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ORIGINAL PAPER

Defining Planktonic Protist Functional Groups on Mechanisms for Energy and Nutrient Acquisition: Incorporation of Diverse Mixotrophic Strategies

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Arranging organisms into functional groups aids ecological research by grouping organisms (irrespective of phylogenetic origin) that interact with environmental factors in similar ways. Planktonic protists traditionally have been split between photoautotrophic "phytoplankton" and phagotrophic "microzooplankton". However, there is a growing recognition of the importance of mixotrophy in euphotic aquatic systems, where many protists often combine photoautotrophic and phagotrophic modes of nutrition. Such organisms do not align with the traditional dichotomy of phytoplankton and microzooplankton. To reflect this understanding, we propose a new functional grouping of planktonic protists in an eco-physiological context: (i) phagoheterotrophs lacking phototrophic capacity, (ii) photoautotrophs lacking phagotrophic capacity, (iii) constitutive mixotrophs (CMs) as phagotrophs with an inherent capacity for phototrophy, and (iv) non-constitutive mixotrophs (NCMs) that acquire their phototrophic capacity by ingesting specific (SNCM) or general non-specific (GNCM) prey. For the first time, we incorporate these functional groups within a foodweb structure and show, using model outputs, that there is scope for significant changes in trophic dynamics depending on the protist functional type description. Accordingly, to better reflect the role of mixotrophy, we recommend that as important tools for explanatory and predictive research, aquatic food-web and biogeochemical models need to redefine the protist groups within their frameworks.

Functional Types in Ecology

In ecology, organism functional categories are often more useful than taxonomic groupings because they can be based on ecological function, rather than evolutionary history. Functional group descriptions are commonly used by scientists to partition the numerous taxonomic classes into categories more relevant to ecology. The concept provides "*a non-phylogenetic classification leading to a grouping of organisms that respond in a similar way to a syndrome of environmental factors*" (Gitay and Noble 1997). Functional group (also referred to as "functional type") classifications thus aid our understanding of ecological processes with applications from fieldwork through to conceptual and mathematical studies.

The functional group approach has been embraced by researchers working on different organisms across biomes. Especially when applied to microorganisms, linking an ecological function to specific members of a community is often challenging, as individual contributions to rate processes are difficult if not impossible to measure in situ. Supplementing the classical plant/animal type dichotomy, one of the earliest categorizations of plankton groups was based on size, driven by practical approaches to plankton sampling (Lohmann 1911; Schütt 1892), as well as conforming to typical predator-prey allometries of 10:1 (Azam et al. 1983). Various later freshwater and marine studies used such allometric classifications (Sieburth et al. 1978), specifically focussing on phytoplankton species (Aiken et al. 2007; Reynolds et al. 2002). Kruk et al. (2010) found easily identifiable

morphological differences among phytoplankton to correlate with functional properties and proposed six functional groups; these were based on volume, maximum linear dimension, surface area, and the presence of mucilage, flagella, gas vesicles, heterocytes or siliceous exoskeletal structures. Further to morphological characteristics, Weithoff (2003) used resource acquisition capabilities, such as phagotrophy (bacterivory), nitrogen fixation, and silica usage, to divide phytoplankton into six functional groups.

In the context of conceptual and mathematical studies of marine ecology, protist functional groups are typically divided simply into "phytoplankton" (phototrophs) and "microzooplankton" (phagotrophs); the former typically include photoautotrophs while the latter represent phagoheterotrophs (e.g., Baretta et al. 1995; Fasham et al. 1990; Plagányi 2007). However, there is now an increasing recognition that many "phytoplankton" and photic-zone "microzooplankton" are, in fact, mixotrophic. A wide range of phytoplankton ingest prey while various microzooplankton retain chloroplasts from their prey or harbour photosynthetic endosymbionts and thus contribute to primary production. Furthermore, many species, when engaging in mixotrophic activity, can attain faster growth than when in photoautotrophic or phagoheterotrophic mode alone (e.g., Adolf et al. 2006; Burkholder et al. 2008; Glibert et al. 2009; Jeong et al. 2010). Thus mixotrophy, defined here as phototrophy plus phagotrophy, is an inherent capability of many planktonic protists rather than being the exception that it was previously considered to be (see reviews by Flynn et al. 2013 and references therein; Stoecker et al. 2009; Jeong et al. 2010).
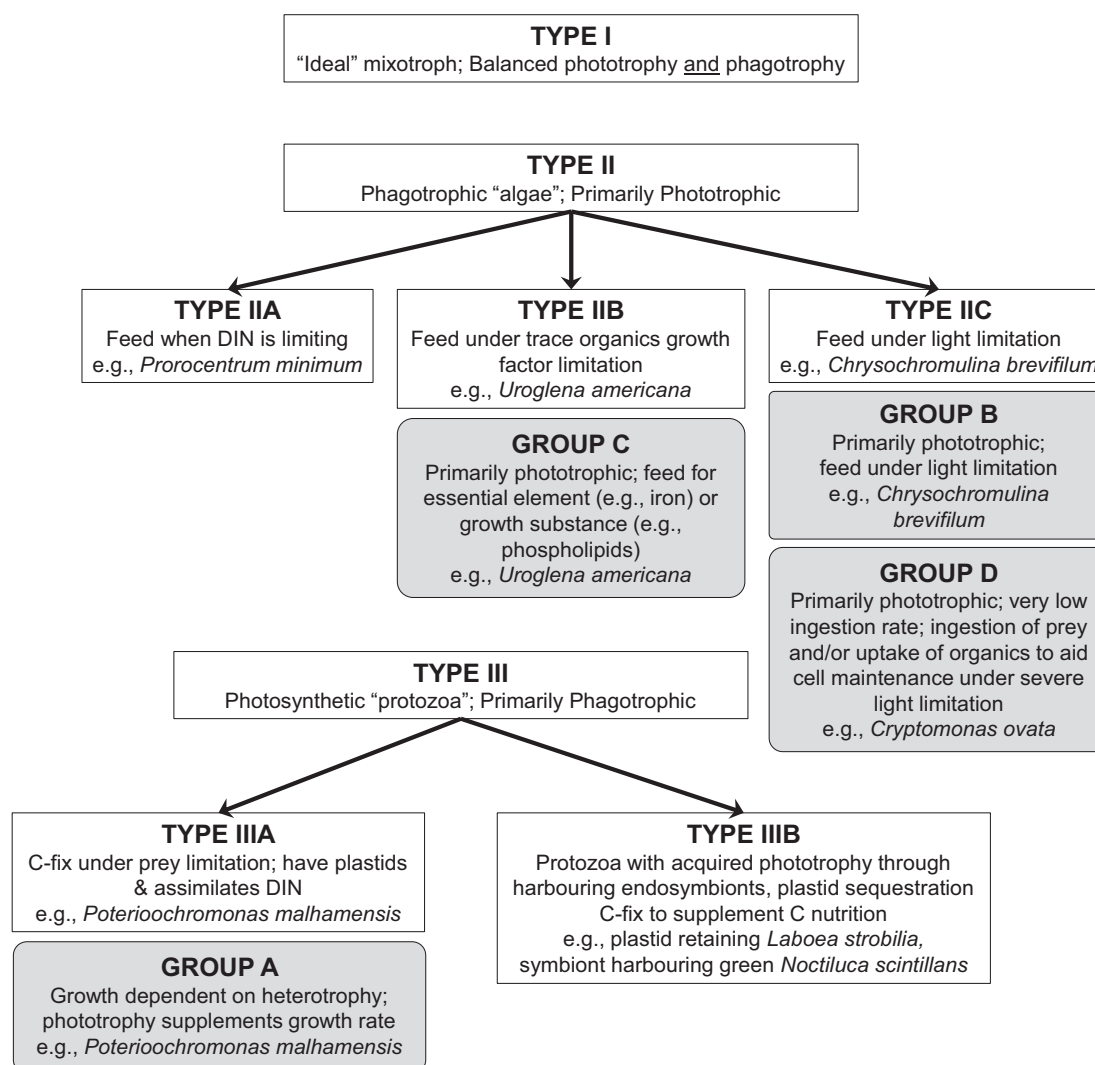
Mixotrophic protists are ubiquitous, and commonly have been found to be dominant in freshwater as well as marine systems from the tropics to the poles (e.g., Jeong et al. 2010; Sanders 1991; Stoecker et al. 2009; Zubkov and Tarran 2008). Yet, most plankton functional type classifications make minimal reference to these mixotrophs. Pratt and Cairns (1985), in their protist-centric functional groupings, emphasised strategies to acquire resources. They, thus, divided protists into six functional groups – (1) *photo-autotrophic primary producers* with no distinction made between those which can employ a level of heterotrophy, (2) *bacti- and detritivores* feeding on bacteria and/or detritus, (3) *saprotrophs* consuming dissolved material, (4) *algivores* primarily feeding on algae, (5) *nonselective omnivores* feeding non-selectively on algae, detritus and bacteria, and, (6) *raptorial predators* feeding primarily on other protozoa and organisms from the higher trophic levels. There is no explicit mention of mixotrophy. In contrast, Jones (1997) and Stoecker (1998) specifically focussed on how groups of protists combine phototrophy and phagotrophy to support growth (Fig. 1). Jones (1997) primarily focussed on the mixotrophic flagellates, proposing four functional groups according to their photosynthetic and heterotrophic capabilities. Stoecker's (1998) classification included a wider group of protists. In addition to flagellates, Stoecker (1998) accounted for ciliates, sarcodines and protists with algal symbionts – groups which had not been included in the studies of Pratt and Cairns (1985) and Jones (1997).

It is now clear that the time has come to abandon the premise that protists are either "little plants" or "little animals", to move away from the misplaced dichotomy of "phytoplankton" versus "microzooplankton" (Flynn et al. 2013). How can we best reclassify them for ecological studies? Depending on the character of interest, there is scope to create many types of functional groups. For the application of functional classifications to mathematical models, however, a simpler approach is required in order to constrain computational loads while prioritising functionality to biogeochemistry and/or to trophic (food web) dynamics. Based on our understanding of aquatic ecology, coupled with improved understanding of how protists have evolved, we suggest that a reappraisal is required of the definition of functional group descriptions for planktonic protists, now explicitly including mixotrophs. We propose a new, ecologically based, functional group classification for aquatic planktonic protists. This is the first time that all the three groups of planktonic protists – phytoplankton, mixotrophs and microzooplankton – have been considered explicitly under the functional group approach. The group divisions we propose are based on both energy and nutrient acquisitions, and are consistent with the main drivers for conceptual and mathematical modelling (i.e., biogeochemistry and trophic dynamics). We discuss the importance of these groups as descriptors for future research on planktonic protist communities.

Classifying Protist Functional Groups

In evolutionary terms, heterotrophy (originally osmotrophic or saprotrophic, later including phagotrophy) is the ancestral state in protists while photo-autotrophy is the derived and more recent state (Raven 1997; Raven et al. 2009). The

TYPE I
"Ideal" mixotroph; Balanced phototrophy <u>and</u> phagotrophy

TYPE II
Phagotrophic "algae"; Primarily Phototrophic

TYPE IIA
Feed when DIN is limiting
e.g., *Prorocentrum minimum*

TYPE IIB
Feed under trace organics growth
factor limitation
e.g., *Uroglena americana*

TYPE IIC
Feed under light limitation
e.g., *Chrysochromulina brevifilum*

GROUP B
Primarily phototrophic;
feed under light limitation
e.g., *Chrysochromulina
brevifilum*

GROUP C
Primarily phototrophic; feed for
essential element (e.g., iron) or
growth substance (e.g.,
phospholipids)
e.g., *Uroglena americana*

GROUP D
Primarily phototrophic; very low
ingestion rate; ingestion of prey
and/or uptake of organics to aid
cell maintenance under severe
light limitation
e.g., *Cryptomonas ovata*

TYPE III
Photosynthetic "protozoa"; Primarily Phagotrophic

TYPE IIIA
C-fix under prey limitation; have plastids
& assimilates DIN
e.g., *Poterioochromonas malhamensis*

TYPE IIIB
Protozoa with acquired phototrophy through
harbouring endosymbionts, plastid sequestration
C-fix to supplement C nutrition
e.g., plastid retaining *Laboea strobilia*,
symbiont harbouring green *Noctiluca scintillans*

GROUP A
Growth dependent on heterotrophy;
phototrophy supplements growth rate
e.g., *Poterioochromonas malhamensis*

Figure 1. Traditional classification of mixotrophic protists according to Stoecker (1998; open boxes) and Jones (1997; grey boxes). "Groups" proposed by Jones (1997) have been aligned against "Types" proposed by Stoecker (1998). DIN, dissolved inorganic nutrients.

developmental lines from phago-heterotrophy to photo-autotrophy have occurred through a series of evolutionary pathways with gains and losses of physiological functionality (Raven et al. 2009). Notably, there have been sequences of acquisition and then loss of the capability to photosynthesize (e.g., Delwiche 1999; Saldarriaga et al. 2001; Van Doorn and Yoshimoto 2010; Wisecaver and Hackett 2010). That capability, and the nature of its expression in extant protists, is of such fundamental importance that it usefully forms the basis of a functional group classification.

Broadly following the direction of protist evolutionary pathways, we propose a division of protists according to the schematic shown in Figure 2;

differences in physiological processes between the different groups are highlighted in Figure 3. All protists appear to be osmotrophic to some degree, if only for certain vitamins and to re-acquire leaked primary metabolites such as protein amino acids (Flynn and Berry 1999). Accordingly, we do not use osmotrophy as a functional group characteristic. In contrast, the presence or absence of phagotrophy and/or of phototrophy are clear defining characteristics that have profound consequences for biogeochemistry and trophic dynamics through the operation of predator-prey interactions (Flynn et al. 2013; Mitra et al. 2014a). In what follows, we thus place emphasis on photo- and phago- trophy as classifying processes.
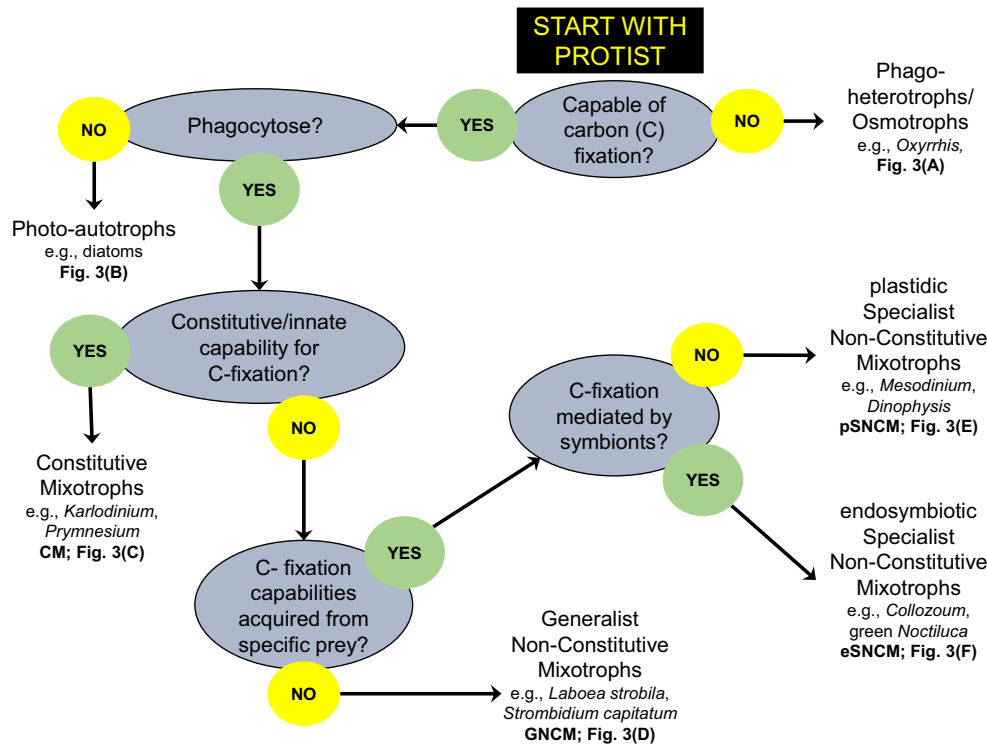
Figure 2. Flowchart showing the pathways used to derive the functional groups we propose to classify the planktonic protists. See also Figure 3.

We identify functional groups at the extreme ends of the spectrum as (i) phagotrophs, which conform to the common concept of "microzoo-plankton" (including heterotrophic nano-flagellates; Figs 2 and 3A), and, (ii) phototrophs incapable of phagocytosis that conform to the common concept of "phytoplankton" (Figs 2 and 3B). The mixotrophic protists, combining phago- and photo- trophy in a single cell, are first divided with respect to phototrophy between constitutive (inherent or innate) versus non-constitutive (acquired) capabilities. Constitutive mixotrophs (CMs; Figs 2 and 3C) have the innate ability to photosynthesize – that is, they have vertical transmission of plastids and, presumably, the ability to regulate plastid function via protist nuclear-encoded genes. Non-constitutive mixotrophs (NCMs, Fig. 2), in contrast, acquire the capability to photosynthesize from consumption of phototrophic prey. They depend on horizontal transmission of plastids or symbionts. The NCMs can then be divided into generalists and specialists. Generalist non-constitutive mixotrophs (GNCMs; Figs 2 and 3D) can use photosystems sequestered from a broad range of phototrophic prey. Specialist non-constitutive mixotrophs (SNCMs; Fig. 2) have developed a need to acquire the capacity for photosynthesis from one or a few specific sources. This

SNCM grouping can then be further divided into those which are plastidic (pSNCMs, Figs 2 and 3E) and those which contain endosymbionts (eSNCMs, Figs 2 and 3F).

Constitutive mixotrophs (CMs, Fig. 3C) conform to the common perception of mixotrophic protists as unicellular algae that can consume other organisms (Sanders and Porter 1988). The CM group includes representatives from a wide range of eukaryotic "phytoplankton" (almost all major phototrophic protist groups excluding diatoms; Flynn et al. 2013; Jeong et al. 2010), ingesting various prokaryotic (e.g., cyanobacteria, bacteria) and eukaryotic prey (e.g., ciliates, dinoflagellates, cryptophytes, amoebae; Burkholder et al. 2008; Jeong et al. 2010; Stoecker et al. 2006; Tillmann 1998; Zubkov and Tarran 2008).

Non-constitutive mixotrophs (NCMs, Fig. 3D-F) lack an inherent (constitutive) ability to fully synthesize, repair and control the photosynthetic machinery (Flynn and Hansen 2013). Mechanisms differ among representatives, but they all engulf photosynthetic prey. They may then retain the prey as symbionts through a process termed endosymbiosis. Alternatively, they retain parts of the ingested prey necessary for photosynthesis – chloroplasts (kleptoplastidy), along with,
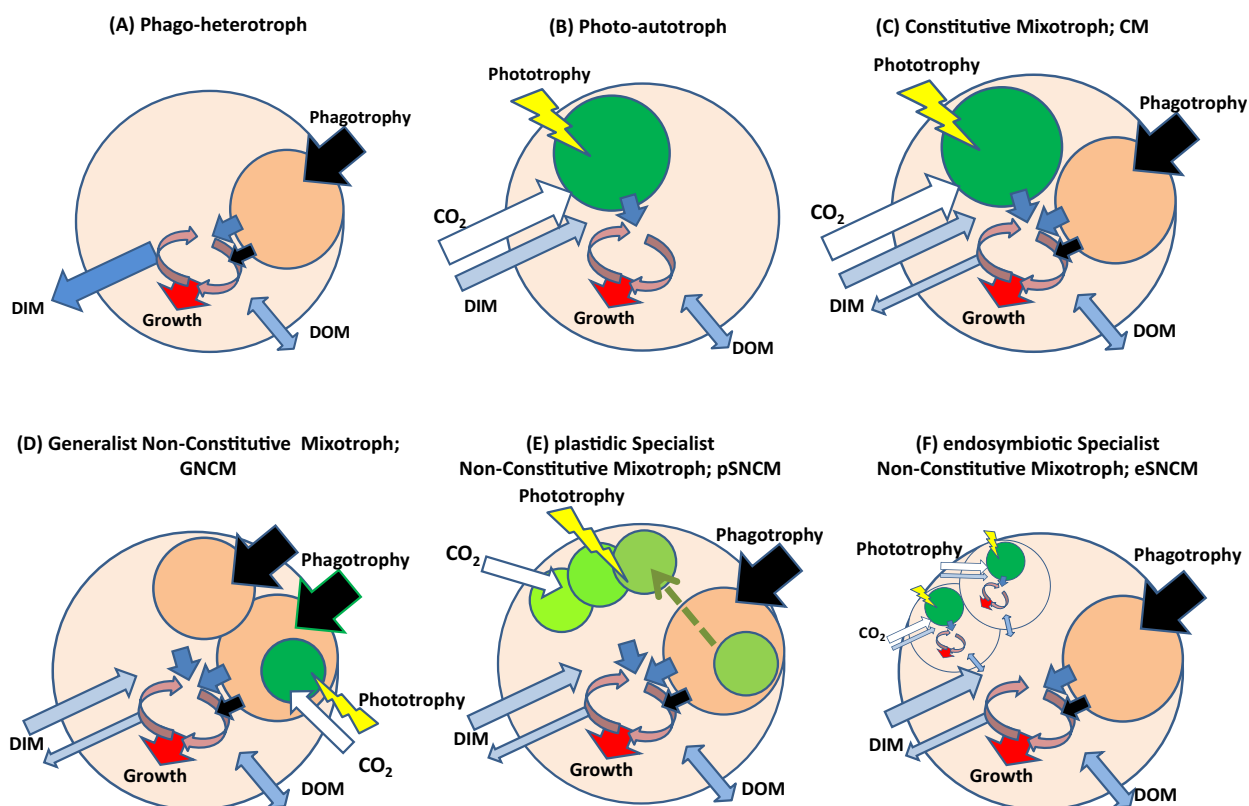
Figure 3. Schematic illustrating the different levels in complexity among different types of protist. (**A**) phagotrophic (no phototrophy); (**B**) phototrophic (no phagotrophy); (**C**) constitutive mixotroph, with innate capacity for phototrophy; (**D**) generalist non-constitutive mixotroph acquiring photosystems from different phototrophic prey; (**E**) specialist non-constitutive mixotroph acquiring plastids from a specific prey type; (**F**) specialist non-constitutive mixotroph acquiring photosystems from endosymbionts. DIM, dissolved inorganic material (ammonium, phosphate etc.). DOM, dissolved organic material. See also Figure 2.

sometimes, the prey nucleus (karyoklepty) and mitochondria, making use of these for a period of time (see reviews by Johnson 2011a, b and references therein; Stoecker et al. 2009). The retention time (for kleptoplastids) varies from hours to days or longer, depending on the mixotroph and the prey.

The GNCM group (Fig. 3D) uses chloroplasts derived from several to many prey types (e.g., Laval-Peuto and Febvre 1986; Laval-Peuto et al. 1986; Schoener and McManus 2012; Stoecker et al. 1988, 1989). About a third of the ciliates (by numeric abundance) inhabiting the marine photic zone fall within this GNCM functional group (Blackbourn et al. 1973; Calbet et al. 2012; Dolan and Pérez 2000; Jonsson 1987; Laval-Peuto and Rassoulzadegan 1988; McManus et al. 2004; Pitta and Giannakourou 2000; Pitta et al. 2001; Stoecker et al. 1987). The ability to maintain an acquired photosynthetic capacity by GNCMs is poor, and frequent re-acquisition is required.

In contrast to the GNCM group, SNCMs acquire photosystems from only specific prey. Specialization ranges from harbouring only plastids to harbouring intact cells (protists or cyanobacteria) as symbionts. Maintenance of the acquired photosystems is usually good, so that SNCMs can modulate photosynthesis (photoacclimate and undertake damage repair) similar to that seen in CMs but absent in GNCMs. Among the SNCMs, the pSNCM sub-group (Fig. 3E) includes ciliates such as *Mesodinium rubrum* (Garcia-Cuetos et al. 2012), which feed on several prey types, but acquire photosynthetic apparati (and nuclei) only from specific cryptophyte clades (Hansen et al. 2012; Johnson 2011a, b; Johnson et al. 2007), the dinoflagellate *Dinophysis*, which sequesters plastids from the ciliate *M. rubrum* (Park et al. 2006), and, an undescribed *Karlodinium*-like dinoflagellate that acquires plastids from the haptophyte *Phaeocystis antarctica* (Gast et al. 2007; Sellers et al. 2014).

The photosymbiotic eSNCM sub-group (Fig. 3F) includes, within marine systems, the biogeochemically important and cosmopolitan Foraminifera and Radiolaria (Acantharia and Polycystinea). These mixotrophs harbour and maintain dinoflagellate, haptophyte, or green algal endosymbionts. The endosymbionts are acquired during the juvenile stages and maintained throughout most of the life cycle (Caron et al. 1995). The presence of these endosymbionts is obligatory for normal growth and reproduction in eSNCMs (Caron et al. 1995; Decelle et al. 2012; Langer 2008; Probert et al. 2014). Freshwater ciliates (*Paramecium bursaria*, *Vorticella* spp, *Stentor* spp, *Frontonia* spp, *Stokesia* spp and *Euplotes* spp) specifically harbour the microalga *Chlorella* as endosymbionts (Berninger et al. 1986) and thus falls within the eSNCM category. However, in contrast to the marine eSNCMs, the freshwater mixotrophs do not require the symbionts for reproduction (Dolan 1992).

Proposed versus Other Functional Group Classifications

Our proposed grouping strikes at the very basis of ecophysiology - whether an organism is a primary producer, a consumer, or some combination of the two (mixotrophic). These are key features affecting contributions to biogeochemistry and/or trophic interactions. We now compare our proposal to earlier classifications of protists that considered mixotrophy.

Comparison of the mixotrophic functional groups of Jones (1997) to our proposed grouping (Fig. 1 versus Fig. 2) reveals that all the groups proposed by Jones are constitutive mixotrophs (CM, Fig. 3C) because they have an innate capability to photosynthesize. In contrast, the groupings by Stoecker (1998) include both constitutive (Stoecker's Types IIA, B, C and IIIA in Fig. 1; CM, Fig. 3C) and non-constitutive mixotrophs (Stoecker's Type IIIB in Fig. 1; NCM, Fig. 3D-F). The Types IIA, B and C of Stoecker (1998, Fig. 1) could thus in essence be phytoflagellates within the constitutive functional group.

The prime discriminators for the functional group descriptions of Jones (1997) and Stoecker (1998) are the balancing of energy and nutrient supply and demand. Thus, groups were split according to the proportion of phototrophy versus phagotrophy, with the lesser activity "topping up" the least abundant resource (carbon, nitrogen, phosphorus, iron etc.). However, now we view mixotrophy as likely performing a synergistic rather than a complementary role in nutrition (Adolf et al. 2006; Wilken et al. 2014a; and as modelled by Flynn and Mitra 2009). Repression and de-repression across the range of nutrient and energy acquisition options modulate expression of phototrophic versus phagotrophic activities. There is great variation across the constitutive mixotrophs (CM group) in this regard, and also in growth rate potential. For example, it has been shown that when conditions are optimal for mixotrophy (i.e., sufficient light and prey are available), some dinoflagellates have a higher growth rate compared to their growth when functioning as phototrophs (low or no prey available) or phagotrophs (under light limitation) (Jeong et al. 2010). The conditions specified by Jones (1997) and Stoecker (1998) could, therefore, be viewed as a secondary level of classification to that we now propose, describing placement of mixotrophs upon sliding scales of phototrophy versus phagotrophy, depending on their physiological capabilities and resource availabilities.

We can thus envision a series of functional group descriptions ranging from the potential to engage mixotrophy (our proposal), to expression of mixotrophy according to physiological stressors (cf. Jones 1997; Stoecker 1998), to utilizing carbon dioxide or particulate food (bacteria/ detritus, algae, heterotrophic protists and animals) as a source of carbon (Pratt and Cairns 1985). The first division, however, must be between protist groups that are non-phagotrophic phototrophs, phagotrophs with no phototrophic capacity, or mixotrophs with some combination of the two (i.e., between the CM, GNCM and SNCM groups).

Ecological Implications – a Demonstration

The purpose in grouping organisms according to functionality is to aid our understanding of ecology. Arguably, the most fundamental ecological division is between primary producers and their consumers. Interactions between these two groups and higher trophic levels form cornerstone components in ecological research and modelling (e.g., Cohen et al. 1993). The mixotrophic protists combine facets of both primary producer and consumer in one organism. The ability to express these facets allows, and may actually require, us to divide them into CM, GNCM and SNCM functional groups (Figs 2 and 3). Each of these groups display different interactions and dynamics with other plankton. As an example, we show here the contrasting behaviour of a simple trophic food web, in which a particular protist is
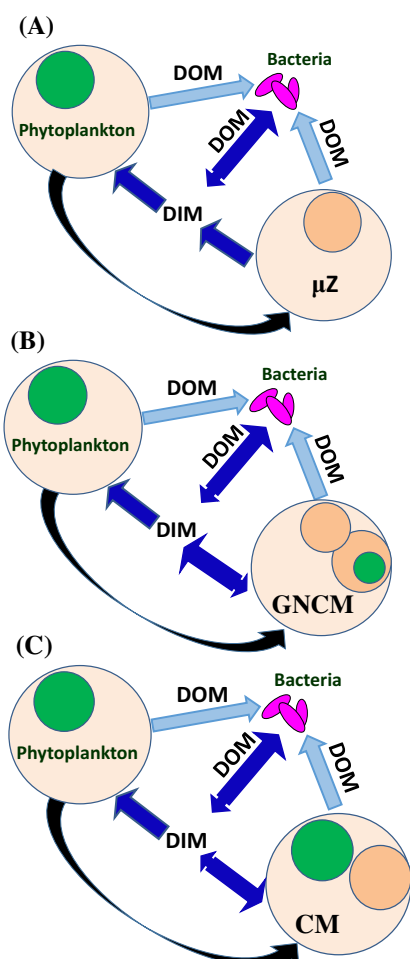
Figure 4. Three contrasting simple food-web structures. Scenario A portrays the classic paradigm where phytoplankton and microzooplankton are the two protist plankton functional types. Within Scenarios B and C, the microzooplankton functional type is replaced by the non-constitutive mixotrophs (NCMs) and constitutive mixotrophs (CMs) respectively. The release and uptake of dissolved inorganic matter (DIM, ammonium, phosphate etc.) is indicated.

operating as a strict phagotroph ("microzooplankton"), as a GNCM, or as a CM.

Method – the foodweb model framework. To demonstrate the potential impact of the different protist functional groups on trophic dynamics, we compare the outputs from three contrasting in silico plankton foodweb structures operating in a mesotrophic setting:

(i) *Scenario A:* traditional foodweb structure (Fig. 4A). This framework includes photo-autotrophic (non-phagotrophic) protist phytoplankton as primary producers, the phago-heterotrophic microzooplankton (µZ) which graze on these phytoplankton and bacteria as decomposers.

(ii) *Scenario B:* an alternative food web framework incorporating GNCMs (Fig. 4B). This food web structure includes the same components as Scenario A, except that the µZ are replaced with GNCMs. The GNCMs demonstrate acquired phototrophy through sequestration of the photosynthetic apparatus from the phytoplankton prey.

(iii) *Scenario C:* the third food web framework incorporates CMs (Fig. 4C). Again, the food web structure includes the same components as Scenario A, except that the µZ are replaced with CMs. The CMs photosynthesize using their constitutive chloroplasts and attain additional nutrition (C,N,P) through the ingestion of the phytoplankton prey.

The food web model structure for the three scenarios was adapted from Flynn and Mitra (2009). In brief, this consists of a mixed layer depth of 25 m, inorganic N of 5 µM, and inorganic P of 0.625 µM, with an effective cross mixing-layer dilution rate of 0.05 d^{-1}. The model includes variable stoichiometric (C:N:P) acclimative descriptions of the plankton. When we first explored modelling the ecophysiology of mixotrophic protists, we found that we had to split the potential for mixotrophy into groupings of what we now term here as CMs and NCMs. The "perfect beast" model of Flynn and Mitra (2009) contained switch functions that could configure between these types, together with acclimation descriptions to enable them to represent all the functional groups described by Jones (1997) and Stoecker (1998). This model is used here. For further details of the description of the model configurations, please see the Supplementary Material.

Results from in silico experiments. The temporal and spatial development of biomass of the different functional groups within the simulated communities are very different under the three scenarios (Fig. 5A-C); additional plots are presented in Supplementary Material Figures S1-S3. In scenario A, the dynamics follow those expected from a typical predator-prey system. However, in both scenarios B and C, the mixotroph functional groups outcompete the phototrophic phytoplankton (hereafter, phytoplankton). Indeed, in scenario C, the CMs ultimately become the dominant functional group (akin to a bloom situation). In scenario B, in contrast, the GNCMs could only attain a limited productivity due to their dependency upon the
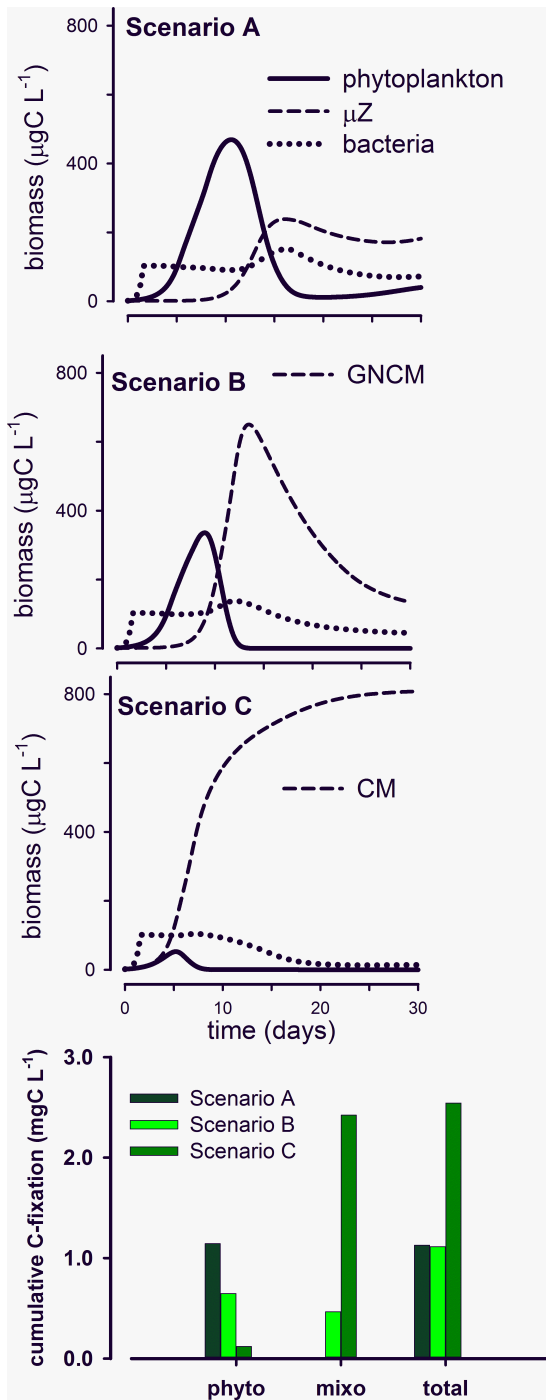
Figure 5. Temporal pattern of the development of the biomass in the simulated communities under the three scenarios. Also shown are the cumulative primary productivities by phytoplankton (phyto) and mixotroph (mixo) over the 30-day simulation period under the three alternative scenarios. No mixotrophs are present in Scenario A. See also Figure 4.

phytoplankton for the supply of plastids to allow photosynthesis.

The implementation of a GNCM versus a CM mixotroph thus generates an interesting dynamic to their phototrophic ecology. The GNCMs can never dominate the system. Due to their dependency on prey to acquire phototrophic capabilities (Figs 2 and 3D), GNCM blooms would always terminate through exhaustion of prey (Supplementary Material Fig. S2). CMs, however, through a combination of phagotrophy and phototrophy have the advantage and the capability to dominate a system, ultimately forming successful blooms. Indeed, harmful algal bloom species are typically CMs (Burkholder et al. 2008). In essence, CMs act as intraguild predators, both feeding on and competing with their prey; thus in contrast to specialist predators (i.e., NCMs) dependent on specific prey items, CMs can suppress their prey much more strongly (Wilken et al. 2014b). For CMs and for phytoplankton, self-shading resulting in light limitation may become of consequence for primary productivity; for the final days of production in a GNCM system, this light limitation is of lesser importance as the pigment content of the water column degrades rapidly during the final stages of the bloom (see Flynn and Hansen 2013).

Comparison of the cumulative primary productivity, by phytoplankton and the mixotrophs (phyto and mixo, respectively, bottom panel in Fig. 5) over the 30-day simulation period under the three alternative scenarios, showed a substantially higher amount of primary production when CMs were implemented (Fig. 5; C-fix in Supplementary Material Figs S1-S3). Production of dissolved organics originating directly from primary production was similarly enhanced for CMs (Supplementary Material Fig. S3; cf. Figs S1 and S2). The regeneration of dissolved inorganics, typically associated with predatory activities, was enhanced where GNCMs were implemented (scenario B; Supplementary Material Fig. S2; cf. Figs S1 and S3).

As yet we know little detail about the mechanisms used by mixotrophs to modulate their photoauto- vs phagohetero- trophic capabilities. Different species may occupy different regions of the continuum from a phototrophic extreme to a phagotrophic extreme, while the ecophysiology of others will be predominantly photoauto- or predominantly phagoheterotrophic. The critical issue, then, is whether the protists are dependent upon other organisms (i.e., NCMs, Fig. 3D-F) for the acquisition and continuation of their mixotrophic potential, or if they possess the full genetic and/or physiological capacity to

undertake both modes of nutrition all of the time (i.e., CMs, Figs 2 and 3C).

Discussion

A major reason for dividing the protists into the proposed functional groups (Figs 2 and 3) is the recognition of the differences in the consequential population dynamics and role of the groups in ecosystems (Figs 4 and 5). The roles of the non-phagotrophic and non-phototropic forms (representative of traditional "phytoplankton" and "microzooplankton") are established. A role of CMs is largely acknowledged in the literature, although discussions are dominated by alternate energy supply options, while we suggest the roles of photo- and phago- trophy are more likely linked to synergy in energy and nutrient supply routes (see also Wilken et al. 2014a, b). The CMs have nonetheless drawn only limited attention of modellers. The scope for a revision of the ecological role of CMs is illustrated by their suggested relationship with bacteria, wherein especially nano-sized CMs promote bacterial growth by release of DOM, and thereby gain nutrients they would otherwise be unable to acquire (Mitra et al. 2014a). The GNCM group is expected to have quite different population dynamics from other mixotrophs, being dependent (within a generation time) on a repeated ingestion of phototrophic prey for chloroplasts. Flynn and Hansen (2013) indicate some differences in these dynamics, but there likely is much more to explore, related to the effects of mixotrophy on assimilation efficiency and of photon dose on the longevity of acquired plastids. The SNCMs at first sight may be considered similar to CMs, but there are sharp contrasts in the nature of the host (ciliate, Foraminifera etc.) in comparison with that of CMs (phytoflagellates, dinoflagellates etc.) and of the main prey types. While the need for donors of phototrophy (as plastids or as endosymbionts) is less frequent for SNCMs than for GNCMs, the specialism in that need places an additional dynamic in their relationship with other planktonic members of the ecosystem.

The ecology of CMs is in some ways relatively simple, as they do not need to acquire their mixotrophic potential (for phototrophy) from another organism. Nevertheless, there are sound reasons to sub-divide CMs into those that consume bacteria (Hartmann et al. 2012; Unrein et al. 2014; Zubkov and Tarran 2008) versus those capable of (also) consuming non-bacterial prey (Burkholder et al. 2008; Stoecker et al. 2006). This is especially true

if the latter are competitors in terms of phototrophy, or even potential predators of the mixotrophs (Thingstad et al. 1996). The simulations run here (Fig. 5) were configured for a mesotrophic system where phagotrophy by μZ, GNCMs and CMs predominantly involves ingestion of phytoplankton prey. Within the three scenarios (Figs 4 and 5), it was assumed that the predators consumed only phytoplankton. The range of size of the protist phytoplankton group in these systems could vary by orders of magnitude (e.g., nano- to micro- sized). Likewise, their protist grazers could occupy a large size spectrum. For example, the size spectrum for the prey for GNCMs (15-60 μm ESD) can vary between 1-40 μm ESD (McManus et al. 2012; Stoecker et al. 1987). Divisions between those capable of consuming different prey sizes may be achieved via allometric considerations. However, there are plenty of examples of mixotrophs feeding on prey larger than themselves and, also, of larger species feeding on bacteria or picocyanobacteria (Glibert et al. 2009; Granéli et al. 2012; Jeong 2011; Jeong et al. 2005, 2012; Lee et al. 2014; Seong et al. 2006). Accordingly, functional group divisions that either partition protists solely according to predator-prey allometrics (e.g., Sieburth et al. 1978), or have a sliding scale for photoauto- versus phagohetero- trophy (e.g., Jones 1997), appear insufficiently robust from an ecological perspective. Below we consider each group in more detail.

CM group. In the photic zone plankton, CMs (Fig. 3C) often dominate eukaryotic microbial biomass (cf. Supplementary Material Fig. S3), both in eutrophic and oligotrophic systems across all climate zones (Burkholder et al. 2008; Hartmann et al. 2012; Havskum and Riemann 1996; Li et al. 2000a, b; Sanders and Gast 2012; Stoecker et al. 2006; Unrein et al. 2007, 2014). Mixotrophic phytoflagellates have accounted for 50% of the pigmented biomass during non-bloom conditions off Denmark (Havskum and Riemann 1996). Constitutive mixotrophy has been identified as a major mode of nutrition for harmful phytoflagellate species in eutrophic coastal waters (Burkholder et al. 2008; Jeong et al. 2010; Stoecker et al. 2006) and CMs can account for significant and occasionally dominant predation pressure on bacteria (Hartmann et al. 2012; Sanders et al. 1989; Unrein et al. 2014; Zubkov and Tarran 2008). For example, it has been shown that mixotrophy plays a vital role in the trophic dynamics of the oligotrophic gyres (Hartmann et al. 2012; Zubkov and Tarran 2008). Modelling this ecosystem using the traditional paradigm (Scenario A, Fig. 4) would fail to portray the correct dynamics and, thus, would be

misleading. Most of the phytoplankton in these systems are bacterivores, and without mixotrophy (photoautotrophy plus bacterivory), primary production would be much lower due to N and P starvation.

The differences between CMs and NCMs are clear and unambiguous. While each group includes examples of the sliding scale and allometrics, we justify the split based on the definition of the source of the phototrophic potential (innate versus acquired), because this has profound impacts for the ecophysiology of the organisms.

GNCM vs SNCM groups. Up to about one-third of photic zone ciliate microzooplankton are GNCMs (Calbet et al. 2012; Dolan and Pérez 2000; McManus et al. 2004; Pitta et al. 2001; Fig. 3D). In summer stratified surface waters, more than 50% of ciliates have on occasion been found to be mixotrophic (Calbet et al. 2012; Putt 1990; Stoecker et al. 1987). The contribution of these GNCMs to "phytoplankton" biomass (as chlorophyll) can thus be substantial (Stoecker et al. 2013; see also Fig. 5 and Supplementary Material Fig. S2). They can comprise a significant proportion of all zooplankton, and their ecology is not only linked to their mixotrophic capabilities, but also limited by their need to consume phototrophic prey in order to acquire chloroplasts.

The SNCMs (Fig. 3D-F), requiring specific prey, present a different ecological dynamic in comparison with the CMs and GNCMs. They do not need to interact with specific prey often, but when they need to do so, they must consume one of only a few prey options in order to acquire phototrophy. That restriction has important implications for the SNCMs and for their specific prey. In blooms, the cosmopolitan SNCMs *Mesodinium rubrum* and *M. major* can account for approaching 100% of plankton biomass (Crawford et al. 1997; Garcia-Cuetos et al. 2012; Herfort et al. 2012; Montagnes et al. 1999; Packard et al. 1978). This is possible as the need for plastids by the red *Mesodinium* species is occasional (Johnson and Stoecker 2005; Johnson et al. 2006), and acquired cryptophyte plastids can even replicate within the ciliate (Hansen et al. 2012; Johnson et al. 2007).

The Foraminifera and Radiolaria (Acantharia and Polycystinea) eSNCMs (Fig. 3F) contribute significantly to primary production and trophic dynamics in oligotrophic oceanic gyres, and thus play a vital role in marine biogeochemistry (Caron et al. 1995; Decelle et al. 2013; Dennett et al. 2002; Gast and Caron 2001; Michaels et al. 1995; Stoecker et al. 2009; Swanberg 1983). These eSNCMs, the dominant large planktonic predators in the subtropical

gyres, probably could not survive and grow in this nutritionally dilute environment without the C supplement from their symbionts (Caron et al. 1995), suggesting a major role of mixotrophy in shaping the trophic structure of subtropical gyre ecosystems.

Conclusion

Functional group descriptors are specifically intended to aid our understanding of ecology (Gitay and Noble 1997; Smith et al. 1997). Arguably the ultimate test of that understanding comes from an ability to construct and use mathematical models which display behaviours that align with expectations gained from empirical knowledge.

Based on experimental and modelling studies, we now realise that mixotrophy in protists can be a synergistic process and does not just provide a "top-up" or "survival" mechanism (Adolf et al. 2006; Mitra and Flynn 2010; Mitra et al. 2014a; Våge et al. 2013). The nature of that synergism between photo- and hetero- trophy is ultimately modulated by whether the phototrophic capacity is constitutive (innate) or acquired. Accordingly, we propose that the functional group descriptors for planktonic protists should align with the innate capacity, or otherwise, for phototrophy and/or phagotrophy (Fig. 2). A division on these grounds makes sense for modelling, both from evolutionary and ecological perspectives, in that these groups are clear and unambiguous. Such traits are important features in functional group definitions. Within this division, the groups described by Jones (1997) and Stoecker (1998) form important secondary functional group descriptions, while those by Pratt and Cairns (1985) form a tertiary level of description for both mixotrophic and heterotrophic protists.

Over the past decade there has been a drive to modify biogeochemical and aquatic food web models through incorporation of the functional group approach. Within these models, plankton functional types are increasingly deployed to aid descriptions of processes from biogeochemistry to fisheries (e.g., end-to-end models; Rose et al. 2010). The primary focus has been on splitting the "phytoplankton" into several groups – for example, into "diatoms" requiring Si, "calcifiers" requiring calcium, etc. – with each group having its own state variables (e.g., Baretta et al. 1995). Far less emphasis has been placed on expanding the "zooplankton" component (see Mitra et al. 2014b and references therein), while "mixotrophic" groups are typically ignored altogether. Modellers typically start with

an attempt to simplify the system sufficiently to enable or assist computation and analysis. That simplification process must not be so great that the critical linkage to reality is lost. Given our renewed appreciation of mixotrophy (Flynn et al. 2013 and references therein), we suggest that a complete overhaul of the core structure of biogeochemical and plankton food web models may be warranted, in order to provide a more accurate ecological perspective on ecosystems functioning that acknowledges the existence of mixotrophs.

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