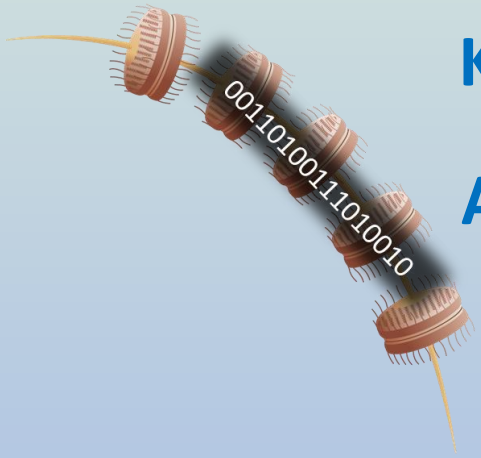
DRAMA – a cybernetic approach for Plankton Digital Twins



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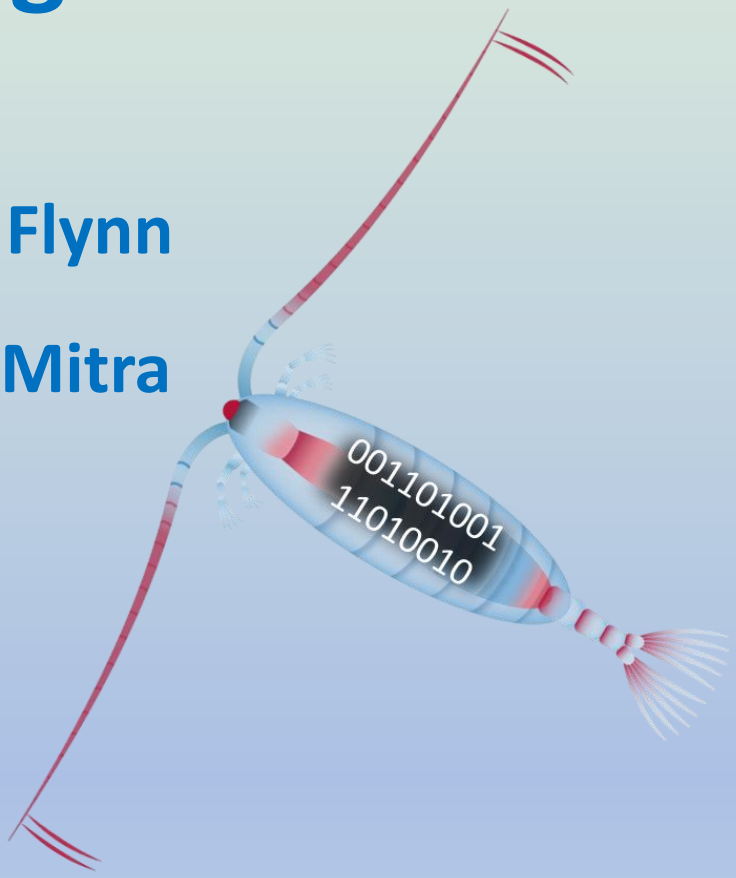

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Executive Summary

A flexible platform for the description of plankton functional types is described that uses a combination of systems biology concepts enacted through the system dynamics modelling of cybernetic (feedback) interactions. Sigmoidal-shaped (allosteric-like) response curves are used to modulate multi-stress interactions controlling growth supported by consumption of different resources. The conceptual framework, *dynamic resource acquisition modulated activity* (DRAMA), provides for a homeostatic description of physiology and behaviour in which cascades of (de)repression processes related to resource availability and satiation are used to control activity and growth of the organism being modelled. Other aspects of behaviour can be similarly controlled. The approach is particularly appropriate for plankton digital twin applications, which demand models that can be configured to align with activities of different species and groups for considerations of ecology with biodiversity. This is because of the ease with which a single structure can be modified and developed to better conform with subtleties between real organisms thus enhancing the ability to simulate the ecophysiology of members of each different group.

Contents

0. Glossary

Term	Explanation
Allosteric	A form of modulation of enzyme activity through modification of an effector at a site other than the active site. The resultant response-curve between substrate concentration and enzyme-catalysed reaction rate is typically sigmoidal, with the modulation changing the curve form between being more (if the effector is a promoter) or less (if the effector is an inhibitor) like a rectangular hyperbola.
Anabolic	Cellular activities, especially respiration, associated with building new biomass. See *SDA*. Cf. *catabolism*.
Assimilation efficiency (AE)	Transfer of food biomass into the metabolism of the consumer. The fraction defined by (1-AE) is voided. Additional losses occur during incorporation of the transferred biomass, notably via *SDA*.
Catabolism	Cellular activities, especially respiration, associated with the generation of energy with the destruction of biomass. See *SDA*. Cf. *anabolism*.
Chemostat	A culture system in which an inflow (dilution) rate of fresh nutrients is matched by an equal outflow of residual (unused) nutrients and organisms. At steady-state, the dilution rate matches the growth rate of the organisms, and the concentration of the limiting nutrient when the dilution rate equals half the maximum growth rate is the half-saturation constant for growth.
CM	Constitutive Mixoplankton; protists that can combine osmotrophy, phagotrophy and innate phototrophy. Cf. *GNCM*, *pSNCM*.
Cybernetics	Communications and automatic control systems in both machines and living things, exploiting circular feedbacks and causality links. Cf. *system dynamics*
Decision Support Tool (DST)	An approach, often supported by outputs from a computer programme, to optimise a management or other operational strategy. DSTs may be as simple as a look-up table or decision tree, or as complex as a *digital twin* enabling near-real-time analysis of options.
Density Dependant Inefficiency	A consequence of a consumer feeding when prey are available at high abundance. Prey are poorly converted into predator biomass (*assimilation efficiency* is low), with a dampening effect on predator-prey oscillations and an increased flow of material to detritus.
(de)repression	A biological control mechanism, often involving *allosteric*-like feedbacks, through which physiological (biochemical, behavioural) processes are modulated by being gradually enabled (de-repressed) or disabled (repressed).
Digital Twin (DT)	An in silico representation of reality which may range from being static (e.g., geological structure) through to highly dynamic (aircraft in flight). Dynamic versions are often associated explicitly or implicitly with a *Decision Support Tool* to guide an intervention. DTs may be differentiated from a *simulation model* by the presence of an interface to enable a non-specialist to interact with the model and to explore features beyond the data originally used in model *validation* in a predictive or 'what-if?' mode.
DRAMA	Dynamic Resource Assimilation Modulated Activity model

Dunning-Kruger effect	Cognitive bias due to an inability to recognize a lack of ability.
Dysfunctional	Applied to models, as per Flynn (2003), to describe a model with behaviour that is contrary to empirical expectations.
Empirical	Based on observation (e.g., field, laboratory) rather than through theory or logic. Empirical data may be subject to subsequent transformations or manipulations based upon theory or logic, but the data are not synthetic as generated by a *model*.
End-product inhibition	Negative feedback inhibition, especially related to the control of a series of enzymatic processes leading to synthesis of a product separated from the source materials by many steps. Often that control will involve an *allosteric* feedback.
Expert Witness Validation (EWV)	A means to validate the performance of a numeric model by exploiting a form of *Turing test*, asking of experts in the real world system 'does this model output so-closely resemble reality that you could be fooled by it?' EWV minimises the risks of *over-fitting* by explicitly considering the full breadth of phenomenological understanding.
GNCM	Generalist Non-Constitutive Mixoplankton; protists that can combine osmotrophy, phagotrophy and phototrophy that is acquired from several different prey types. Cf. *CM*, *pSNCM*.
Grain	A subjective level of modelling detail; fine grain for *Systems Biology* may describe individual biochemical processes, while coarse grain may refer to features such as elemental stoichiometry.
Model	A simplification of reality, ranging from static to dynamic physical structures, mathematical constructs from statistical fits to *numeric models* that may be hypothetical, through to *simulations* and *Digital Twins*. Data generated by a mathematical model are synthetic rather than *empirical*.
Modulation	Exertion of a modifying or controlling influence on a process. (see *allosteric*)
Numeric model	A mathematical model that uses a complex series of equations, often involving differential calculus to described events changing over time as used in *simulations*.
'omics	Molecular biological suffix (e.g., gen*omics*, transcript*omics*, prote*omics*, metabol*omics*) of terms for biological molecules that characterize organismal structures and functions.
Osmotrophy	A means of feeding through which dissolved organic matter (such as sugars, amino acids) are taken into an organism, such as a plankton microbe, to support growth by supplying C, energy and/or other nutrients. Cf. *phagotrophy*.
Over-fitting	A consequence of fitting or *tuning* a model (especially a statistical model) that, because it describes a particular data set too closely, is then unable to well describe the general case.
Phagotrophy	A means of feeding through which particulate matter (food) enters a unicellular organism, such as a protist plankton. Cf. *osmotrophy*.
Phototrophy	A means to acquire C and energy via (light-enabled) photosynthesis. Usually associated with the use of N, P and other nutrients supplied in inorganic forms. Cf. *osmotrophy*, *phagotrophy*.

Phytoplankton	Microbial plankton (cyanobacteria or protist) that can combine *osmotrophy* and *phototrophy*. They are incapable of *phagotrophy*.
Protozooplankton	Protist plankton that can combine *osmotrophy* and *phagotrophy*. They are incapable of *phototrophy*.
pSNCM	plastidic Specialist Non-Constitutive Mixoplankton; protists that can combine osmotrophy, phagotrophy and phototrophy that is acquired from a specific prey species. Cf. *CM*, *GNCM*.
Resource Allocation Model (RAM)	An approach, stemming from finance models, which seeks to balance out model behaviour with respect to the costs of allocating resources towards different activities versus the gain from doing so. See also *Trait Trade-Off*.
Satiation	State of fullness. A replete state (e.g. with respect to food) is fully satiated.
SDA	Specific Dynamic Action; loss of a proportion (typically ca. 30%) of biomass that is assimilated (see *AE*) as a consequence of *catabolic* and *anabolic* respirations. (McCue 2006)
Simulation (model)	A *numeric model* whose outputs provide an imitation of a real world process at least over the conditions under which it was *validated*. (Cf. *Digital Twin*, *validation*)
Systems biology	Computational analysis and modelling of complex biological interactions using a holistic approach (e.g., with explicit inclusion of *(de)repression* feedbacks), rather than the traditional reductionist approach of modelling in which simplification is a dominant feature. Cf. *cybernetics*.
System dynamics	A modelling approach characterised by feed-back, feed-forward interactions developed for simulating especially complex numeric data-poor situations.
Trait Trade-Off (TTO)	A concept, originating from finance models, which seeks to balance out model behaviour with respect to contrasting behaviour traits. Biologically, TTO's can only be considered within an organism (actually reflecting expression of *(de)repression* controls), or from an evolutionary viewpoint between closely related organisms evolving within the same ecosystem. See also *Resource Allocation Model*.
Tuning	Optimising the fit of (minimising differences between) model output and external (usually real world) data series. Cf. *validation*.
Turing test	A test of a machine's (computer programme's) ability to exhibit a level of intelligent behaviour similar to, and thus indistinguishable from, that of a human (Turing 1950). Cf. *Expert Witness Validation*.
Validation	A means of determining the veracity of a model output against external (usually *empirical* numeric) data series independent from those data used for *tuning* or optimisation. Often there are very few data available once those used for tuning have been excluded. See *Expert Witness Validation*.

1. Introduction

This work describes the conceptual basis of a modelling structure to simulate the growth and activities of plankton: ***D**ynamic **R**esource **A**ssimilation **M**odulated **A**ctivity* (DRAMA). The conceptual base of DRAMA is that the physiology and behaviour of real organisms is strongly affected by their current physiological status. For example, an organism that is well fed behaves in a different way and grows faster than one that is less well fed, or is starving. In essence, an organism functions to maximise growth and reproduction through acting to maintain and optimise its homeostasis.

The document provides some example model outputs, but specific applications of DRAMA, with their allied explanations and equations (model code), will be published separately.

The history of this work lays in a series of models developed from Flynn et al. (1997), Flynn (2001), and Flynn & Mitra (2009) which have been deployed in various studies (e.g., John & Flynn 2001, Fasham et al. 2006; Mitra & Flynn 2010; Flynn et al. 2012; Mitra et al. 2014, 2016). Developments of the *Perfect Beast* model of Flynn & Mitra (2009), targeted at describing mixoplankton (protist plankton that engage in phototrophy, osmotrophy and phagotroph - Flynn et al. 2019), such as in Leles et al. (2018, 2021) revealed the potential and also the need for developing a revised plankton model that was at once computationally more efficient, but also a model that could provide an improved description of reality. Initial attempts lead to a model that was deployed for describing commercial algal production in bioreactors (SAPPM; Flynn 2021), and was also implemented for describing protist plankton (Schneider et al. 2021). A radical overhaul of the conceptual and mathematical basis, resulted in DRAMA. The conceptual core of DRAMA has potential to provide a common platform for describing all plankton functional types; bacteria through to mesozooplankton, as well as protist plankton including mixoplankton.

DRAMA is based on concepts from medium/coarse -grain systems biology, and thus lends itself for the development of digital twins of plankton which demands as close a match in behaviour with real organisms as possible while minimising computational effort.

1.1 Plankton Digital Twins

A digital twin (DT) is an *in silico* representation of reality. Applications of DTs include education (e-laboratory), training (e.g. flight simulator, hospital surgery, microbiology growth dynamics), design/testing (engineering, especially when linked to Computed Aided Design, design of a biological studies), real-time operation optimization (e.g., business decision support tool, stock market investment, commercial microalgal cultivation). Many instances replicated as DTs exhibit features of complex adaptive systems, where the emergent behaviour may be counter intuitive and almost certainly non-linear.

Invariably, DTs provide a form of Decision Support Tool (DST) through which the end-user can explore ‘what-if?’ scenarios. The end-user requires an appropriate interface and guidance to use the DT effectively; very often the user must at least be a well-informed novice to use the platform correctly. With that usage, however, there is also a likelihood that empiricists will discover facets of model behaviour that they consider do not live up to the DT ideal. Building a DT thus places

additional demands upon the developer of the model, to ensure that the resultant model output does indeed represent a simulation, in the true meaning of that word.

Applications of DTs, and indeed defining exactly what a DT is in the context of ecological sciences, is a developing arena (Blair 2021). Traditional models of plankton have been simple, if not extremely simple; an empiricist would likely have little difficulty locating aspects of these models that do not conform to a DT standard. Having a flexible core of the model, so that developments and modification can be readily made, is important. DRAMA aims to provide such a core.

The simplest applications of plankton dynamic DTs would be to describe a single microbe clone growing in a flask, to replicate a classic laboratory setup in a digital laboratory. From that, repeated for several species, simple food-webs could be constructed and used to study aspects of theoretical ecology. Placed in ecosystem simulators, plankton digital twins could be used in place of the extant crude descriptions but, because of their providence in development in collaboration with experts in real world systems, these simulations should engender an enhanced level of performance and reliability as true simulators. If the outputs were not reliable, then that would point to errors hitherto undiscovered in the plankton DT, or in the ecological simulator itself. Either way, science is enhanced by using subcomponents (the plankton DTs) which have themselves been rigorously configured and tested. DRAMA provides a framework that facilitates such developments.

1.2 Feedback as a controlling feature of organism trait-expression ('behaviour')

Key traits of living organisms, as taught to students at school, include:

i. They are built on a modular structure comprised of cells.
ii. They have to acquire resources and energy.
iii. They acclimate to the environment to optimize (ii) and also to maintain or optimise homeostasis.
iv. In consequence of (ii) and (iii), they grow (with inevitable impacts on allometry and with (i)) and they reproduce.
v. They adapt (evolve) to environmental change as a long-term selective process linked to (iv).

Of these, (ii) and (iii) and thence (iv) are key for defining the dynamics of life. An important aspect underpinning these features for consideration in DT models is what, in common parlance, is termed 'behaviour' but what is more properly trait expression.

Real organisms react to changes in input variables by altering their behaviour, through processes of (de)repression. These events gradually modulate behaviour by switching processes on and off as a measured response to external and internal stimuli. Such physiological traits have evolved to maintain an optimal homeostatic condition, so maximising growth potential in a given environmental setting. A key common trait here is the modulation of, and of processes by, satiation; satiation defines how close a given process is to being capable of running at its optimum rate. That state may be related to the acquisition of a specific resource, a group of resources, all the way through to growth and reproduction of the whole organism. In biochemical or physiological terms,

satiation elicits a response to down-regulate (repress), or up-regulate (de-repress), different processes.

As an example, the ability or need to exploit increasingly less favoured resources is increased as an organism is stressed (starved) of more optimal 'preferred' resources. Such responses may also incur additional costs, perhaps with an increased risk of mortality, altered motility, with modified life cycle events, and changed allometric and stoichiometric features.

Exploiting this key trait of feedback modulated by satiation in models provides a biologically meaningful, intuitive and robust means to describe and control physiological processes that collectively govern organism activities (behaviour) and growth. Furthermore, where the modulation of satiation incurs choice selections, there may be demonstrable trait-trade-offs (e.g., motility improves the chances of locating food but risks encountering a predator vs staying cryptic and thence balances the risks of starvation versus the risk of being eaten).

The degree to which different organisms operate and exploit feedbacks to environmental and internal stressors provides a whole spectrum of behaviours all centred around a limited number of themes. For example, satiation with respect to N-nutrition (a topic that will be expanded upon in **Section 2.4**) differentially controls the uptake into phytoplankton of amino acids, ammonium, nitrate etc., while being also affected by satiation with respect to P-nutrition and also to C-nutrition. The features required to exploit this approach in modelling, an approach that aligns with how physiology actually functions, are:

i) A means to quantify 'satiation' for appropriate aspects of physiology
ii) A means to describe links from satiation to modulate (up- or down- regulate) processes

The definition of 'satiation' is a state of being full; quantification can thus be provided on a scale of 0 (starved) to 1 (replete). It should be noted, however, that a level of fullness required to be optimal for physiology may likely be less than absolutely full to the maximum extent; the difference provides a reserve to buffer against short-term starvation. For example, the highest cellular N:C is indicative of a lack of C; an organism that is satiated with respect to N may have physiological processes using N running at sub-optimal rates if the supply of C is limiting. We may thus identify states of satiation aligning with 'starved' (minimum), 'sufficient' (optimum), and 'satiated' (maximum). Linkage from satiation to control other facets of physiology can be made in the model using sigmoidal curves relating stimulus to response; this is consistent with the allosteric nature of physiological control mechanisms,.

Figure 1.1 provides an illustration of this concept, a concept that is at the heart of DRAMA. Here, the degree of satiation controls the potential acquisition of 3 alternative nutrient resources. When approaching starvation (satiation tending to 0, and hence the organism is unable to grow), the organism can potentially use all these nutrient sources. As satiation increases, with a consequential increase in growth rate, so the ability to use less-'preferred' nutrients is repressed. Ultimately, only the most 'preferred' resource (here, resource #1) will be used, and at maximum satiation even the use of that resource is modulated.

It should be noted that the degree of satiation controlling resource usage need not be directly linked to growth; enhanced growth is more likely to be the emergent consequence of the simultaneous satiation of several physiological processes providing, for example, energy, protein, fatty acids,

vitamins, etc. Behavioural activities such as prey selectivity, motility, migration and mating are all directly or indirectly related to levels of satiation.

The satiation-linked modulations, described above, need to operate in a dynamic setting; in essence its needs to provide control via a 'Dynamic Resource Assimilation Modulated Activity' (DRAMA).

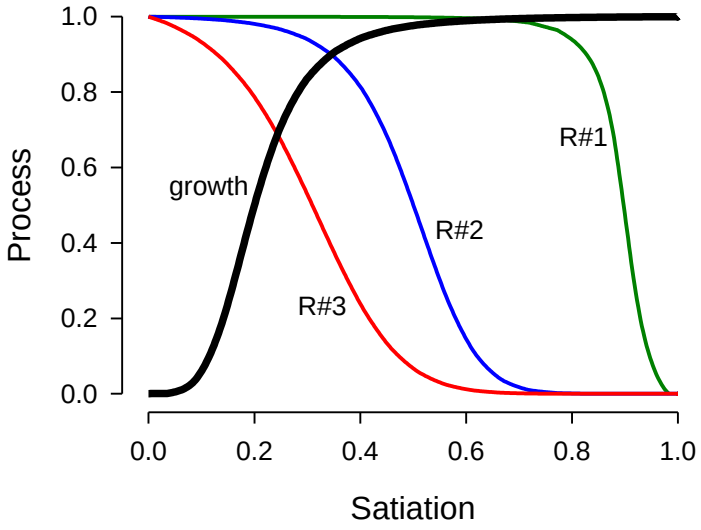

Fig.1.1 Satiation controlling nutrient acquisition from 3 alternative resources (R#1 .. R#3) and also supporting the potential growth of the organism. As shown, growth is at its maximum potential controlled by this nutrient type from a relative state of satiation of ca.>0.7. At full satiation of this resource (satiation =1) the use of all resource options are restricted; growth is limited by other factors such as the evolvable maximum growth rate.

1.3 Additional feedback responses

The behaviour of organisms is not just modulated by feedbacks to the satiation of biochemical processes. There are many other ways in which behaviour is modulated, especially at the level of the whole organism rather than of biosynthetic processes. Changes in the chemico-physical environment, such as temperature, salinity, pH, light-dark, may all simulate behavioural changes. The presence of predators or competitors (signalled through quorum sensing or allelopaths, for example) may also modulate changes in behaviour.

How the organism responds to such stimuli will vary but all those response reflect, and are controlled by, feedbacks. Thus, diel vertical migration in dinoflagellates is modulated by light-dark (and hence to the time of day) and to nutrient availability; this can be described by relating the upwards or downwards swimming of the organism to the balance of cellular C and nutrient status which varies over the day with photosynthetic activity (Flynn 2002).

The DRAMA concept is readily applied to modulating organism behaviours that are not simply related to satiation. The stimulus (independent variable) could be a feature of organism health (such as N:C), or an external feature sensed by the organism (T, light, allelopathic compounds, etc.). These can then be used to control the appropriate facet of behaviour seen in reality, such as motility

towards or away from a stimulus, cell aggregation (clumping) to protect against the presence of a predator.

The starting point is to determine which behavioural traits need to be described, what are the stimuli, and what are the responses. Sigmoidal response curves can then be used to modulate the response. Such curves feature strongly in DRAMA models, being robust, easy to manipulate and usefully also having a basis in biochemistry feedback regulations via allosteric interactions. This use of sigmoidal response curves is not new; the model of Flynn et al. (1997) used such functions to modulate ammonium vs nitrate assimilations into phytoplankton.

Example response curves are shown in **Fig.1.2**; these belong to a family of 4 equation types.

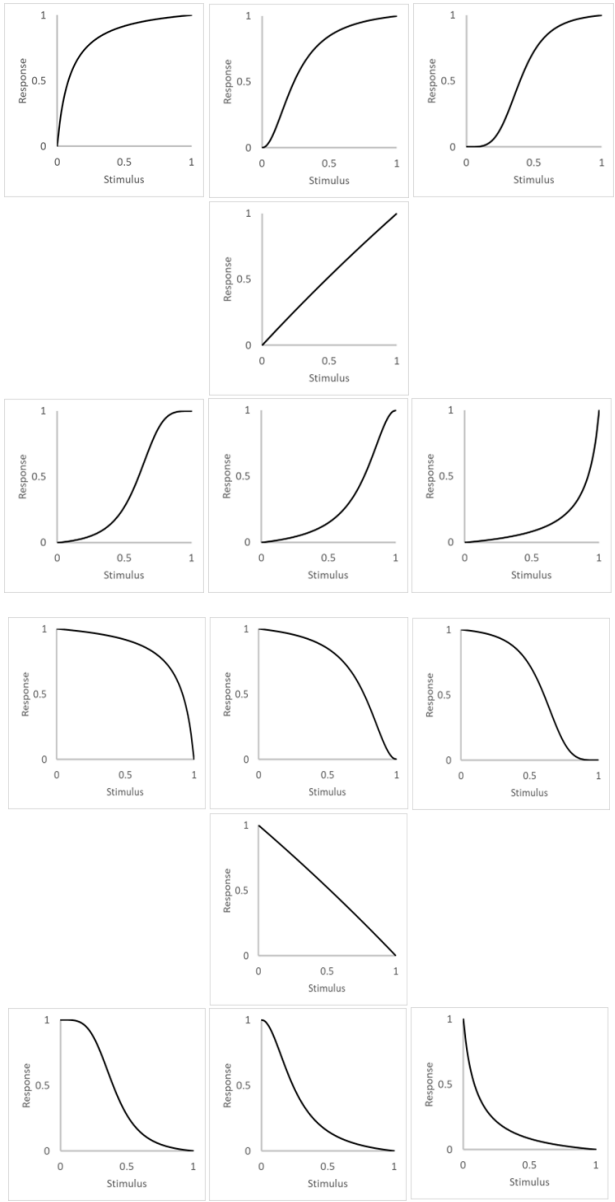

Fig.1.2 Potential relationships between a stimulus (x-axis) and response (y-axis) that can be used to control behaviour. All of these curves are described by forms of 4 basic types of sigmoidal equation based around either increasing-response patterns or decreasing-response patterns. Here the stimuli are all shown as quotients; many external stimuli are effectively open-ended (e.g., chemical concentration).

There are, in addition, more complex relationships, such as with temperature, pH and salinity (**Fig.1.3**). Often only part of such interactions are described (such as the use of the Arrhenius equation to provide a Q_{10} value regulating T with growth rate over the curve with no inhibition by temperature). These types of interaction can be applied to specific process points in a DRAMA model (for example to further modulate a response such as those shown in **Fig.1.1**) or at the level of changing the maximum growth rate potential.

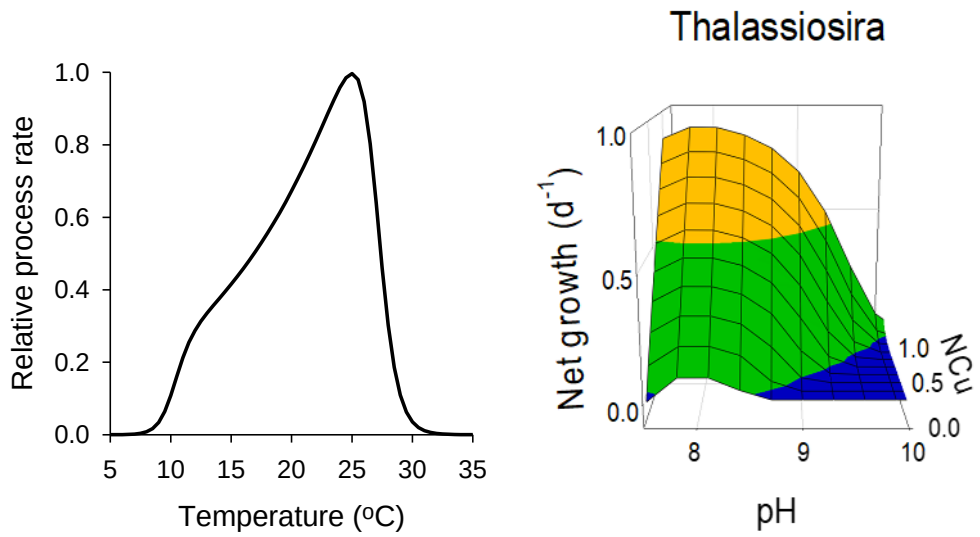

Fig.1.3 Example complex response curves. The left-hand plot shows the initial enhancement of the process rate by temperature, followed by the section described by the Arrhenius equation, and then a rapid decline as damage exceeds enhancement. The right-hand graph shows how the relationship between pH and growth interacts with the diatom *Thalassisosira*'s N-status (NCu); this was used to control a model describing growth patterns of different phytoplankton changing with pH and nutrient status (Flynn et al. 2015b).

1.4 The modelling approach; Systems Biology

Here, for plankton, the ultimate need is for a holistic, whole-organism, simulation platform delivering sufficient fidelity to behave in a realistic fashion, while remaining computationally tractable. This raises question over the modelling approach to be deployed.

Traditional modelling of plankton has used an approach rather akin to a multiple response curve-fitting exercise, in which a combination of inputs delivers the output. Typically, there are no feedbacks controlling modelled physiology within the individual plankton description. The rules for deriving and combining individual functions may differ (allometric scaling, cost-benefit etc.) but a very significant amount of numeric data are required to establish the rules. Like all curve-fitting exercises there are also concerns as to whether the rules will still be followed under conditions outside of those used to acquire the data.

An alternative approach is to marry a relatively new approach to biological thinking, *systems biology*, to a modelling approach that not only aligns well with systems biology but one that operates well in situations where the supply of numeric data is limiting, namely *system dynamics modelling*.

Systems biology seeks to describe biological processes in a holistic (rather than a reductionist) fashion exploiting computational methods (e.g., Voit 2013), from the molecular biology level through to whole organism physiology. The full suite of systems biology tools includes many analytical approaches, of which simulation modelling is just one (Navid 2021). A modelling approach used in systems biology is Flux Balance Analysis (FBA; Orth et al 2010), however that approach is only suitable for steady-state (chemostat-like) conditions; it is not suitable for the dynamic situations relevant to plankton ecology.

An important feature of physiology, and one that is central to systems biology, is the connection of processes through features such as feed-back and feed-forward interactions. Systems biology, for example, considers the active (de)repression controls seen in physiology to modulate the emergent behaviour. Different features of a model are built with different levels of detail, referred to as the level of granulation. A fine-grain component describes high resolution physiology, such as a biochemical pathway with explicit descriptions of individual proteins, enzymes, metabolites etc. At the extreme, whole organism physiology can be described with a fine-grain approach using FBA. At the other extreme, coarse-grain would relate processes to broader biochemical features, such as biochemical or elemental stoichiometry.

Most systems biology applications for whole organisms have coarse-grain components, with deployment of high detailed fine-grain components limited to specific interests, notably in medical and biotechnology sectors to individual biochemical pathways. An example of a medium-fine grain systems biology model of a plankton (cyanobacteria) is given by Sharma & Steuer (2019). For most plankton DTs, coarse/medium-grain systems biology concepts are most likely acceptable. This is also pragmatic given the lack of detailed physiological understanding, the need for flexibility, as well as the need to constrain computational loads for models running under highly dynamic conditions in multi-dimensional ecosystem scenarios.

Plankton modelling approaches that feature (de)repression regulation are not new (e.g., Flynn et al. 1997), but most traditional models of plankton have exploited what we may refer to as passive equilibrium controls (Flynn 2003). Thus, the maximum rate of nutrient uptake aligns with demand at the maximum growth rate (e.g., Geider et al. 1996, 1998), while in reality (and as described by a systems biology approach) the maximum potential rate of uptake may occur under nutrient stress and is modulated as satiation occurs. The former (passive) approach does not lend itself readily to the control of multiple resources which may also interact with each other. The rate of acclimation to stress is also an important facet of physiology and also in models; these rates can be readily modulated in systems biology approaches but not with passive models (e.g. Geider et al. 1996, 1998 vs Flynn et al. 2001).

DRAMA differs from the earlier piecemeal exploitation of systems biology style feedback controls in models (e.g., N-source use by Flynn et al., 1997; satiation feedback of predation and changes in assimilation efficiency by Mitra & Flynn 2007) by integrating whole organism regulations, across many resource types leading through to emergent balanced (i.e. homeostatic) growth. The

maintenance of homeostasis, a key feature of living organisms (**Section 1.2**), and especially when considered in the context of computer control, may be considered as cybernetic.

1.5 Cybernetics

Cybernetics is the science of communication and automatic control, for which feedback mechanisms enacted via circular causality form a defining component. Cybernetics thus describes a form of homeostasis (**Section 1.2**). The origins of cybernetics are often traced back to the mathematician and philosopher Norbert Wiener, and the term itself to the Greek word meaning *to govern*, as in the sense of governing (steering) a ship along its course.

Cybernetics, although often envisaged as relating to engineering, and especially to robotics and artificial intelligence (e.g., in cyborgs), has very broad applications including into ecology and biology. In the 1980'/90's interest developed into exploiting cybernetics to describe microbial growth on multiple resources (Kompala et al. 1986; Ramakrishna et al. 1996). However, perhaps with the rapid developments in molecular biology around the same time, the concept did not take off; those earlier works appears now as exploring a subject before its time.

Systems biology (**Section 1.3**) is now bringing together developments across what would traditionally have been termed 'biochemistry' and '(eco)physiology', to provide a more holistic overview of organismal science. Cybernetics describes much of the dynamic modelling within systems biology, dominated as they are by allosteric feedback processes to modulate the use of resources and internal processing to optimise growth.

The modelling of plankton has typically lacked any cybernetic-like feedback detail within the description of the organisms. Those examples where feedback has been included (e.g. Flynn et al. 1997 for ammonium-nitrate interactions; Geider et al. 1996 for light-N physiology, Flynn & Hipkin 1999 for light-ammonium-nitrate-Fe; Armstrong 1999 for light-ammonium-Fe; Pahlow & Oschilies 2009 for N-P) have targeted specific process rather than providing the holistic control of organism growth that would perhaps be more in line with a 'cybernetic' label. Most modellers have also not used explicit feedback controls with reference to recognisable end-product-feedbacks. Reasons for this are not clear, but likely include concerns over introducing what may be considered as unwarranted complexity. The platform in which models are developed may also affect the design of the model; *system dynamics* is a modelling approach that is particularly well aligned with cybernetic end-product inhibition feedback control.

1.6 System Dynamics

System dynamics is an approach to modelling that originated through the work of the computer and systems analyst Jay Wright Forrester. Feed-backs and feed-forwards are defining features of system dynamics models, often describing active multiple interactions to control the whole process. The approach is especially useful in data-poor situations where phenomenological understanding nonetheless informs us of where we may expect trends of individual components to run, but where the complexity of the linkages, with all its non-linearities, prevents a simple prediction of emergent behaviour for the whole system.

The concepts of system dynamics are supportive of the notion of '*it is better to be roughly right than precisely wrong*' (attributed to the economist, John Maynard Keynes). That does not mean that system dynamics models cannot be tuned, or optimised, to real (empirical) data sets, just that such models do not need to be tuned to such complete data series in order to be useful. This is of particular importance in ecophysiology and ecology where we know very little for certain but we often do have a good grasp of how things work in general. Such functionality is also of critical importance in considerations of making digital twins of plankton.

As an example of how the system dynamics approach differs from the common alternative in modelling plankton, consider the control of zooplankton feeding:

In a traditional approach the maximum feeding rate is typically set as a constant so that the desired maximum growth rate is emergent, net of all losses. Feeding is not controlled by satiation; it is just conveniently set with a maximum value that supports the maximum growth rate net of the losses. The linkage between feeding and growth is thus passive (with no feedback control) and there is no easy way to include other facets of how a real zooplankton may respond to changing environmental conditions. In some ways, this passive approach is similar to statistical regression fits through data used to identify trends. There is, in consequence, a risk of such models misbehaving when they are used in conditions beyond those considered during their construction and testing. The suggestion that '*all models are wrong, but some are useful*', attributed to the statistician George Box when referring specifically to statistical models, may thus be considered as applicable to most models of plankton (see Skogen et al. 2021).

Contrasting with the traditional approach, in a system dynamics description of zooplankton feeding, the level of satiation feeds back to modulate the grazing activity. As in a real organism, the maximum instantaneous rate of feeding may far exceed that needed to balance demands at the maximum growth rate. If, for some reason, the zooplankton expends more or less energy, then the amount of feed required to satiate it changes and hence the model modulates the feeding rate accordingly in order to optimise growth to the maximum. Concurrently, the level of satiation may also feedback on food preference (a satiated grazer tends to display greater discrimination between potential food items). The level of motility may also change; satiated grazers often decrease their motility, saving energy and also minimising the likelihood of encountering its own predator. Such controls are akin to those seen in reality, and also lend themselves readily to a systems biology, system dynamics approach.

Conceptually, a system dynamics model built in the vein of systems biology should never, to misquote Box (as he was referring to statistical models, not to simulation models), be '*wrong*'. Incomplete, yes, almost certainly; but not wrong unless the feed-back and feed-forward linkages are incorrect.

1.7 Aim of this work

What follows is a description of a platform for plankton digital twins. The approach uses a combination of systems biology concepts enacted through the system dynamics modelling of cybernetic interactions that exploit allosteric response curves (**Fig.1.1**). The approach provides a highly flexible, robust platform of intermediate computational cost.

The following sections work through a justification for the rationale behind the concept (**Section 2**), a description of the core concept itself (**Section 3**), an overview of the functionality (**Section 4**), functional equations (**Section 5**), example configurations for different plankton types (**Section 6**) and deployment (**Section 7**), and comparisons with some other models used for describing plankton (**Section 8**).

This work does not give a detailed mathematical treatment. The description given here is deliberately mathematics-light, intended to introduce the concept equally to biologists and to modellers.

2. Rationale – an introduction to DRAMA

DRAMA (**D**ynamic **R**esource **A**ssimilation with **M**odulated **A**ctivity) is an approach for simulating organism ecophysiology, that builds from well-established modelling concepts, but enhances them by inclusion of a C-metabolite quota. It deploys extensive multi-nutrient feedback mechanisms to modulate resource acquisitions and other aspects of behaviour such as motility. It provides a comprehensive and realistic, yet computationally tractable, approach to describe organism growth exploiting multiple resource types.

All organisms have to acquire resources from amongst those different options available to them in their environment in order to grow. External resource availability limits the acquisition (uptake, ingestion), but internal resource availability limits growth. The internal abundance of acquired resources also (via biochemical feedbacks) control physiology through modulating the ability to acquire different resources. So, for example, an organism that is approaching satiation will have down-regulated the ability to use less preferred nutrients, and perhaps at the extreme halt ingestion of all resources and (if applicable) cease motility while it digests its meal.

Some resources are biochemically more expensive to acquire, to process and then to assimilate, than are other resources even when they are provided in excess. Organisms have thus evolved to best manage what they acquire to support their growth and proliferation. The way that organisms achieve these ends, and are more-or-less successful under different conditions, are key facets that defines their ecology and competitive scope. The challenge for a simulation modeller of such organisms and of their ecology is to describe these processes in a meaningful yet computationally efficient fashion.

2.1 Simplification vs the risks of dysfunctionality

Models are simplifications of reality. They range from crude regression lines at one extreme to digital twins at the other extreme. Simulations, by definition, reflect reality in an artificial construct, in this instance a wholly *in silico* construct. A simulation model is at the heart of a digital twin (**Section 1.1**).

Simple models of plankton assume a single limiting nutritional factor, with the maximum growth rate defined by (constrained to) the maximum acquisition rate of that limiting factor. In the quest for simplicity, the consumption of other nutrients is often assumed to follow that of the limiting factor. In reality, biology does not work like this. And assuming it does do so has serious repercussions for model behaviour which most likely hinders or prevents the output from conforming to the term ‘simulation’ except when run under tightly constrained conditions.

In reality, the growth potential is constrained by factors other than the maximum acquisition rate of resources. Examples of such complexities are shown in **Fig.2.1**. The way that resources are handled changes as the growth rate approaches the maximum. For example, for various reasons the assimilation efficiency of food by a consumer decreases as the degree of satiation increases, while motility may decrease (minimising encounters with predators, and conserving energy).

Another aspect that is really important, in consideration of the modelling of plankton growth, is the use and fate of non- or lesser- limiting nutrients. If the use of these resources is not handled correctly

then their removal from the ecosystem and incorporation into the organism affects the growth and ecological linkages in other parts of the model later in the model run (Flynn 2005).

Feature	Response to stress	Genus	Reference
a. Growth rate	Down	all	e.g. Sunda and Huntsman (1997)
	Greater response in NO_3^- cells	*Thalassiosira* spp.	Maldonado and Price (1996)
b. Fe:C	Down to ca. 8% of Fe-replete cells	*Gymnodinium*	Doucette and Harrison (1991a)
		Actinocyclus; Emiliania	Muggli et al. (1996)
	e.g. Q_{max} = 62 μg Fe·g^{-1} C	*Thalassiosira*	from Harrison and Morel (1986)
	Q_0 = 5 μg Fe·g^{-1} C		Maldonado and Price (1996)
	1.8-fold higher in NO_3^- than NH_4^+	*Thalassiosira* spp.	Raven (1988)
c. chl:C	Down significantly	*Gymnodinium*	Doucette and Harrison (1991a)
		Synechococcus	Rueter and Unsworth (1991)

Limiting resource	Primary response	Potential secondary response
Light	[Chl:C & P_{max}]↑, mixotrophy↑	N:C↑, P:C↑, Fe:C↑, diatom Si:C↑, C:cell↓
N	N:C↓, use of alternates ↑, e.g. (NO_3, DON, N_2) mixotrophy↑	P:C↑, diatom Si:C↑, [Chl:C & P_{max}]↓, C:cell↓
P	P:C↓ use of DOP ↑, mixotrophy↑	C:cell↑, diatom Si:C↑, N:C↓, [Chl:C P_{max}]↓
Fe	Fe:C↓, siderophores ↑, mixotrophy↑	P:C↑, diatom Si:C↑, [Chl:C P_{max}]↓, C:cell↓
Si (diatoms)	Si:C↓, thin frustules, short setae	C:cell↑, P:C↑, [Chl:C P_{max}]↓

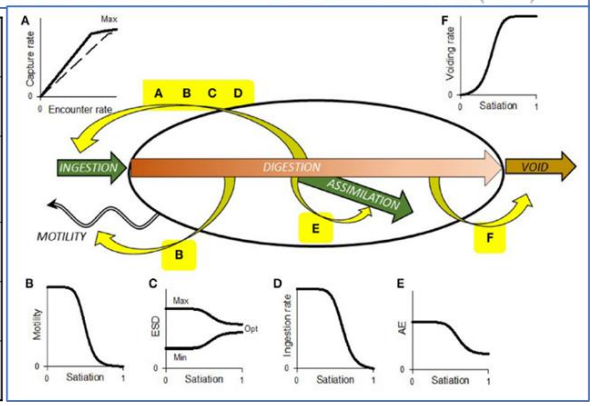

Fig.2.1 Examples showing interactions between different physiological processes. Top: part of a table listing characteristics of Fe-limited phytoplankton used for model construction (Flynn & Hipkin 1999). Lower left: primary and secondary stress responses for phytoplankton. Lower right: response curves for planktonic grazers (Flynn & Mitra 2016).

A failure to correctly account for salient features of real organisms can easily lead to a model that displays dysfunctionality, that is, the model output is contrary to expectation, not as expected or even neutral, but actually contrary (Flynn 2003).

Dysfunctionality may be primary, apparent in the description of the autecology of the organism itself, or secondary, apparent elsewhere in the model output. An example of dysfunctionality in plankton models is seen as the consequence of incorrectly describing the assimilation of Si by diatoms (Flynn 2005); an inappropriate description leaves too much Si in the water column when the N resource is exhausted, which enables a bloom of diatoms to occur later in the simulation when it should not. That modelled event then adversely affects the growth of non-diatoms and of zooplankton that express different prey preferences.

The difference between a simple model of a plankton, and a model suitable for simulations, comes down to how these physiological processes (all of which are subjected in reality to feedback modulations) are handled and thence projected in the results.

Even in simple models, changes to the conceptual base to bring the functioning of the construct more in line with reality can have significant impacts on the output of the model (Flynn et al. 2021). Sometimes, accounting for such matters, trying to make the model 'more realistic', may make the

model output worse (e.g., Mitra et al. 2007). Assuming that the modification is itself sound, a resultant worse output will be due to a failing elsewhere in the model (an example of secondary dysfunctionality). Tracking down and resolving such problems takes time, but for sure the situation is helped by having sub-models for each organism that individually provide robust outputs consistent with empirical understanding.

2.2 The role of expert witness validation (EWV)

To establish whether a model can indeed provide a simulation, perhaps as a model that could provide a component in a digital twin, requires validation of the model behaviour against data. The problem for plankton modellers is the paucity of good numeric data available in a suitable form. Often, the few data series of utility for modelling are used in configuring the model, leaving none for validation. However, we do have access to 'expert witnesses'; these are scientists who work with real-world plankton and thus have the required phenomenological knowledge ('natural history data', so to speak) to be able to judge if a model is producing a plausible output. Engagement with experts of empirical plankton science can take many forms, but at the least it could include the development of check lists of how a specific plankton type or species does and does not respond (e.g., **Fig.2.1**).

To fully engage with experts requires an iterative process through which they can interact, ideally with a digital twin -like platform. In such a setting they can experiment with the digital plankton, running models under different scenarios as they desire. If the models fail to behave as expected then the model developer will need to further develop and enhance the *in silico* description. While the traditional use of models has been the preserve of the modeller, by definition digital twins have input/output interfaces than facilitate their usage by the non-modeller. No longer will a plankton model be judged solely by the appearance of selected output demonstrating a specific point in a scientific paper; with digital twins the model can be tested in any and all ways, including by the experts who are best equipped to judge whether the output is plausible, as a 'digital twin'.

In essence, EWV employs a type of Turing-test (Turing 1950). The Turing test (after Alan Turing) describes an approach used to judge whether behaviour of an artificial intelligence (AI) aligns to that of a human. In crude terms, can the human be fooled into thinking that the AI is actually a human? For the application of EWV for digital twins, the question is whether the expert in empirical science could be fooled into thinking that the synthetic model output was actually real. There are two important caveats in configuring the test for such an analysis. One is that typically the output from a model is too 'clean', lacking in the noise seen in data from real world biological and ecological systems. The other concerns whether the model seeks to describe a very specific system, or a generality. As any empiricist will appreciate, even in highly controlled systems, biological events rarely follow the exact same path. Some level of subjectivity is thus required during EWV. Nonetheless, an model which displays dysfunctionality will likely be rejected quite quickly during EWV.

This subject is considered further in Flynn et al. (2022).

2.3 Developing plankton models

The earliest models describing organism growth in a form that could claim to be mechanistic, have their history in the approach of Monod (1949). This recognised that growth by a microbe in a chemostat culture system when constrained by the external availability of the limiting resource can, by making an analogy with the enzyme kinetics equation of Michaelis-Menten, be described using a rectangular hyperbolic relationship.

$$\mu = \mu_{max} \cdot \frac{S}{S+K_g} \qquad \text{Eq.2.1}$$

Here, μ is the growth rate, μ_{max} is the maximum potential growth rate, S is the limiting nutrient concentration, and K_g is the concentration of S that enables μ to be $\mu_{max}/2$. In a chemostat at steady-state, the value of μ is set by the dilution rate, and the value of S is the residual (i.e. remaining) concentration of the limiting nutrient. Substrate S is typically transported into a microbial organism by an active process that is under tight metabolic control. By analogy, the activity of consumers grazing on prey has been described using an identical equation, but making reference to a half saturation for predation (i.e., K_{pred}), even though this approach is inappropriate when set against how real predators function (Flynn & Mitra 2016).

While models of plankton capable of describing quite complex facets of physiology can be described from simple concepts (e.g., Anschütz & Flynn 2020), and are ideal for introductory teaching (Flynn & Mitra 2021), it is not possible to include any of the detail that is so important in defining how real organisms behave. The growth of real organisms is limited by the abundance of resources inside of them, as free metabolites supporting and driving synthetic pathways. Of course, internal resource levels are related to external resource availability, but resource acquisition is invariably decoupled for periods of time from internal processing. Furthermore, different external resources are converted internally to a common suite of biochemicals that are then used by cellular metabolism for growth.

The Droop quota model, originating in the 1960's, recognised that control of growth is by internal resource availability. Droop (1968) defined growth as a function of the internal nutrient resource, the 'quota', using a curvi-linear relationship between the concentration of intracellular vitamin B_{12} and the growth of protist phytoplankton. Droop described the quota in terms of the cell; thus it was referred to as a {resource}-cell quota, such as B_{12}:cell. Many other researchers have exploited the concept, some using the cell-quota (e.g., N:cell) and others referencing the {resource}-C quota (e.g., N:C). Thingstad (1987) used a cell-quota approach to describe bacterial growth on C, N and P substrates; this then used quotas of C:cell, N:cell and P:cell. Flynn (2008a) reviewed 40 years of the use of the Droop quota concept, arguing that the cell quota was itself problematic as cell size varies greatly with temperature (Atkinson et al. 2003), and with different nutrient limitations (sometimes cells becoming larger, sometimes smaller; e.g., John & Flynn 2002). And, of course, organisms increase in size simply with growth itself; microbes double and then halve in size during cell proliferation. Temperature also affects elemental quotas (e.g. Simonds et al. 2010)

Despite the popularity of the quota concept, there are remarkably few full data series of multiple nutrient quotas. Arguably the best of them is the data series of Elrifi & Turpin (1985), as shown in **Fig.2.2**. This also shows how the non-limiting quota may vary when growth is limited by another resource; here P:C attains extremely high values when growth is N-limited, while N:C falls from its optimum level when growth is P-limited.

Although, in conversation to the authors, Michael Droop was adamant that he saw only an empirical relationship between the (cell) quota and the potential for growth, it is quite easy to make the

argument for some form of mechanistic relationship. Considering N, an organism uses this element for DNA, RNA and proteins (which includes enzymes). These may be categorised into structural components (DNA and structural proteins) and rate-controlling components (RNA, enzyme proteins etc.). The minimum quota (Q_{min}), at which point growth rate is zero, thus defines an organism containing only the minimum configuration of structural components plus house-keeping functional components. As the concentration of functional components increases so the capacity for growth increases towards a maximum. For N that relationship is essentially linear, while it is curvi-linear for P:C (**Fig.2.2**).

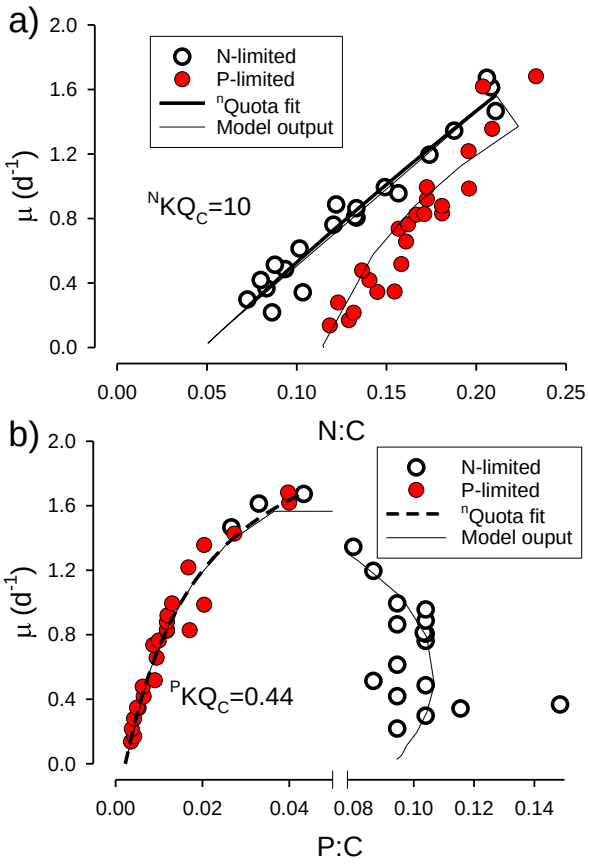

Fig.2.2 Data of Elrifi & Turpin (1985) as plotted in Flynn (2008b) with N:C and P:C quota relationships and simulation model outputs overlain. Note the split x-axis in (b).

It should be noted that the value of the minimum quota (and by inference likely also the optimum quota value) may vary with temperature (Simonds et al. 2010; Lewington-Pearce et al. 2019), as well as with other nutrient stresses (Elrifi & Turpin 1985).

There are various forms of the quota-growth equation. The original (Droop) version contained a hypothetical maximum growth rate value, and used a curve form that was fixed. Flynn (2008a, 2008b) provides an equation that yields a growth rate that is normalised (i.e., it reports a nutrient status ranging between 0 and 1) operating against a normalised quota (minimum quota =0,

optimum quota = 1); this approach simplifies its use in any function that requires reference to an index of nutrient status, and its linkage with any required maximum growth rate value. The revised formula also contains a curve-shaping constant that can be readily altered to conform to expectations for a particular combination of nutrient and organism (Flynn 2008b).

This normalised equation is –

$$Qu = \frac{(1+KQ)\cdot(Q-Q_{min})}{(Q-Q_{min})+KQ\cdot(Q_{opt}-Q_{min})} \qquad \text{Eq.2.2}$$

Here, *Q* is the current quota value that ranges between Q_{min} and $Q_{max,}$ but the controlling part of the curve ends at an optimal value of Q that is here termed Q_{opt}. For especially P:C, but also to a lesser extent for N:C, the maximum quota, Q_{max}, may be much higher than Q_{opt} and a value of $Q > Q_{opt}$ represents an imbalance in physiology accumulating too much of the nutrient or conversely not enough C (e.g., **Fig.2.2** – see also the discussion about satiation in **Section 1.2**). *KQ* is a curve shaping constant, and *Qu* is the resultant quotient describing the nutrient-status for nutrient quota *Q*. For a near-linear relationship, *KQ* needs to be larger than 5; when considering the quota in terms of N:C a value of 10 is suitable, while if we were considering P:C not only would the values of Q_{min} and Q_{max} be very different but also *KQ* would be much lower (ca. 0.5) giving the response curve a distinctly curvi-linear form (Flynn 2008a, 2008b; **Fig.2.2**).

In Eq.2.2, the value of $(Q-Q_{min})$ takes the role of a substrate in what is a modified rectangular hyperbolic equation (Cf., Eq.2.1). The value of $(Q_{opt}-Q_{min})$ scales the value of this 'substrate', describing the availability of the internal resource across the potential limiting range of Q. The involvement of (1+*KQ*) in the numerator ensures that the output is a quotient, such that $Qu=1$ when $Q=Q_{opt}$, irrespective of the value of *KQ.*

It must be stressed that quota values above optimum (i.e., $Q>=Q_{opt}$, such that Qu = 1) do not assure a maximum growth rate is actually attained; growth may be limited by another factor such as light for photosynthesis, or availability of another nutrient. Combining N and P limitations has been done usually using a Liebig approach; whichever is most limiting defines the growth rate. Alternatively, the quotas may be combined in various ways (e.g. Flynn 2001) or priority given to one over the other (as in the chain model of Pahlow & Oschlies 2009, or as in Flynn 2008b).

Another type of dual resource limitation model is the light-N limitation as described by Geider et al. (1996), and also developed with a different conceptual basis by Flynn (2001). Both those approaches involve another quota, for photosystems described by the Chl:C quota, that controls the acquisition of energy and fixed C through photosynthesis. The approach of Geider et al. used a combination of N:C and the incident irradiance to modulate Chl:C. In contrast, Flynn (2001) used the N:C level to modulate Chl:C; if N:C is high then this signals an insufficient amount of C and thence stimulates the synthesis of photosystems. Either way, growth proceeds at a given rate not because of the direct description of growth rate as a function of N:C (as it would in a Droop formulation), but because a high N:C enables a suitably high Chl:C which at a given irradiance brings in C at a rate that then defines the organism growth rate. The Geider et al. (1996) model used a passive approach to the control of the acquisition of N that enters the N:C quota. This approach is satisfactory when considering the exploitation of a single N-source, though the dynamics of the whole model is rather slower than is expected from empirical evidence (Flynn et al. 2001). More realistically, and as

needed to provide the types of output seen in **Fig.2.2**, an active feedback control of resource acquisitions is required (Flynn 2003).

A real organism has to modulate many different resources, not just 1, 2 or even 3. The most complex physiologies to represent in models are those of bacteria and of mixoplankton (photo-phagotrophic protists – Mitra et al. 2016; Flynn et al. 2019). Both these organism groups require coordination of the acquisitions and assimilations of many inorganic and organics sources of C, N, P and other nutrients. This requires a more complex, comprehensive approach than using a Liebig minimum term. An important question is, how to involve a C-quota which is not organism-based (i.e., in this instance, not C:cell). As there is C within structures as well as within metabolites, a solution is to describe the C-quota in reference to the contribution made to the total organism-C by the C in the metabolic pool. One may then question what a {metabolite-C}:{total-C} quota control actually looks like and, because of the multiplicity of C-resources, also how to modulate the exploitation of different forms of those C-resources. Analogous questions, of course, apply to modulating exploitation of different N and P resources.

In this work, we propose a model structure that has general utility for the description of the control of organism growth and modulation of the acquisition of different resources and other activities. We start by drawing parallels with control processes in real organisms.

2.4 Metabolic regulation: from fine-grain reality to coarse-grain practicality

The way that organisms control their growth physiology with the acquisition of resources is often associated with the repression (switching 'off') and de-repression (switching 'on') of biochemical pathways associated with consumption of alternative, or additional resources. Thus, the presence or absence of metabolites within the organism act as triggers prompting the organism to respond in different ways.

As an example, consider the ways by which a phototrophic microalga controls its use of different sources of dissolved inorganic-N, as ammonium, nitrate, or (for diazotrophic cyanobacteria) N_2-gas. Ultimately the product of all these N-source assimilations flows through the same initial entry point for organic nitrogenous biochemicals, namely into amino acids. If we consider the simplest example, for the assimilation of ammonium (**Fig.2.3**), we see firstly the involvement of a transporter protein to bring in ammonium from outside of the cell. The internally accumulated ammonium combines with the 1-N amino acid glutamate (Glu) to give the 2-N amino acid glutamine (Gln) via the activity of the enzyme glutamine-synthatase. In turn, the Gln combines with a product of C-fixation, 2-oxoglutaric acid (2OG) to give 2 molecules of Glu, one of which goes back around the loop, and the other carries on into N-metabolism to make all other cellular N-components. And it is that balance of Gln:2OG that likely indicates to the cell whether it has a high or low balance of N or C. If there is too much N relative to C (Gln:2OG is high) then the transport of ammonium is restricted (repressed), or at extreme even shut down. If there is insufficient N (Gln:2OG is low), then other pathways for N-resource acquisition are de-repressed, *de facto* 'turned on'.

This process is shown in **Fig.2.4**; with an increase in N-stress (signalled as a decline in Gln:2OG) the ability to transport ammonium is increased by the synthesis of more transporter proteins to increase cellular affinity for the N-source. If there is insufficient N flowing through the system to meet demand then the ability to use nitrate is de-repressed ('turned on'), and if supply of that N-source

is insufficient, and assuming the cell can synthesise the enzyme nitrogenase (diazotrophs), then N_2-fixation is de-repressed.

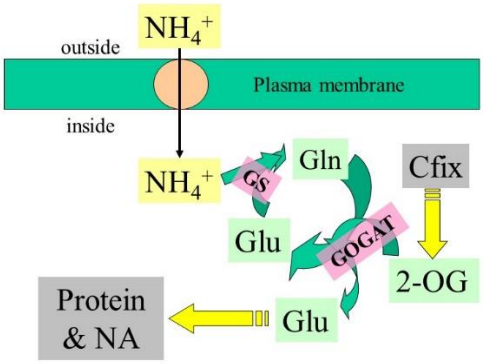

Fig.2.3 Ammonium assimilation. Ammonium (NH_4^+) is transported into the cell where it combines with the amino acid glutamate (Glu) to give the amino acid glutamine (Gln); this is enabled via the enzyme glutamine synthetase (GS). C is supplied, as shown here via C-fixation from photosynthesis, as 2-oxoglutaric acid (2-OG). Supported by the enzyme glutamine-oxoglutaric acid-amino transferase (GOGAT), 2-OG combines with Gln to produce 2 molecules of Glu; 1 Glu is syphoned off to support the synthesis of other amino acids, proteins and nucleic acids (NA), while the other Glu cycles around to assimilate the next molecule of NH_4^+.

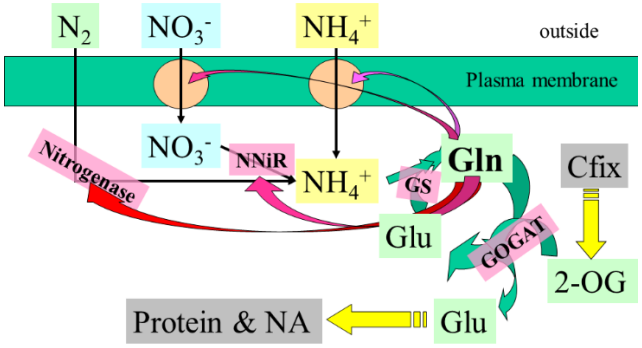

Fig.2.4 (De)-repressive regulation of N-source acquisition. Through the scheme shown, the internal concentration of the first organic product of inorganic-N assimilation, glutamine (Gln), likely allied to the concentration of a C-substrate such as 2-oxoglutaric acid (2-OG) is implicated in the control of the transport of ammonium and nitrate, and the synthesis of enzymes for N_2-fixation (nitrogenase) and nitrate+nitrite reductases (indicated here as NNiR, though they are often separated within the cell, nitrite reductase being closely associated with chloroplasts in protist microalgae). The ability to use nitrate is only de-repressed (enabled) if there is insufficient ammonium assimilation to sufficiuently raise levels of Gln. Nitrogenase is only present in some cyanobacteria; by this scheme it would only then be expressed if there was insufficient ammonium or nitrate available to repress its synthesis. See also **Fig.2.3**.

In **Fig.2.5** we see values for Gln:Glu (a surrogate for Gln:2OG; see **Fig.2.3**; Flynn et al. 1989, Flynn 1991) in a phytoplankter grown on ammonium or nitrate to different levels of N-stress. As we expect from **Fig.2.4**, the Gln:Glu values in ammonium-grown cells are higher than those growing using

nitrate. Note though that the maximum growth rates are not very different; even though N-replete nitrate-grown cells must be more stressed than N-replete ammonium-grown cells (else, nitrate usage would not be de-repressed), the realised growth rate is not affected greatly, and certainly not by the 20-30% level expected from the great energetic cost in reducing nitrate to ammonium. Not only do we see signs of that stress in the lower values of Gln:Glu, but we also see a lower cellular N:C in the nitrate-grown cells (**Fig.2.5**); the extra N-stress in using nitrate thus impacts gross cellular physiology even though it does not affect the growth rate.

We can also see, in **Fig.2.6**, how the potential activity for nutrient transporters to bring in ammonium and nitrate varies with N-stress. The explanation for the enhancement in transport can be seen in **Fig.2.4** and extended in **Fig.2.5**. As N-stress develops, indicated by a falling cellular N:C ratio, the ability to transport in ammonium increases steeply. Then the ability to bring in nitrate develops and also increases. However, as the organisms become more seriously N-stressed (N:C becomes lower and lower, approaching the minimum N:C quota) the transport potential decreases; the cell redistributes its N-resources to more critical components. Note that the point where the transport potential crosses the light-grey lines in **Fig.2.6** indicates the cellular needs for N at that growth rate; only where the transport potential exceeds the demand is there the potential for satiation by that N-source.

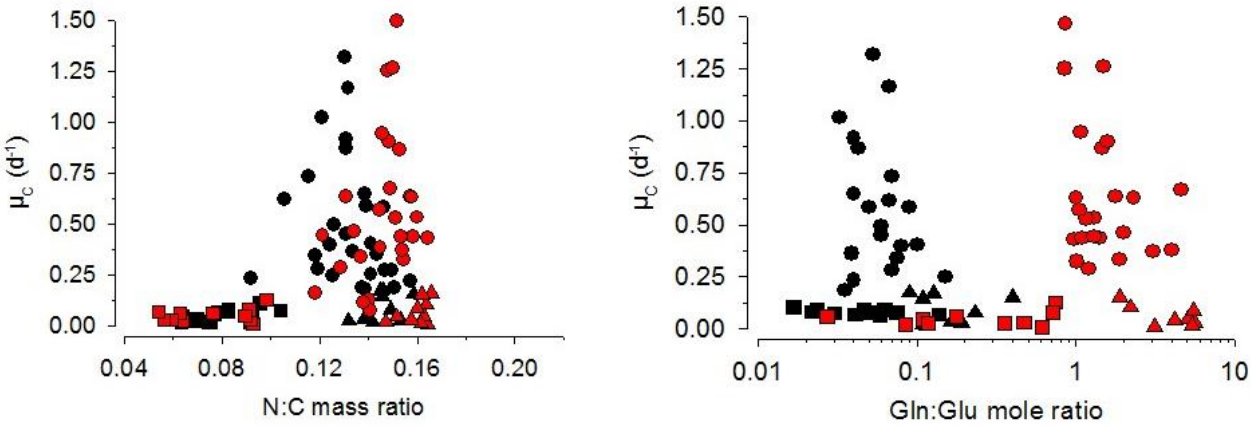

Fig.2.5 Experimental data for the coccolithophorid phytoplankter *Emiliania huxleyi*, relating cellular N:C and Gln:Glu to C-specific growth rate (μ_C). The vertical scatter in μ_C is due to sampling during growth in a light:dark cycle. Cells were either growing with surplus nutrients (circles), as Dissolved Inorganic C (DIC) and Dissolved Inorganic N (DIN), or limited by DIC (triangles) or limited by DIN (squares). Black symbols indicate cells grown on DIN supplied as nitrate or, as red symbols, grown using ammonium. Note that peak growth rates are similar between sources of DIN, but that nitrate-grown cells have slightly lower N:C but also greatly decreased Gln:Glu in comparison with ammonium-grown cells. See also **Fig.2.4**. Data from Flynn, unpublished.

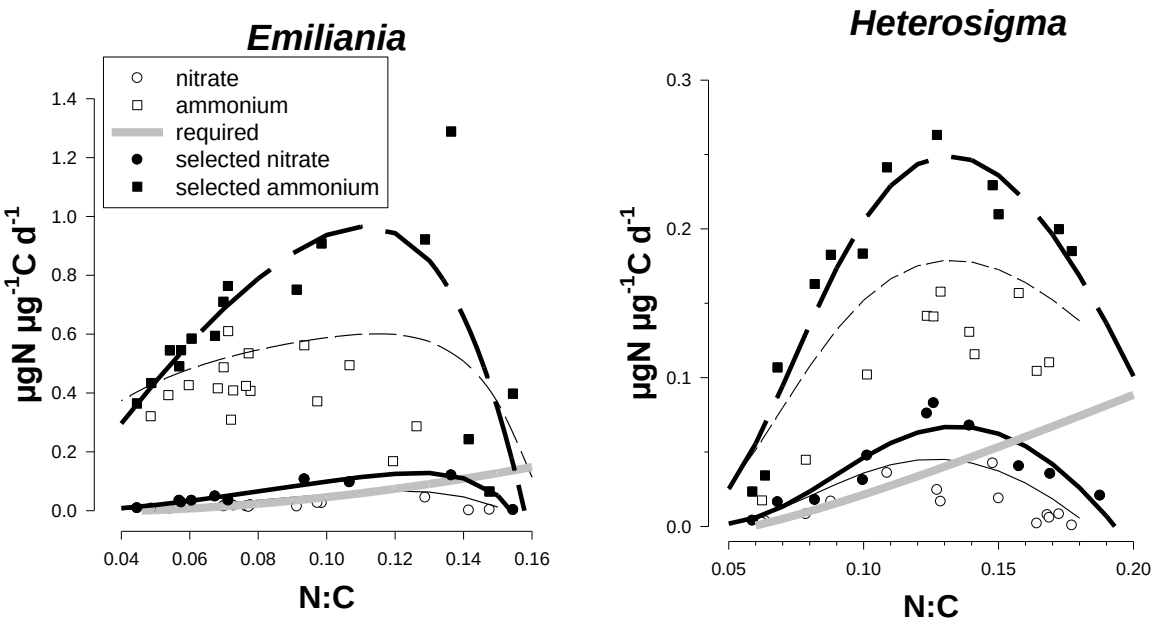

Fig.2.6 Relationships between estimates of the maximum nutrient transport rate (T_{max}) for ammonium or nitrate, for two different phytoplankters. N-stress is indicated by the mass ratio of N:C; high values indicate a good N-status (see also **Fig.2.5**). The "required" lines indicate the rate of transport required to support steady state growth at a given level of N-stress (given N:C). Note how the transport capacity increases as stress develops (e.g., N:C decreases from high to mid values) and then decreases at extreme stress (i.e., N:C falls to minimum values). Also note that the ammonium T_{max} values greatly exceed those for nitrate, and at high N:C (possible only when growing using ammonium) the transport of nitrate is repressed (see **Fig.2.3**). See Flynn et al. (1999).

In total, then, the physiological status of the organism's control the growth processes using different resources, including the ability to bring in and exploit those resources. In doing so there are a series of interactions that develop to provide the organism with a differential preference for resources. These modulations also affect whether the exploitation of some resources in sufficient amounts controls, or perhaps totally inhibits, the use of other resources. Here, the supply vs demand for assimilation of sufficient ammonium prevents the need and thence the ability to use nitrate. In reality, most of these interactions are more properly termed (de)repression processes; satiation in demand represses the need to increase the potential to exploit additional resources.

Such interactions are core to the functioning of organisms. However, although such processes can be described explicitly in models (for example, the ammonium-nitrate interaction is described in the fine-grain model of Flynn et al. 1997), this is not possible in general terms across the whole organism biochemistry and physiology as it would be far too complex and we also lack sufficient information and data to configure such models. Nonetheless, the conceptual basis at a coarse-grain level can be deployed in models to control the use of different resources and define the resultant behaviour and growth of the organism.

It is important to note that a low level of stress experienced by an organism can be mitigated against without necessarily causing a decrease in growth rate. This is shown diagrammatically in **Fig.2.7**,

with an organism growing under optimal conditions having the greatest scope for growth. Thus, growth of a microalga using nitrate may be no slower than that using ammonium (Thompson et al. 1989; Wood & Flynn 1995), although nitrate-grown cells by definition (see **Figs.2.4 – 6**) are more stressed in terms of inorganic N supply for physiology. The scope for growth shown in **Fig.2.7** provides a head-space for an organism to counter different stresses, such as those stemming from adverse temperature, pH etc.

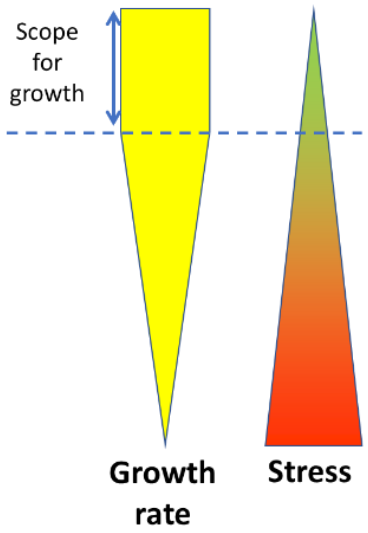

Fig.2.7 Growth rate is not affected by low levels of stress, but at some point (dashed line) stress becomes sufficiently great that growth rate is adversely affected. Increasing levels of stress cause de-repression of metabolic pathways and resource acquisition routes that may mitigate stress, but also decreases the scope of growth so that other factors such as pH cannot be mitigated against so effectively (see **Fig.1.3**, righthand panel).

If we now extend the examples given above (**Figs. 2.4** to **2.7**), to how an organism may respond to different resource types and associated stresses encountered when those resources are not available, we can begin to more fully appreciate the complexity of the situation (**Fig.2.8**).

In **Fig.2.8**, it is noteworthy that there is a hierarchy in resource acquisition potential. In addition, some resources are only accessed when the level of stress are such that maximum growth rates cannot be attained, a situation that may be exacerbated by the need to expend more energy in obtaining and utilizing those resources. Alternatively, a resource may be acquired at a lower stress level but the chemical nature of the resource may nonetheless prevent its use to support a rapid growth rate. Thus, the amino acids arginine (4N), histidine (3N) and lysine (2N) may all transported by the same cationic amino acid transporter, but histidine is a very poor N-source for growth as it has a biochemical pathway for synthesis and catabolism that is very different to those of other amino acids.

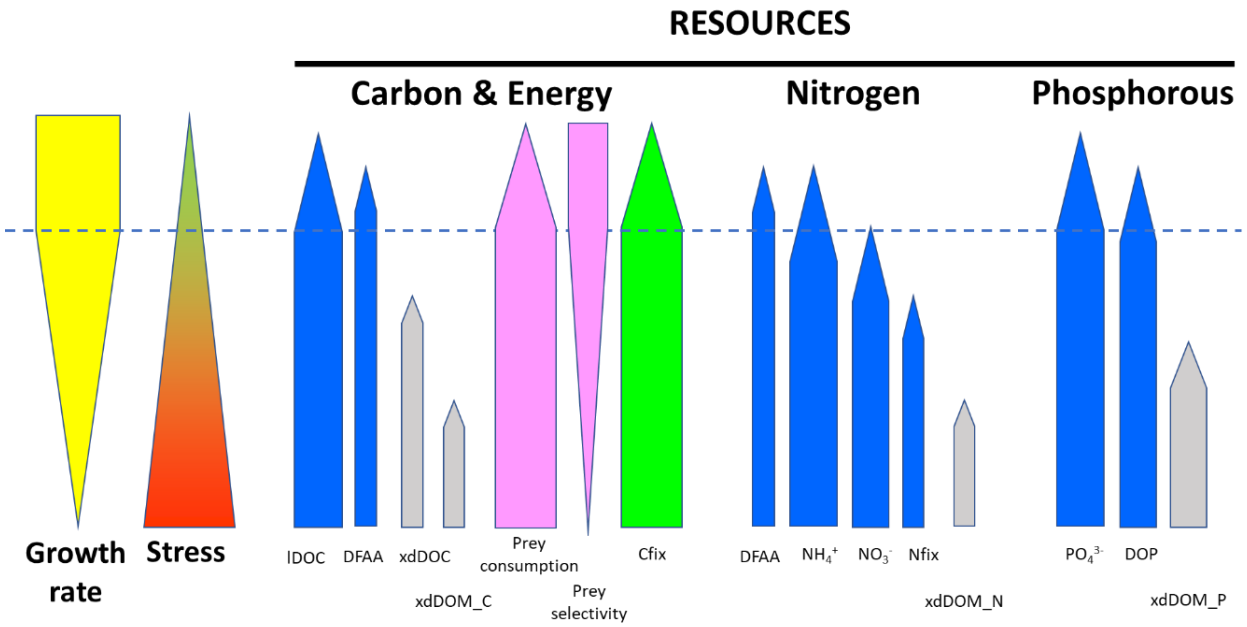

Fig.2.8 Schematic of the (de)repression of the acquisition of different resources changing with stress. Note that some resources cannot support the maximum growth rate (dashed line). No individual organism will express all these options; thus, bacteria express extra-cellular digestion (grey symbols), while predators ingest whole particles and digest them internally (pink symbols). lDOC – labile DOC such as glucose; DFAA – dissolved free amino acids, but similar for free nucleic acids; DOP – phosphorylated primary metabolites, such as glucose-6P; xdDOC – polymeric DOC which requires extracellular digestion prior to transport of the liberated small molecules; xdDOM_C, xdDOM_N, xdDOM_P – as for xdDOC but for more complex molecules including proteins, lipids, DNA through to exotic secondary metabolites. For predation, note that the potential to eat more rapidly increases with stress (i.e., with hunger) while prey selectivity also decreases (i.e. more prey types may be considered for exploitation).

In reference to the changes in acquisition potential shown in **Fig.2.6**, it is worth explaining that the elevation in that potential above the rate needed provides an effective lowering in the half-saturation value for the process. The explanation for this is shown in **Fig.2.9**; in essence each transporter molecule has a half-saturation value of K_t and a maximum process rate of K_{cat}. By synthesising more transporter molecules, the effective emergent half-saturation for growth (K_g) is decreased, so enabling the organism to exploit nutrients at lower substrate concentrations (limited by diffusion gradients).

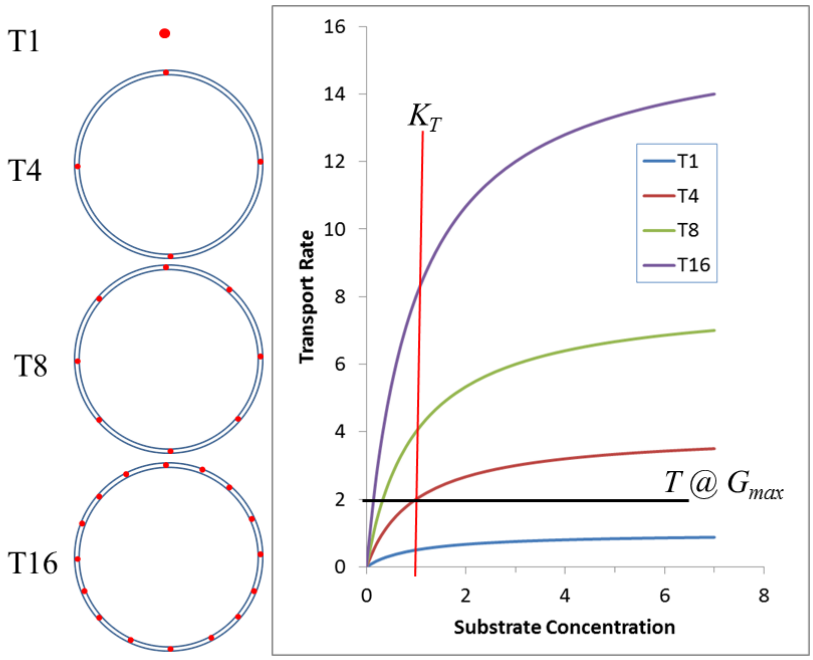

Fig.2.9 Schematic showing the theoretical relationship between substrate concentration at the site of the transporter, the activity of a single transporter protein (T1), with k_{cat}=1 (units of transporter-specific activity per time) and half saturation K_t=1 (units of substrate concentration at the transporter site), and the collective activity of 4, 8 or 16 of such transporter proteins within the external membrane of a cell (T4, T8, T16). Note that K_t remains the same, while the effective maximum transport rate (T_{max}, as represented by the plateau value of the transport rate) is a product of k_{cat} and the number of transporter proteins. Consider now the instance where the organism can attain its maximum growth rate (μ_{max}) through a transport rate of 2 (marked by the line at T@ μ_{max}), then the substrate concentration that would support $\mu_{max}/2$ (i.e., the value of K_g) can be seen to be lower than K_t by a margin related to the number of transporter proteins. Where T=16, K_g can be seen to be a very small fraction of K_t. All units are arbitrary. See Flynn et al. (2018).

The number of resource types and the ways that they are regulated are vast. **Fig.2.8** gives only some indication of this complexity, although it must be stressed that no organism expresses all of these particular options. For example, only diazotrophs express the potential to perform N_2-fixation, and that capability will be repressed if N-stress is compensated by sufficient assimilation of NO_3^-, NH_4^+ or DFAA. The most complex permutations are expressed by mixoplankton and by bacteria, both of which play critical roles in plankton ecology and neither of which is often included in simulation models in a way that reflects their real potential. In part this may be explained by the nutritional complexity itself that hinders development of tractable models. But, there is a way of simplifying these interactions (**Section 2.5**).

2.5 Simplifying the control interactions

Simple model descriptions often see individual processes either ignored, or merged together and handled in an additive rather than an interactive fashion that is consistent with reality. Thus, one may construct a model based upon a single state variable (e.g., C-biomass), where growth is simply

the sum of all resource inputs minus all associated losses. No real organism behaves in such a fashion, and thus a model based on a crude additive structure has a clear potential to be dysfunctional (to behave contrary to expectations with the simulation outputs significantly awry). From the foregoing, however, we can consider exploiting the fact that real organisms control their physiology through modelling the action of feedback systems relating resource supply to their demand for growth.

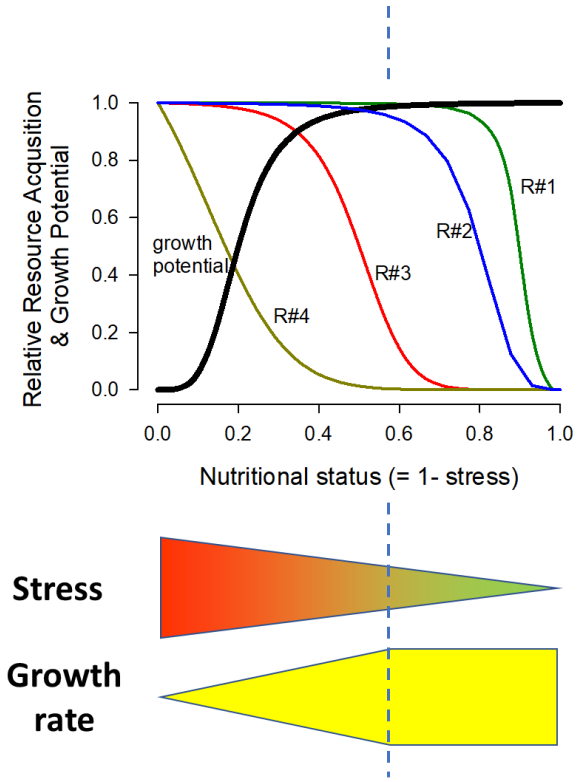

Fig.2.10 Schematic of the progressive (de)repression of resource acquisition systems with nutrient status. At zero stress, where the nutritional status is maximum, growth rate is also maximum. As stress develops, with the nutritional status declining, initially the acquisition potential for resource R#1, and then R#2, are de-repressed. Use of these resources have the potential to support a maximum growth rate, though R#1 provides greater scope for growth (see **Fig.2.7**). If demand cannot be satisfied by R#1 and/or R#2, stress develops further leading to expression (de-repression) of the potential to use resources R#3 and finally R#4. Note that use of R#4 alone can only support ca. 50% of the maximum growth rate, although some minor use of R#4 may occur when growth is ca. 90% of maximum with a nutritional status of ca. 0.4.

In **Fig.2.10**, we consider a conceptual mechanism controlling a hierarchy of different resource acquisitions. It is important to note that potential acquisition can only be considered as satisfying demand if sufficient of that resource is in the environment to support that demand. Thus, all four of the resources shown in **Fig.2.10** (i.e. resources R#1 - #4) may be exploited simultaneously if all are only available at sufficiently low concentrations such that the nutritional status, as indicated here, is below ca. 0.4.

If there was evidence that a resource acquisition potential was shut down at low levels of stress, then the curve form would decrease at high nutritional status (e.g. **Fig.2.6**; modelled in Flynn et al. 1997). For some resources that is less likely than for others. Hence, for ammonium and nitrate

assimilations, the N from nitrate passes through the same biochemical pathway once the nitrate has been converted (reduced) to ammonium inside the cell. On the other hand, in a mixoplankton which is expressing both phototrophy and phagotrophy, a level of stress that de-represses (switches 'on' or up-regulates) the ability to feed may perhaps also depress (switch 'off' or down-regulate) the ability to photosynthesise.

The form of the curves shown in **Fig.2.10** are sigmoidal. Such curve forms are consistent with the synergistic modes of allosteric biochemical regulation. As a curve form, sigmoidal curves also provide robust response descriptions in models, with a low rate of change at both ends of the curve. In contrast, the response curve given by the Michaelis-Menten rectangular hyperbola (Eq.2.1) gives a very abrupt rate of change that typically require very small integration step-sizes to function reliably in models at low substrate levels. Examples of different response curve types are shown in **Fig.1.2**.

The equation for the resource acquisition control curves in **Fig.2.10** is:

$$RA = (1 + K^H) \cdot \frac{(1-S)^H}{(1-S)^H + S^H} \qquad \text{Eq.2.3}$$

Here, RA is relative resource acquisition rate, S is the relative nutritional state (thus, [1-S] is a quotient for stress), and K and H are constants. Usually H, the Hill number, is a value between 4 and 8. K is less than 1.

The equation for the growth rate control curve is:

$$GR = (1 + K^H) \cdot \frac{S^H}{S^H + S^H} \qquad \text{Eq.2.4}$$

Here, GR is the relative growth rate; other variables are as for Eq.2.3.

These are normalised curve functions, making reference to the nutritional state expressed as a quotient (0 to 1), and yielding a response that is also a quotient (0 to 1); see also **Fig.1.2**.

Thus far, we have considered how complex the control is for individual resource acquisitions, and a means by which (**Fig.2.10**) we can radically simply the description. We now turn to consider further facets of the building of a model using these cybernetic approaches (**Section 3**).

3. The DRAMA Concept

The concepts of homeostatic (cybernetic) control used to simultaneously control the exploitation of different nutrient streams in support of organism growth are central to the DRAMA framework, through ***D**ynamic **R**esource **A**ssimilation with **M**odulated **A**ctivity*.

3.1 Controlling resource acquisition and growth

We have seen above (**Section 2**) how resource acquisitions are modulated by and, at least empirically, how growth rate can be made a function of, the nutrient status of the organism. In **Fig.3.1**, is a schematic showing generic resource acquisition and growth interactions for an organism. The biochemical, physiological, control is based upon the size of the metabolite and biochemical machinery components, ^{M}**C**, which comprises part of the total carbon biomass, **C**. The structural carbon is ^{C}**C**.

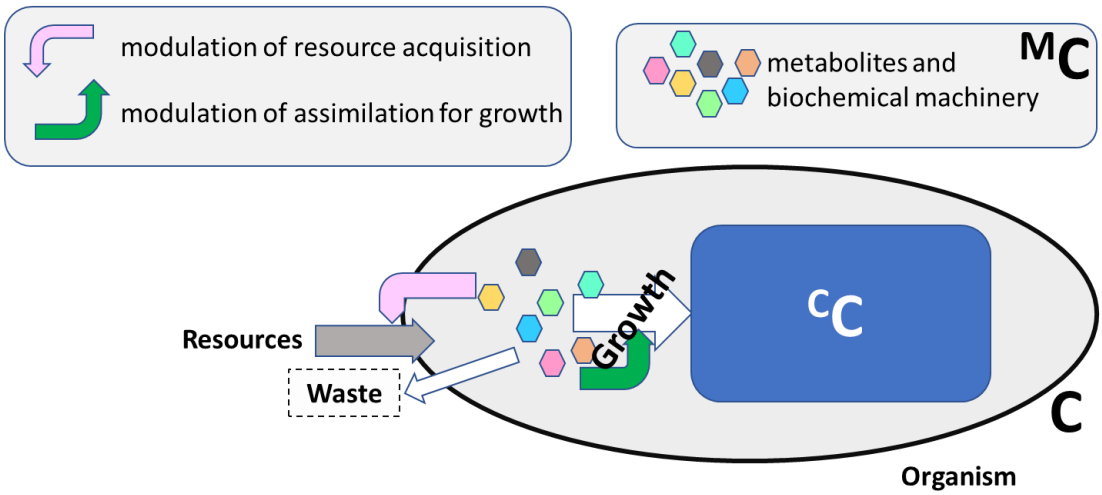

Fig.3.1 Schematic showing the means by which the internal nutrient status of the organism, reflected by the size of the pool of metabolites and allied biochemical machinery, modulates both resource acquisition and growth rate. The whole organism comprises the total carbon-biomass of **C**; a large fraction of **C** comprises core structural components, ^{C}**C**. The metabolic contribution to **C** is ^{M}**C**.

Also shown in **Fig.3.1** are feedbacks from the size of the ^{M}C pool to modulate resource acquisition (pink arrow in **Fig.3.1**; cf. control of resource acquisition potential at high "Nutritional Status" in **Fig.2.10**) and also the support of a high growth rate (green arrow; cf. "Growth potential" curve in **Fig.2.10**).

As the organism is starved of resources, becoming resource deplete (**Fig.3.2**), the size of the metabolite and machinery pool, ^{M}**C**, decreases. The feedback to repress expression of different resources (pink arrow) is now lessened, so more resource types may be exploited (cf. **Figs. 2.8** & **2.10**). The support of growth rate (green arrow in **Fig.3.1**, and the similar green arrows in **Fig.3.2**) is lessened.

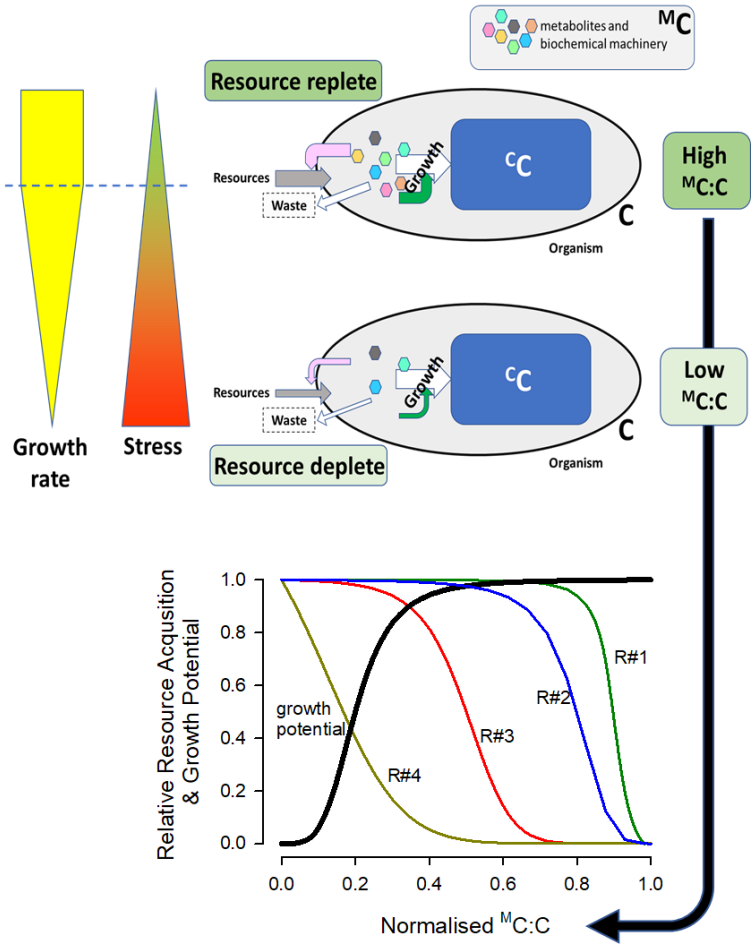

Fig.3.2 The normalised value of $^{M}C:C$ controls growth and resource acquisition. The value of $^{M}C:C$ is *de facto* a quota simultaneously defining C-resource and nutritional status (see **Fig.3.1**). A C-resource-replete organism (one which is under low physiological stress) has a high $^{M}C:C$, while a resource-deplete organism (higher stress) has a low $^{M}C:C$. The value of $^{M}C:C$ controls the expression of resource acquisition modes (e.g., resources *R*#1 .. *R*#4), and also defines the potential growth rate. Note that some level of stress (such that normalised $^{M}C:C$ declines down to the dashed blue line, top left) can be mitigated against without affecting the growth rate.

From **Fig.3.2**, we see the derivation of what amounts to a C-quota, $^{M}C:C$, that represents the carbon and energy status of the organism. These C-quota curves complement those for N and P used for controlling the acquisition of N and P and also controlling growth, as the transfer of C from ^{M}C to ^{C}C. Example forms of these functions are shown in **Fig.3.3**.

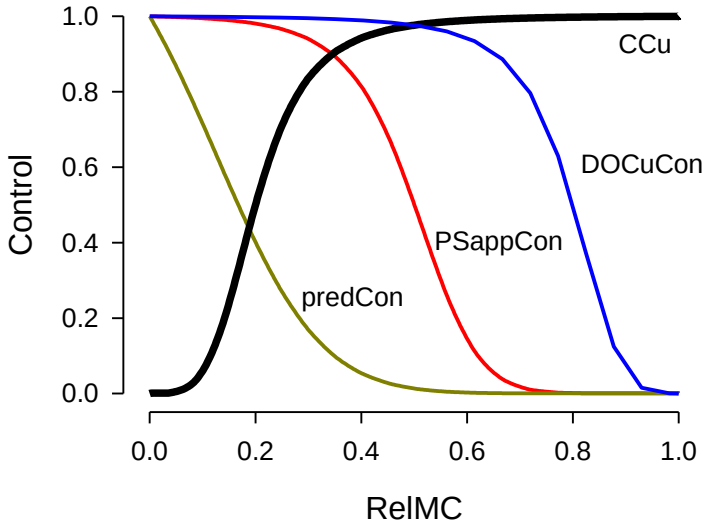

Fig.3.3 The value of RelMC (which is given as the value of the ratio ^{M}C:C) in a mixoplankton model can be used to control the use of labile DOC (DOCuCon), photosystem construction (PSappCon), predation (predCon), while the C-quota (Ccu) is in turn involved in the control of C-specific growth. As RelMC decreases, so more resource acquisition options are enabled (their control values increasing to 1). In this instance, predation is shown as only enabled as an extreme stress response to a lack of C. See Flynn & Mitra (2023).

3.2 Justifying two state variables for carbon

The need for two state variables (core and metabolite C, as **^{C}C** and **^{M}C**), rather than just the one for total carbon biomass, begs the question whether the additional state variable (and its allied computational cost) is warranted.

A variable stoichiometric plankton model typically uses 1 SV for each component. Thus, a model capable of describing variable C:N will have two SVs, with (for example) units of mgC m^{-3} and mgN m^{-3}, respectively. A C,N based model provides scope to control the acquisition of N-sources by reference to the N:C quota by developing response curves that mimic the form of the relations shown for example in **Fig.2.6**. Likewise, P resource acquisition can be modulated by reference to a P:C quota in a C,P-based model. The need for the acquisition of sugar (as a form of C) could also be modulated by reference to the N:C quota, but with a curve form opposite of that for N-sources. But there is a problem: In a conventional quota model, the quotas are used to control growth by resource availability of the limiting nutrient. We can thus have, for example, an organism with the maximum N:C but incapable of growth if C was limiting.

In a simple N-limited quota model the ratio of N:C is decreased by the assimilation of C being in excess of the assimilation of N. In the concept shown in **Figs.3.1** & **3.2**, organism growth sees the transfer of carbon from ^{M}C to ^{C}C (with some losses due to respiration). As that event occurs, so the ^{M}C:C quota declines. By analogy to the use of the N:C quota being used to control both N-limitation of growth and the acquisition potential for different N-sources, so the changing ^{M}C:C quota can be used, as per **Fig.2.10**, to not only control growth but also to modulate the acquisition of different C-

resources. Additional levels of interaction are possible, perhaps involving coupled interactivity between P:C and N:C (e.g. **Fig.2.2**).

This level of fidelity cannot be achieved without involving additional state variables. Those models that use a single state variable for the main model currency (cell or C) can at best use a passive means to relate growth to resource availability and require a considerable level of prognostic assumptions to control interactions between different resource type acquisitions. Aside from there being too few degrees of freedom for differential control of physiology and growth in using a single C state variable approach, circular arguments can be generated in the mathematical formulation between the processes of acquisition, control of acquisition and growth. Such circularities can be resolved by the use at least one additional state variable to handle immediate post-acquisition processing of resources (e.g., by inclusion of what could be considered as a 'gut' in a consumer model – Mitra & Flynn 2007).

3.3 What biologically is the ^{M}C:C quota?

From the above, we conclude that the minimum number of state variables required in a model that can modulate both acquisition and growth is thus two: one that describes the non-structural materials involved in making the organism work and the other (usually larger in its mass) is the core physical structure of the organism. For the control of contrasting routes of carbon acquisition, we have proposed the ^{M}C:C quota. The quotas of N:C, P:C and Chl:C are readily determined chemically, and these quotas are widely used in plankton models. But for ^{M}C:C we must ask:

- What is ^{M}C?
- Is it readily measurable?
- Do we need to actually measure it?

Biochemically, the two state variables ^{M}C and ^{C}C (**Fig.3.2**) broadly align with materials that are, or are not, (respectively) extractable in hot tricarboxylic acid (TCA) and alcohol (Roberts et al. 1955). In consumers with a gut or feeding vacuoles, the digestate also contributes to ^{M}C.

The relative size of the metabolic (^{M}C) pool to the total enables growth through assimilation of materials into the structural core (^{C}C, which may also include materials to support production). The size of the TCA-alcohol soluble fraction is greater in cells that are rapidly growing. As with other quotas, N:C, P:C, Chl:C etc, there will be a minimum ^{M}C:C which describes an organism with a bare minimum physiology. There is always material within the TCA-alcohol soluble fraction. An example of a specific component of ^{M}C is the intracellular amino acid pool (InAA; Flynn 1990), of which the amino acids Gln and Glu have already been discussed (**Fig.2.5**; Flynn 1991).

Whether it is necessary to explicitly define the range of ^{M}C:C from experimental work is an important question. For sure, it is necessary to know the total carbon-biomass ($C=^{C}C+^{M}C$), as this also affects the other quota values, based as they are on C. The range of the ^{M}C:C quota can be set pragmatically to enable the integration step size to be suitably large to facilitate the smooth operation of the simulation calculations; having a small ^{M}C pool would inevitably require the use of a small integration step size. It is the normalised value of the quota, bound between minimum and optimum and absolute maximum ($^{M}C:C_{min}$, $^{M}C:C_{opt}$, $^{M}C:C_{max}$) values that is used to control the functionality of

the model; the values themselves do not affect the rate of flow of C through to make ^{C}C, and hence drive growth. This pragmatism sees the setting of $^{M}C:C_{opt}$ with a value of around 0.2, which is rather conveniently also a plausible value seen in TCA-alcohol extraction experiments of the diatom *Phaeodactylum* (Flynn, unpublished). The maximum percentage of total C present in a zooplankton gut is ca. 5-10% (Mitra & Flynn 2007) to which ^{M}C would also include metabolite C within the actual organism biomass.

Perhaps of equal, if not greater importance, is the need to relate the form of the growth-potential curve within the $^{M}C:C$ quota range (e.g., see **Fig.2.10**, considering "Nutritional status" as a normalised $^{M}C:C$ quota value between $^{M}C:C_{min}$ and $^{M}C:C_{opt}$). This needs to be done both with respect to the growth potential and the relative controls of different resources; these define the overall dynamics of growth and resource usage. However, in general terms all we really need to know are:

- the order of resource acquisition (de)repression
- the degree to which each resource can support growth when supplied in excess

How well the resultant model meets with expectations can be judged via expert witness validation (**Section 2.2**), with modifications made to the controlling curve forms (**Fig.3.3**) as required. Within a system dynamics modelling framework, and because sigmoidal response curves enables mathematically robust cybernetic control, the whole model is stable and easy to modify.

3.4 Alternative realities; simplifications to provide tractable empirical descriptions

The means through which acclimations are described in any model include a high level of empirical simplification, and there are different approaches possible for each challenge. Different types of simplification can be readily built into DRAMA-based models; the DRAMA approach does not prevent additional mechanisms from being deployed by the modeller. Si acquisition in diatoms is a case in point; this is not controlled by quotas but by interrelationships between the cell cycle (setting the period of Si deposition) and the availability of silicate (Flynn & Martin-Jézéquel 2000). Two other examples are given below.

3.4.1 Nutrient transport and predator-prey encounters

Often models describe the rate of resource acquisition (V) using a simple (Michaelis-Menten, Monod -like) rectangular hyperbola, with reference to the resource abundance (S), the half saturation constant (K), and the maximum rate (V_{max}).

$$V = V_{max} \cdot \frac{S}{S + K}$$

While intuitively, because of the links between this equation in biochemistry and Monod growth kinetics, it may seem appropriate to use this equation, in reality its use in a dynamic setting controlling organism growth is questionable. This is because of the changes in expression of the transport systems with nutrient stress and satiation, and complications are caused by diffusion at the cell surface that limit transport at very low substrate concentrations (see **Section 2.1**; Flynn et al. 2018).

From a modelling perspective there are two other undesirable consequences:

i) very small changes in substrate concentration have disproportionate effects on the transport rate, which in modelling can require small integration timesteps to resolve,
ii) the maximum transport rate is only possible at extremely high (and perhaps impossible) substrate concentrations.

Because of these complications it may be argued that it is better to deploy a simple linear description of transport:

$$V = MIN\left(V_{max}, V_{max} \cdot \frac{S}{2 \cdot K}\right)$$

Using this equation effectively overcomes the pragmatic challenges in modelling the process, while behaviour at very low resource abundances is suppressed akin to that which occurs through diffusion limitation (Flynn et al. 2018).

At the other extreme, if desired, the DRAMA model may explicitly describe the changes in transport protein abundance in the membrane of the organism that occur during acclimation to stress, and diffusion limitation (Flynn et al. 2018), or the empirical consequences of interactions between P and N metabolism (Helliwell et al. 2021).

The description of predator-prey encounters can similarly be described by any one of various simple mathematical approaches (Gentleman et al. 2003), through to explicitly considering organism sizes and motilities with differential prey preferences (Mitra & Flynn 2006; Flynn & Mitra 2016).

3.4.2 Photoacclimation

The control of photoacclimation is highly complex and also incompletely understood, if not from a physiological view point then certainly in terms of the dynamics (Wilhelm et al. 2014). For modelling, the process of photoacclimation can be simplified in different ways. The approach taken by Geider et al. (1996, 1998) is consistent with evidence that the irradiance value itself is sensed by the cell. However, this approach cannot be used to modulate photoacclimation with concurrent mixotrophy; some form of feedback from the inflow of C from other sources (osmotrophy, phagotrophy) is required.

The approach suggested by Flynn (2001), which related photoacclimation to supply and demand for C offers a route that provides the same empirical description (Flynn et al. 2001) as the Geider et al. approach, but which also suits the needs in modelling mixotrophs. The approaches are not mutually exclusive either; the interplay between the light and dark reactions of photosynthesis that likely plays a role in regulation (Wilhelm et al. 2014) would involve both a direct PFD linkage and also a modulation via the flow of fixed C. DRAMA provides scope for deploying such different mechanisms.

3.5 DRAMA and ‘omics

The interface between data revealed by genomics, transcriptomics and proteomics (‘omics in general) and dynamic modelling is highly problematic (Flynn et al. 2022; Strzepek et al. 2022). In essence: ‘omics provides highly detailed data for the potential of physiological processes, but no rates; modelling requires rate data but typically cannot include much detail.

The meeting of these key methodologies occurs most readily in steady-state flux balance analyses (Orth et al. 2010), but such modelling is not compatible with dynamic modelling as required for ecological research. However, where there is an overlap is in the time-course of the development (de-repression, 'switching-on') of physiological processes in response to changing stresses that impact an organism. These processes are controlled and enacted by very many individual metabolic processes, each of which will link to different 'omic signal; in a model the net emergent property is described. For digital twin applications it is necessary to describe the sequence of de-repressions of key physiological processes.

DRAMA provides a platform to describe these events both with respect to the relative sequence (timing) of them and also the magnitude of the consequences. Just as 'omics describes the potential for physiological processes and may flag the relative rates in some instances (especially when coupled with metabolomics), so the de-repression of physiological processes described by DRAMA (see **Section 2**) indicates potential and not actual rates. The actual rates come from the operation of the model with all of the interconnecting feedbacks.

The level of complexity required in a DRAMA model is dictated by the need. The origins of DRAMA lay in the model of ammonium-nitrate interaction developed by Flynn et al. (1997); that model described transporter and enzyme synthesis for the initial stages of inorganic-N assimilation not phytoplankton. Subsequent analysis of the operation of that model revealed the level to which the model could be simplified (Flynn & Fasham 1997). Similarly, the full DIN-Fe-light model of Flynn & Hipkin (1999) was simplified by Flynn (2001). The building of detailed DRAMA models can thus facilitate and analysis of how best to simplify models to make them computationally compact while retaining critical functionality.

4. DRAMA functionality overview – an example

As an example of the main physiological processes described in a DRAMA model, here we specifically consider the application for a constitutive mixoplankton as that structure is suitably complex (**Fig.4.1)**. A constitutive mixoplankton is a protist that can simultaneously exploit osmotrophy (dissolved organics), phagotrophy (feed on prey), and phototrophy (photosynthesis including the use of internally recycled and externally provided inorganic nutrients). The following sections provide an overview of the components and how their operation is modulated in DRAMA; this model was used in Mitra & Flynn (2023), and for various protist plankton types including phytoplankton and zooplankton in Flynn & Mitra (2023).

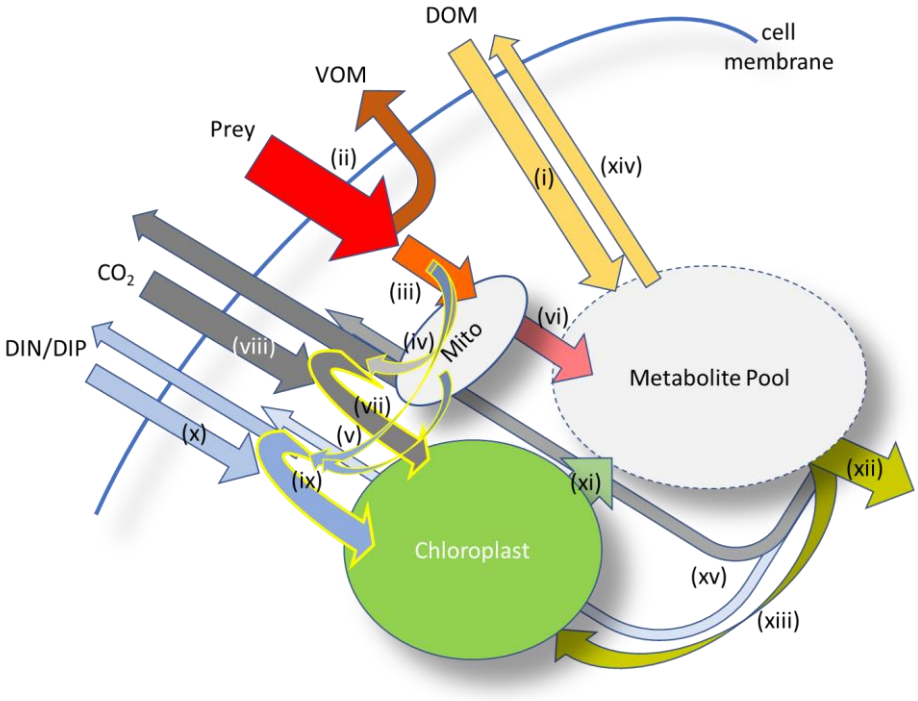

Fig.4.1 Protist plankton resource acquisition mechanisms. Dissolved organic matter (DOM; sugars, amino acids etc.) is taken up (i) and enters the metabolite pool; this action supports osmotrophy. Prey (or other forms of particulate organic matter) are engulfed, and a fraction (ca. 20-40%) egested as voided organic matter (VOM) during digestion (ii). The retained fraction is broken down and a fraction (ca. 30%) is lost through specific dynamic action (iii) as CO_2 (iv) and as dissolved inorganic N (DIN, as ammonium) and dissolved inorganic P (DIP) (v). This anabolic activity is associated with the mitochondria (Mito) and other sub-cellular compartments. The resultant remaining material enters the metabolite pool (vi); this activity, with (ii) and (iii) constitutes phagotrophy. The CO_2 and DIN/DIP lost through specific dynamic action contribute to CO_2 demands for photosynthesis in chloroplasts (yellow edged arrow, vii), with the balance of the CO_2 demand being brought in from outside of the cell (viii). Similarly, any additional demand for DIN & DIP over that supplied by recycling (yellow edged arrow, ix) is brought in from outside (x). Products from phototrophy contribute to the metabolite pool (xi). The total metabolite pool supports biomass growth (xii) including that of the chloroplasts (xiii). Excess metabolites are leaked (xiv), and there are additional losses of CO_2 through respiration, with allied regeneration of DIN (as ammonium) and DIP to maintain cellular stoichiometric balance (xv). The metabolite pool equates to ^{M}C in the model.

4.1 Dissolved resources

The actual description of the kinetics of acquisition, whether it is made a simple hyperbolic function of bulk concentration, considers diffusion etc., may be defined as required by the modeller. DRAMA is used to modulate the expression of the maximum potential for acquisition of a particular resource in organisms of a given physiological state.

All microplankton exhibit uptake of various dissolved resources. The core DRAMA control modulates the expression of the ability to use such resources. Thus, the use of nitrate is repressed as N:C increases, while even the use of ammonium is repressed at a higher value of N:C (see **Section 2.4**). Both inorganic N-sources may be able to support the maximum growth rate. As N becomes limiting, so the ability to use these N-sources (and others) is at first enhanced. In a real organism the transport rate potential may be increased many fold; for ammonium, and for DIP during P-limitation, that enhancement may be an order of magnitude above that needed to support the average needs at the maximum growth rate. This is shown in **Figs. 2.6, 2.9**; in **Fig.2.9** we see that further enhancement in the transport potential can be achieved by the synthesis of more transport porters, and the net consequence is that the half saturation for growth (K_g) is decreased.

In DRAMA, the maximum acquisition potential does not exceed that required to support the maximum growth rate on that nutrient when supplied as the sole source. To then simulate the emergent increase in substrate affinity, the value of cellular Ks is decreased. Examples are given below for controls of the usage of DIP (**Fig.4.2**), ammonium (**Fig.4.3**) and nitrate (**Fig.4.4**).

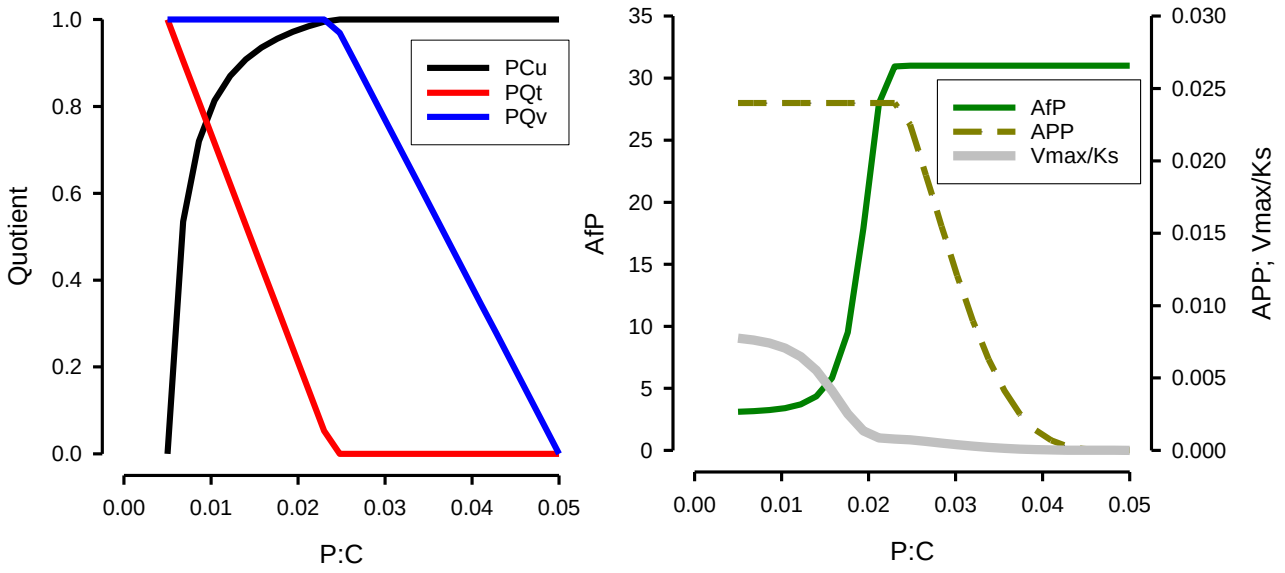

Fig.4.2 Control of DIP acquisition. The value of P:C is used to generate a quotient to define the P-status (PCu, which declines as P:C decreases) and also modulates the potential assimilation rate (PQv) and substrate affinity for transport (PQt) of DIP. In turn, these are used to define the operational maximum acquisition rate (i.e., a Vmax value, APP) and half saturation value Ks (AfP; mgP m^{-3}), with resultant changes in substrate affinity (Vmax/Ks).

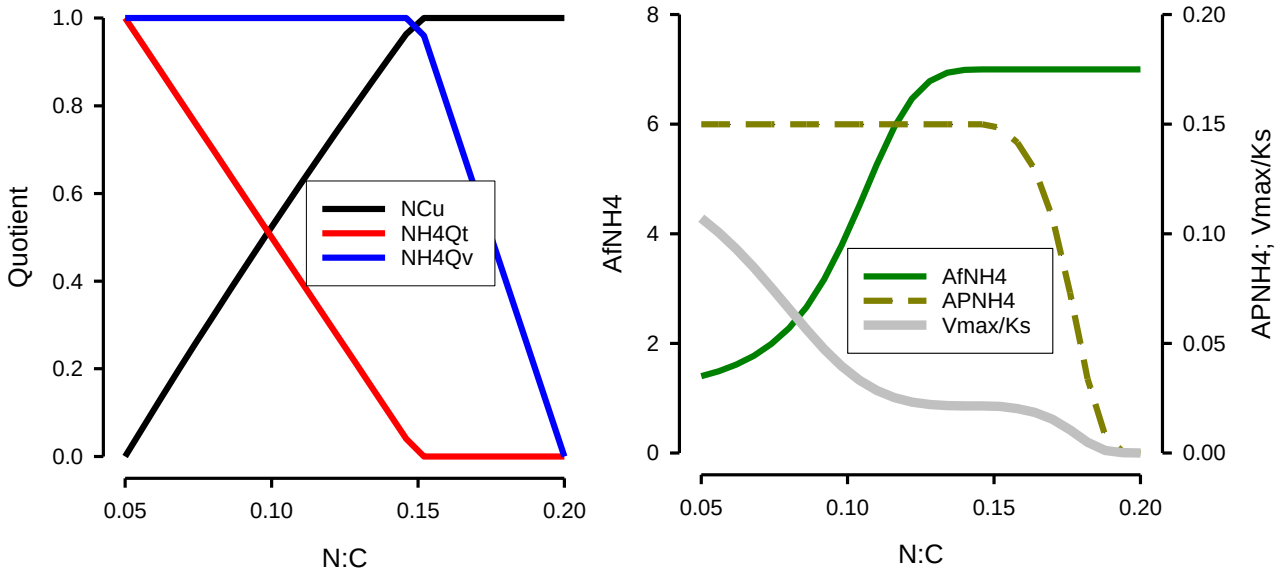

Fig.4.3 Control of ammonium acquisition. The value of N:C is used to generate a quotient that define the N-status (NCu, declining as N:C decreases) and also modulates the potential assimilation rate (NH4Qv) and substrate affinity for transport (NH4Qt). In turn, these are used to define the operational maximum acquisition rate (i.e., a Vmax value, APNH4) and half saturation value Ks (AfNH4; mgN m^{-3}), with resultant changes in substrate affinity (Vmax/Ks).

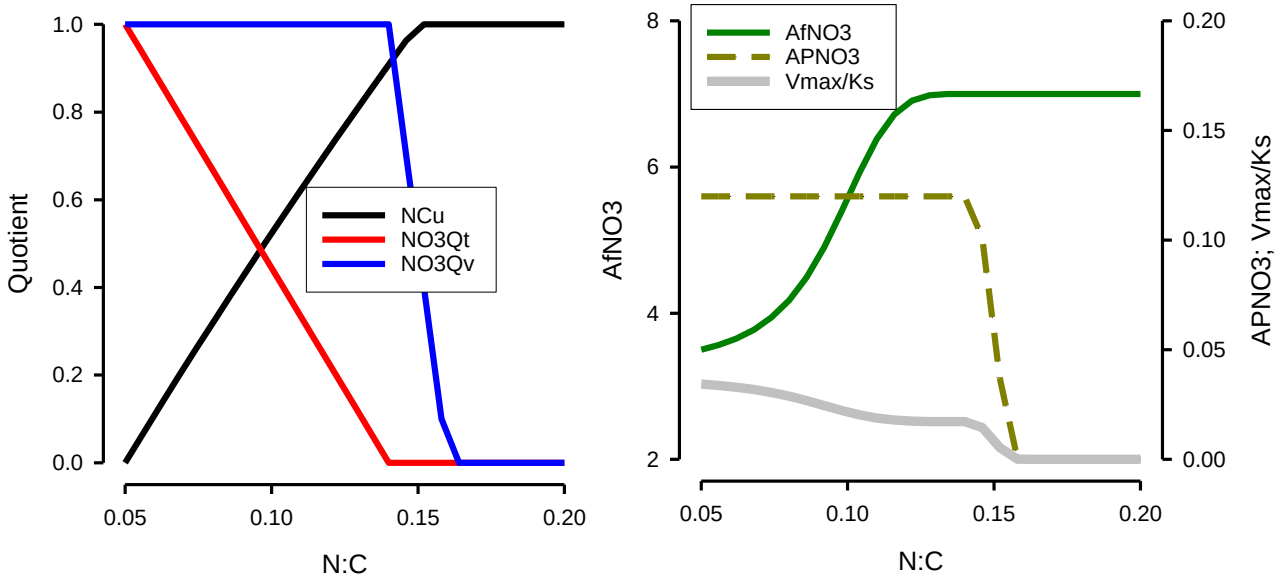

Fig.4.4 Control of nitrate acquisition. This is similar to that for ammonium (**Fig.4.3**), but the maximum nitrate uptake is slower and is enabled at a greater N-stress level (i.e. at a lower N:C).

The control for amino acid acquisition potential is different as this substrate is a source of both C and N (**Fig.4.5**). Here, N:C is used to modulate both a lack of N (low N:C) and also a lack of C (high N:C).

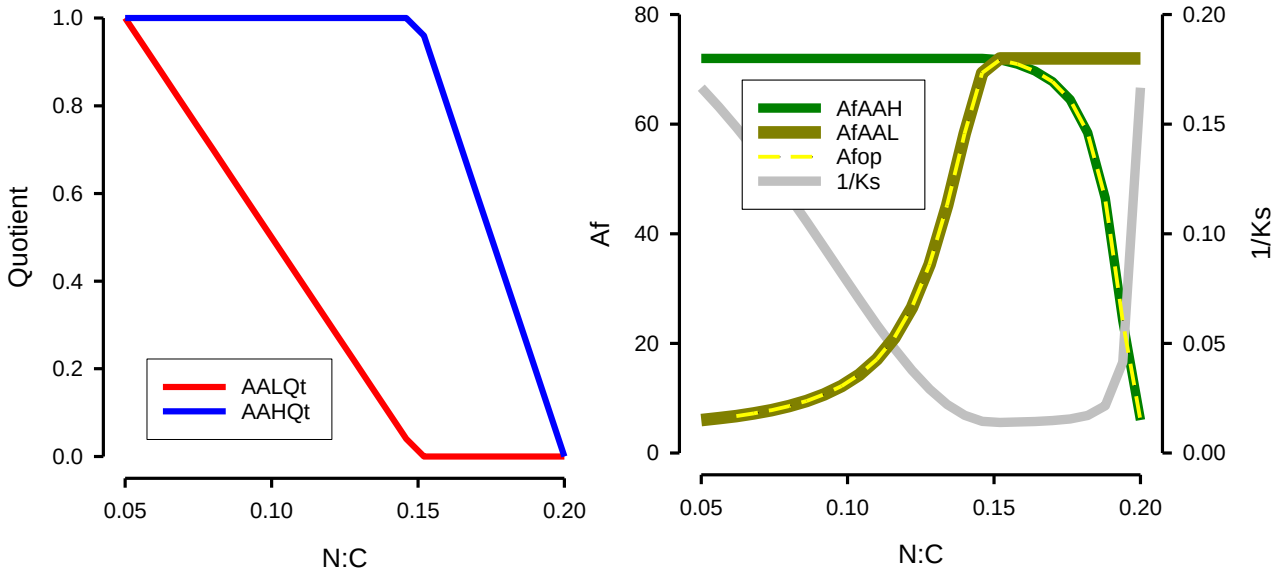

Fig.4.5 Control of amino acid acquisition. The NC-status changes as N:C varies. The value of N:C is used to generate quotients to modulate the potential assimilation rate in response to a lack of N (AALQt) or a lack of C (i.e. too much N; AAHQt). These in turn are used to define the operational half saturation value AfAA for low N-status (AfAAL) and high N-status (AfAAH); the operational value is the lower of these values (Afop = MIN[AfAAL, AfAAH]; mgC m^{-3}), with changes in affinity (1/ Ks) also shown.

4.2 Predation

There are various interactions between resource acquisition via predation and the state of satiation (**Fig.4.6**).

All prey (food) items bring C,N,P into the organism and thus, depending on the driver for predation, this may be modulated by any/all of the quotients describing C, N and/or P status. Because of the homeostatic action of DRAMA, the modulation of each and every potential prey item can be configured as required and the model will respond accordingly. Thus, prey switching in consequence of changes in predator satiation or in nutrient status of the prey items (food quality) can be readily configured and the model will automatically modulate other physiological and behavioural actions as appropriate. For example, with reference to **Fig.3.2**, each of the potential resources R#1 .. #4 could be considered as a different prey type.

The actual description of the kinetics of prey acquisition, whether it is made a simple linear of hyperbolic function of prey availability, explicitly considers encounter rates etc., may be defined as required (see Flynn & Mitra 2016). DRAMA can be used to modulate the expression of the maximum potential for acquisition of a particular resource. This can be achieved explicitly by using different response curves from RelMC for each prey type; this would be required if there was a 'taste' difference, for example. The parameter values (especially the value of K in Eq.2.3) controlling those curves could themselves be made a function of the prey palatability; the curve for an increasingly unpalatable prey items would be shifted to the left, so that it would only be consumed when the predator was more starved. Changing the value of K from ca. 0.2 to 0.8, with H=8, achieves such a

goal. Alternatively (or perhaps, additionally), changes in selection could be made by altering the size range that is predated (**Fig.4.6, plot C**); typically a satiated predator will take larger prey items, and only exploit smaller items as starvation sets in.

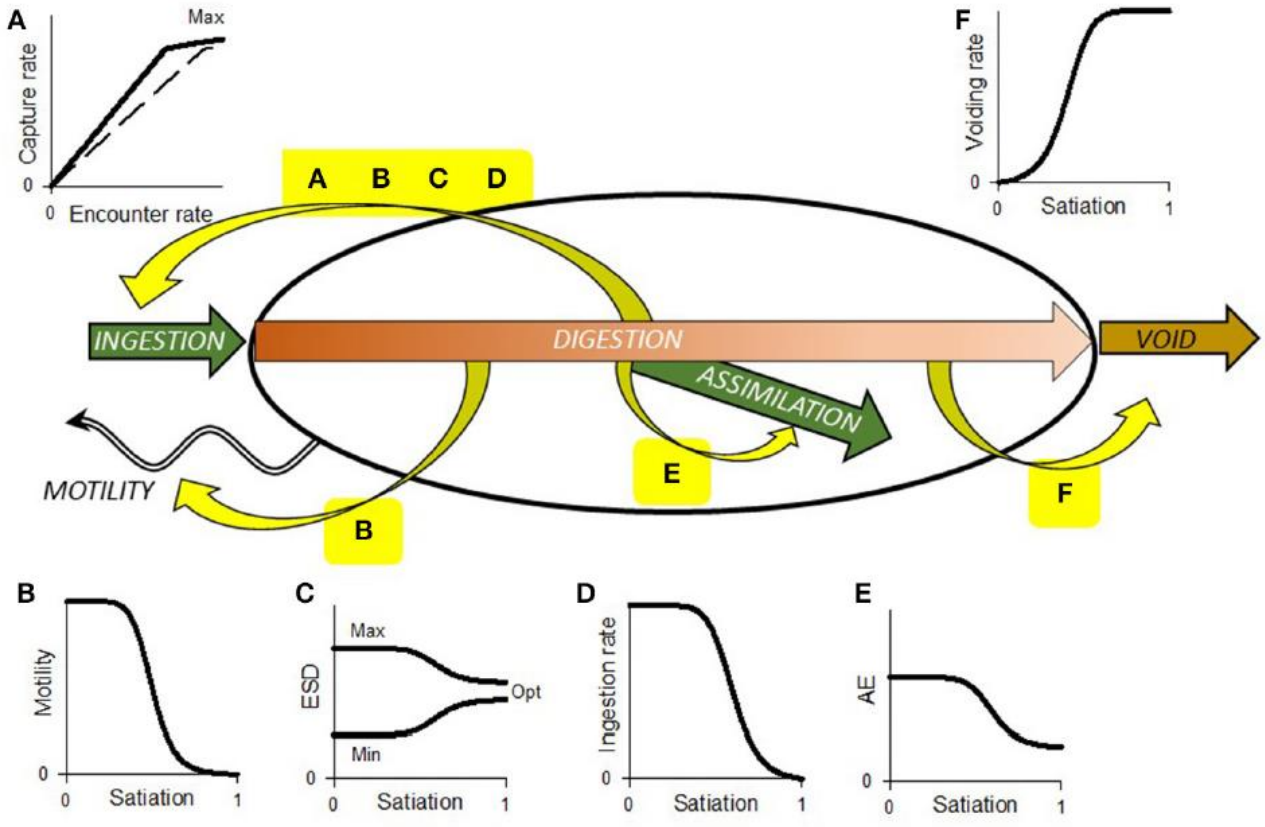

Fig.4.6 Schematic showing a range of interactions between prey (food) encounter, ingestion, digestion, assimilation, and voiding. Yellow curved arrows indicate feedbacks; the sinusoidal arrow indicates consumer motility. Plot (A) shows the relationship between encounter and capture, which is initially linear but in the absence of any other feedbacks would be ultimately limited by handling at the point of capture or ingestion. The dashed line is for a prey type of lesser preference. Depending on preference, several prey types may be consumable over a period of time; consumption of any/all prey types contribute to satiation. Plot (B) shows satiation affecting motility, which in turn affects encounter rate (A). Set against this feedback is the need for some consumers to move for reasons other than feeding, and motion of the prey relative to the consumer caused by their own motility and/or turbulence. Plot (C) shows how prey preference, indicated here by their size selectivity, may be expected to tighten as satiation develops. Plot (D) shows how gut satiation itself also controls ingestion rate, effectively decreasing the maximum level (plateau value) of capture indicated in plot (A). Plot (E) shows assimilation efficiency (AE) declining with satiation, so that in the presence of abundant food digestion is less complete. Plot (F) shows how, especially in a peristaltic gut, satiation promotes voiding. This works with €, to counter (D) to enhance the flow of food through the digestive system. The relationship between prey abundance (per unit of space) and ingestion rate is an emergent property of all these interactions, coupled to other facets such as food quality and prior nutritional history of the consumer. From Flynn & Mitra (2016).

Because growth is a function of the combined levels of organismal C,N,P satiation (defined by CCu, NCu and PCu), and ingestion is also a function of RelMC, the addition of a food quantity function to decrease assimilation efficiency (AE, as in Mitra & Flynn, 2007; Flynn 2009) is simple to implement. This may also include decreasing the rate of motion (swimming; **Fig.4.6, plot B**). At high prey availability, decreasing AE leads to density-dependant inefficiency (Flynn 2009), which has important ramifications for the conversion of prey-biomass to that of the predator and for the production of voided waste materials. This increased loss of material with a lower AE (**Fig.4.6, plot E**) will delay the onset of the satiation control of ingestion. Again, akin to reality, these changes can be enacted and the DRAMA model will react through feedbacks to balance supply and demand.

The use of the ingested material may also be considered as applying to the whole diet (e.g., with respect to the bulk ingested C:N:P) or with reference to each component, if that was required. By adding another state variable for essential fatty acids (with units of mgC m^{-3}), the growth rate can be further modulated by reference to fatty acids in addition to C,N,P.

4.3 Photosynthesis

Photosynthesis is typically defined in plankton models with reference to the initial slope of the photosynthesis-irradiance (PE) curve, 'alpha', which is associated with photopigment levels, the plateau of the PE curve, 'P_{max}', associated with RuBisCO and other enzymatic activities, and at high light with photodamage. In addition to the description of photopigment content (at least with reference to Chl), DRAMA enables inclusion of an explicit description of RuBisCO and other structural parts of the photosystem. This inclusion comes at the computational cost of adding another state variable.

The control of the configuration of the photosystems, and hence the rate of photosynthesis, can be modulated directly from the value of RelMC (**Fig.3.3**), used to control the synthesis and decay of photosystems (developed from Flynn & Hipkin 1999). Photodamage can be described in a dynamic sense, with the recycling of resources between state variables for the metabolite pool (^{M}C) and those for the photosystem components. These processes are not considered further here due to its inherent complexity, but it can be seen from **Fig.3.3**, and the allied text, that the control of the relative priority of C acquisitions from osmotrophy, phagotrophy (for mixoplankton) and photosynthesis can be achieved by suitable configuration of the functions relating to the value of RelMC (i.e. to ^{M}C:C; see also Mitra & Flynn 2023).

4.4 Loss functions

Net resource acquisition, as indexed to the values of CCu, NCu and PCu, together with the value of RelMC controls growth. There are also various loss processes to be considered as well.

The leakage of metabolites can be made a function of the value of RelMC, with appropriate stoichiometric drains also on N and P. Such materials released from the plankton are available for recovery through osmotrophy, permitting a description of the leak-recovery dynamics described with feedback functionality by Flynn & Berry (1999).

Voiding of particulate material from predation (**Fig.4.6 plots E/F**) likewise can be made functions of satiation, while recovery of part-consumed materials (as noted by Flynn & Davidson 1993) will be induced by a widening of the prey size spectrum for starved consumers (**Fig.4.6, plot C**).

In all instances, changes in loss rates may be simulated as being countered or exacerbated by changing the physiological dynamics, just as they may in reality.

4.5 Controlling physiological processes

The normalised values of the nutrient quotas (i.e., 0 at minimum quota, 1 at optimal quota) for C, N and P (i.e., CCu, NCu, PCu) are used to control physiological processes. In essence these controls replicate the biochemical events of (de)repression and, consistent with allosteric controls, exploit sigmoidal curve functions. For example, the N:C quota value is used to controls the use of ammonium and nitrate (Flynn et al. 1997; Flynn 2001).

The example described here applies to a mixoplankton modulating phototrophy and phagotrophy (deployed in Flynn & Mitra 2023); the value of RelMC (defined as $^{M}C:^{T}C$) is used to modulate the (de)repression of these processes (**Fig.4.7**).

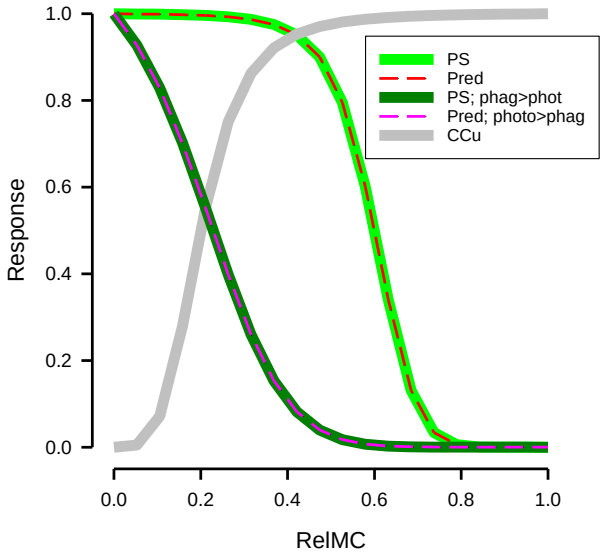

Fig.4.7 Control of growth, resources acquisition via phototrophy and phagotrophy through reference to the value of RelMC. The relative availability of C for growth is defined by CCu, increasing as the relative size of the metabolite pool (RelMC) increases. Curves describing the (de)repression of phototrophy and phagotrophy are shown for when they are similar (PS and Pred respectively), and also for when either of these processes is de-repressed when the cell is of a lower C-status (i.e. RelMC is lower). Shifting these curves provides a mechanism to alter the balance of phagotrophy vs phototrophy ('phag>phot', or 'phot>phag'). Note that the cross-over point between CCu and the resource acquisition trophic control curves affects the potential maximum growth rate attainable when solely exploiting that trophic mode. For example, when phagotrophy provides a backup mechanism for when phototrophy cannot supply sufficient C to maintain a high enough RelMC ('Pred; photo>phag'), this aligns with a growth rate maximum exploiting phagotrophy of ca. 50% (Response ≈0.5). From Flynn & Mitra (2023).

The curve form for CCu in **Fig.4.7** is:

$$Ccu = (1 + CCuK^{CCuH}) \cdot \frac{RelMC^{CcuH}}{RelMC^{CcuH} + CcuK^{CcuH}}$$

For the curve shown in **Fig.4.7**, CCuH=4, CcuK=0.2. The model is not sensitive to the value of these parameters.

The generic curve form for the resource acquisition controls is:

$$Con = (1 + K^H) \cdot \frac{(1 - RelMC)^H}{(1 - RelMC)^H + K^H}$$

For the PS and Pred curves shown in **Fig.4.7**, H=8 and K=0.4. For the more repressed curves ('phag>phot', or 'phot>phag'), H=8 and K=0.8.

For phagotrophy (which brings in a complete package of C,N,P) the above described control dominates regulation of the activity. However, phototrophy is also controlled by the acquisition of N (as ammonium and nitrate) and P (as phosphate); those interactions are modulated by N:C and P:C respectively. Phototrophy is further affected by synthesis and operation of the photosystems, which are modulated via RelMC and N:C.

5. Functional equations – an example

As an example, the following provides functional equations for a generic constitutive mixoplankton model that considers C,N,P,Chl. This example is given because in the one organism there is the coupling of osmotrophy, phagotrophy and phototrophy (as per **Fig.4.1**). For descriptions of phytoplankton (osmotrophy + phototrophy), protistan zooplankton (osmotrophy + phagotrophy), or metazoan zooplankton (feeding in place of phagotrophy), the unused components are deleted.

These equations are textual, to provide an initial level of conceptual understanding prior to the development of mathematical equations to suit the purpose at hand.

5.1 State variables

The model comprises the following state variables and associated flows:

Core structure: $prot^{C}C$; mgC m^{-3}

d $prot^{C}C$/dt = {anabolism} – {catabolism when ^{M}C:^{T}C is critically low}

Metabolic pool of C: $prot^{M}C$; mgC m^{-3}

d $prot^{M}C$/dt = {osmotrophy(C)} + {phagotrophy(C)} + {phototrophy} + {degradation of $prot^{PM}C$} – {synthesis of $prot^{PM}C$} – {anabolism} – {catabolism} – {NO3-assimilation(reductant)} – {DOM(C)-leak}

C in photosystems : $prot^{PM}C$; mgC m^{-3} (this sets the maximum rate of photosynthesis, Pmax)

d $prot^{PM}C$/dt = {synthesis of ^{PM}C} – {degradation of ^{PM}C}

Photopigments : protChl; mgChl m^{-3}

d protChl/dt = {synthesis of Chl} – {degradation of Chl}

Organism-P : protP ; mgP m^{-3}

d protP/dt = {osmotrophy(P)} + {phagotrophy(P)} + {DIP-assimilation} – {P-regeneration} – {DOM(P)-leak}

Organism-N : protN; mgN m^{-3}

d protN/dt = {osmotrophy(N)} + {phagotrophy(N)} = {NH4-assimilation} + {NO3-assimilation} – {N-regeneration} – {DOM(N)-leak}

Depending on the application, additional state variables are included, together with associated equations for resource acquisition and exploitation :

- **protCells** (cells m^{-3}): cells, required for a dynamic description of cell-size with nutrient status, diel light cycle and temperature
- **protSi** (mgSi m^{-3}); organism-Si, required for diatoms
- **protANA** (mgNA m^{-3}); acquired nucleic acid material from phototrophic prey, required to support acquired phototrophy in plastidic specialist non-constitutive mixoplankton (pSNCM)
- **protFe** (mgFe m^{-3}); organism Fe required to involve Fe-limitation

5.2 Rate processes and controls

NOTE: In what follows, for brevity, state variable names (noted in **Section 5.1**) in the equations do not include the prefix 'prot'.

Functional-equation description of the model are given as text strings, with the form:

result = *f*{comma delimited list of factors involved in deriving the result}

Underlined terms in the equations are rates. Those in bold donate terms that provide a positive interaction (i.e., the result increases when the term increases; these are usually enacted via a curvi-linear function); terms not in bold may involve negative or more complex interactions (such as bell-shaped for prey allometry affecting capture). See **Section 4** for more information.

The equations are provided working backwards from the emergent organism growth rate, with descriptions of the steps enabling that rate to be attained.

Total protist biomass C (^{T}C) is given as, $^{M}C+^{PM}C+^{C}C$.

5.2.1 Growth and nutrient status

Ultimately growth is a function of the nutritional status of the organism (in terms of elements C, N and P) and the maximum growth rate potential. The latter varies with temperature, T, around the value of μ_{max} at a reference temperature, μ_{maxRT}.

$\underline{\text{Growth}}$ = *f*{**C-status**, **N-status**, **P-status**, $\underline{\boldsymbol{\mu_{max}}}$, $\underline{\text{losses}}$}

$\underline{\boldsymbol{\mu_{max}}}$ = *f*{$\underline{\boldsymbol{\mu_{maxRT}}}$, **T**}

The nutrient quotas define the health of the organism in terms of C ($^{M}C:^{T}C$), N ($N:^{T}C$) and P ($P:^{T}C$), and is a function of various inputs and outputs. Inputs are associated with the use of dissolved organic substrates via osmotrophy, prey via phagotrophy, and also the use of inorganics via phototrophy. Losses occur through respiration and regeneration, and also through the leakage of

metabolites as dissolved organic matter (DOM), some of which may be recovered via osmotrophy (**Fig.4.1**).

C-status = *f*{**osmotrophy**, **phagotrophy**, **phototrophy**, C-respiration, DOM-leak}

N-status = *f*{**osmotrophy**, **phagotrophy**, **phototrophy**, **DIN assimilation**, N-regeneration, DOM-leak}

P-status = *f*{**osmotrophy**, **phagotrophy**, **phototrophy**, **DIP assimilation**, P-regeneration, DOM-leak}

Losses = *f*{**C-respiration**, **N-regeneration**, **P-regeneration**, **DOM-leak**}

Growth is associated with catabolic (including basal) and anabolic respiration, part of which is associated with specific dynamic action (SDA, McCue 2006) during prey digestion and assimilation. Anabolic respiration is affected by the flows of resources via the different trophic mechanisms. Nitrate assimilation incurs an additional cost for reduction of nitrate to nitrite to ammonium. There are also losses of C, N, P required to preserve organism stoichiometry within the bounds of acceptable C:N:P.

C-respiration = f{μ_{max}, **basal respiration**, prot-C:N, **C-assimilation**, **NO3-assimilation**}

C-assimilation = f{**osmotrophy**, **phagotrophy**, **phototrophy**}

N-regeneration = f{**C-respiration**, **prot-C:N**, **prey-C:N**, **digestion**, SDA}

P-regeneration = f{**C-respiration**, **prot-C:P**, **prey-C:P**, **digestion**, SDA}

DOM-leakage is closely associated with osmotrophy (see further below).

Cell division occurs when the cell reaches a critical size (which varies with nutrient status and temperature affecting the growth rate), and typically occurs in phototrophs within a specific part of the diel light:dark (LD) cycle.

Division = *f*{**size**, critical size, LD}

critical size = *f*{T, C-status, N-status, **P-status**, **growth**}

The size of the organism affects predation for phagotrophy, and whether it itself is likely to encounter its own predator.

5.2.2 Osmotrophy

Osmotrophy depends on the concentration of the substrate, [DOM], the C:N:P status of that material, and the uptake kinetics parameters of the maximum uptake rate ($^{DOM}V_{max}$) and the

substrate affinity (i.e., $^{DOM}V_{max}/K_{DOM}$). The uptake kinetics depend on the nutrient status of the organism; cells that are nutrient-stressed have a higher uptake potential and a higher affinity.

Osmotrophy = *f*{**[DOM]**, DOM-C:N:P, **$^{DOM}V_{max}$**, **$^{DOM}V_{max}/K_{DOM}$**}

$^{DOM}V_{max}$ = *f*{C-status, N-status, **μ_{max}**}

$1/K_{DOM}$ = *f*{C-status, N-status}

Against the gains from osmotrophy, there are losses with the leakage of DOM. At especially high growth rates, which require a high nutrient status and hence a replete internal metabolite pool containing mM concentrations of dissolved organics, DOM inevitably leaks. Osmotrophy may recover some of that leakage. The net leakage of N-containing DOM (as amino acids) is most significant during N-replete growth conditions, while leakage of DOC (sugars) occurs especially with high rates of phototrophy, including when N becomes exhausted and the cell has yet to down-regulate photosynthesis.

DOM-leak = f{C-status, N-status, **osmotrophy**, **phagotrophy**, **phototrophy**, **μ_{max}**}

5.2.3 Phagotrophy (feeding) and voiding

Here the term 'phagotrophy' is used, but feeding by metazoan zooplankton would be handled in a similar way.

Phagotrophy brings in resources from the assimilation of prey biomass. Prey need to be encountered (which depends on the sizes of the predator organism and of the prey, their respective motilities and turbulence), captured (which, like the predator motility, varies with satiation, and also with the 'taste' of the prey as affected by its stoichiometric quality) and then ingested. These processes are prey-species specific; the collective biomass from many ingestions, perhaps of different prey organisms, is then digested. During digestion a fraction of the ingested prey is subjected to voiding; this depends on the assimilation efficiency (AE), predator satiation and the food quality. Another fraction is lost associated with specific dynamic action (SDA, McCue 2006) as the prey biomass is subjected to catabolic and then anabolic processes. The internal recycling of regenerated inorganic nutrients is a critical physiological feature of mixoplankton; see *Inorganic nutrient assimilations*, below.

Phagotrophy = *f*{**prey-assimilations**}

prey-assimilation = *f*{**digestion**, SDA}

digestion = *f*{**ingestion**, voiding, **prey C:N:P**, **μ_{max}**}

ingestion = *f*{**capture**, **μ_{max}**}

capture = *f*{C-status, N-status, P-status, **prey quality**, prey allometry}

encounter = *f*{**[prey]**, allometry, **motility**, **prey motility**, **turbulence**}

motility = *f*{C-status, N-status, P-status}

voiding = *f*{**ingestion**, **C-status**, **N-status**, **P-status**, prey quality, AE}

5.2.4 Phototrophy

Photosynthesis depends on light, the availability of dissolved organic C (DIC, especially as CO_2 and HCO_3^-), photopigment content (Chl:C), the value of alpha governing the initial slope of the light-photosynthesis curve, and the maximum rate of C-fixation (P_{max}). Traditional simple plankton models resort to describing the empirical photosynthesis-irradiance curve (Jassby & Platt 1976), while limitation by DIC can be described using a rectangular-hyperbolic function (Clark & Flynn 2000). To describe the process in any detail, however, requires inclusion of numerous feedback interactions.

The value of P_{max} is set by the size of $^{PM}C:^{T}C$. For organisms with a constitutive ability to photosynthesise, both Chl:C and P_{max} are modulated by the demand for C and energy, reflected by the organisms nutritional status and growth rate potential. For non-constitutive mixoplankton (NCM), phototrophy is acquired from captured phototrophic prey. Light is a function of the photon flux density at the water surface and of attenuation within the water (which varies with the biomass of the pigmented organisms). Light also varies over the diel light:dark cycle; this imparts a diel cycle on phototrophy and hence the size of RelMC (C-status), that then works through to affect osmotrophy and phagotrophy.

Phototrophy = *f*{light, **[DIC]**, **Chl:C**, **alpha**, **P_{max}**}

Chl:C = *f*{C-status, **N-status**, (for NCM, prey-Chl:C, capture)}

P_{max} = *f*{C-status, **N-status**, P-status, **μ_{max}** (for NCM, prey-P_{max}, capture)}

5.2.5 Inorganic nutrient assimilations

For mixoplankton, inorganic nutrients are sourced both internally, as regenerative products of prey assimilation, and externally; use of the former takes priority and will be affected by prey C:N:P. The use of external nutrients depends on the substrate concentrations ([DIP], [NH4], [NO3]) and the respective uptake kinetics (V_{max}, affinity). The latter vary with the nutritional state of the organism, with uptake potential enhanced when nutrient-stressed and, at the extreme, shut down if nutrient-replete (i.e. V_{max} tends to zero at elevated nutrient status).

DIP assimilation = f{prey-assimilation, prey C:N:P, SDA, **[DIP]**, **$^{DIP}V_{max}$**, **$^{DIP}V_{max}/K_{DIP}$**}

$^{DIP}V_{max}$ = f{P-status, **μ_{max}**}

$1/K_{DIP}$ = f{P-status}

The uptake of DIN is affected also by the P-status of the organism. The uptake kinetics for ammonium (NH4) provide for development of a much enhanced uptake capability over that for

nitrate (NO3), with that development also commencing at a higher N-status. The latter results in ammonium being taken up 'in preference' to nitrate. There is no 'inhibition' term controlling NO3 assimilation by [NH4]; if the supply of ammonium from internal recycling plus external sources cannot meet the demand, such that organism N:C declines, then the ability to use nitrate is de-repressed.

DIN assimilation = f{prey-assimilation, prey C:N:P, SDA, **NH4-assimilation**, **NO3-assimilation**}

NH4-assimilation = f{**[NH4]**, $^{NH4}\mathbf{V_{max}}$, $^{NH4}\mathbf{V_{max}/K_{NH4}}$}

NO3-assimilation = f{**[NO3]**, $^{NO3}\mathbf{V_{max}}$, $^{NO3}\mathbf{V_{max}/K_{NO3}}$ }

$^{NH4}V_{max}$ = f{N-status, P-status, $\boldsymbol{\mu_{max}}$}

$1/K_{NH4}$ = f{N-status}

$^{NO3}V_{max}$ = f{N-status, P-status, $\boldsymbol{\mu_{max}}$}

$1/K_{NO3}$ = f{N-status}

6. Example configurations of the DRAMA concept for different applications

The above sets the basis for the Dynamic Resource Assimilation & Modulated Activity (DRAMA) model. The DRAMA model concept seeks to provide a flexible form for describing growth of an organism exploiting different types of resources in a fashion that meets with empirical evidence and is consistent with the mechanistic underpinnings of biochemistry.

A DRAMA model contains at least 2 state variables, defining the metabolic and core structural biomass. Usually these will be defined in terms of C as carbon is the base unit in biochemistry of Earth organisms. To these state variables are then added 1 state variable for each additional element or nutrient type that is described in the stoichiometry of the organism. An overview of the form for a C,N,P application is given in **Fig.6.1**. To this structure could include additional nutrient types that the organism cannot synthesise; for example for essential fatty acids or a vitamin.

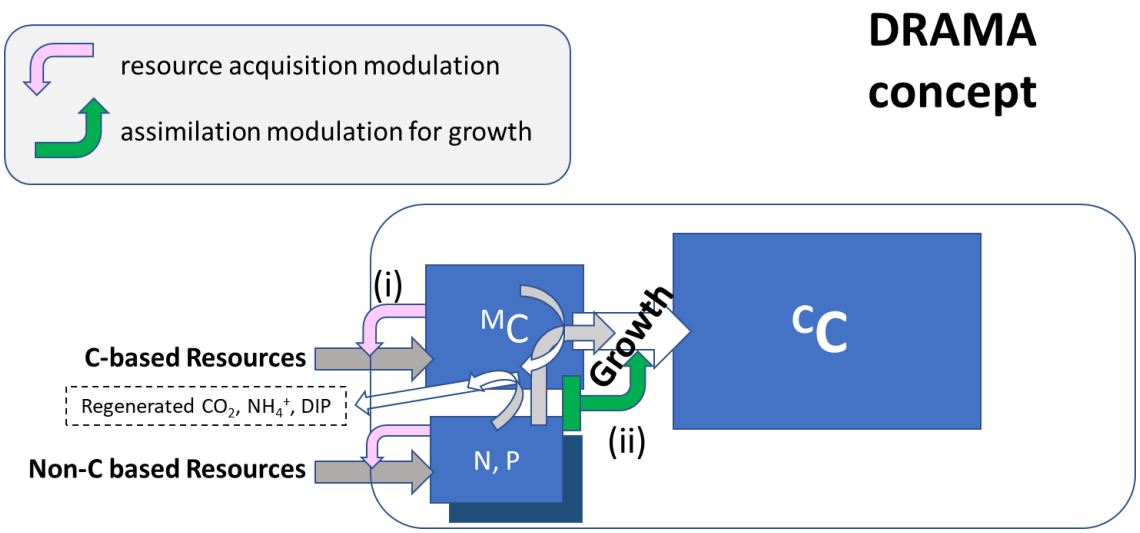

Fig.6.1 Schematic of the Dynamic Resource Assimilation & Modulated Activity (DRAMA) model. The C-biomass of the organism is described as comprising a metabolic pool (^{M}C) plus a core structural pool (^{C}C). Each of these comprises a state variable in mathematical terms. The relative size of ^{M}C compared with the total ($^{M}C+^{C}C$), as the ^{M}C:C quota, defines the level of satiation with respect to C and feeds back (i) to modulate the potential to acquire different sources of C, and feeds forwards (ii) to modulate growth of ^{C}C. Additional stoichiometric needs require an additional state variable each; N and P are shown, but this could include Si, Fe, fatty acids or others. The relative presence of these additional components to total C (e.g., N:C) is used to modulate the potential to acquire those components and also to module growth, as indicated. The rate and efficiency of assimilation (ii) may also be vary between resource types, differentially affecting emergent growth and loss rates.

The DRAMA structural concept readily lends itself to descriptions of:

- Heterotrophic bacteria and archaea
- Cyanobacteria (diazotrophic nitrogen-fixers and otherwise)
- Protist zooplankton (osmo-phago-trophic)
- Phytoplankton (osmo-photo-trophic; e.g. diatoms and also certain other microalgae)

- Mixoplankton (protists that exploit osmo-phago-photo-trophic nutritional routes)
- Zooplankton (with addition of appropriate age-stage descriptions)
- Higher consumers (with addition of appropriate age-stage descriptions)

Some example configuration schematics are shown in **Fig.6.2**. In all instances, as befits the DRAMA concept, the organism is considered as comprising two main components; a core component (^{C}C) and a metabolic component (^{M}C). C-based resources initially enter ^{M}C, from whence losses are incurred associated with the reconfiguration of those resources into products that build the core. For photosynthetic organisms, such losses include building photosystems (^{PM}C), though that can be converted (recycled) back to ^{M}C if the need for photosynthate decreases.

Typically the units for the state variables will be mgC m^{-3}, mgN m^{-3}, mgP m^{-3}, mgChl m^{-3}, as appropriate.

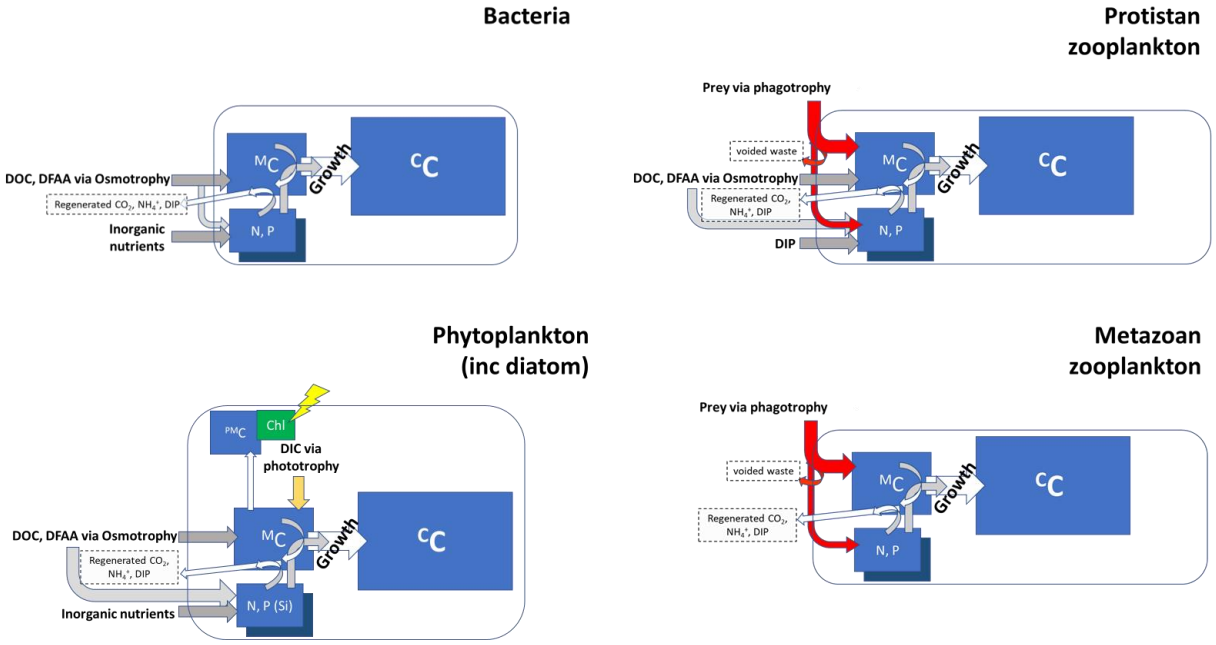

Fig.6.2 Schematic of the DRAMA concept as configured for bacteria (osmotrophy and use of inorganics), protozooplankton (phagotrophy plus osmotrophy), phytoplankton (osmotrophy, use of inorganics and phototrophy), and metazooplankton (food ingestion). In all instances, carbon first enters a metabolic pool (^{M}C) the size of which affects acquisition of resources and growth (see **Figs.3.1** & **3.2**). For phytoplankton, C is also used to synthesise the photosynthetic machinery (^{PM}C) which includes RuBisCO and also the synthesis of Chl. The size of ^{M}C relative to the total (as ^{M}C:C) controls growth of the core biomass (^{C}C), affected also by N and P (and for diatoms, Si) acquisition. Materials are voided as particulates as a by-product of phagotrophy, and regenerated as inorganics from respiratory processes. Not shown is the leakage of excess production of DOC and DFAA.

A schematic of a single DRAMA model that can be configured to describe any of constitutive mixoplankton, generalist non-constitutive or specialist non-constitutive mixoplankton is shown in **Fig.6.3**.

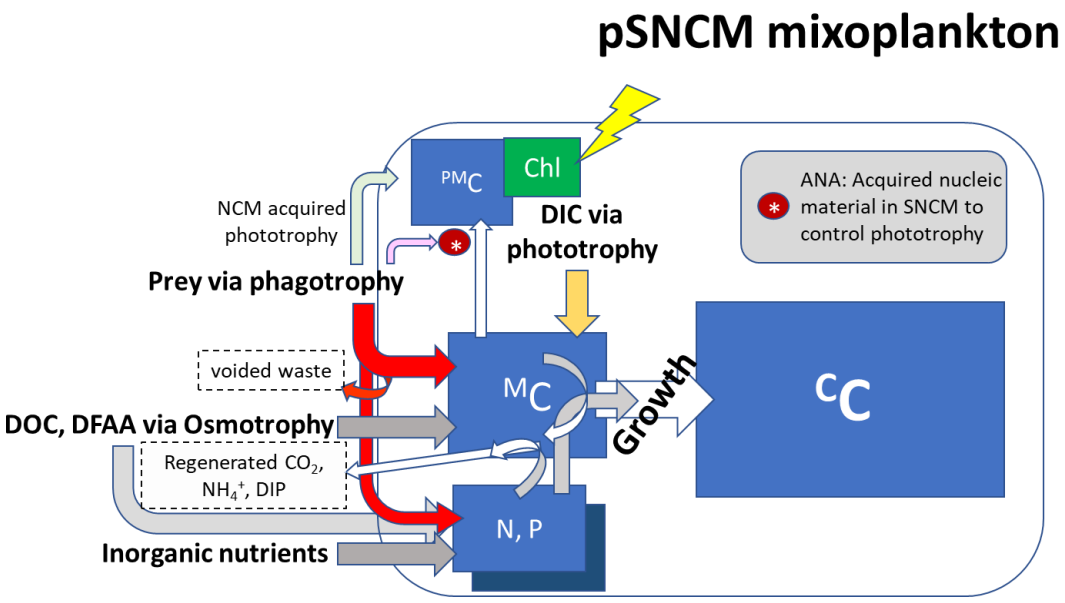

Fig.6.3 Schematic of the DRAMA concept as configured for a plastidic specialist non-constitutive mixoplankton (pSNCM). Carbon enters a metabolic pool (^{M}C) via osmotrophy, phagotrophy and/or phototrophy. While constitutive phototrophs have full control over the synthesis of the photosynthetic machinery (^{PM}C) and Chl, pSNCMs also acquire some level of control over phototrophy by acquisition of nucleic material (ANA) from their special prey. Acquired phototrophy decays over time in pSNCM. The size of ^{M}C feeds back to control resource acquisition and also, for constitutive phototrophs and specialist non-constitutive mixoplankton, to control synthesis of ^{PM}C and Chl. The size of ^{M}C then controls growth of the core biomass (^{C}C), affected also by N and P acquisition. Materials are voided as particulates as a by-product of phagotrophy, and regenerated as inorganics from respiratory processes. Not shown is the leakage of excess production of DOC and DFAA.

7. Example DRAMA deployment; protist-DRAMA

Shown here are some example model output for protist plankton described using the DRAMA concept. The models were run in a testbed for consideration of autecology; thus prey could be included, but they were not modelled as growing (i.e., they were are akin to particulate resources analogous to dissolved nutrients) and there was no scope for the description of trophic interactions in the testbed. There are 2 prey types described here, which could be independently configured (including their motility and photosystem status). The prey could be configured as bacteria, for example, with no photosystems and an equivalent spherical diameter (ESD) of ca. 1µm. However, none of these prey grow; they are *de facto* present as a form of nutrient resource. For the GNCM and pSNCM they are also the source of acquire phototrophy. For more information on mixoplankton types, see Flynn et al. (2019) and Mitra et al. (2023a).

The following pages provide example output plots from the protist-DRAMA model run to simulate different protist types.

1. **Protozooplankton** (osmotrophy + phagotrophy) (**Fig.7.1**)
2. **Generalist Non-Constitutive Mixoplankton – GNCM** (osmotrophy + phagotrophy + acquired phototrophy; it needs to eat phototrophic prey and the photosystems of the prey set the potential for phototrophy in the GNCM – that ability decays rapidly) (**Fig.7.2**)
3. **plastidic Specialist Non-Constitutive Mixoplankton - pSNCM** (osmotrophy + phagotrophy + acquired phototrophy; it needs to eat a special phototrophic prey {here, that is prey#2} to acquire an ability for phototrophy – that ability decays relatively slowly) (**Fig.7.3**)
4. **Constitutive Mixoplankton - CM** (osmotrophy + phagotrophy + phototrophy) (**Fig.7.4**)
5. **Phytoplankton** (osmotrophy + phototrophy); may be configured as a non-diatom (**Fig.7.5**) or diatom (**Fig.7.6**)

Parameter names are as follows, arranged in the order of encountering them in the figures, working left-to-right and top-to-bottom through the panels. DL – dimensionless.

Name	**Unit**	**Explanation**
sysC	mgC m^{-3}	total system C
DOC	mgC m^{-3}	labile dissolved organic C
AA_C	mgC m^{-3}	dissolved amino acid C
DIC	mgC m^{-3}	dissolved inorganic C
C_prey1	mgC m^{-3}	prey#1 C
C_prey2	mgC m^{-3}	prey#2 C
VOC	mgC m^{-3}	voided organic C
ProtCTot	mgC m^{-3}	total protist C (i.e., $^{M}C + {}^{C}C$)
ChlC	g g^{-1}	chlorophyll : total protist C content
Rubpc	g g^{-1}	photosystem : total protist C content
sysN	mgN m^{-3}	total system N
AA_N	mgN m^{-3}	dissolved amino acid N
NH4	mgN m^{-3}	ammonium-N
NO3	mgN m^{-3}	nitrate-N
N_prey1	mgN m^{-3}	prey#1 N
N_prey2	mgN m^{-3}	prey#2 N
VON	mgN m^{-3}	voided organic N
protN	mgN m^{-3}	total protist N
sysP	mgP m^{-3}	total system N
DIP	mgP m^{-3}	dissolved inorganic P
P_prey1	mgP m^{-3}	prey#1 P
P_prey2	mgP m^{-3}	prey#2 P
VOP	mgP m^{-3}	voided organic P
protP	mgP m^{-3}	total protist P
GCu	gC gC^{-1} d^{-1}	gross C-specific growth
Cfix	gC gC^{-1} d^{-1}	photosynthesis
BR	gC gC^{-1} d^{-1}	basal respiration
mavgCu	gC gC^{-1} d^{-1}	24hr moving average growth
UmRT	gC gC^{-1} d^{-1}	maximum growth rate at reference temperature
UmT	gC gC^{-1} d^{-1}	maximum growth rate at current temperature
Cfix	gC gC^{-1} d^{-1}	photosynthesis
mavgnetPS	gC gC^{-1} d^{-1}	24hr moving average photosynthesis
NCu	DL	N-status (1 being optimal)
PCu	DL	P-status (1 being optimal)
SCu	DL	diatom Si-status (1 being optimal)
NPSiCu	DL	N,P,Si,C-status (1 being optimal)
relU	DL	relative growth rate (1 being = UmT)
CCu	DL	C-status (1 being optimal)
cells	cell L^{-1}	cell abundance

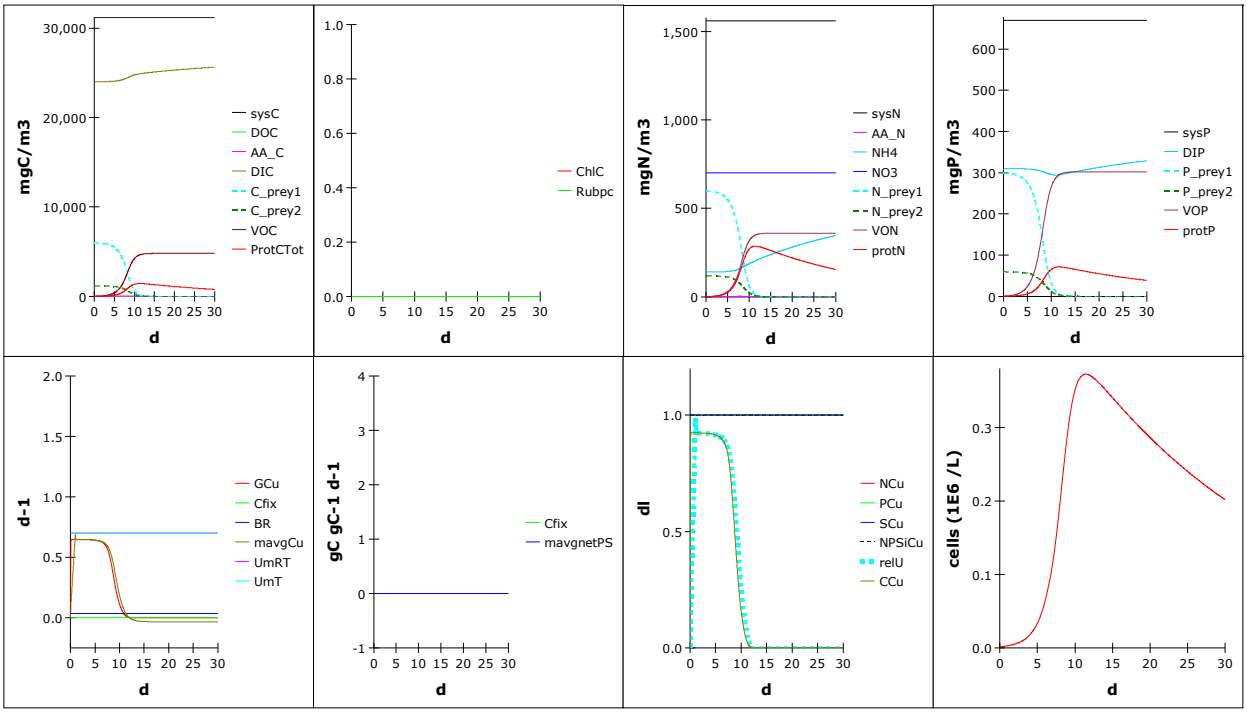

Fig.7.1 Example output for a protozooplankton. There is no photosynthesis and hence no ChlC nor RuBisCO. The two prey types are consumed, with the accumulation of voided waste and regenerated nutrients. On exhaustion of the prey, the protozooplankton respires itself to death.

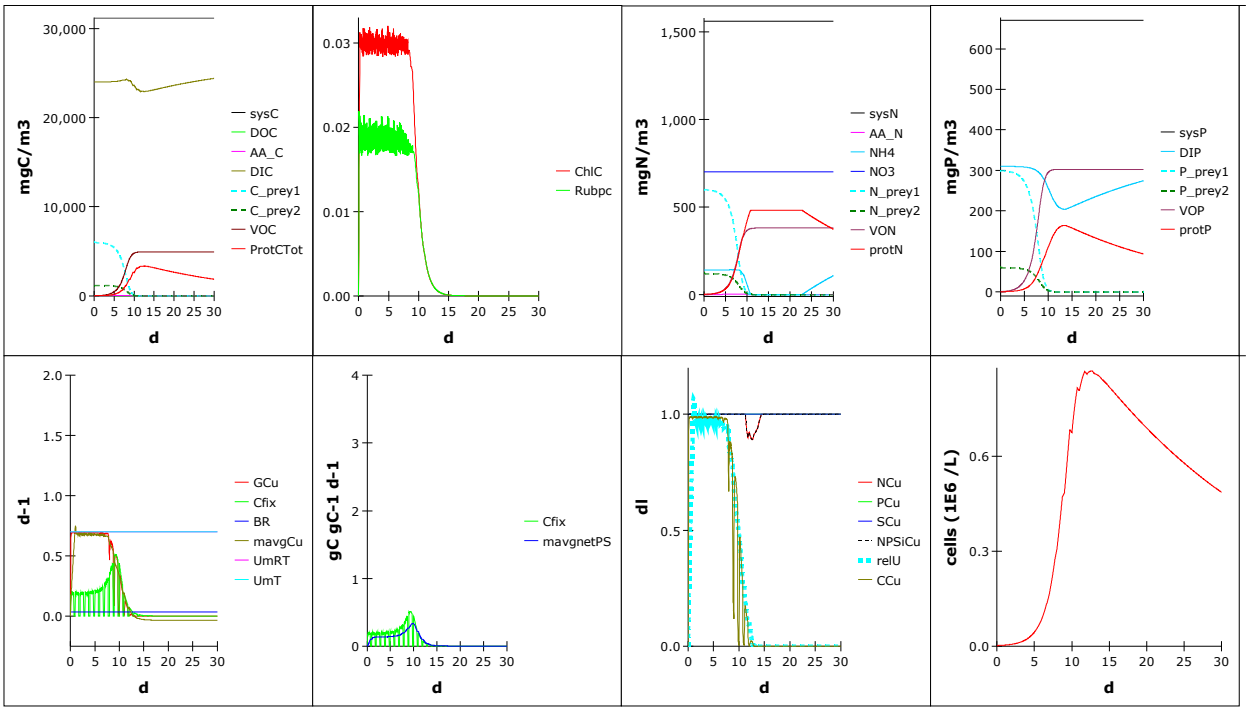

Fig.7.2 Example output for a GNCM. The ability to photosynthesize is acquired through consumption of the prey. Nitrate cannot be used, though (in this instance) external ammonium can. Note that photosynthesis becomes more important (Cfix is higher) at the end of the prey consumption; this is because less light is shaded out by the prey. Ultimately, on exhaustion of the prey, the GNCM respires itself to death. Oscillations reflect the consequences of growing in a light:dark illumination cycle.

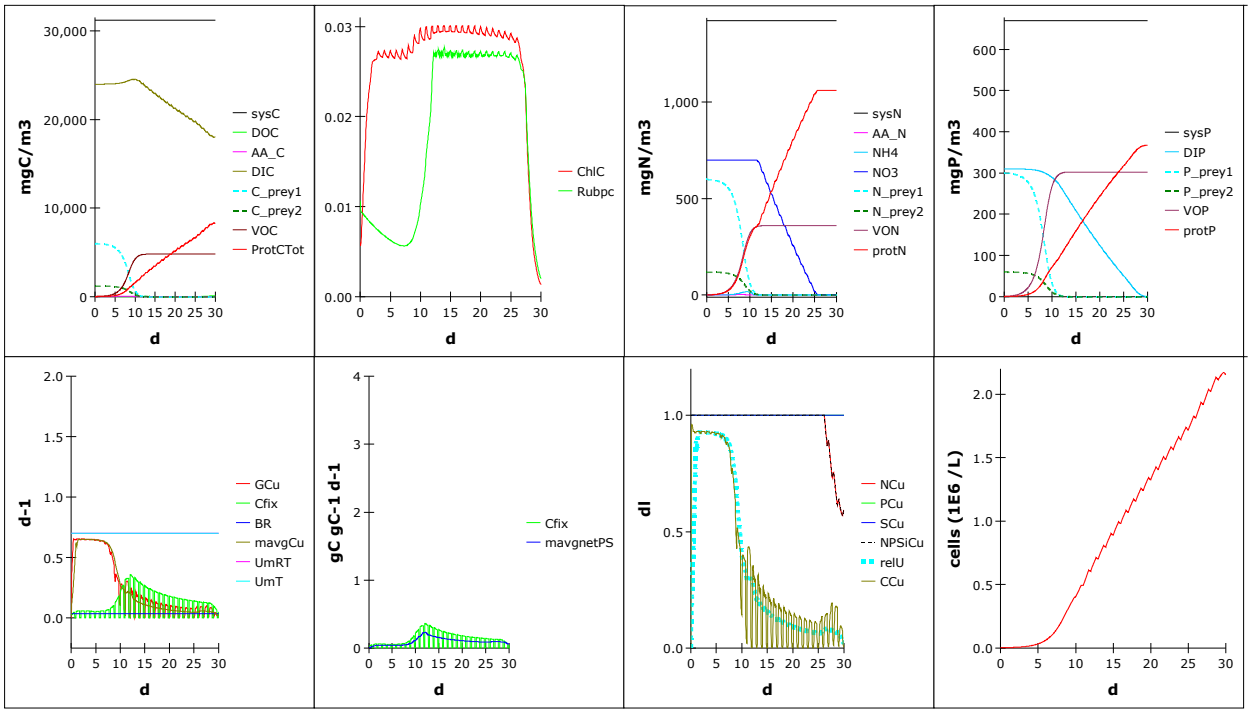

Fig.7.3 Example output for a pSNCM. These acquire the ability to photosynthesize from a specific prey (here, prey2). In contrast to the GNCM, pSNCM continue to photosynthesize after the prey have been consumed, though at the end of the simulation this ability is degrading rapidly. Growth is linear due to increasing self-shading in the culture. Oscillations reflect the consequences of growing in a light:dark illumination cycle.

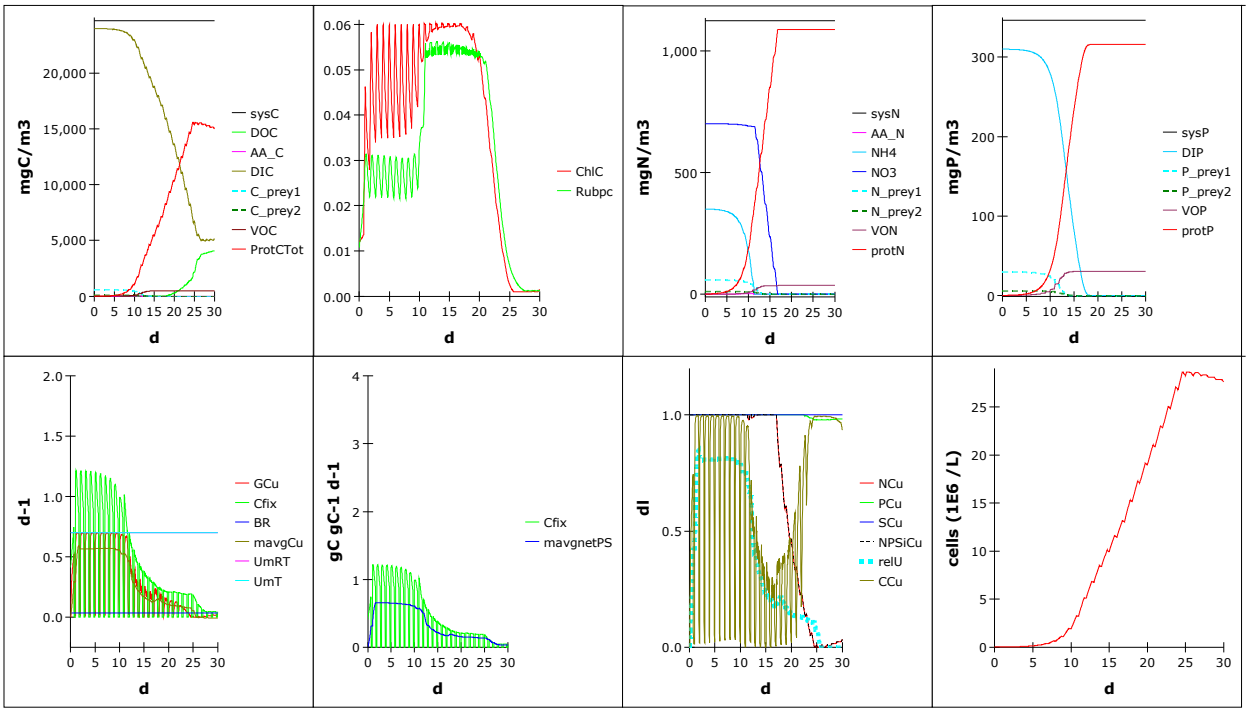

Fig.7.4 Example output for a CM. These have their own photosynthetic capabilities. They grow like phytoplankton but also consume prey. Here the prey are of low abundance. With increasing self-shading and the use of nitrate rather than ammonium after d10, ChlC and RuBisCO increase, falling on exhaustion of the DIN. Oscillations reflect the consequences of growing in a light:dark illumination cycle.

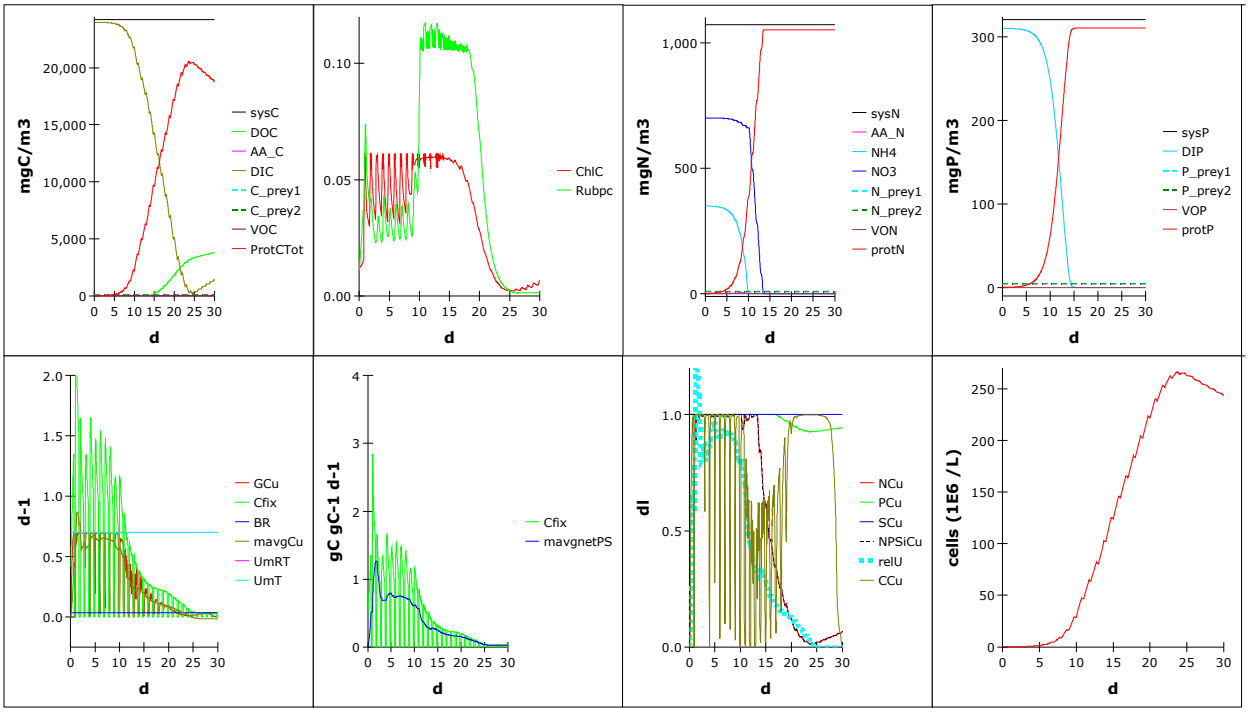

Fig.7.5 Example output for a (non-diatom) phytoplankton. Rather like the CM, but with no prey to consume.

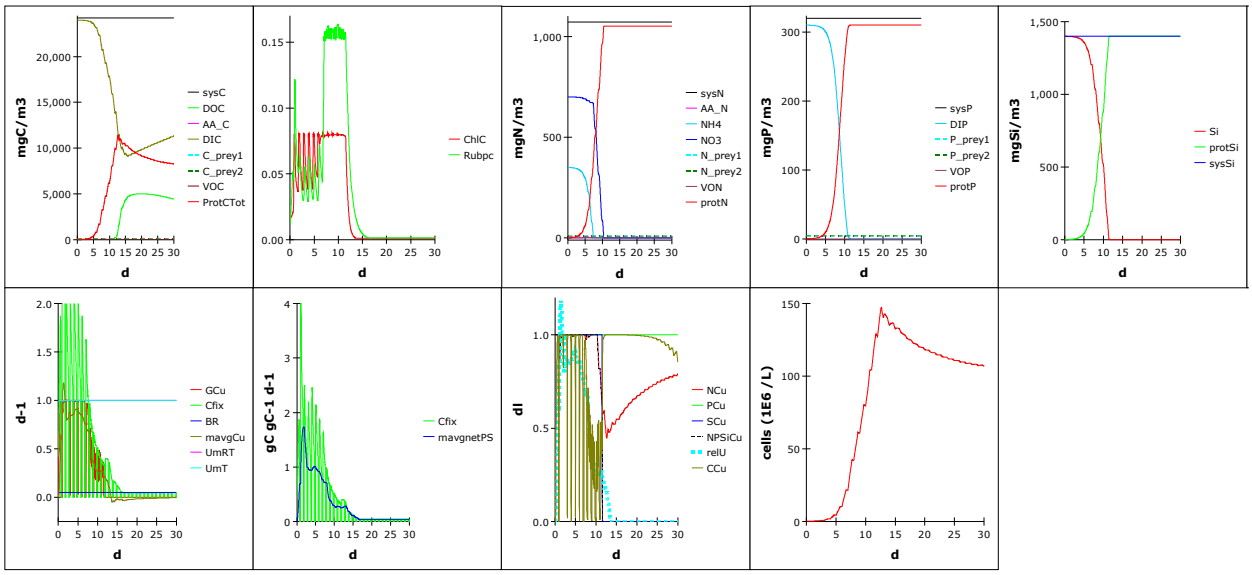

Fig.7.6 Example output for a diatom. Rather like the phytoplankton but with higher growth rate potential and the consumption of (and here, ultimately limitation by) silicate. Again, oscillations reflect the consequences of growing in a light:dark illumination cycle.

8. Comparison of the DRAMA concept with some alternative model forms

Below are given comparisons between the DRAMA concept with some other approaches. While DRAMA was designed to provide a homeostatic description for the description of plankton growth using, and potentially limited by, multiple resources, most of the alternative approaches were designed for a limited combination of resources.

8.1 Droop Quota

The Droop Quota models (Droop 1968), and nutrient-quota-style models in general (reviewed in Flynn 2008a), relate growth limited by the least available resource to the cellular content of that nutrient. The quota may be defined in terms of C or (as originally described) per cell. Growth is thus not limited directly by the external resource availability, but by the internal availability, for example as N $cell^{-1}$ or N C^{-1}. Modified quota-style models (but not the original Droop variant) have the potential to describe full variable C:N:P stoichiometry, though in practice few describe more than 2 interacting limitations. Linkage between multiple limiting resources (e.g. light, N, P, Si) is not possible using a simple extension of the quota concept, and various mechanisms are used to achieve that end (e.g., Liebig, chain-model). For example, growth may be limited by whichever is the most limiting of N:C and P:C.

Quota models usually use a passive control of resource acquisition; that is the maximum acquisition rate aligns with the maximum rate of growth (Flynn 2003). Quota models were designed, and are generally used, for descriptions of phytoplankton; they do not readily lend themselves to descriptions of heterotrophy or mixotrophy. The quota model in its basic form can only invoke C,N,P limited-growth by involving another state variable. Thus, Thingstad (1987) used cell-quotas for a simple quota-based model of bacterial growth. Nonetheless, in general terms the quota concept has a solid pedigree in providing an empirical description of simple nutrient interactions. The Si:C quota should not be used to control growth (Flynn & Martin-Jézéquel 2000).

The DRAMA model uses a normalised quota structure for N:C and P:C to actively modulate acquisition of dissolved inorganic N and P. C-limited growth is described with reference to the ^{M}C:C quota. In this context, the empirical basis for that aspect of the DRAMA model is well founded, and similar to the MAP-series of models (see **Section 8.6**). Otherwise, however, the DRAMA description is quite different due to the way the components are coupled. The incorporation of Si using DRAMA follows the approach described in the short form model of Flynn & Martin-Jézéquel (2000).

8.2 Shuter & *EVE*

The Shuter model (Shuter 1979) has some similarities with the quota model except that it divides the phytoplankton cell into a core with fixed C:N:P stoichiometry, plus a C-reserve. The format cannot thus describe full variable C:N:P stoichiometry, as N:P is fixed. The model was designed for descriptions of phytoplankton, although it has been used for descriptions of heterotrophy and mixotrophy (Ghyoot et al. 2017).

The Shuter model is also used as the core of the EVE model (Daines et al. 2014), which is a cell-based construct that makes specific reference to the role of RNA in controlling growth. The model simulates changes in N:P stoichiometry related to temperature induced increases in growth rate. The basis is that as temperature and thence growth rate changes so the investment of cellular-P in RNA changes. Rees & Raven (2021) note that this investment is associated with a general increase in P-containing moieties; accordingly the fine-grain description of RNA in EVE can be replaced by reference to the whole-organism P quota. That cell size changes with both temperature and P-status conflicts with the use of a P:cell quota approach.

The DRAMA model, like Shuter, has two carbon-based state variables, but in the DRAMA model the non-core component defines the C in the metabolic (not the reserve) pool. The size of the reserve C pool in a DRAMA model is computed with reference to the excess of whole organism C:N to the C:N in a nutrient replete organism; there is not a specified C-reserve. While such a reserve could be described explicitly, it would require another state variable. If desired, a specific state variable for RNA could be included in DRAMA (as in EVE), but the commentary by Rees & Raven (2021) indicates that this would not be justified, while in contrast a specific linkage to cell size changes may be warranted. DRAMA is a C-based construct which can be coupled (as/if required) to a cell-base. Cell-based models, such as EVE (see comments in **Section 8.1**), risk confounding C and cell-based changes in quota and temperature affects due to changes in cell size. DRAMA has the scope to change stoichiometry and cell size with different resource limitations and with temperature using approaches similar to that in Flynn (2001).

8.3 *Chain* Model

Traditionally the stresses incurred due to the lack of more than one resource have been handled either in a multiplicative or minimum (Liebig) fashion. As an alternative, Pahlow & Oschilis (2009) developed what they termed the ‘chain model’ to link P, N and light limitations. In their model, using the terminology deployed in this current work, PCu limits N assimilation, and then NCu affects growth via affecting how the phytoplankton responds to light availability. These authors refer to the approach used by Flynn (2008) as an ad hoc arrangement, while actually it functions via a shift by P-stress of the (de)repression control of N source uptake. Thus, the effect is that P-stress alters the regulation of N assimilation and then invokes N-stress at the whole cell level, which is exactly the same as that argued as the rational for the chain model by Pahlow & Oschilis (2009). The chain model only considers a generic DIN source, and is suitable (as originally published) for DIP-DIN-light interactions in phytoplankton.

DRAMA retains the modification to the (de)repression control of N-source usage by P-status (see **Fig.2.2**) that originated in Flynn (2008), but further develops it to include nitrate and ammonium. It is not clear how P-stress interacts on other facets of physiology, though it affects cell size amongst other things. DRAMA may be adapted to handle such interactions as data become available, most obviously by altering the form of the (de)repression controls acting via combinations of CCu, NCu and PCu.

8.4 *Dynamic Energy Budget*

Dynamic Energy Budget (DEB) models (Kooijman 1993) are based on energy and structural components, with recently acquired materials assumed to first enter a 'reserve' pool. There is an explicit description of a division between the allocation of those resources between the support of growth or maturation.

The concept was designed to describe a generic life form but, while it has been widely used across many different types of organisms, it does not lend itself to readily permit the modulation of different types of resource acquisitions (be those acting positively or negatively) as required especially for describing plankton activity on multiple resources. Despite criticisms that measuring key components of the model are problematic, the DEB concept enjoys a loyal following.

DRAMA does not describe energy per se; energy is derived from respiration of C, which is itself the core unit in the model. Unlike DEB, DRAMA is specifically designed to readily describe multiple-resource acquisitions. Arguably DRAMA, like DEB, also has a challenge in its parameterisation. However, as noted earlier, $^{M}C:^{C}C$, and thence $^{M}C:C$, can be estimated by hot-TCA/alcohol extraction protocols, at least for microbes. More fundamentally, DRAMA exploits (de)repression to define how the model responds.

8.5 Trait-based models and trait-trade-offs

Many plankton models claim to be trait-based. In reality, other than Lotka–Volterra models (and similar simple constructs), all plankton models describe traits. What is implicitly meant by trait-based is often trait-rule-based; this is an approach to provide a continuum in trait expression with respect to a master trait, typically organism size. Trait-rules include, that small organisms grow faster than do large organisms (West et al. 2001), and that plankton size controls resource acquisition routes (Andersen et al. 2016).

The derivation of trait rules requires the establishment of cause-and-effect relationships; often the relationship itself carries poor explanatory power (a low R^2). Trait-based models are often linked to trait-trade-off concepts (often via resource allocation arguments) which, while they undoubtedly have an appeal, are also extremely problematic from an evolutionary point of view. This is because the organisms whose evolution is argued to be being directed by trait-trade-offs must be closely related and growing in the same environment. Some of the pitfalls that can be thrown up by such considerations are considered in Flynn et al. (2015a) and Mitra et al. (2023b).

Trait-based models provide a useful approach when considering gross changes of trait expression over a wide organism size range. Whether the steepness of the gradient describing the trait is sufficient to provide for the 'devious strategies' suggested by May (1972) as required to provide stability in ecology (Allesina & Tang 2012) is another matter.

May's 'devious strategies' (May 1972) include the facets of behaviour (both at the level of cell physiology and whole organism activity) that are usually omitted in plankton models but which form the cornerstone for most empirical studies. DRAMA provides the means to include such behavioural traits in plankton models. Thus, while DRAMA could be configured to support trait-rule approaches (including those associated with resource allocations), it can also support descriptions of other traits

that make real organisms different from each other. DRAMA is also well placed to explore trait-trade-offs in the physiology of an organism, such as whether to 'limit motility and starve' vs 'move to acquire resource and risk being eaten'.

8.6 Model of Algal Physiology (MAP) and Perfect Beast

These models use active controls of resource acquisition (e.g. Flynn 2003), based upon normalised quota response functions, with photoacclimation, in descriptions of phytoplankton (MAP; Flynn 2001) and mixoplankton ('Perfect Beast'; Flynn & Mitra 2009). Both MAP and Perfect Beast contain combinations of feed -forwards and –backward functions, but they also use prognostic terms, rather than enabling self-regulation throughout. Perfect Beast also allows an exploration of trait-trade-off theory, including descriptions of protist functions that are theoretical (not existing in nature – Mitra et al. 2023b).

Though complex, these models permit high levels of flexibility in describing protist plankton types in considerable detail, including for MAP Fe limitation. Including Fe in Perfect Beast would give an even more complex structure, requiring additional developmental effort. Modulation of photo-physiology when operating in a light-dark cycle makes reference to a history function describing growth over the previous 24h; this history function may cause problems when the model is used in a multi-dimensional setting as its value cannot be readily divided between physical zones as its dimensions do not include water volume.

The DRAMA model also uses normalised quota responses for some functions, but employs a normalised quota-like satiation function (based on ^{M}C:C) as an over-riding modulator of resource acquisition control and also of growth. In consequence, the model is significantly less complex (fewer state variables) than Perfect Beast, also replacing implicit prognostic terms with explicit satiation-linked homeostatic controls that are more easily controlled. Importantly, there are no history functions; all state variables have dimensions including m^{-3}. The structure also overcomes various other shortcomings in these and other plankton models. As described here, Fe-limitation is not included in DRAMA, though it has been incorporated along the lines used in MAP with inclusion of another state variable and controls related to Fe:C (see Flynn & Hipkin 1999).

8.7 *SAPPM*

SAPPM (Switchable Acclimative Protist Plankton -Model) was designed as a more inclusive protist-plankton description to succeed Perfect-Beast (Flynn & Mitra (2009). SAPPM is described by Flynn (2021), forming the base for a Decision Support Tool for the commercial-facing EnhanceMicroAlgae project (https://www.enhancemicroalgae.eu/) which provides a digital laboratory description of a bioreactor containing microalgae. As a description of protist plankton, SAPPM was used by Schneider et al. (2021).

SAPPM employs a different approach to controlling resource management, and also to describing the interactions between phagotrophy versus phototrophy. It includes an explicit description of osmotrophy and provides various advances compared with MAP (Flynn 2001) and Perfect Beast (Flynn & Mitra 2009) for the description of different mixoplankton types. Key parts of SAPPM require

reference to history functions, recording the average growth rate and C-fixation rate over the previous 24h, and the description of the status of acquired phototrophic components for non-constitutive mixoplankton (NCMs). These history terms can present some challenges in placing the model in ecosystem simulation platforms; for this reason the version of SAPPM used by Schneider et al. (2021) did not display the full features of the original form (Flynn 2021). DRAMA was developed in part to overcome these challenges but ultimately resulted in a very different solution that provides both an improved description of biology and a better computational solution.

8.8 Summary of advantages of DRAMA

The advantages of DRAMA over the above mentioned model types are :

- Provision of stoichiometric description suitable for all plankton types based on a common conceptual framework.
- A platform to handle multiple resource forms in a dynamic fashion with ready scope for resource switching and concurrent resource usage under multi-stressor scenarios.
- Overcome challenges in extant constructions with respect to the description of resource selectivity and priority, relationship between L:D and daily C-fixation, different forms of mixotrophy, density dependant inefficiency, etc.
- Cost effective in computation terms (low state variable count, moderate integration step size of ca. 10-15min).

DRAMA allows for a much simplified approach in comparison with other constructs that can be considered to describe trait expression, with all resources being funnelled through a common interface, allowing a sequential control of resource acquisition and exploitation.

The provision of an explicit description of the photosynthetic machinery (^{PM}C in **Fig. 6.3**), represents an important difference. In most models the maximum photosynthetic rate (P_{max}) is fixed and so the modelled rate of photosynthesis cannot acclimate as it should be able to by altering not only the pigment content (Geider et al. 1996) but crucially also the value of P_{max} (Richardson et al. 1983). Indeed, it appears that acclimation is most commonly achieved by changing the RuBisCO content (Vandenhecke et al. 2015).

Being able to independently consider the impacts of prey (food) quality on prey selectivity and assimilation efficiency (AE) is an important advantage in considering consumer growth dynamics. With DRAMA it is easy to consider different AE values for each prey type (perhaps most appropriate for protist zooplankton, where each prey is digested within a separate vacuole), or averaged over the whole diet (perhaps more appropriate for a consumer with a gut). Allied features related to the nature of the voided material (Mayor et al. 2020) may be readily included, all with feedbacks to gut passage time as modulated by the relative size of ^{M}C.

9. Conclusions

This work provides a broad description of the DRAMA concept. The flexibility afforded by DRAMA, of using a C-quota together with quotas for other nutrient types to modulate acquisitions of resources from different mechanisms and ultimately to control growth, provides a common platform for the description of different organism types, and specifically (as originated) for plankton. That flexibility in configuration is critical for describing growth of the individual and of allied trophic and biogeochemical dynamics. In addition other facets of behaviour can be readily modulated. The control of trait expression is as important a defining feature for an organism as is possession of the trait; DRAMA provides a means to control trait expression.

Built using system dynamics concepts, the model structure is robust to the exact value of parameters used to configure the response curves that are used to provide the cybernetic feedback controls. Problems of over-fitting are less easily directed towards system dynamics models due to their feed-back/forward connectivities. Those connectivities also align with those seen in real biological systems, thus also facilitating the use of the system dynamics together with systems biology concepts in the DRAMA approach.

Collectively, DRAMA is a low-state-variable robust flexible structure with which to explore the digital twinning of plankton models. Specific application of DRAMA, with their allied explanations and equations (model code) will be published separately. Example applications include, Mitra & Flynn (2023), and Flynn & Mitra (2023).

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11. Appendix 1 - published applications of DRAMA

Mitra A, Flynn KJ (2023) Low rates of bacterivory enhances phototrophy and competitive advantage for mixoplankton growing in oligotrophic waters. *Scientific Reports* 13, 6900. https://doi.org/10.1038/s41598-023-33962-x **[this work uses DRAMA to describe a constitutive mixoplankton]**

Flynn KJ, Mitra A (2023) Feeding in mixoplankton enhances phototrophy increasing the potential for coastal water bloom-induced pH changes with ocean acidification. *Journal of Plankton Research* 45, https://doi.org/10.1093/plankt/fbad030 **[this work uses DRAMA to describe zooplankton, phytoplankton, generalist-non-constitutive mixoplankton, specialist-plastidic-non-constitutive mixoplankton, and constitutive mixoplankton]**