (gC^-1)</td>
</tr>
<tr>
<td>Has</td>
<td>Hill number for digestion rate</td>
<td>24</td>
<td>2</td>
</tr>
<tr>
<td>Heq</td>
<td>Hill number for quantity-linked AE</td>
<td>29</td>
<td>4</td>
</tr>
<tr>
<td>Hhet</td>
<td>Hill number for control of FCmax</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>Hing</td>
<td>Hill number for ingestion control</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>Hpbal</td>
<td>Hill number for digestion link to critical C-fixation</td>
<td>22</td>
<td>4</td>
</tr>
<tr>
<td>Hpd</td>
<td>Hill number for digestion suppression</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td>Hq</td>
<td>Hill number for uptake control</td>
<td>37</td>
<td>4</td>
</tr>
<tr>
<td>Hreg</td>
<td>Hill number for regeneration</td>
<td>43</td>
<td>4</td>
</tr>
<tr>
<td>K_A</td>
<td>Half saturation for NH4^+ uptake</td>
<td>33</td>
<td>14 \mu gN L^{-1}</td>
</tr>
<tr>
<td>Kmax</td>
<td>Half saturation for digestion rate</td>
<td>24</td>
<td>0.5</td>
</tr>
<tr>
<td>Kreq</td>
<td>Response control to ingestion quantity; 10^{-6} to turn off</td>
<td>29</td>
<td>0.4</td>
</tr>
<tr>
<td>Krest</td>
<td>Half saturation for FCmax</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>Kreg</td>
<td>Half saturation for ingestion control</td>
<td>17</td>
<td>0.2</td>
</tr>
<tr>
<td>KSl</td>
<td>Half saturation for NO3^- uptake</td>
<td>34</td>
<td>14 \mu gN L^{-1}</td>
</tr>
<tr>
<td>Kp</td>
<td>Half saturation for PO4^- uptake</td>
<td>38</td>
<td>1.55 \mu gP L^{-1}</td>
</tr>
<tr>
<td>Kpbal</td>
<td>Half saturation for digestion link to critical C-fixation</td>
<td>22</td>
<td>0.1</td>
</tr>
<tr>
<td>Kpd</td>
<td>Half saturation for digestion suppression</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>Kq</td>
<td>Half saturation for uptake control</td>
<td>37</td>
<td>0.1</td>
</tr>
<tr>
<td>Kreg</td>
<td>Half saturation for regeneration</td>
<td>43</td>
<td>1</td>
</tr>
<tr>
<td>Kec</td>
<td>Response control to prey quality; 10^{-6} to turn off</td>
<td>28</td>
<td>10</td>
</tr>
<tr>
<td>KQX</td>
<td>Half saturation for quota curve</td>
<td>6</td>
<td>10 (N), 0.1 (P)</td>
</tr>
<tr>
<td>M</td>
<td>Multiplier for Chl synthesis (see Flynn et al., 2001)</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>MR</td>
<td>Metabolic respiration</td>
<td>23, 41</td>
<td>0.2 gC (gC)^{-1}</td>
</tr>
<tr>
<td>NC</td>
<td>Absolute maximum N:C</td>
<td>37, 44</td>
<td>0.25 gN (gC)^{-1}</td>
</tr>
<tr>
<td>NCmax</td>
<td>Maximum N:C affecting growth rate</td>
<td>6, 8, 27, 32, 39, 44, 45</td>
<td>0.2 gN (gC)^{-1}</td>
</tr>
<tr>
<td>NCmin</td>
<td>Minimum N:C</td>
<td>6</td>
<td>0.05 gN (gC)^{-1}</td>
</tr>
<tr>
<td>Pbalcrit</td>
<td>Minimum critical proportion of growth supported by photosynthesis</td>
<td>21, 22</td>
<td>0.1</td>
</tr>
<tr>
<td>PC</td>
<td>Absolute maximum P:C</td>
<td>38, 44</td>
<td>0.04 gP (gC)^{-1}</td>
</tr>
<tr>
<td>PCmax</td>
<td>Maximum P:C affecting growth rate</td>
<td>6, 27, 32, 39, 44, 45</td>
<td>0.02 gP (gC)^{-1}</td>
</tr>
<tr>
<td>PCmin</td>
<td>Minimum P:C</td>
<td>6</td>
<td>0.005 gP (gC)^{-1}</td>
</tr>
<tr>
<td>PrefA</td>
<td>Relative preference for NH4^+; controls surge</td>
<td>33</td>
<td>2</td>
</tr>
<tr>
<td>redco</td>
<td>Cost of NO3^- reduction to NH4^+</td>
<td>34</td>
<td>1</td>
</tr>
<tr>
<td>S_{mix}</td>
<td>Switch to mix C input; 0 if substitional; 1 if additive mixotrophic interaction</td>
<td>10, 14</td>
<td>0.1</td>
</tr>
<tr>
<td>S_{PD}</td>
<td>Switch to relate C demand to digestion; 0 if C-fixation does not affect digestion; 1 otherwise</td>
<td>19</td>
<td>0.1</td>
</tr>
<tr>
<td>S_{vol}</td>
<td>Switch to share cell volume; 0 if feeding vacuole does not compromise ChlCmax</td>
<td>12</td>
<td>0.1</td>
</tr>
<tr>
<td>a_{Chl}</td>
<td>Initial slope of PE curve</td>
<td>Text near equation 8</td>
<td>7.10^{-6}</td>
</tr>
<tr>
<td>b</td>
<td>Control constant for nutrient uptake</td>
<td>37, 38</td>
<td>0.05</td>
</tr>
<tr>
<td>m_{max}</td>
<td>Maximum possible growth rate</td>
<td>15, 23</td>
<td>1 day^{-1}</td>
</tr>
<tr>
<td>m_{phot}</td>
<td>Maximum phototrophic growth rate</td>
<td>8, 11, 32</td>
<td>0.5 day^{-1}</td>
</tr>
</tbody>
</table>

*Units for a_{Chl} are m^2 g^{-1} Chl at-\mu g C \mu mol^{-1} photon.*

Table III: Constants for the mixotroph model and equations for their use
little stress initially.

\[ \text{XCu} = \begin{cases} \frac{\text{XC}}{\text{XCmax}} & \text{if } \text{XC} \leq \text{XCmax} \\ \frac{1 + \text{KQ}_X}{\text{XCmin}} \cdot \left( \frac{\text{XC} - \text{XCmin}}{\text{XC} - \text{XCmax}} \right) & \text{if } \text{XC} > \text{XCmax} \end{cases} \]

The minimum of the values of the levels of N and P stress, NCu and PCu, sets the value of NPCu [equation (7)], which gives a quotient for overall nutrient status.

\[ \text{NPCu} = \min(\text{NCu, PCu}) \]  

Photosynthesis

The description of photosynthesis and photoacclimation within the core mixotroph photosystem follows that in Flynn (Flynn, 2001), with increasing ChlC up to a maximum set by ChlCmax developing with decreasing photon flux density (PFD), and decreasing ChlC occurring on nutrient depletion and/or at higher PFD.
This formulation references the development of ChlC to the cellular demand for C; the greater the demand, the greater the synthesis of ChlC. For a mixotroph, this demand for C may be satisfied by C-fixation alone or with (perhaps, sole) inclusion of ingested C. Thus, C assimilated from prey digestion could substitute for C-fixation (decreasing the need for photosynthesis). Alternatively, the two processes could occur independently, being additive in their action.

The maximum rate of C-fixation required to support phototrophy at the current nutrient status [NPCu; equation (7)] is given by Pq max [equation (8)]; the basis of this equation is given in Flynn (Flynn, 2001), but in essence the rate of C-fixation needs to cover the costs of basal respiration (BR) and of respiration associated with growth at the maximum N:C (NCmax), including reduction of nitrate to ammonium (redco) and subsequent amino acid synthesis etc. ([AA syn], to support a maximum phototrophic growth rate of μphot.

\[ P_{q_{\text{max}}} = \left( \mu_{\text{phot}} + \text{BR} + \text{NC}_{\text{max}} \cdot \mu_{\text{phot}} \cdot \text{redco} \cdot [\text{AA syn}] \right) \cdot \text{NPCu} \]  

(8)

The core mixotroph photosynthesis, PS, and that performed by functioning ingested photosystems (kleptochloroplasts), FPS, are computed by reference to their respective photosystem sizes (ChlC and FChlC), initial PE-curve slopes \( \alpha_{\text{Chl}} \) and \( \alpha_{\text{FChl}} \), \( P_{q_{\text{max}}} \) and PFD, using the equations in Flynn (Flynn, 2001). The value of \( P_{q_{\text{max}}} \) applied to the kleptochloroplastic photosynthesis is that accorded to the prey when under ideal nutrient status [i.e. prey NPCu = 1]; the reasoning behind this is that the environment within the mixotroph feeding vacuole is considered to be nutrient rich. This assumption thus gives an optimistic kleptochloroplastic C-fixation rate. FChlC describes the concentration of prey photosystems relative to core mixotroph biomass and C fixed from their operation enters core mixotroph biomass. Total photosynthesis rate \( \text{PS}_{\text{Tot}} \), equation (9), is the sum of that performed by PS and FPS.

\[ \text{PS}_{\text{Tot}} = \text{PS} + \text{FPS} \]  

(9)

Photosystems within captured prey are assumed not to be able to photoacclimate. The values of \( \alpha_{\text{Chl}} \) and Chl:C for photosystems associated with ingested prey are held fixed at the values of the ingested material, and define the rate of C-fixation by reference to the value of FChl:C (i.e. the amount of prey Chl per unit of core mixotroph C, mC). For simplicity, the degradation of these captured photosystems (kleptochloroplasts) is assumed to follow the consumption of prey biomass [equations (25) and (26)]. The value of FChlC decreases as core mixotroph C increases, with dilution of captured photosystems amongst the growing biomass of the consumer [equation (4); Skovgaard, 1998].

Equation (10) describes the C input rate which is involved in regulating photoacclimation. The Boolean logic term in equation (10) \( S_{\text{Mix}} \) enables additive \( S_{\text{Mix}} = 1 \) or substitutional \( S_{\text{Mix}} = 0 \) interactions between phototrophic \( \text{PS}_{\text{Tot}} \) and heterotrophic \( C_{\text{as}} \) [equation (9)] and heterotrophic \( C_{\text{as}} \) interactions \( S_{\text{Mix}} = 0 \) input (interaction Int1 in Fig. 2). In an additive interaction, C entering from heterotrophy has no impact upon the operation of the core photosynthetic activity. In the substitutional interaction, the need for photosynthesis is depressed as the value of \( C_{\text{as}} \) makes up a greater proportion of \( C_{\text{in}} \). The interaction controlled by \( S_{\text{Mix}} \) is a physiological one, operating at the point of photosynthesis. The net interaction between photop- and heterotrophy depends on more than this interaction, as will be explored below.

\[ C_{\text{in}} = \text{PS}_{\text{Tot}} + C_{\text{as}} \cdot (S_{\text{Mix}} = 0) \]  

(10)

The description of the synthesis of core mixotroph photosystems and hence photoacclimation [equation (11)] is derived from that used before (Flynn, 2001; Flynn et al., 2001). In the original version, the synthesis of ChlC is related to the rate of photosynthesis [here
photosynthesis (equation [9]) relative to the maximum required to support growth at the current nutrient status \(P_{\text{m}}\) (equation [8]). For this model, it is related to the rate of total C input, as defined by part A in equation (11); if \(S_{\text{M}}\) entering from feeding substitutes for the need for photosynthesis \(S_{\text{M}}\) then \(C_{\text{a}}\) (equation [10]) plays a role in down-regulating ChlC synthesis.

\[
\frac{d \text{ChlC}}{dt} = (\text{ChlC} < \text{ChlC}_{\text{max}}) \cdot \text{ChlC}_{\text{max}} \cdot \left(1 + 0.05 \cdot \frac{(1 - \text{ChlC} / \text{ChlC}_{\text{max}})}{(1 - \text{ChlC} / \text{ChlC}_{\text{max}}) + 0.05} - \text{ChlC} \cdot (\mu + (1 - NCu) \cdot \mu_{\text{phot}}) \right)
\]

In addition to core mixotroph photosynthesis, photosynthesis from any material held within the food vacuole (associated with FChlC) contributes to the total C input (equation [9]). This activity can depress the synthesis of the mixotrophs’ own photosystems. A significant depression is particularly likely if the feeding vacuole is large and full with photosynthetically active prey [hence FPS, in equation (9), is high].

Another source of interaction between photo- and heterotrophic activity is the potential linkage between ChlC and the size of the food vacuole (interaction Int 2 in Fig. 2); the more space occupied by material within the food vacuole, the less space is available for photosystems. In the standard photoclimatic description for a phototroph, the value of ChlC\(_{\text{max}}\) is a constant (Flynn, 2001). Here, however, the operational value of ChlC\(_{\text{max}}\) may be variable, being related to the current contents of the food vacuole (FC). The value of FC has units of gC within the food vacuole per g C of core mixotroph. Thus, assuming FC is within the core mixotroph cell (i.e. the organism is phagotrophic) and that the C-density of the core mixotroph is similar to that of the food vacuole, then the proportion of cell space as C occupied by FC is given by FC/(1 + FC). If we then consider that the space occupied by photosystems is proportional to the product of ChlC (g Chl per g C\(^{-1}\)) and the C content of chloroplast per unit of Chl (CCchl; gC (g Chl\(^{-1}\))), then the volume available for photosystems decreases as the value of FC/(1 + FC) increases. A value of CCchl = 12 (g chloroplast C (g Chl\(^{-1}\)) would mean that photosystems giving a maximum cellular value of ChlC = 0.06 (g Chl (g C\(^{-1}\)) would occupy 72% of the cell; this seems a not unreasonable estimate from visual inspection. This relationship is described by equation (12), where ChlC\(_{\text{abs}}\) is the absolute maximum possible value of ChlC\(_{\text{max}}\). This linkage can be switched on (for simulating organisms which ingest their prey, hence sharing the cell volume between photosystems and food vacuole; \(S_{\text{M}}\) = 1) or off (for simulating mixotrophs that hold their prey outside of the main cell within a feeding veil; \(S_{\text{M}}\) = 0). For those that use a peduncle (feeding tube; e.g. Berge et al., 2008a, b), the effective value of the internal food vacuole may still be of consequence for photosystem synthesis.

\[
\text{ChlC}_{\text{max}} = \text{ChlC}_{\text{abs}} - (S_{\text{M}} = 1) \cdot \left(\frac{FC/(1 + FC)}{C_{\text{Chl}}}\right)
\]

**Ingestion and digestion of prey C**

Control of the ingestion and digestion of prey is related to demand for heterotrophic nutrition relative to the maximum rate of growth \(\mu_{\text{max}}\). If \(\mu_{\text{max}}\) exceeds the maximum rate of growth attainable under phototrophy alone (\(\mu_{\text{phot}}\)), then there will always be some level of demand though this will be depressed if the food vacuole is full. In the model, the demand for heterotrophy controls the current maximum size of the food vacuole, FC\(_{\text{max}}\), as a value between minimum (FC\(_{\text{min}}\)) and absolute maximum values (FC\(_{\text{abs}}\)). FC\(_{\text{max}}\) may be down-regulated in a substitional interaction with phototrophy (i.e. \(S_{\text{M}}\) = 0); if phototrophy is sufficient then phagotrophy need not be enabled. By default, FC\(_{\text{max}}\) is related to the demand for any all nutrients, as defined by the quotient \(\mu_{\text{rel}}\) [equation (13a)]. In equation (13a), reference is made to the 24 h averaged growth rate, \(\mu_{\text{avg}}\), reflecting the expected developmental lag. The lower the growth rate, the greater the demand is, and hence the capacity, for feeding. Alternatively, \(\mu_{\text{rel}}\) could be linked to a specific nutritional status, such as the P or N status. To enact such a control, equation (13a) is replaced by an equation making reference to the quotient [equation (13b)], or quotients [equation (13c)], describing nutrient status \(\text{NCu} \) or \(\text{PCu}\) (equation (6); NPCu, equation (7)).

\[
\mu_{\text{rel}} = \text{MIN} (1, \frac{\mu_{\text{avg}}}{\mu_{\text{max}}})
\]

\[
\mu_{\text{rel}} = \text{XCu}
\]

\[
\mu_{\text{rel}} = \text{NPCu}
\]

FC\(_{\text{max}}\) is then defined by equation (14). A normalized sigmoidal function is employed to control the size of FC\(_{\text{max}}\) limiting size as growth (perhaps due in part to
photosynthetic activity) or nutrient status approaches maximum; the form of the response factor RF is shown in Fig 3A. Note that \( FC_{\text{max}} \) [equation (14)] defines the maximum possible value of FC, and not its actual size; volume-sharing interactions [equation (12)] only come into play if the vacuole actually contains food.

\[
FC_{\text{max}} = (S_{\text{mix}} = 0)
\]

\[
FC_{\text{min}} + \left( 1 - \left( \frac{1 + \text{H}_\text{Het}}{\mu_{\text{Het}} + \text{H}_\text{Het}} \right) \cdot (FC_{\text{abs}} - FC_{\text{min}}) \right) + (S_{\text{dis}} = 1) \cdot FC_{\text{abs}}
\]

(14)

The value of \( \mu_{\text{rel}} \) is only of consequence if the physiological interaction between photo- and hetero-trophic nutrition is substitutonal \([S_{\text{mix}} = 0; \text{see also equation (10)}]\). Thus if photosynthetic growth supplies sufficient C then there is less need for C from ingestion; in equation (10), the opposite regulation applies with heterotrophic activity substituting (i.e. depressing) for the need for photosystem synthesis. When using either of equations (13b) or (13c), it is necessary to set \( S_{\text{mix}} = 0 \), else ingestion is always enabled rather than being restricted to periods of nutrient stress. If the interaction is additive \([S_{\text{mix}} = 1]\) then \( FC_{\text{max}} = FC_{\text{abs}} \) [equation (14)].

Prey capture \( (\text{Cpi}) \) is a function of prey availability \( (SC; \text{Table V}) \) through equation (15); here this is capped at a rate equating to three times the maximum growth rate \( (\mu_{\text{max}}) \), so allowing a level of surge ingestion. The actual maximum rate is a function of prey handling time by the mixotroph.

\[
\text{Cpi} = \text{MIN}(SC \cdot \text{Cri}, 3 \cdot \mu_{\text{max}})
\]

(15)

The rate of ingestion is down-regulated by reference to the level of food vacuole satiation, \( FC_{\text{relV}} \) as given by equation (16). Ingestion \([\text{IgC}; \text{equation (17)}]\) into the food vacuole \( (FC) \) is then controlled through a normalized sigmoidal feedback function from the level of \( FC_{\text{relV}} \) The form of the response factor RF is shown in Fig 3B.

\[
\text{FC}_{\text{relV}} = \frac{FC}{FC_{\text{max}}}
\]

\[
\text{IgC} = \text{Cpi} \cdot \frac{(1 + \frac{\text{H}_\text{Ing}}{\text{H}_\text{Ing}}) \cdot (1 - FC_{\text{relV}})}{(1 - FC_{\text{relV}}) + \frac{\text{H}_\text{Ing}}{\text{H}_\text{Ing}}} \quad \text{RF}
\]

(17)

The combined interactions between equations (15) and (17) equate to the form of the predation function described in Mitra and Flynn (Mitra and Flynn, 2006b); if additional prey items are to be considered then their capture is described via equation (15), but the feedback controlling ingestion of each prey type is via the RF part in equation (17), making reference to the total material in the food vacuole ("gut" as termed by Mitra and Flynn, 2006b). Subsequent ingestion is thus linked to the rate of digestion removing material from the food vacuole.

The rate of digestion is a function of C-demand. If the rate of C-fixation relieves the level of C-demand sufficiently then digestion can be decreased, if not blocked. Thus, for example, if sufficient prey of high photosynthetic capability are ingested then their digestion (and hence of the kleptochloroplasts) may be slowed and the mixotroph can grow using this source of C, rather than from direct digestion. The relative rate of photosynthesis, \( PS_{\text{rel}} \) [equation (18)], is a function of current total C-fixation \([\text{PS}_{\text{Tot}}, \text{equation (9)}]\) and \( P_{\text{qmax}} \).

\[
PS_{\text{rel}} = \text{MIN}\left(1, \frac{\text{PS}_{\text{Tot}}}{P_{\text{qmax}}}ight)
\]

(18)

This is used to control digestion through the quotient PD [equation (19)], operating through a normalized sigmoidal function of the relative photosynthesis rate \( (PS_{\text{rel}}) \). The form of the response factor RF is shown in Fig 3C. This function for prey digestion is enabled by setting \( S_{\text{PD}} = 1 \); if photosynthesis does not affect digestion \( (i.e. \ S_{\text{PD}} = 0) \), then PD is fixed at 1.

\[
PD = 1 - (S_{\text{PD}} = 1) \cdot \left[ \frac{(1 + \frac{\text{H}_\text{Pd}}{\text{Pd}}) \cdot \frac{\text{S}_{\text{PD}}^{\text{Hpd}}}{\text{RF}}} {\text{S}_{\text{PD}}^{\text{Hpd}} + \frac{\text{H}_\text{Pd}}{\text{Pd}}} \right]
\]

(19)

In addition to the control of digestion based upon the need for C, a second interaction is through the potential need for a critical minimum contribution of photosynthetically derived metabolic activity. This is in reflection that certain nutritional components (such as fatty acids; e.g. Karlodinium micrum, Adolf et al., 2007a), or metabolite transformations, may have to be derived or routed via de novo photosynthesis. In this instance, the rate of digestion needs to be controlled (using parameter \( \text{Pbal}_{\text{Con}} \)) to ensure that a critical proportion of total C entering the system is derived from photosynthesis. \( \text{Pbal} \) [equation (20)] determines the contribution of total photosynthesis \([\text{PS}_{\text{Tot}}, \text{equation (9)}]\) relative to the average growth rate \( (\mu_{\text{avg}}) \); this is compared to the critical contribution, set by \( \text{Pbal}_{\text{crit}} \) in equation (21). If there is no critical value \( (\text{Pbal}_{\text{crit}} = 0) \) then the mixotroph has no obligatory photosynthesis requirement, and \( \text{Pbal}_{\text{Con}} \)
Fig. 3. Response factors (RF) for various (de)repression interactions controlling modelled processes. See equation descriptions.
is set at 1 [equation (22)]. Otherwise the value of PbalCon is set by a normalized sigmoidal function of Bal; this rapidly enables digestion of material from the food vacuole once Pbal > Pbal_crit. The form of the response factor RF is shown in Fig. 3D.

\[
P_{bal} = \text{MIN} \left( 1, \frac{P_{S_{tot}}}{\mu_{avg}} \right) \cdot (\mu_{avg} > 0) \quad (20)
\]

\[
\text{Bal} = (P_{bal} > P_{bal\text{crit}}) \cdot \left( \frac{P_{bal} - P_{bal\text{crit}}}{1 - P_{bal\text{crit}}} \right) \quad (21)
\]

\[
P_{bal\text{Con}} = (P_{bal\text{crit}} = 0) + (P_{bal\text{crit}} > 0) \cdot \frac{(1 + K_{H_{bal}}) \cdot \text{Bal}_{H_{bal}}}{\text{Bal}_{H_{bal}} + K_{H_{bal}}_{RF}} \quad (22)
\]

The actual digestion of material, and hence the rate of removal of C from the food vacuole, is then set by DgC [equation (24)]. This is controlled by quotients PD [equation (19)] and PbalCon [equation (22)], by D_max and by a normalized sigmoidal function of the concentration of material in the food vacuole (FC_{rel,FC} = FC/FC_{abs}); the form of the response factor (RF) is shown in Fig. 3E.

\[
D_{gC} = P_{bal\text{Con}} \cdot PD \cdot D_{\text{max}} \cdot \frac{(1 + K_{H_{as}}) \cdot FC_{H_{as}}}{FC_{H_{as}} + K_{H_{as}}_{RF}} \quad (24)
\]

Changes in the size of the food vacuole are thus given by equation (25); the part, FC·μ, accounts for dilution of the material through growth of the mixotroph [μ is given by equation (47)].

\[
\frac{d FC}{dt} = \text{IgC} - D_{gC} - FC \cdot \mu \quad (25)
\]

The digestion of kleptochloroplast material (FChlC) is assumed to occur pro rata with the digestion of other prey C so that the value of captured prey Chl:C remains the same during digestion. This is the default (simplest) expectation; in reality kleptochloroplasts are likely to be protected from digestion, remaining functional for many days if not weeks (Lewitus et al., 1999). However, this does not mean that their full physiological competence is retained (this topic is considered further in the Discussion). Equation (26) describes the rate of change of kleptochloroplasm chlorophyll. As there is a dilution of the value of FChlC with the decrease (dilution) of FC with C growth [last part of equation (26)], growth using kleptochloroplasm photosynthesis requires the periodic capture of new photosynthetic prey items.

\[
\frac{dF_{ChlC}}{dt} = S_{ChlC} \cdot C - F_{ChlC} \cdot D_{gC} \cdot FC \quad (26)
\]

Equations similar to equation (26) define changes in the N:C and P:C of material in the food vacuole; substitute prey N:C (SNC) or P:C (SPC) for SChlC, and FNC or FPC for FChlC in equation (26).

**Assimilation of ingested C**

The description of prey assimilation was modified from approaches developed by Mitra (Mitra, 2006). This makes reference to the stoichiometric value of the prey, through the description of food quality within FC relative to the optimal core mixotroph values [equation (27)]. Thus, the assimilation efficiency (AE) for food C declines as food N:C or P:C declines. This description operates through two routes. First, there is the expected simple linear relationship with stoichiometric food quality; this is enacted via equation (27). Second, there is scope for an additional relationship reflecting the fact that decreased food quality is often related to other chemical changes (presence of toxins and other secondary metabolites) that may further decrease AE (Mitra and Flynn, 2005). This latter relationship is governed here by the value of Kec in equation (28). The form of the response factor RF is shown in Fig. 3F, with the enhanced change in AE being enabled (Kec = 10); a very small value of Kec disables stoichiometric modulation (see Mitra and Flynn, 2005; Mitra, 2006).

\[
\text{MINup} = \text{MIN} \left( F_{NC} \cdot \text{FPC} \cdot \frac{1}{N_{max} \cdot P_{max}}, 1 \right) \quad (27)
\]

\[
\text{AE}_{\text{equal}} = \text{AE}_{\text{min}} + (\text{AE}_{\text{max}} - \text{AE}_{\text{min}}) \cdot \frac{(1 + \text{Kec}) \cdot \text{MINup}}{\text{MINup} + \text{Kec}} \quad (28)
\]

In addition, there is evidence that AE declines if there is a surfeit of food, with mixotroph growth...
approaching maximum (Fig. 1C). This is described using the normalized sigmoidal function in equation (29) which is disabled by setting $K_{eq}=10^{-6}$. The form of the response factor $RF$ is shown in Fig. 3G.

$$AE_{\text{quan}} = (K_{eq} > 10^{-6}):$$

$$AE_{\text{min}} + \left( \frac{(1 + K_{eq}^{\text{Heq}}) \cdot (1 - \mu_{\text{eq}}^{\text{Heq}})}{(1 - \mu_{\text{eq}}^{\text{Heq}}) + K_{eq}^{\text{Heq}}} + RF \right) \cdot AE_{\text{max}}$$

$$+ (K_{eq} = 10^{-6}) \cdot AE_{\text{max}}$$

(29)

The operational value of $AE$ is then set by equation (30).

$$AE = \text{MINup} \cdot \text{MIN} (AE_{\text{qual}}, AE_{\text{quan}})$$

(30)

The net result is that a variable proportion of material within the feeding vacuole is ultimately assimilated into the core mixotroph biomass [$C_{\text{as}}$, equation (31)]. Of the remainder, a proportion may be used to support basal respiration, with the balance voided (as per Mitra, 2006).

$$C_{\text{as}} = AE \cdot \text{DgC}$$

(31)

Assimilation of N and P

Inorganic N enters the mixotroph as nitrate and/or ammonium. The interaction of these assimilations would be described most appropriately through involvement of the explicit description of a pool of low molecular weight N metabolites (namely glutamine; Flynn, 2001). Here a simplified approach is used in which the f-ratio (frat = ratio of nitrate assimilation: total inorganic N assimilation) is computed by reference to their potential transport rates. Equation (32) gives the maximum required N uptake rate to support growth at a maximum rate of $\mu_{\text{phot}}$, which requires that $NC=NC_{\text{max}}$ [equation (6)]. Equations (33) and (34) give the potential transport rates of ammonium and nitrate, respectively, in the absence of any interaction. The values of Pref$_X$ in equations (33) and (34) are used here as constants defining surge uptake capacities; they could be replaced with curves relating the level of nutrient stress to nutrient transport capacity (see Flynn et al., 1999). The rates including the interaction are given by equations (35) and (36); the uptake of nitrate occurs only if ammonium transport is insufficient to meet demands.

$$\mu_{\text{N}} = \mu_{\text{phot}} \cdot NC_{\text{max}}$$

(32)

$$PV_A = \mu_{\text{N}} \cdot \text{Pref}_A \cdot \frac{A}{A + K_A}$$

(33)

$$PV_{\text{Ni}} = \mu_{\text{N}} \cdot \text{Pref}_{\text{Ni}} \cdot \frac{Ni}{Ni + K_{\text{Ni}}}$$

(34)

$$V_A = (\mu_{\text{N}} < PV_A) \cdot \mu_{\text{N}} + (\mu_{\text{N}} \geq PV_A) \cdot PV_A$$

(35)

$$PV_{\text{P}} = (PV_A < \mu_{\text{N}}) \cdot (PV_A + PV_{\text{Ni}} < \mu_{\text{N}}),$$

$$PV_{\text{Ni}} + (PV_A + PV_{\text{Ni}} \geq \mu_{\text{N}}) \cdot (\mu_{\text{N}} - PV_A)$$

(36)

The actual total inorganic N uptake is given by equation (37), with uptake being stopped by a normalized sigmoidal function as NC approaches the absolute maximum allowed ($NC_{\text{abs}}$). The operation of the equation is described in detail by Flynn (Flynn, 2001, 2003).

$$up_{\text{N}} = (V_A + V_{\text{Ni}}) \cdot ((NCu > PCu) \cdot (NC < NC_{\text{abs}}),$$

$$NPCu^{\beta} + (NCu = NPCu)) \cdot \frac{1 + K_{\text{Heq}}^{\text{Hq}} \cdot (1 - \frac{NC}{NC_{\text{abs}}})^{\text{Hq}}}{1 - \frac{NC}{NC_{\text{abs}}}^{\text{Hq}} + K_{\text{Heq}}^{\text{Hq}}}$$

(37)

The description of inorganic P uptake [equation (38)] is similar to that for N, except there is only one source of P. The value 5 in equation (38) is to provide a surge transport capacity in excess of that required to support maximum steady-state growth (that value being given by $\mu_{\text{phot}} \cdot PC_{\text{max}}$). Flynn (Flynn, 2002) discusses the importance of the surge transport capacity; like the nutrient-stress linked changes in transport capacity for N-sources (Flynn et al., 1999) this capacity is likely linked to P-stress.

$$up_{\text{P}} = (PC < PC_{\text{abs}}) \cdot \mu_{\text{phot}} \cdot PC_{\text{max}} \cdot 5.$$ 

$$((PCu > NPCu) \cdot NPCu^{\beta} + (PCu = NPCu)) \cdot \frac{P}{P + K_P} \cdot \frac{1 + K_{\text{Hq}}^{\text{Hq}} \cdot (1 - \frac{PC}{PC_{\text{abs}}})^{\text{Hq}}}{1 - \frac{PC}{PC_{\text{abs}}}^{\text{Hq}} + K_{\text{Heq}}^{\text{Hq}}}$$

(38)

Inorganic N and P entering the mixotroph contributes to NC and PC, respectively. These elements...
entering in conjunction with C through prey assimilation are assumed to be assimilated at a ratio consistent with core cellular structures, which is according to the value of NC and PC that enables maximum growth (namely, NC\textsubscript{max} and PC\textsubscript{max}). These are described below with reference to element X (N or P). Input of X from ingested material into mixotroph biomass is given by equation (27). The sufficiency of X associated with the assimilated material is ensured by MINup [equation (27)].

\[ \text{Inc}_X = C_{as} \cdot X_{C_{max}} \]  

### Respiration and regeneration

Phototrophic-linked respiration [\( R_{\text{phot}} \), equation (40)] includes the cost of reducing nitrate [part A in equation (40)]. It also includes the cost of re-assimilating ammonium that would otherwise be regenerated through heterotrophic processing; \( N_{\text{reas}} = R_{\text{het}} \cdot X_{C_{max}} (1 - \text{RegN}) \), with RegN described in equation (40). Heterotrophic-related respiration includes basal and metabolic components [\( R_{\text{het}} \), equation (41)], with the total respiration [equation (42)] being the sum of \( R_{\text{phot}} \) and \( R_{\text{het}} \).

\[ R_{\text{phot}} = \text{redco} \cdot X_{\text{frat}} \cdot \text{frat} + (up_N + N_{\text{reas}}) \cdot A_{\text{syn}} \]  

\[ R_{\text{het}} = B_{\text{R}} + C_{as} \cdot MR \]  

\[ R_{\text{Tot}} = R_{\text{phot}} + R_{\text{het}} \]  

The control of nutrient regeneration within a mixotroph is a key difference between these organisms and other grazers. The ability to assimilate inorganic nutrients also enables a re-assimilation of nutrients that would otherwise be lost during normal biochemical cycling (such as protein turnover). If this re-assimilation is not simulated, allowing the release of inorganic N and P from the mixotroph, there would then be a competition between these organisms and other plankton for the liberated nutrient. Rather, \textit{de facto} this material does not leave the organism in the first instance. Thus, C respiration is not associated with a regeneration of N and/or P unless N:C and/or P:C in the organism attains high levels. As the C-quota (X:C) approaches an absolute maximum value (X\textsubscript{C_{abs}}), the likelihood of regeneration rather than retention increases. This control, \( \text{Reg}_X \), is given by equation (43), making reference to the proximity of XC to X\textsubscript{C_{abs}} in the zone between X\textsubscript{C_{max}} and X\textsubscript{C_{abs}} as described by Rep\textsubscript{X} in equation (44). The form of response factor RF in equation (43) is shown in Fig. 3H.

\[ \text{Reg}_X = \frac{(1 + K_{\text{Reg}}) \cdot \text{Rep}_X^{Heg}}{\text{Rep}_X^{Heg} + K_{\text{Reg}}} \]  

\[ \text{Rep}_X = (X_C > X_{C_{max}}) \cdot \left(1 - \frac{X_{C_{abs}} - X_C}{X_{C_{abs}} - X_{C_{max}}} \right) \]  

The actual regeneration of X is then given by equation (45); the expected regeneration rate would be stoichiometrically linked to the respiration rate (i.e. \( R_{\text{het}} \cdot X_{C_{max}} \)), but is now downplayed by \( \text{Reg}_X \) until XC is close to X\textsubscript{C_{abs}}. The overall changes in XC are given by equation (46).

\[ \frac{dX_C}{dt} = up_X + \text{Inc}_X - \text{Res}_X - X_C \cdot \mu \]  

### Growth

Growth rate, \( \mu \), is given by equation (47), with changes in mC by equation (48).

\[ \mu = \frac{C_{as} + P S_{\text{Tot}} - R_{\text{Tot}}}{mC} \]  

\[ \frac{dmC}{dt} = mC \cdot \mu \]  

### Sensitivity analyses

Steady-state sensitivity analyses were conducted on all the constant parameters (for method see Mitra, 2006) in particular to test the sensitivity of the feedback control equations. Doubling and halving of constants for these feedback equations exerted little leverage on state variables. The only significant sensitivity was seen when doubling and halving of \( K_{\text{ass}}, \text{Has}, \) and \( K_{\text{PD}} \), which had a pro rata effect on the value of FC. As these constants affect the rate of flows from FC, this is wholly in line with expectations.

### External factors and testing scenarios

For the purposes of testing the model, it was run under either dynamic or steady-state conditions with various values of photon flux density (PFD), of prey biomass concentration, of prey C:N:P and prey Chl:C, and of inorganic N and P. Under steady-state, with fixed input variables, steady-state rates and state variables for
cellular quotas of nutrients, Chl and food vacuole contents, were achieved typically within a few days of simulation time. The implications of operating the system under different configurations (enabling or disabling substitutional versus additive interactions between phototrophy and heterotrophy) was also tested. Finally, initial investigations of behaviour were made under dynamic conditions when grazing on bacteria or a phototrophic prey. The bacteria model used was that described by Flynn (Flynn, 2005a), consuming ammonium and phosphate with very high affinity uptake systems \((K/K^* = 0.3)\). Figure 4A–C shows the steady-state behaviour of the model against variable DIN (nitrate), with no prey [equations (10) and (14)], no volumetric sharing between these processes \([S_{\text{vol}} = 0; \text{equation (12)}]\), no depression of digestion by the products of photosynthesis \([S_{\text{pp}} = 0; \text{equation (19)}]\) and no absolute requirement for photosynthesis \([P_{\text{bal, crit}} = 0; \text{equations (21) and (22)}]\).

**RESULTS**

**Steady-state simulations**

The default configuration of the model used in the initial set of scenarios described here was of an additive interaction between photo- and heterotrophic nutrition [i.e. no depression of photosynthesis from heterotrophic nutrition; \(S_{\text{Mix}} = 1; \text{equations (10) and (14)}\)], no volumetric sharing between these processes \([S_{\text{vol}} = 0; \text{equation (12)}]\), no depression of digestion by the products of photosynthesis \([S_{\text{pp}} = 0; \text{equation (19)}]\) and no absolute requirement for photosynthesis \([P_{\text{bal, crit}} = 0; \text{equations (21) and (22)}]\).

Figure 4A–C shows the steady-state behaviour of the model against variable DIN (nitrate), with no prey (Fig. 4A) or with a sub-saturating level of prey of good (Fig. 4B) or poor quality (Fig. 4C). The availability of prey removes the inorganic nutrient-growth interaction, although some evidence of this interaction remains when consuming poor quality prey (Fig. 4C). Note that the AE in Fig. 4C is lower than in Fig. 4B, but that the Chl:C value is similar between these fed simulations at high DIN only. C acquisition through heterotrophy depresses photosystem synthesis (Fig. 4B and C; Cf. Fig. 4A). Figure 4D and E shows behaviour with variable prey concentrations but no DIN, either without light (Fig. 4D, Cf Fig. 4A with light but no prey) or at high light (Fig. 4E). Growth is higher in heterotrophic mode (Cf. Fig. 4A–C), but photosynthesis adds to the growth when high quality prey are consumed (Fig. 4E) with the extra C fixed compensating for respiration losses. The depression in Chl:C in the low quality prey scenario (Fig. 4F) reflects the nutrient stress in such conditions. Note also the difference in respiration rates between pure phototrophic and heterotrophic growth (Fig. 4A versus D), giving the 2-fold difference when normalized to the growth rate expected from, for example, Hansen et al. (Hansen et al., 2000).

Figure 5 shows light interactions with different combinations of photo- or hetero-trophic nutrition; prey were supplied at sub-saturating levels of 120 \(\mu g \text{ C L}^{-1}\) (Cf. Fig. 4D–F). Figure 5A shows a typical phototrophic irradiance-growth curve under high (saturating) inorganic nutrient conditions, with a decrease in Chl:C with increasing PFD. With no DIN but in the presence of high-quality prey (Fig. 5B), the additive form of photosynthesis plus heterotrophy to the shape of the irradiance-growth curve is apparent. Also seen is the enhanced suppression of Chl:C through the assimilation of the prey. In the absence of nitrate assimilation, the respiration rate is decreased. If there is an absolute requirement for photosynthesis to provide certain metabolites \((P_{\text{bal, crit}} > 0)\) then the irradiance-growth curve again resembles the classic phototrophic form (Fig. 5C). Figure 5D, in comparison with Fig. 5B, shows the effect of DIN assimilation; growth becomes more typical of phototrophy in its form, with higher photosynthesis, higher respiration (associated with nitrate assimilation), but the effort appears largely futile in this instance. In the presence of low NC prey (Fig. 5E), the contribution of heterotrophic nutrition lessens growth rate, irradiance-growth curve and Chl:C tend more towards the pattern with no prey, as seen in Fig. 5A.

Respiration rates in Figs 4 and 5 are highly variable. Part of that respiration burden is associated with internal recycling, though most is with nitrate assimilation. Mixotrophs, unlike pure heterotrophic protists, would by default be equipped with high affinity ammonium assimilatory pathways and hence (unlike microzooplankton) could re-assimilate rather than release ammonium liberated during catabolic processes. This process can be disabled in the model by removing \(R_{\text{regX}}\) [equations (43) and (44)]. A close comparison between Fig. 6A and B shows a slight difference in respiration rates and hence also in growth rates; the ability to retain ammonium comes at a growth cost. The ability to use nitrate in darkness (Fig. 6C) had no impact because even when feeding on stoichiometrically poor prey, the stoichiometry of the mixotroph in darkness was high (suppressing the consumption of DIN). Figure 6D shows, for completeness, the phosphate
equivalent of Fig. 6B; because phosphate re-assimilation carries no respiratory overhead (or certainly not comparable to that for DIN), there is no impact of employing RegX.

The steady-state configurations used for Figs 4–6 used $S_{\text{Mix}} = 1$, $S_{\text{Vol}} = 0$ and $S_{\text{PD}} = 0$. In Fig. 7, the implications of using alternative configurations are explored. In both Fig. 7A and B, the 0 SC (no prey) configuration is the control; changes in $S_{\text{Mix}}$, $S_{\text{Vol}}$ and $S_{\text{PD}}$ are irrelevant with no prey to ingest. With $S_{\text{Mix}} = 0$ (which results in the suppression of photosynthesis by heterotrophic C assimilation), whether photosynthesis depresses digestion ($S_{\text{PD}} = 1$) or not ($S_{\text{PD}} = 0$) is irrelevant. With $S_{\text{Mix}} = 1$, however, ingestion and hence growth is decreased at high light levels with $S_{\text{PD}} = 1$ because C flowing from photosynthesis depresses the digestion of material held in the food vacuole. Similarly, considering the interaction between $S_{\text{Mix}}$ and $S_{\text{Vol}}$ (Fig. 7B), $S_{\text{Vol}}$ only interacts when $S_{\text{Mix}} = 1$; here most obviously at low light there is a decrease in Chl:C (with volumetric
competition between photosystems and feeding vacuole such that photosynthesis and hence growth can be restricted under light-limiting conditions.

In Fig. 8A, the effect of increasing the critical contribution of photosynthesis (as set by Pbal Crit) is seen. This develops from Fig. 5C, in which $S_{\text{Mix}} = 1$, $S_{\text{PD}} = 0$, $S_{\text{Vol}} = 0$ and Pbal Crit = 0.1. The higher the value of Pbal Crit, the shallower the initial section of the PE curve. In Fig. 8B, the role of photosynthesis from captured photosystems (kleptochloroplasts) is considered; constants were set as $S_{\text{Mix}} = 1$, $S_{\text{PD}} = 1$, $S_{\text{Vol}} = 1$ and FCabs = 1, giving a configuration that would result in the filling of a large food vacuole, competing for space with the core photosystems, and also with C-fixation depressing prey digestion. The result is the retention of prey within the food vacuole, with digestion repressed through photosynthesis originating in part from the ingested material. The plots in the mid-section of Fig. 8B, with SChlC = 0, showing consumption of non-photosynthetic prey; the right-hand section with SChlC = 0.05 shows consumption of photosynthetic prey. The latter has lower levels of growth, but also much lower levels of ingestion and of core mixotroph ChlC; growth proceeds primarily using C fixed by the FChl:C, with ingestion making good the loss of FChl:C through dilution with mixotrophy growth and loss through residual digestion.
Figure 9A shows the impact of enabling AE_{quan} [equation (29)], with a decline in AE and enhanced ingestion at higher light levels. In Fig. 9B, the model has been configured to be broadly consistent with the operation of *Fragilidium*, with \( S_{Mix} = 1 \), \( S_{PD} = 1 \), \( S_{Vol} = 1 \) and \( K_{eq} = 0.4 \) describing the growth of a mixotroph which completely ingests its prey (Cf. Fig. 1C).

**Dynamic simulations**

Figure 10 shows the behaviour of the model feeding upon bacteria in a scenario commencing as a batch-type suspension, but with a slow dilution with fresh medium. In this instance, the material voided by the mixotroph was considered to be constitute semi-labile dissolved organic matter (DOM); the C:N:P of this material depends on mixotroph behaviour. Ten percent of C fixed by the mixotroph was considered to contribute to DOC, while additional DOC was supplied from the feed medium. Where mixotroph grazing on bacteria operated using the standard substitional configuration [equation (13a)], the organisms were in competition for ammonium and DIP and bacteria were grazed throughout (Fig. 10A–C). With feeding enabled only during P-stress [equation (13b), with reference to PCu], the mixotroph only consumed bacteria during the latter part of the simulation (Fig. 10D–F). Although DIP decreased to very low concentrations early in the simulation, significant P-stress is not developed in the mixotroph until later because of the ability to redistribute internal P buffers physiology against the exhaustion of external DIP. While bacterial biomass is low later in the simulations, growth is higher when being grazed.

Figures 11 and 12 are based upon similar systems to that in Fig. 10, but now with a photosynthetic prey in competition for nutrients. For Fig. 11, the mixotroph configuration was substitutional in its interaction between photo- and heterotrophy (\( S_{Mix} = 0 \)). Because of this mode of operation, and in a situation in which the activity of the prey chloroplasts contributes to the interaction between photo- and heterotrophy, there are periods of oscillation between feeding. This is most obvious when the prey are nutrient replete and hence have a good photosynthetic potential (until ca. day 6), and where the mixotroph has a greater capacity for holding prey (i.e. FC_{abs} is high; Fig. 11D–F). In Fig 12, the mixotroph configuration was additive (\( S_{Mix} = 1 \)). This gives a smoother predator–prey interaction and
higher mixotroph growth rates, with higher ingestion rates. Prey are removed more rapidly but while predation proceeds the kleptochloroplastic contribution to mixotroph growth is at least as high as with the substitu-
tional configuration (Fig. 12; CF. Fig. 11). The amount of material voided by the mixotroph (VOC) is, in conse-
quence of the overall grazing burden being decreased, lower when the mixotroph has a large food vacuole
capacity (Fig. 12D–F).

**DISCUSSION**

This paper presents the first attempt to construct a multi-nutrient mechanistic model of a protistan mixo-
troph. In the absence of high quality data for parameter-
ization, the model has sufficient flexibility to enable it to be configured to describe any one of a range of
possible configurations. Different growth rates, bio-
chemical interactions, food vacuole sizes and disposi-
tions, prey type and quality, kleptochloroplastic interac-
tions etc. may be considered, allowing exploration of the mixotroph types shown in Table I. It is thus possible to compare different configurations, to consider some of the evolutionary advantages and dis-
advantages of this mode of nutrition.

The model performs as expected in pure photo-
trophic or heterotrophic guise. The interaction between these modes of nutrition depends on the extent of the photo- and hetero-trophic processes, and on the struc-
tural configuration of the interactions (Figs 4–9). The value
of some of these configurations depends on other factors; for example, differences between configurations $S_{\text{Vol}} = 0$ and $S_{\text{Vol}} = 1$ depends in part on whether the
The challenge of parameterization

Without doubt a great challenge is the parameterization of models of mixotrophs, a situation which is even greater than that for phytoplankton and microzooplankton (Flynn, 2005b). Problems exist both with respect to the types of experiments (combinations of conditions) and the actual parameters measured (most works report cell number-based data, when there are clear indications of changes in cell size; e.g. Hansen and Nielsen, 1997; Smalley et al., 2003). The complexity of mixotrophic nutritional opportunities, coupled with the interactions with their prey, make experiment design extremely challenging. The use of the model described here would help in the design of appropriate experiments. It is also necessary to have a suitably parameterized data series for the prey species; only Adolf et al. (Adolf et al., 2003) go some way in achieving this for a mixotroph–prey combination.

Examples of problem areas include the common challenges of determining predator and prey biomass in mixed suspensions, and in particular of determining nutrient status of different species (e.g. Flynn and Davidson, 1993) when it is clear that stoichiometric disparities can have a profound effect on the interaction. Even if one could readily determine the chemical composition of the mixotrophs separated from their free-living prey, there is the problem of separating the biomass of the core mixotroph from that associated with the material in the food vacuole (though see Li et al., 2001). While a similar problem exists for all predators, it is of particular significance here because of the relative size of the compartments. Then there is the issue of the activity of kleptochloroplasts. It seems likely that all photosynthetic prey will continue to fix C for a while after capture; the relative importance of such activity for not only the growth of the mixotroph but also minimizing subsequent ingestion of more prey warrants consideration.

Switching between photo- and heterotrophy

A fundamental question is which nutritional mode, phototrophy or heterotrophy, exerts the dominant control in mixotrophy? Given the evolutionary history of protistan mixotrophs, that chloroplasts are considered to have their origins as (de facto) kleptochloroplasts, and that heterotrophy is considered the more cost effective form of nutrition (Raven, 1997), one could assume that heterotrophy should have the dominant role. Growth efficiency (net/gross C-fixation) and growth rate is poorer (ca. half) in the mixotroph Karlodinium micrum when growing phototrophically than in the prey Storeatula major (Adolf et al., 2003) because of the dinoflagellate’s higher respiration rate. Chl:C decreases with increased irradiance (as is usual in photosynthetic protists), is lower in nutrient-stressed cells (also as usual), and also is decreased in feeding cells of Karlodinium (Adolf et al., 2006). Fragilidium subglobosum grows quickest in dual feeding mode rather than just through phototrophy, though at high light ingestion declines (Skovgaard, 1996); again with phagotrophy, there is a decrease in photosynthesis, and in Chl:C (Skovgaard et al., 2000).

The freshwater bactivorous mixotroph Poterioochromonas malhamensis also appears primarily configured for heterotrophy (Caron et al., 1990). Growth under mixotrophy typically exceeds that under phototrophy.
In such instances, it appears that photosynthesis acts as a top-up system of value when prey availability is limiting (Table I; Stoecker type III).

It may be argued that adding phototrophy to a primarily heterotrophic organism seems an unlikely evolutionary event because the investment in phagotrophy is minor compared with that required for photosynthesis (ca. <10% versus 50% of cellular energy demands; Raven, 1997). In the model, the operational cost of phototrophy is represented by $R_{\text{phot}}$ [equation (40)]. In comparison, the converse, of adding heterotrophy to a phototroph, is relatively cheap and easy. That may be expected so even for a nutrient-limited phototroph; limitation by nutrients such as N and P leads to the accumulation of excess C, which could supply much of the material and energy for prey capture. That said, Skovgaard (Skovgaard, 1996) suggests that a period of 24 h is required to fully engage phagotrophy in the dinoflagellate *Fragilidium subglobosum*. In contrast, an energy-limited heterotroph is unlikely to be able to readily reallocate cellular resources to chloroplast synthesis; the most cost effect option for these organisms (Raven, 1997) is the use of kleptochloroplasts. One could argue, or expect, that all mixotrophs that do not engage in kleptochloroplasty for their sole support of phototrophy would have an obligatory need for photosynthesis ($P_{\text{bal crit}}$).

**Fig. 10**. Dynamic simulation of the interaction between bacteria and mixotroph under a scenario with a dilution rate of 0.05 day$^{-1}$, bringing in fresh nutrient at 14 μgN L$^{-1}$ for ammonium and nitrate; 3.1 μgP L$^{-1}$ for phosphate and 240 μgC L$^{-1}$ for DOC and flushing out residual nutrient and biomass. Bacteria consumed DOC, ammonium and DIP. In panels A–C, mixotroph feeding on bacteria was enabled throughout [equation (13a)], while in panels D–E it was enabled only during P-stress [equation (13b), with PCu]. Panels (C) and (F) show bacterial growth rate (bac $\mu$) and mixotroph C-fixation, ingestion and growth (mixo $\mu$) rates.

(Keong et al., 2004, 2005a). In such instances, it appears that photosynthesis acts as a top-up system of value when prey availability is limiting (Table I; Stoecker type III).

It may be argued that adding phototrophy to a primarily heterotrophic organism seems an unlikely evolutionary event because the investment in phagotrophy is minor compared with that required for photosynthesis (ca. <10% versus 50% of cellular energy demands; Raven, 1997). In the model, the operational cost of phototrophy is represented by $R_{\text{phot}}$ [equation (40)]. In comparison, the converse, of adding heterotrophy to a phototroph, is relatively cheap and easy. That may be expected so even for a nutrient-limited phototroph; limitation by nutrients such as N and P leads to the accumulation of excess C, which could supply much of the material and energy for prey capture. That said, Skovgaard (Skovgaard, 1996) suggests that a period of 24 h is required to fully engage phagotrophy in the dinoflagellate *Fragilidium subglobosum*. In contrast, an energy-limited heterotroph is unlikely to be able to readily reallocate cellular resources to chloroplast synthesis; the most cost effect option for these organisms (Raven, 1997) is the use of kleptochloroplasts. One could argue, or expect, that all mixotrophs that do not engage in kleptochloroplasty for their sole support of phototrophy would have an obligatory need for photosynthesis ($P_{\text{bal crit}} > 0$), and that these organisms, irrespective of their relative rates of growth and frequencies in engaging phototrophy rather than heterotrophy, should be considered as primarily phototrophs (Table I).
In the model, heterotrophy controls phototrophy through $S_{Mix}$ \cite[equations (10) and (14)]{mix}; the argument here is that the control of the synthesis of photosystems is driven by the supply/demand of C skeletons for growth. In addition, assuming a phagotrophic organism, there is competition for space within the cell that limits the size of Chl:C \cite[X_{Vid}; equation (12)]{vid}. It could be argued that the presence of a replete food vacuole within the cell would restrict chloroplast synthesis, and hence cap the increase of Chl:C at low light. All the evidence suggests that C acquisition via prey digestion is more efficient, and quicker acting, than via photosynthesis, so this possibility does not appear likely. (To enact it would require the space-sharing interaction in the model to be reversed.) There is a link in the model that explains the enhancement of predation at high light, and that is via the need for a critical level of photosynthesis \cite[P_{bal}; equations (21) and (22)]{bal}. At low light, this link depresses heterotrophy to track the rate of photosynthesis, with Chl:C increasing to maintain that rate of C-fixation. At high light, the lessening of photoacclimation decreases the need for high Chl:C. Hansen \textit{et al.} \cite{hansen2000} suggest that in \textit{Fragilidium subglobosum} feeding does not depress photosystem synthesis but that while feeding such synthesis does not keep pace with growth. These dynamics occur with the model described here. To drive an active removal of Chl:C, equation (11) would require an additional degradation term linked to $C_{av}$.

There are also routes enacted in the model for the modulation of heterotrophy by phototrophy. This includes the depression of digestion by a high flow of C-skeletons from photosynthesis \cite[P_{Pphi}; equation (19)]{phi} which is of particular importance in sustaining the role
of kleptochloroplast C-fixation. There is also the issue of having an obligatory requirement for requiring \textit{de novo} photosynthetic products [enacted by setting Pbal\textsubscript{crit}; equations (21) and (22)] (Adolf \textit{et al.}, 2007a, b). What is not clear is the extent to which a need for photosynthesis could be serviced by the operation of kleptochloroplasts, removing the need for the mixotrophs’ own photosynthetic capacity. The growth and physiology of \textit{Myrionecta} (”\textit{Mesodinium}”) suggests this to be possible (Gustafson \textit{et al.}, 2000); to simulate the physiology of such an organism, it would be necessary to set Chl:C\textsubscript{abs} = 0, so that a core mixotrophic photosynthetic capacity could never develop. Johnson \textit{et al.} (Johnson \textit{et al.}, 2007) report that in addition to plastids, \textit{Myrionecta} also retains cryptophyte nuclei to aid control of the captured chloroplasts. That a simple top-up role for photosynthesis is not the mode of interaction is evident where C-fixation declines as prey concentration is increased (Hansen \textit{et al.}, 2000; Adolf \textit{et al.}, 2006). However, at a given (saturating) prey availability, growth is still enhanced by elevated light; ingestion appears even more enhanced, suggesting a decrease in AE of prey-C as growth approaches its maximum (Figs 1G and 9B). At the other extreme, in the haptophyte \textit{Prymnesium parvum}, feeding occurs whether the organism is nutrient replete or not, with no stimulation involved (Skovgaard \textit{et al.}, 2003). To simulate such an event using the model, the value of \( \mu \text{rel} \) [from equations (13)] needs to be set to a value of 1, so that feeding is always enabled. Care should be taken, however, not to use this control if there is some required level of obligatory photosynthesis. The value of kleptochloroplasts for mixotroph nutrition depends on the photosynthetic capacity of the prey (Skovgaard, 1998; Jakobsen \textit{et al.}, 2000), and also on the fate of those photosystems within the food vacuole (how quickly they are degraded). Here we assumed that the
photosystem capacity of the ingested chloroplasts was the same as at the point of ingestion, and that digestion proceeds at the same rate as other ingested material; both of these may be considered unlikely (e.g. Lewitus et al., 1999). An additional complicating issue is the repair of photodamage within kleptochloroplasts, which may be expected to differ from that of the core mixotroph photosystems. Nonetheless, the potential value of kleptochloroplastic activity is clear from Figs 8, 11 and 12.

In contrast with the normal operation of photosynthetic protists, the output of photosystems for organismal growth from kleptochloroplasts is, per unit, higher than that from the core mixotroph chloroplasts. This is because the C fixed by them is not used in the synthesis of new chloroplasts, but only in the enhancement of core mixotroph biomass. In economic terms, this is akin to asset stripping, where the value from the acquired biochemical machinery is stolen and not reinvested. As the acquisition (kleptochloroplast) fails, rather than being repaired, it is pulled apart (digested) and any unwanted material voided. In the model, configuration $S_{pj} = 1$ [equation (19)] controls this interaction, with the proceeds from C-fixation (core and/or kleptochloroplastic) repressing the digestion of material held in the food vacuole.

**Controlling mixotrophy**

There is more to mixotrophy than satisfying the demand for C. The need for N and P is commonly considered a driver for mixotrophy in organisms otherwise seen as primarily phototrophs (Jones, 1997; Stoecker, 1998; Jones, 2000). Thus, N-limitation and P-limitation enhances mixotrophic activity in *Pinnularia minimum* (Stoecker et al., 1997), *Centrum furca* (Smalley et al., 2003) and *Gyrodiunium galatheum* (Li et al., 2000). In contrast, mixotrophy in *Prymnesium parvum* appears to be always expressed (Skovgaard et al., 2003). For a primarily photosynthetic organism, the ingestion of prey to satisfy P-limitation may be achieved at minimal cost in terms of conflict between phot- and heterotrophy. The capture of bacteria (Caron et al., 1990; Nygaard and Tobiessen, 1993) provides a high density P-rich nutrition, food vacuoles for which would present little spatial competition within a photosynthetic mixotroph. Further, because of the shape of the P−C quota curve (Flynn, 2008a, 2008b), significant gain in growth terms can be achieved with little acquisition of P.

When constructing this model, we considered using separate controls between photo- and heterotrophy, linked to C, N and/or P stress. The emphasis here has been placed on linking control to growth rate. This was done because limitation by any nutrient, macro or micro (Table I, Stoecker IIa, IIIb) results in the suppression of growth rate. Secondly, it is not possible during heterotrophy to ingest just C, or N or P, or Fe (etc.); a complete package is ingested. However, in an organism predominantly phototrophic in its operation mixotrophy may be expressed in response specifically to a nutrient stress, linked perhaps to the nutrient quota. Such a link between nutrient quotas and mixotrophy was identified by Smalley et al. (Smalley et al., 2003). The model is easily altered such that the stimulus for mixotrophy is a decline in the quotient describing a specific nutrient status [described using equations (6) and (7)]. These are the same quotients that are used within a normalized-quota description of phytoplankton growth (Flynn, 2008b), and can be related to the control of inorganic nutrient acquisition (Flynn et al., 1999). Thus, to enact P-stress-stimulated mixotrophy, equation (13b) is used making reference to PCu. Figure 10 compares interactions between mixotroph and bacteria using alternate configurations for controlling mixotrophy, with quite different dynamics.

However, even if the evidence is that the driver for the expression of phagotrophy is the supply of a specific nutrient, and not C (e.g. Stoecker et al., 1997). this does not remove the fact that particles of C+N+P+Fe+ (etc.) are ingested. Contrary to models that do not consider the consequential stoichiometric implications (e.g. Thingstad et al., 1996; Jost et al., 2004), models of the type developed here will respond to the ingestion of any/all surplus elements. For C, this results for example in a decrease in Chl:C and photosynthesis concurrent with satisfying P-demand through phagotrophy (data not shown). The involvement of photosynthesis may provide additional differences between phagotrophic activity performed by heterotrophic flagellates in comparison with mixotrophy. Zubkov et al. (Zubkov et al., 2001) report a higher retention of amino acids from ingested bacteria by *Ochromonas* while this may reflect differences in “preference” as suggested by these authors it is also possible that with the differential operation of biochemical systems, and the provision of extra C, less amino acid catabolism occurs in these mixotrophs.

It is apparent that the interactions between mixotrophic modes of nutrition are more complex than an approach of simply adding descriptions of pure phototrophy and heterotrophy (microzooplankton-style) together can attempt to realize. This is so even when attempting to consider a conceptually simple interaction, such as the consumption of bacteria by “phototrophs” to furnish P in the absence of sufficient DIP. This is because ingestion of bacteria will provide C:N:P in proportions not dissimilar to that required to provide a complete nutritional source for the “phytoplankton”.
The implications of using the traditional modelling approach of combining "phytoplankton" and "microzooplankton" descriptors to support the growth of a common C biomass versus that achieved with the model presented here will be considered in a future study.

**Missing processes**

As comprehensive as we have sought to make the model, various processes are not described in this study. Here we consider the means to introduce some of these.

We have not described trace-element involvement (Table I, Stoecker group IIb). The most obvious element to consider here is Fe. It may be expected that predation upon bacteria would provide a source of Fe for mid-ocean mixotrophs. To incorporate this into the control of the core mixotroph simply requires the addition of the Fe-submodel developed previously (Flynn and Hipkin, 1999; Flynn, 2001). The interaction between Fe and phototrophy is primarily enacted through the modulation of photosynthesis and photoacclimation, with secondary interactions to respiration and nitrate assimilation (Flynn and Hipkin, 1999). How Fe nutrition relates to the functioning of kleptochloroplasts (especially when these originated from an organism with a higher affinity Fe-acquisition ability) is unknown.

Protistan grazers show prey selectivity (for mixotrophs, Hansen and Nielsen, 1997; Jakobsen et al., 2000; Jeong et al., 2007; Berge et al., 2008a, b), and even cannibalism (Caron et al., 1990; Martel and Flynn, 2008). Gross selectivity for different types or sizes of prey is easily described by assigning different Cri values [equation (15)]. Changes in selectivity may also occur; for example, the material described as voided here (Figs 11 and 12) may be reabsorbed, just as microfaecal material is re-ingested by starving *Oxyrrhis marina* (Flynn and Davidson, 1993). The model structure can be modified to accommodate such processes using approaches we have already developed and employed to describe changes in prey selectivity with prey quantity and quality (Mitra and Flynn, 2006a, 2006b). Likewise, explicit mechanistic descriptions of inorganic nitrogen interactions, and of dissolved organic fluxes into and out of the cell may be provided by application of established approaches (Flynn and Berry, 1999; Flynn et al., 2008). Interactions with inclusion of DOM uptake may be particularly important in some instances; for example, the lytic action of the haptophyte *Prymnesium parvum* upon other organisms results in the liberation of DOM (Skovgaard et al., 2003). Stoecker and Gustafson (Stoecker and Gustafson, 2003) suggest that cell-surface proteolytic activity provides DOM for uptake, circumventing the need for prey ingestion.

Temperature is suspected to affect protistan phagotrophic activity with a different Q10 to that for phototrophy (Rose and Caron, 2007). Digestion is strongly temperature-linked in the mixotroph *Gyrodiunium galathea-num*, and the suggestion is made that the value of ingested chloroplasts as photosystems would be greater at lower temperatures in consequence (Li et al., 2001). Because of the use of normalized functions throughout, the model differential temperature links can be described by applying different Q10 values to constants \( \mu_{\text{ph}} \) and \( \mu_{\text{max}} \). Li et al. (Li et al., 2001) also suggest that the voiding of part-digested food is enhanced at elevated temperatures. This could be enacted within the model by linking AEquan [equation (29)] to temperature.

**The “perfect beast”?**

If mixotrophy offers such an ideal mix of nutrition then one may question why it is not ubiquitous. First, it must be said that mixotrophy is now considered to be far more important in ecological terms than originally thought (Jeong et al., 2005b; Unrein et al., 2007; Zubkov and Tarran, 2008). Secondly, there is the suspicion that the motto “jack-of-all-trades, and master of none” is applicable, that mixotrophs are only successful under certain conditions (Rothhaupt, 1996; Thingstad et al., 1996; Jost et al., 2004). To be a successful, phototroph requires a decoupling of the cell cycle from the diel cycle (an ability most noted in the diatoms; Nelson and Brand, 1979), a high Chl:C (which may conflict with the need for space for a food vacuole), and high affinity, high surge, nutrient transporters covering the cell membrane (which may compete for space if a significant area is associated with prey capture and ingestion). For organisms for which an important predator (mesozooplankton) may hunt using some level of visual clue or be attracted towards motile prey, being pigmented and motile may also present a high-risk strategy. At the start of the production cycle (classically represented by the temperate spring bloom), being a phototroph offers the greatest rewards; it may be best at that time to either be a pure phototroph or a pure heterotroph. Beyond that time, however, mixotrophy may come into its own.

The implications of some of the mixotroph configurations described by the model for ecology can be judged initially from the dynamic simulations we present in Figs 10–12. It is clear that different configurations have great potential for affecting the overall dynamics of the system. Some of the combinations of conditions that we provide in the steady-state analyses are improbable in an ecological context. For example, being exposed to high nutrient levels simultaneously with low-quality prey (Fig. 5E) is most unlikely; likely scenarios are of high...
inorganic nutrients with few high-quality prey, or of low inorganic nutrients with variable quantity of low- or high-quality prey (the latter would be prey which have access to nutrients otherwise unavailable to the mixotroph). Likewise, although the ability to recycle nutrients that would be otherwise regenerated (employing RegX) appears to be of no benefit, or even of negative consequences (Fig. 6), that should not be taken to mean that under a competitive scenario that would be so. A more comprehensive exploration of such factors will be presented in future papers.

The ability to eat your photosynthetic competitors, to balance stoichiometric demands for growth and hence not waste any ingested material (as must pure heterotrophic protists) are factors that conspire to provide mixotrophs with a competitive edge. For a primarily phototrophic organism, mixotrophy enacted through consumption of bacteria would seem to offer a most effective nutritional balance. Bacteria access a pool of nutrients either unavailable or relatively poorly available to photrophs (either to the mixotroph itself, or to its phototrophic competitors or prey), and they are by virtue of allometric scaling, numerous (cells m$^{-3}$), fast growing, dense (in terms of gC m$^{-3}$ cell volume) and rich in non-C elements. Mixotrophs inhabiting systems in which bacterial activity is high (freshwater lakes and oceans) appear to be dominated by bacterivores (Jones, 2000; Zubkov and Tarran, 2008).

Take a mixotroph and add toxicity to discourage your predators (Tillmann, 2004; Adolf et al., 2007b), and one has a potent red-tide bloom species (Smalley and Coats, 2002; Burkholder et al., 2008; Flynn, 2008c). An interesting potential role for mixotrophy is the acquisition of toxin-synthesizing machinery from the prey. Secondary metabolites, together with the photosynthetic biochemistry associated with their synthesis could be acquired into mixotrophs that are primarily heterotrophs, or indeed into mixotrophs that are primarily phototrophs but that are otherwise non-toxic. Such activity could explain levels of prey selectivity (preferred prey may have a higher anti-grazer metabolite content) while also compensating for the decreased growth rate associated with pure phototrophy. Such an event is consistent with the results of Perez et al. (Perez et al., 1997) where mixotrophs grew slower than pure heterotrophic ciliates, but appeared to be subjected to lower grazing losses from copepods. Mixotrophy through consumption of photrophs rather than bacteria may be less efficient from a pure nutritional point of view, but may be compensated through decreased death rates.

The conceptual models of mixotrophs (Table I) can now be considered making use of a mathematical framework. The essential configurations of the model to conform to previously developed conceptual guidelines (Jones, 1997; Stoecker, 1998) are shown in the last column in Table I. The most notable modification of the original concepts suggested by the model is that the labelling of mixotrophs as primarily heterotrophic requires that they are not obligate phototrophic. The implication is that many organisms that would conform to Stoecker type IIIa (Stoecker, 1998) are actually representatives of organisms at the other end of the mixotrophic spectrum from type II, and are not fundamentally different. There is such plasticity in mixotrophic configuration, both revealed in the literature and also demonstrated by the model, that it would not be appropriate to be more prescriptive. Future experimental work can find a framework in the structure of the model, for example in establishing the form of the feedback functions shown in Fig. 3, determining the values of constants such as Chl:C$_{\text{max}}$, and parameterizing prey selectivity against the nutrient status of both the prey and predator (Mitra and Flynn, 2006b). In turn, such experiments will inform further model development.

ACKNOWLEDGEMENTS

We thank all those who have entered into discussions with us on this subject over the past few years, including the referees and John Dolan who passed comment upon an earlier version of this work.

REFERENCES


