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Modelling mixotrophy in harmful algal blooms: More or less the sum of the parts?

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ABSTRACT

Mixotrophs are significant components of planktonic food webs, are frequently associated with harmful algal bloom events, and thus warrant inclusion in coastal ecosystem models. There are, however, insufficient quantitative data to support the construction and testing of simple empirical descriptions of mixotrophs. Here, a complex mixotroph model based upon phenomenological understanding (Flynn and Mitra, 2009) was used to generate control "realities" against which to compare contrasting simple descriptions of mixotrophy using a Turing Test approach. The simplest description, adding together phototrophic and heterotrophic functions gave the worst output. The best model tested, in keeping with the evolution of these organisms, used phototrophy as a nutritional supplement mechanism for heterotrophy. However, none of the simple models described kleptochloroplasty — an important process in some harmful bloom species. None of the simple models correctly matched the balance of phototrophy and heterotrophy (grazing); while fits to bulk parameters (biomass, nutrients) could be acceptable, rate processes were often completely in error. This is of particular concern because of the difficulty in determining rate processes. A generalised implication is that a fit to bulk data gives no assurance that the model structure is not dangerously dysfunctional; determining model skill should include locating and removing structural dysfunctionality.

1. Introduction

1.1. Role of mixotrophy

Organisms that possess the capability of combining phototrophy and heterotrophy are termed mixotrophs. Depending on light, nutrient and prey availability, mixotrophs display varying proportions of phototrophic and heterotrophic activity. Mixotrophs thus occupy a unique niche affecting trophic levels both below and above them with the potential to change the dynamics of the system. Mixotrophy can, therefore, affect biogeochemical cycling of nutrients.

Mixotrophs do not form a unique group but occur under different physiological guises, amongst different species ranging over a variety of taxonomic groups. From an evolutionary point of view phagotrophy in eukaryote microbes is believed to be the primitive state from whence pure phototrophic protists evolved (Raven, 1997; Raven et al., 2009). Within planktonic organisms, mixotrophy is a common phenomenon in marine as well as freshwater systems (Jones, 1997; Raven, 1997; Stoecker, 1998; Jones, 2000). Indeed, mixotroph populations can be responsible for ecologically catastrophic events such as harmful algal blooms (Kempton et al., 2002; Vaqué et al., 2006; Burkholder et al., 2008).

In the presence of abundant light, nutrients or prey, strict autotrophs and/or heterotrophs dominate. Mixotrophy comes into play in mature systems. In such systems mixotrophs act as conduits for energy and elements from different parts of the food web. Thus, in post-autumn bloom when there is low light as well as low food availability, mixotrophs photosynthesising and engulfing bacteria can channel energy to higher trophic levels (e.g., Myung et al., 2006). This activity also improves the C:N:P ratio of the mixotrophs resulting in these becoming nutritionally replete food for the higher trophic levels (so-called seston upgrading; Ptacnik et al., 2004; Weithoff and Wacker, 2007). In the post spring bloom period, when autotrophs become increasingly reliant upon regenerated nutrients, mixotrophs including HAB species are advantaged through their ability to consume other organisms.

When confronted with unfavourable conditions, mixotrophs possess an advantageous survival strategy, thriving in conditions where food and/or light limits growth of their non-mixotrophic competitors. Therefore, one could expect occurrences of "ideal mixotrophs" in nature capable of balancing autotrophy and phagotrophy to maintain a high growth rate under varying environmental conditions. However, there is no evidence of occurrence of such organisms in reality. Indeed, mixotrophic organisms typically have lower growth rates compared to dedicated autotrophs or heterotrophs (Raven, 1997; Stoecker, 1998). This reflects the compromises required to operate two nutritional modes within one cell type, and is suggestive of a complex regulatory interaction between the processes, rather than them being simply additive. As we shall see, this has implications for modelling mixotrophic activity.

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1.2. Need for models

Given the established importance of mixotrophy, notably in certain HAB events, a need to simulate their involvement in the aquatic ecosystem is clear. However, even with the current trend of including multiple compartments to represent different groups of plankton (i.e., plankton functional types, PFTs) the most complex planktonic food web models often do not include mixotrophs as a separate entity (e.g., Baird and Suthers, 2007; Follows et al., 2007). The implicit assumption is that these organisms do not warrant a "box" of their own within these multi-component ecosystem models. By default the phototrophic activity of mixotrophs is therefore typically assigned to the "phytoplankton" group while the heterotrophic activity is assigned to the "microzooplankton" component.

Where mixotrophy is considered explicitly usually it is to perform some specific role, such as providing an additional nutrient source for a primarily phototrophic form (e.g., through ingestion of bacteria; Thingstad et al., 1996; Myung et al., 2006), or provision of energy/carbon through ingestion of phototrophs (e.g., Stickney et al., 2000; Jost et al., 2004). In such studies inclusion of mixotrophy within models is simplistic, lacking any mechanistic justification or physiological basis for the control of the mixotrophic act (e.g., Thingstad et al., 1996; Hammer and Pitchford, 2005; Troost et al., 2005). For example, the partitioning between autotrophy and phagotrophy may be apportioned using fixed ratios, various features of cell physiology may also either be disregarded or grossly simplified — photoacclimation is not typically included in the description of the photosynthetic component of the mixotroph. Within the description of the heterotrophic activity, the kinetics of predation and growth, effects of nutritional status and availability of food on ingestion, assimilation and the linkages between these processes are ignored. Furthermore, these models assign fixed assimilation efficiencies and ignore all feedback processes which are typical of biological systems. While this latter point is common of microzooplankton models in general, the absence of such descriptions in mixotrophs is a key omission in organisms whose functioning depends on the regulation of the feedback events.

1.3. The challenge and a compromise

Operation of the mechanistic model of protist mixotrophy developed by Flynn and Mitra (2009) gives some insight to the behavioural patterns achievable in models of mixotrophy and how they relate to our knowledge of these organisms. There are various datasets available from freshwater as well as marine studies to guide the construction and testing of such models. Unfortunately, the vast bulk of these experimental studies, while contributing much to the phenomenological data base, have limited value for the purpose of model development and application within steady state as well as dynamic systems. For example, mixotrophic experimentation typically focuses on either impacts of light or nutrient limitation on grazing (Nygaard and Tobiesen, 1993; Stoecker et al., 1997; Li et al., 2000; Smalley et al., 2003; Adolf et al., 2006). Suitably designed experiments are required in particular to parameterize the switching between phototrophic and heterotrophic activities within mixotrophs over a range of nutrient and prey concentrations against a background of variable light availability. It is also necessary to know the fates of the organics and inorganics in the system in order to check whether the system balances; such information is usually not documented in experimental studies.

In short we are left with a challenge. Mixotrophs are known to be important (Raven, 1997; Stoecker, 1998; Jones, 2000; Dolan and Perez, 2000; Raven et al., 2009) and we recognise the importance of modelling them (Zhang et al., 2003; Hood et al., 2006; Flynn and Mitra, 2009). That is especially so in the context of harmful algal blooms (Kempton et al., 2002; Burkholder et al., 2008). However, we lack the parametric data upon which to rigorously test different model formulations, to judge their skill in describing processes (for a discussion on model skill, see Stow et al., 2009). More than likely it will be several if not many years yet before such data become available. The best we can do at present is to construct models using phenomenological data and use these modelling experiments to help identify gaps in available data. This was the undertaking made by Flynn and Mitra (2009) but the outcome is arguably too complex for easy placement within ecosystem models.

Given the above, there is a need to deploy the simplest models of mixotrophs that are fit for purpose. In view of a lack of laboratory and field data upon which to base and test the construction of such models, a compromise is to use the model of Flynn and Mitra (2009), hereafter "F&M09", to generate a series of "realities" against which to compare simplified formulations. This approach is similar to Turing tests (testing an output from a computer programme against the reality of human behaviour to see whether the former demonstrates artificial intelligence), except here the reality is actually generated by a model which has itself been tested and deemed acceptable in its behaviour. While this exercise carries with it some level of risk, we argue that the risk is far less than making assumptions on mixotroph physiology with no physiological mechanistic basis at all.

1.4. Aims

With the caveats raised above, in this study, we test the functionality and fidelity of a range of contrasting models describing protist mixotrophy. The test models range from the most simplistic approach, where a proportion of phototrophic activity is assigned to a phototrophic compartment while the phagotrophic activity is assigned to a heterotrophic component (i.e., no feedbacks between the two components), to more complex structures where there is some level of feedback between the phototrophic and phagotrophic activities within the mixotrophs. These models are all compared against the behaviour of the physiological mechanistic model of Flynn and Mitra (2009) when operating under different scenarios. The aim is thus to determine the level of complexity necessary to describe mixotrophs within ecosystem models (such as those used for studying HABs) using a complex model as the control in Turing tests. Such an approach enables us to determine the optimal balance of complexity and fidelity in model constructs for describing this important PFT.

The conclusions that we reach are that models of mixotrophy must involve some description of metabolic switching. While simplistic models may describe the bulk properties such as biomass, nutrient concentrations adequately, they are incapable of simulating the underlying rate processes that drive the system dynamics correctly, which could result in erroneous prediction of HAB events. This inadequacy of the simple models is especially important due to the difficulties in measuring rate processes required for modelling HAB mixotrophy, in the laboratory as well as in the field.

2. Methods

For reasons given in the Introduction (namely the lack of field and laboratory data suitable for model parameterisation) the fidelity of simplified models has been determined by their ability to match the output of a mechanistic description of mixotroph activity that is capable of describing the different types of mixotrophic behaviour observed in nature (Flynn and Mitra, 2009) especially during HAB events.

2.1. Control mixotroph model

The mixotroph model was that of Flynn and Mitra (2009), hereafter F&M09. This model was operated using the constants as

described in Flynn and Mitra (2009), describing C:N:P physiology with photoacclimation, with a release of 10% of C-fixed as DOC (cf. Flynn et al., 2008). Voided material (excess from phagotrophic activity) was released as inorganics and semilabile organics.

2.2. Simplified mixotroph models

Five different configurations for simple mixotroph models were constructed as test models. Schematics for these, and for F&M09, are given in Fig. 1, with further information given in Table 1. Only the specific descriptions linking the models of Flynn (2001) and Mitra (2006) to generate the 5 test models are given here; the reader is referred to the source papers for the detailed description of the base models. In brief, the phytoplankton component of the mixotroph model was the same as used for the algal description in the ecosystem scenario, as an implementation of that described by Flynn (2001). The model also displays a repression of nitrate use by ammonium assimilation within the mixotroph. The zooplankton model of Mitra (2006) describes the capture of prey (using the ingestion-based prey selectivity (IS) function of Mitra and Flynn, 2006a), and the subsequent stoichiometric-related partitioning of ingested material into biomass and inorganic and organic voided materials. Voided organics were considered here to be semilabile and hence available for consumption by bacteria. No stoichiometric modulation of predation (SMP — Mitra and Flynn, 2005; Mitra, 2006) was described, and neither was it enacted in F&M09. Here prey were consumed according to their C-biomass concentration in the water, with the fate of their C:N:P determined according to the disparity between this and the fixed C:N:P of the predator. (The same zero, neutral, level of SMP was applied by the F&M09 model used here.) Depending on the simulation scenario of interest, grazing by the mixotroph was enabled (with equal C-specific rate) on algae and/or bacteria.

In all instances (both F&M09 and the test models), the contribution of the heterotrophic (zooplankton-like) component of the mixotroph could be enabled all the time, or in response to a specific nutrient limitation. For the F&M09 model application this was achieved as described in Flynn and Mitra (2009). For the test models it was achieved by reference to the value of a quotient, θ. To enable a potential for grazing all the time $\theta = 0$. If the potential for grazing developed in response to a general lowering of growth rate (e.g., nutrient and/or light limitation) then $\theta = {}^{\mu}/_{\mu}max$. If the potential for grazing developed in response to a specific nutrient limitation (e.g., P-stress as defined by the quotient PCu in Flynn, 2001) then θ was defined by that nutrient status (e.g., $\theta = \mathrm{PCu}$, where $\mathrm{PCu} = 0$ for maximum stress, and $\mathrm{PCu} = 1$ for no stress). The maximum value of the heterotrophic growth rate component of the mixotroph, μ^{het}_{max}, was a function of the absolute maximum possible rate under pure heterotrophy, μ^{het}_{absmax}, and θ according to Eq. (1).

$$\mu^{het}_{max} = \mu^{het}_{abs\,max} \cdot (1-\theta) \tag{1}$$

Schematics of the test model configurations are given in Fig. 1, with parameters in Table 1. The following describes these configurations in more detail.

Type I: there was no linkage between the phototrophic and heterotrophic descriptions. Mixotroph biomass was described simply by summing the individual masses attributed to the photosynthetic and heterotrophic components; there was no state-variable describing the total mixotroph biomass.

Type II: phototrophic and heterotrophic components shared a common biomass. Here, C, N and P entering from either or both phototrophic and heterotrophic activities was combined in the descriptions of state variables for C-biomass, N:C and P:C. Apart from that, however, there was no linkage between the modes of physiology, growth being the sum of the two activities. The maximum possible mixotroph growth rate was described by summing the maximum phototrophic (μ^{phot}_{max}) and the maximum heterotrophic (μ^{het}_{max}) activities. Nutrients released (regenerated) by heterotrophic activity entered the external medium and were recovered (against any competition with other organisms) by the phototrophic component.

Type III: this was like Type II except any nutrients regenerated by heterotrophic activity (i.e., via predatory behaviour by the mixotroph) was retained for direct use by the phototrophic component. Excess NH_4^+ and phosphate over that required to raise mixotroph N:C and P:C to the maximum values were released. The descriptor of this function was the same as that employed by Flynn and Mitra (2009), their equation 43; this used a sigmoidal function to progressively increase nutrient release as the nutrient quota (Q, as N:C or P:C) increased above Q_{max} (NC_{max}, PC_{max}) towards the absolute maximum Q_{abs} (NC_{abs}, PC_{abs}).

Type IV: developed from Type III; here the mixotroph growth rate was capped at the maximum possible level μ_{max}. Any shortfall, due to a lack of prey items, was balanced by phototrophy. The activity of the phototrophic component was thus down-regulated by the activity (success) of the heterotrophic component. This down-regulation employed a sigmoidal curve of the form described in Eq. (2) which worked on the value of the absolute maximum phototrophic growth rate, μ^{phot}_{max} giving an operational maximum ($^{op}\mu^{phot}_{max}$) which decreased to zero as the overall mixotroph growth rate (μ) approached the maximum value μ_{max}. K (typically <1) and H (typically between 2 and 8) are constants affecting the form of this control function.

$$^{op}\mu^{phot}_{max} = \mu^{phot}_{max} \cdot \frac{\left(1+K^H\right)\cdot\left(1-{}^{\mu}/_{\mu_{max}}\right)^H}{\left(1-{}^{\mu}/_{\mu_{max}}\right)^H + K^H} \tag{2}$$

Type V: this was like Type IV, except the regulation was reversed, with priority now to the phototrophic component (Eq. (3)). Heterotrophy now acted as the nutritional supplement for when phototrophic activity failed to support the potential growth rate.

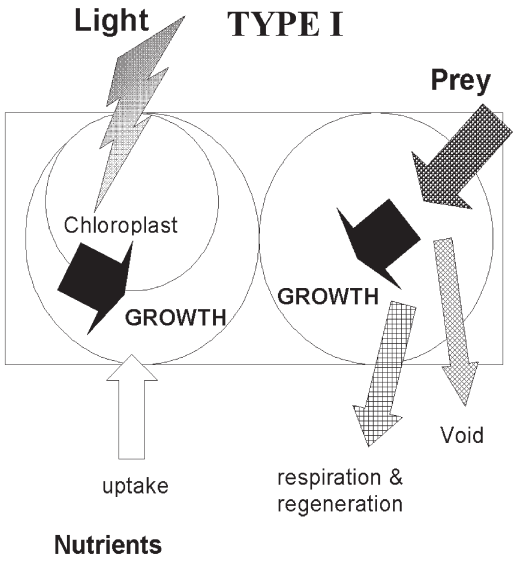
$$^{op}\mu^{het}_{max} = \mu^{het}_{max} \cdot \frac{\left(1+K^H\right)\cdot\left(1-{}^{\mu}/_{\mu_{max}}\right)^H}{\left(1-{}^{\mu}/_{\mu_{max}}\right)^H + K^H} \tag{3}$$

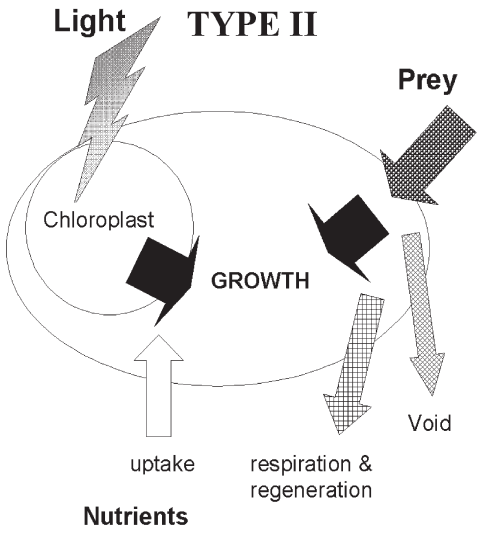
2.3. Simulation scenarios

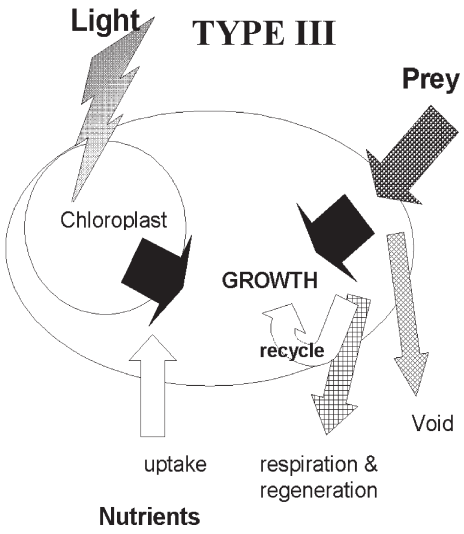
The base model scenario used to generate the control data series was a combination of the algal and bacterial models with the F&M09 mixotroph model as described and used by Flynn and Mitra (2009). Algae and bacteria were thus potential prey species, or under different conditions, competitors for nutrients with the mixotroph. The bacterial model was as described by Flynn (2005), consuming inorganic forms of N and P, and labile and semilabile organics. Test model runs were identical, except for the substitution of the F&M09 model with one of the simplified mixotroph descriptions.

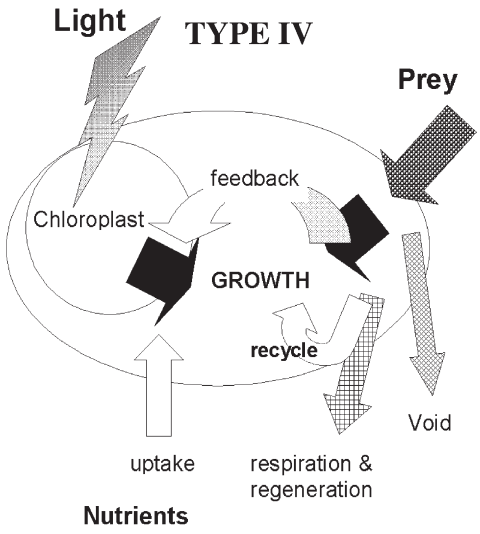
The interactions between algae (consuming inorganics as nitrate, ammonium and phosphate and releasing DOC), bacteria (consuming

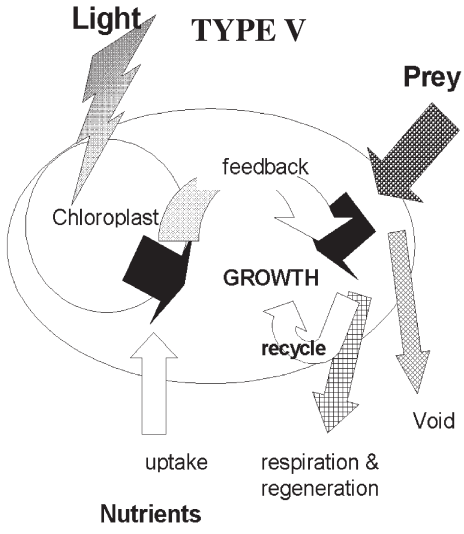
Fig. 1. Schematics of the different model types, together with that of the control model F&M09 described in Flynn and Mitra (2009). See also Table 1. Shown are flows of light, nutrients and prey into the organisms, together with losses from respiration, regeneration and voiding. In Type I the processes of phototrophy and heterotrophy are completely separate. Types III, IV, V and F&M09 recycle nutrients otherwise voided following assimilation of prey material. Interaction "Int1" in F&M09 refers to an interaction at the level of C-supply and demand; interaction "Int2" refers to a spatial interaction between space for chloroplasts versus that for feeding vacuoles — see Flynn and Mitra (2009) for further information.


Light
TYPE I
Prey
Chloroplast
GROWTH
GROWTH
Void
uptake
respiration &
regeneration
Nutrients


Light
TYPE II
Prey
Chloroplast
GROWTH
Void
uptake
respiration &
regeneration
Nutrients


Light
TYPE III
Prey
Chloroplast
GROWTH
recycle
Void
uptake
respiration &
regeneration
Nutrients


Light
TYPE IV
Prey
feedback
Chloroplast
GROWTH
recycle
Void
uptake
respiration &
regeneration
Nutrients


Light
TYPE V
Prey
feedback
Chloroplast
GROWTH
recycle
Void
uptake
respiration &
regeneration
Nutrients

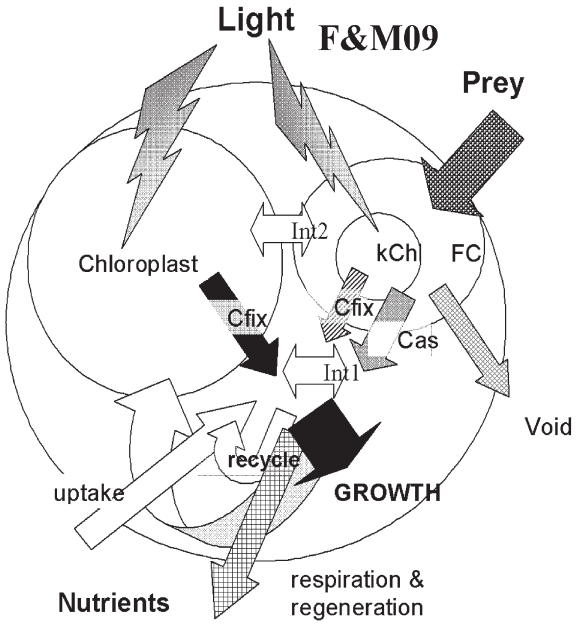

Light
F&M09
Prey
Int2
kCh
FC
Chloroplast
Cfix
Cfix
Cas
Int1
Void
recycle
uptake
GROWTH
respiration &
regeneration
Nutrients

Table 1
Features of the different types of simplified mixotroph models tested, and of the control model, F&M09.

	Model type					
	I	II	III	IV	V	F&M09
Number of state variables	5	4	4	4	4	8
Separate phototrophic and heterotrophic systems; variable N:C, P:C and Chl:C for phototroph, fixed N:C and P:C for heterotroph	✓					
Common state variables describing variable N:C, P:C and Chl:C		✓	✓	✓	✓	✓
Separate state variables describing material and Chl held in the feeding vacuole						✓
Explicit description of kleptochloroplasty						✓
No internal nutrient cycling	✓	✓				
Internal nutrient cycling			✓	✓	✓	✓
$\mu = \mu^{phot} + \mu^{het}$	✓	✓	✓			
$\mu \leq \mu^{het}_{max}$; μ^{phot}_{max} down-regulated				✓		
$\mu \leq \mu^{phot}_{max}$; μ^{het}_{max} down-regulated					✓	
μ a function of integrated interactions						✓
Grazing always enabled, or modulated by general or specific growth-limiting stress	✓	✓	✓	✓	✓	✓

Table 2
Descriptions of scenarios. The operation of kleptochloroplasty was as described in Flynn and Mitra (2009) in which the maximum size of the feeding vacuole was set at a value of 0.2 rather than 0.05 of the mixotroph core biomass, and digestion was linked to the supply of C for support of growth.

	Scenarios				
	A	B	C	D	E
Mixing depth (m)	20	20	5	5	5
Initial NO_3^- concentration (μM)	5	5	20	20	20
Initial PO_4^{3-} concentration (μM)	0.5	0.125	1	0.5	1
Grazing on algae	✓	✓	✓	✓	✓
Grazing on bacteria	✓	✓	✗	✗	✗
Kleptochloroplasty	✗	✗	✗	✗	✓
Limitation stimulating mixotrophy (none = 1; P-stress = 2; N- and/or P-stress = 3)	1	2	1	3	1

Table 3
Constants in test model subjected to tuning, their units, and the range of values in which they were tuned.

Constant	Role	Unit	Range
Cr_a	Capture rate on algal prey	$(C\,C^{-1}\,d^{-1})\cdot(C\,L^{-1})^{-1}$	0.001–0.01
Cr_b	Capture rate on bacterial prey	$(C\,C^{-1}\,d^{-1})\cdot(C\,L^{-1})^{-1}$	0.001–0.01
AE_{max}	Maximum assimilation efficiency	Dimensionless	0.5–0.9
AE_{min}	Minimum assimilation efficiency	Dimensionless	0.1–0.45
H	Hill constant for switch between modes of nutrition	Dimensionless	1–8
K	Half saturation constant for switch between modes of nutrition	Dimensionless	0.01–1
K_I	Half saturation constant for ingestion	$C\,C^{-1}\,d^{-1}$	0.1–0.5
μ^{phot}_{max}	Maximum rate of photosynthesis-driven growth	$C\,C^{-1}\,d^{-1}$	0.5–2
μ^{het}_{max}	Maximum rate of heterotrophic-driven growth	$C\,C^{-1}\,d^{-1}$	0.5–2

ammonium, DOC and semilabile organics) and mixotrophs (consuming nitrate, ammonium, phosphate, algae and/or bacteria, releasing DOC and semilabile organics) were followed within a physical description of a mixed water column of either 5 or 20 m depth. Mixing between the mixed and lower water masses was equivalent to a dilution rate of 0.05 d^{-1}, thus removing organisms and residual nutrients and introducing fresh nutrients. The different scenarios of nutrients, mixed depth and mixotroph configuration (feeding on bacteria and/or algae, in response to general growth limitation or to specific nutrient limitation, and with or without a description of kleptochloroplastic activity) are summarised in Table 2.

2.4. Model operation and tuning

The control model, with the mixotroph described according to F&M09 (Fig. 1), was run under different scenarios (Table 2). The test model configurations were then tuned against the output data from the control. The data used for tuning were concentrations of nitrate, ammonium, phosphate, DOC, algal-C, bacterial-C and mixotroph-C. These are the types of data most likely to be obtained from experimental studies (but are scant and/or non-existent at present; see Introduction and Discussion). The data used for tuning were used with a temporal resolution of 0.125 d.

Tuning was performed using the evolutionary search method supported by Powersim Solver v2 (Isdalstø, Norway). This method maximizes the likelihood of obtaining a global rather than a local minimum (Haefner, 1996). In essence, through what are termed "evolutionary algorithms" (i.e., multiple simulations carried out with different combinations of the constant parameters), Solver identifies values for the chosen constants which produce the fit closest to the presented data series (e.g., biomass). Thus, Solver runs the model with different combinations of parameter values, "crossing" (in a genetic, evolutionary sense) those combinations that give the best fits and rejecting those that are poor; Solver eventually identifies a combination of parameter values that gives the best fit. Typically, some 10,000 or so simulations are run during each model tuning.

The constants in the test models subjected to tuning were only those associated with the test mixotroph configurations; all other constants (e.g., algal, bacterial, and physical descriptors) remained the same as those in the control setup. We also restricted the range of the mixotroph model parameters tuned. Thus parameters describing the minimum and maximum N:C and P:C, those associated with photosynthesis and photoacclimation were kept constant across all descriptions (with values as given in Flynn and Mitra, 2009). We did this because the emphasis here was on judging how the coupling between phototrophic and heterotrophic processes could (in modelling terms) be most economically achieved. The test model constants tuned are listed in Table 3.

To provide some level of objectivity in comparing the output of the model systems, the mean deviation between the outputs of the control model using the F&M09 description and the five test models was calculated for the total carbon biomass of the mixotroph (mC), the rates of mixotroph carbon fixation (C-fix) and ingestion (IgC) and the mixotroph growth rate ($C\mu$). This deviation was calculated by summing the squared differences between the outputs of the scenarios operating the test models versus the F&M09 model at that time, and averaging them for the number of data points considered (Eq. (4)).

$$\text{Mean deviation} = \overline{\sum(\text{test model output} - \text{F\&M09 model output})^2} \quad (4)$$

3. Results

The tuned values of the constants for the five model types for the five different scenarios are listed in Table 4. Coloured versions of the plots are available in the online version of this paper. Only the data for organism biomass and mixotroph rates of photosynthesis, ingestion and growth are shown. Data for nutrients for all but ammonium were very similar between simulations.

Table 4
Tuned values of constants used by the different mixotroph types; see Table 3 for units and definitions; na, not applicable for this model type. The values used for the control, F&M09, model were: $Cr_a = 0.005$; $Cr_b = 0.005$ for Scenarios A and B and $Cr_b = 0$ for Scenarios C–E; $AE_{max} = 0.8$; $AE_{min} = 0.2$; $\mu^{phot}_{max} = 0.35$; $\mu^{het}_{max} = 0.7$.

	Model				
	I	II	III	IV	V
Scenario A					
Cr_a	0.008	0.008	0.003	0.004	0.007
Cr_b	0.003	0.005	0.004	0.003	0.004
AE_{max}	0.734	0.896	0.723	0.703	0.871
AE_{min}	0.337	0.242	0.180	0.104	0.214
H	na	na	na	5.306	4.974
K	na	na	na	0.550	0.685
K_I	0.499	0.484	0.243	0.349	0.262
μ^{phot}_{max}	0.948	0.762	0.798	1.396	0.891
μ^{het}_{max}	0.734	0.136	0.113	0.174	0.218
Scenario B					
Cr_a	0.006	0.008	0.005	0.005	0.010
Cr_b	0.004	0.006	0.004	0.004	0.006
AE_{max}	0.612	0.794	0.899	0.895	0.896
AE_{min}	0.224	0.395	0.404	0.160	0.353
H	na	na	na	4.450	1.192
K	na	na	na	0.734	0.304
K_I	0.124	0.128	0.272	0.389	0.221
μ^{phot}_{max}	1.036	0.658	0.677	1.275	0.679
μ^{het}_{max}	1.988	0.432	0.546	0.970	0.424
Scenario C					
Cr_a	0.004	0.004	0.002	0.002	0.002
Cr_b	0.000	0.000	0.000	0.000	0.000
AE_{max}	0.898	0.733	0.684	0.649	0.777
AE_{min}	0.235	0.341	0.152	0.308	0.333
H	na	na	na	6.618	3.886
K	na	na	na	0.417	0.589
K_I	0.388	0.337	0.195	0.337	0.303
μ^{phot}_{max}	0.857	0.641	0.705	1.011	0.760
μ^{het}_{max}	0.867	0.170	0.116	0.140	0.440
Scenario D					
Cr_a	0.004	0.004	0.005	0.004	0.009
Cr_b	0.000	0.000	0.000	0.000	0.000
AE_{max}	0.721	0.754	0.749	0.725	0.680
AE_{min}	0.426	0.373	0.265	0.294	0.350
H	na	na	na	4.156	5.260
K	na	na	na	0.595	0.186
K_I	0.210	0.260	0.404	0.281	0.285
μ^{phot}_{max}	1.006	0.602	0.633	1.039	0.646
μ^{het}_{max}	2.000	0.611	0.597	0.995	0.484
Scenario E					
Cr_a	0.001	0.0003	0.004	0.002	0.0004
Cr_b	0.000	0.000	0.000	0.000	0.000
AE_{max}	0.559	0.747	0.567	0.688	0.769
AE_{min}	0.258	0.152	0.186	0.239	0.260
H	na	na	na	3.761	4.940
K	na	na	na	0.190	0.812
K_I	0.401	0.487	0.151	0.456	0.387
μ^{phot}_{max}	1.083	0.946	0.820	0.999	0.927
μ^{het}_{max}	0.460	0.057	0.005	0.007	0.033

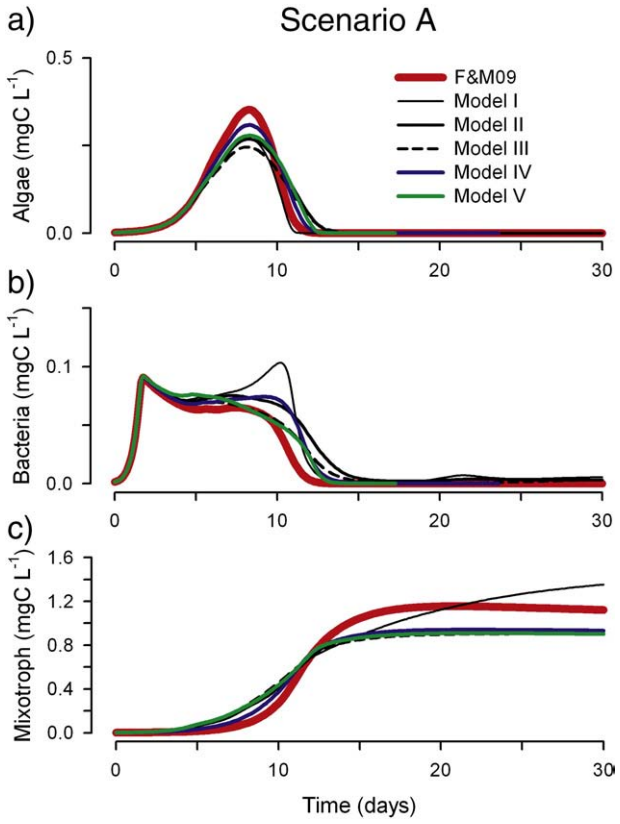


Fig. 2. Model outputs for algal, bacterial and mixotroph biomass in Scenario A, with continuous grazing by the mixotroph on algae and bacteria in a deep mixing layer, low-nutrient setting. The models containing the alternate mixotroph models, Types I–V, were tuned against the control model containing the full mixotroph description (F&M09) described by Flynn and Mitra (2009). The data used for tuning were concentrations of nitrate, ammonium, phosphate, DOC, algal-C, bacterial-C and mixotroph-C. See also Table 2. A colour version of this plot is available online.

3.1. Scenario A: continuous grazing on algae and bacteria

Here grazing on both the algal and bacterial prey was enabled all the time (Table 2), and the outputs for nutrients (not shown) and organism populations of the five test models were not substantially different from the outputs when employing F&M09 (Fig. 2a–c). Simulations using model Type I showed the least overall deviation for the mixotroph biomass (Table 5). However, fits were attained through contrasting predator–prey dynamics (Fig. 3a–c). With the control model F&M09, the initial increase in the mixotroph population was attained through prey ingestion (Fig. 3b); the decline in prey populations (Fig. 2a and b) resulted in a decline in mixotroph growth rate (Fig. 3c) with subsequent growth being sustained through carbon fixation (Fig. 3a). In the test models, however, the initial increase in the growth rate of the mixotroph was associated with high phototrophic activity and low prey ingestion (Fig. 3a versus b). The growth rate patterns for the mixotroph population in all the five test models followed that of carbon fixation and were markedly different from the control model using the F&M09 description. Note in particular the values ascribed to the maximum potential for growth under phototrophy (μ^{phot}_{max}) versus that for heterotrophy (μ^{het}_{max}) in the test models versus F&M09 (Table 4). Of the five test models, Type IV (which used phototrophy as a nutritional supplement to heterotrophy; Table 1) achieved the best fit to those of F&M09 (Table 5).

3.2. Scenario B: grazing on algae and bacteria to compensate for P-stress

Mixotroph grazing was stimulated here by P-stress (Table 2). As in Scenario A, in general terms there was similar temporal agreement between the test model outputs and those from the control model F&M09 for the prey populations (Fig. 4a and b). The initial increase in the mixotroph population, however, was markedly higher (up to 4-fold) for all the test models, and Type I clearly performed worst; Type IV was best (Fig. 4c). Again, however, there was a clear difference in the C-acquisition dynamics between the simplified models and F&M09 (Fig. 5). The high initial growth rates of the mixotroph for the test models were due to high phototrophic activity compared to that from F&M09 (Fig. 5a vs. 5c; see also values for μ^{phot}_{max} in Table 4). With the advent of phosphorus stress (phosphate was nearly exhausted by day 10 of the simulation, not shown), the F&M09 mixotroph switched

Table 5
Deviations of simulations of mixotroph C-biomass (mC), mixotroph C-fixation (C-fix), ingestion (IgC) and growth rate (Cμ) conducted using different mixotroph model Types I–V, in comparison with the output of the control model. Deviations were calculated as according to Eq. (4). Text in bold signifies the lowest deviation.

Model	Scenario A				
	I	II	III	IV	V
mC	**0.013**	0.030	0.035	0.022	0.032
C-fix	0.200	0.172	0.211	**0.117**	0.273
IgC	0.098	0.110	0.110	**0.063**	0.140
Cμ	0.027	0.025	0.032	**0.008**	0.035
Scenario B					
mC	0.033	0.013	0.013	**0.005**	0.014
C-fix	0.241	0.069	0.085	**0.035**	0.087
IgC	0.150	0.075	0.092	**0.052**	0.093
Cμ	0.086	0.031	0.035	**0.013**	0.036
Scenario C					
mC	0.620	0.454	0.390	0.345	**0.306**
C-fix	0.082	**0.070**	0.119	0.101	0.154
IgC	0.069	**0.026**	0.039	0.028	0.047
Cμ	0.051	0.012	0.020	**0.010**	0.020
Scenario D					
mC	0.200	0.005	0.006	**0.004**	**0.004**
C-fix	0.338	**0.056**	0.080	0.067	0.088
IgC	0.447	0.082	0.115	**0.051**	0.129
Cμ	0.107	0.020	0.027	**0.018**	0.031
Scenario E					
mC	0.207	0.114	**0.061**	0.077	0.081
C-fix	0.411	0.334	**0.266**	0.353	0.347
IgC	0.072	0.062	**0.054**	0.065	0.066
Cu	0.077	0.065	**0.046**	0.062	0.065

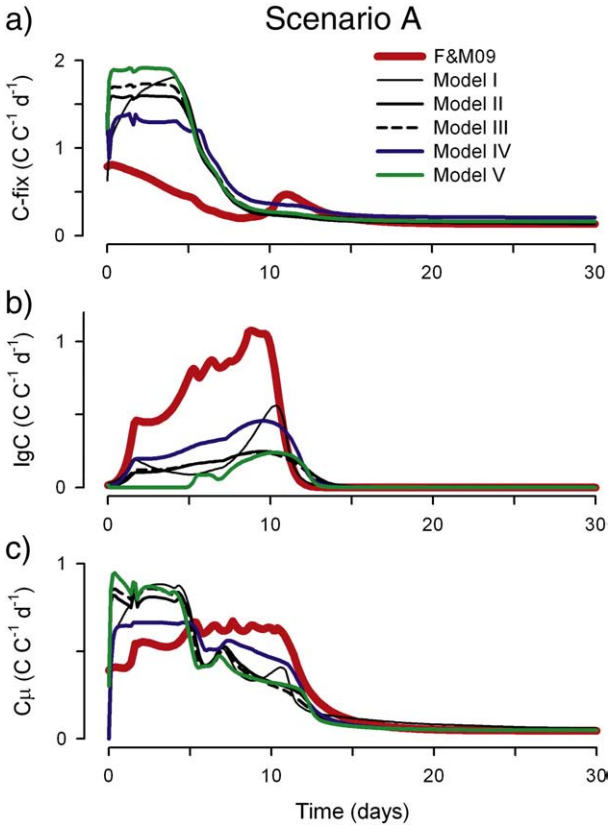


Fig. 3. Model outputs for mixotroph C-fixation (C-fix), ingestion (IgC) and growth rates (Cμ) from the simulations described in Fig. 2. A colour version of this plot is available online.

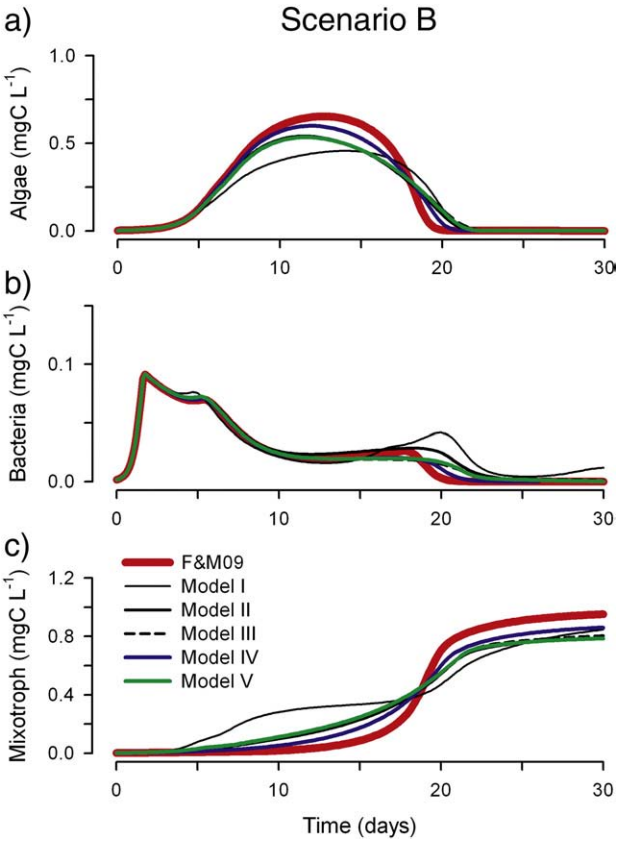


Fig. 4. As for Fig. 2, but for Scenario B, with mixotroph grazing on algae and bacteria to compensate for P-stress in a deep mixing layer, low-nutrient setting. See also Table 2. A colour version of this plot is available online.

over to heterotrophic activity. This resulted in an increase in the mixotroph growth rate and thence population (Figs. 4c and 5c). However, while P-stress was used to trigger feeding in the test Types as well, these did not develop such a level of heterotrophic activity (low IgC, Fig. 5b; see also values for μ_{max}^{het} in Table 4) resulting in a lower growth rate (Fig. 5c) during this phase. Again, the test model Type IV gave the best fits to the control model F&M09 (Table 5), though even this did not give a particularly close fit (Fig. 5).

3.3. Scenario C: continuous grazing on algae only

In this scenario (Table 2), the mixing depth was shallower, and nutrient loading higher (more akin to a coastal area or lagoon, the type of environment where HABs may develop). Here, while the outputs from the model with mixotroph Type I was substantially different compared to the control model F&M09, there was no substantial temporal difference in populations between the four other test models and the control model (Fig. 6; Table 5). The mixotroph population, however, was substantially higher for the control model than for Types II–V (Fig. 6c). Looking at the dynamics of C-acquisition (Fig. 7; Table 4), again one can see marked differences in the modulation of phototrophic and heterotrophic activities between the mixotroph model described by F&M09 and the test Types. Oscillations in C-ingestion (IgC) by F&M09 reflect sequences of feeding and digestion, as described by the filling and emptying of the feeding vacuole (see Flynn and Mitra, 2009). The test Types were chiefly dependent on carbon fixation for the increase in the mixotroph population at the start of the simulation, while F&M09 engaged in feeding from the start and down-regulated photosynthesis (Fig. 7a vs. 7b; Table 4). Unlike Scenarios A and B, the best fits to the data from the control model was not given by any particular test model, though Type I was worst (Table 5). Dynamics for Type I were different to

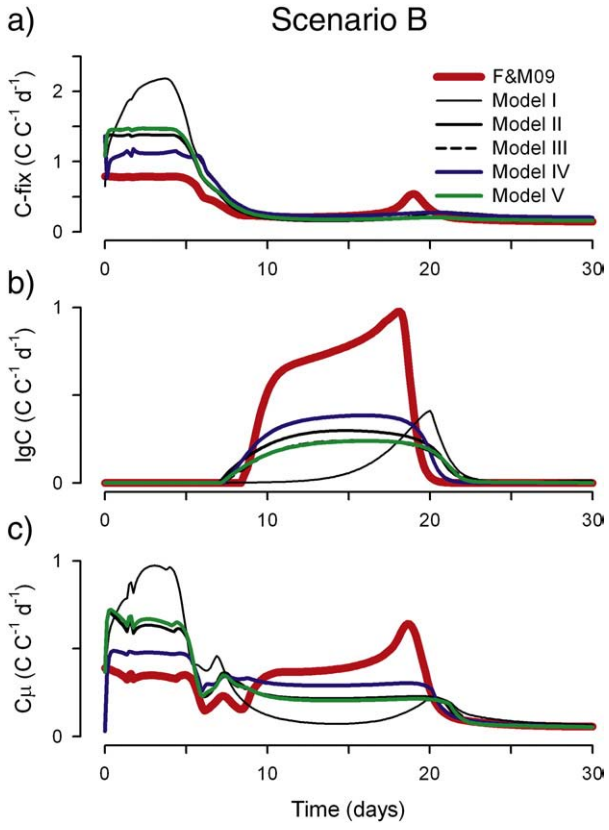


Fig. 5. Model outputs for mixotroph C-fixation (C-fix), ingestion (IgC) and growth rates (Cμ) from the simulations described in Fig. 4. A colour version of this plot is available online.

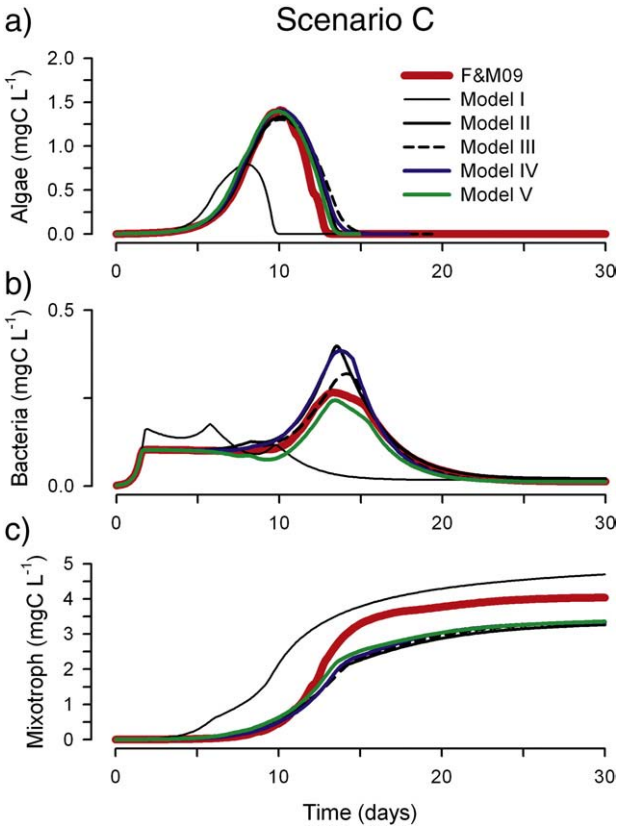


Fig. 6. As for Fig. 2, but for Scenario C, with continual grazing on algae in a shallow water setting. See also Table 2. A colour version of this plot is available online.

the others; grazing contributed little to a relatively high initial growth rate, with the combined phototrophic activity of mixotroph and algae depleting nutrients rapidly after day 5 (not shown). As nutrients were exhausted there was a rapid increase in grazing that led to a rapid decline in prey algal biomass by day 10 (Figs. 6 and 7).

3.4. Scenario D: grazing on algae to compensate for nutritional limitation

Scenario D was the same as Scenario C, except for the involvement of N or P nutrient stress in inducing grazing (Table 2). Again, behaviour of test Type I was clearly different to the others in the way in which it described the overall biomass dynamics (Fig. 8); the others were in close agreement with each other. The C-acquisition dynamics for the mixotroph (Fig. 9; Table 4) were, also, very different; the greatest difference was between Type I and F&M09, but the behaviour of Types II–V were also markedly at variance in comparison with F&M09 (Table 5). The increase in the mixotroph population (Fig. 8c) in the control simulation using F&M09 was achieved with a relatively lower initial growth rate (Fig. 9c) resulting from a low carbon fixation rate (Fig. 9a). P-stress started to build from day 5 onwards, with near exhaustion from day 10 (not shown). Grazing developed in consequence of this, and was higher in the simulation using F&M09 (Fig. 9b). Through different dynamics similar final mixotroph biomasses were achieved (Fig. 8c).

3.5. Scenario E: continuous grazing on algae, with retention and use of kleptochloroplasts

For this scenario, the model of F&M09 was configured with a large feeding vacuole in which algal prey could be held without much digestion so that their kleptochloroplastic contribution to mixotroph C-fixation was protracted (Table 2). Kleptochloroplasty is, for example, important for the HAB species *Dinophysis*. Like other models in the literature, the test Type descriptions have no capacity for explicitly describing such behaviour because there are no state variables describing kleptochloroplasts and hence their photosynthetic activity.

Unlike any of the above scenarios, the test model outputs for Scenario E are markedly different from the control model using F&M09 in all instances (Figs. 10 and 11; Table 5). The mixotroph population in all the test models depend solely on the phototrophic activity for increasing the population; there is only a low level of grazing activity (Fig. 11a vs. b; Table 4). The growth rate of the mixotroph portrayed through F&M09 is chiefly maintained through heterotrophic activity (Fig. 11b) in conjunction with retention of the operational kleptochloroplasts in order to maintain a high growth rate (Fig. 11c) resulting in an increasing mixotroph population under nutrient limiting conditions (Fig. 10c). Type III comes closest to describing the behaviour of the F&M09 model (Table 5), though even this fails totally to describe the C-acquisition dynamics (Fig. 11).

4. Discussion

The target of this work is crucial to the description of many harmful algal bloom events. While mixotrophs may be, and indeed have been, ignored in ocean models, they cannot be justifiably ignored in models dedicated to the study of certain HAB events and indeed in coastal ecosystem models in general. In such situations the activity of mixotrophs can be central to ecosystem dynamics. That is especially so where the success of the HAB is related to the increased abundance of prey species due, for example, to eutrophication and/or removal of benthic (bivalve) predators of those prey.

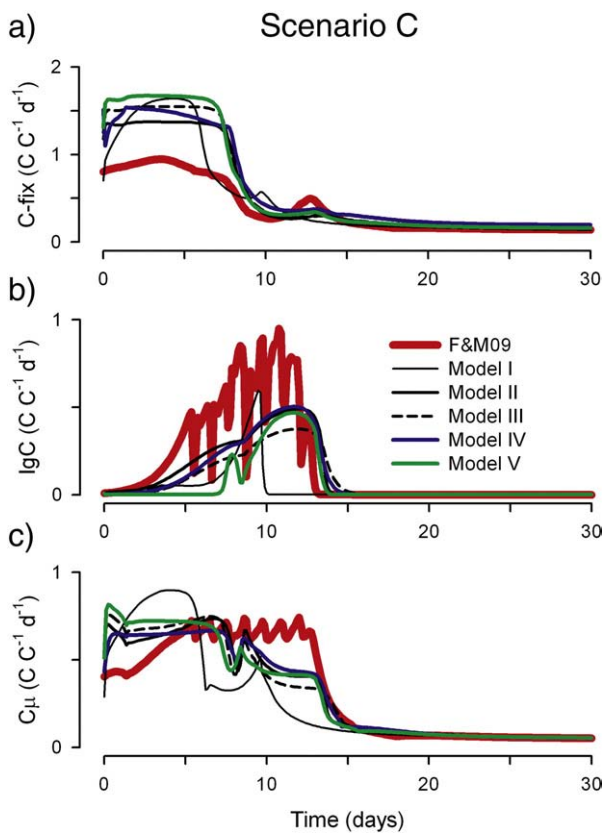


Fig. 7. Model outputs for mixotroph C-fixation (C-fix), ingestion (IgC) and growth rates (Cμ) from the simulations described in Fig. 6. A colour version of this plot is available online.

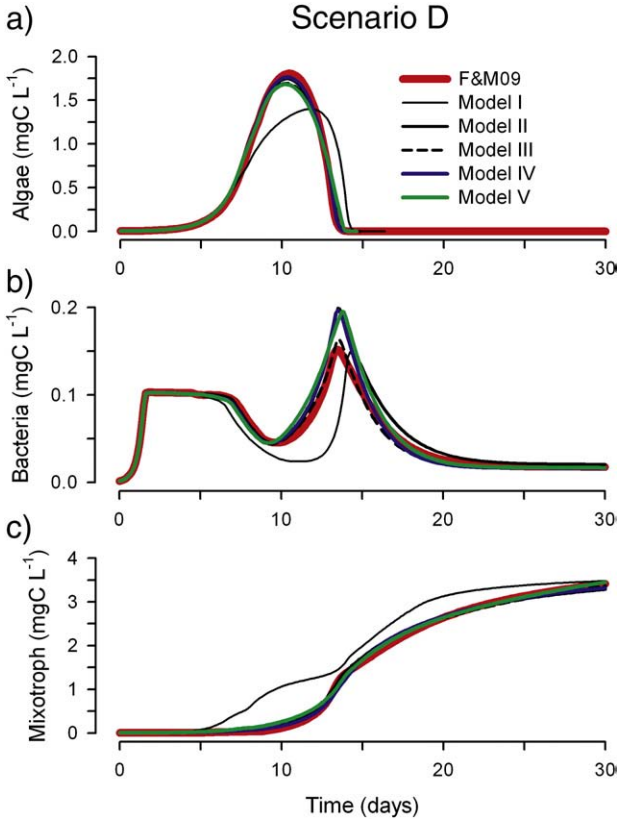


Fig. 8. As for Fig. 2, but for Scenario D, with grazing on algae to compensate for nutritional limitation in a shallow water setting. See also Table 2. A colour version of this plot is available online.

4.1. Overview of results

The simulations of population biomass, and of the nutrients (not shown) produced using the simple descriptions of mixotrophs generally matched those of the multi-nutrient mechanistic description of Flynn and Mitra (2009) against which they were tuned (Figs. 2, 4, 6, 8, and 10). This could be considered as giving some cause for optimism for the deployment of simple models of mixotrophy. However, as ever, the devil is in the detail.

Outputs generated using the Type I description gave the poorest descriptions of the events portrayed by the control model. There is an important difference between Type I and all the others (Table 2). In Type 1 any nutritional advantage gained from consuming prey is not transferable to the phototrophic component, or vice versa. Thus during the operation of Type I in Scenario B grazing was slow to develop (Fig. 5b) because phototrophy over the preceding period had only developed the phototrophic potential (Fig. 5a), leaving the heterotrophic potential as a minor component of the whole. In reality, energy and resources from these processes interact to support organism growth. For example, the consumption of bacteria into a mixotroph (Myung et al., 2006), a food source rich in N and P and relatively poor in C, would be expected to enhance the phototrophic ability (by raising mixotroph N:C and P:C) and simultaneously stimulating C-fixation countering respiratory losses during prey digestion. Additional carbon is also required to compensate for the stoichiometric difference between the prey and mixotroph. At the very least, then, any model of protist mixotrophy requires the integration of phototrophy and heterotrophy. Simply adding the two processes together is inappropriate, and arguably gives a dysfunctional model (see Flynn, 2010 for discussion on dysfunctionality). Type II (Fig. 1; Table 2) achieves at least some level of integration through the sharing of a common biomass descriptor but its performance is still wanting.

Better matches are given by Types III–V (Figs. 6, 8, and 10), that more fully integrate the physiological processes (Table 2), with some degree of feedback to balance and modulate the processes of phototrophy and heterotrophy. This result is important because hitherto most descriptions of mixotrophs have relied on additive descriptions, with no feedback (Thingstad et al., 1996; Baretta-Bekker et al., 1998; Stickney et al., 2000; Hammer and Pitchford, 2005).

The best results from the simple models appeared to require a dominantly heterotrophic description with phototrophy acting as a nutritional supplement. This, Type IV, is perhaps coincidentally consistent with the evolution of these organisms as being primarily heterotrophs. That said, none of the five test types came close to replicating the behaviour most closely matching that of those original mixotrophs, namely a mode of operation involving kleptochloroplasty (Scenario E, Fig. 10; Lewitus et al., 1999; Johnson et al., 2006, 2007; Kim et al., 2008).

There is, however, a very important caveat that must be levelled at even the ability of the best of the simple test types, namely that the fit to the bulk state-variable data (biomass, nutrients) was achieved typically using totally different dynamics of phototrophic and heterotrophic activity to those in the control simulation using the mechanistic description afforded by F&M09 (Figs. 3, 5, 7, 9, and 11; Table 4). This issue will be considered further below, in Section 4.3.

One final issue is that the test models tend to misrepresent the "reality" in the same way — an over-estimation of the importance of C-fixation for the mixotroph which is particularly apparent in the early phases when the numeric values of biomass are lowest. This was so even for Type IV which was configured to give priority to the heterotrophic mode of nutrition. The explanation for this is not obvious, but the operation of mixotroph physiology in nature, and as represented by F&M09, is more complex than just switching between modes because of

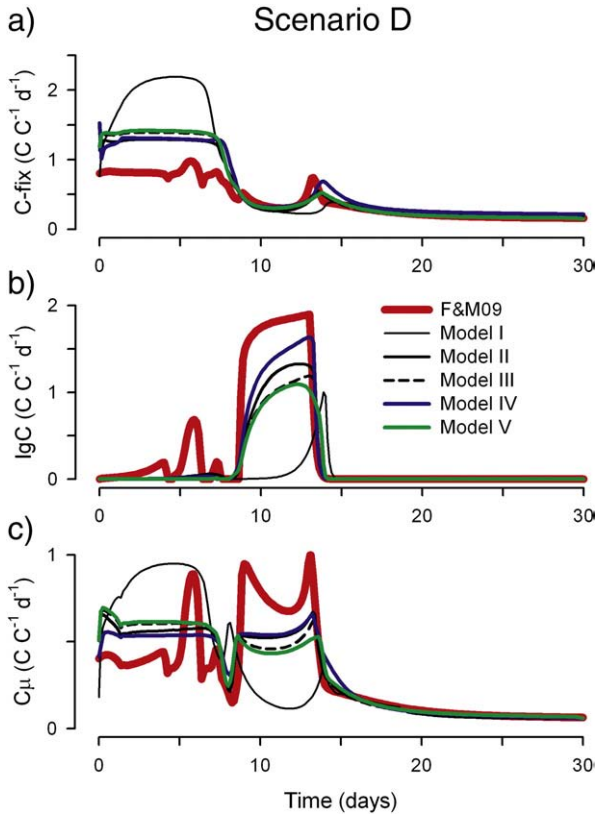


Fig. 9. Model outputs for mixotroph C-fixation (C-fix), ingestion (IgC) and growth rates (Cμ) from the simulations described in Fig. 8. A colour version of this plot is available online.

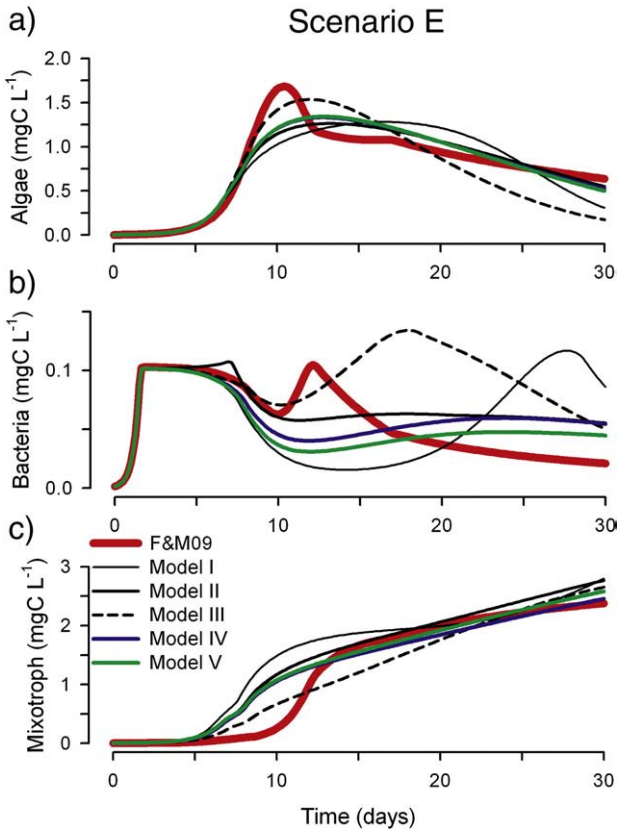


Fig. 10. As for Fig. 2, but for Scenario E, with continual grazing on algae, but with retention and use of kleptochloroplasts, with growth in a shallow water setting. See also Table 2. A colour version of this plot is available online.

the competition for space and resources within the mixotroph (Flynn and Mitra, 2009).

4.2. Can we judge when simple mixotroph models may be acceptable?

Validation of a model is often judged against available laboratory or field data. For example, Friedrichs et al. (2007) conducted a comparison of a range of diverse plankton food web models against field data for both bulk and rate data. There are problems with that approach of assessing model skill (Stow et al., 2009) that apply to laboratory and especially to field data. Problems are primarily based around the presence of errors in the data — some of the data types have greater scope for errors, and most data then require the application of transforms to obtain comparable units to those in the models, adding further scope for error. In addition, the temporal range of data also varies greatly, some being collected with high frequency (e.g., chlorophyll fluorescence), and some rarely (e.g., sedimentation rates). An alternative approach is where one model type is considered as being a "reality", enabling the use of a form of Turing test. This requires that we believe the model "reality". In defence, this is analogous to believing that data series collected in the field or from experiments based on specific species represent typical events. Accepting the concept of using a mechanistic complex model in generation of a data series not only provides data of high temporal resolution which is free of errors associated with laboratory and field sampling but is also free from the need for transforming data with the associated errors. This then provides a platform for exploring various matters that would assist in deriving experiment and sampling design (frequency, and type). In this study, however, we concentrate on the issue of model complexity.

For the application at hand, there are no substantial parametric data for even an initial validation. The model of Flynn and Mitra (2009) as used for the generation of "reality" here was subjected to non-parametric validation using the wealth of information available on the general behaviour of these organisms; the model conforms to expectations. Reasons for the lack of parametric data are explored in Flynn and Mitra (2009), but are primarily due to problems over measurement units (e.g., rates expressed per cell, when cell size varies during the event), the inherent complexity of the organisms, and the interactions with their prey (e.g., pigment allocation between prey, mixotroph and mixotroph food vacuole). Rate data are particularly difficult to obtain, because of the activities of prey within the same water sample (e.g., predation against the background of prey growth; C-fixation by algal prey, the mixotrophic predator and by klepto-chloroplasts; nutrient use and regeneration). In consequence, data from field and/or laboratory experimental studies will typically consist of the nutrient concentrations and population cell numbers or, at most, biomass (i.e., the types of parameters used for tuning the test model types in this study).

However, from the test scenarios here it is evident that the simple models tuned just to bulk data may not necessarily portray the correct system dynamics. For example, in a system where the mixotroph population thrives via ingestion in a period of nutrient depletion (Scenario B; e.g., Caron et al., 1990; Skovgaard, 1996; Adolf et al., 2003, 2006), none of the test Types I–V would be able to portray this phenomenon. Indeed, all the test types show a bias towards carbon fixation over ingestion (Figs. 3, 5, 7, 9, and 11). Knowledge of rate processes appears essential; Friedrichs et al. (2007) reached a similar conclusion in their work on plankton food web models (which, incidentally, ignored the role of mixotrophs). This knowledge is especially required for models which are used to predict HAB events (Mitra and Flynn, 2006b).

Determining key rates in mixotrophs is difficult. If the prey is photosynthetic then only a combined population C-fixation rate would be available. Measuring ingestion rates in microzooplankton is

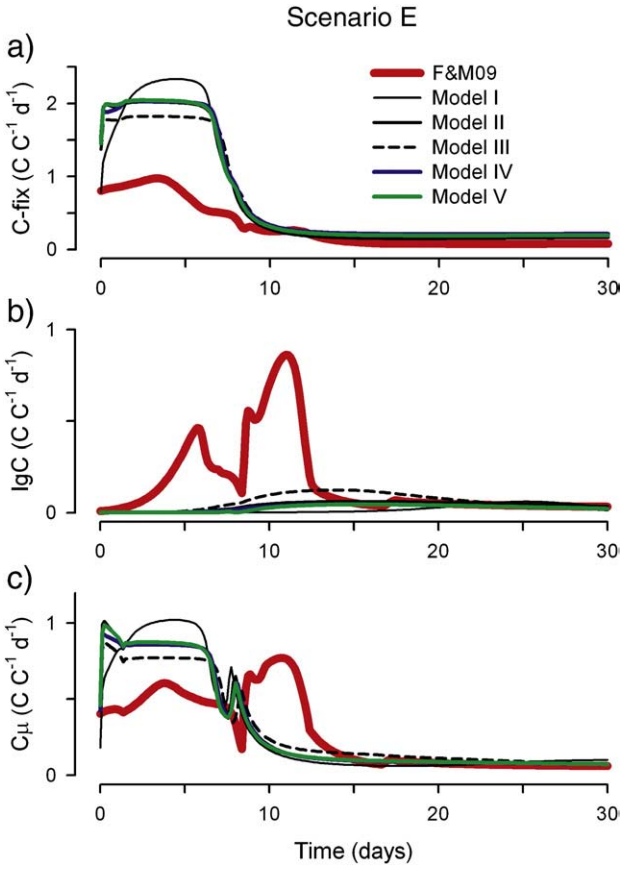


Fig. 11. Model outputs for mixotroph C-fixation (C-fix), ingestion (IgC) and growth rates (Cμ) from the simulations described in Fig. 10. A colour version of this plot is available online.

notoriously difficult but, of the two processes, is more likely to be measured. To test whether the inclusion of rate data during model optimisation could make a difference to our analysis, we have run the tunings with the addition of the data for the mixotroph ingestion rates. The results were the same as those without this inclusion, even when a higher priority was given to fitting the test models to the ingestion rate data (not shown). The basic problem, as mentioned above, is that most of the simple model types are incapable of describing the system dynamics expected, although Type IV does what may be accepted as a good enough job in some scenarios.

The lack of good data for the parameterisation of mixotroph models was highlighted as a major problem by Flynn and Mitra (2009). Even the best experiments in this regard (e.g., Adolf et al., 2003) are inadequate. We need experimental laboratory and field data for rate processes such as ingestion, carbon fixation and nutrient uptake. Until such data become available we only have phenomenological data to test our models against; F&M09 (Flynn and Mitra, 2009) represents a dynamic embodiment of that phenomenological data.

In the absence of adequate data to better validate models, it would appear that at present the placement of simple additive types of mixotroph models within ecosystem scenarios should be avoided. At the least the mixotroph models need to simulate the core activity of these organisms, namely an interactive trade-off between the contrasting physiological processes of phototrophy and heterotrophy.

4.3. Lessons from the study — when two wrongs make a right

In this work we have made the assumption that the behaviour of the F&M09 model accords with reality. Setting aside the question of whether such an assumption is acceptable, there is an important lesson from the work described here.

Let us accept that indeed the output from the control simulation, using F&M09, is real. The fact that model Types III–V do a passable job at matching the bulk behaviour of the "real" data but do so using totally incorrect dynamics is a great cause for concern (Figs. 2–11; Table 4). Indeed, presented only with the output using Type I, which has a structure that is clearly dysfunctional, many would accept the performance of the model as being useful The apparently "good" fits of these simple models to the data is due to the growth rate of the mixotroph being approximately correct even though both the ingestion and C-fixation rates are wrong ($\mu_{max}^{phot}$and μ_{max}^{het}values, Table 4) — these errors thus compensate for each other. We have seen such a situation before where a model apparently fitted the data correctly using incorrect system dynamics; Mitra et al. (2007) demonstrated how two errors in the classic Nutrient–Phytoplankton–Zooplankton (NPZ) model compensated for each other resulting in two wrongs appearing to make a right.

A basic tenet of modelling is to use models that are as simple as possible to describe the process, to answer the question being set, and to validate them against data. Traditionally that judgement has been made against numeric data, by assessing what is now referred to as model skill (Stow et al., 2009). We have argued before (Flynn, 2010; Anderson and Mitra, 2010) that irrespective of the question being set, the functionality of the model structure must be in accordance with the system that it is attempting to mimic. The results here give a demonstration of why that is so. Simple models are usually simple because some sweeping generalisations have been made in their construction; those generalisations have rarely if ever been tested. One is then left wondering about all those models used as drivers of theoretical ecology, many of which are very simple in their structure (not least to aid mathematical tractability). Should we trust them?

5. Conclusions

Mixotrophs are important components of HABs, and of planktonic food webs in general. Their activity warrants appropriate recognition in models. Simply adding "boxes" to depict the two contrasting modes of nutrition within a mixotroph population is inadequate. Mixotrophy is not the sum of the parts but is a complex mechanistic process requiring inclusion of appropriate feedback controls. Of the simple types considered here, that which was most successful was Type IV, which was primarily heterotrophic, using phototrophy to counter shortages in prey. Notably, however, none of the simple types could match the behaviour of a kleptochloroplastic mode of feeding which is an important survival strategy for various mixotrophic species (e.g., *Dinophysis*; Kim et al., 2008).

In defence of simple models, one can argue that such models may match population data adequately. However, such a justification is not a strong enough defence when similar values of state variables can be given with contrasting underpinning rates, a situation made worse as critical rate data are typically missing from the literature. Conforming to phenomenological (non-parametric) data should be just as an important part of determining model skill as fits to parametric data (Stow et al., 2009). Indeed, it should be undertaken first, to remove dysfunctionality (Flynn, 2010). The approach provided in this paper could be utilized to achieve a compromise between the fidelity and complexity of models for their application in describing population and system dynamics.

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