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Review paper

The Challenges of Incorporating Realistic Simulations of Marine Protists in Biogeochemically Based Mathematical Models

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Abstract. Protists are key components of marine microbial communities and hence of the biogeochemical mathematical models that are used to study the interaction between organisms, and the associated cycling of carbon and other nutrients. With increased computing power, models of microbial communities have markedly increased in complexity in the last 20 years, from relatively simple single nutrient currency, nutrient-phytoplankton-zooplankton-detritus (NPZD) models to plankton functional type (PFT) or trait based models of multiple organisms, or individual based models (IBMs) of specific organisms. However, our recognition, if not parameterisation, of the physiological processes that underpin both autotrophic and heterotrophic protist nutrition and growth arguably have increased faster than our modelling capability, generating a wealth of new modelling challenges. This paper therefore reviews historical development, current capability, and the future directions and challenges in protist based mathematical modelling.

Key words: Mathematical modelling, protists, NPZD models, PFT models, trait based models, IBM models.

INTRODUCTION

Mathematical modellers of marine microbial ecosystems seek to produce models that are sufficiently "simple" to be tractable, but still able to give geographically "robust" predictions (Fasham 1993). Models typically incorporate coupled equations that describe the rate of change of the key functional groups and the resultant biogeochemical cycling of carbon and/or other nutrients.

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The open ocean plankton ecosystem model of Fasham *et al*. (1990) was a landmark publication, being an excellent example of so called nutrient-phytoplankton-zooplankton-detritus (NPZD) models (Fig. 1). The model contained a state of the art representation of the nutrient cycling processes of the "microbial loop," and was one of the first robust biological models to be applied within a general circulation model. Protists (single celled eukaryotes) were represented in two functional groups: autotrophic phytoplankton and heterotrophic zooplankton (this second group including both heterotrophic protists and the meso-zooplankton that are characteristic of traditional food chain models). Inorganic nutrients were represented by a single nutrient currency (nitrogen), with production and trophic transfer be-
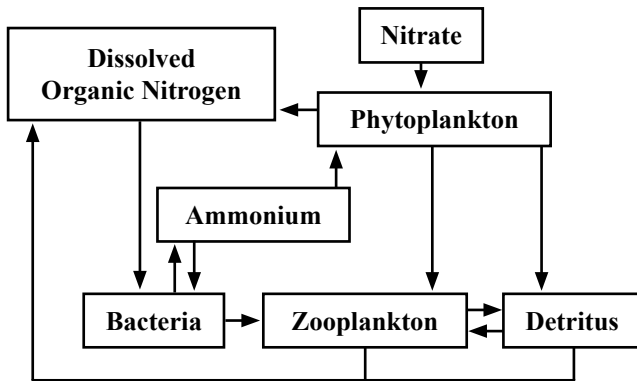
Fig. 1. Representation of the NPZD model of Fasham *et al.* (1990).

ing estimated by assuming a constant carbon:nitrogen (C:N) ratio within organisms' biomass.

In the intervening years since the publication of Fasham's model, considerable steps forward have been made in our conceptual and experimental understanding of the array of interactions exhibited by protistan based communities. Processes such as mixotrophy and alleopathy are now thought to be key components of microbial food webs. Our increasing understanding of microbial diversity also indicates the potential for organisms of the same genus or even species to exhibit different behavioural traits.

Developments in marine microbial food web models have been extensively reviewed (e.g. Davidson 1996, Allen *et al.* 2010). This paper therefore restricts its scope to the developments in "protist modelling" that have occurred in the context of marine biogeochemical modelling since Fasham's seminal paper, discussing the challenges of developing parameterisable mathematical representations of the key properties of autotrophic and heterotrophic protists.

Given the expected continued increase in model complexity, and the recognition that our ability to parameterise models often remains lacking, safeguards are necessary to ensure that modelling the complexity of protist physiology is more than an exercise in fitting multiple free parameters (Anderson 2010). To this end, modellers must ask more searching questions of the skill of their model in terms of rates of change as well as standing stocks. Also, models should not omit key aspects of biological behaviour unless it can be demonstrated that it is safe to do so (Flynn 2005). However, examples from the terrestrial literature (see Allen

et al. 2010) demonstrate that, given good data sets, true physiologically based biological models are achievable. Hence many challenges underpinning advances in marine protist modelling are likely to be, at least partly, experimental. These will require new techniques to study poorly characterised, difficult to isolate, microscopic organisms in a challenging medium.

AUTOTROPHIC PROTISTS

Autotrophic protists are the organisms that underpin marine production, and are the food items for heterotrophic protozooplankton and mesozooplantkon. Their accurate modelling is therefore particularly important as errors introduced at this stage will propagate to higher trophic levels. Given this, it is perhaps surprising that the methods used to model the utilisation of nutrients by, and subsequent growth of, autotrophic protists have remained remarkably enduring over time. Typically these rely on either Monod (Monod 1942), or Quota (Droop 1968) approaches. The former assumes both nutrient uptake and growth are governed by the same rectangular hyperbolic function of the nutrient in the least relative supply (the "limiting" nutrient).

The simplicity of the Monod approach is very appealing for inclusion in large scale biogeochemical models, with examples of general circulation models (GCMs) that use (modified) Monod kinetics for phytoplankton nutrient utilisation including the Hadley Centre Ocean Carbon Cycle model (Palmer and Totterdell 2001). However, the great simplifications of reality inherent in the Monod model lead to issues such as the inability to simulate the temporal decoupling of nutrient uptake and biomass growth/cell division (Davidson *et al.* 1993), or the variability in intra- and extra-cellular nutrient stoichiometry (Geider and La Roche 2002, Davidson *et al.* 2012). This has led some authors, e.g. Flynn (2010), to argue that the use of Monod models for protist simulations is now "unacceptable."

Quota models simulate nutrient uptake in a similar manner to Monod kinetics as a function of external nutrient concentration, potentially modulated by temperature and light (see Flynn 2003). However, growth is made a function of the (variable) intracellular ratio of limiting nutrient to biomass (the cell quota). Models (of autotrophs and heterotrophs) based on this general Quota principal are therefore to be encouraged on grounds of biological realism, but also because the role of vari-

able nutrient stoichiometry in governing trophic transfer is increasingly recognised (Sterner and Elser 2002). However, caution must still be applied when simulating limiting nutrients other than nitrogen or phosphorous. For example the basic Quota model structure is not appropriate to describe silicon (Si) limited growth, as Si cannot be redistributed within the cell to support growth (Flynn 2008). Moreover, proper description of the trophic transfer of nutrients requires the simulation of the utilisation of non-limiting nutrients (Davidson and Gurney 1999; Flynn 2001, 2008).

PHYSIOLOGICAL MODELS

While a mechanistic derivation of the Quota Model has recently been published (Pahlow and Oschlies 2013), the models described above were empirically generated, with the functions governing processes such as nutrient uptake, growth rate and grazing rate being fixed no matter the physiological changes in an organism that may occur in response to its environment. To address this clear lack of biological realism some authors have attempted to relate model parameters to abiotic conditions and/or to develop physiologically based "mechanistic" models that allow greater stoichiometric flexibility.

Examples include the photo-acclimation models of Geider *et al*. (1998) and Ross and Geider (2009) that includes C, N and chlorophyll dynamics, and the model of Flynn (2001), that combined a number of previous formulations to generate a "mechanistic" model of phytoplankton growth incorporating the interaction of five nutrients (ammonium, nitrate, silicon, phosphorous and iron) with temperature and light (Fig. 2). Such models are markedly more complex than "simple" Quota based approaches described above, and hence generate issues related to parameterisation and verification. Flynn (2003) suggested that, given the paucity of test data sets, the output of (correctly parameterised) complex models can be used to guide the construction and performance of simpler models that can then be more widely used.

HETEROTROPHIC PROTISTS

The majority of protist based model development has been directed at autotrophs. Yet the trophic trans-
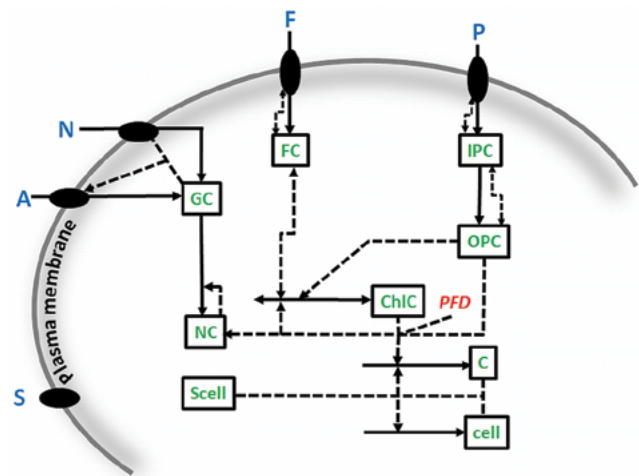
Fig. 2. The mechanistic phytoplankton model of Flynn (2001) that represents multi nutrient uptake and utilisation of N – nitrate; A – ammonium; F – bioavailable iron; P – phosphate; S – silicate; and the interaction with light (PFD). Major flows in and out of state variables (boxes) are depicted by solid arrows, with the major feedback processes depicted by dashed arrows. C – carbon biomass; Cell – cell density; NC – N C-quota; ChlC – chlorophyll C-quota, FC – iron C-quota; IPC – inorganic P C-quota, OPC – organic P C-quota; Scell – silicon cell-quota (reproduced with permission).

fer of primary productivity is clearly important to biogeochemical cycling. Functional and numerical response relationships govern the ingestion of protist and prokaryote prey items by heterotrophs and are therefore central to population models with more than one trophic level. Typically, rectangular hyperbolic or "type II Holling" responses are used to characterize the feeding behaviour of heterotrophic protists. However, even for a single heterotrophic protist (the marine dinoflagellate *Oxyrrhis marina*) a range of different functional response relationships have been found to exist (Davidson *et al*. 2011, Yang *et al*. 2012).

Factors such as temperature (Kimmance *et al*. 2006, Montagnes *et al*. 2008b), turbulence (Peters and Gross 1994), or multiple prey availability (Flynn *et al*. 1996) have been shown to fundamentally modify *in situ* and modelled grazing behaviour. Hence many of the challenges of protist modelling relate to heterotrophs with a clear need for a more mechanistic approach to their simulation. Issues raised by this, and potential solutions to these problems, are discussed in the following sections.

SELECTIVE GRAZING

Active discrimination for or against different prey items may occur, impacting on the functional and numerical response relationships introduced above. Lack of experimental methods to study this process mechanistically make selectivity a particularly difficult process to model. Attempts to include selectivity in models include the study of Strom (1993) who used a model to show discrimination between live and dead prey, Flynn *et al*. (1996) who demonstrated the selection of alternative live prey species, and Mitra *et al*. (2003) who evaluate the theoretical implications for model predictions of processes such as "surge feeding." Recent studies include that of Mariani *et al*. (2013) who allow grazers to switch between either of two feeding modes: ambush feeding on motile cells or current-feeding on non-motile cells. While their work was directed towards grazing by meso- rather than micro-zooplankton, it has clear potential for adaptation to protist grazers.

Montagnes *et al*. (2008b) characterised the mechanistic processes that govern prey selectivity by heterotrophic protists. These are: searching, contact, capture, processing, ingestion and digestion (Fig. 3). An operational model of selective grazing based on these six rate processes would contain a very large number of parameters, with an associated requirement for considerable experimental advances to allow their parameterisation.

Given the perceived importance of prey selection by heterotrophic protists, a pressing challenge is therefore to evaluate the relative importance of the different processes detailed above to assess which are most critical to model performance, and hence to guide future experimental efforts.

ASSIMILATION AND GROWTH EFFICIENCIES

In biogeochemical models trophic transfer of carbon and other nutrients is often assumed to be governed by constant assimilation efficiency. However variable assimilation efficiency, as a function of prey density, and its potential influence on trophic transfer to both heterotrophic protists and meso-zooplankton has been demonstrated. For example Fenton *et al*. (2010) and Montagnes and Fenton (2012) show assimilation efficiency to decrease with increased prey concentration, and Minter *et al*. (2011) demonstrated that (prey dependent) predator mortality can fundamentally alter microbial model response.

Other work on this topic includes that of Mitra (2005) who modelled how the stoichiometric differences (in terms of C:N:P composition) between autotrophic and heterotrophic protists influence growth efficiency of the latter group. Much of the modelling work conducted in this area, is theoretical in nature. There is therefore a clear need for innovative experiments that allow the quantification of the extent of variability and the factors governing variable growth and trophic transfer efficiencies.

MIXOTROPHY

Mixotrophs are capable of combining both phototrophic and heterotrophic modes of nutrition. Heterotrophy includes uptake of dissolved organic substrates (osmatrophy), and/or phagotrophic feeding on particulate
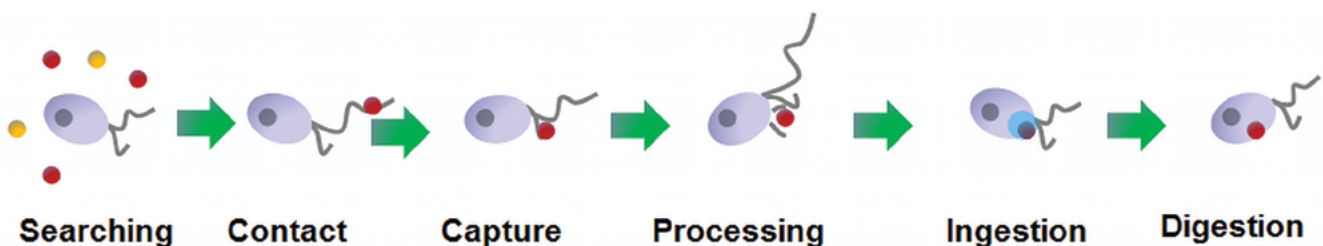
Fig. 3. The six stages of selective grazing, redrawn from Montagnes *et al*. (2008b).

material. While mixotrophy has been relatively long recognised (Jones 1997, Stoecker 1998), it is frequently ignored with protists typically characterised as either "phytoplankton" or "micro-zooplankton" (Flynn *et al.* 2012). Unsurprisingly, given the lack of experimental study of mixotrophy, it is relatively rarely modelled. However, those studies that have been done so have confirmed its importance to microbial communities.

Theoretically based models that do include a representation of mixotrophy suggest it may govern food web stability and short term dynamics (Hammer *et al.* 2001). More complex models such as that of Flynn and Mitra (2009) and Ward *et al.* (2011) consider the interactions between photosynthesis, inorganic nutrient acquisition, the prey consumption, and the potential for switching between different modes of nutrition. Such mechanistic models are currently too complex to parameterise for routine operational use. However, given more tractable based formulations are currently unable to recreate the predictions of the more complex models with, in particular, the process of kleptochloroplasty being difficult to incorporate (Mitra and Flynn 2010), it is clear that substantial model development is required in this sphere.

PLANKTON FUNCTIONAL TYPE (PFT) MODELS

Realistic model predictions of processes that are governed by trophic transfer, such as marine biogeochemical nutrient cycling, require a clear and succinct mathematical description of the biology of, and interactions between, autotrophic and heterotrophic protists and other organisms that make up the marine microbial food web. This cannot be achieved when the myriad of protists are aggregated into "P" and "Z" compartments with fixed stoichiometric ratios between their carbon and nitrogen content.

Increased predictive power can potentially be achieved by elaboration of the basic NPZD structure. For example a 3N–2P–2Z–2D structure was used by Aumont (2003) as the biological component of a global marine ecosystem model. However, the increase in modern computing power has allowed the development of Plankton Functional Type (PFT) modelling approaches. These have allowed a dramatic increase in the number of functional groups modelled and the level of physiology that is included in their simulation (Litchman *et al.* 2006).

A clear representation of the PFT approach is encapsulated within PlankTOM a ten PFT model (Le Quere *et al.* 2005), Table 1. While PFT models include the fundamental distinction, for example, between diatoms and other phytoplankton, PlankTOM and other similar models also group some organisms by their biogeochemical role (e.g. calcifiers, N_2 fixers, DMS producers) allowing a much greater degree of resolution of organism "function" to be incorporated. However, as discussed by Hood *et al.* (2006) there is no overarching theory of marine ecology to guide this approach, and hence such categories cut across the traditional taxonomic (or indeed modern molecular) classification of organisms. This raises questions of whether the aggregation of potentially very different organisms that have one (but perhaps only one) similar function is appropriate, and how appropriate model parameterisation can be achieved (Hoffman 2010). Moreover, the size range within the single functional group of "phytoplankton silicifiers" in the PlankTOM model still encompasses a full order of magnitude, from 20–200 μm. This demonstrates the continued need to aggregate very different organisms as a single entity in even the most highly resolved of current models.

Table 1. The ten functional groups of the PlankTOM model (Le Quere *et al.* 2005) with their size ranges. The six protist functional groups are shown in bold.

Size class	PFT name	Cell size (μm)
Pico		
	Pico-heterotrophic bacteria	0.3–1.0
	Pico-autotrophs	0.7–2.0
	Phytoplankton N_2 fixers	0.5–2.0
Nano		
	Phytoplankton calcifiers	5–10
	Phytoplankton dimethyl sulphide producers	5
	Mixed-phytoplankton	2–200
Micro		
	Phytoplankton silicifiers	20–200
	Proto-zooplankton	5–200
Meso		
	Meso-zooplankton	200–2000
Macro		
	Macro-zooplankton	> 2000

PFT models remain in their relative infancy, and their predictions must therefore be treated with caution. Hood *et al*. (2006) noted that continuing to add complexity and more functional groups to models may even lower predictive ability if models are not sufficient constrained by data. The paucity of validation data for many key groups has led to the practice of parameterising and testing models based on a small subset of organisms about which we know the most. However, these organisms are not necessarily of biogeochemical importance, or representative of those that are. Rather these are species that are accessible to study due to their relative ease of culturing, or monitoring by remote sensing. However, when sufficient data is available, the potential of PFT models is evident, as demonstrated by Friedrichs *et al*. (2007) who, using an adjoint method of data assimilation, compared the predictions of 12 lower trophic level biogeochemical models in an identical physical framework. They found that, in a single pelagic regime, the simplest models fitted the data as well as the more complex multi-functional group formulations. However, those models with multiple protist functional groups produced better simulations of different regimes when identical parameter sets were used.

TRAIT BASED MODELS

The ever increasing number of functional groups included within PFT models, and the difficulty of assigning organisms to these groups, has led some authors to suggest alternative approaches to protist modelling that are capable of capping the potential explosion of model complexity. An example of this is the use of "traits" of organisms to allow their logical combination in groups within models.

In their review of trait based community ecology Litchman and Klausmeier (2008) suggest that as phytoplankton (and other protists) possess a manageable number of well-defined traits these can potentially be used to guide the construction of tractable mechanistic mathematical models capable of deriving community composition on the basis of environmental gradients.

An example of the trait based approach is the work of Bruggeman and Kooijman (2007) who use an "omnipotent population model" to simulate all species within an ecosystem with interspecific differences being defined by differences in values of key parameters (traits) rather than differences in model structure. A second example

of this approach is that of Ward *et al*. (2012) who model 25 size classes of phytoplankton and 30 size classes of zooplankton, but distribute these within a restricted number of functional groups. The number of model parameters are minimised by using published power-law functions that link cell size to physiological traits such as nutrient uptake, cell quota, growth, mortality, sinking and grazing.

MODELS OF INDIVIDUAL PROTISTS

In some applications there is a requirement to simulate the dynamics of a particular genus or species. One approach is to incorporate the key organism within an expanded NPZD or PFT model as a separate category. Vanhoutte-Brunier *et al*. (2008) used this approach to model the harmful dinoflagellate *Karenia mikimotoi* in the western English Channel. In this study, hydrodynamic and sediment models were coupled with a NPZD based biological model to which a specific representation of *K. mikimotoi* was added as a separate functional group. This approach was possible due to the high biomass of *K. mikimotoi*, and is likely to be less appropriate for species that do not produce such large blooms.

An alternative, individual based model (IBM) approach simulates only the organism of interest in detail. A representation of the rest of the food web is not included although important loss processes such as grazing are parameterised. Such models are of particular use for the study of advective populations and/or species that form only a small fraction of the biomass of a trophic level but are important for other reasons. Examples of organism conducive to IBM modelling include a number of important biotoxin producing phytoplankton such as the advective mixotrophic genus *Dinophysis* (Raine *et al*. 2010), or the low biomass high-toxicity dinoflagellate *Alexandrium tamarense* (Touzet *et al*. 2010) fit these criteria.

An example of a protist based IBM relates to outbreaks of paralytic shellfish poisoning (PSP) caused by the dinoflagellate *Alexandrium fundyense* in the Northeastern USA. *A. fundyense* blooms are transported by plume advection and wind driven forcing. These competing mechanisms make prediction of the timing and magnitude of a bloom difficult to determine observationally. A coupled physical/biological IBM based model has therefore been developed to predict the transport of *A. fundyense* with functions to represent

germination, swimming, growth and mortality being included in the biological model. The model has demonstrated good skill at reproducing observations (Stock *et al*. 2005). However, initialisation of the model requires a detailed knowledge of the distribution of *A. fundyense* cysts in the region, prior to the growth season. This information is gathered annually by ship based cyst surveys, with obvious resource implications.

IBM models are clearly not applicable to all modelling scenarios. However, for specific organisms they may be a pragmatic choice. The major challenge with such a modelling approach is correctly parameterising the interaction of the organism of interest with the rest of the food web. This is particularly problematic for low biomass organisms, the abundance of which may be significantly influenced by competition with other organisms for nutrients and/or prey items and (possibly multiple) grazing pressures.

DISCUSSION

In 1990, Fasham's model provided a great step forward providing a mathematical synthesis of our knowledge of protist community interactions in a concise mathematical representation. However, it was clear that the model contained many broad brush assumptions. While modelling sophistication has increased markedly in the subsequent two decades our appreciation of the complexity of protist biology and physiology has perhaps increased even more, and one may argue that the gulf between the two is widening rather than closing.

Mechanistic/physiological, PFT, IBM and trait based models seek to bridge this gap. Anderson (2005) highlighted many issues related to the development of PFT models, including poorly understood ecology, lack of data, the difficulties of aggregating diversity within functional groups into meaningful model state variables and constants, and the sensitivity of model output to the particular parameterisations used and the physical and chemical environment simulated. These issues apply equally to the other modelling approaches discussed here and are of particular relevance to heterotrophic protists and our ability to model them given the difficulty in conducting experiments to determine key rate processes.

A particular concern is the process of "fitting" a marine ecosystem model to a data set. This typically involves setting the parameters that one believes to be correct, either from experiment or from literature. Free (unknown) parameters are then varied until an acceptable "fit" of model to data is achieved, sometimes on the basis of minimising an error function that evaluates the deviation of model and experiment. While such an approach is logical, it also lacks much of the rigour and statistical evaluation that scientists apply to other areas of work. Indeed, when a good "fit" is achieved the modelling process typically ceases. A popular saying of uncertain provenance applied to many different disciplines is "An amateur practices until he/she gets its right. A professional practices until he/she can't get it wrong". By this definition are the modelling efforts of the marine science community more amateur than professional? Franks (2009) discusses this issue and coins the term "a failure to fail" arguing that we must be much more critical of the shortcomings within our models.

Fortunately more data are becoming available for model testing, e.g. Leblanc *et al*. (2012), but a continuing issue is the lack of criteria to evaluate model performance. Franks (2009) uses the study of Friedrichs *et al*. (2007) that was discussed above to present an interesting critique of the methods modellers use to evaluate their models, arguing that not all data are equally powerful for testing and constraining a model. As an example he cites chlorophyll, simulation of which is frequently used to assess field based model performance. He argues that multiple different models can successfully predict the annual cycle of this parameter, as long as the modelled primary productivity and grazing exhibit an appropriate ratio, but crucially correct simulation of chlorophyll can be achieved without the value of either governing parameter having to be correct. Hence, a much stronger test of models would be their ability to simulate multiple rates of change, be their growth, grazing or other rate parameters. Similar arguments were made by Flynn (2005). Adopting such criteria would markedly increase confidence in our ability to predict as well as hindcast, and is therefore to be encouraged. However, progress in this area requires experimentalists to make difficult rate process measurements much more routinely in the laboratory and the field, and modellers to be willing to ask much more difficult questions of their models' skill.

It is easy to be critical of marine ecosystem models. However, they have achieved many clear advances and successes. Models demonstrate much greater skill that at the time of Fasham's 1990 paper. GCMs provide satisfactory simulations of global chlorophyll. Protist

based models such as the ACExR-LESV model of Tett *et al.* (2011), that evaluates sea loch carrying capacity, have been successfully applied to support the sustainable development of coastal aquaculture. The *A. fundyense* IBM discussed above is used operationally and helps to safeguard health and economic wellbeing. However, developments in protist modelling methodology are required to further advance the field and generating better simulations of ocean biogeochemistry and global climate change. Moreover, many important processes such as competition and alleopathic interaction, cell death and evolution are rarely studied experimentally and are hence modelled (if at all) in a superficial manner. Their inclusion is far from a trivial issue, but one that requires addressing to ensure the wide applicability of protist based models.

The experimental techniques that we currently possess to study protists also require model friendly application. Chlorophyll is typically used to estimate phytoplankton biomass, yet correlation of chlorophyll with carbon or nitrogen content of cells is often tenuous (Davidson *et al.* 1991). Few laboratory studies are designed with subsequent model parameterisation or testing in mind, and even those that are, are restricted to those (possibly unrepresentative) species that are currently amenable to culturing. Mesocosms are an under-utilised resource that may aid protist model development and testing. These large scale enclosures allow some degree of decoupling of the ecosystem from the complicating (but better understood) physical oceanographic processes that prevent multiple trophic levels being easily studied over extended periods of time in the open ocean (Davidson *et al.* 2007).

Clearly development/parameterisation of mechanistic models of protists is required. For example Allen *et al.* (2010) discuss physiologically based descriptions of organismal responses to changing light levels, respiration and nutrient limitation. To make these advances it will be necessary to accept that quantities that we previously assumed to be constant in models are clearly not; for example prey assimilation by heterotrophic protists. Recognition that many of the empirically derived functional relationships (for example functional responses) are inadequate is also required. A major challenge will therefore be achieving sufficient physiological detail without generating unmanageable complexity. In their review of modelling practices Allen and Polimene (2011) suggest this requires generic models of cells that capture the essence of key physiological activities; similar to the trait based approach discussed above. Differ-

ent functional types then express different components of these key activities. Advances made in relation to the modelling of meso-zooplankton may be of benefit here, but the clear differences between single celled protists and multi-cellular animals require a cautious approach to be taken.

With the many areas of uncertainly in protist biology that exist, there are many avenues for model development. However, given that mixotrophy may extend to a wide range of protists, outwith of the dinoflagellates in which it was first recognised, perhaps most crucial is the question of how we assign protists to functional groups in PFT (or other) models, and hence how we parameterise cooperatively co-occurring autotrophic and (potentially selective) heterotrophic rate processes within the same organism. The application of developing techniques such as lectin labelling on cell surfaces, flow cytometric separation of prey and predators, the analysis of stable isotope signatures, and stable isotope probing (see Montagnes *et al.* 2008a), will hopefully provide the data sets from which to progress these key areas.

Finally, the arguments presented above are summarised in a set of recommendations:

Modellers should:

- Test models' ability to simulate both standing stocks and the major rate processes that generate these stocks (growth, grazing etc.);
- Quantitatively evaluate model fits to data, and be prepared for models to fail;
- Test models on multiple data sets;
- Not omit key areas of physiology because they are "inconvenient" or difficult to parameterise;
- Use models to set and test hypotheses;
- Periodically re-evaluate basic modelling equations or principals;
- Talk to experimentalists before/while formulating their equations!

Experimentalists should:

- In the field: measure a wider suite of state variables and physiological rate parameters;
- In the laboratory: culture and study a wider suite of environmentally relevant protists;
- Publish full raw data sets;
- Address unknowns that are key to model building and parameterisation – mixotrophy, cell death, alleopathy etc.;
- Include modellers in their experimental design process!

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